UNIVERSITAT DE
BARCELONA

Combinació de l’anàlisi dirigida i no dirigida per a la identificació i determinació de contaminants

Adrià Sunyer Caldú

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Adrià Sunyer Caldú

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Adrià Sunyer Caldú

Programa de doctorat
"Química Analítica i Medi Ambient"

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Memòria presentada en compendi d'articles per optar al títol de
Doctor per la Universitat de Barcelona

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Barcelona, 17 de novembre del 2022

Directora	Director
Dra. M. Silvia Diaz Cruz	Dr. Pablo Gago Ferrero

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"The important thing is not to stop questioning. Curiosity has its own reason for existence. One cannot help but be in awe when he contemplates the mysteries of eternity, of life, of the marvelous structure of reality. It is enough if one tries merely to comprehend a little of this mystery each day."

Albert Einstein

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TABLE OF CONTENTS

ACRONYMS

$logk_{ow}$	Octanol-water partition constant
4HB	4-hydroxybenzophenone
ACN	Acetonitrile
acSMX	Acetylsulfamethoxazole
ADI	Acceptable daily intake
AGR	Antibiotic resistance genes
ARB	Antibiotic resitance bacteria
AVO	Avobenzone
BePB	Benzylparaben
BP	Benzophenone
BP3	Benzophenone-3, oxybenzone
BP4	Sulfisobenzone
BuPB	Butylparaben
BZT	Benzotriazole
BZX	3,4-Dihydro-2H-1,4-Benzoxazin-6-ol
C	Carbon atoms
CECs	Contaminants of emerging concern
CBZ	Carbamazepine
CFF	Caffeine
CLA	Clarithromycin
CLI	Clindamycin
CPX	Ciprofloxacin
DCF	Diclofenac
DCOIT	4,5-dichloro-2-octyl-4-isothiazolin3-one
DDA	Data dependent acquisition
DIA	Data independent acquisition
DIU	Diuron
DMBZT	Dimethylbenzotriazole
ECHA	European Chemicals Agency
EDI	Estimation daily intake
EEA	European Economic Area
EHMC	2-ethyl-hexyl-4-trimethoxycinnamate
ERY	Erythromycin
ESI	Electrospray ionization
EtPABA	Benzocaine
EtPB	Ethylparaben
F	F atoms
FDA	Food and Administration Drug agency
GC	Gas chromatography
GMZ	Gemfibrozil
HILIC	Hydrophilic interaction liquid chromatography
HPLC-MS/MS	High performance liquid chromatography coupled to mass spectrometry
HRAP	High-rate algae ponds
HRMS	High resolution mass spectrometry
IEC	Ion-exchange chromatography
IPC	Ion-pair chromatography
IRG	Irgarol
KPF	Ketoprofen
L-L	Liquid-liquid
LOQ	Limit of quantification
LRMS	Low resolution mass spectrometry
m/z	Mass-to-charge ratio
MeBZT	Methylbenzotriazole
MePB	Methylparaben
MFA	Mefenamic acid
MPY	N-Methyl-2-pyrrilodone
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MSPD	Matrix solid phase dispersion
MTBE	Methyl tert–butyl ether
NDX	Nalidixic acid
NORMAN-SLE	Norman Suspect List Exchange
NPX	Naproxen
OC	Octocrylene
PBs	Paraben preservatives
PCPs	Personal care products
PFAS	Per- and polyfluoroalkyl substances
PFHxS	Perfluorooctanoic acid
PFOA	Perfluorohexanesulfonic acid
PFOS	Perfluorooctanesulfonate
PLE	Pressurized liquid extraction
POCIS	Polar organic chemical integrative samplers
PPCPs	Pharmaceuticals and personal care products
PrPB	Propylparaben
QqQ	Triple quadrupole

ACRONYMS

SAT	Soil aquifer treatment
SFC	Supercritical fluid chromatography
SMX	Sulfamethoxazole
(d-)SPE	(Dispersive) Solid-phase extraction
q-TRAP	Quadrupole-ion trap
QuEChERS	Quick, Easy, Cheap, Efficient, Rugged and Safe
RPLC	Reversed-phase liquid chromatography
RQ	Risk quotients
SCY	Salicylic acid
SER	Sertraline
SI	Supplementary information
SMD	Sulfisomidine
SPY	Sulfapyridine
TMP	Trimethoprim
TOF	Time of flight
TPs	Transformation products
UAE	Ultrasound assisted extraction
UVFs	Ultraviolet filters
WWTPs	Wastewater treatment plants

Abstract

Contaminants of emerging concern (CECs) are those that have been detected in the environment and/or in humans, can cause ecological or human health impacts, and are normally not regulated. Their continuous introduction into the environment (favoured by conventional wastewater treatments' inefficiency in their degradation), pseudo-persistence, and intrinsic ability to interfere with organisms, concern the scientific and public community. Their potential toxic effects can threaten the ecological status of water bodies as well as human health. There is a need to know what these contaminants are and understand their occurrence, fate and transformation processes in the environment. In the current context of climate change, circular economy processes acquire great importance. In this regard, the reuse of waste and reclaimed water is applied as a helpful solution to alleviate water scarcity and enable better use of resources. However, this approach is not exempt from risk, since the water-borne contaminants can be translocated into crops after irrigation, constituting a threat to human health. Likewise, there are other routes of exposure to CECs that should be studied, and human biomonitoring is necessary to address specific exposures.

In this thesis, powerful analytical methodologies were developed and applied in the different stages of the CECs cycle, from their release until they are degraded (transformed) or (bio)accumulated. The presence of different types of CECs including personal care products (PCPs), pharmaceuticals, biocides and per- and polyfluoroalkyl substances (PFAS) was assessed in different environmental compartments. Advanced target and non-target approaches were applied to expand the knowledge of the presence of these chemicals and their derived transformation products in water, sediment, biota, and crops. Special emphasis was placed on evaluating the wastewater reuse feasibility for irrigation purposes in agriculture. Finally, human exposure to CECs was evaluated, putting a special focus on prenatal exposure at the early stage of development through the analysis of umbilical cord blood.

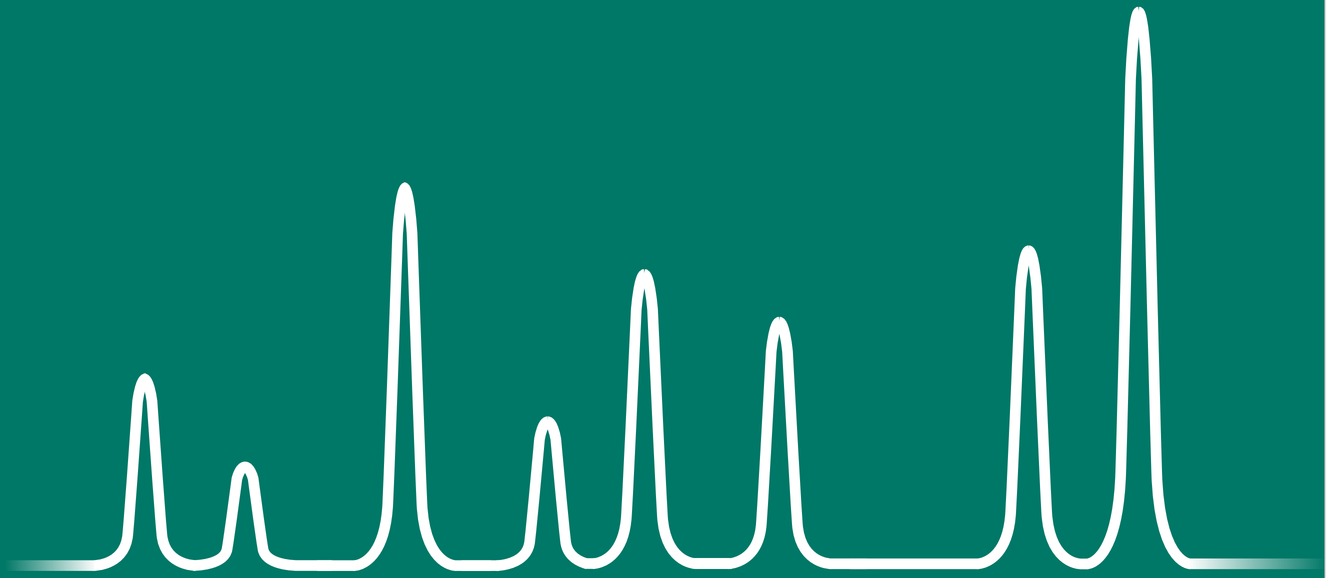
Thus, this thesis has contributed to improving the understanding and knowledge about CECs occurrence, removal, transformation, transfer, and fate in aquatic ecosystems, agrifood environment and, ultimately, humans.

Resum

Els contaminants d'interès emergent (CECs) són aquells que s'han detectat en el medi ambient i/o en humans, poden causar impactes ecològics o en la salut humana i normalment no estan regulats. La seva contínua introducció al medi ambient (afavorida per la ineficiència dels tractaments d'aigües residuals convencionals), la pseudo-persistència i la seva capacitat intrínseca d'interferir amb els organismes, preocupen la comunitat científica i la societat en general. Els seus potencials efectes tòxics poden amenaçar l'estat ecològic de les masses d'aigua així com la salut humana. Cal conèixer quins són aquests contaminants i comprendre la seva presència, destí i processos de transformació en el medi ambient. En el context actual de canvi climàtic, els processos emmarcats en l'economia circular adquireixen una gran importància. En aquest sentit, la reutilització d'aigües residuals i renaturalitzades s'aplica com una solució útil a l'escassetat d'aigua i permeten un millor aprofitament dels recursos. Tanmateix, aquest enfocament no està exempt de risc, ja que els contaminants transmesos per l'aigua es poden traslladar als cultius després del reg, representant una amenaça per a la salut humana. Així mateix, hi ha altres vies d'exposició als CECs que s'han d'estudiar, i és necessari el biomonitoreig en humans per avaluar exposicions específiques.

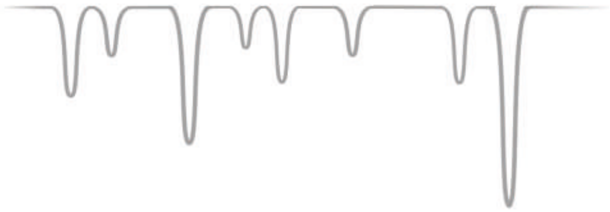
En aquesta tesi, es van desenvolupar i aplicar potents metodologies analítiques en les diferents etapes del cicle dels CECs, des d'el seu alliberament fins a la seva degradació (transformació) o (bio)acumulació. Es va avaluar la presència de diferents tipus de CECs, inclosos productes per a la cura personal (PCP), fàrmacs, biocides i substàncies per- i polifluoroalquilades (PFAS) en diferents compartiments ambientals. Es va aplicar l'anàlisi dirigida i no dirigida per ampliar el coneixement de la presència d'aquests productes químics, i els productes de transformació derivats, a l'aigua, els sediments, la biota i els cultius. Es va posar especial èmfasi en l'estudi de la viabilitat de reutilitzar aigües residuals amb finalitats agrícoles. Finalment, es va avaluar l'exposició humana als CECs, posant especial atenció en l'exposició prenatal, durant fases inicials del desenvolupament, mitjançant l'anàlisi de la sang del cordó umbilical.

Així, aquesta tesi ha contribuït a millorar la comprensió i el coneixement sobre la presència, eliminació, transformació, transferència i destí dels CECs en ecosistemes aquàtics, entorns agrícoles i, en definitiva, humans.



Chapter 1

General introduction



1.1 Contaminants of emerging concern

The term contaminants of emerging concern (CECs) describes a broad range of chemical compounds usually not regulated by any environmental legislation (Dulio et al., 2018). However, they can reach environmental compartments (mostly water bodies, but also biota) and humans, constituting a threat to ecosystems and human health. The lack of regulations covering these contaminants results from the limited information available regarding their occurrence or published health impacts. CECs include different compound classes, namely pharmaceuticals and personal care products (PPCPs), per- and polyfluoroalkyl substances (PFAS), pesticides, biocides, (old and novel) flame retardants, plasticizers, surfactants, and industrial chemicals, among others, as well as their related transformation products (TPs).

Some CECs are massively produced, mainly because they have essential uses by society. For example, ultraviolet filters (UVFs) are necessary to protect us against deleterious ultraviolet radiation from the sun. Since the demand for chemical products in society is only expected to grow with population and industrial development, so will their occurrence in the environment unless action is taken. **Figure 1.1** shows the clear increasing trend in the revenue of the personal care products market between 2013 and 2022 and the estimation for the coming years. Despite the decrease in 2020 and 2021, probably due to the COVID-19 pandemic, the increase between 2015 and the predicted value in 2026 is almost 50%. Regardless of this increasing trend, the production of compounds hazardous to the environment and human health has remained relatively high in recent years, as shown in **Figure 1.2** for the production of chemicals between 2004 and 2020 in the European Union. The significant decrease in 2009 was probably due to the economic recession. Overall, these data demonstrates that, despite a slight decrease trend, the production of hazardous chemicals needs to be reduced in the European Union.

CECs’ properties vary widely among families of compounds. Still, their persistence usually comes from its hydrophobicity (or lipophilicity), which indicates a compound’s tendency to have more affinity for non-aqueous environments over aqueous ones (Kaliszan, 2015). However, most of these contaminants are considered of emerging concern because they are “*pseudo-persistent*” since they are continually released into the environment through different pathways (Rosenfeld and Feng, 2011).

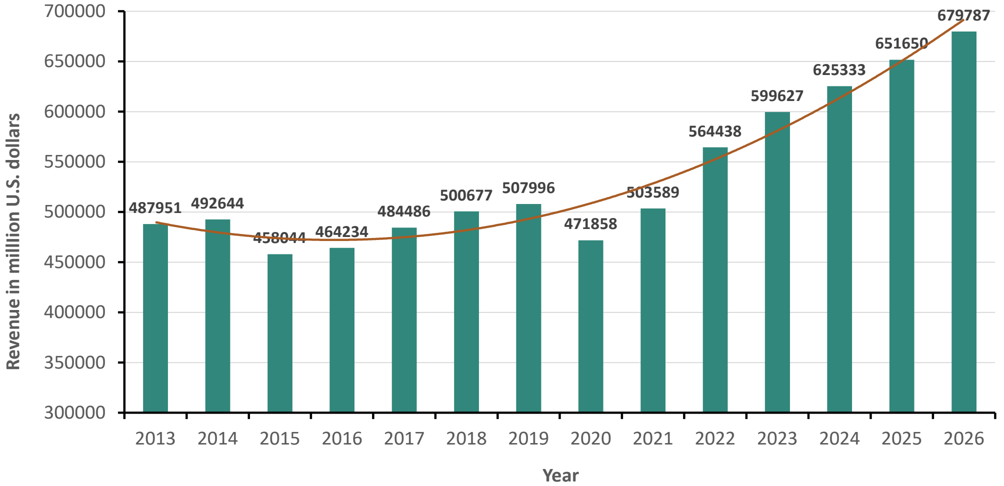


Figure 1.1. Revenue of the beauty and personal care market worldwide from 2013 to 2026 (in U.S. million dollars). (Statista, 2022a)

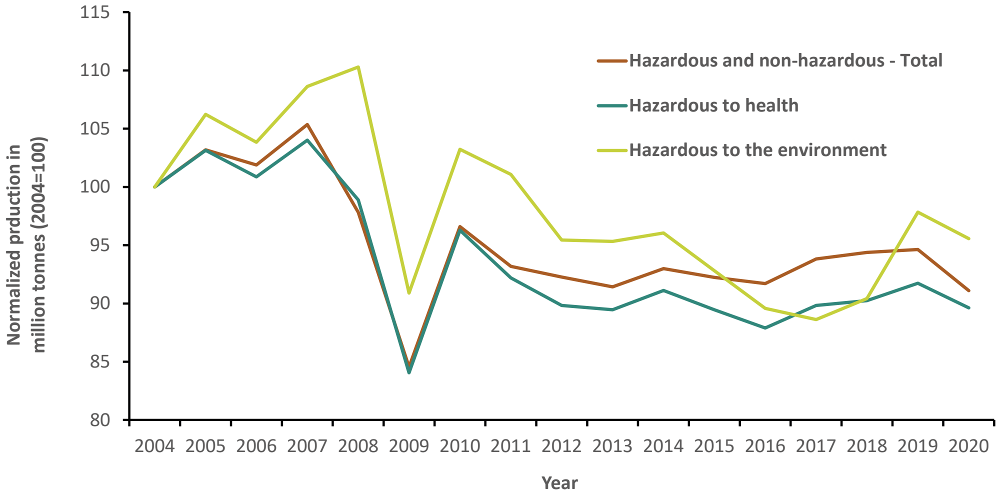


Figure 1.2. Production trends of chemicals between 2004 and 2020 in the European Union. (Eurostat, 2022)

Discharges from wastewater treatment plants (WWTPs) are one of the main causes of aquatic pollution. Conventional WWTPs, primarily operating through biological processes, were designed to protect water resources mainly by promoting microbial community growth. It removes the carbon, nitrogen, and phosphorous present in the influent. However, the increased detection of a wide range of non-regulated anthropogenic CECs in the aquatic environment shows the limitations of WWTPs in removing these compounds, and ultimately, they can reach humans. However, for most CECs, there is little (eco)toxicity information, especially regarding long-term or low-level exposures. Moreover, these compounds are always mixed with others, making it more challenging to evaluate their toxicity with potential synergistic effects that they might display.

Although thousands of CECs are known and have been reported in the literature, they represent a tiny fraction of the universe of environmental contaminants. These unidentified compounds are neglected, ignored, omitted, or overlooked mainly because of limitations of the analytical instrumentation. Nevertheless, recent advances in analytical and especially in high-resolution mass spectrometry (HRMS) techniques have enabled detecting and identifying part of these unknown compounds. With the combination of robust data treatment and in-silico fragmentation tools, data prioritization has been improved considerably. Therefore, with these new possibilities, many new compounds will join the category of CECs soon (Gao, 2022).

This thesis focuses on personal care products (PCPs), pharmaceuticals, biocides, and PFAS, together with some of their most important TPs. PCPs (especially UVFs and paraben preservatives (PBs)) are the most investigated compounds in this thesis since they are suitable candidates to occur in environmental and human matrices based on their extensive use and lipophilic properties. Pharmaceuticals were included mainly in the water reuse and removal studies since their occurrence in waste and reclaimed water is inevitable and concerning. Biocides were included in two studies where the sampling was performed in areas with intensive maritime activity, such as ports and shipyards. Finally, PFAS were included in a study conducted in Sweden, where the environmental occurrence of these substances is extremely concerning (Weiss et al., 2021).

A brief introduction to each studied family is given in the subsequent subsections.

1.1.2 Personal care products (PCPs)

This group encompass any substance intended to be placed in contact with the external parts of the human body (e.g. skin, nails, hair, lips, etc.) or with the teeth and the mucous membranes of the oral cavity to maintain them clean, perfumed, protected, or to change the appearance, keep them in good condition and to correct body odours (Aranaz et al., 2018). It includes sunscreens, household products, perfumes, shampoos, lotions, soaps, and cosmetics, but also products of basic hygiene. Therefore, the most used compounds in this group are UVFs, parabens (PBs), surfactants, plasticisers, hair dyeing products and fragrances. In fact, thousands of compounds with different physicochemical properties can be included in this category.

After the consumer's direct application/use to the body, most of these compounds are washed out (shampoos, toothpaste, or shower gel) and transported by grey waters. Still, some of them are retained by the body and accumulated or excreted later (e.g. lotions, sunscreens, and sanitisers) (Ray et al., 2020). Ultimately, they end up either in the human body or the environment, potentially causing unknown effects. The focus in this thesis has been put mainly on UVFs and PBs because they are extensively used. Still, some works include other families, such as plasticisers, surfactants and hair dyeing compounds.

Ultraviolet filters (UVFs)

As the name suggests, UVFs absorb and/or reflect the sun's ultraviolet radiation protecting humans against its harmful effects. They can be classified into two groups according to their nature. Inorganic UVFs (also called physical UVFs) reflect or scatter the ultraviolet light, while organic ones (also called chemical UVFs) absorb it. The compounds included in the latter grup usually possess aromatic structures, conjugated carbon-carbon double bonds and carbonyl moieties (Chisvert and Salvador, 2007).

Like the other CECs, their use is increasing yearly, mainly because, with climate change and the ozone layer depletion, the receiving radiation intensifies, but also because of population growth and its concern to avoid contracting diseases caused by sun exposure.

Figure 1.3 shows the revenue of the sun protection market worldwide; the increasing trend is very similar to the one previously shown for the personal care product market; the decrease due to the pandemic can also be observed. UVFs are used in sunscreens but also in packages of different products to prevent ultraviolet light from damaging scents, colours, or food, but also in textiles, painting, and building materials (Gao et al., 2015). In humans, once the sun care product is applied, a percentage of it is washed out, but it is mainly absorbed through the skin. It can be further accumulated in the human body, as such or as a metabolite, until further excretion.

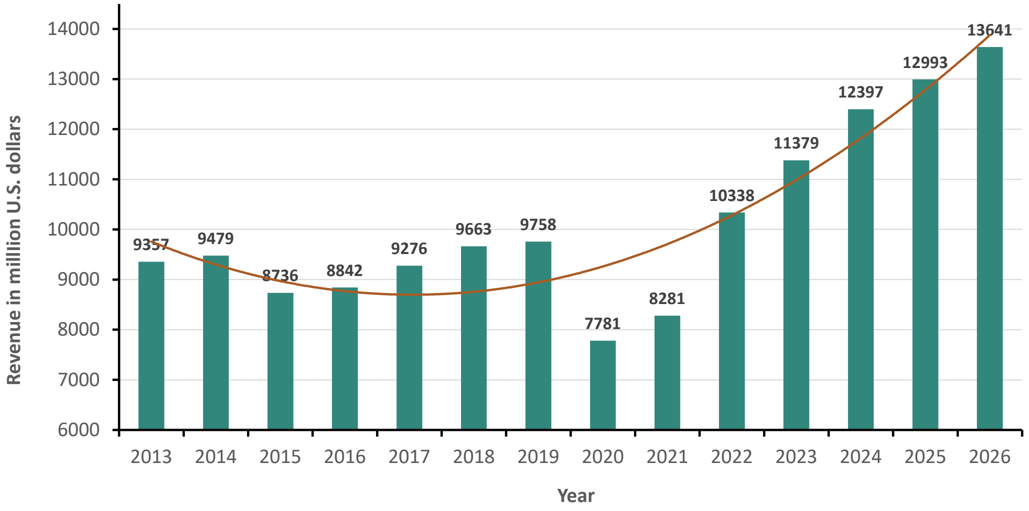


Figure 1.3. Revenue of the sun protection market worldwide from 2013 to 2026 (in U.S. million dollars). (Statista, 2022b)

Within UVFs, the most used family in formulations are benzophenones which are characterised by two aromatic rings linked with a ketone, as shown in **Table 1.1** (Mitchelmore et al., 2021). The parent compound is benzophenone (BP), from which all the benzophenone derivatives are originated. Benzophenone-3 (also known as oxybenzone, BP3) is the most used UVF worldwide in PCPs (it is estimated to be present in 20-30% of commercial products) (Aronson, 2016). Still, some of its TPs (e.g. 4-hydroxybenzophenone (4HB)) are banned in cosmetics in Europe (European Chemicals Agency (ECHA), 2022a). Octocrylene (OC) or avobenzone (AVO) are also benzophenones (or compounds derived from it) extensively used in the PCPs' formulations and are frequently formulated simultaneously with BP3.

Another important family within UVFs are benzotriazoles. These compounds present a benzene nucleus fused to a 1H-1,2,3-triazole ring, making them very versatile. This ring can be combined with many functional groups, resulting in widely different benzotriazoles. The most frequently used are benzotriazole (BZT), simplest member of this class, methyl-benzotriazole (MeBZT), and dimethyl-benzotriazole (DMBZT). Others UVFs included in this list are camphor, cinnamate and salicylate derivatives.

Table 1.1. Most frequently used UVFs and PBs in PCPs' formulations, with family, CAS number, molecular mass, structure, and logK$_{ow}$.

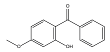
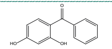
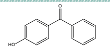
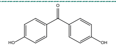
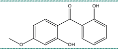
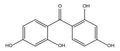
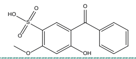
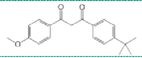
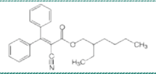
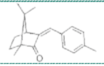
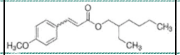
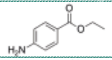
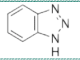
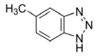
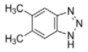
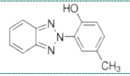
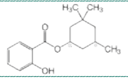
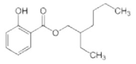
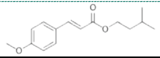
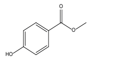
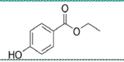
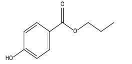
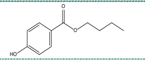
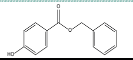
Compound	Other names	Family	CAS number	Molecular mass (g/mol)	Structure	logK$_{ow}$
Ultraviolet filters (UVFs)						
Benzophenone-3 (BP3)	Oxybenzone; 2-Hydroxy-4-methoxybenzophenone	Benzophenones	131-57-7	228.24		3.79
Benzophenone-1 (BP1)	2,4-Dihydroxybenzophenone	Benzophenones	131-56-6	214.22		3.15
4-Hydroxybenzophenone (4HB)	-	Benzophenones	1137-42-4	193.18		2.92
4,4'-Dihydroxy benzophenone (DHB)	-	Benzophenones	611-99-4	214.22		2.19
2,2'-Dihydroxy-4-methoxy benzophenone (DHMB)	Benzophenone-8; Dioxybenzone	Benzophenones	131-53-3	244.25		3.82
Benzophenone-2 (BP2)	2,2',4,4'-Tetrahydroxy benzophenone	Benzophenones	131-55-5	246.22		2.78
Benzophenone-4 (BP4)	5-benzoyl-4-hydroxy-2-methoxy benzene sulfonic acid: HMBS; Sulisobenzone	Benzophenones	4065-45-6	308.31		0.88
Avobenzone (AVO)	1-(4-tert-butylphenyl)-3-(4-methoxyphenyl)propane-1,3-dione	Benzophenones	70356-09-1	310.17		4.51
Octocrylene (OCR)	2-Ethylhexyl 2-cyano-3,3-diphenylacrylate	Benzophenone origin	6197-30-4	261.48		6.1
Enzacame (4MBC)	3-(4-Methylbenzilidene) camphor	Camphor derivatives	36861-47-9	254.17		5.14
Octinoxate (EHMC)	2-Ethylhexyl trans-4-methoxy cinnamate	Cinnamate derivatives	5466-77-3	290.4		5.8
Benzocaine (EtPABA)	Ethyl 4-aminobenzoate	p-aminobenzoic acid derivatives	94-09-7	165.19		1.86
Benzotriazole (BZT or 1H-BZT)	1,2,3-Benzotriazole	Benzotriazoles	95-14-7	119.12		1.44
Methyl-benzotriazole (MeBZT or 5-MeBZT)	5-Methyl-1H-benzotriazole	Benzotriazoles	136-86-5	133.15		Not available
Dimethyl-benzotriazole (DMBZT)	5,6-Dimethyl-1H-benzotriazole monohydrate	Benzotriazoles	4184-79-6	147.18		Not available
Drometrizole (UVP)	Benazol P; Tinuvin P; 2-(2H-Benzotriazol-2-yl)-p-cresol	Benzotriazole derivative	2440-22-4	225.25		4.31
Homosalate (HOM)	Homomenthyl salicylate	Salicylates	118-56-9	262.34		5.82
Octisalate (OCS)	2-Ethylhexyl salicylate	Salicylates	118-60-5	250.33		5.94
Amiloxate	Isoamyl 4-methoxycinnamate	Cinnamate derivatives	71617-10-2	248.32		4.06

Table 1.1. Continued.

Compound	Other names	Family	CAS number	Molecular mass (g/mol)	Structure	logK$_{ow}$
Paraben preservatives (PBs)						
Methyl paraben (MePB)	Methyl 4-hydroxybenzoate	Parabens	99-76-3	152		2
Ethyl paraben (EtPB)	Ethyl p-hydroxybenzoate	Parabens	120-47-8	116.17		2.47
Propyl paraben (PrPB)	Propyl 4-hydroxybenzoate	Parabens	94-13-3	180.2		2.98
Butyl paraben (BuPB)	Butyl 4-hydroxybenzoate	Parabens	94-26-8	194.23		3.47
Benzyl paraben (BePB)	Benzyl 4-hydroxybenzoate	Parabens	94-18-8	228.24		3.7

The octanol-water constant (logK$_{ow}$) indicates a compound's lipophilicity (or hydrophobicity). These values for most of the UVFs shown in **Table 1.1** are considerably high. For example, the extensively used BP3, OC, or AVO present values > 3.7, with OC having the highest value in **Table 1.1** (6.1). It is not surprising, then, that chemical compounds produced by millions of tons yearly with these logK$_{ow}$ values can accumulate in environmental compartments.

Paraben preservatives (PBs)

Parabens are a group of compounds used as preservatives, usually in cosmetics, PCPs, pharmaceuticals and food products. Its function is to prevent the growth of bacteria, fungi and yeast, preventing products from being in poor condition or spoiling. With these properties, PBs contribute to the extension of products' lifetime and make them safer for consumption. The most commonly used PBs are shown in **Table 1.1**, and it is frequent to find more than one combined in the formulations.

PBs' chemical structure consists of a para-hydroxybenzoate linked with an alkyl group of different lengths. The most used in formulations is methylparaben (MePB), and the use frequency decreases when increasing the alkyl chain length (MePB> Ethylparaben (EtPB) > Propylparaben (PrPB) > Butylparaben (BuPB) > Benzylparaben (BePB)). These compounds present logK$_{ow}$ values similar to those of UVFs, but as shown in **Table 1.1**, it also increases with the alkyl chain length, making the longest ones more toxic and lipophilic. As for most CECs, these conditions are ideal for facilitating their entrance into environmental compartments.

1.1.3 Pharmaceuticals

This group is constituted by any chemical compound meant for medical purposes to diagnose, treat, mitigate and prevent sickness or disease. This broad group of compounds can be divided into different families depending on the pharmaceutical purpose: analgesics, anti-epileptics, anti-depressants, anti-inflammatories, lipid regulators, antibiotics, hormonal agents, antivirals, cardiovascular agents, and stimulants, among others. Thousands of compounds fit this description, so only the most relevant in terms of detection frequency, toxicity and intrinsic properties were included for analysis in this thesis (**Table 1.2**).

Among the group of compounds described, pharmaceuticals have the highest industrial production and use, with more than 100000 tonnes consumed globally yearly (German Environment Agency, 2014). Some of them are highly lipophilic ($\log k_{ow}$>4), such as gemfibrozil (GMZ), mefenamic acid (MFA), diclofenac (DCF), and sertraline (SER), others are frequently reported in environmental matrices because of its extensive use, such as caffeine (CFF), carbamazepine (CBZ), and naproxen (NPX), and some antibiotics, such as the sulfonamides family, contribute to the development of antibiotic resistance bacteria (ARB) and genes (AGR). Within this broad group, the physicochemical properties and chemical structures vary considerably (**Table 1.2**), making their simultaneous analysis very complex.

1.1.4 Biocides

Biocides are a group of compounds with a similar purpose to that of PBs because they inhibit the development of active organisms to preserve or extend a product's useful life. This family of compounds can be divided into four groups based on its purpose: disinfectants, preservatives, treatment against pests, and others (such as antifouling products or embalming fluids) (European Chemicals Agency (ECHA), 2012). Antifouling booster biocides are products commonly used in paints and further applied on surfaces submerged in seawater (to avoid marine biofouling).

In this thesis, the focus is only on the latter biocides because they are continuously and directly in contact with seawater, so the probability of ending up in the aquatic environment is higher. The most used antifouling biocides with their main properties are shown in **Table 1.3**.

Table 1.2. Frequently used compounds in pharmaceutical formulations, with family, CAS number, molecular mass, structure, and $\log K_{ow.}$

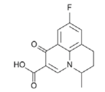
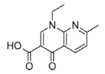
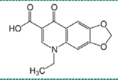
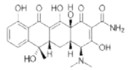
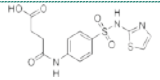
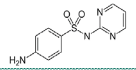
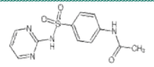
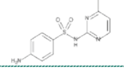
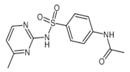
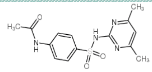
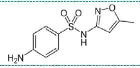
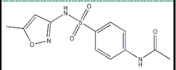
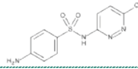
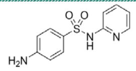
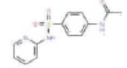
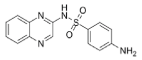
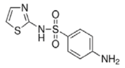
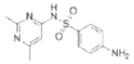
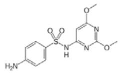
Compound	Other names	Family	CAS number	Molecular mass (g/mol)	Structure	$\log K_{ow}$
	Pharmaceuticals					
Flumequine (FLU)	Flumigal; Apurone	Fluoroquinolones	42835-25-6	261.25		1.6
Nalidixic acid (NDX)	Nalidixate; nalidixin	Quinolones	389-08-2	232.23		1.59
Oxolinic acid (OXL)	Nidantin; dioxacin	Quinolones	14698-29-4	261.23		0.94
Tetracycline (TCY)	Deschlorobiomycin	Tetracyclines	60-54-8	444.4		1.3
Succinylsulfathiazole (Succinyl-STZ)	Cremosuxidine; Colistatin	Sulfonamides	116-43-8	355.4		-
Sulfadiazine (SDZ)	Sulfapyrimidine	Sulfonamides	68-35-9	250.28		-0.09
N^4-acetylsufladiazine (acSDZ)	N-[4-(pyrimidin-2 -ylsulfamoyl)-pheyl] acetamide	Sulfonamides	127-74-2	292.32		Not available
Sulfamerazine (SMZ)	Sulfamethyldiazine	Sulfonamides	127-79-7	264.31		0.14
N4-acetyl sulfamerazine (acSMR)	N(4)-Acetyl sulfamerazine	Sulfonamides	127-73-1	306.34		Not available
N4-acetyl sulfamethazine (acSMZ)	N(4)-Acetyl sulfamethazine	Sulfonamides	100-90-3	320.37		Not available
Sulfamethoxazole (SMX)	Gantanol; sulfisomezole	Sulfonamides	723-46-6	253.28		0.89
Acetylsulfa methoxazole (acSMX)	N4-Acetyl sulfamethoxazole	Sulfonamides	21312-10-7	295.32		0.86
Sulfamethoxy pyridazine (SMPZ)	Sulphametoxy pyridazine	Sulfonamides	80-35-3	280.31		Not available
Sulfapyridine (SPY)	Sulfidin	Sulfonamides	144-83-2	249.29		0.35
Acetylsulfapyiridine (acSPY)	N-(4-(N-(Pyridin-2-yl) -sulfamoyl) -phenyl)acetamide	Sulfonamides	19077-98-6	291.33		Not available
Sulfaquinoxaline (SQX)	Avicocid	Sulfonamides	59-40-5	300.34		1.68
Sulfathiazole (STZ)	2-Sulfanil amidothiazole	Sulfonamides	72-14-0	255.3		0.05
Sulfisomidine (SMD)	-	Sulfonamides	515-64-0	278.33		-0.33
Sulfadimethoxine (SDM)	Sulfadimethoxy diazine	Sulfonamides	122-11-2	310.33		1.63

Table 1.2. Continued.

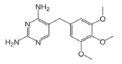
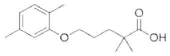
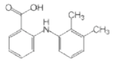
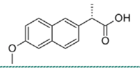
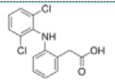
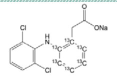
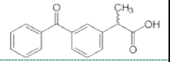
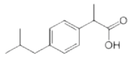
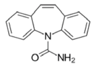
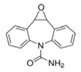
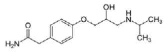
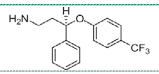
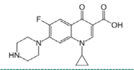
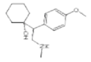
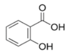
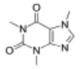
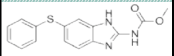
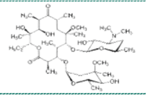
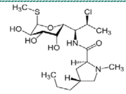
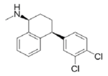
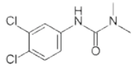
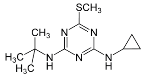
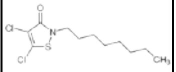
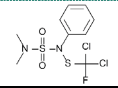
Compound	Other names	Family	CAS number	Molecular mass (g/mol)	Structure	logK$_{ow}$
	Pharmaceuticals					
Trimethoprim (TMP)	Proloprim; Trimpex	Others	738-70-5	290.32		0.91
Gemfibrozil (GFZ)	5-(2,5-Dimethyl phenoxy)-2,2-dimethyl pentanoic acid	Lipid regulators	25812-30-0	250.33		4.39
Mefenamic acid (MFA)	2-[(2,3-dimhetyl phenyl)-amino] benzoic acid	Analgesics	61-68-7	241.28		5.12
Naproxen (NPX)	-	Anti-inflammatories	22204-53-1	230.26		3.18
Diclofenac (DCF)	Voltaren	Anti-inflammatories	15307-86-5	296.15		4.51
Diclofenac-^{13}C (DCF-^{13}C)	Diclofenac- (acetophenyl ring-^{13}C$_6$)	Anti-inflammatories	1261393-73-0	302.15		Not available
Ketoprofen (KPF)	2-(3-Benzoylphenyl) propanoic acid	Analgesics	22071-15-4	254.28		3.12
Ibuprofen (IBU)	2-(4-Isobutylphenyl) propanoic acid	Anti-inflammatories	15687-27-1	206.28		3.97
Carbamazepine (CBZ)	5H-Dibenzo[b,f] azepine-5-carboxamide	Anti-epileptics	298-46-4	236.27		2.77
Carbamazepine-10,11-epoxide (CBZ-E)	-	Anti-epileptics	36507-30-9	252.27		Not available
Atenolol (ATL)	Tenorium; blokium; normiten	B-blockers	29122-68-7	266.34		0.16
Norfluoxetine (norFXT)	Desmethylfluoxetine	Anti-depressants	56161-73-0 83891-03-6	295.3		Not available
Ciprofloxacin (CFX)	-	Fluoroquinolones	85721-33-1	331.34		0.28
N-desmethylvenlafaxine (N-desVFX)	Norvenlafaxine	Anti-depressants	149289-30-5	263.37		Not available
Salicylic acid (SCY)	2-Hydroxybenzoic acid	Anti-septics	69-72-7	138.12		2.26
Caffeine (CFF)	1,3,7-Trimethyl xanthine; guaranine	Stimulants (drug)	58-08-2	194.19		-0.07
Fenbendazole (FBZ)	Panacur	Antihelmintic	43210-67-9	299.35		3.93
Clarithromycin (CFF)	Klaricid; Biaxin	Antibiotic	81103-11-9	747.95		0.69
Clindamycin	Chlolincocin	Antibiotic	18323-44-9	424.98		2.16
Sertraline	-	Anti-depressants	79617-96-2	306.23		4.3

Table 1.3. Frequently used booster biocides, with family, CAS number, molecular mass, structure, and logK$_{ow}$.

Compound	Other names	Family	CAS number	Molecular mass (g/mol)	Structure	logK$_{ow}$
	Biocides					
Diuron	3-(3,4-Dichloro phenyl)-1,1-di -methylurea	Urea	330-54-1	233.09		2.68
Irgarol	Cybutryne	Triazines	28159-98-0	253.37		3.95
4,5-Dichloro -2-octylisothiazol-3(2H)-one (DCOIT)	Kathon 930	Thiazoles	64359-81-5	282.23		2.8
Dichlofluanid	Elvarol; Euparen	Sulfamides	1085-98-9	333.23		1.59

1.1.5 Per- and polyfluoroalkyl substances (PFAS)

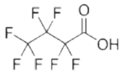
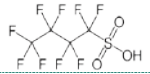
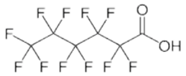
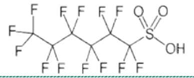
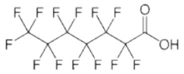
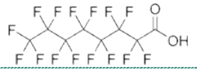
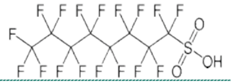
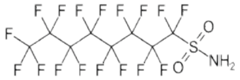
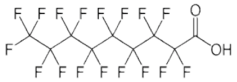
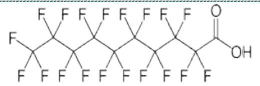
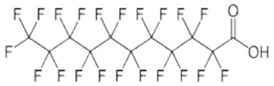
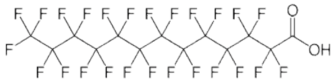
PFAS are human-made compounds with valuable properties, essential in almost all industry branches and many customer products. The most common uses are fire-fighting foam production, water-repellent clothing, and electroplating, but they have many other applications, most related to grease, water, or oil resistance (Glüge et al., 2020).

Thousands of PFAS have been manufactured, but only the most relevant ones are included in this thesis (**Table 1.4**). As logk$_{ow}$ values show, PFAS are highly lipophilic, and this property increases notably when increasing the chain length. For this reason, PFAS are a well-known issue in the scientific community.

PFAS present a characteristic structure consisting of a chain of carbon atoms (C) bonded to fluorine ones (F), with a functional group at the end of the chain. The C-F bond is one of the strongest ever created, and substituting F with another atom is nearly impossible, explaining the strong persistence of these compounds.

As mentioned, this persistence increases with the chain length because more C-F bonds increase the stability of the compound. Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS) (considered long-chain PFAS) are the most widely used, but shorter-chain ones are replacing them due to their environmental persistence.

Table 1.4. Frequently used PFAS, with family, CAS number, molecular mass, structure, and logK$_{ow}$.

Compound	Family	CAS number	Molecular mass (g/mol)	Structure	logK$_{ow}$
	PFAS				
Perfluorobutanoic acid (PFBA)	Carboxylic acid	375-22-4	214.04		2.82
Perfluorobutanesulfonic acid (PFBS)	Sulfonic acid	375-73-5	300.1		3.9
Perfluorohexanoic acid (PFHxA)	Carboxylic acid	307-24-4	314.05		4.06
Perfluorohexanesulfonic acid (PFHxS)	Sulfonic acid	355-46-4	400.12		5.17
Perfluoroheptanoic acid (PFHpA)	Carboxylic acid	375-85-9	364.06		4.67
Perfluorooctanoic acid (PFOA)	Carboxylic acid	335-67-1	414.07		5.3
Perfluorooctanesulfonic acid (PFOS)	Sulfamide	1763-23-1	500.13		6.3
Perfluorooctane-sulfonamide (PFOSA)	Sulfamide	754-91-6	499.15		5.8
Perfluorononanoic acid (PFNA)	Carboxylic acid	375-95-1	464.08		5.92
Perfluorodecanoic acid (PFDA)	Carboxylic acid	335-76-2	514.08		6.5
Perfluoroundecanoic acid (PFUnDA)	Carboxylic acid	2058-94-8	564.09		7.15
Perfluorododecanoic acid (PFDnDA)	Carboxylic acid	307-55-1	614.1		Not available
Perfluorotridecanoic acid (PFTnDA)	Carboxylic acid	72629-94-8	664.1		Not available

1.2 Environmental legislation and regulation in formulations

Environmental legislation

There are regulatory frameworks to monitor and manage potential pollution sources of some priority pollutants in the aquatic environment, but CECs are not subjected to the same regulations (Vargas-Berrones et al., 2020).

A few regulations include the monitoring of specific CECs in drinking water. In the USA, for example, the EPA updated in 2021 the Drinking Water Contaminant Candidate List (up to 97 CECs), where only PFOS and PFOA are regulated (maximum of 70 ng/L of PFOS and PFOA together). The other CECs are listed as “contaminants known or anticipated to occur in public water systems, but currently not subject to any drinking water regulations” (US EPA, 2021). In Europe, the European Monitoring Network (NORMAN) provides occurrence and effects data of CECs, contributing evidence to make policies for identifying, assessing, and prioritising these compounds (von der Ohe et al., 2011). The NORMAN Substance List Exchange contains more than 100000 CECs (Aalizadeh et al., 2022), providing essential data for its monitoring, but they are not yet contemplated within any regulation. Only PFAS have a limit of 0.1 µg/L individually and 0.5 µg/L altogether in drinking water (The European Parliament and the Council of the European Union, 2020). In the rest of the regions worldwide, the situation is very similar, and the guidelines at the environmental level are practically non-existent.

This lack of regulations is caused mainly by the scarce occurrence and toxicological data and analysis complexity (expensive, unavailable standards, low concentration levels, expert personnel, etc.). These difficulties increase considering that new chemical compounds are introduced into the market continuously, and the degradation of actual CECs generates new TPs and by-products that could be more harmful to ecosystems and human health.

Regulation in formulations

For the families of compounds covered in this thesis, the only regulations are related to formulations, limiting the percentage of a compound (or a mixture of compounds from a particular family) in the total mass of ingredients constituting a product. In the case of UVFs in Europe, their individual concentration in formulations is limited to 10% top (European Parliament and the Council of the European Union, 2009). BP3, for example, is limited to 6% of the formulation for face, hand and lip products but only to 2.2% in body products, while OC is only allowed to 0.9% in propellant and spray products but up to 10% in the rest (ECHA EUROPE, 2022). However, this regulation does not specify any limit for a mixture of UVFs, allowing commercial companies to combine them without limitation. This European regulation is very similar to the ones applied in New Zealand, Australia, most of the Asiatic countries (China, Hong Kong, Japan, India, Taiwan, Arabic countries, and ASEAN countries), Turkey, and Canada (Agawin et al., 2022). In the USA, the Food and Drug Administration (FDA) agency considers that almost all organic UVFs ingredients require further investigation to determine if they are safe (generally recognized as safe and effective (GRASE)). Still, no amount limitations are specified for the formulations (FDA, 2019). However, sunscreens containing BP3 have already been banned in Hawaii, Key West (Florida), the Virgin Islands, and Puerto Rico. Furthermore, in Palau, Aruba, and Bonaire, BP3 is officially banned for use.

Regarding PBs, in areas such as Europe, Japan, and Southeast Asia, the total concentration of all PBs in a product cannot be higher than 0.8%, and the individual of any PB cannot be higher than 0.4% of the total (European Parliament and the Council of the European Union, 2009). For the FDA agency, PBs are in the same situation as UVFs, and no limit is specified in the formulations.

Pharmaceutical's case is unique because they are manufactured for direct human consumption, so no limitations are specified in the formulations. Antifouling biocides are neither limited in formulations, but in the case of irgarol (IRG), it was banned in 2019 (Europe) and 2020 (USA) to be used in paints for biocidal purposes (European Chemicals Agency (ECHA), 2019; US EPA, 2020), and the same institutions are evaluating diuron (DIU) with a similar purpose.

In the case of PFAS, PFOS and PFOA were globally banned in 2009 and 2020, respectively. Perfluorohexanesulfonic acid (PFHxS) is being considered for inclusion in Stockholm's Convention, and perfluorinated carboxylic acids (C9-14), their salts, and precursors will be restricted in the EU/European Economic Area (EEA) from February 2023 onwards (European Chemicals Agency (ECHA), 2022b). Some European countries have set national limit values for water and soil (Denmark, Germany, the Netherlands and Sweden), for textiles (Norway) and food contact materials (Denmark). Still, no standard guidelines have been specified at the European level.

1.3 Pathways and occurrence into the environment

After the CECs have been used, or at the end of their useful life, they are disposed of at home (toilet, shower, sink), in industries (effluent), hospitals (effluent), or in the urban run-off. These pathways lead to the nearest WWTP, where they arrive along with other contaminants such as oils, microplastics, and heavy metals (**Figure 1.4**). Nowadays, most WWTPs present the classical organigram consisting of a primary clarifier (solid organic matter-water separation), an aeration step (pumping air encouraging the organic matter degradation), and a secondary clarifier (removing the remaining solid organic matter). Therefore, most CECs passing through these processes are only partially removed because WWTPs are not designed to remove them and, thus, are still present in the effluent discharged into the environment.

Other anthropogenic pathways, such as agricultural activities or UVFs wash-off in tourist zones, can suppose a direct entry of CECs into the environment through rivers, seawater and groundwater. Still, the direct contribution is small compared to the discharge of WWTP effluents.

Due to their easy entry into the environment, their occurrence has been reported in many environmental matrices (**Table 1.5**). In aquatic bodies, CECs have been detected in wastewater, groundwater, surface water, seawater, and drinking water (Čelić et al., 2021; Golovko et al., 2021; K'oreje et al., 2022; Ng et al., 2022; Peng et al., 2018; Wee et al., 2022). The levels reported vary enormously depending on the type of water, sampling site and surrounding conditions, so they have been detected from a few ng/L to hundreds of µg/L.

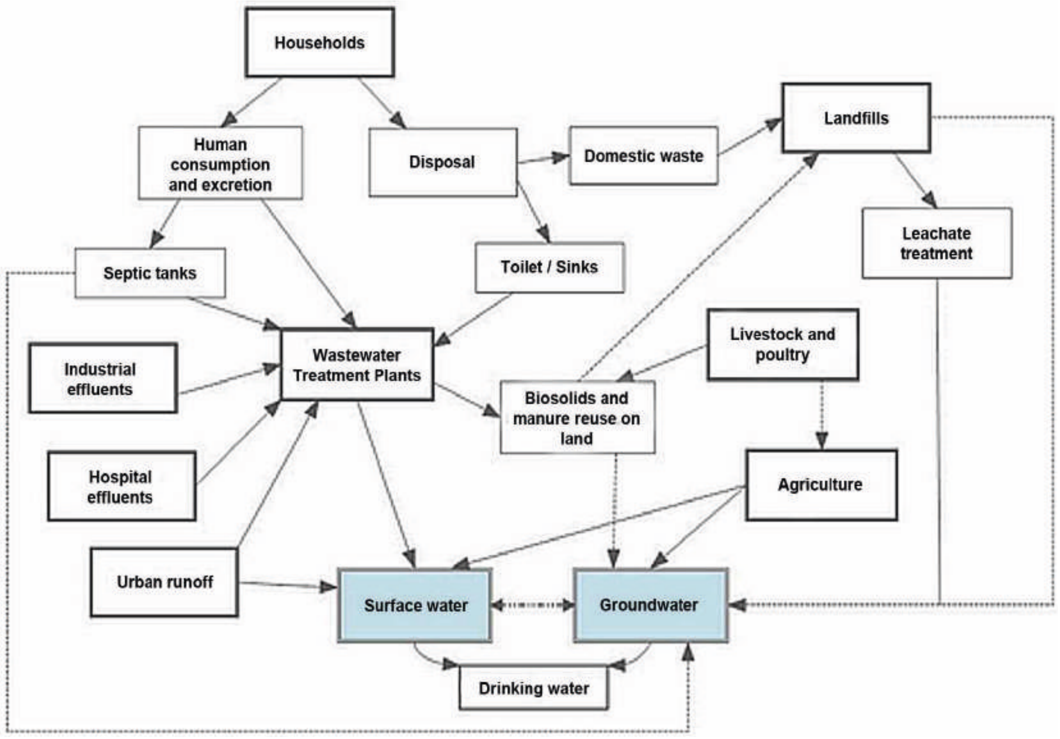


Figure 1.4. CECs' pathways into environmental compartments (Stefanakis and Becker, 2016).

Table 1.5. State-of-the-art summary of reported CECs' occurrence in environmental matrices.

	Water	**Sediment/soil**
Studied matrix	All types of water	Soil, marine and continental sediments
Extraction procedures	Easy, cheap and efficient procedures	Labourious: PLE, SPE
Analysis difficulty	Clean matrix (usually)	Relatively easy matrix
Nº of CECs analyzed	<100	<50
Concentrations range	Very variable (ng/L-ng/mL)	1-2500 ng/g
	Vegetables/Plants	**Animals**
Studied matrix	Any crop or plant	Fish, mussels, dolphins coral, birds, seals, etc
Extraction procedures	Variable: UAE, Soxhlet, QuEChERS	Very variable: PLE, Soxhlet, MAE, SPE, UAE, etc
Analysis difficulty	Complex matrices	Complex matrices
Nº of CECs analyzed	<20	<20
Concentrations range	1-900 ng/g	Variable (1-1500 ng/g)

PLE: Pressurized liquid extraction; SPE: Solid-phase extraction; UAE: Ultrasounds assistex extraction; QuEChERS: Quick, Easy, Cheap, Effective, Rugged, and Safe; MAE: Matrix-assisted extraction

The widespread presence in water and the high lipophilicity of most CECs lead to their bioaccumulation in organic components, such as soils/ sediments, vegetables/plants, and biota. Soils/sediments are in continuous and direct contact with water, but they present a much higher component of organic matter, so CECs present a higher affinity to accumulate on them (Ben Mordechay et al., 2021; León et al., 2020; Nishimuta et al., 2021). The soil/sediment extraction usually requires a cleaning step to remove the lipidic part, which significantly simplifies its analysis. Detected levels of CECs vary from a few to thousands ng/g.

Biota organisms are more lipidic than sediments, increasing the CECs' tendency to bioaccumulate. CECs' uptake in aquatic (e.g. algae) and terrestrial plants and different types of crops (carrots, lettuces, tomatoes, radish, corn, rice, cabbage, cauliflower, etc.) have also been demonstrated (Abril et al., 2021; Mao et al., 2017; Tadić et al., 2019). In animals, CECs' presence has been described at different levels of the trophic chain, showing their biomagnification capacity (McLeod et al., 2014). They have been found in other species, such as fish (Gago-Ferrero et al., 2015; Ojemaye and Petrik, 2019; Oliveira et al., 2017), coral reefs (Mitchelmore et al., 2021), mammals (dolphins, seals) (Ahrens, 2011; Alonso et al., 2015), or birds (Xue and Kannan, 2016), showing CECs' persistence.

The challenges of CECs removal

Implementing new removal treatments in WWTPs would be the ideal solution to avoid this indirect pathway into the environment. However, as explained in section 1.1, this diverse group of contaminants presents a wide range of physicochemical properties, making it much more difficult. Therefore, most degradation technologies tested so far can efficiently remove some CECs, but not others with different properties or behaviour. In addition, most of these technologies are expensive, such as ozonation.

The most tested treatment is the adsorption of CECs to remove them from water streams. Many different materials have been evaluated, namely biochar, granular/powder-activated carbon, silica aerogels, natural clays, ion-exchange resins, and carbon nanotubes, among others (Antunes et al., 2021).

Other tested technologies are biological treatments (activated sludge (most typical), bioreactors, wetlands, algal/fungi-based, etc.), membrane filtration, and advanced oxidation processes (chlorination, UV radiation, ozonation, electrochemical/photochemical degradation, etc.). However, most of these oxidation processes (except ozonation, which is very expensive) are known to generate new TPs that, in most cases, are even more toxic and persistent than the original compound (Chen et al., 2018). Combining technologies is also a common practice to achieve higher removal rates. The investment and public attention to finding and implementing new technologies are increasing, but there is still a long way to go.

Water scarcity and water reuse

Given the ease these compounds enter the environment and the difficulty that, for now, their complete removal entails, the impact they have on aquatic ecosystems is considerably increasing. Combined with climate change's effects, scarcity of freshwater is a real concern that will only worsen with population increase. In fact, by 2030, the global water demand is expected to grow by 50% (Guppy and Anderson, 2017). Therefore, the most viable solution seems to be reusing waste/reclaimed water to cover demands. Agriculture accounts for 70% of all water withdrawals globally (Guppy and Anderson, 2017), so reusing water for irrigation purposes would be ideal for reducing hydric stress.

However, many contaminants (CECs, but also pathogens, heavy metals, etc.) present in the waste/reclaimed water can be uptaken by the crops aimed for human consumption. In recent years, more studies have focused on the feasibility of wastewater reuse in agriculture, but most were developed in hydroponic and greenhouse environments (Abril et al., 2021; Shenker et al., 2011; Wu et al., 2015). In most cases, the growing conditions in these studies are far from real scenarios, hindering to extract sound conclusions applicable to real agrosystems. Some studies have assessed the CECs' uptake by crops in real scenarios (de Santiago-Martín et al., 2020; Liu et al., 2020; Tadić et al., 2021), but they concluded that more information is needed to fully understand the processes behind the uptake and the role of the different variables involved in its cultivation.

1.4 Pathways and occurrence in humans

The extensive occurrence of CECs in the environment implies that humans are also exposed to them via different pathways. These potentially harmful substances enter through various routes of exposure, the main ones being ingestion, inhalation, and direct application on the body (skin, hair, nails, etc.).

Pharmaceuticals, are consumed to treat a disease or an injury, entering directly into the body. UVFs or PBs, are essential ingredients in personal care products that can be applied, for example, on the skin. In both cases, after use, CECs are absorbed by the body and partially metabolised and/or excreted, but they can also be accumulated in different tissues. The other most important input comes from ingesting contaminated food or drinking water. After their entrance and occurrence in the environment, it is difficult to avoid their presence in water or food (such as crops) and thus, their exposure. For example, the principal exposure pathway to PFAS is ingesting contaminated food and water, especially near highly contaminated sites where these compounds are manufactured or where aqueous film-forming foam has been used (Deluca et al., 2022). In the case of antifouling biocides, human exposure is probably produced via the ingestion of aquatic species (e.g. fish).

The determination of compounds in human samples is not widely addressed, and the vast majority of studies in this field are limited to a minimal number of samples and focus on specific families of well-known compounds (e.g. PFAS or PBDEs). They generally provide diffuse and not comprehensive data that do not allow drawing solid conclusions on the overall chemical exposure. There are few systematic studies and sampling campaigns with the objective of determining, from a broad perspective, what the compounds that accumulate in humans are. A good example is the campaigns that are carried out within the national biomonitoring program, led by the Centers for Disease Control and Prevention (CDC, USA), in which 300 organic compounds are analyzed in different human tissues; or the interesting studies that are recently being carried out under the framework of the European program HBM4EU, which focuses on different classes of compounds after a thorough prioritization.

CECs analysis in human matrices is usually performed in biofluids (breast milk, urine, blood, etc.) and hair, although studies exist in various tissues (e.g. placenta). In the case of pharmaceuticals, some studies (Gil-Solsona et al., 2021) report their presence in the placenta, for example. Still, since its main pathway is direct consumption, they are expected to be found in the human body, and exposure studies are meaningless. To the best of the authors' knowledge, antifouling biocides have not been reported in any human matrix. Therefore, the average concentrations reported for PFAS, UVFs and PBs are summarised in **Figure 1.5**. So far, UVFs and PBs have been detected in amniotic fluid (0.02-3.38 ng/mL) (Krause et al., 2018; Song et al., 2020), urine (0.02-10034 ng/mL) (Krause et al., 2018; Meeker et al., 2011; Song et al., 2020; Zhang et al., 2013), breast milk (0.3-780 ng/g) (Molins-Delgado et al., 2018), placenta (0.03-11.77 ng/mL) (Valle-Sistac et al., 2016), mother blood (0.02-3.38 ng/mL) (Kolatorova Sosvorova et al., 2018; Krause et al., 2018; Song et al., 2020; Zhang et al., 2013) and umbilical cord blood (0.02-71.8 ng/mL) (Kolatorova Sosvorova et al., 2018; Krause et al., 2018; Song et al., 2020). PFAS have also been found in urine (0.17-95.35 ng/mL) (Peng et al., 2022), breast milk (0.002-28.2 ng/mL) (Kärrman et al., 2010; Macheka-tendenguwo, 2018), liver (0.20-52.13 ng/g) (Kärrman et al., 2010), placenta (0.05-1.02 ng/g) (Vela-Soria et al., 2021), mother serum/blood (0.03-6.95 ng/mL) (Mcdonough et al., 2021; Poothong et al., 2017) and umbilical cord blood (0.18-65.61 ng/mL) (B. Wang et al., 2016). PFOS and PFOA were the compounds found at higher concentrations in all cases, demonstrating their persistence capacity.

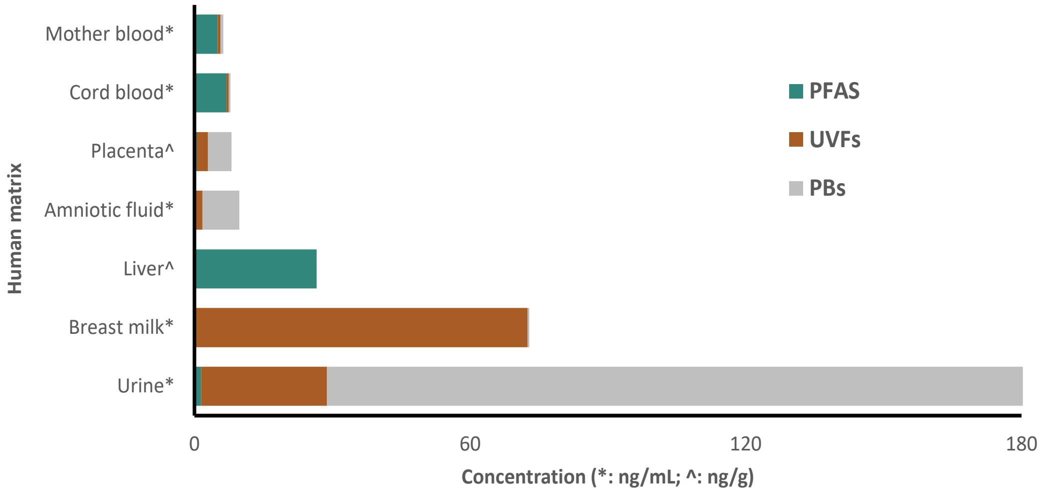


Figure 1.5. Average concentrations of PFAS, UVFs and PBs in human matrices.

1.5 Risk assessment of the exposure to CECs

Considering CECs' persistence in both the environment and humans and the lack of environmental regulations, these contaminants' potential risks are significant. However, the assessment of mixtures of compounds and long-term effects are very complex, especially concerning humans.

UVFs

UVFs' endocrine-disrupting capacity has been reported in different species of fish (Kim et al., 2014; Kinnberg et al., 2015; Krause et al., 2012; Wang et al., 2016) and rats (Axelstad et al., 2011; Morohoshi et al., 2005; Suzuki et al., 2005). Some have also been reported to cause hepatoxicity (Liu et al., 2015), developmental (Balázs et al., 2016) and reproductive outcomes (Kim et al., 2014), and carcinogenicity (Mao et al., 2017) in different organisms. In the environment, they cause coral bleaching (Downs et al., 2016) and interference with metabolic, enzymatic, and reproductive activities in practically all organisms, especially aquatic ones (Agawin et al., 2022; Huang et al., 2021). In humans, scarce information about UVFs association with adverse effects is available, so they are barely known.

PBs

Although their extensive use in PCPs, PBs are considered hazardous to the aquatic environment by ECHA (ECHA Europe, 2022) and are known endocrine disruptors (Charles and Darbre, 2013; Ding et al., 2022; Nowak et al., 2018), with increasing potency with the length of the alkyl chain (Gao et al., 2016; Valle-Sistac et al., 2016). In animals, reproductive (Oishi, 2002a, 2002b) and genotoxic effects have been reported, and in humans, they have been associated with birth outcomes (Baker et al., 2020; Geer et al., 2017), premature birth (Baker et al., 2020), reproductive issues (Smarr et al., 2017) or breast cancer (Amin et al., 2019).

Pharmaceuticals

Human and veterinary pharmaceuticals are extensively present in the environment due to the continuous discharge of wastewater and suppose a major threat to aquatic ecosystems. Some of them, such as DCF, an anti-inflammatory, are a threat to aquatic animals, plants and mammals since they can affect the development, growth and immune system (Sathishkumar et al., 2020). Moreover, some organisms are exposed to these doses for long periods, potentially leading to chronic toxic effects (Fent et al., 2006). Some studies have reported reproductive impairment effects in fish exposed to pharmaceuticals (Nash et al., 2004). But considering the enormous production rate, the primary concern with pharmaceuticals is the contribution to the development and prevalence of AGR caused by released antibiotics, a significant emerging threat to human health (Krzeminski et al., 2019).

Biocides

Once in aquatic ecosystems, biocides can contaminate coastal areas (Thomas and Brooks, 2010), and they have been reported to cause acute toxicity in different species of plankton (Jung et al., 2017), algae, crustaceans, and fish (Bao et al., 2011; Oliveira et al., 2017). Studies assessing their toxicity in humans are as scarce as those of their occurrence (Mohammed et al., 2018; Van Boven et al., 1990).

PFAS

PFAS are one of the most concerning groups of CECs since they have been associated with many adverse health effects in animals and humans. In animals, they have been associated with immunological, developmental, endocrine, reproductive, haematological, neurobehavioral and carcinogenic effects (Kwan et al., 2015; NTP TOX 97: National Toxicology Program. U. S. Department of Health and Human Services, 2019; Pachkowski et al., 2019; Research Triangel Park et al., 2020). In humans, PFAS have been related to altered immune and thyroid function, liver disease, lipid and insulin dysregulation, kidney disease, adverse reproductive and developmental outcomes, and cancer (Fenton et al., 2017).

1.6 Analytical methodologies

The type of analysis to detect or quantify CECs depends on the matrix, the compound's properties, and the purpose of the study. Depending on these factors, the sampling and sample preservation, the extraction methodologies, and the instrumental analysis will vary. Each step for the analysis of CECs performed in this thesis is briefly explained in the following subsections.

Sampling and sample preservation

Liquid samples (mainly water) were collected in sterile opaque glass bottles and maintained cool in portable freezers. Later on, the bottles were stored at -20ºC until further analysis. In one of the studies, water sampling was performed with Polar Organic Chemical Integrative Samplers (POCIS). It consists of a sorbent phase, isolated between two membranes, able to sequester and concentrate contaminants with considerable $\log K_{ow}$ values (Magi et al., 2018), and allowed obtaining composite samples over long periods of time.

In the case of solids (biota, crops, plants, etc.) (**Figure 1.6**), they were sampled representatively. Then, they were put in sterile plastic bags and maintained cool in portable freezers. Once in the laboratory, they were thawed, sliced into small pieces, freeze-dried, homogenised, and stored at -20ºC until further analysis.

Figure 1.6. Lettuce sampling in experimental plots located in Palamós WWTP.

Extraction methodologies for liquid matrices

The common extraction technique for liquid samples is solid-phase extraction (SPE). It consists of a solid material through which the sample is passed, and the compounds of interest are retained by their physical or chemical properties. There are different types of SPE cartridges because it determines the retained compounds. The most commonly used for CECs extraction are normal-phase SPE (retaining polar compounds) and reversed-phase SPE (retaining non-polar compounds). Still, recently mixed-mode SPE (combines properties of both mentioned SPE) has also been extensively used.

Other SPE techniques are HILIC (Hydrophilic interaction liquid chromatography) or ion-exchange. SPE technique can be performed manually, automatically or online. Its purpose is to preconcentrate (more volume of sample passed, more concentration) and to clean up (most of the interferences are not retained, and the final extract is cleaner). Another valuable technique for CECs extraction in liquids is liquid-liquid (L-L) extraction. L-L extraction is a separation process based on the compound's solubilities into two immiscible liquids, usually water (polar) and an organic solvent (non-polar). Then, once CECs are dissolved in the organic solvent, an evaporation step allows its (pre) concentration and further analysis.

Extraction methodologies for solid matrices

In solid matrices, the extraction step is usually followed by a clean-up to avoid difficulties in the further instrumental analysis, such as matrix effects, interferences or low intensity, among others. A consolidated technique for CECs extraction from solids is pressurised liquid extraction (PLE). In PLE, the sample is dispersed with an inert material and placed in an extraction vessel, through which solvent is passed at high temperature and pressure. The extraction cycle can be repeated to enhance extraction efficiency, even with different solvents/temperatures. The final extract is collected in a vial and further analysed. As it is an automated process, this technique is time-saving and solvent-reducing.

Matrix solid-phase dispersion (MSPD) is another useful technique in food and environmental samples. In MSPD, the sample and appropriate solid material are placed in a mortar and manually blended for a certain time. Thus, abrasion and sample disruption occurs, and the target compounds are dissolved in an organic solvent (Xu and Lee, 2012). This green-chemistry method is speedy and saves large volumes of solvents.


Figure 1.7. QuEChERS extraction of lettuce samples.

QuEChERS technique (abbreviation of Quick, Easy, Cheap, Efficient, Rugged and Safe) was initially developed for the extraction of pesticides from fruits and vegetables (Anastassiades et al., 2003) (**Figure 1.7**). The first step consists of an L-L extraction (usually with acetonitrile (ACN)), followed by a clean-up through dispersive SPE (d-SPE), where a sorbent is used to remove undesired matrix components (organic acids, pigments and sugars) commonly present in foodstuff. Recently, its applicability has been extended to other families of CECs, such as pharmaceuticals or PFAS, in different environmental matrices, such as sediments or biota (Nannou and Boti, 2019; Sznajder-katarzy et al., 2020).

Ultrasound-assisted extraction (UAE) is another easy and useful extraction technique. It uses mechanical energy (ultrasound waves) to favour the compounds of interest to be dissolved in a solvent, and it is commonly combined with other techniques.

For the analysis of CECs in challenging matrices, the usual practice is combining different extraction techniques (for example, PLE and SPE) to improve the extraction efficiency and obtain a cleaner extract for instrumental analysis. Usually, a final (near)evaporation step is performed to reduce the solvent volume, concentrate the analytes of interest, and obtain a suitable volume for chromatographic vials (~ 2 mL).

Instrumental analytical techniques

Sections 1.3 and 1.4 explain the occurrence data of CECs in the environment and human samples, where CECs are usually found within the range ng/L-ng/mL (or ng/kg-ng/g). Only powerful analytical techniques with the required sensitivity, precision, and robustness can be used to achieve these low levels in complex matrices. Thus, high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) is the ideal technique for CECs determination. Through HPLC, the extracted analytes are separated based on their distribution or affinity between two different phases. One of the phases is active and remains motionless (stationary phase), and the other is mobile (mobile phase) and passes (or percolates) through the stationary phase. In HPLC, this separation occurs along a chromatographic column, and the stationary phase material in the column and mobile phase (solvents) passing through vary depending on the analytes' properties. The separation efficiency depends mainly on particle size, diameter, column length, flow rate, pH, and temperature.

For CECs analysis, reversed-phase columns (made of C18 or C8) are the standard ones because they efficiently separate non-polar compounds. CECs have more affinity for the stationary phase and elute later (higher retention times) than polar compounds that do not interact with it. However, other LC-columns can be required for the analysis of specific compounds. For example, polar compounds are weakly retained in reversed-phase columns for their separation. Normal-phase columns (polar stationary phase) with non-polar solvents are used in this case. However, for those analytes that are not soluble enough in polar solvents, the mobile phase is a combination of aqueous-organic phases. This technique is known as hydrophilic liquid chromatography (HILIC), and its popularity has strongly increased over the last few years. Other columns, such as ion-pair (IPC) or ion-exchange (IEC), take advantage of the charge to separate analytes and are helpful in separating ionic substances. Finally, supercritical fluid chromatography (SFC) is a hybrid technique between HPLC and gas chromatography (GC), that uses a supercritical fluid as mobile phase. It allows the retention of compounds with different polarities but requires high operating (the mobile phase has to be maintained in a supercritical state) and equipment costs. A summary of the most suitable technique depending on the polarity of the compounds is shown in **Figure 1.8**.

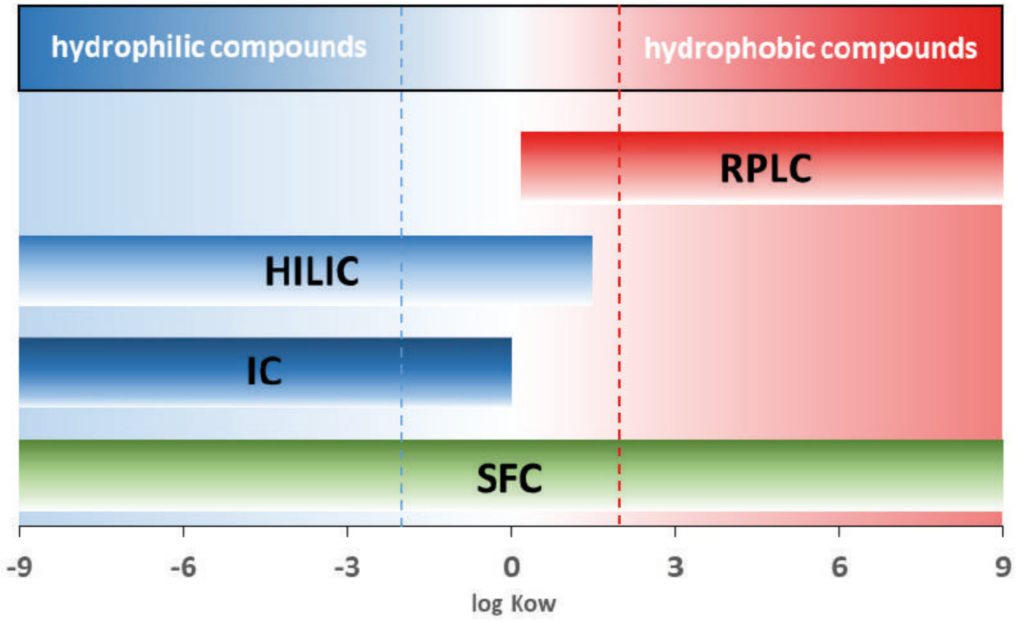


Figure 1.8. Polarity scheme for chromatographic separation techniques (reversed-phase liquid chromatography (RPLC), hydrophilic interaction liquid chromatography (HILIC), ion chromatography (IC) and supercritical fluid chromatography (SPF)) based on $logk_{ow}$ values (based on (Bieber and Letzel, 2020)).

Once the analytes are separated, they are detected with a mass spectrometry (MS) analyser. This technique ionises chemical species and sorts the ions based on their mass-to-charge ratio (m/z). The detection of the ions can be qualitative or quantitative (based on their respective m/z abundance). The common ion source for CECs analysis with HPLC is electrospray ionisation (ESI). It provides a simple and sensitive way to ionise solutions and is effectively coupled to multiple chromatographic techniques.

Mass analysers can be separated into two groups depending on their resolution. Low-resolution mass spectrometers (LRMS) give the nominal m/z values (e.g. CFF = 195 in positive ionisation), while HRMS are able to provide m/z values with up to 5 decimals (e.g. CFF = 195.08765). The most commonly used LRMS are quadrupoles (four cylindrical rods set in parallel to filter the ions by applying electric fields) and ion traps (a combination of electric and magnetic fields to trap ions in a specific region). Concerning HRMS, orbitrap (two external electrodes and one central that filters ions based on its oscillation frequency) and time of flight (TOF) (an electric field that filters the ions based on the time they use to cover a known distance) are the most used.

LRMS are usually combined in tandem with other LRMS (triple quadrupole (QqQ) or quadrupole-ion trap (q-TRAP)) or HRMS (q-Orbitrap or q-TOF)) to achieve more levels of fragmentation of the ion of interest to elucidate or confirm its structure.

Target/Non-target approaches

Depending on the purpose in the analysis of CECs (identification or quantitation), they can be divided between the target or non-target analysis. LRMS can almost only be used for target analysis, as their elucidation power is extremely limited. However, they provide a bit higher sensitivity compared to HRMS instruments. That is why target analysis of CECs is traditionally performed with LRMS instruments under Multiple Reaction Monitoring (MRM). This acquisition mode means that the first quadrupole isolates parent ion m/z, which is fragmented in the second quadrupole, acting as a collision cell, and (at least) two of their fragment ions are then isolated in the third quadrupole. These transitions between the parent ion and the two most intense fragment ions, as well as the correct ratio between both transitions, are used to identify with confidence the CEC of interest. As mentioned, it is a very sensitive approach, helped by really specific extraction methods to reduce interferences and recover the analytes of interest as much as possible. In fact, it permits a reliable quantitation but requires *a priori* selection of the analytes of interest. For this reason, the determination is only of a limited number of target compounds (decided *a priori*), and using reference standards is mandatory. As said before, the extraction method is critical and must be as specific as possible for the compounds to be analysed.

Currently, the performance of target analysis has also been done with HRMS instruments, thanks to the increase in sensitivity, joined to the great selectivity that HRMS provides. Although sensitivity is lower than LRMS, around one order of magnitude, the possibility of doing wide-scope target analysis or suspect analysis provides a broader coverage of compounds present in our samples, which helps to have a comprehensive idea of compounds' presence, which helps to select, in a future target analysis step, those compounds that actually appears there.

When non-target (or suspect) approaches are used, HRMS is required. For CECs analysis, data acquisition is commonly performed under Data Dependent Acquisition (DDA) or Data Independent Acquisition (DIA). In both cases, initial full-scan analyses the precursor ions entering the instrument. Then, in DDA, some of these precursor ions are isolated based on different criteria (e.g. intensity or inclusion list) and further fragmented. With appropriate instrumentation, these fragment ions can also be fragmented as many times as desired (MSn) to obtain extra information about specific moieties' positions. In DIA, all the precursor ions (without pre-selection) are fragmented using a high energy (HE) function, obtaining a combined mass spectrum of all the precursors entering simultaneously. New DIA methodologies have appeared (e.g. SWATH in SCIEX), where more than one HE functions are acquired, obtaining less interfered functions and facilitating further compounds elucidation.

On the one hand, non-target approaches allow comprehensive screening for virtually (as they must be ionizable) any compound present in the samples, independently of the reference standards' availability. In addition, it allows a retrospective analysis, which means reanalysing already acquired data in the future for searching specific information (for example, compounds described in the future that we suspect can be in our already injected samples). On the other hand, HRMS is less sensitive than LRMS, and much more data processing is required for the reliable identification of compounds. Therefore, the use of databases and in-silico fragmentation tools is almost mandatory.

Depending on confidence in compound identifications, different levels are assigned, as proposed by (Schymanski et al., 2014.) **Figure 1.9** shows this scale with confidence levels.

Non-target methodologies, notwithstanding, can also act as "quantitative" techniques, with their limitations. For example, new methods have been developed recently to semi-quantify (quantification without any reference standard). However, they are still in development, and some reference standards are still needed for reliable compound quantification. In this case, SPE is not required to be as selective as in LRMS methodologies but sometimes recommended to remove matrix interferences, such as in the case of biological or solid matrices.

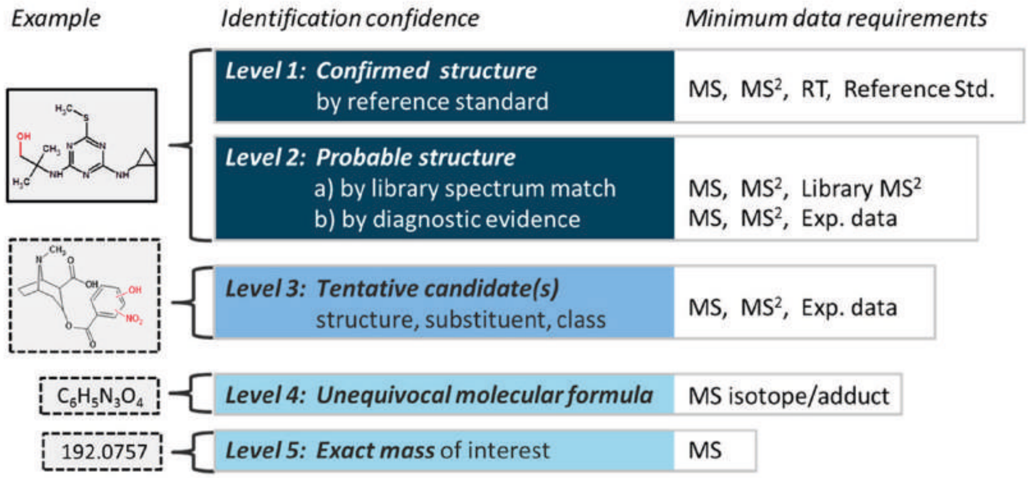


Figure 1.9. Proposed identification levels in high resolution mass spectrometric analysis. (Schymanski et al., 2014)

1.7 References

Aalizadeh, R., Alygizakis, N., Schymanski, E., Slobodnik, J., Fischer, S., Cirka, L., 2022. S0 | SUSDAT | Merged NORMAN Suspect List: SusDat. https://doi.org/10.5281/zenodo.2664077

Abril, C., Santos, J.L., Martín, J., Aparicio, I., Alonso, E., 2021. Uptake and translocation of multiresidue industrial and household contaminants in radish grown under controlled conditions. Chemosphere 268. https://doi.org/10.1016/j.chemosphere.2020.128823

Agawin, N.S.R., Sunyer-Caldú, A., Díaz-Cruz, M.S., Frank-Comas, A., García-Márquez, M.G., Tovar-Sánchez, A., 2022. Mediterranean seagrass Posidonia oceanica accumulates sunscreen UV filters. Mar. Pollut. Bull. 176. https://doi.org/10.1016/j.marpolbul.2022.113417

Ahrens, L., 2011. Polyfluoroalkyl compounds in the aquatic environment: A review of their occurrence and fate. J. Environ. Monit. 13, 20–31. https://doi.org/10.1039/c0em00373e

Alonso, M.B., Feo, M.L., Corcellas, C., Gago-Ferrero, P., Bertozzi, C.P., Marigo, J., Flach, L., Meirelles, A.C.O., Carvalho, V.L., Azevedo, A.F., Torres, J.P.M., Lailson-Brito, J., Malm, O., Diaz-Cruz, M.S., Eljarrat, E., Barceló, D., 2015. Toxic heritage: Maternal transfer of pyrethroid insecticides and sunscreen agents in dolphins from Brazil. Environ. Pollut. 207, 391–402. https://doi.org/10.1016/j.envpol.2015.09.039

Amin, M.M., Tabatabaeian, M., Chavoshani, A., Amjadi, E., Hashemi, M., Ebrahimpour, K., Klishadi, R., Khazaei, S., Mansourian, M., 2019. Paraben Content in Adjacent Normal-malignant Breast Tissues from Women with Breast Cancer. Biomed. Environ. Sci. 32, 893–904. https://doi.org/10.3967/bes2019.112

Anastassiades, M., Lehotay, S.J., Stajnbaher, D., Schenck, F., 2003. Fast and Easy Multiresidue Method Employing Acetonitrile. J. AOAC Int. 86, 412–431.

Antunes, E., Vuppaladadiyam, A.K., Sarmah, A.K., Varsha, S.S. V, Pant, K.K., Tiwari, B., Pandey, A., 2021. Chapter Three - Application of biochar for emerging contaminant mitigation, in: Sarmah Environmental Management and Protection, A.K.B.T.-A. in C.P. (Ed.), Biochar: Fundamentals and Applications in Environmental Science and Remediation Technologies. Elsevier, pp. 65–91. https://doi.org/https://doi.org/10.1016/bs.apmp.2021.08.003

Aranaz, I., Acosta, N., Civera, C., Elorza, B., Mingo, J., Castro, C., Gandía, M. de los L., Caballero, A.H., 2018. Cosmetics and cosmeceutical applications of chitin, chitosan and their derivatives. Polymers (Basel). 10. https://doi.org/10.3390/polym10020213

Aronson, J., 2016. Sunscreens. Elsevier, Oxford. https://doi.org/https://doi.org/10.1016/B978-0-444-53717-1.01491-8

Axelstad, M., Boberg, J., Hougaard, K.S., Christiansen, S., Jacobsen, P.R., Mandrup, K.R., Nellemann, C., Lund, S.P., Hass, U., 2011. Effects of pre- and postnatal exposure to the UV-filter Octyl Methoxycinnamate (OMC) on the reproductive, auditory and neurological development of rat offspring. Toxicol. Appl. Pharmacol. 250, 278–290. https://doi.org/10.1016/j.taap.2010.10.031

Baker, B.H., Wu, H., Laue, H.E., Boivin, A., Gillet, V., Langlois, M.-F., Bellenger, J.-P., Baccarelli, A.A., Takser, L., 2020. Methylparaben in meconium and risk of maternal thyroid dysfunction, adverse birth outcomes, and Attention-Deficit Hyperactivity Disorder (ADHD). Environ. Int. 139, 105716. https://doi.org/10.1016/j.envint.2020.105716

Balázs, A., Krifaton, C., Orosz, I., Szoboszlay, S., Kovács, R., Csenki, Z., Urbányi, B., Kriszt, B., 2016. Ecotoxicology and Environmental Safety Hormonal activity , cytotoxicity and developmental toxicity of UV filters. Ecotoxicol. Environ. Saf. 131, 45–53. https://doi.org/10.1016/j.ecoenv.2016.04.037

Bao, V.W.W., Leung, K.M.Y., Qiu, J.W., Lam, M.H.W., 2011. Acute toxicities of five commonly used antifouling booster biocides to selected subtropical and cosmopolitan marine species. Mar. Pollut. Bull. 62, 1147–1151. https://doi.org/10.1016/j.marpolbul.2011.02.041

Ben Mordechay, E., Mordehay, V., Tarchitzky, J., Chefetz, B., 2021. Pharmaceuticals in edible crops irrigated with reclaimed wastewater: Evidence from a large survey in Israel. J. Hazard. Mater. 416, 126184. https://doi.org/10.1016/j.jhazmat.2021.126184

Bieber, S., Letzel, T., 2020. White Paper – Polarity-Extended Chromatography. AFIN-TS Forum.

Čelić, M., Jaén-Gil, A., Briceño-Guevara, S., Rodríguez-Mozaz, S., Gros, M., Petrović, M., 2021. Extended suspect screening to identify contaminants of emerging concern in riverine and coastal ecosystems and assessment of environmental risks. J. Hazard. Mater. 404. https://doi.org/10.1016/j.jhazmat.2020.124102

Charles, A.K., Darbre, P.D., 2013. Combinations of parabens at concentrations measured in human breast tissue can increase proliferation of MCF-7 human breast cancer cells. J. Appl. Toxicol. 33, 390–398. https://doi.org/10.1002/jat.2850

Chen, W.L., Cheng, J.Y., Lin, X.Q., 2018. Systematic screening and identification of the chlorinated transformation products of aromatic pharmaceuticals and personal care products using high-resolution mass spectrometry. Sci. Total Environ. 637–638, 253–263. https://doi.org/10.1016/j.scitotenv.2018.05.011

Chisvert, A., Salvador, A., 2007. 3.1 - UV Filters in Sunscreens and other Cosmetics. Regulatory Aspects and Analytical Methods. Elsevier, Amsterdam. https://doi.org/https://doi.org/10.1016/B978-044452260-3/50028-0

de Santiago-Martín, A., Meffe, R., Teijón, G., Martínez Hernández, V., López-Heras, I., Alonso Alonso, C., Arenas Romasanta, M., de Bustamante, I., 2020. Pharmaceuticals and trace metals in the surface water used for crop irrigation: Risk to health or natural attenuation? Sci. Total Environ. 705, 135825. https://doi.org/10.1016/J.SCITOTENV.2019.135825

Deluca, N.M., Minucci, J.M., Mullikin, A., Slover, R., Hubal, E.A.C., 2022. Human exposure pathways to poly- and perfluoroalkyl substances (PFAS) from indoor media : A systematic review. Environ. Int. 162, 107149. https://doi.org/10.1016/j.envint.2022.107149

Ding, T., Yan, W., Zhou, T., Shen, W., Wang, T., Li, M., Zhou, S., Wu, M., Dai, J., Huang, K., Zhang, J., Chang, J., Wang, S., 2022. Endocrine disrupting chemicals impact on ovarian aging: Evidence from epidemiological and experimental evidence. Environ. Pollut. 305, 119269. https://doi.org/10.1016/j.envpol.2022.119269

Downs, C.A., Kramarsky-Winter, E., Segal, R., Fauth, J., Knutson, S., Bronstein, O., Ciner, F.R., Jeger, R., Lichtenfeld, Y., Woodley, C.M., Pennington, P., Cadenas, K., Kushmaro, A., Loya, Y., 2016. Toxicopathological effects of the sunscreen UV filter, oxybenzone (benzophenone-3), on coral planulae and cultured primary cells and its environmental contamination in Hawaii and the U.S. Virgin Islands. Arch. Environ. Contam. Toxicol. 70, 265–288. https://doi.org/10.1007/s00244-015-0227-7

Dulio, V., van Bavel, B., Brorström-Lundén, E., Harmsen, J., Hollender, J., Schlabach, M., Slobodnik, J., Thomas, K., Koschorreck, J., 2018. Emerging pollutants in the EU: 10 years of NORMAN in support of environmental policies and regulations. Environ. Sci. Eur. 30. https://doi.org/10.1186/s12302-018-0135-3

ECHA Europe, 2022. Summary of Classification and Labelling Methylparaben [WWW Document]. URL https://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/discli/details/40334

ECHA EUROPE, 2022. European Commission. (2022). Commission Regulation (EU) 2022/1176 of 7 July 2022 amending Regulation (EC) No 1223/2009 of the European Parliament and of the Council as regards the use of certain UV filters in cosmetic products.

European Chemicals Agency (ECHA), 2022a. ECHA substance infocard: 4-methylbenzophenone [WWW Document]. URL https://echa.europa.eu/substance-information/-/substanceinfo/100.004.691

European Chemicals Agency (ECHA), 2022b. Perfluoroalkyl chemicals (PFASs).

European Chemicals Agency (ECHA), 2019. Information on biocides. In European Chemicals Agency.

European Chemicals Agency (ECHA), 2012. Regulation (EU) No 528/2012 of the european parliament and of the council of 22 May 2012 concerning the making available on the market and use of biocidal products.

European Parliament and the Council of the European Union, 2009. Regulation (EC) No 1223/2009 of the european parliament and of the council of 30 November 2009 on cosmetic products.

Eurostat, 2022. Consumption of chemicals by hazardousness - EU aggregate [WWW Document]. Prod. code sdg_12_10. URL https://ec.europa.eu/eurostat/databrowser/view/sdg_12_10/default/table?lang=en

Fent, K., Weston, A.A., Caminada, D., 2006. Ecotoxicology of human pharmaceuticals 76, 122–159. https://doi.org/10.1016/j.aquatox.2005.09.009

Fenton, S.E., Ducatman, A., Boobis, A., Dewitt, J.C., Lau, C., Ng, C., Smith, J.S., Roberts, S.M., 2017. Per- and Polyfluoroalkyl substance toxicity and human health review: current state of knowledge and strategies for informing future research. Physiol. Behav. 176, 139–148. https://doi.org/10.1002/etc.4890.Per-

Food And Drug Administration (FDA), 2019. Fact sheet proposed rule : Sunscreen drug products for over-the-counter-human use 80.

Gago-Ferrero, P., Díaz-Cruz, M.S., Barceló, D., 2015. UV filters bioaccumulation in fish from Iberian river basins. Sci. Total Environ. 518–519, 518–525. https://doi.org/10.1016/j.scitotenv.2015.03.026

Gao, B., 2022. Emerging contaminants in soil and groundwater systems: Occurrence, impact, fate, and transport. Chapter 1 - Introduction. Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-12-824088-5.00002-1

Gao, C.J., Liu, L.Y., Ma, W.L., Zhu, N.Z., Jiang, L., Li, Y.F., Kannan, K., 2015. Benzonphenone-type UV filters in urine of Chinese young adults: Concentration, source and exposure. Environ. Pollut. 203, 1–6. https://doi.org/10.1016/j.envpol.2015.03.036

Gao, Y., Ji, Y., Li, G., An, T., 2016. Theoretical investigation on the kinetics and mechanisms of hydroxyl radical-induced transformation of parabens and its consequences for toxicity: Influence of alkyl-chain length. Water Res. 91, 77–85. https://doi.org/https://doi.org/10.1016/j.watres.2015.12.056

Geer, L.A., Pycke, B.F.G., Waxenbaum, J., Sherer, D.M., Abulafia, O., Halden, R.U., 2017. Association of birth outcomes with fetal exposure to parabens, triclosan and triclocarban in an immigrant population in Brooklyn, New York. J. Hazard. Mater. 323, 177–183. https://doi.org/10.1016/j.jhazmat.2016.03.028

German Environment Agency, 2014. Pharmaceuticals in the environment – the global perspective.

Gil-Solsona, R., Nika, M.C., Bustamante, M., Villanueva, C.M., Foraster, M., Cosin-Tomás, M., Alygizakis, N., Gómez-Roig, M.D., Llurba-Olive, E., Sunyer, J., Thomaidis, N.S., Dadvand, P., Gago-Ferrero, P., 2021. The potential of sewage sludge to predict and evaluate the human chemical exposome. Environ. Sci. Technol. Lett. 8, 1077–1084. https://doi.org/10.1021/acs.estlett.1c00848

Glüge, J., Scheringer, M., Cousins, I.T., Dewitt, J.C., Goldenman, G., Herzke, D., Lohmann, R., Ng, C.A., Trier, X., Wang, Z., 2020. An overview of the uses of per- and polyfluoroalkyl substances (PFAS). Environ. Sci. Process. Impacts 22, 2345–2373. https://doi.org/10.1039/d0em00291g

Golovko, O., Örn, S., Sörengård, M., Frieberg, K., Nassazzi, W., Lai, F.Y., Ahrens, L., 2021. Occurrence and removal of chemicals of emerging concern in wastewater treatment plants and their impact on receiving water systems. Sci. Total Environ. 754, 142122. https://doi.org/https://doi.org/10.1016/j.scitotenv.2020.142122

Guppy, L., Anderson, K., 2017. Global Water Crisis : the Facts. United Nations Univ. Inst. Water, Environ. Heal. 1–3.

Huang, Y., Law, J.C.F., Lam, T.K., Leung, K.S.Y., 2021. Risks of organic UV filters: a review of environmental and human health concern studies. Sci. Total Environ. 755, 142486. https://doi.org/10.1016/j.scitotenv.2020.142486

Jung, S.M., Bae, J.S., Kang, S.G., Son, J.S., Jeon, J.H., Lee, H.J., Jeon, J.Y., Sidharthan, M., Ryu, S.H., Shin, H.W., 2017. Acute toxicity of organic antifouling biocides to phytoplankton Nitzschia pungens and zooplankton Artemia larvae. Mar. Pollut. Bull. 124, 811–818. https://doi.org/10.1016/j.marpolbul.2016.11.047

K'oreje, K., Okoth, M., Van Langenhove, H., Demeestere, K., 2022. Occurrence and point-of-use treatment of contaminants of emerging concern in groundwater of the Nzoia River basin, Kenya. Environ. Pollut. 297, 118725. https://doi.org/10.1016/j.envpol.2021.118725

Kaliszan, R., 2015. Quantitative structure–retention relationships (QSRRs) in chromatography, Molecular Sciences and Chemical Engineering. Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-12-409547-2.11535-5

Kärrman, A., Domingo, J.L., Llebaria, X., Nadal, M., Bigas, E., Bavel, B. Van, Lindström, G., 2010. Biomonitoring perfluorinated compounds in Catalonia, Spain: concentrations and trends in human liver and milk samples 750–758. https://doi.org/10.1007/s11356-009-0178-5

Kim, S., Jung, D., Kho, Y., Choi, K., 2014. Effects of benzophenone-3 exposure on endocrine disruption and reproduction of japanese medaka (Oryzias latipes)-A two generation exposure study. Aquat. Toxicol. 155, 244–252. https://doi.org/10.1016/j.aquatox.2014.07.004

Kinnberg, K.L., Petersen, G.I., Albrektsen, M., Minghlani, M., Awad, S.M., Holbech, B.F., Green, J.W., Bjerregaard, P., Holbech, H., 2015. Endocrine-disrupting effect of the ultraviolet filter benzophenone-3 in zebrafish, Danio rerio. Environ. Toxicol. Chem. 34, 2833–2840. https://doi.org/10.1002/etc.3129

Kolatorova Sosvorova, L., Vitku, J., Hampl, R., Adamcova, K., Skodova, T., Simkova, M., Parizek, A., Starka, L., Duskova, M., 2018. Exposure to bisphenols and parabens during pregnancy and relations to steroid changes. Environ. Res. 163, 115–122. https://doi.org/10.1016/j.envres.2018.01.031

Krause, M., Frederiksen, H., Sundberg, K., Jørgensen, F.S., Jensen, L.N., Nørgaard, P., Jørgensen, C., Ertberg, P., Juul, A., Drzewiecki, K.T., Skakkebaek, N.E., Andersson, A.M., 2018. Presence of benzophenones commonly used as UV filters and absorbers in paired maternal and fetal samples. Environ. Int. 110, 51–60. https://doi.org/10.1016/j.envint.2017.10.005

Krause, M., Klit, A., Blomberg Jensen, M., Søeborg, T., Frederiksen, H., Schlumpf, M., Lichtensteiger, W., Skakkebaek, N.E., Drzewiecki, K.T., 2012. Sunscreens: are they beneficial for health? An overview of endocrine disrupting properties of UV-filters. Int. J. Androl. 35, 424–436. https://doi.org/10.1111/j.1365-2605.2012.01280.x

Krzeminski, P., Tomei, M.C., Karaolia, P., Langenhoff, A., Almeida, C.M.R., Felis, E., Gritten, F., Andersen, H.R., Fernandes, T., Manaia, C.M., Rizzo, L., Fatta-Kassinos, D., 2019. Performance of secondary wastewater treatment methods for the removal of contaminants of emerging concern implicated in crop uptake and antibiotic resistance spread: A review. Sci. Total Environ. 648, 1052–1081. https://doi.org/10.1016/J.SCITOTENV.2018.08.130

Kwan, C., Goo, S., Tae, J., Lee, S., Ho, J., Hwan, D., Chul, B., Hyung, K., Hui, C., Yeong, S., Beom, Y., 2015. Molecular and cellular endocrinology effects of perfluorooctane sulfuric acid on placental PRL-family hormone production and fetal growth retardation in mice. Mol. Cell. Endocrinol. 401, 165–172. https://doi.org/10.1016/j.mce.2014.10.026

León, V.M., Viñas, L., Concha-Graña, E., Fernández-González, V., Salgueiro-González, N., Moscoso-Pérez, C., Muniategui-Lorenzo, S., Campillo, J.A., 2020. Identification of contaminants of emerging concern with potential environmental risk in Spanish continental shelf sediments. Sci. Total Environ. 742, 140505. https://doi.org/10.1016/j.scitotenv.2020.140505

Liu, Hui, Sun, P., Liu, Hongxia, Yang, S., Wang, L., Wang, Z., 2015. Ecotoxicology and environmental safety hepatic oxidative stress biomarker responses in freshwater fish Carassius auratus exposed to four benzophenone UV filters. Ecotoxicol. Environ. Saf. 119, 116–122. https://doi.org/10.1016/j.ecoenv.2015.05.017

Liu, Xianjing, Liang, C., Liu, Xiaohui, Zhao, F., Han, C., 2020. Occurrence and human health risk assessment of pharmaceuticals and personal care products in real agricultural systems with long-term reclaimed wastewater irrigation in Beijing, China. Ecotoxicol. Environ. Saf. 190, 110022. https://doi.org/10.1016/j.ecoenv.2019.110022

Macheka-tendenguwo, L.R., 2018. Per- and polyfluoroalkyl substances in human breast milk and current analytical methods 36064–36086.

Mao, F., He, Y., Kushmaro, A., Gin, K.Y.H., 2017. Effects of benzophenone-3 on the green alga Chlamydomonas reinhardtii and the cyanobacterium Microcystis aeruginosa. Aquat. Toxicol. 193, 1–8. https://doi.org/10.1016/j.aquatox.2017.09.0294

Mcdonough, C.A., Choyke, S., Barton, K.E., Mass, S., Starling, A.P., Adgate, J.L., Higgins, C.P., 2021. Unsaturated PFOS and other PFASs in human serum and drinking water from an AFFF-impacted community. https://doi.org/10.1021/acs.est.1c00522

McLeod, A.M., Paterson, G., Drouillard, K.G., Haffner, G.D., 2014. Ecological factors contributing to variability of persistent organic pollutant bioaccumulation within forage fish communities of the Detroit River, Ontario, Canada. Environ. Toxicol. Chem. 33, 1825–1831. https://doi.org/10.1002/etc.2606

Meeker, J.D., Yang, T., Ye, X., Calafat, A.M., Hauser, R., 2011. Urinary concentrations of parabens and serum hormone levels, semen quality parameters, and sperm DNA damage. Environ. Health Perspect. 119, 252–257. https://doi.org/10.1289/ehp.1002238

Mitchelmore, C.L., Burns, E.E., Conway, A., Heyes, A., Davies, I.A., 2021. A critical review of organic ultraviolet filter exposure, hazard, and risk to corals. Environ. Toxicol. Chem. 40, 967–988. https://doi.org/10.1002/etc.4948

Mohammed, A.M., Karttunen, V., Huuskonen, P., Huovinen, M., Auriola, S., Vähäkangas, K., 2018. Transplacental transfer and metabolism of diuron in human placenta. Toxicol. Lett. 295, 307–313. https://doi.org/https://doi.org/10.1016/j.toxlet.2018.07.012

Molins-Delgado, D., Olmo-Campos, M. del M., Valeta-Juan, G., Pleguezuelos-Hernández, V., Barceló, D., Díaz-Cruz, M.S., 2018. Determination of UV filters in human breast milk using turbulent flow chromatography and babies' daily intake estimation. Environ. Res. 161, 532–539. https://doi.org/10.1016/j.envres.2017.11.033

Morohoshi, K., Yamamoto, H., Kamata, R., Shiraishi, F., Koda, T., Morita, M., 2005. Estrogenic activity of 37 components of commercial sunscreen lotions evaluated by in vitro assays. Toxicol. Vitr. 19, 457–469. https://doi.org/https://doi.org/10.1016/j.tiv.2005.01.004

Nannou, C., Boti, V., 2019. A modified QuEChERS approach for the analysis of pharmaceuticals in sediments by LC-Orbitrap HRMS 1383–1396.

Nash, J.P., Kime, D.E., Van der Ven, L.T.M., Wester, P.W., Brion, F., Maack, G., Stahlschmidt-Allner, P., Tyler, C.R., 2004. Long-term exposure to environmental concentrations of the pharmaceutical ethynylestradiol causes reproductive failure in fish. Environ. Health Perspect. 112, 1725–1733. https://doi.org/10.1289/ehp.7209

Ng, K., Alygizakis, N., Androulakakis, A., Galani, A., Aalizadeh, R., Thomaidis, N.S., Slobodnik, J., 2022. Target and suspect screening of 4777 per- and polyfluoroalkyl substances (PFAS) in river water, wastewater, groundwater and biota samples in the Danube River Basin. J. Hazard. Mater. 436, 129276. https://doi.org/10.1016/j.jhazmat.2022.129276

Nishimuta, K., Ueno, D., Takahashi, S., Kuwae, M., Kadokami, K., Miyawaki, T., Matsukami, H., Kuramochi, H., Higuchi, T., Koga, Y., Matsumoto, H., Ryuda, N., Miyamoto, H., Haraguchi, T., Sakai, S.I., 2021. Use of comprehensive target analysis for determination of contaminants of emerging concern in a sediment core collected from Beppu Bay, Japan. Environ. Pollut. 272, 115587. https://doi.org/10.1016/j.envpol.2020.115587

Nowak, K., Ratajczak-Wrona, W., Górska, M., Jabłońska, E., 2018. Parabens and their effects on the endocrine system. Mol. Cell. Endocrinol. 474, 238–251. https://doi.org/10.1016/j.mce.2018.03.014

NTP TOX 97: National Toxicology Program. U. S. Department of Health and Human Services, 2019. NTP Technical report on the toxicity studies of perfluoroalkyl carboxylates (perfluorohexanoic acid, perfluorooctanoic acid, perfluorononanoic acid and perfluorodecanoic acid) administered by gavage to sprague dawley rats.

Oishi, S., 2002a. Effects of propyl paraben on the male reproductive system. Food Chem. Toxicol. 1807–1813.

Oishi, S., 2002b. Effects of butyl paraben on the male reproductive system in mice. Arch. Toxicol. 76, 423–429. https://doi.org/10.1007/s00204-002-0360-8

Ojemaye, C.Y., Petrik, L., 2019. Occurrences, levels and risk assessment studies of emerging pollutants (pharmaceuticals, perfluoroalkyl and endocrine disrupting compounds) in fish samples from Kalk Bay harbour, South Africa. Environ. Pollut. 252, 562–572. https://doi.org/10.1016/j.envpol.2019.05.091

Oliveira, I.B., Groh, K.J., Schönenberger, R., Barroso, C., Thomas, K. V., Suter, M.J.F., 2017. Toxicity of emerging antifouling biocides to non-target freshwater organisms from three trophic levels. Aquat. Toxicol. 191, 164–174. https://doi.org/10.1016/j.aquatox.2017.07.019

Pachkowski, B., Post, G.B., Stern, A.H., 2019. The derivation of a Reference Dose (RD) for perfluorooctane sulfonate (PFOS) based on immune suppression. Environ. Res. 171, 452–469. https://doi.org/10.1016/j.envres.2018.08.004

Peng, L., Xu, W., Zeng, Q., Sun, F., Guo, Y., Zhong, S., Wang, F., 2022. Exposure to perfluoroalkyl substances in waste recycling workers : Distributions in paired human serum and urine. Environ. Int. 158, 106963. https://doi.org/10.1016/j.envint.2021.106963

Peng, Y., Fang, W., Krauss, M., Brack, W., Wang, Z., Li, F., Zhang, X., 2018. Screening hundreds of emerging organic pollutants (EOPs) in surface water from the Yangtze River Delta (YRD): Occurrence, distribution, ecological risk. Environ. Pollut. 241, 484–493. https://doi.org/10.1016/j.envpol.2018.05.061

Poothong, S., Thomsen, C., Padilla-sanchez, J.A., Papadopoulou, E., 2017. Distribution of novel and well-known poly- and perfluoroalkyl substances (PFASs) in human serum, plasma, and whole blood 13388–13396. https://doi.org/10.1021/acs.est.7b03299

Ray, S.S., Gusain, R., Kumar, N., 2020. Carbon nanomaterial-based adsorbents for water purification. Chapter two - Classification of water contaminants, Micro and Nano Technologies. Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-12-821959-1.00002-7

Research Triangel Park, National Toxicology Program, U. S. Department of Health and Human Services, 2020. NTP Technical report on the toxicology and carcinogenesis studies of perfluorooctanoic acid (CAS No. 335-67-1) administered in feed to sprague dawley rats. Technical report 598.

Rosenfeld, P.E., Feng, L.G.H., 2011. Risks of Hazardous Wastes. Chapter 16 - Emerging Contaminants. William Andrew Publishing, Boston. https://doi.org/10.1016/B978-1-4377-7842-7.00016-7

Sathishkumar, P., Anu, R., Meena, A., Palanisami, T., 2020. Occurrence, interactive effects and ecological risk of diclofenac in environmental compartments and biota - a review. Sci. Total Environ. 698, 134057. https://doi.org/10.1016/j.scitotenv.2019.134057

Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P., Hollender, J., 2014. Identifying small molecules via high resolution mass spectrometry: Communicating confidence. Environ. Sci. Technol. 48, 2097–2098. https://doi.org/10.1021/es5002105

Shenker, M., Harush, D., Ben-Ari, J., Chefetz, B., 2011. Uptake of carbamazepine by cucumber plants - A case study related to irrigation with reclaimed wastewater. Chemosphere 82, 905–910. https://doi.org/10.1016/j.chemosphere.2010.10.052

Smarr, M.M., Sundaram, R., Honda, M., Kannan, K., Buck Louis, G.M., 2017. Urinary concentrations of parabens and other antimicrobial chemicals and their association with couples' fecundity. Environ. Health Perspect. 125, 730–736. https://doi.org/10.1289/EHP189

Song, S., He, Y., Huang, Y., Huang, X., Guo, Y., Zhu, H., Kannan, K., Zhang, T., 2020. Occurrence and transfer of benzophenone-type ultraviolet filters from the pregnant women to fetuses. Sci. Total Environ. 726, 138503. https://doi.org/10.1016/j.scitotenv.2020.138503

Statista, 2022a. Revenue of the beauty & personal care market worldwide from 2013 to 2026 (in million U.S. dollars).

Statista, 2022b. Market revenue of the sun protection market worldwide from 2013 to 2026 [WWW Document]. URL https://www.statista.com/forecasts/812522/sun-care-market-value-global

Stefanakis, A.I., Becker, J.A., 2016. A review of emerging contaminants in water: classification, sources, and potential risks, Impact of Water Pollution on Human Health and Environmental Sustainability. IGI Global, Hershey, PA, USA. https://doi.org/10.4018/978-1-4666-9559-7.ch003

Suzuki, T., Kitamura, S., Khota, R., Sugihara, K., Fujimoto, N., Ohta, S., 2005. Estrogenic and antiandrogenic activities of 17 benzophenone derivatives used as UV stabilizers and sunscreens. Toxicol. Appl. Pharmacol. 203, 9–17. https://doi.org/https://doi.org/10.1016/j.taap.2004.07.005

Sznajder-katarzy, K., Surma, M., Pisku, M., 2020. Determination of perfluoroalkyl substances (PFASs) in fats and oils by QuEChERS/micro-HPLC-MS/MS 137. https://doi.org/10.1016/j.foodres.2020.109583

Tadić, Đ., Bleda Hernandez, M.J., Cerqueira, F., Matamoros, V., Piña, B., Bayona, J.M., 2021. Occurrence and human health risk assessment of antibiotics and their metabolites in vegetables grown in field-scale agricultural systems. J. Hazard. Mater. 401, 123424. https://doi.org/10.1016/j.jhazmat.2020.123424

Tadić, Đ., Matamoros, V., Bayona, J.M., 2019. Simultaneous determination of multiclass antibiotics and their metabolites in four types of field-grown vegetables. Anal. Bioanal. Chem. 411, 5209–5222. https://doi.org/10.1007/s00216-019-01895-y

The European Parliament and the Council of the European Union, 2020. Directive (EU) 2020/2184 of the European Parliament and of the Council. Off. J. Eur. Union 2019, 1–62.

Thomas, K. V, Brooks, S., 2010. The environmental fate and effects of antifouling paint biocides. Biofouling 26, 73–88. https://doi.org/10.1080/08927010903216564

US EPA, 2021. Drinking Water Contaminant Candidate List 4- Final.

US EPA, 2020. Irgarol: Proposed Interim Registration Review Decision Case Number 5031 1–77.

Valle-Sistac, J., Molins-Delgado, D., Díaz, M., Ibáñez, L., Barceló, D., Silvia Díaz-Cruz, M., 2016. Determination of parabens and benzophenone-type UV filters in human placenta: First description of the existence of benzyl paraben and benzophenone-4. Environ. Int. 88, 243–249. https://doi.org/10.1016/j.envint.2015.12.034

Van Boven, M., Laruelle, L., Daenens, P., 1990. HPLC Analysis of Diuron and Metabolites in Blood and Urine. J. Anal. Toxicol. 14, 231–234. https://doi.org/10.1093/jat/14.4.231

Vargas-Berrones, K., Bernal-Jácome, L., Díaz de León-Martínez, L., Flores-Ramírez, R., 2020. Emerging pollutants (EPs) in Latin América: A critical review of under-studied EPs, case of study -Nonylphenol-. Sci. Total Environ. 726, 138493. https://doi.org/10.1016/j.scitotenv.2020.138493

Vela-soria, F., García-villanova, J., Mustieles, V., Haro, T. De, Antignac, J.P., 2021. Assessment of perfluoroalkyl substances in placenta by coupling salt assisted liquid-liquid extraction with dispersive liquid-liquid microextraction prior to liquid chromatography-tandem mass spectrometry. Talanta 221, 121577. https://doi.org/10.1016/j.talanta.2020.121577

von der Ohe, P.C., Dulio, V., Slobodnik, J., De Deckere, E., Kühne, R., Ebert, R.U., Ginebreda, A., De Cooman, W., Schüürmann, G., Brack, W., 2011. A new risk assessment approach for the prioritization of 500 classical and emerging organic microcontaminants as potential river basin specific pollutants under the European Water Framework Directive. Sci. Total Environ. 409, 2064–2077. https://doi.org/10.1016/j.scitotenv.2011.01.054

Wang, B., Chen, Q., Shen, L., Zhao, S., Pang, W., Zhang, J., 2016. Perfluoroalkyl and polyfluoroalkyl substances in cord blood of newborns in Shanghai , China: Implications for risk assessment. Environ. Int. 97, 7–14. https://doi.org/10.1016/j.envint.2016.10.008

Wang, J., Pan, L., Wu, S., Lu, L., Xu, Y., Zhu, Y., Guo, M., Zhuang, S., 2016. Recent advances on endocrine disrupting effects of UV filters. Int. J. Environ. Res. Public Health 13, 1–11. https://doi.org/10.3390/ijerph13080782

Wee, S.Y., Ismail, N.A.H., Haron, D.E.M., Yusoff, F.M., Praveena, S.M., Aris, A.Z., 2022. Pharmaceuticals, hormones, plasticizers, and pesticides in drinking water. J. Hazard. Mater. 424, 127327. https://doi.org/10.1016/j.jhazmat.2021.127327

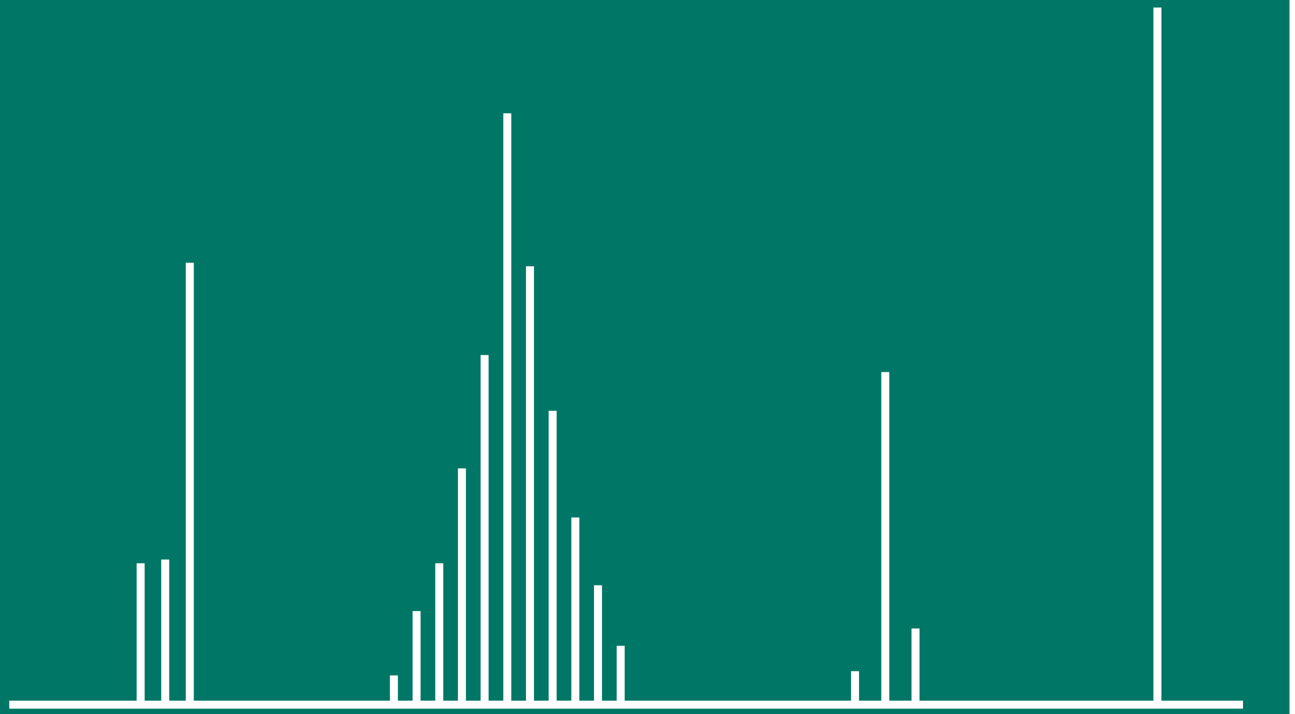
Weiss, J.M., Jones, B., Koekkoek, J., Bignert, A., Lamoree, M.H., 2021. Per- and polyfluoroalkyl substances (PFASs) in Swedish household dust and exposure of pet cats. Environ. Sci. Pollut. Res. 28, 39001–39013. https://doi.org/10.1007/s11356-021-13343-5

Wu, X., Dodgen, L.K., Conkle, J.L., Gan, J., 2015. Plant uptake of pharmaceutical and personal care products from recycled water and biosolids: A review. Sci. Total Environ. 536, 655–666. https://doi.org/10.1016/j.scitotenv.2015.07.129

Xu, L., Lee, H.K., 2012. Comprehensive sampling and sample preparation. Section 3.27 - Sorbent-phase sample preparation in environmental analysis. Academic Press, Oxford. https://doi.org/10.1016/B978-0-12-381373-2.00100-9

Xue, J., Kannan, K., 2016. Accumulation profiles of parabens and their metabolites in fish, black bear, and birds, including bald eagles and albatrosses. Environ. Int. 94, 546–553. https://doi.org/10.1016/j.envint.2016.06.015

Zhang, T., Sun, H., Qin, X., Wu, Q., Zhang, Y., Ma, J., Kannan, K., 2013. Benzophenone-type UV filters in urine and blood from children, adults, and pregnant women in China: Partitioning between blood and urine as well as maternal and fetal cord blood. Sci. Total Environ. 461–462, 49–55. https://doi.org/10.1016/j.scitotenv.2013.04.074



Chapter 2

Thesis objectives and structure

2.1 Objectives

The primary objective of this thesis is **to develop, validate and apply target and non-target analytical methodologies for the determination of CECs in environmental and human samples**. The attainment of this main objective implies other specific objectives:

- Develop and validate target and non-target (U)HPLC-MS-based methods for a wide range of samples, including water, sediments, soils, biota, vegetables and umbilical cord blood.

- Evaluate the presence and potential impact of CECs in diverse environmental ecosystems.

- Monitor CECs to evaluate the performance of well-established and novel elimination/degradation treatments for their efficient removal from wastewater.

- Assess the feasibility of water reuse in agriculture by determining CECs translocation from irrigation water to crops.

- Investigate the risk posed by CECs to humans under diet and maternal transfer exposure.

- Apply non-target strategies to broaden the scope of the CECs analysed and identify related transformation products.

- Demonstrate that analytical target and non-target strategies are complementary and necessary to obtain a more accurate picture of the CEC’s occurrence and impact.

2.2 Structure

This thesis is presented as a compendium of articles. It is divided into seven chapters, and the structure summary is shown in **Figure 2.1**. Chapter 1 introduces the definition of CECs, the different families within this group, and the specific ones on which this thesis focuses. The regulations on CECs worldwide are summarised, and the potential pathways and occurrence in the environment and humans are also described. Then, the most used analytical methodologies and techniques for CECs analysis are explained, together with the ones used in this thesis. Finally, target and non-target approaches, as well as their role in TPs' identification are described.

Chapter 2, this chapter, explains the main and specific objectives of the thesis and its structure. Chapters 3, 4, 5 and 6 include the experimental work, the relevant articles, the results achieved and discussions. The four chapters are separated by topic, following a circular structure, i.e. since CECs are determined in the environment (Chapter 4), followed by the implementation of degradation technologies (Chapter 5), the reuse of reclaimed water for agriculture (Chapter 6), and, ultimately, human exposure through diet and maternal transfer (Chapter 7). All the chapters follow the same structure, with an initial introduction of the subject and an explanation of the methodologies used (when necessary), followed by the articles included in the chapter and a final joint discussion.

In Chapter 3, novel data on the occurrence of CECs (mostly PPCPs and biocides) in different environmental matrices are presented and discussed. It includes four publications reporting the occurrence of CECs in *Posidonia oceanica,* seawater, sediments, and fish. These are the corresponding published works:

- Publication #1: N. S. R., Agawin., **Sunyer-Caldú, A.**, Diaz-Cruz, M. S., Frank-Comas, A., García-Marquez, M. G., Tovar-Sánchez, A., "Mediterranean seagrass Posidonia oceanica accumulates sunscreen UV filters", 2022, *Marine Pollution Bulletin*, https://doi.org/10.1016/j.marpolbul.2022.113417

- Publication #2: Fenni, F., **Sunyer-Caldú, A.**, Ben, M. H., Diaz-Cruz, M. S., "Contaminants of emerging concern in marine areas: first evidence of UV filters and paraben preservatives in seawater and sediment in Eastern coast of Tunisia", 2022, *Environmental Pollution*, https://doi.org/10.1016/j.envpol.2022.119749

- Publication #3: Lotz, K., **Sunyer-Caldú, A.**, Caldas, S., Gilberto, E., Fillmann, G., Diaz-Cruz, M. S., "Rapid and cost-effective multiresidue analysis of pharmaceuticals, personal care products, and antifouling booster biocides in marine sediments using matrix solid phase dispersion", 2021, *Chemosphere*, https://doi.org/10.1016/j.chemosphere.2020.129085

- Publication #4: Lotz, K., **Sunyer-Caldú, A.**, Caldas, S., Gilberto, E., Fillmann, G., Diaz-Cruz, M. S., "Distribution in marine fish and EDI estimation of contaminants of emerging concern by vortex-assisted matrix solid-phase dispersion and HPLC-MS/MS", *Submitted to Marine Pollution Bulletin*.

In Chapter 4, CECs' degradation novel technologies are tested and evaluated to achieve higher removal rates for PPCPs than those by conventional techniques in wastewater. Degradation pathways and transformation products' identification are also presented and discussed. Four publications are included in this chapter, two based on the removal with algae, one on soil aquifer treatment, and the last one on oxidative degradation.

- Publication #5: Vassalle, L., **Sunyer-Caldú, A.**, Uggetti, E., Díez-Montero, R., Diaz-Cruz, M. S., García, J., García-Galán, M. J., "Bioremediation of emerging micropollutants in irrigation water. The alternative of microalgae-based treatments", 2020, *Journal of Environmental Management*, https://doi.org/10.1016/j.jenvman.2020.111081

- Publication #6: Vassalle, L., **Sunyer-Caldú, A.**, Uggetti, E., Díez-Montero, R., Diaz-Cruz, M. S., García, J., García-Galán, M. J., "Behavior of UV Filters, UV Blockers and Pharmaceuticals in High Rate Algal Ponds Treating Urban Wastewater", 2020, *Water*, https://doi.org/10.3390/w12102658

- Publication #7: **Sunyer-Caldú, A.**, Benedetti, B., Valhondo, C., Martínez-Landa, L., Carrera, J., Di Carro, M., Magi, E., Diaz-Cruz, M. S., "Using integrative samplers to estimate the removal of pharmaceuticals and personal care products in a WWTP and by soil aquifer treatment enhanced with a reactive barrier", *Submitted to Science of the Total Environment.*

- Publication #8: Calzadilla, W., Espinoza, C., Diaz-Cruz, M. S., **Sunyer-Caldú, A.**, Aranda, M., Peña-Farfal, C., Salazar, R., "Simultaneous degradation of 30 pharmaceuticals by anodic oxidation: Main intermediaries and by-products", 2021, *Chemosphere*, https://doi.org/10.1016/j.chemosphere.2020.128753

In Chapter 5, the feasibility of reusing wastewater and reclaimed water in agriculture is evaluated, with the development of new fast and simple analytical methodologies and the determination of CECs and TPs in different types of crops. It consists of four papers, three in the real field (in Palamós, Girona) and one in hydroponics at laboratory scale.

- Publication #9: **Sunyer-Caldú, A.**, Diaz-Cruz, M. S., "Development of a QuEChERS-based method for the analysis of pharmaceuticals and personal care products in lettuces grown in field-scale agricultural plots irrigated with reclaimed water", 2021, *Talanta*,
  https://doi.org/10.1016/j.talanta.2021.122302

- Publication #10: **Sunyer-Caldú, A.**, Sepúlveda-Ruiz, P., Salgot, M., Folch-Sánchez, M., Barceló, D., Diaz-Cruz, M. S., "Reclaimed water in agriculture: a plot-scale study assessing crop uptake of emerging contaminants and pathogens", 2022, *Journal of Environmental Chemical Engineering*,
  https://doi.org/10.1016/j.jece.2022.108831

- Publication #11: **Sunyer-Caldú, A.**, Quintana, G., Diaz-Cruz., M. S., "Pharmaceuticals and personal care products uptake by crops irrigated with reclaimed water and human health implications", *Submitted to Science of the Total Environment.*

- Publication #12: **Sunyer-Caldú, A.**, Golovko, O., Kaczmarek, M., Asp, H., Bergstrand, KJ., Gil-Solsona, R., Gago-Ferrero, P., Diaz-Cruz, M. S., Ahrens, L., Hultberg, M., "Occurrence and fate of contaminants of emerging concern and their transformation products after uptake by Pak Choi vegetable", *Submitted to Environmental Pollution.*

In Chapter 6, the studies assessing human exposure to CECs and their intake capacity are included. The two papers included consist of the method development and assessment of prenatal exposure to CECs with a target and suspect screening of umbilical cord blood. The risk assessment of the exposure through the diet (fish and crops) from Publications #4 and #11 is also included in this chapter to discuss the estimation of daily intake values (EDI).

- Publication #13: **Sunyer-Caldú, A.**, Perió, A., Díaz, M., Ibáñez, L., Gago-Ferrero, P., Diaz-Cruz, M. S., "Development of a sensitive analytical method for the simultaneous analysis of benzophenone-type UV filters and paraben preservatives in umbilical cord blood", 2021, *MethodsX*,
  https://doi.org/10.1016/j.mex.2021.101307

- Publication #14: **Sunyer-Caldú, A.**, Perió, A., Díaz, M., Ibáñez, L., Gil-Solsona, R., Gago-Ferrero, P., Diaz-Cruz, M. S., "Target analysis and suspect screening of UV filters, parabens and other chemicals used in personal care products in human cord blood: prenatal exposure by mother-fetus transfer", *Submitted to Environment International.*

Finally, the general conclusions of this thesis are collected in Chapter 7.

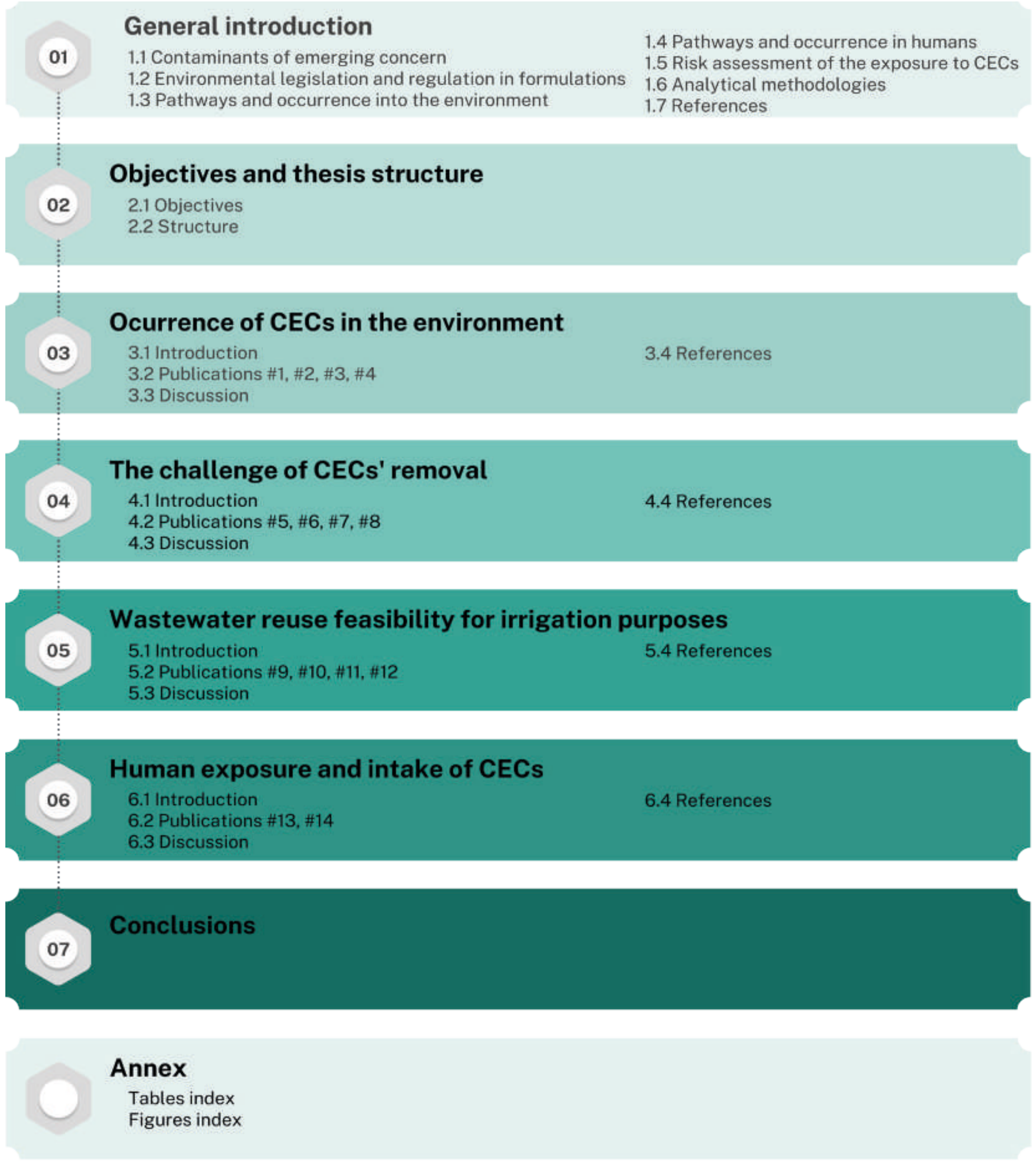
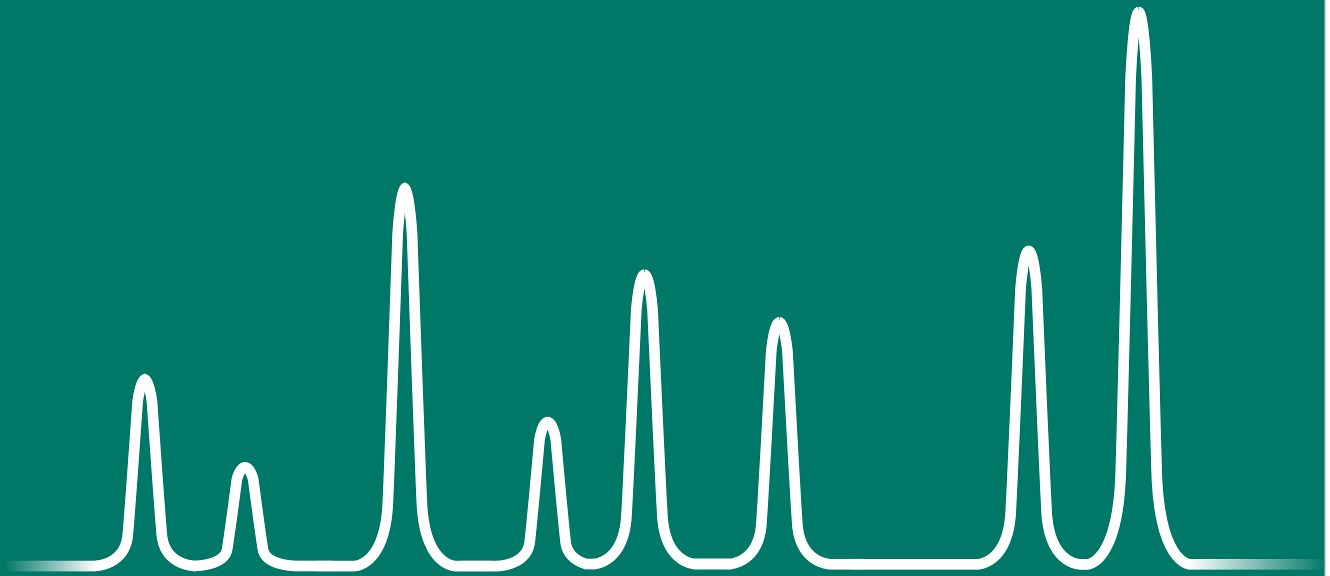
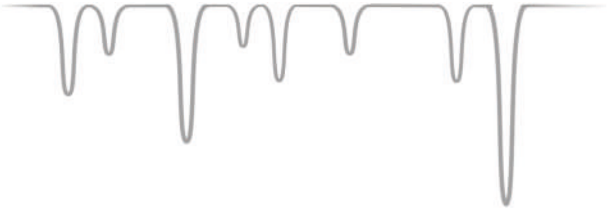


Figure 2.1. Thesis structure.



Chapter 3

Occurrence of CECs in the marine environment



3.1 Introduction

The marine environment is an essential component of the global life-support system. Oceans cover 71% of the Earth's surface and sustain humans with food and oxygen (United Nations Environment Programme, 2022). Despite the wealth of fauna and flora, they are probably the least understood and most undervalued of all ecosystems. The population growth worldwide is constant, as well as the industrial production to fulfil the needs of this population (Gorito et al., 2017). This is especially evident in natural areas where tourism is massive, and thousands of people enjoy natural paradises and visit small regions. In many cases, it causes very stressful conditions for the marine fauna and flora in the area, and CECs play an essential role in this pressure (León et al., 2020). For example, commercial cruises and other kinds of ships represent a significant source of pollution because they are like floating cities that evacuate the wastewater produced on board directly to the sea without any treatment (Brumovský et al., 2017). Furthermore, antifouling biocides in the hull’s painting of the ships represent an environmental issue. Antifouling emissions are one of the most significant marine traffic environmental impacts recognised as a threat to coastal environmental health (Karlsson et al., 2010). They have been reported to be toxic for many aquatic species (Bao et al., 2011), and their monitoring is crucial to assess the potential adverse effects in the ecosystems. After two years of pause, mass tourism has resumed, so this problem is only expected to worsen.

CECs’ occurrence in continental compartments, such as rivers, soils, or biota (Starling et al., 2019), has been extensively studied in recent years. However, since water is CECs’ main vehicle, it represents a continuous discharge of these contaminants, via WWTPs’ effluents or freshwater, to the marine environment. Despite the dilution effect, all marine species are continuously exposed to them, producing unknown effects on ecosystems and marine organisms.

Thus, this chapter of the thesis focuses on the marine environment rather than continental compartments; it represents the ultimate sink of CECs, and the biota and ecosystems' diversity is considerably richer than in freshwater bodies. New methodologies were developed to comprehensively understand the PPCPs' and biocides' fate in the marine environment. The CECs' occurrence was studied in many different matrices from different locations (remote areas, but also stressed touristic places).

A wide range of locations could be selected to assess the impact of tourism and anthropogenic activities on the environment and the consequent occurrence of contaminants. For example, a representative area of massive tourism is Mallorca island, which received 17 million tourists in 2019. Considering that the island has only ~210 km^2 of territory, but counting more than 600,000 touristic accommodations, the stress on the surrounding ecosystems is noticeable. It is an ideal monitoring area since it is located in the Mediterranean area, particularly susceptible to climate change and human activities (Celma et al., 2022).

To assess the impact of tourism over the years, an endemic Mediterranean seagrass named *Posidonia oceanica* (**Figure 3.1**) was selected in Publication #1. Posidonia plays important ecological roles in the Mediterranean coasts; they maintain oxygenated water, stabilise sediments, and supports a high diversity of micro and macro-organisms. Furthermore, it is ideal because it is a good bioindicator of the ecosystems' quality status and allows, at the same time, a time-trend evaluation of the contaminants accumulated in them.

Figure 3.1. *Posidonia oceanica* fields.

This measurement can be done by taking advantage of the fact that internodal lengths follow oscillating year cycles. Three sampling points were selected to ensure the real anthropogenic impact, one close to populated cities or industrial activities and another in a remote area, as shown in **Figure 3.2**. In this case, only UVFs and PBs were analysed because of the usual correlation with their occurrence in highly touristic areas.

Another touristic zone in the Mediterranean coastline is the coast of Madhia, in Tunisia. It is characterised by big coastal resorts, and it is the fourth most visited country in Africa. Despite being a touristic destiny, scarce information about CECs' occurrence in this area is available. Therefore, to assess the time-trend patterns and contamination in this area, an extensive sampling of seawater and coastal sediments was conducted, presented in Publication #2. The sampling points were selected following the procedure described in Publication #1, selecting two points close to urban centres and one away from anthropogenic activities (**Figure 3.2**). UVFs and PBs were analysed, as in the study of Mallorca island.

Finally, another interesting region to monitor is Patos Lagoon (Rio Grande, Brazil) because, besides the known touristic activity, it presents high maritime activity, with different harbours and a shipyard. Different points along the estuary and lagoon were selected in Publications #3 and #4 (**Figure 3.2**). The sampling consisted of sediments at different depths and fish (*Micropogonias furnieri*) to evaluate the bioaccumulation of antifouling biocides and PPCPs in both matrices. Sediments were sampled mainly in Sao Paulo's estuary and, to a lesser extent, in Patos Lagoon (Rio Grande). Fish was only sampled in the latter. This publication also includes a risk assessment with the CECs concentrations found in the fish, but it will be further discussed in Chapter 6 (human exposure to CECs)

Methodologies

For the analysis of the different matrices included in this chapter, new methodologies were developed in the case of Publications #3 and 4#, and previous ones were optimized and directly applied in the case of Publications #1 and #2.

In the first case, matrix solid phase extraction was combined with vortex-assisted extraction to develop a simple, fast and eco-friendly methodology, ideal for small amounts of sample. Lately, this technique has been applied for many different purposes and matrices in multi-residue analysis, showing its robustness even with complex matrices (Caldas et al., 2018). Therefore, the extraction parameters (extraction solvent and solid support) and analysis conditions were adapted and optimised to analyse sediment and fish samples.

In the second case, the seawater and sediment samples from Publication #2 were analysed based on previous methodologies (Gago-Ferrero et al., 2013, 2011), and the *Posidonia oceanica* from Publication #1 was analysed with a QuEChERS-based extraction methodology developed in this thesis, included in Chapter 5.

Figure 3.2. Sampling sites selected for all the publications in this chapter (Samples colour code : Grey= fish, Red=fish and sediments, Blue=seawater and sediments, Black= sediments, Green=*Posidonia oceanica*).

3.2 Results

This chapter includes four articles (three published and one submitted for publication). The occurrence of CECs in the marine environment is described in seawater, sediment, fish and seagrass from different parts of the world under tourism and industrial impacts. The publications are:

- Publication #1: N. S. R., Agawin., **Sunyer-Caldú, A.**, Diaz-Cruz, M. S., Frank-Comas, A., García-Marquez, M. G., Tovar-Sánchez, A., "Mediterranean seagrass Posidonia oceanica accumulates sunscreen UV filters", 2022, *Marine Pollution Bulletin*, https://doi.org/10.1016/j.marpolbul.2022.113417

- Publication #2: Fenni, F., **Sunyer-Caldú, A.**, Ben, M. H., Diaz-Cruz, M. S., "Contaminants of emerging concern in marine areas: first evidence of UV filters and paraben preservatives in seawater and sediment in Eastern coast of Tunisia", 2022, *Environmental Pollution*, https://doi.org/10.1016/j.envpol.2022.119749

- Publication #3: Lotz, K., **Sunyer-Caldú, A.**, Caldas, S., Gilberto, E., Fillmann, G., Diaz-Cruz, M. S., "Rapid and cost-effective multiresidue analysis of pharmaceuticals, personal care products, and antifouling booster biocides in marine sediments using matrix solid phase dispersion", 2021, *Chemosphere*, https://doi.org/10.1016/j.chemosphere.2020.129085

- Publication #4: Lotz, K., **Sunyer-Caldú, A.**, Caldas, S., Gilberto, E., Fillmann, G., Diaz-Cruz, M. S., "Distribution in marine fish and EDI estimation of contaminants of emerging concern by vortex-assisted matrix solid-phase dispersion and HPLC-MS/MS", *Submitted to Marine Pollution Bulletin*.

3.2.1 Publication #1

Mediterranean seagrass Posidonia oceanica accumulates sunscreen UV filters

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Mediterranean seagrass *Posidonia oceanica* accumulates sunscreen UV filters

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ABSTRACT

Certain ultra-violet filter (UVF) components of solar creams have negative impacts on coral reefs and have been prohibited in international tourism destinations (i.e., Hawaii, Florida, and Palau) to protect coral reefs. In the Mediterranean coasts which are also hotspots of international tourism and where endemic seagrass *Posidonia oceanica* forms extensive meadows, the accumulation of UVF components have not been studied. We report for the first time, that the rhizomes of *P. oceanica* internally accumulated UVFs BP3, BP4, AVO, 4MBC and MeBZT and the paraben preservative MePB. The components BP4 and MePB occurred in higher concentrations reaching up to 129 ng g^{-1} dw and 512 ng g^{-1} dw, respectively. This work emphasizes the need for more experimental studies on the effects of UVFs on seagrasses and check if we should follow suit to prohibit certain UVFs to protect this species as what has been done in other regions to protect corals.

1. Introduction

Sunscreen products are contaminants of emerging concern (CECs) composed of a wide range of organic (e.g., benzophenones, p-aminobenzoates, and camphors among others) and inorganic compounds [e.g., nanoparticle oxides: titanium dioxide (TiO_2) and zinc oxide (ZnO)] ultra-violet filters (UVFs) with light absorption and reflection, respectively, in the range of UV-A and/or UV-B. Sunscreen components enter the marine environment in both the overlying water and sediments as a consequence of direct inputs from beach-goers, cruise travels in marine waters and indirectly through treated domestic and industrial wastewater discharges (Giokas et al., 2007; Tovar-Sánchez et al., 2013; Gondikas et al., 2014). In coastal ecosystems which are subject to very high tourism pressure such as in the Southern Mediterranean Sea (Tovar-Sánchez et al., 2019), high concentrations of UVFs [organic UVFs, particularly, benzophenone-3 (BP3) and 4-methylbenzylidene camphor (4-MBC), as well as inorganic TiO_2 and ZnO] in the surface microlayer were reported during summer (Sánchez-Quiles et al., 2020) and may pose a threat to Mediterranean coastal ecosystems. The few studies that are published on the accumulation and effects of these emerging pollutants have focused on higher components of the marine food web (e.g., zooplankton, pelagic fishes, dolphins, and birds; Sánchez-Quiles et al., 2020) and for primary producers the focus was on microalgae (Wang et al., 2016; Haynes and Ward, 2020) and lately on macroalgae (Pacheco-Juárez et al., 2019; Montesdeoca-Esponda et al., 2021). Most of the research analyzed inorganic components of UVFs and very few studies have been done on seagrasses despite that sedimentation is one of the pathways of UVFs in coastal environments (Sánchez-Quiles et al., 2020). To our knowledge, there are only two studies on seagrasses, one reporting the effects of TiO_2 in *Halophila stipulacea* (Mylona et al., 2020) and the other, on the effects of ZnO in *Cymodocea nodosa* (Malea et al., 2019). These were done in experimental set-ups and did not report accumulation UVF accumulation in the seagrass tissues in the natural environment. In coastal Mediterranean, the endemic seagrass *Posidonia oceanica* forms extensive meadows occupying 12,247 km^2. They form a very important ecosystem which maintains the health of the sea and they are classified as priority habitat for conservation under the Habitats Directive (Dir. 92/43/CEE). Given the importance of *P. oceanica* meadows, there is an urgent need for data and information on the accumulation and effects of CECs (i.e., UVFs) in these meadows so that environmental policymakers can include in their monitoring programs these emerging pollutants. This is necessary to have sustainable tourism in the Mediterranean coasts. In other international tourism destinations, such as in Hawaii, the Hawaiian Parliament, recently

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approved on April 2018, a bill prohibiting the use of solar protection products containing BP3 (also known as oxybenzone) and ehtylhexylmethoxycinnamate (EHMC, also known as octinoxate), after researchers gave evidence that these components have negative effects on their corals (Corinaldesi et al., 2018). Other states in the USA (Florida, Key West, U.S. Virgin Islands, and Puerto Rico) also presented similar regulations. Nation-wide, the Pacific Nation of Palau was the first country in restricting the market of solar protection products containing components, octinoxate, oxybenzone and octocrylene (OC) starting on January 1, 2020 and prohibits foreign tourists to use these products to the country (Senate Bill No. 10-135, SD1, HD1, available in https://www.palaugov.pw/documents/rppl-no-10-30-the-responsible-tourism-education-act-of-2018/; Final Report on Sunscreen Pollution Analysis In Jellyfish Lake Coral Reef Research Foundation (Palau) available in https://coralreefpalau.org/wp-content/uploads/2017/10/CRRF-UNESCO-Sunscreen-in-Jellyfish-Lake-no.2732.pdf).

In Mediterranean countries, in spite of the high tourism pressure and high risk (Diaz-Cruz and Molins-Delgado, 2018; Tovar-Sánchez et al., 2019) on their coasts, there is no legislation of these types, mainly because there is no scientific evidence yet which demonstrates the accumulation and effects and impact of these emergent pollutants on EU marine coastal waters. Here, we report for the first time, evidences of internal accumulation of organic UVFs in the rhizomes of *P. oceanica* in the coastal sites in one of the top summer island destinations in the Mediterranean Sea, Mallorca.

2. Materials and methods

The plant materials were taken from three sites in Mallorca: (1) near the port of the capital Palma at two sampling points (39.547372° N, 2.599127°E and 39.531129° N, 2.591346° E), (2) near the port of Alcudia (39.833796° N, 3.148142° E) and (3) in a pristine site in Ses Salines (39.263284° N, 3.051308° E) (Supplementary Fig. S1). The port of Palma is the busiest port in Mallorca receiving ca. 2 million tourist cruise passengers yearly and the city offers 49,000 tourist beds while the port of Alcudia receives less tourist cruise passengers (some 2000) but also offers high number of hotel tourist beds (28,000) with 90% hotel occupancy rate during peak summer months (http://dadesdelturisme.caib.es). In both ports, the seawater also receives wastewater treatment discharges and the *P. oceanica* meadows near these ports are reported to be in an unfavourable state of conservation (with plant shoot densities significantly lower) compared with the pristine site Ses Salines which have plant shoot densities >800 shoots m^{-2} (Supplementary Table S1) and are less subject to urban and tourism pressure (Agawin et al., 2018).

In each of the sampling points, between 3 and 4 vertical rhizomes (the longest) of *P. oceanica* have been collected by SCUBA diving at 6 to 14 m depth in July 2018. The rhizomes were carefully peeled of hairy remains of old degrading leaf sheaths baring the scales or nodes (i.e., the proximal parts that remain attached to the rhizome when leaves die, Supplementary Fig. S2). The age of the rhizome pieces was determined by dating techniques based on measurement of internodal lengths (i.e., the length of two successive nodes) described in Duarte et al. (1994). The method takes advantage of the observation that internodal lengths follow oscillating yearly cycles. The internodal length was measured using a binocular microscope LEICA MZ16 coupled with an image analysis system. The average number of leaves annually produced per shoot was estimated from the seasonal variability in vertical internodal length as described in Marbà et al. (2002). Knowledge of the mean number of leaves produced annually per shoot during the shoot lifespan allowed calculation of the annual mean leaf plastochron interval (which is the time elapsed between the formation of two consecutive leaves; Erickson and Michelini, 1957), and consequently allowing the conversion of time in plastochron interval units to absolute chronological time (i.e., years). Selected internodes of different periods (dating from 2001 to 2014, Supplementary Table S1) were cut and pooled and were analyzed for different organic components of UVFs (BP3, benzophenone-1 [BP1], benzophenone-2 [BP2], benzophenone-4 [BP4], 4-hydroxybenzophenone [4HB], 4,4′-dihydroxybenzophenone [4DHB], 2,2′-dihydroxy-4-methoxybenzophenone (benzophenone 8, dioxybenzone, DHMB, avobenzone [AVO], 3-(4-methylbenzylidene) camphor [4-MBC], 2-ethylhexyl-4-methoxycinnamate [EHMC], ethyl-4-aminobenzoate [EtPABA], benzotriazole [BZT], 5-methyl benzotriazole [MeBZT], dimethylbenzotriazole [DMBZT], benzotriazol-2-yl)-4-methylphenol UVP), and the paraben preservatives benzylparaben [BePB], butylparaben [BuPB], propylparaben [PrPB], and methylparaben [MePB]. A total of ten rhizome segments were analyzed.

For the analysis of UVFs and paraben preservatives, an already developed quick, easy, cheap, effective, rugged, and safe (QuEChERS)-based methodology used originally to analyze pharmaceuticals and personal care products (PPCPs) in lettuces was adapted and used (Sunyer-Caldú and Diaz-Cruz, 2021). Shortly, *P. oceanica* was freeze dried and 1 g dw (dry-weight) was used for the analysis. The QuEChERS extraction methodology was applied using the commercial citrate and the PSA-Kit-02 kits from BEKOlut® as extraction and dispersive phases. Next, an aliquot (5 mL) from the supernatant was evaporated until near dryness and reconstituted to 1 mL. This extract was injected with a Symbiosis™ Pico from Spark Holland (Emmen, The Netherlands) to a liquid chromatography (LC) analytical column Hibar Purosher® STAR® HR R-18 (50 mm × 2.0 mm, 5 μm) coupled to a 4000 QTRAP mass spectrometer from Applied Biosystems-Sciex (Foster City, USA). Selected reaction monitoring (SRM) using the two most intense transitions in both positive and negative electrospray ionization (ESI+, ESI−) was the operating mode. Calibration curves were prepared at 10 different concentrations with a mix of the target compounds in the range 1–700 ng mL^{-1}. The limits of detection of the method (MLODs) ranged from 0.1 to 1.44 ng g^{-1} dw and the coefficient of determination ranged from 0.979 to 0.999 in the detected compounds. Limits of detection and quantification, and precision (relative standard deviation, RSD) of the analyses of the different organic UVFs and paraben conservatives are provided in Supplementary Table S2. Different steps in the analysis were performed as quality assistance or quality control: (1) different blank methods were included (procedural blank, without matrix or sample); (2) use of a surrogate standard (BP-13C) as evaluation of the extraction performance and isotopically labelled standards for the quantification of the target analytes; (3) use of quality controls at known concentrations of the target analytes, that were included in the analysis sequence randomly; (4) following the EU normative (Commission Decision 2002/657/EC), all compounds were identified with the tR compared with the standards at a maximum tolerance of 2.5% and ion intensities between the selected SRM transitions were below 15%; (5) matrix matched standard solutions were used to correct potential matrix effects, and (6) cleaning of glass material in the laboratory with ethanol and acetone and muffling (400 °C) of the non-volumetric one overnight.

3. Results and discussion

The *P. oceanica* rhizome pieces analyzed dated back from 10 to 22 years (Figure 1). Of the various organic UVFs and parabens analyzed, the rhizomes of *P. oceanica* internally accumulated UVF filters BP3, BP4, AVO, 4-MBC and MeBZT and the paraben conservative MePB (Table 1). In all of the rhizome segments analyzed, BP4 and MePB occurred in higher concentrations particularly from the waters of the urbanized capital of Mallorca (Palma), where these compounds reached up to 129 ng g^{-1} dw and 512 ng g^{-1} dw, respectively (Table 1). These values correspond approximately to accumulation rates of 103.2 ng g^{-1} dw yr^{-1} and 409.6 ng g^{-1} dw yr^{-1} respectively, assuming it takes 1.25 yr for seven internodes to grow (based on our data, an average of 5.6 leaves yr^{-1} have been produced on *P. oceanica* rhizome or it takes 0.18 yr for an internode to elongate). Based on the oldest age of the rhizome segment analyzed from Alcudia (Sample 4, Supplementary Table S1), these compounds have been internally accumulated since 2 decades ago. This

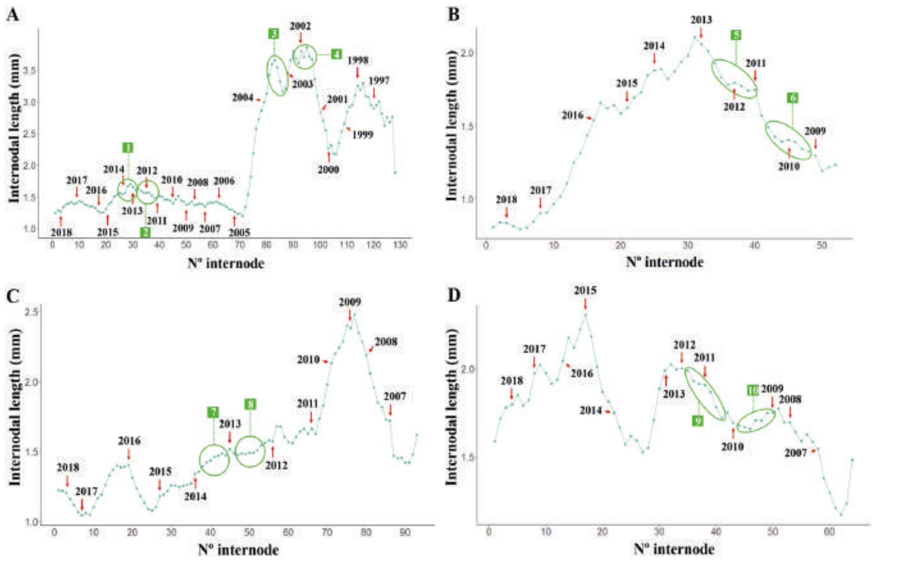

Fig. 1. Internodal lengths of the rhizome samples of *Posidonia oceanica* collected in (A) Alcudia, (B) Palma 1, (C) Palma 2 and (D) Ses Salines. The green circles correspond with the rhizome segments analyzed (seven internodes each) for the different organic components of ultra-violet filters (UVFs) and paraben conservatives. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Concentrations of organic components of ultra-violet filters (UVFs) * and paraben conservatives* in *Posidonina oceanica* rhizome samples analyzed.

Sample	Site	UV Filters (UVFs) (ng g^{-1} dw)					Parabene conservatives (PB) (ng g^{-1} dw)	∑UVFs (ng g^{-1} dw)	∑PBs (ng g^{-1} dw)
		BP3	BP4(–)	AVO	4MBC	MeBZT	MePB(–)		
1	Alcudia	–	21.3	17.8	–	2.3	29.5	41.3	29.5
2	Alcudia	–	51.3	16.9	2.9	–	185.5	71.0	185.5
3	Alcudia	–	24.4	–	1.3	–	34.5	25.6	34.5
4	Alcudia	–	33.9	12.8	2.8	–	161.0	49.5	161.0
5	Palma 1	5.2	129.0	48.1	–	4.2	512.0	186.4	512.0
6	Palma 1	–	91.7	–	–	6.3	148.5	98.0	148.5
7	Palma 2	8.6	103.1	–	–	–	205.5	111.7	205.5
8	Palma 2	3.3	91.0	–	–	–	185.5	94.2	185.5
9	Ses Salines	–	44.4	–	–	–	60.6	44.4	60.6
10	Ses Salines	–	66.8	–	–	1.0	90.8	67.8	90.8

[*] UVFs BP1, BP2, 4HB. 4DHB, DHMB, EHMC, EtPABA, BZT, DMBZT, UVP and paraben conservatives BePB(–), BuPB(–) and PrPB(–) were not detected in the samples.

study is the first to report on the accumulation of organic UVFs in seagrass tissues, hence, comparison of the concentrations determined was only possible with organisms of different phyla. The maximum concentration of BP4 estimated (129 ng g^{-1} dw) was higher than the reported by Rodil et al. (2019) in bivalves (87 ng g^{-1} dw) from the Galician coast, Spain. Although the MePB exhibited the highest concentrations in the rhizomes of *P. oceanica*, the comparison of these was not possible due to the lack of data available on its occurrence in aquatic species. For the other UVFs analyzed, the range of BP3 content in the rhizome samples (from undetectable to 8.6 ng g^{-1} dw) was considerably lower than those detected in coral species by Tsui et al. (2017) in the Pearl River Estuary, South China Sea (2–31.8 ng g^{-1} dw), and Mitchelmore et al. (2019) in Oahu, Hawaii (0–600 ng g^{-1} dw). The concentrations of BP3 and 4MBC we found here (from undetectable to 2.9 ng g^{-1} dw) were also below those determined in bivalve species by Rodil et al. (2019; 0–63 ng g^{-1} dw of BP3 and 0–49 ng g^{-1} dw of 4MBC), Castro et al. (2018) along the Portuguese coastline (undetectable-622.1 ng g^{-1} dw of BP3 and undetectable-88.3 ng g^{-1} dw of 4MBC), and Cunha et al. (2018) with specimens commercialized as seafood in Europe (from undetectable to 85.5 and 56.2 ng g^{-1} dw of BP3 and 4MBC, respectively). The levels of AVO (from undetectable to 48.1 ng g^{-1} dw) and MeBZT (from undetectable to 6.3 ng g^{-1} dw) were similar to those described by Peng et al. (2015) in the filet and belly of farmed red snappers from the Pearl River Estuary (filet: 33 ± 12 ng g^{-1} dw; belly: 52 ± 14 ng g^{-1} dw), and Díaz-Cruz et al. (2019) in the cyprinid *Squalius keadicus* from the Evrotas River, Greece (3.5–6.2 ng g^{-1} dw), respectively. In general, given that *P. oceanica* occupies a lower trophic level as a primary producer compared with consumer species, it would be expected for it to accumulate less of these pollutants in its tissues (LeBlanc, 1995). However, it should be noted that the extraction methods for these chemicals and the water quality of the study sites considered in different researches may affect the estimations of UVFs concentrations in biological samples.

In general, the current legislation regulating sunscreen products around the world includes, depending on the geographical region, a set of UVFs allowed in their formulations with specified regulations related to the concentration ranges of these substances. For instance, among the UVF detected, BP3, BP4, AVO, and 4-MBC are listed in the EU Regulation CE/1223/09 (Annex VI), which is also applied in New Zealand, some of the Middle East/Arabic countries, Turkey, and ASEAN countries, and is similar to the list of UVFs permitted in Australia, Canada, China, Hong Kong, India, Japan and Taiwan. In the USA, BP3, BP4 and AVO were recently classified as ingredients that require further investigation to determine if they are generally recognized as safe and effective (GRASE) under a proposed rule of the US Food and Drug Administration (FDA) in 2019 (Federal Register 84FR6204, 2019-03019). However, sunscreens containing BP3 are actually prohibited for sale in Hawaii (S.B. NO. 2571, A Bill for an Act, 2018), Florida (Key West), the Virgin Islands, and Puerto Rico, following scientific reports regarding its negative impacts on the marine environment (Paredes et al., 2014; Downs et al., 2016). Similarly, in other locations like Palau (Responsible Tourism Education Act 2018), Aruba and Bonaire, this chemical is officially banned for use in sunscreen products. Previous researchers have demonstrated that BP3 and 4-MBC can cause a variety of effects on the marine biota (e.g., bacteria, microalgae, corals, echinoderms, mollusks, crustaceans, fish, turtles, and mammals), such as bioaccumulation and biomagnification through the food web, high levels of toxicity, disruption of the endocrine and reproductive systems, developmental impairments, oxidative stress, and viral infections (Danovaro et al., 2008; Paredes et al., 2014; Downs et al., 2016; Corinaldesi et al., 2017; Chen et al., 2018; Barone et al., 2019; Cocci et al., 2020). Specifically for primary producers such as phytoplankton, BP3 is mainly associated with cell growth inhibition, decreased photosynthetic pigments production, damage in the photosynthetic and respiratory membrane structures, increased reactive oxygen species generation, decreased metabolic activity and cell morphology alterations (Danovaro et al., 2008; Rodil et al., 2009; Tovar-Sánchez et al., 2013; Paredes et al., 2014; Zhong et al., 2019; Thorel et al., 2020). These UVF components (BP3 and 4-MBC) we found here to accumulate in *P. oceanica* rhizomes and it remains to be investigated experimentally whether these compounds actually have deleterious effects on the plant the same way as was reported for phytoplankton or just accumulated in the tissues. Although BP4 accumulated at higher concentrations in the rhizome samples of *P. oceanica* compared with BP3 and 4-MBC, so far it has been regarded as a less toxic substance in the marine environment than the other two (Paredes et al., 2014; Zhang et al., 2017; He et al., 2019) and scarce information is available in relation to the effects of AVO and MeBZT (Pillard et al., 2001; Fel et al., 2019). Moreover, the only studies available on the effects of sunscreen components on seagrasses and macroalgae were done recently for inorganic UVFs, such as Malea et al. (2019), who determined that exposure to ZnO nanoparticles disrupted the functioning of the photosystem II in *Cymodocea nodosa*, resulting in a reduced photosynthetic efficiency, and also increased the generation of H_2O_2. Later on, Mylona et al. (2020) assessed the toxicity of TiO_2 nanoparticles in *Halophila stipulacea* and reported significant leaf elongation inhibition, along with impairments in the leaf cell structure and viability. Liu et al. (2019) also concluded that TiO_2 nanoparticles can induce oxidative stress responses, disturbances in the antioxidant defense system and decreased photosynthetic pigments content in the macroalgae *Gracilaria lemaneiformis*.

Clearly, now that we know that the key component of Mediterranean coastal ecosystems, *P. oceanica* accumulates UVFs in their tissues, there should be more experimental studies done on their effects and check if we should follow suit to prohibit certain UVFs to protect this species as what has been done in other regions to protect corals.

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Nona S.R. Agawin: Writing – original draft, Writing – review & editing, Validation. **Adrià Sunyer-Caldú:** Writing – original draft, Writing – review & editing, Validation. **M. Silvia Díaz-Cruz:** Writing – original draft, Writing – review & editing, Validation. **Aida Frank-Comas:** Writing – original draft, Writing – review & editing, Validation. **Manuela Gertrudis García-Márquez:** Writing – original draft, Writing – review & editing, Validation. **Antonio Tovar-Sánchez:** Writing – original draft, Writing – review & editing, Validation.

Declaration of competing interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

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References

Agawin, N.S.R., Frank, A., Sureda, A., Mateu-Vicens, G., Ruiz-Pérez, M., Pinya, S., Cabrer-Rosselló, X., 2018. In: Análisis del estado de conservación de la Posidonia oceanica y evaluación de posibles amenazas. Desarrollo e Implantación de un software de alerta en zonas potencialmente críticas por la presencia de praderas de Posidonia en la Zona II de los puertos de la APB (P.O.94.17). Autoritat Portuària de Balears, Fundació Universitat Empresa de les Illes Balears & Universitat de les Illes Balears, Palma, p. 62. Unpublished Report.

Barone, A.N., Hayes, C.E., Kerr, J.J., Lee, R.C., Flaherty, D.B., 2019. Acute toxicity testing of TiO2-based vs. oxybenzone-based sunscreens on clownfish (Amphiprion ocellaris). Environ. Sci. Pollut. Res. 26, 14513–14520. https://doi.org/10.1007/s11356-019-04769-z.

Castro, M., Fernandes, J.O., Pena, A., Cunha, S.C., 2018. Occurrence, profile and spatial distribution of UV-filters and musk fragrances in mussels from portuguese coastline. Mar. Environ. Res. 138, 110–118. https://doi.org/10.1016/j.marenvres.2018.04.005.

Chen, L., Li, X., Hong, H., Shi, D., 2018. Multigenerational effects of 4-methylbenzylidene camphor (4-MBC) on the survival, development and reproduction of the marine copepod Tigriopus japonicus. Aquat. Toxicol. 194, 94–102. https://doi.org/10.1016/j.aquatox.2017.11.008.

Cocci, P., Mosconi, G., Palermo, F.A., 2020. Sunscreen active ingredients in loggerhead turtles (Caretta caretta) and their relation to molecular markers of inflammation, oxidative stress and hormonal activity in wild populations. Mar. Pollut. Bull. 153, 111012 https://doi.org/10.1016/j.marpolbul.2020.111012.

Corinaldesi, C., Damiani, E., Marcellini, F., Falugi, C., Tiano, L., Brugè, F., Danovaro, R., 2017. Sunscreen products impair the early developmental stages of the sea urchin Paracentrotus lividus. Sci. Rep. 7, 7815. https://doi.org/10.1038/s41598-017-08013-x.

Corinaldesi, C., Marcellini, F., Nepote, E., Damiani, E., Danovaro, R., 2018. Impact of inorganic UV filters contained in sunscreen products on tropical stony corals (Acropora spp.). Sci. Total Environ. 637–638, 1279–1285. https://doi.org/10.1016/j.scitotenv.2018.05.108.

Cunha, S.C., Trabalón, L., Jacobs, S., Castro, M., Fernandez-Tejedor, M., Granby, K., Verbeke, W., Kwadijk, C., Ferrari, F., Robbens, J., Sioen, I., Pocurull, E., Marques, A., Fernandes, J.O., Domingo, J.L., 2018. UV-filters and musk fragrances in seafood commercialized in Europe union: occurrence, risk and exposure assessment. Environ. Res. 161, 399–408. https://doi.org/10.1016/j.envres.2017.11.015.

Danovaro, R., Bongiorni, L., Corinaldesi, C., Giovannelli, D., Damiani, E., Astolfi, P., Greci, L., Pusceddu, A., 2008. Sunscreens cause coral bleaching by promoting viral infections. Environ. Health Perspect. 116, 441–447. https://doi.org/10.1289/ehp.10966.

Diaz-Cruz, M.S., Molins-Delgado, D., 2018. Risk assessment of organic UV filters in aquatic ecosystems. In: Hauser-Davis, R.A., Parente, T.E. (Eds.), Ecotoxicology: Perspectives on Key Issues. CRC Taylor & Francis Group, LLC, Boca Raton, pp. 111–126.

Díaz-Cruz, M.S., Molins-Delgado, D., Pau Serra-Roig, M., Kalogianni, E., Skoulikidis, N. Th., Barceló, D., 2019. Personal care products reconnaissance in EVROTAS river (Greece): water-sediment partition and bioaccumulation in fish. Sci. Total Environ. 651 (Pt 2), 3079–3089. https://doi.org/10.1016/j.scitotenv.2018.10.008.

Downs, C., Kramarsky-Winter, E., Segal, R., Fauth, J., Knutson, S., Bronstein, O., Ciner, F. R., Jeger, R., Lichtenfeld, Y., Woodley, C.M., Pennington, P., Cadenas, K., Kushmaro, A., Loya, Y., 2016. Toxicopathological effects of the sunscreen UV filter, oxybenzone (benzophenone-3), on coral planulae and cultured primary cells and its environmental contamination in Hawaii and the U.S. Virgin Islands. Arch. Environ. Contam. Toxicol. 70, 265–288. https://doi.org/10.1007/s00244-015-0227-7.

Duarte, C.M., Marbà, N., Agawin, N., Cebrian, J., Enriquez, S., Fortes, M.D., Gallegos, M. E., Merino, M., Olesen, B., Sand-Jensen, K., Uri, J., Vermaat, J., 1994. Reconstruction of seagrass dynamics: age determinations and associated tools for the seagrass ecologist. Mar. Ecol. Prog. Ser. 107, 195–209. https://doi.org/10.3354/meps107195.

Erickson, R.O., Michelini, F.J., 1957. The plastochron index. Am. J. Bot. 44 (4), 297–305. https://doi.org/10.1002/j.1537-2197.1957.tb10544.x.

Fel, J., Lacherez, C., Bensetra, A., Mezzache, S., Béraud, E., Léonard, M., Allemand, D., Ferrier-Pagès, C., 2019. Photochemical response of the scleractinian coral Stylophora pistillata to some sunscreen ingredients. Coral Reefs 38, 109–122. https://doi.org/10.1007/s00338-018-01759-4.

Giokas, D.L., Salvador, A., Chisvert, A., 2007. UV filters: from sunscreens to human body and the environment. TrAC Trends Anal. Chem. 26, 360–374. https://doi.org/10.1016/j.trac.2007.02.012.

Gondikas, A.P., von der Kammer, F., Reed, R.B., Wagner, S., Ranville, J.F., Hofmann, T., 2014. Release of TiO2 nanoparticles from sunscreens into surface waters: a one-year survey at the old Danube recreational Lake. Environ. Sci. Technol. 48, 5415–5422. https://doi.org/10.1021/es405596y.

Haynes, V., Ward, J., 2020. The interactive effects of titanium dioxide nanoparticles and light on heterotrophic bacteria and microalgae associated with marine aggregates in nearshore waters. Mar. Environ. Res. 161, 105146 https://doi.org/10.1016/j.marenvres.2020.105146.

He, T., Tsui, M.M.P., Tan, C.J., Ng, K.Y., Guo, F.W., Wang, L.H., Chen, T.H., Fan, T.Y., Lam, P.K.S., Murphy, M.B., 2019. Comparative toxicities of four benzophenone ultraviolet filters to two life stages of two coral species. Sci. Total Environ. 651 (Pt 2), 2391–2399. https://doi.org/10.1016/j.scitotenv.2018.10.148.

LeBlanc, G.A., 1995. Trophic-level differences in the bioconcentration of chemicals: implications in assessing environmental biomagnification. Environ. Sci. Technol. 29 (1), 154–160. https://doi.org/10.1021/es00001a020.

Liu, J., Yin, P., Zhao, L., 2019. Adverse effect of TiO2 nanoparticles on antioxidant system and antitumor activities of macroalgae Gracilaria lemaneiformis. J. Ocean Univ. China 18 (5), 1130–1138. https://doi.org/10.1007/s11802-019-3819-4.

Malea, P., Charitonidou, K., Sperdouli, I., Mylona, Z., Moustakas, M., 2019. Zinc uptake, photosynthetic efficiency and oxidative stress in the seagrass Cymodocea nodosa exposed to ZnO nanoparticles. Materials 12 (13), 2101. https://doi.org/10.3390/ma12132101.

Marbà, N., Duarte, C.M., Holmer, M., Martínez, R., Basterretxea, G., Orfila, A., Jordi, A., Tintoré, J., 2002. Assessing the effectiveness of protection on Posidonia oceanica populations in the Cabrera National Park (Spain). Environ. Conserv. 29 (4), 509–518. https://doi.org/10.1017/S037689290200036X.

Mitchelmore, C.L., He, K., Gonsior, M., Hain, E., Heyes, A., Clark, C., Younger, R., Schmitt-Kopplin, P., Feerick, A., Conway, A., Blaney, L., 2019. Occurrence and distribution of UV-filters and other anthropogenic contaminants in coastal surface water, sediment, and coral tissue from Hawaii. Sci. Total Environ. 670, 398–410. https://doi.org/10.1016/j.scitotenv.2019.03.034.

Montesdeoca-Esponda, S., Torres-Padrón, M., Sosa-Ferrera, Z., Santana-Rodríguez, J., 2021. Fate and distribution of benzotriazole UV filters and stabilizers in environmental compartments from gran Canaria Island (Spain): a comparison study. Sci. Total Environ. 756, 144086 https://doi.org/10.1016/j.scitotenv.2020.144086.

Mylona, Z., Panteris, E., Kevrekidis, T., Malea, P., 2020. Effects of titanium dioxide nanoparticles on leaf cell structure and viability, and leaf elongation in the seagrass Halophila stipulacea. Sci. Total Environ. 719, 137378 https://doi.org/10.1016/j.scitotenv.2020.137378.

Pacheco-Juárez, J., Montesdeoca-Esponda, S., Torres-Padrón, M.E., Sosa-Ferrera, Z., Santana-Rodríguez, J.J., 2019. Analysis and occurrence of benzotriazole ultraviolet stabilisers in different species of seaweed. Chemosphere 236, 124344. https://doi.org/10.1016/j.chemosphere.2019.124344.

Paredes, E., Perez, S., Rodil, R., Quintana, J., Beiras, R., 2014. Ecotoxicological evaluation of four UV filters using marine organisms from different trophic levels Isochrysis galbana, Mytilus galloprovincialis, Paracentrotus lividus, and Siriella armata. Chemosphere 104, 44–50. https://doi.org/10.1016/j.chemosphere.2013.10.053.

Peng, X., Jin, J., Wang, C., Ou, W., Tang, C., 2015. Multi-target determination of organic ultraviolet absorbents in organism tissues by ultrasonic assisted extraction and ultra-high performance liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1384, 97–106. https://doi.org/10.1016/j.chroma.2015.01.051.

Pillard, D., Cornell, J., Dufresne, D., Hernandez, M., 2001. Toxicity of benzotriazole and benzotriazole derivatives to three aquatic species. Water Res. 35, 557–560. https://doi.org/10.1016/s0043-1354(00)00268-2.

Rodil, R., Moeder, M., Altenburger, R., Schmitt-Jansen, M., 2009. Photostability and phytotoxicity of selected sunscreen agents and their degradation mixtures in water. Anal. Bioanal. Chem. 395, 1513–1524. https://doi.org/10.1007/s00216-009-3113-1.

Rodil, R., Villaverde-de-Sáa, E., Cobas, J., Quintana, J.B., Cela, R., Carro, N., 2019. Legacy and emerging pollutants in marine bivalves from the galician coast (NW Spain). Environ. Int. 129, 364–375. https://doi.org/10.1016/j.envint.2019.05.018.

Sánchez-Quiles, D., Blasco, J., Tovar-Sánchez, A., 2020. Sunscreen components are a new environmental concern in coastal waters: an overview. In: Tovar-Sánchez, A., Sánchez-Quiles, D., Blasco, J. (Eds.), Sunscreens in Coastal Ecosystems, The Handbook of Environmental Chemistry, Vol. 94. Springer, Cham, pp. 1–14. https://doi.org/10.1007/698_2019_439.

Sunyer-Caldú, A., Diaz-Cruz, M.S., 2021. Development of a QuEChERS-based method for the analysis of pharmaceuticals and personal care products in lettuces grown in field-scale agricultural plots irrigated with reclaimed water. Talanta 230, 122302. https://doi.org/10.1016/j.talanta.2021.122302.

Thorel, E., Clergeaud, F., Jaugeon, L., Rodrigues, A.M.S., Lucas, J., Stien, D., Lebaron, P., 2020. Effect of 10 UV filters on the brine shrimp Artemia Salina and the marine microalga tetraselmis sp. Toxics 8, 29. https://doi.org/10.3390/toxics8020029.

Tovar-Sánchez, A., Sánchez-Quiles, D., Basterretxea, G., Benedé, J.L., Chisvert, A., Salvador, A., Moreno-Garrido, I., Blasco, J., 2013. Sunscreen products as emerging pollutants to coastal waters. PLoS One 8 (6), e65451. https://doi.org/10.1371/journal.pone.0065451.

Tovar-Sánchez, A., Sánchez-Quiles, D., Rodríguez-Romero, A., 2019. Massive coastal tourism influx to the Mediterranean Sea: the environmental risk of sunscreens. Sci. Total Environ. 656, 316–321. https://doi.org/10.1016/j.scitotenv.2018.11.399.

Tsui, M.M.P., Lam, J.C.W., Ng, T.Y., Ang, P.O., Murphy, M.B., Lam, P.K.S., 2017. Occurrence, distribution, and fate of organic UV filters in coral communities. Environ. Sci. Technol. 51 (8), 4182–4190. https://doi.org/10.1021/acs.est.6b05211.

Wang, Y., Zhu, X., Lao, Y., Lv, X., Tao, Y., Huang, B., Wang, J., Zhou, J., Cai, Z., 2016. TiO2 nanoparticles in the marine environment: physical effects responsible for the toxicity on algae Phaeodactylum tricornutum. Sci. Total Environ. 565, 818–826. https://doi.org/10.1016/j.scitotenv.2016.03.164.

Zhang, Q., Ma, X., Dzakpasu, M., Wang, X., 2017. Evaluation of ecotoxicological effects of benzophenone UV filters: luminescent bacteria toxicity, genotoxicity and hormonal activity. Ecotoxicol. Environ. Saf. 142, 338–347. https://doi.org/10.1016/j.ecoenv.2017.04.027.

Zhong, X., Downs, C., Che, X., Zhang, Z., Li, Y., Liu, B., Li, Q., Li, Y., Gao, H., 2019. The toxicological effects of oxybenzone, an active ingredient in suncream personal care products, on prokaryotic alga arthrospira sp. and eukaryotic alga chlorella sp. Aquat. Toxicol. 216, 105295 https://doi.org/10.1016/j.aquatox.2019.105295.

3.2.1 Publication #1 Supplementary information

Mediterranean seagrass Posidonia oceanica accumulates sunscreen UV filters

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Supplementary Tables 1 and 2
Supplementary Figures 1 and 2

Table S1. List of *Posidonia oceanica* rhizome samples analyzed for the different organic components of ultra-violet filters (UVFs) and paraben conservatives.

Sample	Site	Density (shoots m^{-2})	No. of internodes analyzed	Year corresponding to the rhizome piece analyzed	Vertical growth of the rhizome piece (mm)
1	Alcudia	73.1 ± 46.6	7	2012-2014	11.41
2	Alcudia	73.1 ± 46.6	7	2011-2012	10.97
3	Alcudia	73.1 ± 46.6	7	2003-2004	24.42
4	Alcudia	73.1 ± 46.6	7	2001-2003	24.11
5	Palma 1	68.3 ± 14.7	7	2011-2013	13.68
6	Palma 1	68.3 ± 14.7	7	2009-2011	11.50
7	Palma 2	169.2 ± 30.7	7	2013-2014	10.13
8	Palma 2	169.2 ± 30.7	7	2012-2013	9.53
9	Ses Salines	888.6 ± 221.1	7	2010-2012	14.89
10	Ses Salines	888.6 ± 221.1	7	2009-2010	11.91

Table S2. Limits of detection and quantification, and precision (relative standard deviation, RSD%) of the analyses of the different ultra-violet filters (UVFs) and paraben conservatives. LOD: limit of detection; LOQ: limit of quantification; r^2: determination coefficient.

UV Filters (UVFs)	LOD (ng g^{-1} dw)	LOQ (ng g^{-1} dw)	r^2	RSD (%)
BP3	0.79	2.64	0.9919	10.17
BP1	1.00	3.34	0.9870	13.97
BP2	0.46	1.53	0.9972	12.53
BP4 (-)	0.40	1.33	0.9979	15.53
4HB	1.02	3.41	0.9865	11.00
4DHB	0.59	1.96	0.9955	15.53
DHMB	0.79	2.62	0.9920	7.33
AVO	0.33	1.10	0.9986	10.20
4MBC	0.11	0.37	0.9998	11.20
EHMC	0.10	0.35	0.9999	14.60
EtPABA	0.56	1.88	0.9959	21.93
BZT	1.36	4.53	0.9863	9.10
MeBZT	1.30	4.33	0.9882	19.03
DMBZT	1.38	4.61	0.9904	20.80
UVP	1.44	4.80	0.9786	12.83
Parabene conservatives (PB)				
BePB (-)	0.78	2.61	0.9920	8.00
BuPB (-)	0.72	2.41	0.9932	7.60
PrPB (-)	1.02	3.40	0.9866	10.00
MePB (-)	0.59	1.98	0.9954	10.60

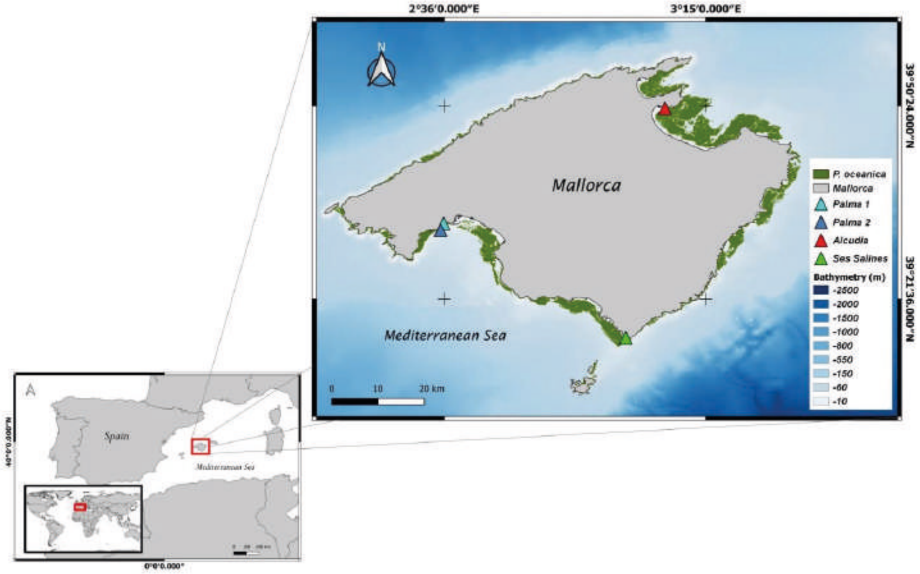

Figure S1. Map of the study site indicating the location of the sampling points.

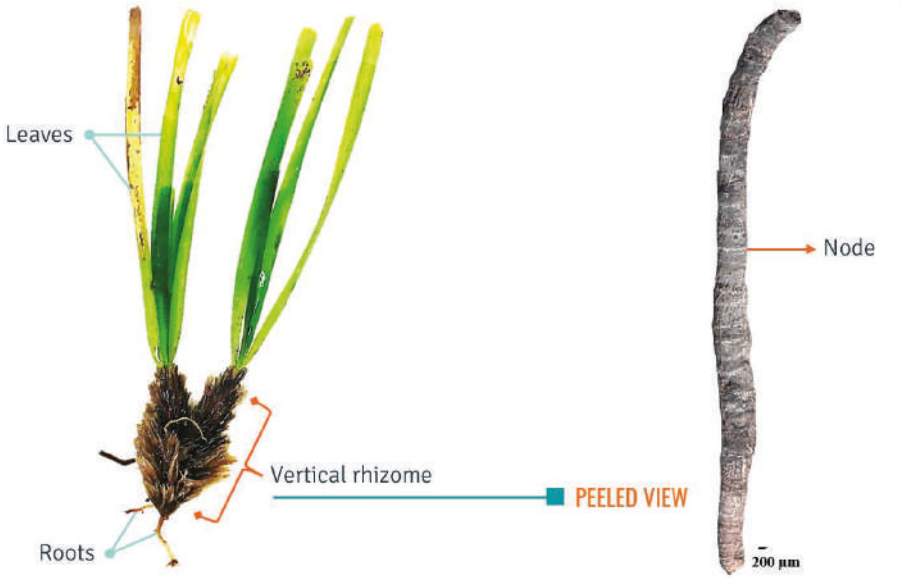

Figure S2. Morphology of the seagrass *Posidonia oceanica*, detailing the structure of the peeled rhizome.

3.2.2 Publication #2

Contaminants of emerging concern in marine areas: first evidence of UV filters and paraben preservatives in seawater and sediment in Eastern coast of Tunisia

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Contaminants of emerging concern in marine areas: First evidence of UV filters and paraben preservatives in seawater and sediment on the eastern coast of Tunisia[☆]

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ABSTRACT

UV filters (UVFs) and paraben preservatives (PBs) are widely used components in many personal care products. However, there has been a rising concern for their endocrine-disrupting effects on wildlife once they reach aquatic ecosystems via recreative activities and wastewater treatment plants effluents. This study addresses UVFs and PBs occurrence in seawater and sediment impacted by tourism and sewage discharges along the coast of Mahdia, center East Tunisia. Samples of water and sediment were collected for 6 months from 3 coastal areas. Among the 14 investigated UVFs, 8 were detected in seawater and 4 were found in sediment. All PBs were present in seawater and only methylparaben (MePB) was detected in sediment. Benzophenone-3 (oxybenzone, BP3), benzocaine (EtPABA), and MePB were present in all water samples with concentrations in the ranges 16.4–66.9, 7.3–37.7, and 17.6–222 ng/L, respectively. However, the highest value, 1420 ng/L, corresponded to octinoxate (EHMC). In sediments, avobenzone (AVO), 4-methyl benzylidene camphor (4MBC), EHMC, 5-methyl-1-H-benzotriazole (MeBZT), and MePB were detected at concentrations within the range 1.1–17.6 ng/g dw, being MePB the most frequently detected (89%). MePB and MBZT presented the highest sediment-water partition coefficients and MePB also showed a positive correlation with total suspended solids' water content. Overall, pollutants concentrations remained rather constant along the sampling period, showing little seasonal variation. This study constitutes the first monitoring of UVFs and PBs on the Tunisian coastline and provides occurrence data for reference in further surveys in the country.

1. Introduction

Personal care products (PCPs) constitute a wide variety of items commonly used by individuals for hygiene, health or cosmetic reasons (European Parliament and the Council of the European Union, 2009). Over the past decade, PCPs were considered high concern environmental pollutants because many of them can induce toxicity to wildlife at environmentally relevant concentrations (Carve et al., 2021) and are continuously released into the aquatic environment. PCPs are usually released in effluents from wastewater treatment plants (WWTPs) (Afsa et al., 2020; Golovko et al., 2021). Their presence has been documented from parts-per-trillion (ng/L) to parts-per-billion (μg/L) concentration in WWTP effluents (Ali et al., 2017), rivers (Díaz-Cruz et al., 2019), lakes (Malnes et al., 2022), seawater (Lu et al., 2021), and groundwater (Yang et al., 2017). Specifically, organic ultraviolet filters (UVFs) have attracted much attention in the last years. They are chemicals with the capacity to absorb UV radiation to protect human skin, nails and hair from its deleterious outcomes (Carstensen et al., 2022). The increased use of UVFs has been directly related to the uprising skin cancer awareness and the growing concern about skin damage such as photoaging (Matouskova and Vandenberg, 2022). Nowadays, there is a widespread demand for sunlight protection products in which UVFs concentration varies between 0.5% and 10% of the total formula (Kim and Choi, 2014).

Benzophenone-3 (oxybenzone, BP3) and 2-ethylhexyl 4-methoxycinnamate (octinoxate, EHMC) are the two most commonly used UVFs

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in sunscreens worldwide (Glanz et al., 2022), and they have been extensively detected in wastewater (e.g. BP3 average concentrations of 12,000 ng/L) (Mao et al., 2019) and environmental matrices (Agawin et al., 2022; Cadena-Aizaga et al., 2022; Wang et al., 2022). For example, BP3 highest concentration found was 1037 ng/g dw in cod livers, and EHMC was frequently detected with even higher concentrations in bivalves, fish and crustaceans (Huang et al., 2021). The discharge of UVFs in the environment has been correlated to multiple toxicological effects manifested in marine organisms (Carvalhais et al., 2021; da Silva et al., 2022).

Paraben preservatives (PBs) constitute another group of PCPs extensively used for their anti-microbial and anti-fungal capacities. They are used as preservatives in food, beverages, and cosmetic products due to their wide spectrum of antimicrobial activity, low cost, and high stability at different pH. Lately, the application of PBs has been extended to pharmaceuticals and drug-associated products being present in nearly 80% of them (Li et al., 2020) and in 32% of baby care products (Gao and Kannan, 2020). Methyl paraben (MePB) and propyl paraben (PrPB) are the most abundant PBs found in raw wastewaters with high average concentration of 30.000 ng/L and 20.000 ng/L, respectively (Haman et al., 2015). PBs' estrogenic effects have been extensively reported (Bachelot et al., 2012; Chebbi et al., 2022; Kang et al., 2019; Mikula et al., 2009).

In the Mediterranean Sea, Tunisia occupies a coastline of 2290 km, with over 1566 km of continental coastline, 457 km of linear island, and 267 km of artificial coastline. Mahdia, located in the Eastern center of Tunisia, stands on over 75 km of shoreline, and represents a suitable installation for the most active offshore fish farms. Tunisia, and specifically Mahdia, is a very touristic place. In this area, WWTPs are usually over-exploited and the majority of industries discharge their wastewater directly into the marine environment. However, additional discharges of untreated (likely illicit) urban wastewater into the environment also occur. These factors combined with the lack of regulations about UVFs and PBs concentrations in surface water and the lack of monitoring of these compounds, make it an interesting area to investigate their occurrence.

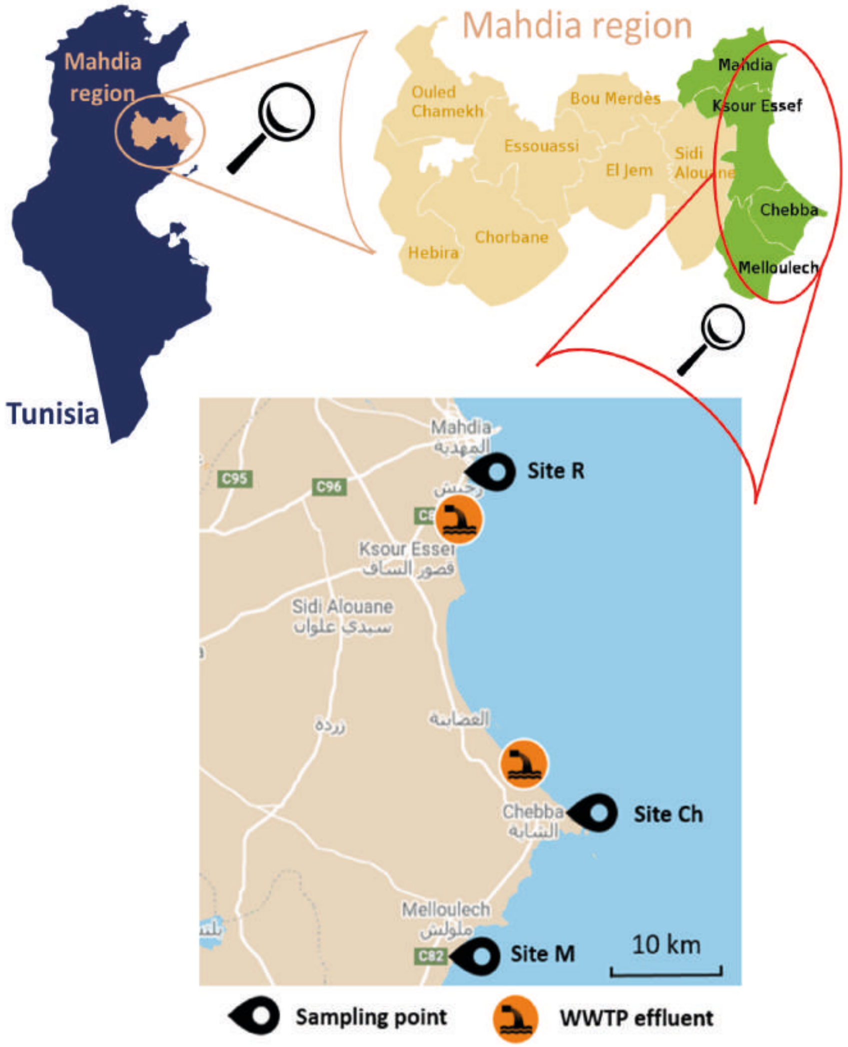

Fig. 1. Sampling sites and WWTP locations along Mahdia shore line.

In this work, a 6 months extensive sampling of seawater and coastal shore sediments was carried out in 3 touristic coastal cities located in Eastern Tunisia to monitor the occurrence and fate of 14 UVFs and 4 PBs. To the best of the authors' knowledge, this work provides the first data on UVFs and PBs occurrence in the marine environment of Tunisia.

2. Materials and methods

2.1. Sampling area

The coastal area from Ras Kaboudia to Zarzis manifests a high tide and it is characterized by a clay sediment and shallow depth (Béjaoui et al., 2019). The three selected sampling sites cover two of the most geographically important and topographically different bays of Tunisia, which are noted as R, Ch and M in Fig. 1. Sampling sites details are shown in Table S1 of the Supplementary Information (SI).

Briefly, **Site R** is a wide sandy swimming beach close to two big cities (Mahdia and Rejiche). **Site Ch** is a sandy beach characterized by a deep sea and considerable recreational activities. It is also influenced by a strong north-south current with an Atlantic origin that travels through the Tunisian-Sicily channel. **Site M** is characterized by remarkable tidal movement, shallow depth, a light current and clayey sediments. It is close to a very touristic city (Melloulech) that lacks of sanitation program, so wastewater is discharged directly into the sea. Sampling collection details are described in **Section S1** of SI. Seawater samples and sediments were monthly collected in each site in triplicate between October 2018 and March 2019.

2.2. Standards and reagents

UVFs and PBs standards were of high purity ($>$98%). Benzophenone-1 (BP1), benzophenone-3 (oxybenzone, BP3), benzophenone-4 (BP4), 4-hydroxybenzophenone (4HB), 4,4′dihidroxybenzophenone (4DHB), ethyl 4-aminobenzoate (EtPABA), 2-(2-hydroxy-5-methylphenyl)-benzotriazole (UVP), avobenzone (AVO), benzylparaben (BePB), propylparaben (PrPB), butylparaben (BuPB) and methylparaben (MePB) were purchased from Sigma Aldrich (Steinheim, Germany). 4-methyl benzylidene camphor (4MBC) was supplied by Dr Ehrenstorfer (Augsburg, Germany) while benzophenone-2 (BP2), 2,2-dihydroxy-4-methoxybenzophenone (DHMB, BP8), and 2-ethylhexyl 4-methoxycinnamate (octinoxate, EHMC) were supplied by Merck (Darmstadt, Germany). 5-methyl-1-H-benzotriazole (MeBZT) was purchased from TCI (Zwijndrecht, Belgium). Isotopically labelled standards, solvents, reagents, and solutions used are described in **Section S2** of SI.

2.3. Total suspended solids determination

Seawater samples were analyzed for total suspended solids content (TSSs) using a UV analyzer (Pastel UV, Secomam, Alès, France). The analytical method applied was developed and validated by the group previously and can be found elsewhere (Afsa et al., 2021; Oueslati et al., 2021).

2.4. Samples pre-treatment for UVFs and PBs determination

2.4.1. Seawater

Waters were filtered through a nylon membrane filter and a glass fiber filter to remove all suspended particulate matter. After filtration, 50 μl of the internal standards mix solution at 50 ng/mL were added to 50 mL of the water samples for further online solid-phase extraction.

2.4.2. Sediment

The target PCPs were determined in sediments following the method developed by Gago-Ferrero et al. (2011) . Briefly, sediment samples were homogenized, frozen, and lyophilized. Aliquots of 1 g were weighed, spiked with a surrogate standard solution and extracted by pressurized liquid extraction (PLE) in an accelerated solvent extractor (ASE-350) from Dionex Corporation (Thermo Fisher Scientifics, Sunnyvale, CA, USA) in two cycles, first with MeOH and then with MeOH/H_2O. The resulting extract was brought to 25 ml with MeOH.

Aliquots of 2 ml of the extract's solution were loaded onto a 0.45 μm nylon syringe filter and eluted in an LC-vial. Subsequently, the filtrate was evaporated to dryness under a nitrogen flow. Finally, the dry residue was reconstituted with 50 μl of the internal standards mix solution and MeOH, and then stored at −20 °C until analysis.

2.5. HPLC-MS/MS analysis and quality assurance

2.5.1. Water samples

Extraction and chromatographic separation of filtered seawater samples were performed by using the on-line SPE–LC instrument Symbiosis™ Pico from Spark Holland (Emmen, The Netherlands). The on-line SPE of the samples, standard solutions, and blanks was performed by loading 5 mL of the corresponding solutions at 1 mL/min through a PLRP-s cartridge previously conditioned with 1 mL of MeOH, 1 mL of ACN, and 1 mL of HPLC-water (flow rate 5 mL/min). After loading the samples, all cartridges were washed with 0.5 ml of HPLC-water and then eluted to the LC-column by the chromatographic mobile phase. The chromatographic separation was achieved using a Hiber Purospher ®STAR® HR R-18ec (50 × 2.0 mm, 2 μm) liquid chromatography column (Merck). Detection was performed with a 4000 QTRAP™ mass spectrometer from Applied Biosystems-Sciex (Foster City, CA, USA). Analyses were carried out under both positive and negative ionization using an electrospray ionization probe (ESI+, ESI-). The injection volume was 5 mL. Additional experimental conditions are listed in Tables S2 and S3 of SI. The performance of the applied method is elsewhere (Gago-Ferrero et al., 2013).

2.5.2. Sediment samples

The analysis of sediments was achieved following the method by Gago-Ferrero et al. (2011). The HPLC-MS/MS experimental conditions were the same ones used for the water analysis, except for the extraction that was carried out off-line. The injection volume was 20 μl.

The target compounds, retention time, transitions selected and internal standards used are compiled in Table S4 of the SI. The analytical parameters of the applied methods including limits of detection (LOD) limits of quantification (LOQ), and correlation coefficients (r^2) are summarized in Table S5 of SI.

2.6. UVFs and PBs sediment-water distribution

For a reliable risk estimation, it is important to consider the concentration of contaminants in the different environmental compartments. In aquatic ecosystems the distribution of a chemical into water and sediment is expressed in terms of the sediment-water-partition coefficient (Kd), which is the ratio between the concentration of the substance absorbed onto the sediment and its concentration in the surrounding water (Equation S1). It informs about the contaminants' accumulation potential by aquatic organisms (Díaz-Cruz et al., 2019).

2.7. Quality assurance/quality control and data analysis

The multiple measures applied to ensure the quality of the analysis from the sampling to the final analysis are described in **Section S3** of the SI. For data analysis, one-way analysis of variance (ANOVA) and t-tests were performed to TSS data, to assess their similarity or difference. Correlation analysis between TSS, UVFs and PBs levels in seawater and UVFs and PBs levels in sediment were performed through Pearson correlation tests. Additional information is provided in **Section S4 of the SI**.

3. Results and discussion

3.1. Statistical results

ANOVA of the TSS values showed difference of variances among sites. T-tests assuming unequal variances indicated significant differences between TSS from site M and from site R, but the other sites' relationships (M-Ch and Ch- R) were not significantly different.

T-tests for the UVFs and PBs seawater concentrations and sampling site showed that values in site M and site Ch were significantly different for EHMC, MeBZT, DMBZT and UVP and values in site R and site CH were significantly different for MeBZT and UVP. The other sites and compounds combinations showed no significant differences. Regarding families, UVFs total concentration was significantly different between site M and site R, and between site M and Ch. PBs total concentration was significantly different between sites M and Ch, and between sites R and Ch. The total load of contaminants (UVFs and PBs together) was significantly different between sites M and Ch, and between sites R and Ch, like PBs. In sediments, sites M and Ch were significantly different with respect to EHMC and MeBZT concentrations, and values in site R and site Ch were also significantly different for EHMC. Finally, UVFs, PBs, and total concentration showed no differences among sites.

Correlation tests for all combinations of TSS, UVFs and PBs concentrations in seawater and sediments are compiled in Tables S6 and S7 of the SI. Overall, the highest correlation (0.91) was found between TSS and MePB concentrations. Other significant correlations found were between EHMC and AVO (0.81), EHMC and MeBZT (0.69), and EHMC and DMBZT (0.68) concentrations in seawater. All values from the other tests, along with all the combinations in sediments showed poor or no correlation (<0.65).

3.2. Total suspended solids

TSSs in seawater was quite different in the three sites (Fig. S1). However, t.-tests revealed that only in sites M and R significantly different TSS values were obtained. Along the sampling campaign, the detected concentrations were very similar in sites R and Ch (they did not exceed 14.0 mg/L and 14.5 mg/L, respectively). Monthly distribution of TSS in sites Ch and R remained within the acceptable levels (Fig. S2 and Fig. S3). In contrast, TSSs levels in site M remained higher than those from sites R and Ch (Fig. S4), while the majority of data recorded kept under acceptable values, the average level in December 2018 was 43.7 mg/L, surpassing the legislation value of 30.0 mg/L (Conseil bibliographique de Ministères, 1989; Ministre de l'Economie Nationale, 1989). High TSS in water bodies may cause decrease in dissolved oxygen and increase in water temperature creating an unfavorable environment for aquatic life. Moreover, high TSS is usually related to higher concentrations of bacteria, nutrients, organic pollutants and metals in water.

3.3. PCPs in seawater samples

Eight out of the fourteen investigated UVFs were detected in seawater, and all four investigated PBs, as shown in Fig. 2. Concentration values are listed in Table S6 of SI. Accumulated concentrations (calculated using Equation S2) in seawater are shown in Fig. S5 and numerical data are compiled in Table S8, and the average values, concentrations range and frequency are listed in Table S9. UVFs was the group with the highest accumulated concentration (6923 ng/L). Indeed, the highest average concentration per compound throughout the sampling campaign (494 ng/L) was for UVFs. Considering that UVFs concentrations were significantly different between sites, it suggests that the conditions for accumulating UVFs or the receiving amount of UVFs between sites were also different. PBs concentration showed the lowest values in the total accumulated (1147 ng/L) and average per compound of (287 ng/L). There were not significant differences among sites. This suggests that the accumulation pattern for UVFs and PBs is quite different. As an example, the chromatogram recorded for the seawater sample collected in site M on November 2018 is shown in Fig. S6.

3.4. PCPs in sediment samples

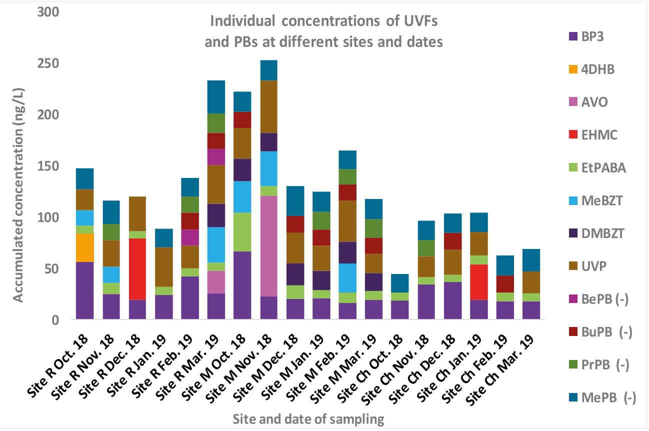

Fig. 2. Individual concentrations of UVFs and PBs in the seawater samples at the different sampling sites and dates.

In the sediments, the accumulation pattern was very similar to that observed in the waters (Fig. S7). Five compounds were detected in sediments; 4 UVFs (AVO, 4 MB, EHMC, and MeBZT) and the preservative MePB. Concentrations found are represented in Fig. 3, the corresponding numerical values in Table S11, and additional information in Table S12 and Table S13 of the SI. UVFs presented the highest total accumulated concentration (181 ng/g dry weight (dw)), but not on average per compound (12.9 ng/g dw). PBs total accumulated concentration (69.6 ng/g dw) was lower, but the average concentration per compound was higher (17.4 ng/g dw). Concerning sediments, there were no significant differences among sites, suggesting that the accumulation in this matrix depends mostly on the contamination level in the water rather than on the site characteristics. An example of the chromatogram obtained for a sediment sample collected in site M on October 2018 is depicted in Fig. S8.

3.5. TSS-CECs correlation in seawater

The comparison of accumulated TSSs and detected compounds in seawater revealed that MePB and TSS were strongly correlated (0.91). The PBs group also showed a positive correlation with TSS (0.67). The correlation between TSSs, UVFs and PBs in seawater could be an indicator of insufficient WWTPs' capacity to extensively retain solids and remove chemical micropollutants from wastewater, being ultimately released in the marine environment. Only few studies focused on PCPs adsorption on suspended matter from surface water due to many difficulties encountered in terms of sampling treatment (Silva et al., 2011). However, to fully understand the relationship between TSSs and the actual amount of PCPs released in seawater, it is necessary to determine those PCPs that preferentially tend to adsorb onto the suspended-solid phase (Terasaki et al., 2012). Therefore, this would explain the highest concentrations of some of the compounds (BP3, EHMC, MeBZT and UVP) that were detected in the periods of higher TSS levels (between October and December of 2018 in site M), probably because of a higher organic content of the suspended matter or a better contact and exchange along the water column (Molins-Delgado et al., 2017). Moreover, compounds adsorbed on suspended solids that later settle down onto sediments will show higher accumulation rate in sediments, as is the case of MePB.

3.6. UVFs and PBs occurrence

BP3 was detected in all water samples in a range between 16.4 and 66.9 ng/L. The highest concentration was found in site M, followed by 56.7 ng/L in site R, and 36.6 ng/L in site Ch. However, no significant differences were found between sites. These concentrations are much lower than BP3 levels reported in coastal seawater samples from touristic areas such as West Indies (1230 ng/L), Spain (3317 ng/L) or Japan (1258 ng/L) (Horricks et al., 2019; Sánchez Rodríguez et al., 2015). According to the octanol-water partition coefficient (logK_{ow} 3.79), BP3 is a lipophilic compound with tendency to adsorb onto suspended matter and sediment and to bioaccumulate in tissues (fish, shellfish, etc) (Gago-Ferrero et al., 2015). In the sediment samples analyzed, however, BP3 was not detected, showing a low sorption affinity to sediment particles (Rostvall et al., 2018). With a pKa of 7.5, BP3 is a mostly basic compound that remains in its anionic form in solution, at the pH of seawater (8.1), which can explain its absence in sediment samples (Díaz-Cruz et al., 2019). Considering that BP3 occurrence in seawater is partially related to the wash-off of sunscreen products from swimmers and bathers, the low values found in a less touristic area seems to be in agreement. BP3 transformation products (TPs) were sporadically or not detected in any of the water or sediment samples, probably due to the low levels of concentration found for BP3, which generate even lower concentrations of TPs.

4-MBC was absent in the water samples and was below the LOQ in sites R and M, but present in the sediments from site Ch, ranging from <LOQ to 17.10 ng/g dw. In previous marine studies, 4-MBC was not detected in seawater samples, but with a relatively high logK_{OW} value of 4.95, it is expected to accumulate in sediments, and it has already been detected in muscle samples of marine organisms (Horricks et al., 2019) or in *Posidonia Oceanica* seagrass (frequently used as coastal water quality indicator) (Agawin et al., 2022).

EHMC was the compound found at the highest concentration in seawater (1420 ng/L) and the highest average concentration (296 ng/L). It was also considerably detected in sediment samples, mostly in site M (where the concentrations in the water samples were also high). Both seawater and sediment concentrations were significantly different between sites M and Ch, suggesting a different accumulation as a result of the site M characteristics. EHMC showed considerable correlation with AVO, MeBZT and DMBZT, indicating similar accumulation patterns for these compounds in water. Measured values are slightly higher than those reported in previous studies on seawater in Spain (756 ng/L) (Sánchez Rodríguez et al., 2015) and Norway (390 ng/L) (Langford and Thomas, 2008). However, EHMC has been detected at similar concentrations in rivers (1040 ng/L) (Kameda et al., 2011).

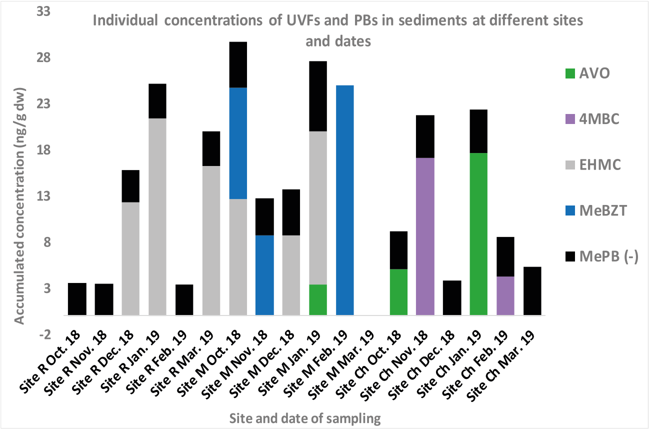

Fig. 3. Individual concentrations of UVFs and PBs in sediments at the different sampling sites and dates.

EtPABA was detected in all water samples, but it was absent in the respective sediment samples, following the same behaviour as BP3. The concentrations in water were quite constant among samples, ranging from 7.3 to 37.7 ng/L, showing no significant differences between sites. These concentrations are in line with those reported in beach waters of Japan, where EtPABA was measured up to 143 ng/L (Tashiro and Kameda, 2013), or in Spanish rivers (38.6 ng/L) (Serra-Roig et al., 2016).

The benzotriazoles detected in this study were MeBZT and DMBZT, mostly in site M and at similar concentrations in the water samples, but only MeBZT was detected in the sediment samples, also in site M. Measured seawater concentrations were significantly different between site M and site Ch, and even for MeBZT, values were also different between both sites. UVP was detected in nearly all water samples at similar levels as MeBZT and DMBZT, but it was not detected in sediment samples, showing a similar behaviour as BP3 and EtPABA. Highest detected concentrations for MeBZT, DMBZT, and UVP in water were 34.6 ng/L, 22.5 ng/L and 50.9 ng/L, respectively. The first two were detected in seawater offshore samples of the Adriatic Sea at slightly lower concentrations (9.2 ng/L and 18.5 ng/L) (Loos et al., 2013), and UVP's levels in water are slightly higher than others found in seawater (4 ng/L) (Fent et al., 2014). In sediment samples, MeBZT was exclusively detected in site M, suggesting a different accumulation and distribution along sediment structures. The levels observed are low compared with the ones reported in sediments from China and USA (<165 ng/g dw) (Zhang et al., 2011).

MePB was the only PB detected in all water samples with a 100% frequency, but also the only one detected in sediments. The levels found barely variated among samples (20 ng/L in water and 4 ng/g dw in sediments) suggesting that the concentrations found do not depend on the characteristics of the bay. Furthermore, statistical analysis showed no differences among sites. Some studies report the occurrence of this compound in rivers at similar and much higher concentrations (Díaz-Cruz et al., 2019; Serra-Roig et al., 2016). Other works have reported similar levels of MePB in sludge (Golovko et al., 2021) or sediments (Soares et al., 2021). Despite MePB is the least lipophilic paraben (shorter side chain), it has been reported to accumulate in fish, marine mammals, invertebrates, and birds (Xue et al., 2015).

BuPB was the second most frequently detected PB (58%), followed by PrPB (39%) and BePB (11%). This pattern in the frequency of detection may be explained by the frequency of use, as MePB is the most commonly used followed by PrPB, BuPB, and eEtPB as stated by the Federal Food Drug and Cosmetic Act (FD&C Act). The maximum average concentrations of MePB, PrPB and BuPB were very similar within the same site and mostly detected in site R with a maximum average of 55.6 ng/L, 8.4 ng/L and 5.1 ng/L respectively. This can be explained by the higher WWTP effluents pressure on site R. A similar work reported lower seawater concentrations than those measured in this study for BuPB (0.5 ng/L), PrPB (1.96 ng/L), and BePB (0.1 ng/L) (Zhao et al., 2017), although, reported concentration in wastewater (Haman et al., 2015) and rivers (Serra-Roig et al., 2016) are higher, as expected because of the lower dilution factor.

All detected UVFs present endocrine-disrupting activity ((Blüthgen et al., 2012; Christen et al., 2011; He et al., 2021; Ramos et al., 2015; Sang and Leung, 2016), and some of them (e.g. EHMC) demonstrated bioaccumulation and biomagnification capacity along the trophic chain (Fent et al., 2010). Being highly lipophilic compounds, PBs can also be absorbed by human skin, being linked to cause endocrine system and female reproductive disorders (Gao and Kannan, 2020) and breast cancer (Jagne et al., 2016).

3.7. *Sediment-water distribution*

The sediment-water partition coefficient, Kd, could only be calculated for the compounds detected in paired seawater and sediment samples. Therefore, Kd was estimated for AVO, EHMC, MeBZT and MePB using the average values of each month and site (Table S14). All Kd values were >30, showing that the mentioned compounds tend to adsorb onto sediment. The highest Kd value corresponded to MePB (183 L/kg), followed by MeBZT (103 L/kg), AVO (35 L/kg) and EHMC (23 L/kg), showing the highest values for the compounds with lower logKow (1.9 and 2 for MeBZT and MePB, respectively). However, to estimate the compounds mobility in environmental compartments, it is preferably to use the combination of Kow and pKa (Jurado et al., 2014). MePB and MeBZT present very high pKa values (8.5 and 8.85, respectively), indicating that they exist in the protonated form in most aqueous systems, tending to bind strongly to sediments. Furthermore, both compounds have been reported at similar concentrations in seawater sediments (Soares et al., 2021). These results highlight the need to consider other sedimentary phases, to account for the observed, stronger retention of the compounds investigated according to the expected as regards their logKow.

3.8. *Spatial distribution pattern*

The behavior of the contaminants in seawater and sediment was similar, as site M was the one with higher accumulated values, followed by site R and site Ch. Some compounds as EHMC, MeBZT, DMBZT and UVP showed significant differences between sites in seawater, suggesting more affinity with site M conditions. In sediments, only EHMC and MeBZT showed differences between sites, being M the most contaminated site o. These compounds have the most highest logKow, indicating large affinity to low polar bodies. As site M is known principally for small fishing activities, the entrance pathway of these compound to this site was less expected. However, it could possibly come from nearby beaches or even from a WWTP, as sea currents in this site facilitate the accumulation of contaminants from the Northern coasts and they can be accumulated over long periods of time (Béjaoui et al., 2019). Furthermore, this site is close to a very touristic city where wastewater is most likely discharged directly into the sea.

The next most contaminated site was R, which could be easily explained by the close proximity of a WWTP discharging point. Moreover, between 2011 and 2013, 84 reported violations were filed against the WWTP in regards to the Tunisian norm (NT106-04-1994). Meanwhile, WWTP is sometimes over-exploited or poorly sized to the point that the treatment cannot satisfy the NT 106-02 standard relating to the dumping of discharges into the receiving environments.

Finally, it was expected to find site Ch to be the most contaminated site by UVFs, since the ecosystem services from this area contribute to 61% of the total marine production of Mahdia. However, results showed that site Ch was the less contaminated site by both UVFs and PBs. This could be explained by the topographic and hydrodynamics properties of site Ch, as it is influenced by strong North-South water currents and presents a very extensive continental shelf, that probably offer certain protection. Individual accumulation values among sites are shown in Fig. 4 for seawater samples and in Fig. S9 for sediment samples.

No available information has been found about the consumption rates of products containing UVFs and PBs in Tunisia. Instead, a study reporting their presence in urine samples from Tunisian women (Jiménez-Díaz et al., 2016) was found. BP3 and BP1 (major metabolite of BP3) were frequently detected in the cohort (65% and 91%, respectively). Regarding PBs, MePB was detected in 94% of the cohort, followed by PrPB (71%) and BePB (38%). These results are in accordance with the concentration patterns found in seawater and sediments in this work, as BP3 was the only benzophenone-type UVF detected in all seawater samples, and detection frequency of PBs followed the order MePB > PrPB > BePB, like in urine. Furthermore, MePB was detected in all the sediment samples, giving a suitable explanation for its frequent detection (94%) in the urine. Considering that the urine samples were collected in two hospitals at least at 100 km from the sampling points in the coastline of this study, the ubiquity and presence of these compounds in the Tunisian environment appears to be in clear connection.

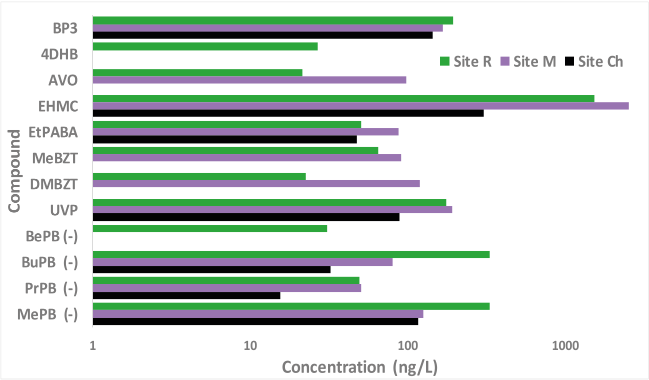

Fig. 4. Accumulated concentrations of detected compounds according to sites and groups in the seawater samples (ng/L).

3.9. *Current regulation*

The European Union restricts the concentration of PBs and UVFs in cosmetics to a maximum concentration of 0.4–0.8% and from 5% to 10%, respectively (Regulation No 1223/2009) and similar measures are applied worldwide through different agencies, depending on the country (e.g. FDA in the USA) (U.S. FDA, 2022). Forbidden and allowed compounds in the PCPs' formulation also varies a lot among countries. For example, 27 compounds are allowed in Europe, while only 16 and 32 are permitted in the USA and Africa, respectively. Even the allowed proportion of each compound varies among regions (Díaz-Cruz, 2020).

Considering that Tunisia is a subtropical touristic country with a significant sun exposure, there is high consumption of products containing UVFs or PBs, therefore a local policy is required to assess the efficiency of UVFs removal from the Tunisian marine domain. This study presents the first attempt to monitor PCPs (UVFs and PBs) in seawater and sediments in Tunisia's eastern coast and provides the first data on their occurrence in its marine environment, which might be considered to foster future regulations and promote intensive surveys.

4. Conclusions

To the best of the authors' knowledge, UVF and PBs were investigated for the first time in seawater and sediment of the Eastern Tunisian coast line. Over a period of 6 months, detected concentrations of UVF and PBs in seawater and sediment samples showed their widespread occurrence in all locations and seasons. Overall, 13 compounds were detected in the water phase (BP3, AVO, 4DHB, EHMC, EtPABA, MeBZT, DMBZT, UVP, BePB, BePB, BuPB, PrPB, and MePB) and 5 in sediment (AVO, 4MBC, EHMC, MeBZT and MePB). Three UVFs (BP3, EtPABA, UVP), and one paraben (MePB) were ubiquitous. Detected values remained rather constant along the six-months sampling period, showing little seasonal variation. Some compounds as EHMC, MeBZT, DMBZT or UVP showed significant differences among sites, evidencing the particular pressures in each area. EHMC was the compound with the highest concentration in seawater (1420 ng/L) and MeBZT in sediments (24.9 ng/g dw). Also, EHMC was the compound with highest average concentrations in both seawater (296 ng/L) and sediment (4.8 ng/g dw).

MePB and MeBZT showed the highest sediment-water partition coefficients, tending to accumulate in sediments. MePB were strongly correlated with TSS; adsorption to the suspended particulate matter that further settle down onto sediments would contribute to the high concentration of MePB in the sediment samples. Unexpectedly, site M presented the highest load of pollutants in seawater (6374 ng/L) and sediment (140 ng/g dw), as site M is the only one that has no WWTP discharge, suggesting uncontrolled/illicit dumping of wastes to the sea. Considering the still limited number of ecotoxicological studies, it is important to fill the current knowledge gaps, some about occurrence data, to fully understand the PCPs fate and effects after released into the environment. With improved understanding, appropriate regulations might be developed based on environmental monitoring and ecotoxicity data from long-term exposure to aquatic organisms for a reliable risk assessment and management.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.119749.

References

Afsa, S., Hamden, K., Lara Martin, P.A., Mansour, H. Ben, 2020. Occurrence of 40 pharmaceutically active compounds in hospital and urban wastewaters and their contribution to Mahdia coastal seawater contamination. Environ. Sci. Pollut. Res. 27, 1941–1955. https://doi.org/10.1007/s11356-019-06866-5.

Afsa, S., Sallem, O.F., Abdeljelil, N. Ben, Feriani, A., Najjar, M.F., Mansour, H. Ben, 2021. In vivo toxicities of the hospital effluent in Mahdia Tunisia. J. Water Health 19, 499–511. https://doi.org/10.2166/wh.2021.024.

Agawin, N.S.R., Sunyer-Caldú, A., Díaz-Cruz, M.S., Frank-Comas, A., García-Márquez, M. G., Tovar-Sánchez, A., 2022. Mediterranean seagrass Posidonia oceanica accumulates sunscreen UV filters. Mar. Pollut. Bull. 176 https://doi.org/10.1016/j.marpolbul.2022.113417.

Ali, A.M., Rønning, H.T., Al Arif, W.M., Kallenborn, R., Kallenborn, R., 2017. Occurrence of pharmaceuticals and personal care products in effluent-dominated Saudi Arabian coastal waters of the Red Sea. Chemosphere 175, 505–513. https://doi.org/10.1016/j.chemosphere.2017.02.095.

Bachelot, M., Li, Z., Munaron, D., Le Gall, P., Casellas, C., Fenet, H., Gomez, E., 2012. Organic UV filter concentrations in marine mussels from French coastal regions. Sci. Total Environ. 420, 273–279. https://doi.org/10.1016/j.scitotenv.2011.12.051.

Béjaoui, B., Ben Ismail, S., Othmani, A., Ben Abdallah-Ben Hadj Hamida, O., Chevalier, C., Feki-Sahnoun, W., Harzallah, A., Ben Hadj Hamida, N., Bouaziz, R., Dahech, S., Diaz, F., Tounsi, K., Sammari, C., Pagano, M., Bel Hassen, M., 2019. Synthesis review of the Gulf of Gabes (eastern Mediterranean Sea, Tunisia): morphological, climatic, physical oceanographic, biogeochemical and fisheries features. Estuar. Coast Shelf Sci. 219, 395–408. https://doi.org/10.1016/j.ecss.2019.01.006.

Blüthgen, N., Zucchi, S., Fent, K., 2012. Effects of the UV filter benzophenone-3 (oxybenzone) at low concentrations in zebrafish (Danio rerio). Toxicol. Appl. Pharmacol. 263, 184–194. https://doi.org/10.1016/j.taap.2012.06.008.

Cadena-Aizaga, M.I., Montesdeoca-Esponda, S., Sosa-Ferrera, Z., Santana-Rodríguez, J.J., 2022. Occurrence and environmental hazard of organic UV filters in seawater and wastewater from Gran Canaria Island (Canary Islands, Spain). Environ. Pollut. 300 https://doi.org/10.1016/j.envpol.2022.118843.

Carstensen, L., Beil, S., Börnick, H., Stolte, S., 2022. Structure-related endocrine-disrupting potential of environmental transformation products of benzophenone-type UV filters: a review. J. Hazard Mater. 430 https://doi.org/10.1016/j.jhazmat.2022.128495.

Carvalhais, A., Pereira, B., Sabato, M., Seixas, R., Dolbeth, M., Marques, A., Guilherme, S., Pereira, P., Pacheco, M., Mieiro, C., 2021. Mild effects of sunscreen agents on a marine flatfish: oxidative stress, energetic profiles, neurotoxicity and behaviour in response to titanium dioxide nanoparticles and oxybenzone. Int. J. Mol. Sci. 22, 1–25. https://doi.org/10.3390/ijms22041567.

Carve, M., Nugegoda, D., Allinson, G., Shimeta, J., 2021. A systematic review and ecological risk assessment for organic ultraviolet filters in aquatic environments. Environ. Pollut. 268, 115894 https://doi.org/10.1016/j.envpol.2020.115894.

Chebbi, M., Beltifa, A., Alibi, S., Di Bella, G., Loturco, V., Sire, O., Ben Mansour, H., Le Tilly, V., 2022. Estrogenic hazards of short chain phthalates and bisphenols found in cosmetic products. Int. J. Environ. Health Res. 32, 252–263. https://doi.org/10.1080/09603123.2020.1749573.

Christen, V., Zucchi, S., Fent, K., 2011. Effects of the UV-filter 2-ethyl-hexyl-4-trimethoxycinnamate (EHMC) on expression of genes involved in hormonal pathways in fathead minnows (Pimephales promelas) and link to vitellogenin induction and histology. Aquat. Toxicol. 102, 167–176. https://doi.org/10.1016/j.aquatox.2011.01.013.

Conseil bibliographique de Ministères, 1989. relative aux rejets d'effluents dans le milieu hydrique (Protection de l'environnement) (Agriculture, E., Nationale, S.P. et E., 1989. Norme Tunisienne NT. 106.002.

da Silva, A.C.P., Santos, B.A.M.C., Castro, H.C., Rodrigues, C.R., 2022. Ethylhexyl methoxycinnamate and butyl methoxydibenzoylmethane: toxicological effects on marine biota and human concerns. J. Appl. Toxicol. 42, 73–86. https://doi.org/10.1002/jat.4210.

Díaz-Cruz, M.S., 2020. Chemical UV Filters: Analysis in Marine Waters, Sunscreens in Coastal Ecosystems. Occurrence, Behavior, Effect and Risk, the Handbook of Environmental Chemistry. Springer International Publishing. https://doi.org/10.1007/978-3-030-56077-5.

Díaz-Cruz, M.S., Molins-Delgado, D., Serra-Roig, M.P., Kalogianni, E., Skoulikidis, N.T., Barceló, D., 2019. Personal care products reconnaissance in EVROTAS river (Greece): water-sediment partition and bioaccumulation in fish. Sci. Total Environ. 651, 3079–3089. https://doi.org/10.1016/j.scitotenv.2018.10.008.

European Parliament and the Council of the European Union, 2009. REGULATION (EC) No 1223/2009 of the EUROPEAN PARLIAMENT and of the COUNCIL of 30 November 2009 on Cosmetic Products.

Fent, K., Chew, G., Li, J., Gomez, E., 2014. Benzotriazole UV-stabilizers and benzotriazole: antiandrogenic activity in vitro and activation of aryl hydrocarbon receptor pathway in zebrafish eleuthero-embryos. Sci. Total Environ. 482–483, 125–136. https://doi.org/10.1016/j.scitotenv.2014.02.109.

Fent, K., Zenker, A., Rapp, M., 2010. Widespread occurrence of estrogenic UV-filters in aquatic ecosystems in Switzerland. Environ. Pollut. 158, 1817–1824. https://doi.org/10.1016/j.envpol.2009.11.005.

Gago-Ferrero, P., Díaz-Cruz, M.S., Barceló, D., 2015. UV filters bioaccumulation in fish from Iberian river basins. Sci. Total Environ. 518–519, 518–525. https://doi.org/10.1016/j.scitotenv.2015.03.026.

Gago-Ferrero, P., Díaz-Cruz, M.S., Barceló, D., 2011. Fast pressurized liquid extraction with in-cell purification and analysis by liquid chromatography tandem mass spectrometry for the determination of UV filters and their degradation products in sediments. Anal. Bioanal. Chem. 400, 2195–2204. https://doi.org/10.1007/s00216-011-4951-1.

Gago-Ferrero, P., Mastroianni, N., Díaz-Cruz, M.S., Barceló, D., 2013. Fully automated determination of nine ultraviolet filters and transformation products in natural waters and wastewaters by on-line solid phase extraction-liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1294, 106–116. https://doi.org/10.1016/j.chroma.2013.04.037.

Gao, C.J., Kannan, K., 2020. Phthalates, bisphenols, parabens, and triclocarban in feminine hygiene products from the United States and their implications for human exposure. Environ. Int. 136, 105465 https://doi.org/10.1016/j.envint.2020.105465.

Glanz, K., Kwong, P.L., Avelis, J., Cassel, K., 2022. Development of a survey of sunscreen use and attitudes among adults in two coastal states, 2019. Int. J. Environ. Res. Publ. Health 19. https://doi.org/10.3390/ijerph19052677.

Golovko, O., Örn, S., Sörengård, M., Frieberg, K., Nassazzi, W., Lai, F.Y., Ahrens, L., 2021. Occurrence and removal of chemicals of emerging concern in wastewater treatment plants and their impact on receiving water systems. Sci. Total Environ. 754, 142122 https://doi.org/10.1016/j.scitotenv.2020.142122.

Haman, C., Dauchy, X., Rosin, C., Munoz, J.F., 2015. Occurrence, fate and behavior of parabens in aquatic environments: a review. Water Res. 68, 1–11. https://doi.org/10.1016/j.watres.2014.09.030.

He, K., Hain, E., Timm, A., Blaney, L., 2021. Bioaccumulation of estrogenic hormones and UV-filters in red swamp crayfish (Procambarus clarkii). Sci. Total Environ. 764, 142871 https://doi.org/10.1016/j.scitotenv.2020.142871.

Horricks, R.A., Tabin, S.K., Edwards, J.J., Lumsden, J.S., Marancik, D.P., 2019. Organic ultraviolet filters in nearshore waters and in the invasive lionfish (Pterois volitans) in Grenada, West Indies. PLoS One 14, 1–11. https://doi.org/10.1371/journal.pone.0220280.

Huang, Y., Law, J.C.F., Lam, T.K., Leung, K.S.Y., 2021. Risks of organic UV filters: a review of environmental and human health concern studies. Sci. Total Environ. 755, 142486 https://doi.org/10.1016/j.scitotenv.2020.142486.

Jagne, J., White, D., Jefferson, F., 2016. Endocrine-disrupting chemicals: adverse effects of bisphenol A and parabens to women's health. Water, Air, Soil Pollut. 227 https://doi.org/10.1007/s11270-016-2785-3.

Jiménez-Díaz, I., Artacho-Cordón, F., Vela-Soria, F., Belhassen, H., Arrebola, J.P., Fernández, M.F., Ghali, R., Hedhili, A., Olea, N., 2016. Urinary levels of bisphenol A, benzophenones and parabens in Tunisian women: a pilot study. Sci. Total Environ. 562, 81–88. https://doi.org/10.1016/j.scitotenv.2016.03.203.

Jurado, A., Gago-Ferrero, P., Vàzquez-Suñé, E., Carrera, J., Pujades, E., Díaz-Cruz, M.S., Barceló, D., 2014. Urban groundwater contamination by residues of UV filters. J. Hazard Mater. 271, 141–149. https://doi.org/10.1016/j.jhazmat.2014.01.036.

Kameda, Y., Kimura, K., Miyazaki, M., 2011. Occurrence and profiles of organic sun-blocking agents in surface waters and sediments in Japanese rivers and lakes. Environ. Pollut. 159, 1570–1576. https://doi.org/10.1016/j.envpol.2011.02.055.

Kang, H.M., Kim, M.S., Hwang, U.K., Jeong, C.B., Lee, J.S., 2019. Effects of methylparaben, ethylparaben, and propylparaben on life parameters and sex ratio in the marine copepod Tigriopus japonicus. Chemosphere 226, 388–394. https://doi.org/10.1016/j.chemosphere.2019.03.151.

Kim, S., Choi, K., 2014. Occurrences, toxicities, and ecological risks of benzophenone-3, a common component of organic sunscreen products: a mini-review. Environ. Int. 70, 143–157. https://doi.org/10.1016/j.envint.2014.05.015.

Langford, K.H., Thomas, K.V., 2008. Inputs of chemicals from recreational activities into the Norwegian coastal zone. J. Environ. Monit. 10, 894–898. https://doi.org/10.1039/b806198j.

Li, C., Cui, X., Chen, Y., Liao, C., 2020. Paraben concentrations in human fingernail and its association with personal care product use. Ecotoxicol. Environ. Saf. 202, 110933 https://doi.org/10.1016/j.ecoenv.2020.110933.

Loos, R., Tavazzi, S., Paracchini, B., Canuti, E., Weissteiner, C., 2013. Analysis of polar organic contaminants in surface water of the northern Adriatic Sea by solid-phase extraction followed by ultrahigh-pressure liquid chromatography-QTRAP® MS using a hybrid triple-quadrupole linear ion trap instrument. Anal. Bioanal. Chem. 405, 5875–5885. https://doi.org/10.1007/s00216-013-6944-8.

Lu, S., Lin, C., Lei, K., Xin, M., Wang, B., Ouyang, W., Liu, X., He, M., 2021. Endocrine-disrupting chemicals in a typical urbanized bay of Yellow Sea, China: distribution, risk assessment, and identification of priority pollutants. Environ. Pollut. 287, 117588 https://doi.org/10.1016/j.envpol.2021.117588.

Malnes, D., Ahrens, L., Köhler, S., Forsberg, M., Golovko, O., 2022. Occurrence and mass flows of contaminants of emerging concern (CECs) in Sweden's three largest lakes and associated rivers. Chemosphere 294. https://doi.org/10.1016/j.chemosphere.2022.133825.

Mao, F., He, Y., Gin, K.Y.H., 2019. Occurrence and fate of benzophenone-type UV filters in aquatic environments: a review. Environ. Sci. Water Res. Technol. 5, 209–223. https://doi.org/10.1039/c8ew00539g.

Matouskova, K., Vandenberg, L.N., 2022. Towards a paradigm shift in environmental health decision-making: a case study of oxybenzone. Environ. Heal. A Glob. Access Sci. Source 21, 1–12. https://doi.org/10.1186/s12940-021-00806-y.

Mikula, P., Kružíková, K., Dobšíková, R., Haruštiaková, D., Svobodová, Z., 2009. Influence of propylparaben on vitellogenesis and sex ratio in juvenile zebrafish (Danio rerio). Acta Vet. 78, 319–326. https://doi.org/10.2754/avb200978020319.

Ministre de l'Economie Nationale, 1989. relative aux rejets d'effluents dans le milieu hydrique (Protection de l'environnement). Norme homologuée par arrêté du Ministre de l'Economie Nationale du 20 juillet 1989, 1989. Norme Tunisienne NT. 106.002. JORT no 59.

Molins-Delgado, D., Távora, J., Silvia Díaz-Cruz, M., Barceló, D., 2017. UV filters and benzotriazoles in urban aquatic ecosystems: the footprint of daily use products. Sci. Total Environ. 601–602, 975–986. https://doi.org/10.1016/j.scitotenv.2017.05.176.

Oueslati, A., Montevecchi, G., Antonelli, A., Mansour, H. Ben, 2021. Short-time irrigation on young olive tree (Olea europaea L. cv. Chemlali) with untreated industrial poultry wastewater: investigation of growth parameters and leaves chemical composition. Environ. Sci. Pollut. Res. Int. 28, 50420–50429. https://doi.org/10.1007/s11356-021-14261-2.

Ramos, S., Homem, V., Alves, A., Santos, L., 2015. Advances in analytical methods and occurrence of organic UV-filters in the environment — a review. Sci. Total Environ. 526, 278–311. https://doi.org/10.1016/j.scitotenv.2015.04.055.

Rostvall, A., Zhang, W., Dürig, W., Renman, G., Wiberg, K., Ahrens, L., Gago-Ferrero, P., 2018. Removal of pharmaceuticals, perfluoroalkyl substances and other micropollutants from wastewater using lignite, Xylit, sand, granular activated carbon (GAC) and GAC+Polonite® in column tests – role of physicochemical properties. Water Res. 137, 97–106. https://doi.org/10.1016/j.watres.2018.03.008.

Sánchez Rodríguez, A., Rodrigo Sanz, M., Betancort Rodríguez, J.R., 2015. Occurrence of eight UV filters in beaches of Gran Canaria (Canary Islands). An approach to

environmental risk assessment. Chemosphere 131, 85–90. https://doi.org/10.1016/j.chemosphere.2015.02.054.

Sang, Z., Leung, K.S.-Y., 2016. Environmental occurrence and ecological risk assessment of organic UV filters in marine organisms from Hong Kong coastal waters. Sci. Total Environ. 566–567, 489–498. https://doi.org/10.1016/j.scitotenv.2016.05.120.

Serra-Roig, M.P., Jurado, A., Díaz-Cruz, M.S., Vázquez-Suñé, E., Pujades, E., Barceló, D., 2016. Occurrence, fate and risk assessment of personal care products in river–groundwater interface. Sci. Total Environ. 568, 829–837. https://doi.org/10.1016/j.scitotenv.2016.06.006.

Silva, B.F. da, Jelic, A., López-Serna, R., Mozeto, A.A., Petrovic, M., Barceló, D., 2011. Occurrence and distribution of pharmaceuticals in surface water, suspended solids and sediments of the Ebro river basin, Spain. Chemosphere 85, 1331–1339. https://doi.org/10.1016/j.chemosphere.2011.07.051.

Soares, K.L., Sunyer-Caldú, A., Barbosa, S.C., Primel, E.G., Fillmann, G., Diaz Cruz, M.S., 2021. Rapid and cost-effective multiresidue analysis of pharmaceuticals, personal care products, and antifouling booster biocides in marine sediments using matrix solid phase dispersion. Chemosphere 267. https://doi.org/10.1016/j.chemosphere.2020.129085.

Tashiro, Y., Kameda, Y., 2013. Concentration of organic sun-blocking agents in seawater of beaches and coral reefs of Okinawa Island. Japan. Mar. Pollut. Bull. 77, 333–340. https://doi.org/10.1016/j.marpolbul.2013.09.013.

Terasaki, M., Takemura, Y., Makino, M., 2012. Paraben-chlorinated derivatives in river waters. Environ. Chem. Lett. 10 https://doi.org/10.1007/s10311-012-0367-1.

U.S. FDA, 2022. Parabens in cosmetics. WWW Document]. URL. https://www.fda.gov/cosmetics/cosmetic-ingredients/parabens-cosmetics.

Wang, W., Lee, I.S., Oh, J.E., 2022. Specific-accumulation and trophic transfer of UV filters and stabilizers in marine food web. Sci. Total Environ. 825 https://doi.org/10.1016/j.scitotenv.2022.154079.

Xue, J., Sasaki, N., Elangovan, M., Diamond, G., Kannan, K., 2015. Elevated accumulation of parabens and their metabolites in marine mammals from the United States coastal waters. Environ. Sci. Technol. 49, 12071–12079. https://doi.org/10.1021/acs.est.5b03601.

Yang, Y., Ok, Y.S., Kim, K.H., Kwon, E.E., Tsang, Y.F., 2017. Occurrences and removal of pharmaceuticals and personal care products (PPCPs) in drinking water and water/sewage treatment plants: a review. Sci. Total Environ. 596–597, 303–320. https://doi.org/10.1016/j.scitotenv.2017.04.102.

Zhang, Z., Ren, N., Li, Y., Kunisue, T., Gao, D., Kannan, K., 2011. Determination of Benzotriazole and Benzophenone UV Filters in Sediment and Sewage Sludge, pp. 3909–3916.

Zhao, X., Zhang, Z.-F., Xu, L., Liu, L.-Y., Song, W.-W., Zhu, F.-J., Li, Y.-F., Ma, W.-L., 2017. Occurrence and fate of benzotriazoles UV filters in a typical residential wastewater treatment plant in Harbin, China. Environ. Pollut. 227, 215–222. https://doi.org/10.1016/j.envpol.2017.04.072.

3.2.2 Publication #2 Supplementary information

Contaminants of emerging concern in marine areas: first evidence of UV filters and paraben preservatives in seawater and sediment in Eastern coast of Tunisia

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Section S1. Sampling collection.
Section S2. Isotopically labelled standards, solvents, reagents, and solution preparations.
Section S3. Quality Assurance/Quality control.
Section S4. Data analysis
Section S4. Tables. (S1-S14)
Section S5. Figures (S1-S9)
Section S6. Equations (S1-S2)

Section S1. Sampling collection.

Seawater samples were monthly collected along the Mahdia coastline from October 2018 until March 2019. All water samples were collected from the surface, in triplicate each month in brown glass sterile bottles and kept at -20°C in the dark until analysis. Likewise, sediment samples were monthly collected at 1.5 m depth with a Van Veen grab samplers, at the same sites as seawater, also in triplicate in sterile glass containers, and stored at -20 °C until analysis. Site R and site Ch presented the same phase of sedimentation, with fine moderately sized grain. Site M, however, presented higher clay composition.

Section S2. Isotopically labeled standards, solvents, reagents, and solution preparations.

Isotopically labelled standards 2-hydroxy-4-methoxy-2',3',4',5',6'-d5 (BP3-d5), 3-(4-methylbenzylidene-d4) camphor (4MBC-d4) and, benzyl-4-hydroxybenzoate-d4 (BePB-d4) were supplied by CDN Isotopes Inc. (Quebec, Canada) (>99%). Working solvents, including methanol (MeOH), acetonitrile (ACN), HPLC-grade water (Lichrosolv), and reagents formic acid and alumina (aluminum oxide, 99%) were purchased from Merck. Nitrogen (>99%) used for evaporation was provided by Air liquid (Barcelona, Spain). Glass fiber filters (0.2 µm), nylon membranes (0.45 µm), and Puradisc syringe filters were obtained from Whatman International Ltd. (Maidstone, UK).

Standards stock solutions, including isotopically labeled standards, were prepared in MeOH and stored in the dark at -20°C. A mixture standard solution was prepared weekly at 20 mg/L. Working mixture standard solutions were prepared daily by appropriate dilution of the mixture stock standard solution.

Section S3. Quality Assurance/Quality control.

Several measures were applied to prevent contamination and degradation of samples and standard solutions. Personal care and hygiene products that could contain UVFs or PBs were avoided during sampling and sample treatment by the analysts. All glass material containing samples was opaque and the working material was cleaned with MeOH and acetone. The non-volumetric glassware was muffled at 400º C overnight. A blank of the method was performed and quality control samples were introduced randomly in the analysis' sequence to ensure the quality of the determination. All the compounds were identified using the chromatography retention time (t_R) and with the two most intense transitions (first to quantify and second to confirm the analyte), as recommended by the European Commission ("2002/657/EC: Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (Text with EEA relevance) (notified under document number C(2002) 3044)," 2002). Surrogate standard recoveries were within the range of 80-120% in all the extracted samples. Limits of detection and quantification can be consulted in Table S5 of this document.

References

2002/657/EC: Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (Text with EEA relevance) (notified under document number C(2002) 3044), 2002.

Section S4. Data analysis

One-way analysis of variance (ANOVA) was performed on TSS values, to evaluate if site variance was different or not, and consequently, t-tests were individually performed to find out which sites were statistically different (or not) from others. The same procedure was followed for UVFs and PBs values found in seawater and sediment samples, individually, as groups, and with the total value. Finally, correlation analysis between TSS, UVFs, and PBs levels in seawater and UVFs and PBs levels in sediment was performed with Pearson correlation tests. Data below the limits of detection were changed to numerical values corresponding to LOD/sqrt(2) to consider the variance as well. ANOVA, t-tests, and Pearson correlation tests were performed for α = 0.05 with RStudio open software, v. 1.2.5001 (2019) Rstudio. Inc. (the USA).

Section S4. Tables

Table S1. Sampling sites location, beach dimensions, distance to interesting locations and relevant details.

Sampling site	GPS location	Bay location	Beach dimension	Distance to WWTP	Other interesting locations	Relevant information
Site R	35°28'39" N 11°03'13" E	Between Hammamet and Gabes Bay	4.7 km	<900 m	Large fishing port to the North (1.7 km distance)	Very close to two cities (Mahdia and Rejiche)
Site Ch	35°14'18" N 11°08'48" E	Hammamet Bay	2.4 km	5 km	Highly active fishing port to the South (1 km)	Huge recreational and swimming activities. City (Chebba) at 3 km
Site M	35°08'21" N 11°02'31" E	Gabes Bay	12.3 km	14.7 km	Small fishing port (1.5 km)	Very touristic city at 3 km (Melloulech)

Table S2. Gradient, mobile phases and flow used in positive ionization.

Time (min)	Flow	Positive ionization	
		%A (MeOH 0.1% F.A.)	%B (H_2O 0.1% F.A.)
0	0.3 mL/min	5	95
7	0.3 mL/min	75	25
10	0.3 mL/min	100	0
17	0.3 mL/min	100	0
18	0.3 mL/min	5	95
23	0.3 mL/min	5	95

F.A.: Formic acid

Table S3. Gradient, mobile phases and flow used in negative ionization.

Time (min)	Flow	Negative ionization	
		%A (MeOH 5 mM Ac.NH_4)	%B (H_2O 5 mM Ac.NH_4)
0	0.3 mL/min	5	95
3	0.3 mL/min	50	50
6	0.3 mL/min	90	10
13	0.3 mL/min	100	0
17	0.3 mL/min	100	0
18	0.3 mL/min	5	95
20	0.3 mL/min	5	95

Ac.NH_4: Ammonium acetate

Table S4. Chemical properties, optimized HPLC-MS/MS conditions and internal standards of the investigated compounds.

Acronym	Name	Molecular formula	CAS Number	pka	Logk$_{ow}$	Water solubility (25°C)	Retention time (min)	1st Transition	2nd Transition	Internal standard
BP1	Benzophenone-1	$C_{13}H_{10}O_3$	131-56-6	7.09	2.96	390 mg/L	9.94	215>137	215>105	BP-3d$_5$
BP2	Benzophenone-2	$C_{13}H_{10}O_5$	131-55-5	6.75	2.78	98 mg/L	8.74	247>137	247>109	BP-3d$_5$
BP3	Benzophenone-3	$C_{14}H_{12}O_3$	131-57-7	7.56	3.79	210 mg/L	11.14	229>151	229>105	BP-3d$_5$
BP4(-)	Benzophenone-4	$C_{14}H_{12}O_6S$	4065-45-6	-2.4	0.37	650 mg/L	6.89	307>227	307>211	Gemfibrozil-d$_6$
4HB	4-Hydroxybenzophenone	$C_{13}H_{10}O_2$	1137-42-4	7.85	3.02	410 mg/L	9.39	199>121	199>105	BP-3d$_5$
4DHB	4,4-Dihydroxybenzophenone	$C_{13}H_{10}O_3$	611-99-4	7.55	2.55	600 mg/L	8.25	215>121	215>93	BP-3d$_5$
DHMB	2,2-Dihydroxy-4-methoxybenzophenone	$C_{14}H_{12}O_4$	131-53-3	6.78	3.82	52 mg/L	10.25	245>121	245>151	BP-3d$_5$
AVO	Avobenzone	$C_{20}H_{22}O_3$	70356-09-1	3.23	4.51	1517 mg/L	12.79	311>135	311>161	BP-3d$_5$
4-MBC	4-Methylbenzylidene camphor	$C_{18}H_{22}O$	36861-47-9	-	4.95	0.16 mg/L	11.80	255>105	255>212	4-MBC-d$_4$
EHMC	2-Ethylhexyl 4-methoxycinnamate	$C_{18}H_{26}O_3$	5466-77-3	8.13	5.8	0.42 mg/L	13.06	291>179	291>161	4-MBC-d$_4$
EtPABA	Ethyl 4-aminobenzoate	$C_{18}H_{18}N_2O_5$	94-09-7	2.51	1.71	1.71 g/L	8.50	166>138	166>120	4-MBC-d$_4$
MeBZT	5-Methyl-1H-benzotriazole	$C_7H_7N_3$	136-85-6	8.85	8.74	3 g/L	8.13	134>79	134>95	BZT-d$_4$
BZT	1,2,3-Benzotriazole	$C_6H_5N_3$	95-14-7	8.37	1.44	6 g L	6.63	120>65	120>92	BZT-d$_4$
DMBZT	2,2′-Dihydroxy-4-methoxybenzophenone	$C_{14}H_{12}O_4$	131-53-7	8.92	2.26	914 mg/L	8.84	148>77	148>93	BZT-d$_4$
UVP	Drometrizole	$C_{13}H_{11}N_3O$	2440-22-4	8.15	2.26	25.59 mg/L	12.37	226>120	226>92	BZT-d$_4$
BePB (-)	Benzyl 4-hydroxybenzoate	$C_{14}H_{12}O_3$	94-18-8	8.18	3.56	0.135 mg/L	8.21	227>92	227>136	BePB-d$_4$
BuPB (-)	Butyl 4-hydroxybenzoate	$C_{11}H_{14}O_3$	94-26-8	-+3.5 0.02	3.24	0.466 mg/L	8.21	193>137	193>92	BePB-d$_4$
PrPB (-)	Propyl 4-hydroxybenzoate	$C_{10}H_{12}O_3$	94-13-3	-+ 2.94 0.01	3.04	0.96 mg/L	7.90	179>92	179>137	BePB-d$_4$
MePB (-)	Methyl 4-hydroxybenzoate	$C_8H_8O_3$	99-76-3	-+1.91 0.01	1.96	3.69 mg/L	6.57	151>92	151>136	BePB-d$_4$

Table S5. Limits of detection (LOD) and quantification (LOQ) of the method, and determination coefficient (r^2) for the target compounds.

Compound	BP3	BP1	BP2	BP4 (-)	4HB	4DHB	DHMB	AVO	4MBC
LOD water (ng/L)	0.74	1.07	1.17	0.58	1.04	0.99	1.13	0.93	0.40
LOQ water (ng/L)	2.47	3.56	3.90	1.94	3.47	3.30	3.75	3.09	1.32
r^2 water	0.9944	0.9885	0.9862	0.9965	0.9891	0.9901	0.9873	0.9913	0.9984
LOD sediments (ng/g dw)	0.51	0.76	1.02	2.16	0.91	1.00	0.93	0.51	1.19
LOQ sediments (ng/g dw)	1.69	2.54	3.40	7.21	3.02	3.33	3.11	1.69	3.97
r^2 sediments	0.9972	0.9937	0.9886	0.9519	0.9910	0.9892	0.9905	0.9972	0.9846
Compound	**EHMC**	**EtPABA**	**MeBZT**	**DMBZT**	**UVP**	**BePB (-)**	**BuPB (-)**	**PrPB (-)**	**MePB (-)**
LOD water (ng/L)	1.71	0.29	0.73	0.90	1.03	0.74	0.72	0.73	0.86
LOQ water (ng/L)	5.69	0.97	2.42	2.99	3.42	2.47	2.42	2.44	2.85
r^2 water	0.9714	0.9991	0.9946	0.9918	0.9894	0.9944	0.9947	0.9945	0.9926
LOD sediments (ng/g dw)	1.43	1.33	1.11	0.93	0.80	0.64	0.62	0.45	0.12
LOQ sediments (ng/g dw)	4.78	4.43	3.70	3.08	2.68	2.14	2.06	1.50	0.39
r^2 sediments	0.9780	0.9810	0.9866	0.9906	0.9929	0.9954	0.9958	0.9978	0.9998

MLOD: Limit of detection of the method; MLOQ: Limit of quantification of the method: r^2: determination coefficient; (-) negative mode

Table S6. Pearson correlation values of the different combinations between concentrations of TSS, UVFs, and PBs in seawater samples.

Correlation values	TSS	BP3	4DHB	AVO	EHMC	EtPABA	MeBZT	DMBZT	UVP	BePB (-)	BuPB (-)	PrPB (-)	MePB (-)
TSS	**1.00**	-0.10	-0.01	-0.15	-0.21	-0.14	-0.15	-0.29	0.14	0.05	0.18	-0.06	**0.91**
BP3	-0.10	**1.00**	0.50	-0.10	-0.02	0.63	0.30	0.01	0.02	0.15	-0.04	-0.11	-0.17
4DHB	-0.01	0.50	**1.00**	-0.07	-0.20	-0.07	0.12	-0.19	-0.11	-0.09	-0.09	-0.19	-0.06
AVO	-0.15	-0.10	-0.07	**1.00**	**0.81**	-0.03	0.56	0.32	0.56	0.06	-0.09	-0.11	-0.07
EHMC	-0.21	-0.02	-0.20	**0.81**	**1.00**	0.31	**0.69**	**0.68**	0.59	0.12	0.05	0.06	-0.13
EtPABA	-0.14	0.63	-0.07	-0.03	0.31	**1.00**	0.45	0.44	0.14	-0.11	0.05	-0.19	-0.11
MeBZT	-0.15	0.30	0.12	0.56	**0.69**	0.45	**1.00**	0.61	0.60	0.23	0.14	0.16	-0.14
DMBZT	-0.29	0.01	-0.19	0.32	**0.68**	0.44	0.61	**1.00**	0.49	0.12	-0.13	0.33	-0.16
UVP	0.14	0.02	-0.11	0.56	0.59	0.14	0.60	0.49	**1.00**	0.13	0.01	0.09	0.17
BePB (-)	0.05	0.15	-0.09	0.06	0.12	-0.11	0.23	0.12	0.13	**1.00**	-0.04	0.47	-0.06
BuPB (-)	0.18	-0.04	-0.09	-0.09	0.05	0.05	0.14	-0.13	0.01	-0.04	**1.00**	0.32	-0.08
PrPB (-)	-0.06	-0.11	-0.19	-0.11	0.06	-0.19	0.16	0.33	0.09	0.47	0.32	**1.00**	-0.18
MePB (-)	**0.91**	-0.17	-0.06	-0.07	-0.13	-0.11	-0.14	-0.16	0.17	-0.06	-0.08	-0.18	**1.00**

Table S7. Pearson correlation values of the different combinations between concentrations of UVFs, and PBs in sediment samples.

	AVO	4MBC	EHMC	MeBZT	MePB (-)
AVO	**1.00**	-0.10	-0.13	-0.14	0.23
4MBC	-0.10	**1.00**	-0.19	-0.12	0.11
EHMC	-0.13	-0.19	**1.00**	-0.09	0.34
MeBZT	-0.14	-0.12	-0.09	**1.00**	-0.44
MePB (-)	0.23	0.11	0.34	-0.44	**1.00**

Table S8. Concentrations (ng/L) in the seawater samples of the PPCPs detected at least in one sample.

Site and date	BP3	4DHB	AVO	EHMC	EtPABA	MeBZT	DMBZT	UVP	BePB (-)	BuPB (-)	PrPB (-)	MePB (-)
Site R 10/2018	56.7	26.9	n.d.	n.d.	8.3	15.2	n.d.	20.0	n.d.	n.d.	n.d.	20.1
Site R 11/2018	25.4	n.d.	n.d.	338	10.9	15.4	n.d.	26.1	n.d.	300	15.5	22.6
Site R 12/2018	19.3	n.d.	n.d.	60.0	7.3	n.d.	n.d.	33.2	n.d.	n.d.	n.d.	222
Site R 01/2019	24.5	n.d.	n.d.	291	7.9	n.d.	n.d.	37.9	n.d.	n.d.	n.d.	18.7
Site R 02/2019	42.1	n.d.	n.d.	n.d.	8.3	n.d.	n.d.	21.7	15.4	17.1	15.5	17.6
Site R 03/2019	26.1	n.d.	21.6	836	8.2	34.6	22.5	37.9	15.3	16.2	18.8	31.9
Site M 10/2018	66.9	n.d.	n.d.	640	37.7	30.2	22.0	29.6	n.d.	16.0	n.d.	19.5
Site M 11/2018	22.8	n.d.	97.7	1420	10.0	33.5	18.1	50.9	n.d.	n.d.	n.d.	19.4
Site M 12/2018	20.5	n.d.	n.d.	474	13.5	n.d.	21.1	29.3	n.d.	16.5	n.d.	29.7
Site M 01/2019	21.5	n.d.	n.d.	255	7.7	n.d.	18.8	23.8	n.d.	16.0	17.6	19.4
Site M 02/2019	16.4	n.d.	n.d.	188	10.5	27.8	21.7	39.6	n.d.	15.9	15.2	17.6
Site M 03/2019	19.8	n.d.	n.d.	520	8.4	n.d.	17.7	18.5	n.d.	16.0	17.7	19.8
Site Ch 10/2018	18.7	n.d.	n.d.	n.d.	7.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	18.6
Site Ch 11/2018	34.2	n.d.	n.d.	n.d.	7.6	n.d.	n.d.	20.3	n.d.	n.d.	15.6	19.1
Site Ch 12/2018	36.6	n.d.	n.d.	n.d.	7.4	n.d.	n.d.	24.2	n.d.	16.3	n.d.	19.4
Site Ch 01/2019	19.6	n.d.	n.d.	34.6	8.2	n.d.	n.d.	23.3	n.d.	n.d.	n.d.	18.9
Site Ch 02/2019	17.8	n.d.	n.d.	271	8.8	n.d.	n.d.	n.d.	n.d.	16.3	n.d.	19.6
Site Ch 03/2019	18.2	n.d.	n.d.	n.d.	7.7	n.d.	n.d.	21.2	n.d.	n.d.	n.d.	21.6

Units: ng/L; n.d.: not detected; (-): analyzed in negative mode

Table S9. Accumulated concentrations of each group of compounds (UVF and PB) in the seawater samples (ng/L).in the sediment samples (ng/g dw).

Site and date	∑UVFs	∑PBs	∑TOTAL	∑UVFs	∑PBs	∑TOTAL
Site R 10/2018	127	20.1	147	2082	746	2829
Site R 11/2018	415	338	753			
Site R 12/2018	119	222	341			
Site R 01/2019	361	18.7	379			
Site R 02/2019	72.1	65.6	137			
Site R 03/2019	986	82.2	1069			
Site M 10/2018	826	35.5	861	4252	256	4509
Site M 11/2018	1652	19.4	1672			
Site M 12/2018	558	46.2	604			
Site M 01/2019	326	53	379			
Site M 02/2019	304	48.7	352			
Site M 03/2019	584	53.5	637			
Site Ch 10/2018	26.5	18.6	45.1	587	165	752
Site Ch 11/2018	62.1	34.7	96.8			
Site Ch 12/2018	68.2	35.7	103			
Site Ch 01/2019	85.7	18.9	104			
Site Ch 02/2019	297	35.9	333			
Site Ch 03/2019	47.1	21.6	68.7			

Units: ng/L; UVFs: Ultra violet filters; PCs: Paraben conservatives

Table S10. Average, range and frequency of detection of UVF and PB in seawater samples (ng/L).

Location		UV Filters								Paraben Conservatives			
		BP3	4DHB	AVO	EHMC	EtPABA	MeBZT	DMBZT	UVP	BePB (-)	BuPB (-)	PrPB (-)	MePB (-)
site M	Average	28.0	<LOD	16.3	583	14.6	15.3	19.9	32.0	<LOD	13.4	8.4	20.9
	Range	16.4-66.9	–	<LOD-97.7	188-1420	7.7-37.7	<LOD-33.5	17.7-22.0	18.5-50.9	–	<LOD-16.5	<LOD-17.7	17.6-29.7
	Frequency	100%	0	5.56%	100%	100%	50%	100%	100%	0%	83%	50%	100%
Site R	Average	35.4	4.5	3.6	254	44.1	10.9	3.8	29.5	5.1	55.6	8.3	55.5
	Range	19.3-56.7	<LOD-26.9	<LOD-21.6	<LOD-836	7.3-10.9	<LOD-34.6	<LOD-22.5	20-37.9	<LOD-15.4	<LOD-300	<LOD-18.8	17.6-222
	Frequency	100%	5.56%	5.56%	66%	100%	50%	6%	100%	33.00%	50.00%	50.00%	100.00%
Site CH	Average	24.1	<LOD	<LOD	50.9	7.9	<LOD	<LOD	14.8	<LOD	5.4	2.6	19.5
	Range	17.8-36.6	–	–	<LOD-271	7.4-8.8	–	–	<LOD-24.2	–	<LOD-16.3	<LOD-15.6	18.6-21.6
	Frequency	100%	0	0	33%	100%	0%	0%	66%	0%	33%	6%	100%

<LOD: Below the limit of detection; (-): analyzed in negative mode

Table S11. Concentrations (ng/g dw) in the sediment samples of the compounds detected at least in one sample.

Site and date	AVO	4MBC	EHMC	MeBZT	MePB (-)
Site R 10/2018	n.d.	< LOQ	n.d.	n.d.	3.6
Site R 11/2018	n.d.	< LOQ	< LOQ	n.d.	3.4
Site R 12/2018	n.d.	< LOQ	12.3	n.d.	3.5
Site R 01/2019	n.d.	< LOQ	21.4	n.d.	3.7
Site R 02/2019	n.d.	< LOQ	n.d.	n.d.	3.4
Site R 03/2019	n.d.	n.d.	16.2	n.d.	3.7
Site M 10/2018	n.d.	n.d.	12.6	12.1	4.9
Site M 11/2018	n.d.	n.d.	< LOQ	8.7	4.0
Site M 12/2018	n.d.	n.d.	8.7	< LOQ	5.0
Site M 01/2019	3.4	n.d.	16.6	< LOQ	7.6
Site M 02/2019	n.d.	< LOQ	< LOQ	24.9	n.d.
Site M 03/2019	n.d.	< LOQ	n.d.	n.d.	n.d.
Site Ch 10/2018	5.0	< LOQ	n.d.	< LOQ	4.1
Site Ch 11/2018	n.d.	17.1	n.d.	n.d.	4.6
Site Ch 12/2018	n.d.	n.d.	n.d.	n.d.	3.9
Site Ch 01/2019	17.6	n.d.	n.d.	n.d.	4.8
Site Ch 02/2019	n.d.	4.3	n.d.	n.d.	4.2
Site Ch 03/2019	n.d.	n.d.	n.d.	n.d.	5.3

Units: ng/g dw; n.d.: Not detected; <LOQ: Under the limit of quantification (-): analyzed in negative mode

Table S12. Accumulated concentrations of each group of compounds (UVF and PB) separated by sites and date.

Site and date	∑UVFs	∑PBs	∑TOTAL	∑UVFs	∑PBs	∑TOTAL
Site R 10/2018	0.0	3.6	3.6	49.9	21.3	71.2
Site R 11/2018	0.0	3.4	3.4			
Site R 12/2018	12.3	3.5	15.8			
Site R 01/2019	21.4	3.7	25.1			
Site R 02/2019	0.0	3.4	3.4			
Site R 03/2019	16.2	3.7	19.9			
Site M 10/2018	24.7	4.9	29.6	87.0	21.5	108.5
Site M 11/2018	8.7	4.0	12.7			
Site M 12/2018	8.7	5.0	13.7			
Site M 01/2019	20.0	7.6	27.6			
Site M 02/2019	24.9	0.0	24.9			
Site M 03/2019	0.0	0.0	0.0			
Site Ch 10/2018	5.0	4.1	9.1	44.0	26.8	70.8
Site Ch 11/2018	17.1	4.6	21.7			
Site Ch 12/2018	0.0	3.9	3.9			
Site Ch 01/2019	17.6	4.8	22.4			
Site Ch 02/2019	4.3	4.2	8.5			
Site Ch 03/2019	0.0	5.3	5.3			

Units: ng/g dw; UVFs: Ultra violet filters; PCs: Paraben conservatives

Table S13. Average, range and frequency of detection of UVF and PB in the sediment samples (ng/g dw).

Location		UVF				PC
		AVO	4MBC	EHMC	MeBZT	MePB(-)
Site M	Average	-	-	6.3	7.6	3.6
	Range	-	-	<LOD-16.60	<LOD-24.9	<LOD-7.6
	Frequency	0%	0.00%	50.00%	50%	100%
Site R	Average	0.6	-	8.3	-	3.6
	Range	<LOD-3.4	-	<LOD-21.4	-	3.4-3.8
	Frequency	6%	0	50.00%	0%	100%
Site Ch	Average	3.8	3.7	-	-	4.5
	Range	0-17.6	<LOD-17.1	-	-	3.9-5.3
	Frequency	33%	33%	0	0%	100%

<LOD: Below the limit of detection; (-): Analyzed in negative

Table S14. Distribution coefficient (Kd) of each compound (L/kg) in each site, with its mean values and octanol/water partition coefficient (logKow).

	Kd (L/Kg)			
	AVO	EHMC	MeBZT	MePB (-)
Site R	-	24	-	64
Site M	35	22	103	257
Site Ch	-	-	-	228
Mean	35	23	103	183
log(Kow)	4.5	5.8	1.9	2

log(Kow): octanol/water partition coefficient

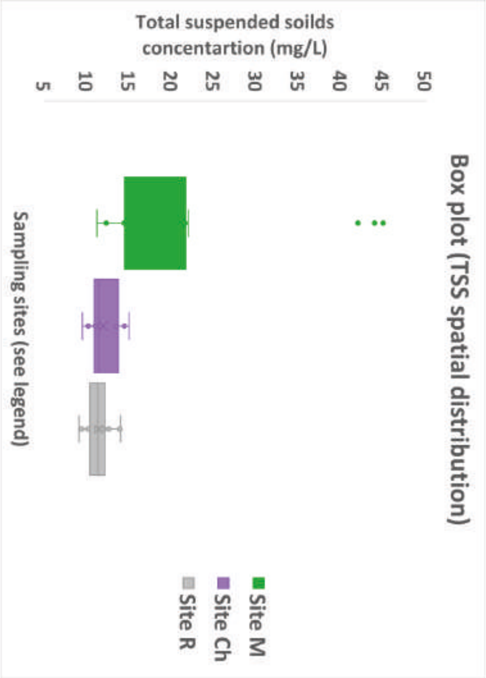

Figure S1. Box plot of total suspended solids' special distribution in sites M, R and Ch.

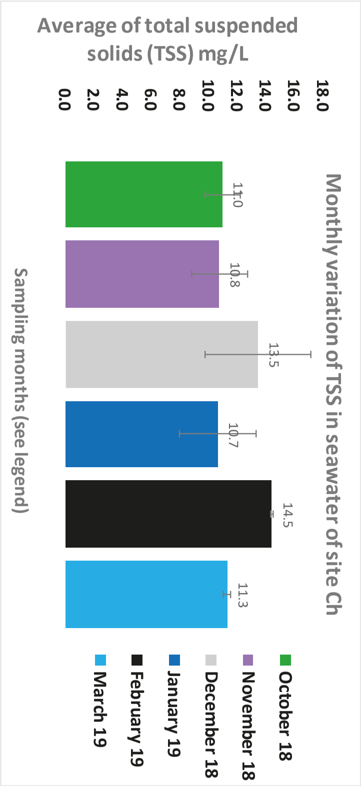

Figure S2. Monthly variation of suspended matter in seawater of site Ch (mg/L).

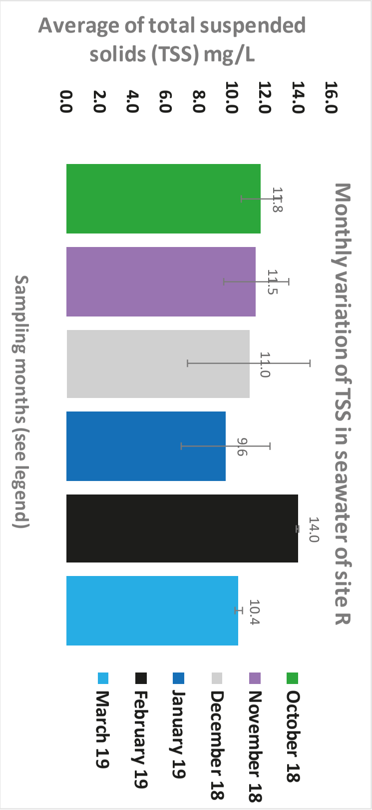

Figure S3. Monthly variation of suspended solids in seawater of site R (mg/L).

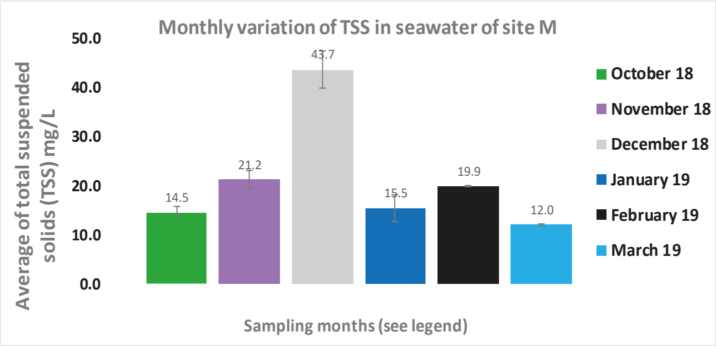

Figure S4. Monthly variation of suspended solids in seawater of site M (mg/L).

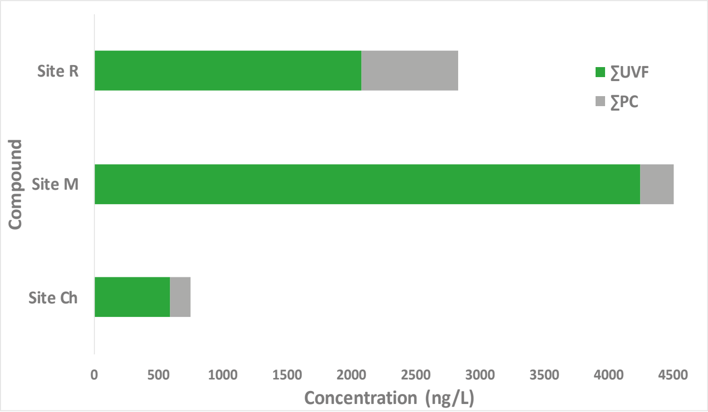

Figure S5. Accumulated concentrations of each group of compounds separated by sites in the seawater samples (ng/L).

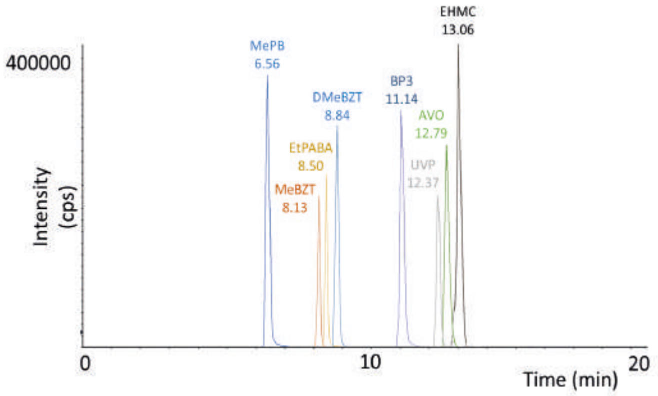

Figure S6. Reconstructed ion chromatograms showing the 1st transition obtained for the seawater sample taken in site M on 11/2018.

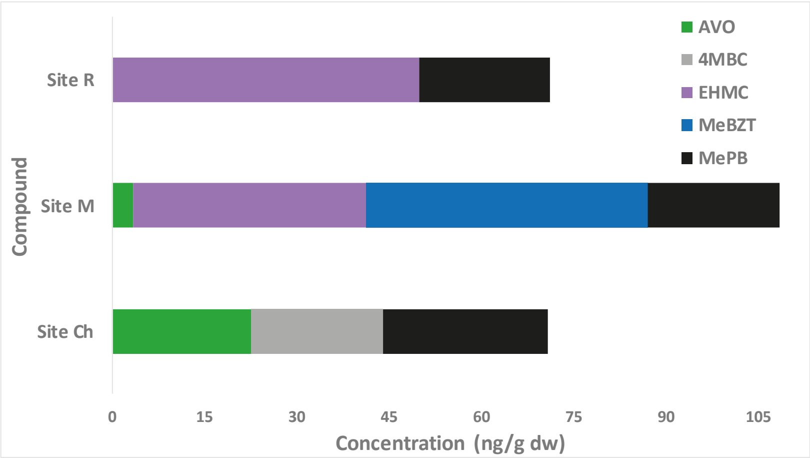

Figure S7. Accumulated concentrations of each group of compounds separated by sites in the sediment samples (ng/g dw).

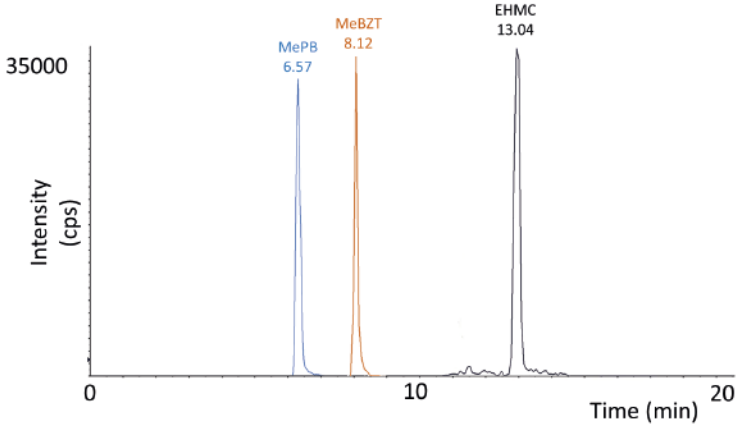

Figure S8. Reconstructed ion chromatograms showing the 1st transition obtained for the sediment sample taken in site M on 10/2018.

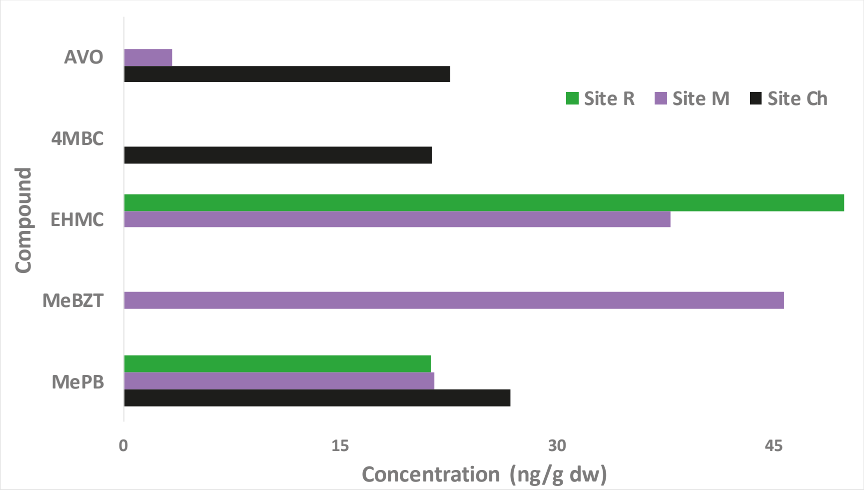

Figure S9. Accumulated concentrations of detected compounds separated by sites and groups in the sediment samples (ng/g dw).

Section S6. Equations.

$$\text{Partition coefficient (Kd) (L/kg)} = [\text{CECs}]_{\text{sediment}}\ (\text{ng/g})/([\text{CECs}]_{\text{water}}\ (\text{ng/L})*1000\ (\text{g/kg}))$$

Equation S1. Partition coefficient equation.

$$\text{Concentration (UVFs)} = \sum(\text{UVFs})\text{i}$$

$$\text{Concentration (PBs)} = \sum(\text{PBs})\text{i}$$

$$\text{Total accumulated concentration} = \text{Concentration (UVFs)} + \text{Concentration (PBs)}$$

Equation S2. Total accumulated concentration equation.

3.2.3 Publication #3

Rapid and cost-effective multiresidue analysis of pharmaceuticals, personal care products, and antifouling booster biocides in marine sediments using matrix solid phase dispersion.

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Rapid and cost-effective multiresidue analysis of pharmaceuticals, personal care products, and antifouling booster biocides in marine sediments using matrix solid phase dispersion

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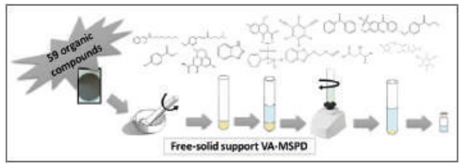
HIGHLIGHTS

- A method for 59 emerging organic contaminants analysis in sediments was developed.
- Matrix solid phase dispersion allowed limits of quantification of 0.42 ngg^{-1}dw.
- The validated method was applied to analyze marine sediments from Brazil.
- Detected analytes were measured at concentrations between 1.44 and 69.69 ng g^{-1}dw.

GRAPHICAL ABSTRACT



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ABSTRACT

Currently, there are many contaminants of concern that need to be accurately determined to help assess their potential environmental hazard. Despite their increasing interest, yet few environmental occurrence data exist, likely because they are present at low levels and in very complex matrices. Therefore, multiresidue analytical methods for their determination need to be highly sensitive, selective, and robust. Particularly, due to the trace levels of these chemicals in the environment, an extensive extraction procedure is required before determination. This work details the development of a fast and cheap vortex-assisted matrix solid-phase dispersion-high performance liquid chromatography tandem-mass spectrometry (VA-MSPD-HPLC-MS/MS) method for multiresidue determination of 59 contaminants of emerging concern (CECs) including pharmaceuticals, personal care products, and booster biocides, in sediment. The validated method provided high sensitivity (0.42–36.8 ngg^{-1} dw quantification limits), wide and good linearity ($r^2 > 0.999$), satisfactory accuracy (60–140%), and precision below 20% for most target analytes. In comparison with previous methods, relying on traditional techniques, the proposed method demonstrated to be more environmentally friendly, cheaper, simpler, and faster.

The method was applied to monitor the occurrence of these compounds in sediments collected in Brazil, using only 2 g dw sediment samples, free-solid support, and 5 mL methanol as extraction solvent. The UV filter avobenzone, the UV stabilizer and antifreeze methylbenzotriazole, the preservative

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methylparaben, the fluoroquinolone antibiotic ciprofloxacin, and the biocides irgarol and 4,5-dichloro-2-octyl-4-isothiazolin-3-one were determined at concentrations in the range 1.44–69.7 ngg^{-1} dw.

1. Introduction

Pharmaceuticals and personal care products (PPCPs), and antifouling booster biocides comprise a large group of contaminants worldwide studied which are currently considered environmental contaminants of emerging concern (CECs). These substances have been increasingly investigated in water (Köck-Schulmeyer et al., 2019), sediment (Batista-Andrade et al., 2016, 2018), sludge (Cerqueira et al., 2018), and biota (Vieira et al., 2018b). Due to their physicochemical properties, some compounds have more affinity for the organic phase, having a greater tendency to be adsorbed onto sediments and the suspended particulate matter of the water column (Martins et al., 2018). Besides, they tend to bioaccumulate in aquatic organisms and thus have been investigated for potential ecotoxicity (Molins-Delgado et al., 2018a).

In recent years, PPCPs have been regarded as an important environmental issue since these compounds are widely used in human and veterinary medicine and represent an important group of high volume production products (Čelić et al., 2018). Within this group, sunscreen agents, also known as UV filters (UVFs) deserve major attention for their increasing use as a protection against the harmful effects of the UV solar radiation (Molins-Delgado et al., 2018b). They are present in numerous hygiene and beauty consumer goods (cosmetics, sunscreens, and hair-style products, among others), and have many additional industrial applications (plastic, rubber, textile materials, etc) to protect the polymeric materials.

Concerning antifouling booster biocides, their use is directly related to marine biofouling, defined as a biological phenomenon characterized by the colonization and/or growth of organisms over surfaces submerged in seawater. To minimize the problems caused by biofouling, paints containing chemicals with biocidal properties were actively developed. More recently, booster biocides, such as diuron, irgarol, dichlofluanid, 4,5-dichloro-2-octyl-4-isothiazolin-3-one (DCOIT) and [(1,3-benzothiazol-2-yl)sulfanyl]methyl thiocyanate (TCMTB) deserve particular attention due to its potential to contaminate coastal areas (Thomas and Brooks, 2010), already under the pressure of tourism and its associated PPCPs' release.

To determine traces of organic contaminants in complex solid environmental matrices, the development of analytical methods is undoubtedly one of the current greatest needs. In this regard, analytical sample preparation procedures employed so far for organic contaminants determination in environmental solid samples apply techniques using a high amount of sample and solvents' volume, generating a lot of waste and requiring long and tedious extraction methods. Therefore, the application of multi-residue analytical methods, simple, cost-effective, fast, and minimizing the amount of reagents, sample, and solvents are preferred. The major challenge for the simultaneous determination of a wide range of organic contaminants in the environment results from the broad spectrum of physicochemical properties combined with the high complexity of the matrix sample. Furthermore, usually, they are present at low concentrations, thus requiring a preconcentration step (Caldas et al., 2016).

In this regard, an interesting approach is the application of the matrix solid phase dispersion (MSPD) for extraction and purification in CECs analysis. This technique consists of mixing and blending solid or semi-solid samples with abrasive solid support and subsequent elution of the target compounds with a small volume of a suitable organic solvent (Barker et al., 1989). However, to make the technique more robust, less susceptible to errors, and environmentally friendly, last years, the original MSPD technique underwent some modifications. One of these modifications improving the selectivity of the original technique is vortex-assisted matrix solid phase dispersion (VA-MSPD). This approach consists of vortexing the mixture (sample plus solid support) with a few mL of an organic solvent. This approach reduces some drawbacks of the original technique such as the package of the mixture, making the technique simpler, cheaper, and more robust (Caldas et al., 2013). Besides, this miniaturization version of the technique can still be optimized to further reduce reagent and solvent consumption and waste generation (Soares et al., 2017).

In this context, the present study aimed to develop and validate a simple, rapid, and cost-effective method based on VA-MSPD and HPLC-MS/MS for the simultaneous multiclass analysis of 59 organic compounds of sound environmental relevance (www.epa.gov) and quite different physicochemical properties in sediment samples. The selected organic compounds encompass 3 categories: pharmaceuticals and metabolites, personal care products and degradation products, and antifouling booster biocides. A particular aim of this work was to highlight the advantages of the combination of VA-MSPD-HPLC-MS/MS as a compound-selective tool for the trace determination of CECs environmental contaminants. Finally, the validated method was applied to the determination of the target CECs in sediment samples from one of the largest South American Ports in Brazil.

2. Experimental

2.1. Standards and reagents

Analytical standards (Table 1S of the Supporting Information) of high purity (96–99.9%) were purchased from Sigma-Aldrich (Germany) and TCI (Belgium). Isotopically labeled analytical standards were obtained from CDN isotopes (Canada) and Sigma Aldrich (Germany). Florisil, alumina, silica, formic acid (98%), and ammonium acetate (≥96%) were supplied by Merck (Germany), C18 cartridges (500 mg) by Isolute (Spain), methanol (MeOH), ethanol (EtOH), and acetonitrile (ACN) ultra-gradient HPLC grade, ethyl acetate (EtAc) and dichloromethane (DCM) (organic residue analysis grade) by J.T. Backer (The Netherlands) and nitrogen (99,999%) and argon (99.995%) by Air Liquid (Spain).

Stock solutions of individual standards (1000 mg L^{-1}) and an intermediate stock solution containing all analytes (1 mg L^{-1}) were prepared in MeOH. Daily, standard work solutions were prepared at appropriate concentrations. All solutions were stored in the dark at −20 °C.

2.2. HPLC-(QqLIT)-MS/MS analysis

For the analytical determination, a 4000 Q TRAP™ hybrid triple quadrupole-linear ion-trap mass spectrometer (Applied Biosystems-Sciex; Foster City, Ca, USA), equipped with an HPLC system with an Alias autosampler was used. Target analytes were monitored in electrospray ionization under positive (ESI+) and negative (ESI-) modes.

The initial conditions selected to develop the method were based on previous studies (Gago-Ferrero et al., 2011a, 2013a; Serra-Roig et al., 2016). Chromatographic separation was performed using a Purosher® STAR® HR R-18 (50 mm × 2.0 mm, 5 μm) (Merck) column. The elution was performed at a flow rate of 0.3 mL min^{-1}. For the analysis in ESI+, a mixture of HPLC-grade water and MeOH, both with 0.1% formic acid, was used. In the ESI- mode, the mobile phase consisted of the same binary solvent combination containing 5 mM of ammonium acetate. The injection volume was 20 μL in both modes.

For improve sensitivity and selectivity, the tandem-MS detection was performed under selected reaction monitoring (SRM) mode, targeting the two major characteristic fragments of the precursor molecular ion for each analyte. The most abundant and the second most abundant transitions were used for quantification and confirmation, respectively, in line with the EU recommendation (Commission Decision, 2002/657/EC).

General operation conditions for the analysis were as follows: ESI+: capillary voltage, 5000 V; source T^{a}, 700 °C; curtain gas, 30 psi; ion source gas 1, 50 psi; ion source gas 2, 60 psi, and entrance potential, 10 V. ESI-: capillary voltage, −4000 V; source T^{a}, 500 °C; curtain gas, 20 psi; ion source gas 1, 50 psi; ion source gas 2, 60 psi, an entrance potential, −10 V (Gago-Ferrero et al., 2013b). All data were processed using the Analyst software V 1.4.2 (Applied Biosystems).

Once the chromatographic conditions were established, a chromatogram under total ion mode in both positive and negative electrospray ionization modes were recorded for a mixture standards solution in MeOH at 700 ng mL^{-1}. Fig. 1S illustrates the chromatogram in the positive mode and Fig. 2S in the negative mode, respectively. According to Figs. 1S and 2S, it is possible to observe that many compounds obtained close retention times or else, they co-eluted. The lack of resolution in a chromatogram can be a problem in liquid chromatography when coupled with traditional detectors such as UV since they are specific enough when spectral differences are small. However, the use of LC-MS/MS can circumvent these problems of chromatographic separation, since MS/MS and SRM mode used are highly selective.

2.3. Sample collection and TOC determination

Eight sediment samples were collected in Santos-São Vicente Estuarine System (SSES) (São Paulo) and a shipyard at Patos Lagoon Estuary (Rio Grande do Sul). Samples were collected at different depths, using a stainless steel *Ekman* grab, in areas under influence of maritime anthropogenic activities.

The sediments were lyophilized, homogenized and stored at −20 °C for subsequent analysis. The granulometry was determined according to Gray and Elliott (2014), whereas total organic carbon (%TOC) was measured, after de-carbonation of the sediment samples, using a TOC-L SSM 5000 A (Shimadzu) instrument (Kristensen and Andersen, 1987).

2.4. VA-MPSD extraction

To develop the method, the initial conditions were selected based on a previous study where VA-MSPD was applied for diuron, irgarol, TCMTB, and DCOIT extraction from sediments (Batista-Andrade et al., 2016). Considering that it was a multi-residue method, VA-MSPD was optimized to obtain recovery rates of 100 ± 40%, with relative standard deviation (RSD) below 20%.

2.4.1. Extraction solvent selection

The appropriate selection of the extraction solvent is a key factor in the development of sample preparation methods. In addition to its effectiveness, human and environmental toxicity must be considered as well (Anastassiades et al., 2003). In this work due to the different polarity of the selected compounds and based on a literature review, MeOH, EtOH, EtAC, and ACN were evaluated as extraction solvents.

2.4.2. Solid support selection

Materials used as solid support can have a simple abrasive role to ensure complete matrix disruption or can be selective materials enhancing the MSPD selectivity, allowing purification and extraction in the same step. A solid support is considered one of the most studied variables in the MSPD technique and its choice depends on the matrix, analytes of interest, and extraction solvent.

The success of C18 material as solid support is due to its removal capability for non-polar compounds, such as fatty substances and lipids, being recommended for matrices with fat content >2%. The potential interaction among non-polar analytes and the solid support occurs through van der Waals forces and the use of C18 or its combination with other materials, such as Primary Secondary Amine (PSA), to turn the sample preparation of matrices with high contents of fat more effectively.

Florisil is one of those materials used in MSPD to retain nonpolar lipids, dyes, amines, hydroxyls, and carbonyls through polar interaction mechanisms, such as hydrogen bonds (Kurz et al., 2019).

In the present study, and according to a literature review, the solid supports C18, florisil, alumina, silica, and polymeric material (Strata-X) were tested.

In the optimized procedure, an aliquot of 2 g of freeze-dried sediment was spiked with 100 μL of a solution containing the isotopically labeled surrogate standard benzophenone-C13, left 30 min. to equilibrate and then manually ground in a mortar and pestle for 5 min. This mixture was transferred to a polypropylene tube, and 5 mL of the extraction solvent was added, vortexed for 1 min and centrifuged at 4000 rpm for 10 min. Before injection, the IS mixture (50 ng mL^{-1}) was added. Analyses were performed in triplicate, and each replica was measured 3 times.

2.5. Analytical validation

The method was validated following SANTE (2017) and INMETRO (2018). Method limits of detection and quantification (LODm and LOQm), calibration curves and linearity, accuracy, precision, and matrix effect (ME %) were evaluated. LODm and LOQm, were determined as the lowest compound concentration that yielded a signal-to-noise (S/N) ratio of 3 and 10, respectively. Analytical calibration curves were constructed and adjusted according to the individual response range of each compound. Accuracy was assessed by the recovery efficiency of each standard spiked in the blank sediment, determined in triplicate at three concentration levels (1xLOQ, 5xLOQ, and 10xLOQ), and measured 3 times (n = 9). Precision was calculated as the RSD (in %) for each concentration level, analyzed intra-day and inter-day. Matrix effect was evaluated by comparing the slopes of the analytical calibration curves prepared in MeOH and in the sediment extracts (matrix-matched calibration standards). All analytical calibration curves, as well as all validation tests, were carried out in the presence of the isotopically labeled IS mixture at 50 ng mL^{-1} (Table 1S).

3. Results and discussion

3.1. VA-MSPD extraction optimization

3.1.1. Selection of the extraction solvent

Five mL of MeOH, EtOH, EtAc, and ACN were individually tested in combination with 0.25 g of C18 material as solid support. The recovery efficiency for each solvent tested is shown in Fig. 1. It shows that when ACN and EtAc were evaluated, even though both have similar polarities, some compounds were scarcely recovered. When ACN was used as extraction solvent, average recoveries were below 70% for about 60% of the analytes and only 17% were between 60 and 140%. Besides, RSD were higher than 20% for most compounds. EtAc is often used in sample preparation procedures, since this solvent is considered non-mutagenic, non-bioaccumulative and more environmental friendly, however, when we used it, many matrix components were co-extracted. In addition, it was unsuitable for the extraction of more polar compounds, especially those with Log Kow values below 3. For moderately polar analytes, such as BP3, BP1, 4HB, 4DHB, DHMB, gemfibrozil, mefenamic acid, naproxen, diclofenac, atenolol and diuron, recovery rates ranged between 60 and 140%. It is known that polar compounds do not readily partition in this solvent, and significant amounts of Na_2SO_4 and/or polar co-solvents, such as MeOH and EtOH, have been used to improve the recovery rates (Anastassiades et al., 2003). However, to keep the method as simple and environmentally friendly as possible solvent mixtures were not tested in the present study.

When MeOH was employed in our optimization, average recoveries between 60 and 140% were obtained for 64% of the compounds, providing the best extraction efficiency. This is likely because of the majority of the analytes in this study have medium to high polarity, from the most polar one (SPY: Log Kow0.05) to the most apolar one (EHMC: Log Kow 4.95). The effectiveness of MeOH as an extraction solvent in sample preparation techniques is linked to its ability to interact with polar compounds because it is a polar protic solvent. MeOH has high dielectric constant (ε), which is a good indicator of the higher probability of interacting with analytes of polar nature, favoring the extraction through the capacity of hydrogen bonding. In general, the dielectric constant is considered an important parameter to predict the behavior and to measure the polarity of a solvent (Cerqueira et al., 2018).

In previous studies, reported recovery rates for UVFs from sediments ranged from 80 to 125% when using pressurized liquid extraction (PLE) and MeOH as the extraction solvent. However, a larger volume of MeOH was used (25 mL) because the PLE technique requires a relatively large volume of extraction solvents as typically employs various extraction cycles for extended isolation (Gago-Ferrero et al., 2011).

When EtOH was evaluated, obtained recoveries were between 60 and 140% for 40% of the compounds. UVFs showed good recoveries, with RSD below 19%. Similarly to MeOH, EtOH is a protic polar solvent (with ε of 24.5), and likely because of this slightly less polar character, it provided lower recovery rates than MeOH.

Concerning antifouling booster biocides, MeOH, EtOH, ACN, DCM, and/or acetone have been used for the isolation of organic chemicals from sediment samples. However, the number of compounds as well as the chemical classes studied in these works is much lower than those included in our developed method. Moreover, the solvent volumes used were also larger than the MeOH volume used in the present study (5 mL).

In light of these results, MeOH was finally selected as the extraction solvent in the present study due to the best performance, reaching recovery rates between 79 and 120% for about 60% of the target analytes. To further improve the number of compounds with recoveries within this range, different solid supports were also assessed using MeOH as the extraction solvent.

3.1.2. Selection of solid support

Fig. 2 shows the recovery rates of each compound when different solid supports were evaluated. Besides, a sample without added solid support was also tested.

The obtained average recovery rates were between 60 and 140% for 22% of the compounds when Florisil was used. Some compounds, such as BP2, DHMB, and succinyl-sulfathiazole, showed average recoveries below 70%, and 61% of compounds showed values above 140%. Recovery rates between 75 and 114% for 17% of analytes were obtained when alumina was used as solid support. As observed for Florisil, most of the compounds showed recoveries out of the recommended range. Thus, considering that these compounds had not been extracted, the explanation could be that i) these materials may be selectively interacting with the sample or, ii) the mechanical force applied during the technique was not able to break the original structure of the sample.

When silica was employed, the average recoveries were between 60 and 140% for 25% of analytes. Recovery rates above 140% were obtained for most polar compounds, such as some UVFs. The use of Strata-X yielded similar average recoveries than silica for some compounds (35%). Strata-X is a polymeric material with the presence of the pyrrolidine group and styrene in its structure. The presence of these two groups may favor the interaction with other compounds by hydrogen bonding or the possibility of interaction by π-π and dipole-dipole bonds, thus reducing extraction efficiency. Sulfonamides and booster biocides were recovered well beyond the acceptable range.

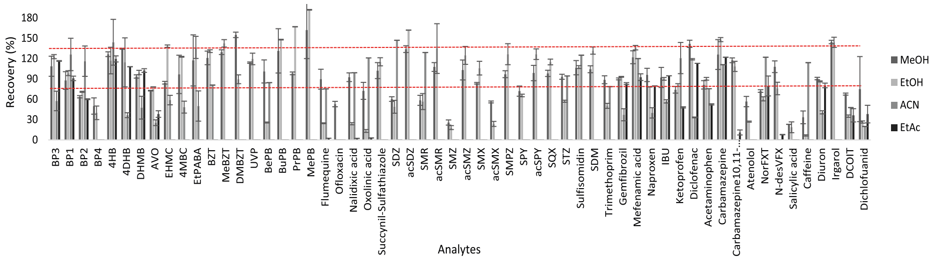

Fig. 1. Recovery rates of the target compounds after VA-MSPD extraction with four extraction solvents. Error bars represent RSD (%). Experimental conditions: 2 g dw sediment sample; 0.25 g C18 material as the solid support, and 5 mL of extraction solvent. One min. of vortex agitation and 10 min centrifugation at 4000 rpm, n = 9.

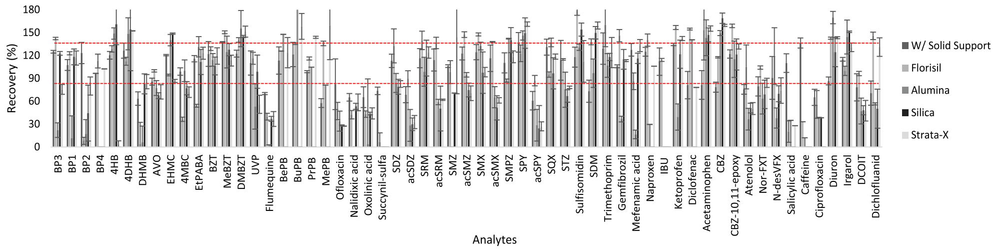

Fig. 2. Recovery rates of the target compounds after VA-MSPD extraction with four solid supports, and without solid support. Error bars represent RSD (%). Experimental conditions: 2 g dw sediment sample; 0.25 g C18 material as the solid support, and 5 mL of MeOH as extraction solvent. One min. vortex agitation and 10 min centrifugation at 4000 rpm, n = 9.

Overall, in the present study, we obtained recoveries between 70 and 120% for 71% of compounds when only the sediment sample (2 g dw) was macerated and MeOH (5 mL) was used as extraction solvent. This solid support-free VA-MSPD approach, assuming that the matrix itself, when macerated, can be considered abrasive, such as dried sediment, does not need the addition of solid support. This alternative extraction has scarcely been investigated so far, despite it appears an attractive alternative in sample preparation since it reduces the consumption of reagents and generates lower wastes.

Thus, according to the results, the solid support-free VA-MSPD was selected as the extraction technique since, besides providing the best recoveries among the tested configurations, it consumes lower volume of reagents, generates fewer residues, consumes less time, and has the lower operative cost.

Despite the good performance obtained in this case, we must consider that the effectiveness of solid support-free extraction is directly related to the particular characteristics of the sediments analyzed. The sediment sample used in this study likely presents the content of inorganic substances such as carbon, magnesium, calcium, iron, aluminum, and silicon, which provide it with abrasive characteristics (Cerqueira et al., 2018). Also, there were practically no organic compounds that recovered below 60% when no solid support was used.

As the recommended recovery range was reached for 71% of compounds, and considering that it is a multiresidue method based on an extraction technique for analytes with very different physicochemical properties, other parameters that might influence the extraction and purification of the sediments were not further optimized. However, if the main goal of the study would be a specific chemical group of compounds with similar characteristics, we recommend for the improvement in the acceptable recovery range the optimization of other variables, such as the solvent volume, the amount of sample and solid support, and the blending time of the mixture solid sample plus solid support.

3.2. Method performance

The performance parameters of the validated method are shown in Table 1. Overall, LOQm ranged from 0.42 to 36.8 ng g^{-1} dw following those previously reported in studies analyzing PPCPs in soil and sediment (Batista-Andrade et al., 2016; Caldas et al., 2018). Regarding antifouling booster biocides, LOQm were comparable to the sediment quality guideline thresholds limit (Maximum Permissible Concentration – MPC) established by restrictive legislation on sediment quality criteria, Dutch authorities for instance, for diuron (9 ng g^{-1}) and irgarol (1.4 ng g^{-1}) (Crommentuijn et al., 2000). Depending on where the booster biocide is found, it can be classified as class IV "bad" for contamination.

The linear range was evaluated from 1 to 1000 ng mL^{-1}. Correlation coefficients (r^2) of analytical calibration curves ranged from 0.9979 to 0.9999 in MeOH, and from 0.9888 to 0.9999 in the sediment extract (matrix-matched standards), indicating good and wide linearity for all compounds (INMETRO, 2018).

The extraction efficiency of VA-MSPD was satisfactory, obtaining recovery rates between 60 and 140% (SANTE, 2017; INMETRO, 2018). Lower recoveries, below 60% were obtained for BP4, EtPABA, UVP, SPY, and ketoprofen. Generally, RSD values for intraday and inter-day tested at three concentration levels were below 20% except for BP4 at 10xLOQm and nalidixic acid at 1xLOQm.

Concerning ME, by comparing the calibration curves in pure solvent and the sediment extract, 60% of compounds presented low to medium ME, mainly suppression of the signal. ME deemed to be low for signal suppression/enhancement of ±20%, the medium between ±20% and ±50%, and high for values higher than 50% or lower than −50% (Economou et al., 2009). Whenever ME is considered insignificant (less than or equal to 20%), calibration curves in the solvent can be used, avoiding the need for more laborious calibrations. However, in the presence of ME, some strategies should be done to appropriately compensate for signal changes and/or minimize the variability of results (Martins et al., 2016). The use of isotopically labeled IS provides a practical way of correction for any bias caused by the matrix that may affect the reliability of the instrument response factors. For multi-residue methods, as the developed one, the more deuterated internal standard used the better. However, deuterated standards are not available for every analytes and, whenever available are very expensive (SANTE, 2017). Thus, the quantification in the present study was done using at least one deuterated internal standard for each class of compound (Table 1S).

The developed VA-MSPD based method was intended for the analysis of a large number of analytes in sediment. Nevertheless, it may be applied to other solid environmental samples, such as soil and sewage sludge, once the characteristics of each matrix are considered. In general, and in comparison, with the previously published methods, the one developed in this work can simultaneously analyze 59 organic compounds while the previous ones limit the analysis to a specific and small group of substances with similar structures and/or properties. Furthermore, the method described in the present study is faster and cheaper, and more environmentally friendly.

Table 1
Method limits of detection (LODm) and quantification (LOQm), recovery rates (R%) and Relative Standard Deviations (±RSD)D%), for intra- and inter-day precision (% R) a the three concentration levels evaluated (1xLOQ, 5xLOQ, and 10xLOQ).

Analyte	LODm	LOQm	Precision (intra-day) R (%) ± RSD (%)			Precision (inter-day) R (%) ± RSD (%)		
	(ng g^{-1})	(ng g^{-1})	LOQ	5 LOQ	10 LOQ	LOQ	5 LOQ	10 LOQ
BP3	0.67	2.23	72.5 ± 23.4	75.8 ± 9.7	125.9 ± 0.7	111.2 ± 3.4	61.4 ± 0.6	102.2 ± 11.0
BP1	1.31	4.35	128.9 ± 20.7	129.8 ± 13.3	102.8 ± 7.5	128.5 ± 1.2	60.3 ± 10.1	115.0 ± 4.8
BP2	1.49	4.95	65.4 ± 1.2	74.0 ± 1.6	121.4 ± 4.9	79.0 ± 8.8	115.5 ± 0.3	34.0 ± 1.1
BP4	2.50	8.31	69.4 ± 21.5	69.3 ± 25.7	123.5 ± 33.1	56.2 ± 38.6	70.7 ± 29.4	121.5 ± 24.6
4HB	2.44	8.13	95.2 ± 20.0	130.8 ± 18.1	81.8 ± 5.0	86.1 ± 4.8	111.2 ± 1.5	124.6 ± 6.2
4DHB	2.02	6.71	63.4 ± 22.3	106.6 ± 26.1	68.8 ± 11.1	77.9 ± 6.7	101.3 ± 5.9	121.9 ± 6.3
DHMB	1.03	3.44	71.7 ± 21.1	64.8 ± 5.6	70.7 ± 8.6	112.9 ± 4.1	115.8 ± 9.8	149.9 ± 5.1
AVO	0.96	3.19	69.7 ± 10.0	94.0 ± 15.9	121.8 ± 12.2	91.5 ± 5.4	70.6 ± 12.2	123.5 ± 4.2
4MBC	0.49	1.65	120.7 ± 11.6	79.7 ± 14.8	66.2 ± 0.9	71.5 ± 1.1	100.2 ± 2.4	136.5 ± 3.8
EHMC	11.06	36.83	123.2 ± 4.5	87.2 ± 21.3	100.7 ± 1.1	62.2 ± 1.8	100.3 ± 1.2	80.7 ± 6.4
EtPABA	0.94	3.14	74.7 ± 19.4	61.3 ± 8.7	107.5 ± 10.3	53.6 ± 4.5	111.6 ± 12.0	69.6 ± 5.4
BZT	0.62	2.07	69.4 ± 17.6	124.9 ± 26.5	103.8 ± 26.0	116.6 ± 8.8	114.1 ± 1.5	113.6 ± 16.9
MeBZT	0.96	3.20	72.6 ± 12.8	78.6 ± 4.7	116.0 ± 23.7	88.4 ± 8.0	74.8 ± 7.5	69.9 ± 13.0
DMBZT	0.88	2.94	81.2 ± 3.4	65.5 ± 1.4	84.1 ± 49.8	95.9 ± 14.7	113.4 ± 4.0	81.3 ± 15.5
UVP	0.80	2.67	86.9 ± 16.5	75.8 ± 4.6	112.5 ± 8.9	55.0 ± 11.8	69.2 ± 21.5	118.4 ± 17.4
BePB	1.35	4.50	73.7 ± 8.1	111.8 ± 8.4	74.5 ± 7.0	63.7 ± 9.9	84.5 ± 2.2	110.3 ± 5.6
BuPB	0.94	3.13	73.0 ± 4.7	68.6 ± 7.5	70.2 ± 6.1	79.0 ± 9.5	95.8 ± 8.6	82.0 ± 0.8
PrPB	1.33	4.43	89.1 ± 5.0	94.5 ± 22.4	86.5 ± 11.1	86.0 ± 9.4	111.2 ± 8.3	53.1 ± 0.1
MePB	1.40	4.66	85.0 ± 6.8	78.4 ± 11.5	65.8 ± 17.2	69.9 ± 6.7	103.5 ± 0.3	89.1 ± 2.0
Flumequine	1.99	6.64	71.3 ± 7.7	75.6 ± 10.5	60.8 ± 9.7	77.5 ± 18.3	84.0 ± 8.0	124.8 ± 2.8
Ofloxacin	3.09	10.3	80.3 ± 4.2	130.5 ± 26.6	124.9 ± 12.4	99.6 ± 1.8	78.4 ± 15.2	78.7 ± 2 .2
Ciprofloxacin	0.62	2.07	136.5 ± 4.6	82.2 ± 10.0	72.2 ± 9.0	89.6 ± 19.0	122.2 ± 3.9	80.8 ± 2.0
Nalidixic acid	0.82	2.73	121.6 ± 6.0	123.3 ± 9.6	75.6 ± 11.5	122.8 ± 31.4	87.8 ± 7.3	95.8 ± 6.6
Oxolinic acid	0.54	1.79	80.7 ± 18.0	62.2 ± 24.8	75.0 ± 7.7	95.1 ± 21.0	121.4 ± 4.8	81.8 ± 1.8
Succinyl-sulfathiazole	0.32	1.07	84.8 ± 10.0	79.9 ± 4.2	122.8 ± 4.7	77.4 ± 1.5	69.4 ± 2.2	120.3 ± 4.6
SDZ	0.60	1.99	78.8 ± 18.9	102.9 ± 29.2	121.7 ± 20.5	129.9 ± 10.8	63.2 ± 12.5	104.2 ± 7.9
acSDZ	1.82	6.07	66.2 ± 10.8	124.7 ± 6.1	85.6 ± 13.8	115.7 ± 4.2	80.5 ± 13.0	97.7 ± 6.3
SMR	1.13	3.76	68.3 ± 17.3	122.4 ± 23.6	81.9 ± 24.4	80.2 ± 4.0	84.5 ± 12.2	122.4 ± 6.9
acSMR	0.22	0.72	121.3 ± 15.3	69.4 ± 5.3	99.9 ± 22.0	91.2 ± 25.4	54.9 ± 3.3	117.9 ± 21.3
SMZ	0.13	0.42	74.8 ± 9.0	70.0 ± 7.0	83.3 ± 11.3	99.8 ± 14.8	77.9 ± 11.2	95.8 ± 2.0
acSMZ	0.33	1.09	129.7 ± 20.6	89.5 ± 3.7	129.7 ± 5.8	80.6 ± 12.0	82.0 ± 16.6	125.7 ± 8.7
SMX	0.18	0.60	132.7 ± 6.3	78.3 ± 9.1	131.0 ± 5.8	68.6 ± 14.3	97.1 ± 12.4	145.7 ± 24.5
acSMX	0.41	1.37	124.4 ± 0.2	70.0 ± 6.6	105.4 ± 7.0	128.5 ± 23.2	73.0 ± 16.3	110.6 ± 8.1
SMPZ	0.16	0.53	68.7 ± 18.4	88.0 ± 3.0	122.3 ± 5.4	79.3 ± 9.9	89.5 ± 4.6	129.9 ± 20.4
SPY	0.26	0.86	57.9 ± 1.0	74.4 ± 3.3	111.6 ± 14.9	120.9 ± 2.9	88.8 ± 25.3	138.1 ± 18.9
acSPY	0.21	0.70	77.5 ± 4.4	49.3 ± 6.8	71.3 ± 0.1	117.2 ± 25.3	94.4 ± 5.6	81.1 ± 3.2
SQX	0.43	1.43	86.7 ± 8.5	97.6 ± 11.1	105.6 ± 20.0	88.8 ± 14.3	108.2 ± 17.6	156.5 ± 7.4
STZ	5.19	17.28	112.5 ± 2.4	72.6 ± 26.2	115.7 ± 8.7	128.7 ± 13.5	90.0 ± 23.5	101.7 ± 18.0
Sulfisomidin	5.70	18.99	75.4 ± 11.9	74.4 ± 26.3	65.7 ± 3.8	64.1 ± 3.7	79.0 ± 3.8	122.0 ± 0.2
SDM	1.54	5.14	62.4 ± 1.6	103.7 ± 4.3	91.8 ± 4.3	80.3 ± 16.1	99.9 ± 15.0	141.3 ± 15.3
Trimethoprim	1.56	5.20	88.9 ± 17.5	78.5 ± 16.7	98.8 ± 21.5	93.3 ± 8.1	120.7 ± 3.8	103.8 ± 11.5
Gemfibrozil	0.65	2.16	66.5 ± 11.2	68.3 ± 15.3	68.5 ± 5.8	131.3 ± 21.1	168.2 ± 2.0	136.3 ± 6.4
Mefenamic acid	1.16	3.86	135.7 ± 10.1	95.8 ± 9.3	123.1 ± 25.3	62.3 ± 2.5	83.1 ± 14.4	117.7 ± 17.0
Naproxen	0.47	1.58	123.5 ± 17.1	65.3 ± 5.4	112.0 ± 18.9	126.4 ± 5.1	69.6 ± 20.1	116.0 ± 12.9
IBU	1,66	5.54	84.4 ± 5.4	78.9 ± 17.5	111.8 ± 17.9	87.9 ± 25.3	131.3 ± 10.8	63.9 ± 10.5
Ketoprofen	0.36	1.21	57.6 ± 3.7	74.0 ± 10.9	106.5 ± 11.0	68.4 ± 7.4	68.3 ± 3.2	121.3 ± 22.7
Diclofenac	3.50	11.64	120.1 ± 4.2	111.5 ± 25.7	59.4 ± 17.9	62.0 ± 16.4	78.1 ± 23.1	89.2 ± 3.9
Acetaminophen	1.11	3.70	75.4 ± 7.4	74.8 ± 24.2	102.6 ± 27.2	86.8 ± 6.9	74.1 ± 16.5	111.9 ± 16.1
CBZ	1.87	6.24	64.0 ± 1.0	103.6 ± 12.8	103.2 ± 9.4	88.1 ± 1.4	93.4 ± 1.3	127.0 ± 24.7
CBZ-10,11-epoxy	5.04	16.80	63.0 ± 13.0	73.3 ± 13.5	109.3 ± 2.1	89.1 ± 1.8	82.1 ± 2.7	116.4 ± 5.2
Atenolol	0.41	1.38	117.3 ± 0.6	92.0 ± 1.7	138.1 ± 1.5	69.8 ± 0.5	87.6 ± 7.3	53.2 ± 0.5
NorFXT	2.40	8.00	120.9 ± 17.7	93.4 ± 0.3	70.1 ± 14.7	129.5 ± 6.5	49.9 ± 1.6	101.3 ± 6.6
N-desVFX	1.17	3.91	71.0 ± 4.2	131.4 ± 16.4	66.3 ± 13.3	66.9 ± 5.1	51.8 ± 1.9	94.1 ± 6.5
Salicylic acid	1.42	4.72	87.9 ± 8.0	80.5 ± 4.4	98.8 ± 12.5	110.0 ± 20.0	67.4 ± 24.4	89.6 ± 11.0
Caffeine	0.89	2.96	88.9 ± 10.0	115.3 ± 0.4	122.3 ± 13.7	128.4 ± 0.4	90.0 ± 7.9	134.6 ± 4.7
Diuron	0.86	2.87	74.6 ± 6.3	123.9 ± 26.8	108.6 ± 3.2	77.2 ± 3.7	120.4 ± 0.9	102.4 ± 6.9
Irgarol	0.43	1.44	119.8 ± 5.0	92.3 ± 4.4	111.8 ± 27.8	81.2 ± 15.0	106.6 ± 19.4	138.1 ± 28.3
DCOIT	0.53	1.78	125.4 ± 2.3	116.9 ± 17.8	100.2 ± 2.4	95.0 ± 4.2	112.5 ± 4.2	93.1 ± 18.7
Dichlofluanid	0.75	2.48	71.6 ± 14.8	104.4 ± 26.3	86.6 ± 6.0	123.9 ± 2.7	85.7 ± 0.4	122.8 ± 4.3

4. Applicability of the method to the analysis of coastal sediment samples

Once validated, the analytical method was applied to analyze marine sediment samples. The analyzed sediments had TOC contents ranging from 0.9 to 3.5%, percentage of fines from 17.4 to 65.1%, and slightly distinct profiles of use (Table 2). Among the 59 analytes, only AVO (<3.2–7.8 ng g^{-1} dw), MeBZT (<3.2–3.5 ng g^{-1} dw), MePB (<4.7–69.7 ng g^{-1} dw), ciprofloxacin (<2.1–9.5 ng g^{-1} dw), irgarol (<1.4–2.2 ng g^{-1} dw) and DCOIT (5.0–41.1 ng g^{-1} dw) were detected (Table 3), although AVO, MeBZT, and ciprofloxacin were below LOQm only in one site. Disregarding any local input sources, since all of these sites are under the influence of maritime activities, sediment characteristics may also influence the amount of each contaminant accumulated in the matrix. The most contaminated sample (Alemoa) had the highest % of fines (65%) and

Table 2
Location, percentage of fines (% Fines), and total organic carbon (% TOC) of the sediment samples collected in Brazil at Santos-São Vicente Estuarine System (SESS) (São Paulo), Patos Lagoon Estuary (Rio Grande do Sul) and Rio Grande shipyard (Rio Grande do Sul).

Sampling site	Longitude (W)	Latitude (S)	Depth (m)	Brief description	% Fines	% TOC
Pier do Pescador	46° 18′ 10.82″	23° 59′ 30.75″	3.2	Entrance of the estuary (fishing boats) (by the main navigation channel)	17.4	0.9
Balsa	46° 17′ 40.27″	23° 59′ 15.40″	2.0	Intensive traffic of ferryboat used for vehicles and passengers (by the main navigation channel)	22.2	1.3
Terminal 1	46° 18′ 22.85″	23° 56′ 55.88″	7.0	Main mooring area of Santos Port	37.2	3.0
Terminal 2	46° 19′ 39.83″	23° 55′ 31.16″	6.0	Main mooring area of Santos Port/shipyard	32.5	2.7
Terminal 3	46° 22′ 9.11″	23° 55 ′7.56″	7.0	Main mooring area of Santos Port	39.4	2.8
Alemoa	46° 17′ 11.80″	23° 58′ 34.55″	4.5	Mooring area for Oil-based Transport Vessels	65.1	3.5
São Vicente 2	46° 25′ 30.36″	23° 57′ 7.24″	3.0	São Vicente estuary channel (near the mangrove swamps). Small fishing and leisure boats	14.8	1.1
Rio Grande shipyard	52° 04′ 07.04″	32° 03′ 15.09″	0.3	Oldest shipyard in the city. Few repair activities today and the presence of a fuel supply station	20.5	1.6

Table 3
Concentrations (ng g^{-1} dw) of the target compounds in sediment samples collected in Brazil at Santos-São Vicente Estuarine System (SESS) (São Paulo), Patos Lagoon Estuary (Rio Grande do Sul), and Rio Grande (Rio Grande do Sul). LOQm, method limit of quantification for each analyte.

Analytes		Sediment samples							
	LOQm (ng g^{-1})	São Vicente	Alemoa	Pier pesca	Balsa	Tersminal 1	Terminal 2	Terminal 3	RG shipyard
AVO	3.19	<LOD$_m$	<LOD$_m$	<LOD$_m$	<LOD$_m$	<LOD$_m$	<LOD$_m$	<LOD$_m$	7.75
MeBZT	3.20	<LOD$_m$	3.48	<LOD$_m$	<LOD$_m$	<LOD$_m$	<LOD$_m$	<LOD$_m$	<LOD$_m$
MePB	4.66	12.63	69.69	44.19	12.09	38.00	21.30	36.56	5.79
Ciprofloxacin	2.07	<LOD$_m$	<LOD$_m$	<LOD$_m$	9.54	<LOD$_m$	<LOD$_m$	<LOD$_m$	<LOD$_m$
Irgarol	1.44	<LOD$_m$	<LOD$_m$	1.51	<LOQ$_m$	1.63	<LOD$_m$	1.55	2.18
DCOIT	1.78	11.55	6.50	5.02	6.70	10.42	7.46	41.14	9.18
∑Total		24.18	79.67	50.72	28.33	50.05	28.76	79.25	24.90

<LODm - below the limit of detection of the method; <LOQm - below the limit of quantification of the method for Irgarol.

TOC content (3.4%), whereas the less contaminated sediment (São Vicente 2) had one of the lowest percentage of fines (14%) and TOC (1.1%) (Table 2).

AVO is a UVF present in many sunscreens, often employed as a substitute for the endocrine-disrupting BP3. Measurable levels of this contaminant were only detected in the sediment of Rio Grande shipyard (7.8 ng g^{-1} dw). The observed concentration was similar to previously reported values in soil and sediments, for instance from coastal areas of Hawaii, (<7 ng g^{-1} dw) (Mitchelmore et al., 2019).

Benzotriazoles are high production volume chemicals used in a wide range of industrial applications; as UV stabilizers in different plastic products, as corrosion inhibitors in detergents, and antifreeze or antifogging agents in automotive fluids (Molins-Delgado et al., 2015). Benzotriazole and MeBZT are the two UV-stabilizers most frequently detected in the environment, as they are poorly volatile and only partially removed during conventional wastewater treatment because of its high polarity and poor biodegradability (Liu et al., 2012; Asimakopoulos et al., 2013; Molins-Delgado et al., 2015, 2017). In this study, only MeBZT was detected in a single sediment sample, SSES (Alemoa - 3.5 ng g^{-1}dw), at the comparatively low levels to those found by Zhang et al. (2011) in sediments from China and the USA Similarly, MeBZT was detected but not quantifiable in river sediment samples from Iguaçu watershed, also in Brazil (Mizukawa et al., 2017). In contrast, they observed quite high concentrations of BZT (<LODm – 630 ng g^{-1}dw).

Due to the use and consumption of personal care products, pharmaceuticals, beverages, and other foodstuff containing parabens as preservatives, there is a continuous introduction into the environment, and thus parabens are ubiquitous in surface water and sediments worldwide. Methylparaben (MeP) and propylparaben (PrP) predominate, reflecting the composition of paraben mixtures in common consumer products (Haman et al., 2015). Their efficiency as fungicidal and bactericidal agent combined with its low cost, likely explains why parabens are so widely used (Soni et al., 2005). Many studies reported links between paraben preservatives and adverse effects observed in aquatic organisms (Dobbins et al., 2009), and highlighted its endocrine disrupting activity at environmentally relevant concentrations (Darbre et la., 2003; Golden et al., 2005).

In the present study, 100% of the sediments contained MePB (E number E218) in concentrations from 5.8 to 69.7 ng g^{-1}dw. MeP as well as EtP (E214), PrP (E216), BuP, and BzP have been found in sediment and sewage sludge samples of the USA, Japan, and Korea at a wider concentration range, from 0.70 to 95.7 ng g^{-1}dw (Liao et al., 2013).

Fluoroquinolones (FQs) are a class of antibiotics used in human and veterinary medicine. Due to their widespread application, and considering that they are only partially metabolized in the organisms and not completely removed in wastewater treatment plants, FQs such as ciprofloxacin, are one of the most detected antibiotics in the environment (Ziarrusta et al., 2018). Ciprofloxacin inhibits microorganisms and, therefore, can represent an important risk for the environment, especially for microbial ecology (Girardi et al., 2011). In the present study, ciprofloxacin was the only FQs detected in one single site of SSES (Balsa - 9.5 ng g^{-1}dw), which is an area of ferryboat operation and a mooring pier for boats and fishing boats. Also in Brazil, in mangrove sediment from the Paciencia River, (Maranhão Island) ciprofloxacin was the only antibiotic found in three out of the six samples analyzed, but at higher concentrations (56.55–70.45 ng g^{-1}) (Neves at al., 2018). These values are far below the recently reported concentrations of this antibiotic in river sediments from Kenia (4125-1275 ng g^{-1}) (Kairigo et al., 2020). This high pollution can be explained by the lower dilution effect and the higher impact of wastewater treatment plants discharges in rivers in comparison with the sea.

Regarding antifouling booster biocides, irgarol and DCOIT were detected in 75% and 100% of the samples, respectively, and at concentrations between 1.5 and 2.2 ng g^{-1} dw, and 5.0–41.1 ng g^{-1} dw, respectively. All sampling sites are within an estuarine system under the direct influence of maritime activities. This suggests that the presence of antifouling booster biocides may be related to the large flow of vessels that may be using these compounds in antifouling paint formulations. The current use of DCOIT is well known (Abreu et al., 2020). Its low water solubility (0.0065 mg L^{-1} a 25 °C) and high log K_{oc} (2.6–4.2) suggests the preferential partition in the suspended particulate matter from the water column and sediments. Although DCOIT has a short half-life in aquatic environments (Chen and Lam, 2017; Jacobson and Willingham, 2000) its presence may be explained by the continuous inputs in the region (Abreu et al., 2020; Chen and Lam, 2017), behaves as a pseudo-persistent contaminant due to its continuous release. Diuron and irgarol are more persistent than DCOIT in the environment (half-lives of 14 and 100 days in sediment, respectively), and their moderate log K_{oc} (2.3 and 3.3, respectively) indicate partition in both water and sediment. As a consequence of its toxicity for primary producers, their use has been restricted in many countries. In a previous study in Panamá, irgarol, diuron, and DCOIT were measured at concentrations between below 0.25 and 2.8 ng g^{-1} dw; 2.4 and 14.1 ng g^{-1} dw; and 2.4 and 81.6 ng g^{-1} dw, respectively (Batista-Andrade et al., 2016b). Lower values were reported by Abreu et al. (2020) for DCOIT, diuron, and irgarol ranging from below 0.2–75 ng g^{-1}, below 0.5–9.9 ng g^{-1}, and below 0.2 ng g^{-1}, respectively.

5. Conclusions

The present study developed and validated a multiresidue method based on vortex-assisted matrix solid-phase dispersion in combination with liquid chromatography-tandem mass spectrometry for the trace determination of 59 organic contaminants of emerging concern, including pharmaceuticals, personal care products, and biocides, in sediment. This techniques combination can be considered innovative and efficient in determining organic contaminants with a wide range of physicochemical properties in solid environmental matrices. The method used solely 2 g of sediment sample and 5 mL of extraction solvent. This approach provided limits of detection and quantification in the low ng g^{-1} dw range, which are below or similar to those reported in the literature for these analytes.

The method was applied to investigate the target contaminants in marine sediment samples collected at two major ports in Brazil. The application in the environmental samples evidenced that solely 4 out of the 59 analytes of interest were found in the marine sediments. The preservative MePB and the antifouling booster biocide DCOIT were present in 100% of the samples. Irgarol was also frequently detected (75%). The sunscreen agents AVO and MeBZT, and the antibiotic ciprofloxacin were eventually detected (only in one sample each). The occurrence of these commonly detected contaminants, even at low ng g^{-1} levels, indicates the prevalent impact of the direct maritime activities and some urban inputs on the quality status of the selected coastal aquatic ecosystems of Brazil, which appears to be similar to those reported worldwide.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.129085.

References

Abreu, F.E.L., Silva, J.N.L., Castro, I.B., Fillmann, G., 2020. Are antifouling residues a matter of concern in the largest South American port? J. Hazard Mater. https://doi.org/10.1016/j.jhazmat.2020.122937.

Anastassiades, M., Lehotay, S.J., Štajnbaher, D., Schenck, F.J., 2003. Fast and easy multi-residue method employing ACN extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. J. AOAC Int. 86, 412–431.

Asimakopoulos, A.G., Ajibola, A., Kannan, K., Thomaidis, N.S., 2013. Occurrence and removal efficiencies of benzotriazoles and benzothiazoles in a wastewater treatment plant in Greece. Sci. Total Environ. 452–453, 163–171.

Barker, S.A., Long, A.R., Short, C.R., 1989. Isolation of drug residues from tissues by solid phase dispersion. J. Chromatogr. A 475, 353–361.

Batista-Andrade, J.A., Caldas, S.S., de Oliveira Arias, J.L., Castro, I.B., Fillmann, G., Primel, E.G., 2016. Antifouling booster biocides in coastal waters of Panama: first appraisal in one of the busiest shipping zones. Mar. Pollut. Bull. 112, 415–419.

Batista-Andrade, J.A., Caldas, S.S., Batista, R.M., Castro, I.B., Fillmann, G., Primel, E.G., 2018. From TBT to booster biocides: levels and impacts of antifouling along coastal areas of Panama. Environ. Pollut. 234, 243–252.

Caldas, S.S., Bolzan, C.M., de Menezes, E.J., Escarrone, A.L.V., Martins, C.d.M.G., Bianchini, A., Primel, E.G., 2013. A vortex-assisted MSPD method for the extraction of pesticide residues from fish liver and crab hepatopancreas with determination by GC–MS. Talanta 112, 63–68.

Caldas, S.S., Rombaldi, C., De Oliveira Arias, J.L., Marube, L.C., Primel, E.G., 2016. Multi-residue method for determination of 58 pesticides, pharmaceuticals and personal care products in water using solvent demulsification dispersive liquid-liquid microextraction combined with liquid chromatography-tandem mass spectrometry. Talanta 146, 676–688.

Caldas, S.S., Soares, B.M., Abreu, F., Castro, Í.B., Fillmann, G., Primel, E.G., 2018. Antifouling booster biocide extraction from marine sediments: a fast and simple method based on vortex-assisted matrix solid-phase extraction. Environ. Sci. Pollut. Control Ser. 25, 7553–7565.

Čelić, M., Gros, M., Farré, M., Barceló, D., Petrović, M., 2018. Pharmaceuticals as Chemical Markers of Wastewater Contamination in the Vulnerable Area of the Ebro Delta (Spain). Science of The Total Environment.

Cerqueira, M.B., Soares, K.L., Caldas, S.S., Primel, E.G., 2018. Sample as solid support in MSPD: a new possibility for determination of pharmaceuticals, personal care and degradation products in sewage sludge. Chemosphere 211, 875–883.

Chen, L., Lam, J.C.W., 2017. SeaNine 211 as antifouling biocide: a coastal pollutant of emerging concern. J. Environ. Sci. 61, 68–79.

Crommentuijn, T., Sijm, D., Bruijn, J. De, Leeuwen, K. Van, Plassche, E. Van De, 2000. Maximum Permissible and Negligible Concentrations for Some Organic Substances and Pesticides, pp. 297–312.

Darbre, P., Byford, J., Shaw, L., Hall, S., Coldham, N., Pope, G., Sauer, M., 2003. Oestrogenic activity of benzylparaben. J. Appl. Toxicol. 23, 43–51.

Dobbins, L.L., Usenko, S., Brain, R.A., Brooks, B.W., 2009. Probabilistic ecological hazard assessment of parabens using Daphnia magna and Daphnia magna and Pimephales promelas. Environ. Toxicol. Chem. 28, 2744–2753.

Economou, A., Botitsi, H., Antoniou, S., Tsipi, D., 2009. Determination of multi-class pesticides in wines by solid-phase extraction and liquid chromatography-tandem mass spectrometry. J. Chromatogr., A 1216, 5856–5867.

Gago-Ferrero, P., Díaz-Cruz, M.S., Barceló, D., 2011. Fast pressurized liquid extraction with in-cell purification and analysis by liquid chromatography tandem mass spectrometry for the determination of UV filters and their degradation products in sediments. Anal. Bioanal. Chem. 400, 2195–2204.

Gago-Ferrero, P., Díaz-Cruz, M.S., Barceló, D., 2013a. Multi-residue method for trace level determination of UV filters in fish based on pressurized liquid extraction and liquid chromatography–quadrupole-linear ion trap-mass spectrometry. J. Chromatogr. A 1286, 93–101.

Gago-Ferrero, P., Mastroianni, N., Díaz-Cruz, M.S., Barceló, D., 2013b. Fully automated determination of nine ultraviolet filters and transformation products in natural waters and wastewaters by on-line solid phase extraction–liquid chromatography–tandem mass spectrometry. J. Chromatogr., A 1294, 106–116.

Girardi, C., Greve, J., Lamshöft, M., Fetzer, I., Miltner, A., Schäffer, A., Kästner, M., 2011. Biodegradation of ciprofloxacin in water and soil and its effects on the microbial communities. J. Hazard Mater. 198, 22–30.

Golden, R., Gandy, J., Vollmer, G., 2005. A review of the endocrine activity of parabens and implications for potential risks to human health. CRC Crit. Rev. Toxicol. 35, 435–458.

Gray, J.S., Elliott, M., 2014. Ecology of Marine Sediments. Oxford University Press.

Haman, C., Dauchy, X., Rosin, C., Munoz, J.F., 2015. Occurrence, fate and behavior of parabens in aquatic environments: a review. Water Res. 68, 1–11.

INMETRO, 2018. Orientação sobre validação de métodos analíticos DOQ-CGCRE-008, Revisão em julho de 2018.

Jacobson, A.H., Willingham, G.L., 2000. Sea-nine antifoulant: an environmentally acceptable alternative to organotin antifoulants. Sci. Total Environ. 258, 103–110.

Kairigo, P., Ngumba, E., Sundberg, L., Gachanja, A., Tuhkanen, T., 2020. Contamination of surface water and river sediments by antibiotic and antiretroviral drug cocktails in low and middle-income countries: occurrence, risk and mitigation strategies. Water 12, 1376.

Köck-Schulmeyer, M., Postigo, C., Farré, M., Barceló, D., de Alda, M.L., 2019. Medium to highly polar pesticides in seawater: analysis and fate in coastal areas of Catalonia (NE Spain). Chemosphere 215, 515–523.

Kristensen, E., Andersen, F., 1987. Determination of organic carbon in marine sediments: a comparison of two CHN-analyzer methods. J. Exp. Mar. Biol. Ecol. 109, 15–23.

Kurz, M.H., Batista, J.L.d.S., de Oliveira, L.G., Hoff, R., Martins, M.L., Gonçalves, F.F., 2019. Clean-up procedure development and method validation for pesticide residues analysis in carrots. Food Analytical Methods 12, 282–292.

Liu, Y.S., Ying, G.G., Shareef, A., Kookana, R.S., 2012. Occurrence and removal of benzotriazoles and ultraviolet filters in a municipal wastewater treatment plant. Environ 165, 225–232.

Martins, M.L., Rizzetti, T.M., Kemmerich, M., Saibt, N., Prestes, O.D., Adaime, M.B., Zanella, R., 2016. Dilution standard addition calibration: a practical calibration strategy for multi-residue organic compounds determination. J. Chromatogr. A 1460, 84–91.

Martins, S.E., Fillmann, G., Lillicrap, A., Thomas, K.V., 2018. Ecotoxicity of organic and organo-metallic antifouling co-biocides and implications for environmental hazard and risk assessments in aquatic ecosystems. Biofouling 34, 34–52.

Mitchelmore, C.L., He, K., Gonsior, M., Hain, E., Heyes, A., Clark, C., Younger, R., Schmitt-Kopplin, P., Feerick, A., Conway, A., 2019. Occurrence and distribution of UV-filters and other anthropogenic contaminants in coastal surface water, sediment, and coral tissue from Hawaii. Sci. Total Environ.

Mizukawa, A., Molins-Delgado, D., de Azevedo, J.C.R., Fernandes, C.V.S., Díaz-Cruz, S., Barceló, D., 2017. Sediments as a sink for UV filters and benzotriazoles: the case study of Upper Iguaçu watershed, Curitiba (Brazil). Environ. Sci. Pollut. Res. 24, 18284–18294.

Molins-Delgado, D., Díaz-Cruz, M.S., Barceló, D., 2015. Removal of polar UV stabilizers in biological wastewater treatments and ecotoxicological implications. Chemosphere 119 (874), S51–S57.

Molins-Delgado, D., Távora, J., Díaz-Cruz, M.S., Barceló, D., 2017. UV filters and benzotriazoles in urban aquatic ecosystems: the footprint of daily use products. Sci. Total 877 Environ. 601– 602, 975–986.

Molins-Delgado, D., del Mar Olmo-Campos, M., Valeta-Juan, G., Pleguezuelos-Hernández, V., Barceló, D., Díaz-Cruz, M.S., 2018a. Determination of UV filters in human breast milk using turbulent flow chromatography and babies' daily intake estimation. Environ. Res. 161, 532–539.

Molins-Delgado, D., Muñoz, R., Nogueira, S., Alonso, M.B., Torres, J.P., Malm, O., Ziolli, R.L., Hauser-Davis, R.A., Eljarrat, E., Barceló, D., 2018b. Occurrence of organic UV filters and metabolites in lebranche mullet (Mugil liza) from Brazil. Sci. Total Environ. 618, 451–459.

Neves, M., Silva, G., Brito, N., Araújo, K., Marquesa, E., Silva, l., 2018. Aqueous ultrasound-assisted extraction for the determination of fluoroquinolones in mangrove sediment by high-performance liquid chromatography and fluorescence detector. J. Braz. Chem. Soc. 29, 1 São Paulo Jan. 2018.

SANTE, 2017. Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticide Residues and Analysis in Food and Feed.

Serra-Roig, M.P., Jurado, A., Díaz-Cruz, M.S., Vázquez-Suñé, E., Pujades, E., Barceló, D., 2016. Occurrence, fate and risk assessment of personal care products in river–groundwater interface. Sci. Total Environ. 568, 829–837.

Soares, K.L., Cerqueira, M.B.R., Caldas, S.S., Primel, E.G., 2017. Evaluation of alternative environmentally friendly matrix solid phase dispersion solid supports for the simultaneous extraction of 15 pesticides of different chemical classes from drinking water treatment sludge. Chemosphere 182, 547–554.

Soni, M., Carabin, I., Burdock, G., 2005. Safety assessment of esters of p-hydroxybenzoic acid (parabens). Food Chem. Toxicol. 43, 985–1015.

Thomas, K.V., Brooks, S., 2010. The environmental fate and effects of antifouling paint biocides. Biofouling 26, 73–88.

Vieira, A.A., Caldas, S.S., Kupski, L., Tavella, R.A., Primel, E.G., 2018. Extraction of chlorothalonil, dichlofluanid, DCOIT, and TCMTB from fish tissues employing the vortex assisted matrix solid-phase dispersion. Microchem. J. 143, 92–98.

Zhang, Z., Ren, N., Li, Y.F., Kunisue, T., Gao, D., Kannan, K., 2011. Determination of benzotriazole and benzophenone UV filters in sediment and sewage sludge. Environ. Sci. Technol. 45, 3909–3916. https://doi.org/10.1021/es2004057.

Ziarrusta, H., Mijangos, L., Irazola, M., Prieto, A., Etxebarria, N., Anakabe, E., Olivares, M., Zuloaga, O., 2018. Ciprofloxacin by-products in seawater environment in the presence and absence of gilt-head bream. Chemosphere 197, 560–568. https://doi.org/10.1016/j.chemosphere.2018.01.069.

3.2.3 Publication #3 Supplementary information

Rapid and cost-effective multiresidue analysis of pharmaceuticals, personal care products, and antifouling booster biocides in marine sediments using matrix solid phase dispersion.

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https://doi.org/10.1016/j.chemosphere.2020.129085

Content:
Table 1S: Analyte name, respective acronym, class, logk$_{ow}$ (octanol-water partition constant), solubility in water at 25 ºC (S) (from ChemSpider; DrugBank; Pubchem).
Figure 1S: Total ion chromatogram of the target compounds monitored in positive ionization mode (ESI +).
Figure 2S: Total chromatogram of the target compounds monitored in negative ionization mode (ESI -).

TABLE 1S

	Analyte	Acronym	Class	Log Kow	S (mg mL^{-1})	Corresponding IS
UV filters	2-hydroxy-4-methoxybenzophenone	BP3	Benzophenone	3.79	0.128	BP3-d5
	2,4-dihydroxybenzophenone	BP1	Benzophenone	3.17	0.39	BP3-d5
	2,2’,4,4’-tetrahydroxybenzophenone	BP2	Benzophenone	2.78	0.09842	BP3-d5
	5-benzoyl-4-hydroxy-2-methoxybenzene sulfonic acid	BP4	Benzophenone	0.39	0.214	Gemfibrozil-d6
	4-hydroxybenzophenone	4HB	Benzophenone	3.82	N.f	BP3-d5
	4,4′-dihydroxybenzophenone	4DHB	Benzophenone	3.02	0.41	BP3-d5
	2,2’-dihydroxy-4-methoxybenzophenone	DHMB	Benzophenone	2.55	0.60	BP3-d5
	1-(4-tert-butylphenyl)-3-(4-methoxyphenyl)propane-1,3-dione	AVO	Benzophenone	N.f	N.f	BP3-d5
UV filters	3-(4-methylbenzilidene)camphor	4MBC	Camphor derivative	4.50	0.00015	4MBC-d4
	2-ethylhexyltrans-4-methoxycinnamate	EHMC	Cinnamate derivative	4.95	0.00510	4MBC-d4
	ethyl-4- aminobenzoate	EtPABA	*p*-aminobenzoic acid derivatives	1.86	131	4MBC-d4
	1,2,3-benzotriazole	BZT	Benzotriazoles	1.23	20	BZT-d4
	4-methyl-1H-benzotriazole	MeBZT	Benzotriazoles	1.89	5	BZT-d4
	5,6-dimethyl-1H-benzotriazole monohydrate	DMBZT	Benzotriazoles	2.06	N.f	BZT-d4
	2-(2-benzotriazolyl-*p*-cresol	UVP	Benzotriazole derivates	3.24	0.01359	BZT-d4
						BePB-d4

	Analyte	Acronym	Class	Log Kow	S (mg mL^{-1})	Corresponding IS
Parabens	benzyl-4-hydroxybenzoate	BePB	Parabens	N.f	N.f	
	butyl-4-hydroxybenzoate	BuPB	Parabens	N.f	N.f	BePB-d4
	propyl-4-hydroxybenzoate	PrBP	Parabens	N.f	N.f	BePB-d4
	methyl-4-hydroxybenzoate	MePB	Parabens	N.f	N.f	BePB-d4
Pharmaceuticals	9-fluoro-5-methyl-1-oxo-1,5,6,7-tetrahydropyrido[3,2,1-ij]quinoline-2-carboxylic aci	FLU	Fluorquinolones	N.f	2.19	Flumequine-13C
	9-fluoro-3-methyl-10(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid	ofloxacin	Fluoroquinolones	1.41	0.1	Sulfametazine-d4
	1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid	ciprofloxacin	Fluoroquinolones	N.f	N.f	Trimethoprim-d3
	1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid	nalidixic acid	Quinolones	N.f	0.0514	Flumequine-13C
Pharmaceuticals	5-ethyl-8-oxo-5,8-dihydro-[1,3]dioxolo[4,5-g]quinoline-7-carboxylic acid	oxolinic acid	Quinolones	N.f	0.077	Flumequine-13C
	4-oxo-4-[4-(1,3-thiazol-2-ylsulfamoyl)anilino]butanoic acid	succynil-Sulfatiazole	Sulfonamides	N.f	N.f	Sulfametazine-d4
	4-amino-N-pyrimidin-2-ylbenzenesulfonamide	SDZ	Sulfonamides	N.f	0.202 (at 20 °C)	Sulfametazine-d4
	N-[4-(pyrimidin-2-	acSDZ	Sulfonamides	N.f	>0.046	Sulfametazine-d4

	Analyte	Acronym	Class	Log Kow	S (mg mL^{-1})	Corresponding IS
Pharmaceuticals	ylsulfamoyl)phenyl]acetamide					
	4-amino-N-(4-methylpyrimidin-2-yl)benzenesulfonamide	SMR	Sulfonamides	N.f	N.f	Sulfametazine-d4
	N-[4-(4-methylpyrimidin-2-yl)sulfamoyl]phenyl]acetamide	acSMR	Sulfonamides	N.f	0.61 (at 37 °C)	Sulfametazine-d4
	4-amino-N-(6-mathoxypyridazin-3-yl)benzenesulfonamide	SMZ	Sulfonamides	N.f	N.f	Sulfametazine-d4
	N-[4-[(4,6-dimethylpyrimidin-2-yl)sulfamoyl]phenyl]acetamide	acSMZ	Sulfonamides	N.f	N.f	Sulfametazine-d4
	4-amino-N-(5-methyl1,2-oxazol-3-yl)benzenesulfonamide	SMX	Sulfonamides	N.f	0.268	Sulfametazine-d4
	4-acetylamino-N-(5-methyl-3-isoxazoyl)benzenesulfonamide	acSMX	Sulfonamides	N.f	N.f	Sulfametazine-d4
	4-amino-N-(6-methoxypyridazin-3-yl)benzenesulfonamide	SMPZ	Sulfonamides	1.68	0.0075	Sulfametazine-d4
Pharmaceuticals	4-amino-N-pyridin-2-ylbenzenesulfonamide	SPY	Sulfonamides	0.05	0.373	Sulfametazine-d4
	N-[4-(pyridine-2-ylsulfamoyl)phenyl]acetamide	acSPY	Sulfonamides	N.f	N.f	Sulfametazine-d4
	4-amino-N-quinoxalin-2-ylbenzenesulfonamide	SQX	Sulfonamides	N.f	0.343	Sulfametazine-d4
	4-amino-N-(1,3-thiazol-2-yl)benzenesulfonamide	STZ	Sulfonamides	0.91	0.4	Sulfametazine-d4
	4-amino-N-(2,6-dimethylpyrimidin-4-	sulfisomidin	Sulfonamides	N.f	14	Sulfametazine-d4

	Analyte	Acronym	Class	Log Kow	S (mg mL^{-1})	Corresponding IS
Pharmaceuticals	yl)benzenesulfonamide					
	4-amino-N-(2,6-dimethoxypyrimidin-4-yl)benzenesulfonamide	SDM	Sulfonamides	0.16	13.3	Sulfametazine-d4
	5-[(3,4,5-trimethoxyphenyl)methyl]pyrimidine-2,4-diamine	trimethoprim	Lipid regulators	4.77	0.0278	Trimetoprim-d3
	5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid	gemfibrozil	Lipid regulators	N.f	0.0213	Gemfibrozil-d6
	2-(2,3-dimethylanilino)benzoic acid	mefenamic acid	Analgesics	N.f	0.02 (at 30 °C)	Mefenamic acid -d3
	(2S)-2-(6-methoxynaphthalen-2-yl)propanoic acid	naproxen	Anti-inflammatorys	3.18	0.0159	Naproxen-d3
	2-[4-(2-methylpropyl)phenyl]propanoic acid	IBU	Anti-epileptics	2.45	0.0177	IBU-d3
	2-(3-benzoylphenyl)propanoic acid	ketoprofen	Analgesic	N.f	N.f	BP3-d5
	diclofenac soduim salt	diclofenac	Anti-depressants	N.f	N.f	Diclofenac-d4
	N-(4-hydroxyphenyl)acetamide	acetaminophen	Anti-ammatorys	-0.39	0.0283	Trimetoprim-d3
	benzo[b][1]benzazepine-11-carboxamide	CBZ	Anti-epileptics	-0.07	21.6	Carbamazepine-d10
	3-oxa-11-azatetracyclo[10.4.0.02,4.05,10]hexadeca-1(16),5,7,9,12,14-hexaene-11-carboxamide	CBZ-10,11-epoxy	Anti-epileptics	0.28	30 (at 20 °C)	Carbamazepine-d10

	Analyte	Acronym	Class	Log Kow	S (mg mL^{-1})	Corresponding IS
	2-[4-[2-hydroxy-3-(propan-2-ylamino)proproxy]phenyl]acetamide	atenolol	B-blocker	N.f	N.f	Trimethoprim-d3
	3-phenyl-3-[4-trifluoromethyl)phenoxy]propan-1-amine	Nor-FXT	Anti-depressive	N.f	N.f	Trimethoprim-d3
	N-desmethylvenlafaxine	N-desVFX	Anti-depressive	N.f	N.f	Trimethoprim-d3
	2-hydroxybenzoic acid	Salicylic acid	Anti-septic	N.f	N.f	Salicylic acid-d6
Booster biocides	3-(3,4-dichlorophenyl)-1,1-dimethylurea	diuron	Triazines	2.85	36.4	BP3-d5
	2-N-tert-butyl-4-N-cyclopropyl-6-methylsulfanyl-1,3,5-triazine-2,4-diamine	irgarol	Triazines	3.95	7	BZT-d4
	4,5-dichloro-2-n-octyl-3-(2H)-isothiazolin-3-one	DCOIT	Organochlorine	2.85	0.0065	BP3-d5
	N-[dichloro(fluoro)methyl]sulfanyl-N-(dimethylsulfamoyl)aniline	dichlofluanid	Organochlorine	3.70	0.0060	Carbamazepine-d10

FIGURE 1S

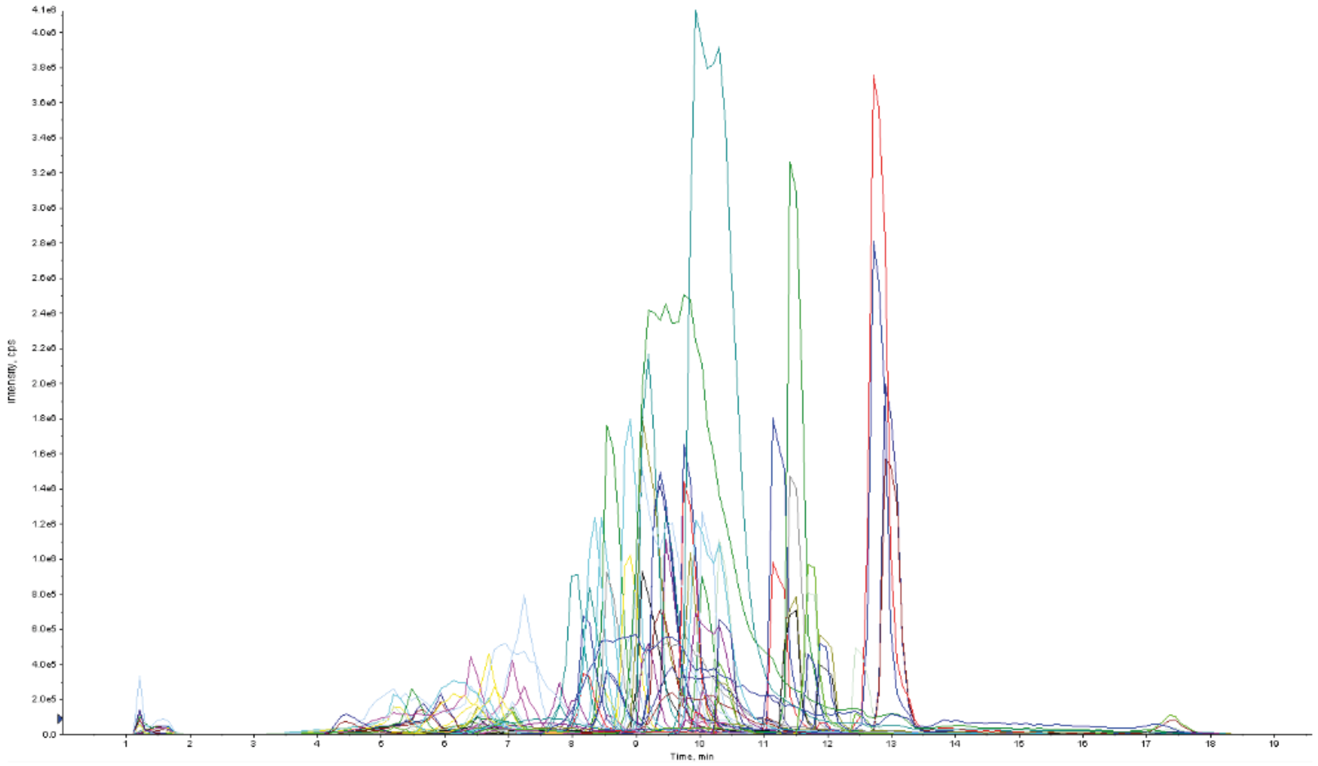
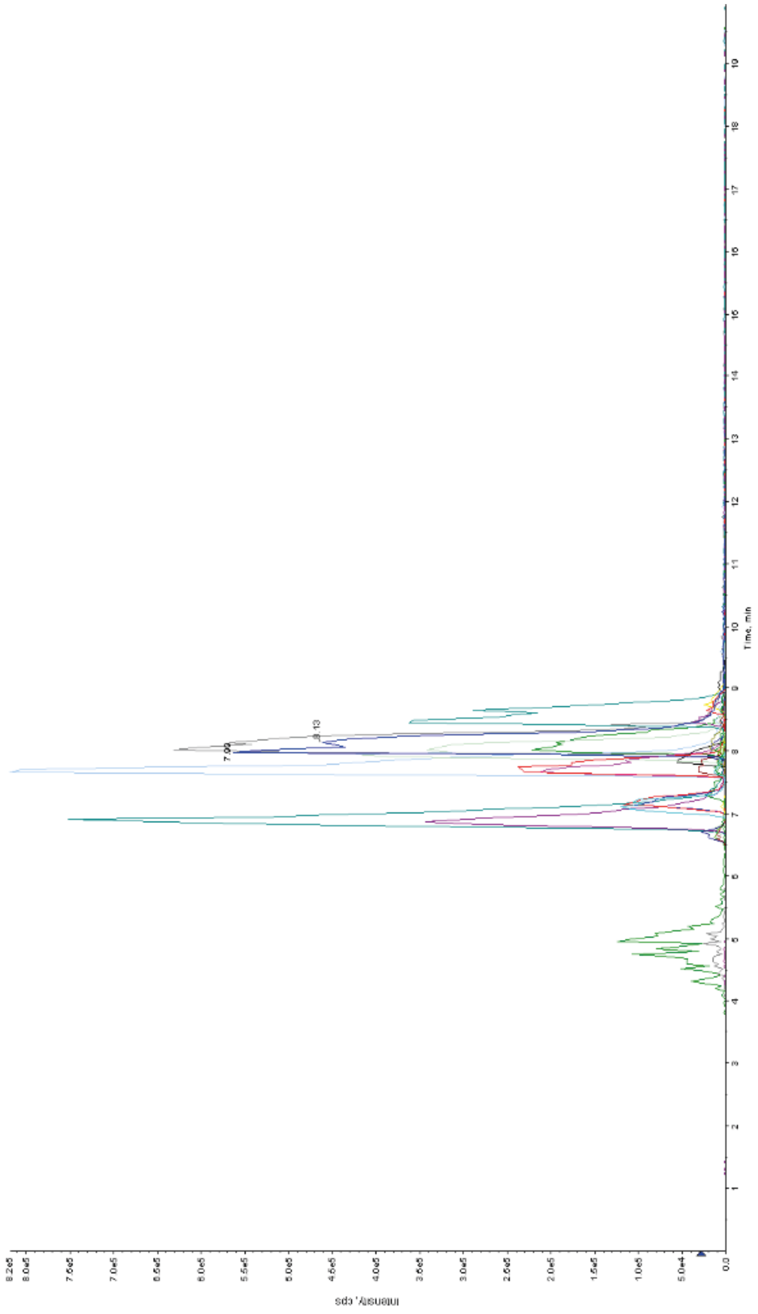


FIGURE 2S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4.0e5
3.5e5
3.0e5
2.5e5
2.0e5
1.5e5
1.0e5
5.0e4
0.0
Time, min
1
2
3
4
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16
17
18
19
7.99
8.13

3.2.4 Publication #4

Distribution in marine fish and EDI estimation of contaminants of emerging concern by vortex-assisted matrix solid-phase dispersion and HPLC-MS/MS

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Distribution in marine fish and EDI estimation of contaminants of emerging concern by vortex-assisted matrix solid-phase dispersion and HPLC-MS/MS

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Abstract

Due to their persistence or continuous discharge, toxic substances are present in the aquatic environment, and can bioaccumulate and biomagnify in the food web, generating a significant ecological risk and a threat to human health. The present study assess the occurrence and tissue (muscle, liver, stomach and gills) distribution of 59 anthropogenic contaminants of emerging concern (CECs) in marine fish tissues from Brazil. Thus, a simpler and faster analytical methodology based on vortex-assisted matrix solid-phase dispersion (VA-MSPD) and LC-MS/MS was developed and validated. Limits of quantification ranged from 3.31 to 114 ng g^{-1} dw with recovery rates between 60 and 140% and RSD below 20%. The UV filters 4HB (benzophenone-3 metabolite) and Et-PABA, and the antibacterial salicylic acid were frequently accumulated in muscle and liver at concentrations between 39.5 and 21.0 ngg^{-1} dw. The determined concentrations resulted to be lower than the tolerable daily intake recommended by the EFSA.

Keywords: Biocides; EDI; fish; pharmaceuticals; personal care products; VA-MSPD.

Highlights:

- VA-MSPD was employed for the extraction of PPCPs and biocides from fish organs.
- The developed method was efficient, fast, sensitive and environmentally friendly.
- The method allowed to identify distribution patterns of the selected CECs in fish.
- Et-PABA, 4HB, and salicylic acid were frequently accumulated in muscle and liver.
- EDI of salicylic acid was lower than tolerable daily intake recommended by EFSA.

Introduction

Classified as contaminants of emerging concern (CECs), pharmaceuticals and personal care products (PPCPs) have been used worldwide for decades and, consequently, ending up in the aquatic environment due to their poor removal in current wastewater treatments and limited natural environmental degradation (Golovko et al., 2021). PPCPs' group comprises several classes of organic substances including ultraviolet filters (UVFs), fragrances, preservatives, analgesics, anti-inflammatories, hormones, antibiotics, their metabolites, and other degradation products (Gopal et al., 2020). In general, these compounds, as well as their metabolites and other transformation products, are polar organic compounds, usually identified in the marine environment (Chen et al., 2021).

Antifouling booster biocides (ABBs) are incorporated into antifouling paint formulations to prevent marine biofouling, constituting another important group of organic contaminants commonly found in the marine environment (Abreu et al., 2020; Batista-Andrade et al., 2018).

Due to their widespread use, PPCPs and ABBs have been extensively studied in different environmental compartments, namely water (Downs et al., 2021, Dominguez-Morueco et al., 2020; Batista-Andrade et al., 2016; Liu et al., 2019), sediment and sludge (Abreu et al., 2020; Cerqueira et al., 2018; Soares et al., 2021) and, less frequently, in biological samples (Diaz-Cruz et al., 2019; Mitchelmore et al., 2019; Molins-Delgado et al., 2018). These organic contaminants are present at trace levels in the environment (µg L^{-1} to ng L^{-1} for water and ng g^{-1} for sediment and biota) (Caldas et al., 2016), requiring extremely sensitive analytical techniques to detect them with accuracy, precision and, preferably, at a low cost. In fish, previous works have reported PPCPs and ABBs in species from Brazil (Martins et al., 2020; Molins-Delgado et al., 2018; Vieira et al., 2018), reporting levels within the range 0.33-41.9 ng/g dry weight (dw).

However, extraction techniques applied in the determination of CECs involve a large amount of sample, a high volume of organic solvents, and time-consuming extraction procedures. Although relatively fast, pressurized liquid extraction (PLE), for instance, needs 1-10 g of sample, up to 50 mL of organic solvent, and specific equipment (Gago-Ferrero et al., 2013; Molins-Delgado et al., 2018). In this sense, vortex-assisted matrix solid-phase dispersion (VA-MSPD) is a technique currently gaining prominence because of the several advantages provided over other usually used extraction techniques. VA-MSPD, needs less sample mass and a small volume of solvents, reducing waste generation and cost. In addition, this technique replaces the elution step carried out in solid-phase extraction (SPE) by adding the solvent through polypropylene tubes (Souza Caldas et al., 2013). This minimizes the issues caused by the compactness of the cartridges, making the technique simpler, cheaper, and more robust (Sebastià et al., 2010; Souza Caldas et al., 2013). So far, it has been successfully applied to the determination of several organic contaminants in sediment (Caldas et al., 2018), sewage sludge (Cerqueira et al., 2018; Soares et al., 2017), biota (Duarte et al., 2013; Hertzog et al., 2015), and food (dos Santos et al., 2019), demonstrating its robustness, versatility, and accuracy.

The combination of the miniaturization provided by the VA-MSPD with the sensitivity and selectivity achieved by liquid chromatography tandem mass spectrometry (LC-MS/MS) makes this analytical methodology a promising tool for determining organic contaminants in environmental solid matrices (Soares et al., 2021). The present work combined these two techniques to optimize and develop a new analytical methodology to evaluate CECs' occurrence in fish tissues. The target CECs were selected based on their environmental occurrence, bioaccumulative potential, and endocrine disrupting capacity. The suitability of the developed method was tested by determining 59 anthropogenic CECs in muscle, liver, stomach, and gill tissues from *Micropogonias furnieri* fish *specimens* collected in Southern Brazil. The variable distribution of CECs within tissues helped to evaluate the potential human risk by diet through fish consumption, expressed in terms of estimated daily intake (EDI). Thus, this work provides a new routine methodology and novel occurrence data in marine fish that could help in the revision of regulations aimed at the protection of the marine environment.

1. Experimental section

1.1 Chemicals, materials, and standard solutions

Information about the standards used is provided in the **Supplementary Information (Section S1)**. Acronyms of all compounds are showin in Section S1, but also in Table 1.

Stock solutions of individual standards (1000 mg L^{-1}) and an intermediate stock solution of a mix of standards (1 mg L^{-1}) were prepared in MeOH. Fresh standards working solutions were prepared daily at appropriate concentrations. All solutions were preserved in the dark at -20 °C.

1.2 Sample collection and characterization

Fish samples of *Micropogonias furnieri* (mouth croaker) were collected with trawl in December 2017 in the Patos Lagoon estuary at a site of intense ship and boat traffic and under the influence of the Rio Grande harbour (Rio Grande City, RS, Brazil). Four specimens (11.25 ± 1.99 cm) were dissected and tissues (muscle, liver, stomach, and gills) were individually frozen. Tissues were freeze-dried and stored at -20 ºC in the dark until analysis. *Micropogonias furnieri* is a well-known species due to its wide geographic distribution in the western Atlantic, that presents estuarine-dependent behavior, living locally in the early stages of life, growth, and feeding (Mulato et al., 2015). Its eating habits are benthic; it is a carnivorous fish that feeds on crustaceans, bivalve mollusks (mussels and clams), plankton, and juvenile fish such as anchovy. In this study, the muscle was used to optimize sample preparation and validation steps. The other organs were exclusively used for CECs' determination.

1.3 VA-MSPD

The VA-MSPD extraction procedure was optimized regarding the two main variables of the technique, solvent, and solid support. In the optimized procedure, an aliquot of 0.5 g of freeze-dried muscle tissue was spiked with 100 µL of a solution containing the isotopically labeled surrogate standards, left for 30 min to equilibrate, and then blended in a mortar and pestle for 5 min with 2 g of C18 as solid support. C18 was used in the initial optimization tests as it is one of the most used supports in sample preparation. The blended mixture was transferred to a polypropylene tube and 5 mL of the organic solvent were added, vortexed for 1 min, and centrifuged at 4000 rpm for 10 min. Before injection, the internal standard mix solution (50 ng mL^{-1}) was added. Finally, 20 µL were injected into the chromatographic system, as shown in Figure 1.

Analyses were performed in triplicate and each replica was measured 3 times. Freeze-dried fish tissues used in all analyses were selected as the matrices routinely used as blanks, which were previously analyzed for the target analytes.

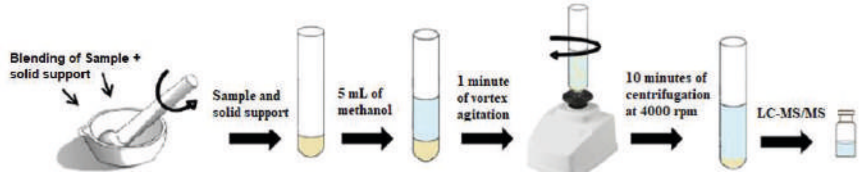

Figure 1 - VA-MSPD extraction procedure of 59 CECs from 0.5 g freeze-dried fish muscle.

1.4 HPLC-MS/MS analysis

An LC Symbiosis™ Pico instrument from Spark Holland (Emmen, The Netherlands) equipped with a 4000 Q TRAP™ hybrid quadrupole-linear ion-trap mass spectrometer (Applied Biosystems-Sciex; Foster City, Ca, USA), a Purosher® STAR® HR R-18 (50 mm × 2.0 mm, 5 µm) (Merck) column and an Alias autosampler was employed for the LC-MS/MS analysis. Relevant parameters of the analysis are presented in Table 1. Analytes were performed in electrospray ionization under positive (ESI+) and negative (ESI-) modes. The selected reaction monitoring (SRM) mode was tuned to record the two more intense transitions for quantification and confirmation purposes (Commission Decision 2002/657/EC). The Analyst Software V 1.4.2 (Applied Biosystems) was used for data processing.

For the chromatographic separation, the elution was achieved at a flow rate of 0.3 mL min^{-1}. For the analysis in ESI+, a mixture of HPLC-grade water and MeOH, both 0.1% in formic acid, was used. In the ESI- mode, the mobile phase consisted of the same binary solvent combination containing 5 mM of ammonium acetate instead of formic acid. The injection volume was set to 20 µL in both modes and the gradient of the mobile phase was explained elsewhere (Soares et al., 2021).

Table 1 - Selected compounds, chromatographic retention time (t_R), the two most intense MS/MS transitions, MS/MS parameters (Cone voltage (CV), collision energy (CE), collision cell exit potential (CxP), precursor ion, and labeled internal standard (IS) used for each compound. *transition selected for quantification.

	Compounds	**t_R (min)**	**MS/MS Transition (m/z)**	**CV (V)**	**CE (eV)**	**CxP (eV)**	**Precursor Ion**	**Corresponding IS**
UV filters	BP3	11.18	229>151*	40	25	12	$[M+H]^+$	BP3-d5
			229>105	40	27	16		
	BP1	10.05	215>137*	40	27	10	$[M+H]^+$	BP3-d5
			215>105	40	29	6		
	BP2	8.89	247>137*	46	25	8	$[M+H]^+$	BP3-d5
			247>109	46	45	8		
	BP4	6.60	307>227*	-50	-34	-15	$[M-H]^-$	GMZ-d6
			307>211	-50	-46	-9		
	4HB	9.49	199>121*	40	25	8	$[M+H]^+$	BP3-d5
			199>105	40	27	8		
	4DHB	8.47	215>121*	45	27	8	$[M+H]^+$	BP3-d5
			215>93	45	45	60		
	DHMB	10.33	245>121*	43	27	12	$[M+H]^+$	BP3-d5
			245>151	43	29	8		
	AVO	12.94	311>135*	40	25	15	$[M+H]^+$	BP3-d5
			311>161	40	25	15		
	4MBC	9.85	255>105*	61	41	6	$[M+H]^+$	4MBC-d4
			255>212	61	29	14		
	EHMC	12.75	291>179*	51	13	4	$[M+H]^+$	4MBC-d4
			291>161	51	25	10		
	Et-PABA	8.57	166>138*	41	20	10	$[M+H]^+$	4MBC-d4
			166>120	41	25	28		
	BZT	7.24	120>65*	56	31	4	$[M+H]^+$	BZT-d4
			120>92	56	25	16		
	MeBZT	8.27	134>79*	46	29	10	$[M+H]^+$	BZT-d4
			134>95	46	35	14		
	DMBZT	8.89	148>77*	56	39	2	$[M+H]^+$	BZT-d4
			148>93	56	27	6		
	UVP	12.47	226>120*	46	25	10	$[M+H]^+$	BZT-d4
			226>107	46	19	18		
Preservatives	BePB	7.99	227>92*	-65	-26	-9	$[M-H]^-$	BePB-d4
			227>136	-65	-22	-1		
	BuPB	8.03	193>137*	-55	-22	-5	$[M-H]^-$	BePB-d4
			193>92	-55	-34	-13		
	PrBP	7.68	179>92*	-60	-30	-13	$[M-H]^-$	BePB-d4
			179>137	-60	-24	-5		
	MePB	6.90	151>92*	-45	-28	-7	$[M-H]^-$	BePB-d4
			151>136	-45	-20	-9		
Pharmaceuticals	FLU	9.37	262>244*	51	29	14	$[M+H]^+$	FLU-13C
			262>202	51	45	10		
	OFX	7.63	362>318*	56	29	18	$[M+H]^+$	SMZ-d4
			362>261	56	41	18		
	CPX	6.80	332>288*	91	27	14	$[M+H]^+$	TMP-d3

	Compounds	tR (min)	MS/MS Transition (m/z)	CV (V)	CE (eV)	CxP (eV)	Precursor Ion	Corresponding IS
Pharmaceuticals			332>231	91	51	4		
	NDX	9.13	233>215*	61	19	14	$[M+H]^+$	FLU-13C
			233>187	61	37	12		
	OXL	9.37	262>244*	36	25	12	$[M+H]^+$	FLU-13C
			262>216	36	41	16		
	S-STZ	6.68	356>256*	71	25	16	$[M+H]^+$	SMZ-d4
			356>192	71	33	16		
	SDZ	5.23	251>156*	46	27	10	$[M+H]^+$	SMZ-d4
			251>108	46	30	8		
	acSDZ	6.42	293>134*	65	30	12	$[M+H]^+$	SMZ-d4
			293>198	65	30	12		
	SMR	5.93	265>156*	61	27	8	$[M+H]^+$	SMZ-d4
			265>92	61	47	6		
	acSMR	6.77	307>134*	60	35	8	$[M+H]^+$	SMZ-d4
			307>110	60	35	8		
	SMZ	8.73	279>59*	36	27	4	$[M+H]^+$	SMZ-d4
			279>87	36	19	14		
	acSMZ	7.04	321>134*	86	35	4	$[M+H]^+$	SMZ-d4
			321>124	86	35	4		
	SMX	7.06	254>108*	56	27	10	$[M+H]^+$	SMZ-d4
			254>156	56	25	10		
	acSMX	7.80	296>198*	60	30	10	$[M+H]^+$	SMZ-d4
			296>134	60	30	10		
	SMPZ	6.68	281>126*	66	27	12	$[M+H]^+$	SMZ-d4

	Compounds	tR (min)	MS/MS Transition (m/z)	CV (V)	CE (eV)	CxP (eV)	Precursor Ion	Corresponding IS
Pharmaceuticals			281>156	66	27	14		
	SPY	5.65	250>156*	51	28	12	$[M+H]^+$	SMZ-d4
			250>92	51	31	6		
	acSPY	6.52	292>134*	70	30	8	$[M+H]^+$	SMZ-d4
			292>198	70	30	8		
	SQX	8.20	301>156*	76	25	10	$[M+H]^+$	SMZ-d4
			301>92	76	47	12		
	STZ	5.49	256>156*	40	25	14	$[M+H]^+$	SMZ-d4
			256>92	40	25	10		
	SMD	6.42	279>124*	76	33	8	$[M+H]^+$	SMZ-d4
			279>186	76	23	14		
	SDM	8.03	311>356*	76	31	8	$[M+H]^+$	SMZ-d4
			311>92	76	31	6		
	TMP	6.15	291>230*	76	33	20	$[M+H]^+$	TMP-d3
			291>261	76	35	12		
	GMZ	8.47	249>121*	-50	-26	-23	$[M-H]^-$	GMZ-d6
			249>127	-50	-12	-21		
	MFA	11.46	242>224*	46	23	22	$[M+H]^+$	MFA -d3
			242>209	46	41	14		
	NPX	7.18	229>169*	-30	-40	-9	$[M-H]^-$	NPX-d3
			229>170	-30	-22	-1		
	IBU	7.86	205>159*	-30	-10	-41	$[M-H]^-$	IBU-d3
			205>161	-30	-14	-35		
	KPF	9.78	255>105*	66	33	18	$[M+H]^+$	BP3-d5

	Compounds	t_R (min)	MS/MS Transition (m/z)	CV (V)	CE (eV)	CxP (eV)	Precursor Ion	Corresponding IS
	DCF	7.70	255>209	66	25	10	$[M-H]^-$	DCF-d4
			295>251*	-40	-16	-7		
			295>215	-40	-30	-19		
	APH	5.21	152>110*	71	23	18	$[M+H]^+$	TMP-d3
			152>65	71	45	10		
	CBZ	9.18	237>194*	61	29	14	$[M+H]^+$	CBZ-d10
			237>192	61	31	12		
	CBZ-E	8.34	253>180*	36	35	12	$[M+H]^+$	CBZ-d10
			253>151	36	109	10		
	ATL	4.43	267>116*	46	31	8	$[M+H]^+$	TMP-d3
			267>133	46	39	22		
	Nor-FXT	5.6	296>134*	31	11	8	$[M+H]^+$	TMP-d3
			296>59	31	33	10		
	N-desVFX	6.93	264>58*	31	57	18	$[M+H]^+$	TMP-d3
			264>133	31	39	46		
	SCY	4.76	137>93*	-50	-18	-1	$[M-H]^-$	SCY-d6
			137>65	-50	-40	-3		
	CFF	6.79	195>109*	56	31	8	$[M+H]^+$	TMP-d3
			195>138	56	29	10		
	DIU	9.80	233>72*	81	39	12	$[M+H]^+$	BP3-d5
			233>46	81	37	0		
Biocides	IRG	9.96	254>198*	61	25	12	$[M+H]^+$	BZT-d4
			254>68	61	69	10		
			254>83*	61	41	4		
	DCOIT	11.76	282>169	66	21	10	$[M+H]^+$	BP3-d5
			282>43*	66	51	0		
			282>57	66	33	8		
	DFL	10.50	333>123*	61	37	8	$[M+H]^+$	CBZ-d10
			333>223	61	17	8		

2. Analytical performance and QA&QC

The present analytical method was validated in accordance with standard analytical procedures (SANTE, 2017; INMETRO, 2018). Method limits of detection (LODm) and quantification (LOQm), linearity, accuracy, precision, and matrix effect (ME%) were evaluated. LODm and LOQm were determined as the lowest compounds concentration yielding a signal-to-noise (S/N) ratio of 3 and 10, respectively.

Analytical calibration curves were constructed and adjusted according to the individual response range of each target compound. Accuracy was assessed by the recovery efficiency of each standard spiked in the blank tissue, determined in triplicate at three concentration levels (1xLOQ, 5xLOQ, and 10xLOQ), and measured 3 times (n = 9). Precision was calculated as the RSD (in %) for each concentration level, and analyzed intra-day and inter-day.

ME was evaluated by comparing the slopes of the analytical calibration curves prepared in MeOH and the fish muscle tissue extracts (matrix-matched calibration standards). All analytical calibration curves, as well as all validation tests, were carried out in the presence of the isotopically labeled IS mixture (50 ng mL^{-1}).

The quality assurance (QA) and quality control (QC) criteria used in the present study comprised analyses of laboratory blanks (pure solvent and reactive without sample to test for instrument contamination). These analyses were made to document laboratory background levels. The daily set of samples under analysis was processed together with a blank extract that eliminates possible false positives by contamination in the extraction process. Calibration standards were prepared daily to test both sensitivity and linearity in the working range of concentrations. A control standards mixture solution (to check for instrumental drift in response factors) was included in the analysis sequences.

Quantification was based on isotope dilution and measuring the area of the peaks obtained. Through this approach, the ME from the complex matrices analysed was overcomed.

3 Results and discussion

3.1 VA-MSPD optimization

Considering that biota is a complex matrix and that its different tissue types are relatively small, in the present study we used 0.5 dw of fish tissue blended with 2 g of solid support and 5 mL of extraction solvent in all the analyses except blanks (only solvents and reactives). MeOH and EtOH were tested as extraction solvents due to the polarity range of most of the target analytes, and in combination with C18 as solid support, since it is the support used in the original VA-MSPD technique. Blending time, vortexing time, and stirring speed in the centrifugation step were the same in all the experiments.

3.1.1 Solvent selection

The solvent used for extraction of CECs plays a crucial role, and its selection is key to having a reliable and robust methodology.

MeOH was the solvent used in previous studies investigating the presence of PPCPs in biota samples. State et al., (2009) employed this solvent for the liquid-liquid extraction (LLE) of 17 PPCPs from fish liver samples, where SPE was used in the purification of the extract and LC-MS/MS for analysis. Reported recovery rates varied between 70 and 120% and LOQm ranged from 4.2 to 12.3 ng g^{-1} dw. (Zenker et al., (2008) used the combination of MeOH, EtOH, and acetonitrile (ACN) to analyze polar and mid-polar UV filters in fish tissues. Although different combinations among the three solvents were tested, MeOH was chosen since it provided recovery rates between 60 and 140% for 75% of the 59 target contaminants. Gago-Ferrero et al., (2013) tested different solvents, including MeOH, water, ethyl acetate, and DCM, for the extraction of UV filters from fish tissue using pressurized liquid extraction (PLE) and MeOH provided cleaner extracts and lower matrix effects, not requiring further clean-up. Other similar works such as Ramirez et al., (2007), Du et al., (2012), and Huerta et al., (2013) also reported satisfactory recoveries with the selection of MeOH as extraction solvent.

Hertzog et al., (2015) applied VA-MSPD with different extraction solvents (MeOH, acidified MeOH, MeOH:water, MeOH:acidified water, and ACN) for the extraction of 15 pharmaceuticals from fish tissues and the best recoveries (50 to 128%) were obtained using MeOH. Although EtOH was also successfully applied by Vieira et al., (2018) to extract the booster biocides DIU and IRG from fish muscle using VA-MSPD and LC-MS/MS (recoveries between 81 and 110%), the analytical challenge was much more simple since only two compounds were analyzed. Therefore, based on the literature, only MeOH and EtOH were evaluated as extraction solvents, and their selection was based on the recoveries obtained when tested.

Figure 2 shows the recovery rates achieved when MeOH and EtOH were individually tested using C18 as solid support. The results are presented by the percentage of compounds that showed recoveries within 3 ranges (< 60%, between 60 and 140%, and > 140%).

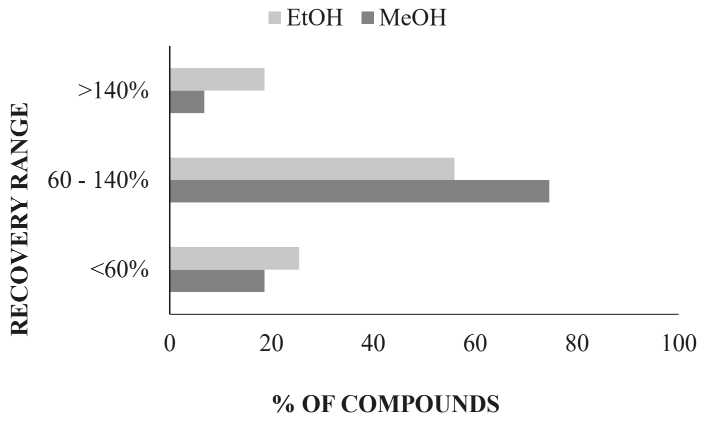

Figure 2 – Percentage of compounds within recovery ranges <60%, between 60 and 140%, and >140% after VA-MSPD extraction with EtOH and MeOH. Experimental conditions: 0.5 of freeze-dried fish muscle tissue; 2.0 g of C18 and 5 mL of extraction solvent. Vortex mixing for 1 min. and centrifugation at 4000 rpm for 10 min.; n=9.

According to the results, the solvent that presented a greater recovery range for most contaminants was MeOH. This result is supported by the fact that MeOH is a polar solvent (polarity index of 5.1 P), with a high capacity to extract polar to medium-polar analytes from complex matrices (eluent power 0.95), but also because of its low viscosity (0.54 cP) which favors blending. Although EtOH (polarity index 4.3 P; eluent power 0.88; viscosity 1.08 cP) has shown lower recoveries in the main range, the use of this solvent must be considered as it has received great attention from green chemistry (Tekin et al., 2018). Furthermore, the final VA-MSPD extract when using MeOH appeared cleaner, less pigmented, and less cloudy; relevant features to consider when choosing the solvent for solid support-based extractions.

3.1.2 Solid support optimization

Once MeOH was chosen as the solvent for the multi-residue extraction of the selected analytes, the other relevant decision in the extraction process was the selection of the solid support for the VA-MSPD extraction because it is an important parameter that affects the efficiency of extraction.

In a previous study using MPSD for the extraction of organic compounds from fish tissues, Hertzog et al., (2015) tested several solid supports such as C18, florisil, diatomaceous earth, and alternative materials derived from renewable sources (chitin and chitosan). Diatomaceous earth was selected as solid support since it presented the highest efficiency. Duarte et al., (2013) also tested several solid supports for the analysis of organic mercury in fish tissue using VA-MSPD. Silica provided the best recoveries most likely due to the combination of the polar nature of this abrasive material and the physical strength applied in blending.

In this work, to further increase the percentage of compounds within an acceptable recovery range and based on literature background, solid supports other than traditional C18 were also tested. Florisil, silica, alumina, and solid support-free were tested using MeOH as extraction solvent.

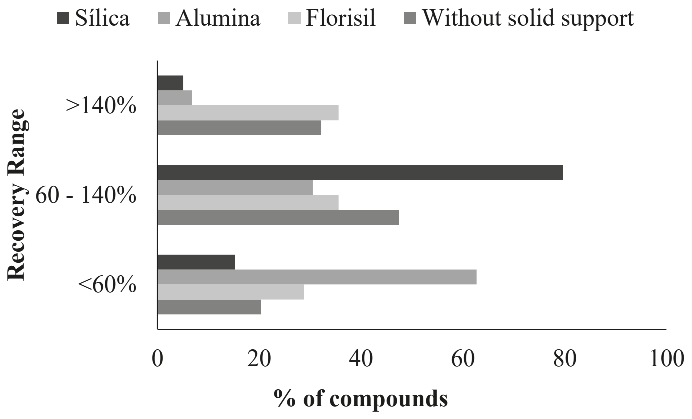

Figure 3 – Percentage of compounds within the recovery ranges <60%, between 60 and 140%, and >140%. Experimental conditions: 0.5 g dw of freeze-dried fish muscle tissue, 2.0 g of solid support, and 5 mL of MeOH. Vortex mixing for 1 min. and centrifugation at 4000 rpm for 10 min.; n=9.

Recoveries were <60% or >140% for most compounds when the extraction was carried out without any solid support and exceeded 140% when florisil and alumina were used. In contrast, recovery values between 60% and 140% were obtained for 80% of the analytes when silica was used. Despite being a synthetic material, silica is a chemical considered cheaper than those traditionally used, such as C18. The best results achieved with this material are likely due to its small particle size and porous structure that provides a high surface area.

3.2 Method performance

The method performance was assessed through linearity, sensitivity, accuracy, and precision (Table S1) determination. Optimized conditions involved the use of 0.5 g of freeze-dried fish muscle tissue, 2 g of silica as a solid support, and 5 mL of MeOH as extraction solvent.

Calibration range and linearity

Eleven calibration standard solutions at concentrations ranging from 1 to 1000 ng mL^{-1} were prepared, in both, MeOH and tissue extract. The correlation coefficients (r^2) of the calibration curves prepared in the solvent ranged from 0.9974 to 0.9999, whereas those corresponding to those the matrix extract ranged between 0.970 and 0.9999. These results are in good agreement with the established by regulatory agencies (SANTE, 2017).

Sensitivity

LODm and LOQm were estimated by the injection of the mixture of compounds prepared in MeOH and the fish tissue extracts. Values of LODm and LOQm varied between 0.99 and 34.4 ng g^{-1} dw and 3.31 and 114 ng g^{-1} dw, respectively (Table S1). These values are similar to or below those reported in the literature for solid environmental samples analysis. Gago-Ferrero et al., (2013) obtained LODm between 0.1 and 6 ng g^{-1} dw using PLE and LC-MS/MS to determine UVFs in fish, whereas Hertzog et al., (2015) achieved LOQm between 5 and 1000 ng g^{-1} using VA-MSPD and LC-MS/MS to determine 15 pharmaceuticals in fish. For ABBs, Vieira et al., (2018) obtained LOQm ranging from 5 to 625 ng g^{-1} using VA-MSPD and LC-MS/MS to analyze fish samples.

Accuracy and precision

The VA-MSPD extraction efficiency was evaluated according to recovery responses, where values between 60% and 140% were considered satisfactory (INMETRO, 2018). Therefore, repeatability was evaluated within this expected range. Fish samples were also spiked at three concentration levels, namely 1xLOQm, 5xLOQm, and 10xLOQm for each target compound.

RSD was calculated to assess the dispersion of replicates in calculating recoveries. Precision and repeatability were calculated through intra-day and inter-day analyses. Although few compounds showed recoveries >120% or <70%, and RSD >20%, these were a minority considering the large number and range of physicochemical properties of the compounds analyzed, and the analytical challenge that comprises a multi-residue method of this magnitude.

Matrix effect (ME)

ME is usually observed for sample matrices that present high analytical complexity, such as fish tissue, and is reported in the analysis of biological tissues. In the case of fish, ME is habitually produced by the high-fat content of the sample, which results in a dirty and difficult to analyze extract. Therefore, its evaluation in any analytical method is crucial. In the present study, ME was evaluated through the comparison of analytical calibration curves prepared in MeOH and matrix extract (usually known as matrix-matched standards). For the quantification stage of the study analytes, the analytical curve selected to compensate for potential issues caused by the matrix itself was matrix-matched calibration.

Results showed low or medium ME for 58% of the compounds, while the rest presented high ME, with values >50% or <-50%. Pavlovic *et al.* (2012) added some additional cleaning steps, such as SPE, after the extraction to mitigate ME. Ramirez et al., (2007) observed ME higher than that obtained in the present study, despite applying a more time-consuming and costly method.

In addition to the analytical performances shown in this study, it is possible to state that the robustness of the VA-MSPD is also a positive and interesting factor. Previous studies have demonstrated the robustness of the technique in several fish species and tissues (Hertzog et al., 2015; Vieira et al., 2018), supporting its applicability in the present study.

4 Distribution profile of the selected CECs in fish tissues

Once developed and validated, the method was applied to determine the bioaccumulated concentrations of the target compounds in samples of the marine benthic fish *Micropogonias furnieri,* collected in the southern part of Patos Lagoon estuary (Southern Brazil). To assess the distribution of the bioaccumulated compounds in the fish, four tissues (namely muscle, liver, stomach, and gills) were analyzed. Table 3 lists the results obtained for the analyzed samples.

Out of the 59 compounds investigated, 13 were detected. All analysed tissues contained at least Et-PABA, SCY, or the BP3 metabolite 4HB. BP3 was only detected in the life (<LOQm), where 4HB was present at a quantifiable concentration (39.5 ng g^{-1} dw). This compound was also detected in all the samples, but <LOQm in muscle, stomach, and gills. This could be expected since the liver is the organ where the metabolism of toxic substances takes place.

Table 3 - Concentrations of target analytes (ng g^{-1} dw) measured in different tissues of *Micropogonias furnieri* from the Patos Lagoon estuary (Brazil). *<LODm - below the limit of detection of the method; <LOQm – below the limit of quantification of the method.

Analytes	**Muscle (ng g^{-1} dw)**	**Liver (ng g^{-1} dw)**	**Stomach (ng g^{-1} dw)**	**Gills (ng g^{-1} dw)**
BP3	<LODm	<LOQm	<LODm	<LODm
4HB	<LOQm	39.5	<LOQm	<LOQm
4DHB	<LOQm	<LODm	<LODm	<LODm
AVO	<LOQm	<LODm	<LODm	<LODm
Et-PABA	2840	8320	21.0	13.2
FLU	<LOQm	<LODm	<LODm	<LODm
OFX	<LOQm	<LODm	<LODm	<LODm
OXL	<LOQm	<LODm	<LODm	<LOQm
SQX	<LODm	<LOQm	<LODm	<LODm
SDM	<LOQm	<LODm	<LODm	<LODm
SCY	124	67.7	63.8	57.3
IRG	<LOQm	<LOQm	<LODm	<LODm
DCOIT	<LOQm	<LODm	<LODm	<LOQm

Previous studies have reported 4HB in fish samples. For example, Molins-Delgado et al., (2018) found it in *Mugil liza* from highly urbanized areas in Guanabara Bay, Rio de Janeiro (Brazil). Results showed that target UV-Fs and metabolites were ubiquitous in the analyzed tissues, including quite high concentrations of 4HB (40.1-152 ng g^{-1} dw) or the estrogenic UV filter Et-PABA (13.2-2840 ng g^{-1} dw). These results are in support of the UV filters' bioaccumulation capacity reported so far in marine ecosystems (Agawin et al., 2022; Downs et al., 2022; Fenni et al., 2022; Gago-Ferrero et al., 2015).

Et-PABA is currently used as a substitute for 4-aminobenzoate (PABA) in sunscreens but also in anesthetic products. Moreover, its distribution within the organs was somewhat unexpected as the bioaccumulation followed the order of *liver* > *muscle* > *stomach* > *gills*. As mentioned, the liver is the organ where the metabolism of toxic substances takes place, so the highest levels in this organ are logical. The same applies to the muscle, a tissue with a high fat content that favors the accumulation of lipophylic compounds such as Et-PABA.

Due to its widespread use, Et-PABA has been found in different environmental matrices worldwide, i.e. sediment, water, and fish samples (Díaz-Cruz et al., 2019; Li et al., 2017). These studies focused on different fish species (Fent et al., 2008; Kunz and Fent, 2006; Zenker et al., 2008), and raise the question of whether UV filter residues found in the environment are potentially able to adversely affect aquatic organisms and/or cause interferences in metabolic processes depending on the tissue selected for analysis. Although Díaz-Cruz et al. (2019) found Et-PABA in surface water from Greece (up to 956 ng L^{-1}) and related the concentrations detected with temporal and spatial distribution, Et-PABA was only detected (<LOQm) in one *S. keadicus* fish. In contrast, BP3 was found in 50% of the analyzed samples.

Another organic compound present in the samples analyzed in the present study was SCY, accumulated in 100% of the analyzed fish tissues. Its distribution across the organs differs from that observed for Et-PABA. Concentrations accumulated followed the order of *muscle* > *liver* > *stomach* > *gills*. The levels in muscle were two-fold those determined in the liver, stomach, and gills, which were quite similar (67.74, 63.8, and 57.3, respectively). This could be explained by their different physicochemical properties (Table 4).

Table 4 - Physicochemical properties of Et-PABA and SCY (ChemSpider | Search and share chemistry, © Royal Society of Chemistry 2022, Registered charity number: 207890). K_{ow}, octanol-water partition constant; Ka, dissociation constant.

Acronym	**CAS Nº**	**Log Kow**	**pKa**	**Solubility (mg L^{-1}, in water at 25ºC)**
Et-PABA	94-09-7	1.86	2.51	1671
SCY	69-72-7	2.26	2.97	3808

According to the physicochemical properties of SCY and Et-PABA, it is possible to perceive that the most differentiating factor between these two compounds is their solubility in water. As SCY is much more soluble, its concentration in the water column is high. Furthermore, this factor causes also a higher transfer potential through the skin of the fish, while for Et-PABA, being less soluble, diet would constitute the only exposure pathway.

SCY is an organic compound widely used in pharmaceutical and cosmetic formulations (ingredients of acne treatment, shampoos, facial cleansers, and moisturizers) and it is a metabolite of the acetylsalicylic acid (Cerqueira et al., 2019). This compound, as for Et-PABA has also been detected in different environmental matrices, including seawater, groundwater, and river water (Lopes et al., 2016; Paíga et al., 2015), sediments (Martín et al., 2010; Sadutto et al., 2020), and sewage sludge (Albero et al., 2014; Cerqueira et al., 2019), demonstrating its environmental persistency. This organic contaminant is considered a bio-persistent compound toxic to aquatic organisms (Zenker et al., 2008) and has been previously found up to 490 ng g^{-1} in *Mytilus edulis* bivalve(Wille et al., 2011).

Some pharmaceuticals, such as FLU, OFX, OXL, SQX, and SDM were found in the different tissues, but <LOQm. Regarding ABBs, only IRG and DCOIT were detected in the analyzed tissues, but < LOQm. Although they have been detected but not quantified, their detection was expected because these two biocides are the most prevalent worldwide (Thomas and Brooks, 2010).

Overall, it is worth mentioning that pollutants bioaccumulation levels found may differ according to the species of fish analyzed. Our study focused on the *Micropogonias furnieri* species, but other studies also report the determination of PCPs and ABBs in species such as *Mugil liza Cynoscion, Guatucupa* and *Mugil liza* tissues from Brazil (Molins-Delgado et al., 2018; Vieira et al., 2018). Alteration in reproduction is a common feature repoprted, along with physiological changes in organs such as the liver, brain, and gills. There are studies in the literature that lead to the belief that compounds, such as pharmaceuticals, at high concentrations in the environment can cause adverse effects to humans, by water consumption and/or through their accumulation in aquatic organisms. In short, the process of metabolizing contaminants can be different depending on the species, even distinct in the different tissues, since each species has a particular content of lipids, proteins, and other components. In addition, the bioaccumulation of an individual can vary significantly and is dependent on factors such as species, age, weight, natural habitat, and time of capture, among others (McLeod et al., 2014).

5. Estimated daily intake (EDI) of bioaccumulated pollutants

Three compounds were detected in muscle and/or liver samples at concentrations exceeding LOQms: Et-PABA, SCY, and the metabolite 4HB. Considering that SCY has maximum residue limit (MRL) values in certain organisms, for instance, 400 ng g^{-1} in muscle and 200 ng g^{-1} in the liver of turkey (Commission Regulation (EU), 2010), it was relevant to calculate the EDI by fish consumption of Brazilian residents, according to the concentrations determined in the muscle and liver of the analyzed fish.

According to the Fish and Aquaculture Ministry, the average daily consumption of fish in Brazil is 25 g per capita (Ministério da Pesca e Aquicultura, 2010). With this diet information and the concentrations determined, EDI values were estimated for the three compounds following equation (1):

$$EDI = (p \cdot C_{\sum s}) \cdot M^{-1} \qquad (1)$$

where p is the average daily weight of fish consumed, $C_{\sum s}$ is the concentration of the substance in the fish liver and muscle, expressed in wet weight (ww), and M is the mean human body weight, 68 kg, corresponding to the average of South American population (Walpole et al., 2012). According to (FAO, 1991) the overall water content of fish is 72%, the value that we used to calculate the weight of the fish to be consumed from the dry weight considered in the analyses. Considering fish muscle and liver as the only edible parts, and that <LOQm usually is estimated as half of the LOQm value, this translates into EDIs of 18.2, 4.10, and 70.3 ng (kg body weight)$^{-1}$d^{-1} for 4HB, EtPABA, and SCY, respectively.

For this estimation, muscle and liver concentrations of SCY expressed in fish wet weight in the tissues analyzed were 123 ng g^{-1} and 67.7 ng g^{-1}, respectively. These concentrations were far less than the stablished MRL for turkey, and no other matrix was found to compare with, suggesting that the adverse health effects of SCY associated with fish diet are minimal. Nevertheless, given that the MRL corresponds to another animal food, the possibility of ingesting multiple foods, water, and considering other potential sources to SCY, it is imperative to continue investigating the SCY levels in foodstuff, water, and other potential exposure pathways. SCY has been classified by the European Food Safety Authority (EFSA) (Silano et al., 2020) as one of the three high-priority substances (FCM No 121) based on teratogenic properties shown in animals (ECHA, 2016). Based on the lowest No-Observed-Adverse-Effect Level (NOAEL) of 75 mg (kg body weight)$^{-1}$d^{-1} from a developmental toxicity study carried out in rats (Tanaka et al., 1973), a tolerable daily intake (TDI) below 1 mg (kg body weight)$^{-1}$d^{-1} could be derived (with uncertainty factor of 100 for inter- and intra-species differences). Therefore, the EDI value calculated in this study for SCY is far below the TDI considered by the EFSA (70.35 ng (kg body weight)$^{-1}$d^{-1}) and thus, health risk associated solely with fish consumption is unexpected.

However, information on specific species of fish in Brazil is scarce to make a comparison of data. Generally, EDIs are calculated individually and compared with those reported for persistent organic pollutants in fish. Previous studies have found EDI in line with those reported in fish in the present study (Berger et al., 2009; Fent et al., 2008; Kunz and Fent, 2006; Molins-Delgado et al., 2018). Therefore, we can conclude that the estimated daily intake of SCY through fish consumption in Brazil is significantly lower than the tolerable daily intake recommended by the EFSA for this compound.

Conclusions

In the present study, a multi-residue method based on sample preparation by VA-MSPD and analysis by LC-MS/MS for the determination of 59 CECs including pharmaceuticals, personal care products, and biocides, in fish tissues was developed and validated. The proposed method allowed to investigate the differential distribution of selected CECs in fish tissue samples, proving to be easy to execute, cheaper, and more robust than traditional techniques applied, being suitable for laboratory routine analyses. Et-PABA, 4HB, and SCY were frequently accumulated in muscle and liver. A preliminary risk assessment of the target compounds showed that exposure to SCY from fish consumption is unlikely to pose a health risk. Nevertheless, it is important to monitor its levels in other potential exposure routes and foodstuff. Further studies should also focus on the occurrence, behaviour, and fate of CECs in the environment to fully understand their bioaccumulation and biomagnification in living organisms, as well as their potential synergistic toxic effects because all occurrence data could be used as a reference in further regulation revisions.

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References

Abreu, F.E.L., Silva, J.N.L., Castro, I.B., Fillmann, G., 2020. Are antifouling residues a matter of concern in the largest South American port?. J. Hazard. Mater. https://doi.org/10.1016/j.jhazmat.2020.122937

Agawin, N.S.R., Sunyer-Caldú, A., Díaz-Cruz, M.S., Frank-Comas, A., García-Márquez, M.G., Tovar-Sánchez, A., 2022. Mediterranean seagrass Posidonia oceanica accumulates sunscreen UV filters. Mar. Pollut. Bull. 176. https://doi.org/10.1016/j.marpolbul.2022.113417

Albero, B., Sánchez-Brunete, C., Miguel, E., Aznar, R., Tadeo, J.L., 2014. Determination of selected pharmaceutical compounds in biosolids by supported liquid extraction and gas chromatography-tandem mass spectrometry. J. Chromatogr. A 1336, 52–58. https://doi.org/10.1016/j.chroma.2014.02.020

Batista-Andrade, J.A., Caldas, S.S., Batista, R.M., Castro, I.B., Fillmann, G., Primel, E.G., 2018. From TBT to booster biocides: Levels and impacts of antifouling along coastal areas of Panama. Environ. Pollut. 234, 243–252. https://doi.org/10.1016/j.envpol.2017.11.063

Batista-Andrade, J.A., Caldas, S.S., de Oliveira Arias, J.L., Castro, I.B., Fillmann, G., Primel, E.G., 2016. Antifouling booster biocides in coastal waters of Panama: First appraisal in one of the busiest shipping zones. Mar. Pollut. Bull. 112, 415–419. https://doi.org/10.1016/j.marpolbul.2016.07.045

Berger, U., Glynn, A., Holmström, K.E., Berglund, M., Ankarberg, E.H., Törnkvist, A., 2009. Fish consumption as a source of human exposure to perfluorinated alkyl substances in Sweden - Analysis of edible fish from Lake Vättern and the Baltic Sea. Chemosphere 76, 799–804. https://doi.org/10.1016/j.chemosphere.2009.04.044

Caldas, S.S., Rombaldi, C., De Oliveira Arias, J.L., Marube, L.C., Primel, E.G., 2016. Multi-residue method for determination of 58 pesticides, pharmaceuticals and personal care products in water using solvent demulsification dispersive liquid-liquid microextraction combined with liquid chromatography-tandem mass spectrometry. Talanta 146, 676–688. https://doi.org/10.1016/j.talanta.2015.06.047

Caldas, S.S., Soares, B.M., Abreu, F., Castro, Í.B., Fillmann, G., Primel, E.G., 2018. Antifouling booster biocide extraction from marine sediments: a fast and simple method based on vortex-assisted matrix solid-phase extraction. Environ. Sci. Pollut. Res. 25, 7553–7565. https://doi.org/10.1007/s11356-017-0942-x

Cerqueira, M.B.R., Kupski, L., Caldas, S.S., Primel, E.G., 2019. Golden mussel shell and water in matrix solid phase dispersion: A suitable combination for the extraction of acetylsalicylic and salicylic acids from sewage sludge. Microchem. J. 148, 102–107. https://doi.org/10.1016/j.microc.2019.04.046

Cerqueira, M.B.R., Soares, K.L., Caldas, S.S., Primel, E.G., 2018. Sample as solid support in MSPD: A new possibility for determination of pharmaceuticals, personal care and degradation products in sewage sludge. Chemosphere 211, 875–883. https://doi.org/10.1016/j.chemosphere.2018.07.165

ChemSpider | Search and share chemistry [WWW Document], 2022.

Chen, H., Chen, W., Guo, H., Lin, H., Zhang, Y., 2021. Pharmaceuticals and personal care products in the seawater around a typical subtropical tourist city of China and associated ecological risk. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-020-12335-1

Commission Regulation (EU), 2010. (Commission Regulation (EU) No. 914/2010) [WWW Document]. Annex to Regul. No 37/2010 Pharmacol. Act. Subst. their Classif. regarding maximum residue limits Foodst. Anim. Orig. as regards Subst. sodium salicylate.

Díaz-Cruz, M.S., Molins-Delgado, D., Serra-Roig, M.P., Kalogianni, E., Skoulikidis, N.T., Barceló, D., 2019. Personal care products reconnaissance in EVROTAS river (Greece): Water-sediment partition and bioaccumulation in fish. Sci. Total Environ. 651, 3079–3089. https://doi.org/10.1016/j.scitotenv.2018.10.008

dos Santos, E.O., Gonzales, J.O., Ores, J.C., Marube, L.C., Caldas, S.S., Furlong, E.B., Primel, E.G., 2019. Sand as a solid support in ultrasound-assisted MSPD: A simple, green and low-cost method for multiresidue pesticide determination in fruits and vegetables. Food Chem. 297, 124926. https://doi.org/10.1016/j.foodchem.2019.05.200

Downs, C.A., Bishop, E., Diaz-Cruz, M.S., Haghshenas, S.A., Stien, D., Rodrigues, A.M.S., Woodley, C.M., Sunyer-Caldú, A., Doust, S.N., Espero, W., Ward, G., Farhangmehr, A., Tabatabaee Samimi, S.M., Risk, M.J., Lebaron, P., DiNardo, J.C., 2022. Oxybenzone contamination from sunscreen pollution and its ecological threat to Hanauma Bay, Oahu, Hawaii, U.S.A. Chemosphere 291. https://doi.org/10.1016/j.chemosphere.2021.132880

Du, B., Perez-Hurtado, P., Brooks, B.W., Chambliss, C.K., 2012. Evaluation of an isotope dilution liquid chromatography tandem mass spectrometry method for pharmaceuticals in fish. J. Chromatogr. A 1253, 177–183. https://doi.org/10.1016/j.chroma.2012.07.026

Duarte, F.A., Soares, B.M., Vieira, A.A., Pereira, E.R., MacIel, J. V., Caldas, S.S., Primel, E.G., 2013. Assessment of modified matrix solid-phase dispersion as sample preparation for the determination of CH 3 Hg + and Hg 2+ in fish. Anal. Chem. 85, 5015–5022. https://doi.org/10.1021/ac4002436

ECHA, 2016. Committee for Risk Assessment (RAC), Opinion proposing harmonised classification and labelling at EU level of Salicylic acid, EC Number: 200-712-3, CAS Number: 69-72-7, CLH-O-0000001412-86-110/F, Adopted 10 March 2016. [WWW Document]. Eur. Chem. Agency.

FAO, 1991. Fish for Food and Development. Food and Agriculture Organization of the United Nations. https://rsmag.org/en/Food_and_Agriculture_Organization_of_the_United_Nations-0840802862.

Fenni, F., Sunyer-Caldú, A., Ben, H., Diaz-cruz, M.S., 2022. Contaminants of emerging concern in marine areas : First evidence of UV filters and paraben preservatives in seawater and sediment on the eastern coast of Tunisia ☆ 309. https://doi.org/10.1016/j.envpol.2022.119749

Fent, K., Kunz, P.Y., Gomez, E., 2008. UV filters in the aquatic environment induce hormonal effects and affect fertility and reproduction in fish. Chimia (Aarau). 62, 368–375. https://doi.org/10.2533/chimia.2008.368

Gago-Ferrero, P., Díaz-Cruz, M.S., Barceló, D., 2015. UV filters bioaccumulation in fish from Iberian river basins. Sci. Total Environ. 518–519, 518–525. https://doi.org/10.1016/j.scitotenv.2015.03.026

Gago-Ferrero, P., Díaz-Cruz, M.S., Barceló, D., 2013. Multi-residue method for trace level determination of UV filters in fish based on pressurized liquid extraction and liquid chromatography-quadrupole-linear ion trap-mass spectrometry. J. Chromatogr. A 1286, 93–101. https://doi.org/10.1016/j.chroma.2013.02.056

Golovko, O., Örn, S., Sörengård, M., Frieberg, K., Nassazzi, W., Lai, F.Y., Ahrens, L., 2021. Occurrence and removal of chemicals of emerging concern in wastewater treatment plants and their impact on receiving water systems. Sci. Total Environ. 754, 142122. https://doi.org/https://doi.org/10.1016/j.scitotenv.2020.142122

Gopal, C.M., Bhat, K., Praveenkumarreddy, Y., Shailesh, Kumar, V., Basu, H., Joshua, D.I., Singhal, R.K., Balakrishna, K., 2020. Evaluation of selected pharmaceuticals and personal care products in water matrix using ion trap mass spectrometry: A simple weighted calibration curve approach. J. Pharm. Biomed. Anal. 185, 113214. https://doi.org/10.1016/j.jpba.2020.113214

Hertzog, G.I., Soares, K.L., Caldas, S.S., Primel, E.G., 2015. Study of vortex-assisted MSPD and LC-MS/MS using alternative solid supports for pharmaceutical extraction from marketed fish. Anal. Bioanal. Chem. 407, 4793–4803. https://doi.org/10.1007/s00216-015-8685-3

Huerta, B., Jakimska, A., Gros, M., Rodríguez-Mozaz, S., Barceló, D., 2013. Analysis of multi-class pharmaceuticals in fish tissues by ultra-high-performance liquid chromatography tandem mass spectrometry. J. Chromatogr. A 1288, 63–72. https://doi.org/10.1016/j.chroma.2013.03.001

INMETRO, 2018. Orientação sobre validação de métodos analíticos DOQ-CGCRE-008, Revisão em julho de 2018. [WWW Document].

Kunz, P.Y., Fent, K., 2006. Multiple hormonal activities of UV filters and comparison of in vivo and in vitro estrogenic activity of ethyl-4-aminobenzoate in fish. Aquat. Toxicol. 79, 305–324. https://doi.org/10.1016/j.aquatox.2006.06.016

Li, A.J., Sang, Z., Chow, C.H., Law, J.C.F., Guo, Y., Leung, K.S.Y., 2017. Environmental behavior of 12 UV filters and photocatalytic profile of ethyl-4-aminobenzoate. J. Hazard. Mater. 337, 115–125. https://doi.org/10.1016/j.jhazmat.2017.04.067

Liu, M., Yin, H., Wu, Q., 2019. Occurrence and health risk assessment of pharmaceutical and personal care products (PPCPs) in tap water of Shanghai. Ecotoxicol. Environ. Saf. 183, 109497. https://doi.org/10.1016/j.ecoenv.2019.109497

Lopes, V.S.A., Riente, R.R., da Silva, A.A., Torquilho, D.F., Carreira, R. da S., Marques, M.R. da C., 2016. Development of a solid-phase extraction system modified for preconcentration of emerging contaminants in large sample volumes from rivers of the lagoon system in the city of Rio de Janeiro, Brazil. Mar. Pollut. Bull. 110, 572–577. https://doi.org/10.1016/j.marpolbul.2016.05.059

Martín, J., Santos, J.L., Aparicio, I., Alonso, E., 2010. Multi-residue method for the analysis of pharmaceutical compounds in sewage sludge, compost and sediments by sonication-assisted extraction and LC determination. J. Sep. Sci. 33, 1760–1766. https://doi.org/10.1002/jssc.200900873

Martins, M.F., Costa, P.G., Bianchini, A., 2020. Contaminant screening and tissue distribution in the critically endangered Brazilian guitarfish Pseudobatos horkelii. Environ. Pollut. 265, 114923. https://doi.org/10.1016/j.envpol.2020.114923

McLeod, A.M., Paterson, G., Drouillard, K.G., Haffner, G.D., 2014. Ecological factors contributing to variability of persistent organic pollutant bioaccumulation within forage fish communities of the Detroit River, Ontario, Canada. Environ. Toxicol. Chem. 33, 1825–1831. https://doi.org/10.1002/etc.2606

Ministério da Pesca e Aquicultura, 2010. Boletim Estatístico da Pesca e Aquicultura. Brasil 2008 - 2009. Bol. Estatístisco da Pesca e Aquicultura 129.

Mitchelmore, C.L., He, K., Gonsior, M., Hain, E., Heyes, A., Clark, C., Younger, R., Schmitt-Kopplin, P., Feerick, A., Conway, A., Blaney, L., 2019. Occurrence and distribution of UV-filters and other anthropogenic contaminants in coastal surface water, sediment, and coral tissue from Hawaii. Sci. Total Environ. 670, 398–410. https://doi.org/10.1016/j.scitotenv.2019.03.034

Molins-Delgado, D., Muñoz, R., Nogueira, S., Alonso, M.B., Torres, J.P., Malm, O., Ziolli, R.L., Hauser-Davis, R.A., Eljarrat, E., Barceló, D., Díaz-Cruz, M.S., 2018. Occurrence of organic UV filters and metabolites in lebranche mullet (Mugil liza) from Brazil. Sci. Total Environ. 618, 451–459. https://doi.org/10.1016/j.scitotenv.2017.11.033

Mulato, I.P., Corrêa, B., Vianna, M., 2015. Time-space distribution of Micropogonias furnieri (Perciformes, Sciaenidae) in a tropical estuary in southeastern Brazil. Bol. do Inst. Pesca 41, 1–18.

Paíga, P., Lolić, A., Hellebuyck, F., Santos, L.H.M.L.M., Correia, M., Delerue-Matos, C., 2015. Development of a SPE-UHPLC-MS/MS methodology for the determination of non-steroidal anti-inflammatory and analgesic pharmaceuticals in seawater. J. Pharm. Biomed. Anal. 106, 61–70. https://doi.org/10.1016/j.jpba.2014.06.017

Ramirez, A.J., Mottaleb, M.A., Brooks, B.W., Chambliss, C.K., 2007. Analysis of pharmaceuticals in fish using liquid chromatography-tandem mass spectrometry. Anal. Chem. 79, 3155–3163. https://doi.org/10.1021/ac062215i

Sadutto, D., Álvarez-Ruiz, R., Picó, Y., 2020. Systematic assessment of extraction of pharmaceuticals and personal care products in water and sediment followed by liquid chromatography–tandem mass spectrometry. Anal. Bioanal. Chem. 412, 113–127. https://doi.org/10.1007/s00216-019-02207-0

SANTE, 2017. Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticide Residues and Analysis in Food and Feed. [WWW Document].

Sebastià, N., Soler, C., Soriano, J.M., Mães, J., 2010. Occurrence of aflatoxins in tigernuts and their beverages commercialized in Spain. J. Agric. Food Chem. 58, 2609–2612. https://doi.org/10.1021/jf903818x

Silano, V., Barat Baviera, J.M., Bolognesi, C., Chesson, A., Cocconcelli, P.S., Crebelli, R., Gott, D.M., Grob, K., Lambré, C., Lampi, E., Mengelers, M., Mortensen, A., Steffensen, I.L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Benfenati, E., Castle, L., Di Consiglio, E., Franz, R., Hellwig, N., Milana, M.R., Pfaff, K., Civitella, C., Lioupis, A., Pizzo, F., Rivière, G., 2020. Review and priority setting for substances that are listed without a specific migration limit in Table 1 of Annex 1 of Regulation 10/2011 on plastic materials and articles intended to come into contact with food. EFSA J. 18, 1–104. https://doi.org/10.2903/j.efsa.2020.6124

Soares, K.L., Cerqueira, M.B.R., Caldas, S.S., Primel, E.G., 2017. Evaluation of alternative environmentally friendly matrix solid phase dispersion solid supports for the simultaneous extraction of 15 pesticides of different chemical classes from drinking water treatment sludge. Chemosphere 182, 547–554. https://doi.org/10.1016/j.chemosphere.2017.05.062

Soares, K.L., Sunyer-Caldú, A., Barbosa, S.C., Primel, E.G., Fillmann, G., Diaz Cruz, M.S., 2021. Rapid and cost-effective multiresidue analysis of pharmaceuticals, personal care products, and antifouling booster biocides in marine sediments using matrix solid phase dispersion. Chemosphere 267. https://doi.org/10.1016/j.chemosphere.2020.129085

Souza Caldas, S., Marian Bolzan, C., Jaime De Menezes, E., Laura Venquiaruti Escarrone, A., De Martinez Gaspar Martins, C., Bianchini, A., Gilberto Primel, E., 2013. A vortex-assisted MSPD method for the extraction of pesticide residues from fish liver and crab hepatopancreas with determination by GC-MS. Talanta 112, 63–68. https://doi.org/10.1016/j.talanta.2013.03.054

State, M., State, M., State, M., State, M., California, S., Water, C., Mesa, C., State, M., State, M., 2009. Determination of 17 a -Ethynylestradiol, Carbamazepine, Diazepam, Simvastatin, and Oxybenzone in Fish Livers 359–370.

Tanaka, S., Kawashima, K., Nakaura, S., Nagao, S., Kuwamura, T., Omori, Y., 1973. Studies on the Teratogenicity of Food Additives (1): Effects of Saccharin Sodium on the Development of Rats and Mice. Food Hyg. Saf. Sci. (Shokuhin Eiseigaku Zasshi) 14, 371–379. https://doi.org/10.3358/shokueishi.14.371

Tekin, K., Hao, N., Karagoz, S., Ragauskas, A.J., 2018. Ethanol: A Promising Green Solvent for the Deconstruction of Lignocellulose. ChemSusChem 11, 3559–3575. https://doi.org/https://doi.org/10.1002/cssc.201801291

Thomas, K. V, Brooks, S., 2010. The environmental fate and effects of antifouling paint biocides. Biofouling 26, 73–88. https://doi.org/10.1080/08927010903216564

Vieira, A.A., Caldas, S.S., Escarrone, A.L.V., Arias, J.L. de O., Primel, E.G., 2018. Environmentally friendly procedure based on VA-MSPD for the determination of booster biocides in fish tissue. Food Chem. 242, 475–480. https://doi.org/10.1016/j.foodchem.2017.09.085

Walpole, S.C., Prieto-Merino, D., Edwards, P., Cleland, J., Stevens, G., Roberts, I., 2012. The weight of nations: An estimation of adult human biomass. BMC Public Health 12, 1. https://doi.org/10.1186/1471-2458-12-439

Wille, K., Kiebooms, J.A.L., Claessens, M., Rappé, K., Vanden Bussche, J., Noppe, H., Van Praet, N., De Wulf, E., Van Caeter, P., Janssen, C.R., De Brabander, H.F., Vanhaecke, L., 2011. Development of analytical strategies using U-HPLC-MS/MS and LC-ToF-MS for the quantification of micropollutants in marine organisms. Anal. Bioanal. Chem. 400, 1459–1472. https://doi.org/10.1007/s00216-011-4878-6

Zenker, A., Schmutz, H., Fent, K., 2008. Simultaneous trace determination of nine organic UV-absorbing compounds (UV filters) in environmental samples. J. Chromatogr. A 1202, 64–74. https://doi.org/10.1016/j.chroma.2008.06.041

3.2.4 Publication #4 Supplementary information

Distribution in marine fish and EDI estimation of contaminants of emerging concern by vortex-assisted matrix solid-phase dispersion and HPLC-MS/MS

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Submitted to Marine Pollution Bulletin

Section S1. Standards purchase and purity
Section S2. Tables (Table S1).

Section S1. Standards purchase and purity

High purity (96-99.9%) analytical standards of 5-benzoyl-4-hydroxy-2-methoxybenzene sulfonic acid (BP4), 2-hydroxy-4-methoxybenzophenone (BP3), 2,2’,4,4’-tetrahydroxybenzophenone (BP2), 2,4-dihydroxybenzophenone (BP1), 4-hydroxybenzophenone (4HB), 4,4′-dihydroxybenzophenone (4DHB), 2,2’-dihydroxy-4-methoxybenzophenone (DHMB), 1-(4-tert-butylphenyl)-3-(4-methoxyphenyl)propane-1,3-dione (AVO), 3-(4-methylbenzilidene)camphor (4MBC), 2-ethylhexyltrans-4-methoxycinnamate (EHMC), ethyl-4-aminobenzoate (Et-PABA), 1,2,3-benzotriazole (BZT), 4-methyl-1H-benzotriazole (MeBZT), 5,6-dimethyl-1H-benzotriazole monohydrate (DMBZT), 2-(2-benzotriazolyl)-*p*-cresol (UVP), benzyl-4-hydroxybenzoate (BePB), butyl-4-hydroxybenzoate (BuPB), propyl-4-hydroxybenzoate (PrBP), methyl-4-hydroxybenzoate (MePB), 9-fluoro-5-methyl-1-oxo-1,5,6,7-tetrahydropyrido[3,2,1-ij]quinoline-2-carboxylic acid (flumequine, FLU), 9-fluoro-3-methyl-10(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid (ofloxacin, OFX), 1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid (ciprofloxacin, CPX), 1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (nalidixic acid, NDX), 5-ethyl-8-oxo-5,8-dihydro-[1,3]dioxolo[4,5-g]quinoline-7-carboxylic acid (oxolinic acid, OXL), 4-oxo-4-[4-(1,3-thiazol-2-ylsulfamoyl)anilino]butanoic acid (Succynil-Sulfatiazole, S-STZ), 4-amino-N-pyrimidin-2-ylbenzenesulfonamide (SDZ), N-[4-(pyrimidin-2-ylsulfamoyl)phenyl]acetamide (acSDZ), 4-amino-N-(4-methylpyrimidin-2-yl)benzenesulfonamide (SMR), N-[4-(4-methylpyrimidin-2-yl)sulfamoyl]phenyl]acetamide (acSMR), 4-amino-N-(6-mathoxypyridazin-3-yl)benzenesulfonamide (SMZ), N-[4-[(4,6-dimethylpyrimidin-2-yl)sulfamoyl]phenyl]acetamide (acSMZ), 4-amino-N-(5-methyl1,2-oxazol-3-yl)benzenesulfonamide (SMX), 4-acetylamino-N-(5-methyl-3-isoxazoyl)benzenesulfonamide (acSMX), 4-amino-N-(6-methoxypyridazin-3-yl)benzenesulfonamide (SMPZ), 4-amino-N-pyridin-2-ylbenzenesulfonamide (SPY), N-[4-(pyridine-2-ylsulfamoyl)phenyl]acetamide (acSPY), 4-amino-N-quinoxalin-2-ylbenzenesulfonamide (SQX), 4-amino-N-(1,3-thiazol-2-yl)benzenesulfonamide (STZ), 4-amino-N-(2,6-dimethylpyrimidin-4-yl)benzenesulfonamide (sulfisomidine, SMD), 4-amino-N-(2,6-dimethoxypyrimidin-4-yl)benzenesulfonamide (SDM), 5-[(3,4,5-trimethoxyphenyl)methyl]pyrimidine-2,4-diamine (trimethoprim, TMP), 5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid (gemfibrozil, GMZ), 2-(2,3-dimethylanilino)benzoic acid (mefenamic acid, MFA), (2S)-2-(6-methoxynaphthalen-2-yl)propanoic acid (naproxen, NPX), 2-[4-(2-methylpropyl)phenyl]propanoic acid (IBU), 2-(3-benzoylphenyl)propanoic acid (ketoprofen, KPF), diclofenac soduim salt (diclofenac, DCF), N-(4-hydroxyphenyl)acetamide (acetaminophen, APH), benzo[b][1]benzazepine-11-carboxamide (CBZ), 3-oxa-11-azatetracyclo[10.4.0.02,4.05,10]hexadeca-1(16),5,7,9,12,14-hexaene-11-carboxamide (CBZ-10,11-epoxy, CBZ-E), 2-[4-[2-hydroxy-3-(propan-2-ylamino)proproxy]phenyl]acetamide (atenolol, ATL), 3-phenyl-3-[4-trifluoromethyl)phenoxy]propan-1-amine (Nor-FXT), N-desmethylvenlafaxine (N-desVFX), 2-hydroxybenzoic acid (Salicylic acid, SCY), caffeine (CFF), 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron, DIU), 2-N-tert-butyl-4-N-cyclopropyl-6-methylsulfanyl-1,3,5-triazine-2,4-diamine (irgarol, IRG), 4,5-dichloro-2-n-octyl-3-(2H)-isothiazolin-3-one (DCOIT), N-[dichloro(fluoro)methyl]sulfanyl-N-

(dimethylsulfamoyl)aniline (dichlofluanid, DFL) were purchased from Sigma-Aldrich (Germany), Sigma-Aldrich (Brazil) and TCI (Belgium).

BP3-d5, GMZ-d6, 4MBC-d4, BZT-d4, BePB-d4, FLU-13C, SMZ-d4, TMP-d3, MFA-d3, NPX-d3, IBU-d3, DCF-d4, CBZ-d10 and SCY-d6 were obtained from CDN isotopes (Canada) and Sigma-Aldrich (Germany). Florisil, alumina, silica, formic acid (98%), and ammonium acetate (≥ 96%) were supplied by Merck (Germany), SPE C18 cartridges (500 mg) by Isolute (Spain), methanol (MeOH) and acetonitrile (ACN) ultra-gradient HPLC grade, ethyl acetate (EtAc) and dichloromethane (DCM) (both for organic residue analysis grade) by J.T. Backer (The Netherlands), nitrogen (99,999%) and argon (99.995%) by Air Liquid (Spain).

Section S2. Tables.

Table S1. Correlation coefficients (r^2) of the calibration curves in solvent (MeOH)) and in fish extract (tissue)), method limits of detection (LODm) and quantification (LOQm), and intra- and inter-day precision of recovery rates (R%) ± Relative Standard Deviation (RSD%) at three concentration levels (1xLOQm, 5xLOQm, and 10xLOQm) for each target analyte.

Compound	r^2	r^2	LODm	LOQm	Precision (intra-day) R % ± RSD (%)			Precision (inter-day) R % ± RSD (%)		
	(MeOH)	(tissue)	(ng g^{-1} dw)	(ng g^{-1} dw)	1-LOQm	5-LOQm	10-LOQm	1-LOQm	5-LOQm	10-LOQm
BP3	0.9999	0.9997	3.98	13.3	78.9 ± 23.5	110 ± 24.0	121± 2.4	82.1 ± 3.7	111 ± 18.8	105 ± 0.21
BP1	0.9994	0.9999	9.81	32.7	89.0 ± 20.9	79.0 ± 15.1	118± 8.1	76.5 ± 4.9	119 ± 3.0	128 ± 3.1
BP2	0.9999	0.9999	1.76	5.87	65.8 ± 7.4	62.6 ± 16.8	91.2 ± 3.1	68.1 ± 4.9	99.9 ± 7.9	86.2 ± 18.6
BP4	0.9995	0.9999	6.70	22.3	70.2 ± 2.0	113 ± 2.5	91.1 ± 7.0	84.3 ± 3.2	72.2 ± 2.1	68.6 ± 0.14
4HB	0.9996	0.9999	6.03	20.1	92.7 ± 6.6	121 ± 1.10	120 ± 1.4	93.6 ± 0.1	97.9 ± 5.3	85.6 ± 4.2
4DHB	0.9995	0.9994	4.68	15.6	97.9 ± 14.2	100 ± 15.7	114 ± 15.4	81.3 ± 3.8	86.3 ± 1.7	95.8 ± 1.6
DHMB	0.9989	0.9999	6.95	23.1	98.5 ± 24.2	91.9 ± 22.2	124 ± 0.7	71.0 ± 3.4	83.2 ± 5.7	71.0 ± 0.41

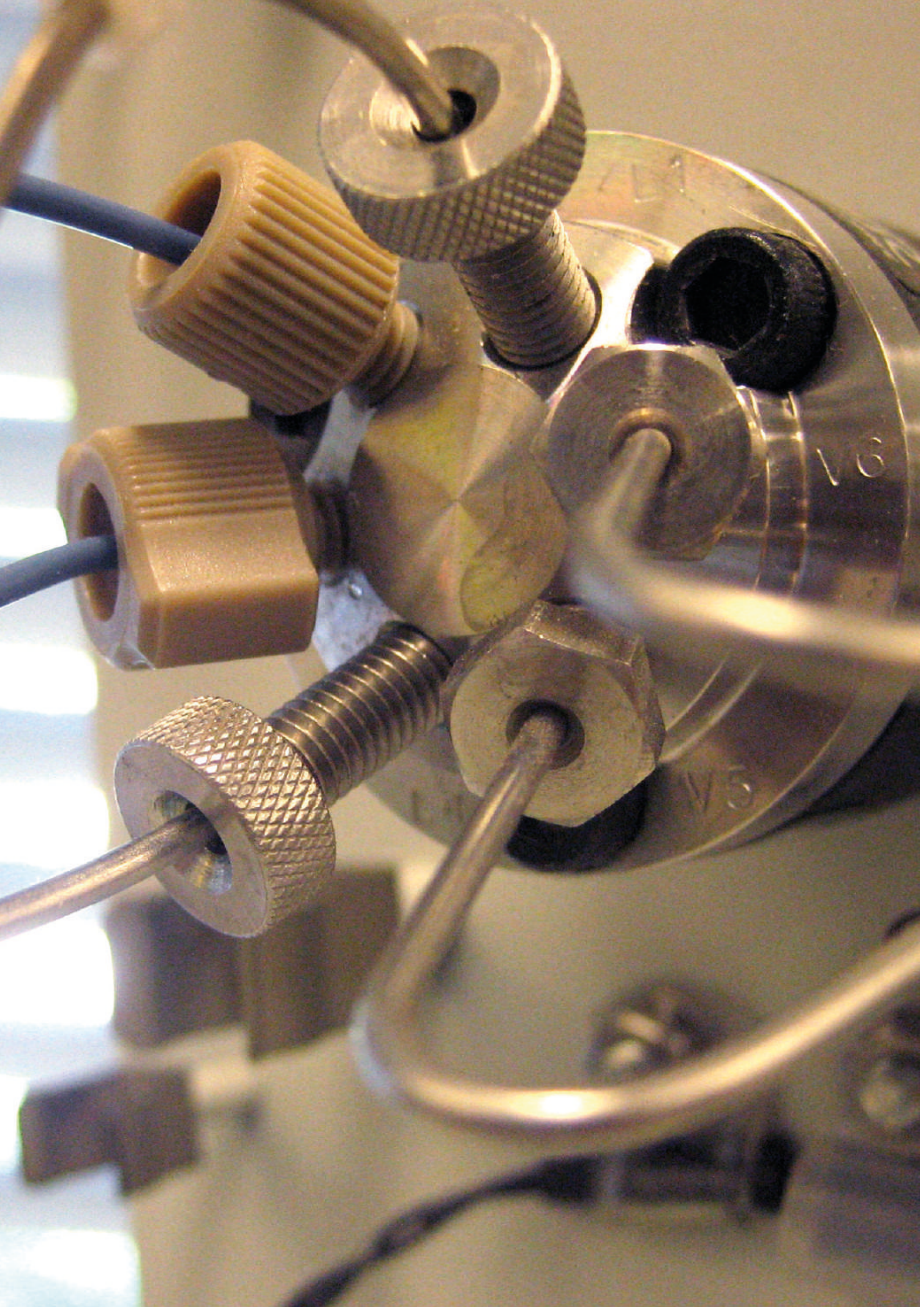
Compound	r²	r²	LODm	LOQm	Precision (intra-day) R % ± RSD (%)			Precision (inter-day) R % ± RSD (%)		
	(MeOH)	(tissue)	(ng g^{-1} dw)	(ng g^{-1} dw)	1-LOQm	5-LOQm	10-LOQm	1-LOQm	5-LOQm	10-LOQm
AVO	0.9999	0.9700	4.68	15.6	86.7 ± 16.9	89.3 ± 4.0	99.4 ± 11.5	121 ± 10.9	121 ± 12.9	127 ± 7.1
4MBC	0.9998	0.9994	6.70	22.3	95.5 ± 13.1	97.6 ± 17.1	110 ± 7.5	71.6 ± 21.7	113 ± 3.7	120 ± 16.8
EHMC	0.9967	0.9998	16.8	56.0	83.6 ± 4.1	108 ± 9.1	73.9 ± 4.5	86.4 ± 14.3	99.0 ± 9.9	90.4 ± 10.2
Et-PABA	0.9998	0.9999	6.93	23.1	112 ± 11.3	127± 22.7	122 ± 22.4	109 ± 9.8	102 ± 23.0	85.4 ± 24.0
BZT	0.9999	0.9989	4.61	15.34	69.4 ± 15.8	75.3 ± 14.5	81.1 ± 3.10	77.1 ± 5.40	69.6 ± 17.0	102.2 ± 19.3
MeBZT	0.9999	0.9999	4.34	14.46	124.6 ± 6.60	129.8 ± 20.9	129.9 ± 13.4	111.9 ± 0.40	101.8 ± 1.60	91.7 ± 2.90
DMBZT	0.9998	0.9982	2.81	9.37	62.7 ± 9.90	64.9 ± 19.2	68.9 ± 4.30	72.0 ± 0.30	91.9 ± 1.00	88.8 ± 1.20
UVP	0.9992	0.9830	2.95	9.83	93.0 ± 4.60	99.2 ± 5.20	88.7 ± 9.70	72.0 ± 1.30	72.0 ± 0.50	91.9 ± 3.90
BePB	0.9996	0.9999	2.45	8.17	63.4 ± 10.5	104.0 ± 3.40	125.5 ± 0.50	76.1 ± 4.20	118.3 ± 3.30	115.3 ± 0.10
BuPB	0.9998	0.9999	3.14	10.44	66.7 ± 10.5	119.1 ± 5.70	111.7 ± 2.60	69.8 ± 4.20	87.3 ± 0.30	70.8 ± 0.30
PrPB	0.9990	0.9999	4.77	15.9	84.2 ± 8.30	113 ± 4.70	115 ± 0.30	84.5 ± 3.60	84.1 ± 0.90	88.7 ± 0.10
MePB	0.9998	0.9999	3.76	12.5	94.8 ± 4.0	124 ± 3.60	108 ± 6.20	71.7 ± 0.10	76.9 ± 0.46	67.0 ± 1.50

Compound	r²	r²	LODm	LOQm	Precision (intra-day) R % ± RSD (%)			Precision (inter-day) R % ± RSD (%)		
	(MeOH)	(tissue)	(ng g^{-1} dw)	(ng g^{-1} dw)	1-LOQm	5-LOQm	10-LOQm	1-LOQm	5-LOQm	10-LOQm
FLU	0.9998	0.9986	2.36	7.85	86.3 ± 1.8	79.7 ± 9.60	118 ± 14.5	81.7 ± 2.50	61.5 ± 1.80	91.1 ± 1.30
OFX	0.9998	0.9998	3.07	10.2	83.8 ± 19.1	119 ± 12.3	110 ± 14.5	110.5 ± 23.2	112 ± 0.60	92.0 ± 0.50
CPX	0.9986	0.9979	20.8	69.3	129 ± 11.2	120 ± 7.40	110 ± 4.6	121.5 ± 12.6	111 ± 7.70	99.6 ± 0.68
NDX	0.9999	0.9997	3.06	10.2	66.4 ± 11.7	83.1 ± 7.50	119 ± 1.60	111.6 ± 3.8	71.5 ± 7.90	111 ± 1.00
OXL	0.9996	0.9985	3.23	10.8	94.3 ± 0.7	79.4 ± 8.80	121 ± 15.1	91.7 ± 2.20	81.5 ± 1.00	94.1 ± 1.20
S-STZ	0.9999	0.9997	3.58	11.9	74.4 ± 4.5	71.2 ± 9.30	123 ± 8.8	81.9 ± 1.2	91.8 ± 3.1	91.6 ± 0.6
SDZ	0.9999	0.9998	3.86	12.9	63.1 ± 4.50	80.7 ± 24.4	114 ± 22.4	61.9 ± 0.80	81.7 ± 0.30	71.5 ± 6.60
acSDZ	0.9999	0.9998	3.33	11.1	73.3 ± 9.90	107 ± 10.9	144 ± 11.5	77.8 ± 0.30	91.7 ± 7.50	82.4 ± 6.30
SMR	0.9998	0.9998	3.07	10.20	91.6 ± 13.30	60.4 ± 22.0	114 ± 12.8	92.8 ± 0.10	71.6 ± 1.30	92.3 ± 9.00
acSMR	0.9999	0.9999	2.89	9.60	79.6 ± 18.2	123 ± 12.2	96.9 ± 19.7	111 ± 0.20	101 ± 3.90	81.3 ± 10.4
SMZ	0.9994	0.9989	11.1	36.9	66.4 ± 7.10	90.7 ± 3.40	95.2 ± 2.40	69.6 ± 2.20	78.6 ± 5.40	77.6 ± 0.60
acSMZ	0.9999	0.9999	3.20	10.6	78.3 ± 4.60	115 ± 11.9	101 ± 11.4	71.8 ± 0.60	91.6 ± 14.1	84.1 ± 14.7

Compound	r²	r²	LODm	LOQm	Precision (intra-day) R % ± RSD (%)			Precision (inter-day) R % ± RSD (%)		
	(MeOH)	(tissue)	(ng g^{-1} dw)	(ng g^{-1} dw)	1-LOQm	5-LOQm	10-LOQm	1-LOQm	5-LOQm	10-LOQm
SMX	0.9997	0.9998	3.77	12.54	89.9 ± 2.8	73.9 ± 3.8	144 ± 26.2	88.6 ± 1.0	61.6 ± 3.7	72.3 ± 12.7
acSMX	0.9998	0.9998	4.27	14.22	90.3 ± 12.2	99.8 ± 10.6	127.8 ± 9.1	97.6 ± 1.4	81.7 ± 4.9	101.5 ± 5.3
SMPZ	0.9999	0.9999	2.66	8.87	81.7 ± 3.5	75.6 ± 12.8	118.4 ± 14.6	111.0 ± 0.6	71.6 ± 9.8	61.2 ± 7.9
SPY	0.9999	0.9998	2.95	9.83	65.3 ± 10.2	67.0 ± 14.7	118.3 ± 7.5	95.4 ± 0.5	81.6 ± 1.5	81.3 ± 9.4
acSPY	0.9999	0.9999	2.67	8.90	70.1 ± 16.1	106 ± 13.5	128 ± 6.90	81.8 ± 1.30	71.6 ± 4.60	101.1 ± 6.60
SQX	0.9998	0.9999	4.19	14.0	71.8 ± 0.80	116 ± 34.6	118 ± 1.60	61.8 ± 0.50	91.7 ± 6.10	77.4 ± 7.20
STZ	0.9999	0.9994	5.56	18.5	82.3 ± 2.30	84.6 ± 12.9	117 ± 11.9	81.9 ± 0.50	61.7 ± 1.20	77.5 ± 8.60
SMD	0.9998	0.9999	2.90	9.66	99.9 ± 22.8	94.1 ± 9.40	112 ± 24.9	71.9 ± 0.20	71.7 ± 2.20	91.5 ± 3.90
SDM	0.9996	0.9999	3.32	11.0	72.3 ± 0.60	116 ± 20.6	140 ± 5.00	102 ± 0.60	101 ± 8.30	111.1 ± 10.4
TMP	0.9996	0.9968	8.86	29.5	95.7 ± 8.90	91.1 ± 7.40	71.4 ± 9.3	91.5 ± 2.70	89.6 ± 0.70	77.9 ± 9.70
GFZ	0.9999	0.9997	2.59	8.62	94.9 ± 23.1	110 ± 6.70	128 ± 13.4	85.1 ± 13.4	104 ± 5.80	130 ± 0.60
MFA	0.9997	0.9999	4.06	13.5	117 ± 5.20	91.3 ± 1.20	87.9 ± 13.2	112 ± 5.00	91.6 ± 0.60	91.2 ± 9.90

Compound	r²	r²	LODm	LOQm	Precision (intra-day) R % ± RSD (%)			Precision (inter-day) R % ± RSD (%)		
	(MeOH)	(tissue)	(ng g^{-1} dw)	(ng g^{-1} dw)	1-LOQm	5-LOQm	10-LOQm	1-LOQm	5-LOQm	10-LOQm
NPX	0.9998	0.9987	3.18	10.6	81.3 ± 4.50	77.3 ± 0.70	119 ± 7.10	85.6 ± 14.5	92.7 ± 7.00	127 ± 4.50
IBU	0.9998	0.9998	3.07	10.2	123 ± 8.60	101 ± 2.80	85.4 ± 4.70	121 ± 21.1	76.7 ± 14.2	107 ± 12.1
KPF	0.9997	0.9998	4.99	16.6	64.1 ± 3.60	104 ± 6.20	125 ± 3.70	111 ± 0.10	92.0 ± 0.30	91.4 ± 1.50
DCF	0.9980	0.9959	34.4	114	77.6 ± 14.2	76.3 ± 13.8	112 ± 2.10	86.9 ± 14.2	104 ± 17.4	67.3 ± 6.70
APH	0.9998	0.9909	13.2	43.9	89.5 ± 6.30	83.6 ± 5.30	82.0 ± 1.40	96.4 ± 0.50	88.6 ± 0.20	99.6 ± 1.60
CBZ	0.9999	0.9999	0.99	3.31	87.0 ± 4.90	101 ± 16.5	137 ± 22.0	91.8 ± 0.20	71.4 ± 8.90	81.0 ± 2.50
CBZ-E	0.9996	0.9999	1.72	5.74	99.9 ± 0.90	81.8 ± 12.5	129 ± 3.40	71.8 ± 1.10	61.4 ± 2.90	101 ± 2.20
ATL	0.9992	0.9991	14.1	47.0	65.5 ± 3.50	61.8 ± 0.10	73.1 ± 12.9	110 ± 5.30	101 ± 20.1	85.0 ± 12.5
NorFXT	0.9974	0.9993	6.58	21.9	88.2 ± 3.90	65.2 ± 1.60	110 ± 10.8	91.8 ± 0.80	71.6 ± 4.50	99.3 ± 3.30
N-desVFX	0.9991	0.9979	9.62	32.0	86.4 ± 3.20	87.8 ± 5.20	79.8 ± 13.4	111 ± 23.6	98.6 ± 7.60	77.5 ± 1.70
SCY	0.9998	0.9999	3.07	10.0	102 ± 24.5	104 ± 22.1	120 ± 26.9	121 ± 20.8	85.1 ± 14.6	112 ± 13.7
CFF	0.9994	0.9951	14.5	48.3	66.5 ± 5.90	61.8 ± 13.8	111 ± 11.2	61.7 ± 0.30	78.5 ± 4.30	101 ± 4.30

Compound	r^2	r^2	LODm	LOQm	Precision (intra-day) R % ± RSD (%)			Precision (inter-day) R % ± RSD (%)		
	(MeOH)	(tissue)	(ng g^{-1} dw)	(ng g^{-1} dw)	1-LOQm	5-LOQm	10-LOQm	1-LOQm	5-LOQm	10-LOQm
DIU	0.9996	0.9996	8.81	29.3	70.1 ± 1.10	111 ± 36.6	126 ± 19.0	112 ± 0.20	71.9 ± 0.70	82.8 ± 0.40
IRG	0.9996	0.9992	3.32	11.1	71.0 ± 3.90	113 ± 23.9	85.1 ± 11.9	71.9 ± 5.70	81.8 ± 2.20	91.7 ± 1.70
DCOIT	0.9985	0.9991	7.00	23.3	102 ± 9.50	86.5 ± 5.80	104 ± 19.0	81.3 ± 4.20	77.6 ± 4.90	88.1 ± 0.50
DFL	0.9997	0.9984	3.67	12.2	104 ± 9.80	109 ± 10.8	68.3 ± 13.0	77.9 ± 2.60	80.3 ± 5.20	99.7 ± 10.1



3.3 Discussion

The results compiled in this chapter represent novel data on CECs' occurrence, fate and behaviour in the environment, showing their ubiquity and concern, especially in stressed coastal areas. In some of the published works, the first reported levels of PCPs were found in specific matrices and regions. Although the individual conclusions of each work are detailed in the manuscripts, this section discusses the general findings on the occurrence of CECs in the marine environment studied.

CECs' distribution among matrices

UVFs and PBs are reported for the first time on the Tunisian coastline, and the levels found in the seawater and sediments are similar to reported values in other regions (Díaz-Cruz et al., 2019; Loos et al., 2013; Tashiro and Kameda, 2013). However, the compounds found in the water column and the sediments show different patterns since some that are not detected in water accumulate in the sediments. A pattern that seem to follow the sediments and fish from Brazil, too, since the compounds found at the highest concentrations in the fish were not detected in the sediments, and vice versa. In theory, the concentration of these compounds in lipophilic matrices (such as sediments, fish, or seagrass) should be higher than in matrices with lower organic/lipidic content, such as water. Actually, compounds such as sulfisobenzone (BP4) with low logK_{ow} values can reach lipophilic organisms (such as *Posidonia oceanica*) and bioaccumulate up to high concentrations. And other highly lipophilic such as 2-ethyl-hexyl-4-trimethoxycinnamate (EHMC), are extensively present in water but barely in sediments. There are more complex mechanisms behind bioaccumulation, but in some cases, lipophilicity is still valuable to have an estimation of the behaviour in the environment.

The concentrations and compounds detected in the different locations and environmental matrices seem logical. For example, BP3 was present in all the seawater samples from Tunisia but not detected in the sediments (neither from Tunisia nor Brazil), barely detected in the seagrass and below the limit of quantification (<LOQ) in the fish, showing that despite its lipophilicity, its presence in sediments and biota is not related with the levels in the surrounding water. Methyl benzotriazole (MeBZT) was not detected in water or fish. Still, it presented similar concentrations in the sediments from Tunisia and Brazil (7.6 and 3.5 ng/g dw average, respectively), and it was also present in the *Posidonia oceanica* at similar rates (1-6.3 ng/g dw). Very similar patterns were observed for the ultraviolet filter AVO and the preservative MePB since both were present in the sediments from Tunisia and Brazil and also in the *Posidonia oceanica* but absent or barely present in the fish. The occurrence of these CECs in the sediments and seagrass could be justified by its high $\log K_{ow}$ (4.51 for AVO) and extensive use (MePB and MeBZT, a benzotriazole derivative, probably with a common origin), but its absence in the fish suggests that other mechanisms intervene in the bioaccumulation processes.

Indeed, the fish accumulation pattern was completely different from the "similar" matrices (sediments or seagrass) in terms of hydrophobicity. The UVFs 4HB and benzocaine (EtPABA) were accumulated at high concentrations (> 39 ng/g dw) in fish tissues, but they were absent in the sediments or the seagrass. EtPABA was detected at high concentrations in fish muscle and liver (>2800 ng/g dw) and lower ones in the stomach and gills (> 10 ng/g dw), while 4HB was only detected in the fish liver. These results demonstrate a concerning bioaccumulation tendency in fish aimed for human consumption by compounds usually left out in CECs monitoring.

Finally, in both works from Brazil, many pharmaceuticals and four biocides not analysed in the works from Tunisia and Mallorca were included. Regarding pharmaceuticals, ciprofloxacin (CPX) was the only one detected above the LOQ in sediments (9.5 ng/g dw average), and salicylic acid (SCY) the only one in fish, but it was detected in all tissues at considerable concentrations (> 57 ng/g dw). Regarding biocides, IRG and 4,5-dichloro-2-octyl-4-isothiazolin3-one (DCOIT) were detected in the sediments (1.7 ng/g dw and 12.2 ng/g average, respectively), with DCOIT present in all analysed samples. In fish, however, both were detected only <LOQ. Therefore, pharmaceuticals and biocides occurrence also supports the theory that accumulation in sediments and bioaccumulation in fish follows different mechanisms.

CECs' temporal trends

The temporal evaluation of CECs' occurrence is not easy since most of them have been analysed for a short period of time due to the recent advances in analytical techniques and the novel findings about their concerning occurrence. However, the analysis of *Posidonia oceanica* supposed a unique opportunity to study the accumulated concentrations of UVFs and PBs over time in zones with different tourism impacts. As explained, this was possible thanks to the internodal length of seagrass following oscillating year cycles.

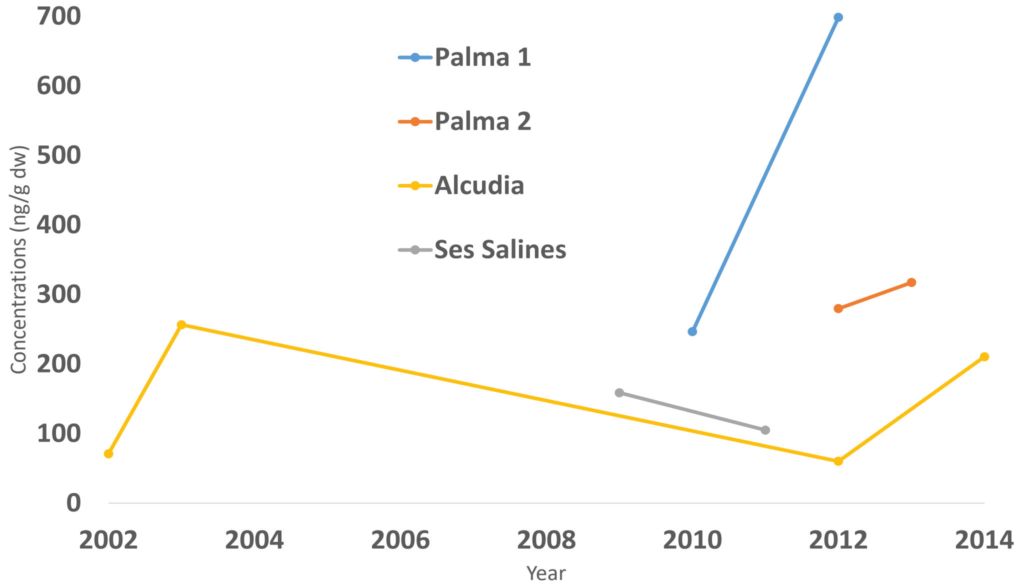


Figure 3.3. Temporal trend of the cumulative concentrations of UVFs and PBs in the selected sampling sites.

Thus, the oldest samples dated from 2002 (20 years ago) and the most recent from 2014, demonstrating that UVFs and PBs were already accumulated in the seagrass two decades ago. The temporal trends are shown in **Figure 3.3**, where it can be observed that the highest concentrations were found in sampling points 1 and 2 of Palma. It could be expected since these are the closest points to an urban centre. Even though only two points per sampling site are available, both present a concerning increase in concentration with time, especially site 1. Alcudia point showed lower contamination values (understandable since 20 times fewer inhabitants live there). The trend is variable, but a big gap of missing data between 2003 and 2012 makes it challenging to extract sound conclusions.

Finally, the Ses Salines point presented similar levels to the ones found in Alcudia, showing a similar trend (slightly decreasing). This tendency could indicate that old concentration peaks are slowly decreasing due to the degradation and diffusion of the contaminants. However, more sampling periods are needed to support these arguments, even though an increase in concentrations is observed in the last years.

Antrophogenic influence

The correlation between the UVFs and PBs present in the marine environment and the population living near the sampling point was evaluated. To do so, the data collected in Publication #1 and #2 were used because both studies had sampling sites with very different anthropogenic influences (Palma city-Ses Salines in Publication #1, Site M-Site Ch in Publication #2). The sampling points selected in both publications (#3 and #4) from Brazil were in zones strongly influenced by human activities, but no other point was far from anthropogenic focus. Therefore, they were not included in this discussion.

Palma and Site M sampling points were very close to large urban centres (Palma and Melloulech cities, respectively). Palma has 410,000 and Melloulech 24,600 censed inhabitants (National Statistics Offices, 2022), which are the values used for the statistical analysis. However, it must be considered that both cities are very tourist, so these numbers increase considerably in periods of good weather. On the other hand, the scenario was different in remote areas such as Ses Salines or Site R, where the sampling point was far away from any urban centre or population numbers were much lower (11,000 and 5,000 inhabitants, respectively) (National Statistics Offices, 2022); the levels of contamination in these sites were considerably lower.

Statistically, the correlation between the sum of the concentrations (ng/g in the case of solid matrices, ng/mL in the case of seawater) and the population in the area was evaluated with a Pearson test (α > 0.05). A strong positive correlation (r2 = 0.807 or α = 0.000412) was found. This indicates that the concentrations of UVFs and PBs found in the environmental matrices were closely related to intense human activity. This is clearer when observing **Figure 3.4**, where the normalised values are represented.

The concentration values (bars) match the number of inhabitants in each site. It is also remarkable that sites like Ses Salines and Alcudia, with a low population (compared with Palma), still present considerable contamination levels.

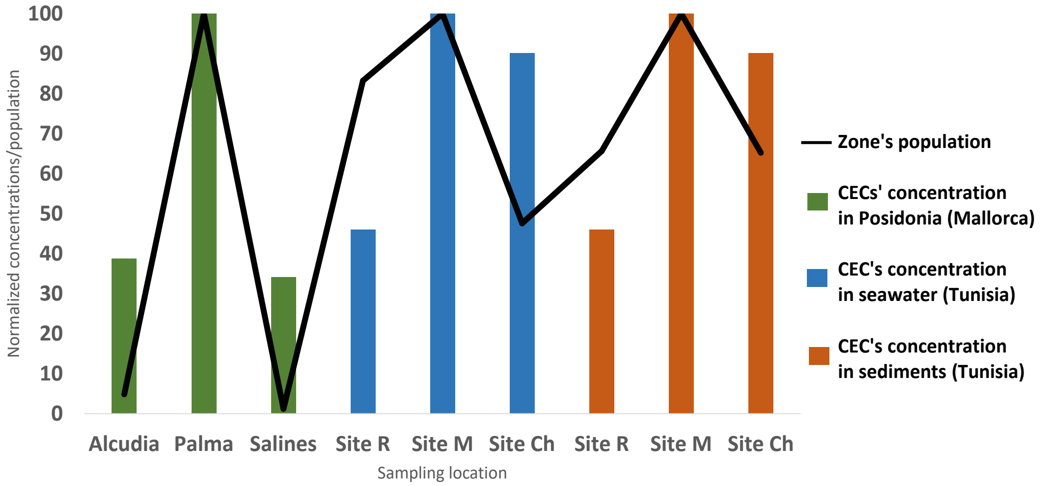


Figure 3.4. Normalized CECs' concentrations (ng/g in the case of solid matrices, ng/mL in the case of seawater) in *Posidonia oceanica*, seawater and sediments (bar graph) and normalized inhabitants of each sampling region (trend line).

These outcomes could be explained by the fact that, even if pollution is mainly produced in urban centres, some of these compounds can spatially spread, contaminating nearby zones. However, as mentioned, this contamination could also be a product of the tourist population, not included in the statistical analysis since its variability makes it very difficult.

Thus, after the concerning correlation observed between the contaminants' occurrence and anthropogenic activity, it is necessary to continue investigating new ways to reduce these concentrations in the environment. It could be done either with more efficient elimination techniques in the WWTPs or by implementing new regulations that limit or prohibits their use, putting the focus on the contamination source.

3.4 References

Bao, V.W.W., Leung, K.M.Y., Qiu, J.W., Lam, M.H.W., 2011. Acute toxicities of five commonly used antifouling booster biocides to selected subtropical and cosmopolitan marine species. Mar. Pollut. Bull. 62, 1147–1151. https://doi.org/10.1016/j.marpolbul.2011.02.041

Brumovský, M., Bečanová, J., Kohoutek, J., Borghini, M., Nizzetto, L., 2017. Contaminants of emerging concern in the open sea waters of the Western Mediterranean. Environ. Pollut. 229, 976–983. https://doi.org/https://doi.org/10.1016/j.envpol.2017.07.082

Caldas, S.S., Soares, B.M., Abreu, F., Castro, Í.B., Fillmann, G., Primel, E.G., 2018. Antifouling booster biocide extraction from marine sediments: a fast and simple method based on vortex-assisted matrix solid-phase extraction. Environ. Sci. Pollut. Res. 25, 7553–7565. https://doi.org/10.1007/s11356-017-0942-x

Celma, A., Gago-Ferrero, P., Golovko, O., Hernández, F., Lai, F.Y., Lundqvist, J., Menger, F., Sancho, J. V., Wiberg, K., Ahrens, L., Bijlsma, L., 2022. Are preserved coastal water bodies in Spanish Mediterranean basin impacted by human activity? Water quality evaluation using chemical and biological analyses. Environ. Int. 165. https://doi.org/10.1016/j.envint.2022.107326

Díaz-Cruz, M.S., Molins-Delgado, D., Serra-Roig, M.P., Kalogianni, E., Skoulikidis, N.T., Barceló, D., 2019. Personal care products reconnaissance in EVROTAS river (Greece): Water-sediment partition and bioaccumulation in fish. Sci. Total Environ. 651, 3079–3089. https://doi.org/10.1016/j.scitotenv.2018.10.008

Gago-Ferrero, P., Díaz-Cruz, M.S., Barceló, D., 2011. Fast pressurized liquid extraction with in-cell purification and analysis by liquid chromatography tandem mass spectrometry for the determination of UV filters and their degradation products in sediments. Anal. Bioanal. Chem. 400, 2195–2204. https://doi.org/10.1007/s00216-011-4951-1

Gago-Ferrero, P., Mastroianni, N., Díaz-Cruz, M.S., Barceló, D., 2013. Fully automated determination of nine ultraviolet filters and transformation products in natural waters and wastewaters by on-line solid phase extraction-liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1294, 106–116. https://doi.org/10.1016/j.chroma.2013.04.037

Gorito, A.M., Ribeiro, A.R., Almeida, C.M.R., Silva, A.M.T., 2017. A review on the application of constructed wetlands for the removal of priority substances and contaminants of emerging concern listed in recently launched EU legislation. Environ. Pollut. 227, 428–443. https://doi.org/10.1016/j.envpol.2017.04.060

Karlsson, J., Ytreberg, E., Eklund, B., 2010. Toxicity of anti-fouling paints for use on ships and leisure boats to non-target organisms representing three trophic levels. Environ. Pollut. 158, 681–687. https://doi.org/10.1016/j.envpol.2009.10.024

León, V.M., Viñas, L., Concha-Graña, E., Fernández-González, V., Salgueiro-González, N., Moscoso-Pérez, C., Muniategui-Lorenzo, S., Campillo, J.A., 2020. Identification of contaminants of emerging concern with potential environmental risk in Spanish continental shelf sediments. Sci. Total Environ. 742, 140505. https://doi.org/10.1016/j.scitotenv.2020.140505

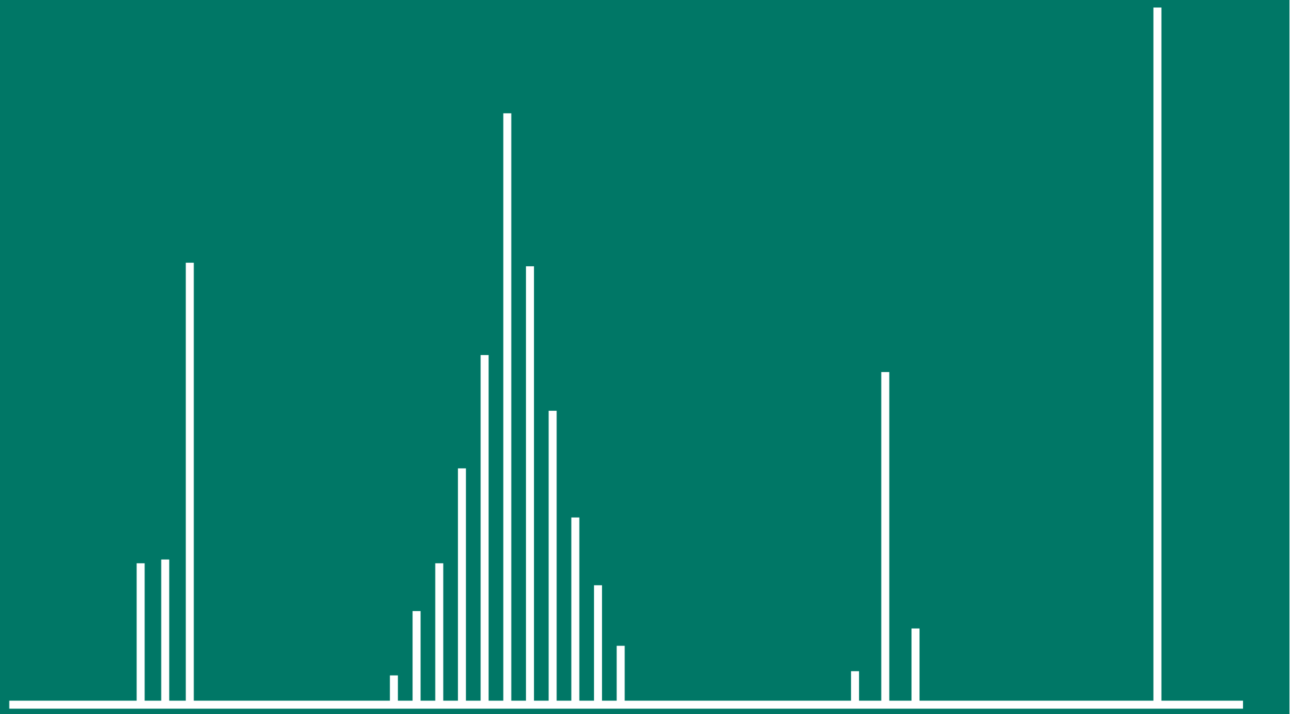
Loos, R., Tavazzi, S., Paracchini, B., Canuti, E., Weissteiner, C., 2013. Analysis of polar organic contaminants in surface water of the northern Adriatic Sea by solid-phase extraction followed by ultrahigh-pressure liquid chromatography-QTRAP® MS using a hybrid triple-quadrupole linear ion trap instrument. Anal. Bioanal. Chem. 405, 5875–5885. https://doi.org/10.1007/s00216-013-6944-8

National Statistics Offices, 2022, 2022. Population statistics [WWW Document]. URL https://www.citypopulation.de/

Starling, M.C.V.M., Amorim, C.C., Leão, M.M.D., 2019. Occurrence, control and fate of contaminants of emerging concern in environmental compartments in Brazil. J. Hazard. Mater. 372, 17–36. https://doi.org/10.1016/j.jhazmat.2018.04.043

Tashiro, Y., Kameda, Y., 2013. Concentration of organic sun-blocking agents in seawater of beaches and coral reefs of Okinawa Island, Japan. Mar. Pollut. Bull. 77, 333–340. https://doi.org/10.1016/j.marpolbul.2013.09.013

United Nations Environment Programme, 2022. The marine environment is an essential component of the global life-support system [WWW Document]. URL https://www.unep.org/news-and-stories/story/marine-environment-essential-component-global-life-support-system



Chapter 4

The challenge of CECs' removal

4.1 Introduction

The occurrence of CECs in the environment, as explained in Chapter 3, demonstrates that conventional WWTPs are inefficient in removing them. Ideally, the solution to reduce the occurrence of CECs in the environment would be the introduction of elimination/removal technologies complementary to those currently in use that would altogether remove these contaminants from wastewater. However, the solution is more complex than that. First, implementing new methodologies capable of removing CECs is usually very expensive as they present a wide range of physicochemical properties (Morin-crini et al., 2021). It means that the implemented technologies need to be robust and provide the required conditions for the degradation or retention of the contaminants. Furthermore, the maintenance of these technologies needs to be minimal since they are designed to treat large volumes of (waste) water continuously; frequent maintenance would make them expensive and useless in the long term.

A common problem when eliminating CECs is the formation of new TPs (Starling et al., 2019). This is particularly remarkable when induced or drastic degradation processes, such as chlorine disinfection, are used. These new TPs, sometimes unknown, can present different physicochemical properties and, in some cases, are even more toxic than the parent compounds (Menger et al., 2021). Therefore, an efficient elimination technology for CECs must remove them from the wastewater by degrading or retaining them, but the same applies to the formed TPs.

Since this problem with CECs has been known for years, a wide variety of technologies and materials have been tested. **Table 4.1** summarises the most commonly applied technologies for CECs removal from wastewater. In general, the technologies that achieve higher removal efficiencies present high investment costs (in terms of installation, maintenance, and operation) and certain issues related to their continuous operation at a real scale.

Table 4.1. Summary of the advantages and disadvantages of the most common elimination/removal technologies used for CECs removal. (Morin-crini et al., 2021; Norton-Brandão et al., 2013)

Technology	Advantages	Disadvantages
Adsorption processes		
Activated carbons	No formation of by-products, flexible and easy process operation	High operability, moderate investment costs, rapid saturation
Clay-based adsorbents	No formation of by-products, low investment costs	Low adsorption capacity, moderate operating costs
Polymers	No formation of by-products, high removal rates	High operability, high investment costs, no real-scale studies
Metal-organic frameworks	Capable to remove and degrade, high removal rates	Low stability material, high operability, high operating costs
Chitosan-based materials	No formation of by-products, great performance	No real-scale studies, commercial limitations
Nanocellulose	No formation of by-products, low cost, high removal rates	No real-scale studies, commercial limitations
Biological processes		
Wetlands	Low maintenance costs and energy usage, no formation of by-products	Large footprint, efficiency depending on meteorological conditions
Algal/fungi based strategies	Low maintenance costs and energy usage, no formation of by-products	Large footprint, difficult to control conditions on large scale
Membrane filtration	No formation of by-products, wide range of membranes	High investment costs, high operating costs, fast clogging
Advanced processes		
Wastewater desinfection (e.g. chlorination)	Low operating costs	High operability, high formation of by products
UV radiation	No formation of by-products, low operating costs	High operability, moderate investment costs
Ozonation	Low formation of by-products	High operability, high investment costs
Electrochemical	Low maintenance costs, low investment costs	High formation of by products, energy usage

Otherwise, lower-cost technologies usually require more substantial degradation steps that produce high quantities of by-products.

From the information in **Table 4.1**, it seems that electrochemical processes are the most promising degradation technology. It requires a very low initial investment and maintenance, but the main drawback is the extensive formation of TPs. Thus, the first degradation technology tested in this thesis was anodic oxidation at a lab scale (Publication #8). It was applied to degrade 30 pharmaceuticals and generate hydroxyl radicals that favour the mineralisation of the formed TPs. The feasibility of this technique was assessed in a secondary effluent from a WWTP, and a non-target screening of the CECs present was carried out to search for new TPs formed during the oxidation process.

Another interesting CECs’ degradation technology, according to **Table 4.1**, are algal ponds (as well as wetlands) thanks to their low cost, efficiency, and no by-product formation. The only drawback is the difficulty in controlling the conditions (temperature, pH, dissolved oxygen, etc.) at a field scale. A study on a pilot high-rate algae pond (HRAP) system was carried out in this thesis to evaluate the removal efficiency of PPCPs from different types of water (Publications #5 and #6). The pilot HRAP system is shown in **Figure 4.1**. The system was located in Agropolis, an experimental area belonging to the Polytechnic University of Catalonia facilities (Barcelona Spain), consisting of two tanks continuously homogenised with paddle wheels. These tanks were connected by 16 horizontal tubes, facilitating the flow by gravity between them. The system was inoculated with a mix of microalgae and bacteria. Further information about this system can be found elsewhere (García-Galán et al., 2018; Uggetti et al., 2018).

In recent years, the trend in removal technologies has been to couple them on line to combine their advantages and achieve higher CECs’ removal rates. This was the purpose when constructing a pilot soil aquifer treatment (SAT) system with reactive barriers. It was located in the WWTP of Palamós (Spain) to evaluate its performance to improve the removal rate of the remaining CECs in the secondary effluent of the WWTP. One of the barriers was built only with sand and used as a reference, and the other five were made with different combinations of natural materials, such as sand, compost, woodchips, or clay. Therefore, the wastewater infiltrated through the barrier is renaturalized when it reaches the aquifer, where additional natural processes occur, improving the chemical quality of the aquifer water. A general view and a representative scheme of the system are presented in **Figure 4.2**. Further details of this experimental system can be consulted elsewhere (Valhondo et al., 2020).

The system's performance in removing PPCPs from the secondary WWTP effluent was evaluated, constituting Publication #7. This paper also incorporates the assessment of POCIS, a novel sampling approach in a SAT system that allows obtaining composite samples over long periods, achieving more representative data of the actual status of the water. This is crucial in systems with a continuous water flow, such as WWTPs, since the contamination in the sample will vary very quickly and will differ depending on the season. With the help of these integrative samplers, the information obtained is from the entire load of contaminants present in the (waste)water during, for example, a week, giving much more representative information data.

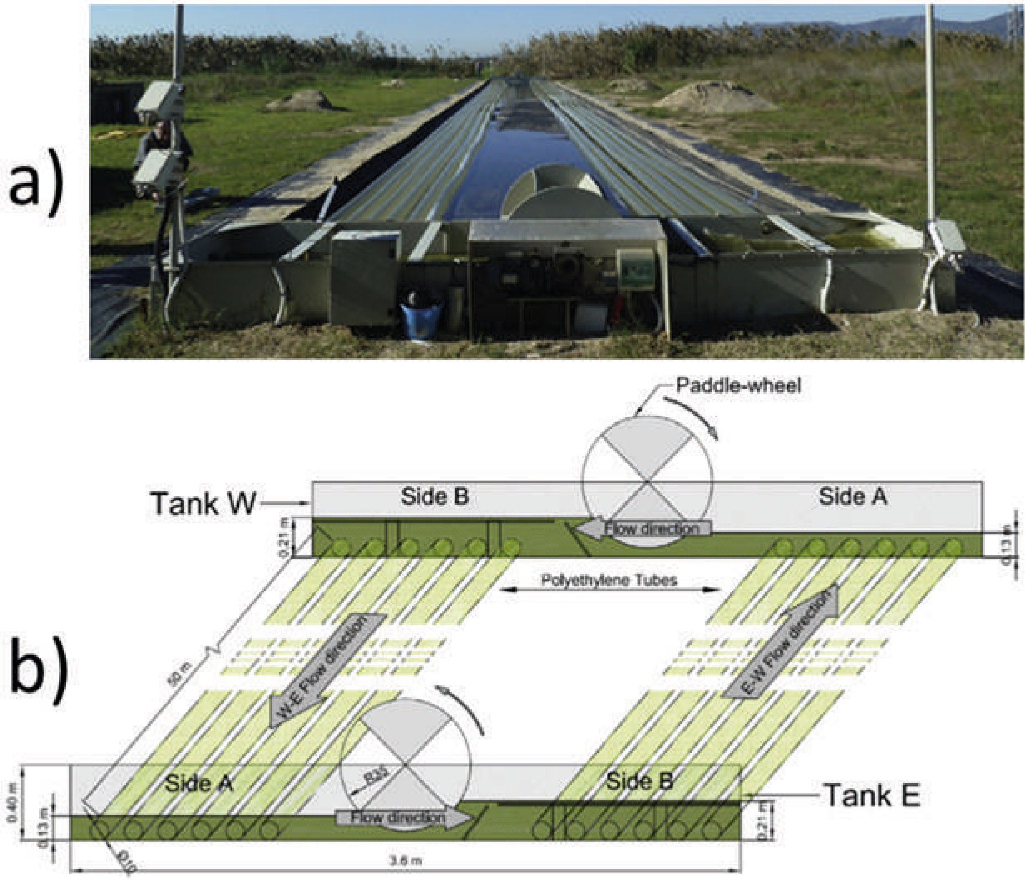


Figure 4.1. . a) View of the full-scale HRAP system operating at full capacity. b) Flow diagram and sketches of different parts of the full-scale HRAP system. (García-Galán et al., 2018)

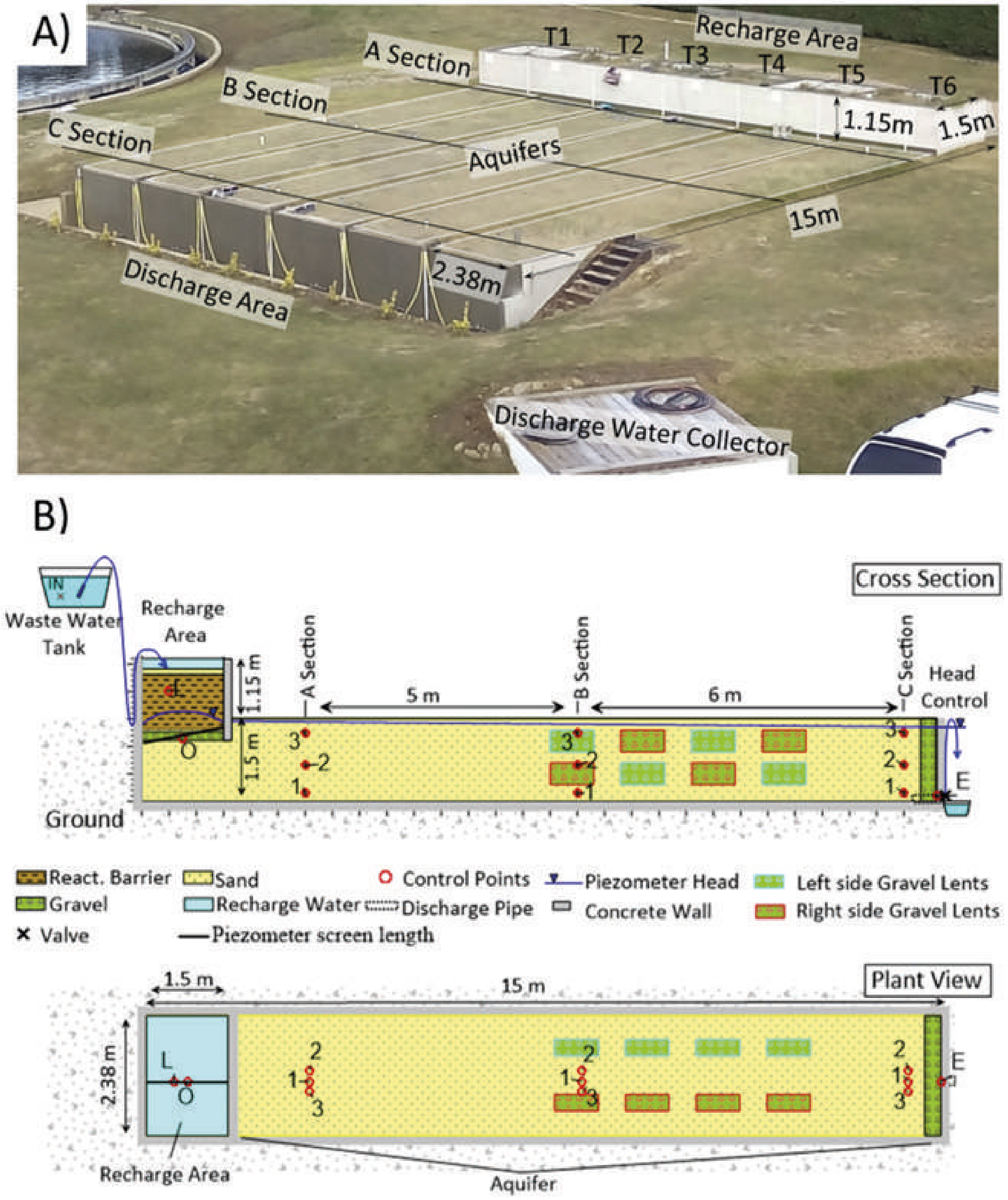


Figure 4.2. a) General view of pilot system, with the recharge and discharge areas, piezometer sections and replicates T1 through T6. B) Cross section and plant view scheme of one of the systems with the recharge area, heterogeneity, and monitoring points. (Valhondo et al., 2020)

Methodologies

For analysing the different waters included in this chapter (wastewater and SAT-reclaimed water), our previous published methodologies were adapted or directly applied.

In Publication #8, the suspect screening of wastewater was performed with a database from the Norman Network (Taha et al., 2022). In Publications #5 and #6, the water was analysed following the same online-SPE-HPLC-MS/MS methodology explained in Chapter 3 for the seawater samples, based on Gago-Ferrero et al., (2013).

Finally, in Publication #7, it is important to remark that the water samples were obtained with POCIS. The obtained extracts from the passive samplers were analysed with a target analysis of PPCPs by HPLC-MS/MS under the same conditions used in the previous publications.

4.2 Results

The results of this chapter are described in three published and one submitted for publication articles. The publications are:

- Publication #5: Vassalle, L., **Sunyer-Caldú, A.**, Uggetti, E., Díez-Montero, R., Diaz-Cruz, M. S., García, J., García-Galán, M. J., “Bioremediation of emerging micropollutants in irrigation water. The alternative of microalgae-based treatments”, 2020, *Journal of Environmental Management*, https://doi.org/10.1016/j.jenvman.2020.111081

- Publication #6: Vassalle, L., **Sunyer-Caldú, A.**, Uggetti, E., Díez-Montero, R., Diaz-Cruz, M. S., García, J., García-Galán, M. J., “Behavior of UV Filters, UV Blockers and Pharmaceuticals in High Rate Algal Ponds Treating Urban Wastewater”, 2020, *Water*, https://doi.org/10.3390/w12102658

- Publication #7: **Sunyer-Caldú, A.**, Benedetti, B., Valhondo, C., Martínez-Landa, L., Carrera, J., Di Carro, M., Magi, E., Diaz-Cruz, M. S., “Using integrative samplers to estimate the removal of pharmaceuticals and personal care products in a WWTP and by soil aquifer treatment enhanced with a reactive barrier”, *Submitted to Science of the Total Environment.*

- Publication #8: Calzadilla, W., Espinoza, C., Diaz-Cruz, M. S., **Sunyer-Caldú, A.**, Aranda, M., Peña-Farfal, C., Salazar, R., “Simultaneous degradation of 30 pharmaceuticals by anodic oxidation: Main intermediaries and by-products”, 2021, *Chemosphere*, https://doi.org/10.1016/j.chemosphere.2020.128753

4.2.1 Publication #5

Bioremediation of emerging micropollutants in irrigation water. The alternative of microalgae-based treatments

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Research article

Bioremediation of emerging micropollutants in irrigation water. The alternative of microalgae-based treatments

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ABSTRACT

The present study evaluated the efficiency of a semi-closed horizontal tubular photobioreactor (PBR) at demonstrative scale to remove a total of 35 target compounds, including benzotriazoles, benzophenones, antibiotics and different pharmaceuticals present in irrigation water in a peri-urban rural area. This water run through an open channel and was a mixture of reclaimed wastewater from a nearby wastewater treatment plant (WWTP) and run-off from the different agricultural fields in the area. Most of the compounds studied are usually not fully eliminated during conventional wastewater treatment, which justifies the need to investigate alternative treatment strategies. A total of 21 of these compounds were detected in the irrigation water. Benzotriazoles were only partially removed after the microalgae treatment, with elimination rates similar to those of conventional WWTPs. The UV filter benzophenone-3 (BP3) showed variable removals, ranging from no elimination to 51%, whereas 4-methylbenzilidenecamphor (4MBC) was completely eliminated. Regarding pharmaceuticals, average removals were higher, in the range of 60–100%, with the exception of the antibiotics sulfamethoxazole (46%) and sulfapyridine, which was not removed. Despite the low biomass productivity of the PBR, parameters such as the size of the reactors, the specific mixed cultures developed and the high temperatures and pH in the closed system may account for the overall good results, The efficiency and sustainability of these systems make them a solid, feasible treatment choice.

1. Introduction

Pharmaceuticals and personal care products (PPCPs) have become an integral part of our daily life. Their frequent consumption and usage have led to their regular entrance into the environment, being the aquatic ecosystems the most vulnerable. Indeed, they are constantly receiving these inputs from both point sources such as wastewater treatment plant (WWTP) effluents (urban, rural or industrial) (García-Galán et al., 2011; Molins-Delgado et al., 2015; Verlicchi et al., 2010) or from non-point sources such as urban or agricultural run-off waters, intensive cattle farming or biosolids application from WWTPs (Dolliver and Gupta, 2008; Sabourin et al., 2009). In rural areas (including also peri-urban croplands), irrigation or strong precipitation events can lead to the loss of different pollutants from the soils such as pesticides, inorganic fertilizers or residues of different PPCPs contained in the applied biosolids. Drainage and open irrigation channels can receive a large amount of this rural run-off, but they usually discharge into rivers and not in other main collectors towards WWTPs. In consequence, a huge variety of these organic micropollutants eventually reach surface waters and groundwater bodies, and may indirectly affect to different non-target organisms and the ecological status of the receiving aquatic ecosystems (Langdon et al., 2010; Postigo et al., 2016; Proia et al., 2013).

During the last two decades, intensive monitoring campaigns on the occurrence of PPCPs have been carried out, demonstrating the inefficient removal for most of them during conventional activated sludge (CAS) wastewater treatment , and their ubiquity in basically all type of environmental matrices, including tap water (Díaz-Cruz et al., 2012;

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Dolar et al., 2012; Gros et al., 2012; Serra-Roig et al., 2016). Apart from pharmaceuticals, personal care products (PCPs) comprise a wide group of chemicals of daily use such as soaps and detergents, toothpastes, sunscreens, cosmetics, biocides, fragrances and insect repellents amongst others. Their continuous release into the environment has led to their classification as *pseudo*-persistent contaminants (Tolls et al., 2009) and understanding their fate and behaviour in the aquatic ecosystems shoud be a priority, considering their potential to bioaccumulate and biomagnify through the trophic chain, and their subsequent negative impact on the receiving ecosystems (Fent et al., 2010). For instance, it has been demonstrated that two metabolites of benzophenone-3 (BP3), 2,4-dihydroxybenzophenone (BP1) and 4-hydroxybenzophenone (4HB) show a high estrogenicity against rainbow trout (Kunz and Fent, 2009). Nevertheless, available ecotoxicity data for many sunscreen agents is still scarce.

Similarly, benzotriazoles are high production volume chemicals which have become crucial in many industrial processes. They are used as UV blockers or stabilizers of different plastic products, as corrosion inhibitors in detergents, antifreezing or antifogging agents in photography or airplane fluids (Asimakopoulos et al., 2013; Gatidou et al., 2019; Liu et al., 2012, 2011a). 1H-benzotriazole (BZT, also found as BTri) and 5-methyl-1H-benzotriazole (5-MeBZT, also found as TTri) are the two UV blockers most frequently detected in environmental samples, as they are poorly volatile and only partially removed during CAS treatment due to their high polarity and low biodegradability (Asimakopoulos et al., 2013; Molins-Delgado et al., 2015; Reemtsma et al., 2010). Some studies have shown that benzotriazoles can ultimately accumulate in humans, being detected in human adipose tissues, urine and amniotic fluid samples (Li et al., 2018; Wang et al., 2015). Regarding their ecotoxicity, there are only a few studies and yet all have evidenced that these chemicals have endocrine disrupting properties, impairing oxidative stress, hepatotoxicity and neurotoxicity in freshwater and marine fish (Liang et al., 2017, 2016; 2014; Tangtian et al., 2012).

Regarding pharmaceuticals, thousands of tons of different classes are consumed regularly in both human and veterinary medicine. It is estimated that pharmaceuticals usage will reach 4.5 trillion doses per day in adults worldwide by 2020 (Patel et al., 2019). After usage and excretion, both the metabolites and the remnants of the original drug are released into the environment where they may resist biodegradation and bioaccumulate, depending on their physical and chemical properties (Daughton, 2013). In these cases, they could also pose a toxicological risk to different non-target organisms, altering the ecosystem dynamics, as is the case of antibiotics and the development of antibiotic resistant bacteria and genes (including pathogens) (Kümmerer, 2009, 2004; Rodriguez-Mozaz et al., 2015).

Taking all this information into account, the need to find alternative and more efficient treatments is evident. Nature-based, low-cost treatment systems such as microalgae-based systems or constructed wetlands are currently being intensively investigated and, so far, with promising results regarding PPCPs removal (Ávila et al., 2014; García-Galán et al., 2018; Matamoros et al., 2015; Vassalle et al., 2020a). In particular, microalgae-based treatments, despite having been operative since the 50's, are recently gaining a renewed popularity due to their high efficiency removing nutrients and organic matter within a much more sustainable frame than conventional treatments. Indeed, these systems can operate maintaining low operation and maintenance (O&M) costs, as they do not require external aeration due to photosynthesis, or any chemical input (García et al., 2006; Muñoz and Guieysse, 2006). Microalgae biomass grows fixating CO_2 and assimilating the nutrients (mostly nitrogen (N) and phosphorus (P)) present in the influent wastewater. Through photosynthesis, microalgae generate the oxygen needed by heterotrophic bacteria to aerobically degrade the organic matter present in the water (including organic micropollutants). These systems have the dual advantage of treating wastewater efficiently and, simultaneously, producing microalgae biomass which, after an appropriate harvesting/separation technique from the aqueous phase, can be further profited to produce bioenergy (biogas) or other added-value products such as pigments, biofertilizers or even bioplastics (Arashiro et al., 2018; Rueda et al., 2020; Vassalle et al., 2020b). If managed sustainably (with proper use), the waste generated in this overall process is considerably reduced, as well as the energy requirement for this system, when compared to conventional systems. For microalgae bioremediation, there are two basic types of systems: open and closed systems. Open systems or high rate algal ponds (HRAPs) have already been used for decades not only for wastewater treatment but in industrial microalgae production (Chisti, 2013; Oswald, 1995). HRAPs are the most frequently used systems, mainly due to their lower energy requirements, as well as O&M costs, but cultures can be easily contaminated and the control of the different growth and environmental parameters (temperature, sunlight) is worse than that of closed systems (Park and Craggs, 2010). Closed systems avoid these drawbacks and usually yield higher biomass productions. However, the costs of O&M are higher (higher energy requirements for mixing), dissolved oxygen may accumulate to toxic levels and biofouling may appear in the inner walls. It seems consistent that a new design of a hybrid, semi-closed PBR, combining the advantages and avoiding the shortcomings of both open and closed systems, could yield the highest efficiencies in biomass yield and wastewater bioremediation. Regarding their efficiency in PPCPs removal, studies on open systems are predominant, but yet scarce. In recent studies, removal efficiencies (RE%) between 40% and >90% have been reported in HRAPs treating sewage (García-Galán ET AL., 2020b; Vassalle et al., 2020a). Regarding closed or semi-closed systems, to the authors knowledge only Hom-Diaz et al. (2017) evaluated the fate of 17 pharmaceuticals in a closed tubular reactor treating wastewater. More recently, two other studies from our research group evaluated PPCPs and pesticides removal in water by microalgae semi-closed systems (García-Galán et al., 2020, 2018).

The present study aims to evaluate the removal capacity of a semi-closed, horizontal tubular PBR for 35 different PPCPs, including UV-filters and parabens (10), benzotriazoles (4), antibiotics (15) and other pharmaceuticals (6), in a mixed water from an irrigation channel To the best of the author's knowledge, it is the first time that a hybrid, (open/close) PBR is investigated regarding the removal of a UV filters in this type of water matrix.

2. Materials and methods

2.1. Microalgae-based treatment system description and operation

2.1.1. Hybrid tubular horizontal photobioreactor (PBR)

The sampling campaign was carried out in one of the three PBRs designed, built and operated by the Environmental Engineering and Microbiology research group ((GEMMA) - Universitat Politècnica de Catalunya-BarcelonaTech) in collaboration with Disoltech S.L. (Tarragona, Spain), within the framework of the H2020 EU project INCOVER "Innovative Eco-technologies for Resource Recovery from Wastewater" (http://incover-project.eu/GA 689242). These PBRs were the core of a more complex pilot plant at demonstrative scale, which main objective was to use wastewater as a valuable resource to produce different added-value products within the biorefinery concept and circular economy paradigm. A detailed description of the PBRs can be found elsewhere (García et al., 2018; Uggetti et al., 2018). Briefly, each PBR consisted of two open tanks of polypropylene connected by 16 horizontal tubes (Fig. 1). The useful volume of each PBR was 11.7 m^3. Eight-blade paddlewheels were installed in the middle of each open tank to ensure and favor the homogeneous distribution and mixing of the liquor and also the release of the excess dissolved oxygen (DO) accumulated along the closed tubes. Paddle wheels also contributed to create a water level difference (0.2 m) which made the mixed liquor flow by gravity from one tank to the other. The PBRs were inoculated in April with a mixed culture of microalgae and bacteria grown in urban wastewater and operating since then (Uggetti et al., 2018). Irrigation water derived from a drainage-irrigation channel near the facilities, containing a mix of agricultural run-off and reclaimed wastewater from a WWTP nearby, was fed to the PBR daily, under a HRT regime of 5 d (2.3 m^3 d^{-1} approximately). Previously, this water was mixed with urban wastewater from a septic tank (7:1, v:v) (to provide nutrients for the biomass growth) in a homogenization tank with constant stirring, right before the feeding operation (it was filled up anew every day). Online sensors of pH (Hach Lange Spain S.L.), dissolved oxygen (Neurtek, Spain) and temperature (Campbell Scientific Inc., USA) were installed in one of the two open tanks of each PBR.

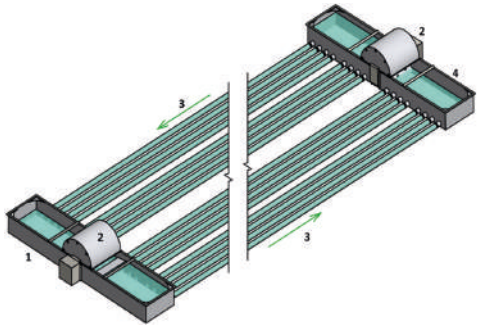

Fig. 1. Scheme of the hybrid tubular closed photobioreactor used in this study. 1:inflow from the homogenization tank; 2: paddle wheel; 3: direction of the flow within the tubes; 4: outflow to the storage tanks. Samples were taken in 1 and 4.

2.2. Sampling strategy

The PBR was already in operation for two full months before the sampling campaign in July, and had already reached the steady state (for complete-mix reactors, from 3 to 5 HRTs are needed to reach this steady state). Sampling was carried out during two weeks in July, three days per week. Influent samples were taken from the homogenization tank and effluent samples were taken from one of the open tanks (as mixed liquor) (n = 12 samples). The PBR was treated as a complete-mix reactor, so the HRT was not considered when taking the influent and effluent samples. For chemical characterization of the water, samples were taken in PVC bottles and directly analyzed in the laboratory on the same day. For the analysis of environmental levels of PPCPs, samples were collected and immediately filtered through 0.45 µm PVDF membrane filters (Millipore, USA) and frozen upon arrival to the laboratory (amber glass bottles).

2.3. Chemicals and reagents

High purity standards (>99%) for 4 benzotriazoles (1H-benzotriazole (BZT), its two metabolites 5-methyl-1-H-benzotriazole (MeBZT) and 5,6-dimethyl-1H-benzotriazole (DMBZT), and 2-(2′-hydroxy-5′-methylphenyl) benzotriazol (UVP), 6 benzophenones (benzophenone-1 (BP1), benzophenone-2 (BP2), benzophenone-3 (BP3), two metabolites of BPR, 4-hydroxybenzophenone (4HB) and 4,4 –dihydroxybenzophenone (4DHB), and 2,2′-dihydroxy-4-methoxybenzophenone (DHMB)), 1 camphor derivative (4-methylbenzilidenecamphor (4MBC)), 1 cinnamate derivative (ethylhexyl methoxycinnamate (EHMC)), 2 *p*-aminobenzoic acid derivatives (benzocaine (EtPABA), and ethylhexyl-4-(dimethylamino)benzoate (ODPABA)) and their corresponding isotopically labelled compounds were purchased from were purchased from Sigma Aldrich (Augsburg, Germany) and Merck (Darmstadt, Germany). Regarding pharmaceuticals, 2 macrolides (clarithromycin and tylosin), 2 fluoroquinolone (flumequine and ofloxacine), 1 quinolone (oxolinic acid), 9 sulfonamides, trimethoprim, 3 anti-inflammatories (ketoprofen, naproxen and mefenamic acid), the lipid regulator gemfibrozil, the β-blocking agent atenolol and the stimulant caffeine, and their corresponding isotopically labelled compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA) and TRC (Toronto Research Chemicals Inc., Ontario, Canada). Detailed information for all the studied compounds is given in Table S1 of the Supplementary Information (SI). Standard solutions of the mixtures of all compounds were made at appropriate concentrations and used to prepare the aqueous calibration curve and also to perform the recovery studies. Similarly, stock standard solutions for the internal standards were prepared. Aqueous standard solutions always contained <0.1% of methanol (MeOH).

2.4. Analytical methodologies and statistical analysis

2.4.1. Samples characterization

Both influent and effluent samples were analyzed on the following conventional wastewater quality parameters: dissolved oxygen (DO) and temperature (EcoScan DO 6, ThermoFisher Scientific, USA) and pH (portable pH-meter 506, Crison Instruments, Spain). These parameters were also measured on-site in the mixed liquor of the PBR by means of online sensors submerged in one of the open tanks and connected to a Multimeter 44 (Crison Instruments, Spain); turbidity (Hanna HI 93703, USA); total suspended solids (TSS), volatile suspended solids (VSS), alkalinity, chemical oxygen demand (COD), following Standard Methods (APHA-AWWA-WEF, 2012); Ammonium (NH_4^+-N) according to Solórzano method (Solórzano, 1969). The ions nitrite (NO_2^--N), nitrate (NO_3^--N) and phosphate (PO_4^{3-}P) were measured by ion chromatography (ICS-1000, Dionex Corporation, USA). Total carbon (TC), total phosphorus (TP) and total nitrogen (TN) were measured by a TOC analyzer (multi N/C 2100 S, Analytik Jena, Germany). All the analyses were done in triplicate and results are given as average values. Mixed liquor samples were examined under bright light microscope (Motic, China) equipped with a camera (Fi2, Nikon, Japan) for qualitative evaluation of microalgae populations, employing taxonomic books and databases for their identification (Streble and Krauter, 2018).

Average biomass productivity (gVSS $m^{-2}{\cdot}d^{-1}$) in the PBR was calculated based on the VSS concentration in the mixed liquor of both systems, using equation (1):

$$\textit{Biomass productivity} = \frac{VSS\ (Q - Q_E + Q_P)}{A} \tag{1}$$

where *VSS* is the volatile suspended solids concentration of the PBR mixed liquor (g VSS L^{-1}); Q is the wastewater flow rate (L d^{-1}); Q_E is the evaporation rate (L d^{-1}); Q_P is the precipitation rate (L d^{-1}); and A is the surface area of the system (for the PBR, it was calculated including both tanks surfaces and half of the surface of the 16 tubes). The evaporation rate was calculated using equation (2):

$$Q_E = E_p\, A \tag{2}$$

where E_p is the potential evaporation (mm d^{-1}), calculated using equation (3) (Fisher and Pringle, 2013).

$$E_p = a\ \frac{T_a}{(T_a + 15)}(R + 50) \tag{3}$$

where a is a dimensionless coefficient which varies depending on the sampling frequency (0.0133 for daily samples); R is the average solar radiation in a day (MJ m^{-2}), and T_a is the average air temperature (°C). Meteorological data (solar radiation, temperature and precipitation) were obtained from the network of local weather stations in the metropolitan area of Barcelona (www.meteo.cat), and are given in Table S2 in SI.

2.4.2. Online-SPE-HPLC-MS/MS analysis of the target compounds

The target analytes were analyzed using a methodology adapted from Gago-Ferrero et al. (2013) and García-Galán et al. (2010). Briefly, pre-concentration and chromatographic separation was performed by automated on-line solid phase extraction coupled to liquid chromatography (SPE–LC), by means of a Symbiosis™ Pico instrument from Spark Holland (Emmen, The Netherlands). On-line SPE pre-concentration of all samples, including the calibration curve (5 mL volume), were performed using PLRP-s cartridges (Agilent, St. Clara, CA, US). HPLC-MS/MS analyses were performed using a 4000 Q TRAP™ MS/MS system (Applied Biosystems-Sciex (Foster City, CA, US). MS/MS detection was performed in both positive and negative ionization modes, under the selected reaction monitoring (SRM) mode. Table S3 summarizes the HPLC-MS/MS conditions for the targeted compounds. Linearity and limits of detection (LOD) of the methodology are given in Table S4.

2.4.3. Statistical analysis

The Mann-Whitney U-Statistical test was used for independent samples to confirm the statistical difference between the concentration I influent and effluent samples, regarding both physical-chemical parameters and to PPCPs. Statistica 10.0® software was used, using a significance level for all tests of 95%.

3. Results

3.1. Conventional water quality parameters and PBR performance

The physical-chemical properties of the feed water (irrigation water from the open channel) and the PBR effluent are summarized in Table 1. Point and continuous measurements of temperature are given in more detail in SI (Figure S1). The VSS/TSS ratio in the PBR mixed liquor was 74%. In these systems, pH values > 8 promote precipitation of inorganic salts of different nature, leading to an increase of the VSS/TSS ratio (Gutiérrez et al., 2016). Average COD was 92 mg L^{-1} in the irrigation water, with no removal but an increase of 16% in the PBR. This increase has been also observed in previous works on closed systems (Arbib et al., 2013; García-Galán et al., 2018; García et al., 2006) and can be related to the microalgae biomass produced in the system releasing a fraction of the carbon fixed during photosynthesis as dissolved organic carbon (DOC). It has been demonstrated that 5–30% of the carbon photosynthetically fixed is released during microalgae growth as dissolved organic matter or carbon (DOC) (Arbib et al., 2013). In the present study, using the relation given by Dubber and Gray (2010)[4] to convert COD into total organic carbon (TOC, and in consequence DOC), we would obtain an increase of 18% in the PBR.

$$COD = 7.25 + 2.99 \times TOC \tag{4}$$

Furthermore, indicative of carbon fixation is the increase of the total carbon (TC) on the effluent, from 162 mg L^{-1} to 246 mg L^{-1}. In addition, there is also an increase in VSS (from 20 mg L^{-1} to 215 mg L^{-1}) indicating the growth of biomass in the system. On the other hand, the higher COD values in the effluent from PBR may be related to the low organic matter biodegradability of the feed water and consequently, to carbon limitation that affected the algal growth. The same was observed in the work by Arbib et al. (2013). The Mann-Whitney U statistical test applied not showed significant statistical difference for temperature and TN parameters.

The average concentration of N–NH_4^+ in the irrigation water was 4.4 mg [N–NH_4^+] L^{-1}, which was removed up to 83%. Microalgae biomass assimilation is the main removal pathway of N–NH4$^+$ in algae systems, but nitrification and volatilization (in the open tanks of the reactor) as secondary routes should also be considered (García et al., 2006).

Table 1
Physical-chemical characterization of the feed water (PBR inf) and PBR effluent (mixed liquor).

Parameters	Sample type	
	PBR_{inf}	PBR_{eff}
	Mean ± SD	Mean ± SD
TSS (mg L^{-1})	73.70 ± 58.81	291.18 ± 200.91
VSS (mg L^{-1})	20.43 ± 13.85	215.35 ± 124.95
COD (mgO_2 L^{-1})	92.50 ± 50.06	107.64 ± 81.06
pH	8.3 ± 0.3	9.1 ± 1.0
DO (mg L^{-1})	–	8.97 ± 0.86
Temperature (°C)	24.18 ± 2.1	24.87 ± 1.6
N–NH^{4+} (mg L^{-1})	4.4 ± 1.5	0.3 ± 0.5
TN (mg L^{-1})	23.8 ± 2.7	23.8 ± 1.9
TIN (mg L^{-1})	14.4 ± 8.9	6.6 ± 5.3
TC (mg L^{-1})	162.0 ± 19.9	246.3 ± 34.4
N–NO_2^- (mg L^{-1})	1.1 ± 1.0	2.2 ± 3.4
N–NO_3^- (mg L^{-1})	9.3 ± 1.8	4.3 ± 5.3
P-PO_4^{3-} (mg L^{-1})	1.6 ± 1.0	0.0 ± 0.0
S–SO_4^{2-} (mg L^{-1})	79.5 ± 18.8	63.9 ± 17.9

TSS – Total Suspended Solids; VSS – Volatile Suspended Solids; COD – Chemical oxygen demand; DO – Dissolved Oxygen; TN – Total Nitrogen; N–NH_4^+ - Ammonium - TN – Total Nitrogen – TC – Total Carbon.

3.1.1. Biomass productivity

The average biomass productivity in the PBR was 61.18 ± 6 mg VSS L^{-1} d^{-1} (equivalent to 6.88 g VSS m^{-2} d^{-1}). Similar results, ranging from 4.4 g m^{-2} d^{-1} to 8.26 g m^{-2} d^{-1}, were reported by Arbib et al. (2013) in a small scale PBR (380 L) and also by Park and Craggs (2010), with an an average volumetric productivity ranging from 53 to 69 mg VSS L^{-1} d^{-1} treating domestic wastewater in an open system. However, values between 20 and 40 g m^{-2} d^{-1} are considered typical in closed systems (García-Galán et al., 2018). As expected, the concentration of total inorganic nitrogen (TIN) and total phosphorus (P-PO_4^{3-}) in the feed water were low, explaining the overall low productivity. Higher productivities (55–79 mg_{dcw} L^{-1} d^{-1}), were obtained in previous studies using pure cultures and synthetic culture medium (Troschl et al., 2018). The BG-11 medium used In this study contained 65.8 mg L^{-1} of TIN, whereas the average concentration in the water feedstock of the present study was 15 mg L^{-1} of TIN. Furthermore, biomass development involves the assimilation of NH_4^+ (Arashiro et al., 2019), and indeed nearly all the available NH_4^+ was assimilated by microalgae, but again the input in the PBR influent was low. Last of all, despite the good maintenance of the PBR system during the experiment, the development of biofilm was unavoidable. Its attachment to the inner walls of the tubes probably hindered partially the penetration of sunlight within the tubes and the mixed liquor, affecting to the full growth of microalgae. Nevertheless, its detachment due to the shearing stress produced by the turbulent flow within the tubes together with its regular maintenance aided to keep a correct operation of the PBR.

Regarding the different microalgae species present in the PBR, the cyanobacteria *Synechocystis* sp. Was the most abundant (Fig. 2). Arias et al. (2018) reported the same cyanobacteria species in a lab-scale PBR system, and explained its predominance in terms of the nutrients concentrations in the liquor. These authors showed that in PBR systems operating with 4 days of HRT and with TN concentration <11.72 mg N L^{-1} d^{-1}, cyanobacteria predominated. These conditions are similar to the PBR system of this work.

3.2. Occurrence of PPCPs in irrigation/agricultural run-off water

3.2.1. UV-filters and benzotriazoles

Out of the 14 UV filters and benzotriazoles (UV blockers) targeted, 6 were detected in the feedwater to the PBR. For the 10 UV filters evaluated, none of the *p*-aminobenzoic acid and cinnamate derivatives were detected. The benzophenone BP3 was detected in all the influent samples of the PBR at concentrations ranging from 7 ng L^{-1} to 75 ng L^{-1}. Its metabolites 4DHB and DHMB were not present, and 4HB was only detected in one sample. The camphor derivative 4MBC was found in 3 of the 6 samples analyzed (21 ng L^{-1}- 86 ng L^{-1}). To the best of the

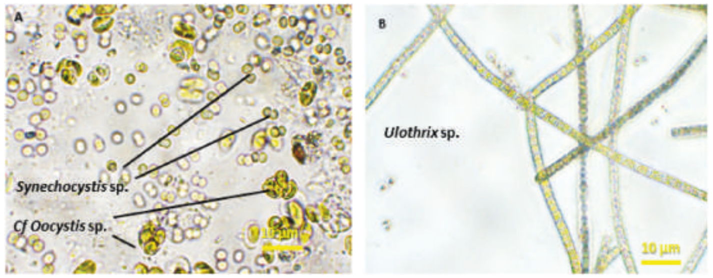

Fig. 2. Microscope images of mixed liquor of the hybrid PBR (A–B), observed in bright light microscopy (x1000).

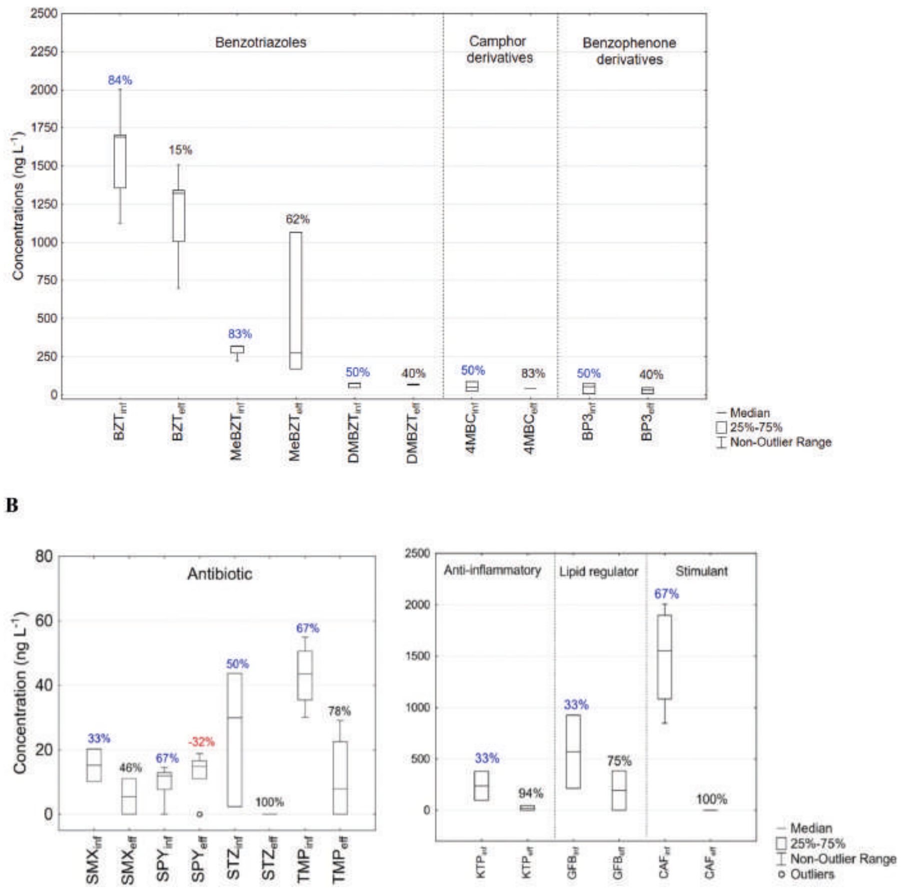

Fig. 3. Concentrations of benzotriazoles and UV-filters (A) and pharmaceuticals (B) detected in the PBR system. For practical purposes, only compounds with frequencies of detection (F%) ≥ 30% (2 samples out of 6) are represented. The percentage value placed on top of the influent box-plots refers to the frequency of detection in the influent water of the PBR; the percentage value placed on top of the effluent box-plots corresponds to the average removal observed (RE%). For pharmaceuticals: SPY: sulfapyridine; STZ: sulfathiazole; TMP: trimethoprim; KTP: ketoprofen; GFB: gemfibrozil.

authors' knowledge, there is no previous data on the presence of these compounds in agricultural run-off, nor irrigation water.

Regarding the 4 benzotriazoles targeted, BZT and MeBZT were the two compounds detected at the highest concentration (1098 ng L^{-1}- 2003 ng L^{-1} for BZT and 220 ng L^{-1}- 1982 ng L^{-1} for MeBZT, respectively) (see Fig. 3). DMBZT was detected in the 50% of the samples and at lower concentrations (45 ng L^{-1}- 77 ng L^{-1}). In a previous campaign on the same site, BZT was also one of the contaminants of emerging concern detected at the highest concentration in the irrigation water, despite at lower levels (420 ng L^{-1}) (García-Galán et al., 2018).

3.2.2. Pharmaceuticals

Thirteen out of the 21 PhACs targeted were also detected in the feed water of the PBR (Fig. 3). For antibiotics, only sulfonamides and trimethoprim were detected. Caffeine was detected in 5 of the 6 samples and at the highest concentrations, ranging from 850 ng L^{-1} to 2008 ng L^{-1}. These levels are higher than those detected in the same location and season in a previous study (150 ng L^{-1}) (García-Galán et al., 2018). Median concentrations of 384 ng L^{-1} and up to 29,300 ng L^{-1} were detected in agricultural run-off in Singapore (Tran et al., 2019) and up to 5200 ng L^{-1} in storm water run-off in Australia (Sidhu et al., 2013), with frequencies of detection near or 100% in both cases. High concentrations were observed also for gemfibrozil (214 ng L^{-1} and 925 ng L^{-1}), and naproxen (774 ng L^{-1}), although they were detected only in 2 and 1 samples, respectively. Similar levels of gemfibrozil were reported in agricultural run-off from effluent-irrigated crop fields in Southern California, ranging from 190 ng L^{-1} to 790 ng L^{-1} (Pedersen et al., 2005). Ketoprofen was found in 50% of the samples, at levels ranging from 98 ng L^{-1} to 379 ng L^{-1}, similar to those detected by Moeder et al. (2017) in agricultural run-off in Mexico (18 ng L^{-1}-230 ng L^{-1}). Regarding antibiotics, sulfapyridine and trimethoprim were detected with the highest frequencies and average values of 43 ng L^{-1} and 15 ng L^{-1}, respectively, followed by sulfathiazole. Sulfamethoxazole was present only 2 out of the 6 days of sampling, at concentrations between 10 ng L^{-1}-20 ng L^{-1}. Similar results were obtained by Bailey et al. (2015), who also obtained average concentrations of 22 ng L^{-1} for sulfapyridine in agricultural run-off. These authors pointed out the use of sulfonamides as fertilizers in agriculture, which justifies their presence in the run-off. The β-blocker atenolol was not detected in any sample.

3.3. Removal of PPCPs in microalgae-based systems

Aqueous phase removal in the PBR was calculated according to equation (5):

$$RE\% = 100x\left(1 - \frac{C_{eff}}{C_{inf}}\right) \tag{5}$$

where C_{inf} and C_{eff} are the concentrations (ng L^{-1}) in the influent and effluent waters, respectively.

3.3.1. Removal efficiency of PBRs

3.3.1.1. Benzotriazoles and UV filters. Fig. 3A and B shows the influent and effluent concentrations of the different compounds evaluated, indicating also their frequency of detection and removal after the treatment in the PBR. Removal of BZT in the PBR ranged from 3% to 25%. To the author's knowledge, only García-Galán et al. (2018) had previously studied the removal of BZT in closed (semi-closed) systems. These authors obtained a 50% elimination in a PBR of smaller capacity, and in the same location. But as mentioned in the previous section, the concentrations detected in the influent water of the PBR were much lower than those obtained in the present study. Matamoros et al. (2015) also studied the removal of BZT in microalgae-based treatments, specifically in an open system. The authors obtained eliminations in the range of 33–74% working under an HRT of 4 d during summer in the city of Barcelona, but again the concentrations at the influent were lower. Regarding RE% of BZT in different conventional WWTPs, results are highly variable and can range from negative values up to 99% (Molins-Delgado et al., 2017); for instance, Liu et al. (2012) obtained a RE% of 7%, Asimakopoulos et al. (2013) obtained RE% in the range of 25–37%, and Reemtsma et al. (2010) observed eliminations of 20–59%. Nevertheless, the frequent presence of BZT in different aquatic ecosystems (Molins-Delgado et al., 2017; Serra-Roig et al., 2016) reinforces the predominance of low removals in conventional WWTPs. Photodegradation, which is usually enhanced in microalgae-based treatment systems (García-Galán et al., 2020; Matamoros et al., 2015), was not significant for BZT. Yet, its photodegradability was demonstrated by Liu et al. (2012, 2011b), who obtained removals in stabilization ponds of 47% but only after long HRTs (27 d). BZT is highly soluble and with a low log K_{ow}, which together with its high ionization tendency (high pk_a, see Table S1), indicates a poor retention/sorption tendency on the microalgae biomass and a low biodegradability. Elimination of MeBZT was more efficient, ranging from 15% to 48%, and the second metabolite DMBZT was barely removed, with variable RE% from 3% to 17%, but also 100% elimination one of the sampling days (average RE% of 40%) (Fig. 3A). Comparing these RE% to those obtained in conventional WWTPs, a high variability is again observed for these two compounds, with removals in the range of 0–72% for MeBZT and 0–16% for DMBZT (Asimakopoulos et al., 2013; Molins-Delgado et al., 2015; Reemtsma et al., 2010). The presence of other benzotriazole derivatives in the irrigation water (not included in the scope of the present study) that could biotransform into BZT or MeBZT, could also explain the low removals obtained for these compounds. For instance, xylyltriazole demethylates are known to release both MeBZT and BZT as transformation products, and 5-chloro-benzotriazole can lose the chlorine moiety to transform back into BZT (Liu et al., 2011a).

Regarding benzophenone derivatives, BP3 was eliminated up to a 43% (39.9% in average). These results are similar to those obtained by Díaz-Garduño et al. (2017) in an open microalgae system. The authors used an HRAP as tertiary treatment for WWTPs effluents, with RE% ranging from −50% to 70%. Removal rates found in the literature after conventional wastewater treatments are also variable and in the range of 58%–91% for BP3 (Molins-Delgado et al., 2017). As expected, due to its nature and end use, the resilience of BP3 to photodegradation has been demonstrated (Gago-Ferrero et al., 2012). The metabolite 4HB was detected only once in the inlet of the PBR and was 100% removed (Figure S2); its removal in WWTPs is also efficient, and has been detected in sewage sludge at concentrations of 0.15 μg g^{-1} (Gago--Ferrero et al., 2011). The other two metabolites evaluated, 4DHB and 4DHMB, were not detected in the inlet. Regarding the camphor derivative 4MBC, it was efficiently removed in the PBR system (50–100%). 4MBC is a highly lipophilic compound, and due to its low solubility and high log K_{ow}, biosorption to microalgae biomass seems to be the main removal pathway in the pond. Its removal in conventional WWTPs is also usually high, being frequently detected in the sewage sludge (Gago-Ferrero et al., 2011). To the author's knowledge, this is the first study evaluating the fate of benzothiazoles in closed microalgae-based systems.

The Mann-Whitney U statistical test applied showed significant statistical difference for all benzotriazoles and UV filters found in the analyzed matrix.

3.3.1.2. Pharmaceuticals. Efficient removals were obtained for sulfathiazole (100%) and trimethoprim (78%). Sulfapyridine was not removed (−32% average). The higher pK_a of sulfapyridine compared to the other two antibiotics could explain that difference, meaning a lower adsorption tendency to the microalgae biomass and lower bioavailability (see Table S1). Moreover, previous studies have demonstrated that the acetylated metabolite of this compound, N^4-acetylsulfapyridine (out of the scope of this study) can revert back into the parent compound

during wastewater treatment (García-Galán et al., 2012); this back-transformation could explained the frequent higher concentrations of sulfapyridine found in the effluent wastewaters of different reactors and incomplete removals in CAS WWTPs (García-Galán et al., 2012, 2011). Lower removals were obtained also for sulfamethoxazole, ranging from no elimination (−8%) to 100% removal. The PBR showed a good performance in the elimination of the anti-inflammatory ketoprofen (88%–100%) and also for naproxen, which was fully removed. Lower elimination rates for ketoprofen (36%–85%) and naproxen (10%–70%) were obtained by Hom-Diaz et al. (2017) in a smaller PBR used to treat urban wastewater. These differences are probably due to the different season of the year in that study (autumn), with lower solar irradiation and fewer light hours. Temperature may also have a relevant role in the treatment efficiency of these systems; the high temperatures in the PBR (see Figure S1), especially inside the tubes, could enhance not only biodegradation and bioassimilation routes in the mixed liquor, but could also alter the structure and stability of the compound itself (Cirja et al., 2008).

Regarding the lipid regulator gemfibrozil, an average elimination of 75% was observed. The log K_{ow} of this drug, 4.77, indicates its high tendency to adsorb onto the biomass and probably accounts for these results, as it is generally only moderately biodegradable. Lower eliminations for gemfibrozil are usually obtained during conventional wastewater treatment, and may be related to a competition for adsorption sites with the humic substances (Maeng et al., 2011). A greater availability of active sites in the microalgae biomass could also be responsible for this high removal rates (Vassalle et al., 2020a). Nevertheless, worse removals (20.6%) were reported in a PBR operating under controlled conditions with artificial light and a HRT of 4 days (Kang et al., 2018). Last of all, caffeine was fully eliminated in the PBR. These results contrast with RE% values previously obtained in the same location (<50%) (García-Galán et al., 2018), indicating an enhanced efficiency of the studied PBR. The Mann-Whitney U statistical test applied showed significant statistical difference for all pharmaceuticals found in the analyzed matrix.

Considering these results, it should be noted that PBRs are biological, complex systems. In consequence, different mixed cultures can grow under different reactors configurations and/or conditions, leading to different elimination routes and pathways in the systems. Indeed, different publications on the elimination of a single compound using different microalgae cultures have yielded dissimilar results (de Wilt et al., 2016; Matamoros et al., 2016; Xiong et al., 2016). Biomass production in the reactors can also be determinant in terms of bioadsorption. In our case, however, despite the low biomass productivity in the PBR, its removal efficiency was high. The scale of the reactor studied may also influence the outcome. High temperature within the tubes of the PBR (Figure S1) may have led to faster biodegradation processes and removal routes. It has been observed in different studies in WWTPs that seasonal variations in the temperature do influence the removal efficiency for different contaminants (Matamoros et al., 2015). Other factors such as the transparency of the tubes in the closed system (and indirectly the material) or its roughness regarding biofouling can also be determinant (Harris et al., 2013).. The control of pH could also aid to the removal via bioadsorption of the target analytes by changing their protonation state. Taking all this into consideration, further studies should be conducted, including the analysis of biomass to make a complete mass balance of the evaluated compounds and thus, better clarify the main removal routes in systems similar evaluated in this study.

4. Conclusions

The efficiency of a semi-closed, tubular horizontal PBR to treat a mix of agricultural run-off and reclaimed wastewater used for irrigation was evaluated, focusing on their capacity to remove different PPCPs, including benzotriazoles, benzothiazoles and pharmaceuticals amongst others. BZT and BP3 were detected in all the influent samples investigated, and MeBZT in all except one. The PBR was not efficient in removing BZT and MeBZT, with average elimination not better than those obtained in conventional WWTPs. The limited photodegradability and low sorption to biomass tendency of these compounds could account for this lack of improvement, with elimination rates only attributable to biodegradation. The removal of BP3 was better than that of the benzotriazoles, but yet not higher than 40%. On the other hand, pharmaceuticals were efficiently removed, with full elimination in most cases. The sulfonamide antibiotic sulfapyridine was the exception, with no removal and negative eliminations in all cases, probably due to metabolite deconjugation. High temperatures and pH could be determinant parameters in the elimination rates observed. Nevertheless, further studies should be developeded to confirm this hypothesis, including biomass analysis in order to establish complete mass balances. Other factors potentially affecting PPCP removals in these systems, such as the presence of heavy metals, should also be considered, as well as biomass growth inhibition by other microorganisms present in the mixed liquor, such as protozoa. Overall, data on the removal capacity of microalgae-based systems under real conditions is still scarce, especially in closed or semi-closed systems, as most of the studies are developed under laboratory controlled conditions and, more importantly, not considering the concentration adsorbed in the biomass. This data would provide a more complete picture of the predominant removal mechanisms within these systems.

CRediT authorship contribution statement

Lucas Vassalle: Investigation, Writing - original draft, Writing - review & editing, Supervision. **Adrià Sunyer-Caldú:** Methodology, Formal analysis. **Enrica Uggetti:** Resources, Writing - review & editing. **Rubén Díez-Montero:** Writing - review & editing. **M. Silvia Díaz-Cruz:** Methodology, Resources, Writing - review & editing, Funding acquisition, Visualization. **Joan García:** Project administration, Funding acquisition, Visualization. **M. Jesús García-Galán:** Conceptualization, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2020.111081.

References

Apha-Awwa-Wef, 2012. Standard Methods for the Examination of Water and Wastewater. In: Published Jointly by the American Water Works Association, twenty-second ed. the American Public Health Association, and the Water Environment Federation., Washington, D.C.

Arashiro, L.T., Ferrer, I., Rousseau, D.P.L., Van Hulle, S.W.H., Garfí, M., 2019. The effect of primary treatment of wastewater in high rate algal pond systems: biomass and bioenergy recovery. Bioresour. Technol. 280, 27–36. https://doi.org/10.1016/j.biortech.2019.01.096.

Arashiro, L.T., Montero, N., Ferrer, I., Acién, F.G., Gómez, C., Garfí, M., 2018. Life cycle assessment of high rate algal ponds for wastewater treatment and resource recovery. Sci. Total Environ. 622 (623), 1118–1130. https://doi.org/10.1016/j.scitotenv.2017.12.051.

Arbib, Z., Ruiz, J., Álvarez-Díaz, P., Garrido-Pérez, C., Barragan, J., Perales, J.A., 2013. Long term outdoor operation of a tubular airlift pilot photobioreactor and a high rate algal pond as tertiary treatment of urban wastewater. Ecol. Eng. 52, 143–153. https://doi.org/10.1016/j.ecoleng.2012.12.089.

Arias, D.M., Uggetti, E., García-Galán, M.J., García, J., 2018. Production of polyhydroxybutyrates and carbohydrates in a mixed cyanobacterial culture: effect of nutrients limitation and photoperiods. N. Biotech. 42, 1–11. https://doi.org/10.1016/j.nbt.2018.01.001.

Asimakopoulos, A.G., Ajibola, A., Kannan, K., Thomaidis, N.S., 2013. Occurrence and removal efficiencies of benzotriazoles and benzothiazoles in a wastewater treatment plant in Greece. Sci. Total Environ. 452–453, 163–171. https://doi.org/10.1016/j.scitotenv.2013.02.041.

Ávila, C., Matamoros, V., Reyes-Contreras, C., Piña, B., Casado, M., Mita, L., Rivetti, C., Barata, C., García, J., Bayona, J.M., 2014. Attenuation of emerging organic contaminants in a hybrid constructed wetland system under different hydraulic loading rates and their associated toxicological effects in wastewater. Sci. Total Environ. 470–471, 1272–1280. https://doi.org/10.1016/j.scitotenv.2013.10.065.

Bailey, C., Spielmeyer, A., Frings, R.M., Hamscher, G., Schüttrumpf, H., 2015. From agricultural fields to surface water systems: the overland transport of veterinary antibiotics. J. Soils Sediments 15, 1630–1634. https://doi.org/10.1007/s11368-015-1140-4.

Chisti, Y., 2013. Raceways-based production of algal crude oil. Greenpeace 3, 195–216. https://doi.org/10.1515/green-2013-0018.

Cirja, M., Ivashechkin, P., Schäffer, A., Corvini, P.F.X., 2008. Factors affecting the removal of organic micropollutants from wastewater in conventional treatment plants (CTP) and membrane bioreactors (MBR). Rev. Environ. Sci. Biotechnol. 7, 61–78. https://doi.org/10.1007/s11157-007-9121-8.

Daughton, C.G., 2013. Pharmaceuticals in the environment: sources and their management. Comprehensive Analytical Chemistry, pp. 37–69. https://doi.org/10.1016/B978-0-444-62657-8.00002-1.

de Wilt, A., Butkovskyi, A., Tuantet, K., Leal, L.H., Fernandes, T.V., Langenhoff, A., Zeeman, G., 2016. Micropollutant removal in an algal treatment system fed with source separated wastewater streams. J. Hazard Mater. 304, 84–92. https://doi.org/10.1016/j.jhazmat.2015.10.033.

Díaz-Cruz, M.S., Gago-Ferrero, P., Llorca, M., Barceló, D., 2012. Analysis of UV filters in tap water and other clean waters in Spain. Anal. Bioanal. Chem. 402, 2325–2333. https://doi.org/10.1007/s00216-011-5560-8.

Díaz-Garduño, B., Pintado-Herrera, M.G., Biel-Maeso, M., Rueda-Márquez, J.J., Lara-Martín, P.A., Perales, J.A., Manzano, M.A., Garrido-Pérez, C., Martín-Díaz, M.L., 2017. Environmental risk assessment of effluents as a whole emerging contaminant: efficiency of alternative tertiary treatments for wastewater depuration. Water Res. 119, 136–149. https://doi.org/10.1016/j.watres.2017.04.021.

Dolar, D., Gros, M., Rodriguez-Mozaz, S., Moreno, J., Comas, J., Rodriguez-Roda, I., Barceló, D., 2012. Removal of emerging contaminants from municipal wastewater with an integrated membrane system, MBR-RO. J. Hazard Mater. 239–240, 64–69. https://doi.org/10.1016/j.jhazmat.2012.03.029.

Dolliver, H., Gupta, S., 2008. Antibiotic losses in leaching and surface runoff from manure-amended agricultural land. J. Environ. Qual. 37, 1227–1237. https://doi.org/10.2134/jeq2007.0392.

Dubber, D., Gray, N.F., 2010. Replacement of chemical oxygen demand (COD) with total organic carbon (TOC) for monitoring wastewater treatment performance to minimize disposal of toxic analytical waste. J. Environ. Sci. Heal. Part A Toxic/Hazardous Subst. Environ. Eng. 45, 1595–1600. https://doi.org/10.1080/10934529.2010.506116.

Fent, K., Zenker, A., Rapp, M., 2010. Widespread occurrence of estrogenic UV-filters in aquatic ecosystems in Switzerland. Environ. Pollut. 158, 1817–1824. https://doi.org/10.1016/j.envpol.2009.11.005.

Fisher, D.K., Pringle III, H.C., 2013. Evaluation of alternative methods for estimating reference evapotranspiration. Agric. Sci. 51–60. https://doi.org/10.4236/as.2013.48A008, 04.

Gago-Ferrero, P., Badia-Fabregat, M., Olivares, A., Piña, B., Blánquez, P., Vicent, T., Caminal, G., Díaz-Cruz, M.S., Barceló, D., 2012. Evaluation of fungal- and photo-degradation as potential treatments for the removal of sunscreens BP3 and BP1. Sci. Total Environ. 427–428, 355–363. https://doi.org/10.1016/j.scitotenv.2012.03.089.

Gago-Ferrero, P., Díaz-Cruz, M.S., Barceló, D., 2011. Occurrence of multiclass UV filters in treated sewage sludge from wastewater treatment plants. Chemosphere 84, 1158–1165. https://doi.org/10.1016/j.chemosphere.2011.04.003.

Gago-Ferrero, P., Mastroianni, N., Díaz-Cruz, M.S., Barceló, D., 2013. Fully automated determination of nine ultraviolet filters and transformation products in natural waters and wastewaters by on-line solid phase extraction-liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1294, 106–116. https://doi.org/10.1016/j.chroma.2013.04.037.

García-Galán, M.J., Díaz-Cruz, M.S., Barceló, D., 2011. Occurrence of sulfonamide residues along the Ebro river basin. Removal in wastewater treatment plants and environmental impact assessment. Environ. Int. 37, 462–473. https://doi.org/10.1016/j.envint.2010.11.011.

García-Galán, M.J., Díaz-Cruz, M.S., Barceló, D., 2010. Determination of 19 sulfonamides in environmental water samples by automated on-line solid-phase extraction-liquid chromatography-tandem mass spectrometry (SPE-LC-MS/MS). Talanta 81, 355–366. https://doi.org/10.1016/j.talanta.2009.12.009.

García-Galán, M.J., Frömel, T., Müller, J., Peschka, M., Knepper, T., Díaz-Cruz, S., Barceló, D., 2012. Biodegradation studies of N 4-acetylsulfapyridine and N 4-acetylsulfamethazine in environmental water by applying mass spectrometry techniques. Anal. Bioanal. Chem. 402, 2885–2896. https://doi.org/10.1007/s00216-012-5751-y.

García-Galán, M.J., Gutiérrez, R., Uggetti, E., Matamoros, V., García, J., Ferrer, I., 2018. Use of full-scale hybrid horizontal tubular photobioreactors to process agricultural runoff. Biosyst. Eng. 166, 138–149. https://doi.org/10.1016/j.biosystemseng.2017.11.016.

García, J., Green, B.F., Lundquist, T., Mujeriego, R., Hernández-Mariné, M., Oswald, W. J., 2006. Long term diurnal variations in contaminant removal in high rate ponds treating urban wastewater. Bioresour. Technol. 97, 1709–1715. https://doi.org/10.1016/j.biortech.2005.07.019.

García, J., Ortiz, A., Álvarez, E., Belohlav, V., García-Galán, M.J., Díez-Montero, R., Antonio, J., Uggetti, E., 2018. Nutrient removal from agricultural run-o ff in demonstrative full scale tubular photobioreactors for microalgae growth. Ecol. Eng. 120, 513–521. https://doi.org/10.1016/j.ecoleng.2018.07.002.

García-Galán, M.J., Monllor-Alcaraz, L.S., Postigo, C., Uggetti, E., López de Alda, M., Díez-Montero, R., García, J., 2020. Microalgae-based bioremediation of water contaminated by pesticides in peri-urban agricultural areas. Environ. Pollut. 265 (Part B), 114579. https://doi.org/10.1016/j.envpol.2020.114579.

García-Galán, M.J., Arashiro, L., Santos, L.H.M.L.M., Insa, S., Rodríguez-Mozaz, S., Barceló, D., Ferrer, I., Garfí, M., 2020. Fate of priority pharmaceuticals and their main metabolites and transformation products in microalgae-based wastewater treatment systems. J. Hazard Mater. 390 (121771) https://doi.org/10.1016/j.jhazmat.2019.121771.

Gatidou, G., Anastopoulou, P., Aloupi, M., Stasinakis, A.S., 2019. Growth inhibition and fate of benzotriazoles in Chlorella sorokiniana cultures. Sci. Total Environ. 663, 580–586. https://doi.org/10.1016/j.scitotenv.2019.01.384.

Gros, M., Rodríguez-Mozaz, S., Barceló, D., 2012. Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem. J. Chromatogr. A 1248, 104–121. https://doi.org/10.1016/j.chroma.2012.05.084.

Gutiérrez, R., Ferrer, I., González-Molina, A., Salvadó, H., García, J., Uggetti, E., 2016. Microalgae recycling improves biomass recovery from wastewater treatment high rate algal ponds. Water Res. 106, 539–549. https://doi.org/10.1016/j.watres.2016.10.039.

Harris, L., Tozzi, S., Wiley, P., Young, C., Richardson, T.M.J., Clark, K., Trent, J.D., 2013. Potential impact of biofouling on the photobioreactors of the Offshore Membrane Enclosures for Growing Algae (OMEGA) system Linden. Biores. Technol. 144, 420–428. https://doi.org/10.1016/j.biortech.2013.06.125.

Hom-Diaz, A., Jaén-Gil, A., Bello-Laserna, I., Rodríguez-Mozaz, S., Vicent, T., Barceló, D., Blánquez, P., 2017. Performance of a microalgal photobioreactor treating toilet wastewater: pharmaceutically active compound removal and biomass harvesting. Sci. Total Environ. 592, 1–11. https://doi.org/10.1016/j.scitotenv.2017.02.224.

Kang, D., Zhao, Q., Wu, Y., Wu, C., Xiang, W., 2018. Removal of nutrients and pharmaceuticals and personal care products from wastewater using periphyton photobioreactors. Bioresour. Technol. 248, 113–119. https://doi.org/10.1016/j.biortech.2017.06.153.

Kümmerer, K., 2009. Antibiotics in the aquatic environment - a review - Part I. Chemosphere 75, 417–434. https://doi.org/10.1016/j.chemosphere.2008.11.086.

Kümmerer, K., 2004. Resistance in the environment. J. Antimicrob. Chemother. 54, 311–320. https://doi.org/10.1093/jac/dkh325.

Kunz, P.Y., Fent, K., 2009. Estrogenic activity of ternary UV filter mixtures in fish (Pimephales promelas) - an analysis with nonlinear isobolograms. Toxicol. Appl. Pharmacol. 234, 77–88. https://doi.org/10.1016/j.taap.2008.09.032.

Langdon, K.A., Warne, M.S.T.J., Kookanaz, R.S., 2010. Aquatic hazard assessment for pharmaceuticals, personal care products, and endocrine-disrupting compounds from biosolids-amended land. Integrated Environ. Assess. Manag. 6, 663–676. https://doi.org/10.1002/ieam.74.

Li, X., Wang, L., Asimakopoulos, A.G., Sun, H., Zhao, Z., Zhang, J., Zhang, L., Wang, Q., 2018. Benzotriazoles and benzothiazoles in paired maternal urine and amniotic fluid samples from Tianjin, China. Chemosphere 1999, 524–530. https://doi.org/10.1016/j.chemosphere.2018.02.076.

Liang, X., Martyniuk, C.J., Zha, J., Wang, Z., 2016. Brain quantitative proteomic responses reveal new insight of benzotriazole neurotoxicity in female Chinese rare minnow (Gobiocypris rarus). Aquat. Toxicol. 181, 67–75. https://doi.org/10.1016/j.aquatox.2016.10.030.

Liang, X., Wang, M., Chen, X., Zha, J., Chen, H., Zhu, L., Wang, Z., 2014. Endocrine disrupting effects of benzotriazole in rare minnow (Gobiocypris rarus) in a sex-dependent manner. Chemosphere 112, 154–162. https://doi.org/10.1016/j.chemosphere.2014.03.106.

Liang, X., Zha, J., Martyniuk, C.J., Wang, Z., Zhao, J., 2017. Histopathological and proteomic responses in male Chinese rare minnow (Gobiocypris rarus) indicate hepatotoxicity following benzotriazole exposure. Environ. Pollut. 229, 459–469. https://doi.org/10.1016/j.envpol.2017.06.013.

Liu, Y.S., Ying, G.G., Shareef, A., Kookana, R.S., 2012. Occurrence and removal of benzotriazoles and ultraviolet filters in a municipal wastewater treatment plant. Environ. Pollut. 165, 225–232. https://doi.org/10.1016/j.envpol.2011.10.009.

Liu, Y.S., Ying, G.G., Shareef, A., Kookana, R.S., 2011a. Biodegradation of three selected benzotriazoles under aerobic and anaerobic conditions. Water Res. 45, 5005–5014. https://doi.org/10.1016/j.watres.2011.07.001.

Liu, Y.S., Ying, G.G., Shareef, A., Kookana, R.S., 2011b. Photolysis of benzotriazole and formation of its polymerised photoproducts in aqueous solutions under UV irradiation. Environ. Chem. 8, 174–181. https://doi.org/10.1071/EN10141.

Maeng, S.K., Sharma, S.K., Lekkerkerker-Teunissen, K., Amy, G.L., 2011. Occurrence and fate of bulk organic matter and pharmaceutically active compounds in managed aquifer recharge: A review. Water Res. 45 (10), 3015–3033. https://doi.org/10.1016/j.watres.2011.02.017.

Matamoros, V., Gutiérrez, R., Ferrer, I., García, J., Bayona, J.M., 2015. Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: a pilot-scale study. J. Hazard Mater. 288, 34–42. https://doi.org/10.1016/j.jhazmat.2015.02.002.

Matamoros, V., Uggetti, E., García, J., Bayona, J.M., 2016. Assessment of the mechanisms involved in the removal of emerging contaminants by microalgae from wastewater: a laboratory scale study. J. Hazard Mater. 301, 197–205. https://doi.org/10.1016/j.jhazmat.2015.08.050.

Moeder, M., Carranza-Diaz, O., López-Angulo, G., Vega-Aviña, R., Chávez-Durán, F.A., Jomaa, S., Winkler, U., Schrader, S., Reemtsma, T., Delgado-Vargas, F., 2017. Potential of vegetated ditches to manage organic pollutants derived from agricultural runoff and domestic sewage: a case study in Sinaloa (Mexico). Sci. Total Environ. 598, 1106–1115. https://doi.org/10.1016/j.scitotenv.2017.04.149.

Molins-Delgado, D., Silvia Díaz-Cruz, M., Barceló, D., 2015. Removal of polar UV stabilizers in biological wastewater treatments and ecotoxicological implications. Chemosphere 119, S51–S57. https://doi.org/10.1016/j.chemosphere.2014.02.084.

Molins-Delgado, D., Távora, J., Silvia Díaz-Cruz, M., Barceló, D., 2017. UV filters and benzotriazoles in urban aquatic ecosystems: the footprint of daily use products. Sci. Total Environ. 601–602, 975–986. https://doi.org/10.1016/j.scitotenv.2017.05.176.

Muñoz, R., Guieysse, B., 2006. Algal-bacterial processes for the treatment of hazardous contaminants: a review. Water Res. 40, 2799–2815. https://doi.org/10.1016/j.watres.2006.06.011.

Oswald, W.J., 1995. Ponds in the twenty-first century. Water Sci. Technol. 31, 1–8. https://doi.org/10.1016/0273-1223(95)00487-8.

Park, J.B.K., Craggs, R.J., 2010. Wastewater treatment and algal production in high rate algal ponds with carbon dioxide addition. Water Sci. Technol. 61, 633–639. https://doi.org/10.2166/wst.2010.951.

Patel, M., Kumar, R., Kishor, K., Mlsna, T., Pittman Jr., C.U., Mohan, D., 2019. Pharmaceuticals of emerging concern in aquatic systems: Chemistry, occurrence, effects, and removal methods. Chem. Rev. 119, 3510–3673. https://doi.org/10.1021/acs.chemrev.8b00299.

Pedersen, J.A., Soliman, M., Suffet, I.H., 2005. Human pharmaceuticals, hormones, and personal care product ingredients in runoff from agricultural fields irrigated with treated wastewater. J. Agric. Food Chem. 53, 1625–1632. https://doi.org/10.1021/jf049228m.

Postigo, C., García-Galán, M.J., Köck-Schulmeyer, M., Barceló, D., 2015. Occurrence of Polar Organic Pollutants in Groundwater Bodies of Catalonia. In: Munné, A., Ginebreda, A., Prat, N. (Eds.), Experiences from Ground, Coastal and Transitional Water Quality Monitoring. The Handbook of Environmental Chemistry. https://doi.org/10.1007/698_2015_343 vol 43. Springer, Cham.

Proia, L., Osorio, V., Soley, S., Köck-Schulmeyer, M., Pérez, S., Barceló, D., Romaní, A.M., Sabater, S., 2013. Effects of pesticides and pharmaceuticals on biofilms in a highly impacted river. Environ. Pollut. 178, 220–228. https://doi.org/10.1016/j.envpol.2013.02.022.

Reemtsma, T., Miehe, U., Duennbier, U., Jekel, M., 2010. Polar pollutants in municipal wastewater and the water cycle: occurrence and removal of benzotriazoles. Water Res. 44, 596–604. https://doi.org/10.1016/j.watres.2009.07.016.

Rodriguez-Mozaz, S., Chamorro, S., Marti, E., Huerta, B., Gros, M., Sànchez-Melsió, A., Borrego, C.M., Barceló, D., Balcázar, J.L., 2015. Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. Water Res. 69, 234–242. https://doi.org/10.1016/j.watres.2014.11.021.

Rueda, E., García-Galán, M.J., Díez-Montero, R., Vila, J., Grifoll, M., García, J., 2020. Polyhydroxybutyrate and glycogen production in photobioreactors inoculated with wastewater borne cyanobacteria monocultures. Bioresour. Technol. 295, 122233. https://doi.org/10.1016/j.biortech.2019.122233.

Sabourin, L., Beck, A., Duenk, P.W., Kleywegt, S., Lapen, D.R., Li, H., Metcalfe, C.D., Payne, M., Topp, E., 2009. Runoff of pharmaceuticals and personal care products following application of dewatered municipal biosolids to an agricultural field. Sci. Total Environ. 407, 4596–4604. https://doi.org/10.1016/j.scitotenv.2009.04.027.

Serra-Roig, M.P., Jurado, A., Díaz-Cruz, M.S., Vázquez-Suñé, E., Pujades, E., Barceló, D., 2016. Occurrence, fate and risk assessment of personal care products in river–groundwater interface. Sci. Total Environ. 568, 829–837. https://doi.org/10.1016/j.scitotenv.2016.06.006.

Sidhu, J.P.S., Ahmed, W., Gernjak, W., Aryal, R., McCarthy, D., Palmer, A., Kolotelo, P., Toze, S., 2013. Sewage pollution in urban stormwater runoff as evident from the widespread presence of multiple microbial and chemical source tracking markers. Sci. Total Environ. 463–464, 488–496. https://doi.org/10.1016/j.scitotenv.2013.06.020.

Solórzano, L., 1969. Determination of ammonia in natural seawater by the phenol-hypochlorite method. Limnol. Oceanogr. 14, 799–801. https://doi.org/10.4319/lo.1969.14.5.0799.

Streble, H., Krauter, D., 2018. Das Leben im Wassertropfen: Mikroflora und Mikrofauna des Süsswassers. Kosmos, Stuttgart.

Tangtian, H., Bo, L., Wenhua, L., Shin, P.K.S., Wu, R.S.S., 2012. Estrogenic potential of benzotriazole on marine medaka (Oryzias melastigma). Ecotoxicol. Environ. Saf. 80, 327–332. https://doi.org/10.1016/j.ecoenv.2012.03.020.

Tolls, J., Berger, H., Klenk, A., Meyberg, M., Müller, R., Rettinger, K., Steber, J., 2009. Environmental safety aspects of personal care products - a European perspective. Environ. Toxicol. Chem. 28, 2485–2489. https://doi.org/10.1897/09-104.1.

Tran, N.H., Reinhard, M., Khan, E., Chen, H., Nguyen, V.T., Li, Y., Goh, S.G., Nguyen, Q. B., Saeidi, N., Gin, K.Y.H., 2019. Emerging contaminants in wastewater, stormwater runoff, and surface water: application as chemical markers for diffuse sources. Sci. Total Environ. 676, 252–267. https://doi.org/10.1016/j.scitotenv.2019.04.160.

Troschl, C., Meixner, K., Fritz, I., Leitner, K., Romero, A.P., Kovalcik, A., Sedlacek, P., Drosg, B., 2018. Pilot-scale production of poly-β-hydroxybutyrate with the cyanobacterium *Synechocytis sp.* CCALA192 in a non-sterile tubular photobioreactor. Algal Res 34, 116–125. https://doi.org/10.3390/biom9050198.

Uggetti, E., García, J., Álvarez, J.A., García-Galán, M.J., 2018. Start-up of a microalgae-based treatment system within the biorefinery concept: from wastewater to bioproducts. Water Sci. Technol. 78, 114–124. https://doi.org/10.2166/wst.2018.195.

Vassalle, L., Díez-Montero, R., Machado, A.T.R., Moreira, C., Ferrer, I., Mota, C.R., Passos, F., 2020. Upflow anaerobic sludge blanket in microalgae-based sewage treatment: Co-digestion for improving biogas production. Bioresour. Technol. 300, 9. https://doi.org/10.1016/j.biortech.2019.122677.

Vassalle, L., García-Galan, M.J., de Aquino, S.F., Afonso, R.J., de, C.F., Ferrer, I., Passos, F., Filhoa, C.R.M., 2020. Can high rate algal ponds be used as post-treatment of UASB reactors to remove micropollutants? Chemosphere 248, 125969. https://doi.org/10.1016/j.chemosphere.2020.125969.

Verlicchi, P., Galletti, A., Petrovic, M., BarcelÓ, D., 2010. Hospital effluents as a source of emerging pollutants: an overview of micropollutants and sustainable treatment options. J. Hydrol. 389, 416–428. https://doi.org/10.1016/j.jhydrol.2010.06.005.

Wang, L., Asimakopoulos, A.G., Kannan, K., 2015. Accumulation of 19 environmental phenolic and xenobiotic heterocyclic aromatic compounds in human adipose tissue. Environ. Int. 78, 45–50. https://doi.org/10.1016/j.envint.2015.02.015.

Xiong, J.Q., Kurade, M.B., Abou-Shanab, R.A.I., Ji, M.K., Choi, J., Kim, J.O., Jeon, B.H., 2016. Biodegradation of carbamazepine using freshwater microalgae Chlamydomonas mexicana and Scenedesmus obliquus and the determination of its metabolic fate. Bioresour. Technol. 205, 183–190. https://doi.org/10.1016/j.biortech.2016.01.038.

4.2.1 Publication #5 Supplementary information

Bioremediation of emerging micropollutants in irrigation water. The alternative of microalgae-based treatments

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Supplementary Tables S1, S2, S3 and S4
Supplementary Figures S1 and S2

Table S1. Target PPCPs, classified by their chromatographic retention time, and their optimized UPLC–QqLIT-MS/MS parameters by positive ionization mode.

Family	Abbreviation	Compound	CAS no.	Formula	Molecular weight	Solubility (25ºC mg L^{-1})	Log Kow	pKa
Benzotriazoles	**BZT/Btri**	**1H-benzotriazole**	95-14-7	$C_6H_5N_3$	119,120	20000	1,23	8,2
	MeBZT	**5-methyl-1-H-benzotriazole**	136-85-6	$C_7H_7N_3$	133,150	5	1,89	8,66
	DMBZT/ DMeBTri	**5,6-Dimethyl-1H-benzotriazole monohydrate**	4184-79-6	$C_8H_9N_3$	147,177	914,2	2,26	-
	UVP	**2-(2'-Hydroxy-5'-methylphenyl)benzotriazol**	2440-22-4	$C_{13}H_{11}N_3O$	225.25	-	-	-
Benzophenones	**BP1**	**Benzophenone-1 2,4-Dihhydroxybenzophenone***	131-56-6	$C_{13}H_{10}O_3$	214,220	240	3,17	7,1
	BP2	**Benzophenone-2 2,2',4,4'-Tetrahydroxybenzophenone***	131-55-5	$C_{13}H_{10}O_5$	246,053	98,42	2,78	-
	BP3	**Benzophenone-3/Oxybenzone 2-Hydroxy-4-methoxybenzophenone***	131-57-7	$C_{14}H_{12}O_3$	228,240	0,21	3,79	-
	4HB	**4-hydroxybenzophenone**	1137-42-4	$C_{13}H_{10}O_2$	198,220	405,8	3,02	-
	4DHB	**4,4 -dihydroxybenzophenone**	611-99-4	$C_{13}H_{10}O_3$	214,220	1905	2,55	-
	DHMB	**2,2'-Dihydroxy-4-methoxybenzophenone**	131-53-3	$C_{14}H_{12}O_4$	244,074	52,73	3,82	-
Camphor derivatives	**4MBC**	**Enzacamene 3-(4-Methylbenzilidene) camphor***	36861-47-9	$C_{18}H_{22}O$	254,400	0,1966	4,95	-
Cinnamate derivatives	**EHMC**	**Ethylhexyl methoxycinnamate**	5466-77-3	$C_{18}H_{26}O_3$	290,400	0,1548	5,8	-
***p*-aminobenzoic acid derivatives**	**EtPABA**	**Benzocaine Ethyl 4-aminobenzoate**	94-09-7	$C_9H_{11}NO_2$	165,189	1671	1,86	-
	ODPABA	**Padimate-O2-Ethylhexyl-4-(dimethylamino)benzoate**	21245-02-3	$C_{17}H_{27}NO_2$	277,400	0,1977	6,15	-
Macrolide		**Clarithromycin**	81103-11-9	$C_{38}H_{69}NO_{13}$	747,953	0.33	3.16	8.99
		Tylosin	1401-69-0	$C_{46}H_{77}NO_{17}$	916,100	5	1.63	7.73
Fluoroquinolone		**Flumequine**	42835-25-6	$C_{14}H_{12}FNO_3$	261,248	308,4	2,6	6.5
		Ofloxacin	82419-36-1	$C_{18}H_{20}FN_3O_4$	361,367	6,76E+05	-2	2.1
Quinolone		**Oxolinic acid**	14698-29-4	$C_{13}H_{11}NO_5$	261.23	8007	0.94	8.77
Sulfonamides		**Sulfabenzamide**	127-71-9	$C_{13}H_{12}N_2O_3S$	276,311	1316	1,3	4.32
		Sulfadimethoxine	122-11-2	$C_{12}H_{14}N_4O_4S$	310,329	433,1	1.63	6.91
		Sulfamerazine	127-79-7	$C_{11}H_{12}N_4O_2S$	264,304	202	0.14	7.12
		Sulfamethizole	144-82-1	$C_9H_{10}N_4O_2S_2$	270,331	1050	0.54	2.1 / 5.3
		Sulfamethoxazole	723-46-6	$C_{10}H_{11}N_3O_3S$	253,278	610	0.89	9.1
		Sulfanitran	122-16-7	$C_{14}H_{13}N_3O_5S$	335,335	36,57	2,26	7.44
		Sulfapyridine	144-83-2	$C_{11}H_{11}N_3O_2S$	249,289	268	0.35	8.43
		Sulfathiazole	72-14-0	$C_9H_9N_3O_2S_2$	255.317	373	0.05	7.2
		Sulfisomidin	515-64-0	$C_{12}H_{14}N_4O_2S$	278,330	1620	-0,33	6.12
Dihydrofolate reductases		**Trimethoprim**	738-70-5	$C_{14}H_{18}N_4O_3$	290,318	400	0.91	7.2
Analgesic/ anti-inflammatories		**Ketoprofen**	22071-15-4	$C_{16}H_{14}O_3$	254,281	51	3,12	4.45
		Mefenamic acid	61-68-7	$C_{15}H_{15}NO_2$	241,285	20 (30ºC)	5.12	4.2
		Naproxen	22204-53-1	$C_{14}H_{14}O_3$	230,259	15.9	3.18	4.15
Lipid regulators		**Gemfibrozil**	25812-30-0	$C_{15}H_{22}O_3$	250,333	4964	4.77	4.5
B-blocking agent		**Atenolol**	29122-68-7	$C_{14}H_{22}N_2O_3$	266,336	685,2	0.16	9.6
Stimulants		**Caffeine**	58-08-2	$C_8H_{10}N_4O_2$	194,191	21600	-0,07	14

Experimental data obtained from Chemspider database and the US Environmental Protection Agency's EPISUITE^{TM}

Table S2. Solar irradiation and air temperatures registered during the days of the experiment.

Date	Irradiance (MJ/m²)	Temperature (ºC)
10/07	23.3	24.9
11/07	26.6	25.5
12/07	28.9	26
13/07	25.3	25.6
14/07	7.7	24.6
15/07	27	25.9
16/07	28.1	25.8
17/07	28.3	25.8
18/07	27.1	26.2
19/07	25.3	25.9
20/07	24.6	25.1

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Table S3. Chromatographic retention time and optimized MS/MS transitions for the target analytes

	COMPOUND	RT	MRM1	DP (V)	CE (eV)	CXP (eV)	MRM2	DP (V)	CE (eV)	CxP (eV)	Internal standard
Benzotriazoles	**BZT**	4,6	120>65	56	31	4	120>92	56	25	16	BZT-d4
	MeBZT	5,3	134>79	46	29	10	134>95	46	35	14	
	DMBZT	5,8	148>77	56	39	2	148>93	56	27	6	
	UVP	10,8	226>120	46	25	10	226>107	46	19	18	
Benzophenones	**BP1**	7,7	215>137	40	27	10	215>105	40	29	6	BP3-d5
	BP2	6,6	247>137	46	25	8	247>109	46	45	8	
	BP3	9,2	229>151	40	25	12	229>105	40	27	16	
	4HB	7,1	199>121	40	25	8	199>105	40	27	8	
	4DHB	5,9	215>121	45	27	8	215>93	45	45	6	
	DHMB	8,2	245>121	43	29	8	245>151	43	27	12	
Camphor derivatives	**4MBC**	10,8	255>105	61	45	6	255>212	61	29	14	4MBC-d4
Cinnamate derivatives	**EHMC**	11,9	291>179	51	19	4	291>161	51	25	10	
***p*-aminobenzoic acid derivatives**	**EtPABA**	6,5	166>138	41	20	10	166>120	41	25	28	
	ODPABA	12,3	278>166	86	43	42	278>151	86	27	40	
Macrolide	**Clarithromycin**	7,2	749>158	76	39	10	749>83	76	84	5	Clarithromycin-N-methyl-d3
	Tylosin	5,74	917>174	106	53	10	917>773	106	46	12	
Quinolone	**Oxolinic acid**	5,8	262>244	36	25	12	262>216	36	41	16	Flumequine-^{13}C
Fluoroquinolone	**Flumequine**	6,7	262>244	51	29	14	262>202	51	45	10	
	Ofloxacin	4,5	362>318	56	29	18	362>261	56	41	18	Sulfamethazine-d4
Sulfonamides	**Sulfabenzamide**	5,8	277>156	56	17	10	257>92	56	41	6	Sulfamethazine-d4
	Sulfadimethoxine	6,1	311>156	76	31	8	311>92	76	31	6	
	Sulfamerazine	4,4	265>156	61	27	8	265>92	61	47	6	
	Sulfamethizole	4,4	271>156	56	25	10	271>108	56	27	10	
	Sulfamethoxazole	5,6	254>108	56	27	10	254>156	56	25	10	

	Sulfanitran	6,6	336>156	66	17	12	336>198	66	29	14	
	Sulfapyridine	4,2	250>156	51	28	12	250>92	51	31	6	
	Sulfathiazole	4,1	256>156	40	25	14	256>92	40	25	10	
	Sulfisomidin	4,6	279>124	76	33	8	279>186	76	23	14	
Dihydrofolate reductases	**Trimethoprim**	3,9	291>230	76	33	20	291>261	76	35	12	Trimethoprim-d$_3$
Analgesic/ antiinflammatories	**Ketoprofen**	7,3	255>105	66	33	18	255>209	66	25	10	BP3-d$_5$
	Mefenamic acid	9,2	242>224	46	23	22	242>209	46	41	14	Mefenamic acid-d$_3$
	Naproxen	7,4	231>185	56	17	14	231>115	56	77	10	Flumequine-^{13}C
Lipid regulators	**Gemfibrozil**	9,2	251>233	31	7	14	251>129	31	19	12	BP3-d$_5$
B-blocking agent	**Atenolol**	3,1	267>116	46	31	8	267>133	46	39	22	Trimethoprim-d$_3$
Stimulants	**Caffeine**	4	195>109.960	56	31	8	195>138	56	29	10	Trimethoprim-d$_3$
Internal standards	***BZT-d$_4$***	4,5	124>96	81	25	6					
	BP3-d$_5$	9,2	234>151	36	27	12					
	4MBC-d$_4$	10,8	259>216	76	27	14					
	Flumequine-^{13}C	6,7	265>247	51	31	20					
	Sulfamethazine-d$_4$	4,6	283>96	26	35	5					
	Trimethoprim-d$_3$	3,9	294>230	72	35	10					
	Gemfibrozil-d$_6$	6,7	257.416>239	26	9	14					
	Mefenamic acid-d$_3$	5,4	245>227.4	66	17	8					
	Caffeine-d$_3$	4	198>140	71	25	8					
	Atenolol-d$_7$	3,1	274>107	51	69	4					

Table S4. Linearity and method limits of quantification (LOQ), given in ng L^{-1}, for the targeted analytes.

Family	Compound	Linearity	r2	Urban sewage	Agricultural runoff	HRAP$_{eff}$	PBR$_{eff}$
Benzotriazoles	**BZT**	1-700	0,9939	29,59	31,57	39,91	51,02
	5-MeBZT	5-700	0,9978	9,01	5,44	31,44	3,36
	DMBZT	30-500	0,9985	13,99	22,47	20,50	11,15
	UVP	3-700	0,9932	282,02	81,04	209,00	94,60
Benzophenones	**BP1**	30-700	0,9988	15,18	-	11,70	5,11
	BP2	10-700	0,9982	-	-	-	-
	BP3	1-700	0,9928	27,83	6,43	27,91	7,91
	4HB	5-700	0,9989	8,05	1,18	-	0,65
	4DHB	3-700	0,9907	16,51	-	20,09	-
	DHMB	10-700	0,9957	-	-	-	-
Camphor derivatives	**4MBC**	30-700	0,997	3,76	15,77	3,68	4,26
Cinnamate derivatives	**EHMC**	5-700	0,9958	48,58	-	0,02	-
***p*-aminobenzoic acid derivatives**	**EtPABA**	50-700	0,9919	-	-	-	-
Macrolide	**Clarithromycin**	30-700	0,9989	-	4,19	8,00	13,24
	Tylosin	50-700	0,9957	-	-	-	-
Fluoroquinolone	**Flumequine**	30-700	0,9955	5,63	9,97	9,21	8,38
	Ofloxacin	30-700	0,9997	613,78	1011,09	1036,76	188,17
Quinolone	**Oxolinic acid**	1-700	0,9926	-	-	-	-
Sulfonamides	**Sulfabenzamide**	1-700	0,9919	8,91	-	-	-
	Sulfadimethoxine	3-700	0,9926	11,04	7,43	1,15	1,83
	Sulfaguanidine	30-700	0,9958	-	-	-	-
	Sulfamerazine	10-700	0,9961	-	-	7,69	7,59
	Sulfamethizole	30-700	0,9927	17,50	-	13,41	-
	Sulfamethoxazole	3-700	0,9929	18,98	7,35	2,25	5,77
	Sulfamethoxypyridazine	1-700	0,9958	-	-	7,71	-
	Sulfanitran	3-700	0,9956	-	-	-	-
	Sulfapyridine	1-700	0,9981	6,71	1,50	7,27	2,39
	Sulfaquinoxaline	3-700	0,9979	9,23	-	-	-
	Sulfathiazole	10-700	0,9968	10,29	3,78	30,38	5,97
	Sulfisomidin	5-700	0,9968	-	-	-	15,95
Dihydrofolate reductases	**Trimethoprim**	3-700	0,9931	11,83	10,68	16,41	3,43
Analgesic/ antiinflammatories	**Ketoprofen**	10-700	0,9955	113,15	60,35	67,78	41,18
	Mefenamic acid	1-700	0,9959	-	-	-	-
	Naproxen	10-700	0,9946	80,29	29,89	90,21	10
Lipid regulators	**Gemfibrozil**	30-700	0,9878	30	1,05	765,63	5,49
B-blocking agent	**Atenolol**	30-700	0,9983	80,02	27,29	8,89	3,05
Stimulants	**Caffeine**	50-700	0,9944	291,00	69,72	183,53	34,52

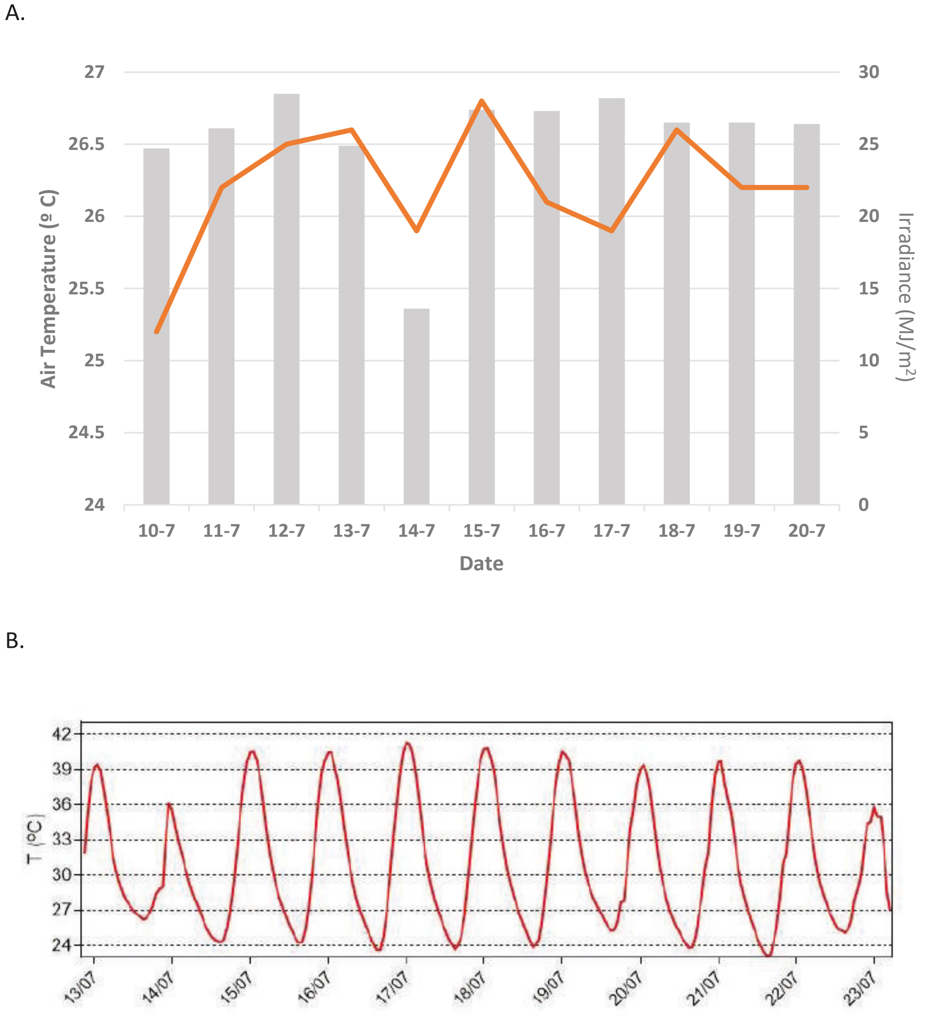


Figure S1. Air temperature (ºC) registered at 10 am (sampling time) and average solar radiation registered during the sampling days (A); diurnal temperature variations in the PBR (B). It should be considered that higher temperatures would be expected within the tubes of the PBR (measurements were performed in the open tanks).

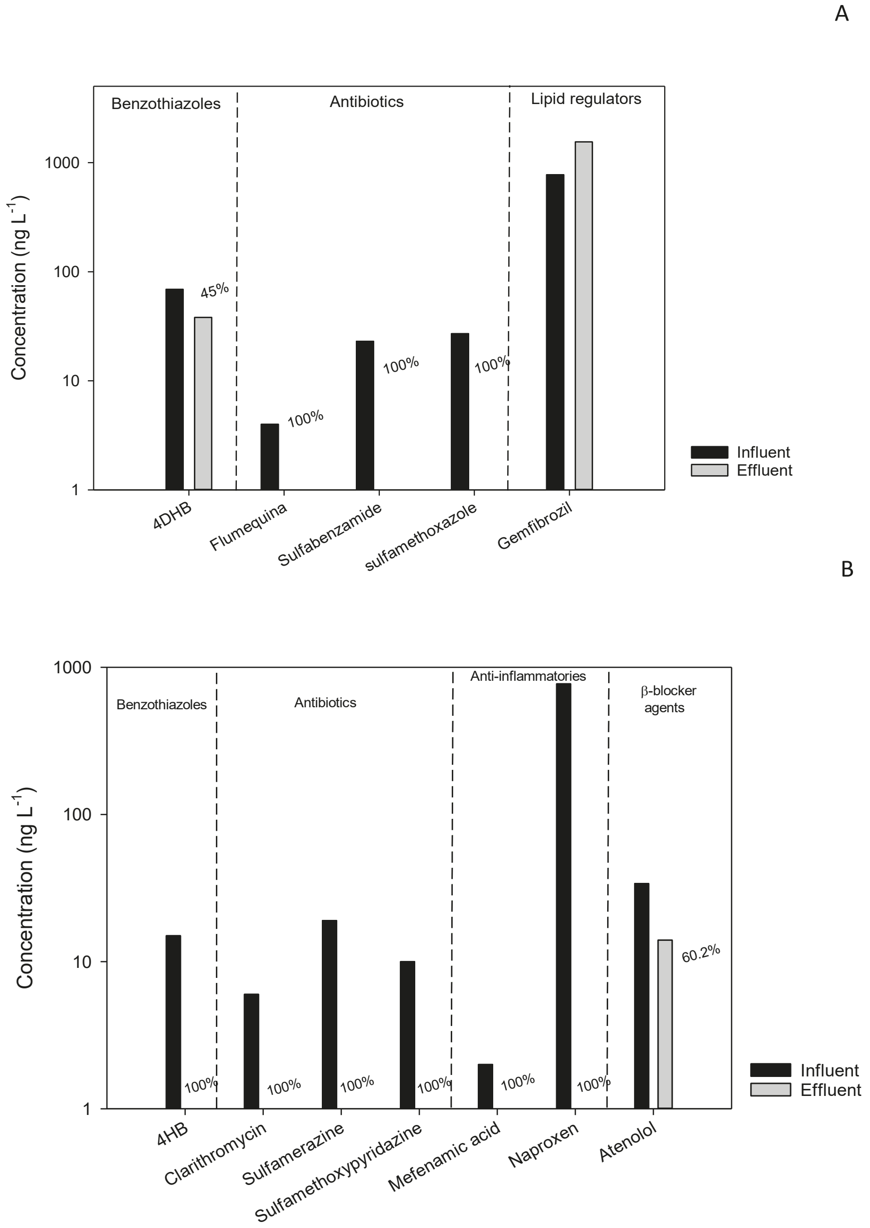
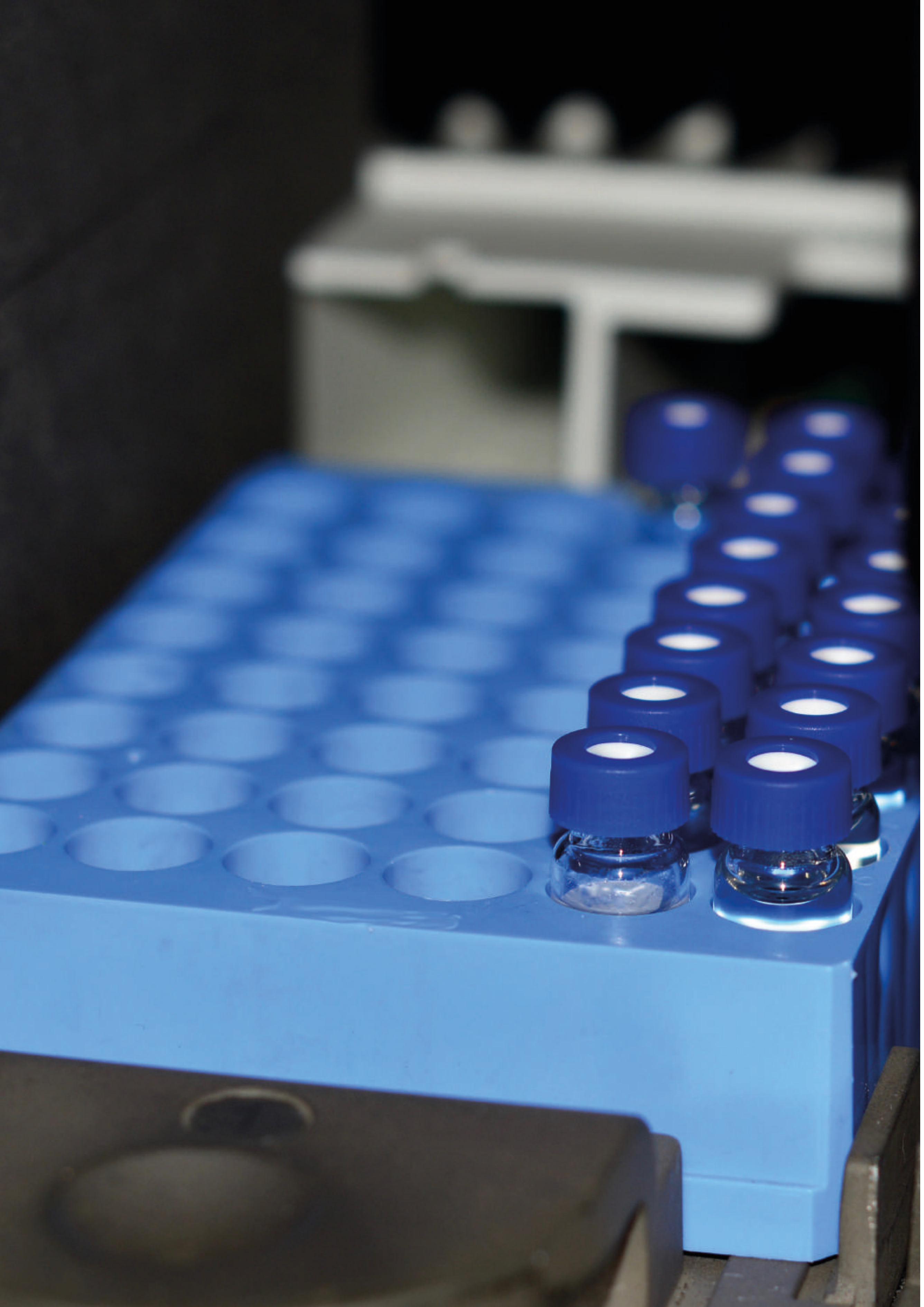


Figure S2. Concentrations of the targeted compounds detected with frequencies of detection (F%) ≤ 17% (1 samples out of 6) in the HRAP (A) and PBR (B)



4.2.2 Publication #6

Behavior of UV filters, UV blockers and pharmaceuticals in high rate algal ponds treating urban wastewater

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Article

Behavior of UV Filters, UV Blockers and Pharmaceuticals in High Rate Algal Ponds Treating Urban Wastewater

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Abstract: The present study evaluated the efficiency of a high rate algal pond (HRAP) at pilot scale to remove pharmaceuticals and personal care products (PPCPs) from urban wastewater, including UV-filters and parabens (10), benzotriazoles (4), antibiotics (15), anti-inflammatories (3) and other pharmaceuticals (3). A total of 35 compounds were targeted, of which 21 were detected in the influent wastewater to the HRAP. Removals (RE%) for pharmaceuticals were variable, with efficient eliminations for atenolol (84%) and sulfathiazole (100%), whereas the anti-inflammatories naproxen and ketoprofen were only partially removed <50%. Benzotriazoles showed elimination rates similar to those of conventional WWTPs, with RE% ranging from no elimination to 51% for the UV filter benzophenone-3 (BP3) and 100% for 4-methylbenzilidenecamphor (4MBC). Hazard quotients (HQs) were estimated for those compounds not fully eliminated in the HRAP, as well as the cumulative ecotoxicity in the resulting effluent. The majority of the compounds yielded HQs < 0.1, meaning that no environmental risk would be derived from their discharge. Overall, these results clearly indicate that HRAPs are a reliable, green and cost-effective alternative to intensive wastewater treatment, yielding promising results removing these contaminants.

Keywords: emerging contaminants; sunscreens; metabolites; microalgae; wastewater treatment; green treatments

1. Introduction

Nature-based, low-cost treatment systems are gradually becoming feasible alternatives to conventional secondary/biological treatment (activated sludge) in wastewater treatment plants (WWTP), especially to cover the needs of small populations (up to 10,000 inhabitants) with no access to sanitary facilities. Microalgae-based systems, and specifically high rate algal ponds (HRAP), have already been used for decades for wastewater treatment [1,2]. They were introduced to improve the efficiency of stabilization ponds, being generally shallower to ensure light penetration, having a higher rate of oxygen production and operating under shorter retention times. As a result, the area requirements were also reduced.

Compared to conventional WWTPs, HRAPs do not require external aeration due to microalgae photosynthesis nor any chemical inputs during the treatment process [3,4]. Microalgae biomass

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grows fixing CO_2 and assimilating the nutrients (mostly nitrogen (N) and phosphorus (P)) present in the influent wastewater. Oxygen is generated through photosynthesis and used by heterotrophic aerobic bacteria to degrade the organic matter present in the water. This way, these systems have the capacity of treating wastewater efficiently whereas producing microalgae biomass which, after an appropriate harvesting/separation technique from the aqueous phase, can be further profited to produce bioenergy (biogas) or other added-value bioproducts such as pigments, biofertilizers or bioplastics [5–9]. In consequence, if this biomass is managed properly, the waste generated during microalgae treatment (biomass) is considerably reduced, as well as the operation and maintenance costs when compared to conventional WWTPs.

Several recent studies have demonstrated the efficiency of HRAPs in terms of nutrients, organic matter and pathogens elimination [10–12]. The mechanisms to eliminate these pollutants in HRAPs are similar to those occurring in stabilization ponds. Specifically for particulated organic matter, sorption to the algae/bacteria flocs is the main elimination pathway [13]. HRAPs become more efficient when they operate at pH below 9. Organic matter removal (in terms of BOD_5) of up to 94% has been reported in HRAP systems with pH control (by means of CO_2 injection) below 9 [14]. Regarding nutrients (N and P), volatilization and photosynthetic assimilation are the main removal pathways for N, and precipitation in the case of P, with a strong correlation between pH values and residual levels of $N\text{-}NH_4$ and phosphates [15].

Currently, these systems are being intensively investigated regarding their capacity to eliminate organic micropollutants such as pharmaceuticals and personal care products (PPCPs), obtaining promising results [11,16,17]. PPCPs comprise a broad number and variety of compounds, which are neither routinely monitored in wastewaters, nor regulated [18,19]. These compounds are used on a daily basis and, after usage, they enter the environment regularly mostly via WWTP effluents. Despite their low concentrations, their bioactivity and recalcitrance can cause natural imbalances in the receiving water bodies, including aquatic toxicity, genotoxicity, endocrine disruption and selection of resistant pathogenic bacteria amongst others [18]. The reduction of microinvertebrates diversity in rivers and behavioral changes in the ichthyofauna have also been reported [20]. Ponsatí et al. [21] evaluated the effect on the biodiversity of the presence of inorganic and organic compounds, including pesticides and pharmaceuticals, and observed a considerable decrease in biodiversity and simplification of the biological structure of both biofilms (algae, bacteria and fungi) and invertebrates. However, the lack of long-term studies and chronic effects is evident in the literature and further research should be carried out [22].

On the other hand, personal care products are less frequently studied than pharmaceuticals, and the research on this topic is generally focused on some types of disinfectants (triclosan), insect repellents (DEET) and fragrances, whereas there is little data on the occurrence and fate of other compounds such as UV filters and/or UV blockers [23,24]. Based on these gaps in the literature and knowing that the main entrance pathway of PPCPs in the aquatic environment are WWTP effluents, the need to find alternative and more efficient treatments is evident. Within this context, HRAPs are gaining a renewed popularity due to their demonstrated high efficiency treating wastewater [10–12] within a more sustainable frame than activated sludge treatments. Efficient removal of different PPCPs such as hormones, anti-inflammatories and other pharmaceuticals in HRAPs has recently been demonstrated [11,16], yet the number of studies on the removal capacity of microalgae-based systems against these organic contaminants is scarce, especially in real scale systems.

Thus, the present study aims to evaluate the removal capacity of a HRAP at pilot scale, as secondary treatment, for 35 different PPCPs, including UV-filters and parabens (10), benzotriazoles (4), antibiotics (15) and other pharmaceuticals (6). The different removal pathways have been discussed for each pollutant, and the potential ecotoxicity of the HRAP effluent has been evaluated, estimating the risk quotients (HQs) associated to the PPCPs to ascertain a safe discharge in the receiving surface waters. To the best of the author's knowledge, it is the first time that an open algae-based system (HRAP) is investigated regarding the specific removal of UV filters and UV Blockers in wastewater.

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2. Materials and Methods

2.1. Microalgae-Based Treatment System Description and Operation

Samples were taken from a pilot HRAP installed outdoors the UPC premises (Barcelona, Spain). The pilot plant has been previously described in detail elsewhere [10]. Briefly, the wastewater treatment line was composed by a screening pre-treatment, a primary settler in a cylindrical PVC settling tank (effective volume: 3 L, HRT: 41 min), a HRAP and a secondary settler (effective volume: 3.3 L, HRT: 46 min) to separate the biomass produced from the treated effluent (Figure 1). The system was continuously fed with wastewater directly pumped from the public sewer to a homogenization tank (1.2 m^3), which was constantly stirred to avoid solids sedimentation. The HRAP had a volume of 470 L (surface area of 1.54 m^2, 0.3 m depth) and was equipped with a paddle wheel (working at 5 rpm approx.) for mixed liquor mixing. The system was operating continuously for almost one year with an HRT of 4.5 days.

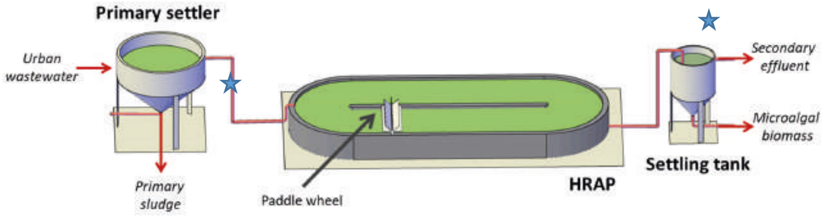


Figure 1. Diagram of the high rate algal pond (HRAP) pilot plant used in the study. Samples were taken after the primary settler and after the secondary settler (indicated with a star).

2.2. Sampling Campaign

The sampling campaign was carried out when the HRAP had already been in operation for almost one year and reached steady state (which requires from 3 to 5 HRT in completely mixed systems). Sampling was carried out during two consecutive weeks in July, three days per week. Grab samples of the HRAP influent were taken after primary treatment, right before entering the pond, and from the effluent after the secondary settler (Figure 1). As the HRAP works as a completely mixed reactor, the HRT was not considered when taking influent and effluent samples. For physicochemical characterization of the water, samples were taken in PVC bottles and directly analyzed in the laboratory on the same day. For the analysis of environmental levels of PPCPs, samples were collected, immediately filtered through 0.45 μm PVDF membrane filters (Millipore, Burlington, MA, USA) and frozen (amber glass bottles) until analysis.

2.3. Chemicals and Reagents

High purity standards (>99%) for 4 benzotriazoles (1H-benzotriazole (BZT), 5-methyl-1-H-benzotriazole (MeBZT), 5,6-dimethyl-1H-benzotriazole (DMBZT) and 2-(2′-hydroxy-5′-methylphenyl)benzotriazol (UVP), 6 benzophenones (benzophenone-1 (BP1), benzophenone-2 (BP2), benzophenone-3 (BP3), 4-hydroxybenzophenone (4HB), 4,4–dihydroxybenzophenone (4DHB) and 2,2′-dihydroxy-4-methoxybenzophenone (DHMB)), 1 camphor derivative (4-methylbenzilidenecamphor (4MBC)), 1 cinnamate derivative (ethylhexyl methoxycinnamate (EHMC)), 2 *p*-aminobenzoic acid derivatives (benzocaine (EtPABA), and ethylhexyl-4-(dimethylamino)benzoate (ODPABA)) and their corresponding isotopically labelled compounds were purchased from Sigma Aldrich (Augsburg, Germany) and Merck (Darmstadt, Germany). Regarding pharmaceuticals, 2 macrolides (clarithromycin and tylosin), 2 fluoroquinolones (flumequine and ofloxacine), 1 quinolone (oxolinic acid), 9 sulfonamides (sulfabenzamide, sulfadimethoxine, sulfamerazine, sulfamethizole, sulfamethoxazole, sulfanitran, sulfapyridine, sulfathiazole and sulfisomidin), trimethoprim, 3 antiinflammatories (ketoprofen, naproxen and mefenamic acid), the lipid regulator gemfibrozil, the β-blocking agent atenolol and the stimulant caffeine and their corresponding isotopically labelled compounds were obtained from Sigma-Aldrich (St. Louis, MO, USA) and TRC (Toronto Research Chemicals Inc., Ontario, Canada). Detailed information for all the studied compounds is given in Table S1 of the Supplementary Materials. Standard solutions of the mixtures of all compounds were made at appropriate concentrations and used to prepare the aqueous calibration curve and to perform the recovery studies. Similarly, stock standard solutions for the internal standards were prepared. Aqueous standard solutions always contained <0.1% of methanol (MeOH).

2.4. Analytical Methodologies

2.4.1. Physico-Chemical Characterization

Both HRAP influent and effluent samples were analyzed for the following wastewater quality parameters: DO and temperature (EcoScan DO 6, ThermoFisher Scientific, Waltham, MA, USA); pH (portable pH-meter 506, Crison Instruments, Barcelona, Spain); turbidity (Hanna HI 93703, Woonsocket, RI, USA); total suspended solids (TSS), volatile suspended solids (VSS), alkalinity and chemical oxygen demand (COD) following Standard Methods [25]; NH_4^+-N according to the Solórzano method [26]. The ions $N\text{-}NO_2^-$, $N\text{-}NO_3^-$ and $P\text{-}PO_4^{3-}$ were measured by ion chromatography (ICS-1000, Dionex Corporation, Sunnyvale, CA, USA). Total organic carbon (TOC), total phosphorus (TP) and total nitrogen (TN) were measured by a TOC analyzer (multi N/C 2100S, Analytik Jena, Germany). All the analyses were done in triplicate and results are given as average values. Mixed liquor samples were examined under an optic microscope (Motic, China) for qualitative evaluation of microalgae populations, employing taxonomic books and databases for their identification [27,28].

Average biomass productivity (g VSS $m^{-2}\cdot day^{-1}$) in the HRAP was calculated based on the VSS concentration in the mixed liquor, using Equation (1):

$$\text{Biomass productivity} = \frac{\text{VSS}\ (Q - Q_E + Q_P)}{A} \tag{1}$$

where VSS is the volatile suspended solids concentration of the HRAP mixed liquor (g VSS L^{-1}); Q is the wastewater flow rate (L d^{-1}); Q_E is the evaporation rate (L d^{-1}); Q_P is the precipitation rate (L d^{-1}), and A is the surface area of the system. The evaporation rate was calculated using Equation (2):

$$Q_E = E_p\ A \tag{2}$$

where E_p is the potential evaporation (mm d^{-1}), calculated using Equation (3) [29].

$$E_p = a\ \frac{T_a}{(T_a + 15)}(R + 50) \tag{3}$$

where a is a dimensionless coefficient which varies depending on the sampling frequency (0.0133 for daily samples); R is the average solar radiation in a day (MJ m^{-2}), and T_a is the average air temperature (°C). Meteorological data (solar radiation, temperature and precipitation) were obtained from the network of local weather stations in Barcelona Metropolitan Area (*www.meteo.cat*) and are given in Table S2 of the Supplementary Materials.

2.4.2. Online-SPE-HPLC-MS/MS Analysis of the Target Compounds

The target analytes were analyzed using an adapted methodology based on previous analytical strategies, by means of high resolution liquid chromatography coupled to mass spectrometry in tandem (HPLC-MS/MS) [30,31]. Briefly, pre-concentration and chromatographic separation were done using on-line solid phase extraction (SPE) coupled to liquid chromatography (on-lineSPE–LC) (Symbiosis^{TM} Pico, Spark Holland (Emmen, The Netherlands)). On-line SPE pre-concentration of all samples, including the calibration curve (5 mL volume), was carried out using PLRP-s cartridges (Agilent, St. Clara, CA, USA). HPLC-MS/MS analyses were performed using a 4000 Q TRAP^{TM} MS/MS system (Applied Biosystems-Sciex (Foster City, CA, USA). MS/MS detection was performed in both positive and negative ionization modes, under the selected reaction monitoring (SRM) mode. Table S3 summarizes the HPLC-MS/MS conditions for the targeted compounds. Linearity and limits of quantification (LOQ) of the methodology are given in Table S4.

2.5. Environmental Risk Assessment

In order to evaluate the potential ecotoxicological risk of those PPCPs still present in the HRAP effluent, hazard quotients (HQs) were estimated as indicated in Equation (4), following the guidelines of the European Medicines Agency (EMA),

$$\text{HQ} = \frac{\text{MEC}}{\text{PNEC}} \tag{4}$$

where MEC is the measured environmental concentration, and PNEC is the predicted-no effect concentration. When PNEC data are not available, alternative PNECs can be derived by dividing the toxicity endpoint values found in the literature (EC_{50} or LC_{50}) by an uncertainty factor of up to 1000 [32]. HQ values < 0.1 mean that no adverse effects are expected. When $0.1 < \text{HQ} < 1$, the risk is low, but it should not be neglected; when $1 < \text{HQ} < 10$, a moderate risk is implied, and $\text{HQ} > 10$ mean a relevant ecological hazard.

Eventually, for the purpose of evaluating the overall ecotoxicity risk of the HRAP effluent, cumulative HQs were calculated for each trophic level considered, adding all HQs calculated for each individual PPCP detected in the effluent.

3. Results

3.1. Physico-Chemical Water Quality Parameters

The physico-chemical properties of the influent and effluent from the HRAP are summarized in Table 1. The VSS/TSS ratio in the mixed liquor was 85% in average, which is in accordance with previous studies [10,33]. The TSS removal efficiency of the HRAP was 77.8%, which is higher than other results obtained previously (52.6%) in bigger HRAPs [34]. The average influent COD was 199 mg L^{-1}, with an average removal of 60% in the HRAP. The COD removal observed in the HRAP (60%) was in accordance with the results obtained in the same system operated and monitored for one year [10] and also with those observed in different HRAPs [35,36]. The removal for $N\text{-}NH_4^+$ was 97%. Microalgae biomass assimilation is the main removal pathway of $N\text{-}NH_4^+$ in algae systems but also nitrification and volatilization as secondary routes [3].

3.2. Biomass Productivity

The average biomass productivity in the HRAP was 20.71 ± 6 g VSS m^{-2} day^{-1} (equivalent to 67.9 ± 17 mg VSS L^{-1} day^{-1}), which is in accordance with results obtained in previous studies (ranging from 5 to 33 g VSS $m^{-2}\cdot day^{-1}$ [6,13]. Regarding the different microalgae species identified in the HRAP, *Chlorella* sp. was predominant in the HRAP (Figure 2).

3.3. Occurrence of PPCPs in the HRAP Influent

3.3.1. Benzotriazoles and UV-Filters

Eight UV filters and benzotriazoles (UV blockers) were detected in the urban wastewater feeding the HRAP (Figure 3A). The metabolite of BP3, 4DHB, was detected only one day of sampling (frequency of detection ≤ 17%) and has been included in Figure S2 of the SM. For UV filters, none of the *p*-aminobenzoic acid and cinnamate derivatives were detected. The highest concentrations corresponded to BZT (935 ng L^{-1}–2292 ng L^{-1}) followed by its derivative, MeBZT (220 ng L^{-1}–1982 ng L^{-1}) (see Figure 3A). Both were detected in 5 of the 6 samples, and these concentrations were in agreement with levels reported in previous studies of urban wastewaters [30,37–40]. Another derivative of BZT, DMBZT was also detected in 50% of the samples, but at much lower concentrations (45 ng L^{-1}–107 ng L^{-1}). Benzotriazoles are generally used as UV-blockers in industrial products such as plastics and paints to protect polymers and pigments against photodegradation [41], but also in combination with UV-filters such as BP3 to extend the UV protection range of sunscreens and other cosmetics. Regarding UV filters, BP3 was present in all samples (119 ng L^{-1}–480 ng L^{-1}), whereas its main metabolite, BP1, was detected in 3 of the 6 samples collected and at concentrations similar to BP3 (113–480 ng L^{-1}). These levels are in agreement with those previously found in urban wastewaters in the Barcelona Metropolitan Area (Spain) and other European regions [30,40,42]. Concerning the other BP3 metabolites, 4HB was present only in two samples of the HRAP influent, at concentrations <48 ng L^{-1}, and 4DHB and DHMB were detected only in one of the 6 samples. These three metabolites of BP3 are not usually included in monitoring studies, and their frequency of detection and concentration levels are generally very low [30,40]. On the contrary, BP4 was not detected in any of the influent samples, despite being frequently found in urban wastewater at concentrations >1000 ng L^{-1} [30]. Regarding the camphor derivatives, 4MBC was detected in 3 of the 6 samples analyzed, in the range of 26–106 ng L^{-1}, which also agrees with previous studies in the area [30,40,43].

Table 1. Physicochemical characterization of the influent and effluent of HRAP.

Parameters (n = 6)	**Sample Type**	
	HRAP$_{influent}$	**HRAP$_{effluent}$**
	Mean ± SD	**Mean ± SD**
TSS (mg L^{-1})	192.44 ± 114.65	323.15 ± 88.4 */42.59 ± 12.8
VSS (mg L^{-1})	154.67 ± 102.9	274.30 ± 77.5 */42.89 ± 13.9
COD (mgO$_2$ L^{-1})	199.92 ± 75.11	78.85 ±33.13
pH	8.2 ± 0.2	8.4 ± 0.4
DO (mg L^{-1})	-	8.80 ± 1.7
Temperature (°C)	19.5 ± 1.3	18.9 ± 1.6
N-NH$_4$$^+$ (mg L^{-1})	24.1 ± 3.0	0.6 ± 0.4
TN (mg L^{-1})	56.9 ± 17.8	38.7 ± 9.4
TIN (mg L^{-1})	29.3 ± 9.94	17.4 ± 6.5
TC (mg L^{-1})	253.5 ± 95.6	160.6 ± 21.6
N-NO$_2$$^-$ (mg L^{-1})	5.2 ± 4.5	1.8 ± 2.1
N-NO$_3$$^-$ (mg L^{-1})	0.15 ± 0.36	15.2 ± 8.6
P-PO$_4$$^{3-}$ (mg L^{-1})	3.9 ± 2.1	3.2 ± 2.1
S-SO$_4$$^{2-}$ (mg L^{-1})	76.2 ± 84.9	39.9 ± 20.1

TSS—Total Suspended Solids; VSS—Volatile Suspended Solids; COD—Chemical oxygen demand (soluble); DO—Dissolved Oxygen; TN—Total Nitrogen; N-NH$_4$$^+$—Ammonium; TN—Total Nitrogen; TC—Total Carbon. (*) values measured in the mixed liquor of the reactors.

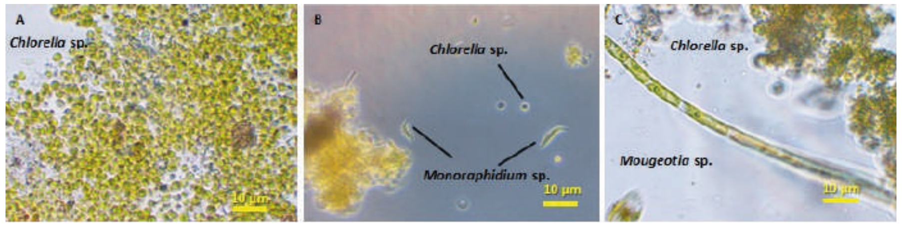
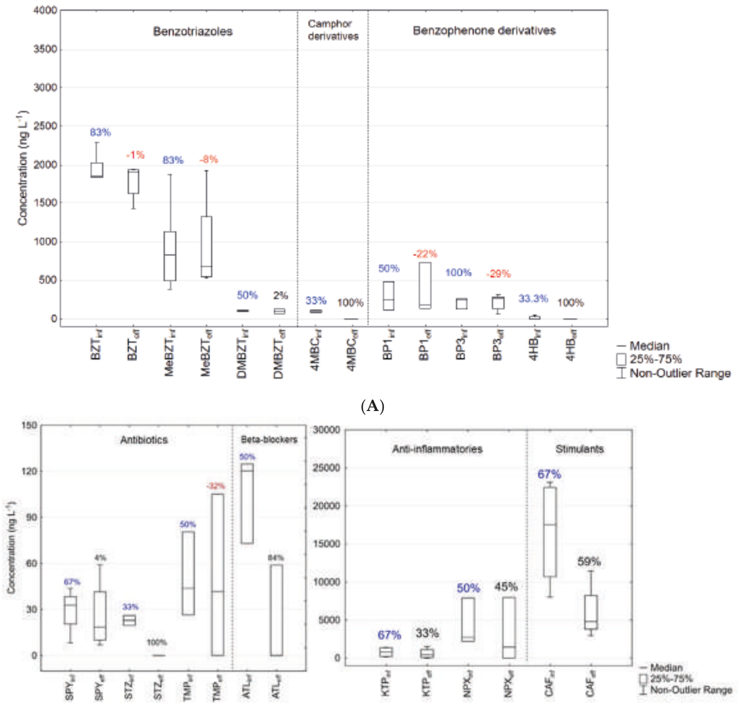


Figure 2. Microscope images of mixed liquor of the HRAP observed in bright light microscopy (×1000).



(**A**)

(**B**)

Figure 3. Concentrations of benzotriazoles and UV-filters (**A**) and pharmaceuticals (**B**) detected in the HRAP. For practical purposes, only compounds with frequencies of detection (F%) ≥ 33% (2 samples out of 6) are presented. The percentage value placed on top of the influent box plots refers to the frequency of detection in HRAP influent; the percentage value placed on top of the effluent boxplots corresponds to the average removal observed (RE%). Negative RE% values are depicted in red. For pharmaceuticals: SPY: sulfapyridine; STZ: sulfathiazole; TMP: trimethoprim, ATL: atenolol; KTP: ketoprofen; NPX: naproxen.

3.3.2. Pharmaceuticals and Stimulants

Eleven out of the 21 pharmaceuticals targeted were detected in HRAP effluent. For antibiotics, only sulfonamides and trimethoprim were detected. The highest concentrations corresponded to the stimulant caffeine (8050 ng L^{-1}–23,126 ng L^{-1}), one of the most consumed worldwide and frequently used as tracking marker for wastewater pollution [44,45] due to its low metabolization in the human body [46]. These results are in accordance with previous monitoring studies in HRAPs [17,47]. High concentrations were detected also for the non-steroidal anti-inflammatory drugs (NSAID) naproxen and ketoprofen, with concentrations in the range of 1529–7900 ng L^{-1} and 165–1406 ng L^{-1}, respectively, in agreement with the levels reported in previous monitoring studies on wastewater [48–50]. Antibiotics were present at much lower concentrations, generally <50 ng L^{-1}. Six out of the nine sulfonamides targeted were detected, but only one or two days of sampling. Only sulfapyridine was detected in the raw wastewater 5 of the 6 days of sampling, with average concentrations of 25 ng L^{-1}. Sulfapyridine is usually found at levels ranging from 10 ng L^{-1} to 100 ng L^{-1} in wastewater [51,52] but also at concentrations higher than 1000 ng L^{-1} [53]. Sulfathiazole was present (two days of sampling) at average concentrations of 15 ng L^{-1}. Frequencies of detection for sulfathiazole are generally low in urban wastewater, as its main use is veterinary [51,54]. Nevertheless, it was detected in 50% of the influent samples analyzed by García-Galán et al. [55] in different WWTPs in Catalonia, at levels in the range of 7.3–142 ng L^{-1}, and also at concentrations up to 300 ng L^{-1} in an Australian WWTP [56]. On the contrary, sulfamethoxazole is clearly the sulfonamide most frequently detected in urban wastewater, considered even as a water quality indicator by different authors due to its environmental ubiquity and recalcitrance. However, in the present study, it was determined only in one sample and at a very low concentration (27 ng L^{-1}), similarly to sulfaquinoxaline and sulfabenzamide (20 ng L^{-1} and 23 ng L^{-1}, respectively). Trimethoprim was frequently present (5 of the 6 days of sampling) at an average concentration of 76 ng L^{-1}, which is in accordance with the levels reported in previous studies for raw wastewater [48,51]. The β-blocker atenolol was detected in 3 out of the 6 samples analyzed, at an average concentration of 107 ng L^{-1}. Results in previous studies were more than one order of magnitude higher (μg L^{-1}), which may be related to local consumption trends [46,48,57].

3.4. Removal Efficiency in the HRAP

The removal efficiency in the HRAP was calculated according to Equation (5):

$$\mathrm{RE}(\%) \;=\; 100 \times \left(1 - \frac{C_{\mathrm{eff}}}{C_{\mathrm{inf}}}\right) \tag{5}$$

where RE(%) is the removal efficiency, and C_{inf} and C_{eff} are the concentrations (ng L^{-1}) in the influent and effluent water, respectively.

Figure 3A,B shows the removal efficiencies (RE%) obtained for different families of contaminants evaluated.

3.4.1. Benzotriazoles

BZT was not efficiently removed in the HRAP, with RE not higher than 22% and obtaining also negative eliminations (see Figure 3A). To the author's knowledge, only Matamoros et al. [17] had previously studied the removal of BZT in HRAPs, obtaining eliminations in the range of 33–74% under the same HRT (4 d). Vassalle et al. [58] investigated the removal of this compound in closed photobioreactors, obtaining similar efficiencies (15% removal in average). The results obtained in different conventional WWTPs are highly variable and can range from negative RE up to 70% [40]. For instance, Liu et al. obtained a RE of 7% [38], Asimakopoulos et al. obtained RE values in the range of 25–37% [37], Reemtsma et al. achieved eliminations of 20–59% [59] and Liu et al. obtained removals of 47% [60]. The low removal of this compound in WWTP explains their ubiquity and impact in the receiving surface waters [61,62],

Removal of MeBZT was similar (0–19%), and higher concentrations in the effluent than in the influent were frequently observed. MeBZT is also frequently found in surface waters and groundwaters in urban areas, at concentrations up to 7181 ng L^{-1} and 1980 ng L^{-1}, respectively [40,43]. Another benzotriazole derivative, DMBZT, was also only barely removed, with RE% between 0% and 8%. RE% in conventional WWTP are again highly variable for these two compounds, with removals in the range of 0–72% for MeBZT and 0–16% for DMBZT [37,59,63].

3.4.2. UV Filters

The elimination of BP3 was variable, with removals ranging from −28% to 51%. This results agree with those obtained by Díaz-Garduño et al. [47], using HRAP as tertiary treatment for WWTPs effluents, with RE% ranging from −50% to 70%, and also with those obtained in a closed photobioreactor operating as tertiary treatment (40% removal on average) [58]. BP3 has been even found in tap water at concentrations up to 290 ng L^{-1} [64] and also in sewage sludge (0.79 μg g^{-1}) [65]. Its metabolite BP1 was not removed in the HRAP, with only one positive value (44%) out of 3. Eliminations reported in the literature after activated sludge treatments are also variable and in the range of 48%–97% for BP1 and 58%–91% for BP3 [40]. Regarding the other BP3 metabolites, 4HB was completely eliminated, and 4DHB was removed by 45% (Figure S2). The environmental occurrence of these metabolites is not as relevant as that of BP1 or BP3, as their occurrence in wastewater is less frequent and at much lower levels. Their removal in WWTPs is also efficient, and both metabolites have been detected in sewage sludge at concentrations of 0.15 μg g^{-1} for 4HB and up to 0.62 μg g^{-1} for 4DHB, despite their low K_{ow} [65,66].

The camphor derivative 4MBC was fully removed in the HRAP. High removal efficiencies are usually registered in conventional WWTPs for this compound (given its high K_{ow}), where it is frequently detected in sewage sludge [65]. Nevertheless, it is still also in surface water and groundwater at low concentrations [40,43] and even in tap water (10 ng L^{-1}–35 ng L^{-1}) [64]. Table S5A of the Supplementary Materials. gives more detailed information on the levels detected on each sampling day.

3.4.3. Pharmaceuticals

Negative RE% were obtained for trimethoprim in the HRAP (−32% in average), meaning that higher concentrations were found in the effluent than in the influent. Previous studies also obtained negative and low RE% for trimethoprim (3.7%) in HRAPs [47], but elimination efficiencies up to 78% have been obtained in a closed photobioreactor working as tertiary treatment [58,67,68]; in that same study, however, the elimination of sulfapyridine was negative, whereas a 4% removal in average has been obtained in his work. On the other hand, sulfathiazole was fully removed within the HRAP.

A removal efficiency of 84% for atenolol was observed, which is in agreement with previous studies in HRAPs [47,68]. Regarding NSAIDs, RE values were highly variable. Ketoprofen was eliminated by 33% in average, with RE ranging from negative to 100%. Previous studies also obtained elimination efficiencies, ranging from 0% to 50% in HRAPs [47], also in closed photobioreactors [58]. Similarly, naproxen was only moderately removed (45% on average), with RE% ranging from −3% to 100%. Díaz-Garduño et al. [47] reported RE% in the range of 0–35% in laboratory-scale reactors; Vassalle et al. [11] obtained a RE% of 54% in a pilot HRAP (205 L) operating with a HRT of 8 days, and Matamoros et al. [17] obtained removals in the range 60–90%, with better efficiencies during the warm season and with longer HRTs (8 vs. 4 days).

Last of all, caffeine was efficiently removed (63–80%), except for one day (14%). Matamoros et al. [17] obtained RE% > 90% for this compound, but lower and even negative RE% have been also reported [47,58]. Table S5B gives more detailed information on the pharmaceutical levels detected on each sampling day.

3.5. Ecotoxicity in the HRAP Effluent

The environmental risk estimated for those PPCPs still present in the HRAP effluent (FD ≥ 33%) was evaluated by means of HQs, as indicated in Section 2.5. This quotient estimates the potential adverse effects of the drug concentration detected in the effluent on non-target organisms present in the receiving water body. Environmental assessments based on the calculation of HQs are frequently carried out [47,55]. Microalgae, invertebrates (*Daphnia magna*) and fish were considered to calculate HQs as representative species of three of the main trophic levels in aquatic ecosystems. Table 2 summarizes the results obtained, as well as the endpoints used. Given that the different considered species are exposed regularly to low concentrations of the PPCPs studied, PNECs were calculated using EC_{50}-LC_{50} as indicators of acute toxicity, divided by an uncertainty factor of 1000 to transform these endpoints into values more representative of the real situation under environmental conditions (longer periods of exposure) [32,55].

Table 2. Average HRAP effluent concentrations (mg L^{-1}) (used as measured environmental concentrations, MEC), ecotoxicity endpoints used for the different trophic levels considered (mg L^{-1}) and hazard quotients (HQ) estimated. HQs have been estimated for those PPCP present in the effluent with a frequency of detection ≥33.3% ($n \geq 2$ over 6).

			Toxicity Endpoints (mg L^{-1})				Hazard Quotients (HQs)		
		Effluent Concentration (mg L^{-1})	**Green Algae**	**Crustaceans**	**Fish**	**Ref.**	**Green Algae**	**Crustaceans**	**Fish**
Benzotriazoles	**1H-benzotriazole (BZT)**	0.001769	2.51	107	654	[1]	7.07×10^{-1}	1.65×10^{-2}	2.72×10^{-2}
	5-methyl-1-H-benzotriazole (MeBZT)	0.000998	2.861	51.6	224	[1]	3.49×10^{-1}	1.93×10^{-2}	4.54×10^{-2}
Benzophenones	**BP1**	0.000346	10.52	3.9	49,194	[2]	3.3×10^{-2}	8.87×10^{-2}	7.03×10^{-5}
	BP3	0.000239	0.961	1.6	7495	[2]	2.49×10^{-1}	1.49×10^{-1}	3.19×10^{-4}
Camphor derivatives	**4MBC***	0.000049	–	2.3	4155	[2]	–	2.13×10^{-2}	1.18×10^{-4}
Antibiotics	**Sulfapyridine (SPY)**	0.000026	1912	–	<39.836	[1]	1.29×10^{-4}	–	6.21×10^{-4}
	Trimethoprim (TMP)	0.000073	80.32	92	>1007	[1]	9×10^{-4}	7.98×10^{-4}	7.34×10^{-4}
Analgesics/Anti-inflammatories	**Ketoprofen (KPF)**	0.000954	1642	248	32	[3]	5.8×10^{-3}	3.85×10^{-3}	2.40×10^{-2}
	Naproxen (NPX)	0.005373	223	15	34	[3]	2.44×10^{-1}	3.583×10^{-2}	1.58×10^{-1}
Stimulants	**Caffeine (CAF)**	0.004924	>1502	160	1514	[1]	3.28×10^{-2}	3.08×10^{-2}	3.26×10^{-2}
	Cumulative HQs						**1.62**	**0.36**	**0.27**

(1) Desmodesmus subspicatus; (2) Pseudokirchneriella subcapicata; (3) Selenastrum capricornotum; (4) Pimephales promelas; (5) Oncorhynchus mykiss; (6) Danio rerio. (7) Orizyas latypes. [1] ECOTOX database of the Environmental Protection Agency (EPA); [2] Molins-Delgado et al. (2017); [3] Sanderson et al. (2003).

4. Discussion

The role of WWTP effluent discharges as main contributors to the presence of benzotriazoles in aquatic ecosystems is generally highlighted in the bibliography [37,38,59], and the low biodegradability of these substances makes these concentrations cumulative in sediments and also in living organisms at different trophic levels [62]. Indeed, concentrations up to 8529 ng L^{-1} for BZT have been found in highly-impacted rivers in the Barcelona Metropolitan area [40], but it is generally detected at lower concentrations in surface waters. Generally, as demonstrated by Loos et al. [61], BZT is frequently detected (>94%) in surface waters at average concentrations of 493 ng L^{-1}. On the other hand, Liu et al. demonstrated that BZT was photodegradable, and obtained removals in stabilization ponds (with HRT of 27 days) of 47% [38,60]. Nevertheless, photodegradation, which is usually enhanced in microalgae-based treatment systems [16,17], was not significant for BZT under the HRAP configuration in the present study. Given its high solubility and low K_{ow}, its retention/sorption tendency on the microalgae biomass was also low. Regarding its two main metabolites, MeBZT and DMBZT, better RE% were obtained in a closed photobioreactor for both metabolites [58], reaching average eliminations up to 62% and 40%, respectively, compared to the results <19% in the studied HRAP. The presence of other benzotriazole derivatives in the influent wastewater (not considered as target compounds of the present study) that could biotransform into BZT or MeBZT could also explain the overall low removals obtained for these compounds in the pond. For instance, xylyltriazole demethylates are known to release both MeBZT and BZT as transformation products, and 5-chloro-benzotriazole can lose the chlorine moiety to transform back into BZT [39].

Regarding UV-filters and the variable and low RE obtained for BP3, Gago-Ferrero et al. demonstrated that photodegradation was not a relevant degradation pathway for this compound [66]. If photodegradation of BP3 is discarded in the HRAP, the higher concentrations of BP1 in the effluent of the pond could be explained in terms of biodegradation of BP3 and new synthesis of BP1 as a by-product. Indeed, in the same study by Gago et al., the authors identified BP1 as a biodegradation by-product of BP3 by the white-rot fungi *Trametes versicolor*. The camphor derivative 4MBC was fully removed in the HRAP. It is a highly lipophilic compound, and due to its low solubility and high K_{ow}, biosorption to microalgae biomass seems to be the main removal pathway in the pond.

The low biodegradability of the antibiotics trimethoprim and sulfapyridine, combined with their high ionization potential and hydrophilicity, could explain the poor RE% obtained. Indeed, the high pK_a values of these substances and their low K_{ow} (Table S1) explain their low tendency to adsorb to the biomass and their presence in the aqueous phase. A recent study evaluated the bioconcentration of trimethoprim and different sulfonamides in microalgae biomass, not finding any of the compounds in the cell wall [67]. These authors in that study also stated that sulfonamides and trimethoprim were resistant to microalgae-based treatments. However, sulfathiazole was fully eliminated. Generally, sulfonamides are not prone to photodegradation [69], so biodegradation seems the most feasible removal pathway for these antibiotics.

Similarly, atenolol is highly hydrophilic and shows a very low tendency to sorption, but different studies have demonstrated that it is susceptible to photodegradation [70], which could be the main removal pathway for this compound (84% removal). Indeed, it shows low and variable RE% in WWTPs, which leads to its frequent presence in river and even coastal waters.

Naproxen is generally considered an easily biodegradable drug, with RE% >80% in conventional WWTPs [71,72]. Its photodegradability has also been demonstrated [73] and should be considered as one of the main removal pathways within the pond. Sorption processes are not considered due to its low K_{ow} value [74–76].

Last of all, and based on the established methodologies [77,78], the HQs calculated for the compounds detected in the effluent showed that no environmental risk would be derived from the discharge of the HRAP effluent. Amongst the different PPCP still present in the HRAP effluent, none of them yielded HQ > 1. HQ values between 0.1 and 1 (low risk) were obtained for BZT and MeBZT against green microalgae, for BP3 against green microalgae and *Daphnia magna* and for naproxen in the three trophic levels. As it can be observed, the majority of the compounds yielded HQs < 0.1, meaning that no environmental risk would be derived from their discharge after HRAP treatment.

Final Remarks

Considering the results obtained, it should be noted that the comparison of the removal efficiency among microalgae-based wastewater treatment systems is complex. First of all, different mixed cultures can lead to different elimination routes and pathways in the systems. Indeed, different publications on the elimination of a single compound using different microalgae cultures have yielded very different results [20,79,80]. Biomass productivity in the photobioreactors can also be determinant in terms of bioadsorption, and so does the HRT, and higher temperatures may lead to faster biodegradation processes and removal routes [17]. This should be considered when comparing, for instance, closed and open systems, as the former will usually keep higher temperatures. The control of pH could also aid to the removal via bioadsorption of the target analytes by changing their protonation state.

5. Conclusions

The efficiency of an innovative natural and low-cost treatment system, based on microalgae biomass productivit and operating as secondary treatment of urban wastewater, was evaluated focusing on their capacity to remove different PPCPs, including benzotriazoles, benzothiazoles and pharmaceuticals amongst others. BP3 was detected in all the HRAP influent wastewater samples investigated and also the benzotriazoles BZT and MeBZT in all except one and at concentrations usually >1000 ng L^{-1}. The HRAP was not efficient in removing these two compounds, with average elimination not better than those obtained in conventional WWTPs. Their limited photodegradability and low sorption to biomass tendency could account for this lack of improvement, with elimination rates only attributable to biodegradation. The removal of BP3 was better than that of the benzotriazoles but still not higher than 51%. Nevertheless, further studies should be developed to confirm these results, including biomass analysis in order to establish complete mass balances. The ecotoxicity of the resulting effluent was neglectable; on the other hand, the role of the HRAP in reducing the environmental risk of the treated wastewater was not relevant either, considering either the low concentrations in the influent or the low removal efficiencies for some of the contaminants studied. Overall, data on the removal capacity of microalgae-based systems under real conditions is still scarce, with most of the studies developed under laboratory-controlled conditions and not considering the concentration in the biomass, which would help to understand the main removal mechanisms and predominant routes within these systems.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/12/10/2658/s1. Table S1: Target PPCPs, classified by their chromatographic retention time, and their optimized UPLC–QqLIT-MS/MS parameters by positive ionization mode. Table S2: Solar irradiation and air temperatures registered during the days of the experiment. Table S3: Chromatographic retention time and optimized MS/MS transitions for the target analytes. Table S4: Linearity and method limits of quantification (LOQ), given in ng L^{-1}, for the targeted analytes. Table S5: Concentrations detected in the HRAP Influent and effluent on every day of sampling. -_ not detected. Figure S1: Concentrations of the targeted compounds detected with frequencies of detection (F%) ≤ 17% (1 sample out of 6) in the HRAP.

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References

1. Chisti, Y. Raceways-based production of algal crude oil. *Green* **2013**, *3*, 195–216. [CrossRef]
2. Oswald, W.J. Ponds in the twenty-first century. *Water Sci. Technol.* **1995**, *31*, 1–8. [CrossRef]
3. García, J.; Green, B.F.; Lundquist, T.; Mujeriego, R.; Hernández-Mariné, M.; Oswald, W.J. Long term diurnal variations in contaminant removal in high rate ponds treating urban wastewater. *Bioresour. Technol.* **2006**, *97*, 1709–1715. [CrossRef] [PubMed]
4. Muñoz, R.; Guieysse, B. Algal-bacterial processes for the treatment of hazardous contaminants: A review. *Water Res.* **2006**, *40*, 2799–2815. [CrossRef] [PubMed]
5. Arashiro, L.T.; Ferrer, I.; Pániker, C.C.; Gómez-Pinchetti, J.L.; Rousseau, D.P.L.; Van Hulle, S.W.H.; Garfí, M. Natural pigments and biogas recovery from microalgae grown in wastewater. *ACS Sustain. Chem. Eng.* **2020**. [CrossRef] [PubMed]
6. Arashiro, L.T.; Ferrer, I.; Rousseau, D.P.L.; Van Hulle, S.W.H.; Garfí, M. The effect of primary treatment of wastewater in high rate algal pond systems: Biomass and bioenergy recovery. *Bioresour. Technol.* **2019**, 27–36. [CrossRef] [PubMed]
7. Khan, S.A.; Sharma, G.K.; Malla, F.A.; Kumar, A.; Rashmi; Gupta, N. Microalgae based biofertilizers: A biorefinery approach to phycoremediate wastewater and harvest biodiesel and manure. *J. Clean. Prod.* **2019**, *211*, 1412–1419. [CrossRef]
8. Rueda, E.; García-Galán, M.J.; Ortiz, A.; Uggetti, E.; Carretero, J.; García, J.; Díez-Montero, R. Bioremediation of agricultural runoff and biopolymers production from cyanobacteria cultured in demonstrative full-scale photobioreactors. *Process Saf. Environ. Prot.* **2020**. [CrossRef]
9. Rueda, E.; García-Galán, M.J.; Díez-Montero, R.; Vila, J.; Grifoll, M.; García, J. Polyhydroxybutyrate and glycogen production in photobioreactors inoculated with wastewater borne cyanobacteria monocultures. *Bioresour. Technol.* **2020**, *295*, 122233. [CrossRef] [PubMed]
10. Arashiro, L.T.; Montero, N.; Ferrer, I.; Acién, F.G.; Gómez, C.; Garfí, M. Life cycle assessment of high rate algal ponds for wastewater treatment and resource recovery. *Sci. Total Environ.* **2018**. [CrossRef]
11. Vassalle, L.; García-Galan, M.J.; De Aquinoc, S.F.; Afonso, R.J.; FrancoAfonso, R.J.d.; Ferrer, I.; Passos, F.; Filho, C.R.M. Can high rate algal ponds be used as post-treatment of UASB reactors to remove micropollutants? *Chemosphere* **2020**, 125969. [CrossRef] [PubMed]
12. Vassalle, L.; Díez-Montero, R.; Machado, A.T.R.; Moreira, C.; Ferrer, I.; Mota, C.R.; Passos, F. Upflow anaerobic sludge blanket in microalgae-based sewage treatment: Co-digestion for improving biogas production. *Bioresour. Technol.* **2020**, *300*, 9. [CrossRef] [PubMed]
13. De Godos, I.; Arbib, Z.; Lara, E.; Rogalla, F. Evaluation of High Rate Algae Ponds for treatment of anaerobically digested wastewater: Effect of CO_2 addition and modification of dilution rate. *Bioresour. Technol.* **2016**, *220*, 253–261. [CrossRef] [PubMed]
14. Craggs, R.J.; Tanner, C.C.; Sukias, J.P.S.; Davies-Colley, R.J. Dairy farm wastewater treatment by an advanced pond system. *Water Sci. Technol.* **2003**, *48*, 291–297. [CrossRef] [PubMed]
15. Solimeno, A.; García, J. Microalgae and bacteria dynamics in high rate algal ponds based on modelling results: Long-term application of BIO_ALGAE model. *Sci. Total Environ.* **2019**, *650*, 1818–1831. [CrossRef]
16. García-Galán, M.J.; Arashiro, L.; Santos, L.H.M.L.M.; Insa, S.; Rodríguez-Mozaz, S.; Barceló, D.; Ferrer, I.; Garfi, M. Fate of priority pharmaceuticals and their main metabolites and transformation products in microalgae-based wastewater treatment systems. *J. Hazard. Mater.* **2020**, 121771. [CrossRef]
17. Matamoros, V.; Gutiérrez, R.; Ferrer, I.; García, J.; Bayona, J.M. Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: A pilot-scale study. *J. Hazard. Mater.* **2015**, *288*, 34–42. [CrossRef]

18. Kümmerer, K. Antibiotics in the aquatic environment—A review—Part I. *Chemosphere* **2009**, *75*, 417–434. [CrossRef]
19. Van Wezel, A.P.; Van den Hurk, F.; Sjerps, R.M.A.; Meijers, E.M.; Roex, E.W.M.; Ter Laak, T.L. Impact of industrial waste water treatment plants on Dutch surface waters and drinking water sources. *Sci. Total Environ.* **2018**, *640–641*, 1489–1499. [CrossRef]
20. Matamoros, V.; Uggetti, E.; García, J.; Bayona, J.M. Assessment of the mechanisms involved in the removal of emerging contaminants by microalgae from wastewater: A laboratory scale study. *J. Hazard. Mater.* **2016**, *301*, 197–205. [CrossRef]
21. Ponsatí, L.; Corcoll, N.; Petrović, M.; Picó, Y.; Ginebreda, A.; Tornés, E.; Guasch, H.; Barceló, D.; Sabater, S. Multiple-stressor effects on river biofilms under different hydrological conditions. *Freshw. Biol.* **2016**, *61*, 2102–2115. [CrossRef]
22. Kuzmanović, M.; Ginebreda, A.; Petrović, M.; Barceló, D. Risk assessment based prioritization of 200 organic micropollutants in 4 Iberian rivers. *Sci. Total Environ.* **2015**, *503–504*, 289–299. [CrossRef] [PubMed]
23. Liu, J.L.; Wong, M.H. Pharmaceuticals and personal care products (PPCPs): A review on environmental contamination in China. *Environ. Int.* **2013**, *59*, 208–224. [CrossRef] [PubMed]
24. Brausch, J.M.; Rand, G.M. A review of personal care products in the aquatic environment: Environmental concentrations and toxicity. *Chemosphere* **2011**, *82*, 1518–1532. [CrossRef]
25. APHA-AWWA-WEF. *Standard Methods for the Examination of Water and Wastewater*, 22nd ed.; Rice, E.W., Baird, R.B., Eaton, A.D., Clesceri, L.S., Eds.; American Water Works Association: Washington, DC, USA; American Public Health Association: Washington, DC, USA; Water Environment Federation: Washington, DC, USA, 2012; ISBN 9780875530130.
26. Solórzano, L. Determination of ammonia in natural seawater by the phenol-hypochlorite method. *Limnol. Oceanogr.* **1969**, *14*, 799–801. [CrossRef]
27. Bourrelly, P. *Les Algues d'eau Douce. Les Algues Vertes*; Boubée, Ed.; Editions N. Boubee & Cie: Paris, French, 1990.
28. Palmer, C.M. *Algas en Abastecimientos de Agua: Manual Ilustrado Acerca de la Identificación, Importancia y Control de Las Algas en Los Abastecimientos de Agua*; Interamericana: Puerto Rico, México, 1962.
29. Fisher, D.K.; Pringle, H.C., III. Evaluation of alternative methods for estimating reference evapotranspiration. *Agric. Sci.* **2013**, *4*, 51–60. [CrossRef]
30. Gago-Ferrero, P.; Mastroianni, N.; Díaz-Cruz, M.S.; Barceló, D. Fully automated determination of nine ultraviolet filters and transformation products in natural waters and wastewaters by on-line solid phase extraction-liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A* **2013**. [CrossRef]
31. García-Galán, M.J.; Díaz-Cruz, M.S.; Barceló, D. Determination of 19 sulfonamides in environmental water samples by automated on-line solid-phase extraction-liquid chromatography-tandem mass spectrometry (SPE-LC-MS/MS). *Talanta* **2010**, *81*, 355–366. [CrossRef]
32. Sanderson, H.; Johnson, D.J.; Reitsma, T.; Brain, R.A.; Wilson, C.J.; Solomon, K.R. Ranking and prioritization of environmental risks of pharmaceuticals in surface waters. *Regul. Toxicol. Pharmacol.* **2004**, *39*, 158–183. [CrossRef]
33. Santiago, A.F.; Calijuri, M.L.; Assemany, P.P.; Calijuri, M.D.C.; Reis, A.J.D. Dos Algal biomass production and wastewater treatment in high rate algal ponds receiving disinfected effluent. *Environ. Technol.* **2017**, *34*, 1877–1885. [CrossRef]
34. Villar-Navarro, E.; Baena-Nogueras, R.M.; Paniw, M.; Perales, J.A.; Lara-Martín, P.A. Removal of pharmaceuticals in urban wastewater: High rate algae pond (HRAP) based technologies as an alternative to activated sludge based processes. *Water Res.* **2018**, *139*, 19–29. [CrossRef] [PubMed]
35. Young, P.; Taylor, M.; Fallowfield, H.J. Mini-review: High rate algal ponds, flexible systems for sustainable wastewater treatment. *World J. Microbiol. Biotechnol.* **2017**, *33*. [CrossRef] [PubMed]
36. Sutherland, D.L.; Turnbull, M.H.; Craggs, R.J. Increased pond depth improves algal productivity and nutrient removal in wastewater treatment high rate algal ponds. *Water Res.* **2014**, *53*, 271–281. [CrossRef] [PubMed]
37. Asimakopoulos, A.G.; Ajibola, A.; Kannan, K.; Thomaidis, N.S. Occurrence and removal efficiencies of benzotriazoles and benzothiazoles in a wastewater treatment plant in Greece. *Sci. Total Environ.* **2013**, *452–453*, 163–171. [CrossRef] [PubMed]
38. Liu, Y.S.; Ying, G.G.; Shareef, A.; Kookana, R.S. Occurrence and removal of benzotriazoles and ultraviolet filters in a municipal wastewater treatment plant. *Environ. Pollut.* **2012**, *165*, 225–232. [CrossRef]

39. Liu, Y.S.; Ying, G.G.; Shareef, A.; Kookana, R.S. Biodegradation of three selected benzotriazoles under aerobic and anaerobic conditions. *Water Res.* **2011**, *45*, 5005–5014. [CrossRef]
40. Molins-Delgado, D.; Távora, J.; Silvia Díaz-Cruz, M.; Barceló, D. UV filters and benzotriazoles in urban aquatic ecosystems: The footprint of daily use products. *Sci. Total Environ.* **2017**. [CrossRef]
41. Langford, K.H.; Reid, M.J.; Fjeld, E.; Øxnevad, S.; Thomas, K.V. Environmental occurrence and risk of organic UV filters and stabilizers in multiple matrices in Norway. *Environ. Int.* **2015**, *80*, 1–7. [CrossRef]
42. Wick, A.; Fink, G.; Ternes, T.A. Comparison of electrospray ionization and atmospheric pressure chemical ionization for multi-residue analysis of biocides, UV-filters and benzothiazoles in aqueous matrices and activated sludge by liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A* **2010**. [CrossRef]
43. Serra-Roig, M.P.; Jurado, A.; Díaz-Cruz, M.S.; Vázquez-Suñé, E.; Pujades, E.; Barceló, D. Occurrence, fate and risk assessment of personal care products in river–groundwater interface. *Sci. Total Environ.* **2016**. [CrossRef]
44. Kurissery, S.; Kanavillil, N.; Verenitch, S.; Mazumder, A. Caffeine as an anthropogenic marker of domestic waste: A study from Lake Simcoe watershed. *Ecol. Indic.* **2012**, *23*, 501–508. [CrossRef]
45. Sidhu, J.P.S.; Ahmed, W.; Gernjak, W.; Aryal, R.; McCarthy, D.; Palmer, A.; Kolotelo, P.; Toze, S. Sewage pollution in urban stormwater runoff as evident from the widespread presence of multiple microbial and chemical source tracking markers. *Sci. Total Environ.* **2013**, *463–464*, 488–496. [CrossRef]
46. Luo, Y.; Guo, W.; Hao, H.; Duc, L.; Ibney, F.; Zhang, J.; Liang, S.; Wang, X.C. Science of the Total Environment A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Sci. Total Environ.* **2014**, *473–474*, 619–641. [CrossRef] [PubMed]
47. Díaz-Garduño, B.; Pintado-Herrera, M.G.; Biel-Maeso, M.; Rueda-Márquez, J.J.; Lara-Martín, P.A.; Perales, J.A.; Manzano, M.A.; Garrido-Pérez, C.; Martín-Díaz, M.L. Environmental risk assessment of effluents as a whole emerging contaminant: Efficiency of alternative tertiary treatments for wastewater depuration. *Water Res.* **2017**, *119*, 136–149. [CrossRef] [PubMed]
48. Gros, M.; Rodríguez-Mozaz, S.; Barceló, D. Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem. *J. Chromatogr. A* **2012**, *1248*, 104–121. [CrossRef] [PubMed]
49. Gros, M.; Petrovic, M.; Ginebreda, A.; Barceló, D. Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. *Environ. Int.* **2010**, *36*, 15–26. [CrossRef] [PubMed]
50. López-Serna, R.; Marín-de-Jesús, D.; Irusta-Mata, R.; García-Encina, P.A.; Lebrero, R.; Fdez-Polanco, M.; Muñoz, R. Multiresidue analytical method for pharmaceuticals and personal care products in sewage and sewage sludge by online direct immersion SPME on-fiber derivatization—GCMS. *Talanta* **2018**, *186*, 506–512. [CrossRef]
51. Gros, M.; Rodríguez-Mozaz, S.; Barceló, D. Rapid analysis of multiclass antibiotic residues and some of their metabolites in hospital, urban wastewater and river water by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. *J. Chromatogr. A* **2013**, *1292*, 173–188. [CrossRef]
52. Szymańska, U.; Wiergowski, M.; Sołtyszewski, I.; Kuzemko, J.; Wiergowska, G.; Woźniak, M.K. Presence of antibiotics in the aquatic environment in Europe and their analytical monitoring: Recent trends and perspectives. *Microchem. J.* **2019**, *147*, 729–740. [CrossRef]
53. García-Galán, M.J.; Díaz-Cruz, M.S.; Barceló, D. Occurrence of sulfonamide residues along the Ebro river basin. Removal in wastewater treatment plants and environmental impact assessment. *Environ. Int.* **2011**, *37*, 462–473. [CrossRef]
54. Paíga, P.; Correia, M.; Fernandes, M.J.; Silva, A.; Carvalho, M.; Vieira, J.; Jorge, S.; Silva, J.G.; Freire, C.; Delerue-Matos, C. Assessment of 83 pharmaceuticals in WWTP influent and effluent samples by UHPLC-MS/MS: Hourly variation. *Sci. Total Environ.* **2019**, *648*, 582–600. [CrossRef] [PubMed]
55. García-Galán, M.J.; González Blanco, S.; López Roldán, R.; Díaz-Cruz, S.; Barceló, D. Ecotoxicity evaluation and removal of sulfonamides and their acetylated metabolites during conventional wastewater treatment. *Sci. Total Environ.* **2012**, *437*, 403–412. [CrossRef] [PubMed]
56. Niu, X.Z.; Glady-Croué, J.; Croué, J.P. Photodegradation of sulfathiazole under simulated sunlight: Kinetics, photo-induced structural rearrangement, and antimicrobial activities of photoproducts. *Water Res.* **2017**, *124*, 576–583. [CrossRef]

57. Dolar, D.; Gros, M.; Rodriguez-Mozaz, S.; Moreno, J.; Comas, J.; Rodriguez-Roda, I.; Barceló, D. Removal of emerging contaminants from municipal wastewater with an integrated membrane system, MBR-RO. *J. Hazard. Mater.* **2012**, *239–240*, 64–69. [CrossRef] [PubMed]
58. Vassalle, L.; Sunyer Caldú, A.; Uggetti, E.; Díez-Montero, R.; Díaz-Cruz, M.S.; García, J.; García-Galán, M.J. Bioremediation of emerging micropollutants in irrigation water. The alternative of microalgae-based treatments. *J. Environ. Manag.* **2020**. [CrossRef]
59. Reemtsma, T.; Miehe, U.; Duennbier, U.; Jekel, M. Polar pollutants in municipal wastewater and the water cycle: Occurrence and removal of benzotriazoles. *Water Res.* **2010**, *44*, 596–604. [CrossRef]
60. Liu, Y.S.; Ying, G.G.; Shareef, A.; Kookana, R.S. Photolysis of benzotriazole and formation of its polymerised photoproducts in aqueous solutions under UV irradiation. *Environ. Chem.* **2011**, *8*, 174–181. [CrossRef]
61. Loos, R.; Gawlik, B.M.; Locoro, G.; Rimaviciute, E.; Contini, S.; Bidoglio, G. EU-wide survey of polar organic persistent pollutants in European river waters. *Environ. Pollut.* **2009**, *157*, 561–568. [CrossRef]
62. Mizukawa, A.; Molins-Delgado, D.; De Azevedo, J.C.R.; Fernandes, C.V.S.; Díaz-Cruz, S.; Barceló, D. Sediments as a sink for UV filters and benzotriazoles: The case study of Upper Iguaçu watershed, Curitiba (Brazil). *Environ. Sci. Pollut. Res.* **2017**, *24*, 18284–18294. [CrossRef]
63. Molins-Delgado, D.; Silvia Díaz-Cruz, M.; Barceló, D. Removal of polar UV stabilizers in biological wastewater treatments and ecotoxicological implications. *Chemosphere* **2015**, *119*, S51–S57. [CrossRef]
64. Díaz-Cruz, M.S.; Gago-Ferrero, P.; Llorca, M.; Barceló, D. Analysis of UV filters in tap water and other clean waters in Spain. *Anal. Bioanal. Chem.* **2012**, *402*, 2325–2333. [CrossRef] [PubMed]
65. Gago-Ferrero, P.; Díaz-Cruz, M.S.; Barceló, D. Occurrence of multiclass UV filters in treated sewage sludge from wastewater treatment plants. *Chemosphere* **2011**. [CrossRef] [PubMed]
66. Gago-Ferrero, P.; Badia-Fabregat, M.; Olivares, A.; Piña, B.; Blánquez, P.; Vicent, T.; Caminal, G.; Díaz-Cruz, M.S.; Barceló, D. Evaluation of fungal- and photo-degradation as potential treatments for the removal of sunscreens BP3 and BP1. *Sci. Total Environ.* **2012**, *427–428*, 355–363. [CrossRef] [PubMed]
67. Bai, X.; Acharya, K. Removal of trimethoprim, sulfamethoxazole, and triclosan by the green alga *Nannochloris* sp. *J. Hazard. Mater.* **2016**, *315*, 70–75. [CrossRef] [PubMed]
68. Gentili, F.G.; Fick, J. Algal cultivation in urban wastewater: An efficient way to reduce pharmaceutical pollutants. *J. Appl. Phycol.* **2017**, *29*, 255–262. [CrossRef]
69. Tolboom, S.N.; Carrillo-Nieves, D.; De Jesús Rostro-Alanis, M.; De la Cruz Quiroz, R.; Barceló, D.; Iqbal, H.M.N.; Parra-Saldivar, R. Algal-based removal strategies for hazardous contaminants from the environment—A review. *Sci. Total Environ.* **2019**, *665*, 358–366. [CrossRef]
70. Salgado, R.; Pereira, V.J.; Carvalho, G.; Soeiro, R.; Gaffney, V.; Almeida, C.; Cardoso, V.V.; Ferreira, E.; Benoliel, M.J.; Ternes, T.A.; et al. Photodegradation kinetics and transformation products of ketoprofen, diclofenac and atenolol in pure water and treated wastewater. *J. Hazard. Mater.* **2013**, *244–245*, 516–527. [CrossRef]
71. Suarez, S.; Lema, J.M.; Omil, F. Removal of Pharmaceutical and Personal Care Products (PPCPs) under nitrifying and denitrifying conditions. *Water Res.* **2010**, *44*, 3214–3224. [CrossRef]
72. Mira, Č.; Gros, M.; Farré, M.; Barceló, D.; Petrovi, M. Pharmaceuticals as chemical markers of wastewater contamination in the vulnerable area of the Ebro Delta (Spain). *Sci. Total Environ.* **2019**, *652*, 952–963.
73. Guzik, U. Naproxen in the environment: Its occurrence, toxicity to nontarget organisms and biodegradation. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 1849–1857.
74. Kasprzyk-Hordern, B.; Dinsdale, R.M.; Guwy, A.J. The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. *Water Res.* **2009**, *43*, 363–380. [CrossRef] [PubMed]
75. Maeng, S.K.; Sharma, S.K.; Abel, C.D.T.; Magic-Knezev, A.; Amy, G.L. Role of biodegradation in the removal of pharmaceutically active compounds with different bulk organic matter characteristics through managed aquifer recharge: Batch and column studies. *Water Res.* **2011**, *45*, 4722–4736. [CrossRef] [PubMed]
76. Kang, D.; Zhao, Q.; Wu, Y.; Wu, C.; Xiang, W. Removal of nutrients and pharmaceuticals and personal care products from wastewater using periphyton photobioreactors. *Bioresour. Technol.* **2018**, *248*, 113–119. [CrossRef]

77. Ávila, C.; Matamoros, V.; Reyes-Contreras, C.; Piña, B.; Casado, M.; Mita, L.; Rivetti, C.; Barata, C.; García, J.; Bayona, J.M. Attenuation of emerging organic contaminants in a hybrid constructed wetland system under different hydraulic loading rates and their associated toxicological effects in wastewater. *Sci. Total Environ.* **2014**, *470–471*, 1272–1280. [CrossRef] [PubMed]
78. Valcárcel, Y.; González Alonso, S.; Rodríguez-Gil, J.L.; Gil, A.; Catalá, M. Detection of pharmaceutically active compounds in the rivers and tap water of the Madrid Region (Spain) and potential ecotoxicological risk. *Chemosphere* **2011**, *84*, 1336–1348. [CrossRef]
79. De Wilt, A.; Butkovskyi, A.; Tuantet, K.; Leal, L.H.; Fernandes, T.V.; Langenhoff, A.; Zeeman, G. Micropollutant removal in an algal treatment system fed with source separated wastewater streams. *J. Hazard. Mater.* **2016**, *304*, 84–92. [CrossRef]
80. Xiong, J.Q.; Kurade, M.B.; Abou-Shanab, R.A.I.; Ji, M.K.; Choi, J.; Kim, J.O.; Jeon, B.H. Biodegradation of carbamazepine using freshwater microalgae *Chlamydomonas mexicana* and *Scenedesmus obliquus* and the determination of its metabolic fate. *Bioresour. Technol.* **2016**, *205*, 183–190. [CrossRef]

4.2.2 Publication #6 Supplementary information

Behavior of UV filters, UV blockers and pharmaceuticals in high rate algal ponds treating urban wastewater

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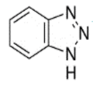
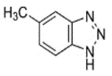
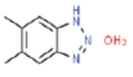
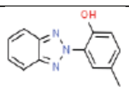
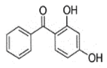
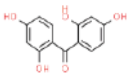
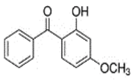
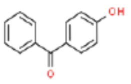
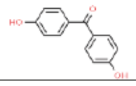
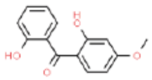
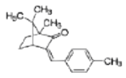
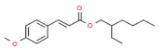
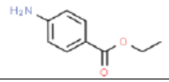
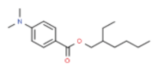
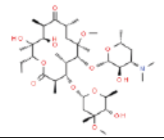
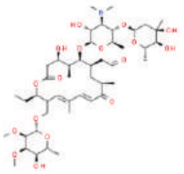
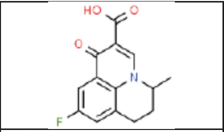
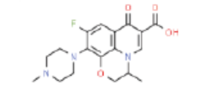
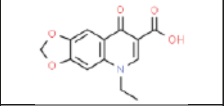
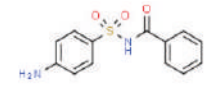
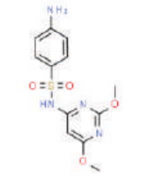
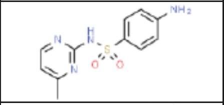
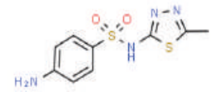
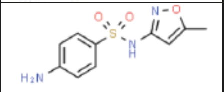
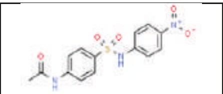
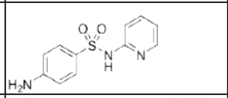
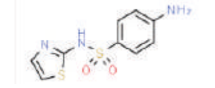
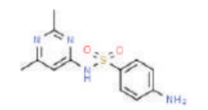
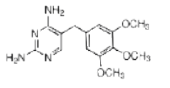
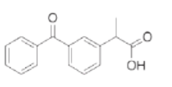
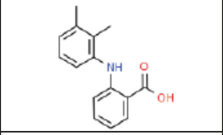
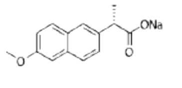
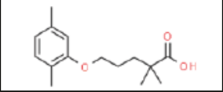
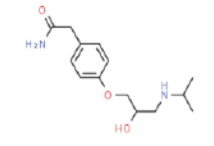
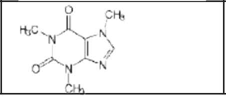
Supplementary Tables S1, S2, S3 and S4
Supplementary Figures S1

Table S1. Target PPCPs, classified by their chromatographic retention time, and their optimized UPLC–QqLIT-MS/MS parameters by positive ionization mode.

Family	Abbreviation	Compound	Molecular Structure	CAS no.	Formula	Molecular weight	Solubility (25ºC) (mg L⁻¹)	Log K_{ow}	pK_a
Benzotriazoles	BZT	1H-benzotriazole		95-14-7	$C_6H_5N_3$	119.120	20000	1.23	8.2
	MeBZT	5-methyl-1-H-benzotriazole		136-85-6	$C_7H_7N_3$	133.150	5	1.89	8.66
	DMBZT	5,6-Dimethyl-1H-benzotriazole monohydrate		4184-79-6	$C_8H_{11}N_3O$	165.192	914.2	2.26	-
	UVP	2-(2'-Hydroxy-5'-methylphenyl)benzotriazol		2440-22-4	$C_{13}H_{11}N_3O$	225.25	-	-	-
Benzophenones	BP1	Benzophenone-1 2,4-Dihhydroxybenzophenone*		131-56-6	$C_{13}H_{10}O_3$	214.220	240	3.17	7.1
	BP2	Benzophenone-2 2,2',4,4'-Tetrahydroxybenzophenone*		131-55-5	$C_{13}H_{10}O_5$	246.053	98.42	2.78	-
	BP3	Benzophenone-3/Oxybenzone 2-Hydroxy-4-methoxybenzophenone*		131-57-7	$C_{14}H_{12}O_3$	228.240	0.21	3.79	-
	4HB	4-hydroxybenzophenone		1137-42-4	$C_{13}H_{10}O_2$	198.220	405.8	3.02	-
	4DHB	4,4 -dihydroxybenzophenone		611-99-4	$C_{13}H_{10}O_3$	214.220	1905	2.55	-
	DHMB	2,2'-Dihydroxy-4-methoxybenzophenone		131-53-3	$C_{14}H_{12}O_4$	244.074	52.73	3.82	-
Camphor derivatives	4MBC	Enzacamene 3-(4-Methylbenzilidene) camphor*		36861-47-9	$C_{18}H_{22}O$	254.400	0.1966	4.95	-
Cinnamate derivatives	EHMC	Ethylhexyl methoxycinnamate		5466-77-3	$C_{18}H_{26}O_3$	290.400	0.1548	5.8	-
p-aminobenzoic acid derivatives	EtPABA	Benzocaine Ethyl 4-aminobenzoate		94-09-7	$C_9H_{11}NO_2$	165.189	1671	1.86	-
	ODPABA	Padimate-O2-Ethylhexyl-4-(dimethylamino)benzoate		21245-02-3	$C_{17}H_{27}NO_2$	277.400	0.1977	6.15	-
Macrolide		Clarithromycin		81103-11-9	$C_{38}H_{69}NO_{13}$	747.953	0.33	3.16	8.99
		Tylosin		1401-69-0	$C_{46}H_{77}NO_{17}$	916.100	5	1.63	7.73

Fluoroquinolone	**Flumequine**		42835-25-6	$C_{14}H_{12}FNO_3$	261.248	308.4	2.6	6.5
	Ofloxacin		82419-36-1	$C_{18}H_{20}FN_3O_4$	361.367	6.76E+05	-2	2.1
Quinolone	**Oxolinic acid**		14698-29-4	$C_{13}H_{11}NO_5$	261.23	8007	0.94	8.77
Sulfonamides	**Sulfabenzamide**		127-71-9	$C_{13}H_{12}N_2O_3S$	276.311	1316	1.3	4.32
	Sulfadimethoxine		122-11-2	$C_{12}H_{14}N_4O_4S$	310.329	433.1	1.63	6.91
	Sulfamerazine		127-79-7	$C_{11}H_{12}N_4O_2S$	264.304	202	0.14	7.12
	Sulfamethizole		144-82-1	$C_9H_{10}N_4O_2S_2$	270.331	1050	0.54	2.1 / 5.3
	Sulfamethoxazole		723-46-6	$C_{10}H_{11}N_3O_3S$	253.278	610	0.89	9.1

	Sulfanitran		122-16-7	$C_{14}H_{13}N_3O_5S$	335.335	36.57	2.26	7.44
	Sulfapyridine		144-83-2	$C_{11}H_{11}N_3O_2S$	249.289	268	0.35	8.43
	Sulfathiazole		72-14-0	$C_9H_9N_3O_2S_2$	255.317	373	0.05	7.2
	Sulfisomidin		515-64-0	$C_{12}H_{14}N_4O_2S$	278.330	1620	-0.33	6.12
Dihydrofolate reductases	**Trimethoprim**		738-70-5	$C_{14}H_{18}N_4O_3$	290.318	400	0.91	7.2
Analgesic/ anti-inflammatories	**Ketoprofen**		22071-15-4	$C_{16}H_{14}O_3$	254.281	51	3.12	4.45
	Mefenamic acid		61-68-7	$C_{15}H_{15}NO_2$	241.285	20 (30ºC)	5.12	4.2
	Naproxen		22204-53-1	$C_{14}H_{14}O_3$	230.259	15.9	3.18	4.15
Lipid regulators	**Gemfibrozil**		25812-30-0	$C_{15}H_{22}O_3$	250.333	4964	4.77	4.5

B-blocking agent		**Atenolol**		29122-68-7	$C_{14}H_{22}N_2O_3$	266.336	685.2	0.16	9.6
Stimulants		**Caffeine**		58-08-2	$C_8H_{10}N_4O_2$	194.191	21600	-0.07	14

Experimental data obtained from Chemspider database and the US Environmental Protection Agency's EPISUITE™

S2. Solar irradiation and air temperatures registered during the days of the experiment.

Date	Irradiance (MJ/m²)	Temperature (ºC)
10/07	23.3	24.9
11/07	26.6	25.5
12/07	28.9	26
13/07	25.3	25.6
14/07	7.7	24.6
15/07	27	25.9
16/07	28.1	25.8
17/07	28.3	25.8
18/07	27.1	26.2
19/07	25.3	25.9
20/07	24.6	25.1

Table S3. Chromatographic retention time and optimized MS/MS transitions for the target analytes

	COMPOUND	RT	MRM1	DP (V)	CE (eV)	CXP (eV)	MRM2	DP (V)	CE (eV)	CxP (eV)	Internal standard
Benzotriazoles	**BZT**	4.6	120>65	56	31	4	120>92	56	25	16	BZT-d_4
	MeBZT	5.3	134>79	46	29	10	134>95	46	35	14	
	DMBZT	5.8	148>77	56	39	2	148>93	56	27	6	
	UVP	10.8	226>120	46	25	10	226>107	46	19	18	
Benzophenones	**BP1**	7.7	215>137	40	27	10	215>105	40	29	6	BP3-d_5
	BP2	6.6	247>137	46	25	8	247>109	46	45	8	
	BP3	9.2	229>151	40	25	12	229>105	40	27	16	
	4HB	7.1	199>121	40	25	8	199>105	40	27	8	
	4DHB	5.9	215>121	45	27	8	215>93	45	45	6	
	DHMB	8.2	245>121	43	29	8	245>151	43	27	12	
Camphor derivatives	**4MBC**	10.8	255>105	61	45	6	255>212	61	29	14	4MBC-d_4
Cinnamate derivatives	**EHMC**	11.9	291>179	51	19	4	291>161	51	25	10	
***p*-aminobenzoic acid derivatives**	**EtPABA**	6.5	166>138	41	20	10	166>120	41	25	28	
	ODPABA	12.3	278>166	86	43	42	278>151	86	27	40	
Macrolide	**Clarithromycin**	7.2	749>158	76	39	10	749>83	76	84	5	Clarithromycin-N-methyl-d_3
	Tylosin	5.74	917>174	106	53	10	917>773	106	46	12	
Quinolone	**Oxolinic acid**	5.8	262>244	36	25	12	262>216	36	41	16	Flumequine-^{13}C
Fluoroquinolone	**Flumequine**	6.7	262>244	51	29	14	262>202	51	45	10	
	Ofloxacin	4.5	362>318	56	29	18	362>261	56	41	18	Sulfamethazine-d_4
Sulfonamides	**Sulfabenzamide**	5.8	277>156	56	17	10	257>92	56	41	6	Sulfamethazine-d_4
	Sulfadimethoxine	6.1	311>156	76	31	8	311>92	76	31	6	
	Sulfamerazine	4.4	265>156	61	27	8	265>92	61	47	6	
	Sulfamethizole	4.4	271>156	56	25	10	271>108	56	27	10	
	Sulfamethoxazole	5.6	254>108	56	27	10	254>156	56	25	10	

	Sulfanitran	6.6	336>156	66	17	12	336>198	66	29	14	
	Sulfapyridine	4.2	250>156	51	28	12	250>92	51	31	6	
	Sulfathiazole	4.1	256>156	40	25	14	256>92	40	25	10	
	Sulfisomidin	4.6	279>124	76	33	8	279>186	76	23	14	
Dihydrofolate reductases	**Trimethoprim**	3.9	291>230	76	33	20	291>261	76	35	12	Trimethoprim-d_3
Analgesic/ antiinflammatories	**Ketoprofen**	7.3	255>105	66	33	18	255>209	66	25	10	BP3-d_5
	Mefenamic acid	9.2	242>224	46	23	22	242>209	46	41	14	Mefenamic acid-d_3
	Naproxen	7.4	231>185	56	17	14	231>115	56	77	10	Flumequine-^{13}C
Lipid regulators	**Gemfibrozil**	9.2	251>233	31	7	14	251>129	31	19	12	BP3-d_5
B-blocking agent	**Atenolol**	3.1	267>116	46	31	8	267>133	46	39	22	Trimethoprim-d_3
Stimulants	**Caffeine**	4	195>109.960	56	31	8	195>138	56	29	10	Trimethoprim-d_3
Internal standards	***BZT-d_4***	4.5	124>96	81	25	6					
	BP3-d_5	9.2	234>151	36	27	12					
	4MBC-d_4	10.8	259>216	76	27	14					
	Flumequine-^{13}C	6.7	265>247	51	31	20					
	Sulfamethazine-d_4	4.6	283>96	26	35	5					
	Trimethoprim-d_3	3.9	294>230	72	35	10					
	Gemfibrozil-d_6	6.7	257.416>239	26	9	14					
	Mefenamic acid-d_3	5.4	245>227.4	66	17	8					
	Caffeine-d_3	4	198>140	71	25	8					
	Atenolol-d_7	3.1	274>107	51	69	4					

Table S4. Linearity and method limits of quantification (LOQ), given in ng L^{-1}, for the targeted analytes.

Family	Compound	Linearity	r2	Urban sewage	HRAP$_{eff}$
Benzotriazoles	**BZT**	1-700	0.9939	29.59	39.91
	5-MeBZT	5-700	0.9978	9.01	31.44
	DMBZT	30-500	0.9985	13.99	20.50
	UVP	3-700	0.9932	282.02	209.00
Benzophenones	**BP1**	30-700	0.9988	15.18	11.70
	BP2	10-700	0.9982	-	-
	BP3	1-700	0.9928	27.83	27.91
	4HB	5-700	0.9989	8.05	-
	4DHB	3-700	0.9907	16.51	20.09
	DHMB	10-700	0.9957	-	-
Camphor derivatives	**4MBC**	30-700	0.997	3.76	3.68
Cinnamate derivatives	**EHMC**	5-700	0.9958	48.58	0.02
***p*-aminobenzoic acid derivatives**	**EtPABA**	50-700	0.9919	-	-
Macrolide	**Clarithromycin**	30-700	0.9989	-	8.00
	Tylosin	50-700	0.9957	-	-
Fluoroquinolone	**Flumequine**	30-700	0.9955	5.63	9.21
	Ofloxacin	30-700	0.9997	613.78	1036.76
Quinolone	**Oxolinic acid**	1-700	0.9926	-	-
Sulfonamides	**Sulfabenzamide**	1-700	0.9919	8.91	-
	Sulfadimethoxine	3-700	0.9926	11.04	1.15
	Sulfaguanidine	30-700	0.9958	-	-
	Sulfamerazine	10-700	0.9961	-	7.69
	Sulfamethizole	30-700	0.9927	17.50	13.41
	Sulfamethoxazole	3-700	0.9929	18.98	2.25
	Sulfamethoxypyridazine	1-700	0.9958	-	7.71
	Sulfanitran	3-700	0.9956	-	-
	Sulfapyridine	1-700	0.9981	6.71	7.27
	Sulfaquinoxaline	3-700	0.9979	9.23	-
	Sulfathiazole	10-700	0.9968	10.29	30.38
	Sulfisomidin	5-700	0.9968	-	-
Dihydrofolate reductases	**Trimethoprim**	3-700	0.9931	11.83	16.41
Analgesic/ antiinflammatories	**Ketoprofen**	10-700	0.9955	113.15	67.78
	Mefenamic acid	1-700	0.9959	-	-
	Naproxen	10-700	0.9946	80.29	90.21
Lipid regulators	**Gemfibrozil**	30-700	0.9878	30	765.63
B-blocking agent	**Atenolol**	30-700	0.9983	80.02	8.89
Stimulants	**Caffeine**	50-700	0.9944	291.00	183.53

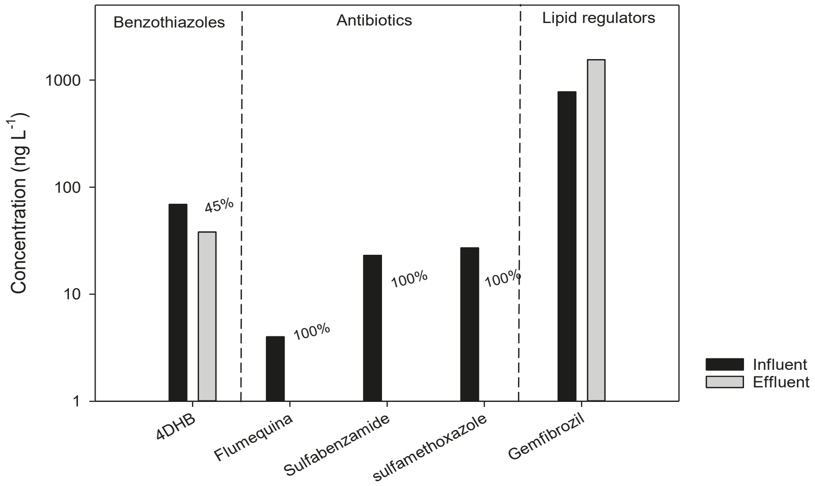


Figure S1. Concentrations of the targeted compounds detected with frequencies of detection (F%) ≤ 17% (1 samples out of 6) in the HRAP

4.2.3 Publication #7

Using integrative samplers to estimate the removal of pharmaceuticals and personal care products in a WWTP and by soil aquifer treatment enhanced with a reactive barrier

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Submitted to Science of the Total Environment

USING INTEGRATIVE SAMPLERS TO ESTIMATE THE REMOVAL OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS IN A WWTP AND BY SOIL AQUIFER TREATMENT ENHANCED WITH A REACTIVE BARRIER

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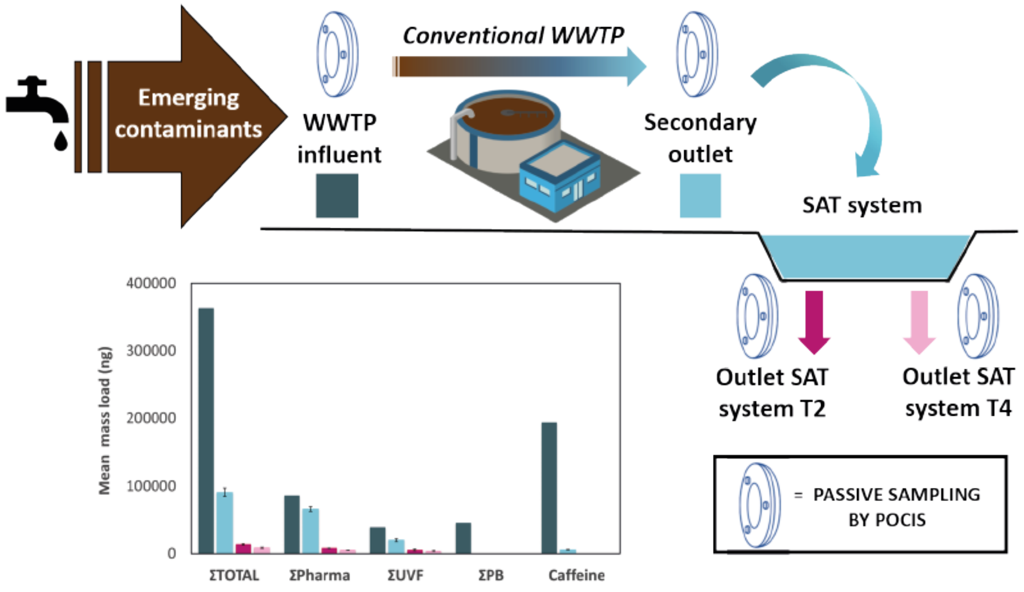
Highlights

- Integrative passive sampling proved useful to assess PPCPs in wastewater
- Soil Aquifer Treatment achieves an efficient removal of PPCPs in conventional WWTPs
- Carbamazepine and its epoxy- metabolite were extensively removed during SAT
- Treatment parameters should be controlled to degrade a broad range of contaminants.
- It can be foreseen that SAT has large application prospects in wastewater reclamation.

Graphical abstract



ABSTRACT

The need and availability of freshwater is a major environmental issue, aggravated by climate change. It is necessary to find alternative sources of freshwater. Wastewater could represent a valid option but requires extensive treatment to remove wastewater-borne contaminants, such as contaminants of emerging concern (CECs). It is urgent to develop not only sustainable and effective wastewater treatment techniques, but also water quality assessment methods. In this study, we used polar organic chemical integrative samplers (POCIS) to investigate the presence and abatement of contaminants in an urban wastewater treatment plant (WWTP) and in soil aquifer treatment (SAT) systems (a conventional one and one enhanced with a reactive layer). This approach allowed us to overcome inter-day and intraday variability of the wastewater composition. Passive sampler extracts were analyzed to investigate contamination from 56 pharmaceuticals and personal care products (PPCPs). Data from the POCIS were used to estimate PPCPs' removal efficiency along the WWTP and the SAT systems. A total of 31 compounds, out of the 56 investigated, were detected in the WWTP influent. Removal rates along WWTP influent were highly variable (16%-100), with benzophenone-3, benzophenone-1, parabens, ciprofloxacin, ibuprofen, and acetaminophen as the most effectively removed chemicals. The two SAT systems yielded much higher elimination rates than those achieved through the primary and secondary treatments together. The SAT system that integrated a reactive barrier, based on sustainable materials to promote enhanced elimination of CECs, was significantly more efficient than the conventional one. The removal of the recalcitrant carbamazepine and its epoxy-metabolite was especially remarkable in SAT, with removal rates between 69-81% and 63-70%, respectively.

KEYWORDS: wastewater, contaminants of emerging concern (CECs), MAR (Managed Aquifer Recharge), passive samplers, UV filters.

1. INTRODUCTION

Incomplete removal of organic pollutants in wastewater treatment plants (WWTPs) is the main reason for the wide distribution of these substances in the environment. Many of these compounds are considered contaminants of emerging concern (CECs), because of their poor (bio)degradability, potential toxicity, (bio)accumulation, and biomagnification through the food web. In fact, these substances are classified as pseudo-persistent pollutants because they are continuously introduced into the environment at rates that cannot be matched by degradation, so that they look as persistent. CECs encompass a wide range of substances, including pharmaceuticals, personal care products (PCPs), hormones, biocides and pesticides, among many others.

Pharmaceutical residues are typically present in the environment, but it is still unclear whether the levels of these compounds in natural waters can cause undesired physiological effects in wildlife. Research has shown that several regularly used pharmaceuticals are endocrine-disrupting compounds (Veldhoen et al., 2014). Still, so far, the major concern regarding environmental contamination from drugs is the occurrence of antibiotics. They contribute to the development of antibiotic resistance, reducing the therapeutic potential against human and animal bacteria pathogens (Kim and Aga, 2007) and are potentially toxic for sensitive organisms (Richardson and Kimura, 2011).

Sunscreens, frequent in wastewater from tourist areas, are also a source of concern. Benzophenone-3 (BP3) is toxic to the larval stages of coral and fish and it is known to induce a multigenerational toxic effect from a single exposure (Downs et al., 2016; Kim et al., 2014; Lucas et al., 2021). Furthermore, BP3 can travel through the blood-brain barrier and accumulate in the white matter of the brain up to 0.32 ng g^{-1} in humans (Van Der Meer et al., 2017; Wang et al., 2015). Rat models evidenced that BP3 induced oxidative stress and apoptosis within the hippocampus and frontal cortex and increased the concentration of extracellular glutamine in the brain (Pomierny et al., 2019), whose high levels can lead to epilepsy, Alzheimer's, Parkinson's, and Huntington's diseases (Cavus et al., 2008).

Benzotriazoles are high-production volume manufactured chemicals that have become essential in diverse industrial applications. They are used as UV stabilizers in plastic materials, as corrosion inhibitors in dishwashing detergents, and as antifreeze in airplane fluids (Asimakopoulos et al., 2013; Gatidou et al., 2019). BZT and MeBZT have been frequently reported in the environment, even in Antarctica (Domínguez-Morueco et al., 2021), as they are poorly volatile and only partially removed during conventional wastewater treatments, due to their high polarity and low biodegradability (Asimakopoulos et al., 2013; Molins-Delgado et al., 2017, 2015). Several studies have shown that BZT can bioaccumulate in humans, being detected in adipose tissues, urine and amniotic fluid (Li et al., 2018; Wang et al., 2015). Ecotoxicity studies showed their endocrine-disrupting properties, impairing neurotoxicity and hepatotoxicity in different fish species (Liang et al., 2017; Tangtian et al., 2012).

The occurrence of parabens (PBs) in the environment is also a source of concern as they have been found to display endocrine-disrupting activity including a decrease in testosterone and alterations in the reproductive tract of male rodents (Oishi, 2002a, 2002b) as well as carcinogenicity (Charles and Darbre, 2013). Several studies also revealed that PBs bioaccumulate in invertebrates and adipose tissue from birds and fishes, and can biomagnificate through the food web (Gago-Ferrero et al., 2013). Thus, removing PPCPs water bodies is important for human and environmental health.

Several factors drive the removal of pollutants in WWTPs, including physicochemical properties of the pollutant, hydraulic retention time (HRT) and configuration and operating conditions (pH, T^{a}) of the WWTP. The need to operate on such a diverse group of contaminants in a broad range of concentrations (from low ng L^{-1} to µg L^{-1}) and physicochemical properties requires the application of non-specific technological solutions. These need to be as effective as possible under different operating conditions (Rodriguez-Narvaez et al., 2017) and for a large number of pollutants. Several wastewater treatment technologies are applied for CECs removal, ranging from conventional activated sludge (CAS) (Tiwari et al., 2017) to membrane bioreactors (MBR) (Besha et al., 2017), or advanced oxidation processes (AOPs) (Grandclément et al., 2017). Nature-based solutions have also been applied, such as constructed wetlands (Gorito et al., 2017).

However, none of them is able to efficiently remove CECs. Removal efficiencies can be highly variable from one WWTP to another and were reported as compound-specific. Consequently, it is still necessary to introduce new strategies in wastewater treatment, to enhance the CECs removal from wastewater.

Advanced treatment technologies for CECs removal require either the addition of chemicals, which often generate toxic disinfection by-products, and/or high costs, which hinder their application (Valhondo and Carrera, 2019). Compared to conventional techniques currently applied, Managed Aquifer Recharge (MAR) through infiltration basins achieves an improvement in the water quality, decreasing the concentration of suspended solids, nutrients, organic matter, pathogens and CECs (Elkayam et al., 2018; Regnery et al., 2015), with low energy demand and no toxic chemicals. Soil Aquifer Treatment (SAT) is a particular case of MAR in which reclaimed water from WWTPs is recharged through the vadose zone, reducing the generation of waste streams (Amy and Drewes, 2007) and supporting a circular economy approach. The implementation of permeable reactive barriers to favour sorption and to promote the growth of diverse microbial communities has proven increase the removal of several CECs during MAR/SAT operations (Valhondo et al., 2014, 2015, 2020a). This allows achieving the improvement of water quality in short times, which facilitates its application as a tertiary treatment (Valhondo et al., 2020b, 2020a). Beyond contaminant removal, MAR helps in recovering aquifer levels and, thus, hyporheic exchange in rivers and the associated ecosystem services.

Demonstrating the effectiveness of any treatment approach in removing pollutants over time is not straightforward. In fact, the analytical methods for the determination of trace contaminants in wastewater are usually based on spot sampling or, at best, 24-h integrated samples. In addition, an in-lab pre-concentration step is generally required before instrumental analysis. These approaches provide the instantaneous or short-time concentrations of the pollutants but suffer from the uncertainty of longer-term fluctuations. An effective alternative is the use of integrative sampling approaches, in particular passive sampling. Its use for continuous pollutant monitoring in water matrices has expanded in recent years. Passive sampling devices get in dynamic contact with large volumes of water, with exposure periods that can reach 4-5 weeks. They allow the *in-situ* pre-concentration of ultra-trace compounds, providing benefits also from a practical point of view (no energy required, easy handling and low cost) (Magi et al., 2018). The final result deriving from passive sampling represents the time weighted average concentration (TWA), which is valuable for ecological risk assessment due to chemical stressors. Instead, obtaining TWA from spot sampling requires frequent sampling and a large number of analyses, thus becoming costly and time-consuming. Passive sampling is based on the diffusive flux of an analyte from water to the receiving phase of the sampler. This flux is driven by the difference between the chemical potential of the analyte in the two media. POCIS (Polar Organic Chemical Integrative Sampler) consists of a sorbent phase, sandwiched between two membranes, able to sequester and concentrate contaminants with $\log K_{OW}$ values in the range 1-4. POCIS have been used for sampling of pharmaceuticals and personal care products (PPCPs) in different water matrices, including wastewater (Alvarez et al., 2004; Di Carro et al., 2018; Liscio et al., 2009; Magi et al., 2018; Tanwar et al., 2015).

In this context, the goal of the present study is to assess the capability of POCIS to help in a more reliable estimation of CECs removal in wastewater treatments. To this end, a WWTP, located in Palamós, Girona (Spain), treating urban wastewater from several municipalities and a hospital, was selected to study the CECs removal throughout the WWTP, by deploying POCIS at the inlet and outlet. This facility is provided with pilot-scale SAT systems fed with the WWTP outflow. The evaluation of CECs removal in two SAT systems (one with and one without reactive barrier) was included in the study to identify the additional pollutants elimination that this technology can provide, compared to the classical secondary treatment.

2. EXPERIMENTAL

2.1 Chemicals and reagents

The 56 CECs selected for investigation in the present study are relevant for treated wastewater reuse in SAT and agricultural irrigation, for public health, and for environmental safety. For the selection, we followed the recommendations of the NEREUS COST Action for urban water reuse (European Cooperation in Science and Technology, 2022) and the EU Watch List (2015/495 and

2015, 2015). In addition, we considered the particular characteristics of the selected WWTP, which treats urban wastewater from 10 municipalities and one hospital. Thus, the target analytes include PPCPs belonging to different compound classes, i.e., antibiotics, anti-inflammatories, UV filters, benzotriazoles, and parabens, among others. The specific target compounds and their physicochemical properties are listed in **Table S1** of the Supporting Information (SI).

The analytical standards of the target PPCPs as well as the isotopically labelled analytical standards, used as surrogate and internal standards, were of analytical grade (> 98%).

Benzophenone-3 (oxybenzone, BP3); benzophenone-1 (BP1); benzophenone-4 (BP4); 4-hydroxybenzophenone (4HB); 4,4'-dihydroxybenzophenone (4DHB); avobenzone (AVO); 2-(2-benzotriazol-2-yl)-p-cresol (UVP); 5,6-dimethyl-1H-benzotriazole (DMBZT); nalidixic acid (NDX); oxolinic acid (OXL); tetracycline (TCY); succynil-sulfathiazole (S-STZ); sulfadiazine (SDZ); N4-acetylsulfadiazidine (acSDZ); sulfamerazine (SMR); N4-acetylsulfamerazine (acSMR); N4-acetylsulfamethazine (acSMZ); sulfamethoxazole (SMX); N4-acetylsulfamethoxazole (acSMX); sulfamethoxypyridazine (SMPZ); sulfapyridine (SPY); N4-acetylsulfapyridine (acSPY); sulfaquinoxaline (SQX); sulfathiazole (STZ); sulfisomidine (SMD); sulfadimethoxine (SDM); trimethoprim (TMP); acetaminophen (APH, also known as paracetamol); atenolol (ATL); gemfibrozil (GFZ); ketoprofen (KPF); mefenamic acid (MFA); carbamazepine (CBZ); norfluoxetine (norFXT); ofloxacin (OFX); ciprofloxacin (CFX); caffeine (CFF); ibuprofen (IBU); salicylic acid (SCY); diclofenac (DCF); diclofenac-13C (DCF-13C); methyl paraben (MePB); propyl paraben (PrPB); benzyl paraben (BePB); butyl paraben (BuPB) and benzophenone-(carbonyl-13C) (BP-13C) were purchased from Sigma Aldrich (Darmstadt, Germany).

Benzophenone-2 (BP2); 2,2'-hydroxy-4-methoxybenzophenone (DHMB); ethyl-4-(dimethyl-amino) benzoate (EtPABA); ethylhexyl methoxycinnamate (EHMC) and 1H-benzotriazole (BZT) were purchased from Merck (Darmstadt, Germany).

2-Hydroxy-4-methoxy-2',3',4',5',6'-d5 (BP3-d5); (±)-3-(4-methylbenzylidene-d4) camphor (4MBC-d4); 1H-benzotriazole-4,5,6,7-d4 (BZT-d4); flumequine-13C3 (FLU-13C3); trimethoprim-d3 (TMP-d3); carbamazepine-d10 (CBZ-d10); mefenamic acid-d3 (MFA-d3);

caffeine-d3 (CFF-d3); ibuprofen-d3 (IBU-d3); salicylic acid-d6 (SCY-d6); diclofenac-d4 (phenyl-d4) (DCF-d4); benzyl paraben-d4 (BePB-d4) and 5-(2,5-dimethylphenoxy)-2,2-bis(trideuteriomethyl)pentanoic acid (GMZ-d6) were supplied by CDN isotopes (Quebec, Canada).

5-Methyl benzotriazole (MeBZT) was obtained from TCI (Zwijndrecht, Belgium). 4-Methylbenzylidene camphor (4MBC) was provided by Dr. Ehrenstorfer (Augsburg, Germany). Flumequine (FLU); N-des-methylvenlafaxine (N-desVFX); carbamazepine 10,11-epoxy (CBZ-E); sulfamethazine-d4 (SMZ-d4) and acetaminophen-d4 (APH-d4) were provided by Toronto Research Chemicals (Toronto, Canada). Oxytetracycline (*O*-TCY) and naproxen (NPX) were purchased from Honeywell Fluka (Wabash, MA, USA).

Water, methanol (MeOH), and acetonitrile (ACN) of MS-grade were obtained from J.T. Baker (Deventer, The Netherlands), and the nitrogen (99.995% purity) and argon (99.99% purity) were supplied by Air Liquide (Barcelona, Spain). Ethanol (EtOH), acetone, formic acid (FA), and ammonium acetate were purchased from Merck (Darmstadt, Germany).

Stock solutions of individual standards and intermediate mix standards solutions containing all analytes (1 mg L^{-1}) were prepared in MeOH. Standard working solutions were daily prepared at appropriate concentrations. Standards solutions were stored in the dark at -20 °C.

2.2. Site description

The selected WWTP is located in Palamós (Girona), on the north of the Spanish Mediterranean coast. The design flow of the facility is 33.000 m^3/day, or 165.450 Eqs/inh., with an HRT of some 24 h. The area is a touristic hotspot, with a daily influent during the summer months (25.000 – 30.000 m^3) that doubles that of the winter months (12.000 – 14.000 m^3). Two pre-treatment lines remove solid waste, grease, and supernatants from inlet water before it flows to the primary treatment. The primary treatment consists of three circular decanters; all operate during the summer, while only two operate during the rest of the year. The secondary biological treatment consists of a conventional activated sludge (CAS) treatment followed by three clarifiers.

A pilot-scale SAT system was installed in the WWTP in 2017 to evaluate the efficiency of reactive barriers to enhance SAT water quality improvement of the secondary treatment effluent. Each SAT system comprises an infiltration basin (1.5 m-long x 2.38 m-wide) overlying a 1.15 m-unsaturated zone, followed by an aquifer (15 m-long x 2.38 m-wide x 1.5 m-high) made up of fine sand (ø=0.1-0.2 mm). A dosing pump (PRIUS) controls the flow into each system and the elevation of the discharge pipe, which collects the water in the deepest part of the aquifer, controls the outlet of each system. One of the systems (SAT system T2) is a conventional SAT, with the unsaturated zone filled with sand. A reactive barrier was installed in SAT system T4. The reactive barriers consist of approximately 50% (in volume) of organic substrates (vegetal compost or woodchips), which provide sorption sites and release dissolved organic carbon into the flowing water to favour the development of diverse redox regions with diverse microbial communities, 50% (in volume) of sand, which provides strength, and a little amount of clay to increase the types of sorption sites. A detailed description of the pilot SAT systems can be found in Valhondo et al., (2020b). SAT systems were operated under the same conditions during the POCIS deployment period, fed with a comparable flow of the effluent of the secondary treatment and allowing plants to grow freely in the recharge areas, thus displaying ideal conditions to compare the removal of CECs between the two systems.

2.3. POCIS assembly

The passive samplers used in this study were developed by Alvarez et al., (2004) to collect and pre-concentrate polar organic compounds. POCIS were assembled in our laboratories by reproducing the characteristics of the commercial type (200 mg sorbent material and 45.8 cm^2 as sampler surface area). Polyethersulfone (PES) membranes (0.1-µm pore size, 150-µm thickness) were obtained from Pall Italia (Buccinasco, Italy) and Oasis hydrophilic-lipophilic balanced (HLB) sorbent phase (60-µm particle size) from Waters (Vimodrone, Italy). PES membranes were washed in water/MeOH (80:20 v/v) for 24 h and with MeOH for 24 h, prior to use. After drying, 200 mg of the HLB sorbent was enclosed between two membranes. The membranes were then compressed between two stainless steel rings, fixed with nuts, and provided with specific holes for the placement (**Figure 1**). POCIS were stored at -20 °C until deployment.

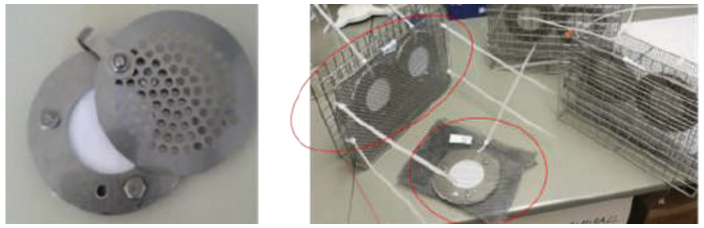


Figure 1: Assembly for the deployment of the passive samplers into the metallic grid to protect the membranes.

2.4. Experiment setup and POCIS deployment

Seven POCIS were placed at different points of the WWTP to monitor the water-borne pollutants along the treatments. Passive sampling could suffer from low reproducibility; therefore, duplicate deployment was performed where possible. Specifically, the following setup was designed (**Figure 2**): one POCIS at the inlet of the plant (referred hereafter as "WWTP influent"), namely where the stream of non-treated wastewater (influent) arrives; two POCIS at the outlet of the secondary treatment (referred hereafter as "Secondary outlet"), which is also the influent to the SAT systems; two POCIS at the outlet of the reference system (referred hereafter as "Outlet SAT T2"), and two POCIS at the outlet of the T4, the one operated with the reactive barrier (referred hereafter as "Outlet SAT T4").

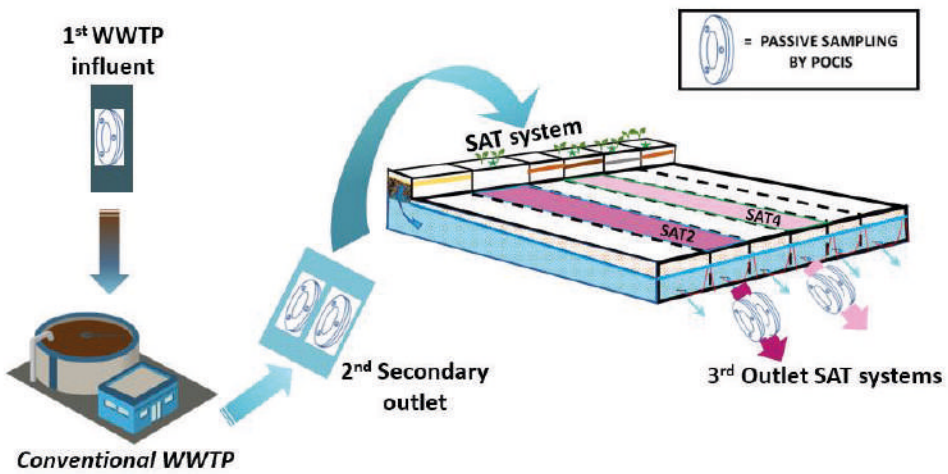


Figure 2: Experimental setup of the POCIS deployment in the WWTP.

All samplers were located inside stainless-steel cages to protect the membranes from accidental impacts. The cages were immersed in the water to obtain a water flow parallel to the membranes; this ensured chemicals accumulated in the samplers by diffusion, avoiding a rapid saturation of the solid phase contained in the POCIS (**Figure S1**). Indeed, the chemicals must diffuse from water through the semipermeable membrane with the only driving force of chemical potential gradient, excluding the “physical filter” behaviour.

The POCIS were deployed for 14 days (from January 18th to February 1st, 2018) to account for temporal fluctuations in concentrations. After the scheduled deployment time, the devices were recovered from the sites (**Figure S2**), removed from the cages, and gently rinsed with deionized water to clean the surfaces. Then, they were wrapped in aluminium foil and stored at -20°C until further processing.

2.5. POCIS processing and HPLC-MS/MS analysis of PPCPs

The samplers were thawed and dismantled; with the aid of HPLC-grade water, the HLB sorbent was carefully transferred into a 1 cm i.d. glass cartridge, provided with a polytetrafluoroethylene (PTFE) frit and glass wool, to prevent phase loss. The solid phase was dried for 30 min under vacuum. An aliquot of 10 µL of a solution of the internal standard diclofenac-C13 at a concentration of 50 mg L^{-1} was added to the sorbent and dried for 30 min under vacuum. Then, an elution with 50 mL of acetone was performed to recover the sorbed analytes and further evaporated to dryness using a rotary evaporator. The residue was reconstituted in 1 mL of MeOH and, if not immediately analyzed, stored at -20°C. An aliquot of this sample was diluted 1:50 with MeOH/water, 50:50 (v/v) and analyzed by HPLC-MS/MS.

Separation and detection of the target PPCPs were performed by online SPE-HPLC-MS/MS in a Symbiosis™ Pico instrument from Spark Holland (Emmen, The Netherlands) coupled to a 4000 Q TRAP™ hybrid quadrupole-linear ion trap-MS analyzer from Applied Biosystems-Sciex (Foster City, California, USA), following the method by Vassalle et al., (2020). Briefly,analytes separation was carried out with a Hibar Purospher® STAR® HR R-18 ec. (50 mm×2.0 mm, 5 µm, Merck) column. The mobile phase consisted of a mixture of HPLC-grade water and ACN, both with 0.1% of formic acid. MS/MS detection was carried out in positive and negative electrospray ionization (ESI+, ESI-) modes under selected reaction monitoring (SRM). Two major characteristic fragments of the precursor molecular ion were recorded per analyte for enhanced sensitivity and selectivity. The most abundant transition was selected for quantification, whereas the second most abundant was used for analyte confirmation. Fragmentation voltage and collision energy were optimised for each transition. The isotope dilution approach was used for quantification. The instrumental limits of detection (ILOD) and instrumental limits of quantification (ILOQs) were calculated as pg injected in the HPLC-MS system using matrix-matched standards. The ILODs were in the range 3.5-11 pg for ultraviolet filters (UVFs), 0.0004-3.7 pg for paraben preservatives (PBs), 0.3-0.8 pg for pharmaceuticals (Pharma) and 0.001 pg for CFF. The complete list of ILODs and ILOQs can be consulted in Table S2.

2.6. Determination of PPCPs removal by the passive sampling approach

The amount of chemicals found in the sorbent phase after POCIS deployment correlates with their TWA concentration in water. To provide this concentration, a kinetic constant, called sampling rate (R_S), needs to be estimated (Di Carro et al., 2014). Still, the knowledge of TWA concentrations was not essential in this work because the interest was in the reduction of TWA along the system, which is proportional to the reduction in mass retained. Thus, only the absolute ng of chemicals sampled per POCIS were considered. By comparing the analytes mass in POCIS deployed in the different sites of a WWTPs, information about the abetment of chemicals after specific treatments was obtained. In particular, the removal ratios (RE%) for the selected PPCPs along the WWTP and in the SAT systems T2 and T4 were calculated as the ratio between analyte masses determined in the POCIS at the inlet and at the outlet of the secondary and SAT systems considered.

3. RESULTS AND DISCUSSION

3.1. Overall results

Data obtained from the analysis of the POCIS extracts were used to determine the presence of the considered contaminants at the various stages of the wastewater treatment. Due to the complex mechanism of absorption of the different chemical classes onto the POCIS sorbent, as well as probable fluctuations in the site's conditions (flow, pH, temperatures), a certain degree of variability was expected in the accumulation of the analytes in the duplicated deployments (Alvarez et al., 2014). Nevertheless, results characterized by a relative standard deviation (RSD) >30%, related to the analyte mass sampled by the duplicated POCIS, had low reliability and were considered with caution.

Thirty-two out of the 56 considered analytes were detected in at least one of the POCIS extracts, 31 of them in the WWTP influent. Full quantitative results are provided in Table S3, expressed as the mass in ng per POCIS of each compound. **Figure 3** displays the chromatogram of the extract deriving from the POCIS deployed at the WWTP influent.

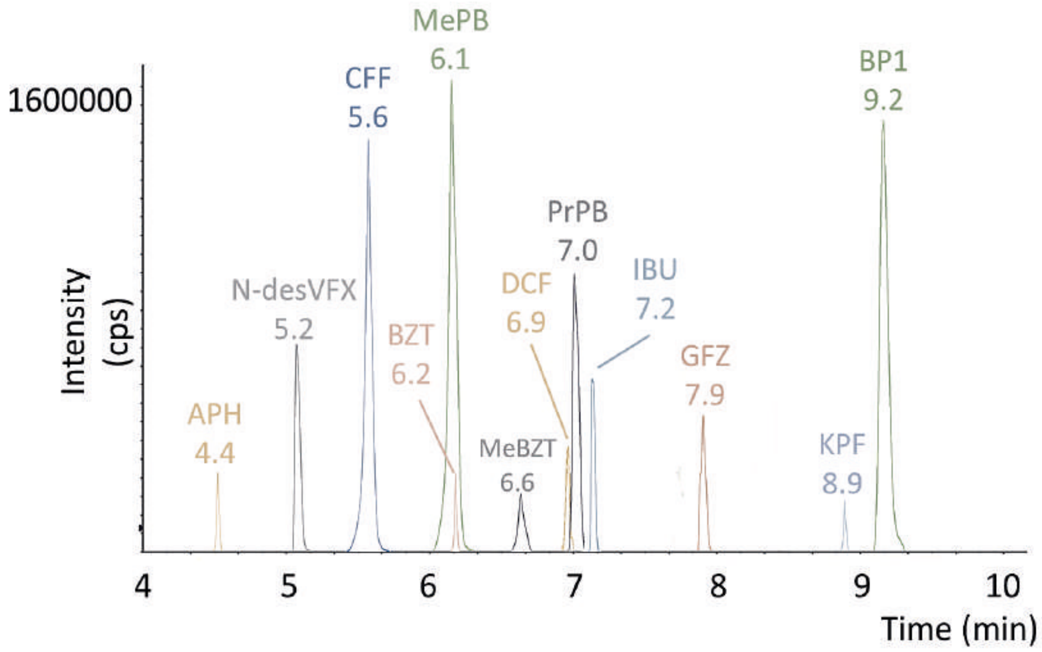


Figure 3: Reconstructed MS/MS chromatogram from the POCIS deployed at the WWTP influent.

It is important to remark that higher sampled amounts of particular contaminants may not necessarily represent a higher concentration in water. Indeed, the amount of a chemical sampled by the POCIS depends on both its concentration in water and the sampling rate (Rs). If two substances have the same water concentration but one has a larger R_S, this will result in a higher sampled amount for the latter. Therefore, direct comparison among the amounts of the different substances detected is possible only for those chemicals characterized by similar R_S.

The contaminant TWA concentrations in the different points of the WWTP could not be calculated, because the specific R_S are not available. Still, by comparing the mass of the analyte adsorbed in the different POCIS deployed, information about the abatement of chemicals after the specific treatments could be retrieved. In fact, since these amounts reflect average concentrations over the sampling period, they are more informative than those obtained by spot sampling (Mirasole et al., 2016).

For the CAS treatment of the selected WWTP, the expected main removal mechanisms of CECs were biodegradation and sorption. Long hydraulic and sludge retention times may foster the growth of slow-growing bacteria, thus improving the removal of many CECs such as DCF and, erythromycin (Fernandez-Fontaina et al., 2012; Suarez et al., 2010). Temperature is also a key factor driving the removal of CECs, as higher removal rates are reported between 15 and 20°C compared to <10°C (Castiglioni et al., 2006; Vieno et al., 2005). In our study the deployment was from January 18th to February 1st, when mean temperatures in the systems were between 12.3 and 13.6 °C, while water in the secondary outlet range between 13 and 15.4ºC.

Figure 4 displays the single CECs mass load calculated for the POCIS at the WWTP influent and the Secondary outlet. Overall, highly variable removal rates were observed for the wide range of selected PPCPs, which is in agreement with the classification of CECs made by Suarez et al., (2010), according to the elimination potential under different biological conditions: highly removed under aerobic and anoxic conditions (e.g. IBU, 95% removed); highly removed under aerobic but hardly removed under anoxic conditions (e.g. DCF, not removed); recalcitrant to biological degradation (e.g. CBZ, not removed). The CECs' abatement was further estimated in the pilot SAT systems T2 and T4 to assess their removal efficiency.

Considering the total mass of contaminants, a significant reduction of the concentration in water was observed going from the WWTP inlet to the secondary treatment outlet and the SAT treatment outlet; a difference was also observed between the residual contamination present after T2 and T4, showing a general higher efficacy of the reactive barrier components to retain and/or degrade the PPCPs. By looking at the total amount grouped by chemical classes, namely Pharma, UVF, PBs and CFF, the same reduction trend was generally observed, as shown in **Figure S3**. However, some compounds inside the classes showed peculiar behaviour **(Figure 4)**. ¡Error! No se encuentra el origen de la referencia. summarizes the calculated RE% for each detected contaminant between the WWTP influent and the secondary outlet, and between the secondary outlet and the Outlet of SAT systems T2, without reactive barrier, and T4, with reactive barrier.

Complete removal from the WWTPs inlet to the outlet of the secondary treatment was observed only for BP3, the parabens group, and APH.

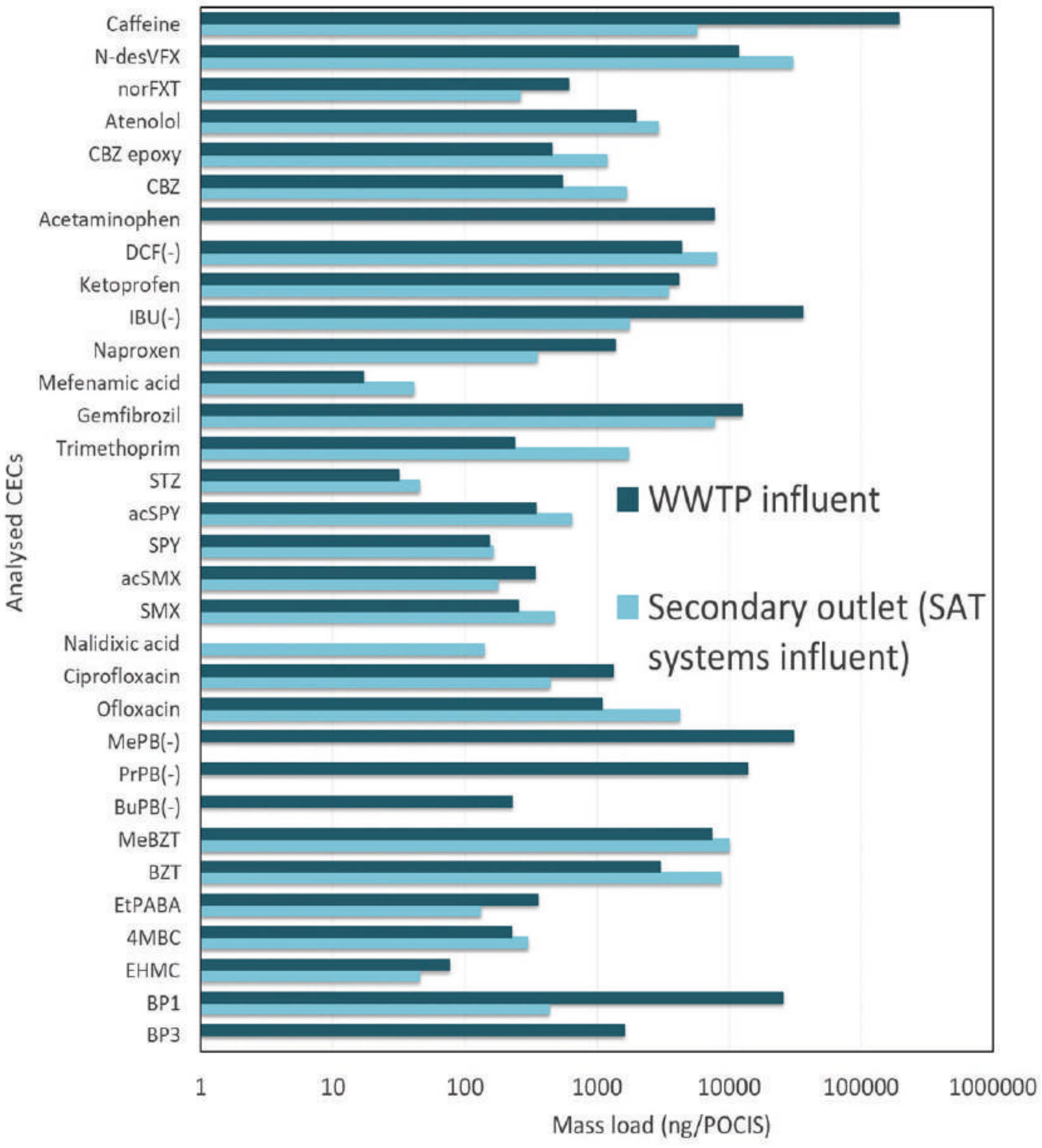


Figure 4: Mass load (in log scale) determined in the POCIS deployed at the WWTP influent and the Secondary outlet (SAT systems influent) streams of the WWTP.

Table 1: Estimated mass removal (RE%) of the single contaminants detected in the WWTP influent through primary + secondary treatments and through SAT systems.

Family group	Compound	Acronym	Inlet/ secondary outlet	Secondary outlet/ MAR outlet T2	Secondary outlet/ MAR outlet T4
Personal care					
Ultraviolet filter	Benzophenone-3	BP3	100	-	-
Ultraviolet filter	Benzophenone-1	BP1	98.3	100	100
Ultraviolet filter	3-(4-Methylbenzilidene) camphor	4MBC	-33.7	100	100
Ultraviolet filter	Ethyl 4-aminobenzoate	EtPABA	63.5	100	100
Ultraviolet filter	1,2,3-Benzotriazole	BZT	-191.1	58	67.7
Ultraviolet filter	Methyl-benzotriazole	MeBZT	-36.1	85	91.5
Paraben	Butylparaben	BuPB(-)	100	-	-
Paraben	Propylparaben	PrPB(-)	100	-	-
Paraben	Methylparaben	MePB(-)	100	-	-
Pharmaceuticals					
Antibiotic	Ofloxacin	OFX	-287.7	100	100
Antibiotic	Ciprofloxacin	CFX	67	100	100
Antibiotic	Nalidixic acid	NDX	-	100	100
Antibiotic	Sulfamethoxazole	SMX	-88	-313.9	-192
Antibiotic	N^4-Acetylsulfamethoxazole	acSMX	48.2	-51.1	41.3
Antibiotic	Sulfapyridine	SPY	-7.6	-0.4	27.5
Antibiotic	Acetylsulfapyiridine	acSPY	-84.2	100	100
Lipid regulator	Sulfathiazole	STZ	-40.1	100	56.4
Anti-inflammatory	Trimethoprim	TMP	-625	100	100
Anti-inflammatory	Gemfibrozil	GFZ	38.3	84.7	89.7
Anti-inflammatory	Mefenamic acid	MFA	-140.5	84.2	87.5
Anti-inflammatory	Naproxen	NPX	74.7	-33	25.1
Anti-inflammatory	Ibuprofen	IBU(-)	95.2	91.3	100
Anti-inflammatory	Ketoprofen	KPF	16	52.2	81.8
Anti-inflammatory	Diclofenac	DCF(-)	-82.6	88.1	91.1
Analgesic	Acetaminophen	APH	100	-	-
Anti-epileptic	Carbamazepine	CBZ	-206.3	69	80.9
Anti-epileptic	Carbamazepine-epoxy	CBZ-E	-161.5	63.3	70.4
β-blocker	Atenolol	ATL	-49.1	100	100
Anti-depressant	Norfluoxetine	norFXT	56.6	-5.9	23.3
Anti-depressant	N-desmethylvenlafaxine	N-desVFX	-156.1	100	100
Stimulant	Caffeine	CFF	97.1	100	100

3.2. Occurrence and fate of pharmaceuticals

Within the pharmaceuticals class, 21 compounds were detected at the WWTP influent, including some fluoroquinolones, sulphonamides, and trimethoprim (antibiotics), the lipid regulator GFZ, the anti-inflammatories MFA, NPX, IBU, KPF, and DCF, the analgesic APH, the anticonvulsant CBZ and its major metabolite CBZ-E, the β-blocker ATL, the antidepressant metabolite norFXT, and the stimulant CFF. A rather wide range of quantities was measured, from tens of ng for Mefenamic acid (MFA) to hundreds of μg for caffeine (CFF), which alone accounted for 53% of the total mass analyzed at the WWTP influent (Figure 4). As already mentioned, this indicates a wide range of water concentrations, as well as POCIS R_S.

Only 23% of the total mass of pharmaceuticals was eliminated by the primary and secondary treatments, thus confirming that conventional WWTPs are not suitable to eliminate pharmaceuticals from wastewater (Krzeminski et al., 2019). The only exception was APH, not detected in the secondary effluent. A wide range of removal rates was observed, from 16% (KPF) to 97% (CFF). For antibiotics, the highest RE% was obtained for CFX, whereas the others were characterized by quite low removals. Anti-inflammatories showed high RE%, especially IBU (>95%). Within the target pharmaceuticals, the anticonvulsant CBZ was the least removed, which was expected, being this neutral substance one of the most critical compounds monitored worldwide. This behaviour has been attributed to its chemical stability and hydrophilicity (log Kow <3) (Nakada et al., 2006). The analytes OFX, several sulphonamides, TMP, CBZ, CBZ-E, ATL, and N-desVFX showed a greater mass at the secondary outlet than at the influent (negative RE%); this phenomenon has been observed in other studies (Behera et al., 2011; Jones et al., 2017). It is usually explained by the reconversion of transformation products into the parent compounds (e.g. by de-conjugation or other transformation processes taking place during the treatment) (Krzeminski et al., 2019).

Effective removal of most pharmaceuticals was observed along the SAT systems: approximately 90% of the total mass measured at the secondary outlet was depleted. The RE% of SAT systems was compound-dependent (**¡Error! No se encuentra el origen de la referencia.**). SAT system T4 performed better than SAT system T2, accounting for 1.2 to 2.6-folds higher removals. **Figure 5** depicts the load of chemicals sampled at the outlet of SAT systems T2 and T4.

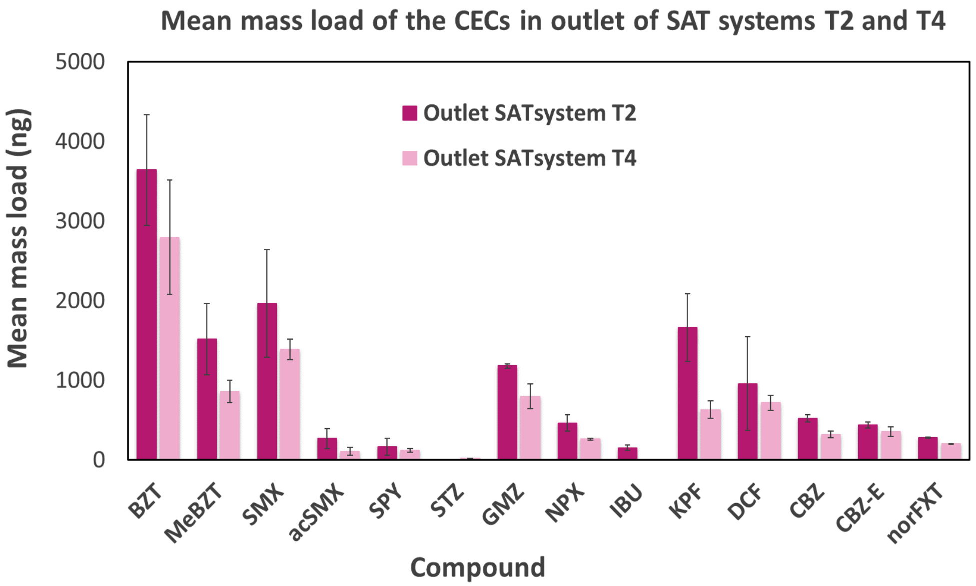


Figure 5: Mean mass load of the selected CECs in the outlet stream of the SAT systems T2 (operated without reactive barrier) and T4 (operated with reactive barrier). Both systems were recharged with the secondary treatment outlet of the WWTP.

In detail, fluoroquinolones, quinolones, the antibiotic TMP, the β-blocker ATL, the anti-depressant N-desVFX, and CFF were completely removed by both T2 and T4 SAT systems, proving the efficiency of such technology to remove many of the pharmaceuticals present in WWTP outflow. For the rest of them, RE% were in the range of 23% - 91%.

Among sulphonamides, the SMX mass significantly increased during SAT, while the acSMX mass slightly increased or remained constant, compared to the secondary outlet. These data might suggest an interconversion between the acetylated and non-acetylated forms of sulphonamides through the steps of the wastewater treatment. A “back transformation” of SMX metabolites was indeed observed in previous studies (Bonvin et al., 2013; Göbel et al., 2005). As for SPY and acSPY, the first remained constant during all WWTP stages, while the second was characterized by a complete removal through the SAT systems. Nevertheless, it is worth noting that the RSD of the two POCIS deployed after the SAT systems were rather high for SMX, acSMX, and SPY. The sulphonamide STZ represents a particular case, being the only compounds for which SAT system T2 showed a better removal efficiency than T4. Its mass was rather low in all samples but completely absent in the POCIS deployed at the outlet of SAT system T2.

The anti-depressant metabolite norFXT decreased from the WWTP influent to the secondary outlet, but was then found at approximately constant mass after SAT. NorFXT is the human metabolism product of the fluorinated antidepressant fluoxetine administered in racemic form. This demethylated active metabolite, even more than the parent compound, is produced in the human body as two enantiomers (Stokes and Holtz, 1997), which act differently, being the (S)-NFLX the more potent regarding its inhibition capacity on serotonin production (Fuller et al., 1992). The apparent inefficacy of the SAT systems might be explained by previously reported evidence showing that the degradation pattern and the enantio-selectivity depend to a large extent on the microorganism phylogenies (Ribeiro et al., 2014). Accordingly, SAT microbial communities would be quite less effective at biodegrading norFXT than the microorganisms from the activated sludge of the secondary treatment. Nevertheless, the slight loss of mass observed in SAT system T4, although not in T2, is consistent with the expected effect of the reactive barrier, which should promote the development of diverse microbial communities, possibly favouring norFXT removal.

Regarding the remaining detected analytes, namely GFZ, all anti-inflammatories, and the two anti-epileptic drugs, the mass sampled at T4 outlet was always lower than at T2, with the highest difference in the abatement extent for KPF. However, the complete removal of these substances was not observed, except for IBU in T4. The removal of NPX in the SAT systems was quite low: only 23% was achieved in T4 (compared to 80-100% of the other NSAIDs), while a negative removal was observed in T2. The NPX degradation extent has been reported to be notoriously poor through conventional wastewater treatments. This is due to its molecular structure with the presence of two aromatic rings, providing high stability and resistance to microbial degradation. Research carried out so far shows that usually only one probable microbiological transformation process occurs for NPX, in which the aromatic rings are not cleaved (Domaradzka et al., 2015). Only a few bacterial strains have provided full NPX degradation (Guzik and Wojcieszyńska, 2020). Thus, most likely, the microbial communities present in the SAT systems did not provide an increased potential to degrade NPX than those present in the activated sludge of the secondary biological treatment. Despite that, removal in T4 was higher than in T2, which again indicates that the reactive barrier installed in T4 induces a change in the microbial community formed (Hellman et al., 2022), making SAT system more efficient. The fate of NPX in the environment is known to be driven not only by biodegradation but also by adsorption (Liu et al., 2019; Martínez-Hernández et al., 2016) which is strongly dependent on pH. As the molecule has a carboxylic acid group that is deprotonated at environmental pH (range 5–8), NPX is present in the environment mostly in its anionic form. In this form, the electrostatic interaction of NPX with the negatively charged natural organic matter and clay of the soil is difficult (Liu et al., 2019). Most likely this low sorption onto the reactive barrier material might contribute to the reduced removal observed for NPX in the SAT system as compared to that in the WWTP. An implication is that the reactive barrier performance might be increased by adding positively charged surfaces, which would act as sorption sites for anions.

Special attention has to be drawn to the removal achieved for the recalcitrant CBZ and its epoxy-metabolite; both showed negative removal rates at the secondary outlet, while SAT systems displayed removals higher than 50%. T4 exhibited an improvement in the removal of these two substances compared to T2, indicating that the presence of the reactive barrier boosted the elimination of these two recalcitrant pharmaceuticals. CBZ is a neutral anticonvulsant known to be recalcitrant at conventional WWTPs (Joss et al., 2006). The increased removal efficiency in the SAT systems might be the result of the largest hydraulic retention time, around 15 days, according to previously reported results in a batch study (Li et al., 2015). Furthermore, at the pH of the system, carbamazepine and derivatives are in their neutral (uncharged) form, which allows a stronger interaction with the organic matter and clay in the barrier material.

3.3. **Occurrence and Fate of Personal Care Products**

Five out of the 10 UVFs analyzed were detected in the WWTP influent, i.e. BP3, BP1, EHMC, 4MBC, and EtPABA. BP1 is the major metabolite of BP3 in different organisms, including humans (Zhang et al., 2021). It is not listed among the permitted UVFs in the EU legislative framework on cosmetic products (European Parliament and the Council of the European Union, 2009), but it can be used as an additive in materials intended to enter in contact with food to protect the product from UV radiation. This application would most likely contribute to its occurrence, as a quite high mass was detected at the WWTP influent (approximately 20 µg), almost 16-fold more than that of the BP3. Still, the value for BP3 was rather high; this result was expected, as it is the most used UVF in sunscreen formulations, constituting up to a 6% (in mass) of the product (European Comission, 2022). It is worth mentioning that the high level of BP3 measured is of concern.

The other detected sunscreens were EtPABA and 4MBC and EHMC, which have been frequently reported globally, being among the most used together with BP3 and octocrylene (Tsui et al., 2014). EHMC is not listed in Table 1 because of the large RSD of the secondary and T2 outlet, but had been fully eliminated after T4. EtPABA and 4MBC were not eliminated during the WWTP, but were fully removed in both SAT systems.

Two benzotriazoles out of the 4 analyzed were detected at the WWTP influent at quite a high level, approximately 3 and 7 µg for BZT and MeBZT, respectively. In this study we considered the paraben preservatives and alkyl esters of the *ρ*-hydroxybenzoic acid (PABA) within the group of personal care and hygiene products, but we could have also included in the pharmaceuticals group. They are present in medicines to prevent the growth of microorganisms and fungi, thus increasing the shelf life of the products. As their alkyl chain increases, so does the antimicrobial activity of the PABA derivative, but its water solubility decreases. Therefore, MePB and PrPB are the most extensively used (Błedzka et al., 2014). This explains the high mass of MePB and PrPB, determined in this study, approximately 30 and 14 µg, respectively. On the other hand, as expected, BuPB was found at a low concentration (around 200 ng) and BePB was not detected.

PBs were fully removed by the primary and secondary treatments. UVFs were characterized by an overall mass reduction of 48% from the WWTP influent to the secondary outlet. BP3 was completely depleted, while 63% and 98% removals were reached for EtPABA and BP1, respectively. 4-MBC showed a negative removal ratio suggesting that derivative species (metabolites, conjugated forms and other transformation products) present in the influent could transform back to the parent compound during the treatment. This was also the case with the two BZTs determined. Compounds such as 5,6-dimethyl-1-benzotriazole and 5-chloro-benzotriazole are biotransformed into MeBZT and ultimately BZT through demethylation and dechlorination consecutive processes (Molins-Delgado et al., 2015) that could have occurred in the influent waters. As already mentioned, EHMC was considered an exception among UVF; a very low amount of it was sampled at the inlet and the high relative standard deviation between the duplicated POCIS in the other sampling points hinders the interpretation of the results.

The RE% of the PCPs at the outlet of the SAT systems is reported in **Table 1**. The 71% and 83% of the overall UVFs mass was depleted by T2 and T4, respectively. Among them, BP3, BP1, 4MBC, and EtPABA were efficiently removed by both T2 and T4. Regarding BZT and MeBZT, a higher depletion was observed along T4, compared to T2 (1.3 and 1.8-folds higher for BZT and MeBZT, respectively). Still, both compounds were present in the outlets of the SAT systems, indicating that the removal was incomplete. In particular, due to an initial negative removal going from the WWTP influent to the secondary outlet, the 100% of the BZT sampled at the WWTP influent was also detected in the outlet of SAT system T4. Overall, this was the only case of concern, due to the effects of this substance in the environment, while a significant decrease in all other substances was observed in the SAT systems.

It is worth noting that the removal achieved in the studied WWTP relies on two treatments, the primary and the biological. In the pilot SAT, the elimination is achieved solely through the soil-aquifer passage, and in the case of T4, upon the stimulation of natural degradation obtained by providing extra organic carbon to the microbial communities grown by means of the reactive barrier.

4. CONCLUSIONS

The application of integrated sampling at WWTPs with POCIS represents a useful approach to assess the removal efficiency of selected PPCPs in an urban WWTP and in a pilot-scale SAT systems and to compare the removals obtained in a conventional SAT (T2) versus a system with a reactive barrier made up of sand, vegetal compost (to favour sorption and as an organic carbon source to foster the growth diverse microbial communities), and clay (T4). The removal rates for PPCPs achieved in the WWTP agreed with reported data in the literature. The contaminants abatement obtained by the SAT systems was notably greater in the system operating with the reactive barrier in comparison to the conventional one, due to the enhanced degradation provided by the microbial communities formed. Overall, the removal efficiency provided by both SAT systems were higher than that achieved in the two wastewater treatments applied in the WWTP. Important improvements were observed for GFZ (from 38% to 90%) and KPF (from 16% to 82%). This improvement was especially remarkable in the case of the recalcitrant CBZ and its major human metabolite (CBZ-E), for which removals of 81% and 70%, respectively, were achieved by SAT system T4. Nevertheless, lower removal was attained for acSMX (48% - 41%), norFXT (57% - 26%), and NPX (74% - 25%).

These outcomes demonstrate that SAT is effective and efficient. Detailed analysis suggests that further improvements can be gained by increasing the variety of sorption sites, ideally including positively charged sites. This could be achieved by creating aerobic zones, so as to favour the precipitation of ferric oxides. We conjecture that increasing residence times, which were of only 15 days in the tested SAT systems, would also help. This could be allowing a longer travel distance in the aquifer (15 m at our pilot sites).

An overall improvement (namely a reduction in the final contaminants mass) was observed by combining the WWTP secondary treatment with SAT, in comparison with the sole secondary treatment. Consequently, SAT systems implemented with a natural reactive barrier constitute a sustainable alternative to advanced tertiary wastewater treatments from both the environmental and economic viewpoints. SAT systems would contribute to combat the continuous release of pharmaceuticals and personal care and hygiene products into the aquatic environment.

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REFERENCES

2015/495, C.I.D. (Eu), 2015, of 20 M., 2015. Comission implementing decission (EU) 2015/495 of 20 March 2015 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council. Off. J. Eur. Union L78/40, 20–30.

Alvarez, D.A., Maruya, K.A., Dodder, N.G., Lao, W., Furlong, E.T., Smalling, K.L., 2014. Occurrence of contaminants of emerging concern along the California coast (2009-10) using passive sampling devices. Mar. Pollut. Bull. 81, 347–354. https://doi.org/10.1016/j.marpolbul.2013.04.022

Alvarez, D.A., Petty, J.D., Huckins, J.N., Jones-Lepp, T.L., Getting, D.T., Goddard, J.P., Manahan, S.E., 2004. Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments. Environ. Toxicol. Chem. 23, 1640–1648. https://doi.org/10.1897/03-603

Amy, G., Drewes, J., 2007. Soil aquifer treatment (SAT) as a natural and sustainable wastewater reclamation/reuse technology: Fate of wastewater effluent organic Matter (EfoM) and trace organic compounds. Environ. Monit. Assess. 129, 19–26. https://doi.org/10.1007/s10661-006-9421-4

Asimakopoulos, A.G., Ajibola, A., Kannan, K., Thomaidis, N.S., 2013. Occurrence and removal efficiencies of benzotriazoles and benzothiazoles in a wastewater treatment plant in Greece. Sci. Total Environ. 452–453, 163–171. https://doi.org/10.1016/j.scitotenv.2013.02.041

Behera, S.K., Kim, H.W., Oh, J.E., Park, H.S., 2011. Occurrence and removal of antibiotics, hormones and several other pharmaceuticals in wastewater treatment plants of the largest industrial city of Korea. Sci. Total Environ. 409, 4351–4360. https://doi.org/10.1016/j.scitotenv.2011.07.015

Besha, A.T., Gebreyohannes, A.Y., Tufa, R.A., Bekele, D.N., Curcio, E., Giorno, L., 2017. Removal of emerging micropollutants by activated sludge process and membrane bioreactors and the effects of micropollutants on membrane fouling: A review. J. Environ. Chem. Eng. 5, 2395–2414. https://doi.org/10.1016/j.jece.2017.04.027

Błedzka, D., Gromadzińska, J., Wasowicz, W., 2014. Parabens. From environmental studies to human health. Environ. Int. 67, 27–42. https://doi.org/10.1016/j.envint.2014.02.007

Bonvin, F., Omlin, J., Rutler, R., Schweizer, W.B., Alaimo, P.J., Strathmann, T.J., McNeill, K., Kohn, T., 2013. Direct photolysis of human metabolites of the antibiotic sulfamethoxazole: Evidence for abiotic back-transformation. Environ. Sci. Technol. 47, 6746–6755. https://doi.org/10.1021/es303777k

Castiglioni, S., Bagnati, R., Fanelli, R., Pomati, F., Calamari, D., Zuccato, E., 2006. Removal of pharmaceuticals in sewage treatment plants in Italy. Environ. Sci. Technol. 40, 357–363. https://doi.org/10.1021/es050991m

Cavus, I., Pan, J.W., Hetherington, H.P., Abi-Saab, W., Zaveri, H.P., Vives, K.P., Krystal, J.H., Spencer, S.S., Spencer, D.D., 2008. Decreased hippocampal volume on MRI is associated with increased extracellular glutamate in epilepsy patients. Epilepsia 49, 1358–1366. https://doi.org/10.1111/j.1528-1167.2008.01603.x

Charles, A.K., Darbre, P.D., 2013. Combinations of parabens at concentrations measured in human breast tissue can increase proliferation of MCF-7 human breast cancer cells. J. Appl. Toxicol. 33, 390–398. https://doi.org/10.1002/jat.2850

Di Carro, M., Bono, L., Magi, E., 2014. A simple recirculating flow system for the calibration of polar organic chemical integrative samplers (POCIS): Effect of flow rate on different water pollutants. Talanta 120, 30–33.

Di Carro, M., Magi, E., Massa, F., Castellano, M., Mirasole, C., Tanwar, S., Olivari, E., Povero, P., 2018. Untargeted approach for the evaluation of anthropic impact on the sheltered marine area of Portofino (Italy). Mar. Pollut. Bull. 131, 87–94. https://doi.org/10.1016/J.MARPOLBUL.2018.03.059

Domaradzka, D., Guzik, U., Wojcieszyńska, D., 2015. Biodegradation and biotransformation of polycyclic non-steroidal anti-inflammatory drugs. Rev. Environ. Sci. Biotechnol. 14, 229–239. https://doi.org/10.1007/s11157-015-9364-8

Domínguez-Morueco, N., Moreno-Merino, L., Molins-Delgado, D., Díaz-Cruz, M.S., Aznar-Alemany, Ò., Eljarrat, E., Farré, M., López-Martínez, J., López de Alda, M., Silva, A., Durán Valsero, J.J., Valcárcel, Y., 2021. Anthropogenic contaminants in freshwater from the northern Antarctic Peninsula region. Ambio 50, 544–559. https://doi.org/10.1007/s13280-020-01404-x

Downs, C.A., Kramarsky-Winter, E., Segal, R., Fauth, J., Knutson, S., Bronstein, O., Ciner, F.R., Jeger, R., Lichtenfeld, Y., Woodley, C.M., Pennington, P., Cadenas, K., Kushmaro, A., Loya, Y., 2016. Toxicopathological Effects of the Sunscreen UV Filter, Oxybenzone (Benzophenone-3), on Coral Planulae and Cultured Primary Cells and Its Environmental Contamination in Hawaii and the U.S. Virgin Islands. Arch. Environ. Contam. Toxicol. 70, 265–288. https://doi.org/10.1007/s00244-015-0227-7

Elkayam, R., Aharoni, A., Vaizel-Ohayon, D., Sued, O., Katz, Y., Negev, Y., Marano R B, M., Cytryn, E., Shtrasler, L., Lev, O., 2018. Viral and Microbial Pathogens, Indicator Microorganisms, Microbial Source Tracking Indicators, and Antibiotic Resistance Genes in a Confined Managed Effluent Recharge System. J. Environ. Eng. 144, 5017011. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001334

European Comission, 2022. European Commission, 2019. 2-hydroxy-4-methoxybenzophenone/oxybenzone. Cosmetic Ingredient Database. [WWW Document]. URL https://ec.europa.eu/growth/tools681 databases/cosing/pdf/COSING_Annex VI_v2.pdf

European Cooperation in Science and Technology, 2022. COST Action ES1403 New and emerging challenges and opportunities in wastewater reuse (NEREUS) [WWW Document]. URL http://www.nereus-cost.eu

European Parliament and the Council of the European Union, 2009. Regulation (EC) No 1223/2009 of the european parliament and of the council of 30 November 2009 on cosmetic products.

Fernandez-Fontaina, E., Omil, F., Lema, J.M., Carballa, M., 2012. Influence of nitrifying conditions on the biodegradation and sorption of emerging micropollutants. Water Res. 46, 5434–5444. https://doi.org/10.1016/j.watres.2012.07.037

Fuller, R.W., Snoddy, H.D., Krushinski, J.H., Robertson, D.W., 1992. Comparison of norfluoxetine enantiomers as serotonin uptake inhibitors in vivo. Neuropharmacology 31, 997–1000. https://doi.org/10.1016/0028-3908(92)90100-4

Gago-Ferrero, P., Díaz-Cruz, M.S., Barceló, D., 2013. Multi-residue method for trace level determination of UV filters in fish based on pressurized liquid extraction and liquid chromatography-quadrupole-linear ion trap-mass spectrometry. J. Chromatogr. A 1286, 93–101. https://doi.org/10.1016/j.chroma.2013.02.056

Gatidou, G., Anastopoulou, P., Aloupi, M., Stasinakis, A.S., 2019. Growth inhibition and fate of benzotriazoles in Chlorella sorokiniana cultures. Sci. Total Environ. 663, 580–586. https://doi.org/10.1016/j.scitotenv.2019.01.384

Göbel, A., Thomsen, A., McArdell, C.S., Joss, A., Giger, W., 2005. Occurrence and sorption behavior of sulfonamides, macrolides, and trimethoprim in activated sludge treatment. Environ. Sci. Technol. 39, 3981–3989. https://doi.org/10.1021/es048550a

Gorito, A.M., Ribeiro, A.R., Almeida, C.M.R., Silva, A.M.T., 2017. A review on the application of constructed wetlands for the removal of priority substances and contaminants of emerging concern listed in recently launched EU legislation. Environ. Pollut. 227, 428–443. https://doi.org/10.1016/j.envpol.2017.04.060

Grandclément, C., Seyssiecq, I., Piram, A., Wong-Wah-Chung, P., Vanot, G., Tiliacos, N., Roche, N., Doumenq, P., 2017. From the conventional biological wastewater treatment to hybrid processes, the evaluation of organic micropollutant removal: A review. Water Res. 111, 297–317. https://doi.org/10.1016/j.watres.2017.01.005

Guzik, U., Wojcieszyńska, D., 2020. Naproxen in the environment: its occurrence, toxicity to nontarget organisms and biodegradation 1849–1857.

Hellman, M., Valhondo, C., Martínez-Landa, L., Carrera, J., Juhanson, J., Hallin, S., 2022. Nitrogen Removal Capacity of Microbial Communities Developing in Compost- and Woodchip-Based Multipurpose Reactive Barriers for Aquifer Recharge With Wastewater. Front. Microbiol. 13, 1–11. https://doi.org/10.3389/fmicb.2022.877990

Jones, S.M., Chowdhury, Z.K., Watts, M.J., 2017. A taxonomy of chemicals of emerging concern based on observed fate at water resource recovery facilities. Chemosphere 170, 153–160. https://doi.org/10.1016/j.chemosphere.2016.11.075

Joss, A., Zabczynski, S., Göbel, A., Hoffmann, B., Löffler, D., McArdell, C.S., Ternes, T.A., Thomsen, A., Siegrist, H., 2006. Biological degradation of pharmaceuticals in municipal wastewater treatment: Proposing a classification scheme. Water Res. 40, 1686–1696. https://doi.org/10.1016/j.watres.2006.02.014

Kim, S., Aga, D.S., 2007. Potential ecological and human health impacts of antibiotics and antibiotic-resistant bacteria from wastewater treatment plants. J. Toxicol. Environ. Heal. - Part B Crit. Rev. 10, 559–573. https://doi.org/10.1080/15287390600975137

Kim, S., Jung, D., Kho, Y., Choi, K., 2014. Effects of benzophenone-3 exposure on endocrine disruption and reproduction of japanese medaka (Oryzias latipes)-A two generation exposure study. Aquat. Toxicol. 155, 244–252. https://doi.org/10.1016/j.aquatox.2014.07.004

Krzeminski, P., Tomei, M.C., Karaolia, P., Langenhoff, A., Almeida, C.M.R., Felis, E., Gritten, F., Andersen, H.R., Fernandes, T., Manaia, C.M., Rizzo, L., Fatta-Kassinos, D., 2019. Performance of secondary wastewater treatment methods for the removal of contaminants of emerging concern implicated in crop uptake and antibiotic resistance spread: A review. Sci. Total Environ. 648, 1052–1081. https://doi.org/10.1016/J.SCITOTENV.2018.08.130

Li, X., de Toledo, R.A., Wang, S., Shim, H., 2015. Removal of carbamazepine and naproxen by immobilized Phanerochaete chrysosporium under non-sterile condition. N. Biotechnol. 32, 282–289. https://doi.org/10.1016/j.nbt.2015.01.003

Li, X., Wang, L., Asimakopoulos, A.G., Sun, H., Zhao, Z., Zhang, J., Zhang, L., Wang, Q., 2018. Benzotriazoles and benzothiazoles in paired maternal urine and amniotic fluid samples from Tianjin, China. Chemosphere 199, 524–530. https://doi.org/10.1016/j.chemosphere.2018.02.076

Liang, X., Zha, J., Martyniuk, C.J., Wang, Z., Zhao, J., 2017. Histopathological and proteomic responses in male Chinese rare minnow (Gobiocypris rarus) indicate hepatotoxicity following benzotriazole exposure. Environ. Pollut. 229, 459–469. https://doi.org/10.1016/j.envpol.2017.06.013

Liscio, C., Magi, E., Di Carro, M., Suter, M.J.F., Vermeirssen, E.L.M., 2009. Combining passive samplers and biomonitors to evaluate endocrine disrupting compounds in a wastewater treatment plant by LC/MS/MS and bioassay analyses. Environ. Pollut. 157, 2716–2721. https://doi.org/10.1016/j.envpol.2009.04.034

Liu, F., Nielsen, A.H., Vollertsen, J., 2019. Sorption and degradation potential of pharmaceuticals in sediments from a stormwater retention pond. Water (Switzerland) 11. https://doi.org/10.3390/w11030526

Lucas, J., Logeux, V., Rodrigues, A.M.S., Stien, D., Lebaron, P., 2021. Exposure to four chemical UV filters through contaminated sediment: impact on survival, hatching success, cardiac frequency, and aerobic metabolic scope in embryo-larval stage of zebrafish. Environ. Sci. Pollut. Res. 28, 29412–29420. https://doi.org/10.1007/s11356-021-12582-w

Magi, E., Di Carro, M., Mirasole, C., Benedetti, B., 2018. Combining passive sampling and tandem mass spectrometry for the determination of pharmaceuticals and other emerging pollutants in drinking water. Microchem. J. 136, 56–60. https://doi.org/10.1016/j.microc.2016.10.029

Martínez-Hernández, V., Meffe, R., Herrera López, S., de Bustamante, I., 2016. The role of sorption and biodegradation in the removal of acetaminophen, carbamazepine, caffeine, naproxen and sulfamethoxazole during soil contact: A kinetics study. Sci. Total Environ. 559, 232–241. https://doi.org/10.1016/j.scitotenv.2016.03.131

Mirasole, C., Di Carro, M., Tanwar, S., Magi, E., 2016. Liquid chromatography–tandem mass spectrometry and passive sampling: powerful tools for the determination of emerging pollutants in water for human consumption. J. Mass Spectrom. 814–820. https://doi.org/10.1002/jms.3813

Molins-Delgado, D., Silvia Díaz-Cruz, M., Barceló, D., 2015. Removal of polar UV stabilizers in biological wastewater treatments and ecotoxicological implications. Chemosphere 119, S51–S57. https://doi.org/10.1016/j.chemosphere.2014.02.084

Molins-Delgado, D., Távora, J., Silvia Díaz-Cruz, M., Barceló, D., 2017. UV filters and benzotriazoles in urban aquatic ecosystems: The footprint of daily use products. Sci. Total Environ. 601–602, 975–986. https://doi.org/10.1016/j.scitotenv.2017.05.176

Nakada, N., Tanishima, T., Shinohara, H., Kiri, K., Takada, H., 2006. Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment. Water Res. 40, 3297–3303. https://doi.org/10.1016/j.watres.2006.06.039

Oishi, S., 2002a. Effects of propyl paraben on the male reproductive system. Food Chem. Toxicol. 1807–1813.

Oishi, S., 2002b. Effects of butyl paraben on the male reproductive system in mice. Arch. Toxicol. 76, 423–429. https://doi.org/10.1007/s00204-002-0360-8

Pomierny, B., Krzyzanowska, W., Broniowska, Z., Strach, B., Bystrowska, B., Starek-Zwiechowicz, B., Maciejska, A., Skórkowska, A., Wesolowska, J., Walczak, M., Budziszewska, B., 2019. Benzophenone-3 Passes through the Blood-Brain Barrier, Increases the Level of Extracellular Glutamate, and Induces Apoptotic Processes in the Hippocampus and Frontal Cortex of Rats. Toxicol. Sci. 171, 485–500. https://doi.org/10.1093/toxsci/kfz160

Regnery, J., Wing, A.D., Alidina, M., Drewes, J.E., 2015. Biotransformation of trace organic chemicals during groundwater recharge: How useful are first-order rate constants? J. Contam. Hydrol. 179, 65–75. https://doi.org/10.1016/j.jconhyd.2015.05.008

Ribeiro, A.R., Maia, A.S., Moreira, I.S., Afonso, C.M., Castro, P.M.L., Tiritan, M.E., 2014. Enantioselective quantification of fluoxetine and norfluoxetine by HPLC in wastewater effluents. Chemosphere 95, 589–596. https://doi.org/10.1016/j.chemosphere.2013.09.118

Richardson, S.D., Kimura, S.Y., 2011. Water analysis: Emerging contaminants and current issues. Anal. Chem. 92, 473–505. https://doi.org/10.1021/acs.analchem.9b05269

Rodriguez-Narvaez, O.M., Peralta-Hernandez, J.M., Goonetilleke, A., Bandala, E.R., 2017. Treatment technologies for emerging contaminants in water: A review. Chem. Eng. J. 323, 361–380. https://doi.org/10.1016/j.cej.2017.04.106

Stokes, P.E., Holtz, A., 1997. Fluoxetine tenth anniversary update: The progress continues. Clin. Ther. 19, 1135–1250. https://doi.org/10.1016/S0149-2918(97)80066-5

Suarez, S., Lema, J.M., Omil, F., 2010. Removal of Pharmaceutical and Personal Care Products (PPCPs) under nitrifying and denitrifying conditions. Water Res. 44, 3214–3224. https://doi.org/10.1016/j.watres.2010.02.040

Tangtian, H., Bo, L., Wenhua, L., Shin, P.K.S., Wu, R.S.S., 2012. Estrogenic potential of benzotriazole on marine medaka (Oryzias melastigma). Ecotoxicol. Environ. Saf. 80, 327–332. https://doi.org/10.1016/j.ecoenv.2012.03.020

Tanwar, S., Di Carro, M., Magi, E., 2015. Innovative sampling and extraction methods for the determination of nonsteroidal anti-inflammatory drugs in water. J. Pharm. Biomed. Anal. 106, 100–106. https://doi.org/10.1016/j.jpba.2014.10.027

Tiwari, B., Sellamuthu, B., Ouarda, Y., Drogui, P., Tyagi, R.D., Buelna, G., 2017. Review on fate and mechanism of removal of pharmaceutical pollutants from wastewater using biological approach. Bioresour. Technol. 224, 1–12. https://doi.org/10.1016/j.biortech.2016.11.042

Tsui, M.M.P., Leung, H.W., Wai, T.C., Yamashita, N., Taniyasu, S., Liu, W., Lam, P.K.S., Murphy, M.B., 2014. Occurrence, distribution and ecological risk assessment of multiple classes of UV filters in surface waters from different countries. Water Res. 67, 55–65. https://doi.org/10.1016/j.watres.2014.09.013

Valhondo, C., Carrera, J., 2019. Chapter 1 - Water as a finite resource: From historical accomplishments to emerging challenges and artificial recharge, in: Galanakis, C.M., Agrafioti, E.B.T.-S.W. and W.P. (Eds.), . Elsevier, pp. 1–17. https://doi.org/https://doi.org/10.1016/B978-0-12-816170-8.00001-6

Valhondo, C., Carrera, J., Ayora, C., Barbieri, M., Nödler, K., Licha, T., Huerta, M., 2014. Behavior of nine selected emerging trace organic contaminants in an artificial recharge system supplemented with a reactive barrier. Environ. Sci. Pollut. Res. 21, 11832–11843. https://doi.org/10.1007/s11356-014-2834-7

Valhondo, C., Carrera, J., Ayora, C., Tubau, I., Martinez-Landa, L., Nödler, K., Licha, T., 2015. Characterizing redox conditions and monitoring attenuation of selected pharmaceuticals during artificial recharge through a reactive layer. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2015.01.030

Valhondo, C., Carrera, J., Martínez-Landa, L., Wang, J., Amalfitano, S., Levantesi, C., Diaz-Cruz, M.S., 2020a. Reactive barriers for renaturalization of reclaimed water during soil aquifer treatment. Water (Switzerland) 12. https://doi.org/10.3390/W12041012

Valhondo, C., Martínez-Landa, L., Carrera, J., Díaz-Cruz, S.M., Amalfitano, S., Levantesi, C., 2020b. Six artificial recharge pilot replicates to gain insight into water quality enhancement processes. Chemosphere 240. https://doi.org/10.1016/j.chemosphere.2019.124826

Van Der Meer, T.P., Artacho-Cordón, F., Swaab, D.F., Struik, D., Makris, K.C., Wolffenbuttel, B.H.R., Frederiksen, H., Van Vliet-Ostaptchouk, J. V., 2017. Distribution of non-persistent endocrine disruptors in two different regions of the human brain. Int. J. Environ. Res. Public Health 14, 1–11. https://doi.org/10.3390/ijerph14091059

Vassalle, L., Sunyer-Caldú, A., Uggetti, E., Díez-Montero, R., Díaz-Cruz, M.S., García, J., García-Galán, M.J., 2020. Bioremediation of emerging micropollutants in irrigation water. The alternative of microalgae-based treatments. J. Environ. Manage. 274. https://doi.org/10.1016/j.jenvman.2020.111081

Veldhoen, N., Skirrow, R.C., Brown, L.L.Y., Van Aggelen, G., Helbing, C.C., 2014. Effects of acute exposure to the non-steroidal anti-inflammatory drug ibuprofen on the developing North American bullfrog (Rana catesbeiana) tadpole. Environ. Sci. Technol. 48, 10439–10447. https://doi.org/10.1021/es502539g

Vieno, N.M., Tuhkanen, T., Kronberg, L., 2005. Seasonal variation in the occurrence of pharmaceuticals in effluents from a sewage treatment plant and in the recipient water. Environ. Sci. Technol. 39, 8220–8226. https://doi.org/10.1021/es051124k

Wang, L., Asimakopoulos, A.G., Kannan, K., 2015. Accumulation of 19 environmental phenolic and xenobiotic heterocyclic aromatic compounds in human adipose tissue. Environ. Int. 78, 45–50. https://doi.org/10.1016/j.envint.2015.02.015

Zhang, H., Li, J., Chen, Y., Wang, D., Xu, W., Gao, Y., 2021. Profiles of parabens, benzophenone-type ultraviolet filters, triclosan, and triclocarban in paired urine and indoor dust samples from Chinese university students: Implications for human exposure. Sci. Total Environ. 798, 149275. https://doi.org/10.1016/j.scitotenv.2021.149275

4.2.3 Publication #7 Supplementary information

Using integrative samplers to estimate the removal of pharmaceuticals and personal care products in a WWTP and by soil aquifer treatment enhanced with a reactive barrier

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Submitted to Science of the Total Environment

Section S1. Figures. (Figures S1, S2 and S3).
Section S2. Tables. (Table S1, S2 and S3).

Section S1. Figures

Figure S1: Installation of the POCIS at the outlet of the SAT system T4.

Figure S2: Appearance of the POCIS once transported to the laboratory and dismantled for subsequent cleaning and extraction.

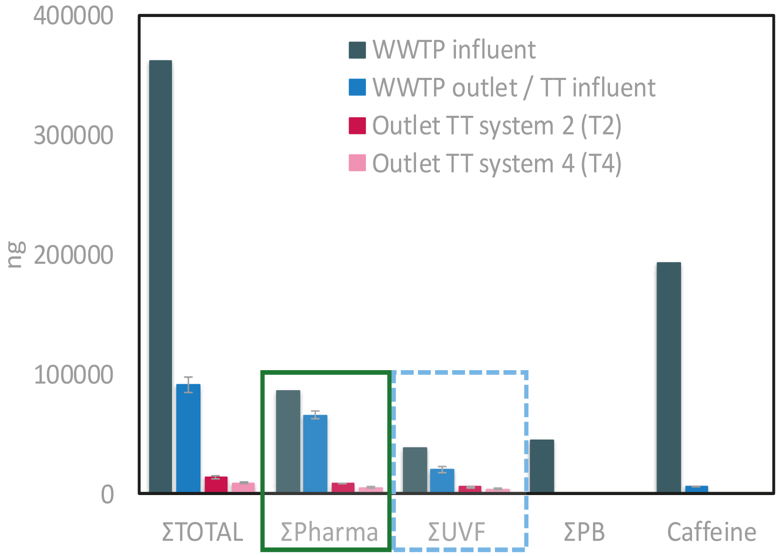


Figure S3. Total amounts of the different chemical classes detected by POCIS sampling in the various steps of wastewater treatment (ST: secondary treatment; TT: tertiary treatment).

Section S2. Tables

Table S1: Selected compounds and their major physicochemmical properties. Kow, octanol-water partition constant; pKa dissociation constant.

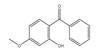
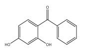
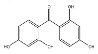
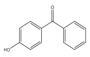
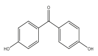
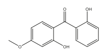
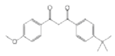
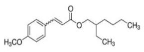
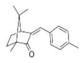
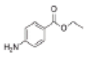
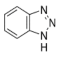
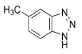
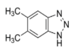
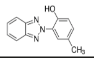
Compound	Synonyms	Structure	Family	Type	CAS number	Molecular mass(g/mol)	Solubility in water (mg/L)	$\log K_{ow}$	pKa
Benzophenone-3 (BP3)	Oxybenzone; 2-Hydroxy-4-methoxybenzophenone		Benzophenones	UV filter (PCP)	131-57-7	228.24	3.7	3.79	7.1
Benzophenone-1 (BP1)	2,4-Dihydroxybenzophenone		Benzophenones	UV filter (PCP)	131-56-6	214.22	Insoluble	3.15	7.1
Benzophenone-2 (BP2)	2,2',4,4'-Tetrahydroxybenzophenone		Benzophenones	UV filter (PCP)	131-55-5	246.22	8.8	2.78	7.1
4-Hydroxybenzophenone (4HB)	-		Benzophenones	UV filter (PCP)	1137-42-4	193.18	Insoluble	2.92	8.14
4,4'-Dihydroxybenzophenone (4DHB)	-		Benzophenones	UV filter (PCP)	611-99-4	214.22	450	2.19	7.55
2,2'-Dihydroxy-4-methoxybenzophenone (DHMB)	Benzophenone-8; Dioxybenzone		Benzophenones	UV filter (PCP)	131-53-3	244.25	Insoluble	3.82	6.78
Avobenzone (AVO)	1-(4-tert-butylphenyl)-3-(4-methoxyphenyl)propane-1,3-dione		Benzophenones	UV filter (PCP)	70356-09-1	310.17	Insoluble	4.51	9.74
Octinoxate (EHMC)	2-Ethylhexyl trans-4-methoxy cinnamate		Cinnamate derivatives	UV filter (PCP)	5466-77-3	290.4	0.2	5.8	-
Enzacame (4MBC)	3-(4-Methylbenzilidene) camphor		Camphor derivatives	UV filter (PCP)	36861-47-9	254.17	Insoluble	5.14	7.5
Benzocaine (EtPABA)	Ethyl 4-aminobenzoate		p-aminobenzoic acid derivatives	UV filter (PCP) UV filter (PCP)	94-09-7	165.19	1310	1.86	2.51
Benzotriazole (BZT or 1H-BZT)	1,2,3-Benzotriazole		Benzotriazoles	UV filter (PCP)	95-14-7	119.12	3000	1.44	8.37
Methyl-benzotriazole (MeBZT or 5-MeBZT)	5-Methyl-1H-benzotriazole		Benzotriazoles	UV filter (PCP)	136-86-5	133.15	6000	Not avaliable	8.85
Dimethyl-benzotriazole (DMBZT)	5,6-Dimethyl-1H-benzotriazole monohydrate		Benzotriazoles	UV filter (PCP)	4184-79-6	147.18	20000	Not avaliable	8.92
Drometrizole (UVP)	Benazol P; Tinuvin P; 2-(2H-Benzotriazol-2-yl)-p-cresol		Benzotriazole derivatives	UV filter (PCP)	2440-22-4	225.25	26	4.31	8.15

Table S1: continued.

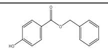
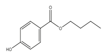
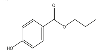
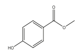
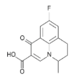
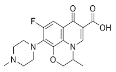
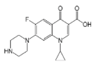
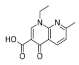
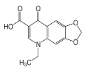
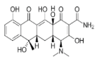
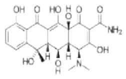
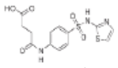
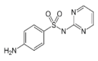
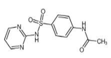
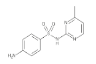
Compound	Synonyms	Structure	Family	Type	CAS number	Molecular mass(g/mol)	Solubility in water (mg/L)	$\log K_{ow}$	pKa
Benzyl paraben (BePB)	Benzyl 4-hydroxybenzoate		Parabens	Preservative (PCP)	94-18-8	228.24	Insoluble	3.7	8.18
Butyl paraben (BuPB)	Butyl 4-hydroxybenzoate		Parabens	Preservative (PCP)	94-26-8	194.23	200	3.47	8.47
Propyl paraben (PrPB)	Propyl 4-hydroxybenzoate		Parabens	Preservative (PCP)	94-13-3	180.2	500	2.98	8.35
Methyl paraben (MePB)	Methyl 4-hydroxybenzoate		Parabens	Preservative (PCP)	99-76-3	152	2500	2	8.17
Flumequine	Flumigal; Apurone		Fluoroquinolones	Pharmaceutical	42835-25-6	261.25	2200	1.6	6.5
Ofloxacin	Floxin; Tarivid		Fluoroquinolones	Pharmaceutical	82419-36-1	361.4	1400	-0.39	5.97
Ciprofloxacin	-		Fluoroquinolones	Pharmaceutical	85721-33-1	331.34	30000	0.28	6.09
Nalidixic acid	Nalidixate; nalidixin		Quinolones	Pharmaceutical	389-08-2	232.23	Insoluble	1.59	8.6
Oxolinic acid	Nidantin; dioxacin		Quinolones	Pharmaceutical	14698-29-4	261.23	3	0.94	6.3
Tetracycline	Deschlorobiomycin		Tetracyclines	Pharmaceutical	60-54-8	444.4	Insoluble	1.3	3.3
Oxytetracycline	Terramycin; Oxymycin		Tetracyclines	Pharmaceutical	79-57-2	460.4	300	-0.9	3.27
Succinylsulfathiazole (Succinyl-STZ)	Cremosuxidine; Colistatin		Sulfonamides	Pharmaceutical	116-43-8	355.4	180	-	3.36
Sulfadiazine (SDZ)	Sulfapyrimidine		Sulfonamides	Pharmaceutical	68-35-9	250.28	67	-0.09	6.99
N^4-acetylsufladiazine (acSDZ)	N-[4-(pyrimidin-2-ylsulfamoyl)phenyl]acetamide		Sulfonamides	Pharmaceutical	127-74-2	292.32	150	Not avaliable	-
Sulfamerazine (SMR)	Sulfamethyldiazine		Sulfonamides	Pharmaceutical	127-79-7	264.31	200	0.14	6.98

Table S1: continued.

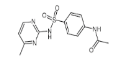
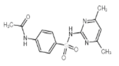
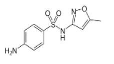
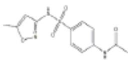
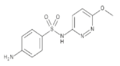
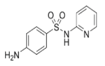
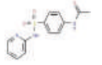
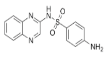
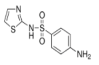
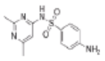
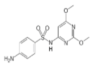
N^4-acetylsulfamerazine (acSMR)	N(4)-Acetylsulfamerazine		Sulfonamides	Pharmaceutical	127-73-1	306.34	46	Not avaliable	-
N^4-acetylsulfamethazine (acSMZ)	N(4)-Acetylsulfamethazine		Sulfonamides	Pharmaceutical	100-90-3	320.37	Not avaliable	Not avaliable	-
Sulfamethoxazole (SMX)	Gantanol; sulfisomezole		Sulfonamides	Pharmaceutical	723-46-6	253.28	Insoluble	0.89	5.81
Acetylsulfamethoxazole (acSMX)	N^4-Acetylsulfamethoxazole		Sulfonamides	Pharmaceutical	21312-10-7	295.32	250	0.86	-
Sulfamethoxypyridazine	Sulphametoxypyridazine		Sulfonamides	Pharmaceutical	80-35-3	280.31	325	Not avaliable	7.19
Sulfapyridine (SPY)	Sulfidin		Sulfonamides	Pharmaceutical	144-83-2	249.29	268	0.35	8.43
Acetylsulfapyiridine (acSPY)	N-(4-(N-(Pyridin-2-yl)sulfamoyl)phenyl)acetamide		Sulfonamides	Pharmaceutical	19077-98-6	291.33	320	Not avaliable	-
Sulfaquinoxaline (SQX)	Avicocid		Sulfonamides	Pharmaceutical	59-40-5	300.34	8	1.68	5.1
Sulfathiazole (STZ)	2-Sulfanilamidothiazole		Sulfonamides	Pharmaceutical	72-14-0	255.3	60	0.05	7.24
Sulfisomidin	-		Sulfonamides	Pharmaceutical	515-64-0	278.33	230	-0.33	7.4
Sulfadimethoxine (SDM)	Sulfadimethoxydiazine		Sulfonamides	Pharmaceutical	122-11-2	310.33	340	1.63	6.21

Table S1: continued.

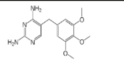
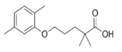
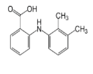
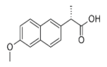
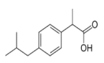
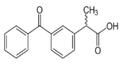
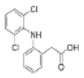
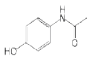
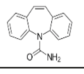
Trimethoprim	Proloprim; Trimpex		Others	Pharmaceutical	738-70-5	290.32	0.4	0.91	7.12
Gemfibrozil	5-(2,5-Dimethylphenoxy)-2,2-dimethylpentanoic acid		Lipid regulators	Pharmaceutical	25812-30-0	250.33	11	4.39	4.5
Mefenamic acid	2-[(2,3-dimhetylphenyl)amino]benzoic acid		Analgesics	Pharmaceutical	61-68-7	241.28	0.1	5.12	4.2
Naproxen	-		Anti-inflammatories	Pharmaceutical	22204-53-1	230.26	0.02	3.18	4.15
Ibuprofen (IBU)	2-(4-Isobutylphenyl)propanoic acid		Anti-inflammatories	Pharmaceutical	15687-27-1	206.28	0.02	3.97	4.91
Ketoprofen (KPF)	2-(3-Benzoylphenyl)propanoic acid		Analgesics	Pharmaceutical	22071-15-4	254.28	51	3.12	4.45
Diclofenac (DCF)	Voltaren		Anti-inflammatories	Pharmaceutical	15307-86-5	296.1	2.4	4.51	4.2
Acetaminophen	Paracetamol		Analgesics	Pharmaceutical	103-90-2	151.16	14000	0.46	9.38
Carbamazepine (CBZ)	5H-Dibenzo[b,f]azepine-5-carboxamide		Anti-epileptics	Pharmaceutical	298-46-4	236.27	18	2.77	15.96

Table S1: continued.

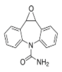
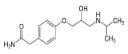
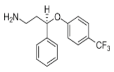
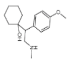
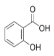
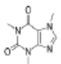
Carbamazepine-10,11-epoxide (CBZ-epoxy)	-		Anti-epileptics	Pharmaceutical	36507-30-9	252.27	1340	Not avaliable	15.96
Atenolol	Tenorium; blokium; normiten		Β-blockers	Pharmaceutical	29122-68-7	266.34	13300	0.16	9.6
Norfluoxetine (norFXT)	Desmethylfluoxetine		Anti-depressants	Pharmaceutical Pharmaceutical	56161-73-0 83891-03-6	295.3	9.2	Not avaliable	9.77
N-desmethylvenlafaxine (N-desVFX)	Norvenlafaxine		Anti-depressants	Pharmaceutical	149289-30-5	263.37	77	Not avaliable	14.42
Salicylic acid	2-Hydroxybenzoic acid		Anti-septics	Pharmaceutical	69-72-7	138.12	2240	2.26	2.98
Caffeine	1,3,7-Trimethylxanthine; guaranine		Stimulants	Drug	58-08-2	194.19	16	-0.07	14

Table S2: Limits of detection (LOD) and limits of quantification (LOQ) of each compound analysed, in pictograms (pg).

Compounds	**BP3**	**BP1**	**BP2**	**4HB**	**4DHB**	**DHMB**	**AVO**	**EHMC**	**4MBC**	**EtPABA**	**BZT**
LOD (pg)	6.410	6.460	7.864	4.313	4.694	8.452	4.068	3.971	6.311	4.559	4.124
LOQ (pg)	21.368	21.535	26.212	14.375	15.646	28.174	13.561	13.237	21.035	15.197	13.747
Compounds	**MeBZT**	**DMBZT**	**UVP**	**BePB(-)**	**BuPB(-)**	**PrPB(-)**	**MePB(-)**	**FLU**	**OFX**	**CFX**	**NDX**
LOD (pg)	3.973	3.479	11.040	0.819	0.315	0.529	0.509	1.085	4.062	2.528	2.065
LOQ (pg)	13.242	11.595	36.800	2.730	1.050	1.763	1.695	3.618	13.541	8.426	6.884
Compounds	**OXL**	**TCY**	**O-TCY**	**S-STZ**	**SDZ**	**acSDZ**	**SMR**	**acSMR**	**acSMZ**	**SMX**	**acSMX**
LOD (pg)	3.251	4.034	2.783	0.949	0.809	0.644	0.387	1.070	0.902	0.751	0.510
LOQ (pg)	10.835	13.446	9.277	3.162	2.698	2.146	1.291	3.566	3.006	2.504	1.701
Compounds	**SMPZ**	**SPY**	**acSPY**	**SQX**	**STZ**	**SMD**	**SDM**	**TMP**	**GFZ**	**MFA**	**NPX**
LOD (pg)	0.809	1.137	0.768	0.870	0.440	1.298	0.706	0.001	0.001	0.001	1.051
LOQ (pg)	2.695	3.789	2.561	2.899	1.466	4.325	2.353	0.004	0.002	0.004	3.504
Compounds	**IBU(-)**	**KPF**	**DCF(-)**	**APH**	**CBZ**	**CBZ-E**	**ATL**	**norFXT**	**N-desVFX**	**SCY (-)**	**CFF**
LOD (pg)	0.639	0.004	3.736	0.464	0.000	0.001	0.001	0.002	0.001	0.001	0.001
LOQ (pg)	2.129	0.015	12.453	1.546	0.001	0.005	0.002	0.008	0.004	0.005	0.003
LOD: Limit of detection; LOQ: Limit of quantification											

Table S3: Concentrations of the single detected CECs in the deployed POCIS, expressed as ng/POCIS ± standard deviation.

Compounds	**BP3**	**BP1**	**EHMC**	**4MBC**	**EtPABA**	**BZT**	**MeBZT**	**BuPB**
WWTP influent (1 POCIS deployed)	1616	25690	76	224	357	2978	7414	227
Secondary outlet (2 POCIS deployed)	n.d.	431 ± 30	*45 ± 64*	299 ± 47	131 ± 6	8670 ± 778	10092 ± 1362	n.d.
Outlet SAT system T2 (2 POCIS deployed)	n.d.	n.d.	*66 ± 94*	n.d.	n.d.	3640 ± 692	1516 ± 447	n.d.
Outlet SAT system T4 (2 POCIS deployed)	n.d.	n.d.	n.d.	n.d.	n.d.	2796 ± 721	859 ± 141	n.d.
Compounds	**PrPB**	**MePB**	**OFX**	**CPX**	**NDX**	**SMX**	**acSMX**	**SPY**
WWTP influent (1 POCIS deployed)	13707	30603	1082	1323	n.d.	253	344	153
Secondary outlet (2 POCIS deployed)	n.d.	n.d.	4195 ± 23	*436 ± 187*	140 ± 8	475 ± 23	178 ± 26	164 ± 23
Outlet SAT system T2 (2 POCIS deployed)	n.d.	n.d.	n.d.	n.d.	n.d.	*1965 ± 677*	*269 ± 124*	*165 ± 105*
Outlet SAT system T4 (2 POCIS deployed)	n.d.	n.d.	n.d.	n.d.	n.d.	1386 ± 128	*105 ± 49*	119 ± 22
Compounds	**acSPY**	**STZ**	**TMP**	**GFZ**	**MFA**	**NPX**	**IBU(-)**	**KPF**
WWTP influent (1 POCIS deployed)	347	32	237	12500	17	1379	36466	4134
Secondary outlet (2 POCIS deployed)	640 ± 10	45 ± 2	1716 ± 104	7706 ± 0	41 ± 3	349 ± 38	1743 ± 104	3472 ± 597
Outlet SAT system T2 (2 POCIS deployed)	n.d.	n.d.	n.d.	1179 ± 28	6 ± 1	464 ± 102	152 ± 32	1659 ± 427
Outlet SAT system T4 (2 POCIS deployed)	n.d.	19 ± 3	n.d.	797 ± 157	5 ± 0	261 ± 12	n.d.	631 ± 110
Compounds	**DCF(-)**	**APH**	**CBZ**	**CBZ-E**	**ATL**	**norFXT**	**N-desVFX**	**CFF**
WWTP influent (1 POCIS deployed)	4397	7759	547	457	1961	608	11767	193103
Secondary outlet (2 POCIS deployed)	8028 ± 714	n.d.	1677 ± 36	1195 ± 23	2924 ± 302	*264 ± 104*	30138 ± 3438	5665 ± 292
Outlet SAT system T2 (2 POCIS deployed)	*956 ± 590*	n.d.	519 ± 46	438 ± 39	n.d.	279 ± 8	n.d.	n.d.
Outlet SAT system T4 (2 POCIS deployed)	718 ± 96	n.d.	320 ± 39	354 ± 60	n.d.	202 ± 1	n.d.	n.d.

n.d.: not detected; (-): analyzed in negative; Units: ng/POCIS ± standard deviation; The results characterized by a RSD >30% are in italics

4.2.4 Publication #8

Simultaneous degradation of 30 pharmaceuticals by anodic oxidation: Main intermediaries and by-products

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Simultaneous degradation of 30 pharmaceuticals by anodic oxidation: Main intermediaries and by-products

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HIGHLIGHTS

- Almost total mineralization of 30 pharmaceuticals by Anodic Oxidation in different electrolytic media.
- Anodic oxidation in NaCl produces chlorinated intermediaries and their formation increase at higher current densities.
- Simultaneous mineralization of 30 pharmaceuticals by AO in a secondary effluent from a wastewater treatment plant.
- More than 25 organic intermediaries generated during electrolysis were identified.

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ABSTRACT

The anodic oxidation (AO) of 30 pharmaceuticals including antibiotics, hormones, antihistaminics, anti-inflammatories, antidepressants, antihypertensives, and antiulcer agents, in solutions containing different supporting electrolytes media (0.05 M Na_2SO_4, 0.05 M NaCl, and 0.05 M Na_2SO_4 + 0.05 M NaCl) at natural pH was studied. A boron-doped diamond (BDD) electrode and a stainless-steel electrode were used as anode and cathode, respectively, and three current densities of 6, 20, and 40 mA cm^{-2} were applied. The results showed high mineralization rates, above 85%, in all the tested electrolytic media. 25 intermediaries produced during the electrooxidation were identified, depending on the supporting electrolyte together with the formation of carboxylic acids, NO_3^-, SO_4^{2-} and NH_4^+ ions. The formation of intermediates in chloride medium produced an increase in absorbance. Finally, a real secondary effluent spiked with the 30 pharmaceuticals was treated by AO applying 6 mA cm^{-2} at natural pH and without addition of supporting electrolyte, reaching c.a. 90% mineralization after 300 min, with an energy consumption of 18.95 kW h m^{-3} equivalent to 2.90 USD m^{-3}. A degradation scheme for the mixture of emerging contaminants in both electrolytic media is proposed. Thus, the application of anodic oxidation generates a high concentration of hydroxyl radicals that favors the mineralization of the pharmaceuticals present in the spiked secondary effluent sample.

1. Introduction

During the last decades, a large amount of many organic micropollutants has been released into the environment as a result of anthropogenic activities (Hernández et al., 2019). Most of these micropollutants have raised particular concern and thus currently they are known as contaminants of emerging concern (CECs). These compounds, are chemical substances not commonly monitored that present potential to enter the environment and cause ecological or human adverse effects, whether known or probable

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(Geissen et al., 2015; Rodriguez-narvaez et al., 2017).

An important group of CECs are pharmaceutically active substances (Gómez et al., 2010; Petrovic, 2014). Pharmaceuticals are designed to prevent or treat human and animal diseases (Ebele et al., 2017; Gracia-lor et al., 2012) by a specific mode of action (Fent et al., 2006); nevertheless, its extensive use worldwide produces bioaccumulation and toxic undesirables effects in aquatic and terrestrial ecosystems (Ebele et al., 2017), such as antibiotic resistance (Ebele et al., 2017; Grenni et al., 2018; Rabbia et al., 2016) and alteration of plasma levels of certain biomolecules (Ebele et al., 2017). On the other hand, in humans the risk of metabolic disorders, neurological disorders, damage to the immune system, hormone levels disorder and alterations in female and male reproductive system has been reported (Barrios-Estrada et al., 2018).

Although risk assessments indicate that it is very unlikely that the trace concentrations found in the water present risks to human health (acute toxicity) (World Health Organization, 2012), the risks associated with prolonged exposure (associated to chronic toxicity) and the combined effects of mixtures of them are unknown and of concern (Hernández et al., 2019; Noguera-oviedo and Aga, 2016; Vasiljevi and Lau, 2009).

Effluents from wastewater treatment plants (WWTPs) are considered one of the most important entry pathways of pharmaceuticals to the aquatic environment (Babic and Horvat, 2007; Gracia-lor et al., 2012; Sancho et al., 2012). WWTPs fail to fully remove most pharmaceuticals (Boix et al., 2015; Hernández et al., 2011, 2019), because they are not designed to remove this type of contaminants (Barbosa et al., 2016; Patel et al., 2019). Thus, many of them have been detected in concentrations from ng L^{-1} to mg L^{-1} in urban wastewater (Alygizakis et al., 2020; Česen et al., 2019; Peña-Guzmán et al., 2019; Racar et al., 2020).

To remove pharmaceuticals more efficiently in the WWTPs, electrochemical advanced oxidation processes (EAOPs) have gained increasing attention as a promising advanced oxidation process (AOP) (Moreira et al., 2017) due to their effectivity to oxidize both organic and inorganic compounds (Rivera-Utrilla et al., 2013).

Anodic Oxidation (AO) is the most popular EAOPs owing to its versatility and ease of scalability (Flores et al., 2017; Martínez-Huitle et al., 2015; Özcan et al., 2008). In AO, the pollutants are oxidized by heterogeneous M(•OH) formed from water electrolysis at the anode surface (Eq. (1)) (Martínez-Huitle and Ferro, 2006).

$$\mathrm{M} + \mathrm{H_2O} \rightarrow \mathrm{M(^{\bullet}OH)} + \mathrm{H^+} + \mathrm{e^-} \tag{1}$$

The hydroxyl radical (•OH) is a powerful oxidant (E^0 (•OH/H_2O) = 2.80 V vs SHE) that reacts with organic compounds by abstraction of a hydrogen atom (dehydrogenation), electrophilic addition to an unsaturated bond (hydroxylation), electron transfer (redox) reaction and ipso-substitution of halogen atom (Mousset et al., 2018) until its complete mineralization to CO_2, water and inorganic ions (Flores et al., 2017; Yu et al., 2014). The electrochemical generation and the chemical reactivity of M(•OH) strongly depend on the anode material (Comninellis et al., 2008). The boron-doped diamond (BDD) electrode is a non-active anode (Espinoza et al., 2018; Panizza and Cerisola, 2009) that favors the indirect oxidation of organic compounds. Additionally, depending on the ions presents in the solution, other oxidants can be produced, for example, persulfate ion ($S_2O_8^{2-}$), peroxodicarbonate ($C_2O_6^{2-}$), peroxodiphosphate ($P_2O_8^{2-}$) (Martínez-Huitle and Brillas, 2009) or active chlorine species (Cl_2, $HClO/ClO^-$) (Contreras et al., 2015). These oxidants facilitate the oxidation of pollutants present near the anode and/or in the bulk of the solution, as well as water disinfection in case of chlorine species (Candia-Onfray et al., 2018).

Several authors have reported the removal of pharmaceuticals by AO (Dirany et al., 2010; García-Montoya et al., 2015; Sopaj et al., 2015). However, there are few studies applying AO to treat a large group of pollutants in both, synthetic or real wastewater (Garcia-Segura et al., 2015; Lan et al., 2017).

This work aims to study the simultaneous degradation of 30 pharmaceuticals, antibiotics, hormones, antihistaminic, anti-inflammatories, antidepressants, antihypertensive, and antiulcer agents by AO using a BDD anode in synthetic solutions, and in a secondary effluent from a WWTP applying different current densities and electrolytic media. Moreover, an exhaustive study by liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) and ion exclusion chromatography was carried out to identify intermediaries and reaction products formed in the process.

2. Materials and methods

2.1. Reagents

Trimebutine maleate, ketorolac trometamol, caffeine, acetaminophen, chlorphenamine maleate, sodium diclofenac, ibuprofen and sodium metamizole (>99% purity) was supplied by Pasteur S.A Laboratory (Santiago de Chile, Chile). Tetracycline hydrochloride (95% purity), norfloxacin, ciprofloxacin, naproxen, β-estradiol, estrone, progesterone, and sulfamethazine (>98% purity) were obtained from Sigma-Aldrich® (Santiago de Chile, Chile). Salicylic acid (>99% purity), analytical grade anhydrous sodium sulfate and sodium chloride used as background electrolyte, were purchased from Merck® Santiago de Chile, Chile). Mefenamic acid, venlafaxine, sertraline, escitalopram, fluoxetine, azithromycin, amoxicillin, losartan, enalapril, famotidine, omeprazole, loratadine and loperamide in commercial tablets, were acquired from the established trademarks in Chile.

Carboxylic acids were purchased from Sigma-Aldrich®, while maleic, formic and acetic acids were from Merck®.

All the other chemicals employed were HPLC grade or analytical grade from Merck® and Sigma-Aldrich®. All solutions were prepared with ultrapure water obtained from a Millipore Milli-Q system (resistivity > 18 mΩ cm).

2.2. Wastewater samples

Secondary effluent samples were collected in July 2019, from “Aguas Andinas, Mapocho/Trebal” WWTP in Padre Hurtado (33°32′82″S/70°50′08″W), Santiago de Chile (Chile). Table 1 reports the main parameters determined for the secondary effluent. The spiked of the wastewater was carried out with a mixture of 30 drugs at the concentrations reported in SM1.

Table 1
Characterization of secondary effluent from the WWTP.

Parameters	Initial value
Color	Light yellow
Odor	strong
Turbidity (NTU)	7.72
Total Dissolved Solid (mg L^{-1})	1202
Conductivity (μS)	2402
Absorbance at 280 nm (U.A)	0.375
Chemical Oxygen Demand (mg L^{-1})	35.50 ± 0.70
Total Organic Carbon (mg L^{-1})	22.68
Total Chlorine (mg L^{-1})	0.16 ± 0.02
Nitrate NO_3^- (mg L^{-1})	7.70 ± 0.14
Sulfate $S_2O_4^-$ (mg L^{-1})	8.17 ± 3.25
Ammonium $NH4^+$ (mg L^{-1})	42.50 ± 2.12

2.3. Electrochemical experiments

Electrochemical system. The electrolysis was carried out in a one compartmental 100 cm^3 electrochemical cell with constant stirring at 25 ± 2 °C. A BDD thin-film electrode from Adamant Technologies® was used as anode and a stainless-steel plate was used as the cathode (BDD/SS system), both with 5.0 cm^2 of area and an inter-electrode gap of 1 cm. The experiments were performed applying constant current densities (j) using an EHQ power supply model PS3010. Cyclic voltammetry was carried out in an Autolab Postentiostat/Galvanostat PGSTAT 204 system using a 10 cm^3 glass electrochemical cell. BDD was used as working electrode (0.19 cm^2 area), Ag/AgCl (1 M) as reference electrode, and a platinum wire as counter electrode. The voltammograms were recorded from 0 to 3 V at 5 mV s^{-1}.

2.4. Instruments and analytical procedures for water analysis

The pH was measured with a pH-meter HANNA HI5222. Conductivity and total dissolved solids were measured using a HI98311 Waterproof EC/TDS/Temperature Tester.

The turbidity of the wastewater was measured with the HANNA instrument HI98703 portable turbidimeter. The chemical oxygen demand (COD) was determined after chemical digestion of the samples during 2 h at 150 °C in a HANNA multiparameter bench photometer for wastewater treatment HI83214. The concentration of ammonium ion was analyzed using the HANNA AHR test tube HI93764B-25 (0–100 mg L^{-1}) and reagent HI93764-0, while nitrate was determined using HI93766-50 test tube (0–30 mg L^{-1}) and nitrate reagent sachet HI93766-0. Then, the concentration of both ions was measured in the spectrophotometer HI83214. The presence of chlorite, chloride, chlorate, nitrate and sulfate ions was determined using a Metrohm Ion Chromatograph, 930 Compact IC Flex model. The separation was carried out in a Metrosep A Supp 5–250/4 (250 × 4.0 mm (i.d), 5 μm) column and mobile phase composed of $Na_2CO_3/NaHCO_3$ (3.2:1 mmol L^{-1}) was used, at a flow rate of 0.7 mL min^{-1}. The injection volume was 20 μL.

The change in the absorbance of the initial wastewater, compounds solution, and secondary effluent during electrolysis was monitored by a spectrophotometer Agilent 8453. The total organic carbon (TOC) variation was monitored by a Shimadzu (TOC-L) analyzer, obtaining an initial average TOC value among the three evaluated electrolytic media of 72.62 ± 0.2 mg L^{-1}. The carboxylic acids generated as intermediaries were monitored and quantified by ionic-exclusion chromatography using an HPLC Prominence 12.770 (Shimadzu) with diode array detector 41.571 model. A Bio-Rad Aminex HPX 87H, 300 × 7.8 mm (i.d.) column at 30 °C was used and the detection was performed at 210 nm. The mobile phase was 4 mM H_2SO_4 with a 0.6 mL min^{-1} flow. The corresponding calibration curves were constructed using pure acid standards.

The energy consumption per volume of electrolyzed solution was obtained from eq. (2):

$$\text{Energy Consumption}\ (\mathrm{kW\,hm^{-3}}) = IE_{cell}t/1000\ Vs \tag{2}$$

where I is the applied current (A), E_{cell} is the average cell voltage (V), t is the electrolysis time (h), and V_s is the volume of the treated solution (m^3) (Candia-Onfray et al., 2018; Contreras et al., 2015; Salazar et al., 2016, 2017).

2.5. LC-ESI- Q-Exactive analysis

To identify aromatic intermediates generated during electro-oxidation by ultra-high performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS), aliquots collected at different electrolysis times were mixed for a total of 10 mL, then they were extracted three times with 30 mL of CH_2Cl_2 and ethyl acetate separately to extract as many intermediates as possible. Each organic fraction collected (90 mL) was dried with anhydrous Na_2SO_4, filtered and evaporated in rotary evaporator. Then, the organic fraction evaporated was reconstituted in 1 mL of methanol and injected into the UHPLC-Q-Exactive (Thermo Fisher Scientific, San Jose, CA, USA) system with a hybrid quadrupole–Orbitrap analyzer.

The ionization was carried out with an electrospray ionization (ESI) source working in positive and negative modes under capillary voltages of + 4 kV and -3kV, respectively. The capillary temperature was 350 °C in both modes, while the probe heater temperatures were 250 °C in positive and 300 °C in negative mode. The sheath gas and the auxiliary gas flows were set at 40 and 10 a.u.

The chromatographic separation was performed using an Acquity BHE-C18 (100 × 2.1 mm, 1.7 μm) column (Waters, Massachusetts, USA), keeping the oven temperature at 40 °C. The mobile phase used in positive mode was: (A) water containing formic acid 0.1% (v/v) and (B) methanol also containing formic acid 0.1% (v/v) at 0.3 mL min^{-1} in gradient mode. The percentage of (A) was changed as follows: 0 min, 95%; 7 min, 25%; 10 min, 0%; 15 min; 0%, 17 min; 95%, 21 min, 95%. In negative mode, the modifications were (A) water containing ammonium acetate 5 mM and (B) methanol containing ammonium acetate 5 mM at 0.3 mL min^{-1} in gradient mode. The percentage of (A) was modified as follows: 0 min, 95%; 3 min, 50%; 6 min, 10%; 13 min; 0%, 17 min; 0%, 18 min, 95%; 20 min, 95%. The analysis run time was 21 and 20 min, respectively, and sample injection volume was 10 μL.

Spectra were acquired in independent data acquisition (DIA) mode. This acquisition mode allows different scans; the first is a full scan at low collision energy (10 eV) in the range m/z 66.70–1000, where all the compounds mass arriving to the analyzer are determined. Then, a MS/MS second scan at high collision energy (40 eV) is performed to all the compounds that had arrived to the analyzer without differentiating the origin and obtaining a MS/MS spectra of all the ions also in the range m/z 66.70–1000.

2.6. Data processing

All the raw data obtained using XcaliburTM 4.1 software (Thermo Scientific), were converted into.mzML files in Msconvert 3.0 software (ProteoWizard) using a threshold of 5000 in ESI (+) and 1000 in ESI (−) and peak picking as filters. These files were exported to the norman-data.eu site. In addition, information was provided on the type of sample, origin, and chromatographic and ionization conditions. A set of standards was introduced for the calibration of the chromatographic retention time (expressed in minutes) obtained under working conditions, in (ESI+) (Guanylurea; 0.91, Amitrol; 0.91, Histamine; 0.81, Chlormequat; 0.94, Methamidophos; 1.99, Vancomycin; 2.95, Cefoperazone; 4.35, Trichlorfon; 4.73, Dichlorvos; 6.23, Tylosin; 7.11, TCMTB; 7.69, Rifaximin; 8.08, Spinosad A; 9.48, Emamectin B1a; 10.57, Avermectin B1a, 10.34, Nigericin; 10.94, Ivermectin B1a; 10.91), and in (ESI-) (Amitrole; 0.96, Benzoic acid; 1.72, Acephate; 2.19, Salicylic acid; 2.37, Simazine 2-Hydroxy; 3.69, Tepraloxydim; 3.75, Bromoxynil; 3.69, MCPA; 4.39, Valproic acid; 4.68, Phenytoin; 4.96, Flamprop; 4.99, Benodanil; 5.35, Dinoterb; 5.2, Inabenfide; 5.88, Coumaphos; 6.59, Triclosan; 7.02, AvermectinB1a; 7.84, and Salinomycin; 6.99).

Screening to find matches was performed using the EXPHRMSMSAVAL list in positive (6828 compounds) and in negative (3042 compounds) mode, from Norman Suspect List Exchange. Norman Substance Database in https://www.normannetwork.com/nds/SLE/(“NORMAN Substance Database,” n.d.). Finally, an .xlsx file is imported from the site containing the possible compounds identified based on identification proofs.

2.7. Identification of intermediaries by-products

The identification was carried out using the identification criteria, mass accuracy (±5 ppm), retention time tolerance (±20%) and MS/MS data. In addition, the mechanisms of interaction of hydroxyl radicals and active chlorine species with pharmaceuticals reported in literature were considered for the selection of possible intermediaries (Cavalcanti et al., 2013; Deborde and von Gunten, 2008; Mousset et al., 2018).

3. Results and discussion

3.1. Effect of the supporting electrolyte and the applied current on the degradation of pharmaceuticals

First, 100 cm^3 of a standard mixture of the 30 pharmaceuticals selected corresponding to 72.62 ± 0.2 mg L^{-1} of TOC in 0.05 M Na_2SO_4 at initial pH 7.80 ± 0.02 were electrolyzed applying 6, 20 and 40 mA cm^{-2} during 300 min at constant stirring (cell potential 7.06, 10.33 and 13.06 V, respectively). Fig. 1A and B shows the TOC abatement and absorbance decay, respectively, as a function of the electrolysis time at different current. In all cases, TOC and absorbance decreased as the electrolysis time elapsed due to the attack of hydroxyl radicals generated on the surface of the BDD electrode.

At the end of the electrolysis, the %TOC removal depends on the applied current density (Fig. 1A), being greater when the current density is higher (Brocenschi et al., 2016), with 73.35, 80.25 and 90.56% of TOC decay applying 6, 20 and 40 mA cm^{-2}, respectively. Similarly, the absorbance of the solutions decreased (Fig. 1B), although at minute 300 a very slight increase occurred for the highest current density applied (40 mA cm^{-2}), suggesting the formation of intermediates with opposite absorptive properties.

Additionally, the mineralization of the pharmaceuticals was evaluated in 0.05 M NaCl medium applying the same current densities and at the same initial pH of the previous experiment. The cell potentials were 8.99, 11.67, and 15.52 V for 6, 20 and 40 mA cm^{-2} , respectively. Fig. 1C shows the TOC decay as a function of the current densities applied. Similar percentage of mineralization were obtained in this electrolytic medium at the three applied current densities. When NaCl is used as supporting electrolyte, in addition to the formation of hydroxyl radicals, other oxidizing species may be generated, for example, active chlorine species as Cl_2 and $HClO/ClO^-$ (Rivera-Utrilla et al., 2013). These active chlorine species also react with pharmaceutical compounds, causing their oxidation, and together with the hydroxyl radicals produced on the surface of the BDD anode, they reach mineralization, both of the initial compounds and of the intermediates produced during electrolysis. The active chlorine species not only contribute to the oxidation of organic contaminants but also to the disinfection of water (Candia-Onfray et al., 2018). In addition, other oxidizing species can be produced from the action of $^{\bullet}OH$ in the presence of Cl^- ion, such as ClO_2^-, ClO_3^- and ClO_4^- (Sirés et al., 2014), which would contribute to the oxidation of organic compounds, but less efficiently than $HClO/ClO^-$ (Martínez-Huitle et al., 2015).

In Fig. 2, cyclic voltammograms in different electrolytic media and the secondary effluent sample spiked with pharmaceuticals are displayed. The oxidation of chlorine begins first at potentials close to 2.25 V (curve A), while oxygen oxidation starts at 2.6 V (versus Ag/AgCl sat.) in sulfate media (curve C). This indicates that the oxidation of chlorine is thermodynamically favored concerning the oxidation of oxygen, which would be in agreement with the slightly higher degradation of the contaminants in the presence of sodium chloride.

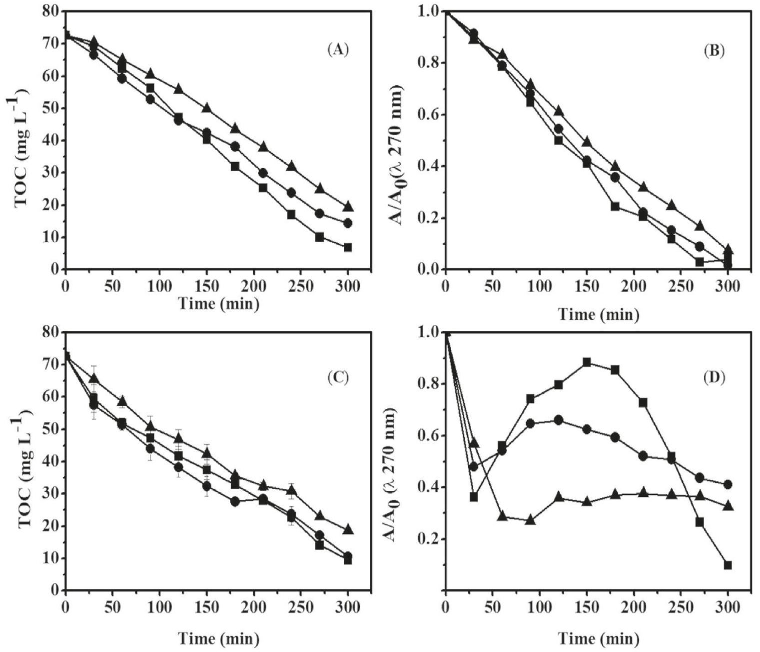

Fig. 1. TOC abatement and absorbance decay at 270 nm as a function of the electrolysis time applied to 100 mL the mixture pharmaceuticals solution, corresponding to 72.62 mg L^{-1} TOC in 0.05 M Na_2SO_4 (A and B) and 0.05 M NaCl (C and D), using a stirred BDD/SS cell at 25 °C and pH 7.8. Intensity density applied was (▲) 6, (●) 20, and (■) 40 mA cm^{-2}.

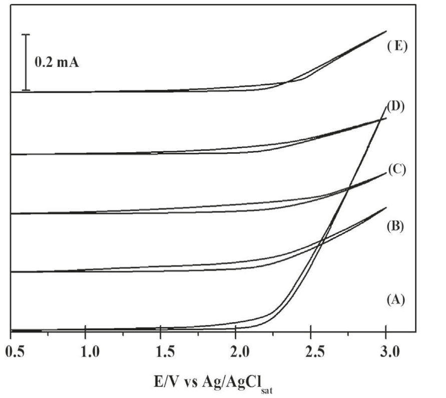

Fig. 2. Cyclic voltammograms of (A) 0.05 M NaCl, (B) 0.05 M NaCl + 0.05 M Na_2SO_4, (C) 0.05 M Na_2SO_4, (D) secondary effluent, (E) secondary effluent spiked with 30 pharmaceuticals corresponding to 72.62 mg L^{-1} TOC, using a BDD working electrode, Ag/AgCl as reference electrode, and a platinum wire as the counter electrode. Sweep rate applied 5 mV s^{-1}.

A large difference in the absorbance change at 270 nm during electrolysis was observed in NaCl medium (Fig. 1B) compared to the obtained in sodium sulfate (Fig. 1D). This difference is consistent with that reported by Jalife-Jacobo et al. (2016) in the study of discoloration of the diazo dye Congo Red in these same electrolytic media at the same concentration (Jalife-Jacobo et al., 2016). When current densities of 20 and 40 mA cm^{-2} were applied, the absorbance at 270 nm increased, almost at the same time. This may be due to the formation of oxychlorides and/or organochlorine intermediaries that have the maximum absorption wavelength close to 270 nm. For example, Murugananthan et al. (2010) have reported a $\lambda_{máx}$ displacement up to 292 nm, ascribed to the formation of the ClO^- ion in ketoprofen's electrolysis conducted in 0.1 M chloride medium using a BDD anode (Murugananthan et al., 2010).

To evaluate the influence of both supporting electrolytes in the same solution, electrolysis of the 30 pharmaceuticals containing a mixture of 0.05 M NaCl and 0.05 M Na_2SO_4 (pH 7.43 ± 0.03) was performed. The cell potentials were 7.32, 10.32 and 10.96 for 6, 20 and 40 mA cm^{-2}, respectively. Under these conditions, besides $^{\bullet}OH$, chlorine and sulfate oxidizing species, additional Cl^- and $SO_4^{-\bullet}$ radicals can be formed from the reaction between $Cl^{\bullet}$ and SO_4^{2-} (Eq. (3)) (Lan et al., 2017). Therefore, the presence of both salts could have a greater effect on the degradation of pollutants.

$$Cl^{\bullet} + SO_4^{2-} \rightarrow (SO_4^-)^{\bullet} + Cl^- + e^- \qquad (3)$$

However, the mineralization rates reached at current densities of 6 and 20 mA cm^{-2} for 0.05 M NaCl (74.28 and 85.50%) and 0.05 M Na_2SO_4 (73.35 and 80.25%) separately were slightly higher than those achieved when the electrolytes were mixed (71.04 and 80.20%) (Fig. 3A). Therefore, the addition of sulfate does not have an additional positive effect on the TOC removal rates achieved at these current densities, but the obtained rates were more similar to that obtained in sulfate alone, even lower, however, the mineralization percentages were similar. This result is very important because in real wastewater, the presence of these two salts is high. In this electrolytic medium, the concentrations of intermediaries are generated in smaller concentrations with respect to the observed in Fig. 1D, since absorbance was lower for each one of the current densities evaluated compared to those found in chloride electrolysis. Specifically, for the electrolysis in 0.05 M NaCl, maximum absorbance values of 0.8837, 0.6597, and 0.3763 (Fig. 1D) were obtained for current densities of 40, 20, and 6 mA cm^{-2}, respectively, while in 0.05 M NaCl and 0.05 M Na_2SO_4 solution, the maximum absorbance values were 0.4271, 0.3368, and 0.2485 for the same j (Fig. 3B). Besides, the oxidation reaction of chloride begins at potentials close to 2.25 V, which is similar to that observed in the presence of sodium chloride (Fig. 2B).

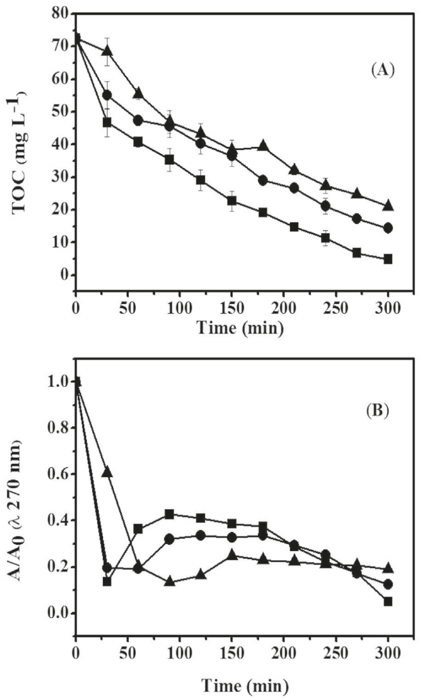

Fig. 3. TOC abatement (A) and absorbance decay at 270 nm (B) with respect to electrolysis time for the treatment of 100 cm^3 of solution containing a mixture of 30 pharmaceuticals in 0.05 M NaCl + Na_2SO_4, using a stirred BDD/SS cell at 25 °C, pH 7.8, applying (▲) 6, (●) 20 and (■) 40 mA cm^{-2}.

Considering that energy consumption is an important factor in an electrochemical treatment, the efficiency of the electro-oxidation of the 30 pharmaceuticals was estimated for each electrolytic media at selected density currents by means of Eq. (2). Considering an electrical energy cost of about CLP$ 110.0 (USD 0,154) (Chilean price, taxes excluded) per kWh consumed (Compañía Chilena de Energía Eléctrica, Chile), the total cost of each electrolysis was calculated in USD. Applying current densities of 6 and 20 mA cm^{-2}, between 70 and 85% of mineralization was reached in all electrolytic media evaluated, with the highest energy consumption corresponding to the electrolysis in chloride of 13.48 and 77.60 kWh^{-3}, which is equivalent to USD 2.10 and USD 12.00, respectively. As expected, the highest energy consumption occurred at the highest current density (40 mA cm^{-2}) for the three electrolytic media; however, the electrolysis in sulfate had the highest energy expenditure (130.60 kWh^{-3}), with a cost of USD 20.00. Consequently, to achieve a 90% mineralization rate the cost of electrolysis is 12.5-folds higher compared to electrolysis in sulfate media, when applying 6 mA cm^{-2}.

3.2. Intermediates and by-products generated during anodic oxidation of pharmaceuticals

To identify the main intermediaries and by-products formed during the electrooxidation of the 30 pharmaceuticals, the samples electrolyzed in 0.05 M NaCl and 0.05 M Na_2SO_4 background were analyzed by HRMS. The short chain carboxylic acids generated were monitored and quantified by ionic exclusion chromatography. Moreover, ions generated at the end of electrolysis were identified and quantified by means of ion chromatography and spectrophotometric analysis.

3.2.1. Intermediates in sulfate background solution

The identification of 15 by-products produced during electrolysis in 0.05 M Na_2SO_4 according to the procedure described in section 2.6 was carried out. Their denominations, molecular formula, retention time, measured exact mass, identifications proofs and suggested chemical structure are reported in SM2.

Intermediaries 1,3,7-trimethyl-9*H*-purine-2,6,8-trione **(1)** and 1,3-diazinane-2,4,5,6-tetrone **(2)** come from caffeine and could be generated from various pathways: (i) hydroxylation of the imidazole ring between N = C followed by oxidation to form **1**; (ii) demethylation of all methyl groups of caffeine and (iii) opening of the imidazole ring followed by decarboxylation at the amino positions and subsequent deamination and oxidation of the hydroxyls that bind to the pyrimidine ring to form intermediate **2**, similar to that reported by (Cavalcanti et al., 2013) for the degradation of omeprazole.

Dehalogenation reactions occur to produce N, *N*-dimethyl-3-phenyl-3-pyridin-2-ylpropan-1-amine **(3)**, 3-phenyl-3-pyridin-2-ylpropanoic acid **(4)**, piperazine-2,5-dione **(5)**, and benzo[g]quinoline **(10)** from chlorpheniramine, ciprofloxacin and loratadine. Furthermore, the abstraction of hydrogen caused by •OH radicals, followed by oxidation at position 1 and 4 in piperazine released from fluoroquinolones must occurs to produce intermediate **5**. On the other hand, further to the dechlorination of loratadine, the contraction of cycloheptane ring can occurs to produce intermediate **10** (Miao and Metcalfe, 2003; Vogna et al., 2004).

The 2-[2-(2,6-dichloro-4-hydroxyanilino) phenyl]acetic acid compound **(6)** could be produced by addition reaction to double bond from diclofenac (hydroxylation). A decarboxylation reaction occurs to give the compound 2-(2,6-dichloroanilino) benzaldehyde **(8)** and a cyclization reaction to give 1-(2,6-dichlorophenyl)-3*H*-indol-2-one **(7)** from the degradation of diclofenac. This last reaction to give rise to intermediate **7** is in accordance with (Zhao et al., 2009).

Compound (8*R*,9*S*,13*S*,14*S*)-3-hydroxy-2-methoxy-13-methyl-7,8,9,11,12,14,15,16-octahydro-6*H*-cyclopenta[a]phenanthren-17-one **(9)** could be generated from estrone or β-estradiol (after oxidation of the hydroxyl at carbon 17); however, the formation of this intermediate with the introduction of a methoxy group at position 2 could be due to reactions among organic radicals produced during the attack of hydroxyl radicals.

Compound 2-Phenylphenol **(11)** comes from the breaking between N–C bond of biphenyl and the imidazole ring in losartan, together a C–C breakdown between the biphenyl and the tetrazole ring.

On the other hand, N-(1,5-dimethyl-3-oxo-2-phenylpyrazol-4-yl) formamide **(12)** could originate due to the attack of hydroxyl radicals to the S position of metamizole, later 1,5-dimethyl-2-phenylpyrazol-3-one **(13)** are formed by the consecutive attack of •OH.

Abstraction of hydrogen at position 11 followed by release of acetyl at position 17 and the subsequent oxidation of the hydroxyl that enters in that position of the progesterone would produce compound (8*S*,9*S*,10*R*,13*S*,14*S*)-10,13-dimethyl-1,2,6,7,8,9,12,14,15,16-decahydrocyclopenta[a]phenanthrene-3,11,17-trione **(14)**.

The 2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl)-*N*, *N*-dimethylethanamine oxide compound **(15)** could be formed from the interaction between oxygen and the protonation of the N moiety in Venlafaxine.

Finally, since the break of the aromatic rings maleic **(26)** and oxamic **(27)** acids are generated, which continue being attacked by •OH forming acetic **(28)** and formic acid **(29)** until the complete mineralization to CO_2 and the release of ions.

3.2.2. Intermediates in chloride background solution

During the electrolysis in 0.05 M NaCl, 11 possible intermediaries were identified. Their denominations, molecular formula, retention time, measured exact mass, identifications proofs and suggested chemical structure are reported in SM3.

The possible generation pathway of the compound 2-Phenylphenol **(11)** was previously discussed for electrolysis in 0.05 M Na_2SO_4 solution. However, in the electrolysis in 0.05 M NaCl medium, additional different compounds were identified.

In this media, in addition to the hydroxyl radicals generated on the surface of the BDD electrode, active chlorine species were produced and reacted with the contaminants (Deborde and von Gunten, 2008).

N-demethylation, dehalogenation reaction and piperazine moiety release caused by attack of the •OH radicals on the structures of ciprofloxacin and norfloxacin could give rise to compound 4-oxo-1H-quinoline-3-carboxylic acid **(16)**. Furthermore, the intermediate 1H-quinolin-4-one **(17)** can be occur from the decarboxylation of **16.**

The 2-(2-chloroanilino) benzaldehyde compound **(18)** could be generated from decarboxylation in phenylacetic moiety and the release of a chlorine in the dichloroaniline moiety from diclofenac.

The quinoline compound **(19)** could originate from the successive attacks of •OH, which led to the opening of the benzene ring in 2-Phenylphenol **(11)** (formed in sulfate background solution) and subsequent decarboxylation.

The addition of an •OH and subsequent oxidation on the pyridine ring of quinoline **(19)** can also produce intermediate 1H-quinolin-4-one **(17)**.

Compounds 2-(3-chloro-2-methylanilino) benzoic acid **(20)** and 2-(2,6-dichloro-3-methylanilino) benzoic acid **(21)** are two possible chlorinated intermediaries from mefenamic acid. Compound **20** could have been formed from the attack of •OH radicals on the methyl group in the *meta* position with respect to the amino moiety of mefenamic acid to form a carboxyl group. Further, the carboxyl group in this molecule could be released and chlorine added to form a bond due to the positive polarization of HClO ($Cl^{\delta+}$-$OH^{\delta-}$). In addition, it could act by electrophilic substitution taking out an H^+ that could previously enter when the decarboxylation took place. Meanwhile, intermediate **21** could originate from the inclusion of a chlorine in an *ortho* position with respect to the N moiety either by addition or electrophilic substitution as explained previously in the formation of intermediate **20**, and by the entry of other chlorine by substitution also in *ortho* position with respect to the N moiety.

On the other hand, due to the consecutives attacks of hydroxyl radicals to the S position of metamizole could originate 4-amino-1,5-dimethyl-2-phenylpyrazol-3-one **(22)**.

O-dealkylation which divided the main structure of trimebutine and the following O-dealkylation of the methoxy groups of the trimethoxy benzoic acid moiety may originate intermediate (3R,4S,5R)-3,4,5-trihydroxycyclohexene-1-carboxylic acid **(23)**.

Compounds 4-[2-(dimethylamino)ethyl]phenol **(24)** and 4-(2-aminoethyl) phenol **(25)** could be generated from cleavage of the C–C bond between the cyclohexanol group and the rest of the venlafaxine molecule. Subsequently, O-dealkylation must have occurred to form **24**, followed by two demethylation on N–C bond to form **25**.

Finally, the breakdown of the simplest aromatic rings yielded short-chain carboxylic acids.

Ionic exclusion chromatography allowed for the identification and quantification of the maleic, formic, oxalic and acetic acids generated. The maximum levels of acetic, oxamic and formic acid reached were 19.7, 2.27 and 2.12 mg L^{-1}, respectively, while the concentration of maleic acid did not exceed 1.7 mg L^{-1}, due to the result of the direct rupture of the aromatic rings present in the pollutants that can be attacked continuously by hydroxyl radicals, creating simpler acids such as formic, acetic and oxalic. In addition, the formation of oxamic acid occurs as a consequence of the breakdown of N-aromatics (Vidal et al, 2018, 2019).

The presence of inorganic ions such as ammonium (NH_4^+) and nitrate (NO_3^-) generated during the electrolysis of the pharmaceuticals in sulfate and chloride media, was determined by spectrophotometry and confirmed by ion chromatography for NO_3^- ion. The spectrophotometric method yielded NO_3^- ion concentrations of 11.5 and 180.1 mg L^{-1} before and after electrolysis, respectively, while NH_4^+ concentrations were 12.0 and 0 mg L^{-1}, respectively. These results were similar in both electrolytic media. With all these results, an abbreviated diagram of intermediates and by-products generated in electrolysis with 0.05 M NaCl and 0.05 M Na_2SO_4 solutions is proposed and showed in Fig. 4.

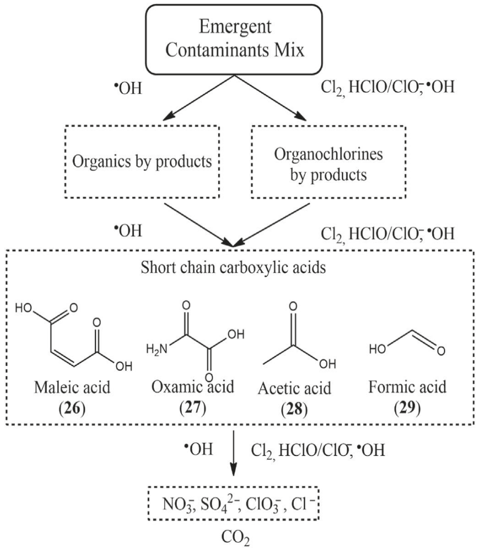

Fig. 4. General diagram of mineralization of a mix of 30 pharmaceuticals in two different electrolytic media by anodic oxidation.

3.3. Mineralization of 30 pharmaceuticals in a secondary effluent water samples

From an applicative standpoint, it is necessary to investigate the mineralization of the pharmaceuticals in a real matrix to evaluate the effect of its composition on the performance of the AO process. Some experiments were carried out in a real secondary effluent from a WWTP at natural pH, without the addition of supporting electrolyte, using BDD anode at 6 mA cm^{-2} (11.48 V cell potential). The application of 6 mA cm^{-2} was based on the results shown above and the lowest energy consumption obtained in all the studied media. Table 1 reports the physicochemical characteristics of the secondary effluent used in this study. As seen in Table 1, the secondary effluent contained 22.68 mg L^{-1} of TOC, which increased to more than 93.3 mg L^{-1} when the solution was spiked with the 30 compounds at the concentration reported in SM1. These concentrations mostly exceed those found for these pollutants in environmental waters, however they were chosen in order to ensure an adequate detection of by-products by LC-HRMS. Fig. 5 illustrates the time course of TOC removal during the degradation of 30 pharmaceuticals spiked into a secondary effluent water sample under the above described conditions. According to Fig. 5A, almost total mineralization occurred after 360 min, with a residual TOC of

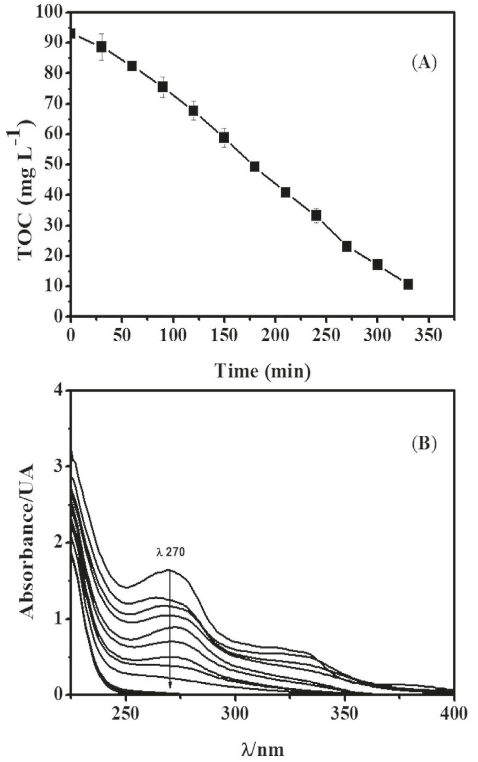

Fig. 5. (A) TOC abatement and (B) spectra of real wastewater containing 30 pharmaceuticals (corresponding to 72.62 mg L^{-1} TOC) with respect to electrolysis time for the treatment of 100 cm^3 of solution without the addition of supporting electrolyte, using a stirred BDD/SS cell at 25 °C, pH 7.66, applying 6 mA cm^{-2}.

10.64 mg L^{-1}. This result shows that through AO, the complete transformation to CO_2 of the pharmaceuticals and the original organic component of the secondary effluent occurs. The remaining TOC in solution could correspond to those short-chain carboxylic acids that were produced in the electrolytic media described above. The percentage of mineralization after 300 min (81.61%) was higher in the secondary effluent than that obtained in the electrolytic media evaluated (0.05 M sulfate, 0.05 M chloride and a mixture of both at 0.05 M) at a current density of 6 mA cm^{-2}, although no support electrolyte was incorporated in this case. The secondary effluent sample was analyzed by ion chromatography, showing levels of sulfate and total chlorine of 0.6 mM and 0.45 mM, respectively. Thus, the composition of the secondary effluent water sample had a positive influence in the mineralization of pharmaceuticals. The highest percentage of mineralization obtained could be mainly due to the action of the hydroxyl radical on the BDD surface, the active chlorine especies, and to a lesser extent, to other oxidants with weak character such as peroxodiphosphate and peroxodicarbonate generated from phosphates and carbonates (in much lower concentration than the other ions) that coexist in the effluent water, they also react with organic pollutants causing their oxidation (Cotillas et al., 2017).

The Fig. 5B shows the noticeable decay of spectral bands intensity ($\lambda_{\text{máx}}$ 270 nm) during the electrooxidation of the 30 pharmaceuticals in the effluent water by applying 6 mA cm^{-2}. Absorbance decreased linearly (results are not shown) over electrolysis time. Therefore, it can be assumed that there was no formation of organochlorine compounds that modified absorption as in the case of electrolysis performed with 0.05 M NaCl and 0.05 M NaCl + Na_2SO_4 electrolyte, due to the low concentration of chloride in the wastewater. A high mineralization rate was achieved without the addition of salts during electrolysis and the pH during electrolysis was close to natural pH (7.66), which is also an advantage for the applicability of AO on a larger scale to treat wastewater.

The electrical consumption in this electrolysis by applying 6 mA cm^{-2} for 330 min was 18.95 kW h m^{-3}, which is equivalent to 2.90 USD m^{-3}, corresponding to 88.6% mineralization. Thus, AO is an economic and effective process for the treatment of wastewater that contains organic emerging contaminants such as pharmaceuticals.

A possible coupling with biological treatments could be attractive in order to further reduce the energy costs associated with the electrooxidation time. In this way, AO could be applied until obtaining lower molecular weight intermediates, or intermediates where antimicrobial activity is lost in the case of antibiotics, and then biologically converting them into CO_2 and CH_4 (Vidal et al., 2018).

Change in concentration of ions after electrolysis was confirmed by ion chromatography and the result is shown in Fig. 6. An increase in NO_3^- concentration close to 16-folds was observed after 300 min of electrolysis, which can be attributed to the degradation of pharmaceuticals containing atoms of N such as famotidine and losartan, among others. The behavior of other ions such as sulfate, chlorine, and chlorate before and after electrolysis is shown as well in the ion chromatogram shown in Fig. 6. SO_4^{2-} ion concentration also increased at the end of the electrolysis and it was attributed to breakdown of S-compounds (Cavalcanti et al., 2013) as sulfamethazine, amoxicillin, omeprazole, and famotidine. Moreover, the concentration of chloride (Cl^-) decreased due to the increase of the chlorate (ClO_3^-) concentration (Sirés et al., 2014). These results confirm the positive influence of the matrix components of the secondary WWTP's effluent in the mineralization of pharmaceuticals.

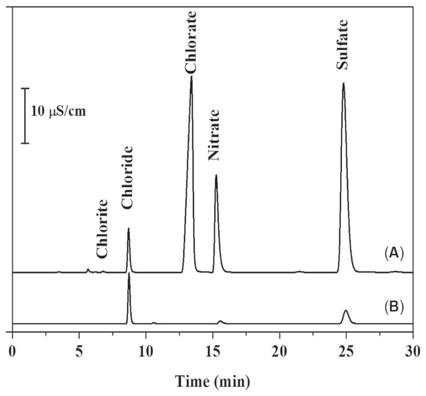

Fig. 6. Ion Chromatogram of the wastewater spiked with a mix of 30 pharmaceuticals (A) before and (B) after electrolysis applying 6 mA cm^{-2} during 330 min in a stirred BDD/SS cell at 25 °C, pH 7.66.

4. Conclusions

The mineralization of 30 pharmaceuticals by AO in three different electrolytic media was achieved. A TOC removal >95% was obtained applying 40 mA cm^{-2}, in the presence of 0.05 M Na_2SO_4 + 0.05 M NaCl, due to the action of hydroxyl radicals and active chlorine species produced on the BDD anode. 25 intermediaries produced during the electrooxidation were identified, obtaining clear differences in the compounds formed when the supporting electrolyte is NaCl or Na_2SO_4. In all electrolytic media, occur the generation of carboxylic acids, NO_3^-, SO_4^{2-} and NH_4^+ ions.

A secondary effluent water sample spiked with 30 pharmaceuticals was treated by AO reaching 88% TOC abatement, applying 6 mA cm^{-2} at natural pH, and without the addition of supporting electrolyte. These results provide an evidence of the suitability AO application for the elimination of organic contaminants of emerging concern, such as pharmaceuticals, because it is not necessary to modify the pH or to add chemicals to the wastewater. Furthermore, the energy consumption for the pharmaceuticals removal was 18.95 kW hm^{-3}, which is equivalent to 2.90 USD m^{-3}. This implies that AO is an economic and effective process for treating wastewater or other water types that contains emergent contaminants such as pharmaceuticals.

CRediT author statement

Wendy Calzadilla: Investigation & Writing - Original Draft. Lidia Carolina Espinoza: Investigation. Silvia Diaz-Cruz: Conceptualization, Resources, Review & Editing. Adrià Sunyer: Investigation. Mario Aranda: Writing - Review & Editing. Carlos Peña-Farfal: Investigation. Ricardo Salazar: Conceptualization, Resources, Writing - Review & Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.128753.

References

Alygizakis, N.A., Urík, J., Beretsou, V.G., Kampouris, I., Galani, A., Oswaldova, M., Berendonk, T., Oswald, P., Thomaidis, N.S., Slobodnik, J., Vrana, B., Fatta-Kassinos, D., 2020. Evaluation of chemical and biological contaminants of emerging concern in treated wastewater intended for agricultural reuse. Environ. Int. 138, 105597. https://doi.org/10.1016/J.ENVINT.2020.105597.

Babic, S., Horvat, A.J.M., 2007. Sample Preparation in Analysis of Pharmaceuticals, vol. 26. https://doi.org/10.1016/j.trac.2007.09.010.

Barbosa, M.O., Moreira, N.F.F., Ribeiro, A.R., Pereira, M.F.R., Silva, A.M.T., 2016. Occurrence and removal of organic micropollutants: an overview of the watch list of EU Decision 2015/495. Water Res. 94, 257–279. https://doi.org/10.1016/j.watres.2016.02.047.

Barrios-Estrada, C., de Jesús Rostro-Alanis, M., Muñoz-Gutiérrez, B.D., Iqbal, H.M.N., Kannan, S., Parra-Saldívar, R., 2018. Emergent contaminants: endocrine disruptors and their laccase-assisted degradation – a review. Sci. Total Environ. 612, 1516–1531. https://doi.org/10.1016/j.scitotenv.2017.09.013.

Boix, C., Ibáñez, M., Sancho, J.V., Rambla, J., Aranda, J.L., Ballester, S., Hernández, F., 2015. Fast determination of 40 drugs in water using large volume direct injection liquid chromatography-tandem mass spectrometry. Talanta 131, 719–727. https://doi.org/10.1016/j.talanta.2014.08.005.

Brocenschi, R.F., Rocha-Filho, R.C., Bocchi, N., Biaggio, S.R., 2016. Electrochemical degradation of estrone using a boron-doped diamond anode in a filter-press reactor. Electrochim. Acta 197, 186–193. https://doi.org/10.1016/j.electacta.2015.09.170.

Candia-Onfray, C., Espinoza, N., Sabino da Silva, E.B., Toledo-Neira, C., Espinoza, L.C., Santander, R., García, V., Salazar, R., 2018. Treatment of winery wastewater by anodic oxidation using BDD electrode. Chemosphere 206, 709–717. https://doi.org/10.1016/j.chemosphere.2018.04.175.

Cavalcanti, E.B., Garcia-Segura, S., Centellas, F., Brillas, E., 2013. Electrochemical incineration of omeprazole in neutral aqueous medium using a platinum or boron-doped diamond anode: degradation kinetics and oxidation products. Water Res. 47, 1803–1815. https://doi.org/10.1016/j.watres.2013.01.002.

Česen, M., Ahel, M., Terzić, S., Heath, D.J., Heath, E., 2019. The occurrence of contaminants of emerging concern in Slovenian and Croatian wastewaters and receiving Sava river. Sci. Total Environ. 650, 2446–2453. https://doi.org/10.1016/J.SCITOTENV.2018.09.238.

Comninellis, C., Kapalka, A., Malato, S., Parsons, S.A., Poulios, I., Mantzavinos, D., 2008. Advanced oxidation processes for water treatment: advances and trends for R&D. J. Chem. Technol. Biotechnol. 83, 769–776. https://doi.org/10.1002/jctb.1873.

Contreras, N., Vidal, J., Berríos, C., Villegas, L., Salazar, R., 2015. Degradation of antihypertensive hydrochlorothiazide in water from pharmaceutical formulations by electro-oxidation using a BDD anode. Int. J. Electrochem. Sci. 10, 9269–9285.

Cotillas, S., Cañizares, L., Muñoz, M., Sáez, C., Cañizares, P., Rodrigo, M.A., 2017. Electrochimica Acta Is it really important the addition of salts for the electrolysis of soil washing effluents? Electrochim. Acta 246, 372–379. https://doi.org/10.1016/j.electacta.2017.06.016.

Deborde, M., von Gunten, U., 2008. Reactions of chlorine with inorganic and organic compounds during water treatment-Kinetics and mechanisms: a critical review. Water Res. 42, 13–51. https://doi.org/10.1016/j.watres.2007.07.025.

Dirany, A., Sirés, I., Oturan, N., Oturan, M.A., 2010. Electrochemical abatement of the antibiotic sulfamethoxazole from water. Chemosphere 81, 594–602. https://doi.org/10.1016/j.chemosphere.2010.08.032.

Ebele, A.J., Abou-Elwafa Abdallah, M., Harrad, S., 2017. Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. Emerg. Contam. 3, 1–16. https://doi.org/10.1016/j.emcon.2016.12.004.

Espinoza, L.C., Henríquez, A., Contreras, D., Salazar, R., 2018. Evidence for the production of hydroxyl radicals at boron-doped diamond electrodes with different sp3/sp2 ratios and its relationship with the anodic oxidation of aniline. Electrochem. Commun. 90, 30–33. https://doi.org/10.1016/j.elecom.2018.03.007.

Fent, K., Weston, A.A., Caminada, D., 2006. Ecotoxicology of human pharmaceuticals. Aquat. Toxicol. 76, 122–159. https://doi.org/10.1016/j.aquatox.2005.09.009.

Flores, N., Sirés, I., Rodríguez, R.M., Centellas, F., Cabot, P.L., Garrido, J.A., Brillas, E., 2017. Removal of 4-hydroxyphenylacetic acid from aqueous medium by electrochemical oxidation with a BDD anode: mineralization, kinetics and oxidation products. J. Electroanal. Chem. 793, 58–65. https://doi.org/10.1016/j.jelechem.2016.07.042.

García-Montoya, M.F., Gutiérrez-Granados, S., Alatorre-Ordaz, A., Galindo, R., Ornelas, R., Peralta-Hernández, J.M., 2015. Application of electrochemical/BDD process for the treatment wastewater effluents containing pharmaceutical compounds. J. Ind. Eng. Chem. 31, 238–243. https://doi.org/10.1016/j.jiec.2015.06.030.

Garcia-Segura, S., Keller, J., Brillas, E., Radjenovic, J., 2015. Removal of organic contaminants from secondary effluent by anodic oxidation with a boron-doped diamond anode as tertiary treatment. J. Hazard Mater. 283, 551–557. https://doi.org/10.1016/j.jhazmat.2014.10.003.

Geissen, V., Mol, H., Klumpp, E., Umlauf, G., Nadal, M., van der Ploeg, M., van de Zee, S.E.A.T.M., Ritsema, C.J., 2015. Emerging pollutants in the environment: a challenge for water resource management. Int. Soil Water Conserv. Res. 3, 57–65. https://doi.org/10.1016/j.iswcr.2015.03.002.

Gómez, M.J., Gómez-Ramos, M.M., Malato, O., Mezcua, M., Férnandez-Alba, A.R., 2010. Rapid automated screening, identification and quantification of organic micro-contaminants and their main transformation products in wastewater and river waters using liquid chromatography-quadrupole-time-of-flight mass spectrometry with an accurate-mass. J. Chromatogr. A 1217, 7038–7054. https://doi.org/10.1016/j.chroma.2010.08.070.

Gracia-lor, E., Sancho, J.V., Serrano, R., Hernández, F., 2012. Chemosphere Occurrence and removal of pharmaceuticals in wastewater treatment plants at the Spanish Mediterranean area of Valencia. Chemosphere 87, 453–462. https://doi.org/10.1016/j.chemosphere.2011.12.025.

Grenni, P., Ancona, V., Barra Caracciolo, A., 2018. Ecological effects of antibiotics on natural ecosystems: a review. Microchem. J. 136, 25–39. https://doi.org/10.1016/j.microc.2017.02.006.

Hernández, F., Calısto-ulloa, N., Gómez-fuentes, C., Gómez, M., Ferrer, J., 2019. Occurrence of antibiotics and bacterial resistance in wastewater and sea water from the Antarctic. J. Hazard Mater. 363, 447–456. https://doi.org/10.1016/j.jhazmat.2018.07.027.

Hernández, F., Ibáñez, M., Gracia-Lor, E., Sancho, J.V., 2011. Retrospective LC-QTOF-MS analysis searching for pharmaceutical metabolites in urban wastewater. J. Separ. Sci. 34, 3517–3526. https://doi.org/10.1002/jssc.201100540.

Jalife-Jacobo, H., Feria-Reyes, R., Serrano-Torres, O., Gutiérrez-Granados, S., Peralta-Hernández, J.M., 2016. Diazo dye Congo Red degradation using a Boron-doped diamond anode: an experimental study on the effect of supporting electrolytes. J. Hazard Mater. 319, 78–83. https://doi.org/10.1016/j.jhazmat.2016.02.056.

Lan, Y., Coetsier, C., Causserand, C., Groenen Serrano, K., 2017. On the role of salts for the treatment of wastewaters containing pharmaceuticals by electrochemical oxidation using a boron doped diamond anode. Electrochim. Acta 231, 309–318. https://doi.org/10.1016/j.electacta.2017.01.160.

Martínez-Huitle, C.A., Brillas, E., 2009. Decontamination of wastewaters containing synthetic organic dyes by electrochemical methods: a general review. Appl. Catal. B Environ. 87, 105–145. https://doi.org/10.1016/j.apcatb.2008.09.017.

Martínez-Huitle, C.A., Ferro, S., 2006. Electrochemical oxidation of organic pollutants for the wastewater treatment: direct and indirect processes. Chem. Soc. Rev. 35, 1324–1340. https://doi.org/10.1039/b517632h.

Martínez-Huitle, C.A., Rodrigo, M.A., Sirés, I., Scialdone, O., 2015. Single and coupled electrochemical processes and reactors for the abatement of organic water pollutants: a critical review. Chem. Rev. 115, 13362–13407. https://doi.org/10.1021/acs.chemrev.5b00361.

Miao, X.S., Metcalfe, C.D., 2003. Determination of carbamazepine and its metabolites in aqueous samples using liquid chromatography - electrospray tandem mass spectrometry. Anal. Chem. 75, 3731–3738. https://doi.org/10.1021/ac030082k.

Moreira, F.C., Boaventura, R.A.R., Brillas, E., Vilar, V.J.P., 2017. Electrochemical advanced oxidation processes: a review on their application to synthetic and real wastewaters. Appl. Catal. B Environ. 202, 217–261. https://doi.org/10.1016/j.apcatb.2016.08.037.

Mousset, E., Oturan, N., Oturan, M.A., Mousset, E., Oturan, N., Oturan, M.A., 2018. An unprecedented route of °OH radical reactivity : ipso-substitution with perhalogenocarbon compounds to cite this version : HAL Id : hal-01712279 electrocatalytical process : ipso-substitution with Paper submitted to Applied Catalysis B - environment fo. Appl. Catal. B Environ. 226, 135–146.

Murugananthan, M., Latha, S.S., Bhaskar Raju, G., Yoshihara, S., 2010. Anodic oxidation of ketoprofen-An anti-inflammatory drug using boron doped diamond and platinum electrodes. J. Hazard Mater. 180, 753–758. https://doi.org/10.1016/j.jhazmat.2010.05.007.

Noguera-oviedo, K., Aga, D.S., 2016. Lessons learned from more than two decades of research on emerging contaminants in the environment. J. Hazard Mater. 316, 242–251. https://doi.org/10.1016/j.jhazmat.2016.04.058.

NORMAN Substance Database [WWW Document], n.d. URL https://www.norman-network.com/nds/susdat/(accessed 6.27.20).

Özcan, A., Şahin, Y., Koparal, A.S., Oturan, M.A., 2008. Propham mineralization in aqueous medium by anodic oxidation using boron-doped diamond anode: influence of experimental parameters on degradation kinetics and mineralization efficiency. Water Res. 42, 2889–2898. https://doi.org/10.1016/j.watres.2008.02.027.

Panizza, M., Cerisola, G., 2009. Direct and mediated anodic oxidation of organic pollutants. Chem. Rev. 109, 6541–6569. https://doi.org/10.1021/cr9001319.

Patel, M., Kumar, R., Kishor, K., Mlsna, T., Pittman, C.U., Mohan, D., 2019. Pharmaceuticals of emerging concern in aquatic systems: chemistry, occurrence, effects, and removal methods. Chem. Rev. 119, 3510–3673. https://doi.org/10.1021/acs.chemrev.8b00299.

Peña-Guzmán, C., Ulloa-Sánchez, S., Mora, K., Helena-Bustos, R., Lopez-Barrera, E., Alvarez, J., Rodriguez-Pinzón, M., 2019. Emerging pollutants in the urban water cycle in Latin America: a review of the current literature. J. Environ. Manag. 237, 408–423. https://doi.org/10.1016/J.JENVMAN.2019.02.100.

Petrovic, M., 2014. Trends in Environmental Analytical Chemistry Methodological challenges of multi-residue analysis of pharmaceuticals in environmental samples. Trends Environ. Anal. Chem. 1, e25–e33. https://doi.org/10.1016/j.teac.2013.11.004.

Rabbia, V., Bello-Toledo, H., Jiménez, S., Quezada, M., Domínguez, M., Vergara, L., Gómez-Fuentes, C., Calisto-Ulloa, N., González-Acuña, D., López, J., González-Rocha, G., 2016. Antibiotic resistance in Escherichia coli strains isolated from Antarctic bird feces, water from inside a wastewater treatment plant, and seawater samples collected in the Antarctic Treaty area. Pol. Sci. 10, 123–131. https://doi.org/10.1016/j.polar.2016.04.002.

Racar, M., Dolar, D., Karadakić, K., Čavarović, N., Glumac, N., Ašperger, D., Košutić, K., 2020. Challenges of municipal wastewater reclamation for irrigation by MBR and NF/RO: physico-chemical and microbiological parameters, and emerging contaminants. Sci. Total Environ. 722, 137959. https://doi.org/10.1016/J.SCITOTENV.2020.137959.

Rivera-Utrilla, J., Sánchez-Polo, M., Ferro-García, M.Á., Prados-Joya, G., Ocampo-Pérez, R., 2013. Pharmaceuticals as emerging contaminants and their removal from water. A review. Chemosphere 93, 1268–1287. https://doi.org/10.1016/j.chemosphere.2013.07.059.

Rodriguez-narvaez, O.M., Peralta-hernandez, J.M., Goonetilleke, A., Bandala, E.R., 2017. Treatment technologies for emerging contaminants in water : a review. Chem. Eng. J. 323, 361–380. https://doi.org/10.1016/j.cej.2017.04.106.

Salazar, C., Contreras, N., Mansilla, H.D., Yáñez, J., Salazar, R., 2016. Electrochemical degradation of the antihypertensive losartan in aqueous medium by electro-oxidation with boron-doped diamond electrode. J. Hazard Mater. 319, 84–92. https://doi.org/10.1016/j.jhazmat.2016.04.009.

Salazar, R., Ureta-Zañartu, M.S., González-Vargas, C., Brito, C. do N., Martinez-Huitle, C.A., 2017. Electrochemical Degradation of Industrial Textile Dye Disperse Yellow 3: role of electrocatalytic material and experimental conditions on the catalytic production of oxidants and oxidation pathway. Chemosphere 198 (2018), 21–29. https://doi.org/10.1016/j.chemosphere.2017.12.092.

Sancho, J.V., Pen, G., Gracia-lor, E., Martı, M., 2012. Talanta Multi-Class Determination of Personal Care Products and Pharmaceuticals in Environmental and Wastewater Samples by Ultra-high Performance Liquid-Chromatography-Tandem Mass Spectrometry, vol. 99, pp. 1011–1023. https://doi.org/10.1016/j.talanta.2012.07.091.

Sirés, I., Brillas, E., Oturan, M.A., Rodrigo, M.A., Panizza, M., 2014. Electrochemical advanced oxidation processes: today and tomorrow. A review. Environ. Sci. Pollut. Res. 21, 8336–8367. https://doi.org/10.1007/s11356-014-2783-1.

Sopaj, F., Rodrigo, M.A., Oturan, N., Podvorica, F.I., Pinson, J., Oturan, M.A., 2015. Influence of the anode materials on the electrochemical oxidation efficiency. Application to oxidative degradation of the pharmaceutical amoxicillin. Chem. Eng. J. 262, 286–294. https://doi.org/10.1016/j.cej.2014.09.100.

Vasiljevi, T., Lau, M., 2009. Determination of multiple pharmaceutical classes in surface and ground waters by liquid chromatography – ion trap – tandem mass spectrometry. J. Chromatogr. A 1216, 4989–5000. https://doi.org/10.1016/j.chroma.2009.04.059.

Vidal, J., Huiliñir, C., Santander, R., Silva-agredo, J., Torres-palma, R.A., Salazar, R., 2018. Science of the total environment effective removal of the antibiotic nafcillin from water by combining the photoelectro-fenton process and anaerobic biological digestion. Sci. Total Environ. 624, 1095–1105. https://doi.org/10.1016/j.scitotenv.2017.12.159.

Vidal, J., Huiliñir, C., Santander, R., Silva-Agredo, J., Torres-Palma, R.A., Salazar, R., 2019. Degradation of ampicillin antibiotic by electrochemical processes: evaluation of antimicrobial activity of treated water. Environ. Sci. Pollut. Res. 26, 4404–4414. https://doi.org/10.1007/s11356-018-2234-5.

Vogna, D., Marotta, R., Andreozzi, R., Napolitano, A., D'Ischia, M., 2004. Kinetic and chemical assessment of the UV/H2O2 treatment of antiepileptic drug carbamazepine. Chemosphere 54, 497–505. https://doi.org/10.1016/S0045-6535(03)00757-4.

World Health Organization, 2012. Pharmaceuticals in drinking-water. Pharm. Drink 1–52. ISBN 978 92 4 150208 5.

Yu, X., Zhou, M., Hu, Y., Groenen Serrano, K., Yu, F., 2014. Recent updates on electrochemical degradation of bio-refractory organic pollutants using BDD anode: a mini review. Environ. Sci. Pollut. Res. 21, 8417–8431. https://doi.org/10.1007/s11356-014-2820-0.

Zhao, X., Hou, Y., Liu, H., Qiang, Z., Qu, J., 2009. Electro-oxidation of diclofenac at boron doped diamond: kinetics and mechanism. Electrochim. Acta 54, 4172–4179. https://doi.org/10.1016/j.electacta.2009.02.059.

4.2.3 Publication #8 Supplementary information

Simultaneous degradation of 30 pharmaceuticals by anodic oxidation: Main intermediaries and by-products

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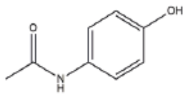
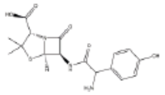
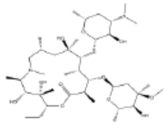
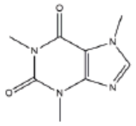
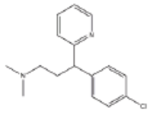
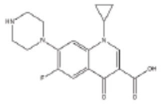
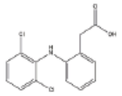
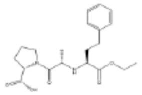
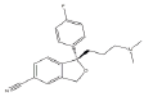
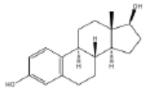
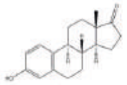
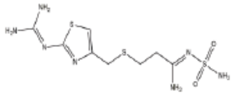
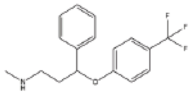
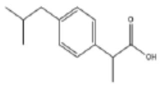
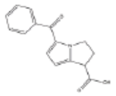
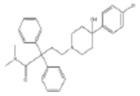
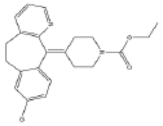
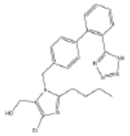
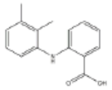
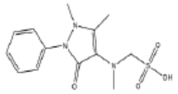
Supplementary Tables SM1, SM2 and SM3

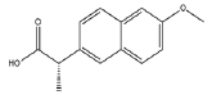
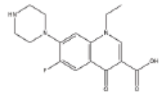
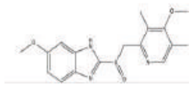
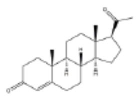
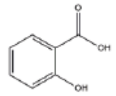
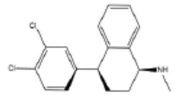
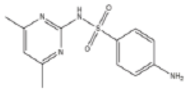
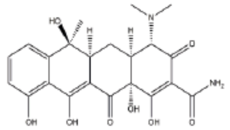
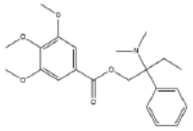
SM1. Pharmaceutical compounds used in this work including molecular formula, chemical structure, exact mass, and concentration.

Compound	*Calculated exact mass*	*Molecular formula*	*Structure*	*Concentration (mg L^{-1})*
Acetaminophen	151.0628	$C_8H_9NO_2$		502.00
Amoxicillin	365.1039	$C_{16}H_{19}N_3O_5S$		15.07
Azithromycin	748.5079	$C_{38}H_{72}N_2O_{12}$		249.50
Caffeine	194.0798	$C_8H_{10}N_4O_2$		500.00
Chlorpheniramine	274.1231	$C_{16}H_{19}ClN_2$		514.00
Ciprofloxacin	331.1327	$C_{17}H_{18}FN_3O_3$		512.00
Diclofenac	295.0161	$C_{14}H_{11}Cl_2NO_2$		14.90
Enalapril	376.1992	$C_{20}H_{28}N_2O_5$		119.20
Escitalopram	324.1632	$C_{20}H_{21}FN_2O$		44.60
β-Estradiol	272.1772	$C_{18}H_{24}O_2$		24.00

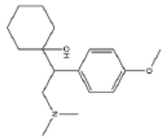
SM1. Continued.

Estrone	270.1614	$C_{18}H_{22}O_2$		18.00
Famotidine	337.0444	$C_8H_{15}N_7O_2S_3$		502.50
Fluoxetine	309.1335	$C_{17}H_{18}F_3NO$		24.80
Ibuprofen	206.1301	$C_{13}H_{18}O_2$		56.00
Ketorolac	255.0889	$C_{15}H_{13}NO_3$		402.00
Loperamide	476.2225	$C_{29}H_{33}ClN_2O_2$		9.95
Loratadine	382.1443	$C_{2}2H_{23}ClN_2O_2$		4.88
Losartan	422.1616	$C_{22}H_{23}ClN_6O$		0.08
Mefenamic acid	241.1097	$C_{15}H_{15}NO_2$		19.04
Metamizol	311.0934	$C_{13}H_{17}N_3O_4S$		498.00

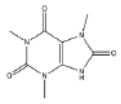
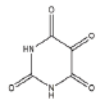
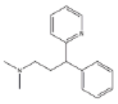
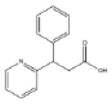
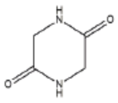
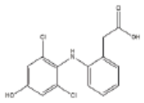
SM1. Continued.

Naproxen	230.0937	$C_{14}H_{14}O_3$		80.00
Norfloxacin	319.1327	$C_{16}H_{18}FN_3O_3$		504.00
Omeprazole	345.1142	$C_{17}H_{19}N_3O_3S$		99.45
Progesterone	314.2240	$C_{21}H_{30}O_2$		0.80
Salicylic acid	138.03115	$C_7H_6O_3$		508.00
Sertraline	305.0733	$C_{17}H_{17}Cl_2N$		3.06
Sulfamethazine	278.0832	$C_{12}H_{14}N_4O_2S$		408.00
Tetracycline	444.1527	$C_{22}H_{24}N_2O_8$		500.00
Trimebutine	387.2040	$C_{22}H_{29}NO_5$		38.00

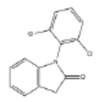
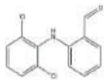
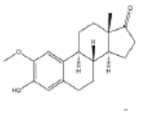
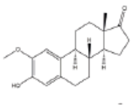
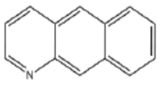
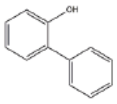
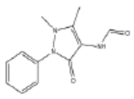
SM1. Continued.

Venlafaxine	277.2036	$C_{17}H_{27}NO_2$		501.20

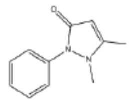
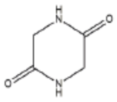
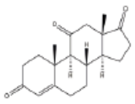
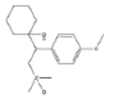
SM2. Identified by-products in 0.05 M Na_2SO_4 background solution

Possible precursor	*Intermediaries*	*Molecular formula*	*RT*	*Ion specie and monoisotopic mass*	*Measured exact mass (m/z)*	*Mass error (ppm)*	*Fragments/ Intensity*	*Identification proof*	*Proposed Structure*	*Number*
Caffeine	1,3,7-trimethyl-9*H*-purine-2,6,8-trione	$C_8H_{10}N_4O_3$	1.56	$[M-H]^-$ 209.0680	209.0679	-0.833	194.0449/38632	Mass accuracy, plausible RT, 1 fragment		1
Caffeine	1,3-diazinane-2,4,5,6-tetrone	$C_4H_2N_2O_4$	1.26	$[M-H]^-$ 140.9941	140.9942	1.414	97.9886/421720	Mass accuracy, plausible RT, 1 fragment		2
Chlorpheniramine	N, *N*-dimethyl-3-phenyl-3-pyridin-2-ylpropan-1-amine	$C_{16}H_{20}N_2$	5.23	$[M+H]^+$ 241.1699	241.1699	0.022	166.0627/59698	Mass accuracy, plausible RT, 1 fragment		3
Chlorpheniramine	3-phenyl-3-pyridin-2-ylpropanoic acid	$C_{14}H_{13}NO_2$	4.00	$[M+H]^+$ 228.1019	228.102	-0.108	210.0913/251450	Mass accuracy, plausible RT, 1 fragment		4
Ciprofloxacin	piperazine-2,5-dione	$C_4H_6N_2O_2$	1.55	$[M-H]^-$ 113.0357	113.0359	2.376	85.0409/161604 83.0253/39863	Mass accuracy, plausible RT, 2 fragments		5
Diclofenac	2-[2-(2,6-dichloro-4-hydroxyanilino) phenyl]acetic acid	$C_{14}H_{11}Cl_2NO_3$	5.37	$[M-H]^-$ 310.0043	310.004	-0.912	115.0556/168918	Mass accuracy, plausible RT, 1 fragment		6

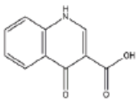
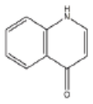
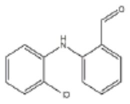
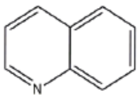
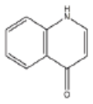
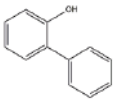
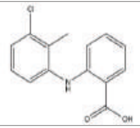
SM2. Continued.

Diclofenac	1-(2,6-dichlorophenyl)-3*H*-indol-2-one	$C_{14}H_9Cl_2NO$	8.86	$[M+H]^+$ 278.0134	278.0135	0.305	95.04917/40142780	Mass accuracy, plausible RT, 1 fragment		7
Diclofenac	2-(2,6-dichloroanilino) benzaldehyde	$C_{13}H_9Cl_2NO$	10.03	$[M+H]^+$ 266.0134	266.0134	-0.095	177.0547/5665852	Mass accuracy, plausible RT, 1 fragment		8
Estradiol	(8*R*,9*S*,13*S*,14*S*)-3-hydroxy-2-methoxy-13-methyl-7,8,9,11,12,14,15,16-octahydro-6*H*-cyclopenta[a]phenanthren-17-one	$C_{19}H_{24}O_3$	8.14	$[M+H]^+$ 301.1798	301.1798	0.031	189.0911/300278 137.0598/294631 103.0543/1238705	Mass accuracy, plausible RT, 3 fragments		9
Estrone	(8*R*,9*S*,13*S*,14*S*)-3-hydroxy-2-methoxy-13-methyl-7,8,9,11,12,14,15,16-octahydro-6*H*-cyclopenta[a]phenanthren-17-one	$C_{19}H_{24}O_3$	8.14	$[M+H]^+$ 301.1798	301.1798	0.031	189.0911/300278 137.0598/294631 103.0543/1238705	Mass accuracy, plausible RT, 3 fragments		9
Loratadine	benzo[g]quinoline	$C_{13}H_9N$	7.01	$[M+H]^+$ 180.0808	180.0804	-1.862	179.0731/93097	Mass accuracy, plausible RT, 1 fragment		10
Losartan	2-Phenylphenol	$C_{12}H_{10}O$	5.46	$[M-H]^-$ 169.0659	169.066	0.716	141.0712/953252	Mass accuracy, plausible RT, 1 fragment		11
Metamizol	N-(1,5-dimethyl-3-oxo-2-phenylpyrazol-4-yl) formamide	$C_{12}H_{13}N_3O_2$	4.45	$[M+H]^+$ 232.1081	232.1081	0.118	146.0602/140559	Mass accuracy, plausible RT, 1 fragment		12

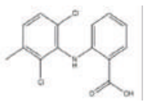
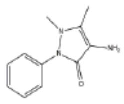
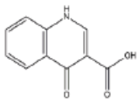
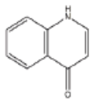
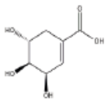
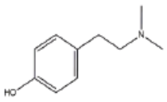
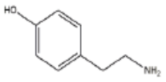
SM2. Continued.

Metamizol	1,5-dimethyl-2-phenylpyrazol-3-one	$C_{11}H_{12}N_2O$	5.31	$[M+H]^+$ 189.1022	189.1023	0.534	149.0710/673174 147.0918/799976 133.0761/439567 106.0652/148623 104.0494/59631 94.0652/2576818 92.0476/73385	Mass accuracy, plausible RT, 7 fragments		13
Norfloxacin	piperazine-2,5-dione	$C_4H_6N_2O_2$	1.55	$[M-H]^-$ 113.0357	113.0359	2.376	85.0409/16160483. 0253/39863	Mass accuracy, plausible RT, 2 fragments		5
Progesterone	(8*S*,9*S*,10*R*,13*S*,14*S*)-10,13-dimethyl-1,2,6,7,8,9,12,14,15,16-decahydrocyclopenta[a]phenanthrene-3,11,17-trione	$C_{19}H_{24}O_3$	8.14	$[M+H]^+$ 301.1798	301.1798	0.031	121.0618/559680	Mass accuracy, plausible RT, 1 fragment		14
Venlafaxine	2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl)-*N*,*N*-dimethylethanamine oxide	$C_{17}H_{27}NO_3$	5.56	$[M+H]^+$ 294.2064	294.2064	0.239	122.0676/163014	Mass accuracy, plausible RT, 1 fragment		15

SM3. Identified by-products in 0.05 M NaCl background solution

Possible precursor	*Intermediaries*	*Molecular formula*	*RT*	*Ion specie and monoisotopic mass*	*Measured exact mass (m/z)*	*Mass error (ppm)*	*Fragments/ Intensity*	*Identification proof*	*Proposed Structure*	*Number*
Ciprofloxacin	4-oxo-1H-quinoline-3-carboxylic acid	$C_{10}H_7NO_3$	4.37	$[M+H]^+$ 190.0499	190.0502	1.952	144.0423/194504 116.0496/36360	Mass accuracy, plausible RT, 2 fragments		16
Ciprofloxacin	1H-quinolin-4-one	C_9H_7NO	3.75	$[M-H]^-$ 144.0455	144.0456	0.709	116.0508/75572	Mass accuracy, plausible RT, 1 fragment		17
Diclofenac	2-(2-chloroanilino) benzaldehyde	$C_{13}H_{10}ClNO$	7.83	$[M+H]^+$ 232.0524	232.0523	-0.378	214.0418/1223128 178.0652/961035	Mass accuracy, plausible RT, 2 fragments		18
Loratadine	quinoline	C9H7N	3.41	$[M+H]^+$ 130.0651	130.0653	1.651	77.0386/1897205	Mass accuracy, plausible RT, 1 fragment		19
Loratadine	1H-quinolin-4-one	C_9H_7NO	3.75	$[M-H]^-$ 144.0455	144.0456	0.709	116.0508/75572	Mass accuracy, plausible RT, 1 fragment		17
Losartan	2-phenylphenol	$C_{12}H_{10}O$	5.37	$[M+H]^+$ 171.0804	171.0813	5.197	153.0685/653001	Mass accuracy, plausible RT, 1 fragment		11
Mefenamic acid	2-(3-chloro-2-methylanilino) benzoic acid (Tolfenamic acid)	$C_{14}H_{12}ClNO_2$	7.54	$[M+H]^+$ 262.0629	262.0631	0.792	246.0491/1046532 245.0558/5006008 244.0522/3076308 229.0289/144770 209.0833/70265	Mass accuracy, plausible RT, 5 fragments		20

SM3. Continued.

Mefenamic acid	2-(2,6-dichloro-3-methylanilino) benzoic acid (Meclofenamic acid)	$C_{14}H_{11}Cl_2NO_2$	9.62	$[M+H]^+$ 296.0239	296.0244	1.385	180.0786/122348	Mass accuracy, plausible RT, 1 fragment		21
Metamizol	4-amino-1,5-dimethyl-2-phenylpyrazol-3-one	$C_{11}H_{13}N_3O$	3.71	$[M+H]^+$ 204.1131	204.1137	2.508	77.0386/2154302	Mass accuracy, plausible RT, 1 fragment		22
Norfloxacin	4-oxo-1H-quinoline-3-carboxylic acid	$C_{10}H_7NO_3$	4.37	$[M+H]^+$ 190.0499	190.0502	1.952	144.0423/194504 116.0496/36360	Mass accuracy, plausible RT, 2 fragments		16
Norfloxacin	1H-quinolin-4-one	C_9H_7NO	3.75	$[M-H]^-$ 144.0455	144.0456	0.709	116.0508/75572	Mass accuracy, plausible RT, 1 fragment		17
Trimebutine	(3R,4S,5R)-3,4,5-trihydroxycyclohexene-1-carboxylic acid	$C_7H_{10}O_5$	1.25	$[M-H]^-$ 173.0455	173.0455	-0.332	73.0294/526412	Mass accuracy, plausible RT, 1 fragment		23
Venlafaxine	4-[2-(dimethylamino)ethyl]phenol	$C_{10}H_{15}NO$	2.9	$[M+H]^+$ 166.1226	166.1228	1.023	77.0386/1789974	Mass accuracy, plausible RT, 1 fragment		24
Venlafaxine	4-(2-aminoethyl) phenol	$C_8H_{11}NO$	2.34	$[M+H]^+$ 138.0913	138.0914	0.724	77.0386/1711873	Mass accuracy, plausible RT, 1 fragment		25

4.3 Discussion

The elimination/removal technologies presented in this chapter are innovative and provide original results about the degradation and removal of CECs in wastewater and the further identification of new TPs formed in the process. As mentioned in Chapter 3, the individual conclusions of each work are discussed in the manuscripts, so only a global comparison and discussion of the performance of the remediation techniques are included in this section.

Removal efficiencies

The best method to compare the efficiency of a degradation technology is comparing the concentrations of each contaminant between the influent and effluent. Therefore, Publication #8 was excluded from further discussion in this section because the removal analysis was evaluated considering the generic behaviour of 30 pharmaceuticals and was performed at a lab scale. Since it was based on the total organic content (TOC), the measurement of the concentrations was qualitative, and each pharmaceutical concentration was unavailable.

The results from Publications #5 and #6 present similar patterns since the same HRAP system was used but applied to different samples (irrigation (#5) and wastewater (#6)). Both studies showed that the HRAP system presented difficulties removing benzotriazoles, especially BZT (<15% removal), but performed better on removing BP3 and some of its TPs. Regarding pharmaceuticals, the removals were considerably high in both matrices. Only trimethoprim (TMP) in the wastewater sample and sulfapyridine (SPY) in both analysed matrices presented low or negative removal rates. Overall, the removals in the irrigation water samples were higher than in the wastewater (8 vs 2, respectively). This was caused, probably, because the HRAP system was more efficient with a cleaner sample, and the removal performance decreased in a more complex matrix like wastewater.

The results from Publication #7 show the removal rates of a reference SAT system (barrier filled only with sand) (T2) and a reactive barrier made of vegetal compost blended with sand (equal volume) and with little clay volume (2%) (T4). In general, the removals from the reference barrier show already a good performance, probably due to the biodegradation in the SAT system. With the incorporation of an active, reactive barrier, T4 presented even higher removal rates, with the barrier's additional degradation/retention area. In general, the performance of the SAT system was very efficient, achieving high removals of very recalcitrant CECs such as CBZ, BZT, and GFZ (81%, 68%, and 90%, respectively) and the complete removal of other contaminants such as 4MBC, TMP or IBU.

Comparing the results from Publications #5, #6, and #7, the compounds with available removal rates are represented in **Figure 4.3**. The specific removal rates of the compounds with missing values are also shown in **Table 4.2**. According to **Figure 4.3**, the best technology for CECs' removal is the SAT system with the T4 barrier, as could be expected since this pilot system combines efficient absorption and biodegradation processes enhanced by the additional organic carbon supplied by the barrier's material. Even the SAT reference showed a better removal performance than HRAP, suggesting that the biological processes in the SAT system provide more adequate conditions for improved CECs' degradation. A clear example are benzotriazoles, which barely degraded in the HRAP system and achieved considerably high removals in the SAT (58-68%).

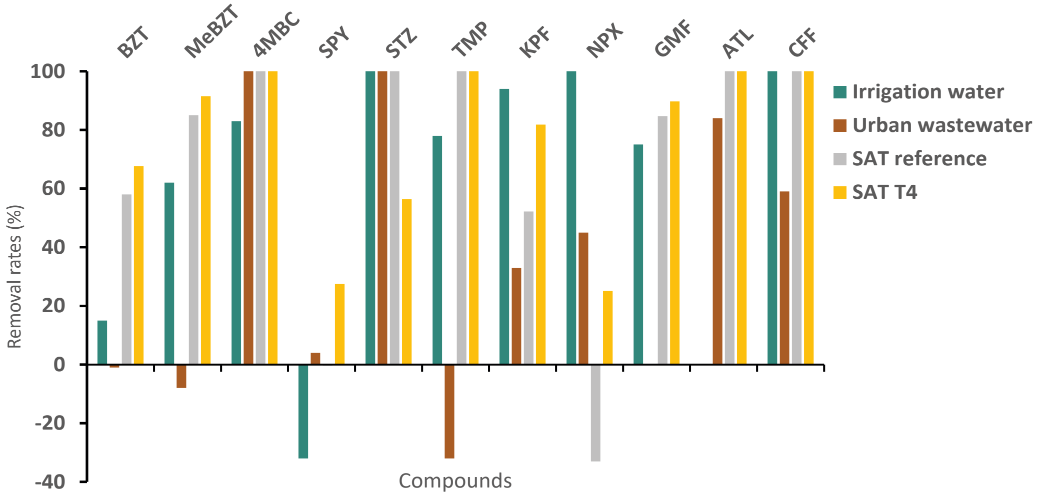


Figure 4.3. Removal rates (%) of each removal approach for the compounds in common in Publications #5, #6 and #7.

Table 4.2. Removal rates (%) of all compounds detected in each pilot system in Publications #5, #6 and #7.

	HRAP system		SAT system	
	Irrigation water	**Urban wastewater**	**SAT reference**	**SAT T4**
BZT	15	-1	58	68
MeBZT	62	-8	85	91
DMBZT	40	2		
BP1	-	-22	100	100
BP3	40	-29	-	-
4HB	-	100		
4MBC	83	100	100	100
EtPABA	-	-	100	100
OFX	-	-	100	100
CFX	-	-	100	100
NDX	-	-	100	100
SMX	46	-	-314	-192
acSMX	-	-	-51	41
SPY	-32	4	-0.4	27
acSPY	-	-	100	100
STZ	100	100	100	56
TMP	78	-32	100	100
KPF	94	33	52	82
MFA	-	-	84	87
NPX	100	45	-33	25
IBU(-)	-	-	91	100
GFZ	75	-	85	90
ATL	-	84	100	100
DCF(-)	-	-	88	91
APH	-	-	-	-
CBZ	-	-	69	81
CBZ-E	-	-	63	70
norFXT	-	-	-6	23
N-desVFX	-	-	100	100
CFF	100	59	100	100

The removal rates represented by "-" mean that the compound was not detected in the influent stream

However, it does not mean that HRAP was ineffective. For some compounds (such as ketoprofen (KPF) or NPX), the removal rate was higher in the HRAP system. For KPF, the difference between removals was small (94% HRAP – 82% SAT), but for NPX was considerably high (100% HRAP - 25% SAT). This makes sense since NPX is highly stable and resistant to microbial degradation due to the presence of two condensed rings in its structure (Domaradzka et al., 2015). Therefore, efficient degradation can only be performed by bacteria, fungi or algae (Guzik and Wojcieszyńska, 2020).

Some sulfonamides also presented interesting behaviour. Sulfamethoxazole (SMX) showed extremely high negative removal rates in the SAT system and was by far the compound that raised the most significant issues to be removed in the SAT systems. As mentioned in Publication #7, a "back transformation" of SMX metabolites has been reported before (Bonvin et al., 2013). However, the HRAP system was able to partially remove SMX (46%), suggesting that HRAP conditions disabled this "back transformation". This difference could be explained by the solar radiation affecting the HRAP system, not present in the SAT system, which could facilitate SMX degradation (Bonvin et al., 2013).

SMX acetylated homologue (acSMX) also presented a negative removal rate in the reference SAT system (-51%), but the removal in the T4 system was acceptable (41%). This significant increase in the removal rate suggests that this compound is mainly degraded/retained due to the materials of the reactive barrier. SPY was the contaminant with lower removal rates when evaluating both systems together. The highest rates were 4% and 27% in HRAP and SAT systems, respectively. Other studies have reported that this contaminant's degradation is favoured by aerobic conditions (Qin et al., 2020). It could explain the lack of degradation in the SAT system but not in the HRAP system, where microalgae generate oxygen through photosynthesis.

These cases exemplify the complexity of degradation processes and the difficulty of completely removing CECs from wastewater. This complexity increases when considering the wide range of physicochemical properties of CECs and the unknown processes happening simultaneously in the degradation/removal of CECs.

So far, the SAT system is more efficient in general terms because of the removal rates obtained and because it was tested receiving a continuous WWTP effluent flow. It shows that it is much closer to applying it under real conditions. However, the HRAP system was more efficient for compounds with specific properties (such as NPX), showing that it can also be beneficial. Thus, the best solution seems to be the combination of different technologies to take advantage of the best parts of each technology. However, there is still a long way to find a system that completely removes these contaminants from wastewater at a low cost and easy implementation.

Generation of transformation products

As discussed above, the removal estimation of the CECs after anodic oxidation was performed with the measurement of the TOC. This electrochemical degradation process generates many TPs, as demonstrated in Publication #8, where 25 compounds were tentatively identified. In Publications #5, #6 and #7, nine out of the 30 CECs detected at least in one sample were TPs, showing the relevance of screening for new TPs in degradation studies. Most of the TPs tentatively identified in Publication #8 are products from parents detected in Publications #5, #6 and #8. Therefore, the identified TPs' presence in the samples is reasonably probable. Regrettably, in the mentioned publications, only a target analysis was performed, providing data exclusively about the previously selected compounds. However, the ideal procedure in the performance evaluation of degradation technologies would be a combination of target and suspect (non-target) analysis.

4.4 References

Bonvin, F., Omlin, J., Rutler, R., Schweizer, W.B., Alaimo, P.J., Strathmann, T.J., McNeill, K., Kohn, T., 2013. Direct photolysis of human metabolites of the antibiotic sulfamethoxazole: Evidence for abiotic back-transformation. Environ. Sci. Technol. 47, 6746–6755. https://doi.org/10.1021/es303777k

Domaradzka, D., Guzik, U., Wojcieszyńska, D., 2015. Biodegradation and biotransformation of polycyclic non-steroidal anti-inflammatory drugs. Rev. Environ. Sci. Biotechnol. 14, 229–239. https://doi.org/10.1007/s11157-015-9364-8

Gago-Ferrero, P., Mastroianni, N., Díaz-Cruz, M.S., Barceló, D., 2013. Fully automated determination of nine ultraviolet filters and transformation products in natural waters and wastewaters by on-line solid phase extraction–liquid chromatography–tandem mass spectrometry. J. Chromatogr. A 1294, 106–116. https://doi.org/10.1016/J.CHROMA.2013.04.037

García-Galán, M.J., Gutiérrez, R., Uggetti, E., Matamoros, V., García, J., Ferrer, I., 2018. Use of full-scale hybrid horizontal tubular photobioreactors to process agricultural runoff. Biosyst. Eng. 166, 138–149. https://doi.org/https://doi.org/10.1016/j.biosystemseng.2017.11.016

Guzik, U., Wojcieszyńska, D., 2020. Naproxen in the environment: its occurrence, toxicity to nontarget organisms and biodegradation 1849–1857.

Morin-crini, N., Lichtfouse, E., Fourmentin, M., Ribeiro, A., Noutsopoulos, C., Mapelli, F., Fenyvesi, É., Vieira, M., Picos-corrales, L., Moreno-piraján, J., Morin-crini, N., Lichtfouse, E., Fourmentin, M., Ribeiro, A., Noutsopoulos, C., Lichtfouse, E., Schwarzbauer, J., Emerg-, D.R., Morin-crini, N., Lichtfouse, E., Fourmentin, M., 2021. Emerging contaminants Vol. 2. Remediation of emerging contaminants, Emerging Contaminants Vol. 2. https://doi.org/10.1007/978-3-030-69090-8_1

Norton-Brandão, D., Scherrenberg, S.M., van Lier, J.B., 2013. Reclamation of used urban waters for irrigation purposes - A review of treatment technologies. J. Environ. Manage. 122, 85–98. https://doi.org/10.1016/j.jenvman.2013.03.012

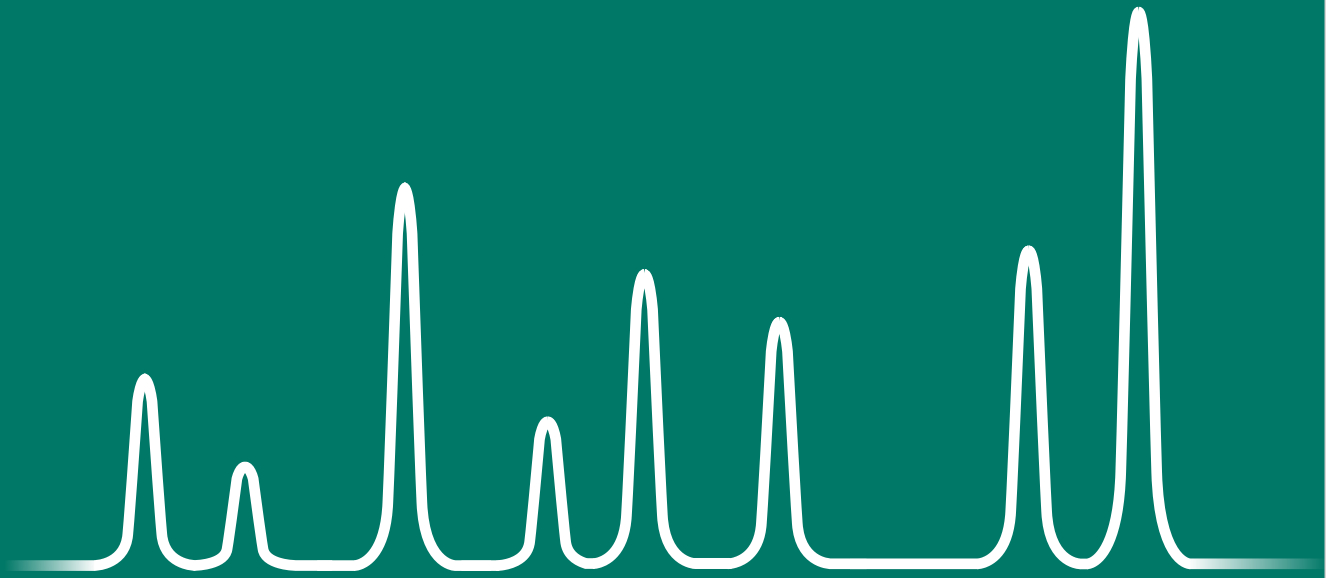
Qin, K., Chen, Y., Li, J., Xue, C., Wei, L., Song, X., Ding, J., Zhao, Q., 2020. Removal trends of sulfonamides and their ARGs during soil aquifer treatment and subsequent chlorination: Effect of aerobic and anaerobic biodegradation. Environ. Sci. Water Res. Technol. 6, 2331–2340. https://doi.org/10.1039/d0ew00270d

Starling, M.C.V.M., Amorim, C.C., Leão, M.M.D., 2019. Occurrence, control and fate of contaminants of emerging concern in environmental compartments in Brazil. J. Hazard. Mater. 372, 17–36. https://doi.org/10.1016/j.jhazmat.2018.04.043

Taha, H.M., Aalizadeh, R., Alygizakis, N., et. al., 2022. The NORMAN Suspect List Exchange (NORMAN-SLE): Facilitating European and Worldwide Collaboration on Suspect Screening in High Resolution Mass Spectrometry. Environ. Sci. Eur. https://doi.org/https://doi.org/10.21203/rs.3.rs-1902466/v1

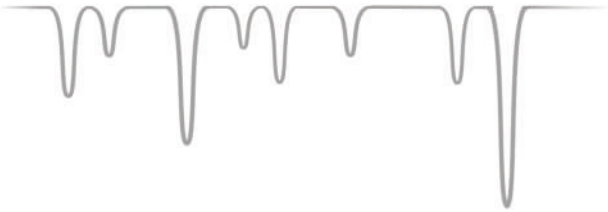
Uggetti, E., García, J., Álvarez, J.A., García-Galán, M.J., 2018. Start-up of a microalgae-based treatment system within the biorefinery concept: from wastewater to bioproducts. Water Sci. Technol. 78, 114–124. https://doi.org/10.2166/wst.2018.195

Valhondo, C., Martínez-Landa, L., Carrera, J., Díaz-Cruz, S.M., Amalfitano, S., Levantesi, C., 2020. Six artificial recharge pilot replicates to gain insight into water quality enhancement processes. Chemosphere 240. https://doi.org/10.1016/j.chemosphere.2019.124826



Chapter 5

Wastewater reuse feasibility for irrigation purposes



5.1 Introduction

Chapter 4 summarises the complexity of removing CECs from wastewater. Therefore, achieving cheap and efficient elimination technologies is still a challenge. Considering the environmental status of CECs' contamination (Chapter 3), the discharge of reclaimed or wastewater directly into the environment should be avoided. Adding the global water scarcity, the exponential population growth and the effects of climate change, reusing this water seems the most straightforward solution for having freshwater availability as required in the near future (Jodar-Abellan et al., 2019).

Thus, an upcoming approach in recent years is reusing reclaimed water for purposes requiring lower quality standards, such as agriculture or industry. Since agriculture accounts for 70% of water withdrawals worldwide (Margenat et al., 2019), wastewater reuse for irrigation seems the most viable solution. Firstly, sustainable reclaimed water reuse would reduce freshwater consumption drastically, which could be saved for higher-quality purposes (e.g. drinking water). Secondly, a continuous irrigation water supply would be precious in arid or semi-arid areas where drought is common (Libutti et al., 2018). Finally, these large volumes of reclaimed water would not reach environmental compartments, avoiding a continuous discharge of contaminants to water bodies and ecosystems.

However, reclaimed or wastewater reuse also presents essential issues to consider. The main problem is that wastewater effluents show high contamination loads of, for example, pathogens, heavy metals, CECs, etc. (Norton-Brandão et al., 2013; Varela and Manaia, 2013). Using this water for irrigation supposes a continuous discharge to the agricultural soil and, ultimately, crops. Some of these contaminants are partially volatilised or infiltrated through the soil following the irrigation water. But due to their persistence, most of them tend to accumulate in the soil and have the capacity to be uptaken by the crops.

Once uptaken, the crop eliminates some contaminants via volatilization or excretion, but others are degraded (forming new TPs) or accumulated in the crop tissues (Tadić et al., 2019). Similar processes take place in the soils, where the formation of new TPs can start the uptake cycle again (**Figure 5.1**). Ultimately, it supposes the presence of unexpected or unknown contaminants in crops aimed for consumption, putting ecosystems and human health at risk.

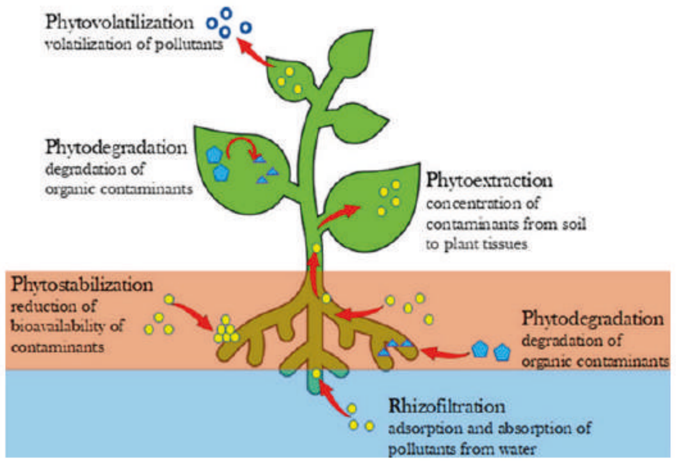


Figure 5.1. Summary of the different processes underwent by contaminants during plant uptake. (Rigoletto et al., 2020)

As explained in Chapter 4, contaminants' accumulation and degradation processes are very complex and mostly not fully understood. Thus, water reuse and the potential uptake of contaminants by crops must be carefully evaluated and monitored. In this thesis, the focus was put on the uptake of CECs. Still, monitoring other contaminants (such as pathogens) is essential to guarantee safe human consumption.

Furthermore, the absorption, uptake, or degradation processes will be different in, for example, a fruit (such as tomatoes), a leaf (such as lettuces), or a root (such as carrots). These crops' feeding and growth mechanisms are entirely distinct, as well as their proper cultivation conditions. For example, some crops need a generous and continuous water supply to grow appropriately (e.g. rice), but others can grow only on rainwater (e.g. pomegranate). Another example is seasonal crops, needing specific weather conditions to achieve proper growth; in Spain, lettuces can be cultivated in cold seasons, but tomatoes are usually grown in summer. Thus, the uptake of CECs between the explained examples will be mechanistically different since they require different cultivation conditions.

So far, many studies have evaluated the uptake of CECs after irrigating with reclaimed or wastewater, but most of them were performed in greenhouses or hydroponic conditions (Abril et al., 2021; Herklotz et al., 2010; Wu et al., 2012). These studies can provide valuable information under controlled conditions, but studies under real-field conditions will be most appropriate. Therefore, studies under real cultivation conditions should be encouraged to fully understand and assess the uptake and fate of CECs in crops when using reclaimed wastewater.

In this thesis, we focused on agricultural field plots to investigate CECs' crop uptake from the wastewater and reclaimed water used for irrigation. For this purpose, two pilot plots were built in the Palamós WWTP, next to the SAT, with the reactive barriers pilot system explained in Chapter 4. It facilitated a continuous supply of reclaimed mater (from the barriers effluent) and wastewater (from the WWTP effluent) to irrigate crops. Briefly, half of both plots were built using sandy soil, and the other half adding 10% of clay. Then, two irrigation lines were installed in each plot, one with sprinklers and the other with drip irrigation tubes. Finally, each plot water supply came from a different source; one from the reactive barriers system and the other from the WWTP effluent. A graphical representation of the plots is shown in **Figure 5.2**.

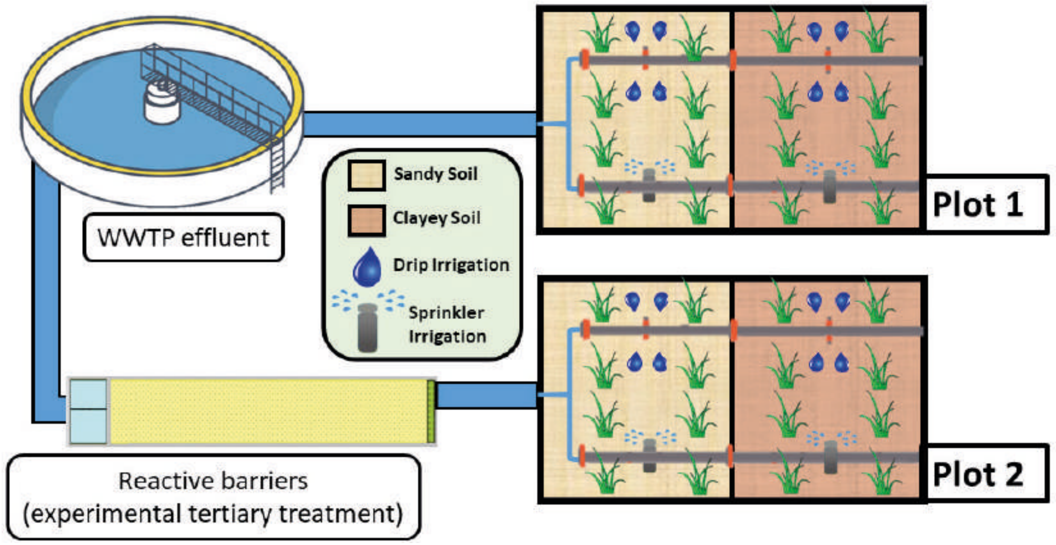


Figure 5.2. Graphical representation of the two plots built in Palamos' WWTP, with the different cultivation variables (namely irrigation water, soil composition and irrigation system).

In these plots, we cultivated lettuces, tomatoes and carrots and evaluated the relevance of different variables in the uptake of CECs by the crops. All the information is provided in Publication #9 (method development and lettuce results), Publication #10 (site description, methodology for soil analysis, and irrigation water characterisation), and Publication #11 (carrots and tomatoes results). Publication #11 also includes a risk assessment with the CECs concentrations, but it will be further discussed in Chapter 6 (human exposure to CECs). Then, complementary work on plant uptake and the generation of TPs was performed through an international collaboration performed in Pak Choi vegetable, fruit of a research stay at the Swedish University of Agricultural Sciences (Sweden) (Publication #12). This study aimed to evaluate the feasibility of reuse digestate (a product of anaerobic digestion) as fertiliser in agriculture. Thus, 18 CECs frequently detected in food waste biogas facilities were selected and spiked in a nutrient solution to evaluate the uptake and fate of these CECs in Pak Choi vegetable. Even though the uptake assessment was considered, the work's main focus was the identification of the TPs produced by the translocation of the spiked CECs from the water to the vegetable.

Methodologies

In Publications #9, #10, and #11, a new QuEChERS-based method was developed for PPCPs analysis in lettuces, tomatoes, carrots, and soil. The method optimisation is included in Publication #9, and a simplified overview is shown in **Figure 5.3**. Basically, it consisted of extracting the analytes of interest with the QuEChERS salts and a further clean-up step to eliminate undesired matrix components, including fatty acids, lipids, and sugars. Then, the target analysis was performed by HPLC-MS/MS with a C18 column and a q-TRAP mass analyser. The irrigation waters were analysed as described in previous papers and based on online-SPE-HPLC-MS/MS. Regarding Publication #12, the extraction and target analysis was performed following a previously developed method by the group from the Swedish University of Agricultural Sciences (Kodešová et al., 2019b, 2019a). The extraction consisted of using an organic/aqueous mixture solution homogenised in a bead-beating tissuelyser, from which the supernatant was filtered.

Finally, the extract was analysed by UHPLC-MS/MS with a C18 column and a triple quadrupole analyser. For the suspect screening (non-target analysis), the extracts were also analysed using a C18 column and a Q-Exactive mass analyser under DDA and DIA modes. The suspect analysis workflow was performed with Compound Discoverer software (v. 3.3.0.550). Further details can be found in the manuscript itself.

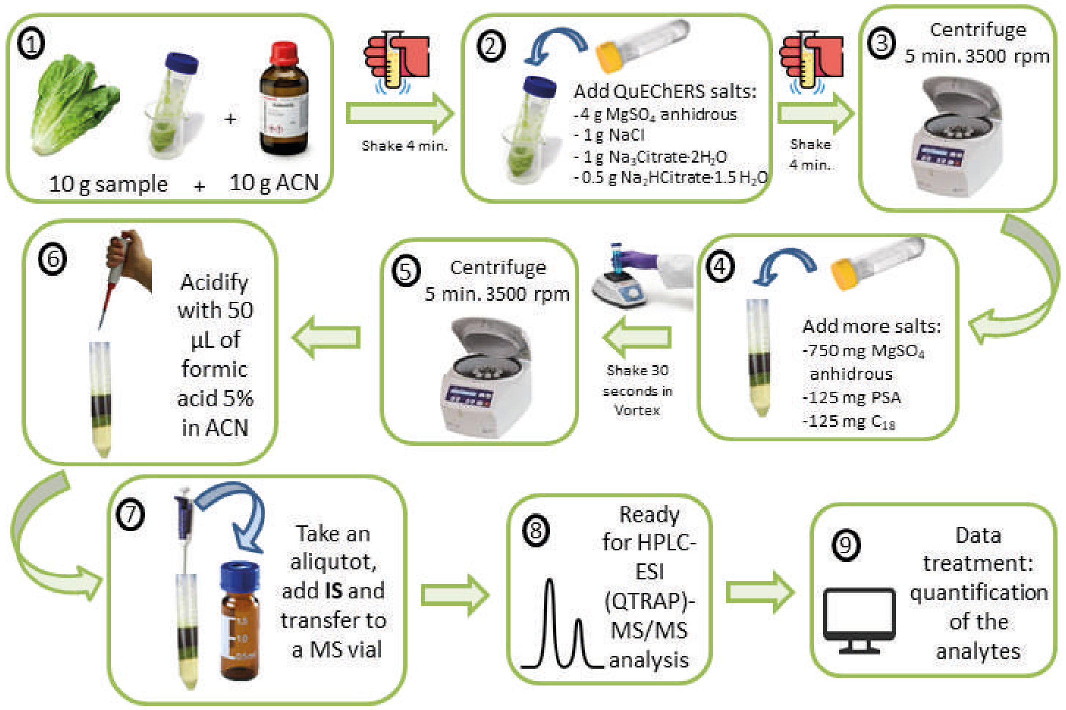


Figure 5.3. Steps followed in the QuEChERS-based methodology for the analysis of PPCPs in different crops.

5.2 Results

The results of this chapter are presented and described in two published articles and two submitted ones. The publications are:

- Publication #9: **Sunyer-Caldú, A.**, Diaz-Cruz, M. S., "Development of a QuEChERS-based method for the analysis of pharmaceuticals and personal care products in lettuces grown in field-scale agricultural plots irrigated with reclaimed water", 2021, *Talanta*, https://doi.org/10.1016/j.talanta.2021.122302

- Publication #10: **Sunyer-Caldú, A.**, Sepúlveda-Ruiz, P., Salgot, M., Folch-Sánchez, M., Barceló, D., Diaz-Cruz, M. S., "Reclaimed water in agriculture: a plot-scale study assessing crop uptake of emerging contaminants and pathogens", 2022, *Journal of Environmental Chemical Engineering*, https://doi.org/10.1016/j.jece.2022.108831

- Publication #11: **Sunyer-Caldú, A.**, Quintana, G., Diaz-Cruz., M. S., "Pharmaceuticals and personal care products uptake by crops irrigated with reclaimed water and human health implications", *Submitted to Science of the Total Environment.*

- Publication #12: **Sunyer-Caldú, A.**, Golovko, O., Kaczmarek, M., Asp, H., Bergstrand, KJ., Gil-Solsona, R., Gago-Ferrero, P., Diaz-Cruz, M. S., Ahrens, L., Hultberg, M., "Occurrence and fate of contaminants of emerging concern and their transformation products after uptake by Pak Choi vegetable", *Submitted to Environmental Pollution.*

5.2.1 Publication #9

Development of a QuEChERS-based method for the analysis of pharmaceuticals and personal care products in lettuces grown in field-scale agricultural plots irrigated with reclaimed water

Adrià Sunyer-Caldú
M. Silvia Diaz-Cruz

Talanta, 2021, 230, 122302
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Development of a QuEChERS-based method for the analysis of pharmaceuticals and personal care products in lettuces grown in field-scale agricultural plots irrigated with reclaimed water

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Plant uptake
HPLC-MS/MS
Managed aquifer recharge
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ABSTRACT

The use of reclaimed water for agricultural irrigation is an increasingly common practice, which recently has found its own European regulatory frame. However, the partial removal of organic contaminants together with other xenobiotic substances in current wastewater treatment plants leads to the occurrence of residues of such pollutants in the treated effluents. Wastewater reclamation techniques are thus required to provide reclaimed water fitting the minimum quality standards set up for irrigation of crops intended for human consumption. This work describes the development and validation of a simple QuEChERS-based extraction and liquid chromatography quadrupole-linear ion trap mass spectrometry (LC-QqLIT-MS/MS) method for the simultaneous quantitative analysis of 55 pharmaceuticals and personal care products (PPCPs) in lettuces irrigated with treated wastewater and reclaimed water. The method showed good recovery rates (80–120%) and low detection limits (0.04–0.8 ng/g dw). In comparison with previous analytical methodologies, this method was simpler, faster and, in most cases, more sensitive. Moreover, is the first one analysing selected personal care products in lettuces. The proposed method was applied to assess the potential transfer of contaminants of urban origin in the use of reclaimed water in agriculture. The case study consisted in the evaluation of the lettuce uptake of the selected contaminants at field scale under two irrigation systems, two soil compositions, and two water types. Benzophenone-2, 4-hydroxybenzophenone, 1H-benzotriazole, 2-(2-Benzotriazol-2-yl)-p-cresol, nalidixic acid, diclofenac, carbamazepine 10,11-epoxy, *N*-des-methylvenlafaxine, and salicylic acid were transferred to all samples. Highest detected values corresponded to 4-hydroxybenzophenone (84.1 ng/g dw), benzophenone-2 (54.4 ng/g dw), and salicylic acid (53.8 ng/g dw). The best combination to minimize the transfer of the target contaminants from the irrigation water to the lettuces was sprinkling irrigation with water reclaimed by soil infiltration through reactive barriers, and clayey soil.

1. Introduction

Currently water scarcity affects 40% of the global population [1] and by 2030, global demand for water is expected to grow by 50% because of population needs [2]. This, together with the unknown consequences of climate change on the water resources, makes it a present-problem that demands immediate solutions to be applied.

Agriculture plays an important role in water balance, as it accounts for 70% of all water withdrawals globally. Around 70% more food will be needed by 2050, increasing exponentially the water needs and putting 2.3 billion of expected people living in areas with severe water stress [3]. Therefore, the obvious solution could be the wastewater reuse for agricultural purposes, leaving the use of available freshwater for other uses. However, and since the occurrence of pollutants in the treated wastewater appears to be unavoidable, the application of reclaimed water in agriculture could constitute a direct pathway of these contaminants to humans, posing their health at risk. To ensure water safety, few months ago, the European Commission launched the Regulation 2020/741 (25 May 2020) on water reuse, where the irrigation water quality standards were set up [4].

An important proportion of the generated wastewater corresponds to urban wastes, and thus from a direct anthropogenic origin. Among urban contaminants, the pharmaceuticals and personal care products (PPCPs) are the most concerning. The personal care products group

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(PCPs) includes compounds that are directly applied to the human body for hygiene and beauty, such as sunscreens, shampoos, and cosmetics. Indeed, some active compounds of these products are also used in other industrial sectors as the UV filters (UVFs), which are added to materials exposed to UV light (i.e. plastics, textiles, and rubbers among others), or paraben preservatives (PBs) that are also present in beverages, food, and medicines. Pharmaceuticals are substances designed exclusively for medicinal purposes and vary as a function of their therapeutic target. Besides, there is an increasing trend in the consumption of pharmaceuticals in the European Union (EU), which has at least doubled in the last decade, and it is expected to increase in the coming years [5] as both population and life expectancy increase, as can be seen in Fig. S1 in Supplementary Material (SM).

Such substances are incompletely removed in wastewater treatment plants (WWTPs), and this is not expected to change in short term because the implementation of new wastewater treatments is expensive and/or complex. As a whole, these compounds are considered contaminants of emerging concern (CECs) since they can accumulate in the environment, bioaccumulating and biomagnificating along the trophic chain. Thus, the occurrence of PPCPs in the environment appears difficult to avoid, and therefore, its abatement constitutes a current big challenge.

Although there is still a lack of information about the potential adverse effects of PPCPs in wildlife and humans, some studies have demonstrated that many of them can alter vertebrate endocrine systems (endocrine-disrupting contaminants (EDCs)) [6] or foster the dissemination of antibiotic resistance genes [7]. Many studies have reported its occurrence in the environment, mostly in waters [8–10]. However, only limited information is available on their transfer from the irrigation waters to plants, likely due to the lack of simple, sensitive, and robust analytical methods to investigate these CECs at low concentrations in complex matrices such as vegetables, with a number of potential interferences caused by chlorophylls, complex carbohydrates and amino acids, among others [11].

A literature review allowed us to identify some studies dealing with the analysis of CECs in crops. In earlier studies, 119 PPCPs were determined in vegetables using ultrasound-assisted extraction (UAE) followed by solid-phase extraction (SPE), but all the results were below the limits of detection of the method (MLOD) [12]. Nineteen PPCPs were determined in crops using the same laborious and time-consuming extraction methodology [13] and analysis by LC-MS using a triple quadrupole (QqQ), but the study was performed in hydroponic conditions. Malchi et al. [14] employed also a quite expense and time-consuming procedure based on pressurized liquid extraction (PLE) followed by filtration to determine 14 pharmaceuticals in carrot and sweet potato irrigated with wastewater and analysis by LC-(QqQ)-MS. More recent studies focused in just a few substances, for instance, five antibiotics in lettuces cultivated in spiked soils with UAE and further SPE clean-up [15] were determined by LC-(QqQ)-MS, obtaining quite high MLODs (3–45 ng/g dw). Beltrán et al. [16] determined carbamazepine, triclosan and atenolol in lettuces, maize and radish irrigated with reclaimed water using UAE followed by QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction and LC-(QqQ)-MS, however, the MLODs were still high (0.3–0.8 ng/g dw). QuEChERS is a sample treatment methodology originally developed for pesticide extraction from vegetables. In short, it consists of a dispersive solid-phase extraction using MgSO4 and further purification with PSA (primary/secondary amine) and a silica sorbent (C18) [17]. Two other recent studies employed QuEChERS in the extraction of pharmaceuticals from crops. De Santiago-Martin et al. [18] cultivated maize using gravity-based irrigation. After the QuEChERS extraction, the extracts were purified by SPE only detecting 4 out of the 25 pharmaceuticals investigated by LC-(QqQ)-MS. Montemurro et al. [19] determined 14 pharmaceuticals in lettuces grown in pots under laboratory controlled conditions irrigating with spiked wastewater, with an adapted QuEChERS method using liquid chromatography followed by quadrupole-time of flight detection (LC-QTOF/MS), a high resolution mass spectrometry detection (HRMS). The MLODs achieved were generally lower than those previously reported (0.01–0.02 ng/g). However, the high cost and complex high resolution mass spectrometry detection applied make it non-viable for routine analysis.

These studies were performed under laboratory controlled conditions, but studies focusing on the occurrence of CECs in real scale agroecosystems, natural conditions with different agricultural practices, are still lacking, but urgently needed for realistic human health risk assessments. The aim of the present work was double: first, to develop a simple, fast and sensitive QuEChERS-based extraction of 55 PPCPs from lettuces cultivated in real field-scale agricultural plots and further high-performance liquid chromatography-quadrupole lineal ion trap-mass spectrometry (HPLC-(QqLIT)-MS/MS), and second, apply the proposed method to evaluate the potential lettuces' uptake of PPCPs in the reuse of two types of water for irrigation; WWTP treated water and reclaimed water through reactive barriers infiltration in a pilot managed aquifer recharge system. Other factors potentially driving the plant uptake i.e. the agricultural soil composition and the irrigation system, were also assessed.

2. Materials and methods

2.1. Standards and reagents

The 55 PPCPs selected for this study encompass different families of compounds including pharmaceuticals, personal care products and stimulants and, therefore, have quite different physicochemical properties, as shown in Table S1.

All the standards used were $>$98% of purity. Benzophenone-3 (BP3), benzophenone-1 (BP1), benzophenone-4 (BP4), 4HB, 4,4′-Dihydroxybenzophenone (4DHB), avobenzone (AVO), UVP, 5,6-dimethyl-1H-benzotriazole (DMBZT), nalidixic acid (NDX), oxolinic acid (OXL), tetracycline (TCY), succynil-sulfathiazole (*S*-STZ), sulfadiazine (SDZ), N4-acetylsulfadiazidine (acSDZ), sulfamerazine (SMR), N4-acetylsulfamerazine (acSMR), N4-acetylsulfamethazine (acSMZ), sulfamethoxazole (SMX), N4-acetylsulfamethoxazole (acSMX), sulfamethoxypyridazine (SMPZ), sulfapyridine (SPY), N4-acetylsulfapyridine (acSPY), sulfaquinoxaline (SQX), sulfathiazole (STZ), sulfisomidine (SMD), sulfadimethoxine (SDM), trimethoprim (TMP), acetaminophen, also known as paracetamol (APH), atenolol (ATL), gemfibrozil (GFZ), ketoprofen (KPF), mefenamic acid (MFA), carbamazepine (CBZ), norfluoxetine (norFXT), ofloxacin (OFX), ciprofloxacin (CFX), caffeine (CFF), ibuprofen (IBU), salicylic acid (SCY), diclofenac (DCF), diclofenac-^{13}C (DCF-^{13}C), methyl paraben (MePB), propyl paraben (PrPB), benzyl paraben (BePB), butyl paraben (BuPB) and benzophenone-(carbonyl-^{13}C) (BP-^{13}C) were purchased from Sigma Aldrich (Darmstadt, Germany). BP2, 2,2′-Dihydroxy-4-methoxybenzophenone (DHMB), ethyl-4-(dimethyl-amino)benzoate (EtPABA), ethylhexyl methoxycinnamate (EHMC), and benzotriazoles (BZT) were obtained from Merck (Darmstadt, Germany). Enzacamen or 4-methyl benzylidene camphor (4MBC) was provided by Dr. Ehrenstorfer (Augsburg, Germany). 5-Methyl benzotriazole (MeBZT) was obtained from TCI (Zwijndrecht, Belgium). Flumequine (FLU), *N*-desmethylvenlafaxine (*N*-desVFX), diclofenac 4-hydroxy (DCF-OH), carbamazepine 10,11-epoxy (CBZ-E) and sulfamethazine-d$_4$ (SMZ-d$_4$) and acetaminophen-d$_4$ (APH-d$_4$) were purchased in Toronto Research Chemicals (Toronto, Canada). Oxytetracycline (*O*-TCY) and naproxen (NPX) were obtained from Honeywell Fluka (Wabash, United States). 2-hydroxy-4-methoxy-2′,3′,4′,5′,6′-d5 (BP3-d$_5$), (±)-3-(4-methylbenzylidene-d$_4$) camphor (4MBC-d4), 1H-benzotriazole-4,5,6,7-d$_4$ (BZT-d$_4$), flumequine-^{13}C$_3$ (FLU-^{13}C$_3$), trimethoprim-d$_3$ (TMP-d$_3$), carbamazepine-d$_{10}$ (CBZ-d$_{10}$), mefenamic acid-d$_3$ (MFA-d$_3$), caffeine-d$_3$ (CFF-d$_3$), ibuprofen-d$_3$ (IBU-d$_3$), salicylic acid-d$_6$ (SCY-d$_6$), diclofenac-d4 (phenyl-d4) (DCF-d$_4$), benzyl paraben-d4 (BePB-d4) and 5-(2,5-dimethylphenoxy)-2,2-bis(trideuteriomethyl)pentanoic acid (GMZ-d6) were from CDN isotopes

(Quebec, Canada).

Water, methanol (MeOH), and acetonitrile (ACN) of MS-grade were obtained from J.T. Baker (Deventer, The Netherlands) and the nitrogen (99.995%) was supplied by Air Liquide (Barcelona, Spain). Ethanol, acetone, formic acid (FA) and ammonium acetate (AcNH$_4$) were from Merck (Darmstadt, Germany). The commercial QuEChERS kits Citrate-Kit-01 and PSA-Kit-02 used were obtained from BEKOlut® (Hauptstuhl, Germany).

Individual stock standard and isotopically labeled internal stock standard solutions were prepared on a weight basis in MeOH at 100 mg/L. The solutions were stored in the dark at −20 °C. A mixture with all the standards was weekly prepared at 10 mg/L in MeOH. Ten working solutions at concentrations between 1 ng/mL and 1 μg/mL were daily prepared by the appropriate dilution of the mixture standard solution with MeOH or with the extract from the lettuce matrix (see section 2.5).

2.2. Agricultural plots and lettuces growing conditions

The lettuces, from the variety *Chicorium Intybus* were grown during the Autumn of 2018 in two experimental plots located in an urban WWTP near Girona (Northeast of Spain), as shown in Fig. S2.

The two plots were designed to have drip and sprinkling irrigation systems, two soil compositions with different proportions of clay and sand, and two types of irrigation water (treated wastewater (the secondary effluent from the WWTP) and reclaimed water by infiltration through a reactive barrier in a managed aquifer recharge (rbMAR) pilot system. A detailed description of this system of wastewater renaturalization can be found elsewhere [20,21].

In addition, humidity, irrigation water volume, conductivity, and temperature in the plots at three different depths were monitored by a series of sensors installed along the plots to ensure that the crops always had available water and to monitor the evolution of the other parameters.

In the case study, three binary variables were evaluated, resulting in eight different sample types. A scheme of the experimental plots set up is shown in Fig. 1 and the variables influencing the crops in each sample type are described in Table S2.

Lettuces were grown in the two experimental plots simultaneously. At harvest, the lettuces had grown enough and were suitable for consumption (typical market size, around three months) in December 2018.

Approximately 50 lettuces per subplot were grown and 10 lettuces per subplot were randomly collected. Each lettuce sample was thoroughly shaken to remove soil particles. Then, the lettuces were stored in a portable freezer until arrival at the laboratory in Barcelona, where they were preserved at −20 °C. The day after sampling, the leaves of the lettuces were thawed, sliced in small pieces, frozen again, lyophilized, and homogenized with a crusher to a fine powder. The 10 lettuces of each subplot were mixed homogeneously, to form composite samples. Finally, the powered lettuce leaves were frozen and stored until analysis.

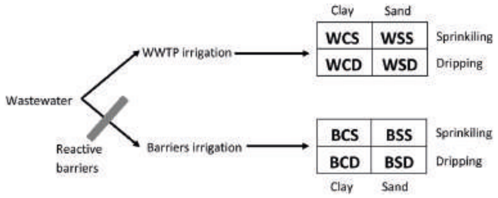

Fig. 1. Distribution of the experimental plots and the variables studied (soil composition, irrigation system, and water type).

2.3. Selection of the QuEChERS salts

Nowadays, there are many alternatives when choosing the salts to be used in QuEChERS extraction, and thus a wide variety of kits are commercially available. The selection is usually carried out depending on the type of crop and the type of clean-up needed. In particular, for the analysis of lettuces, there was one specific type of kit, which was selected to be used in this study. The salts involved have key properties for the analysis; MgSO4 helps the partition of the analytes between the water and the organic phase, favoring the extraction, NaCl enhances extraction efficiency and allows the miscible organic solvent to separate from the water in the sample, Na$_3$Citrate·2H$_2$O and Na$_2$HCitrate·1.5H$_2$O provide buffering conditions (pH 5–5.5). Similarly, for the purification salts, PSA removes sugars, fatty acids and organic acids, and C18 removes lipids and non-polar matrix components from the lettuces that might interfere in the analysis.

2.4. QuEChERS extraction

For the pollutant's extraction, a QuEChERS methodology based on Parrilla Vázquez et al. [22] for the analysis of pesticides in baby food and eggs using a LC-QTOF, was optimized and validated. An aliquot of 1 g dw lettuce sample was weighted in a 50 mL centrifuge tube, where 9 mL of HPLC-grade water and 10 mL of ACN were added. The mixture was manually shaken for 4 min. and the citrate-kit QuEChERS salts was added (containing 4 g of anhydrous magnesium sulfate (MgSO$_4$), 1 g of sodium chloride (NaCl), 1 g of trisodium citrate dihydrate (Na$_3$Citrate·2H$_2$O), and 0.5 g of disodium hydrogencitrate sesquihydrate (Na$_2$HCitrate·1.5H$_2$O) to extract the compounds of interest. Further, it was manually shaken for 4 min and then centrifuged at 3500 rpm for 5 min. Next, the PSA-Kit-02 salts was added to the supernatant (containing 750 mg of MgSO4, 125 mg of primary/secondary amine exchange material (PSA), and 125 mg of a C18 sorbent). The mixture was stirred for 0.5 min. in a vortex and further centrifuged for 5 min at 3500 rpm to complete the dispersive clean-up. Then, the mixture was acidified with 50 μL of a solution of 5% formic acid in ACN. Next, an aliquot of 5 mL of the organic phase was collected and transferred to a 10 mL-vial and then evaporated under a gentle nitrogen stream until near dryness. Then, this extract was transferred to a LC-vial (2 mL), where 100 μL of the isotopically labeled internal standards mixture solution were added and stirred. Finally, this extract was evaporated to dryness and reconstituted with 1 mL of MeOH for further HPLC-MS/MS analysis. The flowchart of the sample pretreatment procedure is shown in Fig. S3.

2.5. Liquid chromatography-tandem mass spectrometry analysis

The target PPCPs were separated by high performance liquid chromatography in a Hibar Purosher® STAR® HR R-18 (50 mm × 2.0 mm, 5 μm) column using a Symbiosis™ Pico instrument from Spark Holland (Emmen, The Netherlands) attached to a 4000 QTRAP mass spectrometer from Applied Biosystems-Sciex (Foster City, USA). Electrospray ionization in positive (ESI+) and negative (ESI-) modes was selected as the ionization source. In positive mode, the mobile phase consisted of a mixture of MeOH and HPLC-grade water, both 0.1% in formic acid. The elution gradient started with 5% of MeOH, increasing to 75% at min. 7 and to 100% at min. 10. After, it was decreased up to initial conditions at min. 17 and maintained until min. 23 for equilibration. In negative mode, the mobile phase consisted of the same solvents, but in this case both were prepared at 5 mM in AcNH$_4$. The elution gradient started with 5% of MeOH, increasing to 50% at min.3, to 90% at min.6, and to 100% at min. 13. Then, it was decreased to 5% at min. 18 and maintained until min. 20 for equilibration. All analyses were performed at a flow rate of 0.3 mL/min and the injection volume was set up to 20 μL.

Tandem-mass spectrometry detection (MS/MS) was performed in the hybrid quadrupole-linear ion trap spectrometer (QqLIT) under the selected reaction monitoring mode (SRM) to enhance the sensitivity and selectivity of the method. For each pollutant, the two most intense transitions were selected and used for quantification (most intense) and confirmation (second most intense). Instrument control, data recording and processing were performed using Analyst v. 1.4.2 (Applied Biosystems).

The chromatographic retention times (t_R) and MS/MS operational parameters of the optimized method are listed in Table 1. The linearity range for all studied compounds was 1–700 ng/mL.

2.6. QA/QC

A common problem in trace analysis is the background contamination. To avoid these problems, a blank of the method was performed (same extraction procedure without matrix). Besides, all the lab glass material was cleaned with ethanol and acetone and the non-volumetric glassware was muffled at 400 °C overnight. The isotopically labeled standard BP-^{13}C was used as surrogate to evaluate the extraction performance of the target analytes. Quality controls at known concentrations of the standards were randomly included along the samples analytical sequences. The standards' chromatographic t_R among calibration curves and samples were compared at a tolerance of 2.5% maximum. The relative ion intensities between the two selected SRM transitions were related with a tolerance <15%. All the compounds were identified with the t_R and with both transitions following the EU normative (Commission Decision 2002/657/EC). Isotopically labeled standards for each group of compounds, as shown in Table 1, were selected depending on different factors including t_R, polarity, and chemical structure, together with the use of matrix matched standards solutions, to overcome matrix effects.

2.7. Data analysis

2.7.1. Correlation analysis

T tests were carried out with the R software [23] to identify correlations among the different variables studied with a 95% of confidence (Table S5).

2.7.2. Principal component analysis

A multivariate analysis of the results was performed with Solo_MIA (Copyright (C) 2007 - 2020 Eigenvector Research, Inc.) to evaluate group interactions and/or correlations that may not be observed with the previous data analysis. Further information about the statistical analysis can be found in Section S3 of SM.

3. Results and discussion

3.1. Optimization of the extraction method

Initially, the extraction procedure reported by Parrilla Vázquez et al. [22] was applied, which involved the use of a citrate buffer. However, during the validation, low recovery rates were obtained (2–25%) for the three spiked concentrations (5, 100, and 350 ng/mL). This could be related with the unclear phase separation obtained after the shaking steps of the process. It must be considered, however, that the original method was developed for fresh samples (not dried), and therefore, they already had a certain water content. In our case, the samples were lyophilized, and thus dehydrated. Moreover, the original QuEChERS method [17] was developed for samples with a high content of water because the organic-aqueous partition greatly facilitates the extraction of the compounds. Therefore, instead of extracting 10 g of sample, and considering that lettuces have 90% of water content, we reproduced this percentage by analyzing 1 g of the lyophilized sample and 9 g (9 mL) of HPLC-grade water. This approach is recommended by most of the kits' suppliers, for the extraction of samples with low water content [24,25].

Under these new experimental conditions, an exothermic reaction was observed after shaking the mixture in the first addition of the QuEChERS salts, and the phases separated. This suggested that the presence of water effectively improved the extraction process. The obtained recoveries improved for all the studied compounds, but still some were somewhat low (40–70%). Thus, an additional concentration step was included by adding 5 mL of the organic phase and evaporating them up to 1 mL. This step, significantly improved the recovery rates, which ranged from 80 to 120% for all the analytes and spike concentration levels. Thus, this extraction procedure was the finally selected to analyze the lettuce samples.

Table 1
Retention time (t_R), selected internal standards, MS/MS transitions and ionization parameters of each compound analyzed. (−) negative ionization mode.

	BP3	BP1	BP2	4HB	4DHB	DHMB	AVO	BP4 (−)	4MBC	EHMC	EtPABA
LOD (ng/ml)	0.12	0.21	0.2	0.19	0.32	0.09	0.26	0.1	0.11	0.12	0.22
LOQ (ng/mL)	0.41	0.72	0.66	0.64	1.07	0.3	0.85	0.35	0.38	0.4	0.74
MLOD (ng/g dw)	0.06	0.1	0.1	0.09	0.16	0.04	0.13	0.05	0.05	0.06	0.11
MLOQ (ng/g dw)	0.2	0.36	0.33	0.32	0.53	0.15	0.42	0.17	0.19	0.2	0.37
r2	0.9992	0.9976	0.9979	0.9981	0.9946	0.9996	0.9966	0.9994	0.9993	0.9993	0.9974
	BZT	**MeBZT**	**DMBZT**	**UVP**	**BePB (−)**	**BuPB (−)**	**PrPB (−)**	**MePB (−)**	**FLU**	**NDX**	**OXL**
LOD (ng/ml)	0.04	0.04	0.11	0.53	0.12	0.06	0.04	0.11	0.04	0.07	0.03
LOQ (ng/mL)	0.14	0.12	0.38	1.76	0.39	0.21	0.14	0.37	0.13	0.22	0.1
MLOD (ng/g dw)	0.02	0.02	0.05	0.26	0.06	0.03	0.02	0.05	0.02	0.03	0.01
MLOQ (ng/g dw)	0.07	0.06	0.19	0.88	0.19	0.1	0.07	0.18	0.06	0.11	0.05
r2	0.9999	0.9999	0.9993	0.9856	0.9993	0.9998	0.9999	0.9994	0.9999	0.9998	1000
	TCY	**Succynil-STZ**	**SDZ**	**acSDZ**	**SMR**	**acSMR**	**acSMZ**	**SMX**	**acSMX**	**SMPZ**	**SPY**
LOD (ng/ml)	0.81	0.28	0.16	0.23	0.09	0.16	0.18	0.12	0.19	0.09	0.1
LOQ (ng/mL)	2.69	0.94	0.55	0.78	0.3	0.52	0.58	0.41	0.63	0.31	0.33
MLOD (ng/g dw)	0.4	0.14	0.08	0.11	0.04	0.08	0.09	0.06	0.09	0.04	0.05
MLOQ (ng/g dw)	1.34	0.47	0.27	0.39	0.15	0.26	0.29	0.2	0.31	0.15	0.16
r2	0.9674	0.9958	0.9986	0.9971	0.9996	0.9987	0.9984	0.9992	0.9982	0.9995	0.9995
	acSPY	**SQX**	**STZ**	**SMD**	**SDM**	**TMP**	**GMZ (−)**	**MFA (−)**	**NPX (−)**	**DCF (−)**	**DCF-13C (−)**
LOD (ng/ml)	0.12	0.05	0.22	0.04	0.09	0.1	0.05	0.41	0.06	0.44	0.47
LOQ (ng/mL)	0.41	0.16	0.73	0.14	0.31	0.32	0.17	1.35	0.21	1.46	1.56
MLOD (ng/g dw)	0.06	0.02	0.11	0.02	0.04	0.05	0.02	0.2	0.03	0.22	0.23
MLOQ (ng/g dw)	0.2	0.08	0.36	0.07	0.15	0.16	0.08	0.67	0.1	0.73	0.78
r2	0.9992	0.9999	0.9975	0.9999	0.9995	0.9995	0.9999	0.9915	0.9998	0.99	0.9887
	KPF (−)	**IBU (−)**	**AAP**	**CBZ**	**CBZ-E**	**ATL**	**norFXT**	**CFX**	***N*-desVFX**	**SCY (−)**	**CFF**
LOD (ng/ml)	0.23	0.15	0.11	0.13	0.05	0.08	0.18	0.57	0.08	0.4	0.07
LOQ (ng/mL)	0.77	0.5	0.38	0.44	0.16	0.26	0.6	1.89	0.27	1.32	0.23
MLOD (ng/g dw)	0.11	0.07	0.05	0.06	0.02	0.04	0.09	0.28	0.04	0.2	0.03
MLOQ (ng/g dw)	0.38	0.25	0.19	0.22	0.08	0.13	0.3	0.94	0.13	0.66	0.11
r2	0.9972	0.9988	0.9993	0.9991	0.9999	0.9997	0.9983	0.9835	0.9996	0.9919	0.9997

LOD: Limit of detection of the instrument; LOQ: Limit of quantification of the instrument; MLOD: limit of detection of the method: MLOQ: limit of quantification of the method.

3.2. Validation of the analytical method

The validation of the method was performed and expressed in terms of calibration range, accuracy, sensitivity, and precision. Matrix effects (ME) were also estimated for each analyte following Eq. (1),

$$\text{ME\%} = (1\text{-(analyte response in matrix extract/analyte response in calibration solvent)) x } 100 \quad (1)$$

3.2.1. Calibration range and matrix effects

Calibration curves at ten different concentrations (1, 3, 5, 10, 30, 50, 100, 300, 500, and 700 ng/mL) were built both in pure MeOH and in lettuce extract (matrix-matched calibration solutions) to study the ME. Examples of these calibration curves are shown in Figs S4 and S5, where BP3 shows 23% of signal enhancement and MePB has a 26% of signal suppression**.** Quite good linearity was observed (0.967–0.999 r^2 values) for most compounds. A high percentage (65%) of the studied compounds presented signal suppression (between 4 and 86%), 33% showed enhanced signal (between 6 and 73%) and 7% did not showed ME. Therefore, according to these results, the most convenient approach for quantification was the use of matrix-matched calibration curves, and additionally, isotopically labeled internal standards.

3.2.2. Accuracy

Fifteen grams of the samples were weighed and thoroughly mixed. Then, 9 validation samples were prepared by spiking 1 g of the lettuce sample at three concentrations (5, 100, and 350 ng/g dw). At each concentration level, 3 replicates were prepared and measures three times each. For the validation blank, we followed the same protocol, but without analytes addition, and the mean value was subtracted from the signals of all the spiked samples. The recovery rates obtained are listed in Table S3 and a bar chart showing the proportion of compounds grouped per recovery percentages is shown in Fig. 2.

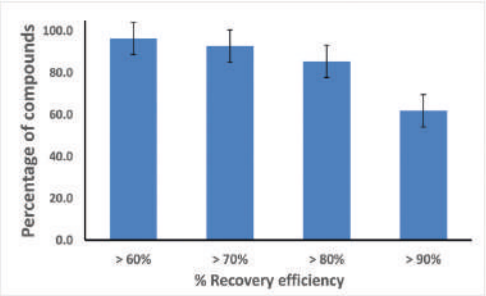

Fig. 2. Percentage of compounds showing recoveries above the specified percentages of recovery rates (60%, 70%, 80%, and 90%).

Recovery rates between 80% and 120% were obtained for most of the 55 analyzed PPCPs. Only three compounds presented some problems; TCY showed good recoveries for the lowest concentration, but values <10% were obtained for 100 ng/g dw and 350 ng/g dw spike levels. However, we decided to include it into the method and consider it only when samples content's in this compound was <100 ng/g dw. This assumption is motivated in light of the concentrations of TCY derivatives in lettuces reported in the literature when it was not detected [26]. Similarly, DCF and CPF, showed low recoveries, <6% and <29%, at 100 ng/g dw and 350 ng/g dw spike levels, respectively. The isotopically labeled standard DCF-^{13}C presented low recoveries at all levels and then it was discarded.

3.2.3. Sensitivity

The method limits of detection (MLODs) and quantification (MLOQs) were calculated as three and ten times, respectively, the standard deviation (SD) of the calibration curve divided by the slope. The MLODs obtained (0.01–0.40 ng/g dw) despite the complex matrix and the simultaneous analysis of the high number of compounds, were lower than those recently reported by Beltrán et al. (2020) (0.3–0.8 ng/g dw), and Albero et al. (2019) (3–45 ng/g dw) for many compounds. Furthermore, both reported methods used much more laborious methodologies, especially Albero et al. [15]. De Santiago-Martín et al. [18] reported MLODs values in the range 0.1-1 ng/g dw (higher than the ones obtained in this study) and Montemurro et al. [19] obtained MLODs between 0.01-0.12 ng/g dw (similar to those achieved with this method), even though they used a much more selective equipment (QTOF) such as HRMS. Linear range, MLODs and MLOQs of each compound are listed in Table 2.

3.2.4. Precision

The precision was evaluated analyzing three replicates of lettuce samples spiked at three concentrations (5, 100, and 350 ng/g dw) in a day and in successive days. Then, the SD of the obtained recoveries was evaluated for each concentration and compound. The inter- and intra-day relative standard deviation (RSD) were between 2 and 35% and from 0.6 to 30%, respectively, for most compounds (Table S4). The variability in the precision may be due to the wide range of compounds analyzed, with highly variable physicochemical properties, and the strong ME observed, which could not have been fully compensated since an internal standard is not included for each compound due to its cost and because it would reduce the sensitivity of the analysis by significantly increasing the number of compounds analyzed, Similar studies only reported intra-day values, that were in the range 4.1–22% [19] or 4–30% [18], quite similar to those provided by the developed method.

3.3. Analysis of lettuces grown at field scale and irrigated with reclaimed water

In order to investigate the potential transfer of the target PPCPs to crops in the reuse of water in agriculture, the developed method was applied to eight types of lettuces, according to the grown conditions in field (see Fig. 1). The total load of PPCPs classified in four groups is represented in Fig. 3. The individual concentrations measured in the lettuces are compiled in Table S5.

The UVFs group was the one having the highest values in the total accumulated (670.6 ng/g dw) and on average per compound (44.8 ng/g dw). All samples individually accumulated the UVFs group the most. Pharmaceuticals had lower total accumulated values (425.4 ng/g dw) despite that a higher number of pharmaceuticals have been analyzed and, besides, the average value per compound (12.5 ng/g dw) was much lower than for UVFs. The PBs group showed the lowest accumulated value (40.3 ng/g dw) and the lowest average value per compound (10.1 ng/g), being the family less bioaccumulated by the lettuces.

4HB, BP2, BZT, UVP, SCY, DCF, NDX, CBZ-E, and *N*-desVFX were present in 100% of the samples. Twenty-three out of the 55 target

Table 2
Linearity range, limit of detection (LOD) and quantification (LOQ) of the instrument, limit of detection (MLOD) and quantification (MLOQ) of the method, and determination coefficient (r^2) for each compound. (−) negative ionization mode.

	BP3	**BP1**	**4HB**	**DHB**	**DHMB**	**BP2**	**BP4 (−)**	**AVO**	**4MBC**	**EHMC**	**EtPABA**	**BZT**	**MeBZT**	**DMBZT**	**UVP**	**BePB (−)**	**BuPB (−)**	**PrPB (−)**	
t_R (min.)	11.95	11.03	9.91	10.52	9.53	11.23	13.5	7.67	12.53	13.31	9.61	8.15	9.27	9.91	13.14	9.07	9.09	8.78	
1st transition	229 > 151	215 > 137	199 > 121	215 > 121	245 > 121	247 > 137	307 > 227	311 > 135	255 > 105	291 > 179	166 > 138	120 > 65	134 > 79	148 > 77	226 > 120	227 > 92	193 > 137	179 > 92	
DP (V)	40	40	40	45	43	46	−50	40	61	51	41	56	46	56	46	−65	−55	−60.0	
CE (eV)	25	27	25	27	29	25	−34	25	45	19	20	31	29	39	25	−26	−22	−30.0	
CxP (eV)	12	10	8	8	8	8	−15	15	6	4	10	4	10	2	10	−9	−5	−13.0	
2nd transition	229 > 105	215 > 105	199 > 105	215 > 93	245 > 151	247 > 109	307 > 211	311 > 161	255 > 212	291 > 161	166 > 120	120 > 92	134 > 95	148 > 93	226 > 107	227 > 136	193 > 92	179 > 137	
DP (V)	40	40	40	45	43	46	−70	40	61	51	41	56	46	56	46	−65	−55	−60.0	
CE (eV)	27	29	27	45	27	45	−46	25	29	25	25	25	35	27	19	−22	−34	−24.0	
CxP (eV)	16	6	8	6	12	8	−9	15	14	10	28	16	14	6	18	−1	−13	−5.0	
IS	BP3-d_5	BP3-d_5	BP3-d_5	BP3-d_5	BP3-d_5	BP3-d_5	GFZ-d_6	4MBC-d_4	4MBC-d_4	4MBC-d_4	BZT-d_4	BZT-d_4	BZT-d_4	BZT-d_4	BZT-d4	BePB-d_4	BePB-d_4	BePB-d_4	
	MePB (−)	**FLU**	**NDX**	**OXL**	**TCY**	**Succinyl-STZ**	**SDZ**	**acSDZ**	**SMR**	**acSMR**	**acSMZ**	**SMX**	**acSMX**	**SMPZ**	**SPY**	**acSPY**	**SQX**	**STZ**	
t_R (min.)	8.03	10.42	10.2	10.42	7.14	7.62	6.03	7.34	6.8	7.69	7.99	7.98	8.82	7.6	6.46	7.47	9.25	6.35	
1st transition	151 > 92	262 > 244	233 > 215	262 > 244	445 > 410	356 > 256	251 > 156	293 > 134	265 > 156	307 > 134	321 > 134	254 > 108	296 > 198	281 > 126	250 > 156	292 > 134	301 > 156	256 > 156	
DP (V)	−45	51	61	36	66	71	46	65	61	60	86	56	60	66	51	70	76	40.0	
CE (eV)	−28	29	19	25	27	25	27	30	27	35	35	27	30	27	28	30	25	25.0	
CxP (eV)	−7	14	14	12	10	16	10	12	8	8	4	10	10	12	12	8	10	14.0	
2nd transition	151 > 136	262 > 202	233 > 187	262 > 216	445 > 427	356 > 192	251 > 108	293 > 198	265 > 92	307 > 110	321 > 124	254 > 156	296 > 134	281 > 156	250 > 92	292 > 198	301 > 92	256 > 92	
DP (V)	−45	51	61	36	66	71	46	65	61	60	86	56	60	66	51	70	76	40.0	
CE (eV)	−20	45	37	41	19	33	30	30	47	35	35	25	30	27	31	30	47	25.0	
CxP (eV)	−9	10	12	16	12	16	8	12	6	8	4	10	10	14	6	8	12	10.0	
IS	BePB-d_4	FLU-13C	FLU-13C	FLU-13C	SMZ-d_4	SMZ-d_4	SMZ-d_4	SMZ-d_4	SMZ-d_4	SMZ-d_4	SMZ-d_4	SMZ-d_4	SMZ-d_4	SMZ-d_4	SMZ-d_4	SMZ-d_4	SMZ-d_4	SMZ-d_4	
	SMD	**SDM**	**TMP**	**GFZ (−)**	**MFA (−)**	**NPX (−)**	**DCF (−)**	**DCF-^{13}C (−)**	**KPF (−)**	**IBU (−)**	**AAP (−)**	**CBZ**	**CBZ-E**	**ATL**	**norFXT**	**CFX**	***N*-desFXT**	**SCY (−)**	**CFF**
t_R (min.)	7.33	9.07	6.58	12.09	9.01	8.2	8.76	8.76	8.16	8.92	5.94	10.2	9.36	4.96	8.82	7.43	7.37	5.47	7.71
1st transition	279 > 124	311 > 156	291 > 230	249 > 121	240 > 196	229 > 169	295 > 251	300 > 256	253 > 209	205 > 159	150 > 106	237 > 194	253 > 180	267 > 116	296 > 134	332 > 288	264 > 58	137 > 93	195 > 109.960
DP (V)	76	76	76	−50	−70	−30	−40	−35	−45	−30	−55	61	36	46	31	91	31	−50	56
CE (eV)	33	31	33	−26	−20	−40	−16	−18	−10	−10	−24	29	35	31	11	27	57	−18	31
CxP (eV)	8	8	20	−23	−13	−9	−7	−9	−21	−41	−19	14	12	8	8	14	18	−1	8
2nd transition	279 > 186	311 > 92	291 > 261	249 > 127	240 > 192	229 > 170	295 > 215	300 > 220	253 > 197	205 > 161	150 > 108	237 > 192	253 > 151	267 > 133	296 > 59	332 > 231	264 > 133	137 > 65	195 > 138
DP (V)	76	76	76	−50	−70	−30	−40	−35	−45	−30	−55	61	36	46	31	91	31	−50	56
CE (eV)	23	31	35	−12	−36	−22	−30	−30	−6	−14	−22	31	109	39	33	51	39	−40	29
CxP (eV)	14	6	12	−21	−3	−1	−19	−9	−41	−35	−7	12	10	22	10	4	46	−3	10
IS	SMZ-d_4	SMZ-d_4	TMP-d3	GFZ-d_6	MFA-d_3	NPX-d_3	DCF-d_4 (phenyl-d_4)	DCF-d_4 (phenyl-d_4)	MFA-d_3	IBU-d_3	TMP-d3	CBZ-d_{10}	CBZ-d_{10}	TMP-d3	CBZ-d10	TMP-d3	TMP-d3	IBU-d3	TMP-d3

DP: Declustering potential in Volts; CE: Collision energy in electronvolts; CxP: Collision cell exit potential in electronvolts; IS: isotopically labeled internal standards.

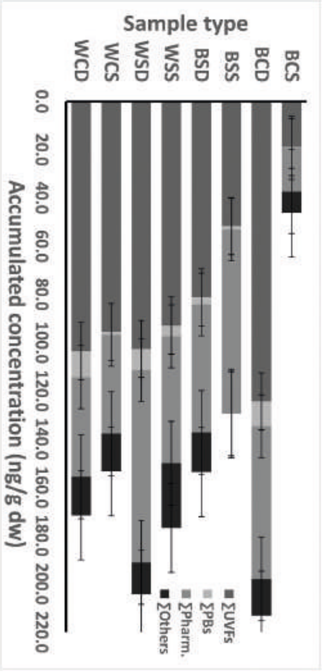

Fig. 3. Accumulated concentrations (∑) of UV filters (UVFs); Parabens (PBs), Pharmaceuticals (Pharm.) and others (CFF) in the different samples (acronyms defined as in Fig. 1).

compounds (42%) were not detected in any sample and 32 (58%) were at least found in one. The highest average concentrations corresponded to 4HB with 60.9 ± 27 ng/g dw, SCY with 18.9 ± 15 ng/g dw (SD), CFF with 16 ± 5 ng/g dw, DCF with 15.5 ± 8 dw, and BP2 with 13.2 ± 17 ng/g dw. As an example, the reconstructed ion chromatograms of the pollutants detected in the lettuce sample BCD are shown in Fig. 4 and Fig. 5.

The compound **4HB** is a metabolite of BP3, one of the most used UVFs in the formulation of PCPs and its occurrence in the aquatic systems is a well-known issue [27,28]. The extended use of BP3 and poor removal of 4HB in WWTPs explain its ubiquity in the reused water and its further transfer to the crops. Several studies reported ecotoxicological implications related to this compound and its endocrine-disrupting effect [29,30]. Urinary levels of 4HB in men were associated with reduced fecundity and in pregnant womens' with endometriosis [33] and higher tax of male births [31,32]. **BP2** is a benzophenone-type UVF, that is used in the formulation of PCPs and its ubiquitous presence in the lettuces may have the same origin as 4HB, especially in a coastal tourism area as that were the WWTP is located.

BZT and UVP benzotriazoles are highly polar and water soluble substances extensively used in three main applications: UV light stabilizer for plastics, rubbers, and textiles, corrosion inhibitor, and antifoggant in photography [34]. Because benzotriazoles are used in large quantities as a corrosion inhibitor, it is mainly through this application that benzotriazoles become very frequent environmental contaminants. A number of studies documented that benzotriazoles impaired morphological changes in a variety of plants. For instance, tomato plants were shown to be sensitive to BZT. In cucumber seedlings, BZT caused 50% repression of root elongation, and in bushbean plants, inhibition of internodal elongation with simultaneous thickening of the stem [35].

Concerning the pharmaceuticals, SCY was ubiquitous. It is used as antiseptic mostly for skincare, and is also the metabolite of the acetylsalicylic acid, which is one of the most consumed analgesics in the world [36]. This antiseptic can cause salicylism at high blood levels [36], and may promote fetal risk as it crosses the placenta [37]. DCF is an anti-inflammatory commonly prescribed for arthritis, and usually present in the aquatic environment [38,39]. The wide range of municipalities covered by the WWTP could explain the high levels found in the lettuces. It produced immobilization and reduced reproduction in *Daphnia magna*, and teratogenicity and mortality in *Zebrafish embryos* [40]. In humans, DCF affects particularly the intestine, liver, and kidney [41]. NDX is a common antibiotic usually prescribed for urinary infections. It produced carcinogenic activity in rats [42] and can cause systemic effects in humans like convulsions, hyperglycemia, sweating, and blood changes in children [43]. It is usually found in water because of its low degradation [44,45], explaining its presence in the irrigation water and, consequently, in the lettuces.

CBZ-E is the most common transformation product of CBZ and both are used as anti-epileptics. Scarce information about CBZ-E toxicity is available, but Fitzgerald et al. [46] demonstrated its capacity to accumulate in humans and cause neurotoxicity, especially when combined with quetiapine pharmaceutical. Its presence in all the studied samples could indicate the original occurrence of CBZ-E in the irrigation water, transformation of the residual CBZ from the water once it is absorbed by the lettuces. *N*-desVFX is a known transformation product of VFX, and both are used as antidepressants. To the author's knowledge no information about its toxicity is available. However, as for CBZ-E, its presence in all the studied lettuces could be an indicator of VFX presence in the irrigation water, and further degraded in the uptake process, or the original occurrence of *N*-desVFX in the water. Venlafaxine, its parental compound, has been associated with mydriasis [47], sleep disturbance [48], neonate complications [49] and hyponatremia in elderly population [50].

CFF is a known stimulant that is present in the coffee and soft drinks, and even in food, like chocolate, and also in many pharmaceuticals. Nowadays, it is consumed in large quantities and its presence in water has been commonly reported [51,52]. This is because, despite it is removed to some extent during wastewater treatment, its levels are so high, that still high concentrations remain in the WWTPs' effluents. Its occurrence at high levels in the irrigation water could explain the uptake of the crops, resulting in the levels found in the lettuces. Although CFF is thought to be safe in moderate amounts in adults, it differs in recommendations for children, adolescents, or pregnant women [53], where it causes alteration in myocardial blood flow [54], hearing [55], cognition indices [56], and increased liver fibrosis risk [57].

The compounds detected in all samples present pk_a values higher than 8, except SCY and DCF. As reported in previous studies [14], pk_a values are a good indicator of the capacity of the PPCPs to be up taken by the crops, since nonionic organic molecules are able to cross cell membranes easily [58]. Once inside the roots, nonionic compounds may be translocated by the water gradient inside the plant and therefore, accumulate more in the crops [59]. Ionic compounds, however, experience more difficulties to cross the permeable membranes, and thus its concentrations are lower. In fact, of the 55 compounds surveyed, the ones having higher pk_a values (alkaline) are CBZ and CBZ-E (15.96), *N*-desVFX (14.42), and CFF (14), found in 100% of the samples, except for CBZ, that could have been degraded to CBZ-E, as already explained.

3.4. Correlation analysis

The tests carried out showed correlations among the groups, except two. These groups are BCS with BCD and BCS with BSS. This statistical difference suggests that two of the studied variables (soil and irrigation

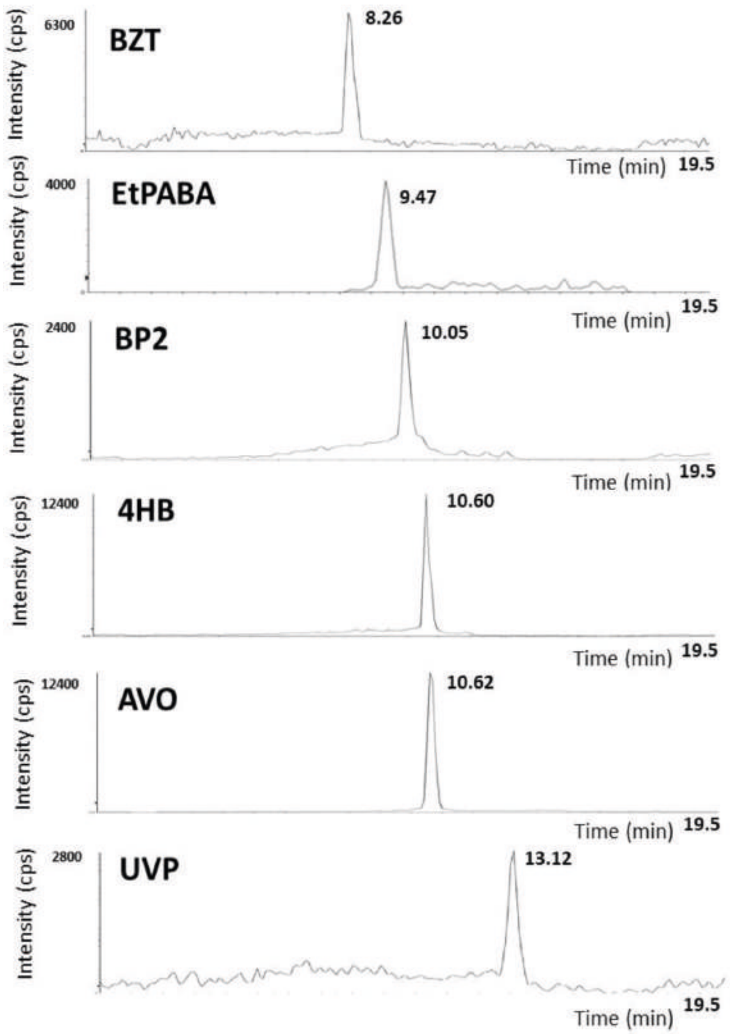

Fig. 4. SRM reconstructed ion chromatograms showing the first selected transition obtained for the lettuce sample BCD showing the content in UVFs.

system) have significant impact on the PPCPs' plant uptake. Unexpectedly, the irrigation water, did not present statistic difference among samples. Concerning the samples irrigated with the WWTP water, they were very similar, suggesting that the change of the variables affects more in the samples irrigated with reclaimed wastewater. Therefore, it appears that the cultivation variables considered in this study have a greater effect when the irrigation water is less contaminated. This fact is in total accordance with two previous studies [18,60], where the reported concentrations of PPCPs in the water did not correspond to the accumulation pattern in the crops.

3.5. *Multivariate analysis*

The scores plot (Fig. S4) of the results confirms that the variables present different behavior on uptake, as they are separately distributed in the plot. The loadings plot (Fig. S5) shows potential correlations among the studied PPCPs (those close among them) and suggests that there might not be a clear pattern within the families of compounds studied, as they are randomly distributed. Finally, the biplot (Fig. S6) shows the main compounds responsible of the variability observed for each combination of variables. For example, the compound causing the major impact in WSD sample is the SCY, because it is the closest one in the plot.

3.6. *Factors driving the PPCPs' lettuce uptake*

The results suggested that the best combination to lower the uptake of PPCPs by lettuces would be to irrigate with the reclaimed water

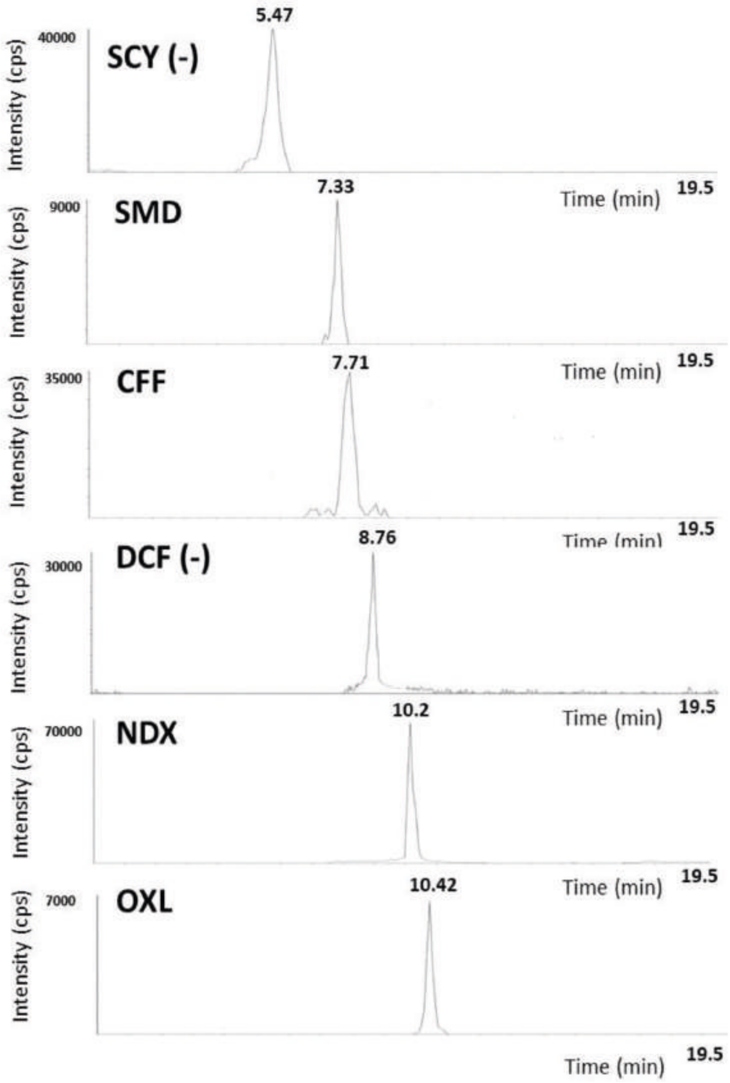

Fig. 5. SRM reconstructed ion chromatograms showing the first selected transition obtained for the lettuce sample BCD showing the content in pharmaceuticals and stimulants.

obtained from the treatment of the reactive barriers, in the soil that had the higher percentage of clay and by sprinkling. Moreover, the most differential factor to reduce the uptake was sprinkling irrigation, because all the lettuces irrigated by this system presented much lower values of accumulated PPCPs. This could be explained because the sprinkling water could have a higher spread throughout the soil around the crop, and therefore, it is exposed to more degrading processes (e.g. photolysis, soil processes, etc). The clayey soil hindered the pharmaceuticals uptake, but this did not affect the other pollutants.

3.7. *Comparison with previous studies*

So far, reported values of PPCPs in lettuces correspond to pharmaceuticals, mainly antibiotics. AAP (<LOD), CFF (3.2 ng/g dw), TMP (1.1 ng/g dw), NPX (<LOD), IBU (<LOD) and GFZ (0.2 ng/g dw) levels in lettuces were reported in two studies where the crops were grown in hydroponic conditions [13,61]. Similar studies reported ATL (1.2 ng/g dw) and CBZ (0.9 ng/g dw) levels [16], CFX (3 ng/g dw) and SMX (12 ng/g dw) [15,62] or DCF (9.05 ng/g dw) [63]. De Santiago-Martín et al. [18] detected APH (8.4–27.5 ng/g dw), IBU (0.2–1.1 ng/g) and CBZ (0.04–0.4 ng/g dw) in corn, Picó et al. [60] reported CFF (48–125 ng/g

dw), GFZ (45–75 ng/g dw), NPX (38 ng/g dw) and ATL (55 ng/g dw) in cabbage and green beans, and Montemurro et al. found CFF (17.9 ng/g dw), GFZ (16.5 ng/g dw), CBZ (18.5 ng/g dw) and CBZ-E (16.5 ng/g dw) in lettuce. All the levels found in these studies are in accordance with the results reported in this study, as can be seen in Table S4. Only APH and IBU values from Ref. [18] and GFZ values in Refs. [19,60] are different, since they were not detected in this study. Picó et al. [60] reported quite high accumulated concentrations compared with literature, but this could be due to the lower sensitivity of the method used. To the best of the authors' knowledge, no studies investigating the bioaccumulation of UV filters or paraben in lettuce have been reported so far, and thus, the present study constitutes the first data documenting the uptake of benzophenone-type UV filters, paraben preservatives, and benzotriazole UV stabilizers by lettuce.

4. Conclusions

Contamination of reclaimed water by CECs and the fate of these substances in agricultural environments are of increasing concern. To afford such knowledge gap, field studies and new simple and sensitive analytical methods are required. We contributed to fill this gap through the development and validation of a new QuEChERS-based method for the analysis of 55 PPCPs, including UVFs, PBs, pharmaceuticals, and stimulants, in lettuces. The new methodology presented good linearity and high sensitivity, low MLODs (0.03–0.8 ng/g dw) and MLOQs (0.1–2.7 ng/g dw). The method was successfully applied to determine the content of the target PPCPs in lettuces grown at field-scale and irrigated with two water types, in two soil compositions, and two irrigation systems. The results achieved allowed us to identify the best cultivation practices for lettuces using reclaimed water, which guarantees the lower contaminants transfer from the water to the plant. BP2, 4HB, BZT, UVP, NDX, DCF, CBZ-E, *N*-desVFX, and SCY were found in the eight groups of samples. The highest PPCP concentration, 84.1 ng/g dw, corresponded to the UV filter 4HB. The measured concentrations of BP2, 4HB, BZT, UVP constitute the first evidence of UV filters and stabilizers lettuces uptake.

Overall, the best conditions to lower the PPCPs' plant uptake appears to be irrigate by sprinkling in a clayey soil with reclaimed water obtained after a reclamation treatment, such as the water treated by soil infiltration through reactive barriers, but statistically there are no differences. In light of these findings, further studies are needed to evaluate the plant uptake under other agricultural systems.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2021.122302.

Author contributions

- **Adrià Sunyer-Caldú**, fieldwork and samples collection, analysis of samples, discussion of results, and writing and review of the manuscript.
- **M. Silvia Díaz Cruz**, direction and supervision of the experimental part, discussion of results, and writing and review of the manuscript.

References

[1] FAO, The State of the World's Land and Water Resources for Food and Agriculture (SOLAW) – Managing Systems at Risk, Rome and London, 2011.

[2] UNESCO, United Nations World Water Development Report 4: Managing Water under Uncertainty and Risk, Paris, 2012. https://unesdoc.unesco.org/ark:/48223/pf0000215644.

[3] L. Guppy, K. Anderson, Global Water Crisis : the Facts, 2017, pp. 1–3. http://inweh.unu.edu.

[4] European Parliament, Regulation (EU) 2020/741 of the European Parliament and of the Council of 25 May 2020 on minimum requirements for water reuse, n.d. http://data.europa.eu/eli/reg/2020/741/oj.

[5] K. Kümmerer, Options for a Strategic Approach to Pharmaceuticals in the Environment, 2018, https://doi.org/10.2779/87838.

[6] E. Archer, B. Petrie, B. Kasprzyk-Hordern, G.M. Wolfaardt, The fate of pharmaceuticals and personal care products (PPCPs), endocrine disrupting contaminants (EDCs), metabolites and illicit drugs in a WWTW and environmental waters, Chemosphere 174 (2017) 437–446, https://doi.org/10.1016/j.chemosphere.2017.01.101.

[7] B.M. Sharma, J. Bečanová, M. Scheringer, A. Sharma, G.K. Bharat, P.G. Whitehead, J. Klánová, L. Nizzetto, Health and ecological risk assessment of emerging contaminants (pharmaceuticals, personal care products, and artificial sweeteners) in surface and groundwater (drinking water) in the Ganges River Basin, India, Sci. Total Environ. 646 (2019) 1459–1467, https://doi.org/10.1016/j.scitotenv.2018.07.235.

[8] S.S. Caldas, J.L.O. Arias, C. Rombaldi, L.L. Mello, M.B.R. Cerqueira, A.F. Martins, E. G. Primel, Occurrence of pesticides and PPCPs in surface and drinking water in southern Brazil: data on 4-year monitoring, J. Braz. Chem. Soc. 30 (2019) 71–80. http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0103-50532019000100071&nrm=iso.

[9] S. Ortiz de García, G. Pinto Pinto, P. García Encina, R. Irusta Mata, Consumption and occurrence of pharmaceutical and personal care products in the aquatic environment in Spain, Sci. Total Environ. 444 (2013) 451–465, https://doi.org/10.1016/j.scitotenv.2012.11.057.

[10] M. Xu, H. Huang, N. Li, F. Li, D. Wang, Q. Luo, Occurrence and ecological risk of pharmaceuticals and personal care products (PPCPs) and pesticides in typical surface watersheds, China, Ecotoxicol, Environ. Saf. 175 (2019) 289–298, https://doi.org/10.1016/j.ecoenv.2019.01.131.

[11] L.J. Carter, M. Williams, C. Böttcher, R.S. Kookana, Uptake of pharmaceuticals influences plant development and affects nutrient and hormone homeostases, Environ. Sci. Technol. 49 (2015) 12509–12518, https://doi.org/10.1021/acs.est.5b03468.

[12] L. Sabourin, P. Duenk, S. Bonte-Gelok, M. Payne, D.R. Lapen, E. Topp, Uptake of pharmaceuticals, hormones and parabens into vegetables grown in soil fertilized with municipal biosolids, Sci. Total Environ. 431 (2012) 233–236, https://doi.org/10.1016/j.scitotenv.2012.05.017.

[13] X. Wu, J.L. Conkle, J. Gan, Multi-residue determination of pharmaceutical and personal care products in vegetables, J. Chromatogr. A 1254 (2012) 78–86, https://doi.org/10.1016/j.chroma.2012.07.041.

[14] T. Malchi, Y. Maor, G. Tadmor, M. Shenker, B. Chefetz, Irrigation of root vegetables with treated wastewater: evaluating uptake of pharmaceuticals and the associated human health risks, Environ. Sci. Technol. 48 (2014) 9325–9333, https://doi.org/10.1021/es5017894.

[15] B. Albero, J.L. Tadeo, M. Del Mar Delgado, E. Miguel, R.A. Pérez, Analysis of multiclass antibiotics in lettuce by liquid chromatography-tandem mass spectrometry to monitor their plant uptake, Molecules 24 (2019), https://doi.org/10.3390/molecules24224066.

[16] E.M. Beltrán, M.V. Pablos, C. Fernández Torija, M.Á. Porcel, M. González-Doncel, Uptake of atenolol, carbamazepine and triclosan by crops irrigated with reclaimed water in a Mediterranean scenario, Ecotoxicol. Environ. Saf. 191 (2020), 110171, https://doi.org/10.1016/j.ecoenv.2020.110171.

[17] M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F. Schenck, Fast and Easy multiresidue method employing acetonitrile, J. AOAC Int. 86 (2003) 412–431.

[18] A. de Santiago-Martín, R. Meffe, G. Teijón, V. Martínez Hernández, I. López-Heras, C. Alonso Alonso, M. Arenas Romasanta, I. de Bustamante, Pharmaceuticals and trace metals in the surface water used for crop irrigation: risk to health or natural attenuation? Sci. Total Environ. 705 (2020), 135825 https://doi.org/10.1016/j.scitotenv.2019.135825.

[19] N. Montemurro, A. Orfanioti, R. Manasfi, N.S. Thomaidis, S. Pérez, Comparison of high resolution mrm and sequential window acquisition of all theoretical fragment-ion acquisition modes for the quantitation of 48 wastewater-borne pollutants in lettuce, J. Chromatogr. A 1631 (2020), 461566, https://doi.org/10.1016/j.chroma.2020.461566.

[20] C. Valhondo, L. Mart, J. Wang, Reactive barriers for renaturalization of reclaimed, Water 12 (4) (2020) 10.

[21] C. Valhondo, L. Martínez-Landa, J. Carrera, S.M. Díaz-Cruz, S. Amalfitano, C. Levantesi, Six artificial recharge pilot replicates to gain insight into water quality enhancement processes, Chemosphere 240 (2020), https://doi.org/10.1016/j.chemosphere.2019.124826.

[22] P. Parrilla Vázquez, A. Lozano, C. Ferrer, M.J. Martínez Bueno, A.R. Fernández-Alba, Improvements in identification and quantitation of pesticide residues in food by LC-QTOF using sequential mass window acquisition (SWATH®), Anal. Meth. 10 (2018) 2821–2833, https://doi.org/10.1039/c8ay00678d.

[23] R Core Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, 2020. https://www.r-project.org/.

[24] Waters Corporation, QuEChERS Procedure for Multi-Residue Pesticide Analysis - DisQuE Dispersive Sample Preparation, Waters, 2011.

[25] J. Stevens, D. Jones, QuEChERS 101:The Basics and beyond, Agil. Technol., 2010, pp. 3–8. https://www.agilent.com/cs/library/eseminars/Public/QuEChERS_101_10_11_01.pdf.

[26] Y. Zhang, J.B. Sallach, L. Hodges, D.D. Snow, S.L. Bartelt-Hunt, K.M. Eskridge, X. Li, Effects of soil texture and drought stress on the uptake of antibiotics and the internalization of Salmonella in lettuce following wastewater irrigation, Environ. Pollut. 208 (2016) 523–531, https://doi.org/10.1016/j.envpol.2015.10.025.

[27] A. Jurado, P. Gago-Ferrero, E. Vàzquez-Suñé, J. Carrera, E. Pujades, M.S. Díaz-Cruz, D. Barceló, Urban groundwater contamination by residues of UV filters, J. Hazard Mater. 271 (2014) 141–149, https://doi.org/10.1016/j.jhazmat.2014.01.036.

[28] M.S. Díaz-Cruz, D. Molins-Delgado, M.P. Serra-Roig, E. Kalogianni, N. T. Skoulikidis, D. Barceló, Personal care products reconnaissance in EVROTAS river (Greece): water-sediment partition and bioaccumulation in fish, Sci. Total Environ. 651 (2019) 3079–3089, https://doi.org/10.1016/j.scitotenv.2018.10.008.

[29] Y. Nakagawa, K. Tayama, Estrogenic potency of benzophenone and its metabolites in juvenile female rats, Arch. Toxicol. 75 (2001) 74–79, https://doi.org/10.1007/s002040100225.

[30] T. Suzuki, S. Kitamura, R. Khota, K. Sugihara, N. Fujimoto, S. Ohta, Estrogenic and antiandrogenic activities of 17 benzophenone derivatives used as UV stabilizers and sunscreens, Toxicol. Appl. Pharmacol. 203 (2005) 9–17, https://doi.org/10.1016/j.taap.2004.07.005.

[31] G.M.B. Louis, K. Kannan, K.J. Sapra, J. Maisog, R. Sundaram, Urinary concentrations of benzophenone-type ultraviolet radiation filters and couples' fecundity, Am. J. Epidemiol. 180 (2014) 1168–1175, https://doi.org/10.1093/aje/kwu285.

[32] J. Bae, S. Kim, K. Kannan, G.M. Buck Louis, Couples' urinary concentrations of benzophenone-type ultraviolet filters and the secondary sex ratio, Sci. Total Environ. 543 (2016) 28–36, https://doi.org/10.1016/j.scitotenv.2015.11.019.

[33] T. Kunisue, Z. Chen, G.M. Buck Louis, R. Sundaram, M.L. Hediger, L. Sun, K. Kannan, Urinary concentrations of benzophenone-type UV filters in U.S. Women and their association with endometriosis, Environ. Sci. Technol. 46 (2012) 4624–4632, https://doi.org/10.1021/es204415a.

[34] L.N. Davis, J. Santodonato, P.H. Howard, Investigations of Selected Potential Environmental Contaminants: Benzotriazoles, 1977.

[35] D. Davis, Benzotriazole, a plant-growth regulator, Science 120 (1954) 989, https://doi.org/10.1126/science.120.3128.989.

[36] R.K. Madan, J. Levitt, A review of toxicity from topical salicylic acid preparations, J. Am. Acad. Dermatol. 70 (2014) 788–792, https://doi.org/10.1016/j.jaad.2013.12.005.

[37] M. Tenenbein, in: J.B.T.-H.T. Descotes (Ed.), 4 - Acute Poisonings in Pregnancy, Elsevier Science B.V., Amsterdam, 1996, pp. 247–257, https://doi.org/10.1016/B978-044481557-6/50006-X.

[38] A. Karnjanapiboonwong, J.G. Suski, A.A. Shah, Q. Cai, A.N. Morse, T.A. Anderson, Occurrence of PPCPs at a wastewater treatment plant and in soil and groundwater at a land application site, Water, Air. Soil Pollut. 216 (2011) 257–273, https://doi.org/10.1007/s11270-010-0532-8.

[39] M.F. Jaramillo, I. Restrepo, Wastewater reuse in agriculture: a review about its limitations and benefits, Sustain 9 (2017), https://doi.org/10.3390/su9101734.

[40] J. Schwaiger, H. Ferling, U. Mallow, H. Wintermayr, R.D. Negele, Toxic effects of the non-steroidal anti-inflammatory drug diclofenac: Part I: histopathological alterations and bioaccumulation in rainbow trout, Aquat. Toxicol. 68 (2004) 141–150, https://doi.org/10.1016/j.aquatox.2004.03.014.

[41] R. Triebskorn, H. Casper, A. Heyd, R. Eikemper, H.R. Köhler, J. Schwaiger, Toxic effects of the non-steroidal anti-inflammatory drug diclofenac: Part II. Cytological effects in liver, kidney, gills and intestine of rainbow trout (Oncorhynchus mykiss), Aquat. Toxicol. 68 (2004) 151–166, https://doi.org/10.1016/j.aquatox.2004.03.015.

[42] R.E. Morrissey, Toxicology and Carcinogenesis Studies of Nalidixic Acid (CAS NO. 389-08-2) in F344/N RATS and B6C3Fi MICE, FEED STUDIES), 1996.

[43] R.J. Lewis, Sax's Dangerous Properties of Industrial Materials, ninth ed. Vo, Van Nostrand Reinhold, New York, 1996.

[44] Z. Li, X. Xiang, M. Li, Y. Ma, J. Wang, X. Liu, Occurrence and risk assessment of pharmaceuticals and personal care products and endocrine disrupting chemicals in reclaimed water and receiving groundwater in China, Ecotoxicol. Environ. Saf. 119 (2015) 74–80, https://doi.org/10.1016/j.ecoenv.2015.04.031.

[45] M. Liu, H. Yin, Q. Wu, Occurrence and health risk assessment of pharmaceutical and personal care products (PPCPs) in tap water of Shanghai, Ecotoxicol. Environ. Saf. 183 (2019), 109497, https://doi.org/10.1016/j.ecoenv.2019.109497.

[46] M.D. Brian J. Fitzgerald, D. Pharm, Anthony J. Okos, Elevation of carbamazepine-10,11-epoxide by quetiapine, Pharmacother 22 (11) (2002) 1500–1503, https://doi.org/10.1592/phco.22.16.1500.33697.

[47] American Society of Health-System Pharmacists, Drug Information 2011, 2011 n.d).

[48] R. Salin-Pascual, L. Galicia-Polo, R. Drucker-Colin, Sleep changes after 4 consecutive days of venlafaxine administratoin in normal volunteers, J. Clin. Psychiatr. 58 (1997) 348–350, https://doi.org/10.4088/JCP.v58n0803.

[49] R.K. Pakalapati, S. Bolisetty, M.-P. Austin, J. Oei, Neonatal seizures from in utero venlafaxine exposure, J. Paediatr. Child Health 42 (2006) 737–738, https://doi.org/10.1111/j.1440-1754.2006.00962.x.

[50] D. Kirby, S. Harrigan, D. Ames, Hyponatraemia in elderly psychiatric patients treated with Selective Serotonin Reuptake Inhibitors and venlafaxine: a retrospective controlled study in an inpatient unit, Int. J. Geriatr. Psychiatr. 17 (2002) 231–237, https://doi.org/10.1002/gps.591.

[51] N.K. Stamatis, I.K. Konstantinou, Occurrence and removal of emerging pharmaceutical, personal care compounds and caffeine tracer in municipal sewage treatment plant in Western Greece, J. Environ. Sci. Health Part B Pestic. Food Contam. Agric. Wastes 48 (2013) 800–813, https://doi.org/10.1080/03601234.2013.781359.

[52] S.S. Verenitch, C.J. Lowe, A. Mazumder, Determination of acidic drugs and caffeine in municipal wastewaters and receiving waters by gas chromatography-ion trap tandem mass spectrometry, J. Chromatogr. A 1116 (2006) 193–203, https://doi.org/10.1016/j.chroma.2006.03.005.

[53] C. Willson, The clinical toxicology of caffeine: a review and case study, Toxicol. Rep. 5 (2018) 1140–1152, https://doi.org/10.1016/j.toxrep.2018.11.002.

[54] R. van Dijk, D. Ties, D. Kuijpers, P. van der Harst, M. Oudkerk, Effects of caffeine on myocardial blood flow: a systematic review, Nutrients 10 (2018) 1–14, https://doi.org/10.3390/nu10081083.

[55] S.Y. Lee, G. Jung, M.J. Jang, M.W. Suh, J.H. Lee, S.H. Oh, M.K. Park, Association of coffee consumption with hearing and tinnitus based on a national population-based survey, Nutrients 10 (2018) 1–12, https://doi.org/10.3390/nu10101429.

[56] S. Haller, M.L. Montandon, C. Rodriguez, F.R. Herrmann, P. Giannakopoulos, Impact of coffee, wine, and chocolate consumption on cognitive outcome and MRI parameters in old age, Nutrients 10 (2018) 1–13, https://doi.org/10.3390/nu10101391.

[57] I. Yaya, F. Marcellin, M. Costa, P. Morlat, C. Protopopescu, G. Pialoux, M.E. Santos, L. Wittkop, L. Esterle, A. Gervais, P. Sogni, D. Salmon-Ceron, M.P. Carrieri, D. Salmon, F. Dabis, L. Esterle, P. Sogni, P. Trimoulet, J. Izopet, L. Serfaty, V. Paradis, B. Spire, P. Carrieri, M.A. Valantin, G. Pialoux, J. Chas, I. Poizot-Martin, K. Barange, A. Naqvi, E. Rosenthal, A. Bicart-See, O. Bouchaud, A. Gervais, C. Lascoux-Combe, C. Goujard, K. Lacombe, C. Duvivier, D. Vittecoq, D. Neau, P. Morlat, F. Bani-Sadr, L. Meyer, F. Boufassa, S. Dominguez, B. Autran, A. M. Roque, C. Solas, H. Fontaine, D. Costagliola, L. Piroth, A. Simon, D. Zucman, F. Boué, P. Miailhes, E. Billaud, H. Aumaître, D. Rey, S. Couffin-Cadiergues, L. Marchand, L. Alagna, B. Terris, A. Krivine, C. Katlama, M.A. Valantin, H. Stitou, Y. Benhamou, F. Charlotte, S. Fourati, A. Simon, P. Cacoub, S. Nafissa, I. Poizot-Martin, O. Zaegel, M. Porcher, C. Tamalet, G. Pialoux, J. Chas, L. Slama, P. Callard, F. Bendjaballah, C. Le Pendeven, B. Marchou, L. Alric, K. Barange, S. Metivier, J. Selves, F. Larroquette, E. Rosenthal, A. Naqvi, J. Haudebourg, M.C. Saint-Paul, C. Partouche, O. Bouchaud, M. Ziol, Y. Baazia, M. Uzan, A. Bicart-See, D. Garipuy, M.J. Ferro-Collados, J. Selves, F. Nicot, A. Gervais, Y. Yazdanpanah, H. Adle-Biassette, G. Alexandre, C. Lascoux-Combe, J.M. Molina, P. Bertheau, J. Duclos, P. Palmer, K. Lacombe, P. Campa, P.M. Girard, D. Wendum, P. Cervera, J. Adam, C. Viala, C. Goujard, E. Teicher, C. Pallier, D. Vittecoq, O. Lortholary, C. Duvivier, C. Rouzaud, J. Lourenco, F. Touam, C. Louisin, A. Mélard, D. Neau, A. Ochoa, E. Blanchard, S. Castet-Lafarie, C. Cazanave, D. Malvy, M. Dupon, H. Dutronc, F. Dauchy, L. Lacaze-Buzy, P. Bioulac-Sage, P. Trimoulet, S. Reigadas, P. Morlat, D. Lacoste, F. Bonnet, N. Bernard, M. Bonarek Hessamfar, J. Roger-Schmeltz, P. Gellie, P. Thibaut, F. Paccalin, C. Martell, M. Carmen Pertusa, M. Vandenhende, P. Mercier, D. Malvy, T. Pistone, M.C. Receveur, S. Caldato, P. Bioulac-Sage, P. Trimoulet, S. Reigadas, J.L. Pellegrin, J.F. Viallard, E. Lazzaro, C. Greib, P. Bioulac-Sage, P. Trimoulet, S. Reigadas, D. Zucman, C. Majerholc, F. Guitard, F. Boué, J. Polo Devoto, I. Kansau, V. Chambrin, C. Pignon, L. Berroukeche, R. Fior, V. Martinez, C. Deback, Y. Lévy, S. Dominguez, J.D. Lelièvre, A.S. Lascaux, G. Melica, E. Billaud, F. Raffi, C. Alavena, A. Rodallec, P. Miailhes, D. Peyramond, C. Chidiac, F. Ader, F. Biron, A. Boibieux, L. Cotte, T. Ferry, T. Perpoint, J. Koffi, F. Zoulim, F. Bailly, P. Lack, M. Maynard, S. Radenne, M. Amiri, T.T. Le-Thi, L. Piroth, P.C.M. Duong Van Huyen, M. Buisson, A. Waldner-Combernoux, S. Mahy, R. Binois, A.L. Simonet-Lann, D. Croisier-Bertin, H. Aumaître, F. Bani-Sadr, D. Lambert, Y. Nguen, C. Rouger, J.L. Berger, D. Rey, M. Partisani, M. L. Batard, C. Cheneau, M. Priester, C. Bernard-Henry, E. De Mautort, P. Gantner, S. Fafi-Kremer, Impact of alcohol and coffee intake on the risk of advanced liver fibrosis: a longitudinal analysis in HIV-HCV coinfected patients (ANRS HEPAVIH CO-13 cohort), Nutrients 10 (2018), https://doi.org/10.3390/nu10060705.

[58] F.C. Hsu, R.L. Marxmiller, A.Y. Yang, Study of root uptake and xylem translocation of cinmethylin and related compounds in detopped soybean roots using a pressure chamber technique, Plant Physiol. 93 (1990) 1573–1578, https://doi.org/10.1104/pp.93.4.1573.

[59] S. Trapp, Plant uptake and transport models for neutral and ionic chemicals, Environ. Sci. Pollut. Res. Int. 11 (2004) 33–39, https://doi.org/10.1065/espr2003.08.169.

[60] Y. Picó, R. Alvarez-Ruiz, A.H. Alfarhan, M.A. El-Sheikh, S.M. Alobaid, D. Barceló, Uptake and accumulation of emerging contaminants in soil and plant treated with wastewater under real-world environmental conditions in the Al Hayer area (Saudi Arabia), Sci. Total Environ. 652 (2019) 562–572, https://doi.org/10.1016/j.scitotenv.2018.10.224.

[61] X. Wu, F. Ernst, J.L. Conkle, J. Gan, Comparative uptake and translocation of pharmaceutical and personal care products (PPCPs) by common vegetables, Environ. Int. 60 (2013) 15–22, https://doi.org/10.1016/j.envint.2013.07.015.

[62] Đ. Tadić, V. Matamoros, J.M. Bayona, Simultaneous determination of multiclass antibiotics and their metabolites in four types of field-grown vegetables, Anal. Bioanal. Chem. 411 (2019) 5209–5222, https://doi.org/10.1007/s00216-019-01895-y.

[63] L.K. Dodgen, J. Li, D. Parker, J.J. Gan, Uptake and accumulation of four PPCP/EDCs in two leafy vegetables, Environ. Pollut. 182 (2013) 150–156, https://doi.org/10.1016/j.envpol.2013.06.038.

5.2.1 Publication #9 Supplementary information

Development of a QuEChERS-based method for the analysis of pharmaceuticals and personal care products in lettuces grown in field-scale agricultural plots irrigated with reclaimed water

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Section S1. Figures. (Figure S1, S2, S3, S4 and S5).
Section S2. Tables. (Table S1, S2, S3, S4 and S5).
Section S3. Statistical analysis.
S3.1 Correlation analysis.
S3.2 Principal component analysis:
Figure S6. Scores plot.
Figure S7. Loadings plot.
Figure S8. Biplot of the scores and loadings.

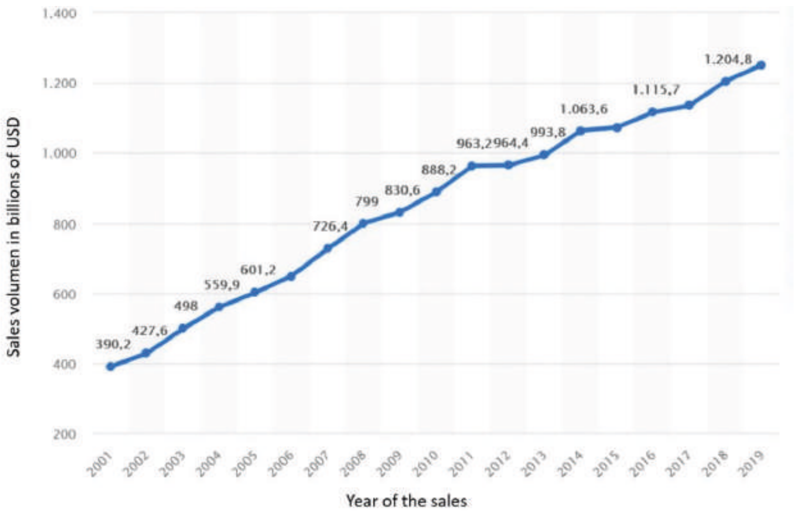


Figure S1. Annual evolution of the revenue volume of the pharmaceutical industry worldwide from 2001 to 2019. (Data from statista.com, August 2020).

Figure S2. Map with the exact location of the WWTP in Palamós (Girona, Spain), where the crops were cultivated.

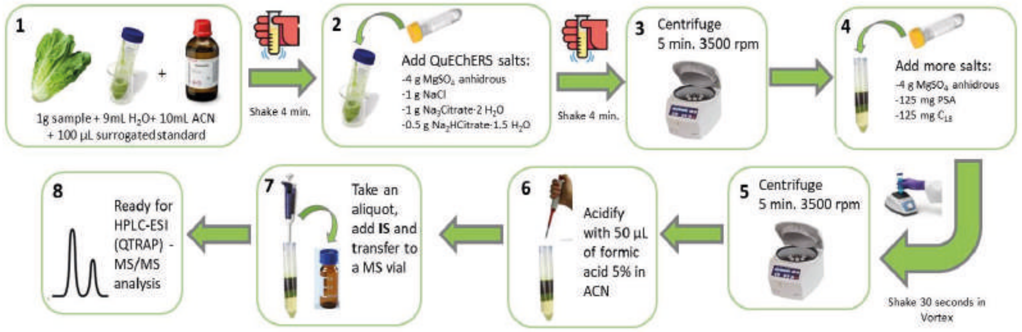


Figure S3. Workflow for analytes extraction, purification and concentration methodology.

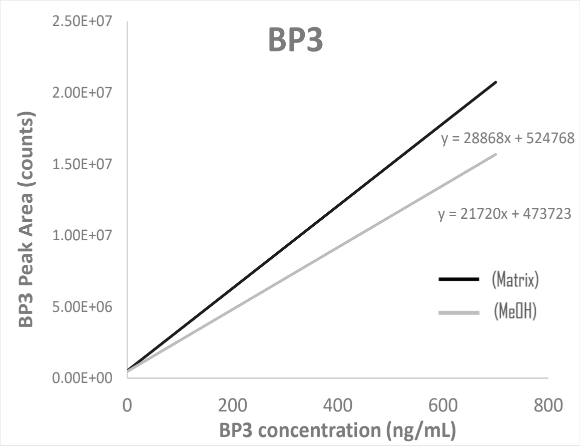


Figure S4. Calibration curves for BP3 compound, showing the enhancement of the signal in the matrix matched calibration curve.

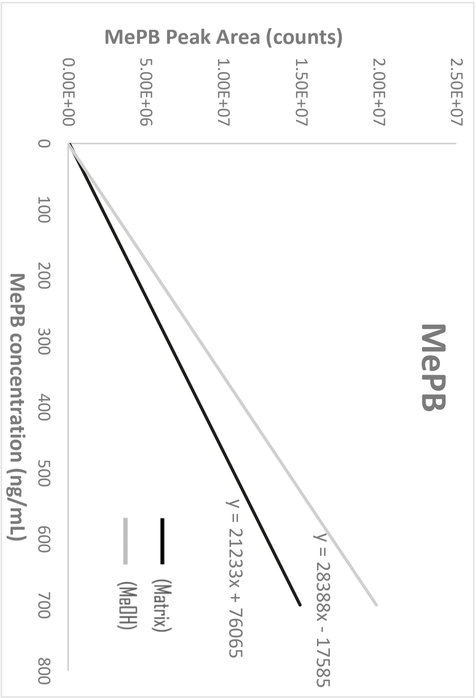
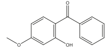
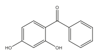
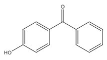
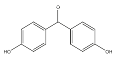
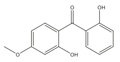
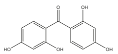
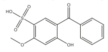
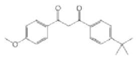
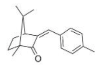
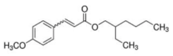
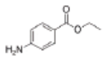
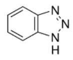
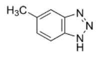
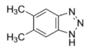
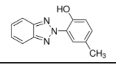
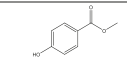
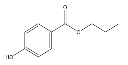
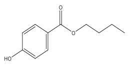
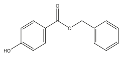
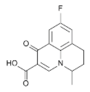
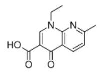
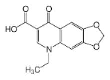
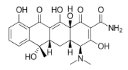
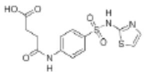
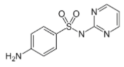
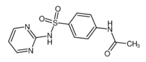
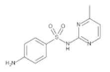
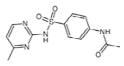
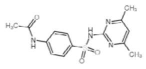
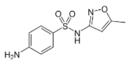
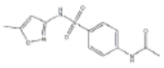
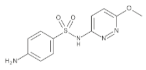
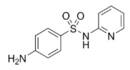
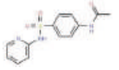
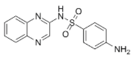
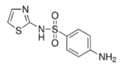
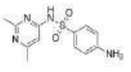
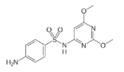


Figure S5. Calibration curves for MePB compound, showing the suppression of the signal in the matrix matched calibration curve.

Table S1. Table that shows all the analyzed compounds, its family group, type of compound, CAS number, molecular mass, structure and octanol-water partition coefficient.

Compound	Other names	Family	Type	CAS number	Molecular mass(g/mol)	Structure	K_{ow}	pKa
Benzophenone-3 (BP3)	Oxybenzone; 2-Hydroxy-4-methoxybenzophenone	Benzophenones	UV filter (PCP)	131-57-7	228.24		3.79	7.1
Benzophenone-1 (BP1)	2,4-Dihydroxybenzophenone	Benzophenones	UV filter (PCP)	131-56-6	214.22		3.15	7.1
4-Hydroxybenzophenone (4HB)	-	Benzophenones	UV filter (PCP)	1137-42-4	193.18		2.92	8.14
4,4'-Dihydroxybenzophenone (DHB)	-	Benzophenones	UV filter (PCP)	611-99-4	214.22		2.19	7.55
2,2'-Dihydroxy-4-methoxybenzophenone (DHMB)	Benzophenone-8; Dioxybenzone	Benzophenones	UV filter (PCP)	131-53-3	244.25		3.82	6.78
Benzophenone-2 (BP2)	2,2',4,4'-Tetrahydroxybenzophenone	Benzophenones	UV filter (PCP)	131-55-5	246.22		2.78	7.1
Benzophenone-4 (BP4)	5-benzoyl-4-hydroxy-2-methoxybenzene sulfonic acid; HMBS; Sulisobenzone	Benzophenones	UV filter (PCP)	4065-45-6	308.31		0.88	-2.4
Avobenzone (AVO)	1-(4-tert-butylphenyl)-3-(4-methoxyphenyl)propane-1,3-dione	Benzophenones	UV filter (PCP)	70356-09-1	310.17		4.51	9.74
Enzacame (4MBC)	3-(4-Methylbenzilidene) camphor	Camphor derivatives	UV filter (PCP)	36861-47-9	254.17		5.14	7.5
Octinoxate (EHMC)	2-Ethylhexyl trans-4-methoxy cinnamate	Cinnamate derivatives	UV filter (PCP)	5466-77-3	290.4		5.8	-
Benzocaine (EtPABA)	Ethyl 4-aminobenzoate	p-aminobenzoic acid derivatives	UV filter (PCP) UV filter (PCP)	94-09-7	165.19		1.86	2.51
Benzotriazole (BZT or 1H-BZT)	1,2,3-Benzotriazole	Benzotriazoles	UV filter (PCP)	95-14-7	119.12		1.44	8.37
Methyl-benzotriazole (MeBZT or 5-MeBZT)	5-Methyl-1H-benzotriazole	Benzotriazoles	UV filter (PCP)	136-86-5	133.15		Not avaliable	8.85
Dimethyl-benzotriazole (DMBZT)	5,6-Dimethyl-1H-benzotriazole monohydrate	Benzotriazoles	UV filter (PCP)	4184-79-6	147.18		Not avaliable	8.92
Drometrizole (UVP)	Benazol P; Tinuvin P; 2-(2H-Benzotriazol-2-yl)-p-cresol	Benzotriazole derivatives	UV filter (PCP)	2440-22-4	225.25		4.31	8.15

Methyl paraben (MePB)	Methyl 4-hydroxybenzoate	Parabens	Preservative (PCP)	99-76-3	152		2	8.17
Propyl paraben (PrPB)	Propyl 4-hydroxybenzoate	Parabens	Preservative (PCP)	94-13-3	180.2		2.98	8.35
Butyl paraben (BuPB)	Butyl 4-hydroxybenzoate	Parabens	Preservative (PCP)	94-26-8	194.23		3.47	8.47
Benzyl paraben (BePB)	Benzyl 4-hydroxybenzoate	Parabens	Preservative (PCP)	94-18-8	228.24		3.7	8.18
Flumequine (FLU)	Flumigal; Apurone	Fluoroquinolones	Pharmaceutical	42835-25-6	261.25		1.6	6.5
Nalidixic acid (NDX)	Nalidixate; nalidixin	Quinolones	Pharmaceutical	389-08-2	232.23		1.59	8.6
Oxolinic acid (OXL)	Nidantin; dioxacin	Quinolones	Pharmaceutical	14698-29-4	261.23		0.94	6.3
Tetracycline (TCY)	Deschlorobiomycin	Tetracyclines	Pharmaceutical	60-54-8	444.4		1.3	3.3
Succinylsulfathiazole (Succinyl-STZ)	Cremosuxidine; Colistatin	Sulfonamides	Pharmaceutical	116-43-8	355.4		-	3.36
Sulfadiazine (SDZ)	Sulfapyrimidine	Sulfonamides	Pharmaceutical	68-35-9	250.28		-0.09	6.99
N⁴-acetylsufladiazine (acSDZ)	N-[4-(pyrimidin-2-ylsulfamoyl)pheyl]acetamide	Sulfonamides	Pharmaceutical	127-74-2	292.32		Not avaliable	-
Sulfamerazine (SMZ)	Sulfamethyldiazine	Sulfonamides	Pharmaceutical	127-79-7	264.31		0.14	6.98

N⁴-acetylsulfamerazine (acSMR)	N(4)-Acetylsulfamerazine	Sulfonamides	Pharmaceutical	127-73-1	306.34		Not avaliable	-
N⁴-acetylsulfamethazine (acSMZ)	N(4)-Acetylsulfamethazine	Sulfonamides	Pharmaceutical	100-90-3	320.37		Not avaliable	-
Sulfamethoxazole (SMX)	Gantanol; sulfisomezole	Sulfonamides	Pharmaceutical	723-46-6	253.28		0.89	5.81
Acetylsulfamethoxazole (acSMX)	N⁴-Acetylsulfamethoxazole	Sulfonamides	Pharmaceutical	21312-10-7	295.32		0.86	-
Sulfamethoxypyridazine (SMPZ)	Sulphametoxypyridazine	Sulfonamides	Pharmaceutical	80-35-3	280.31		Not avaliable	7.19
Sulfapyridine (SPY)	Sulfidin	Sulfonamides	Pharmaceutical	144-83-2	249.29		0.35	8.43
Acetylsulfapyiridine (acSPY)	N-(4-(N-(Pyridin-2-yl)sulfamoyl)phenyl)acetamide	Sulfonamides	Pharmaceutical	19077-98-6	291.33		Not avaliable	-
Sulfaquinoxaline (SQX)	Avicocid	Sulfonamides	Pharmaceutical	59-40-5	300.34		1.68	5.1
Sulfathiazole (STZ)	2-Sulfanilamidothiazole	Sulfonamides	Pharmaceutical	72-14-0	255.3		0.05	7.24
Sulfisomidine (SMD)	-	Sulfonamides	Pharmaceutical	515-64-0	278.33		-0.33	7.4
Sulfadimethoxine (SDM)	Sulfadimethoxydiazine	Sulfonamides	Pharmaceutical	122-11-2	310.33		1.63	6.21

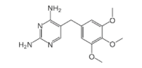
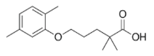
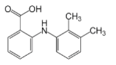
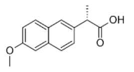
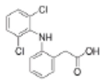
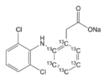
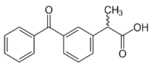
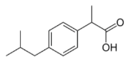
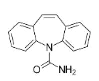
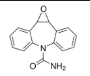
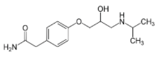
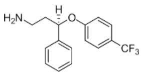
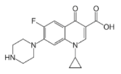
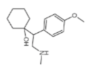
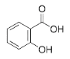
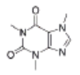
Trimethoprim (TMP)	Proloprim; Trimpex	Others	Pharmaceutical	738-70-5	290.32		0.91	7.12
Gemfibrozil (GFZ)	5-(2,5-Dimethylphenoxy)-2,2-dimethylpentanoic acid	Lipid regulators	Pharmaceutical	25812-30-0	250.33		4.39	4.5
Mefenamic acid (MFA)	2-[(2,3-dimhetylphenyl)amino]benzoic acid	Analgesics	Pharmaceutical	61-68-7	241.28		5.12	4.2
Naproxen (NPX)	-	Anti-inflammatories	Pharmaceutical	22204-53-1	230.26		3.18	4.15
Diclofenac (DCF)	Voltaren	Anti-inflammatories	Pharmaceutical	15307-86-5	296.1		4.51	4.2
Diclofenac-^{13}C (DCF-^{13}C)	Diclofenac-(acetophenyl ring-$^{13}C_6$) sodium salt	Anti-inflammatories	Pharmaceutical	1261393-73-0	810.3		Not avaliable	-
Ketoprofen (KPF)	2-(3-Benzoylphenyl)propanoic acid	Analgesics	Pharmaceutical	22071-15-4	254.28		3.12	4.45
Ibuprofen (IBU)	2-(4-Isobutylphenyl)propanoic acid	Anti-inflammatories	Pharmaceutical	15687-27-1	206.28		3.97	4.91
Carbamazepine (CBZ)	5H-Dibenzo[b,f]azepine-5-carboxamide	Anti-epileptics	Pharmaceutical	298-46-4	236.27		2.77	15.96
Carbamazepine-10,11-epoxide (CBZ-E)	-	Anti-epileptics	Pharmaceutical	36507-30-9	252.27		Not avaliable	15.96
Atenolol (ATL)	Tenorium; blokium; normiten	B-blockers	Pharmaceutical	29122-68-7	266.34		0.16	9.6
Norfluoxetine (norFXT)	Desmethylfluoxetine	Anti-depressants	Pharmaceutical Pharmaceutical	56161-73-0 83891-03-6	295.3		Not avaliable	9.77
Ciprofloxacin (CFX)	-	Fluoroquinolones	Pharmaceutical	85721-33-1	331.34		0.28	6.09
N-desmethylvenlafaxine (N-desVFX)	Norvenlafaxine	Anti-depressants	Pharmaceutical	149289-30-5	263.37		Not avaliable	14.42
Salicylic acid (SCY)	2-Hydroxybenzoic acid	Anti-septics	Pharmaceutical	69-72-7	138.12		2.26	2.98
Caffeine (CFF)	1,3,7-Trimethylxanthine; guaranine	Stimulants	Drug	58-08-2	194.19		-0.07	14

Table S2. Variables studied in each sample.

Acronym	Variables
BCS	Barriers irrigation, clayly soil, sprinkling mode
BCD	Barriers irrigation, clayly soil, dripping mode
BSS	Barriers irrigation, sandy soil, sprinkling mode
BSD	Barriers irrigation, sandy soil, dripping mode
WSS	Secondary irrigation, sandy soil, sprinkling mode
WSD	Secondary irrigation, sandy soil, dripping mode
WCS	Secondary irrigation, clayly soil, sprinkling mode
WCD	Secondary irrigation, clayly soil, dripping mode

Table S3. Recovery efficiency at three concentration levels.

Compound	BP3	BP1	BP2	4HB	4DHB	DHMB	AVO	BP4 (-)	4MBC	EHMC	EtPABA	BZT	MeBZT	DMBZT	UVP	BePB (-)	BuPB (-)	PrPB (-)
5 ng/g dw (1)	5.2	3.2	7.2	4.0	4.8	5.9	4.3	2.8	6.4	4.2	2.8	5.1	4.9	3.3	7.7	5.6	6.0	6.7
5 ng/g dw (2)	4.9	5.4	5.9	4.4	3.6	5.7	3.0	4.1	4.6	4.0	3.4	4.0	3.8	2.0	8.4	4.8	4.6	5.7
5 ng/g dw (3)	4.2	3.5	8.8	3.5	5.6	4.7	2.9	3.2	4.8	2.7	2.3	5.7	2.9	2.6	7.1	4.8	4.9	6.3
100 ng/g dw (1)	100.0	81.0	60.8	98.0	96.7	139.0	110.8	102.0	107.5	78.3	93.0	95.0	110.3	123.0	103.3	107.0	125.4	101.0
100 ng/g dw (2)	97.4	82.8	74.7	95.0	93.5	138.0	106.8	100.0	110.5	73.3	133.0	99.0	118.3	86.0	104.3	114.0	134.4	112.0
100 ng/g dw (3)	109.0	84.4	79.3	103.0	94.8	142.0	112.8	98.2	105.5	79.3	128.0	98.3	115.3	127.7	86.8	118.0	137.4	109.0
350 ng/g dw (1)	311.0	239.9	377.0	255.0	246.0	396.0	392.8	353.0	365.5	266.3	374.0	360.0	453.3	516.7	406.8	345.0	328.4	339.0
350 ng/g dw (2)	320.0	256.9	347.0	272.0	229.0	356.0	354.8	258.0	299.5	308.3	208.0	330.0	256.0	416.7	274.8	325.0	355.4	269.0
350 ng/g dw (3)	396.0	293.9	359.0	351.0	352.0	334.0	376.8	428.0	291.5	369.3	317.0	385.0	423.3	363.0	378.3	396.0	348.4	377.0

Compound	MePB (-)	FLU	NDX	OXL	TCY	Succynil-STZ	SDZ	acSDZ	SMR	acSMR	acSMZ	SMX	acSMX	SMPZ	SPY	acSPY	SQX	STZ
5 ng/g dw (1)	6.4	2.7	6.1	2.6	4.5	5.9	3.5	4.0	2.4	5.8	5.9	2.1	6.7	2.2	1.9	4.2	5.2	5.9
5 ng/g dw (2)	4.3	2.3	7.2	3.1	4.3	5.7	2.4	4.4	1.6	5.6	5.3	2.4	7.8	1.6	1.7	4.9	4.0	5.3
5 ng/g dw (3)	5.7	2.8	5.6	3.4	4.0	4.9	2.5	3.6	1.7	7.7	5.0	1.8	6.1	2.2	1.5	4.7	4.5	5.3
100 ng/g dw (1)	95.2	99.0	108.0	114.5	7.4	119.0	121.3	116.0	109.4	114.0	100.0	124.8	101.2	142.4	123.2	103.0	79.3	113.2
100 ng/g dw (2)	101.7	104.8	92.9	96.0	8.2	100.5	87.7	104.0	75.1	129.0	116.7	94.0	105.2	80.4	78.0	108.5	92.0	101.2
100 ng/g dw (3)	107.8	97.3	105.7	112.8	9.5	108.5	144.0	134.0	136.0	130.0	118.0	139.2	106.2	95.6	117.2	102.5	134.0	158.0
350 ng/g dw (1)	380.0	315.8	270.6	366.8	104.0	332.5	350.0	380.0	385.7	300.0	376.7	456.0	296.0	324.4	391.2	328.5	394.1	424.0
350 ng/g dw (2)	379.0	328.3	325.7	378.2	70.8	330.0	380.0	311.0	410.0	353.0	273.3	389.0	356.0	420.0	357.0	368.0	285.2	286.0
350 ng/g dw (3)	341.7	330.8	305.7	384.0	63.6	382.5	344.0	274.0	315.0	313.0	404.0	399.0	300.0	315.0	300.0	279.0	256.0	293.4

Compound	SMD	SDM	TMP	GMZ (-)	MFA (-)	NPX (-)	DCF (-)	DCF-13C (-)	KPF (-)	IBU (-)	AAP	CBZ	CBZ-E	ATL	norFXT	CFX	N-desVFX	SCY (-)	CFF
5 ng/g dw (1)	3.0	2.1	4.4	4.6	5.2	3.0	6.8	2.2	3.4	6.8	4.9	5.3	5.8	3.3	7.1	5.1	4.2	3.6	7.1
5 ng/g dw (2)	2.9	2.4	3.8	5.1	5.5	2.7	4.7	1.4	3.9	7.5	4.3	5.5	5.2	3.0	6.6	6.0	4.7	5.4	3.9
5 ng/g dw (3)	3.7	1.5	3.5	4.8	3.6	3.7	4.4	2.6	5.8	7.1	6.7	4.9	5.3	4.2	6.4	7.6	6.1	6.1	5.8
100 ng/g dw (1)	112.9	122.9	96.4	108.1	132.2	90.4	67.3	57.8	93.5	94.8	115.1	103.0	93.2	123.0	70.1	10.8	104.5	70.6	87.4
100 ng/g dw (2)	80.9	72.3	96.3	105.7	137.6	86.9	71.2	60.5	92.5	99.2	91.1	106.0	90.6	86.4	72.4	9.7	104.5	88.0	95.0
100 ng/g dw (3)	132.6	94.9	86.8	96.9	141.2	87.5	65.0	62.6	92.8	106.4	92.3	99.3	90.0	82.9	66.3	6.9	82.0	93.8	116.4
350 ng/g dw (1)	360.0	372.0	384.0	388.9	264.0	303.0	95.3	97.8	317.5	352.0	238.6	271.0	254.7	383.0	196.3	109.0	450.0	325.8	408.6
350 ng/g dw (2)	227.4	254.3	390.0	376.9	244.0	290.0	100.3	95.5	347.5	282.0	249.4	269.0	194.7	343.0	131.3	142.0	382.5	325.8	310.0
350 ng/g dw (3)	397.3	225.1	318.0	302.9	246.0	383.0	100.3	94.8	355.0	394.0	368.6	314.0	250.7	375.0	217.5	48.0	331.0	322.8	325.0

(1), (2), (3): Number of replica; (-): analyzed in negative mode.

Table S4. Average of the recovered values at each spiked concentration and relative standard deviation to evaluate inter and intra-day precision.

Compound	Average C1	Average C2	Average C3	RSD% Intra C1	RSD% Intra C2	RSD% Intra C3	RSD% Inter C1	RSD% Inter C2	RSD% Inter C3
BP3	95.1	102.1	97.8	10.9	6.0	13.6	17.3	22.3	34.2
BP1	80.2	82.7	75.3	29.3	2.1	10.5	5.0	24.6	30.0
BP2	146.0	71.6	103.1	19.9	13.5	4.2	29.0	22.6	32.0
4HB	79.3	98.7	83.6	11.4	4.1	17.5	5.1	26.2	12.7
4DHB	93.4	95.0	78.8	20.7	1.7	24.2	3.3	16.9	27.8
DHMB	108.9	139.7	103.4	11.8	1.5	8.7	7.6	3.4	4.1
AVO	67.7	110.1	107.1	22.7	2.8	5.1	15.8	34.5	1.2
BP4 (-)	67.7	100.1	99.0	20.1	1.9	24.6	29.7	18.5	33.2
4MBC	105.6	107.9	91.1	18.6	2.3	12.7	24.6	24.3	28.7
EHMC	72.4	77.0	89.9	23.1	4.2	16.5	32.9	4.4	20.3
EtPABA	56.7	118.0	85.6	19.2	18.5	28.1	3.0	8.1	10.9
BZT	99.0	97.4	102.4	17.4	2.2	7.7	6.6	14.6	19.7
MeBZT	77.2	114.7	107.9	25.4	3.5	28.2	8.4	2.1	17.9
DMBZT	52.4	112.2	123.5	24.1	20.3	18.0	2.2	20.7	30.7
UVP	154.0	98.1	100.9	8.8	10.0	19.7	11.7	14.0	27.6
BePB (-)	101.1	113.0	101.5	8.8	4.9	10.3	17.3	4.2	13.5
BuPB (-)	103.0	132.4	98.3	14.0	4.7	4.1	15.7	10.4	30.2
PrPB (-)	124.7	107.3	93.8	8.0	5.3	16.7	7.8	9.7	8.0
MePB (-)	109.3	101.6	104.8	19.6	6.2	6.0	5.7	31.8	6.6
FLU	52.3	100.4	92.8	10.9	3.9	2.5	24.1	22.4	7.8
NDX	126.4	102.2	85.9	12.4	8.0	9.3	28.4	13.5	29.3
OXL	60.9	107.8	107.5	12.9	9.5	2.3	27.4	1.9	0.1
TCY	85.3	8.4	22.7	6.4	12.5	27.1	12.6	25.4	12.7
Succynil-STZ	110.0	109.3	99.5	9.2	8.5	8.5	8.1	19.4	2.7
SDZ	55.4	117.7	102.3	22.0	24.1	5.4	21.3	12.9	22.5
acSDZ	79.7	118.0	91.9	9.4	12.8	16.7	21.6	24.2	19.2

SMR	37.7	106.9	105.8	22.7	28.6	13.3	22.8	32.5	8.5
acSMR	126.9	124.3	92.0	17.9	7.2	8.6	23.1	34.2	34.9
acSMZ	108.1	111.6	100.4	8.8	9.0	19.6	31.1	23.4	29.0
SMX	41.8	119.3	118.5	13.5	19.3	8.7	2.5	22.2	13.3
acSMX	137.5	104.2	90.7	12.3	2.5	10.6	5.2	22.2	29.3
SMPZ	40.0	106.1	100.9	16.0	30.4	16.5	9.6	27.0	33.1
SPY	33.9	106.1	99.8	11.4	23.1	13.2	31.1	17.0	11.3
acSPY	91.9	104.7	92.9	7.2	3.2	13.7	30.4	2.8	3.3
SQX	91.8	101.8	89.1	12.8	28.1	23.4	34.9	31.1	1.6
STZ	109.9	124.1	95.6	6.1	24.1	23.2	25.4	29.8	2.3
SMD	63.4	108.8	93.8	14.2	24.0	27.2	13.4	5.8	1.5
SDM	40.1	96.7	81.1	22.7	26.2	27.4	22.5	32.2	7.0
TMP	77.9	93.2	104.0	11.5	5.9	11.0	4.4	23.4	4.8
GFZ (-)	96.8	103.6	101.8	4.9	5.7	13.1	24.6	24.2	8.9
MFA (-)	95.6	137.0	71.8	21.3	3.3	4.4	16.9	6.1	30.2
NPX (-)	62.6	88.3	93.0	17.6	2.1	15.5	34.1	26.6	16.9
DCF (-)	106.0	67.8	28.2	24.7	4.6	2.9	33.1	24.5	8.4
DCF-^{13C} (-)	41.9	60.3	27.4	28.7	4.0	1.6	16.7	14.4	26.4
KPF (-)	86.7	92.9	97.1	29.4	0.6	5.8	25.7	24.5	21.7
IBU	142.5	100.1	97.9	5.4	5.8	16.5	32.8	6.3	13.0
APH (-)	106.5	99.5	81.6	23.6	13.6	25.3	19.7	34.8	5.4
CBZ	104.8	102.7	81.3	5.9	3.3	8.9	19.5	31.6	28.9
CBZ-E	108.6	91.2	66.7	5.2	1.9	14.4	12.6	22.0	26.8
ATL	69.7	97.4	104.9	18.4	22.8	5.8	29.2	12.1	28.9
norFXT	134.1	69.6	51.9	5.6	4.5	24.7	19.8	6.2	14.9
CFX	124.6	9.1	28.5	20.1	22.3	47.8	19.7	25.0	28.7
N-desVFX	99.5	97.0	110.8	19.4	13.4	15.4	9.0	32.6	21.7
SCY (-)	100.3	84.1	92.8	25.7	14.4	0.5	31.3	9.3	20.5
CFF	112.3	99.6	99.4	28.3	15.1	15.3	17.6	22.0	31.8

C1: Spiked concentration 1; C2: Spiked concentration 2; RSD%: Relative standard deviation; Intra: Intra-day; Inter: Inter-day

Table S5. Table of the results obtained for all the samples and compounds studied, with its ranges and frequencies of detection.

Compound	BSS	BCS	BCD	BSD	WSD	WCS	WSS	WCD	Range	Positive	Frequency (%)
BP3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
BP1	0.77	<LOD	0.82	2.76	0.69	1.17	3.56	0.42	0.42-3.56	7	87.5
BP2	1.48	54.4	7.03	9.81	5.32	8.14	5.37	13.8	1.48-54.4	8	100
4HB	11.8	64.1	26.6	59.8	80.6	80.1	79.9	84.1	11.8-84.1	8	100
4DHB	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
DHMB	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
AVO	0.85	1.69	6.54	<LOD	1.34	2.13	2.04	0.58	0.58-6.54	7	87.5
BP4 (-)	0.17	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.17	1	
4MBC	<LOD	<LOD	<LOD	0.1	0.71	0.3	1.62	0.48	0.1-1.62	5	62.5
EHMC	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
EtPABA	0.3	1.06	<LOD	<LOD	0.42	<LOD	1.17	0.73	0.3-1.17	5	62.5
BZT	1.52	0.57	5.32	7.05	1.43	6.55	0.49	1.4	0.49-7.05	8	100
MeBZT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
DMBZT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
UVP	1.97	2.51	5.35	1.71	2.39	4.19	1.44	1.94	1.44-5.35	8	100
BePB (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
BuPB (-)	0.4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.4	1	12.5
PrPB (-)	<LOD	<LOD	0.94	1.42	<LOD	0.97	<LOD	<LOD	0.94-1.42	3	37.5
MePB (-)	<LOD	10.24	0.48	1.52	4.52	7.78	1.14	10.9	0.48-10.86	7	87.5
FLU	0.52	1.94	4.25	<LOD	0.62	1.48	1.53	0.52	0.52-4.25	7	87.5
NDX	1.18	19	10.1	2	3.94	3.57	2,078	4.08	1.18-19	8	100
OXL	0.66	1.56	4.91	0.41	0.36	1.91	1.91	<LOD	0.36-4.91	7	87.5
TCY	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
Succynil-STZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SDZ	<LOD	<LOD	4.03	2.23	<LOD	2.95	<LOD	<LOD	2.23-4.03	3	37.5
acSDZ	1.3	<LOD	<LOD	<LOD	<LOD	<LOD	1.44	<LOD	1.3-1.44	2	25
SMR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
acSMR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
acSMZ	0.81	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.81	1	12.5
SMX	2.58	<LOD	<LOD	<LOD	2.97	<LOD	<LOD	<LOD	2.58-2.97	2	25
acSMX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SMPZ	<LOD	2.59	1.55	<LOD	<LOD	<LOD	1.42	<LOD	1.42-2.59	3	37.5
SPY	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
acSPY	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SQX	<LOD	1.02	4.4	<LOD	<LOD	<LOD	<LOD	<LOD	1.02-4.40	2	25
STZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SMD	0.26	2.53	0.93	<LOD	14.8	2.31	3.71	10.6	0.26-14.83	7	87.5
SDM	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
TMP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
GFZ (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
MFA (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
NPX (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
DCF (-)	3.68	19.5	24.9	26.9	9.31	9.76	16.9	12.8	3.68-26.90	8	100
DCF-^{13C} (-)	<LOD	<LOD	<LOD	0.48	<LOD	<LOD	<LOD	<LOD	0.48	1	12.5
KPF (-)	<LOD	<LOD	<LOD	<LOD	<LOD	1.25	<LOD	0.45	0.45-1.25	2	25
IBU	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
APH (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
CBZ	<LOD	<LOD	<LOD	<LOD	0.09	0.27	<LOD	<LOD	0.09-0.27	2	25
CBZ-E	0.38	0.34	0.4	0.33	0.33	0.36	0.42	0.35	0.33-0.42	8	100
ATL	<LOD	<LOD	<LOD	<LOD	1.16	1.24	<LOD	<LOD	1.16-1.24	2	25
norFXT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.23	2.23	1	12.5
CFX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
N-desVFX	0.54	0.87	1	1.29	0.95	0.85	0.87	0.73	0.54-1.29	8	100
SCY (-)	6.14	14	19.9	19.4	18	53.8	10.7	9.46	6.14-53.80	8	100
CFF	8.8	15.24	<LOD	16.5	26.8	13.26	15.7	16	8.80-26.80	7	87.5

Values are in ng/g dw; <LOD: value below the limit of detection

S3.1 Correlation analysis.

The different samples studied (**Table S4**) were tested to find correlations between them with the t-test for paired samples. All of the variables showed to be correlated except two pairs, as it is shown below:

t.test (data$BCS, data$BCD, paired = TRUE, alternative = ”two.sided”)

t = -2.1674, df= 54, p-value = 0.03463 ; alternative hypotesis: true difference in means is not equal to 0

95 percent confidence interval: - 5.847 - 0.227 ; mean of the differences: - 3.03727

t.test (data$BCS, data$BSS, paired = TRUE, alternative = ”two.sided”)

t = -2.5365, df= 54, p-value = 0.01412 ; alternative hypotesis: true difference in means is not equal to 0

95 percent confidence interval: - 2.712 - 0.317 ; mean of the differences: - 1.5145

Both tests have a p-value < 0.05, indicating that the pairs BCS-BCD and BCS-BSS are statistically independent between them.

S3.2 Principal component analysis (PCA):

To perform a PCA, autoscale was chosen as preprocessing of the data, followed by the leave one out cross validation method. After, two principal components were selected and the method was built. Then, scores plot, loadings plot and biplot of scores and loadings were built:

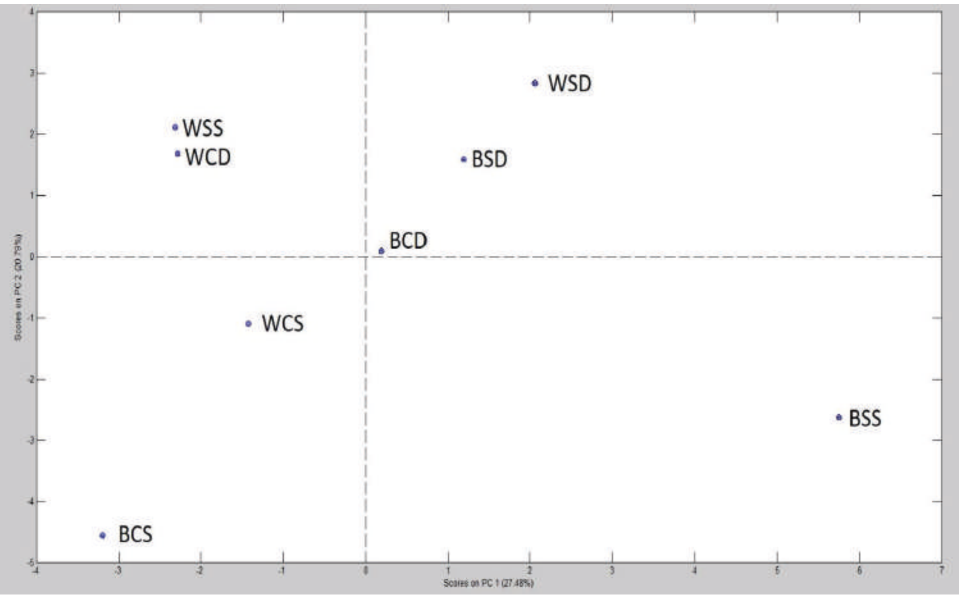


Figure S6. Scores plot

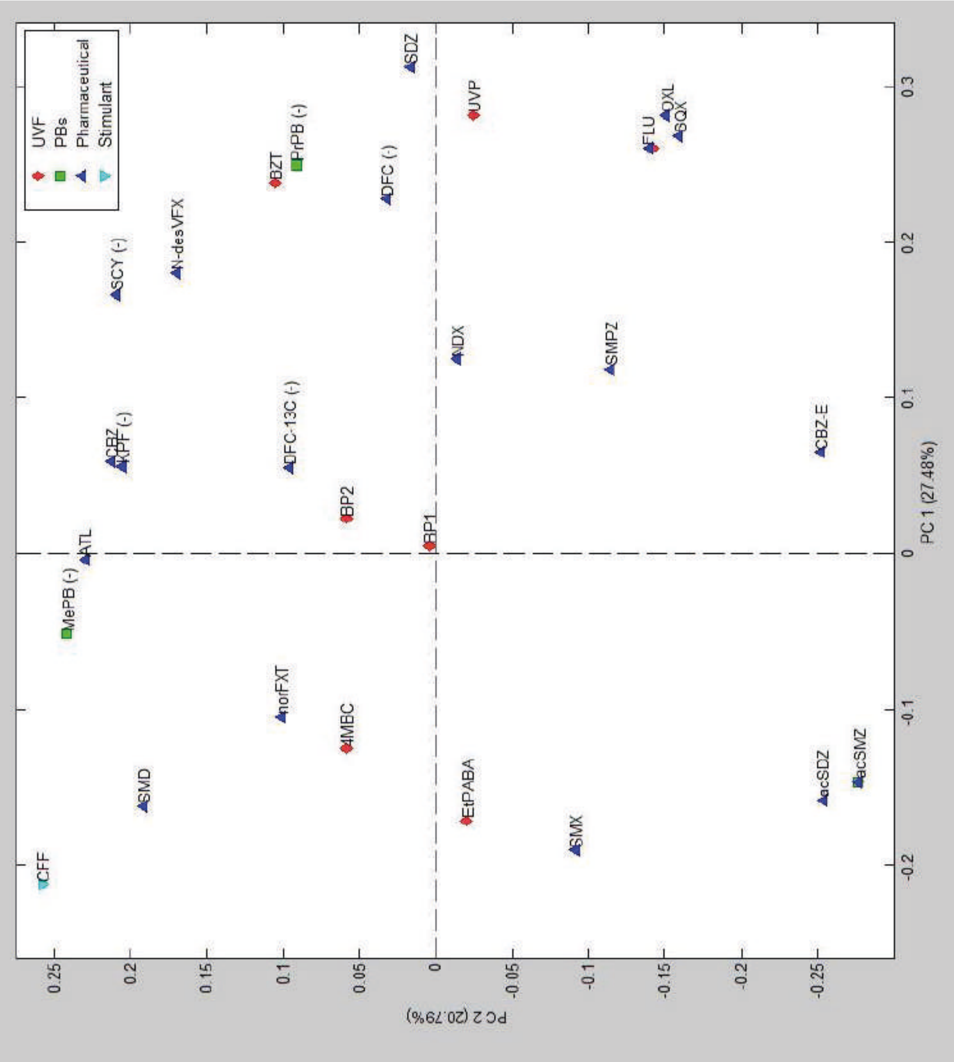


Figure S7. Loadings plot.

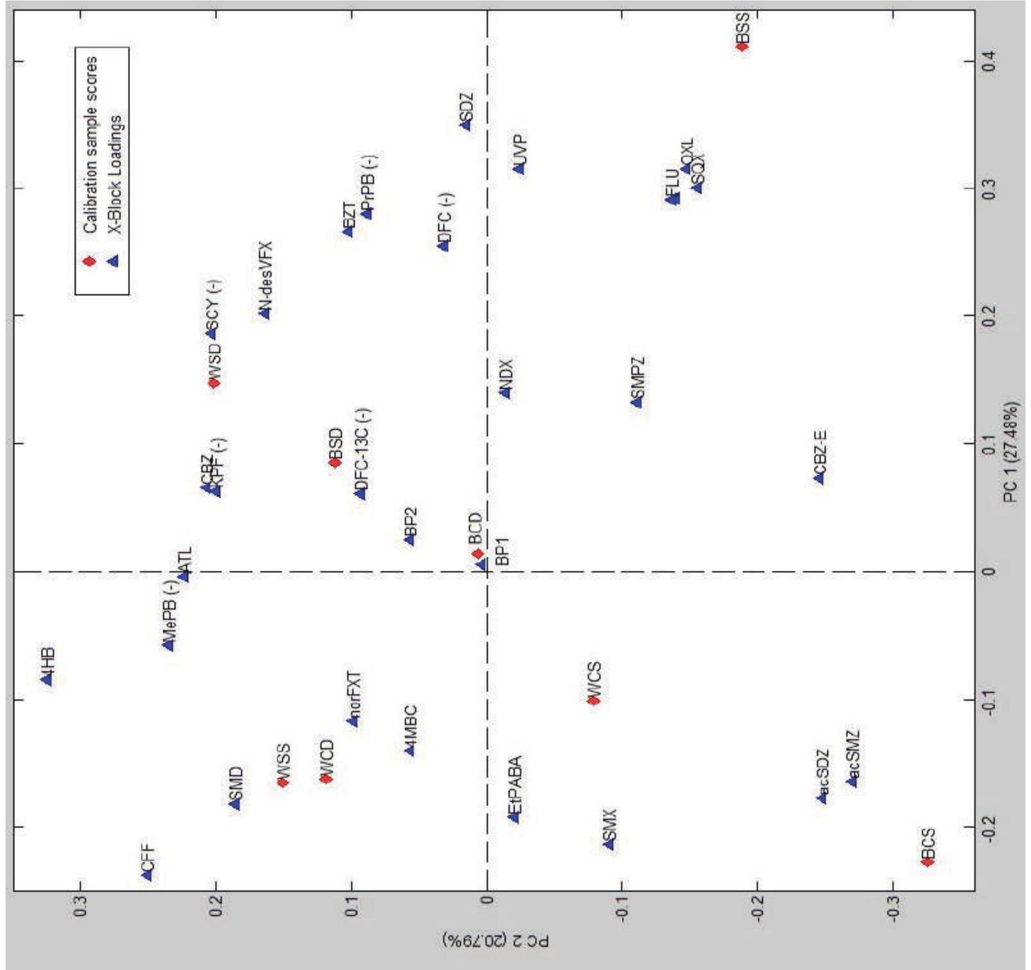


Figure S8. Biplot of the scores and loadings.

5.2.2 Publication #10

Reclaimed water in agriculture: a plot-scale study assessing crop uptake of emerging contaminants and pathogens

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Reclaimed water in agriculture: A plot-scale study assessing crop uptake of emerging contaminants and pathogens

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A B S T R A C T

Nowadays, water is a scarce resource, hence, water management is crucial as demand for agricultural, urban, and industrial purposes increases. The use of reclaimed water in agriculture can be a suitable solution. However, pathogens and chemical contaminants of emerging concern (CECs) present in reclaimed water can accumulate in the soil and ultimately, in the crop. To evaluate the potential transfer of biological and chemical pollutants from water to crop, two plots were designed for the cultivation of lettuce under field conditions. In this study, the influence of water quality, soil composition, and irrigation system on plant uptake of CECs and pathogens was assessed. The applied reclamation process reduced total suspended solids, *E. coli* (3–5 ulog), sulfite-reducing clostridia spores (1 ulog), Helminth eggs, and *Legionella spp* levels (complete removal) in water. Sodium adsorption ratio (SAR) and electric conductivity (EC) in the soils irrigated with reclaimed water were lower, and *E. Coli* was not detected. In lettuces, *E. coli* was only present in the crops irrigated with wastewater. Pharmaceuticals were the most frequently detected CECs in soils and waters, whereas UV filters achieved the highest concentrations. Diclofenac and salicylic acid were the most accumulated in soils, and diclofenac, ofloxacin, and benzophenone-4 were the most prevalent in the WWTP effluent. The irrigation water quality was the factor driving the transfer of CECs to the crops. Results show that the best combination to reduce pathogens and CECs was the use of reclaimed water, soils with high content of clay, and a sprinkling irrigation system.

1. Introduction

Fresh water is essential for human life, yet more than a billion people lack access to water, and by 2025, two-thirds of the world's population could suffer from a lack of water. When water is scarce, people cannot get enough to drink, wash or grow crops, leading to economic decline. Water scarcity is an issue that aggravates with population growth, increased food and energy demands, economic development, and environmental pollution [31]. Water is not only input for economic activities, it provides ecosystem services such as the maintenance of wetlands and river flows, and support for wildlife [53].

Climate change is one of the main causes of the shortage in water and one of the drivers of its changing demand [17]. Current data evidence the need to find alternatives to increase freshwater availability. A possible solution to this issue may be the integrated use of all available water resources, especially reclaimed water, that can be prioritized for purposes that do not require high quality, such as could be agriculture [30].

Wastewater use in agriculture has been a widely studied solution to deal with water shortages with promising benefits such as reduced fertilizer consumption [57]. Nevertheless, wastewater can contain a myriad of substances potentially toxic. Several pollutants ranging from heavy metals to organic chemical compounds - many of them constituting contaminants of emerging concern (CECs) including microplastics, pharmaceuticals, and personal care products (PPCPs) - can be found in wastewater [6], together with pathogens. Most of these chemical contaminants are released in large quantities to the sewerage systems and are only partially removed in wastewater treatment plants (WWTPs), reaching environmental compartments [22]. While waiting for more efficient and economic wastewater treatment technologies

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allowing the complete removal of pathogens and CECs in the WWTPs, their monitoring of the environment is essential. A pathogen is any microorganism that can cause disease once entering the human body, so its occurrence in any matrix (water, soil and especially crops) involved in the production of fresh food intended for human consumption must be avoided. Regarding CECs, many studies have reported adverse health effects on aquatic ecosystems and humans [26], as many of them display endocrine disruption [8] and antibiotic residues contribute to antibiotic resistance dissemination [45]. Therefore, if reclaimed water use is to be implemented, it is urgent to evaluate the occurrence of pathogens and CECs in the reclaimed water and to estimate their potential uptake by crops.

Regulation at the national (Ministerio de la Presidencia, 2007) or European level (The European Parliament and the Council, 2020) do not specify limitations about pathogen levels in crops. Only in some countries, pathogen and certain physicochemical parameters of reclaimed water aimed for agriculture irrigation are regulated for *E. coli*, total coliforms, turbidity, pH, and residual chlorine ([11,51]; the United States Environmental Protection Agency, 2022). Regarding CECs, they are mentioned as “additional requirements”, but no limits of concentration in the irrigation water, soil or crop are specified. It is likely because most of the literature on the assessment of CECs' uptake by plants deals with case studies far from real scenarios [13,16,2,63]. At the global level, the World Health Organization's (WHO) latest guidelines were published in 1987 and followed the same pattern as the other regulations [60].

The scientific knowledge on plant joint exposure to pathogens and CECs under field conditions is yet limited because most studies focus solely on microbiological contamination or chemical contamination and were performed in hydroponic and greenhouse environments. So far, under field conditions, the effects of treated wastewater or reclaimed water use in terms of microbiological safety have only been evaluated in tomato, lettuces, chili, broccoli, and soil ([1,33,36,5]; A M [39]). Regarding CECs, their plant uptake from treated and reclaimed wastewater under real field conditions has been evaluated in carrots, potatoes, cabbage, spinach, radish, corn, rice, tomato, lettuce, cauliflower, broad and long bean, eggplant, cucumber, wheat, and maize [15,43,34,35,40, 50,49].

All these studies proved the potential capacity of pathogens and CECs to reach the crops when irrigating with treated wastewater or reclaimed water, and even some of them show the relevance of the irrigation water quality in the final uptake of contaminants by the crop. Theoretically, other variables such as soil composition and irrigation system may also influence the contaminants' uptake. For example, Forslund et al., [19] and Palese et al., [39] stated that sprinkling irrigation or clayey soils enhance the survival of pathogens, thus increasing the possibility to be transferred to the crops. Drip irrigation has been commonly investigated ([15,1,33,35,36]; A. M. [39]), but there were also several studies using sprinkling irrigation systems [19], wetland columns [5], furrow [37,40] or even gravity irrigation [43,34]. The same applies to soil composition, since sandy soils were the most studied [35], but also combined with clay (from 3% to 40% of the total), loam, or silt ([15,43,1,33,34,37]; A. M. [39]). Due to this marked diversity of setups employed, comparison among results is impeded, and sound conclusions about the role that all these variables may have in the uptake of contaminants by crops cannot be drawn.

To the best of the authors' knowledge, none of the mentioned studies performed under real conditions have evaluated the joint influence of the selected variables. In this scenario, the present study aims to evaluate crop uptake of both microbiological and chemical organic pollutants from irrigation water, as well as the role of the soil, water quality, and the irrigation system in this process in real field conditions.

2. Materials and methods

2.1. Standards and reagents

All the standards used were > 98% of purity. Benzophenone-3 (BP3), benzophenone-1 (BP1), benzophenone-4 (BP4), 4HB, 4,4′-dihydroxybenzophenone (4DHB), avobenzone (AVO), UVP, 5,6-dimethyl-1 H-benzotriazole (DMBZT), nalidixic acid (NDX), oxolinic acid (OXL), tetracycline (TCY), succynil-sulfathiazole (S-STZ), sulfadiazine (SDZ), N4-acetylsulfadiazidine (acSDZ), sulfamerazine (SMR), N4-acetylsulfamerazine (acSMR), N4-acetylsulfamethazine (acSMZ), sulfamethoxazole (SMX), N4-acetylsulfamethoxazole (acSMX), sulfamethoxypyridazine (SMPZ), sulfapyridine (SPY), N4-acetylsulfapyridine (acSPY), sulfaquinoxaline (SQX), sulfathiazole (STZ), sulfisomidine (SMD), sulfadimethoxine (SDM), trimethoprim (TMP), acetaminophen, also known as paracetamol (APH), atenolol (ATL), gemfibrozil (GFZ), ketoprofen (KPF), mefenamic acid (MFA), carbamazepine (CBZ), norfluoxetine (norFXT), ofloxacin (OFX), ciprofloxacin (CFX), caffeine (CFF), ibuprofen (IBU), salicylic acid (SCY), diclofenac (DCF), diclofenac-13 C (DCF-13 C), methyl paraben (MePB), propyl paraben (PrPB), benzyl paraben (BePB), butyl paraben (BuPB) and benzophenone-(carbonyl-13 C) (BP-13 C) were purchased from Sigma Aldrich (Darmstadt, Germany). BP2, 2,2′-dihydroxy-4-methoxybenzophenone (DHMB), ethyl-4-(dimethyl-amino) benzoate (EtPABA), ethylhexyl methoxycinnamate (EHMC), and benzotriazoles (BZT) were obtained from Merck (Darmstadt, Germany). Enzacamen or 4-methyl benzylidene camphor (4MBC) was provided by Dr. Ehrenstorfer (Augsburg, Germany). 5-Methyl benzotriazole (MeBZT) was obtained from TCI (Zwijndrecht, Belgium). Flumequine (FLU), N-desmethylvenlafaxine (N-desVFX), diclofenac 4-hydroxy (DCF-OH), carbamazepine 10,11-epoxy (CBZ-E), and sulfamethazine-d4 (SMZ-d4) and acetaminophen-d4 (APH- d4) were purchased from Toronto Research Chemicals (Toronto, Canada). Oxytetracycline (O-TCY) and naproxen (NPX) were obtained from Honeywell Fluka (Wabash, United States). 2-hydroxy-4-methoxy-2′,3′,4′,5′,6′-d5 (BP3-d5), (±)– 3-(4-methylbenzylidene-d4) camphor (4MBC-d4), 1 H-benzotriazole-4,5,6,7-d4 (BZT-d4), flumequine-13C3 (FLU-13C3), trimethoprim-d3 (TMP-d3), carbamazepine-d10 (CBZ-d10), mefenamic acid-d3 (MFA-d3), caffeine-d3 (CFF-d3), ibuprofen-d3 (IBU- d3), salicylic acid-d6 (SCY-d6), diclofenac-d4 (phenyl-d4) (DCF-d4), benzyl paraben-d4 (BePB-d4) and 5-(2,5-dimethylphenoxy)– 2,2-bis(tri-deuteriomethyl)pentanoic acid (GMZ-d6) were from CDN isotopes (Quebec, Canada).

Information about solvents, stock solutions, extraction and analysis kits can be found in the Supporting Information (SI).

2.2. Field site

Palamós WWTP, the fifth-biggest plant in Catalonia (Spain), was selected to assemble the experimental plots. Detailed information about the facility can be found in the SI.

To improve the quality of the WWTP effluent to be used for agricultural irrigation, a Managed Aquifer Recharge pilot system with reactive barriers (rbMAR) was implemented as advanced tertiary treatment. The rbMAR system aims to improve biodegradation by generating different redox potential zones and enhancing microorganisms' CECs removal and pathogens retention. The pilot rbMAR described by Valhondo et al., [56,54] consists of five soil aquifer treatment systems implemented with a compost/wood ship reactive barrier, and a reference system without barrier; further, the infiltrated water flows along a 15 m simulated aquifer. Finally, the effluent is discharged and stored in a tank to homogenize waters for further use. The water used to feed the rbMAR systems is the WWTP effluent (previously homogenized in a tank to overcome differences in effluent water quality over time).

Two experimental agricultural plots, 4 m x 12 m, were constructed next to the rbMAR system (Fig. 1). Plot 1 was irrigated with rbMAR effluent (B water) and Plot 2 with WWTP effluent (W water). The plots were made up of two types of soil: the first one was the soil from the WWTP terrain, mainly sand (S soil) and the second one was the same soil to which clay was added up to 10% content (C soil). The plots were implemented with two irrigation systems: drip and sprinkler. Different sensors were installed in the plots to control in real-time several parameters: two DataLogger Decagon Em50G to the system ECHO2 were installed for reading, storage, and data transmission via GPRS, and eight 5TE Decagon probes were installed to measure temperature, electrical conductivity, and soil moisture at six depths (10, 20, 30, 40, 50 and 60 cm). These probes were installed in the plots avoiding areas where water could have direct contact with the sensor. Two flowmeters Decagon were also installed for monitoring the flow rate of the irrigation waters. Real-time control of water availability allowed the automated irrigation system to only activate when the crops needed it i.e. after a storm event, the sensors detect large amounts of water at different depths and cancel the scheduled irrigation. On the contrary, in times of drought or hot summer, when water evaporates quickly, the sensors activate irrigation more frequently than the dulled.

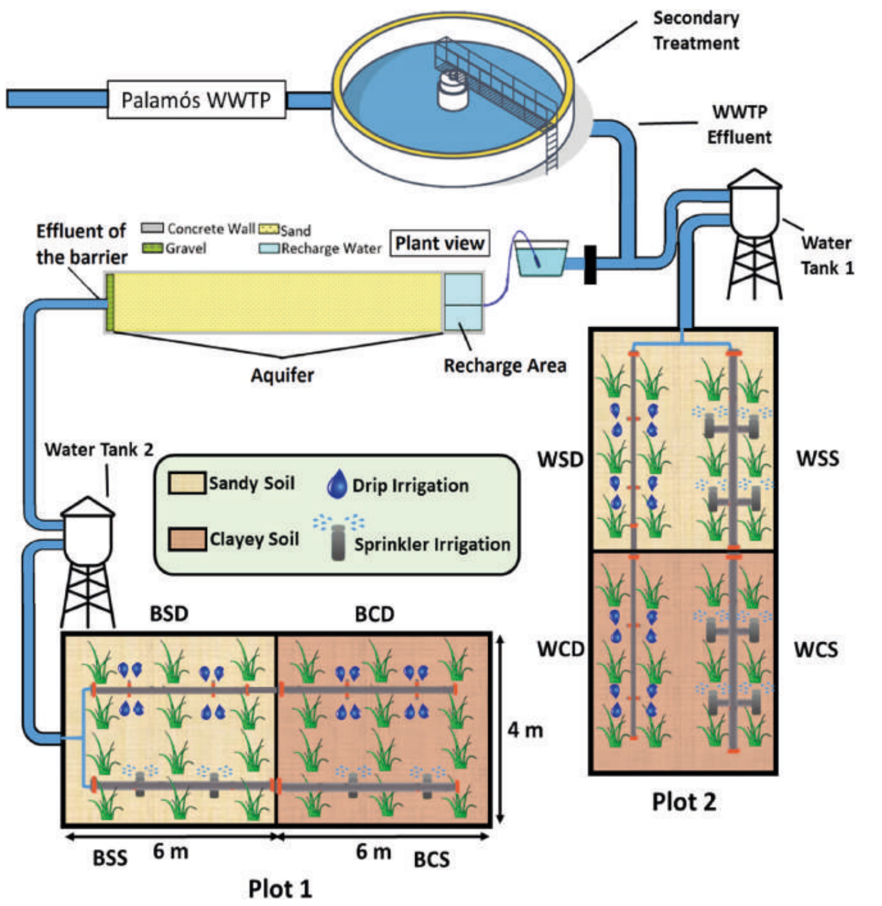

Fig. 1. Diagram of the experimental agricultural plots. BSD: Barriers irrigation, sandy soil, drip irrigation; BCD: Barriers irrigation, clayey soil, drip irrigation; BSS: Barriers irrigation, sandy soil, sprinkler irrigation; BCS: Barriers irrigation, clayey soil, sprinkler irrigation; WSD: Wastewater irrigation, sandy soil, drip irrigation; WCD: Wastewater irrigation, clayey soil, drip irrigation; WSS: Wastewater irrigation, sandy soil, sprinkler irrigation; Wastewater irrigation, clayey soil, sprinkler irrigation.

2.3. Sampling

2.3.1. Water samples

For characterization, water samples were collected throughout each season of the year and additionally at harvest time. Regarding microbiological analysis and physicochemical characterization, W and B water samples were collected in sterilized 1 L glass bottles and transported in portable fridges to the laboratory. The analysis was performed within 24 h after sampling. For CECs analysis, water samples were collected in 1 L brown glass bottles, filled up to ¾ of capacity, and transported under cool conditions to the lab and then frozen.

2.3.2. Soil samples

For microbiological analysis, sterilized plastic bags and glass bottles were used. The microbiological analysis was performed within 24 h after the sampling. Soil samples were collected before sowing, every four months during one year, and at harvest time.

The samples were gathered with a small gardening shovel at the surface, 10 and 20 cm depth around the crops, mixed by subplot and depth, and stored. For physicochemical analysis, soil samples were air-dried, then passed through a 2 mm sieve and stored until analysis. For CECs analysis, soils were freeze-dried and stored in aluminum foil at − 20 °C until analysis.

2.3.3. Lettuces

Chicorium intybus lettuces variety (known as red oak leaf lettuce) was cultivated from October to December 2018. Approximately 50 lettuces per subplot were grown from a previously grown seedling. Once the lettuces were suitable for consumption (market size), 10 specimens per subplot were randomly collected, and further shaken to remove soil particles.

The samples were separated into two groups: one for microbiological analysis and the other for the analysis of CECs. Both were shipped to the laboratories under cool conditions. The roots of the lettuces were very short (few mg), so the whole lettuce had to be used and homogenized. The microbiological analysis was performed within the next 24 h. Upon arrival, the samples for CECs analysis were frozen. The next day, they were thawed, sliced, frozen again, lyophilized, and crushed. Finally, samples were frozen at − 20°C until analysis.

2.4. Analytical methods

2.4.1. Microbiological and physicochemical analysis

Throughout the experiment, physicochemical parameters of water and soil were monitored and are listed in Table 1 [12,7]. For the irrigation water, physicochemical parameters were determined in the four seasons of the year considering the water's variation in quality and quantity. An initial characterization of the soil was carried out before the lettuces were planted.

Microbiological parameters and fecal indicators such as *E. coli* and spores of sulfite-reducing clostridia were analyzed. *Legionella* spp. was monitored because of the risk of transmission by aerosolization. As regards parasites, the presence of helminth eggs was monitored.

Concerning fecal contamination in water and soil, *E. coli* was selected as the indicator and the membrane filter method based on ISO 9308 (2014) [29] was performed. *E. coli* was determined in 30 g of soil and 270 ml of buffer solution, both homogenized for 2 min in a Stomacher to get 10^{-1} dilution [19]. The lettuces were analyzed according to the ISO 6887 (2017) [28]. To have a representative sample, leaves of different parts of the lettuces were cut and 25 g were taken under sterile conditions. Afterward, *E. coli* extraction was performed. Shredded lettuce was placed in sterile bags with 225 ml of peptone water. Subsequently, it was shredded in a Stomacher for 2 min to homogenize the sample and obtain a 10^{-1} dilution. Further, dilutions for the lettuce leaves and soil up to 10^{-4} were made and analyzed in the same way as for the water. The plates were incubated at 36 °C for 24 h and the colony-forming units (cfu) per g dry weight (dw) of soil or vegetable were counted.

Table 1
Methods applied for water and soil analysis (TSS: total suspended solids, COD: chemical oxygen demand, BOD_5: biological oxygen demand, TNK: total Kjeldahl nitrogen, OM: organic matter, EC: electrical conductivity, IC: Ion Chromatography, ICP-OES: Inductively coupled plasma-optical emission spectroscopy).

Parameter	Method for water analysis	Method for soil analysis
TSS	APHA[7], ref. 2540B	–
COD	APHA[7], ref. 5220 C	–
BOD_5	APHA[7], ref. 5220 B	–
TNK	APHA[7], ref. 4500.	ASA (1982)
$N\text{-}NH_4$	APHA[7]	ASA (1982)
NO_3	APHA[7], ref. 4110B. (IC)	–
Cl^-		
SO_4		
OM	–	Wet oxidation by Walkley and Black (1934)
Texture	–	Hydrometer method by Bouyoucos (1962)
pH	pH-meter (Crison GLP21)	ASA (1982) pH-measurement (Crison GLP21)
EC	Conductivity measurement (Crison GLP21)	ASA (1982) Conductivity measurement (Crison GLP21)
Mg^{+2}	APHA[7], ref. 3120B. (ICP-OES)	ASA (1982) (ICP-OES)
Ca^{+2}		
Na^+		

2.4.2. CECs analysis

The determination of CECs in the lettuces and soils was performed following the developed QuEChERS-based method by Sunyer-Caldú and Diaz-Cruz, [48]. Briefly, this method consists of a first extraction step of 1 g dw lettuce using QuEChERS kits (citrate and PSA-Kit-02 kits). Next, an aliquot of 5 ml was evaporated and reconstituted to 1 ml of the final extract. This extract was analyzed in a liquid chromatograph Symbiosis Pico from Spark Holland (Emmen, The Netherlands) using an LC-analytical column Hibar Purosher® STAR® HR R-18 (50 mm × 2.0 mm, 5 µm) coupled to a 4000 QTRAP mass spectrometer from Applied Biosystems-Sciex (Foster City, USA). Analyses were performed in selected reaction monitoring (SRM) mode using the two most intense transitions, in both positive and negative electrospray ionization (ESI+, ESI). The analytes were quantified by isotope dilution using 10 matrix-matched standard solutions to build the calibration curves. For the analysis of soils, the methodology was adapted from our previously developed methods and further validated at three concentrations (10, 50, and 100 ng/g dw). The method limits of detection (MLODs) ranged from 0.01 to 2.92 ng/g dw and the recovery rates were between 60% and 140% for 54 of the 56 determined compounds. Method validation parameters are compiled in the SI.

CECs in water samples were analyzed using on-line solid phase extraction and high-performance liquid chromatography-tandem mass spectrometry (on-line-SPE-HPLC-MS/MS) according to our methodology [21,23,54].

2.5. Quality assurance and quality control

In microbiological analysis, the quality control established by the ISO and Standards methods were followed.

In the CECs analysis, some measures are required to avoid contamination at trace levels. All the glass material was washed with MeOH and acetone and muffled at 400°C for 4 h. Quality control and blank samples were introduced randomly in the sequence of analysis to evaluate the method's performance. The maximum tolerance permitted between chromatographic retention times (t_R) in the calibration curve and the samples was 2.5% and the maximum tolerance permitted for the relative ion intensities between the two selected SRM transitions was 15%. Following the EU normative (Commission Decision 2002/657/EC), all the compounds determined were identified with the t_R and the two selected SRM transitions.

2.6. Uptake factors

Measured CECs concentrations in lettuce, soil, and irrigation water were used to calculate the CECs uptake factors related to soil and water following Eqs. (1)–(3).

$$UF_{SOIL} = \frac{C_{CROP}}{C_{SOIL}} \tag{1}$$

$$UF_{WATER} = \frac{C_{CROP}}{C_{WATER}} \tag{2}$$

$$Kd = \frac{C_{SOIL}}{C_{WATER}} \tag{3}$$

where UF_{soil} is the soil-based uptake factor, UF_{water} is the water-based uptake factor, C_{crop} is the contaminant concentration in the crop, C_{soil} is the contaminant concentration in the soil, C_{water} is the contaminant concentration found in the irrigation water, and Kd is the soil-water sorption coefficient for each contaminant. Uptake factors could only be calculated for contaminants present at least in two matrices. UF_{SOIL}, UF_{WATER} and Kd values are listed in Table S1.

2.7. Statistical methods

T-tests to evaluate individual correlations, principal component analysis (PCA) and partial least squares regression-discriminant analysis (PLS-DA) was performed with RStudio open software, v. 1.2.5001 (2019) RStudio, Inc. The results of the t-tests are included in Table S2.

2. Results and discussion

2.1. Physicochemical parameters

Table 2 lists the quality parameters of W and B waters used for irrigation. W water quality fluctuates notoriously throughout the year. However, the rbMAR system helps to reduce this variability. Beyond, the rbMAR system allows decreasing by 50% of the total suspended solids (TSS), achieving an average concentration of 9,2 mg/L, a COD reduction of 70%, and average removals of 43% of N-NTK and 37% of $N\text{-}NH_4$.

SAR was calculated in irrigation waters according to RD 1620/2007. This parameter informs about the relationship between exchangeable Na^+ and other exchangeable cations. When the concentration of Na^+ is high, it may replace Ca^{2+} and Mg^{+2} which influences soil structure aggregates stability and eventually may minimize soil permeability [59].

W and B waters showed similar SAR and EC, complying with the limits established by RD 1620/2007. Despite SAR values measured in the irrigation waters being high (> 4 meq/L), the also high EC (> 2,5 dS/m) helped to minimize the potential risk of decreasing the infiltration rate [9]. However, high salinity waters may reduce crop yields because of the accumulation of salts in the roots, decreasing water availability to the plant.

Table 2
Quality parameters of W and B waters compared with RD 1620/2007 and Regulation EU 2020/741 standard limits. TSS: total suspended solids, COD: chemical oxygen demand, BOD_5: biological oxygen demand, EC: electrical conductivity, TNK: total Kjeldahl nitrogen, SAR: sodium adsorption ratio. n.d.: Not detected.

Parameter	W water	B water	RD 1620/ 2007 *	Regulation (EU) 2020/ 741 * *
TSS (mg/L)	20 ± 6.5	9.2 ± 4.4	20	10
COD (mg/L O_2)	99 ± 39	28 ± 19		
BOD5 (mg/L O_2)	17 ± 7.1	11 ± 2.4		10
pH	7.8 ± 0.20	7.7 ± 0.30		
EC (dS/m)	2.7 ± 0.80	2.6 ± 0.2	3	
TKN (mg/L)	69 ± 9.2	30 ± 13		
$N\text{-}NH4^+$ (mg/ L)	60 ± 8.2	22 ± 14		
Cl^- (mg/L)	430 ± 26	530 ± 29		
$SO4^{2-}$ (mg/L)	< 0.10	0.60 ± 0.30		
Na^+ (ppm)	230 ± 13	211 ± 10		
Ca^{2+} (ppm)	123 ± 9.8	128 ± 8.5		
Mg^{+2} (ppm)	32 ± 4.4	30 ± 3.6		
SAR (meq/L)	4,79	4,34	6	
***E. coli* (cfu/ 100 ml)**	$4.4 \times 10^6 \pm 1 \times 10^7$	$6.6 \times 10^2 \pm 1 \times 10^3$	1×10^2	1×10^1
Helminth eggs (eggs/ 10 L)	1	n.d.	1	1
Spores of sulfite-reducing clostridia (cfu/100 ml)	$1.9 \times 10^4 \pm 7.0 \times 10^3$	$3.9 \times 10^2 \pm 1.4 \times 10^2$		
***Legionella* spp (cfu/L)**	50	n.d.	1000	1000

* Minimum reclaimed water quality class 2.1; ** Minimum reclaimed water quality class A.

In soils, the organic matter content, total nitrogen, and pH after lettuce harvest did not show significant differences from the values determined before planting. However, soil organic matter content increased after one year of irrigation with both water types (Fig. S1). These results evidenced that B water may increase soil organic matter content minimizing any risk. Increased EC and SAR values of the plot irrigated with W water compared to those of the plot irrigated with B water were observed (Table S3). For the subplots having different sand-clay compositions, significant differences in these two parameters were not observed.

With a specific focus on the soils irrigated with B water, SAR was calculated in triplicate samples collected every four months over one year. As shown in Fig. 2, a significant increase in SAR was observed. Before irrigation, the SAR values in the soil samples were in the range 0,4 – 0,8 meq/L, increasing to 1,1 - 1,2 (150%). This behavior suggests that the use of B water in agriculture might increase soil sodicity and, consequently, alters soil structure and reduces crop production. An excess or deficiency of major plant nutrients, such as Ca^{2+}, can lead to disturbances in the availability, uptake, transport, or distribution of nutrients in the plant [59].

A t-test performed to evaluate if SAR values were influenced by the irrigation system and soil type indicated that both variables influenced SAR. These results agree with those reported by Stevens et al., [47], pointing out an increase in SAR in topsoil irrigated with reclaimed water compared to that in virgin soil. Similarly, Phogat et al., [41] simulated a long-term impact on soil irrigated with reclaimed water and reported a considerable increase in SAR after 7 years of irrigation.

2.2. Pathogen indicators analysis

Concerning microbiological parameters, *E. coli* is considered an indicator of fecal contamination and is regulated by RD 1620/2007 and EU Regulation 2020/741. B water showed reductions between 3 and 5 ulog of *E. coli* and a decrease of 1 ulog of sulfite-reducing clostridia spores compared to the levels measured in the W water. Helminth eggs (1 egg/10 L) and *Legionella spp* (50 cfu/L) were only detected in the W water.

Fig. 3 shows the annual fluctuation of fecal contamination in B water. During tourism's peak season, the *E. coli* rate was found over the limits. All the water samples from March, April, and August exceeded the limits, while 70% of July's samples had less than 1 log of *E. coli*. Nevertheless, in periods when the water input was lower and, consequently, the retention time increased (i.e., in winter), less than 1 log cfu/ 100 ml was detected, which meets the maximum level set up in the RD 1620/2007 (in case there is direct contact of reclaimed water with the edible parts for fresh human food) and with EU Regulation 2020/741.

Regarding fecal contamination in soil, at the time of lettuce harvest, it was observed that the soil irrigated with B water had no *E. coli* (Table 3). In contrast, the plot irrigated with W water by drip in C soil had a higher concentration of *E. coli,* 2,26 × 10^4 cfu/g. The S soil irrigated by drip presented a lower level of fecal contamination (4,3 ×10^2 cfu/g) compared to 5,95 × 10^3 cfu/g when irrigated by the sprinkler system. Similar results were obtained by Forslund et al., [19]. This study showed that the highest values of *E. coli* were obtained in soil irrigated by a micro-sprinkler system using reclaimed water. However, soil properties and weather conditions may stimulate the persistence of *E. coli* in irrigated fields by reclaimed water. Fecal contamination in the soil also depends on the survival capacities of the pathogens [58]. In our study, soil samples were collected in autumn, which might have favored the persistence of the pathogens.

E. coli was not observed in lettuces irrigated with reclaimed water, as

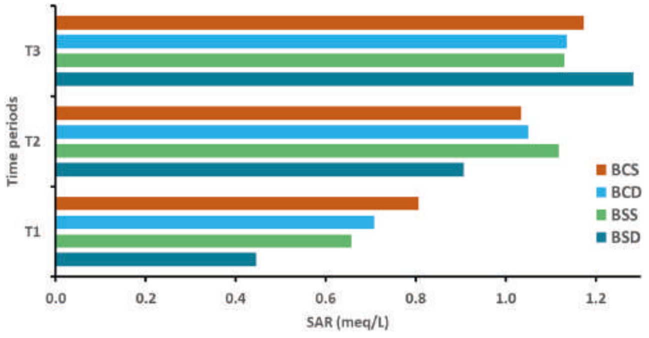

Fig. 2. SAR values in soil irrigated with B water at three time periods. T1: 4 months; T2: 8 months; T3: 12 months. BSD: S soil-drip irrigation; BSS: S soil-sprinkler irrigation; BCD: C soil-drip irrigation; BCS: C soil-sprinkler irrigation.

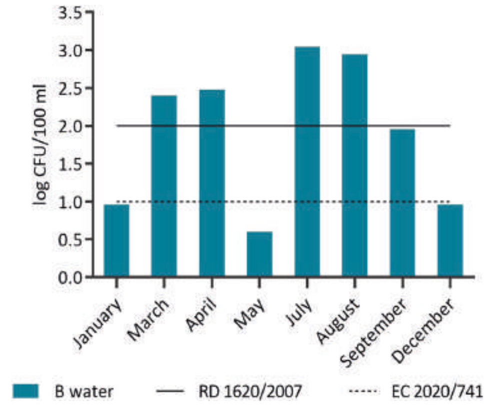

Fig. 3. Seasonal variability of *E. coli* (log cfu/100 ml) in B water compared with RD 1620/2007 and Regulation EU 2020/741 established limits.

Table 3
E. coli levels in soil and lettuces irrigated with W and B waters. S: soil composed of sand, C soil: S soil with 10% clay content.

Irrigation water (cfu/100 ml)	Type of soil	Irrigation system	*E. coli* in soil (cfu/g)	*E. coli* in lettuce (cfu/g)
W	S	Drip	4.3×10^2	n.d.
		Sprinkler	5.9×10^3	9.7×10^3
(2.6×10^5)	C	Drip	2.3×10^4	5.7×10^3
		Sprinkler	1.0×10^3	1.1×10^4
B	S	Drip	n.d.	n.d.
		Sprinkler	n.d.	n.d.
(9.5)	C	Drip	n.d.	n.d.
		Sprinkler	n.d.	n.d.

n.d.: not detected

expected since the B water had less than 10 cfu/100 ml (Table 3). However, its occurrence in W water was considerably higher (2,6 $\times 10^5$ cfu/100 ml), leading to values up to 1,15 $\times$ 10^4 cfu/g in the lettuces irrigated with this water. It is important to emphasize that both soils showed higher *E. coli* value when irrigated by sprinkling, while the lettuces irrigated by dripping presented fecal contamination only when they were cultivated in the C soils. These results are in good agreement with those reported by Mañas et al. [36], who concluded that the use of drip irrigation for ready-to-eat vegetables could avoid microbial contamination. However, in one drip-irrigated lettuce sample of our study, *E. coli* was found, which could be attributed to the wind present in the zone. Nevertheless, the use of B water has not shown any risk of fecal contamination neither in soil nor in lettuce.

2.3. CECs analysis

The occurrence of PPCPs in the lettuces was discussed together with the description of the analytical method developed for their analysis in Sunyer-Caldú and Diaz-Cruz, [48]. In the present work, we focus on soil and water, however, to have the full picture, the concentrations of the PPCPs determined in the lettuces are listed in Table S4. The concentrations for each detected compound, MLODs, method limits of quantification (MLOQs), determination coefficients (r^2), and linear range for soil and water matrices are compiled in Tables S5 and S6, respectively.

The concentrations determined in the three types of samples are shown in Table 4. UVFs presented high bioaccumulation in lettuces and a notorious difference in the total load between the samples irrigated with W water and B water. In contrast, soils and waters had a low concentration of UVFs. As for PBs, the accumulated values were very low in all the matrices. Regarding the pharmaceuticals, the accumulation in the soils was very similar to that in the lettuces, suggesting a direct relationship between soil and plant. As expected, the concentration in waters was much lower, as soils-plants accumulate water-borne contaminants during all the months that the crops were being irrigated.

Of the 55 analyzed compounds, 10 were detected in soil (18%) and 35 were present in the irrigation waters (64%). The pharmaceuticals had the highest accumulated load (50–56 ng/g dw in soils and 3000–7500 ng/L in waters), while the UV filters (UVFs) presented the highest average value per compound (1.7 ng/g dw in soils and 180 ng/L in waters). In soils, PBs and CFF were not detected, despite being present at low concentrations in both types of water.

Table 4
Cumulative concentrations of PPCPs in water, soil, and lettuces. UVFs: UV filters, PBs: paraben preservatives, PhACs: pharmaceuticals, Others (caffeine, CFF)).

	∑UVFs	∑PBs	∑PhACs	∑Others
B water	1.21	n.d.	1.88	n.d.
W water	2.69	n.d.	4.93	n.d.
B soil	2.31	n.d.	50.9	n.d.
W soil	3.15	n.d.	54.6	n.d.
Lettuce Barriers	69.0	3.75	52.7	10.1
Lettuce WWTP	98.6	6.32	53.6	17.9

n.d.: not detected; Units (water): ng/L; Units (soil and lettuce): ng/g dw.

PPCPs concentrations found in S soil and C soil were very similar. In waters, however, there was a significant difference in the amount of PPCPs; the W water was much more contaminated than the B, as the accumulated values of UV filters and pharmaceuticals were two and three-fold higher, respectively.

In soils, DCF showed the highest concentration (37–43 ng/g dw) followed by SCY (7.4–10 ng/g dw). Regarding the other detected compounds, all were found at levels below 1.3 ng/g dw, showing a little affinity for soils. Similar concentrations of DCF and SCY in soil were reported in other studies, as in Carter et al. [14], where DCF was found at 50 and 70 ng/g dw, or Aznar et al. [10] where SCY was reported at 47 ng/g dw.

In W water, the compounds found at the highest concentrations were DCF (1390 ng/L), OFX (1240 ng/L), and BP4 (1030 ng/L), but other compounds were also measured at significant levels: GMZ (916 ng/L), BZT (623 ng/L), MeBZT (601 ng/L) and NPX (454 ng/L). In B water, the values were substantially lower, suggesting that the barriers boost degradation processes. These results are in agreement with reported concentrations in wastewater and reclaimed water of DCF [43,8], OFX [20,27], BP4 [44,8], GMZ [46], BZT [42,8], MeBZT [44] and NPX [46,8].

In lettuces, in contrast to waters and soils, UVFs had the highest accumulated load (671 ng/g dw), showing that the accumulation patterns are not the same in the crops as in the waters and soils for all contaminants. This is also observed in many CECs (namely 4HB, AVO, UVP, SDZ, SMX, SMPZ, SMD, and desVFX) that were uptaken by the lettuces, but were detected neither in the irrigation waters nor in the soils. The compounds found at the highest concentrations in the lettuces were 4HB (61 ng/g dw), SCY (19 ng/g dw), CFF (16) ng/g dw, DCF (15 ng/g dw), and BP2 (13 ng/g dw). 4HB and BP2 are UVFs widely used, but also BP3 metabolites, so their presence in the crops could be due to the degradation of BP3, which was present in the irrigation waters, but absent in the lettuce samples. The presence of CFF and DCF can also be explained by the pretty high levels present in the irrigation waters, as DCF was the compound with the highest concentration, and CFF occurrence in WWTP effluents is also well documented [25,52].

2.4. Statistical analysis

To evaluate potential correlations between CECs concentrations and type of sample, PCA was performed, as shown in Fig. 4. The first two components (PC1 and PC2) explained 77.8% of the variance, being PC1 the one with the greatest contribution (52.7%). PC1 showed high positive loading values for CBZ (0.8) and BP4 (0.7) and high negative ones for BP3 (−0.9) and DCF (−0.9). PC2 had high positive loading values for GMF (0.8) and MFA (0.7) and high negative ones for AVO (−0.9), UVP (−0.9), and N-VFX (−0.9). The larger the value of the contribution, independently of it is positive or negative, the more the variable contributes to a component. Variables that are correlated with PC1 and PC2 are the most important in explaining the variability in the data set. As principal components are designed to explain the variance, the variables that are dimensionally close between them will have more correlation than the ones more separated. In our case, PC1 described the difference between the soil and the other matrices and additionally showed a slight difference between the two types of irrigation waters. PC2, however, described the difference between the lettuce and the B water not explained by PC1.

The three types of samples showed different CECs accumulation patterns, as shown by their separated location in the biplot (Fig. 4). Soils irrigated with B and W waters showed a similar CECs pattern, likewise lettuces. However, there is a higher separation between the two water types, indicating that the infiltration of the water through the barriers modified the CECs content. The different accumulation patterns among the matrices are in accordance with previous works, where the CECs load in the irrigation water was statistically different from that in the soils or the crops. For example, Christou et al., [15] reported that considerable concentrations of DCF, SMX, and TMP (50.6 ng/L, 41.3 ng/L, and 61.8 ng/L, respectively) in the irrigation wastewater did not display a cumulative or increasing pattern. Similarly, Liu et al., [34] found that the concentrations of SMX, SMZ, and TMP in reclaimed water (20 ng/L, 1 ng/L, and 3 ng/L, respectively) were higher than those of many other studied CECs and that their respective values in the irrigated soils and crops did not display a similar trend.

Three well-differentiated groups were observed among the CECs, one for each type of matrix. The largest group of compounds (green ellipse in Fig. 4) is related to the lettuces, the second group (blue ellipse) to the irrigation waters, and the third group (orange ellipse) to the soils. Some of the CECs investigated were not directly correlated with any of the matrices (they are halfway among them), showing similar contributions.

Regarding individual behaviors, the pharmaceutical CBZ is known to be present at high concentrations in wastewater, as we have observed, but its metabolite CBZ-E is mostly accumulated in the lettuces, probably because CBZ is metabolized when it is uptaken by the plant. Concerning UVFs, BZTs, the methylated derivatives are the major contributors to the W water. Conversely, BZT was associated with the lettuces irrigated with the same water. A possible explanation would be the existence of an

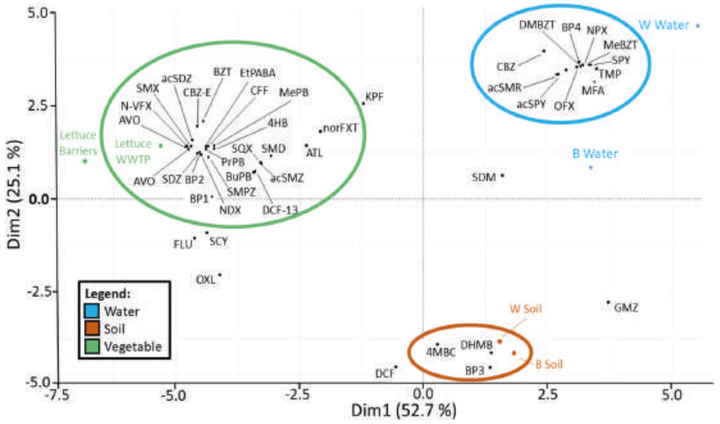

Fig. 4. Biplot of the three studied matrices (lettuce, soil, and water) showing the CEC's contributions.

enzymatic demethylation mechanism in plants. BP3, and some metabolites, are mostly found in soils, although they were also present in the waters, suggesting that it was accumulated thereafter irrigation. Then, soil microorganisms can degrade it, producing DHMB (BP8), and also impacting the soil matrix. Further, the DHMB formed is degraded during the uptaken process by the lettuces, as it was not detected in this matrix. When BP3 was metabolized in the plant, 4HB, BP1, and BP2 were generated, which would explain the high concentrations of these compounds accumulated in the lettuces. Regarding the paraben preservatives, all of them showed the same occurrence pattern in all matrixes, with the greatest correlations observed for the lettuces.

PCA and PLS-DA were also performed to evaluate potential correlations between measured physicochemical properties of the soils and CECs concentrations detected in them. The same analyses were applied to the irrigation waters; however, no correlation was observed in any case (Figs. S2 and S3).

2.5. Soil and lettuces uptake factors

The highest UF_{SOIL} value of 175, corresponded to 4HB, present at a low concentration in soil, but highly accumulated by the lettuces. However, we cannot rule out that part of the 4HB in the plant could be produced by the plant itself in the metabolization of the BP3 accumulated in addition to the 4HB directly transferred from the water to the soil [38]. The concentrations of BP3 measured in the irrigation waters and soils, and their absence in the lettuces would support this hypothesis. Like 4HB, BP1 is another major BP3 metabolite, and thus a similar explanation for its occurrence can be given.

Other compounds with high UF_{SOIL} values were SCY (2.7), FLU (2.4), OXL (1.8), and BP1 (1.3). The UF_{SOIL} values for the rest of the CECs were < 1. SCY is a plant hormone known for mediating host responses upon pathogen infection [32], so its UF_{SOIL} value probably is the result of the natural production of SCY by the lettuces. For the two fluoroquinolone antibiotics, our results are consistent with their reported uptake by lettuces. Tadić et al., [49] found TMP, OFL, and enrofloxacin between 1.9 and 37.8 ng/g dw in lettuces, while we have found FLU and OXL between 0.36 and 4.91 ng/g in this study.

Regarding UF_{WATER}, the compounds with the highest values, but still < 1 were BP2 (0.82) and BP3 (0.83); all other ratios were < 0.4. This could be explained by the relatively low lipophilicity (log Kow <4) of both compounds (log Kow 2.78 and 3.79), which make them preferably accumulate in organic matter components. The Kd values of all compounds were < 0.3, showing low sorption coefficients in soils after irrigation. However, it has to be considered that plant uptake can also contribute to the reduction of CECs in the soil, resulting in low Kd values.

2.6. Variables' role in contaminants' uptake

2.6.1. Irrigation waters

B water had lower levels of TSS, COD, *E. coli*, sulfite-reducing clostridia spores, Helminth eggs, *Legionella spp*, UVFs, and pharmaceuticals than W water. However, SAR, EC, CFF, and PBs were very similar in both. The differences observed evidence that the rbMAR system reduces the levels of pathogens and CECs [55,54]. As shown in Fig. 2, the fluctuation of pathogen indicators and contaminants' load in the influent water of the rbMAR system is an important factor that needs to be considered, especially if a high infiltration flow rate is to be applied, because the reduction of pathogens and CECs can only be effective with long residence times.

2.6.2. Soil

OM, N, and pH of soils were very similar regardless of the irrigation water used, showing that the nutrients present in the wastewater were not altered during infiltration through the reactive barriers and, therefore, B water provided similar nutrient levels to the soil as W water. However, other parameters such as *E. coli* had lower values in the soil irrigated with B water, in accordance with the values found in the irrigation waters. Overall, *E. coli* appears to have more affinity to soils with higher clay content. However, *E. coli* levels varied randomly between drip irrigated and sprinkle irrigated soils. Additionally, SAR and salinity values in the soils increased after a year of irrigation in all the plots, likely lowering the availability of nutrients as well as modifying the soil structure [59]. These negative impacts should be considered for long-term agricultural practices and guarantee further research. Regarding CECs, no significant differences were observed between C and S soils, and between soils irrigated with B water or W water. The poor correlation observed between the CECs occurrence in the water, soil, and crop is an issue reported in previous studies. de Santiago-Martín et al., [43] reported that the concentration of CECs measured in the irrigation water was not in agreement with the bioaccumulation pattern found for the fruit, as observed in both, the sediments and the soils. They showed as an example that APH, IBU, and CBZ (range 0.03–27.5 ng/g) were up taken by crops, but were in the lowest concentration ranges in the irrigation water (range <100–250 ng/L).

2.6.3. Lettuces

Following the same pattern observed in irrigation waters and soils, high values of *E. Coli* were found in lettuces irrigated with W water, but no fecal contamination was present in those irrigated with B water. Considering the difference in *E. coli* levels between W and B water (2.6×10^5 and 9.6 cfu/100 ml, respectively) and W and B soil (7.5×10^3 cfu/g and not detected, respectively), the accumulated levels in lettuces are consistent. Regarding soil composition and irrigation system, the *E. coli* levels found in lettuces (Table 3) suggest that C soil and sprinkling irrigation favor the accumulation of *E. coli* in the lettuces. According to the previous determination of PPCPs in lettuces [48], concentration values were much lower in crops irrigated with B water. But the other variables (soil composition and irrigation system) also appeared to impact the final concentrations of CECs in the plant, being C soils and sprinkling irrigation, the ones leading to a lower CECs' uptake by lettuces.

The irrigation with W water led to higher concentrations of PPCPs and fecal contamination (Fig. S4). However, the other variables seem to affect the levels of pathogen indicators and CECs in the lettuces, but with pretty different behavior. Dripping irrigation capacity to reduce the pathogens' transfer compared with that of sprinkling irrigation has been reported (Banach and Van der Fels-Klerx, 2020; [61]). Sprinkling likely contributes to higher levels of pathogens when they are scattered all over the lettuce surface, meanwhile by dripping the possibility to reach the crop is much lower. CECs, however, could not trespass the crop surface when sprinkled, so they are more uptaken when higher amounts reach the roots of the plant. Regarding soil composition, finer-textured (such as clay), which have better moisture and nutrient retention capacity, support superior pathogens' survival than sandy soils [18,3]. On the contrary, a higher dissipation of CECs in soils with higher clay content has been reported [24,62,64], which could explain the lower values found in the lettuces. However, degradation rates of CECs in soils are determined by different factors and processes and do not exclusively depend on a single factor [4].

Thus, contamination in the irrigation water is crucial to lowering pathogens and CECs levels in the crops. However, other factors such as soil composition and irrigation system have their own influence on the final outcome. According to the results, the best combination to lower the pathogen levels would be to irrigate with B water by drip in S soil, and the best combination to lower the CECs levels would be to irrigate with B water by a sprinkler system in C soil. Therefore, the most favorable conditions to lower pathogens and CECs levels would be the latest (B water, sprinkler, C soil), as pathogens are completely absent when irrigated with B water and the lower uptake of CECs occurs with sprinkler irrigation in C soil.

4. Conclusions

The present study showed that high concentrations of the pathogenic indicator *E. coli* and CECs in irrigation waters could lead to their accumulation in the soil and crops. The quality of the irrigation water turned out to be crucial in reducing the levels of pathogens and CECs in crops. However, other factors such as soil composition and irrigation system also influence the plant uptake of pathogens and CECs. Based on the results, it can be stated that the most favorable conditions to minimize the levels of pathogens and CECs transferred to crops would be the use of reclaimed water with sprinkler irrigation in soil rich in clay. However, the processes leading to this minor transfer of microbiological and chemical contamination were not fully understood. Further studies should be carried out on different types of crops (absorption processes in the crop may be different, as well as metabolism products), in all seasons of the year (fluctuation of contaminants in irrigation waters) and for longer periods (increased salinization) that can lead to a gradual decrease in the availability of nutrients and modification of the soil structure. Beyond the control of the chemical and microbiological quality of the irrigation water, to minimize the risk of fecal contamination in crops irrigated with reclaimed water, continuous monitoring of the irrigation water should be established in the storage tank usually used as a reserve for irrigation water, particularly concerning microbiological quality, since the regrowth of bacteria during water storage is a probable risk.

CRediT authorship contribution statement

Adrià Sunyer-Caldú: Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing. **Paola Sepúlveda-Ruiz**: Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing. **Miquel Salgot**: Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition. **Montserrat Folch-Sánchez**: Term, Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Damià Barceló**: Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition. **M. Silvia Diaz-Cruz**: Term, Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. Adrià Sunyer-Caldú and Paola Sepúlveda-Ruiz contributed equally to this work.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2022.108831.

References

[1] R. Aiello, G.L. Cirelli, S. Consoli, Effects of reclaimed wastewater irrigation on soil and tomato fruits: A case study in Sicily (Italy), Agric. Water Manag 93 (2007) 65–72, https://doi.org/10.1016/j.agwat.2007.06.008.

[2] L. Alcalde-Sanz, B. Gawlik, Minimum quality requirements for water reuse in agricultural irrigation and aquifer recharge, . J. Eur. Union. (2017).

[3] O.O. Alegbeleye, A.S. Sant'Ana, Manure-borne pathogens as an important source of water contamination: An update on the dynamics of pathogen survival/transport as well as practical risk mitigation strategies, Int. J. Hyg. Environ. Health 227 (2020), 113524, https://doi.org/10.1016/j.ijheh.2020.113524.

[4] O.S.A. Al-Khazrajy, E. Bergström, A.B.A. Boxall, Factors affecting the dissipation of pharmaceuticals in freshwater sediments, Environ. Toxicol. Chem. 37 (2018) 829–838, https://doi.org/10.1002/etc.4015.

[5] S.A.A.A.N. Almuktar, M. Scholz, Microbial contamination of Capsicum annuum irrigated with recycled domestic wastewater treated by vertical-flow wetlands, Ecol. Eng. 82 (2015) 404–414, https://doi.org/10.1016/j.ecoleng.2015.05.029.

[6] Antunes, E., Vuppaladadiyam, A.K., Sarmah, A.K., Varsha, S.S.V., Pant, K.K., Tiwari, B., Pandey, A., 2021, Chapter Three - Application of biochar for emerging contaminant mitigation, in: Sarmah Environmental Management and Protection, in C.P. (Ed.), Biochar: Fundamentals and Applications in Environmental Science and Remediation Technologies. Elsevier, pp. 65–91. https://doi.org/https://doi.org/10.1016/bs.apmp.2021.08.003.

[7] APHA, A., and WEF, 2005, Standard methods for the examination of water and wastewater, 21st ed. ed, American Public Works Association. APHA-AWWA-WEF, Washington D.C.

[8] E. Archer, B. Petrie, B. Kasprzyk-Hordern, G.M. Wolfaardt, The fate of pharmaceuticals and personal care products (PPCPs), endocrine disrupting contaminants (EDCs), metabolites and illicit drugs in a WWTW and environmental waters, Chemosphere 174 (2017) 437–446, https://doi.org/10.1016/j.chemosphere.2017.01.101.

[9] Ayers, R.S., Westcot, D.W., 1985, Water Quality for Agriculture. FAO Irrig. Drain. Pap. 29 Rev. 1.

[10] R. Aznar, C. Sánchez-Brunete, B. Albero, J.A. Rodríguez, J.L. Tadeo, Occurrence and analysis of selected pharmaceutical compounds in soil from Spanish agricultural fields, Environ. Sci. Pollut. Res. Int. 21 (2014) 4772–4782, https://doi.org/10.1007/S11356-013-2438-7.

[11] Bahri, A., Drechsel, P., Brissaud, F., 2008, Water reuse in Africa: challenges and opportunities, in: IWMI Conference Proceedings 245271, International.

[12] C.A. Black, D.D. Evans, J.L. White, L.E. Ensminger, F.E. Clark, Methods Soil Anal.: Part 2. Methods Soil Anal. Part 2 Chem. Microbiol. Prop. (2016) 1–1572, https://doi.org/10.2134/AGRONMONOGR9.2.2ED.

[13] D. Calderón-Preciado, V. Matamoros, R. Savé, P. Muñoz, C. Biel, J.M. Bayona, Uptake of microcontaminants by crops irrigated with reclaimed water and groundwater under real field greenhouse conditions, Environ. Sci. Pollut. Res. (2013), https://doi.org/10.1007/s11356-013-1509-0.

[14] L.J. Carter, E. Harris, M. Williams, J.J. Ryan, R.S. Kookana, A.B.A. Boxall, Fate and uptake of pharmaceuticals in soil-plant systems, J. Agric. Food Chem. 62 (2014) 816–825, https://doi.org/10.1021/JF404282Y.

[15] A. Christou, P. Karaolia, E. Hapeshi, C. Michael, D. Fatta-Kassinos, Long-term wastewater irrigation of vegetables in real agricultural systems: Concentration of pharmaceuticals in soil, uptake and bioaccumulation in tomato fruits and human health risk assessment, Water Res 109 (2017) 24–34, https://doi.org/10.1016/j.watres.2016.11.033.

[16] T. Eggen, T.N. Asp, K. Grave, V. Hormazabal, Uptake and translocation of metformin, ciprofloxacin and narasin in forage- and crop plants, Chemosphere 85 (2011) 26–33, https://doi.org/10.1016/j.chemosphere.2011.06.041.

[17] D. Fatta-Kassinos, I.K. Kalavrouziotis, P.H. Koukoulakis, M.I. Vasquez, The risks associated with wastewater reuse and xenobiotics in the agroecological environment, Sci. Total Environ. (2011), https://doi.org/10.1016/j.scitotenv.2010.03.036.

[18] D.R. Fenlon, I.D. Ogden, A. Vinten, I. Svoboda, The fate of Escherichia coli and E. coli O157 in cattle slurry after land application, Symp. Ser. Soc. Appl. Microbiol. 149S -156S (2000), https://doi.org/10.1111/j.1365-2672.2000.tb05343.x.

[19] A. Forslund, J.H.J. Ensink, B. Markussen, A. Battilani, G. Psarras, S. Gola, L. Sandei, T. Fletcher, A. Dalsgaard, Escherichia coli contamination and health aspects of soil and tomatoes (Solanum lycopersicum L.) subsurface drip irrigated with on-site treated domestic wastewater, Water Res 46 (2012) 5917–5934, https://doi.org/doi: 10.1016/j.watres.2012.08.011.

[20] A.M. Franklin, C.F. Williams, J.E. Watson, Assessment of soil to mitigate antibiotics in the environment due to release of wastewater treatment plant effluent, J. Environ. Qual. 47 (2018) 1347–1355, https://doi.org/10.2134/jeq2018.02.0076.

[21] P. Gago-Ferrero, N. Mastroianni, M.S. Díaz-Cruz, D. Barceló, Fully automated determination of UV filters and transformation products in natural waters and wastewaters by online solid-phase extraction-liquid chromatography-tandem mass spectrometry, J. Chromatogr. A 1294 (2013) 106–116, https://doi.org/10.1016/j.chroma.2013.04.037.

[22] P. Gago-Ferrero, M. Gros, L. Ahrens, K. Wiberg, Impact of on-site, small and large-scale wastewater treatment facilities on levels and fate of pharmaceuticals, personal care products, artificial sweeteners, pesticides, and perfluoroalkyl substances in recipient waters, Sci. Total Environ. 601–602 (2017) 1289–1297, https://doi.org/10.1016/j.scitotenv.2017.05.258.

[23] A. García-Gil, E. Garrido Schneider, M. Mejías, D. Barceló, E. Vázquez-Suñé, S. Díaz-Cruz, Occurrence of pharmaceuticals and personal care products in the urban aquifer of Zaragoza (Spain) and its relationship with intensive shallow geothermal energy exploitation, J. Hydrol. 566 (2018) 629–642, https://doi.org/10.1016/J.JHYDROL.2018.09.066.

[24] A. Ghafoor, N.J. Jarvis, T. Thierfelder, J. Stenström, Measurements and modeling of pesticide persistence in the soil at the catchment scale, Sci. Total Environ. 409 (2011) 1900–1908, https://doi.org/10.1016/j.scitotenv.2011.01.049.

[25] O. Golovko, S. Örn, M. Sörengård, K. Frieberg, W. Nassazzi, F.Y. Lai, L. Ahrens, Occurrence and removal of chemicals of emerging concern in wastewater treatment plants and their impact on receiving water systems, Sci. Total Environ. 754 (2021), 142122, https://doi.org/10.1016/j.scitotenv.2020.142122.

[26] K. He, A. Timm, L. Blaney, Simultaneous determination of UV filters and estrogens in aquatic invertebrates by modified quick, easy, cheap, effective, rugged, and safe extraction and liquid chromatography-tandem mass spectrometry, J. Chromatogr. A 1509 (2017) 91–101, https://doi.org/10.1016/j.chroma.2017.06.039.

[27] K. He, A.D. Soares, H. Adejumo, M. McDiarmid, K. Squibb, L. Blaney, Detection of a wide variety of human and veterinary fluoroquinolone antibiotics in municipal wastewater and wastewater-impacted surface water, J. Pharm. Biomed. Anal. 106 (2015) 136–143, https://doi.org/10.1016/j.jpba.2014.11.020.

[28] ISO, 2017b, ISO 6887–1:2017 Microbiología de la cadena alimentaria [WWW Document]. URL https://www.une.org/encuentra-tu-norma/busca-tu-norma/norma?c=N0058878 (accessed 2.15.22).

[29] ISO, 2017a, ISO 9308:2014/A1:2017 Calidad del agua. Recuento de E. coli y de bacterias coliformes.

[30] A. Jodar-Abellan, M.I. López-Ortiz, J. Melgarejo-Moreno, Wastewater treatment and water reuse in Spain. Current situation and perspectives, Water (Switz. 11 (2019) 17–22, https://doi.org/10.3390/w11081551.

[31] Koncagül. E., Tran, M., Connor, R., Uhlenbrook, S., Houngbo, G.F., 2018, The United Nations world water development report 2018: nature-based solutions for water. UNESCO 1–139.

[32] H. Lefevere, L. Bauters, G. Gheysen, Salicylic acid biosynthesis in plants, Front. Plant Sci. 11 (2020) 1–7, https://doi.org/10.3389/fpls.2020.00338.

[33] A. Libutti, G. Gatta, A. Gagliardi, P. Vergine, A. Pollice, L. Beneduce, G. Disciglio, E. Tarantino, Agro-industrial wastewater reuse for irrigation of a vegetable crop succession under Mediterranean conditions, Agric. Water Manag 196 (2018) 1–14, https://doi.org/10.1016/j.agwat.2017.10.015.

[34] Xianjing Liu, C. Liang, Xiaohui Liu, F. Zhao, C. Han, Occurrence and human health risk assessment of pharmaceuticals and personal care products in real agricultural systems with long-term reclaimed wastewater irrigation in Beijing, China, Ecotoxicol. Environ. Saf. 190 (2020), 110022, https://doi.org/10.1016/j.ecoenv.2019.110022.

[35] T. Malchi, Y. Maor, G. Tadmor, M. Shenker, B. Chefetz, Irrigation of root vegetables with treated wastewater: Evaluating uptake of pharmaceuticals and the associated human health risks, Environ. Sci. Technol. 48 (2014) 9325–9333, https://doi.org/10.1021/es5017894.

[36] P. Mañas, E. Castro, J. De Las Heras, Irrigation with treated wastewater: Effects on soil, lettuce (Lactuca sativa) crop and dynamics of microorganisms, J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng. (2009) 44, https://doi.org/10.1080/10934520903140033.

[37] A. Margenat, V. Matamoros, S. Díez, N. Cañameras, J. Comas, J.M. Bayona, Occurrence of chemical contaminants in peri-urban agricultural irrigation waters and assessment of their phytotoxicity and crop productivity, Sci. Total Environ. 599–600 (2017) 1140–1148, https://doi.org/10.1016/j.scitotenv.2017.05.025.

[38] D. Molins-Delgado, R. Muñoz, S. Nogueira, M.B. Alonso, J.P. Torres, O. Malm, R. L. Ziolli, R.A. Hauser-Davis, E. Eljarrat, D. Barceló, M.S. Díaz-Cruz, Occurrence of organic UV filters and metabolites in lebranche mullet (Mugil liza) from Brazil, Sci. Total Environ. 618 (2018) 451–459, https://doi.org/10.1016/j.scitotenv.2017.11.033.

[39] A.M. Palese, V. Pasquale, G. Celano, G. Figliuolo, S. Masi, C. Xiloyannis, Irrigation of olive groves in Southern Italy with treated municipal wastewater: Effects on the microbiological quality of soil and fruits, Agric. Ecosyst. Environ. 129 (2009) 43–51, https://doi.org/10.1016/j.agee.2008.07.003.

[40] M. Pan, C.K.C. Wong, L.M. Chu, Distribution of antibiotics in wastewater-irrigated soils and their accumulation in vegetable crops in the Pearl River Delta, Southern China, J. Agric. Food Chem. 62 (2014) 11062–11069, https://doi.org/10.1021/jf503850v.

[41] V. Phogat, D. Mallants, J.W. Cox, J. Šimůnek, D.P. Oliver, T. Pitt, P.R. Petrie, Impact of long-term recycled water irrigation on crop yield and soil chemical properties, Agric. Water Manag 237 (2020), 106167, https://doi.org/10.1016/j.agwat.2020.106167.

[42] Y. Picó, R. Alvarez-Ruiz, A.H. Alfarhan, M.A. El-Sheikh, S.M. Alobaid, D. Barceló, Uptake and accumulation of emerging contaminants in soil and plant treated with wastewater under real-world environmental conditions in the Al Hayer area (Saudi Arabia, Sci. Total Environ. 652 (2019) 562–572, https://doi.org/10.1016/J.SCITOTENV.2018.10.224.

[43] A. de Santiago-Martín, R. Meffe, G. Teijón, V. Martínez Hernández, I. López-Heras, C. Alonso Alonso, M. Arenas Romasanta, I. de Bustamante, Pharmaceuticals and trace metals in the surface water used for crop irrigation: Risk to health or natural attenuation, Sci. Total Environ. 705 (2020), 135825, https://doi.org/10.1016/J.SCITOTENV.2019.135825.

[44] M.P. Serra-Roig, A. Jurado, M.S. Díaz-Cruz, E. Vázquez-Suñé, E. Pujades, D. Barceló, Occurrence, fate, and risk assessment of personal care products in the river–groundwater interface, Sci. Total Environ. 568 (2016) 829–837, https://doi.org/10.1016/j.scitotenv.2016.06.006.

[45] B.M. Sharma, J. Bečanová, M. Scheringer, A. Sharma, G.K. Bharat, P.G. Whitehead, J. Klánová, L. Nizzetto, Health and ecological risk assessment of emerging contaminants (pharmaceuticals, personal care products, and artificial sweeteners) in surface and groundwater (drinking water) in the Ganges River Basin, India, Sci. Total Environ. 646 (2019) 1459–1467, https://doi.org/10.1016/j.scitotenv.2018.07.235.

[46] N.K. Stamatis, I.K. Konstantinou, Occurrence and removal of emerging pharmaceutical, personal care compounds, and caffeine tracers in municipal sewage treatment plant in Western Greece, J. Environ. Sci. Heal. - Part B Pestic. Food Contam. Agric. Wastes 48 (2013) 800–813, https://doi.org/10.1080/03601234.2013.781359.

[47] D.P. Stevens, M.J. McLaughlin, M.K. Smart, Effects of long-term irrigation with reclaimed water on soils of the Northern Adelaide Plains, South Australia, Soil Res 41 (2003) 933–948, https://doi.org/10.1071/SR02049.

[48] A. Sunyer-Caldú, M.S. Diaz-Cruz, Development of a QuEChERS-based method for the analysis of pharmaceuticals and personal care products in lettuces grown in field-scale agricultural plots irrigated with reclaimed water, Talanta 230 (2021), 122302, https://doi.org/10.1016/j.talanta.2021.122302.

[49] Đ. Tadić, V. Matamoros, J.M. Bayona, Simultaneous determination of multiclass antibiotics and their metabolites in four types of field-grown vegetables, Anal. Bioanal. Chem. 411 (2019) 5209–5222, https://doi.org/10.1007/s00216-019-01895-y.

[50] Đ. Tadić, M.J. Bleda Hernandez, F. Cerqueira, V. Matamoros, B. Piña, J.M. Bayona, Occurrence and human health risk assessment of antibiotics and their metabolites in vegetables grown in field-scale agricultural systems, J. Hazard. Mater. 401 (2021), 123424, https://doi.org/10.1016/j.jhazmat.2020.123424.

[51] A. Tajima, M. Yoshizawa, A. Sakurai, M. Minamiyama, Establishment of guidelines for the reuse of treated wastewater.The European Parliament and the Council, 2020. Regulation (EU) 2020/741, Minimum requirements for water reuse, J. Eur. Union 177/33 (2005) 32–55.

[52] B. Tiwari, B. Sellamuthu, Y. Ouarda, P. Drogui, R.D. Tyagi, G. Buelna, Review on fate and mechanism of removal of pharmaceutical pollutants from wastewater using the biological approach, Bioresour. Technol. 224 (2017) 1–12, https://doi.org/10.1016/j.biortech.2016.11.042.

[53] UNESCO, 2012, United Nations world water development report 4: managing water under uncertainty and risk. Paris.

[54] C. Valhondo, L. Martínez-Landa, J. Carrera, S.M. Díaz-Cruz, S. Amalfitano, C. Levantesi, Six artificial recharge pilot replicates to gain insight into water quality enhancement processes, Chemosphere (2020) 240, https://doi.org/10.1016/j.chemosphere.2019.124826.

[55] C. Valhondo, J. Carrera, C. Ayora, I. Tubau, L. Martinez-Landa, K. Nödler, T. Licha, Characterizing redox conditions and monitoring attenuation of selected pharmaceuticals during artificial recharge through a reactive layer, Sci. Total Environ. (2015), https://doi.org/10.1016/j.scitotenv.2015.01.030.

[56] C. Valhondo, J. Carrera, L. Martínez-Landa, J. Wang, S. Amalfitano, C. Levantesi, M.S. Diaz-Cruz, Reactive Barriers for Renaturalization of Reclaimed Water during Soil Aquifer Treatment, Water 12 (2020) 1012, https://doi.org/10.3390/w12041012.

[57] J.F. Velasco-Muñoz, J.A. Aznar-Sánchez, A. Batlles-delaFuente, M.D. Fidelibus, Rainwater harvesting for agricultural irrigation: An analysis of global research, Water (Switz.) (2019) 11, https://doi.org/10.3390/w11071320.

[58] P. Vergine, R. Saliba, C. Salerno, G. Laera, G. Berardi, A. Pollice, The fate of the fecal indicator Escherichia coli in irrigation with partially treated wastewater, Water Res 85 (2015) 66–73, https://doi.org/10.1016/j.watres.2015.08.001.

[59] G.A. Vivaldi, S. Camposeo, G. Lopriore, C. Romero-Trigueros, F.P. Salcedo, Using saline reclaimed water on almond grown in Mediterranean conditions: Deficit irrigation strategies and salinity effects, Water Sci. Technol. Water Supply 19 (2019) 1413–1421, https://doi.org/10.2166/ws.2019.008.

[60] World Health Organization, 1989, Health guidelines for the use of wastewater in agriculture and aquaculture: report of a WHO scientific group.

[61] World Health Organization, 2006, Guidelines for the safe use of wastewater, excreta, and greywater. Geneva.

[62] X. Wu, J.L. Conkle, J. Gan, Multi-residue determination of pharmaceutical and personal care products in vegetables, J. Chromatogr. A 1254 (2012) 78–86, https://doi.org/10.1016/j.chroma.2012.07.041.

[63] X. Wu, L.K. Dodgen, J.L. Conkle, J. Gan, Plant uptake of pharmaceutical and personal care products from recycled water and biosolids: A review, Sci. Total Environ. 536 (2015) 655–666, https://doi.org/10.1016/j.scitotenv.2015.07.129.

[64] J. Xu, L. Wu, A.C. Chang, Degradation and adsorption of selected pharmaceuticals and personal care products (PPCPs) in agricultural soils, Chemosphere 77 (2009) 1299–1305, https://doi.org/10.1016/j.chemosphere.2009.09.063.

5.2.2 Publication #10 Supplementary information

Reclaimed water in agriculture: a plot-scale study assessing crop uptake of emerging contaminants and pathogens

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Section S1. Solvents, stock solutions, and extraction and analysis kits
specifications.
Section S2. Climatic conditions in the selected WWTP area and operational’s plant conitions.
Section S3. Soils validation parameters.
Section S4. Tables. (Tables S1, S2, S3, S4, S5, S6, S7 and S8).
Section S5. Figures. (Figures S1, S2, S3 and S4).

Section S1. Solvents, stock solutions, and extraction and analysis kits specifications.

Water, methanol (MeOH), and acetonitrile (ACN) of HPLC grade were purchased from J.T. Baker (Deventer, The Netherlands). Nitrogen (99.995%) was supplied by Air Liquide (Barcelona, Spain). Ethanol, acetone, formic acid (FA) and ammonium acetate (NH4Ac) were from Merck (Darmstadt, Germany). The commercial QuEChERS kits Citrate- Kit-01 and PSA-Kit-02 used were obtained from BEKOlut® (Hauptstuhl, Germany).

Individual stock standard and isotopically labeled internal stock standard solutions were prepared on a weight basis in MeOH at 100 mg/L and stored in the dark at −20°C. A mixture with all the standards was weekly prepared at 10 mg/L in MeOH. Ten working solutions at concentrations between 1 ng/mL and 1 µg/mL were daily prepared by the appropriate dilution of the mixture standard solution with MeOH or with the extract from the solid matrix in each case (e.g. soil).

For *E. coli* analysis, Brilliance *E. coli*/Coliform selective medium was purchased from OXOID LTD (Basingstoke, Hampshire, England). For *E. coli* determination in soil, a buffer solution was monthly prepared. The sterile buffer solution consists of 1,25 ml of stock solution I and 5 ml of stock solution II brought up to 1000 ml with distilled water. Stock solution I was composed of 34 g KH2PO4 in 1000 ml of distilled water, adjusting the pH to 7.2 (±0.5) with 1 N NaOH. Stock solution II was contained 38 g of MgCl2 in 1000 ml of distilled water. All solutions were autoclaved at 121°C, under 15 lb pressure for 15 min and subsequently stored at 4°C until use. All the reagents used for APHA/ASA methods were >98% purity.

Section S2. Climatic conditions in the selected WWTP area and operational's plant conitions.

The Palamós WWTP is located on the North-East Spanish Mediterranean coastline and it treats municipal wastewater from 6 municipalities (165.500 inhabitants), that increases by more than 100% in summer (Institue of statistics of Catalonia (Idescat), 2018). WWTP's maximum flow is 33.000 m3/day; during the 2019 summer, the flow rate treated was 709,3 m3 compared with 347,7 m3 treated in February 2019; therefore, the quality of the effluent changes notoriously over the year. The treatment process involves pre-treatment (screening), primary treatment, and biological secondary treatment (activated sludge). The weather of the area is typically Mediterranean, with average temperatures between 8° C in winter and 24°C in summer. July and August are the hottest months whereas December, January and February are the coldest. The annual rainfall is 588 mm, with an average variation of 77 mm between the dry and rainy months. July is the driest month (19 mm precipitation) while October is the rainiest one (96 mm).

Section S3. Soils validation parameters.

For the validation of the method to soil matrices, the same steps used in (Development) were applied. A homogenized pool of soils was spiked at three different concentrations (10, 50 and 100 ng/g dw) by triplicate and the recoveries were evaluated. The values obtained in the spiked samples are shown in Table S7 and the precision parameters are shown in Table S8.

Section S4. Tables

Table S1. Uptake factors and sorption coefficients for the target CECs (WWTP vs Barrier irrigation).

	BP3	BP1	BP2	4HB	DHMB	4MBC	EtPABA	BZT	PrPB	MePB	FLU
UF_{SOIL} WWTP	NA	NA	NA	NA	NA	0.02	NA	NA	NA	NA	2.4
UF_{SOIL} Barrier	NA	1.3	NA	175	NA	0.10	NA	NA	NA	NA	1.4
UF_{WATER} Barrier	NA	0.02	0.13	NA	NA	0.00	0.30	0.01	0.03	0.11	0.17
UF_{WATER} WWTP	NA	0.08	0.82	NA	NA	0.00	NA	0.01	0.03	0.18	0.08
Kd Barrier (L/kg)	0.13	NA	NA	NA	0.03	0.01	NA	NA	NA	NA	0.07
Kd WWTP (L/kg)	0.30	0.06	NA	NA	0.03	0.01	NA	NA	NA	NA	0.06
	NDX	**OXL**	**acSDZ**	**acSMZ**	**GMZ**	**KPF**	**DCF**	**CBZ**	**CBZE**	**SCY**	**CFF**
UF_{SOIL} WWTP	NA	1.8	NA	NA	NA	NA	0.50	NA	NA	1.4	NA
UF_{SOIL} Barrier	NA	1.1	NA	NA	NA	NA	0.35	NA	NA	2.7	NA
UF_{WATER} Barrier	0.81	0.17	0.02	0.03	NA	NA	0.01	NA	0.01	NA	0.25
UF_{WATER} WWTP	NA	0.12	NA	0.02	NA	0.00	0.02	0.00	0.01	NA	0.34
Kd Barrier (L/kg)	NA	0.09	NA	NA	0.00	NA	0.03	NA	NA	NA	NA
Kd WWTP (L/kg)	NA	0.11	NA	NA	0.00	NA	0.07	NA	NA	NA	NA

NA: Not available

Table S2. Results of the t-tests to evaluate the correlation between the different variables.

Studied parameters	Confidence	P-value	Correlated
Physicochemical properties in function of soil composition (sand-clay)	α<0.05	0.88	No
SAR values in function of the irrigation system (drip-sprinkler)	α<0.05	0.87	No
SAR values in function of soil composition (sand-clay)	α<0.05	0.93	No
CECs concentrations in soil in function of soil composition (sand-clay)	α<0.05	0.94	No
CECs concentrations in soil in function of the irrigation water (B water-W water)	α<0.05	0.065	No
CECs concentrations in lettuces in function of soil composition (sand-clay)	α<0.05	0.6	No
CECs concentrations in lettuces in function of the irrigation water (B water-W water)	α<0.05	0.68	No
CECs concentrations in lettuces in function of the irrigation system (drip-sprinkler)	α<0.05	1.0	No

Table S3. Characteristics of soil samples collected during lettuce harvest.

Parameter	Field irrigated by WWTP effluent	Field irrigated by rbMAR effluent
pH	7,9	8,1
EC (µS/cm)	102,6	82,7
N (%)	0,12	0,09
OM (%)	0,9	1
Mg^{+2} (ppm)	18,9	17,7
Ca^{2+} (ppm)	200,4	211,6
Na^{+} (ppm)	52,5	36,9
SAR (meq/L)	0,9	0,6

Table S4. Table of the concentrations detected in the lettuces in the different plots, with ranges and frequencies of detection.

Compound	BSS	BCS	BCD	BSD	WSD	WCS	WSS	WCD	Range	Positive	Frequency (%)
BP3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
BP1	0.77	<LOD	0.82	2.76	0.69	1.17	3.56	0.42	0.42-3.56	7	87.5
BP2	1.48	54.4	7.03	9.81	5.32	8.14	5.37	13.8	1.48-54.4	8	100
4HB	11.8	64.1	26.6	59.8	80.6	80.1	79.9	84.1	11.8-84.1	8	100
4DHB	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
DHMB	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
AVO	0.85	1.69	6.54	<LOD	1.34	2.13	2.04	0.58	0.58-6.54	7	87.5
BP4 (-)	0.17	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.17	1	
4MBC	<LOD	<LOD	<LOD	0.1	0.71	0.3	1.62	0.48	0.1-1.62	5	62.5
EHMC	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
EtPABA	0.3	1.06	<LOD	<LOD	0.42	<LOD	1.17	0.73	0.3-1.17	5	62.5
BZT	1.52	0.57	5.32	7.05	1.43	6.55	0.49	1.4	0.49-7.05	8	100
MeBZT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
DMBZT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
UVP	1.97	2.51	5.35	1.71	2.39	4.19	1.44	1.94	1.44-5.35	8	100
BePB (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
BuPB (-)	0.4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.4	1	12.5
PrPB (-)	<LOD	<LOD	0.94	1.42	<LOD	0.97	<LOD	<LOD	0.94-1.42	3	37.5
MePB (-)	<LOD	10.24	0.48	1.52	4.52	7.78	1.14	10.9	0.48-10.86	7	87.5
FLU	0.52	1.94	4.25	<LOD	0.62	1.48	1.53	0.52	0.52-4.25	7	87.5
NDX	1.18	19	10.1	2	3.94	3.57	2,078	4.08	1.18-19	8	100
OXL	0.66	1.56	4.91	0.41	0.36	1.91	1.91	<LOD	0.36-4.91	7	87.5
TCY	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
Succynil-STZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SDZ	<LOD	<LOD	4.03	2.23	<LOD	2.95	<LOD	<LOD	2.23-4.03	3	37.5
acSDZ	1.3	<LOD	<LOD	<LOD	<LOD	<LOD	1.44	<LOD	1.3-1.44	2	25
SMR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
acSMR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
acSMZ	0.81	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.81	1	12.5
SMX	2.58	<LOD	<LOD	<LOD	2.97	<LOD	<LOD	<LOD	2.58-2.97	2	25
acSMX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SMPZ	<LOD	2.59	1.55	<LOD	<LOD	<LOD	1.42	<LOD	1.42-2.59	3	37.5
SPY	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
acSPY	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SQX	<LOD	1.02	4.4	<LOD	<LOD	<LOD	<LOD	<LOD	1.02-4.40	2	25
STZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SMD	0.26	2.53	0.93	<LOD	14.8	2.31	3.71	10.6	0.26-14.83	7	87.5
SDM	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
TMP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
GFZ (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
MFA (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
NPX (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
DCF (-)	3.68	19.5	24.9	26.9	9.31	9.76	16.9	12.8	3.68-26.90	8	100
DCF-^{13C} (-)	<LOD	<LOD	<LOD	0.48	<LOD	<LOD	<LOD	<LOD	0.48	1	12.5
KPF (-)	<LOD	<LOD	<LOD	<LOD	<LOD	1.25	<LOD	0.45	0.45-1.25	2	25
IBU	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
APH (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
CBZ	<LOD	<LOD	<LOD	<LOD	0.09	0.27	<LOD	<LOD	0.09-0.27	2	25
CBZ-E	0.38	0.34	0.4	0.33	0.33	0.36	0.42	0.35	0.33-0.42	8	100
ATL	<LOD	<LOD	<LOD	<LOD	1.16	1.24	<LOD	<LOD	1.16-1.24	2	25
norFXT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.23	2.23	1	12.5
CFX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
N-desVFX	0.54	0.87	1	1.29	0.95	0.85	0.87	0.73	0.54-1.29	8	100
SCY (-)	6.14	14	19.9	19.4	18	53.8	10.7	9.46	6.14-53.80	8	100
CFF	8.8	15.24	<LOD	16.5	26.8	13.26	15.7	16	8.80-26.80	7	87.5

Values are in ng/g dw; <LOD: value below the limit of detection

Table S5. a) Concentrations in ng/g dw of the compounds present in at least one of the soil samples analysed, with MLOD, MLOQ, r2 and range. b) Total loads by group of compounds (UVFs, PBs and pharmaceuticals).

a)

	BP3	BP1	4HB	DHMB	4MBC	FLU	OXL	GMZ (-)	DCF (-)	SCY (-)
Soil lettuce BCS	0.80	n.d.	n.d.	0.41	1.09	0.70	1.07	1.29	37.40	10.46
Soil lettuce WSD	0.75	n.d.	0.62	n.d.	1.04	0.70	1.07	1.28	42.40	9.92
Soil lettuce WCS	0.74	1.76	n.d.	0.37	1.02	0.70	1.07	1.29	43.40	7.38
MLOD (ng/g dw)	0.06	0.09	0.10	0.09	0.13	0.08	0.05	0.00	0.53	0.04
MLOQ (ng/g dw)	0.21	0.31	0.33	0.30	0.44	0.27	0.16	0.01	1.78	0.12
r^2	0.9991	0.9982	0.9980	0.9983	0.9964	0.9987	0.9995	1.0000	0.9458	0.9997
Range	0.74-0.8	1.76	0.62	0.37-0.41	1.02-1.09	0.70	1.07	1.28-1.29	37.4-43.4	7.38-10.46

b)

	∑UVFs	∑PBs	∑Pharmaceuticals	∑TOTAL
Soil BCS	2.306	0	50.924	53.23
Soil WSD	2.41	0	55.366	57.776
Soil WCS	3.89	0	53.832	57.722

Table S6. a) Concentrations in ng/L of the compounds present in at least one of the water samples analysed, with MLOD, MLOQ, r2 and range. b) Total loads by group of compounds (UVFs, PBs and pharmaceuticals).

a)

	BP3	BP1	BP2	BP4 (-)	DHMB	4MBC	EtPABA	BZT	MeBZT	DMBZT	PrPB (-)	MePB (-)	FLU	OFX	NDX	OXL	acSDZ
Irrigation water WWTP	6.25	47.3	143	1030	15.3	173	1.14	623	601	51.9	21.4	26.9	9.82	1240	9.97	11.4	17
Irrigation water barriers	2.48	14.4	22.6	309	5.59	131	n.d.	487	224	12.5	21.3	27.1	11.7	246	n.d.	9.35	n.d.
MLOD (ng/L)	0.29	0.46	0.86	1.32	0.50	0.44	0.20	0.21	0.28	0.26	1.16	1.43	0.40	2.21	0.40	0.39	0.38
MLOQ (ng/L)	0.98	1.52	2.86	4.41	1.68	1.47	0.66	0.69	0.94	0.88	3.86	4.78	1.32	7.36	1.32	1.30	1.27
r^2	1.00	0.9979	0.9925	0.9825	0.9974	0.9980	0.9996	0.9996	0.9992	0.9993	0.9865	0.9796	0.9984	0.9534	0.9984	0.9984	0.9985

	acSMR	acSMZ	acSMX	SPY	acSPY	SDM	TMP	GMZ (-)	MFA (-)	NPX (-)	KPF	DCF (-)	CBZ	CBZ-E	ATL	norFXT	CFF
Irrigation water WWTP	11.50	7.98	83.1	30	116	<LOQ	35.2	916	23.7	454	157	1390	177	54.1	66.8	122	40.6
Irrigation water barriers	n.d.	8.67	6.94	11.6	n.d.	1.02	21.1	479	23.2	123	122	643	138	37.2	n.d.	n.d.	39.8
MLOD (ng/L)	0.34	0.13	0.07	0.21	0.13	0.19	0.83	0.30	0.24	0.12	0.17	0.99	0.28	0.44	0.23	0.58	0.48
MLOQ (ng/L)	1.14	0.44	0.22	0.71	0.43	0.62	2.76	1.00	0.81	0.41	0.57	3.30	0.94	1.46	0.76	1.93	1.60
r^2	1.00	0.9998	1.0000	0.9995	0.9998	0.9996	0.9930	0.9991	0.9994	0.9998	0.9997	0.9901	0.9992	0.9980	0.9995	0.9966	0.9976

b)

	∑UVFs	∑PBs	∑Pharmaceuticals	∑Others	∑TOTAL
Water WWTP	2691.89	48.3	4932.57	75.8	7748.56
Water barriers	1208.57	48.4	1881.78	60.9	3199.65

Table S7. Detected values in the spiked samples at three concentration levels.

Compound	BP3	BP1	BP2	BP4 (-)	4HB	4DHB	DHMB	AVO	4MBC	EHMC	EtPABA	BZT	MeBZT	DMBZT	UVP	BePB (-)	BuPB (-)	PrPB (-)	
10 ng/g dw (1)	100	125	142	138	106	132	83.3	92.3	100	98.1	108	117	120	79.1	151	135	115	118	
10 ng/g dw (2)	99.4	121	142	110	96.9	132	104	109	102	98.8	85.6	107	98.5	75.2	164	130	111	110	
10 ng/g dw (3)	101	114	141	114	96.4	134	96.8	119	107	95.8	103	117	96.4	81.9	141	140	101	110	
50 ng/g dw (1)	98.4	112.2	103.6	86.4	95	111.6	116.4	46.4	95.4	27.6	89.6	109.2	106.6	118.2	86.4	90.6	83.4	107	
50 ng/g dw (2)	98.8	101.4	106.8	90.8	91.2	102.8	119.8	47.4	92	27.8	93.4	101.4	95.2	117.6	82	108.2	103.2	100.4	
50 ng/g dw (3)	98.2	98	107.4	89.6	100.2	106.8	121.8	49.8	103.2	28.4	113.6	100.4	104.6	109.4	67.4	114.4	103.4	111	
100 ng/g dw (1)	98.7	99	104	92	102	94.8	113	50.2	98.2	16.4	98.5	86.3	100	109	87	77.6	73.8	89.9	
100 ng/g dw (2)	116	112	111	94.9	116	91	103	56.7	105	20.8	103	94.1	103	120	86.7	100	97.7	113	
100 ng/g dw (3)	110	108	110	116	117	104	99.6	48	102	22.5	94.3	113	112	126	85.8	101	103	122	
Compound	MePB (-)	Flumequine	Ofloxacin	Ciprofloxacin	Nalidixic acid	Oxolinic acid	Tetracycline	Oxytetracycline	Succynil-Sulfathiazole	SDZ	acSDZ	SMR	acSMR	acSMZ	SMX	acSMX	SMPZ	SPY	acSPY
10 ng/g dw (1)	95.8	116	82.1	97	109	110	108.5	112.4	137	121	124	101	87	99.9	110	81.6	116	58.1	140
10 ng/g dw (2)	81.2	107	73.8	99	115	137	104.7	89.1	103	82.9	110	67.1	91	117	118	75.6	111	60	108
10 ng/g dw (3)	85.8	129	75	104	109	124	107.5	107.2	84.4	110	133	78	99.4	112	110	114	119	51.8	103
50 ng/g dw (1)	80.6	97.8	120.6	71.2	97.6	103.2	78.7	93.3	102.6	73.8	96.6	92.8	91.6	101.6	108	112.2	137	89.4	97.6
50 ng/g dw (2)	102.8	110.4	119	110.2	124.6	108	97.3	97.2	103.8	69.4	94.2	88.4	93.8	94	92.4	102.8	92.8	109.4	80
50 ng/g dw (3)	82.8	96.8	116	119	125.6	107.2	97.5	118.3	103.2	68.6	97	86.6	93.6	97.2	87.6	90.6	95	103.8	90
100 ng/g dw (1)	73.1	105	106.3	96.6	109	104	69.6	102.6	135	93	99.7	94.1	104	96.8	86.6	90.5	77	105	118
100 ng/g dw (2)	97.1	101	94.4	112	92	89.1	92.1	107.2	134	99.9	106	102	109	110	130	117	110	115	109
100 ng/g dw (3)	106	111	108.6	93.1	88.2	108	97.1	98.2	140	98.2	84.9	102	103	98.1	124	106	111	118	86.4
Compound	SQX	STZ	Sulfisomidin	SDM	Trimethoprim	Gemfibrozil (-)	Mefenamic acid (-)	Naproxen (-)	IBU (-)	Ketoprofen (-)	Diclofenac (-)	Diclofenac-13C (-)	Paracetamol	CBZ	CBZ-epoxy	Atenolol	norFXT	Salicylic acid (-)	Caffeine
10 ng/g dw (1)	88.3	117	92.0	95.3	97.1	164	102	95.1	109	154	134	113.6	120	99.8	121	126	110	108	97
10 ng/g dw (2)	72	70.4	90.8	97.1	97.5	163	110	93.8	108	144	135	98.0	124	105	121	83.4	115	106	96
10 ng/g dw (3)	112	77.4	90.8	104	75.7	155	99.4	93.8	102	109	137	102.0	101	106	122	80	112.9	107	97
50 ng/g dw (1)	91.4	113.4	108.4	103.8	94.6	96.2	129.6	112	79.6	111	100.2	91.0	107.6	95.8	105	95.8	103.4	102.2	94.8
50 ng/g dw (2)	112.4	86.4	97.9	115	89.6	116.2	119.8	101.2	62.8	114.6	127.8	107.2	106.8	121	119.4	88.2	107.8	92.6	91.6
50 ng/g dw (3)	103	88.8	101.6	128.8	114.8	100.4	92.6	105	91	111	109.4	101.8	101.4	113.6	113.2	88.6	94.2	102.2	101.4
100 ng/g dw (1)	104	134	109.4	127	99	86	93.5	113	59.8	96.8	94	124.0	114	94.8	99.4	72	111	126	121
100 ng/g dw (2)	97.2	116	113.2	95.6	103	107	91.5	96.7	78.4	106	99.2	98.4	99.5	104	107.4	93.2	101	118	88.4
100 ng/g dw (3)	96.5	133	112.3	129	116	110	92.8	112	81.2	112	98.8	106	101	106	100.7	104.6	96.8	105	117

(1), (2), (3): Number of replica; (-): analyzed in negative mode

Table S8. Average of the recovered values at the three spiked concentrations and intra-day relative standard deviation.

Compound	BP3	BP1	BP2	BP4 (-)	4HB	4DHB	DHMB	AVO	4MBC	EHMC	EtPABA	BZT	MeBZT	DMBZT	UVP	BePB (-)	BuPB (-)	PrPB (-)	
Average C1	100.1	120.0	141.7	120.7	99.8	132.7	94.7	106.8	103.0	97.6	98.9	113.7	105.0	78.7	152.0	135.0	109.0	112.7	
Average C2	98.5	103.9	105.9	88.9	95.5	107.1	119.3	47.9	96.9	27.9	98.9	103.7	102.1	115.1	78.6	104.4	96.7	106.1	
Average C3	108.2	106.3	108.3	101.0	111.7	96.6	105.2	51.6	101.7	19.9	98.6	97.8	105.0	118.3	86.5	92.9	91.5	108.3	
RSD% Intra C1	0.8	5.6	0.6	15.1	5.4	1.2	10.5	13.5	3.6	1.6	11.8	5.8	13.1	3.4	11.5	5.0	7.2	4.6	
RSD% Intra C2	0.3	7.4	2.0	2.3	4.5	4.4	2.7	1.7	5.7	0.4	12.9	4.8	6.1	4.9	9.9	12.3	11.5	5.4	
RSD% Intra C3	8.8	6.7	3.8	13.1	8.4	6.7	7.0	4.5	3.4	3.1	4.4	13.7	6.2	8.6	0.6	13.2	15.6	16.6	
Compound	MePB (-)	Flumequine	Ofloxacin	Ciprofloxacin	Nalidixic acid	Oxolinic acid	Tetracycline	Oxytetracycline	Succynil-Sulfathiazole	SDZ	acSDZ	SMR	acSMR	acSMZ	SMX	acSMX	SMPZ	SPY	acSPY
Average C1	87.6	117.3	77.0	100.0	111.0	123.7	106.9	102.9	108.1	104.6	122.3	82.0	92.5	109.6	112.7	90.4	115.3	56.6	117.0
Average C2	88.7	101.7	118.5	100.1	115.9	106.1	91.2	102.9	103.2	70.6	95.9	89.3	93.0	97.6	96.0	101.9	108.3	100.9	89.2
Average C3	92.1	105.7	103.1	100.6	96.4	100.4	86.3	102.7	136.3	97.0	96.9	99.4	105.3	101.6	113.5	104.5	99.3	112.7	104.5
RSD% Intra C1	7.5	11.1	4.5	3.6	3.5	13.5	2.0	12.2	26.7	19.6	11.6	17.3	6.3	8.8	4.6	20.7	4.0	4.3	20.1
RSD% Intra C2	12.2	7.6	2.3	25.4	15.9	2.6	10.8	13.4	0.6	2.8	1.5	3.2	1.2	3.8	10.7	10.8	24.9	10.3	8.8
RSD% Intra C3	17.0	5.0	7.6	10.1	11.1	10.0	14.7	4.5	3.2	3.6	10.8	4.6	3.2	7.3	23.5	13.3	19.3	6.8	16.3
Compound	SQX	STZ	Sulfisomidin	SDM	Trimethoprim	Gemfibrozil (-)	Mefenamic acid (-)	Naproxen (-)	IBU (-)	Ketoprofen (-)	Diclofenac (-)	Diclofenac-13C (-)	Paracetamol	CBZ	CBZ-epoxy	Atenolol	norFXT	Salicylic acid (-)	Caffeine
Average C1	90.8	88.3	91.2	98.8	90.1	160.7	103.8	94.2	106.3	135.7	135.3	104.5	115.0	103.6	121.3	96.5	112.6	107.0	96.7
Average C2	102.3	96.2	102.7	115.9	99.7	104.3	114.0	106.1	77.8	112.2	112.5	100.0	105.3	110.1	112.5	90.9	101.8	99.0	95.9
Average C3	99.2	127.7	111.6	117.2	106.0	101.0	92.6	107.2	73.1	104.9	97.3	109.5	104.8	101.6	102.5	89.9	102.9	116.3	108.8
RSD% Intra C1	20.1	25.1	0.7	4.6	12.5	4.9	5.5	0.8	3.8	23.6	1.5	8.1	12.3	3.3	0.6	25.6	2.5	1.0	0.6
RSD% Intra C2	10.5	14.9	5.3	12.5	13.3	10.5	19.2	5.5	14.2	2.1	14.1	8.2	3.4	13.0	7.2	4.3	6.9	5.5	5.0
RSD% Intra C3	4.1	10.1	2.0	18.7	8.9	13.1	1.0	9.1	11.6	7.7	2.9	13.1	8.0	6.0	4.3	16.5	7.3	10.6	17.8

C1: Spiked concentration 1; C2: Spiked concentration C2; C3: Spiked concentration C3; RSD% Intra: Relative standard deviation Intra-day

Section S5. Figures

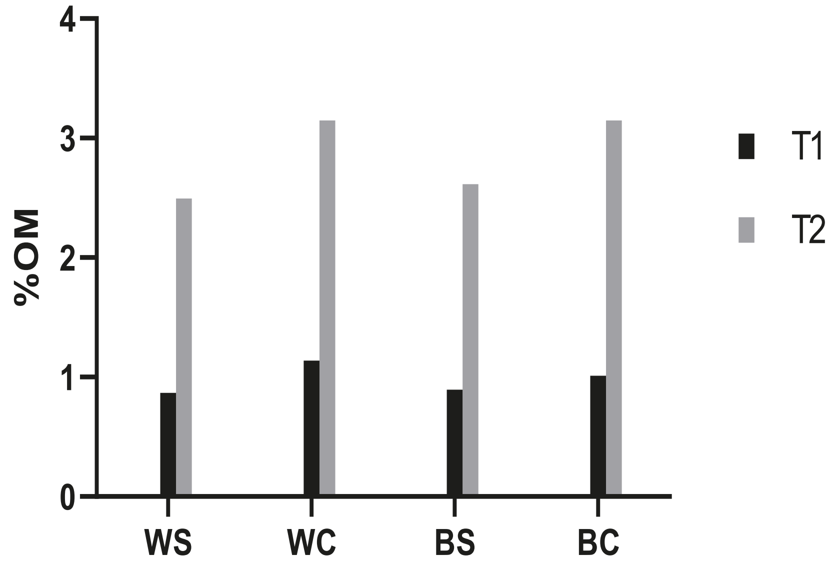

Figure S1. Percentage of organic matter in soil irrigated with W and B waters after one year of irrigation. T1: initial; T2: one year after. WS: S soil-W water; WC: C soil- W water; BS: S soil-B water; BC: C soil-B water.

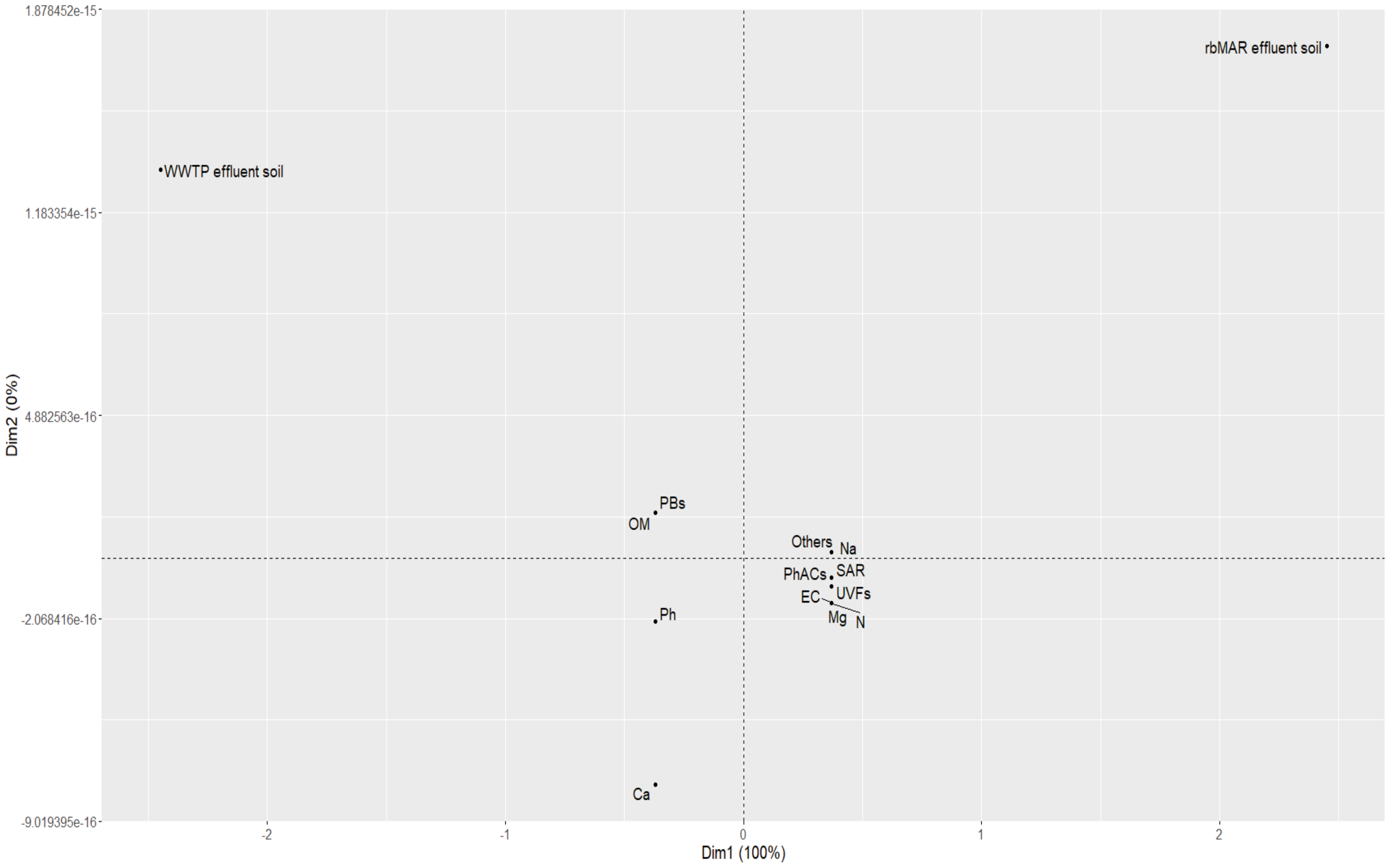

Figure S2. Biplot of the soil results, where the load of PPCPs are represented together with their physicochemical properties.

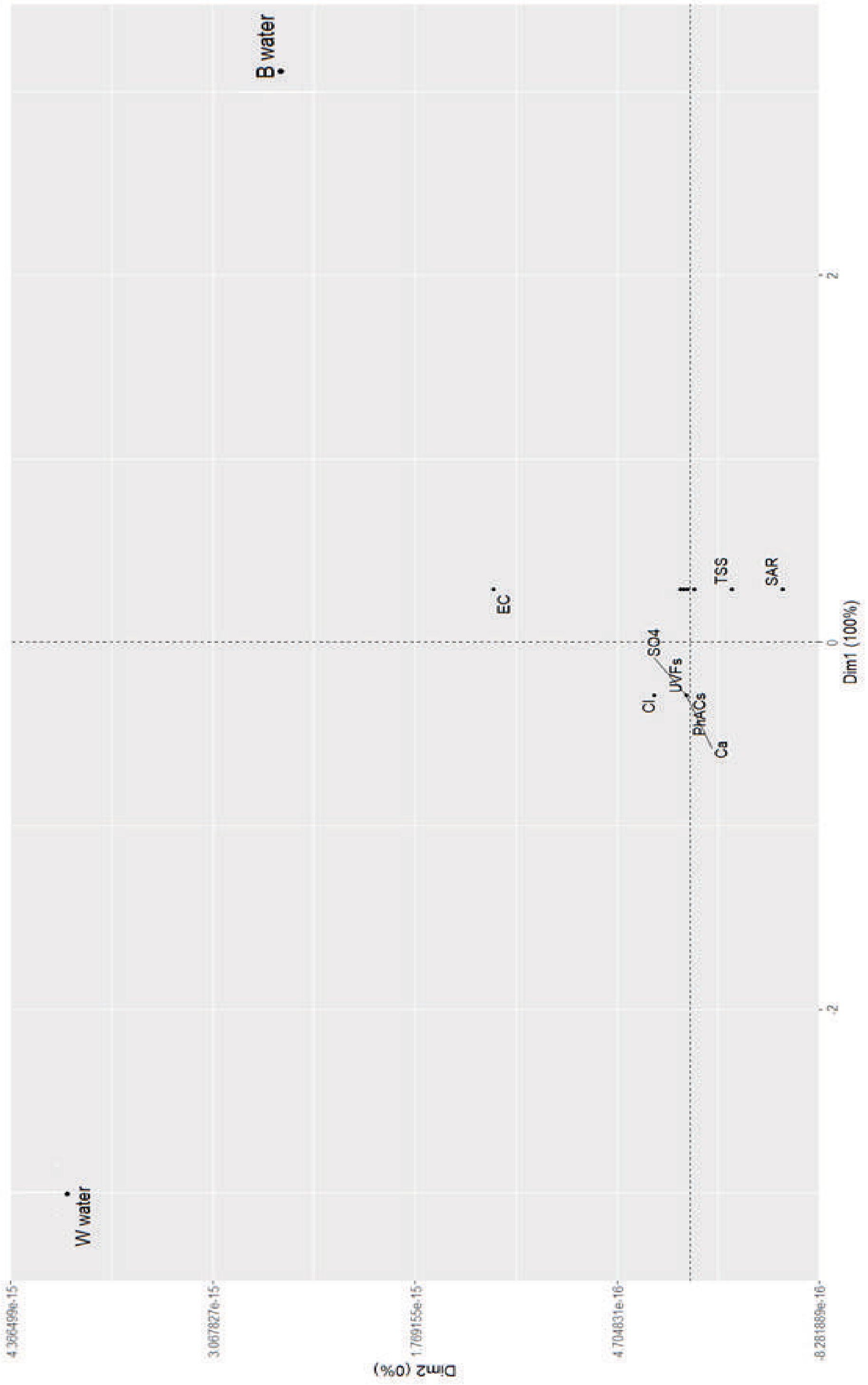

Figure S3. Biplot of the irrigation waters results, where the load of PPCPs are represented together with their physicochemical properties.

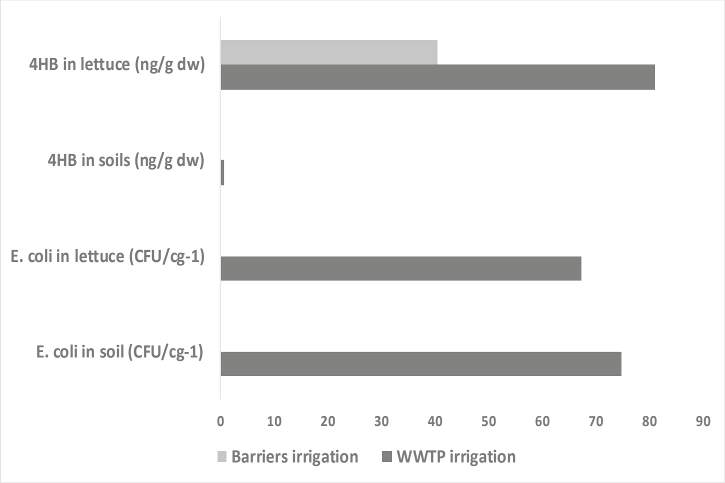

Figure S4. Comparison of *E. coli* and 4HB concentrations between lettuces and soils irrigated with WWTP and barriers effluents water.

5.2.3 Publication #11

Pharmaceuticals' and personal care products' uptake by crops irrigated with reclaimed water and human health implications

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Pharmaceuticals and personal care products uptake by crops irrigated with reclaimed water and human health implications

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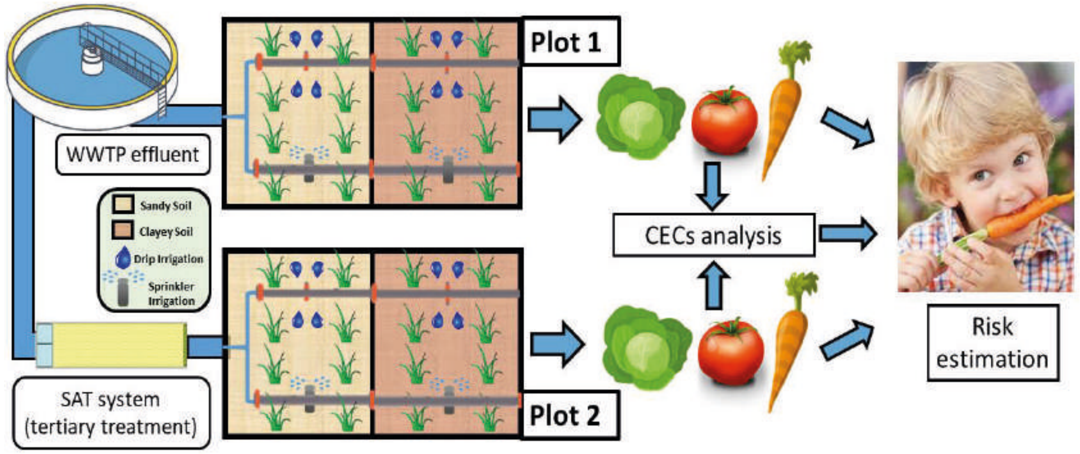
Keywords: PPCPs, water reuse, plant uptake, LC-MS/MS, human health risk, QuEChERS.

Highlights:

- Cultivation conditions (e.g. soil composition) influence PPCPs uptake.
- Diclofenac, norfluoxetine, and salicylic acid show the highest accumulation values.
- PPCPs were more uptaken by carrots than by tomatoes or lettuce.
- Determined PPCPs' concentrations in the crops do not pose a human health risk.

Graphical abstract:



ABSTRACT

In recent years, reclaimed water use for agricultural irrigation has increased worldwide to alleviate water scarcity, boosted by climate change and global population increase. However, emerging contaminants, such as pharmaceuticals and personal care products (PPCPs), are still present in reclaimed waters and can be translocated by crops. In this study, the occurrence of 56 PPCPs in tomatoes, lettuces, carrots, soil, and irrigation water, was evaluated. Secondary effluent of an urban WWTP and the further reclaimed water produced by a coupled soil aquifer treatment (SAT) pilot system were tested for irrigation. Besides water quality, the influence of soil composition and irrigation system (dripping or sprinkling) on the PPCPs' crop uptake was also investigated. Significant and differential bioaccumulation of the PPCPs was observed with a total load of 1248 ng/g dry weight (dw), 1692 ng/g dw, and 7787 ng/g dw in lettuces, tomatoes, and carrots, respectively. Overall, the best conditions to lower the uptake of PPCPs by crops was to irrigate by sprinkling with SAT-reclaimed water in soil with high clay content. According to the risk assessment estimation, the consumption of the crops and the use of reclaimed water for drinking water supply posed no risk to human health.

1. INTRODUCTION

Due to the increasing world population and life expectancy, thousands of tons of pharmaceuticals and personal care products (PPCPs) are consumed yearly, and their use is expected to increase in the following years. PPCPs include a wide range of chemical compounds intended to prevent and treat human and animal diseases and for personal care and hygiene [1]. PPCPs are considered contaminants of emerging concern (CECs) due to their persistence, potential toxicity, and bioaccumulation in the environment. As they present low degradability, they enter massively into the environment. Furthermore, those that can be degraded to some extent are constantly released, making them permanently present in the environment. Many studies show that continuous exposure to these compounds leads living organisms, including humans, to be at risk [2].

PPCPs can be introduced into the environment directly by recreational aquatic activities and sunbathing, and indirectly from urban (excreta, showers…), hospitals, and industrial discharges into the sewage systems [3]. Therefore, wastewater treatment plants (WWTPs) play an essential role in the spread of these contaminants. Still, conventional wastewater treatments are not designed to remove CECs efficiently from the wastewater [4,5]. In particular, many studies have demonstrated the poor removal of PPCP [4,6,7]. Improvements in wastewater treatments are required to prevent/minimize the spread of these pollutants into the environment [8].

Water scarcity is a current problem that will worsen in the coming years due to climate change and the increasing water demand boosted by population growth. Thus, wastewater reuse for agricultural irrigation has been considered a helpful solution, and its implementation is increasing worldwide, not only in arid countries. However, literature reports a wide range of PPCPs transported by wastewater effluents [9,10], whose use as irrigation water could lead to PPCPs uptake by the crop. Only, two studies have evaluated the role of the type of crop or cultivation conditions (e.g. soil composition or irrigation system) on PPCPs uptake [11,12], crops under controlled conditions i.e. hydroponics and greenhouse [13,14]. There is, therefore, an urgent need to evaluate the potential human health implications of using reclaimed water in agriculture intended for human consumption under field agri-food production practices.

This work aimed to identify the agricultural conditions minimizing PPCPs uptake by crops when using treated wastewater and reclaimed water for irrigation, and to assess whether the levels bioaccumulated in the edible parts can constitute a risk to human health. To this end, 56 PPCPs frequently found in WWTP effluents were analyzed in the irrigation waters, soils, and vegetables. Finally, the risk posed to human health through the diet was estimated in terms of risk quotients (HQ).

2. MATERIALS AND METHODS

2.1. Standards and reagents

The 56 PPCPs selected for this study encompassed different families of compounds (anti-inflammatory, antibiotics, analgesics, antidepressants, UV filters, UV blockers, and parabens, among others) that are usually detected in WWTP effluents. Their physicochemical properties are shown in **Table S1** in the Supplementary Information (SI), and data about purity and purchase brands are provided in **Section S1** of SI.

2.2. Agricultural plots and crops description

The WWTP of Palamós (Girona) located in the Nord-East Spanish Mediterranean coast, was selected for the study. The wastewater treatment includes pre-treatment (waste retention) and primary and secondary biologic treatment (activated sludge). In this facility, a water reclamation pilot plant was installed, consisting of six soil aquifer treatment (SAT) systems, five complemented with natural-based reactive barriers to improve chemical degradation (rbSAT). The last one was not implemented with reactive barriers, considered as the reference of the system. Details on this tertiary pilot system can be found elsewhere [15,16].

Two agricultural plots were built next to the rbSAT and the WWTP secondary treatment. One plot was irrigated with the secondary effluent (**W water**), and the other with the reclaimed water from the rbSAT (**B water**). To investigate the effect of soil composition and irrigation system on PPCP uptake by plant, each plot was divided into two subplots containing different proportions of clay (C soil) and sand (S soil) and with two irrigation systems (dripping (D) and sprinkling (S)). These three factors were evaluated in three types of crops, according to what is consumed, i.e., lettuce (the leaf), carrot (the root), and tomato (the fruit). Additional information about the plots design, sensors installed, water parameters, etc., can be found elsewhere [17]. Depending on the cultivation conditions, different acronyms are used (e.g. crops irrigated with wastewater (W) in a clayey soil (C) by sprinkling (S) irrigation are referred to as WCS). All acronyms are summarized in **Figure S1** of SI.

2.3. Samples

Agricultural soils and irrigation waters were collected together with crops in the dates listed in Table 1. Three vegetables were selected as investigated crops. The carrots were from the *Daucus carota sativus* variety, tomatoes from the *Solanum Lycopersicum* variety, and lettuces from the *Chicorium intybus* variety. The crops were harvested when they had grown enough and were suitable for market consumption. For the analysis, ten specimens from each subplot were harvested randomly, shaken to remove soil particles, and stored in zip bags. Soil samples (100 g per subplot) were collected in zip bags from different representative points and mixed. Irrigation waters (500 mL) were collected from the hoses at the connection points with the irrigation systems in 1L brown glass bottles. All samples were transported to the lab in portable refrigerators.

Table 1. Sampling dates and type of samples collected.

Date	Samples collected
10/12/2018	Lettuces, soils, and irrigation waters
30/05/2019	Carrots, soils, and irrigation waters
20/08/2019	Tomatoes, soils, and irrigation waters

In the lab, the vegetables and were sliced into small pieces, and, as well as soils, frozen, freeze-dried, and homogenized in a crusher to a fine powder. Sample belonging to the same subplot were homogenized together and stored at -20ºC. The water samples were frozen upon arrival at the lab until analysis. For lettuces, only the leaves were preserved; for carrots, only the carrot itself, without roots and leaves. The tomatoes were peeled before preservation under the same conditions as the other vegetables. Therefore, only the edible parts were considered for analysis.

2.4. Sample pre-treatment, extraction, and analysis

The extraction of the PPCPs from lettuces, carrots, tomatoes, and soils was performed by optimizing our QuEChERS-based method developed for lettuce analysis [17,18]. QuEChERS is a known extraction method used to perform pesticide analysis on vegetables [19,20]. However, it was optimized for the multi-residue analysis of PPCPs in crops and soil. Details on the HPLC-MS/MS analysis of the extracts can be consulted in **Table S2** in **SI**. Detailed information on the optimization and validation in tomatoes, carrots, and soils is included in **Section S2**. The irrigation water analyses were carried out by our previously developed online solid-phase extraction and HPLC-MS/MS analysis [21].An example of the ion chromatograms of the compounds detected in sample BCS of the tomatoes is shown in **Figure S2** of SI.

2.7 Human health risk assessment

2.7.1. Drinking water

The irrigation water from the rbSAT system was assessed to know whether its use for drinking water production would be safe for human health. Risk quotients (RQ) were calculated for those compounds present in the irrigation water according to Eq. 1

(Eq. 1) $$RQ = {}^{\mathrm{C_{max}}}/_{\mathrm{DWEL}}$$

where C_{max} corresponded to the maximum PPCPs concentration and DWEL to the drinking water equivalent level calculated according to Eq. 2

(Eq. 2) $$DWEL = {}^{\mathrm{ADI\ x\ BW\ x\ 1000}}/_{\mathrm{DWI}}$$

where BWI is the daily water ingestion rate, BW is the body weight, and ADI is the acceptable daily intake from literature [22–27]. BW was considered 70 Kg (average male adult between 18-74 years old) and 24 Kg for children (average child between 3-9 years old).

2.7.2. Crops consumption

To assess the risk posed by the consumption of vegetables, hazard quotients (HQ) were estimated according to Eq. 3

(Eq. 3) $$HQ = \frac{ADI}{EDI}$$

where EDI is the estimated daily intake calculated following Eq. 4

(Eq. 4) $$EDI = \frac{DI \cdot C_e}{BW}$$

where C_e is the average compound concentration and DI is the daily intake from ENALIA and ENALIA2 Surveys from AECOSAN.

Finally, considering a linear approach, the hazard index (HI), considered as the cumulative risk of all the compounds present, was calculated following Eq. 5

(Eq. 5) $$HI = \Sigma HQ$$

2.8. Data Analysis

An exhaustive data analysis was necessary due to the large amount of data. Principal component analysis (PCA) and t-tests ($p<0.05$) were carried out to evaluate the data and compare between conditions and matrices. The PCA and t-tests were performed with R software (v. 4.1.2) using RStudio (v. 2021.09.01+372). The package used for this was factoextra, and the script used is described in **Section S3** of SI.

3. RESULTS AND DISCUSSION

The method used for the analysis of the lettuces [15] was applied for carrots and tomatoes, and a slight modification (5 g of the sample instead of 1 g) was required to be applied to soil analysis. All validation parameters and matrix effect (ME) are discussed in **Section S2** of SI. Information about linearity ranges, coefficients of determination, and limits of detection (LOD) and quantification (LOQ) of tomatoes, carrots, soils, and waters can be consulted in **Tables S3, S4, S5**, and **S6** in **SI**.

3.1. PPCPs' occurrence in crops, soil and irrigation waters

The individual concentrations of the PPCPs determined in the vegetables, soils, and irrigation waters are compiled in **Tables S7, S8, S9, S10,** and **S11** of **SI**, respectively.

3.1.1. Irrigation water

Irrigation waters were analyzed to assess the potential water-crop transfer. **Figure 1** shows that all water samples contained PPCPs residues and that their concentration varied depending on the season collected, as expected. Waters from the secondary effluent of the WWTP were more contaminated than the waters provided by the rbSAT. This demonstrated that reactive barriers are an effective approach to remove efficiently most PPCPs. Forty-two of the fifty-seven target compounds were detected at least in one water sample.

The pharmaceuticals group presented the highest concentrations in the irrigation waters, both in the WWTP (**W water**) and rbSAT effluents (**B water**), with accumulated loads up to 16383 ng/L and 6720 ng/L, respectively. PBs group was, by far, the group with the lowest concentrations, with 232 ng/L (**W water**) and 148.80 ng/L (**B water**) of the total load. Probably it is because paraben preservatives have a high removal rate in conventional wastewater treatments [28]. The highest average concentrations corresponded to the UV filter benzophenone-4 (BP4) with 1673 ng/L, diclofenac (DCF) with 1503 ng/L, ofloxacin (OFX) with 1106.00 ng/L, and gemfibrozil (GFZ) with 1103.67 ng/L, all of them detected in **W water**; DCF with 911.67 ng/L, benzotriazole (BZT) with

482.33 ng/L, methyl-benzotriazole (MeBZT) with 402.33 ng/L, and BP4 with 372.33 ng/L were the highest in **B water**.

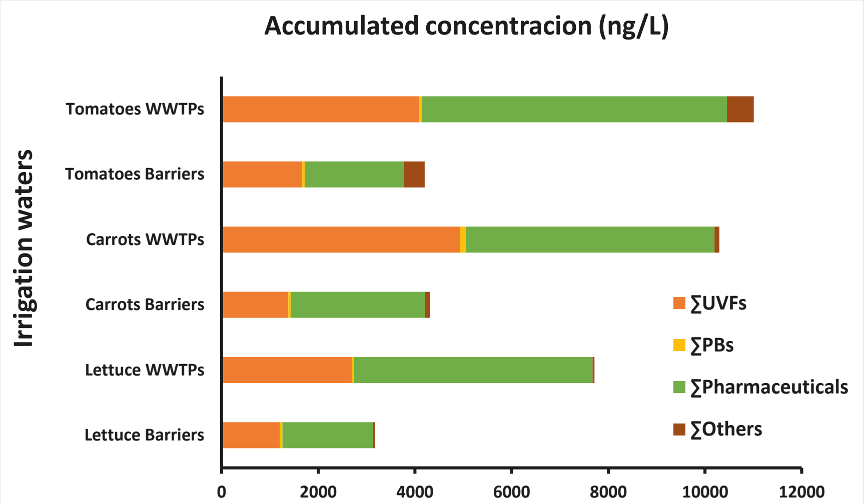


Figure 1. Accumulated concentrations (∑) of UVFs, PBs, pharmaceuticals, and others in irrigation waters (ng/L).

A t-test for independent groups between **W and B waters** was carried out. The results showed statistically significant differences (95% confidence) between them (t(161)=2.7590), demonstrating that the tertiary treatment based on SAT contributed in the PPCPs removal.

3.1.2. PPCPs accumulation in soil

Overall, PPCPs concentrations in the sandy soils (**S**) S soils were higher than in clayey soils (**C soil**). As in irrigation waters, pharmaceuticals showed the highest levels in all soils, but caffeine (CFF), found in all water samples, was never detected. The PBs group was only present in carrot soils. In **S** soils of all the crops, total load of pharmaceuticals was slightly higher than in clayey soils, as shown in **Figure 2**. For example, accumulated concentrations of 46 ng/g dw were found in the tomatoes' **S** soils and 41 ng/g dw in **C** soil. However, in tomatoes and lettuce soils, the UV filters presented higher accumulation values in the C soils than in the S soils. The anti-inflammatory DCF was the most accumulated compound in soils (45 ng/g dw average), far from the next compound, salicylic acid (SCY), 14 ng/g dw.

Regarding crops, tomatoe soils were the ones with the highest accumulated values, followed by carrots, and lettuce. A significant difference is observed among irrigation waters since soils irrigated with W water showed higher accumulated values in all cases.

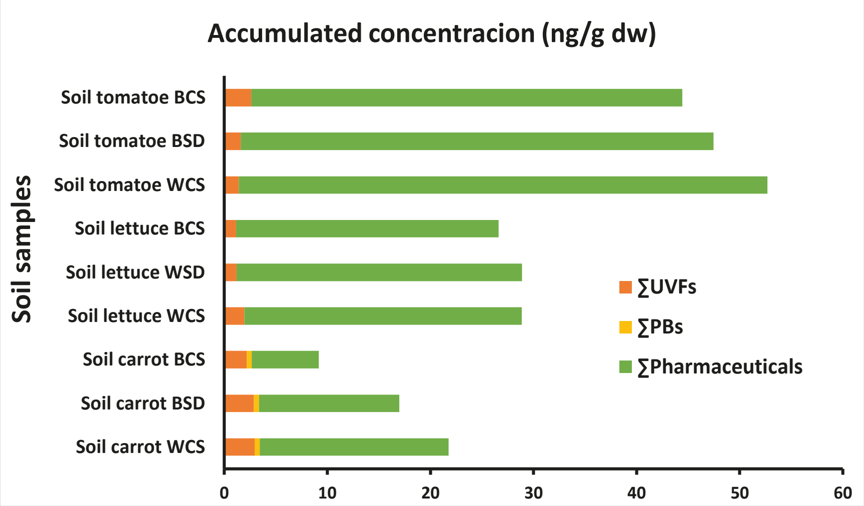


Figure 2. Accumulated concentrations (∑) of UVFs, PBs, pharmaceuticals, and others in soils (ng/g dw).

To evaluate if the crop influenced the levels of PPCPs in the soils, a t-test was performed between the levels found in the same soils, but where different crops were cultivated. All t-tests (tomato and lettuce (t(11)=0.4843), tomato and carrot (t(7)=1.1585), and lettuce and carrot (t(7)=1.0544)) showed no significant differences. Therefore, the accumulated concentrations found in the soils were independent of the type of crop cultivated.

3.1.3. PPCPs bioaccumulation in vegetables

Lettuce

In lettuces, UV filters contributed the most to the total load of PPCPs accumulated (671 ng/g dw), as shown in **Figure 3**. The PBs was the group showing lower values (40 ng/g dw in the total accumulated), as previously observed for waters and soils. Thirty-one out of the fifty-five target compounds were detected at least in one lettuce sample. The highest average concentrations corresponded to 4-hydroxybenzophenone (4HB), metabolite of the UVF oxybenzone (BP3), with 61 ng/g dw, SCY with 19 ng/g dw, CFF with 16 ng/g dw, and benzophenone-2 (BP2) with 13 ng/g dw. The samples cultivated by S irrigation presented lower concentrations of PPCPs than those by D irrigation, likely due to the loose of water when spreading and higher oxidation of the water components. Overall, these results suggest that the lower concentration of PPCPs accumulated by lettuces corresponds to those irrigated with B water by S irrigation in C soil.

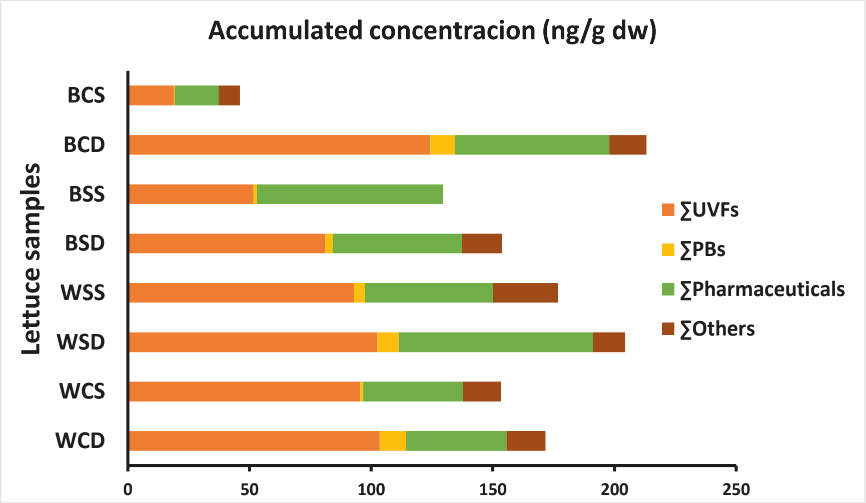


Figure 3. Accumulated concentrations (∑) of UVFs, PBs, pharmaceuticals, and others in lettuces (ng/g dw)

A t-test for the values found in lettuces irrigated with W water and B water (BCS and WCS) showed no statistical differences (t(20)=-1.3650). In contrast, statistical differences where observed when testing the irrigation method (BCS and BCD) (t(11)= 2.1804) or the soil composition (BCS and BSS)(t(23)=2.3406).

Therefore, these results suggest that the PPCPs values found in the lettuces were significantly influenced by the irrigation method and soil composition, but the contamination load in the irrigation waters was not that relevant.

Carrots

In carrots, pharmaceuticals were the group with the highest total load (7125 ng/g dw), as shown in **Figure 4**. Following previous trends, the PBs group was the one that presented the lowest values (81.61 ng/g dw in the total accumulated). The highest average concentrations corresponded to SCY with 678 ng/g dw, norfluoxetine (norFXT) with 132 ng/g dw, AAP with 31 ng/g dw, and CFF with 31 ng/g dw. Only seventeen of the fifty-six target compounds were detected in carrots, but the average concentrations were considerably high. The best combination to lower the PPCPs uptake in carrots was the same as in lettuce; irrigating with B water by sprinkling in C soil.

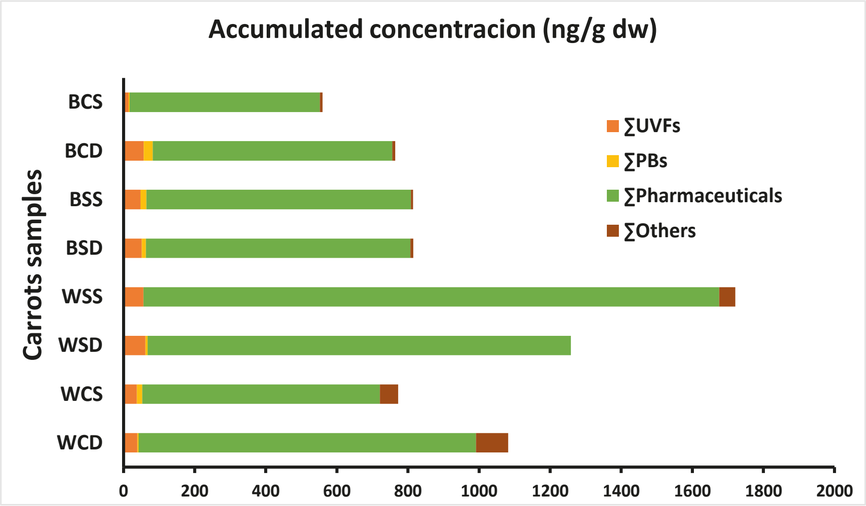


Figure 4. Accumulated concentrations (∑) of UVFs, PBs, pharmaceuticals, and others in carrots (ng/g dw).

Different t-tests were performed with the detected values in carrots, comparing soil composition (BCD or BSD)(t(19)=0.2620), irrigation method (BCS and BCD) (t(20)=0.0699), and water quality (BCS or WSS) (t(12)=0.7803) showing no statistical differences. This suggests that, unlike for lettuce, cultivation variables do not influence the PPCPs' uptake by carrots.

Tomatoes

Tomatoes followed the same trend shown by lettuce and tomatoes, where pharmaceuticals presented higher values in the total load (943.81 ng/g dw) and PBs the lowest (38 ng/g dw total load). However, in tomatoes, pharmaceuticals and UVFs levels were similar in most samples. Twenty-six out of the fifty-seven target compounds were detected at least in one tomato sample. The highest average concentrations corresponded to DCF with 116 ng/g dw, SCY with 48 ng/g dw, BP4 with 34 ng/g dw, and 4HB with 16 ng/g dw. The total load of PPCPs classified by groups is represented in **Figure 5**. The best combination to minimize the PPCPs uptake by tomatoes was the same as previously observed in lettuce and carrots; irrigating with B water by sprinkling in C soil.

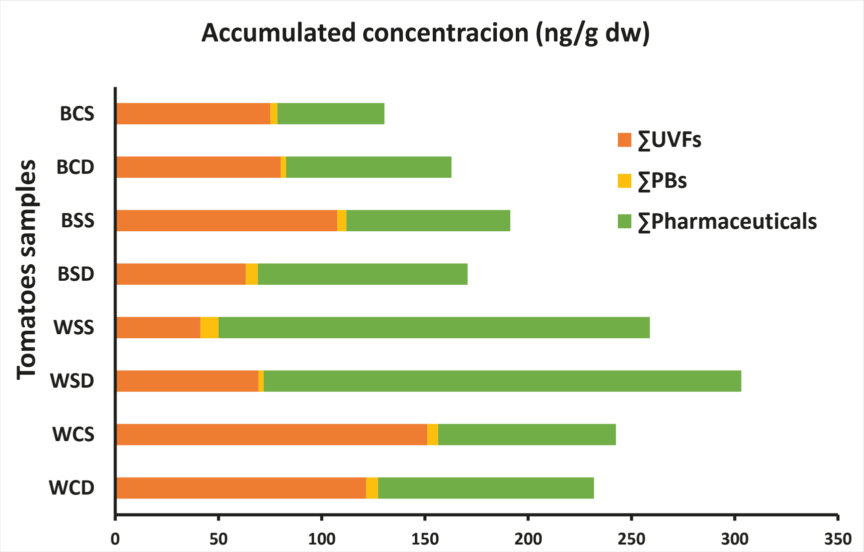


Figure 5. Accumulated concentrations (∑) of UVFs, PBs, pharmaceuticals, and others in tomatoes (ng/g dw).

Similarly, t-tests were carried out to compare the PPCPs values found in tomatoes in order to compare water quality (BCS or WSS) (t(32)=0.5826), irrigation method (BCS and BCD) (t(27)=0.4056), and soil composition (BCS or BSS)(t(30)=0.3049), showing no statistical differences. Likewise in carrots, cultivation conditions did not influence significantly PPCPs uptake by tomatoes.

3.1.4. Vegetables PPCPs uptake comparison

Overall, when comparing lettuce, tomato, and carrot, the latter was found to bioaccumulate the highest levels of PPCPs. As carrots are root crops, they are in continuous contact with the PPCPs present in the water and soil. **Figure 6** shows the total load of PPCPs bioaccumulated in the different types of crops, cultivated under different conditions. Average concentrations ranged from 4.7 to 11.2 ng/g dw for lettuces, 7.3 to 17.2 ng/g dw for tomatoes, 10 to 30.7 ng/g dw for carrots, 1.1 to 5.5 ng/g dw for soils and 129 to 262 ng/L for irrigation waters.

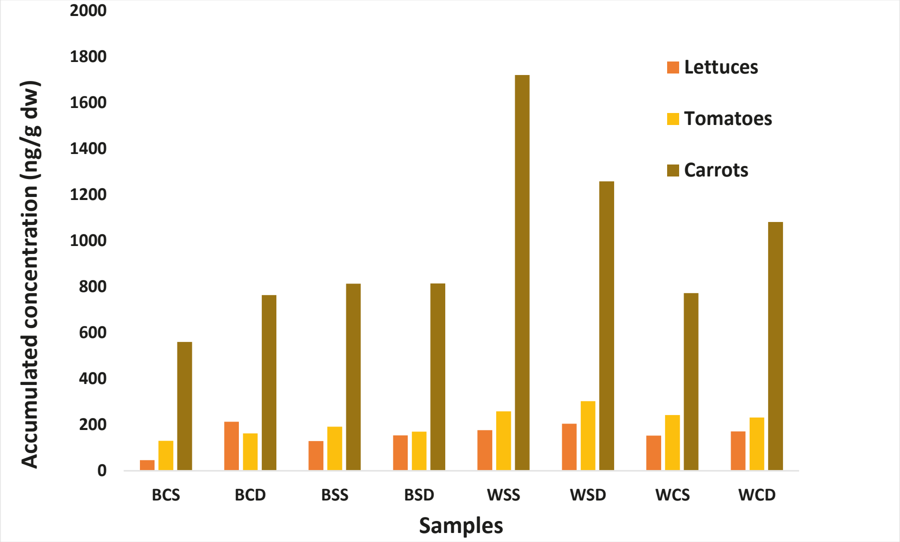


Figure 6. Accumulated concentrations (∑) in the different plots, separated by vegetable (ng/g dw).

Different t-tests on the concentrations found in lettuces, tomatoes, and soils, showed statistical differences between them. Since the comparison was performed under the same cultivation conditions, it evidences that PPCPs were selectively bioaccumulated by crops and soils. It suggests that plant uptake not only depends on the cultivation conditions (as in the case of lettuce) but also on the processes taking place, i.e. absorption/transport, and degradation of these compounds.

Regarding compounds, the pharmaceuticals DCF and SCY and the UV filters 4HB and BP4 were the compounds with the highest concentration in most of the samples.

DCF is an anti-inflammatory commonly used to relieve pain and is prescribed for arthritis [29,30]. The highest concentration of DCF found was 360 ng/g dw in tomatoes, 85 ng/g dw in carrots, and 25 ng/g dw in lettuces. Other studies reported similar levels as 8 ng/g dw [31] or 19 ng/g dw [22] in lettuces. Still, lower in tomatoes,12 ng/d dw [32], but DCF's uptake by carrots was not previously reported, even though a few studies focused on this crop. However, Kovacs *et. al.* [33] demonstrated the concentration increase of DCF over the time with spiking levels in irrigation water and soils similar to the ones in this work. This long exposure to high levels of DCF could explain the levels found in the crops. DCF has been reported to produce immobilization and reduced reproduction in *Daphnia magna* and teratogenicity and mortality in *Zebrafish* embryos [34] and humans, affecting mainly the intestine, liver, and kidney [35].

SCY is the metabolite of acetylsalicylic acid (aspirin), one of the most consumed analgesics in the world. Still, it is also found in various over-the-counter products, such as topical anti-acne products. Besides, SCY is known to be naturally produced in crops and plants [36]. However, tomatoes are known to produce much higher levels of this compound [37], and the measured SCY levels in carrots were much higher (2590 ng/g dw) than in tomatoes (143 ng/g dw). It suggests that SCY levels are not naturally synthetized in this case (tomatoe levels would be higher than carrots'), but rather uptaken. A remarkable fact is that SCY was not found in the irrigation water. Some studies demonstrated SCY uptake in lettuces [38] or tomatoes [39], but it was not found in carrots, such as DCF, since it is not a crop usually studied regarding PPCPs uptake. SCY has been demonstrated to cause salicylism at high blood levels [40] and may promote fetal risk as it crosses the placenta [41].

BP4 is one of the most used UVFs in the formulation of sunscreens and similar products worldwide, and 4HB is a metabolite of BP3, another widely used UVF. The presence of both compounds in aquatic systems is well-studied [42,43]. BP4 showed very low uptake in carrots and lettuces but a very high concentration (140 ng/g dw) in tomatoes. The metabolite 4HB was also present in the three matrices, lettuces being the crop that showed the highest uptake (84 ng/g dw). No data on these compounds have been found in the literature in similar matrices.

The only compound that was present in the water samples but was not detected in any crop or soil was trimethoprim (TMP). Thus, it suggests that it is not bioaccumulated in crops or soil but degraded or infiltrated to deeper surfaces.

In all the crops, the samples that presented the lowest accumulation of PPCPs were the ones that were irrigated by sprinkling with the B water, and cultivated in the C soil. Although the statistical analysis generally showed no influence of the cultivation variables, the uptake values in all crop types suggest that this combination of cultivation conditions is the best way to minimize the uptake of PPCPs.

PCA of the PPCPs in vegetables

All PPCP concentrations in the vegetables were escalated to avoid enormous contributions to the variance, and a PCA analysis was performed. Concentrations not detected or detected below the limits of detection (<LOD) or the limits of quantification (<LOQ) were substituted by LOQ/2 values to avoid statistical issues. Compounds not detected in any sample were also removed to build the PCA. (**Figure 7**).

Three groups of PPCPs had very similar behaviour. The first group includes butylparaben (BuPB), acetylsulfamethazine (acSMZ), sulfamethoxazole (SMX), sulfisomidine (SMD), nalidixic acid (NDX), sulfaquinoxaline (SQX), sulfadiazine (SDZ), benzocaine (EtPABA), BZT, drometrizole (UVP), N-desmethylvenlafaxine (N-desVFX), and carbamazepine-10,11-epoxide CBZ-E, and the second one consisted of naproxen (NPX), DCF, MeBZT, avobenzone (AVO), propylparaben (PrPB), acetylsufladiazine (acSDZ), carbamazepine (CBZ), BP4, gemfibrozil (GMZ), sulfamerazine (SMR), benzylparaben (BePB), and sulfamethoxypyridazine (SMPZ). Methylparaben (MePB), CFF, atenolol (ATL), norFXT, acetaminophen (APH), and SCY formed the last.

Although a similar behaviour between parental and metabolite compounds or compounds with similar chemical structures could be expected, this analysis showed that the translocation and uptake could be very different among vegetables cultivated in similar conditions. It can be confirmed by the sample distribution, as shown in Figure 7. Every matrix of vegetables can be easily differentiated from the others even though the cultivation conditions were the same (soil composition, irrigation system, and irrigation water).

This analysis also provided information about which compounds contributes more to each sample. For example, ATL, norFXT, APH, and SCY are closely related to carrot, probably indicating that these compounds have higher concetrations in the carrots than in the other vegetables. Then, the compounds that are not close to any of the ellipses (such as BP3, BP1, or enzacame (4MBC)) do not have strong correlation with any of the matrices since they present low or similar values in all of them.

PCA of the PPCPs in soil and water

After the comparison among vegetables, the different values for each variable in vegetables and soils were averaged to have only two variables to compare them with the irrigation water (WWTP effluent and rbSAT). Then, a PCA was performed for each matrix of vegetables to study the relationships among them and PPCPs.

Figure 8 shows the tomato's PCA with the soil and irrigation PPCPs levels as a representative example Observing the biplot, a different pattern for each matrix (tomato-soil-water) is observed, as they are far from each other. Soil and tomato points are similar between them since they are close. Still, water samples are pretty distant, suggesting that the rbSAT undergoes specific processes leading to less contaminated water than the secondary effluent.

About the PPCP, there is a big group (MePB, 4,4'-dihydroxybenzophenone (4DHB), SQX, etc.) that seemed to contribute to all the samples, since it is in the center of the plot. However, a few compounds show a different behaviour, i.e., SCY, SMR, AVO, and SMPZ, since they are very close to the vegetables. It suggests that they presented a higher affinity to this matrix than others. Finally, the last group, including 4HB, acSDZ, BP3, and BePB, seemed to have a particular relationship with tomato and the waters, since it is more centered. It is noteworthy that none of the compounds was close to the soil, suggesting that the target compounds did not have a significant relationship with soil.

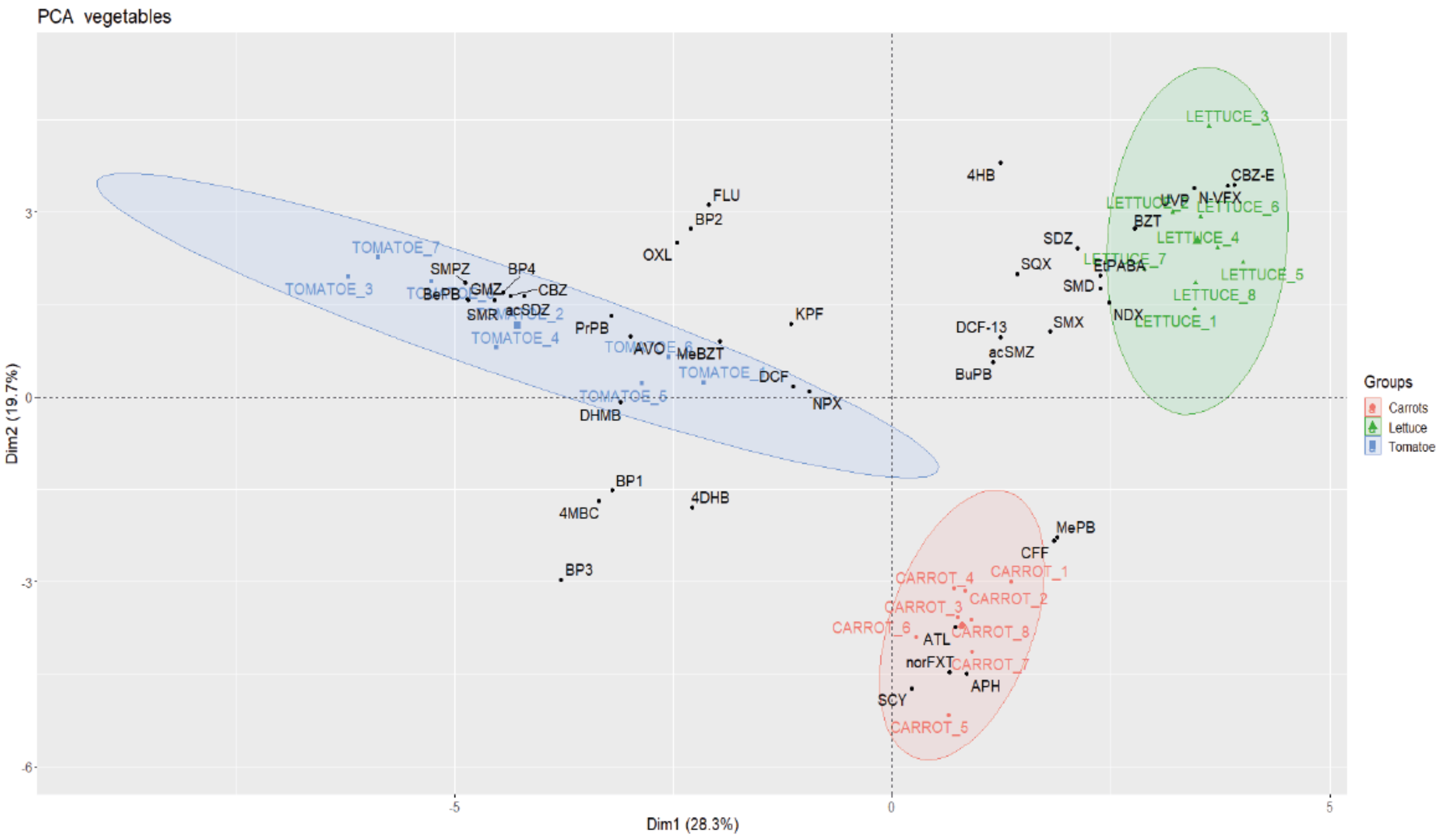


Figure 7. Biplot of the principal components (PC) 1 and 2 that shows the distribution of PPCPs and vegetable samples (ellipses are represented with 95% confidence).

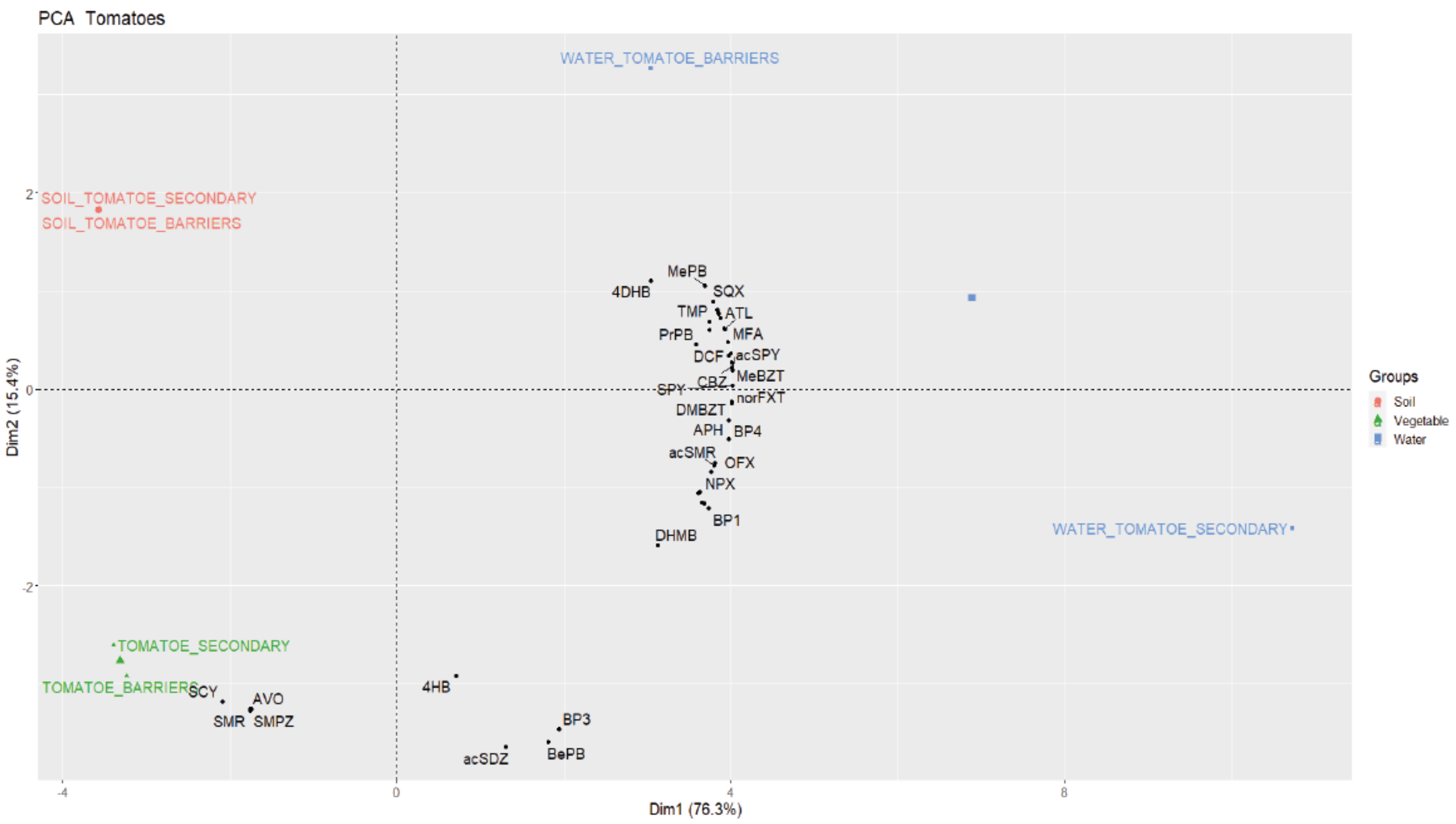


Figure 8. Biplot of the principal components (PC) 1 and 2, showing the PPCPs and samples (water, soils, and vegetables) distribution (ellipses are represented with 95% of confidence).

3.3. Human health risk assessment

In light of the low concentration of PPCPs detected in the reclaimed water (Figure 1), the risk associated with their applicability to the drinking water supply was evaluated. Eight age groups were considered for RQ estimation which are listed in **Table 2,** with the corresponding body weight (BW) and the daily water ingestion rate (DWI).

Table 2. Age groups are considered for estimation.

Age groups	BW (Kg)	DWI (L/day)
0-6 months	7.6	0.68
6-12 months	8.6	1
1-2 years	10.6	1.2
2-3 years	13	1.3
4-8 years	20.4	1.6
9-13 years	35.4	2
14-18 years	58	2.25
Adults	62	2.25

BW: Body weight; DWI: Daily water ingestion

* Data on body weight was extracted from the WHO website and the DWI doses were those published by European Food Safety Authority (EFSA).

The data in Figure 9 show that DCF, BZT, and GFZ were the compounds posing the greatest concern but still far from posing a significant risk for consumers above two years old (RQ < 0.1). The cumulative risk quotient (∑RQ) was also far below the value for medium risk (RQ = 0.5).

In the case of vegetables, SCY was the compound responsible for the highest risk in carrots but still posed a slight threat by consumption. In tomatoes, DCF had the highest HQs, as well as in lettuces, the vegetable with the lowest risk for consumption, according to the results shown in Figure 10. Overall, carrots were the crops with the highest estimated risk.

Although the PPCPs concentrations measured in the vegetables were low, but the intake is continued, the prolonged exposure to PPCPs may cause adverse effects in humans; thus it needs to be monitored and controlled extensively.

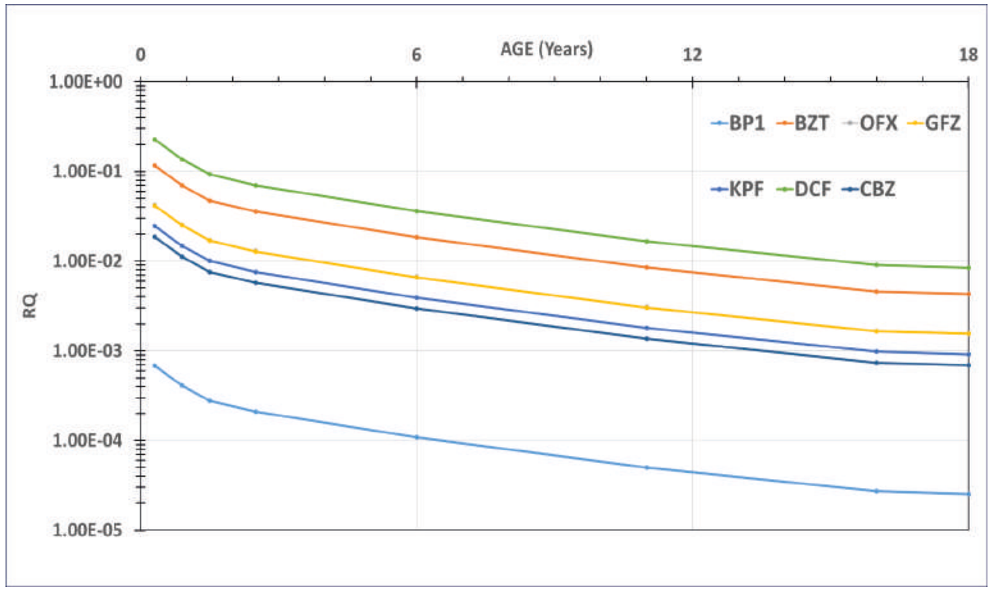


Figure 9. Risk quotients (RQ) estimated for the individual PPCPs for reclaimed water ingestion according to age. Adults are represented by 18 years old.

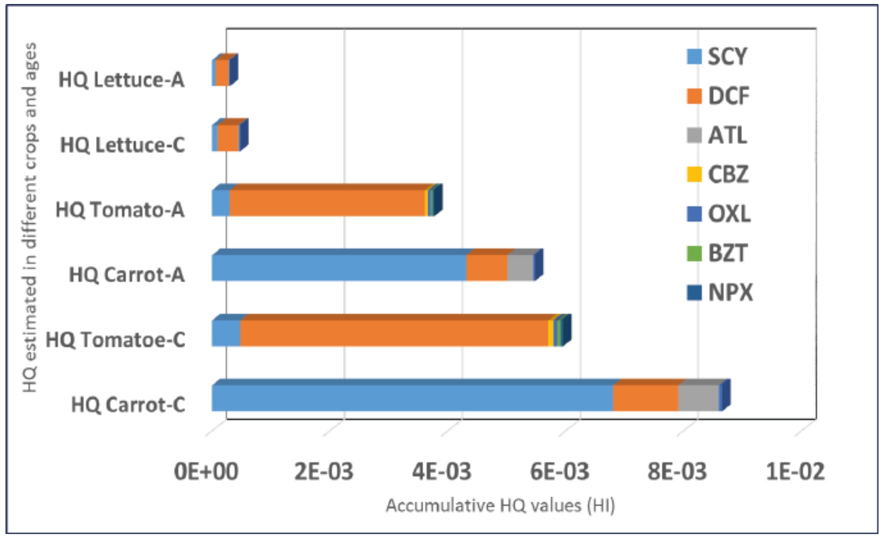


Figure 10. Hazard quotients (HQ) were estimated for the individual PPCPs and the cumulative (HI) values. A: HQ estimated for adults. C: HQ estimated for children.

4. CONCLUSIONS

Fifty-seven PPCPs were determined in lettuces, tomatoes, and carrots, in the agricultural soils used for cultivation, and in the irrigation waters. The multi-residue analytical method based on QuEChERS extraction developed for determining the wide range of PPCPs showed good sensitivity, acceptable accuracy, and precision in all the types of solid matrices analyzed.

The results revealed that the groups with the highest concentrations were generally UVFs or pharmaceuticals (UVFs-lettuces and pharmaceuticals-tomatoes). Specifically, the compounds with higher concentrations in most samples were 4HB, DCF, BP4, and SCY. Furthermore, it was verified that the reactive barriers in the SAT system efficiently reduced PPCPs from the WWTPs effluents. Statistically, only PPCPs values in lettuces were influenced by the soil composition and irrigation system. However, the lowest uptake levels in all crop types were achieved when irrigating by sprinkling, with rbSAT-reclaimed water, and cultivated in clay soil. Overall, PPCPs accumulated more in carrot, followed by tomato, and lettuce. Therefore, under identical cultivation conditions, crop type influenced the uptake levels, ranging between 0.09 to 84 ng/g dw in lettuce, 0.35 to 360 ng/g dw in tomato, 0.79 to 2590 ng/g dw in carrot, 0.4 to 85 ng/g dw in soil and 0.72 to 2800 ng/L in irrigation waters.

Although PPCPs accumulate in the crops when repeatedly irrigated with reclaimed water produced by a SAT complemented with reactive barriers, the use for drinking water production and the consumption of the crops did not pose any potential human health risk ($\sum HQ < 0.1$). However, when exposed to these compounds throughout diet during long periods, chronic toxic effects cannot be ruled out, especially for those consumers with a vegan or vegetarian diet. Therefore, further research is needed to understand long-term exposure to PPCPs through drinking water and agri-food.

Acknowledgements

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References

[1] A.U. Rajapaksha, K. Shashikala, D. Premarathna, V. Gunarathne, A. Ahmed, M. Vithanage, 9. Sorptive removal of pharmaceutical and personal care products from water and wastewater, Elsevier Inc., 2019. https://doi.org/10.1016/B978-0-12-816189-0.00009-3.

[2] E. Touraud, B. Roig, J.P. Sumpter, C. Coetsier, Drug residues and endocrine disruptors in drinking water: Risk for humans?, Int. J. Hyg. Environ. Health. 214 (2011) 437–441. https://doi.org/10.1016/j.ijheh.2011.06.003.

[3] S. Mompelat, B. Le Bot, O. Thomas, Occurrence and fate of pharmaceutical products and by-products, from resource to drinking water, Environ. Int. 35 (2009) 803–814. https://doi.org/10.1016/j.envint.2008.10.008.

[4] Q. Sui, J. Huang, S. Deng, G. Yu, Q. Fan, Occurrence and removal of pharmaceuticals, caffeine and DEET in wastewater treatment plants of Beijing, China, Water Res. 44 (2010) 417–426. https://doi.org/10.1016/j.watres.2009.07.010.

[5] S. Keerthanan, C. Jayasinghe, J.K. Biswas, M. Vithanage, Pharmaceutical and Personal Care Products (PPCPs) in the environment: Plant uptake, translocation, bioaccumulation, and human health risks, Crit. Rev. Environ. Sci. Technol. 51 (2021) 1221–1258. https://doi.org/10.1080/10643389.2020.1753634.

[6] C.I. Kosma, D.A. Lambropoulou, T.A. Albanis, Investigation of PPCPs in wastewater treatment plants in Greece: Occurrence, removal and environmental risk assessment, Sci. Total Environ. 466–467 (2014) 421–438. https://doi.org/10.1016/j.scitotenv.2013.07.044.

[7] X. Yang, R.C. Flowers, H.S. Weinberg, P.C. Singer, Occurrence and removal of pharmaceuticals and personal care products (PPCPs) in an advanced wastewater reclamation plant, Water Res. 45 (2011) 5218–5228. https://doi.org/10.1016/j.watres.2011.07.026.

[8] A. Jelic, M. Gros, A. Ginebreda, R. Cespedes-Sánchez, F. Ventura, M. Petrovic, D. Barcelo, Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment, Water Res. 45 (2011) 1165–1176. https://doi.org/10.1016/j.watres.2010.11.010.

[9] O. Golovko, S. Örn, M. Sörengård, K. Frieberg, W. Nassazzi, F.Y. Lai, L. Ahrens, Occurrence and removal of chemicals of emerging concern in wastewater treatment plants and their impact on receiving water systems, Sci. Total Environ. 754 (2021) 142122. https://doi.org/10.1016/j.scitotenv.2020.142122.

[10] P. Krzeminski, M.C. Tomei, P. Karaolia, A. Langenhoff, C.M.R. Almeida, E. Felis, F. Gritten, H.R. Andersen, T. Fernandes, C.M. Manaia, L. Rizzo, D. Fatta-Kassinos, Performance of secondary wastewater treatment methods for the removal of contaminants of emerging concern implicated in crop uptake and antibiotic resistance spread: A review, Sci. Total Environ. 648 (2019) 1052–1081. https://doi.org/10.1016/J.SCITOTENV.2018.08.130.

[11] A. Libutti, G. Gatta, A. Gagliardi, P. Vergine, A. Pollice, L. Beneduce, G. Disciglio, E. Tarantino, Agro-industrial wastewater reuse for irrigation of a vegetable crop succession under Mediterranean conditions, Agric. Water Manag. 196 (2018) 1–14. https://doi.org/10.1016/j.agwat.2017.10.015.

[12] A.M. Palese, V. Pasquale, G. Celano, G. Figliuolo, S. Masi, C. Xiloyannis, Irrigation of olive groves in Southern Italy with treated municipal wastewater: Effects on microbiological quality of soil and fruits, Agric. Ecosyst. Environ. 129 (2009) 43–51. https://doi.org/10.1016/j.agee.2008.07.003.

[13] R. Kodešová, A. Klement, O. Golovko, M. Fér, M. Kočárek, A. Nikodem, R. Grabic, Soil influences on uptake and transfer of pharmaceuticals from sewage sludge amended soils to spinach, J. Environ. Manage. 250 (2019) 109407. https://doi.org/10.1016/j.jenvman.2019.109407.

[14] M. Goldstein, M. Shenker, B. Chefetz, Insights into the uptake processes of wastewater-borne pharmaceuticals by vegetables, Environ. Sci. Technol. 48 (2014) 5593–5600. https://doi.org/10.1021/es5008615.

[15] C. Valhondo, L. Martínez-Landa, J. Carrera, S.M. Díaz-Cruz, S. Amalfitano, C. Levantesi, Six artificial recharge pilot replicates to gain insight into water quality enhancement processes, Chemosphere. 240 (2020). https://doi.org/10.1016/j.chemosphere.2019.124826.

[16] C. Valhondo, L. Mart, J. Wang, Reactive Barriers for Renaturalization of Reclaimed, Water. 12 (4), 10 (2020).

[17] A. Sunyer-Caldú, P. Sepúlveda-Ruiz, M. Salgot, M. Folch-Sánchez, D. Barcelo, M.S. Diaz-Cruz, Reclaimed water in agriculture: a plot-scale study assessing crop uptake of emerging contaminants and pathogens, J. Environ. Chem. Eng. (2022) 108831. https://doi.org/https://doi.org/10.1016/j.jece.2022.108831.

[18] A. Sunyer-Caldú, M.S. Diaz-Cruz, Development of a QuEChERS-based method for the analysis of pharmaceuticals and personal care products in lettuces grown in field-scale agricultural plots irrigated with reclaimed water, Talanta. 230 (2021) 122302. https://doi.org/10.1016/j.talanta.2021.122302.

[19] B. Kmellár, P. Fodor, L. Pareja, C. Ferrer, M.A. Martínez-Uroz, A. Valverde, A.R. Fernandez-Alba, Validation and uncertainty study of a comprehensive list of 160 pesticide residues in multi-class vegetables by liquid chromatography-tandem mass spectrometry, J. Chromatogr. A. 1215 (2008) 37–50. https://doi.org/10.1016/j.chroma.2008.10.121.

[20] S. Niell, V. Cesio, J. Hepperle, D. Doerk, L. Kirsch, D. Kolberg, E. Scherbaum, M. Anastassiades, H. Heinzen, QuEChERS-based method for the multiresidue analysis of pesticides in beeswax by LC-MS/MS and GC×GC-TOF, J. Agric. Food Chem. 62 (2014) 3675–3683. https://doi.org/10.1021/jf405771t.

[21] L. Vassalle, A. Sunyer-Caldú, E. Uggetti, R. Díez-Montero, M.S. Díaz-Cruz, J. García, M.J. García-Galán, Bioremediation of emerging micropollutants in irrigation water. The alternative of microalgae-based treatments, J. Environ. Manage. 274 (2020). https://doi.org/10.1016/j.jenvman.2020.111081.

[22] R.S. Prosser, P.K. Sibley, Human health risk assessment of pharmaceuticals and personal care products in plant tissue due to biosolids and manure amendments, and wastewater irrigation, Environ. Int. 75 (2015) 223–233. https://doi.org/10.1016/j.envint.2014.11.020.

[23] C. Wang, D. Ye, X. Li, Y. Jia, L. Zhao, S. Liu, J. Xu, J. Du, L. Tian, J. Li, J. Shen, X. Xia, Occurrence of pharmaceuticals and personal care products in bottled water and assessment of the associated risks, Environ. Int. 155 (2021) 106651. https://doi.org/10.1016/j.envint.2021.106651.

[24] G.H. LeFevre, A. Lipsky, K.C. Hyland, A.C. Blaine, C.P. Higgins, R.G. Luthy, Benzotriazole (BT) and BT plant metabolites in crops irrigated with recycled water, Environ. Sci. Water Res. Technol. 3 (2017) 213–223. https://doi.org/10.1039/c6ew00270f.

[25] Y. Ben, M. Hu, F. Zhong, E. Du, Y. Li, H. Zhang, C.B. Andrews, C. Zheng, Human daily dietary intakes of antibiotic residues: Dominant sources and health risks, Environ. Res. 212 (2022). https://doi.org/10.1016/j.envres.2022.113387.

[26] L.J.G. Silva, A.M.P.T. Pereira, H. Rodrigues, L.M. Meisel, C.M. Lino, A. Pena, SSRIs antidepressants in marine mussels from Atlantic coastal areas and human risk assessment, Sci. Total Environ. 603–604 (2017) 118–125. https://doi.org/10.1016/j.scitotenv.2017.06.076.

[27] A. Shahriar, J. Tan, P. Sharma, D. Hanigan, P. Verburg, K. Pagilla, Y. Yang, Modeling the fate and human health impacts of pharmaceuticals and personal care products in reclaimed wastewater irrigation for agriculture, Environ. Pollut. 276 (2021) 116532. https://doi.org/10.1016/j.envpol.2021.116532.

[28] C. Haman, X. Dauchy, C. Rosin, J.F. Munoz, Occurrence, fate and behavior of parabens in aquatic environments: A review, Water Res. 68 (2015) 1–11. https://doi.org/10.1016/j.watres.2014.09.030.

[29] A. Karnjanapiboonwong, J.G. Suski, A.A. Shah, Q. Cai, A.N. Morse, T.A. Anderson, Occurrence of PPCPs at a wastewater treatment plant and in soil and groundwater at a land application site, Water. Air. Soil Pollut. 216 (2011) 257–273. https://doi.org/10.1007/s11270-010-0532-8.

[30] M.F. Jaramillo, I. Restrepo, Wastewater reuse in agriculture: A review about its limitations and benefits, Sustain. 9 (2017). https://doi.org/10.3390/su9101734.

[31] L.K. Dodgen, J. Li, D. Parker, J.J. Gan, Uptake and accumulation of four PPCP/EDCs in two leafy vegetables, Environ. Pollut. 182 (2013) 150–156. https://doi.org/10.1016/j.envpol.2013.06.038.

[32] A. Christou, P. Karaolia, E. Hapeshi, C. Michael, D. Fatta-Kassinos, Long-term wastewater irrigation of vegetables in real agricultural systems: Concentration of pharmaceuticals in soil, uptake and bioaccumulation in tomato fruits and human health risk assessment, Water Res. 109 (2017) 24–34. https://doi.org/10.1016/j.watres.2016.11.033.

[33] E.D. Kovacs, L. Silaghi-Dumitrescu, M.H. Kovacs, C. Roman, Determination of the

Uptake of Ibuprofen, Ketoprofen, and Diclofenac by Tomatoes, Radishes, and Lettuce by Gas Chromatography–Mass Spectrometry (GC–MS), Anal. Lett. 54 (2021) 314–330. https://doi.org/10.1080/00032719.2020.1779278.

[34] J. Schwaiger, H. Ferling, U. Mallow, H. Wintermayr, R.D. Negele, Toxic effects of the non-steroidal anti-inflammatory drug diclofenac: Part I: histopathological alterations and bioaccumulation in rainbow trout, Aquat. Toxicol. 68 (2004) 141–150. https://doi.org/https://doi.org/10.1016/j.aquatox.2004.03.014.

[35] R. Triebskorn, H. Casper, A. Heyd, R. Eikemper, H.R. Köhler, J. Schwaiger, Toxic effects of the non-steroidal anti-inflammatory drug diclofenac: Part II. Cytological effects in liver, kidney, gills and intestine of rainbow trout (Oncorhynchus mykiss), Aquat. Toxicol. 68 (2004) 151–166. https://doi.org/10.1016/j.aquatox.2004.03.015.

[36] H. Lefevere, L. Bauters, G. Gheysen, Salicylic acid biosynthesis in plants, Front. Plant Sci. 11 (2020) 1–7. https://doi.org/10.3389/fpls.2020.00338.

[37] G.L. Robertson, W.J. Kermode, Salicylic acid in fresh and canned fruit and vegetables, J. Sci. Food Agric. 32 (1981) 833–836. https://doi.org/10.1002/jsfa.2740320813.

[38] S. Smoleń, I. Kowalska, P. Kováčik, M. Halka, W. Sady, Biofortification of six varieties of lettuce (Lactuca sativa l.) with iodine and selenium in combination with the application of salicylic acid, Front. Plant Sci. 10 (2019) 1–13. https://doi.org/10.3389/fpls.2019.00143.

[39] E. Guzmán-Téllez, D.D. Montenegro, A. Benavides-Mendoza, Concentration of Salicylic Acid in Tomato Leaves after Foliar Aspersions of This Compound, Am. J. Plant Sci. 05 (2014) 2048–2056. https://doi.org/10.4236/ajps.2014.513220.

[40] R.K. Madan, J. Levitt, A review of toxicity from topical salicylic acid preparations, J. Am. Acad. Dermatol. 70 (2014) 788–792. https://doi.org/10.1016/j.jaad.2013.12.005.

[41] M. Tenenbein, 4 - Acute poisonings in pregnancy, in: J.B.T.-H.T. DESCOTES (Ed.), Elsevier Science B.V., Amsterdam, 1996: pp. 247–257. https://doi.org/https://doi.org/10.1016/B978-044481557-6/50006-X.

[42] A. Jurado, P. Gago-Ferrero, E. Vàzquez-Suñé, J. Carrera, E. Pujades, M.S. Díaz-Cruz, D. Barceló, Urban groundwater contamination by residues of UV filters, J. Hazard. Mater. (2014). https://doi.org/10.1016/j.jhazmat.2014.01.036.

[43] M.S. Díaz-Cruz, D. Molins-Delgado, M.P. Serra-Roig, E. Kalogianni, N.T. Skoulikidis, D. Barceló, Personal care products reconnaissance in EVROTAS river (Greece): Water-sediment partition and bioaccumulation in fish, Sci. Total Environ. 651 (2019) 3079–3089. https://doi.org/10.1016/j.scitotenv.2018.10.008.

[44] Y. Nakagawa, K. Tayama, Estrogenic potency of benzophenone and its metabolites in juvenile female rats, Arch. Toxicol. 75 (2001) 74–79. https://doi.org/10.1007/s002040100225.

[45] T. Suzuki, S. Kitamura, R. Khota, K. Sugihara, N. Fujimoto, S. Ohta, Estrogenic and antiandrogenic activities of 17 benzophenone derivatives used as UV stabilizers and sunscreens, Toxicol. Appl. Pharmacol. 203 (2005) 9–17. https://doi.org/10.1016/j.taap.2004.07.005.

[46] J.O. Tijani, O.O. Fatoba, O.O. Babajide, L.F. Petrik, Pharmaceuticals, endocrine disruptors, personal care products, nanomaterials and perfluorinated pollutants: a review, Environ. Chem. Lett. 14 (2016) 27–49. https://doi.org/10.1007/s10311-015-0537-z.

[47] Z. Li, X. Xiang, M. Li, Y. Ma, J. Wang, X. Liu, Occurrence and risk assessment of pharmaceuticals and personal care products and endocrine disrupting chemicals in reclaimed water and receiving groundwater in China, Ecotoxicol. Environ. Saf. 119 (2015) 74–80. https://doi.org/10.1016/j.ecoenv.2015.04.031.

[48] M. Xu, H. Huang, N. Li, F. Li, D. Wang, Q. Luo, Occurrence and ecological risk of pharmaceuticals and personal care products (PPCPs) and pesticides in typical surface watersheds, China, Ecotoxicol. Environ. Saf. 175 (2019) 289–298. https://doi.org/https://doi.org/10.1016/j.ecoenv.2019.01.131.

5.2.3 Publication #11 Supplementary information

Pharmaceuticals' and personal care products' uptake by crops irrigated with reclaimed water and human health implications

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Gerard Quintana
M. Silvia Diaz-Cruz

Submitted to Science of the Total Environment

Tables. (S1-S11).
Figures. (S1-S2).
Section S1. Standards and reagents.
Section S2. R script used for the statistical analysis (PCA).
Section S3. Validation parameters.

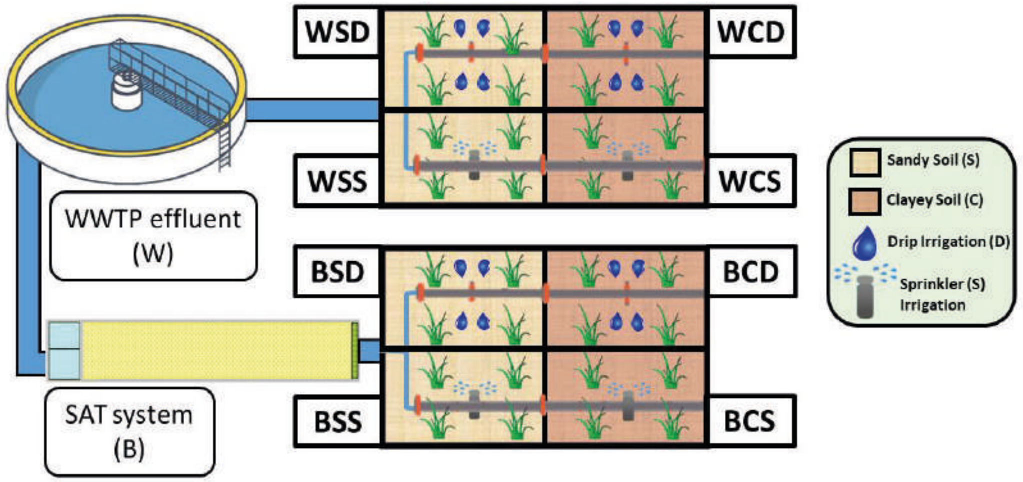


Figure S1. Scheme of the distribution of the experimental plots, the variables studied, and their acronyms.

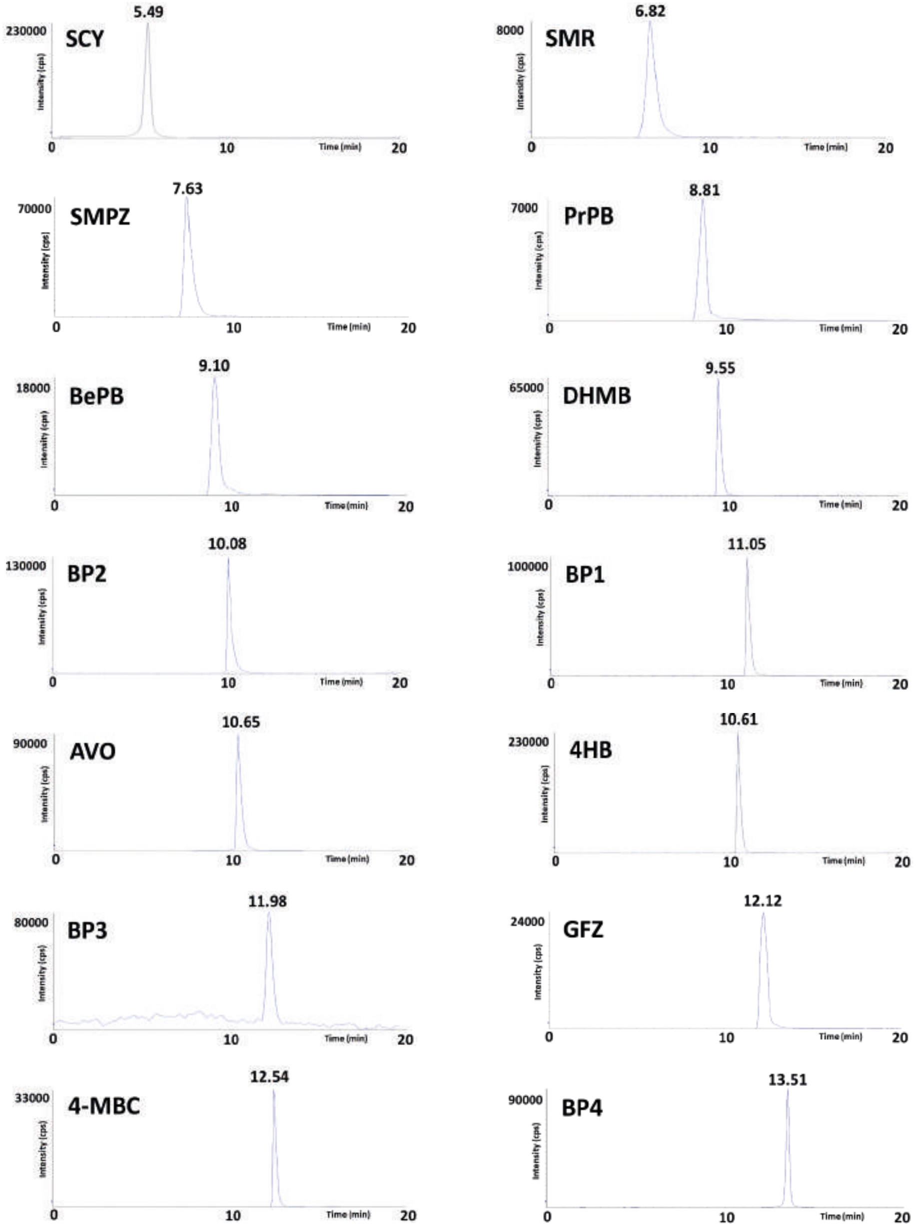
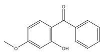
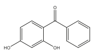
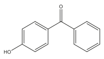
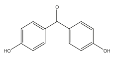
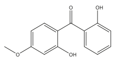
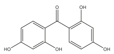
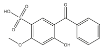
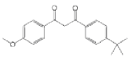
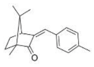
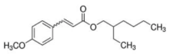
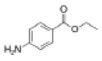
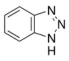
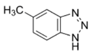
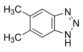
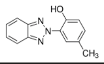
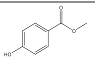
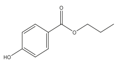
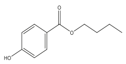
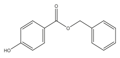
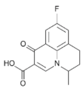
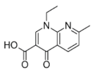
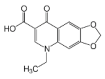
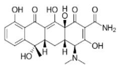
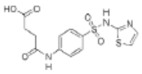
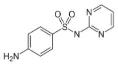
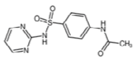
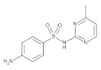
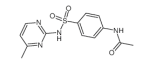
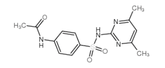
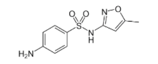
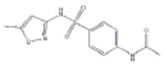
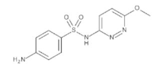
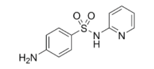
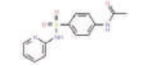
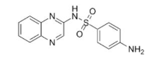
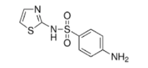
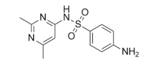
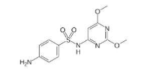


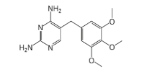
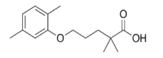
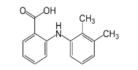
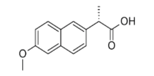
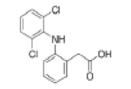
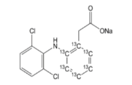
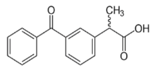
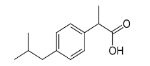
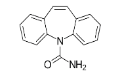
Figure S2. Ion chromatograms of each compound found in sample BCS of the tomatoes in ascending order of retention time.

Tables

Table S1. Target compounds, abbreviations, family, type of compound, CAS number, molecular masss, structure, octanol-water partition constant (log kow) and dissociation constant (pka).

Compound	Other names	Family	Type	CAS number	Molecular mass(g/mol)	Structure	K$_{ow}$	pKa
Benzophenone-3 (BP3)	Oxybenzone; 2-Hydroxy-4-methoxybenzophenone	Benzophenones	UV filter (PCP)	131-57-7	228.24		3.79	7.1
Benzophenone-1 (BP1)	2,4-Dihydroxybenzophenone	Benzophenones	UV filter (PCP)	131-56-6	214.22		3.15	7.1
4-Hydroxybenzophenone (4HB)	-	Benzophenones	UV filter (PCP)	1137-42-4	193.18		2.92	8.14
4,4'-Dihydroxybenzophenone (DHB)	-	Benzophenones	UV filter (PCP)	611-99-4	214.22		2.19	7.55
2,2'-Dihydroxy-4-methoxybenzophenone (DHMB)	Benzophenone-8; Dioxybenzone	Benzophenones	UV filter (PCP)	131-53-3	244.25		3.82	6.78
Benzophenone-2 (BP2)	2,2',4,4'-Tetrahydroxybenzophenone	Benzophenones	UV filter (PCP)	131-55-5	246.22		2.78	7.1
Benzophenone-4 (BP4)	5-benzoyl-4-hydroxy-2-methoxybenzene sulfonic acid; HMBS; Sulisobenzone	Benzophenones	UV filter (PCP)	4065-45-6	308.31		0.88	-2.4
Avobenzone (AVO)	1-(4-tert-butylphenyl)-3-(4-methoxyphenyl)propane-1,3-dione	Benzophenones	UV filter (PCP)	70356-09-1	310.17		4.51	9.74
Enzacame (4MBC)	3-(4-Methylbenzilidene) camphor	Camphor derivatives	UV filter (PCP)	36861-47-9	254.17		5.14	7.5
Octinoxate (EHMC)	2-Ethylhexyl trans-4-methoxy cinnamate	Cinnamate derivatives	UV filter (PCP)	5466-77-3	290.4		5.8	-
Benzocaine (EtPABA)	Ethyl 4-aminobenzoate	p-aminobenzoic acid derivatives	UV filter (PCP) UV filter (PCP)	94-09-7	165.19		1.86	2.51
Benzotriazole (BZT or 1H-BZT)	1,2,3-Benzotriazole	Benzotriazoles	UV filter (PCP)	95-14-7	119.12		1.44	8.37
Methyl-benzotriazole (MeBZT or 5-MeBZT)	5-Methyl-1H-benzotriazole	Benzotriazoles	UV filter (PCP)	136-86-5	133.15		Not avaliable	8.85
Dimethyl-benzotriazole (DMBZT)	5,6-Dimethyl-1H-benzotriazole monohydrate	Benzotriazoles	UV filter (PCP)	4184-79-6	147.18		Not avaliable	8.92
Drometrizole (UVP)	Benazol P; Tinuvin P; 2-(2H-Benzotriazol-2-yl)-p-cresol	Benzotriazole derivatives	UV filter (PCP)	2440-22-4	225.25		4.31	8.15
Methyl paraben (MePB)	Methyl 4-hydroxybenzoate	Parabens	Preservative (PCP)	99-76-3	152		2	8.17
Propyl paraben (PrPB)	Propyl 4-hydroxybenzoate	Parabens	Preservative (PCP)	94-13-3	180.2		2.98	8.35
Butyl paraben (BuPB)	Butyl 4-hydroxybenzoate	Parabens	Preservative (PCP)	94-26-8	194.23		3.47	8.47
Benzyl paraben (BePB)	Benzyl 4-hydroxybenzoate	Parabens	Preservative (PCP)	94-18-8	228.24		3.7	8.18
Flumequine (FLU)	Flumigal; Apurone	Fluoroquinolones	Pharmaceutical	42835-25-6	261.25		1.6	6.5
Nalidixic acid (NDX)	Nalidixate; nalidixin	Quinolones	Pharmaceutical	389-08-2	232.23		1.59	8.6
Oxolinic acid (OXL)	Nidantin; dioxacin	Quinolones	Pharmaceutical	14698-29-4	261.23		0.94	6.3
Tetracycline (TCY)	Deschlorobiomycin	Tetracyclines	Pharmaceutical	60-54-8	444.4		1.3	3.3
Succinylsulfathiazole (Succinyl-STZ)	Cremosuxidine; Colistatin	Sulfonamides	Pharmaceutical	116-43-8	355.4		-	3.36
Sulfadiazine (SDZ)	Sulfapyrimidine	Sulfonamides	Pharmaceutical	68-35-9	250.28		-0.09	6.99
N^4-acetylsufladiazine (acSDZ)	N-[4-(pyrimidin-2-ylsulfamoyl)pheyl]acetamide	Sulfonamides	Pharmaceutical	127-74-2	292.32		Not avaliable	-
Sulfamerazine (SMZ)	Sulfamethyldiazine	Sulfonamides	Pharmaceutical	127-79-7	264.31		0.14	6.98

N^4-acetylsulfamerazine (acSMR)	N(4)-Acetylsulfamerazine	Sulfonamides	Pharmaceutical	127-73-1	306.34		Not avaliable	-
N^4-acetylsulfamethazine (acSMZ)	N(4)-Acetylsulfamethazine	Sulfonamides	Pharmaceutical	100-90-3	320.37		Not avaliable	-
Sulfamethoxazole (SMX)	Gantanol; sulfisomezole	Sulfonamides	Pharmaceutical	723-46-6	253.28		0.89	5.81
Acetylsulfamethoxazole (acSMX)	N^4-Acetylsulfamethoxazole	Sulfonamides	Pharmaceutical	21312-10-7	295.32		0.86	-
Sulfamethoxypyridazine (SMPZ)	Sulphametoxypyridazine	Sulfonamides	Pharmaceutical	80-35-3	280.31		Not avaliable	7.19
Sulfapyridine (SPY)	Sulfidin	Sulfonamides	Pharmaceutical	144-83-2	249.29		0.35	8.43
Acetylsulfapyiridine (acSPY)	N-(4-(N-(Pyridin-2-yl)sulfamoyl)phenyl)acetamide	Sulfonamides	Pharmaceutical	19077-98-6	291.33		Not avaliable	-
Sulfaquinoxaline (SQX)	Avicocid	Sulfonamides	Pharmaceutical	59-40-5	300.34		1.68	5.1
Sulfathiazole (STZ)	2-Sulfanilamidothiazole	Sulfonamides	Pharmaceutical	72-14-0	255.3		0.05	7.24
Sulfisomidine (SMD)	-	Sulfonamides	Pharmaceutical	515-64-0	278.33		-0.33	7.4
Sulfadimethoxine (SDM)	Sulfadimethoxydiazine	Sulfonamides	Pharmaceutical	122-11-2	310.33		1.63	6.21

Trimethoprim (TMP)	Proloprim; Trimpex	Others	Pharmaceutical	738-70-5	290.32		0.91	7.12
Gemfibrozil (GFZ)	5-(2,5-Dimethylphenoxy)-2,2-dimethylpentanoic acid	Lipid regulators	Pharmaceutical	25812-30-0	250.33		4.39	4.5
Mefenamic acid (MFA)	2-[(2,3-dimhetylphenyl)amino]benzoic acid	Analgesics	Pharmaceutical	61-68-7	241.28		5.12	4.2
Naproxen (NPX)	-	Anti-inflammatories	Pharmaceutical	22204-53-1	230.26		3.18	4.15
Diclofenac (DCF)	Voltaren	Anti-inflammatories	Pharmaceutical	15307-86-5	296.1		4.51	4.2
Diclofenac-^{13}C (DCF-^{13}C)	Diclofenac-(acetophenyl ring-^{13}C$_6$) sodium salt	Anti-inflammatories	Pharmaceutical	1261393-73-0	810.3		Not avaliable	-
Ketoprofen (KPF)	2-(3-Benzoylphenyl)propanoic acid	Analgesics	Pharmaceutical	22071-15-4	254.28		3.12	4.45
Ibuprofen (IBU)	2-(4-Isobutylphenyl)propanoic acid	Anti-inflammatories	Pharmaceutical	15687-27-1	206.28		3.97	4.91
Carbamazepine (CBZ)	5H-Dibenzo[b,f]azepine-5-carboxamide	Anti-epileptics	Pharmaceutical	298-46-4	236.27		2.77	15.96

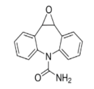
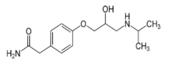
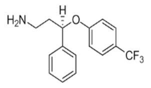
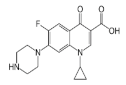
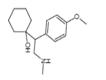
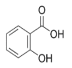
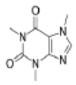
Carbamazepine-10,11-epoxide (CBZ-E)	-	Anti-epileptics	Pharmaceutical	36507-30-9	252.27		Not avaliable	15.96
Atenolol (ATL)	Tenorium; blokium; normiten	B-blockers	Pharmaceutical	29122-68-7	266.34		0.16	9.6
Norfluoxetine (norFXT)	Desmethylfluoxetine	Anti-depressants	Pharmaceutical Pharmaceutical	56161-73-0 83891-03-6	295.3		Not avaliable	9.77
Ciprofloxacin (CFX)	-	Fluoroquinolones	Pharmaceutical	85721-33-1	331.34		0.28	6.09
N-desmethylvenlafaxine (N-desVFX)	Norvenlafaxine	Anti-depressants	Pharmaceutical	149289-30-5	263.37		Not avaliable	14.42
Salicylic acid (SCY)	2-Hydroxybenzoic acid	Anti-septics	Pharmaceutical	69-72-7	138.12		2.26	2.98
Caffeine (CFF)	1,3,7-Trimethylxanthine; guaranine	Stimulants	Drug	58-08-2	194.19		-0.07	14

Table S2. Retention time (t_R), selected internal standards, MS/MS transitions and ionization parameters (declustering potential (DP), collision energy (CE), collision cell exit potential (CxP)) of each compound analyzed. (–) negative ionization mode.

	BP3	BP1	4HB	DHB	DHMB	BP2	BP4 (-)	AVO	4MBC	EHMC	EtPABA	BZT	MeBZT	DMBZT	UVP	BePB (-)	BuPB (-)	PrPB (-)
t_R (min.)	11.98	11.05	10.63	10.55	9.55	10.08	13.51	10.65	12.54	13.35	9.49	8.28	9.3	9.94	13.15	9.1	9.12	8.81
1st transition	229>151	215>137	199>121	215>121	245>121	247>137	307>227	311>135	255>105	291>179	166>138	120>65	134>79	148>77	226>120	227>92	193>137	179>92
DP (V)	40	40	40	45	43	46	-50	40	61	51	41	56	46	56	46	-65	-55	-60.0
CE (eV)	25	27	25	27	29	25	-34	25	45	19	20	31	29	39	25	-26	-22	-30.0
CxP (eV)	12	10	8	8	8	8	-15	15	6	4	10	4	10	2	10	-9	-5	-13.0
2nd transition	229>105	215>105	199>105	215>93	245>151	247>109	307>211	311>161	255>212	291>161	166>120	120>92	134>95	148>93	226>107	227>136	193>92	179>137
DP (V)	40	40	40	45	43	46	-70	40	61	51	41	56	46	56	46	-65	-55	-60.0
CE (eV)	27	29	27	45	27	45	-46	25	29	25	25	25	35	27	19	-22	-34	-24.0
CxP (eV)	16	6	8	6	12	8	-9	15	14	10	28	16	14	6	18	-1	-13	-5.0
IS	BP3-d_5	BP3-d_5	BP3-d_5	BP3-d_5	BP3-d_5	BP3-d_5	GFZ-d_6	4MBC-d_4	4MBC-d_4	4MBC-d_4	BZT-d_4	BZT-d_4	BZT-d_4	BZT-d_4	BZT-d4	BePB-d_4	BePB-d_4	BePB-d_4

	MePB (-)	FLU	NDX	OXL	TCY	Succinyl-STZ	SDZ	acSDZ	SMR	acSMR	acSMZ	SMX	acSMX	SMPZ	SPY	acSPY	SQX	STZ
t_R (min.)	8.05	10.44	10.22	10.45	7.15	7.64	6.06	7.36	6.82	7.72	8.01	8	8.84	7.63	6.48	7.49	9.28	6.37
1st transition	151>92	262>244	233>215	262>244	445>410	356>256	251>156	293>134	265>156	307>134	321>134	254>108	296>198	281>126	250>156	292>134	301>156	256>156
DP (V)	-45	51	61	36	66	71	46	65	61	60	86	56	60	66	51	70	76	40.0
CE (eV)	-28	29	19	25	27	25	27	30	27	35	35	27	30	27	28	30	25	25.0
CxP (eV)	-7	14	14	12	10	16	10	12	8	8	4	10	10	12	12	8	10	14.0
2nd transition	151>136	262>202	233>187	262>216	445>427	356>192	251>108	293>198	265>92	307>110	321>124	254>156	296>134	281>156	250>92	292>198	301>92	256>92
DP (V)	-45	51	61	36	66	71	46	65	61	60	86	56	60	66	51	70	76	40.0
CE (eV)	-20	45	37	41	19	33	30	30	47	35	35	25	30	27	31	30	47	25.0
CxP (eV)	-9	10	12	16	12	16	8	12	6	8	4	10	10	14	6	8	12	10.0
IS	BePB-d_4	FLU-13C	FLU-13C	FLU-13C	SMZ-d_4	SMZ-d_4	SMZ-d_4	SMZ-d_4	SMZ-d_4	SMZ-d_4	SMZ-d_4	SMZ-d_4	SMZ-d_4	SMZ-d_4	SMZ-d_4	SMZ-d_4	SMZ-d_4	SMZ-d_4

	SMD	SDM	TMP	GFZ (-)	MFA (-)	NPX (-)	DCF (-)	DCF-^{13}C (-)	KPF (-)	IBU (-)	AAP (-)	CBZ	CBZ-E	ATL	norFXT	CFX	N-desFXT	SCY (-)	CFF
t_R (min.)	7.35	9.09	6.59	12.12	9.01	8.22	8.79	8.79	8.18	8.94	5.96	10.22	9.38	4.98	8.84	7.44	7.39	5.49	7.73
1st transition	279>124	311>156	291>230	249>121	240>196	229>169	295>251	300>256	253>209	205>159	150>106	237>194	253>180	267>116	296>134	332>288	264>58	137>93	195>109.960
DP (V)	76	76	76	-50	-70	-30	-40	-35	-45	-30	-55	61	36	46	31	91	31	-50	56
CE (eV)	33	31	33	-26	-20	-40	-16	-18	-10	-10	-24	29	35	31	11	27	57	-18	31
CxP (eV)	8	8	20	-23	-13	-9	-7	-9	-21	-41	-19	14	12	8	8	14	18	-1	8
2nd transition	279>186	311>92	291>261	249>127	240>192	229>170	295>215	300>220	253>197	205>161	150>108	237>192	253>151	267>133	296>59	332>231	264>133	137>65	195>138
DP (V)	76	76	76	-50	-70	-30	-40	-35	-45	-30	-55	61	36	46	31	91	31	-50	56
CE (eV)	23	31	35	-12	-36	-22	-30	-30	-6	-14	-22	31	109	39	33	51	39	-40	29
CxP (eV)	14	6	12	-21	-3	-1	-19	-9	-41	-35	-7	12	10	22	10	4	46	-3	10
IS	SMZ-d_4	SMZ-d_4	TMP-d3	GFZ-d_6	MFA-d_3	NPX-d_3	DCF-d_4 (phenyl-d_4)	DCF-d_4 (phenyl-d_4)	MFA-d_3	IBU-d_3	TMP-d3	CBZ-d_{10}	CBZ-d_{10}	TMP-d3	CBZ-d10	TMP-d3	TMP-d3	IBU-d3	TMP-d3

DP: Declustering potential in Volts; CE: Collision energy in electronvolts; CxP: Collision cell exit potential in electronvolts; IS: isotopically labeled internal standards

Table S3. Linearity range, coefficient of determination, limits of detection and limits of quantification for the tomatoe matrix.

	BP3	BP1	BP2	BP4 (-)	4HB	4DHB	DHMB	AVO	4MBC	EHMC	EtPABA	BZT	MeBZT	DMBZT	UVP	BePB (-)	BuPB (-)	PrPB (-)	
Linearity range (ng/mL)	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	
r2	0.9991	0.9982	0.9919	0.9984	0.9980	0.9896	0.9983	0.9990	0.9964	0.9769	0.9975	1.0000	0.9997	0.9998	0.9997	0.9995	0.9997	0.9999	
MLOD (ng/g)	0.32	0.46	0.99	0.44	0.49	1.13	0.46	0.34	0.66	1.70	0.55	0.07	0.20	0.14	0.18	0.24	0.20	0.10	
MLOQ (ng/g)	1.07	1.54	3.32	1.48	1.63	3.77	1.52	1.15	2.21	5.67	1.82	0.24	0.66	0.48	0.62	0.81	0.68	0.32	
	MePB (-)	**FLU**	**OFX**	**CPX**	**NDX**	**OXL**	**TCY**	**OCY**	**S-STZ**	**SDZ**	**acSDZ**	**SMR**	**acSMR**	**acSMZ**	**SMX**	**acSMX**	**SMPZ**	**SPY**	**acSPY**
Linearity range (ng/mL)	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700
r2	0.9991	0.9987	0.9995	0.9993	0.9981	0.9965	0.9884	0.9896	0.9992	0.9996	0.9972	0.9996	0.9994	0.9999	0.9995	0.9999	0.9981	0.9996	0.9995
MLOD (ng/g)	0.32	0.40	0.24	0.28	0.47	0.65	1.20	1.13	0.31	0.22	0.59	0.21	0.27	0.10	0.25	0.12	0.48	0.22	0.23
MLOQ (ng/g)	1.08	1.35	0.80	0.95	1.58	2.18	3.98	3.77	1.03	0.72	1.95	0.70	0.90	0.32	0.85	0.40	1.61	0.73	0.78
	SQX	**STZ**	**SMD**	**SDM**	**TMP**	**GMZ (-)**	**MFA (-)**	**NPX (-)**	**IBU**	**KPF (-)**	**DCF (-)**	**DCF-13C (-)**	**AAP**	**CBZ**	**CBZ-E**	**ATL**	**norFXT**	**SCY (-)**	**CFF**
Linearity range (ng/mL)	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700
r2	0.9980	0.9997	0.9994	0.9999	0.9999	1.0000	0.9996	0.9994	0.9997	0.9742	0.9458	0.9360	0.9996	0.9993	0.9965	0.9999	0.9989	0.9997	0.9996
MLOD (ng/g)	0.49	0.20	0.27	0.12	0.08	0.02	0.21	0.26	0.20	1.80	2.67	2.92	0.22	0.29	0.65	0.13	0.36	0.18	0.23
MLOQ (ng/g)	1.63	0.66	0.90	0.42	0.27	0.07	0.69	0.88	0.66	6.01	8.90	9.75	0.73	0.97	2.16	0.42	1.19	0.60	0.77

r2: determination coefficient: MLOD: limit of determination of the method; MLOQ: limit of quantification of the method

Table S4. Linearity range, coefficient of determination, limits of detection and limits of quantification for the carrot matrix.

	BP3	BP1	BP2	BP4 (-)	4HB	4DHB	DHMB	AVO	4MBC	EHMC	EtPABA	BZT	MeBZT	DMBZT	UVP	BePB (-)	BuPB (-)	PrPB (-)	
Linearity range (ng/mL)	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	
r2	0.9639	0.9538	0.9830	0.9998	0.9748	0.9684	0.9088	0.9484	0.9470	0.9085	0.9457	0.9441	0.9399	0.9365	0.9442	0.9996	0.9999	0.9993	
MLOD (ng/g)	0.96	1.10	0.65	0.05	0.80	0.90	1.60	1.17	1.18	1.61	1.20	1.22	1.27	1.31	1.22	0.07	0.03	0.09	
MLOQ (ng/g)	3.22	3.66	2.17	0.17	2.66	3.00	5.34	3.89	3.95	5.35	4.00	4.06	4.23	4.36	4.06	0.22	0.11	0.29	
	MePB (-)	**FLU**	**OFX**	**CPX**	**NDX**	**OXL**	**TCY**	**OCY**	**S-STZ**	**SDZ**	**acSDZ**	**SMR**	**acSMR**	**acSMZ**	**SMX**	**acSMX**	**SMPZ**	**SPY**	**acSPY**
Linearity range (ng/mL)	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700
r2	0.9999	0.9836	0.9659	0.9977	0.9895	0.9931	0.9930	0.9860	0.9639	0.9665	0.9705	0.9675	0.9641	0.9694	0.9674	0.9660	0.9658	0.9693	0.9669
MLOD (ng/g)	0.02	0.64	0.94	0.24	0.51	0.41	0.41	0.59	0.96	0.93	0.87	0.91	0.96	0.88	0.91	0.93	0.94	0.89	0.92
MLOQ (ng/g)	0.08	2.13	3.12	0.79	1.70	1.38	1.38	1.97	3.21	3.09	2.89	3.04	3.21	2.95	3.04	3.11	3.12	2.95	3.07
	SQX	**STZ**	**SMD**	**SDM**	**TMP**	**GMZ (-)**	**MFA (-)**	**NPX (-)**	**IBU**	**KPF (-)**	**DCF (-)**	**DCF-13C (-)**	**AAP**	**CBZ**	**CBZ-E**	**ATL**	**norFXT**	**SCY (-)**	**CFF**
Linearity range (ng/mL)	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700
r2	0.9621	0.9677	0.9751	0.9514	0.9918	0.9999	0.9987	0.9999	0.9994	0.9970	0.9991	0.9997	0.9979	0.9794	0.9806	0.9944	0.9724	0.9998	0.9887
MLOD (ng/g)	0.99	0.91	0.79	1.13	0.45	0.04	0.11	0.03	0.08	0.18	0.10	0.05	0.23	0.72	0.70	0.37	0.84	0.05	0.53
MLOQ (ng/g)	3.30	3.03	2.65	3.77	1.50	0.12	0.38	0.10	0.27	0.58	0.32	0.18	0.75	2.40	2.33	1.24	2.79	0.16	1.76

r2: determination coefficient: MLOD: limit of determination of the method; MLOQ: limit of quantification of the method

Table S5. Linearity range, coefficient of determination, limits of detection and limits of quantification for the soil matrix.

	BP3	BP1	BP2	BP4 (-)	4HB	4DHB	DHMB	AVO	4MBC	EHMC	EtPABA	BZT	MeBZT	DMBZT	UVP	BePB (-)	BuPB (-)	PrPB (-)	
Linearity range (ng/mL)	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	
r2	0.9991	0.9982	0.9919	0.9984	0.9980	0.9896	0.9983	0.9990	0.9964	0.9769	0.9975	1.0000	0.9997	0.9998	0.9997	0.9995	0.9997	0.9999	
MLOD (ng/g)	0.06	0.09	0.20	0.09	0.10	0.23	0.09	0.07	0.13	0.34	0.11	0.01	0.04	0.03	0.04	0.05	0.04	0.02	
MLOQ (ng/g)	0.21	0.31	0.66	0.30	0.33	0.75	0.30	0.23	0.44	1.13	0.36	0.05	0.13	0.10	0.12	0.16	0.14	0.06	
	MePB (-)	**FLU**	**OFX**	**CPX**	**NDX**	**OXL**	**TCY**	**OCY**	**S-STZ**	**SDZ**	**acSDZ**	**SMR**	**acSMR**	**acSMZ**	**SMX**	**acSMX**	**SMPZ**	**SPY**	**acSPY**
Linearity range (ng/mL)	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700
r2	0.9991	0.9987	0.9995	0.9993	0.9981	0.9965	0.9884	0.9896	0.9992	0.9996	0.9972	0.9996	0.9994	0.9999	0.9995	0.9999	0.9981	0.9996	0.9995
MLOD (ng/g)	0.06	0.08	0.05	0.06	0.09	0.13	0.24	0.23	0.06	0.04	0.12	0.04	0.05	0.02	0.05	0.02	0.10	0.04	0.05
MLOQ (ng/g)	0.22	0.27	0.16	0.19	0.32	0.44	0.80	0.75	0.21	0.14	0.39	0.14	0.18	0.06	0.17	0.08	0.32	0.15	0.16
	SQX	**STZ**	**SMD**	**SDM**	**TMP**	**GMZ (-)**	**MFA (-)**	**NPX (-)**	**IBU**	**KPF (-)**	**DCF (-)**	**DCF-13C (-)**	**AAP**	**CBZ**	**CBZ-E**	**ATL**	**norFXT**	**SCY (-)**	**CFF**
Linearity range (ng/mL)	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700
r2	0.9980	0.9997	0.9994	0.9999	0.9999	1.0000	0.9996	0.9994	0.9997	0.9742	0.9458	0.9360	0.9996	0.9993	0.9965	0.9999	0.9989	0.9997	0.9996
MLOD (ng/g)	0.10	0.04	0.05	0.02	0.02	0.00	0.04	0.05	0.04	0.36	0.53	0.58	0.04	0.06	0.13	0.03	0.07	0.04	0.05
MLOQ (ng/g)	0.33	0.13	0.18	0.08	0.05	0.01	0.14	0.18	0.13	1.20	1.78	1.95	0.15	0.19	0.43	0.08	0.24	0.12	0.15

r2: determination coefficient: MLOD: limit of determination of the method; MLOQ: limit of quantification of the method

Table S6. Linearity range, coefficient of determination, limits of detection and limits of quantification for the water matrix.

	BP3	BP1	BP2	BP4 (-)	4HB	4DHB	DHMB	AVO	4MBC	EHMC	EtPABA	BZT	MeBZT	DMBZT	UVP	BePB (-)	BuPB (-)	PrPB (-)	
Linearity range (ng/mL)	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	
r2	0.9991	0.9979	0.9925	0.9825	0.9963	0.9950	0.9974	0.9908	0.9980	0.9613	0.9996	0.9996	0.9992	0.9993	0.9883	0.9981	0.9978	0.9865	
MLOD (ng/L)	0.29	0.46	0.86	1.32	0.60	0.70	0.50	0.95	0.44	2.00	0.20	0.21	0.28	0.26	1.08	0.44	0.46	1.16	
MLOQ (ng/L)	0.98	1.52	2.86	4.41	2.00	2.35	1.68	3.18	1.47	6.67	0.66	0.69	0.94	0.88	3.59	1.45	1.54	3.86	
	MePB (-)	**FLU**	**OFX**	**CPX**	**NDX**	**OXL**	**TCY**	**OCY**	**S-STZ**	**SDZ**	**acSDZ**	**SMR**	**acSMR**	**acSMZ**	**SMX**	**acSMX**	**SMPZ**	**SPY**	**acSPY**
Linearity range (ng/mL)	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700
r2	0.9796	0.9984	0.9534	0.9836	0.9984	0.9984	0.9970	0.9979	0.9982	0.9995	0.9985	1.0000	0.9988	0.9998	0.9995	1.0000	0.9998	0.9995	0.9998
MLOD (ng/L)	1.43	0.40	2.21	1.28	0.40	0.39	0.54	0.45	0.42	0.22	0.38	0.04	0.34	0.13	0.22	0.07	0.15	0.21	0.13
MLOQ (ng/L)	4.78	1.32	7.36	4.26	1.32	1.30	1.79	1.50	1.40	0.74	1.27	0.15	1.14	0.44	0.73	0.22	0.49	0.71	0.43
	SQX	**STZ**	**SMD**	**SDM**	**TMP**	**GMZ (-)**	**MFA (-)**	**NPX (-)**	**IBU**	**KPF (-)**	**DCF (-)**	**DCF-13C (-)**	**AAP**	**CBZ**	**CBZ-E**	**ATL**	**norFXT**	**SCY (-)**	**CFF**
Linearity range (ng/mL)	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700	1-700
r2	0.9996	0.9991	0.9931	0.9996	0.9930	0.9991	0.9994	0.9998	0.9988	0.9997	0.9901	0.9846	0.9996	0.9992	0.9980	0.9995	0.9793	0.9985	0.9976
MLOD (ng/L)	0.21	0.29	0.83	0.19	0.83	0.30	0.24	0.12	0.35	0.17	0.99	1.24	0.21	0.28	0.44	0.23	1.44	0.39	0.48
MLOQ (ng/L)	0.69	0.98	2.75	0.62	2.76	1.00	0.81	0.41	1.15	0.57	3.30	4.13	0.70	0.94	1.46	0.76	4.81	1.29	1.60

r2: determination coefficient: MLOD: limit of determination of the method; MLOQ: limit of quantification of the method

Table S7. Concentration of each compound in ng/g dw of the lettuces analyzed in each plot, with its range, positive samples and frequency of detection.

Compound	BSS	BCS	BCD	BSD	WSD	WCS	WSS	WCD	Range	Positive	Frequency (%)
BP3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
BP1	0.77	<LOD	0.82	2.76	0.69	1.17	3.56	0.42	0.42-3.56	7	87.5
BP2	1.48	54.4	7.03	9.81	5.32	8.14	5.37	13.8	1.48-54.4	8	100
4HB	11.8	64.1	26.6	59.8	80.6	80.1	79.9	84.1	11.8-84.1	8	100
4DHB	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
DHMB	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
AVO	0.85	1.69	6.54	<LOD	1.34	2.13	2.04	0.58	0.58-6.54	7	87.5
BP4 (-)	0.17	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.17	1	
4MBC	<LOD	<LOD	<LOD	0.1	0.71	0.3	1.62	0.48	0.1-1.62	5	62.5
EHMC	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
EtPABA	0.3	1.06	<LOD	<LOD	0.42	<LOD	1.17	0.73	0.3-1.17	5	62.5
BZT	1.52	0.57	5.32	7.05	1.43	6.55	0.49	1.4	0.49-7.05	8	100
MeBZT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
DMBZT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
UVP	1.97	2.51	5.35	1.71	2.39	4.19	1.44	1.94	1.44-5.35	8	100
BePB (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
BuPB (-)	0.4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.4	1	12.5
PrPB (-)	<LOD	<LOD	0.94	1.42	<LOD	0.97	<LOD	<LOD	0.94-1.42	3	37.5
MePB (-)	<LOD	10.24	0.48	1.52	4.52	7.78	1.14	10.9	0.48-10.86	7	87.5
FLU	0.52	1.94	4.25	<LOD	0.62	1.48	1.53	0.52	0.52-4.25	7	87.5
NDX	1.18	19	10.1	2	3.94	3.57	2,078	4.08	1.18-19	8	100
OXL	0.66	1.56	4.91	0.41	0.36	1.91	1.91	<LOD	0.36-4.91	7	87.5
TCY	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
S-STZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SDZ	<LOD	<LOD	4.03	2.23	<LOD	2.95	<LOD	<LOD	2.23-4.03	3	37.5
acSDZ	1.3	<LOD	<LOD	<LOD	<LOD	<LOD	1.44	<LOD	1.3-1.44	2	25
SMR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
acSMR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
acSMZ	0.81	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.81	1	12.5
SMX	2.58	<LOD	<LOD	<LOD	2.97	<LOD	<LOD	<LOD	2.58-2.97	2	25
acSMX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SMPZ	<LOD	2.59	1.55	<LOD	<LOD	<LOD	1.42	<LOD	1.42-2.59	3	37.5
SPY	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
acSPY	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SQX	<LOD	1.02	4.4	<LOD	<LOD	<LOD	<LOD	<LOD	1.02-4.40	2	25
STZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SMD	0.26	2.53	0.93	<LOD	14.8	2.31	3.71	10.6	0.26-14.83	7	87.5
SDM	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
TMP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
GMZ (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
MFA (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
NPX (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
DCF (-)	3.68	19.5	24.9	26.9	9.31	9.76	16.9	12.8	3.68-26.90	8	100
DCF-13C (-)	<LOD	<LOD	<LOD	0.48	<LOD	<LOD	<LOD	<LOD	0.48	1	12.5
KPF (-)	<LOD	<LOD	<LOD	<LOD	<LOD	1.25	<LOD	0.45	0.45-1.25	2	25
IBU	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
AAP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
CBZ	<LOD	<LOD	<LOD	<LOD	0.09	0.27	<LOD	<LOD	0.09-0.27	2	25
CBZ-E	0.38	0.34	0.4	0.33	0.33	0.36	0.42	0.35	0.33-0.42	8	100
ATL	<LOD	<LOD	<LOD	<LOD	1.16	1.24	<LOD	<LOD	1.16-1.24	2	25
norFXT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.23	2.23	1	12.5
CPX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
N-desVFX	0.54	0.87	1	1.29	0.95	0.85	0.87	0.73	0.54-1.29	8	100
SCY (-)	6.14	14	19.9	19.4	18	53.8	10.7	9.46	6.14-53.80	8	100
CFF	8.8	15.24	<LOD	16.5	26.8	13.26	15.7	16	8.80-26.80	7	87.5

Values are in ng/g dw; <LOD: value below the limit of detection

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Table S8. Concentration of each compound in ng/g dw of the tomatoes analyzed in each plot, with its range, positive samples and frequency of detection.

Compound	BSS	BCS	BCD	BSD	WSD	WCS	WSS	WCD	Range	Positive	Frequency (%)
BP3	4.25	4.45	3.92	4.76	4.36	4.02	4.23	3.45	3.45-4.76	7	87.5
BP1	13.9	6.64	12	7.18	6.6	5.94	16.4	24	5.94-24	7	87.5
BP2	28.8	16.8	22.9	17.1	19.6	24.4	31.1	31.4	16.8-31.4	7	87.5
BP4 (-)	42.6	74.4	99.6	22.9	7.32	61.9	101	140	7.32-140	7	87.5
4HB	39.2	14.7	22.2	20.1	21.9	23	85.7	29.8	14.7-85.7	7	87.5
4DHB	<LOD	19.1	<LOD	20.8	<LOD	<LOD	18.9	<LOD	18.9-20.8	3	37.5
DHMB	2.17	4.09	15.5	2.6	<LOD	4.26	6.44	<LOD	2.17-15.5	5	62.5
AVO	10.3	6.37	13.9	0.772	4.07	2.7	12.4	14.4	0.772-14.4	7	87.5
4MBC	9.21	13.3	25.2	30.4	18.6	12.6	19.4	<LOD	9.21-30.4	6	75
EHMC	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
EtPABA	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
BZT	<LOD	0.392	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOQ-0.392	1	12.5
MeBZT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	6.72	<LOD	6.72	1	12.5
DMBZT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
UVP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
BePB (-)	4.96	4.93	5.03	4.97	4.99	5	4.99	5.01	4.93-5.03	7	87.5
BuPB (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
PrPB (-)	1.55	0.353	3.71	6.46	12.7	<LOD	5.52	6.63	0.353-12.7	6	75
MePB (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
FLU	<LOD	3.58	3.49	3.45	<LOD	3.69	3.72	<LOD	3.45-3.72	5	62.5
OFX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
CPX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
NDX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
OXL	<LOD	5.45	5.39	5.32	<LOD	<LOD	5.94	<LOD	5.32-5.94	4	50
TCY	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
OCY	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
S-STZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SDZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
acSDZ	<LOD	13.4	23	14.4	<LOD	<LOD	10.9	16.8	10.9-23	5	62.5
SMR	3.91	12	8.05	5.25	5.12	4.54	4.77	13.1	3.91-13.1	7	87.5
acSMR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
acSMZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SMX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
acSMX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SMPZ	11.6	21.3	26.7	17.5	14.6	14.7	19.7	14.2	11.6-26.7	7	87.5
SPY	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
acSPY	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SQX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
STZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SMD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SDM	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
TMP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
GMZ (-)	6.41	6.37	6.4	6.45	6.49	6.44	6.43	6.48	6.37-6.49	7	87.5
MFA (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
NPX (-)	<LOD	<LOD	<LOD	<LOD	10.5	<LOD	<LOD	<LOD	10.5	1	12.5
IBU	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
KPF (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3.63	3.63	1	12.5
DCF (-)	<LOD	<LOD	<LOD	<LOD	269	360	<LOD	65.3	65.3-360	3	37.5
DCF-13C (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
AAP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
CBZ	<LOD	7.6	7.65	7.91	<LOQ	<LOD	7.74	7.95	<LOQ-7.95	5	62.5
CBZ-E	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
ATL	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
norFXT	<LOD	8.89	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	8.89	1	12.5
SCY (-)	81.9	81.7	78	143	112	73.2	113	81.6	73.2-143	7	87.5
CFF	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0	0	0

Values are in ng/g dw; <LOD: value below the limit of detection

Table S9. Concentration of each compound in ng/g dw of the carrots analyzed in each plot, with its range, positive samples and frequency of detection.

Compound	BSS	BCS	BCD	BSD	WSD	WCS	WSS	WCD	Range	Positive	Frequency (%)
BP3	7.52	<LOD	8.31	9.22	7.84	9.61	9.61	9.02	7.52-9.61	7	87.5
BP1	<LOD	30.4	40.6	<LOD	26.8	26.4	22.9	<LOD	22.9-40.6	5	62.5
BP2	< LOQ	9.13	4.53	25.4	< LOQ	< LOQ	< LOQ	9.03	4.53-25.4	4	50
BP4 (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
4HB	20.8	< LOQ	16.4	9.31	8	5.41	6.38	5.64	5.41-20.8	7	87.5
4DHB	<LOD	<LOD	25.1	25.1	25.1	26.6	25.1	<LOD	25.1-26.6	5	62.5
DHMB	<LOD	10.7	<LOD	<LOD	< LOQ	< LOQ	10.7	11.4	10.2-11.4	3	37.5
AVO	< LOQ	36.1	< LOQ	< LOQ	< LOQ	11.8	<LOD	< LOQ	3.17-36.1	2	25
4MBC	<LOD	26.8	<LOD	32.8	44	43.5	<LOD	42.4	26.8-44	5	62.5
EHMC	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
EtPABA	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
BZT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
MeBZT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
DMBZT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
UVP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
BePB (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
BuPB (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
PrPB (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
MePB (-)	4.88	51.5	33.6	23.7	<LOD	12	30.4	7.13	4.88-51.5	7	87.5
FLU	<LOD	<LOD	<LOD	<LOD	<LOD	< LOQ	<LOD	< LOQ	3.23-3.75	0	0
OFX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
CPX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
NDX	<LOD	15.6	<LOD	<LOD	<LOD	14.7	<LOD	<LOD	14.7-15.6	2	25
OXL	<LOD	<LOD	<LOD	<LOD	<LOD	3.99	<LOD	<LOD	3.99	1	12.5
TCY	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
OCY	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
S-STZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SDZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
acSDZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SMR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
acSMR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
acSMZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SMX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
acSMX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SMPZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SPY	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
acSPY	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SQX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
STZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SMD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
SDM	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
TMP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
GMZ (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
MFA (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
NPX (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
IBU	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
KPF (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
DCF (-)	75.3	95.6	72.8	18.8	0.79	<LOD	44.7	33.8	0.79-95.6	7	87.5
DCF-13C (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
AAP	113	34.9	60.5	39.7	67.7	64.3	87.7	28.9	28.9-113	8	100
CBZ	<LOD	< LOQ	<LOD	<LOD	< LOQ	< LOQ	< LOQ	<LOD	3.86-4.46	0	0
CBZ-E	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0
ATL	32.4	139	43	<LOD	60.5	31.2	68	38.3	31.2-139	7	87.5
norFXT	225	84.8	192	350	521	407	137	197	84.8-521	8	100
SCY (-)	625	977	1120	1080	2590	1860	999	1600	625-2590	8	100
CFF	16.1	16.7	11.5	16.4	89.9	<LOD	103	181	11.5-181	7	87.5

Values are in ng/g dw; <LOD: value below the limit of detection

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Table S10. Concentration of each compound in ng/g dw of the soils analyzed in certain plots, with its range, positive samples and frequency of detection.

Compound	Tomatoe BSS	Tomatoe BSD	Tomatoe WSS	Lettuce BSS	Lettuce WCS	Lettuce WCS	Carrot BSS	Carrot BSD	Carrot WSS	Range	Positive	Frequency (%)
BP3	1.1	0.8	0.8	0.8	0.8	0.7	1.2	0.9	1.0	0.7-1.2	7	77.8
BP1	<LOD	0.7	0.9	<LOD	<LOD	1.8	<LOD	1.3	1.9	0.7-1.9	4	44.4
BP2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
BP4 (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
4HB	<LOD	0.6	<LOD	<LOD	0.6	<LOD	<LOD	<LOD	<LOD	0.6	1	11.1
4DHB	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
DHMB	<LOD	<LOD	<LOD	0.4	<LOD	0.4	<LOD	0.6	<LOD	0.4-0.6	3	33.3
AVO	<LOQ	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	0	0	0.0
4MBC	<LOD	1.1	1.2	1.1	1.0	1.0	1.4	1.1	1.3	1.0-1.4	7	77.8
EHMC	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.9	1.9	1.8	1.8-1.9	3	33.3
EtPABA	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
BZT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
MeBZT	4.1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	4.1	0	0.0
DMBZT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
UVP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
BePB (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.0	1.0	1.0	1	3	33.3
BuPB (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
PrPB (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
MePB (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
FLU	0.7	0.8	0.8	0.7	0.7	0.7	0.7	0.7	0.7	0.7-0.8	7	77.8
OFX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
CPX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
NDX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
OXL	1.1	1.1	1.2	1.1	1.1	1.1	1.1	1.1	<LOD	1.1-1.2	6	66.7
TCY	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
OCY	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
S-STZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
SDZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0

acSDZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
SMR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
acSMR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
acSMZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
SMX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
acSMX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
SMPZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
SPY	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
acSPY	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
SQX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
STZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
SMD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
SDM	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
TMP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
GMZ (-)	1.4	1.3	1.4	1.3	1.3	1.3	1.3	1.3	1.3	1.3-1.4	7	77.8
MFA (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
NPX (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
IBU	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
KPF (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
DCF (-)	66.0	76.2	85.2	37.4	42.4	43.4	5.3	21.0	30.4	5.3-85.2	7	77.8
DCF-13C (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
AAP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
CBZ	0.8	0.5	0.8	<LOD	<LOQ	<LOD	1.6	<LOQ	<LOQ	0.5-1.6	2	22.2
CBZ-E	<LOD	2.4	2.4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.4	1	11.1
ATL	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
norFXT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0
SCY (-)	13.6	9.3	10.8	10.5	9.9	7.4	3.1	3.2	4.2	3.1-13.6	7	77.8
CFF	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.0

Values are in ng/g dw; <LOD: value below the limit of detection

Table S11. Concentration of each compound in ng/L in the irrigation waters of the different vegetables, with its range, positive samples and frequency of detection.

Compound	Tomatoes WWTP	Tomatoes Barriers	Carrots WWTP	Carrots Barriers	Lettuces WWTP	Lettuces Barriers	Range	Positive	Frequency (%)
BP3	6.55	<LOD	8.2	0.722	6.25	2.48	0.72-8.2	5	83.33
BP1	106	15.5	42.7	12.2	47.3	14.4	12.2-106	6	100.00
BP2	403	30.6	83.5	35.4	143	22.6	22.6-403	6	100.00
BP4 (-)	1190	394	2800	414	1030	309	309-2800	6	100.00
4HB	22.3	18	17.4	13.8	<LOD	<LOD	13.8-22.3	4	66.67
4DHB	32.2	48.9	22.1	13.5	<LOD	<LOD	13.5-48.9	4	66.67
DHMB	8.48	5.68	106	35.5	15.3	5.59	5.59-106	6	100.00
AVO	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.00
4MBC	350	17.5	230	10.2	173	131	10.2-350	6	100.00
EHMC	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.00
EtPABA	2.78	<LOD	64.2	<LOD	1.14	<LOD	1.14-64.2	3	50.00
BZT	711	506	902	454	623	487	454-902	6	100.00
MeBZT	1170	601	622	382	601	224	224-1170	6	100.00
DMBZT	86.5	32.4	28.5	7.03	51.9	12.5	7.03-86.5	6	100.00
UVP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.00
BePB (-)	6.99	<LOD	7.08	<LOD	<LOD	<LOD	6.99-7.08	2	33.33
BuPB (-)	7.33	<LOD	<LOD	<LOD	<LOD	<LOD	7.33	1	16.67
PrPB (-)	21.7	21.7	22.7	22.5	21.4	21.3	21.2-22.7	6	100.00
MePB (-)	27.6	26.8	90.7	29.4	26.9	27.1	26.8-90.7	6	100.00
FLU	17.4	15.9	12.3	9.86	9.82	11.7	9.82-17.4	6	100.00
OFX	1230	163	848	313	1240	246	163-1240	6	100.00
CPX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.00
NDX	7.7	4.24	10.4	5.73	9.97	<LOD	4.24-10.4	5	83.33
OXL	17.5	15.7	27	9.37	11.4	9.35	9.35-27	6	100.00
TCY	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.00
OCY	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.00
S-STZ	4.66	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOQ-4.66	1	16.67
SDZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.00

acSDZ	11.3	<LOD	<LOQ	<LOQ	17	<LOD	<LOQ-17	2	33.33
SMR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.00
acSMR	81.1	9.91	25.3	15.6	11.5	<LOD	9.91-81.1	5	83.33
acSMZ	11.6	9.31	10.4	9.28	7.98	8.67	7.98-11.6	6	100.00
SMX	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.00
acSMX	17.8	14.5	86.1	23.4	83.1	6.94	6.94-86.1	6	100.00
SMPZ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.00
SPY	49.2	22.1	45.6	25.9	30	11.6	11.6-49.2	6	100.00
acSPY	26.2	13.5	106	19.6	116	<LOD	13.5-116	5	83.33
SQX	4.7	4.55	3.69	<LOD	<LOD	<LOD	3.69-4.7	3	50.00
STZ	7.85	<LOD	10.7	6.91	<LOD	<LOD	6.91-10.7	3	50.00
SMD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.00
SDM	<LOQ	<LOD	0.725	<LOQ	<LOQ	1.02	<LOQ-1.02	2	33.33
TMP	26.4	22.8	31.2	25.8	35.2	21.1	21.1-35.2	6	100.00
GMZ (-)	1680	19.7	715	156	916	479	19.7-1680	6	100.00
MFA (-)	60.8	39.2	40.5	<LOD	23.7	23.2	23.2-60.8	5	83.33
NPX (-)	449	44.5	365	198	454	123	44.5-454	6	100.00
IBU	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.00
KPF (-)	638	506	547	448	157	122	122-638	6	100.00
DCF (-)	1470	892	1650	1200	1390	643	643-1650	6	100.00
DCF-13C (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.00
AAP	75.3	23.1	19.5	20.1	<LOD	<LOD	19.5-75.3	4	66.67
CBZ	277	153	280	154	177	138	138-280	6	100.00
CBZ-E	48.1	26	53.4	57.4	54.1	37.2	26-57.4	6	100.00
ATL	65.2	46.2	87.9	47.4	66.8	<LOD	46.2-87.9	5	83.33
norFXT	24.2	11.2	174	36.3	122	<LOD	11.2-174	5	83.33
SCY (-)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0	0	0.00
CFF	556	428	102	99.8	40.6	39.8	39.8-556	6	100.00

Values are in ng/L; <LOD: value below the limit of detection

Section S1. Standards and reagents

The following standards used were of analytical grade (>98%); methyl paraben (MePB), propyl paraben (PrPB), benzyl paraben (BePB) butyl paraben (BuPB), succynil-sulfathiazole (S-STZ), sulfadiazine (SDZ), N4-acetylsulfadiazidine (acSDZ), sulfamerazine (SMR), N4-acetylsulfamerazine (acSMR), N4-acetylsulfamethazine (acSMZ), sulfamethoxazole (SMX), N4-acetylsulfamethoxazole (acSMX), sulfamethoxypyridazine (SMPZ), sulfapyridine (SPY), N4-acetylsulfapyridine (acSPY), sulfaquinoxaline (SQX), sulfathiazole (STZ), sulfisomidine (SMD), sulfadimethoxine (SDM), benzonphenone-3 (BP3), benzophenone-1 (BP1), benzophenone-4 (BP4), 4-hydroxybenzophenone (4HB), 4,4'-dihydroxybenzophenone (4DHB), avobenzone (AVO), 2-(2-benzotriazol-2-yl)-p-cresol (UVP), 5,6-dimethyl-1H-benzotriazole (DMBZT), nalidixic acid (NDX), oxolinic acid (OXL), tetracycline (TCY), trimethoprim (TMP), acetaminophen (AAP), atenolol (ATL), gemfibrozil (GFZ), ketoprofen (KPF), mefenamic acid (MFA), carbamazepine (CBZ), norfluoxetine (norFXT), ofloxacin (OFX), ciprofloxacin (CFX), caffeine (CFF), ibuprofen (IBU), salicylic acid (SCY), diclofenac (DCF), and diclofenac-^{13}C (DCF-13C) were purchased from Sigma Aldrich (Darmstadt, Germany).

The isotopically labeled internal standards 5-(2,5-dimethylphenoxy)-2,2-bis(trideuteriomethyl)pentanoic acid (GMZ-d6), diclofenac-d4 (phenyl-d4) (DCF-d4), benzyl paraben-d4 (BePB-d4), caffeine-d3 (CFF-d3), ibuprofen-d3 (IBU-d3), salicylic acid-d6 (SCY-d6), 2-Hydroxy-4-methoxy-2',3',4',5',6'-d5 (BP3-d5), (±)-3-(4-methylbenzylidene-d4) camphor (4MBC-d4), 1H-benzotriazole-4,5,6,7-d4 (BZT-d4), flumequine-13C3 (FLU-13C3), trimethoprim-d3 (TMP-d3), carbamazepine-d10 (CBZ-d10), and mefenamic acid-d3 (MFA-d3) were acquired from CDN isotopes (Quebec, Canada).

The following UV filters were purchased from Merck (Darmstadt, Germany); 1H-benzotriazole (BZT), ethylhexyl methoxycinnamate (EHMC), benzophenone-2 (BP2), 2,2'-dihydroxy-4-methoxybenzophenone (DHMB), and ethyl-4-(dimethyl-amino) benzoate (EtPABA).

Naproxen (NPX) and oxytetracycline (OTC) were obtained from Honeywell Fluka (Wabash, United States). Sulfamethazine-d_4, flumequine (FLU), carbamazepine 10,11-epoxy (CBZ-E) and acetaminophen-d_4 were purchased from Toronto Research Chemicals (TRC) (Toronto, Canada). 5-Methyl benzotriazole (MeBZT) was obtained from TCI (Zwijndrecht, Belgium) and 4-methylbenzylidene camphor (4MBC) was provided by Dr. Ehrenstorfer (Augsburg, Germany).

The commercial QuEChERS kits used in the extraction method were from BEKOlut®.

Water, methanol (MeOH) and acetonitrile (ACN) of HPLC- grade were purchased from J.T. Backer (Deventer, The Netherlands) and the nitrogen (99.99% purity) was supplied by Air Liquide (Barcelona, Spain). Formic acid (FA) and ammonium acetate ($AcNH_4$), as well as ethanol and acetone cleaning solvents were supplied by Merck.

Section S2. R script used for the statistical analysis (PCA).

After opening Rstudio, the following code was used to perform the statistical analysis for the tomates. The same code was applied for all the other matrices:

```
install.packages("factoextra")
library(factoextra)
Statistics_crops <- read_excel("file_directory", sheet = "Tomatoe")
Tom.pca < -prcomp(Statistics_crops[,c(-1,-47)], scale=TRUE)
dimnames(Tom.pca$x)[[1]] <- Statistics_crops$name
fviz_pca_biplot(Tom.pca, labels = c(1,2), geom = c("point","text"), labelsize=5,
habillage=Statistics_crops$Group, ggtheme = theme_gray(),
        col.var = "black", repel=TRUE, geom.var = c("point", "text"),
        title = "PCA  Tomatoes") +theme(text = element_text(size=15))
```

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Section S3. Validation parameters

In order to validate the method for the new matrices (carrots, tomatoes, and soils) MLODs and MLOQs, were determined as the lowest compound concentration that yielded a signal-to-noise (S/N) ratio of 3 and 10, respectively. Analytical calibration curves were constructed according to the individual response range of each analyte. Accuracy was evaluated by the recovery rates of each standard spiked in the blank sediment or vegetable, determined in five replicate spiked extracts at three concentration levels; 5, 100, and 350 ng/mL in standard solutions for tomatoes, and 10, 50, and 100 ng/mL in standard for carrots and soils, and measured 3 times (n=9). Precision was expressed as percentage of relative standard deviation (RSD (%)), for each concentration level. The ME was evaluated by comparing the slopes of the analytical calibration curves prepared in MeOH and in sample extracts (matrix-matched calibration standards).

Calibration range and matrix effects

For tomatoe, carrot and soil extracts analyses, calibration curves at ten different concentrations (1, 3, 5, 10, 30, 50, 100, 300, 500, and 700 ng/mL) were built, both in pure MeOH and in the matrix extract (matrix-matched standards solutions) to evaluate the potential matrix effect (ME). Correlation coefficients (r^2) of analytical calibration curves were $r^2 > 0.98$ in both MeOH and in the matrix-matched standards, indicating good linearity for all compounds. A high percentage (65%) of the studied com- pounds presented signal suppression (between 4 and 86%), 33% showed enhanced signal (between 6 and 73%) and 7% did not showed ME. That's why matrix matched calibration curves were used for all the matrices. For example, the calibration curves of BP3 and MePB in tomatoe are shown in Figures S3 and S4. According to this, BP3 presents enhancement (20%) and MePB presents medium suppression (27%), as happens with most of the compounds of the method.

Quality assurance

To ensure a reliable analysis and prevent the problems related to background contamination, blanks of the methods were performed. To avoid the cross-contamination, all the glass material was cleaned several times with ethanol and acetone, and the non-volumetric glassware was muffled at 3500 ºC overnight. To ensure extraction efficiency, a surrogate standard (BP-^{13}C) was added to the samples. For a reliable HPLC-MS/MS analysis, quality controls (mixture standard solution at known concentration) and solvent blanks were randomly included along the analysis sequences. Isotopically labelled internal standards were used for quantification to overcome matrix effects. Stock solutions of individual standards (1000 mg/L) and an intermediate stock solution containing all analytes (1 mg/L) were prepared in MeOH. Weekly, standard working solutions were prepared at appropriate concentrations. All standards solutions were stored in the dark at -20 °C.

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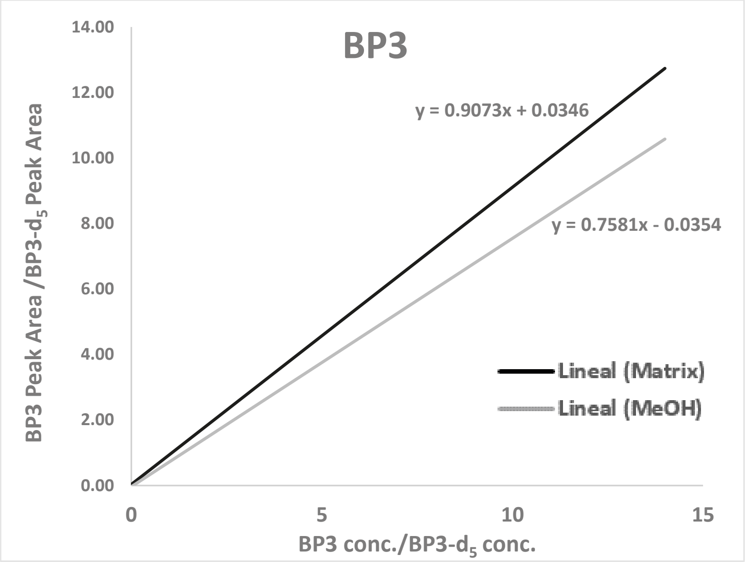

Figure S3. Calibration curves for BP3 in MeOH (grey line) and in tomatoe extract (black line).

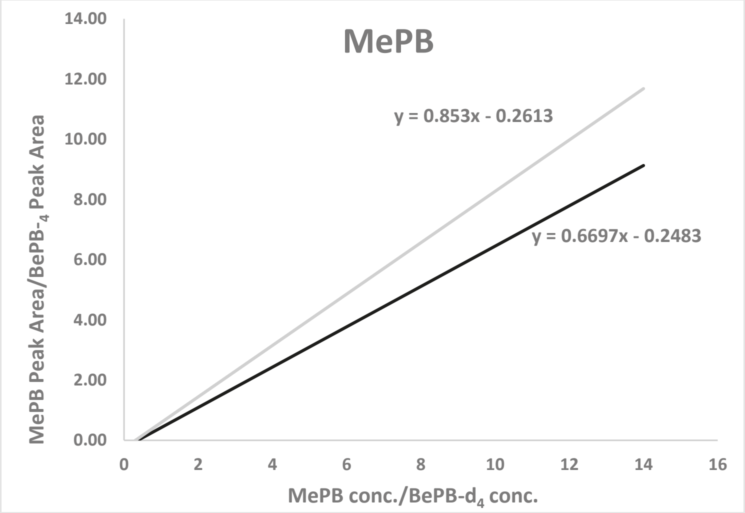

Figure S4. Calibration curves for MePB in MeOH (grey line) and in tomatoe extract (black line).

3.1.2. Accuracy

For tomatoes, the recovery rates ranged between 21% and 145%. The lowest recovery value was obtained for the metabolite acSMZ and the highest for norFXT.

For carrots, the efficiency of the extraction was also satisfactory, obtaining recovery rates between 42% and 154%. Lowest recoveries rates, 42% and 43% were obtained for OTC and 4DHB, respectively, and the high value corresponded to OFX. OTC recovery rates are usually low because, as is well-known, it tends to form metal complexes, and undergo epimerization.

For soils, similar recovery rates were obtained, with values between 15% for EHMC and 154% for ketoprofen.

An overview of the recovery rate values obtained for all the compounds, in all matrices and at the three concentrations levels considered is shown in Figure S5. Overall, about 15% of the recovery rates estimated was below 50%. Only in lettuces, values below 10% were obtained for 3 compounds at 100 and 350 ng/g dw spike levels. The complete list of recovery values is listed in the end of this section in tables S12, S13 and S14.

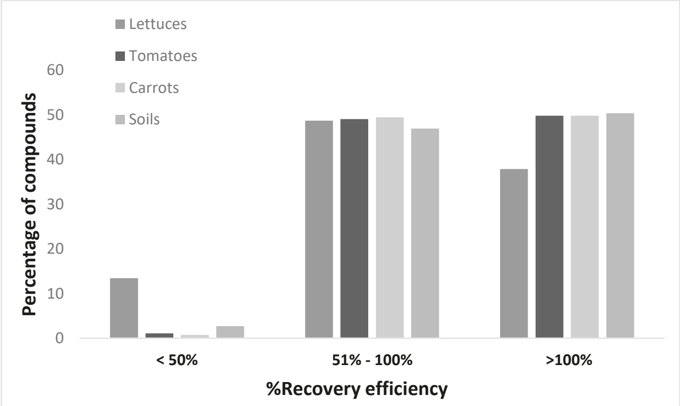

Figure S5. Percentage of compounds grouped by percentage of recovery rates of <50%, 51% - 100% and >100%.

3.1.3. Sensitivity

The MLOD and MLOQ obtained are listed in Tables S8, S9, S10 and S11.

Higher values than the ones reported for lettuces were obtained for carrots, with MLODs and MLOQs in the ranges 0.02- 1.61 ng/g and 0.08-5.35 ng/g, respectively, and similarly for tomatoes, that were in the range 0.01-1.46 ng/g dw for MLODs and 0.03-4.87 ng/g dw for MLOQs.

The MLODs and MLOQs obtained for soil were lower than those achieved in the vegetables. MLODs ranged from 0.01 to 0.29 ng/g dw and MLOQs from 0.03 to 0.97 ng/g dw. For irrigation waters MLODs were between 0.04 ng/L and 2.21 ng/L, and MLOQs between 0.15 ng/L and 7.36 ng/L.

3.1.4. Precision

Five replicates of spiked samples at three concentrations (the spike levels selected; 5, 100 and 350 ng/g dw for lettuces and 10, 50 and 100 ng/g dw for tomatoes, carrots and soils) were analyzed. The RSD values are listed in Tables S15, S16, and S17 below this section.

For some compounds very different RSD were obtained depending on the concentration. %. For tomatoes samples, RSD variated between 0.3% and 36%, for carrots RSD variated between 5% and 46%, and for soils RSD variated between 0.5% and 38%.

3.1.5 Recoveries

Table S12. Recoveries obtained in the tomatoes for each compound in the different replicates.

Concentration level (replicate number)	BP3	BP1	BP2	BP4 (-)	4HB	4DHB	DHMB	AVO	4MBC	EHMC	EtPABA
5 ng/mL (1)	106	107	142	88	81	133	83	103	115	136	95
5 ng/mL (2)	99	114	149	102	95	133	81	125	67	117	114
5 ng/mL (3)	101	71	147	120	94	134	69	98	77	128	120
5 ng/mL (4)	108	93	142	109	93	133	97	125	94	131	105
5 ng/mL (5)	96	111	145	42	91	133	67	59	93	122	83
100 ng/mL (1)	114	123	114	100	110	95	112	110	76	61	112
100 ng/mL (2)	123	82	103	90	109	96	102	121	93	101	125
100 ng/mL (3)	111	117	110	99	123	100	126	89	72	71	101
100 ng/mL (4)	120	120	118	105	123	100	141	88	97	83	96
100 ng/mL (5)	123	126	114	93	121	89	136	106	75	64	91
350 ng/mL (1)	98	91	92	92	108	121	99	107	105	63	116
350 ng/mL (2)	89	84	94	111	118	111	88	84	89	65	126
350 ng/mL (3)	90	114	100	111	118	116	119	68	80	49	93
350 ng/mL (4)	91	104	85	99	88	113	88	85	97	41	93
350 ng/mL (5)	105	114	129	102	111	107	115	100	82	38	81

Concentration level (replicate number)	BZT	MeBZT	DMBZT	UVP	BePB (-)	BuPB (-)	PrPB (-)	MePB (-)	FLU	OFX	CFX
5 ng/mL (1)	64	117	122	93	97	104	131	82	90	126	116
5 ng/mL (2)	96	134	106	130	103	111	132	78	107	81	119
5 ng/mL (3)	84	89	91	85	96	102	127	101	103	85	106
5 ng/mL (4)	99	78	119	66	105	114	135	95	94	90	119
5 ng/mL (5)	106	77	132	72	81	82	80	76	81	77	103
100 ng/mL (1)	112	96	117	100	99	110	98	81	94	74	75
100 ng/mL (2)	111	108	95	92	103	106	115	98	94	148	138
100 ng/mL (3)	90	85	110	81	96	101	96	104	101	96	105
100 ng/mL (4)	102	107	113	113	105	106	102	103	94	124	153
100 ng/mL (5)	91	104	108	108	99	104	109	98	117	144	137
350 ng/mL (1)	94	94	117	64	105	105	109	109	101	126	114
350 ng/mL (2)	110	114	107	129	106	112	104	95	103	101	139
350 ng/mL (3)	90	119	98	94	98	100	108	91	95	72	77
350 ng/mL (4)	91	92	108	80	90	91	107	81	89	81	80
350 ng/mL (5)	104	128	123	87	103	111	116	99	119	98	87

	NDX	OXL	TCY	OTC	S-STZ	SDZ	acSDZ	SMR	acSMR	acSMZ	SMX
5 ng/mL (1)	111	118	77	120	127	114	104	107	116	64	85
5 ng/mL (2)	149	127	90	128	82	64	115	76	102	23	69
5 ng/mL (3)	113	119	89	79	92	81	145	84	102	23	86
5 ng/mL (4)	99	90	87	105	85	77	113	88	97	21	67
5 ng/mL (5)	93	77	86	124	84	77	98	85	100	57	77
100 ng/mL (1)	104	95	103	138	94	84	89	77	107	108	85
100 ng/mL (2)	124	95	103	93	115	126	96	112	106	96	106
100 ng/mL (3)	113	107	116	131	85	64	98	70	100	89	93
100 ng/mL (4)	117	92	116	135	123	102	88	41	112	88	138
100 ng/mL (5)	117	110	114	142	124	92	96	110	93	85	84
350 ng/mL (1)	108	96	101	102	114	121	108	93	95	94	114
350 ng/mL (2)	121	106	111	94	98	116	100	97	90	84	80
350 ng/mL (3)	107	98	111	128	74	89	80	71	84	80	56
350 ng/mL (4)	81	81	83	116	117	63	85	89	121	89	74
350 ng/mL (5)	127	108	104	127	119	71	99	97	97	75	68

Concentration level (replicate number)	acSMX	SMPZ	SPY	acSPY	SQX	STZ	SMD	SDM	TMP	GFZ (-)	MFA (-)
5 ng/mL (1)	103	108	64	134	75	66	94	69	84	90	106
5 ng/mL (2)	112	89	64	137	70	61	103	60	121	94	74
5 ng/mL (3)	129	109	79	109	82	102	118	69	63	88	72
5 ng/mL (4)	143	83	85	110	95	82	131	75	78	93	99
5 ng/mL (5)	59	90	92	77	28	81	54	58	58	81	79
100 ng/mL (1)	93	103	66	85	49	82	85	71	114	93	123
100 ng/mL (2)	104	106	112	116	92	90	96	120	102	101	113
100 ng/mL (3)	94	72	66	83	43	88	86	74	117	96	85
100 ng/mL (4)	106	134	115	124	120	92	97	147	128	102	87
100 ng/mL (5)	127	158	99	124	64	98	117	88	122	96	99
350 ng/mL (1)	125	91	104	108	100	118	115	52	106	107	104
350 ng/mL (2)	107	69	109	114	101	84	98	61	106	103	115
350 ng/mL (3)	62	94	98	92	133	57	57	54	82	93	87
350 ng/mL (4)	118	88	108	99	114	84	108	94	100	95	96
350 ng/mL (5)	105	85	62	90	121	85	96	80	106	106	118
	NPX (-)	**KPF (-)**	**DCF (-)**	**DCF-13C (-)**	**AAP**	**CBZ**	**CBZ-E**	**ATX**	**norFXT**	**SCY (-)**	**CFF**
5 ng/mL (1)	99	139	119	97	72	95	106	77	112	114	114
5 ng/mL (2)	88	67	158	76	73	100	107	109	113	85	115
5 ng/mL (3)	81	75	124	70	127	95	94	55	180	106	111
5 ng/mL (4)	65	128	144	168	149	113	99	96	119	115	111
5 ng/mL (5)	110	106	78	84	133	77	144	50	145	131	111
100 ng/mL (1)	84	103	102	101	92	111	65	125	101	102	110
100 ng/mL (2)	83	128	91	138	122	94	95	136	80	110	114
100 ng/mL (3)	76	117	108	140	96	103	83	94	144	89	126
100 ng/mL (4)	81	106	113	81	115	111	84	114	122	90	90
100 ng/mL (5)	87	91	140	104	94	103	77	93	88	58	140
350 ng/mL (1)	96	112	105	117	100	106	49	105	125	118	109
350 ng/mL (2)	98	115	127	97	123	98	63	120	89	98	92
350 ng/mL (3)	85	108	88	98	79	98	68	76	88	93	92
350 ng/mL (4)	80	115	131	82	86	95	89	136	113	97	94
350 ng/mL (5)	86	127	104	108	97	105	93	97	109	112	132

Table S13. Recoveries obtained in the carrots for each compound in the different replicates.

Concentration level (replicate number)	BP3	BP1	BP2	BP4 (-)	4HB	4DHB	DHMB	AVO	4MBC	EHMC	EtPABA
10 ng/mL (1)	74	100	63	105	113	78	84	119	113	89	94
10 ng/mL (2)	99	133	82	90	106	56	95	85	140	73	80
10 ng/mL (3)	101	53	80	83	120	43	65	113	94	66	52
10 ng/mL (4)	91	57	127	131	72	55	75	47	100	115	139
10 ng/mL (5)	93	132	119	115	98	25	60	105	135	99	95
50 ng/mL (1)	99	145	107	114	101	138	87	119	103	101	98
50 ng/mL (2)	104	99	92	119	112	122	100	119	113	134	106
50 ng/mL (3)	90	92	103	105	102	100	103	102	88	106	89
50 ng/mL (4)	97	120	127	112	98	103	89	137	87	99	101
50 ng/mL (5)	84	125	92	99	75	68	66	107	76	105	116
100 ng/mL (1)	105	112	122	83	98	95	99	85	92	109	103
100 ng/mL (2)	91	100	115	119	120	96	116	122	114	96	81
100 ng/mL (3)	90	103	98	103	125	95	73	106	123	99	86
100 ng/mL (4)	81	113	95	101	96	93	96	104	92	93	123
100 ng/mL (5)	109	110	94	94	107	109	112	97	117	104	68
	BZT	**MeBZT**	**DMBZT**	**UVP**	**BePB (-)**	**BuPB (-)**	**PrPB (-)**	**MePB (-)**	**FLU**	**OFX**	**CFX**
10 ng/mL (1)	92	81	89	121	81	77	70	87	83	122	77
10 ng/mL (2)	110	133	131	115	90	126	90	121	77	84	105
10 ng/mL (3)	105	126	104	107	122	111	77	131	109	154	110
10 ng/mL (4)	114	115	83	154	119	73	91	84	96	141	55
10 ng/mL (5)	86	74	99	173	131	131	127	93	55	154	55
50 ng/mL (1)	109	122	106	106	92	141	142	120	88	62	82
50 ng/mL (2)	107	83	128	84	74	80	131	84	73	59	55
50 ng/mL (3)	108	64	106	93	101	111	147	73	101	123	58
50 ng/mL (4)	110	91	91	118	75	145	127	97	96	61	73
50 ng/mL (5)	67	100	107	93	81	139	118	108	128	67	80
100 ng/mL (1)	85	85	81	52	88	105	84	103	102	95	100
100 ng/mL (2)	91	79	92	113	96	118	80	99	102	87	76
100 ng/mL (3)	86	94	81	83	91	119	108	88	109	82	85
100 ng/mL (4)	86	84	94	75	84	121	80	120	92	102	111
100 ng/mL (5)	69	115	101	68	87	79	109	97	105	89	120

Concentration level (replicate number)	NDX	OXL	TCY	OTC	S-STZ	SDZ	acSDZ	SMR	acSMR	acSMZ	SMX
10 ng/mL (1)	110	89	118	99	124	104	102	123	141	107	131
10 ng/mL (2)	76	74	45	86	97	76	71	103	83	80	96
10 ng/mL (3)	61	45	88	112	85	85	83	82	127	60	91
10 ng/mL (4)	103	71	175	99	151	64	123	102	135	128	91
10 ng/mL (5)	75	153	127	80	151	91	127	112	121	113	177
50 ng/mL (1)	99	103	93	31	142	128	75	98	125	136	100
50 ng/mL (2)	97	106	118	57	84	70	74	87	89	102	93
50 ng/mL (3)	89	91	127	71	114	116	122	98	139	142	121
50 ng/mL (4)	101	74	91	42	98	90	113	135	145	58	100
50 ng/mL (5)	99	81	120	146	94	122	82	117	84	105	82
100 ng/mL (1)	107	88	107	93	99	111	71	98	103	114	89
100 ng/mL (2)	127	118	85	30	120	129	76	92	86	71	97
100 ng/mL (3)	75	66	116	117	111	90	113	69	89	74	108
100 ng/mL (4)	112	123	89	14	101	125	106	106	139	113	101
100 ng/mL (5)	138	137	101	112	130	98	85	90	106	91	83
	acSMX	**SMPZ**	**SPY**	**acSPY**	**SQX**	**STZ**	**SMD**	**SDM**	**TMP**	**GFZ (-)**	**MFA (-)**
10 ng/mL (1)	96	81	123	114	89	121	98	104	84	95	96
10 ng/mL (2)	113	70	95	106	67	80	71	84	117	125	83
10 ng/mL (3)	119	99	89	65	126	111	30	111	115	69	132
10 ng/mL (4)	100	52	105	159	111	57	65	86	83	100	77
10 ng/mL (5)	33	113	160	73	112	193	102	132	77	88	67
50 ng/mL (1)	98	70	112	115	117	82	132	100	92	98	87
50 ng/mL (2)	75	58	72	88	58	80	73	75	115	85	94
50 ng/mL (3)	153	97	120	106	94	130	96	121	96	96	92
50 ng/mL (4)	89	108	142	126	143	106	89	105	103	136	81
50 ng/mL (5)	97	102	84	85	93	139	111	67	132	145	103
100 ng/mL (1)	119	105	88	81	104	76	120	89	117	106	90
100 ng/mL (2)	72	113	83	96	105	127	75	127	98	91	120
100 ng/mL (3)	90	75	70	131	84	107	118	103	120	79	104
100 ng/mL (4)	55	91	125	88	83	132	113	120	103	85	89
100 ng/mL (5)	79	67	91	75	85	66	86	85	76	118	89

Concentration level (replicate number)	NPX (-)	IBU (-)	KPF (-)	DCF (-)	DCF13C	AAP	CBZ	CBZ-E	ATL	norFXT	SCY	SCY (-)
10 ng/mL (1)	75	101	111	120	85	111	94	61	71	93	116	103
10 ng/mL (2)	130	99	146	99	95	82	79	57	111	152	108	115
10 ng/mL (3)	170	104	138	92	116	104	70	47	97	147	109	134
10 ng/mL (4)	103	126	133	84	70	108	80	50	59	122	106	131
10 ng/mL (5)	49	120	128	119	120	82	66	38	123	114	124	100
50 ng/mL (1)	100	147	88	127	78	125	113	104	107	115	71	87
50 ng/mL (2)	127	102	96	148	112	105	87	71	65	133	144	75
50 ng/mL (3)	151	106	75	105	114	143	111	44	92	129	78	109
50 ng/mL (4)	78	95	69	118	109	60	212	105	81	103	136	76
50 ng/mL (5)	131	82	80	129	78	144	67	59	92	71	137	102
100 ng/mL (1)	117	122	69	82	92	107	115	132	110	90	79	109
100 ng/mL (2)	119	98	84	122	107	78	97	77	127	117	83	97
100 ng/mL (3)	76	101	83	104	103	98	65	55	101	78	106	79
100 ng/mL (4)	106	104	61	116	114	107	101	109	71	120	102	97
100 ng/mL (5)	119	101	93	104	115	126	91	74	118	84	91	129

Table S14. Recoveries obtained in the soils for each compound in the different replicates.

Concentration level (replicate number)	BP3	BP1	BP2	BP4 (-)	4HB	4DHB	DHMB	AVO	4MBC	EHMC	EtPABA
10 ng/mL (1)	98	125	142	138	106	132	83	67	88	98	108
10 ng/mL (2)	99	142	143	153	96	132	104	53	100	98	85
10 ng/mL (3)	101	114	142	110	96	135	96	92	102	91	74
10 ng/mL (4)	112	150	142	114	114	137	142	109	107	93	80
10 ng/mL (5)	100	121	141	133	176	134	133	119	117	95	103
50 ng/mL (1)	95	112	103	89	100	111	116	42	95	27	89
50 ng/mL (2)	104	101	106	115	89	102	119	45	92	27	93
50 ng/mL (3)	98	98	125	86	95	106	121	46	103	27	80
50 ng/mL (4)	98	120	107	90	91	112	136	47	121	28	113
50 ng/mL (5)	98	126	120	82	114	121	135	49	84	24	86
100 ng/mL (1)	98	99	92	85	102	112	130	50	88	14	81
100 ng/mL (2)	116	112	94	92	116	94	113	56	98	16	98
100 ng/mL (3)	110	108	104	94	118	91	103	48	105	15	116
100 ng/mL (4)	117	112	111	116	138	136	121	42	119	20	103
100 ng/mL (5)	122	117	110	138	117	104	99	47	102	22	94

Concentration level (replicate number)	BZT	MeBZT	DMBZT	UVP	BePB (-)	BuPB (-)	PrPB (-)	MePB (-)	FLU	OFX	CFX
10 ng/mL (1)	69	120	79	151	145	115	118	95	134	82	145
10 ng/mL (2)	78	98	66	164	135	111	110	81	116	73	114
10 ng/mL (3)	117	77	75	141	130	101	110	85	107	70	97
10 ng/mL (4)	107	85	81	80	152	129	138	81	149	68	99
10 ng/mL (5)	117	96	61	54	140	114	121	64	129	75	104
50 ng/mL (1)	109	106	118	63	90	83	107	80	93	120	67
50 ng/mL (2)	110	117	117	86	108	103	126	102	97	119	71
50 ng/mL (3)	101	95	91	82	86	82	100	82	93	93	110
50 ng/mL (4)	84	115	70	67	114	103	111	63	110	116	64
50 ng/mL (5)	100	104	109	37	78	73	90	71	96	76	119
100 ng/mL (1)	86	92	109	87	70	70	83	70	105	106	81
100 ng/mL (2)	94	100	120	59	73	70	81	71	91	94	96
100 ng/mL (3)	81	103	85	86	77	73	89	73	81	89	83
100 ng/mL (4)	113	112	126	137	100	97	113	97	101	74	112
100 ng/mL (5)	73	87	86	85	101	103	122	106	111	108	93
	NDX	**OXL**	**TCY**	**OTC**	**S-STZ**	**SDZ**	**acSDZ**	**SMR**	**acSMR**	**acSMZ**	**SMX**
10 ng/mL (1)	109	110	108	112	137	138	124	101	80	99	110
10 ng/mL (2)	84	137	104	89	43	121	110	65	87	117	118
10 ng/mL (3)	66	124	95	77	80	82	79	67	91	119	110
10 ng/mL (4)	115	170	121	83	103	110	133	61	116	112	97
10 ng/mL (5)	109	154	107	107	84	162	149	78	99	79	137
50 ng/mL (1)	97	103	78	93	102	125	96	92	118	88	108
50 ng/mL (2)	124	108	97	97	103	73	83	88	91	101	92
50 ng/mL (3)	89	93	77	83	88	69	94	72	91	84	68
50 ng/mL (4)	125	107	97	118	103	68	97	86	93	94	77
50 ng/mL (5)	90	93	69	89	112	61	82	66	93	97	87
100 ng/mL (1)	109	104	66	85	51	44	53	40	82	76	52
100 ng/mL (2)	92	89	66	102	84	76	99	65	112	117	86
100 ng/mL (3)	88	82	69	120	135	93	106	94	104	96	130
100 ng/mL (4)	128	82	92	107	134	99	128	102	109	110	124
100 ng/mL (5)	135	108	97	98	140	98	84	102	103	98	74

Concentration level (replicate number)	acSMX	SMPZ	SPY	acSPY	SQX	STZ	SMD	SDM	TMP	GFZ (-)	MFA (-)	
10 ng/mL (1)	81	144	131	148	42	117	92	95	69	164	102	
10 ng/mL (2)	75	116	60	108	72	70	87	97	97	163	110	
10 ng/mL (3)	114	111	51	103	112	77	70	72	75	155	79	
10 ng/mL (4)	151	121	50	160	138	145	90	104	142	179	99	
10 ng/mL (5)	140	119	58	140	88	47	90	86	97	165	53	
50 ng/mL (1)	112	137	88	97	91	113	85	82	94	96	88	
50 ng/mL (2)	86	92	89	78	112	70	108	103	77	116	129	
50 ng/mL (3)	102	95	82	74	83	62	97	115	89	100	92	
50 ng/mL (4)	90	87	109	80	84	86	82	128	86	126	119	
50 ng/mL (5)	90	72	103	90	103	88	101	84	114	86	92	
100 ng/mL (1)	90	77	49	77	58	58	109	72	99	77	93	
100 ng/mL (2)	117	141	105	135	104	87	113	127	103	77	91	
100 ng/mL (3)	122	110	115	118	97	134	93	95	116	86	92	
100 ng/mL (4)	130	111	118	109	95	116	108	129	128	107	126	
100 ng/mL (5)	106	131	144	86	96	133	112	141	123	110	114	
	NPX (-)	**IBU (-)**	**KPF (-)**	**DCF (-)**	**DCF13C**	**AAP**	**CBZ**	**CBZ-E**	**ATL**	**norFXT**	**SCY (-)**	**CFF**
10 ng/mL (1)	95	109	134	79	113	131	99	124	79	110	124	97
10 ng/mL (2)	90	93	144	135	98	124	114	121	83	115	108	98
10 ng/mL (3)	72	94	109	137	101	149	105	137	80	112	106	97
10 ng/mL (4)	93	108	155	181	114	120	106	121	148	120	107	96
10 ng/mL (5)	93	102	154	134	126	101	132	122	126	127	95	99
50 ng/mL (1)	88	59	111	100	91	91	95	105	62	129	102	90
50 ng/mL (2)	112	79	122	87	120	107	121	119	50	103	92	94
50 ng/mL (3)	101	62	114	127	113	106	89	128	95	87	102	91
50 ng/mL (4)	85	91	84	109	107	101	113	89	88	107	81	88
50 ng/mL (5)	105	62	111	92	101	92	81	113	88	94	91	101
100 ng/mL (1)	113	55	96	94	124	115	94	108	72	118	126	121
100 ng/mL (2)	117	59	81	99	126	80	94	99	93	111	118	88
100 ng/mL (3)	96	58	106	119	98	114	104	107	70	101	105	132
100 ng/mL (4)	112	78	134	98	80	99	106	107	70	120	129	117
100 ng/mL (5)	116	81	112	91	106	101	111	100	104	96	83	78

Table S15. Relative standard deviations among replicates of each spiking level in the different compounds in the tomatoes.

Compound	RSD% Intra 10 ng/g	RSD% Intra 50 ng/g	RSD% Intra 100 ng/g
BP3	4.72	4.46	7.06
BP1	17.76	15.65	13.14
BP2	2.13	5.15	16.79
BP4 (-)	32.94	5.82	7.86
4HB	5.96	6.15	11.06
4DHB	0.34	4.84	4.64
DHMB	15.35	13.41	14.15
AVO	26.44	13.88	16.82
4MBC	20.23	14.00	11.77
EHMC	5.89	21.34	23.85
EtPABA	14.08	13.00	18.03
BZT	18.12	10.44	8.75
MeBZT	25.44	9.43	14.23
DMBZT	13.64	7.71	8.55
UVP	27.93	12.83	26.18
BePB (-)	9.68	3.58	6.43
BuPB (-)	12.36	3.07	8.11
PrPB (-)	18.73	7.42	4.08
MePB (-)	12.45	9.58	10.45
Flumequine	10.85	10.21	11.32
Ofloxacin	21.13	26.94	21.47
Ciprofloxacin	6.72	25.79	26.57
Nalidixic acid	19.12	6.33	16.10
Oxolinic acid	20.24	8.00	10.87
Tetracycline	5.96	6.15	11.06
OTC	17.76	15.65	13.14
Succynil-Sulfathiazole	19.72	16.42	18.07
SDZ	22.09	24.14	27.81
acSDZ	15.74	4.87	11.87
SMR	12.99	35.78	12.04
acSMR	6.92	6.92	14.27
acSMZ	55.30	9.54	8.90
SMX	11.10	22.19	27.64
acSMX	29.28	13.29	23.73
SMPZ	12.09	28.50	11.35
SPY	16.04	26.31	19.98
acSPY	21.21	19.48	9.89
SQX	36.02	43.00	12.30
STZ	19.93	6.66	24.99
Sulfisomidin	29.28	13.29	23.73
SDM	10.77	32.51	26.59
Trimethoprim	30.18	8.36	10.12
Gemfibrozil (-)	5.74	3.54	6.34
Mefenamic acid	17.72	16.00	12.11
Naproxen (-)	19.33	4.99	8.54
IBU (-)	13.74	10.06	11.54
Ketoprofen (-)	30.28	12.97	6.15
Diclofenac (-)	24.19	16.47	15.77
Diclofenac-13C (-)	39.78	22.27	12.71
AAP	32.11	13.27	17.02
CBZ	13.46	6.67	4.84
CBZ-epoxy	17.94	13.18	25.34
Atenolol	32.28	16.78	21.08
norFXT	21.75	24.16	15.03
Salicylic acid (-)	15.20	21.85	10.38
Caffeine	1.73	15.94	16.43

Table S16. Relative standard deviations among replicates of each spiking level in the different compounds in the carrots.

Compound	RSD% Intra 10 ng/g	RSD% Intra 50 ng/g	RSD% Intra 100 ng/g
BP3	11.58	8.13	12.01
BP1	40.67	18.21	5.34
BP2	28.90	13.64	12.04
BP4 (-)	18.48	7.27	12.94
4HB	17.83	13.76	11.77
4DHB	37.17	24.82	6.58
DHMB	18.69	16.16	16.89
AVO	30.73	11.62	12.94
4MBC	17.60	15.56	13.66
EHMC	22.09	13.03	6.27
EtPABA	33.89	9.62	22.81
BZT	11.55	18.70	10.06
MeBZT	25.20	23.52	15.13
DMBZT	18.25	12.09	9.35
UVP	21.04	13.23	28.39
BePB (-)	19.83	13.92	5.02
BuPB (-)	26.15	22.42	16.15
PrPB (-)	24.40	8.77	16.12
MePB (-)	20.55	18.93	11.43
Flumequine	23.87	20.83	5.84
Ofloxacin	22.41	36.55	8.76
Ciprofloxacin	32.81	17.49	18.32
Nalidixic acid	23.99	4.87	21.31
Oxolinic acid	46.49	15.23	26.62
Tetracycline	43.31	15.03	12.34
OTC	12.76	65.06	64.72
Succynil-Sulfathiazole	24.65	21.20	11.51
SDZ	17.84	23.28	14.83
acSDZ	23.91	24.11	20.13
SMR	14.17	17.80	14.99
acSMR	18.42	24.33	20.05
acSMZ	27.59	30.75	21.93
SMX	31.72	14.15	10.19
acSMX	37.17	28.81	28.17
SMPZ	28.66	24.83	21.35
SPY	24.66	26.55	22.17
acSPY	36.11	16.88	23.12
SQX	22.81	31.16	11.99
STZ	45.78	24.79	28.73
Sulfisomidin	39.27	22.40	19.75
SDM	18.99	23.87	17.30
Trimethoprim	20.08	15.25	16.98
Gemfibrozil (-)	21.36	23.77	16.16
Mefenamic acid (-)	27.56	9.06	13.77
Naproxen (-)	44.62	24.35	16.97
IBU (-)	10.89	23.03	9.23
Ketoprofen (-)	9.99	12.96	16.35
Diclofenac (-)	15.93	12.49	14.47
Diclofenac-13C (-)	21.44	18.62	8.99
AAP	14.26	30.17	16.78
CBZ	14.06	47.00	19.19
CBZ-epoxy	17.71	35.36	34.06
Atenolol	28.97	17.99	20.36
norFXT	19.36	22.36	19.55
Salicylic acid (-)	6.38	31.57	12.87
Caffeine	13.38	16.65	17.76

Table S17. Relative standard deviations among replicates of each spiking level in the different compounds in the soils.

Compound	RSD% Intra 10 ng/g	RSD% Intra 50 ng/g	RSD% Intra 100 ng/g
BP3	5.46	3.15	7.92
BP1	11.54	10.79	6.14
BP2	0.50	8.56	8.46
BP4 (-)	13.68	13.90	20.56
4HB	28.25	10.27	10.87
4DHB	1.58	6.42	16.62
DHMB	22.18	7.29	11.09
AVO	31.33	5.87	10.61
4MBC	10.21	14.34	10.86
EHMC	3.11	5.12	18.61
EtPABA	16.10	13.78	12.60
BZT	22.90	10.27	16.70
MeBZT	16.93	8.24	9.60
DMBZT	11.69	20.25	17.81
UVP	40.49	28.41	30.75
BePB (-)	6.10	15.64	17.35
BuPB (-)	8.82	15.23	19.21
PrPB (-)	9.62	12.59	18.79
MePB (-)	14.00	18.50	19.91
Flumequine	12.81	7.10	11.87
Ofloxacin	7.05	18.66	14.82
Ciprofloxacin	17.61	30.04	12.98
Nalidixic acid	21.20	17.16	18.93
Oxolinic acid	17.09	7.02	12.88
Tetracycline	8.82	15.23	19.21
OTC	16.10	13.78	12.60
Succynil-Sulfathiazole	38.19	8.19	35.99
SDZ	24.18	32.48	27.96
acSDZ	21.96	7.84	29.36
SMR	21.37	13.85	33.75
acSMR	14.45	11.65	11.32
acSMZ	15.42	7.20	15.50
SMX	12.72	17.25	35.04
acSMX	30.02	11.19	13.56
SMPZ	10.44	24.78	21.54
SPY	48.67	12.01	33.02
acSPY	19.05	11.44	22.17
SQX	40.32	13.29	19.84
STZ	42.67	23.42	30.87
Sulfisomidin	10.56	11.50	7.41
SDM	13.11	19.33	25.05
Trimethoprim	29.47	14.98	11.00
Ketoprofen (-)	13.59	13.04	18.42
Diclofenac (-)	27.08	15.32	10.70
Diclofenac-13C (-)	10.26	10.51	17.51
AAP	13.94	7.71	13.55
CBZ	11.32	16.58	7.22
CBZ-epoxy	5.46	13.38	4.12
Atenolol	30.76	25.49	19.36
norFXT	5.77	15.62	9.35
Salicylic acid (-)	9.33	9.43	16.40
Caffeine	1.36	5.36	21.26

5.2.4 Publication #12

Occurrence and fate of contaminants of emerging concern and their transformation products after uptake by Pak choi (Brassica rapa subsp. chinensis)

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Submitted to Environmental Pollution

Occurrence and fate of contaminants of emerging concern and their transformation products after uptake by pak choi (*Brassica rapa* subsp. *chinensis*)

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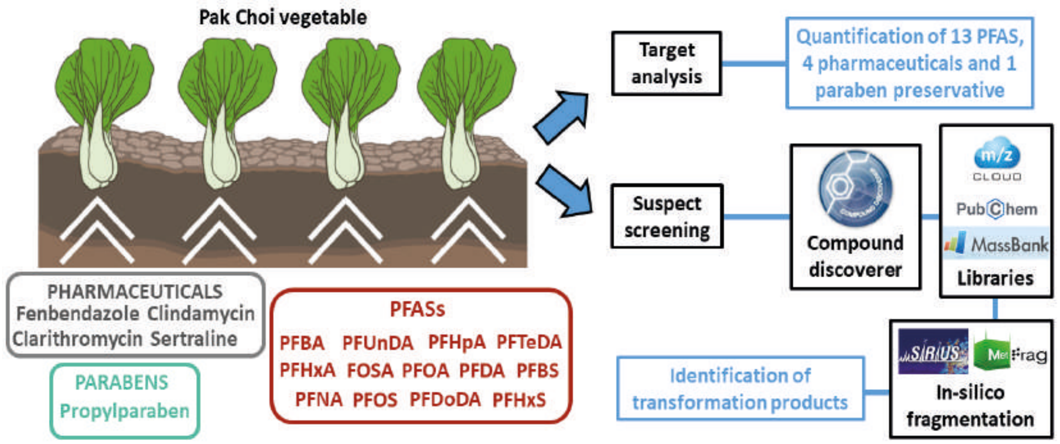
Graphical abstract:



Abstract

Recovery and reuse of nutrients is a major challenge in agriculture. A new process contributing to a circular economy is anaerobic digestion of food waste, which is a sustainable way of recycling nutrients as the digestate can be used as fertiliser in agriculture and horticulture. However, the digestate may be polluted with contaminants of emerging concern (CECs) that can be circulated back into the food chain, posing a risk to the environment and human health. In this work, nutrient solution was spiked with 18 selected CECs frequently detected in food waste biogas facilities, and subsequent uptake and fate of these CECs were evaluated in pak choi grown in two different nutrient solutions (mineral and organic). All spiked compounds except two (propylparaben, fenbendazole) were taken up by pak choi plants, with perfluorobutanoic acid (PFBA) and sertraline displaying the highest concentrations (270 and 190 µg/kg fresh weight, respectively). There were no statistically significant differences in uptake between mineral and organic nutrient solution. Uptake of per- and polyfluoroalkyl substances (PFAS) was negatively correlated with perfluorocarbon chain length and dependent on the functional group (r = -0.73). Sixteen transformation products (TPs) were tentatively identified using suspect screening, most of which were Phase II or even Phase III metabolites. Six of these TPs were identified for the first time in plant metabolism and their metabolic pathways were considered.

Key words: CECs, uptake, crops, transformation products, suspect screening, PFAS, pharmaceuticals, parabens

Highlights

- PFAS uptake depended on perfluorocarbon chain length and functional group
- Sixteen TPs were tentatively identified, most of which were Phase II or III TPs
- Six TPs were reported for the first time in plant metabolism
- Nutrient solution (mineral, organic) did not affect CEC uptake

1. Introduction

Huge quantities of food are lost or wasted in Europe (~15% of the total) and even greater quantities in Asia (~20%) and the United States (~35%) (United Nations Environment Programme, 2021). This is a pertinent issue as the global population increases, so new ways are needed to recover and reuse nutrients in a circular economy. Anaerobic digestion is an eco-friendly solution to closing the nutrient loop as it allows reuse of food waste (Balagurusamy and Chandel, 2020). Apart from biogas, large amounts of an organic residue (digestate) containing useful nutritional components are produced and can be used as crop fertiliser (Bergstrand et al., 2020). However, undesirable components can also be present in the digestate, such metals or contaminants of emerging concern (CECs) (Golovko et al., 2022a).

Thousands of chemicals can be categorised as CECs, but most are mainly unmonitored compounds with potential to reach environmental compartments and cause known or suspected adverse ecological or human health effects (Rosenfeld and Feng, 2011). These include pharmaceuticals, personal care products, per- and polyfluoroalkyl substances (PFAS), pesticides, industrial chemicals, flame retardants, hormones etc. Some CECs are persistent and can potentially enter the environment with wastewater treatment plant (WWTP) effluent or with anaerobically digested sewage sludge used as fertiliser. WWTP effluent makes a great contribution to CEC occurrence in surface water bodies, where CECs are frequently detected at concentrations between ng/L and µg/L (Archer et al., 2017). The side-effects of using wastewater for irrigation in agriculture have been evaluated (Mañas et al., 2009; Sunyer-Caldú et al., 2022), but research is urgently needed on the relevance of anaerobic digestate for CEC concentrations in crops and ultimately in the environment and humans.

Presence of CECs in edible crops may have many undesirable effects in humans, e.g. low doses of antibiotics (clarithromycin, clindamycin etc.) can result in development of antibiotic resistance in bacteria, which is an emerging concern for human health (Golovko et al., 2022b; Nazaret and Aminov, 2014). Other CECs, such as PFAS, can have adverse effects on the environment (Ahrens and Bundschuh, 2014) and immunotoxicity (NTP, 2016), reproductive or carcinogenic effects (ATSDR, 2020) in humans. Parabens have known endocrine-disrupting capacity and estrogenic activity, with increasing potency with perfluorocarbon chain length (Boberg et al., 2010).

During anaerobic digestion, plant uptake and translocation within plants, CECs may be partly or completely metabolised into new compounds, some unknown, with different physicochemical properties and in some cases more toxic effects than the parent compounds (Menger et al., 2021). Identification and monitoring of these transformation products (TPs) is critical in order to gain an overall picture of the contaminants present in crops fertilised with food waste digestate. Recent advances in high-resolution mass spectrometry (HRMS) and associated data treatment workflows have facilitated identification of TPs (Menger et al., 2020).

Pak choi (*Brassica rapa* subsp. *chinensis*, also known as Chinese cabbage) was selected as a model crop in this study because of three relevant characteristics: (i) it is a commonly consumed crop in Sweden; (ii) it is a fast-maturing crop that can be harvested in 30 days; and (iii) the whole plant is edible and can be consumed raw or cooked. Despite it being a widely consumed crop in Asia, America and Northern Europe, little information is available regarding CEC uptake in pak choi and whether this poses a potential risk to the environment and human health.

The aim of this study was to assess uptake and fate of CECs by pak choi, using well established target methodologies, and to identify TPs of any CECs present using HRMS-based suspect screening. Specific objectives were to: i) evaluate the influence of nutrient solution on CEC uptake in pak choi; ii) identify TPs formed following uptake; and iii) investigate the fate of CECs and TPs in the plants.

2. Materials and methods

2.1 Chemicals and reference standards

All analytical standards used for analysis were of high purity grade (>95%). Propylparaben (PrPB), fenbendazole (FBZ), clarithromycin (CLA), clindamycin (CLI), sertraline (SER), perfluorobutanoic acid (PFBA), perfluoroundecanoic acid (PFUnDA), perfluoroheptanoic acid (PFHpA), perfluorotetradecanoic acid (PFTeDA), perfluorohexanoic acid (PFHxA), perfluorododecanoic acid (PFDoDA), perfluorooctanoic acid (PFOA), perfluorodecanoic acid (PFDA), perfluorononanoic acid (PFNA), perfluorooctanesulfonic acid (PFOS), perfluorooctanesulfonamide (FOSA), perfluorohexanesulfonic acid (PFHxS) and perfluorobutanesulfonic acid (PFBS) were purchased from Sigma-Aldrich (USA). Isotopically labelled standards (IS) (D_5-oxazepam-, D_5-diazepam, D_7-propylparaben, $^{13}C_2$-PFHxA, $^{13}C_2$-PFDA, $^{13}C_2$-PFDoDA, $^{13}C_4$-PFOA, PFHxS-18O2, $^{13}C_5$-PFNA, $^{13}C_4$-PFOS, $^{13}C_2$-PFUnDA, $^{13}C_2$-PFBA, $^{13}C_8$-FOSA) were obtained from Wellington Laboratories (Canada), Teknolab AB (Kungsbacka, Sweden), Sigma-Aldrich (St. Louis, Missouri, USA) and Toronto Research Chemicals (Toronto, Canada).

Ultrapure water was produced by a Milli-Q Advantage Ultrapure water purification system and filtered through a 0.22 µm Millipak Express membrane and LC-Pak polishing unit (Merk Millipore, Billerica, MA). Methanol, acetonitrile, ammonium acetate, formic acid, ammonia and ethyl acetate, all of high analytical grade, were obtained from Sigma-Aldrich.

2.2 Plant material

Seeds of pak choi (var. 'Joi Choi', Impecta Förhandel, Julita, Sweden) were sown in rock wool plugs (ø 20 mm, Grodan, Roermond, the Netherlands) in a greenhouse chamber (temperature set-points: heating at 19°C during night, 22°C during day). Artificial light (high-pressure sodium lamps) was used for 16 h/day. Shading screens closed when outside radiation exceeded 700 W m^{-2}. The seedlings were fed with a complete mineral nutrient solution consisting of YaraTera Kristalon Purple + Calcinit (Yara, Oslo, Norway) at electric conductivity (EC) of 1.2 mS/cm. Fourteen days after sowing (DAS), the plants were transferred to experimental vessels as described below.

2.3 Hydroponic cultivation system and nutrition solution

The experiment was performed in a 50 m^2 greenhouse chamber in Alnarp, Sweden, during March-April 2021. The greenhouse climate set-points were as described above. The plants were cultivated under hydroponic conditions in plastic vessels containing 1.5 L of constantly aerated nutrient solution. Two different nutrient solutions were used, an organic solution produced from liquid biogas digestate (for details, see Bergstrand et al., 2020) and mineral solution containing mineral salts with similar nutrient content to the digestate solution (**Table S1** in Supplementary Information (SI)).

2.4 Experimental set-up

Plants growing on each nutrition solution were subjected to three treatments with increasing exposure (nominal concentration of 1, 10 and 100 µg/L) to 18 CECs (four pharmaceuticals, 13 PFAS, one paraben preservative) selected based on frequency of detection in food waste facilities in Sweden (Golovko et al., 2022a). A control treatment without addition of CECs was also included (experimental blank). Nutrient solution was exchanged once a week and sampling (50 mL) was performed to determine initial and final concentration of CECs in each experimental vessel (**Table S2** in SI). The plant material was harvested at 42 DAS and fresh weight (fw) of shoots was recorded. The roots were washed three times in distilled water and left to drain for 2 min before fresh weight determination. The full plants were lyophilised before analysis, in order to minimise degradation during storage.

2.5 Analysis

2.5.1 Sample preparation for micropollutant (MP) analysis

Samples of pak choi shoots were extracted using a validated in-house method described elsewhere (Kodešová et al., 2019a, 2019b). In brief, 0.2 g dry weight (dw) of shoot sample was weighed into a 7-mL tissue homogenisation tube (Precellys, Bertin Technologies, France), followed by addition of IS mixture (20 ng absolute per compound) and 2 mL of extraction solvent mixture (acetonitrile and 2-propanol (3:1, v/v) + 0.1% of formic acid). After 45 min, the samples were homogenised for two 40-s rounds at 5000 rpm, with a 20-s break in between. The tubes were then centrifuged at 3900 rpm for 15 min at 20 °C. The supernatant was collected and filtered through 0.2 µm regenerated cellulose filters into a 2 mL Eppendorf tube, which was stored at -20 °C for 24 hours. Around 30 min before analysis, the supernatant samples were removed from the freezer and kept at room temperature, after which they were centrifuged at 10,000 rpm for 3 min at 4 °C. A 200 µL aliquot was used for analysis. Further information about the blanks and quality controls is included in **section S**1 in SI.

2.5.2 Instrumental analysis of CECs

Target analysis

Extracts were analysed for the presence of the 18 CECs using a DIONEX UltiMate 3000 ultra-high-performance liquid chromatography (UHPLC) system (Thermo Scientific, Waltham, MA, USA) coupled to a triple quadrupole (TSQ) mass spectrometer (TSQ QUANTIVA, Thermo Scientific, Waltham, MA, USA). An Acquity UPLC BEH-C18 column (2.1 x 50 mm, 1.7 µm particle size; Waters Corporation, Manchester, UK) was used as an analytical column for chromatographic separation. The temperature of the column oven was set at 40 ± 2°C. The system was equipped with a heated electrospray ion source (ESI) with static spray voltage set at 3500 V positive mode and 2500 V negative mode. The temperature of the ion transfer tube and the vaporiser was set at 325°C and 400°C, respectively. Data acquisition was performed under multiple reaction monitoring (MRM) using Xcalibur software (Thermo Fisher Scientific, San Jose, CA, USA) and the data were evaluated using TraceFinder^{TM} 4.1. software (Thermo Fisher Scientific).

The mobile phase consisted of Milli-Q water with addition of 5 mM ammonium acetate (phase A) and acetonitrile (phase B). The same linear gradient was used in both ionisation modes, with a flow rate of 0.5 mL/min. The gradient started at 2% of phase B and increased to 99% from 0.5 min to 10.0 min. This composition of the mobile phase was maintained for 3 min, until 13.0 min, after which it returned to initial conditions at 13.1 min and remained there until the end of the analytical run, which took 15 min.

Suspect screening

The highest nominal concentration extracts (100 µg/L), controls (0 µg/L) and blanks were analysed for identification of TPs using a Vanquish Binary Pump H with TriPlus autosampler UHPLC system coupled to a Q-Exactive Focus Orbitrap mass spectrometer. An Acquity UPLC BEH-C18 (2.1 x 50 mm, 1.7 µm particle size) was used as an analytical column for chromatographic separation. The temperature of the column oven was set at 40 ± 2°C. The mobile phases for the positive ionisation (PI) mode (ESI$^+$) consisted of Milli-Q water (phase A) and MeOH (phase B) with the addition of 0.1% of formic acid in both phases. In negative ionisation (NI) mode (ESI$^-$), the same mobile phases were used, but with 5 mM ammonium acetate instead of formic acid as modifier. The same linear gradient was used in both ionisation modes, with a flow rate of 0.3 mL/min. The gradient started at 5% of phase B and increased to 95% from 1 min to 10.0 min. This composition of the mobile phase was maintained for 3 min, until 13.0 min, after which it returned to initial conditions at 13.1 min and remained there until the end of the analytical run, which took 15 min. The acquisition modes used were data-dependent (DDA), selecting the five most intense ions, and data-independent (DIA) at 35 eV of collision energy (CE), with a 53-800 m/z range with 70000/70000 and 70000/35000 resolving power, respectively.

Suspect screening workflow

Data processing was performed using Compound Discoverer software (v 3.3.0.550) (for parameters and workflow used, see **section S2** and **Figure S1** in SI). From the features obtained, those present in the blanks were removed and the remaining features were verified or discarded manually in DDA data with Thermo Xcalibur software v. 3.1.66.7.

2.5.3 Quality assurance and quality control in CEC analysis

Method performance in terms of linearity in calibration, blanks, limit of quantification (LOQ), absolute recovery, precision and matrix effect was assessed. Overall, LOQ ranged between 0.2 and 25 ng/g fw and all recovery rates were above 100%. Specific data on each parameter are provided in **section S1** in SI and recovery rates, LOQs and matrix effect values in **Table S3** in SI.

2.5.4 Plant characterisation

Plant photosynthetic performance was measured by i) chlorophyll fluorescence and ii) gas exchange (leaf photosynthesis). Chlorophyll fluorescence parameters (basic fluorescence F_0, maximum fluorescence F_m and variable fluorescence F_v/F_m) were determined at 30 DAS using a Walz PAM-2500 instrument (Heinz Walz GmbH, Effeltrich, Germany). Leaf photosynthesis was measured at 30 DAS using a leaf-chamber photosynthesis instrument (LCPro, ADC Bioscientific, Hoddesdon, UK). Maximum photosynthesis was measured at a light intensity of 1000 µmol/s·m^2 and ambient CO_2 concentration. Leaf chlorophyll concentration was measured at the end of the experiment using a chlorophyll meter (Apogee MC-100, Apogee Instruments, Logan, UT USA). Fresh and dry weight (after lyophilisation) of shoots and roots were determined. Content of vitamin C, glucosinolates and carotenoids in leafy plant parts was analysed using a high-performance liquid chromatograph coupled to a UV-vis detector (Esteve et al., 1997; Humphries and Khachik, 2003; Maldini et al., 2012; Panfili et al., 2004).

2.6 Uptake factor

Measured concentrations of the CECs in shoots and in nutrient solution were used to calculate the uptake factor (UF) for each compound **(Equation 1)**. For the solution concentrations, an average value for weeks 1-3 (pak choi cultivation period) was calculated. The average plant CEC content for the three different spiking (exposure) levels tested was used to calculate UFs (**Table S4** in SI).

$$UF = \frac{C_{PLANT}}{C_{SOLUTION}} \qquad \text{(Equation 1)}$$

where UF is uptake factor, C_{PLANT} is CEC concentration in shoots and $C_{SOLUTION}$ is CEC concentration in the nutrient solution.

2.7 Statistical analyses

The experiment was performed with three replicates and mean values and standard deviation are reported. Statistical analyses were carried out using Minitab (v.19) and data were tested for individual correlations with Pearson's tests and for significant differences ($p<0.05$) using ANOVA and Fisher's LSD method (Minitab). Values below LOQ were considered as LOQ/2 in all statistical analyses.

3. Results

3.1 Plant parameters

The different parameters related to plant development (plant photosynthetic performance, leaf photosynthesis, leaf chlorophyll concentration, content of vitamin C, glucosinolates and carotenoids, and fresh and dry weight) were measured in order to obtain more information about processes that can occur during plant uptake of CECs. The results are summarised in **Table S5** in SI. Overall, no statistically significant correlations (Pearson correlation coefficient (r) <0.8, confidence interval (p) >0.05) were observed between detected levels of the target CECs and any plant parameter measured. However, for both nutrition solutions (mineral, digestate), a similar pattern of decreasing fresh weight of shoots when exposed to CECs was observed and plants exposed to the highest concentration of CECs (100 µg/L) had significantly lower shoot fresh weight compared with the controls. This suggests that CEC uptake can affect certain processes and mechanisms in pak choi development, indicating a need for monitoring.

3.2 Uptake of CECs by pak choi shoots

Total CEC content in shoots of plants exposed to 1 and 10 µg/L of CECs ranged from 14 to 39 µg/kg fw in both nutrient solutions and no significant differences were observed between the solutions (p>0.05) (**Table S6** in SI). In plants exposed to the highest concentration of CECs (nominal concentration 100 µg/L) the total content in shoots was considerably higher (680±41 and 620±130 µg/kg fw for the mineral and digestate nutrient solution, respectively).

The concentrations observed for each treatment and compound are shown in **Figure 1**. Among the pharmaceuticals, very different behaviours were observed. For example, FBZ was not detected at any exposure level, whereas shoot CLA concentration increased with spiking level. CLI was only detected at the highest spiking level in the digestate substrate, suggesting that substrate characteristics were important for CLI uptake. SER showed the second highest shoot concentrations among the target compounds, while PrPB was not detected in any treatment. All PFAS showed a similar pattern at the 1 and 10 µg/L spiking levels in that they were mostly detected in shoots of plants grown in the digestate solution, but were not detected in the mineral solution. At 100 µg/L, all PFAS were found at notable levels in shoots of plants grown in both solutions.

Overall, the CEC concentrations differed significantly between shoots exposed to 100 µg/L and the other exposure levels (1 and 10 µg/L), suggesting that higher concentrations in the nutrient solution resulted in significantly different uptake by the crop (p<0.05). However, no significant differences were observed with regard to use of mineral or digestate nutrient solution (p>0.05). Thus, type of nutrient solution used appears to be less important for CEC uptake in plants exposed to high concentrations of CECs. The compound present in the highest concentration in shoots was PFBA (272 µg/kg fw), followed by SER (190 µg/kg fw) and PFHxA (70 µg/kg fw). The compound families tested (PFAS, pharmaceuticals, paraben) showed similar average levels of bioaccumulation.

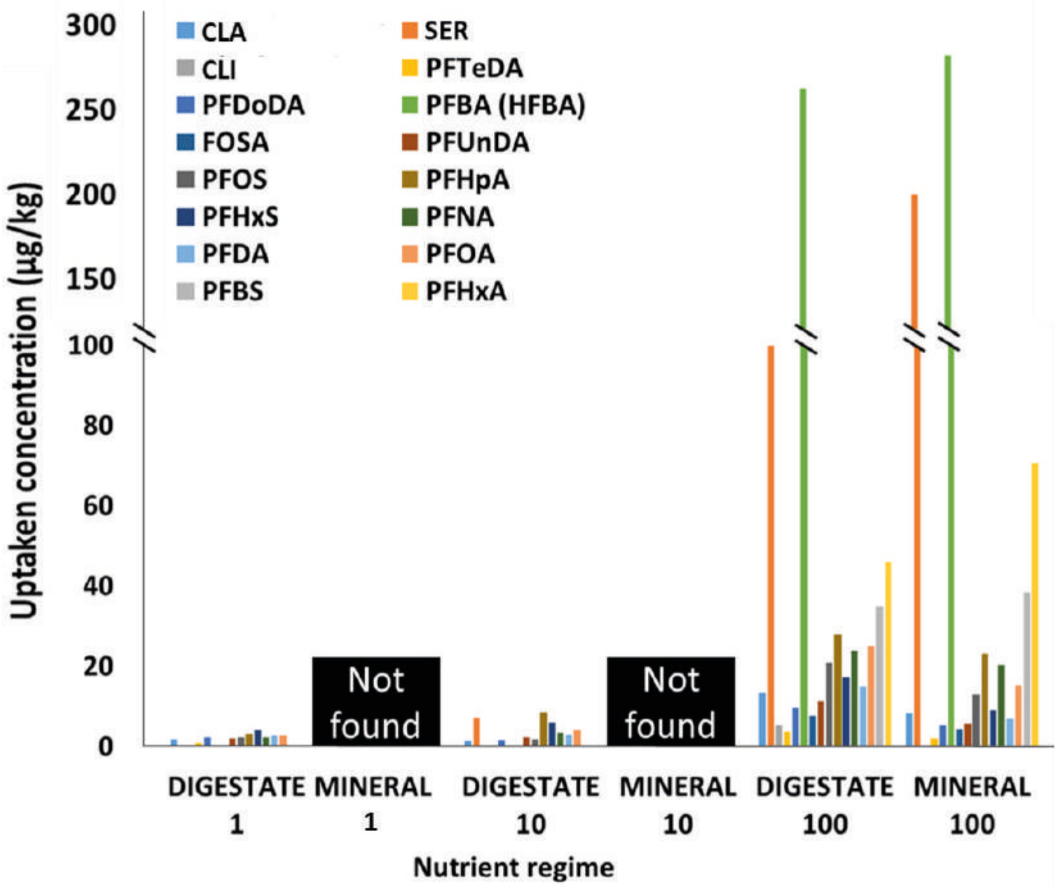


Figure 1. Concentration (µg/kg fresh weight (fw)) of different target compounds detected in pak choi shoots grown on nutrient solution (digestate or mineral) spiked with 1, 10 or 100 µg/L of contaminants of emerging concern (CECs).

Uptake factor

At high CEC concentrations in the nutrient solution, UFs did not differ significantly between shoots of pak choi plants grown in the mineral and digestate solutions (p>0.05), confirming that type of nutrient solution did not influence the concentrations of CECs n shoots. The compound that showed the highest tendency to accumulate in shoots was SER, in plants grown both in the mineral and digestate solution (UF= 33 and 2.4 L/kg, respectively). The next highest UF with both solutions was obtained for PFBA, the PFAS with the shortest perfluorocarbon chain (n=3) in this study, with UF of 1.8 (mineral solution) and 1.6 (digestate). All other compounds had UF <1, indicating low uptake by pak choi.

In a previous study on sewage sludge by Kodešová et al., (2019a), SER was the compound with the highest UF, with similar values (1-37.9) for spinach (*Spinacia oleracea*) as found here for pak choi. A high tendency for the short-chain PFBA to bioaccumulate from soil/sludge has been reported previously (Blaine et al., 2013; Krippner et al., 2015; Lesmeister et al., 2021).

3.3 Identification of transformation products

In data processing for identification of TPs using Compound Discoverer, 340 features in PI and 224 in NI were obtained. The number of features of interest was reduced by (i) filtering out features present in the blanks; (ii) discarding features without MS/MS data; (iii) checking plausible retention time and fragments; and (iv) manual inspection. Only 16 features remained as tentatively identified compounds (14 in PI and two in NI). All TPs were found in plants grown on both nutrient solutions (digestate, mineral). Only four reference standards (oxfenbendazole (oxFBZ), FBZ sulfone, CLI sulfoxide, CLA N-oxide) are commercially available for these 16 compounds. This is consistent with previous findings that the greatest limitation in identification of TPs is lack of reference standards (Menger et al., 2021). The 16 tentatively identified TPs, along with their retention time, parent compound, level of confidence (Schymanski et al., 2014) and evidence supporting their identification, are summarised in **Table 1**.

Most of the 16 TPs were generated from pharmaceuticals (six TPs of SER, five TPs of FBZ, two TPs of CLI, one TP of CLA), while one PFAS generated two TPs. No TPs from the paraben preservative were identified. Glucoside conjugation and hydroxylation were the most common transformation pathways, but other transformations such as acetylation, (des)methylation and acid lactic conjugation were also observed. Most of the TPs identified corresponded to one-step transformation (Phase I metabolites, e.g. CLA), but some TPs were formed through two or even three transformation steps, mostly from FBZ or SER as parent compounds. **Figure 2** shows the metabolic pathways from each parent compound to all TPs identified.

The different TPs identified and their occurrence were considered based on the parent origin (**Table 1**) and on findings in previous works.

SER. Desmethylsertraline (norsertraline, norSER)) and N-hydroxysertraline (N-hydroxySER) are the most commonly reported TPs of SER and have been detected in activated sludge and the helminth *Haemonchus contortus* (Gornik et al., 2020; Zajíčková et al., 2021). These TPs were both tentatively identified in pak choi in the present study. Additional TPs potentially identified in pak choi included SER ketone (exact mass and retention time (R_T)), acetyl SER (characteristic fragments and R_T) and acetyl hydroxysertraline (acetyl hydroxySER) (characteristic fragments and R_T). These three TPs have been reported previously in residues after biological degradation and human metabolism (Gornik et al., 2020; Zajíčková et al., 2021). An additional two SER TPs, SER oxime and N-methyl SER, were detected for the first time in this study, probably because SER oxime and SER are very similar in terms of mass (m/z 306.0447 and m/z 306.0811, respectively) and can only be differentiated with a very sensitive HRMS instrument. The same applies for N-methyl SER and dihydroxy SER (m/z 320.0967 and m/z 320.0603, respectively).

Table 1. Compounds tentatively identified and/or confirmed and their parent compound, level of confidence and supporting evidence

TP: Transformation product; RT: Retention time; PI: Positive ionization; NI: Negative ionization

TP	RT (min)	Parent compound	Level of confidence	Evidences
Norsertraline (norSER)	8.46	Sertraline	2a	Characteristic fragments m/z: 91.0542 $[C_7 H_7]$; 129.0694 $[C_{10} H_9]$; 158.9758 $[C_7 H_5 Cl_2]$; Plausible RT in PI and in accordance with sertraline (8.35) 3 Similarity with mzCloud [Creference3373#T5606#c#650869]
Acetyl sertraline (Acetyl SER)	9.78	Sertraline	3	Characteristic fragments m/z: 91.0542 $[C_7 H_7]$; 129.0694 $[C_{10} H_9]$; 158.9758 [C7 H5 Cl2]; 275.0389 [C16 H13 Cl2] Plausible RT in PI and in accordance with sertraline (8.35) and isotopic profile match
4-hydroxy sertraline (4-hydroxy SER) (two isomers)	7.52/7.96	Sertraline	2b (both isomers)	Characteristic fragments m/z: 273.0223 $[C_{16} H_{11} Cl_2]$ (https://doi.org/10.1002/elps.201700482); 238.0536 [C16 H11 Cl] Plausible RT in PI and in accordance with fenbendazole (8.35)
SER ketone	9.84	Sertraline	4	Plausible RT in PI according to the QSRR model and in accordance with sertraline (8.35)
SER oxime	9.91	Sertraline	4	Plausible RT in PI according to the QSRR model and in accordance with sertraline (8.35)
Acetyl hydroxysertraline (Acetyl hydroxySER)	9.02	Sertraline	3	Characteristic fragments m/z: 273.0225 $[C_{16} H_{11} Cl_2]$; 238.0536 $[C_{16} H_{11} Cl]$ Plausible RT in PI and in accordance with sertraline (8.35) and acetyl sertraline (9.78)
N-methylsertraline (N-methylSER)	8.22	Sertraline	3	Presence of characteristic fragments m/z: 129.0694 $[C_{10} H_9]$; 158.9758 [C7 H5 Cl2]; 275.0389 [C16 H13 Cl2] Plausible RT in PI and in accordance with sertraline (8.35)
Oxfendazole (OxFBZ)	7.08	Fenbendazole	1	Characteristic fragments m/z: 159.0422 $[C_8 H_5 N_3]$; 191.0320 $[C_8 H_5 N_3 O_3]$; 223.05811 $[C_{15} H_{11} S]$; 267.0452 $[C_{14} H_9 N_3 O S]$; 284.0478 $[C_{14} H_{10} N_3 O_2 S]$ Similarity with mzCloud [Creference1614#T2472#c#276359] Confirmed with reference standard
Fenbendazole sulfone (FBZ sulfone)	7.25	Fenbendazole	1	Characteristic fragments m/z: 159.0421 $[C_8 H_5 N_3]$; 191.0319 [C8 H5 N3 O3]; 300.0428 [C14 H10 N3 O3 S] Similarity with mzCloud [Creference4902#T8036#c#1386225] Confirmed with reference standard

Table 1. (continued)

Fenbendazole glucoside (FBZ glucoside)	7.8	Fenbendazole	2a	Characteristic fragments m/z: 159.0421 [$C_8 H_5 N_3$]; 191.0319 6 [C8 H5 N3 O3]; 300.0428 [C14 H10 N3 O3 S] Plausible RT in PI and in accordance with fenbendazole (8.57). Neutral loss of 162 (glucoside) Similarity with bibliographic spectra (Figure 3, E, from https://doi.org/10.1016/j.ijpddr.2019.09.0
Oxfenbendazole+hexose (OxFBZ hexose)	6.25	Fenbendazole	2a	Characteristic fragments m/z of oxfenbendazole: 159.0422 [$C_8 H_5 N_3$]; 191.0320 [$C_8 H_5 N_3 O_3$]; 223.05811 [C15 H11 S]; 267.0452 [C14 H9 N3 O S]; 284.0478 [C14 H10 N3 O2 S]; 299.0713 [$C_{15} H_{13} N_3 O_2$ S]; 316.0140 [$C_{15} H_{14} N_3 O_3$ S] 7 Plausible RT in PI and in accordance with oxfenbendazole (7.08) 8 Similarity with mzCloud [Creference1614#T2472#c#276359]
Fenbendazole sulfone + hexose (FBZ sulfone hexose)	6.38	Fenbendazole	2a	Characteristic fragments m/z of fenbendazole: 159.0421 [$C_8 H_5 N_3$]; 191.0319 [$C_8 H_5 N_3 O_3$]; 300.0428 [$C_{14} H_{10} N_3 O_3$ S]; 332.0700 [$C_{15} H_{14} N_3 O_4$ S] 9 Plausible RT in PI and in accordance with oxfenbendazole (7.25) Similarity with mzCloud [Creference4902#T8036#c#1386225]
Clindamycin glucoside (CLI glucoside)	7.08	Clindamycin	3	Plausible RT in PI and in accordance with clindamycin (7.25) Match of 0.999 score with Metfrag, being the only TP that contains clindamycin original structur
Clindamycin sulfoxide (CLI sulfoxide)	6.42	Clindamycin	1	Characteristic fragments m/z: 126.1273 [$C_8 H_{11}$ N]; 377.1826 [$C_{18} H_{32}$ Cl N O_3 S]; Similarity with MassBank of North America [CCMSLIB00005716876] Confirmed with reference standard
Clarithromycin N-oxide (CLA N-oxide)	8.52	Clarithromycin	1	Characteristic fragments m/z: 83.0494 [$C_5 H_7$ O]; 113.0596 [$C_6 H_9 O_2$]; 123.0802 [$C_8 H_{11}$ O]; 137.0958 [$C_9 H_{13}$ O]. Similarity with mzCloud [Creference3138#T5031#c#600039] Confirmed with reference standard
FOSA hexose	9.2	FOSA	3	Characteristic fragments m/z of PFOSA: 77.9655 [NO_2S]; 118. 9925 [C_2F_5]; 168.9894 [C_3F_7]; 218.9863 [C_4F_9]; 268.9829 [C_5F_{11}]; 497.94568 [C_8 H N O_2 S F_{17}] Neutral losses of 120.0421 [sulfo-hexoside] and 162.0546 [hexose] Plausible RT in NI and in accordance with PFOSA (9.63)
Lactic acid conjugation of FOSA (FOSA lactate)	9.55	FOSA	3	Characteristic fragments m/z of FOSA: 77.9655 [NO_2S]; 497.94568 [C_8 H N O_2 S F_{17}] Plausible RT in NI and in accordance with FOSA (9.63) Similarity with mzCloud [Creference3204#T5238#c#618600]

(Reichl et al., 2018; Stasiuk et al., 2019)

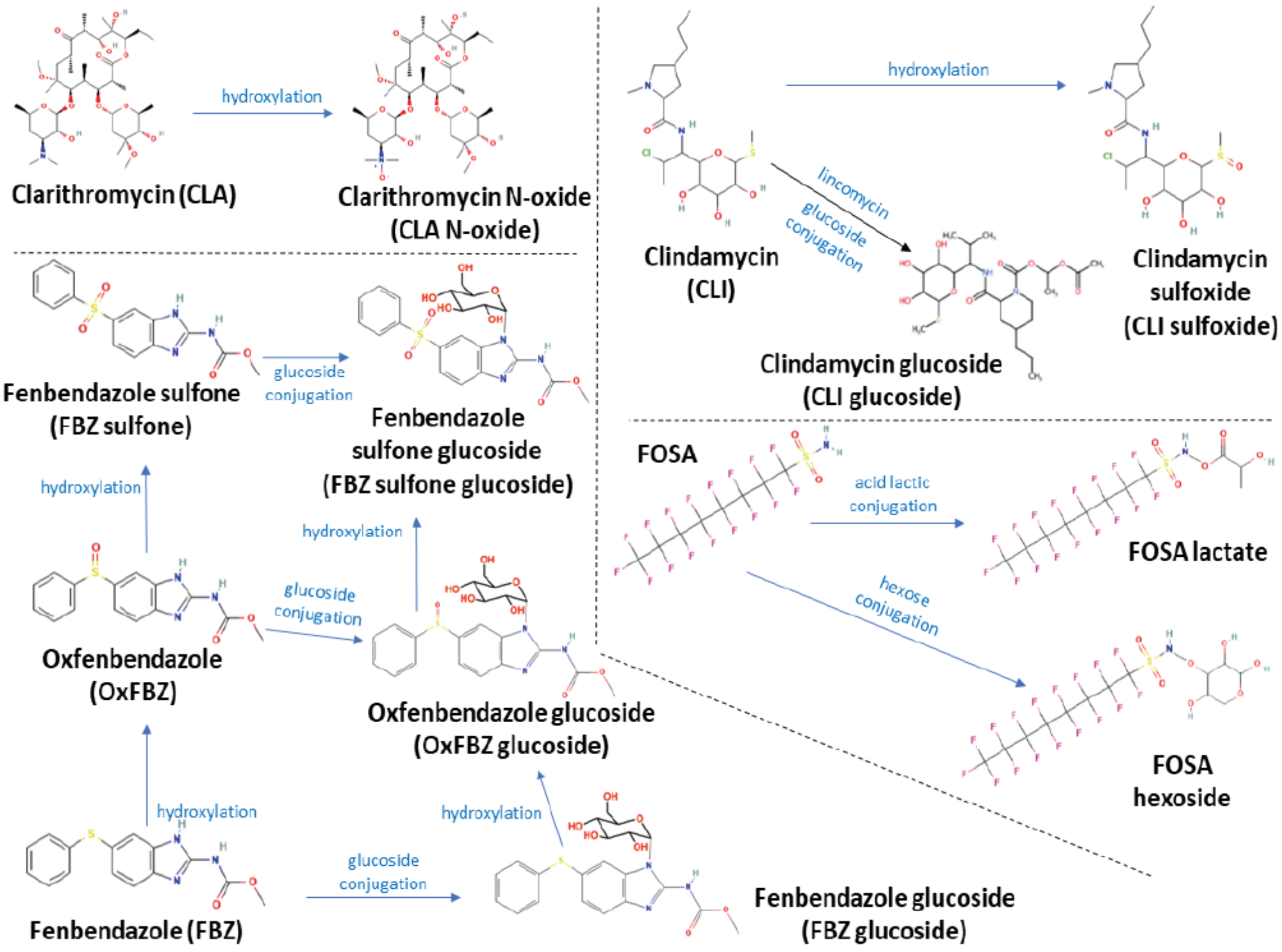


Figure 2. Proposed metabolic pathways of the transformation products (TPs) tentatively identified in pak choi.

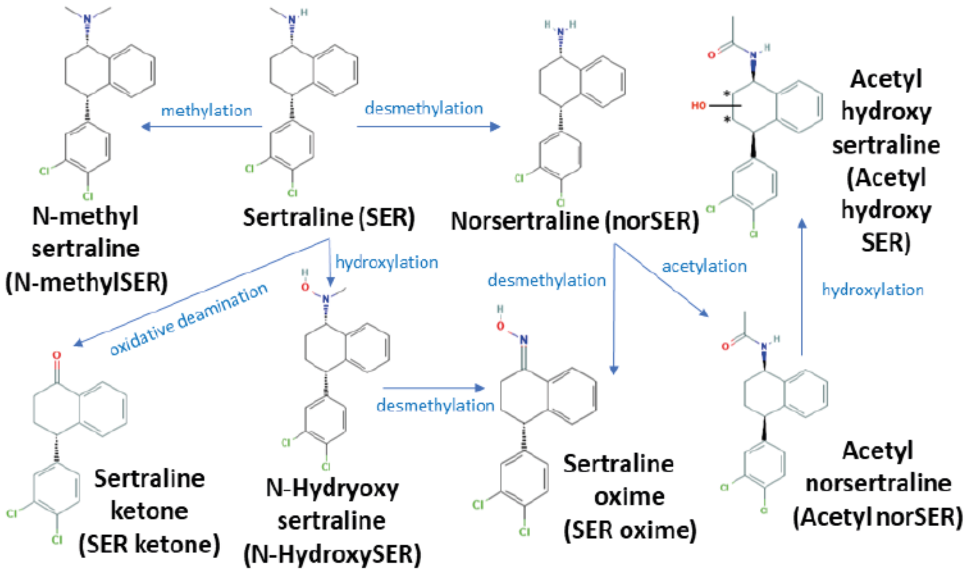


Figure 2. continued.

FBZ. FBZ sulfoxide (oxFBZ) and FBZ sulfone are the TPs from FBZ reported most commonly in the literature (Stuchlíková et al., 2016) and were confirmed with respective reference standards in the present study. FBZ glucoside spectra displayed the common neutral losses (162 and 192) for glucosidation, and the exact same spectra have been reported recently in other plants (Stasiuk et al., 2019). These are the outcome of simple Phase I (hydroxylation) and Phase II transformations (double hydroxylation and glucosidation). All TPs from FBZ have been reported previously, without structure, in studies in vivo in harebell (*Campanula rotundifolia*) (Stuchlíková et al., 2016, 2018), but to our knowledge the present study is the first to detect oxFBZ glucoside and FBZ sulfone glucoside in plants after degradation of FBZ. Both these TPs are formed through a combination of hydroxylation and glucosidation (**Figure 2**). There is strong evidence to support these identifications, e.g. the spectral profile is practically identical to that of oxFBZ and FBZ sulfone, it has the two characteristic neutral losses (162 and 192) for glucoside addition and the retention times are plausible (**Figure 3**). These two TPs are also reported here for the first time in plant metabolism.

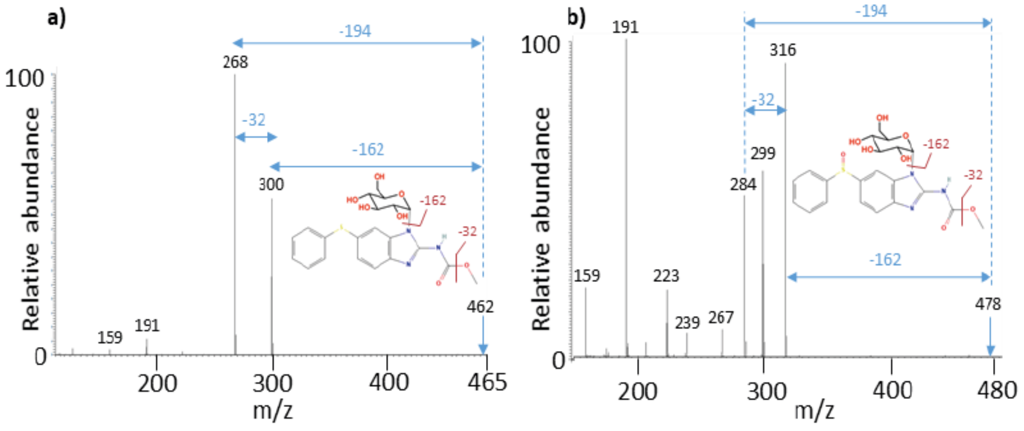


Figure 3. Experimental mass spectra of (a) tentatively identified fenbendazole (FBZ) glucoside and (b) oxfenbendazole (oxFBZ) glucoside, with their neutral losses.

CLI and CLA. The most commonly reported TPs for both CLI and CLA are their respective hydroxylated form (Calza et al., 2012; Ooi et al., 2017), and both were confirmed here with their respective reference standard. CLI glucoside was tentatively identified at level 3, because it was not clear which hexose structure was added in which position. However, the structure (see **Figure 2)** showed a high match with Metfrag (0.999, representing CLI complete structure) as it is a lincomycin derivative and CLI differs from lincomycin only in addition of chlorine on the parent molecule.

FOSA. This PFAS is a major intermediate usually transformed to PFOS but two TPs were tentatively identified, the only TPs from PFAS among the 13 investigated. The PFAS, in particular perfluoroalkyl acids (PFAA), are not expected to degrade during uptake or within plants, due to their high persistence (Ahrens, 2011). However, one TP was observed corresponding to conjugation with acid lactic, probably attached to the nitrogen of FOSA (**Figure 2**), the most nucleophilic part. The reported confidence level was kept to 3 since no spectral evidence could be obtained, as only common PFAS fragments (m/z 77.9655 and m/z 497.94568) were present. More spectral information was available for the other TP, which had many fragments in common with FOSA (m/z 77.9655, 118. 9925, 168.9894, 218.9863, 268.9829 and 497.94568 (FOSA)). Characteristic neutral losses of sulfo-hexoside (m/z of 120.0421) and hexose (m/z of 162.0546) were also detected (**Figure 2**). FOSA has been reported previously in Swedish plants (leaves of birch trees) due to soil contamination

(Gobelius et al., 2017), but the FOSA TPs were detected as plant metabolites for the first time in the present study.

PrPB. The most commonly detected TPs from the paraben family are their hydroxylated derivatives (Penrose and Cobb, 2022). However, no hydroxylation or any other TPs from PrPB were found in this study. Moreover, PrPB itself was not detected in pak choi plants, suggesting extremely low or no uptake, although further rapid degradation of potential TPs could not be ruled out.

4. Discussion

4.1 Occurrence and fate of target CECs

Overall, pak choi plants cultivated on the two different nutrient solutions did not differ significantly in uptake of CECs, although some differences were observed at low spiking levels (1 and 10 µg/L). At these spiking levels, more of the target compounds were detected when pak choi was cultivated on digestate solution compared with mineral solution, possibly because the higher organic matter content in the digestate solution favoured pre-concentration of CECs, affecting uptake by plant roots. This trend was not observed at the highest spiking concentration (100 µg/L), probably because the processes in the nutrient solution and in the plant that govern uptake of the compounds were unable to cope with the high CEC concentrations in the solution. Considering that all TPs tentatively identified were detected in pak choi cultivated in both solutions, type of nutrient solution provided does not seem to affect the degradation processes that most probably take place inside the crop.

PrPB. PrPB was the only target compound that was not detected, unaltered or as TPs, in pak choi, indicating very different uptake capacity or high degradability. In previous studies, PrPB has been found in sewage sludge (Golovko et al., 2022a; Haman et al., 2014) and in lettuce (*Lactuca sativa*) (Sunyer-Caldú and Diaz-Cruz, 2021), oyster mushrooms (*Pleurotus ostreatus*) (Golovko et al., 2022b) and radish (*Raphanus sativus*) (Abril et al., 2021), although at very low levels. Other studies investigating paraben uptake have not found this compound (Margenat et al., 2019; Sabourin et al., 2012). These results are in agreement with its octanol-water partition constant (logk_{ow} of 3.04), which indicates moderate lipophilic behaviour. However, logk_{ow} values alone have limited accuracy as bioconcentration predictors (Dowdy and Mckone, 1997), and it is preferable to use a combination of logk_{ow} and other properties such as pKa, pH, ionic strength, biodegradation or sorption capacity (Jurado et al., 2014).

FBZ. This antiparasitic drug was not detected in pak choi, but up to five TPs from FBZ were tentatively identified. FBZ has been detected previously in sewage sludge (Golovko et al., 2022a) and at low levels in ribwort plantain (*Plantago lanceolata*) (Stuchlíková et al., 2018) and harebell (Stuchlíková et al., 2016). FBZ has high logk_{ow} (3.93) and can be expected to be taken up by pak choi, so its low occurrence in shoots can probably be explained by its tendency for biotransformation into new TPs. Little information is available about the toxicity of most FBZ TPs.

SER. This antidepressant was the pharmaceutical that accumulated most in pak choi and seven TPs were tentatively identified. Potential of SER to bioaccumulate in the environment has been reported previously (Boström et al., 2017; Grabicova et al., 2017). It has been detected in sewage sludge (Golovko et al., 2022a) and in garden cress (*Lepidium sativum*) (Reichl et al., 2018) and spinach (Kodešová et al., 2019a). In both cases, SER was the compound that accumulated most, reaching 190 µg/kg fw in spinach leaves exposed to 10 mg/L in water and 550 µg/kg fw in spinach roots exposed to 285-305 ng/g in soil in the study by Kodešová et al., (2019a). Similar levels were found in pak choi shoots in the present study. Considering the high log k_{ow} of SER (5.29), high accumulation in plants can be expected. However, only a few studies have examined uptake of this common pharmaceutical in crops, and even fewer have investigated formation of TPs from SER during plant metabolism.

CLA. CLA is an antibiotic prescribed to treat bacterial infections, such as pneumonia or bronchitis. It was found to be present at low levels in pak choi shoots (~10 µg/kg fw) and only its hydroxylate derivative was confirmed as a TP. CLA has been detected previously in lettuce (*Lactuca sativa*) (Tian et al., 2019) and oyster mushrooms (Golovko et al., 2022b). Tian et al. (2019) found extremely high concentrations of CLA, probably because they spiked it at 1 mg/L in the irrigation water, while Golovko et al. (2022b) found average levels similar to those reported here (~5 µg/kg fw). These results are in agreement with the logk_{ow} value of CLA (3.16), which is lower than that of SER and FEN, indicating moderate hydrophilic behaviour. Considering that only one TP was found, it appears that CLA is not readily taken up by crops and also that it does not generate many TPs. An alternative explanation is rapid elimination of any TPs back to the substrate, depending on their physicochemical properties.

CLI. This antibiotic was taken up at a low level (5.2 µg/kg fw) in pak choi plants grown on organic nutrient solution, but two TPs from CLI were detected in both the digestate and mineral solution. Previous works have detected CLI in spinach, lettuce and carrot (*Daucus carota*) (Jones-Lepp et al., 2010), but we found no further information on CLI detection in crops. In the study by Jones-Lepp et al. (2010), the level was below the limit of detection in lettuce and spinach and was 53 µg/kg fw in carrots, indicating a low tendency for translocation from soil to crop. The logk$_{ow}$ of CLI was the lowest of all pharmaceuticals investigated here (2.16). Therefore, CLI showed very similar behaviour to CLA, i.e. it was present in low levels and had fewer than three TPs.

PFAS. PFAS uptake was negatively correlated with perfluorocarbon chain length (r = -0.73, $p>0.05$; test performed only with carboxylic acids), as observed previously (Ghisi et al., 2019; Golovko et al., 2022b; Zhang et al., 2021). This was not unexpected, as perfluorocarbon chain length in PFAS is usually correlated with hydrophobic characteristics (Rostvall et al., 2018). Different uptake levels for PFAS with different functional groups were also observed, with sulphonamide and sulfonic acid PFAS showing lower uptake than carboxylic acid PFAS (Joerss, 2020). Both trends were clearly apparent when the PFAS were arranged in order of perfluorocarbon chain length (**Table S7** in SI). Many studies have reported PFAS uptake in crops such as carrots (Bizkarguenaga et al., 2016; Blaine et al., 2013; Lechner and Knapp, 2011), cucumber (*Cucumis sativus*) (Lechner and Knapp, 2011), potatoes (*Solanum tuberosum*) (Lechner and Knapp, 2011), radish (Blaine et al., 2013), spinach (Navarro et al., 2017), tomato (*Solanum lycopersicum*) (Blaine et al., 2013; Navarro et al., 2017) and oyster mushrooms (Golovko et al., 2022b), in levels very similar to those detected in this work. The two most relevant PFAS cases were PFBA and FOSA.

PFBA. This short-chain PFAS was taken up most, reaching a shoot concentration of 250 and 270 µg/kg fw in pak choi grown in the digestate and mineral solution, respectively. PFBA is also the PFAS with the highest plant concentrations or UF in most previous studies assessing plant uptake, e.g. in carrots, tomatoes or radish (Bizkarguenaga et al., 2016; Blaine et al., 2014, 2013). Even though it showed high bioaccumulation in plants, no TPs of PFBA were identified, indicating low degradation and long lifetime once translocated in the crop.

FOSA. The levels of FOSA detected in pak choi shoots were low (7.6 and 4.4 µg/kg fw) compared with those of other PFAS, probably because the sulfamide in the end of the chain allows this compound to degrade more readily than other PFAS. This may explain why it was the only PFAS for which TPs were tentatively identified (two TPs, after conjugation with lactic acid and hexose).

5. Conclusions and Outlook

The CEC concentrations in pak choi plants cultivated in different nutrient solutions (mineral and digestate) did not differ significantly, but there was a trend for higher levels of bioaccumulation in plants grown in the digestate solution. This could be due to higher organic matter content in the nutrient solution favouring pre-concentration in the substrate, leading to higher concentrations in plant material. Among the target CECs analysed, uptake of pharmaceuticals was found to be compound-dependant, e.g. PrPB was not taken up, while PFAS uptake was negatively correlated with perfluorocarbon chain length and functional group.

From the 18 selected CECs used for spiking, 16 TPs were tentatively identified (four confirmed with standard) regardless of nutrient solution used. This indicates that transformation processes occur when the compounds are translocated by the plant and do not depend on the cultivation solution. For the TPs reported here for the first time, no toxicity information is available and further studies are needed to identify potential harmful effects on human health.

The levels of CECs detected demonstrated uptake and transformation in pak choi. Risk assessment based solely on monitoring the parent compound may lead to underestimation of TP concentrations (and their toxicity), which need special monitoring. Identification and quantification of CECs and their TPs in edible plants is necessary to clarify their effects on human health before digestate reuse as a crop fertiliser as part of food recovery and nutrient recycling in a circular economy.

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References

(ATSDR), A. for T.S. and D.R., 2020. Per- and polyfluoroalkyl substances (PFAS) and your health [WWW Document].

Abril, C., Santos, J.L., Martín, J., Aparicio, I., Alonso, E., 2021. Uptake and translocation of multiresidue industrial and household contaminants in radish grown under controlled conditions. Chemosphere 268. https://doi.org/10.1016/j.chemosphere.2020.128823

Ahrens, L., 2011. Polyfluoroalkyl compounds in the aquatic environment: A review of their occurrence and fate. J. Environ. Monit. 13, 20–31. https://doi.org/10.1039/c0em00373e

Ahrens, L., Bundschuh, M., 2014. Fate and effects of poly- and perfluoroalkyl substances in the aquatic environment: A review. Environ. Toxicol. Chem. 33, 1921–1929. https://doi.org/10.1002/etc.2663

Archer, E., Petrie, B., Kasprzyk-Hordern, B., Wolfaardt, G.M., 2017. The fate of pharmaceuticals and personal care products (PPCPs), endocrine disrupting contaminants (EDCs), metabolites and illicit drugs in a WWTW and environmental waters. Chemosphere 174, 437–446. https://doi.org/https://doi.org/10.1016/j.chemosphere.2017.01.101

Balagurusamy, N., Chandel, A.K., 2020. Biogas production : from anaerobic digestion to a sustainable bioenergy industry , 1st ed. ed. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-030-58827-4

Bergstrand, K.J., Asp, H., Hultberg, M., 2020. Utilizing anaerobic digestates as nutrient solutions in hydroponic production systems. Sustain. 12, 1–12. https://doi.org/10.3390/su122310076

Bizkarguenaga, E., Zabaleta, I., Prieto, A., Fernández, L.A., Zuloaga, O., 2016. Uptake of 8:2 perfluoroalkyl phosphate diester and its degradation products by carrot and lettuce from compost-amended soil. Chemosphere 152, 309–317. https://doi.org/10.1016/j.chemosphere.2016.02.130

Blaine, A.C., Rich, C.D., Hundal, L.S., Lau, C., Mills, M.A., Harris, K.M., Higgins, C.P., 2013. Uptake of perfluoroalkyl acids into edible crops via land applied biosolids: Field and greenhouse studies. Environ. Sci. Technol. 47, 14062–14069. https://doi.org/10.1021/es403094q

Blaine, A.C., Rich, C.D., Sedlacko, E.M., Hundal, L.S., Kumar, K., Lau, C., Mills, M.A., Harris, K.M., Higgins, C.P., 2014. Perfluoroalkyl acid distribution in various plant compartments of edible crops grown in biosolids-amended soils. Environ. Sci. Technol. 48, 7858–7865. https://doi.org/10.1021/es500016s

Boberg, J., Taxvig, C., Christiansen, S., Hass, U., 2010. Possible endocrine disrupting effects of parabens and their metabolites. Reprod. Toxicol. 30, 301–312. https://doi.org/https://doi.org/10.1016/j.reprotox.2010.03.011

Boström, M.L., Ugge, G., Jönsson, J.Å., Berglund, O., 2017. Bioaccumulation and trophodynamics of the antidepressants sertraline and fluoxetine in laboratory-constructed, 3-level aquatic food chains. Environ. Toxicol. Chem. 36, 1029–1037. https://doi.org/10.1002/etc.3637

Calza, P., Medana, C., Padovano, E., Giancotti, V., Baiocchi, C., 2012. Identification of the unknown transformation products derived from clarithromycin and carbamazepine using liquid chromatography/high-resolution mass spectrometry. Rapid Commun. Mass Spectrom. 26, 1687–1704. https://doi.org/10.1002/rcm.6279

Dowdy, D.L., Mckone, T.E., 1997. Predicting plant uptake of organic chemicals from soil or air using octanol/water and octanol/air partition ratios and a molecular connectivity index. Environ. Toxicol. Chem. 16, 2448–2456. https://doi.org/10.1002/etc.5620161203

Esteve, M.J., Farré, R., Frigola, A., Garcia-Cantabella, J.M., 1997. Determination of ascorbic and dehydroascorbic acids in blood plasma and serum by liquid chromatography. J. Chromatogr. B Biomed. Appl. 688, 345–349. https://doi.org/10.1016/S0378-4347(96)00248-4

Ghisi, R., Vamerali, T., Manzetti, S., 2019. Accumulation of perfluorinated alkyl substances (PFAS) in agricultural plants: A review. Environ. Res. 169, 326–341. https://doi.org/10.1016/j.envres.2018.10.023

Gobelius, L., Lewis, J., Ahrens, L., 2017. Plant Uptake of Per- and Polyfluoroalkyl Substances at a Contaminated Fire Training Facility to Evaluate the Phytoremediation Potential of Various Plant Species. Environ. Sci. Technol. 51, 12602–12610. https://doi.org/10.1021/acs.est.7b02926

Golovko, O., Ahrens, L., Schelin, J., Sörengård, M., Bergstrand, K.J., Asp, H., Hultberg, M., Wiberg, K., 2022a. Organic micropollutants, heavy metals and pathogens in anaerobic digestate based on food waste. J. Environ. Manage. 313. https://doi.org/10.1016/j.jenvman.2022.114997

Golovko, O., Kaczmarek, M., Asp, H., Bergstrand, K.J., Ahrens, L., Hultberg, M., 2022b. Uptake of perfluoroalkyl substances, pharmaceuticals, and parabens by oyster mushrooms (Pleurotus ostreatus) and exposure risk in human consumption. Chemosphere 291, 1–8. https://doi.org/10.1016/j.chemosphere.2021.132898

Gornik, T., Kovacic, A., Heath, E., Hollender, J., Kosjek, T., 2020. Biotransformation study of antidepressant sertraline and its removal during biological wastewater treatment. Water Res. 181, 115864. https://doi.org/10.1016/j.watres.2020.115864

Grabicova, K., Grabic, R., Fedorova, G., Fick, J., Cerveny, D., Kolarova, J., Turek, J., Zlabek, V., Randak, T., 2017. Bioaccumulation of psychoactive pharmaceuticals in fish in an effluent dominated stream. Water Res. 124, 654–662. https://doi.org/10.1016/j.watres.2017.08.018

Haman, C., Dauchy, X., Rosin, C., 2014. Occurrence , fate and behavior of parabens in aquatic environments : A review 8, 1–11. https://doi.org/10.1016/j.watres.2014.09.030

Humphries, J.M., Khachik, F., 2003. Distribution of lutein, zeaxanthin, and related geometrical isomers in fruit, vegetables, wheat, and pasta products. J. Agric. Food Chem. 51, 1322–1327. https://doi.org/10.1021/jf026073e

Joerss, H.K., 2020. Legacy and Emerging Per- and Polyfluoroalkyl Substances in the Aquatic Environment – Sources , Sinks and Long-Range Transport to the Arctic.

Jones-Lepp, T.L., Sanchez, C.A., Moy, T., Kazemi, R., 2010. Method development and application to determine potential plant uptake of antibiotics and other drugs in irrigated crop production systems. J. Agric. Food Chem. 58, 11568–11573. https://doi.org/10.1021/jf1028152

Jurado, A., Gago-Ferrero, P., Vàzquez-Suñé, E., Carrera, J., Pujades, E., Díaz-Cruz, M.S., Barceló, D., 2014. Urban groundwater contamination by residues of UV filters. J. Hazard. Mater. https://doi.org/10.1016/j.jhazmat.2014.01.036

Kodešová, R., Klement, A., Golovko, O., Fér, M., Kočárek, M., Nikodem, A., Grabic, R., 2019a. Soil influences on uptake and transfer of pharmaceuticals from sewage sludge amended soils to spinach. J. Environ. Manage. 250, 109407. https://doi.org/10.1016/j.jenvman.2019.109407

Kodešová, R., Klement, A., Golovko, O., Fér, M., Nikodem, A., Kočárek, M., Grabic, R., 2019b. Root uptake of atenolol, sulfamethoxazole and carbamazepine, and their transformation in three soils and four plants. Environ. Sci. Pollut. Res. 26, 9876–9891. https://doi.org/10.1007/s11356-019-04333-9

Krippner, J., Falk, S., Brunn, H., Georgii, S., Schubert, S., Stahl, T., 2015. Accumulation Potentials of Perfluoroalkyl Carboxylic Acids (PFCAs) and Perfluoroalkyl Sulfonic Acids (PFSAs) in Maize (Zea mays). J. Agric. Food Chem. 63, 3646–3653. https://doi.org/10.1021/acs.jafc.5b00012

Lechner, M., Knapp, H., 2011. Carryover of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) from soil to plant and distribution to the different plant compartments studied in cultures of carrots (Daucus carota ssp. Sativus), potatoes (Solanum tuberosum), and cucumber. J. Agric. Food Chem. 59, 11011–11018. https://doi.org/10.1021/jf201355y

Lesmeister, L., Lange, F.T., Breuer, J., Biegel-Engler, A., Giese, E., Scheurer, M., 2021. Extending the knowledge about PFAS bioaccumulation factors for agricultural plants – A review. Sci. Total Environ. 766, 142640. https://doi.org/10.1016/j.scitotenv.2020.142640

Maldini, M., Baima, S., Morelli, G., Scaccini, C., Natella, F., 2012. A liquid chromatography-mass spectrometry approach to study “glucosinoloma” in broccoli sprouts. J. Mass Spectrom. 47, 1198–1206. https://doi.org/10.1002/jms.3028

Mañas, P., Castro, E., De Las Heras, J., 2009. Irrigation with treated wastewater: Effects on soil, lettuce (Lactuca sativa L.) crop and dynamics of microorganisms. J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng. 44, 1261–1273. https://doi.org/10.1080/10934520903140033

Margenat, A., Matamoros, V., Díez, S., Cañameras, N., Comas, J., Bayona, J.M., 2019. Occurrence and human health implications of chemical contaminants in vegetables grown in peri-urban agriculture. Environ. Int. 124, 49–57. https://doi.org/10.1016/j.envint.2018.12.013

Menger, F., Boström, G., Jonsson, O., Ahrens, L., Wiberg, K., Kreuger, J., Gago-Ferrero, P., 2021. Identification of Pesticide Transformation Products in Surface Water Using Suspect Screening Combined with National Monitoring Data. Environ. Sci. Technol. 55, 10343–10353. https://doi.org/10.1021/acs.est.1c00466

Menger, F., Gago-Ferrero, P., Wiberg, K., Ahrens, L., 2020. Wide-scope screening of polar contaminants of concern in water: A critical review of liquid chromatography-high resolution mass spectrometry-based strategies. Trends Environ. Anal. Chem. 28, e00102. https://doi.org/10.1016/j.teac.2020.e00102

Navarro, I., de la Torre, A., Sanz, P., Porcel, M.Á., Pro, J., Carbonell, G., Martínez, M. de los Á., 2017. Uptake of perfluoroalkyl substances and halogenated flame retardants by crop plants grown in biosolids-amended soils. Environ. Res. 152, 199–206. https://doi.org/10.1016/j.envres.2016.10.018

Nazaret, S., Aminov, R., 2014. Role and prevalence of antibiosis and the related resistance genes in the environment . Front. Microbiol. .

NTP, 2016. Monograph- Immunotoxicity Associated with Exposure to Perfluorooctanoic Acid or Perfluorooctane Sulfonate. Natl. Inst. Environ. Heal. Sci. 1–147.

Ooi, G.T.H., Escola Casas, M., Andersen, H.R., Bester, K., 2017. Transformation products of clindamycin in moving bed biofilm reactor (MBBR). Water Res. 113, 139–148. https://doi.org/10.1016/j.watres.2017.01.058

Panfili, G., Fratianni, A., Irano, M., 2004. Improved normal-phase high-performance liquid chromatography procedure for the determination of carotenoids in cereals. J. Agric. Food Chem. 52, 6373–6377. https://doi.org/10.1021/jf0402025

Penrose, M.T., Cobb, G.P., 2022. Identifying potential paraben transformation products and evaluating changes in toxicity as a result of transformation. Water Environ. Res. 94, 1–19. https://doi.org/10.1002/wer.10705

Reichl, B., Himmelsbach, M., Emhofer, L., Klampfl, C.W., Buchberger, W., 2018. Uptake and metabolism of the antidepressants sertraline, clomipramine, and trazodone in a garden cress (Lepidium sativum) model. Electrophoresis 39, 1301–1308. https://doi.org/10.1002/elps.201700482

Rosenfeld, P.E., Feng, L.G.H., 2011. 16 - Emerging Contaminants, in: Rosenfeld, P.E., Feng, L.G.H.B.T.-R. of H.W. (Eds.), . William Andrew Publishing, Boston, pp. 215–222. https://doi.org/https://doi.org/10.1016/B978-1-4377-7842-7.00016-7

Rostvall, A., Zhang, W., Dürig, W., Renman, G., Wiberg, K., Ahrens, L., Gago-Ferrero, P., 2018. Removal of pharmaceuticals, perfluoroalkyl substances and other micropollutants from wastewater using lignite, Xylit, sand, granular activated carbon (GAC) and GAC+Polonite® in column tests – Role of physicochemical properties. Water Res. 137, 97–106. https://doi.org/10.1016/j.watres.2018.03.008

Sabourin, L., Duenk, P., Bonte-Gelok, S., Payne, M., Lapen, D.R., Topp, E., 2012. Uptake of pharmaceuticals, hormones and parabens into vegetables grown in soil fertilized with municipal biosolids. Sci. Total Environ. 431, 233–236. https://doi.org/10.1016/j.scitotenv.2012.05.017

Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P., Hollender, J., 2014. Identifying small molecules via high resolution mass spectrometry: Communicating confidence. Environ. Sci. Technol. 48, 2097–2098. https://doi.org/10.1021/es5002105

Stasiuk, S.J., MacNevin, G., Workentine, M.L., Gray, D., Redman, E., Bartley, D., Morrison, A., Sharma, N., Colwell, D., Ro, D.K., Gilleard, J.S., 2019. Similarities and differences in the biotransformation and transcriptomic responses of Caenorhabditis elegans and Haemonchus contortus to five different benzimidazole drugs. Int. J. Parasitol. Drugs Drug Resist. 11, 13–29. https://doi.org/10.1016/j.ijpddr.2019.09.001

Stuchlíková, L., Jirásko, R., Skálová, L., Pavlík, F., Szotáková, B., Holčapek, M., Vaněk, T., Podlipná, R., 2016. Metabolic pathways of benzimidazole anthelmintics in harebell (Campanula rotundifolia). Chemosphere 157, 10–17. https://doi.org/10.1016/j.chemosphere.2016.05.015

Stuchlíková, L.R., Skálová, L., Szotáková, B., Syslová, E., Vokřál, I., Vaněk, T., Podlipná, R., 2018. Biotransformation of flubendazole and fenbendazole and their effects in the ribwort plantain (Plantago lanceolata). Ecotoxicol. Environ. Saf. 147, 681–687. https://doi.org/10.1016/j.ecoenv.2017.09.020

Sunyer-Caldú, A., Diaz-Cruz, M.S., 2021. Development of a QuEChERS-based method for the analysis of pharmaceuticals and personal care products in lettuces grown in field-scale agricultural plots irrigated with reclaimed water. Talanta 230, 122302. https://doi.org/https://doi.org/10.1016/j.talanta.2021.122302

Sunyer-Caldú, A., Sepúlveda-Ruiz, P., Salgot, M., Folch-Sánchez, M., Barcelo, D., Diaz-Cruz, M.S., 2022. Reclaimed water in agriculture: a plot-scale study assessing crop uptake of emerging contaminants and pathogens. J. Environ. Chem. Eng. 108831. https://doi.org/https://doi.org/10.1016/j.jece.2022.108831

Tian, R., Zhang, R., Uddin, M., Qiao, X., Chen, J., Gu, G., 2019. Uptake and metabolism of clarithromycin and sulfadiazine in lettuce. Environ. Pollut. 247, 1134–1142. https://doi.org/10.1016/j.envpol.2019.02.009

United Nations Environment Programme, 2021. Food Waste Index Report 2021, Unep. Nairobi.

Zajíčková, M., Prchal, L., Navrátilová, M., Vodvárková, N., Matoušková, P., Vokřál, I., Nguyen, L.T., Skálová, L., 2021. Sertraline as a new potential anthelmintic against Haemonchus contortus: toxicity, efficacy, and biotransformation. Vet. Res. 52, 143. https://doi.org/10.1186/s13567-021-01012-x

Zhang, L., Wang, Q., Chen, H., Yao, Y., Sun, H., 2021. Uptake and translocation of perfluoroalkyl acids with different carbon chain lengths (C2–C8) in wheat (Triticum acstivnm L.) under the effect of copper exposure. Environ. Pollut. 274, 116550. https://doi.org/10.1016/j.envpol.2021.116550

5.2.4 Publication #12 Supplementary information

Occurrence and fate of contaminants of emerging concern and their transformation products after uptake by pak choi (Brassica rapa subsp. chinensis)

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Section S1. Quality assurance and quality control.
Section S2. Suspect screening (compound discoverer) workflow
Section S3. Figures. Figure S1.
Section S4. Tables. Tables S1-S7.

Section S1. Quality assurance and quality control

Preparation of blank samples (n=6) included all the same steps, but with no shoot material. Spiked quality control samples (n=6) were prepared in the same way as the main samples, but with a native standard mix (100 ng per sample aliquot of native standard) in addition to the IS mix. For post-extraction spiking samples, no native or internal standards were added before or during extraction. Both the IS mixture and the native standard mix were added to the whole volume of the final extract.

The performance of the method was assessed with regard to its linearity, blanks, limits of detection (LOD), limits of quantification (LOQ), absolute recovery, precision, and matrix effect (ME). The blank levels were directly subtracted from the values obtained for the samples.

For testing the linearity of the method, an initial nine-point calibration curve in the concentration range from 0 ng/mL to 1000 ng/mL was created. For each separate analysis, the calibration curve was measured two times, at the beginning and at the end of the sequence, to check instrument stability. The linearity was evaluated by means of the coefficient of determination (R2). The evaluation of the results of the blank samples, demonstrated that no native compounds were detected, which demonstrates that no contaminants of emerging concern (CECs) were introduced during the sample preparation process.

LOQ were calculated as one half of the lowest calibration point in the calibration curve where the relative standard deviation of average response factor was < 30%. LOQs for each analyte in each sample were calculated by using the peak area of the lowest calibration point in the calibration curve. LOD were established as a third part of the corresponding LOQ. The absolute recovery was determined by comparison of the averaged signal (n = 3) of samples spiked at the concentration of 100 ng/mL with the signal of corresponding post-extraction spiked samples at the concentration level of 100 ng/mL.

The extent of ME was determined quantitatively based on the instrumental responses and is an estimate of ionization suppression effects. This approach is based on comparing the peak area of the analyte from the post-extraction spiked sample with the peak area of the analyte recorded from the calibration solution (analyte in pure solvent).

If the ME value yields 100%, such result corresponds to no effect detected, a value less than 100% indicates an ionization suppression, while ME above 100% corresponds to an ionization enhancement. The lower the ME value, the greater is the extent of ionization suppression, the higher ME is, the greater is the extent of ionization enhancement.

Evaluation of results of blank samples, demonstrated that no native compounds were detected, which demonstrates that no CECs were introduced during the sample preparation process.

The suitability of sample preparation methods was assessed based on the values of absolute recoveries and LOQ for each matrix. For each compound and each type of matrix average values (n=3) of absolute recoveries has been calculated. Determination of the relative standard deviation (RSD) of concentrations between substrate and vegetable replicates, was based on the results obtained from the substrate spiked at the concentration level of 100 ng/g, and vegetables grew on such substrate. Relative recoveries, LOQs and ME values can be consulted in **Table S3**.

Section S2. Compound discoverer workflow

The workflow followed in the data treatment of suspect screening, using Compound Discoverer software, is represented in Figure S1 and the parameters of each function are explained below. After selecting the input files, the spectra between 0 and 15 min R_T (our working gradient) was selected and the polarity mode was selected as "any". The R_T was aligned with an adaptive curve model, with a maximum mass deviation of 2 mDa / 5 ppm. In the *generate compounds* function, the spiked compounds were selected (CLA, CLI, FBZ, SER, PrPB, PFBA, PFUnDA, PFHpA, PFTeDA, PFHxA, PFDoDA, PFOA, PFDA, PFNA, PFOS, FOSA, PFHxS and PFBS). Dealkylation and dearylation were specified as "True", the maximum steps were set at 2, and the minimum mass was set at 60 Da. The potential transformations available of phase I and phase II were all selected, the maximum steps of phase II were selected as 1, and the maximum steps in total were 3. The adduct ions allowed were $[M+H]^+$, $[M+Na]^+$ and $[M+NH_4]^+$ in positive and $[M-H]^-$, $[M+Cl]^-$ and $[M-H-H_2O]^-$ in negative. In the *find expected compounds* function, the mass tolerance was set at 5 ppm, intensity tolerance at 30%, intensity threshold at 0.1, minimum isotopes at 2, minimum peak intensity at 1000 a.u. and the most intense isotope was used. In the *group expected compounds* function, the R_T tolerance was set at 0.1 min, align peaks as "False", preferred ions as $[H+M]^+$, area integration as "most common ion", area contribution at 3, CV contribution at 10, FWHM to base at 5, Jaggedness contribution at 5, Modality contribution at 5, Zig-zag index at 5, peak rating threshold 0 and number of files at 0. In the *mark background compounds* function, maximum sample/blank was set at 3, maximum blank/sample at 0, and hide background as "True". In the *merge features* function, mass tolerance was set at 5 ppm and R_T tolerance was set at 0.1 min. In the *FISH scoring* function, annotate full tree was set as "True", match transformation as "True", S/N threshold at 3, high accuracy mass tolerance at 0.0001 Da, low accuracy mass tolerance at 5 ppm, use general rules as "True", use libraries as "True", maximum depth at 5, aromatic cleavage as "True" and minimum fragment *m/z* at 50. In the *detect compounds* function, mass tolerance was set at 5 ppm, minimum peak intensity at 80000 in positive and 50000 in negative, use most intense peak as "True", group isotopes for Br and Cl, and compound detection as [M+H]+, $[M+Na]^+$ and $[M+NH_4]^+$ in positive and $[M-H]^-$, $[M+Cl]^-$ and $[M-H-H_2O]^-$ in negative. In the group compounds function, mass tolerance was set at 5 ppm, R_T tolerance was set at 0.1 min, align peaks as "False", preferred ions as $[M+H]^+$, $[M+Na]^+$ and $[M+NH_4]^+$ in positive and $[M-H]^-$, $[M+Cl]^-$ and $[M-H-H_2O]^-$ in negative, area integration as "Most common ion", area contribution at 3, CV contribution at 10, FWHM to base at 5, Jaggedness contribution at 5, Modality contribution at 5, Zig-zag index at 5, peak rating threshold 0 and number of files at 0. In the *fill gaps* function, mass tolerance was set at 5 ppm and S/N threshold at 3. In the *neutral loss search* function, the most common neutral losses of xenobiotics in plant metabolism reported in the literature were introduced (it can be consulted in spreadsheet "Neutral losses" in the SI), and high accuracy mass tolerance was set at 0.0001 Da, low accuracy mass tolerance at 5 ppm, and S/N threshold at 3. In the *pattern scoring* function, isotope patterns were selected for Cl, Br and S, mass tolerance was set at 5 ppm, intensity tolerance at 30, S/N threshold at 3, and minimum spectral fit at 0%. In the *predict compositions* function, mass tolerance was set at 5 ppm, minimum element count as C H, maximum element count as C90 H190 Br3 Cl4 N10 O18 P3 S5, minimum RDBE at 1, maximum RDBE at 40, minimum H/C at 0.2, maximum H/C at 3.1, maximum candidates at 5, intensity tolerance at 30, intensity threshold at 0.1, S/N threshold at 3, use dynamic recalibration as "true", use fragments as "true", mass tolerance at 5 ppm and S/N threshold at 3. In the *assign compound annotations* function, mass tolerance was set at 5 ppm, four data sources were selected: mzCloud search, prediction compositions, masslist search and Chemspider search, use mzlogic was set as "true", use spectral distance as "true", SFit threshold at 20, SFit range at 20 and clear names as "false". In the *search Chemspider* function, databases was set as KEGG, search mode as by formula or mass, mass tolerance at 5 ppm, maximum results at 10 and maximum predictions at 3. In the *apply mz logic* function, maximum compound was set at 0, max mzcloud at 5, and match factor threshold at 70.

Section S3. Figures

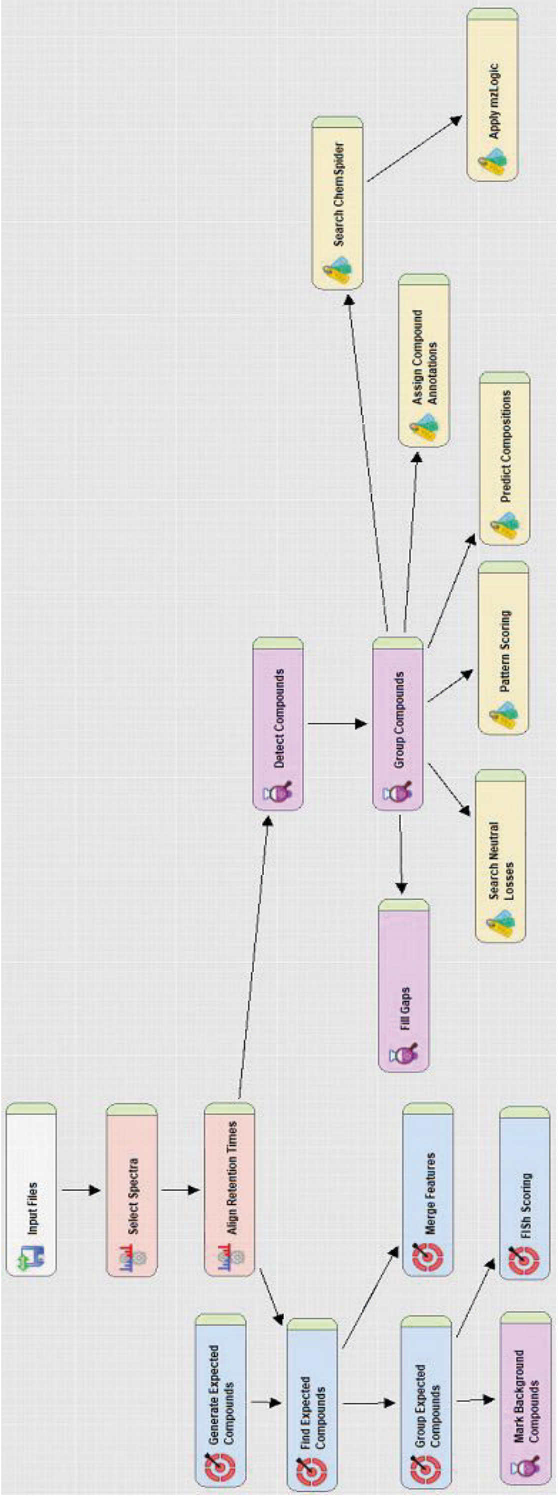


Figure S1. Compound discoverer workflow used for the suspect screening data treatment.

Section S4. Tables

Table S1. Composition of the two different nutrient solutions used in the study. The organic solution was prepared from liquid digestates and the mineral solution was prepared from pure salts to resemble the organic solution, both diluted in miliQ water

Nutrient	Organic mg/L	Mineral mg/L
NO_3^-	140	141
NH_4^+	28	28
P^+	8.2	7.8
K^+	190	189
Mg^{2+}	6.7	6.6
Ca^{2+}	93	160
S^{2-}	16	34
Fe^{3+}	2.1	2.1
Mn^{2+}	0.46	0.44
Zn^{2+}	0.68	0.65
Cl^-	120	120
Mo^{2+}	0.006	0.006
Cu^{2+}	0.006	0.006
B^{3+}	0.16	0.16

Table S2. Concentration (µg/L) of contaminants of emerging concern (CECs) in the nutrient solution. W3 is the third week of Pak Choi cultivation. The nutrient solution was changed each week and initial (start) and final (end) concentration of CECs for each week are presented. The experiment was performed both in mineral nutrients solution (Min) and organic (BG) nutrient solution. Mean±std, n=3

Compound	W3 Min 0 start	W3 Min 0 end	W3 BG 0 start	W3 BG 0 end
Propylparaben	<LOD	<LOD	<LOD	<LOD
Fenbendazole	<LOD	<LOD	<LOD	<LOD
Clarithromycin	<LOD	<LOD	<LOD	<LOD (3.4 in rep 3)
Sertraline	<LOD	<LOD	<LOD	<LOD
Clindamycin	<LOD	<LOD	<LOD	<LOD
PFTeDA	<LOD	<LOD	<LOD	<LOD
PFDoDA	<LOD	<LOD	<LOD	<LOD
PFBA (HFBA)	<LOD	<LOD	<LOD	<LOD
FOSA	<LOD	<LOD	<LOD	<LOD
PFUnDA	<LOD	<LOD	<LOD	<LOD
PFOS	<LOD	<LOD	<LOD	<LOD
PFHpA	<LOD	<LOD	<LOD	<LOD
PFHxS	<LOD	<LOD (2.0 in rep 3)	<LOD	<LOD
PFNA	<LOD	<LOD	<LOD	<LOD (2.7 in rep 3)
PFDA	<LOD	<LOD	<LOD	<LOD
PFOA	<LOD	<LOD (2.3 in rep 3)	<LOD	<LOD (2.9 in rep 3)
PFBS	<LOD	<LOD	<LOD	<LOD
PFHxA	<LOD	<LOD	<LOD	<LOD
ΣCECs	-	-	-	-

Compound	W3 Min 1 start	W3 Min 1 end	W3 BG 1 start	W3 BG 1 end
Propylparaben	<LOD	<LOD	<LOD	<LOD
Fenbendazole	<LOD	<LOD	<LOD	<LOD
Clarithromycin	<LOD	<LOD	1.7±0.6	3.8±3.2
Sertraline	<LOD	<LOD	<LOD	<LOD
Clindamycin	<LOD	<LOD	<LOD	1.8±0.8
PFTeDA	<LOD	<LOD	<LOD	<LOD
PFDoDA	<LOD	<LOD	<LOD	<LOD
PFBA (HFBA)	2.1±0.1	<LOD	<LOD	<LOD
FOSA	<LOD	<LOD	<LOD	<LOD
PFUnDA	<LOD	<LOD	<LOD	<LOD
PFOS	<LOD	1.7±1.0	<LOD	<LOD
PFHpA	<LOD	2.2±1.3	<LOD	2.3±1.2
PFHxS	<LOD	3.6±3.0	<LOD	2.8±2.2
PFNA	<LOD	3.4±4.0	<LOD	<LOD
PFDA	<LOD	1.8±1.1	<LOD	<LOD
PFOA	<LOD	3.6±3.4	<LOD	2.7±2.2
PFBS	2.5±0.1	2.4±0.3	2.2±0.1	2.3±0.3
PFHxA	2.4±0.2	1.2±0.1	2.1±0.2	2.2±0.3
ΣCECs	7.1±0.3	21±13.5	6.1±0.8	18±9.5

Compound	W3 Min 10 start	W3 Min 10 end	W3 BG 10 start	W3 BG 10 end
Propylparaben	11±3.6	<LOD	<LOD	<LOD
Fenbendazole	2.9±0.6	<LOD	<LOD	<LOD
Clarithromycin	4.5±0.8	13±8.3	23±0.6	13±7.1
Sertraline	3.9±0.9	<LOD	<LOD	<LOD
Clindamycin	19±3.2	8.9±10	19±0.6	8.2±9.2
PFTeDA	<LOD	<LOD	<LOD	<LOD
PFDoDA	<LOD	<LOD	<LOD	<LOD
PFBA (HFBA)	16±5.2	14±2.2	18±1.7	14±1.1
FOSA	<LOD	<LOD	<LOD	<LOD
PFUnDA	<LOD	<LOD	<LOD	<LOD
PFOS	2.6±0.6	3.1±1.6	7.4±0.6	3.1±1.8
PFHpA	15±1.7	15±1.9	16±0.6	14±1.3
PFHxS	13±0.0	12±1.9	12±0.6	12±1.9
PFNA	12±1.0	8.8±4.1	12.3±0.6	8.5±4.3
PFDA	2.9±1.2	3.9±2.6	8.6±1.4	3.8±2.9
PFOA	15±0.0	13±4.9	15±0.0	12±4.8
PFBS	20±5.3	21±1.8	22±1.5	20±0.8
PFHxA	20±2.9	21±1.8	22±1.1	20±0.8
ΣCECs	181±28	134±31	180±3.4	128±26

Compound	W3 Min 100 start	W3 Min 100 end	W3 BG 100 start	W3 BG 100 end
Propylparaben	127±5.8	<LOD	49±4.9	<LOD
Fenbendazole	21±1.0	2.8±1.6	9.2±1.6	5.2±1.7
Clarithromycin	137±5.8	130±120	200±21	32±24
Sertraline	43±1.5	<LOD	7.4±0.3	<LOD
Clindamycin	190±10	51±70	170±21	15±7.9
PFTeDA	<LOD	51±37	7.4±2.1	12±11
PFDoDA	<LOD	9.3±7.0	<LOD	4.4±3.7
PFBA (HFBA)	160±0.0	130±21	160±10	130±9.6
FOSA	<LOD	<LOD	<LOD	6.8±3.9
PFUnDA	<LOD	12±13	<LOD	17±12
PFOS	34±11.5	49±30	71±13	25±22
PFHpA	130±0.0	112±8.7	130±5.8	110±47
PFHxS	130±5.8	99±10	110±5.8	79±56
PFNA	130±11	77±62	120±5.8	35±31
PFDA	46±14	61±41	80±17	30±27
PFOA	130±0.0	97±31	130±0.0	59±44
PFBS	170±0.0	160±4.2	163±5.8	146±9.1
PFHxA	180±0.0	160±10.0	173±5.8	152±13
ΣCECs	1800±60	1200±360	1800±129	860±310

Table S3. Method limit of quantification (LOQ) fw, relative recovery and matrix effect for each target compound

	Propylparaben	Fenbendazole	Clarithromycin	Sertraline	Clindamycin	PFTeDA	PFDoDA	PFBA (HFBA)	FOSA
LOQ (ng/g)	25	0.64	1.0	1.6	1.0	0.87	1.0	51	1.5
Relative recovery	152%	151%	123%	126%	130%	123%	112%	198%	128%
Matrix effect	-36%	17%	95%	45%	82%	-23%	59%	-73%	-23%
	PFUnDA	**PFOS**	**PFHpA**	**PFHxS**	**PFNA**	**PFDA**	**PFOA**	**PFBS**	**PFHxA**
LOQ (ng/g)	0.20	1.0	1.6	0.99	0.63	1.0	0.81	3.2	5.8
Relative recovery	112%	115%	152%	172%	137%	110%	108%	130%	128%
Matrix effect	68%	83%	28%	64%	80%	71%	77%	19%	10%

Table S4. Uptake factors (L/kg) of the different compounds in each substrate

Name	Acronym	UF DIGESTATE (L/kg)	UF MINERAL (L/kg)
Propylparaben	**PrPB**	Not available	Not available
Fenbendazole	**FEN**	Not available	Not available
Clarithromycin	**CLA**	0.19	0.26
Sertraline	**SER**	2.4	33
Clindamycin	**CLI**	0.03	Not available
PFBA	**PFBA**	1.8	1.6
PFHxA	**PFHxA**	0.29	0.40
PFHpA	**PFHpA**	0.41	0.18
PFOA	**PFOA**	0.24	0.12
PFNA	**PFNA**	0.23	0.18
PFDA	**PFDA**	0.38	0.14
PFUnDA	**PFUnDA**	Not available	Not available
PFDoDA	**PFDoDA**	Not available	Not available
PFTeDA	**PFTeDA**	Not available	0.23
PFBS	**PFBS**	0.24	0.23
PFHxS	**PFHxS**	0.32	0.09
FOSA	**FOSA**	0.85	Not available
PFOS	**PFOS**	0.49	0.28

UF: Uptake factor

Table S5. The fresh weight of shoots and roots of Pak Choi produced in the different treatments. Also the effect of the treatments on secondary metabolites (C-vitamin (mg g^{-1} dw), carotenoids and glucosinolate (µg g^{-1} dw)), maximum photosynthetic capacity (A_{max}, µmol m^{-2} s^{-1}), variable fluorescence (F_v/F_m), and relative chlorophyll content (Chl) in the shoots are shown. Mean±std, n=3

Nutrient regime	Concentration*	Fresh weight (g)		Secondary metabolites in shoots			A_{max}	F_v/F_m	Chl
		Shoots	Roots	C-vitamin	Carotenoids	Glucosinolates			
Mineral	0	103.1±5.2a**	15.8±1.9a	4.5±0.4b	539.7±37.5a	1.2±0.1b	21.9 ± 0.9 a	0.73 ± 0.01 a	27.4 ± 3.5 a
Mineral	1	91.5±11.7ab	10.1±1.4ab	4.4±0.3b	514.2±89.3a	1.6±0.6ab	22.7 ± 1.9 a	0.75 ± 0.01 a	24.0 ± 1.0 a
Mineral	10	76.6±26.7ab	9.4±4.8ab	5.1±0.2a	504.1±44.1a	2.1±0.1a	21 ± 2.1 a	0.72 ± 0.03 a	27.2 ± 5.4 a
Mineral	100	54.1±38.0b	7.3±5.0b	3.5±0.2c	418.2±107.8a	1.8±0.2a	21 ± 4.1 a	0.74 ± 0.02 a	26.6 ± 3.2 a
Organic	0	95.2±28.0a	11.7±6.3a	6.1±0.8a	496.5±53.5a	2.5±0.9a	25.6 ± 1.9 a	0.76 ± 0.01 a	28.7 ± 2.1 a
Organic	1	62.3±19.2ab	11.3±6.9a	4.6±0.5b	521.4±26.3a	2.2±0.6a	26.7 ± 3.1 a	0.74 ± 0.01 a	27.6 ± 8.5 a
Organic	10	75.1±15.7ab	12.6±3.0a	4.6±0.1b	612.4±59.1a	2.1±0.4a	22.9 ± 0.5 a	0.71 ± 0.07 a	20.2 ± 2.5 ab
Organic	100	41.2±27.3b	10.7±9.4a	4.6±0.2b	459.9±162.2a	1.8±0.6a	23.7 ± 4.2 a	0.69 ± 0.04 a	17.9 ± 2.7 b

* Initial nominal concentration of CECs in the nutrient solution

** Values for each nutrient regime within columns followed by different letters (a, b or ab) are significantly different (p≤0.05).

Table S6. Concentration (µg/kg fresh weight) of CECs in the shoots of Pak Choi. The plant was exposed to three different concentration of CECs (nominal concentration of 1, 10 and 100 µg/L) during cultivation. Mean ±sd, n=3.

Compound	Organic	Mineral	Organic	Mineral	Organic	Mineral
	1 µg/L		10 µg/L		100 µg/L	
Propylparaben	<LOD*	<LOD	<LOD	<LOD	<LOD	<LOD
Fenbendazole	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Clarithromycin	1.8±1.8	1.3±1.4	1.4±0.8	<LOD	13.3±4.0	8.2±3.8
Sertraline	<LOD	<LOD	7.1±2.0	17.5±2.1	100.3±26.5	190.0±21.6
Clindamycin	<LOD	<LOD	<LOD	<LOD	5.2±2.0	<LOD
PFTeDA	0.8±0.5	<LOD	<LOD	<LOD	3.6±1.8	2.0±1.0
PFDoDA	2.2±2.4	<LOD	1.5±0.9	<LOD	9.6±6.0	5.2±3.5
PFBA (HFBA)	<LOD	<LOD	<LOD	<LOD	253.3±23.1	272.5±22.5
FOSA	<LOD	<LOD	<LOD	<LOD	7.6±3.2	4.4±0.3
PFUnDA	2.0±2.6	<LOD	2.2±0.7	<LOD	11.4±5.8	5.8±1.6
PFOS	2.3±2.5	<LOD	1.8±1.1	<LOD	20.7±13.6	13.0±2.8
PFHpA	3.2±3.4	1.9±1.0	8.4±0.8	<LOD	28±4.6	23.3±2.2
PFHxS	4.1±5.0	2.9±1.5	6.0±2.5	<LOD	17.3±7.5	8.9±2.6
PFNA	2.3±2.8	<LOD	3.5±1.0	<LOD	23.7±9.1	20.3±3.8
PFDA	2.7±3.0	<LOD	2.9±0.3	<LOD	15.0±4.0	6.8±1.6
PFOA	2.8±3.4	<LOD	4.2±2.2	<LOD	25.0±12.2	15.3±1.7
PFBS	<LOD	<LOD	<LOD	<LOD	35±11.4	38.3±7.5
PFHxA	<LOD	<LOD	<LOD	<LOD	46.0±3.6	70.5±12.4
ΣCECs	24.0±27.5b**	14.0±8.1b	39.0±6.9b	17.5±2.1b	615.1±127.3a	682.0±41.5a

* <LOD, below detection limit

**Values within rows followed by different letters (a, b or ab) are significantly different (p≤0.05).

Table S7. Concentration (µg/kg fresh weight) of PFAS in the shoots of Pak Choi ordered in function of the chain length

Compound	Concentration BG 100	Concentration MIN 100	Tendency	Chain length	Functional group
PFBA	250	270	⬆	3	Carboxylic acid
PFHxA	46	70	⬆	5	Carboxylic acid
PFHpA	28	23	⬆	6	Carboxylic acid
PFOA	25	15	⬆	7	Carboxylic acid
PFNA	24	20	⬆	8	Carboxylic acid
PFDA	15	6.8	⬆	9	Carboxylic acid
PFUnDA	11	5.8	⬆	10	Carboxylic acid
PFDoDA	9.6	5.2	⬆	11	Carboxylic acid
PFTeDA	3.6	2	⬆	13	Carboxylic acid
PFBS	35	38	⬇	4	Sulfonic acid
PFHxS	17	8.9	⬇	6	Sulfonic acid
PFOS	21	13	⬇	8	Sulfonic acid
FOSA	7.6	4.4	⬇	8	Sulfamide

5.3 Discussion

The studies compiled in this chapter contribute to broadening the knowledge about the feasibility of wastewater reuse in agriculture, the potential uptake of CECs by crops and the formation of new TPs during the processes. Since the individual discussion and conclusions are presented in each manuscript, a general comparison and discussion of the results are included in this section.

Methodology

First, studies regarding CEC's uptake are very scarce (compared with other environmental matrices), mainly because of the difficulties in analysing crops. The analysis of CECs in complex matrices has represented a challenge in recent years. Lipids, non-polar substances, and fatty or organic acids can interfere with or complicate the analysis. So far, most of the methodologies to analyse CECs in crops, especially PPCPs, consist of different laborious steps trying to obtain the cleaner extract possible. However, these methods are time-consuming and require personnel for routine analysis. Therefore, once the field plots were built in Palamós' WWTP, an efficient and fast methodology was necessary to analyse the cultivated crops. Thus, Publication #9 describes the optimisation and development of a QuEChERS-based methodology to analyse CECs in lettuces, which was further applied to the analysis of soils (Publication #10, supplementary information (SI)), carrots and tomatoes (Publication #11, SI). As the QuEChERS acronym indicates, the methodology was fast, cheap, and easy to be performed by personnel without specific laboratory formation.

Uptake patterns and differences among crops

The theory behind the selection of crops to evaluate the CECs' uptake was to analyse different parts of a plant intended for human consumption, namely leaves (lettuce), fruit (tomatoes), and roots (carrots). As explained in the introduction of this section, with the cultivation of diverse crops, it was expected to see different accumulation patterns. **Table 5.1** summarises the results obtained for the crops, soils and irrigation waters, included in Publications #9, #10 and #11.

As expected, in all cases (even in summer, when the CECs amount in the WWTP influent was considerably higher), the reactive barrier lowered the levels of CECs present in the water.

Table 5.1. Levels of CECs determined in the different matrices included in Publications #9, #10 and #11, expressed by families (UVFs, PBs, pharmaceuticals and caffeine).

Crop	UVFs	Pharm.	PBs	Caffeine	Total
Lettuces barriers	69	53	4	10	136
Lettuces secondary	99	54	6	18	177
Tomaotes barriers	163	157	8	0	328
Tomatoes secondary	192	315	11	0	518
Carrots barriers	42	674	14	8	738
Carrots secondary	48	1107	6	47	1208
Units: ng/g dw; UVFs: Ultraviolet filters; Pharm.: Pharmaceuticals; PBs: Parabens					

Soil	UVFs	Pharm.	PBs	Caffeine	Total
Soil barriers (lettuces cultivation)	2	51	0	0	53
Soil secondary (lettuces cultivation)	3	55	0	0	58
Soil barriers (tomatoes cultivation)	4	88	0	0	92
Soil secondary (tomatoes cultivation)	3	103	0	0	105
Soil barriers (carrots cultivation)	5	20	1	0	26
Soil secondary (carrots cultivation)	6	37	1	0	44
Units: ng/g dw; UVFs: Ultraviolet filters; Pharm.: Pharmaceuticals; PBs: Parabens					

Water	UVFs	Pharm.	PBs	Caffeine	Total
Water barriers (lettuces cultivation)	1209	1882	48	40	3179
Water secondary (lettuces cultivation)	2692	4933	48	41	7713
Water barriers (tomatoes cultivation)	1670	2056	49	428	4202
Water secondary (tomatoes cultivation)	4089	6301	64	556	11009
Water barriers (carrots cultivation)	1378	2782	52	100	4312
Water secondary (carrots cultivation)	4927	5150	120	102	10299
Units: ng/L; UVFs: Ultraviolet filters; Pharm.: Pharmaceuticals; PBs: Parabens					

These results are in accordance with those obtained in Publication #8, where the reactive barrier showed efficient removals for most of the CECs studied. Definitely, CECs levels in the irrigation water strongly influence soils and crops since the levels found in the plots irrigated with the WWTP effluent were higher in all cases. Besides, the high levels found in the irrigation waters of the carrots and tomatoes were very similar, probably because they were cultivated in months of good weather (May and August, respectively). The WWTP receives the most significant volumes of wastewater (and amounts of CECs) in a very tourist area because of population increase. Indeed, the lettuces' irrigation waters, cultivated in winter (December), showed the lowest values.

Considering the levels found in the irrigation waters, it could be expected that the CECs present in the tomatoes and carrots and their soils would be higher. However, carrots soil presents the lowest values (much lower than lettuce soil). This suggests that, since carrots are root vegetables and grow inside the soil, most of the CECs reaching the soil were uptaken by the crop, leaving a low CECs levels in the soil. This is consistent with the uptaken CECs found in carrots, which are the highest by far. Since the levels in irrigation waters were very similar to tomatoes, it suggests that carrots have a greater tendency to accumulate CECs. As tomatoes are a fruit, the CECs will likely have more difficulty reaching them than with carrots, which are in direct contact with the soil and, subsequently, the irrigation water.

According to the type of plant cultivated, significant accumulation trends were also observed among crops. For example, although the UVFs concentration in water was similar in carrots and tomatoes, they were strongly accumulated in the tomatoes. BP3 and all its TPs were detected at considerable concentrations (2.6-77.5 ng/g dw), but other UVFs, such as AVO or 4MBC, were also detected. A similar trend was observed with the accumulation of pharmaceuticals in carrots; the irrigation water concentrations were very similar to those in tomatoes, but the uptaken values were much higher in the carrots. The accumulated concentrations in carrots (1107 ng/g dw) were much higher than in lettuces (54 ng/g dw) when irrigating with the WWTP effluent. Notably, the major contributor to the pharmaceuticals' total load was SCY, naturally generated by plants. However, it presented much lower levels in tomatoes (that naturally biosynthesise more SPY than carrots). Thus, carrots seem to have more affinity to uptake pharmaceuticals, probably due to the direct contact with the soil and irrigation water.

In Publication #12, the study was not performed under real field conditions to help investigate TPs formed by Pak Choi when translocating certain CECs. Since it is a leafy vegetable, the uptaken levels can be compared with the lettuce's regarding behaviour trends. Indeed, the considerable concentration of PBs in the irrigation water (or in the nutrient solution as it was a hydroponic system) did not influence the uptake; PBs were detected in lettuces at low levels (0.1-6.1 ng/g dw range) and were not detected in any of the exposure treatments applied to Pak Choi. These results are in accordance with previous works that targeted PBs in crops (Sabourin et al., 2012). Regarding pharmaceuticals, different behaviours were observed. Compounds with high $logk_{ow}$ values, such as SER or DCF (5.29 and 4.51, respectively), were the pharmaceuticals with the highest concentrations. But others such as clindamycin (CLI), clarithromycin (CLA), sulfisomidine (SMD) and nalidixic acid (NDX) were also detected (> 5 ng/g), even though their lipophilicity varies widely (from -0.33 to 3.16).

This confirms that $logk_{ow}$ values alone have limited accuracy as bioconcentration predictors (Dowdy and Mckone, 1997), and it is preferable to combine them with other properties (e.g. pKa, pH, or ionic strength) (Jurado et al., 2014) to reach reliable predictions. Other families of compounds, such as UVFs or PFAS, were analysed only in one of the two publications, but they were considerably uptaken. This demonstrates that a complementary suspect screening to the target analysis would be needed to provide a holistic picture of the contaminants transferred from the irrigation water to the edible part of crops intended for human consumption.

Influence of cultivation conditions

Regardless of the crop type, other cultivation conditions, as already described for irrigation water quality, can influence the CECs' uptake. The conditions that vary most significantly among studies (besides water quality) are the cultivation soil composition (sandy is the most common, but others use clay, loam or silt) and the irrigation system (dripping, sprinkling, furrow, wetland columns or gravity) (Aiello et al., 2007; de Santiago-Martín et al., 2020; Malchi et al., 2014; Margenat et al., 2017; Palese et al., 2009). In the agricultural plots built in Palamós, it was possible to assess the relevance and impact of each of these variables in the uptake of CECs in lettuces, tomatoes and carrots (**Table 5.2**), presented in Publications #9, #10 and #11.

Table 5.2. Average levels of CECs detected in the different crops separated by cultivation variables (water quality, soil composition and irrigation system) and families (UVFs, PBs, pharmaceuticals and caffeine (CFF)).

Lettuces*						
	Secondary irrigation	**Barriers irrigation**	**Sandy soil**	**Clayey soil**	**Sprinkling irrigation**	**Dripping irrigation**
UVFs	98.8	70.7	83.3	86.6	65.6	104.1
PBs	7.0	5.7	4.7	7.8	3.4	8.8
Pharma.	62.5	60.5	75.9	47.5	56.8	67.9
CFF	17.9	13.5	18.8	13.9	17.1	15.3
Total	186.3	150.3	182.7	155.9	142.9	196.1

*Average values; Units: ng/g dw; UVFs: Ultraviolet filters; Pharm.: Pharmaceuticals; PBs: Parabens

Tomatoes*						
	Secondary irrigation	**Barriers irrigation**	**Sandy soil**	**Clayey soil**	**Sprinkling irrigation**	**Dripping irrigation**
UVFs	217.8	173.4	181.3	210.2	202.8	193.1
PBs	13.3	8.0	11.5	8.6	7.4	12.4
Pharma.	401.0	171.6	448.7	373.3	515.1	352.2
CFF	0	0	0	0	0	0
Total	632.0	353.0	641.6	592.1	725.3	557.8

*Average values; Units: ng/g dw; UVFs: Ultraviolet filters; Pharm.: Pharmaceuticals; PBs: Parabens

Carrots*						
	Secondary irrigation	**Barriers irrigation**	**Sandy soil**	**Clayey soil**	**Sprinkling irrigation**	**Dripping irrigation**
UVFs	141.5	174.1	144.1	156.6	151.1	141.4
PBs	16.5	28.4	19.7	26.1	24.7	21.5
Pharma.	2234.5	1378.2	1797.3	1806.0	1562.3	2040.5
CFF	124.6	15.2	56.4	69.7	45.3	74.7
Total	2517.2	1595.8	2017.4	2058.4	1783.4	2278.0

*Average values; Units: ng/g dw; UVFs: Ultraviolet filters; Pharm.: Pharmaceuticals; PBs: Parabens

Based on the values shown in **Table 5.2**, irrigation water quality was the most relevant factor in lowering the uptake; the crops irrigated with reclaimed water showed the lowest CECs' concentrations in all cases. This difference is especially remarkable in the pharmaceuticals group in the carrots and tomatoes; the irrigation with reclaimed water considerably reduces the uptake in tomatoes (57%) and carrots (38%).

CFF uptake is also significantly reduced in carrots when irrigated with reclaimed water (88%), while in tomatoes, it was not detected in any of the samples.

Soil composition had a moderate influence on the lettuce and tomato CEC's uptake since the clayey soil showed a lower capacity to retain the contaminants (15% and 8%, respectively). In carrots, the concentration values of each family and the total load are very similar for both soil compositions, showing no significant influence of clay on the uptake. As mentioned, this could be explained because they are root vegetables and grow inside the soil. It supposes continuous contact with the contaminants, which can skip the soil processes and reach the crop directly. In general, the crops cultivated in the clayey soil have shown lower uptake values, suggesting a higher dissipation of the CECs in clayey soil (Ghafoor et al., 2011; Wu et al., 2012).

The irrigation system also influenced CECs' uptake; sprinkling irrigation reduced the uptake in lettuces and carrots (27% and 22%, respectively), while dripping irrigation reduced it in tomatoes (23%). A possible explanation for the different behaviours under the same irrigation system is the nature of the crops; lettuces and carrots are in direct contact with the soil, while tomatoes hang from the rack (and avoid direct contact, CECs are transported through the stems). Therefore, sprinkling irrigation helps dissipate CECs in lettuces and carrots, which translocate higher loads of CECs when using dripping irrigation, and thus, higher concentrations of CECs reach their roots. In tomatoes, otherwise, the tomato rack may act as a barrier for CECs uptake, while sprinkling irrigation facilitates the direct contact of the contaminants with the tomato.

Overall, the best combination to lower the CECs uptake in crops was to irrigate with the reclaimed water from the rbSAT system by sprinkling in clayey soil. Since the lowest CECs' values were always found with these variables (independent if lettuce, tomatoes, or carrots were cultivated), it suggests that the water quality, soil composition, and irrigation methodology play an important role in CECs' accumulation. In Publication #12, two nutrient solutions (mineral and organic) were used to cultivate Pak Choi, thus, not a real soil. Still, no statistically significant differences in the CECs uptake or the detected TPs were observed. Indeed, most interaction/degradation processes between the soil and the crop do not occur when cultivating under hydroponic conditions.

Matrix influence and correlations

Comparing crops, the highest uptake was found in carrots, with accumulated values seven and two times higher than in lettuce and tomatoes, respectively. These different bioaccumulation trends, as explained, could be justified by the intrinsic nature of each crop. Carrots are in direct contact with the soil and require large quantities of water (compared to tomatoes and lettuce) during cultivation. Tomatoes are the fruit grown from the tomato rack, deeply rooted plants that probably serve to lower the final uptake. Finally, lettuce are leafy crops, which also seemed to help in reducing the uptake of CECs.

The different matrices analysed in the publications included in this chapter were tested for individual correlations with Pearson's tests for $\alpha = 0.05$ with RStudio open software, v. 1.2.5001 (2019) (R Core Team, 2020). Overall, no statistically significant correlations (Pearson correlation coefficient (r) <0.8, confidence interval (p) >0.05) were observed between the CECs levels found in the irrigation waters, soil and crops (Publications #9, #10, and #11), and nutrient solution and crop (Publication #12). This lack of correlation between CECs occurrence is an issue previously reported in the literature (de Santiago-Martín et al., 2020). This demonstrates that the CECs with the highest concentrations in the irrigation waters or soils are not necessarily the ones that are preferably uptaken by the crops. Several processes and variables are involved. Thus, the only viable solution to know CECs occurrence in crops is their continuous monitoring.

Transformation products

As shown in **Figure 5.1**, a process happening during plant uptake (in both plant and soil) is the degradation of the contaminants. Depending on the stability, residence time, and load, CECs can be degraded in the soil or crop after/during their bioaccumulation. This degradation can lead to their further elimination or formation of TPs that can also be uptaken/bioaccumulated by the crops. As explained in previous chapters, screening these unknown TPs is mandatory to provide outcomes closer to reality, as demonstrated when comparing the results from Publications #9, #10 and #11 with Publication #12. In the first case, the quantification of CECs was performed with a target approach comprising 55 compounds (of which 17 were TPs).

In the second case, from the 18 CECs initially present in the nutrient solution, 17 new TPs were formed (15 from the 4 pharmaceuticals). This suggests that the production of TPs during these processes is highly probable, especially for pharmaceuticals, which are usually degraded/metabolized after human consumption. Most of the found TPs were simple (Phase-I) transformations. Still, others presented complex conjugations (e.g. glucoside), such as Phase II or III transformations, challenging to predict and to be included in target analysis approaches. Indeed, most of the TPs from Publication #12 are reported for the first time in plant metabolism. Thus, the only way to detect and identify these TPs is with a suspect screening. No toxicity information is available for nearly all new TPs, and, thus, further studies are needed to identify potential harmful effects on human health.

5.4 References

Abril, C., Santos, J.L., Martín, J., Aparicio, I., Alonso, E., 2021. Uptake and translocation of multiresidue industrial and household contaminants in radish grown under controlled conditions. Chemosphere 268. https://doi.org/10.1016/j.chemosphere.2020.128823

Aiello, R., Cirelli, G.L., Consoli, S., 2007. Effects of reclaimed wastewater irrigation on soil and tomato fruits: A case study in Sicily (Italy). Agric. Water Manag. 93, 65–72. https://doi.org/10.1016/j.agwat.2007.06.008

de Santiago-Martín, A., Meffe, R., Teijón, G., Martínez Hernández, V., López-Heras, I., Alonso Alonso, C., Arenas Romasanta, M., de Bustamante, I., 2020. Pharmaceuticals and trace metals in the surface water used for crop irrigation: Risk to health or natural attenuation? Sci. Total Environ. 705, 135825. https://doi.org/10.1016/J.SCITOTENV.2019.135825

Ghafoor, A., Jarvis, N.J., Thierfelder, T., Stenström, J., 2011. Measurements and modeling of pesticide persistence in soil at the catchment scale. Sci. Total Environ. 409, 1900–1908. https://doi.org/10.1016/j.scitotenv.2011.01.049

Herklotz, P.A., Gurung, P., Vanden Heuvel, B., Kinney, C.A., 2010. Uptake of human pharmaceuticals by plants grown under hydroponic conditions. Chemosphere 78, 1416–1421. https://doi.org/10.1016/j.chemosphere.2009.12.048

Jodar-Abellan, A., López-Ortiz, M.I., Melgarejo-Moreno, J., 2019. Wastewater treatment and water reuse in Spain. Current situation and perspectives. Water (Switzerland) 11, 17–22. https://doi.org/10.3390/w11081551

Kodešová, R., Klement, A., Golovko, O., Fér, M., Kočárek, M., Nikodem, A., Grabic, R., 2019a. Soil influences on uptake and transfer of pharmaceuticals from sewage sludge amended soils to spinach. J. Environ. Manage. 250, 109407. https://doi.org/10.1016/j.jenvman.2019.109407

Kodešová, R., Klement, A., Golovko, O., Fér, M., Nikodem, A., Kočárek, M., Grabic, R., 2019b. Root uptake of atenolol, sulfamethoxazole and carbamazepine, and their transformation in three soils and four plants. Environ. Sci. Pollut. Res. 26, 9876–9891. https://doi.org/10.1007/s11356-019-04333-9

Libutti, A., Gatta, G., Gagliardi, A., Vergine, P., Pollice, A., Beneduce, L., Disciglio, G., Tarantino, E., 2018. Agro-industrial wastewater reuse for irrigation of a vegetable crop succession under Mediterranean conditions. Agric. Water Manag. 196, 1–14. https://doi.org/10.1016/j.agwat.2017.10.015

Malchi, T., Maor, Y., Tadmor, G., Shenker, M., Chefetz, B., 2014. Irrigation of root vegetables with treated wastewater: Evaluating uptake of pharmaceuticals and the associated human health risks. Environ. Sci. Technol. 48, 9325–9333. https://doi.org/10.1021/es5017894

Margenat, A., Matamoros, V., Díez, S., Cañameras, N., Comas, J., Bayona, J.M., 2017. Occurrence of chemical contaminants in peri-urban agricultural irrigation waters and assessment of their phytotoxicity and crop productivity. Sci. Total Environ. 599–600, 1140–1148. https://doi.org/10.1016/j.scitotenv.2017.05.025

Norton-Brandão, D., Scherrenberg, S.M., van Lier, J.B., 2013. Reclamation of used urban waters for irrigation purposes - A review of treatment technologies. J. Environ. Manage. https://doi.org/10.1016/j.jenvman.2013.03.012

Palese, A.M., Pasquale, V., Celano, G., Figliuolo, G., Masi, S., Xiloyannis, C., 2009. Irrigation of olive groves in Southern Italy with treated municipal wastewater: Effects on microbiological quality of soil and fruits. Agric. Ecosyst. Environ. 129, 43–51. https://doi.org/10.1016/j.agee.2008.07.003

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing.

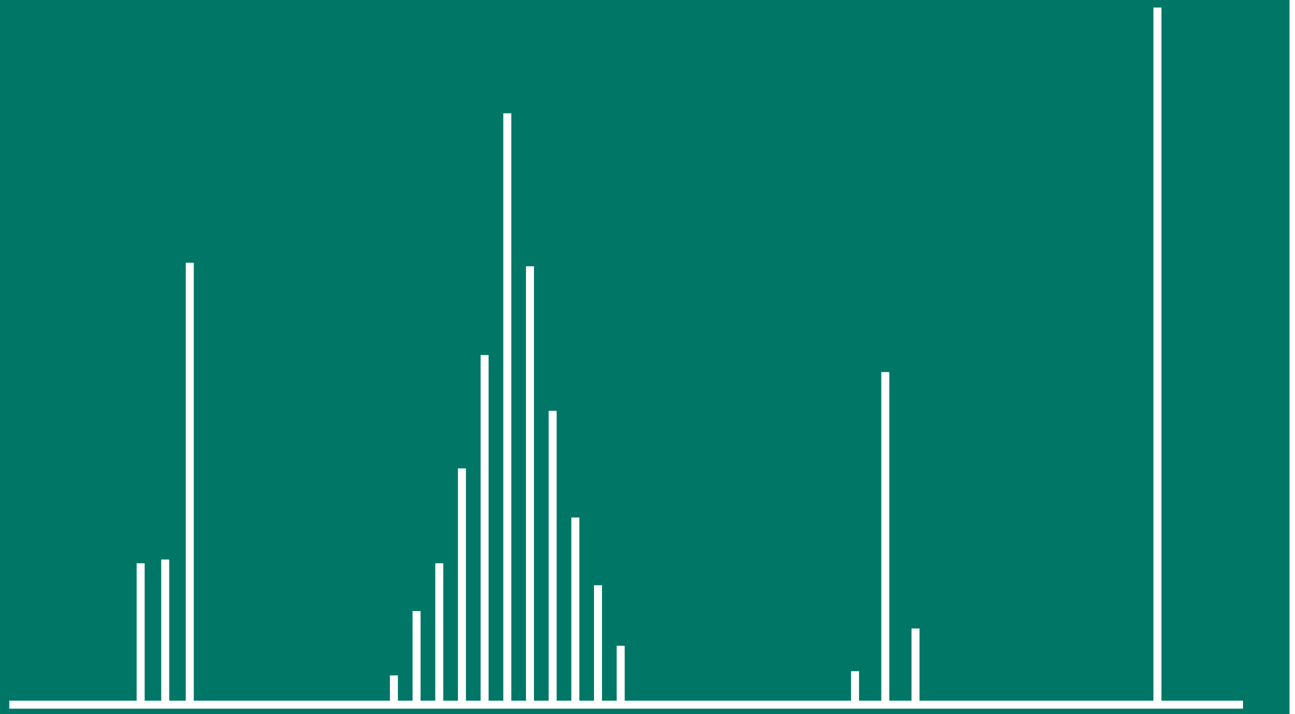
Rigoletto, M., Calza, P., Gaggero, E., Malandrino, M., Fabbri, D., 2020. Bioremediation methods for the recovery of lead-contaminated soils: A review. Appl. Sci. 10. https://doi.org/10.3390/app10103528

Sabourin, L., Duenk, P., Bonte-Gelok, S., Payne, M., Lapen, D.R., Topp, E., 2012. Uptake of pharmaceuticals, hormones and parabens into vegetables grown in soil fertilized with municipal biosolids. Sci. Total Environ. 431, 233–236. https://doi.org/https://doi.org/10.1016/j.scitotenv.2012.05.017

Tadić, Đ., Matamoros, V., Bayona, J.M., 2019. Simultaneous determination of multiclass antibiotics and their metabolites in four types of field-grown vegetables. Anal. Bioanal. Chem. https://doi.org/10.1007/s00216-019-01895-y

Varela, A.R., Manaia, C.M., 2013. Human health implications of clinically relevant bacteria in wastewater habitats. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-013-1594-0

Wu, X., Conkle, J.L., Gan, J., 2012. Multi-residue determination of pharmaceutical and personal care products in vegetables. J. Chromatogr. A 1254, 78–86. https://doi.org/10.1016/j.chroma.2012.07.041



Chapter 6

Human exposure to CECs

6.1 Introduction

So far, this thesis has described how CECs can easily reach environmental compartments (Chapter 4), their mostly low extent of elimination from wastewater in WWTPs (Chapter 5), and their occurrence in crops intended for human consumption (Chapter 6). Therefore, humans are continuously exposed to these contaminants through different pathways (inhalation, ingestion, or direct contact)(Gustafsson et al., 2022).

As already explained in the introductory chapter of this thesis, a wide range of CECs are endocrine disruptors (Wang et al., 2016), display chronic toxicity (Fent et al., 2006) and carcinogenic effects (Amin et al., 2017; Pachkowski et al., 2019). Thus, human exposure to CECs should be minimised. Especially concern deserves exposure during sensitive periods of life to contaminants associated with birth outcomes (e.g. premature birth) or reproductive toxicity (malformations) (Baker et al., 2020; Geer et al., 2017; Kim and Choi, 2014). When the foetus develops during pregnancy, any external factor with adverse but also unknown effects is wholly discouraged.

So far, limited studies are available aiming at monitoring CECs in humans, and thus, little is known about human exposure to CECs and the mechanisms behind it. This lack of data is due, first, to the fact that obtaining human samples is a complicated process that implies ethical issues and, second, to the complexity of the chemical analysis. The most common human samples obtained by non-invasive means are summarised in **Figure 6.1**. Understandably, it is difficult to obtain large cohorts that allow sound conclusions on the relationship between the detected compounds and their potential adverse effects.

Like other biological samples, the chemical analysis of human tissues or fluids is complex due to matrix effects caused by co-extracted matrix components, such as lipids and proteins, which can decrease the analysis' sensibility and the reliability of the results. A commonly used alternative is estimating the daily intake (EDI) of the contaminants found. It uses the quantified concentration of the specific contaminants found, for example, in drinking water or edible organisms such as fish or vegetables. Then, the amount of contaminants ingested through diet is estimated using the average consumption rates of fish, vegetables or water. Finally, a risk assessment of the potential adverse effects on the human body is performed with the calculated daily intake. Since it is an estimation, the obtained values are indicative but constitute the basis for setting maximum values for those contaminants in the corresponding matrix.

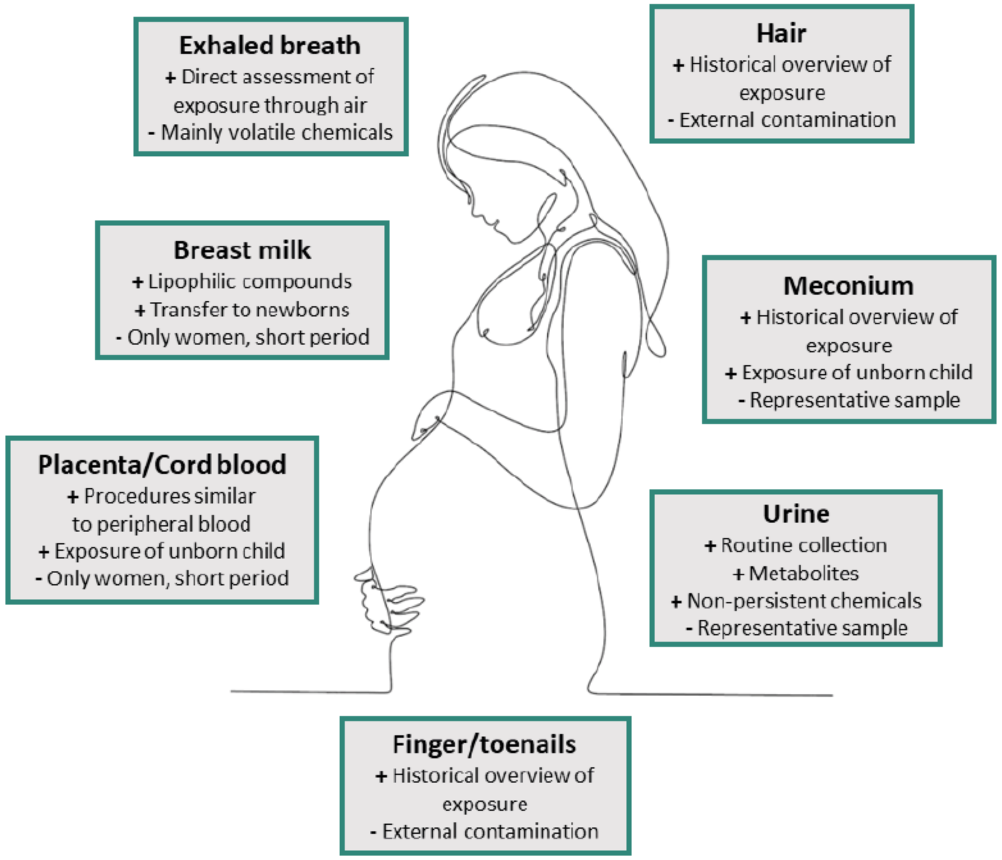


Figure 6.1. Advantages (+) and disadvantages (-) of different non-invasive analysis for human monitoring of CECs. Adapted from Smolders et al., (2009).

Therefore, in this thesis, human exposure to CECs' was addressed through two different pathways. First, exposure through diet was estimated in adults. Secondly, exposure after topical application and mother-foetus transfer. Each case is explained below:

The risk assessment was performed with the calculation of risk quotients, as shown in equation 6.1

(Eq. 6.1)

$$HQ = \frac{ADI}{EDI}$$

where EDI is the estimated daily intake and ADI is the acceptable daily intake. ADI values can be obtained from toxicological studies in the literature, where no observed adverse effect levels (NOAEL) are determined and EDI values are calculated with equation 6.2

(Eq. 6.2)

$$EDI = \frac{DI \cdot C_e}{BW}$$

where Ce is the average compound concentration, DI is the daily intake, and BW is the body weight (population average). The DI is the average quantity of a particular product (e.g. lettuce) consumed daily by an individual, and the BW is the average weight of the population in each study region. Once the HQ are calculated, the highest the value, the more risk to human health. Usually, the HQ values are considered to threaten the health when they are close to or higher than 1.

On the other hand, pre-natal exposure assessment was performed with the biomonitoring of CECs in umbilical cord blood. PCPs were the family selected for the monitoring based on several aspects: (I) they are extensively used, very persistent and present poor degradability, (II) previous studies in biological matrices have shown its widespread occurrence and persistence, (III) humans are exposed to them by multiple pathways, (IV) and scarce information about their biomonitoring in humans is available in the literature (Table 6.1).

Traditional biomonitoring considers only those compounds selected *a priori*, whereas the potential number of compounds to which humans are exposed is higher. Also, developing such targeted methods generally consumes time and resources, which underlines the need for more holistic, high-throughput analytical approaches.

Table 6.1. State-of-the-art on PCPs analysis in human matrices.

State-of-the-arth on PCPs analysis in human matrices					
Reference	**Analysed compounds**	**Cohort**	**Analysis approach**	**Studied matrix***	**Concentrations range**
Casas et. al., (2011)	PBs and phthalates	120 pregnant women, 30 male children	Target analysis	Urine	1.2-755 ng/mL
Meeker et. al., (2011)	PBs	190 men	Target analysis	Urine	0.5-1037 ng/mL
Frederiksen et. al., (2013)	Phenols, PBs and phthalates	848 children, 1311 adolescents, 901 young men, 565 pregnant women	Target analysis	Urine	0.1- 226 ng/mL
Hond et. al., (2013)	Polycyclic musks, PBs and triclosan	210 adolescents, 204 adults	Target analysis	Urine and peripheral blood	0.4-1436 ng/mL and 0.1-0.9 ng/mL
Zhang et. al., (2013)	UVFs	101 adults	Target analysis	Urine and peripheral blood	0.1-46.1 ng/mL and 0.09-3.38 ng/mL
Valle-Sistac et. al., (2016)	UVFs and PBs	12 mothers	Target analysis	Breast milk	0.02-11.8 ng/g
Fisher et. al., (2017)	PBs	31 pregnant women	Target analysis	Urine and breast milk	0.01-1751 ng/mL and 0.1-16.3 ng/mL
Vela-Soria et. al., (2017)	UVFs and PBs	15 mothers	Target analysis	Placenta	0.3-3.6 ng/g
Kolatorova et. al., (2018)	Bisphenols and PBs	27 pregnant women	Target analysis	Peripheral and cord blood	0.02-1.6 ng/mL and 0.05-4.1 ng/mL
Krause et. al., (2018)	UVFs	200 pregnant women	Target analysis	Urine, amniotic fluid, peripheral and cord blood	0.5-10,034 ng/mL, 0.2-11.6 ng/mL, 0.6-71.8 ng/mL and 0.3-10.1 ng/mL
Molins-Delgado et. al., (2018)	UVFs	79 women	Target analysis	Breast milk	3.9-800 ng/g
Li et. al. (2019)	UVFs	53 children	Target analysis	Urine	0.9-336 ng/mL
Park et. al., (2019)	PBs	260 mothers	Target analysis	Breast milk	0.1-42.8 ng/mL
Dualde et. al., (2020)	PBs	120 mothers	Target analysis	Breast milk	0.1-49 ng/mL
Song et. al., (2020)	UVFs	97 pregnant women	Target analysis	Peripheral and cord blood	0.01-34.9 ng/mL and 0.02-56.6 ng/mL
Fernández et. al., (2021)	UVFs and PBs	20 mothers	Target analysis	Placenta	0.2-16.4 ng/g
Frederiksen et. al. (2021)	UVFs	4867 men	Target analysis	Urine, semen and peripheral blood	0.6-644 ng/mL, 0.1-16.3 ng/mL and 0.2-10.7 ng/mL
Gil-Solsona et. al., (2021)	Wide-scope screening	19 pregnant women	Target and non-target	Peripheral blood and placenta	0.01-86 ng/mL and 0.03-840 ng/g
Reimann et. al., (2021)	PBs	229 mothers	Target analysis	Placenta	0.2-169 ng/mL
Wang et. al., (2021)	Wide-scope screening	60 pregnant women	Non-target screening	Peripheral and cord blood	15 confirmed compounds
Uldbjerg et. al., (2022)	Phenols, PBs and phthalates	200 pregnant women	Target analysis	Urine, peripheral blood and amniotic fluid	0.4-52.5 ng/mL, 0.6-1.5 ng/mL and 0.3-5.7 ng/mL

***Serum and blood were considered the same matrix, and were speficied as blood;**
PBs: Parabens; UVFs: Ultraviolet filters; PFAS: per- and polyfluoroalkyl substances

The advances in HRMS have allowed for more rapid characterization and prioritization of compound exposures in humans through non-target and suspect techniques. Therefore, a combination of both approaches was selected; the development of an analytical method was performed to analyse UVFs and PBs (prevalent compounds in PCPs formulations) in the cord blood samples (Publication #13) with a target analysis, and an additional screening of 3,426 PCPs was performed with suspect strategies (Publication #14).

Methodologies

In Publication #13, the development of a new analytical method to analyse PCPs in umbilical cord blood is described. A simplified overview of the developed method is shown in **Figure 6.2**. In brief, the samples were centrifuged, the supernatant was collected and then mixed with a solution of NaCl in MeOH and methyl tert–butyl ether (MTBE) to perform a L-L extraction. Extracts were then frozen (to solidify the aqueous phase), and the recovered organic extract was brought to dryness and further reconstituted with a buffer solution of NH4HCO2 (pH ~ 10) and MeOH. The HPLC-MS/MS methodology was the same applied in Publication #9.

The non-target method applied in Publication #14 followed the method used by Gil-Solsona et al., (2021). Briefly, the extracts were analysed using a C18 column and a Q-Exactive mass analyser under DDA and DIA modes. The suspect screening was performed by uploading the files to the Norman Digital Sample Freezing Platform (Alygizakis et al., 2019) and searching for the compounds included in the database S13 EUCOSMETICS (von der Ohe and Aalizadeh, 2020) from the NORMAN Suspect List Exchange-(NORMAN-SLE) (Taha et al., 2022).

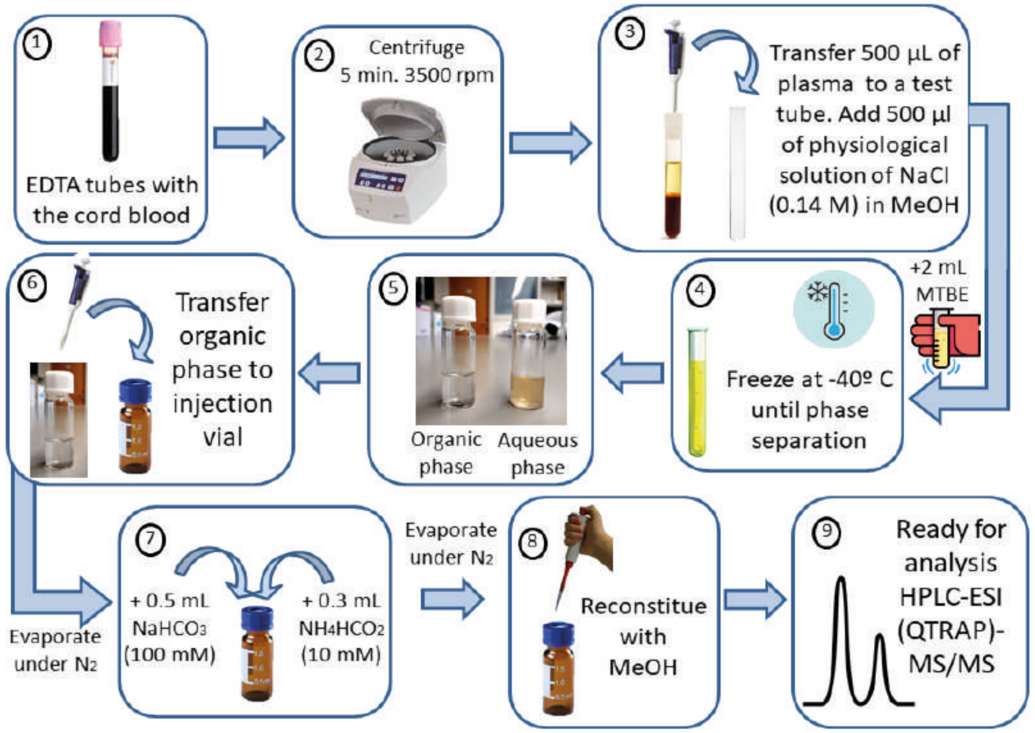


Figure 6.2. Steps followed in the developed methodology for the analysis of PCPs in human umbilical cord blood.

6.2 Results

This Chapter is composed of one published article and one submitted manuscript. In addition to the already discussed results in their corresponding chapters, it includes a CECs' risk assessment that fits better in this chapter, from Publications #4 and #11. Thus, it is provided in the following. The two publications herein included are:

- Publication #13: **Sunyer-Caldú, A.**, Perió, A., Díaz, M., Ibáñez, L., Gago-Ferrero, P., Diaz-Cruz, M. S., "Development of a sensitive analytical method for the simultaneous analysis of benzophenone-type UV filters and paraben preservatives in umbilical cord blood", 2021, *MethodsX*, https://doi.org/10.1016/j.mex.2021.101307

- Publication #14: **Sunyer-Caldú, A.**, Perió, A., Díaz, M., Ibáñez, L., Gil-Solsona, R., Gago-Ferrero, P., Diaz-Cruz, M. S., "Target analysis and suspect screening of UV filters, parabens and other chemicals used in personal care products in human cord blood: prenatal exposure by mother-fetus transfer", *Submitted to Environment International.*

6.2.1 Publication #13

Development of a sensitive analytical method for the simultaneous analysis of benzophenone-type UV filters and paraben preservatives in umbilical cord blood

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Method Article

Development of a sensitive analytical method for the simultaneous analysis of Benzophenone-type UV filters and paraben preservatives in umbilical cord blood

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ABSTRACT

UV filters and parabens are compounds used in large quantities in modern societies and have become ubiquitous in the environment. They are considered compounds of emerging concern due to the unwanted effects they cause in the environment and their bioaccumulation potential in humans. Considering their endocrine disrupting activity and their so far unknown effects in newborns, a continuous monitoring of these substances is required. In this work, we developed and validated a new sensitive methodology for the analysis of 8 UV filters and metabolites, and 4 parabens in umbilical cord blood samples. The method consisted of a liquid-liquid extraction and phase separation by freezing. Then, the organic extract was further analyzed at alkaline pH using liquid chromatography coupled to tandem-mass spectrometry (LC-MS/MS) using a QqLIT hybrid mass spectrometer as analyzer. The low limits of detection achieved (0.01–0.42 ng/mL) allowed the reliable simultaneous quantification of UV filters and parabens in this complex biological matrix.

- Simple, fast and sensitive analysis of UV filters and parabens in cord blood samples.
- First simultaneous analysis of UV filters and parabens in cord blood.
- Allows the evaluation of perinatal transfer of UV filters and parabens from the mother to the fetus.

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Resource availability:	*NA*

Method details

Background

The industrial production and the use of personal care products (PCPs) have increased in recent years. Among these compounds are UV filters and parabens, which are extensively used as sunscreens and as preservatives, respectively. They are present in cosmetics, sunscreens, lotions, hygiene products, but also in foodstuff, plastics, rubbers, and textiles [9]. These compounds are considered contaminants of emerging concern (CECs) for the negative effects they can cause in the environment, including their potential for bioaccumulation [5,6] and biomagnification through the food web [3]. Their bioaccumulation in humans has also been reported [8,12,15,19,20]. This fact combined with their endocrine disrupting activity [1,2,10,13,14] make a regular monitoring of their occurrence necessary. This is even more important in crucial stages of life like pregnancy [4], where the exposure of the unborn to these substances might have short- and long-term consequences in the development of the fetuses. Similar works are reported for the analysis of benzophenones or parabens in cord blood [7,12,16–18]. However, most of these methods use a solid-phase extraction [7,16,18] implying time-consuming and tedious steps, in addition to other steps such as long incubation, and derivatization. Kruse et al. used a laborious method, including an incubation of 3 h. This method was developed to detect benzophenone derivatives in serum (from the mothers and the fetuses) but all the cord blood samples analyzed were below the method limit of detection. Recently Song et al. presented a simpler method, however, it included time-consuming steps (incubation of 12 h and shaking for 60 min). Despite that, only benzophenone-type compounds could be determined. This work describes a sensitive method for the simultaneous analysis of UV filters and paraben preservatives in umbilical cord blood in order to achieve a better understanding of the bioaccumulation of these compounds and their maternal transfer. To this end, a method used for the analysis of bisphenols and parabens [11] was adapted and significantly simplified for the simultaneous analysis of eight benzophenone-type UV filters and metabolites, and four parabens in human cord blood.

Chemicals and reagents

Table 1 lists the selected compounds. The UV filters avobenzone (AVO), benzophenone-2 (BP2), benzophenone-4 (BP4), benzophenone-3 (BP3) and their main metabolites namely benzophenone-1 (BP1), 4-hydroxybenzophenone (4HB), 4,4′-dihydroxybenzophenone (4DHB), and 2,2′-dihydroxy-4-methoxybenzophenone (DHMB, BP8), and the paraben preservatives methyl paraben (MePB), propyl paraben (PrPB), benzyl paraben (BePB), and butyl paraben (BuPB) were purchased from Sigma Aldrich (Darmstadt, Germany). The isotopically labelled compounds 2–hydroxy-4–methoxy-2′,3′,4′,5′,6′-d5 (BP3-d5), benzyl paraben-d4 (BePB-d4), and 5-(2,5-dimethylphenoxy)–2,2-bis(trideuteriomethyl)pentanoic acid (Gemfibrozil-d6) were purchased from CDN isotopes (Quebec, Canada). Water and methanol (MeOH) of high performance liquid chromatography (HPLC) grade were obtained from J.T. Backer (Deventer, The Netherlands) and the nitrogen (99.995% purity) was supplied by Air Liquide (Barcelona, Spain). Formic acid (HCOOH) and ammonium acetate (AcNH4) were from Merck (Darmstadt, Germany). For the extraction process methyl tert–butyl ether (MTBE), sodium chloride (NaCl), sodium hydrogen-carbonate (NaHCO3) from Sigma Aldrich, and ammonium formate (NH4HCO2) from Fisher Scientific (Fair Lawn, New Jersey, EEUU) were used.

A mix of isotopically labelled internal standards containing BP3-d5, BePB-d4 and gemfibrozil-d6 was prepared in MeOH with the appropriate volume of the standard stock solutions at a concentration of 200 ng/mL, and was stored at -20º C.

Ethical aspects

Cord blood samples were provided by the Sant Joan de Déu Hospital (Barcelona, Spain), and were donated voluntarily by the mothers, who were asked to sign an informed consent to participate in the study, well before delivery. The present study was approved by the Ethics Review Board of the University of Barcelona and Sant Joan de Déu Hospital. All the data compiled were saved following the current regulation on Protection of Personal Data and guarantee of digital rights (Ley Orgánica 3/2018).

Sampling and sample extraction

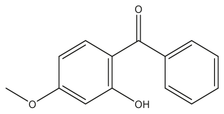
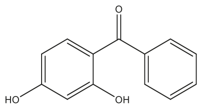
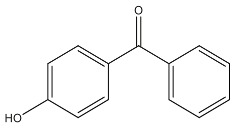
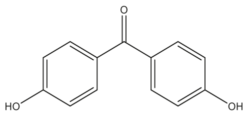
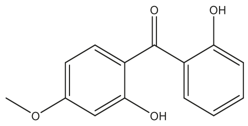
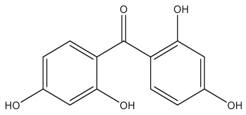
Umbilical cord blood samples were collected in metal-free serum tubes (to have the serum component of the blood) after direct extraction by venipuncture from the umbilical cords obtained immediately after delivery. The biological samples were stored at Sant Joan de Déu Hospital following the Spanish Law of Biomedical Investigation of 2007 (Law 14/2007) until shipment via urgent courier to the IDAEA-CSIC laboratories for analysis. All samples were received in perfect conditions and correctly codified, and were preserved frozen until analysis.

Samples were centrifuged at 3500 rpm during 5 min to remove cell devris and the serum was collected with a Pasteur pipette for further analysis. Then, 500 µL of each serum sample were spiked with 100 µL of the mix of internal standards solution and 500 µL of a physiological solution of NaCl previously prepared with MeOH (0.137 M). Isolation of the target analytes was carried out by liquid-liquid extraction adding 2 mL of MTBE. The mix was vigorously shaken and the samples were frozen until the organic and aqueous phases were separated. The organic phases were transferred with a Pasteur pipette to 2 mL HPLC-vials and further evaporated until almost dryness under a gentle current of nitrogen. Then, 0.5 mL of $NaHCO_3$ (100 mM) were added up to pH 10.5 and the samples were incubated at 60 ºC for 5 min. Further, the samples were evaporated again under a stream of nitrogen until near dryness and then, 0.3 mL of the buffer NH_4HCO_2 (10 mM) and 0.3 mL of MeOH were added to dilute the samples up to 1:1 (v:v) proportion. Finally, the extracts were brought to dryness and further reconstituted with 1 mL of MeOH. The extracts were stored at -20ºC until HPLC-MS/MS analysis.

Instrumental analysis

The chromatographic separation of the compounds was performed in a Hibar Purosher® STAR® HR R-18 (50 mm × 2.0 mm, 5 μm) column using a Symbiosis^{TM} Pico instrument from Spark Holland (Emmen, The Netherlands). Detection was carried out in a 4000 Q TRAP^{TM} hybrid quadrupole-linear ion trap mass spectrometer from Applied Biosystems-Sciex (Foster City, CA, USA). Mobile phases consisted of MeOH and H_2O 0.1% HCOOH in positive ionization mode determination, and MeOH and H_2O 5 mM AcNH4 in negative ionization mode, respectively. The detailed gradient profiles are shown in Tables 2 and 3. The injection volume was set up to 20 µL. Electrospray ionization in positive (ESI+) and negative (ESI-) modes were selected. Tandem-mass spectrometry detection (MS/MS) was performed under selected reaction monitoring (SRM) mode for improved sensitivity and selectivity. The two most intense transitions were selected and used for the quantification (most intense, 1st

Table 1
Target compounds name, acronym, family, CAS number, molecular mass, chemical structure and log octanol-water partition coefficient.

Compound	Other names	Family	CAS number	Molecular mass (g/mol)	Structure	log k_{ow}
Benzophenone-3 (BP3)	Oxybenzone; 2-Hydroxy-4-methoxybenzophenone	Benzophenones	131-57-7	228.24		3.79
Benzophenone-1 (BP1)	2,4-Dihhydroxybenzophenone	Benzophenones	131-56-6	214.22		3.15
4-Hydroxybenzophenone (4HB)	–	Benzophenones	1137-42-4	193.18		2.92
4,4′-Dihydroxybenzophenone (DHB)	–	Benzophenones	611-99-4	214.22		2.19
2,2′-Dihydroxy-4-methoxybenzophenone (DHMB, BP8)	Benzophenone-8; Dioxybenzone	Benzophenones	131-53-3	244.25		3.82
Benzophenone-2 (BP2)	2,2′,4,4′-Tetrahydroxybenzophenone	Benzophenones	131-55-5	246.22		2.78

(continued on next page)

Table 1 (*continued*)

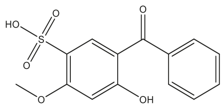
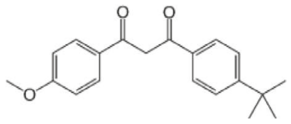
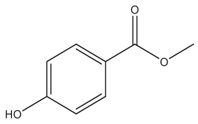
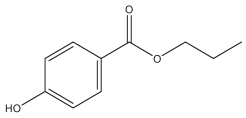
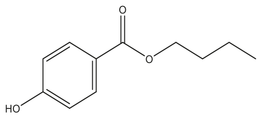
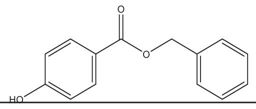
Compound	Other names	Family	CAS number	Molecular mass (g/mol)	Structure	log k_{ow}
Benzophenone-4 (BP4)	5-benzoyl-4-hydroxy-2-methoxybenzene sulfonic acid; HMBS; Sulisobenzone	Benzophenones	4065-45-6	308.31		0.88
Avobenzone (AVO)	1-(4-tert-butylphenyl)-3-(4-methoxyphenyl)propane-1,3-dione	Benzophenones	70,356-09-1	31,017		4.51
Methyl paraben (MePB)	Methyl 4-hydroxybenzoate	Parabens	99-76-3	152		2
Propyl paraben (PrPB)	Propyl 4-hydroxybenzoate	Parabens	94-13-3	180.2		2.98
Butyl paraben (BuPB)	Butyl 4-hydroxybenzoate	Parabens	94-26-8	194.23		3.47
Benzyl paraben (BePB)	Benzyl 4-hydroxybenzoate	Parabens	94-18-8	228.24		3.7

Table 2
Mobile phases used in positive mode, with its gradient flow and time.

Positive ionization			
Time (min)	% Mobile Phase A*	% Mobile Phase B^	Flow (mL/min)
0	95	5	0.3
7	25	75	0.3
10	0	100	0.3
15	0	100	0.3
17	95	5	0.3
23	95	5	0.3

* A: H_2O 0,1% in HCOOH;.
^ B: MeOH 0,1% in HCOOH.

Table 3
Mobile phases used in negative mode, with its gradient flow and time.

Negative ionization			
Time (min)	% Mobile Phase A*	% Mobile Phase B^	Flow (mL/min)
0	95	5	0.3
3	50	50	0.3
6	10	90	0.3
13	0	100	0.3
17	0	100	0.3
18	95	5	0.3
20	95	5	0.3

* A: H_2O 5 mM AcNH4.
^ B: MeOH 5 mM AcNH4.

Table 4
Retention time (t_R), selected MS/MS transitions, internal standard (IS) used, and ionization parameters for each compound. (-) for those analyzed in negative ionization mode.

Compound	t_R	1st transition	DP (V)	CE (eV)	CxP (eV)	2nd transition	DP (V)	CE (eV)	CxP (eV)	IS
BP3	12.12	229>151	40	25	12	229>105	40	27	16	BP3-d_5
BP1	11.39	215>137	40	27	10	215>105	40	29	6	BP3-d_5
4HB	11.36	199>121	40	25	8	199>105	40	27	8	BP3-d_5
4DHB	10.41	215>121	45	27	8	215>93	45	45	6	BP3-d_5
DHMB	11.93	245>121	43	29	8	245>151	43	27	12	BP3-d_5
BP2	10.89	247>137	46	25	8	247>109	46	45	8	BP3-d_5
BP4 (-)	8.42	307>227	-50	-34	-15	307>211	-70	-40	-9	Gemfibrozil-d_6
AVO	13.04	311>135	40	25	15	311>161	40	25	15	BP3-d_5
BePB (-)	9.5	227>92	-65	-26	-9	227>136	-65	-22	-1	BePB-d_4
BuPB (-)	9.54	193>137	-55	-22	-5	193>92	-55	-34	-13	BePB-d_4
PrPB (-)	9.22	179>92	-60	-30	-13	179>137	-60	-24	-5	BePB-d_4
MePB (-)	8.5	151 > 92	-45	-28	-7	151>136	-45	-20	-9	BePB-d_4

DP: Declustering potential (V); CE: Collision energy (eV); CxP: Collision cell exit potencial (eV);

transition) and confirmation (second most intense, 2nd transition) of each compound. The principal parameters of the developed HPLC-MS/MS method, including chromatographic retention time (t_R), selected transitions and ionization parameters are compiled in Table 4. Analytical standards, reagent blank samples, and quality control solutions were included in each analysis batch together with the serum extracts. The Analyst v. 1.4.2 software package (Applied Biosystems) was used for acquisition and data analysis processing

Table 5
Limits of detection (MLODs) and quantification (MLOQs) of the method (expressed in ng/ml blood sample) and determination coefficient (r^2) for each compound.

	MLOD (ng/ml)	MLOQ (ng/mL)	r2
BP3	0.3	1.01	0.9997
BP1	0.08	0.28	0.9984
4HB	0.42	1.39	0.9982
DHB	0.05	0.18	0.9982
DHMB	0.14	0.48	0.9995
BP2	0.16	0.53	0.9974
BP4 (-)	0.26	0.85	0.9988
AVO	0.35	1.17	0.9992
MePB (-)	0.41	1.38	0.9993
PrPB (-)	0.23	0.75	0.9991
BuPB (-)	0.18	0.61	0.9969
BePB (-)	0.01	0.04	0.9986

MLOD: Limit of detection of the method; MLOQ: Limit of quantification of the method.

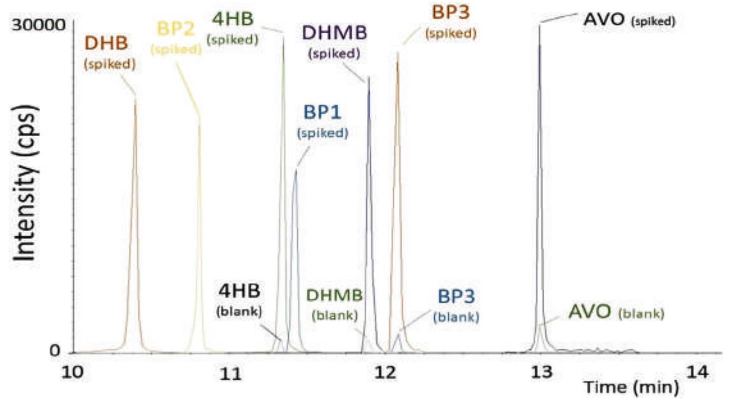


Fig. 1. Reconstructed ion chromatograms showing the SRM 1st transition obtained in the spiked samples at 5 ng/mL and in the procedural blanks using positive ionization (ESI+).

Quality assurance and quality control

One of the most common problems in trace analysis is background contamination. Therefore, procedural blanks were processed and analyzed. The procedural blanks were prepared using 500 µL of HPLC water and submitted to all the steps in the sample analysis. No quantifiable peaks of the target analytes were measured, as shown in Figs. 1 and 2, where the peak area of the spiked samples at 5 ng/mL are notably higher than those of the blanks. Furthermore, all the glass material was cleaned with MeOH and acetone and dried at 400 ºC overnight before use. Quality controls (mix of standards at known concentrations) were randomly measured along the samples' analysis sequence to ensure a reliable determination. The t_R of the compounds were compared at a tolerance of 2.5% maximum, and the relative ion intensities of the two SRM transitions (1st transition / 2nd transition) were compared at a tolerance level below 15% with those of the standards. The target compounds were identified following EU normative (Commission Decision 2002/657/EC). Isotopically labelled standards for each family of compounds were used to overcome potential matrix effects and thus, for proper quantification. The calibration curves were built through ten mix standard solutions at 1, 3, 5, 10, 30, 50, 100, 300, 500 and 700 ng/mL spiked in the matrix (matrix matched standards).

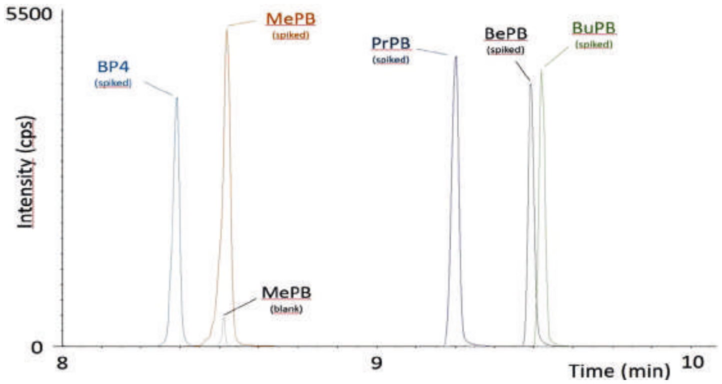


Fig. 2. Reconstructed ion chromatograms showing the SRM 1st transition obtained in the spiked samples at 5 ng/mL and in the procedural blanks using negative ionization (ESI-).

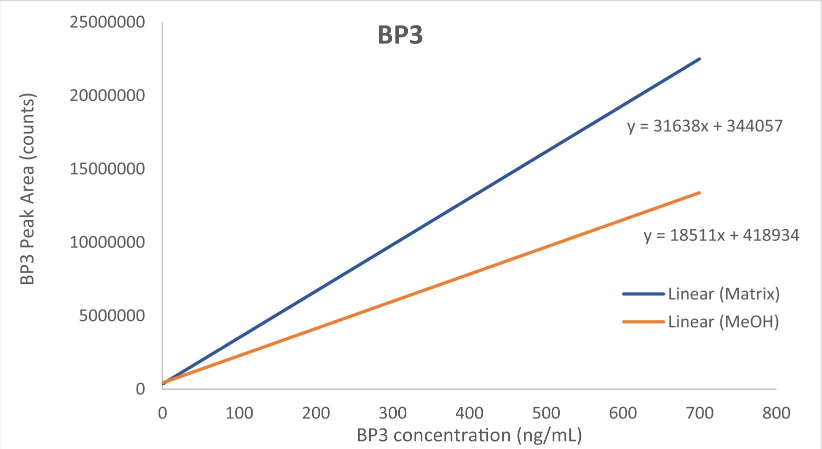


Fig. 3. Calibration curves for BP3 showing the enhancement of the signal in the serum matrix in comparisson with MeOH.

Method validation

A number of the received samples was pooled to obtain a representative mixture of the umbilical cord serum, that was needed to validate the proposed method. Ten aliquots of 500 µL of the pool samples were collected to elaborate the validation samples. These 10 samples were spiked at two concentrations (50 and 400 ng/mL) with the mix of the target compounds. The developed method was evaluated under optimized conditions in terms of linearity range, sensitivity, accuracy, precision, and matrix effects.

The method limits of detection (MLODs) and quantification (MLOQs), and the coefficient of determination (r^2) are listed in Table 5. MLODs and MLOQs were calculated as the concentration of each compound giving a signal-to noise ratio of 3 and 10, respectively. A wide linearity interval 1–700 ng/mL was obtained for all the compounds, with $r^2 > 0.9969$. The method was highly sensitive, with MLODs in the range 0.01–0.42 ng/mL blood.

Considering the high complexity of the sample composition, matrix effects were expected, and consequently, evaluated. Two representative examples of the calibration curves in the matrix extract

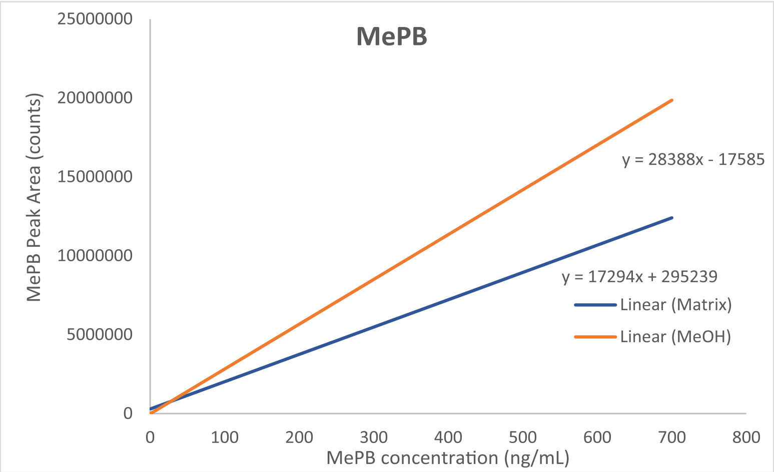


Fig. 4. Calibration curves for MePB showing the suppression of the signal in the serum matrix in comparisson with MeOH in comp.

Table 6
Recovery rates (%) obtained from the spiked samples at the two concentration levels tested.

Validation sample	BP3	BP1	4HB	DHB	DHMB	BP2	BP4 (-)	AVO	BePB (-)	BuPB (-)	PrPB (-)	MEPB (-)
50 ng/mL (1)	89.8	134.4	109.4	94.2	107.4	94.4	17.08	31.2	86.4	100.2	111	121.6
50 ng/mL (2)	83.4	101.2	110.4	87	114.4	72.6	28.4	31.4	87.8	97.4	108	110.6
50 ng/mL (3)	83.8	142.8	123.6	128.8	82.6	82.8	14.28	24.8	81.6	93.8	105.4	114.2
50 ng/mL (4)	85.6	131.2	136.4	101.2	110.8	100.4	19.3	23.6	81.4	93.2	103.4	111.6
50 ng/mL (5)	90.6	146.4	120.4	121.2	121.4	106.4	16.58	21.6	80.4	92.4	104.8	118.6
400 ng/mL (1)	91.5	95	104	98.75	74.5	21.6	68.25	15.52	85.75	101.2	91	77
400 ng/mL (2)	106	96.25	97.5	99.75	65	27.25	66.25	21.77	84.5	103.5	91.75	74.5
400 ng/mL (3)	110	92	104.2	103.2	57.75	20.5	68	25.25	88.75	100	95.25	75.75
400 ng/mL (4)	106.5	93	94.5	84.75	63.5	14.75	82.75	15.8	90.5	97.75	97.75	72.25
400 ng/mL (5)	113.7	96	103.7	105.2	74.5	18.57	74.25	19.17	86.75	101.5	92.75	74.75
Average 50 ng/mL	86.6	131.2	120.0	106.5	107.3	91.3	19.1	26.5	83.5	95.4	106.5	115.3
Average 400 ng/mL	105.5	94.45	100.8	98.35	67.05	20.53	71.9	19.50	87.25	100.8	93.7	74.85

(1), (2), (3), (4), (5): Number of replica; (-); Analyzed in negative mode.

Table 7
Relative standard deviation (RSD%) for inter- and intra-day precision.

	RSD% Intra C1	RSD% Intra C2	RSD% Inter C1	RSD% Inter C2
BP3	1.68	33.8	8.45	10.41
BP1	8.93	7.5	2.56	15.07
4HB	5.51	18.05	0.72	12.09
DHB	8.91	32.17	2.7	2.8
DHMB	7.38	29.28	0.46	15.47
BP2	6.81	18.3	7.04	18.49
BP4 (-)	1.66	9.56	4.45	15.77
AVO	2.25	16.47	1.59	0.82
MePB (-)	1.64	8.47	3.1	7.68
PrPB (-)	1.5	11.1	7.21	14.21
BuPB (-)	2.34	7.02	5.99	11.61
BePB (-)	2.74	27.11	9.25	17.84

C1: Spiked concentration 1; C2: Spiked concentration 2; RSD%: Relative standard deviation; Intra: Intra-day; Inter: Inter-day.

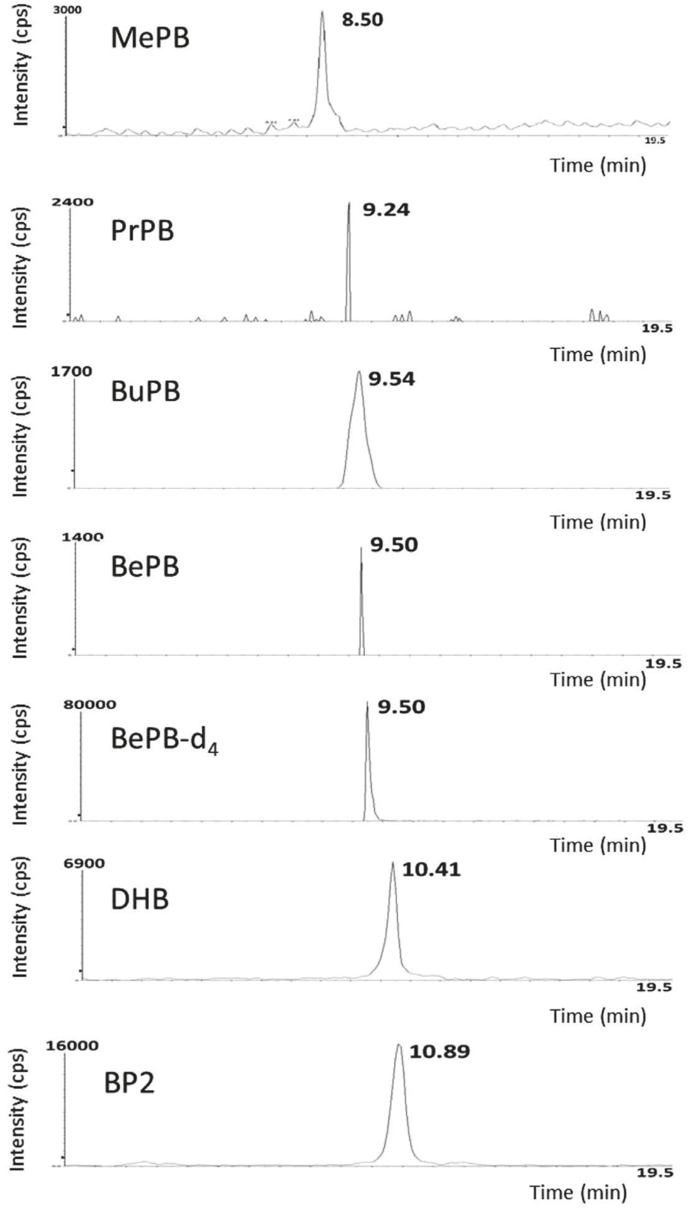


Fig. 5. Reconstructed ion chromatograms showing the SMR first selected transition, in ascendant chromatographic retention time (t_R) order, corresponding to a cord serum sample.

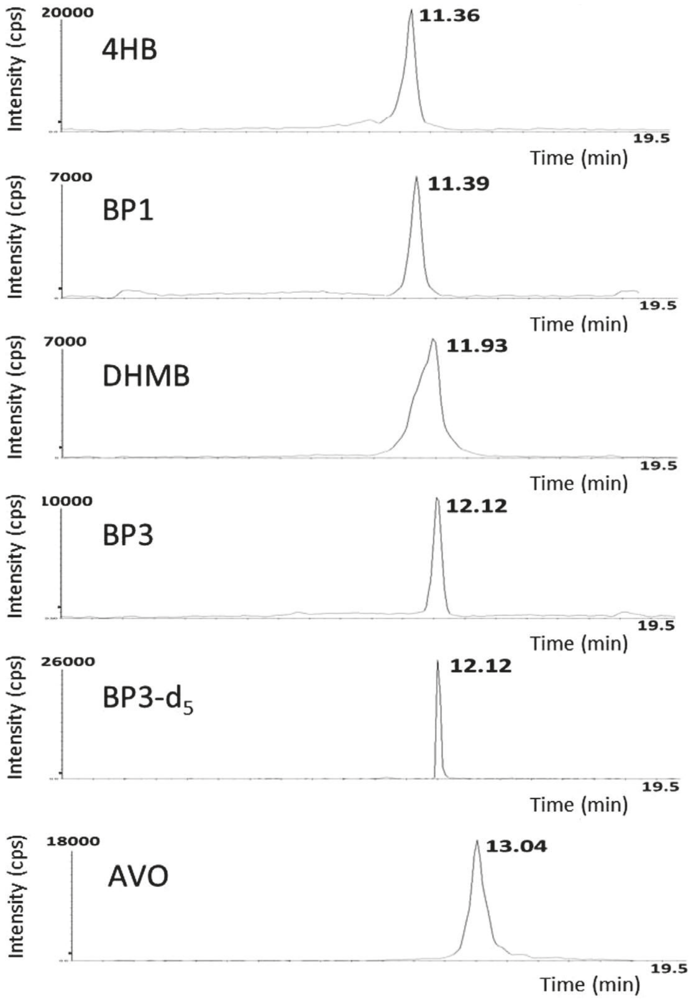


Fig. 5. Continued

(matrix-matched standards) and in the organic solvent are shown in Figs. 3 and 4. The large differences in the slope of the curves showed that, in these cases, BP3 signal suffers from signal enhancement in the presence of the matrix, and MePB experienced signal suppression, as indicated by the different slope obtained in the two media. Therefore, the matrix effects observed were significant, and demanded consideration. Thus, matrix matched calibration curves were used for all the analytes studied and were prepared using the pool of the samples created for the validation of the method.

Table 6 lists the recovery rates obtained at the two spiked concentration levels. Despite generally good recoveries were obtained, between 80 and 120%, BP2 was scarcely recovered (c.a. 20%) at the higher spike level (400 ng/mL); however, at low concentration the recovery was quite good (≈ 91.3%). Considering the complexity of the samples analyzed, occurrence levels are not expected to reach this high concentration, and thus BP2 was also included in the method. AVO, on the other hand, presented medium-to-low recoveries (15.5–31.4%) at both concentrations, so it was also included in the method, but the obtained concentration values were considered semi-quantitative. Finally, BP4 had the opposite behavior than BP2. It showed a good recovery at high concentration (c.a. 71.9%), but small recovery at the low concentration (c.a.19%), and, therefore we decided to proceed as for AVO.

Repeatability and reproducibility were evaluated (Table 7). Intra-day RSD values (1.5–32%) and inter-day RSD values (0.5–18%) indicated quite good precision for the complex matrix.

As an applicability example of the developed method, Fig. 5 shows the reconstructed ion chromatograms corresponding to the UV filters and parabens detected in the serum of a cord blood sample. All the target compounds were detected and quantifiable, at concentrations from 0.20 to 53.3 ng/mL.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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References

[1] J. Boberg, C. Taxvig, S. Christiansen, U. Hass, Possible endocrine disrupting effects of parabens and their metabolites, Reprod. Toxicol. 30 (2) (2010) 301–312, doi:10.1016/j.reprotox.2010.03.011.

[2] P.D. Darbre, P.W. Harvey, Paraben esters: review of recent studies of endocrine toxicity, absorption, esterase and human exposure, and discussion of potential human health risks, J. Appl. Toxicol. 28 (5) (2008) 561–578, doi:10.1002/jat.1358.

[3] K. Fent, A. Zenker, M. Rapp, Widespread occurrence of estrogenic UV-filters in aquatic ecosystems in Switzerland, Environ. Pollut. 158 (5) (2010) 1817–1824, doi:10.1016/j.envpol.2009.11.005.

[4] T.P. Fleming, A.J. Watkins, M.A. Velazquez, J.C. Mathers, A.M. Prentice, J. Stephenson, M. Barker, R. Saffery, C.S. Yajnik, J.J. Eckert, M.A. Hanson, T. Forrester, P.D. Gluckman, K.M. Godfrey, Origins of lifetime health around the time of conception: causes and consequences, Lancet 391 (10132) (2018) 1842–1852, doi:10.1016/S0140-6736(18)30312-X.

[5] P. Gago-Ferrero, M.S. Díaz-Cruz, D. Barceló, An overview of UV-absorbing compounds (organic UV filters) in aquatic biota, Anal. Bioanal. Chem. 404 (9) (2012) 2597–2610, doi:10.1007/s00216-012-6067-7.

[6] P. Gago-Ferrero, M.S. Díaz-Cruz, D. Barceló, UV filters bioaccumulation in fish from Iberian river basins, Sci. Total Environ. 518–519 (2015) 518–525, doi:10.1016/j.scitotenv.2015.03.026.

[7] L.A. Geer, B.F.G. Pycke, J. Waxenbaum, D.M. Sherer, O. Abulafia, R.U Halden, Association of birth outcomes with fetal exposure to parabens, triclosan and triclocarban in an immigrant population in Brooklyn, New York, J. Hazard. Mater. 323 (2017) 177–183, doi:10.1016/j.jhazmat.2016.03.028.

[8] E.P. Hines, P. Mendola, O.S. von Ehrenstein, X. Ye, A.M. Calafat, S.E. Fenton, Concentrations of environmental phenols and parabens in milk, urine and serum of lactating North Carolina women, Reprod. Toxicol. 54 (2015) 120–128, doi:10.1016/j.reprotox.2014.11.006.

[9] Y. Kameda, K. Kimura, M. Miyazaki, Occurrence and profiles of organic sun-blocking agents in surface waters and sediments in Japanese rivers and lakes, Environ. Pollut. 159 (6) (2011) 1570–1576, doi:10.1016/j.envpol.2011.02.055.

[10] K.L. Kinnberg, G.I. Petersen, M. Albrektsen, M. Minghlani, S.M. Awad, B.F. Holbech, J.W. Green, P. Bjerregaard, H. Holbech, Endocrine-disrupting effect of the ultraviolet filter benzophenone-3 in zebrafish, Danio rerio, Environ. Toxicol. Chem. 34 (12) (2015) 2833–2840, doi:10.1002/etc.3129.

[11] L. Kolatorova, J. Vitku, R. Hampl, K. Adamcova, T. Skodova, M. Simkova, A. Parizek, L. Starka, M. Duskova, Exposure to bisphenols and parabens during pregnancy and relations to steroid changes, Environ. Res. 163 (2018) 115–122 Februaryhttps://doi.org/, doi:10.1016/j.envres.2018.01.031.

[12] M. Krause, H. Frederiksen, K. Sundberg, F.S. Jørgensen, L.N. Jensen, P. Nørgaard, C. Jørgensen, P. Ertberg, A. Juul, K.T. Drzewiecki, N.E. Skakkebaek, A.M. Andersson, Presence of benzophenones commonly used as UV filters and absorbers in paired maternal and fetal samples, Environ. Int. 110 (2018) 51–60 April 2017https://doi.org/, doi:10.1016/j.envint.2017.10.005.

[13] M. Krause, A. Klit, M. Blomberg Jensen, T. Søeborg, H. Frederiksen, M. Schlumpf, W. Lichtensteiger, N.E. Skakkebaek, K.T. Drzewiecki, Sunscreens: are they beneficial for health? An overview of endocrine disrupting properties of UV-filters, Int. J. Androl. 35 (3) (2012) 424–436, doi:10.1111/j.1365-2605.2012.01280.x.

[14] P.Y. Kunz, H.F. Galicia, K. Fent, Comparison of *in vitro* and *in vivo* estrogenic activity of UV filters in fish, Toxicol. Sci. 90 (2) (2006) 349–361, doi:10.1093/toxsci/kfj082.

[15] D. Molins-Delgado, M.d.M. Olmo-Campos, G. Valeta-Juan, V. Pleguezuelos-Hernández, D. Barceló, M.S. Díaz-Cruz, Determination of UV filters in human breast milk using turbulent flow chromatography and babies' daily intake estimation, Environ. Res. 161 (2018) 532–539 September 2017https://doi.org/, doi:10.1016/j.envres.2017.11.033.

[16] B.F.G. Pycke, L.A. Geer, M. Dalloul, O. Abulafia, R.U Halden, Maternal and fetal exposure to parabens in a multiethnic urban U.S. population, Environ. Int. 84 (2015) 193–200, doi:10.1016/j.envint.2015.08.012.

[17] S. Song, Y. He, Y. Huang, X. Huang, Y. Guo, H. Zhu, K. Kannan, T. Zhang, Occurrence and transfer of benzophenone-type ultraviolet filters from the pregnant women to fetuses, Sci. Total Environ. 726 (2020) 138503, doi:10.1016/j.scitotenv.2020.138503.

[18] C.V. Towers, P.D. Terry, D. Lewis, B. Howard, W. Chambers, C. Armistead, B. Weitz, S. Porter, C.J. Borman, R.C.M. Kennedy, J Chen, Transplacental passage of antimicrobial paraben preservatives, J. Expo. Sci. Environ. Epidemiol. 25 (6) (2015) 604–607, doi:10.1038/jes.2015.27.

[19] J. Valle-Sistac, D. Molins-Delgado, M. Díaz, L. Ibáñez, D. Barceló, M.S. Diaz-Cruz, Determination of parabens and benzophenone-type UV filters in human placenta: first description of the existence of benzyl paraben and benzophenone-4, Environ. Int. 88 (2016) 243–249, doi:10.1016/j.envint.2015.12.034.

[20] T. Zhang, H. Sun, X. Qin, Q. Wu, Y. Zhang, J. Ma, K. Kannan, Benzophenone-type UV filters in urine and blood from children, adults, and pregnant women in China: partitioning between blood and urine as well as maternal and fetal cord blood, Sci. Total Environ. 461–462 (2013) 49–55, doi:10.1016/j.scitotenv.2013.04.074.

6.2.2 Publication #14

Target and suspect screening of UV filters and parabens personal care products (PCPs) in human cord blood: prenatal exposure after mother-foetus transfer

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Submitted to Environment International

Target analysis and suspect screening of UV filters, parabens and other chemicals used in personal care products in human cord blood: prenatal exposure by mother-fetus transfer

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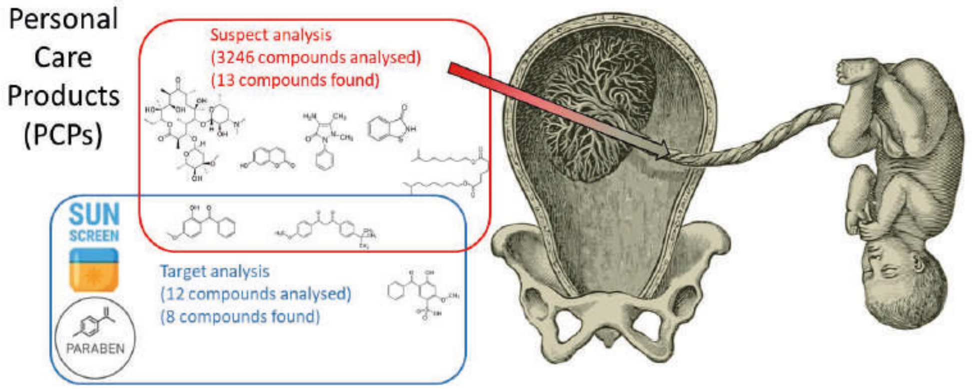
Graphical abstract:



Keywords:

Exposome, umbilical cord blood, personal care products, HRMS, UV filters, parabens

Highlights:

· First study reporting cord blood concentrations of avobenzone UV filter.

· The most frequently detected and predominant paraben was methylparaben.

· Eight compounds are reported in cord blood for the first time (level 1).

· UVFs' and PBs' presence in the umbilical cord blood demonstrates mother-fetus transfer.

· PCPs are capable to cross the placental barrier and suppose an exposure to the fetus.

Abstract

Prenatal exposure to certain organic chemicals like pesticides and phenols has been lifelong associated with birth outcomes and health disorders. Many personal care product (PCP) ingredients have similar properties or structures to those chemicals. Previous studies have documented the occurrence of UF filters (UVFs) and parabens in the placenta, but observational studies concerning PCPs and humans are particularly scarce. Thus, this work aimed to assess the presence of a wide range of PCPs using target and suspect screening in the umbilical cord blood of pregnant women to evaluate their potential transfer to the fetus. To do so, we analysed 69 umbilical cord blood plasma from a mother-child cohort from Barcelona (Spain). We quantified 8 benzophenone-type UVFs and their metabolites, and 4 parabens using validated analytical methodologies based on target screening using liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Then, we screened for additional 3246 substances using high-resolution mass spectrometry (HRMS) and advanced suspect strategies. Six UV filters and three parabens were detected in the plasma with frequencies between 1.4% and 17.4% and concentrations up to 53.3 ng/mL (benzophenone-2). Thirteen additional chemicals were tentatively identified in the suspect screening, and ten were further confirmed with the corresponding standards. Among them, we found the organic solvent N-methyl-2-pyrrolidone, the chelating agent 8-hydroxyquinoline, and the antioxidant 2,2'-methylenebis(4-methyl-6-tert-butylphenol), which have been demonstrated to display reproductive toxicity. UVFs and PBs presence in the umbilical cord blood demonstrates mother-fetus transfer through the placental barrier and prenatal exposure to these PCPs, which may lead to adverse effects in the early stages of fetal development. Considering the small cohort used in this study, the reported results should be interpreted as a preliminary reference for the background umbilical cord transfer levels of the target PCPs. Further research is needed to determine the long-term consequences of prenatal exposure to PCPs.

Introduction

Pregnancy is a critical period in human life as the human fetus is being developed. Chemical exposure is especially concerning as it is well-known that intrauterine life is a highly vulnerable period and sensitive to any external change because of the immaturity of fetal organs and undeveloped detoxification systems (Braun et al., 2014). During this time, the xenobiotics that women have been exposed to may accumulate in their tissues, cross the placenta, and reach the fetus. Despite the placenta being expected to constitute a barrier for chemical xenobiotics (Zhang et al., 2013), it has been demonstrated that it cannot fully protect the fetus from the entrance of all the contaminants (Towers et al., 2015). This early life exposure to pollutants, parental dietary habits and lifestyle can influence not only the development and growth of the fetus during pregnancy but also the newborn's health throughout their entire lifetime (Fleming et al., 2018).

For this reason, although the substantial industrial growth experienced in the last decades has boosted the economic and social development that we enjoy today, the manufacture, use, and later release of a wide range of potentially dangerous chemicals to the environment needs to be carefully controlled to avoid subsequent human health issues. Within a large number of chemicals of emerging concern (CECs) identified so far, some of them deserve special attention. In the current context of high UV radiation reaching the planet, most personal care products (PCPs) contain sunscreen agents to minimise the deleterious effect of sunlight on humans, such as photoaging and melanoma. In addition, some materials also include these chemicals to protect them against photodegradation, yellowing, lowing and improve their stability. Among them, organic UV filters (UVFs) are high-production chemicals (global production of around 10,000 tons per year) used as UV sunlight absorbers in beauty and hygiene products, food packaging materials, plastics, textiles, paints, rubbers, and many more daily-use products (Wang et al., 2010). However, in the product formulations, other chemicals are included. Among them, preservative substances are one of the most important and coexist with UVFs in many consumer products;

being included in pharmaceuticals, beverages, and foodstuffs, they improve product stability against fungi and bacteria growth and make them last longer in proper conditions.

UVFs and PBs are known estrogenic and anti-androgenic endocrine disruptors (Boberg et al., 2010; Kolatorova Sosvorova et al., 2018), and many other adverse effects derived from these products, such as genotoxicity (Zhao et al., 2013), endometriosis (Frederiksen et al., 2017), or cancer promotion (In et al., 2015; Phiboonchaiyanan et al., 2017) have been reported. Some studies have reported disorders of fetal development associated with prenatal exposure to benzophenone-type UVFs, such as Hirschsprung's disease (Balmer et al., 2005), cellular pathologies in brain cells in both fetuses and infants (Sang and Leung, 2016) and development of overweight in childhood (Leppert et al., 2020).

Humans are exposed to UVFs and PBs through multiple pathways. In some cases, as for UVFs, their absorption produces the highest exposure after direct application on the skin (Matta et al., 2019). But their ubiquity in the environment (Agawin et al., 2022; Díaz-Cruz et al., 2019; Fenni et al., 2022; Gago-Ferrero et al., 2013) indicates that humans are also exposed to them (and their metabolites) to a lesser extent, through drinking water and dietary intake. However, despite documented evidence in human matrices exists (breast milk, urine, or semen) (Meeker et al., 2011; Molins-Delgado et al., 2018; Song et al., 2020; Zhang et al., 2013) of external exposure, little is known about prenatal exposure, i.e. maternal transfer to the fetus, and only a few studies have reported concentrations of UVFs and PBs in placenta (Gil-Solsona et al., 2021; Reimann et al., 2021; Song et al., 2020; Valle-Sistac et al., 2016; Zhang et al., 2013) or cord blood (Kolatorova Sosvorova et al., 2018; Krause et al., 2018; Song et al., 2020; Wang et al., 2021; Zhang et al., 2013).

Apart from the UVF and PBs studied, there is a wide range of PCPs to which pregnant women may have been exposed with the potential to transfer to the fetus. Conventional target methodologies, such as the ones used here, are powerful and precise for analysing chemicals. However, to have a broader picture of exposure to PCPs, it is necessary to complement these studies with high-resolution mass spectrometry (HRMS)-based analysis and non-target strategies.

In this context, the objective of the present study was to explore a barely studied group of xenobiotics, such as some PCPs, that can be transferred from the mother to the fetus by analysing the umbilical cord blood. Specifically, eight UVFs and four PBs commonly present in PCPs formulations were analysed and quantified in the samples. In addition, to identify a broader range of chemicals transported through the umbilical cord, 3,246 compounds related to PCPs formulations were investigated through suspect screening.

Methods

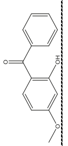
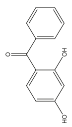
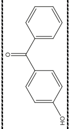
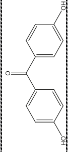
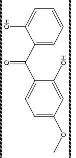
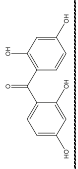
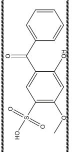
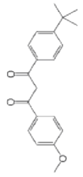
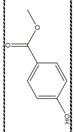
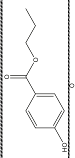
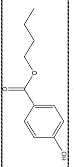
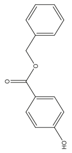
Standards and reagents

Detailed information about the standards and reagents used in the target analysis can be found in **Supplementary Information (SI).** A summary of the target PCPs is shown in **Table 1**. The chemicals purchased to confirm the tentatively identified compounds for the suspect screening are listed in **Table S1 of SI**.

Studied population and sample collection

The samples were collected at *Sant Joan de Déu* hospital in Barcelona (Spain) between July 2004 and April 2005. The mothers were asked to sign the informed consent to participate in the study. Delivery room staff from the hospital measured length, weight, and head circumference at birth using standard anthropometric procedures. Gestational age was calculated based on the last menstrual period. Information about the mother (smoking habits, diabetes, etc.) was obtained through a questionnaire given to pregnant women. The mother's registered anthropometric characteristics, including age, type of delivery, and parity, are compiled in **Table S2**.

Table 1. Selected compounds, family, CAS number, molecular mass, chemical structure and octanol-water partition constant. ("ChemSpider | Search and share chemistry," 2022; Pubchem, 2022)

Compound	Other names	Family	CAS number	Molecular mass (g/mol)	Structure	log K_{ow}
Benzophenone-3 (BP3)	Oxybenzone; 2-Hydroxy-4-methoxybenzophenone	Benzophenones	131-57-7	228.24		3.79*
Benzophenone-1 (BP1)	2,4-Dihhydroxy benzophenone	Benzophenones	131-56-6	214.22		2.96*
4-Hydroxy benzophenone (4HB)	-	Benzophenones	1137-42-4	193.18		3.07^{γ}
4,4'-Dihydroxy benzophenone (DHB)	-	Benzophenones	611-99-4	214.22		2.19^{γ}
2,2'-Dihydroxy-4-methoxy benzophenone (DHMB)	Benzophenone-8; Dioxybenzone	Benzophenones	131-53-3	244.25		3.82*
Benzophenone-2 (BP2)	2,2',4,4'-Tetrahydroxy benzophenone	Benzophenones	131-55-5	246.22		2.78*
Benzophenone-4 (BP4)	5-benzoyl-4-hydroxy-2-methoxybenzene sulfonic acid; HMBS; Sulisobenzone	Benzophenones	4065-45-6	308.31		0.37*
Avobenzone (AVO)	1-(4-tert-butylphenyl)-3-(4-methoxyphenyl)propane-1,3-dione	Benzophenones	70356-09-1	31017		4.51*
Methyl paraben (MePB)	Methyl 4-hydroxybenzoate	Parabens	99-76-3	152		1.96*
Propyl paraben (PrPB)	Propyl 4-hydroxybenzoate	Parabens	94-13-3	180.2		3.04*
Butyl paraben (BuPB)	Butyl 4-hydroxybenzoate	Parabens	94-26-8	194.23		3.57*
Benzyl paraben (BePB)	Benzyl 4-hydroxybenzoate	Parabens	94-18-8	228.24		3.7^{γ}

***Values obtained from Pubchem database; $^{\gamma}$ Values obtained from Chemspider database**

According to the information provided, the average age of the mothers was 30.5 years (4.6 SD). Out of the 69 participants with available samples, 58% were primiparous. Most pregnant women (91%) did not have gestational diabetes, and only five (7%) were smokers. The newborns included in the present study (55% females and 45% males) were born at the term of 39 weeks (1.25 SD), had 3.35 kg weight (4.2 SD), and 49.7 cm length (1.6 SD).

Cord blood samples were stored in metal-free EDTA tubes after collection by venipuncture of the umbilical cords performed immediately after delivery. The biological samples were stored following the Spanish Law of Biomedical Investigation until shipment via urgent courier to the IDAEA laboratories for analysis. All samples were received in perfect conditions, correctly codified, and preserved at -80º C until sample treatment for further instrumental analysis. Procedural blanks were performed by adding HPLC-grade water to the sampling tubes and then preserved under the same storage conditions as the cord blood samples.

The present study was approved by the Ethics Review Board of the University of Barcelona and *Sant Joan de Déu* hospital. All the data compiled were saved following the current regulation on the Protection of Personal Data and guarantee of digital rights.

Analysis of UVFs and PBs

The determination of UVFs and PBs in the cord blood samples was performed by optimizing an existing method (Kolatorovoptimisinga et al., 2018) with the conditions of the method published elsewhere (Sunyer-Caldú et al., 2021). Briefly, samples were centrifuged, supernatant collected, and mixed with a solution of NaCl in MeOH and methyl tert–butyl ether (MTBE) to perform a liquid-liquid extraction. Extracts were then frozen (to solidify the aqueous phase), and the recovered organic extract was brought to dryness and further reconstituted with buffer solution (pH ~ 10) and MeOH before analysis.

The HPLC-MS/MS conditions for the target analysis can also be found elsewhere (Sunyer-Caldú et al., 2021). Summarizing, the chromatographic separation was performed in a Hibar Purosher

STAR HR R-18 (50 mm × 2.0 mm, 5 µm) column and a pre-column of the same material from Waters (Dublin, Ireland) using a Symbiosis TM Pico instrument from Spark Holland (Emmen, The Netherlands). Detection was carried out in a 4000 Q TRAP^{TM} hybrid quadrupole-linear ion trap mass spectrometer from Applied Biosystems-Sciex (Foster City, CA, USA) under selected reaction monitoring (SRM) in positive and negative electrospray ionization modes (ESI+, ESI-) in two different injection sequences.

The LC-HRMS analysis of the samples for suspect screening was carried out following the conditions used in previous works (Gil-Solsona et al., 2021). Briefly, separation was performed with an Acquity UHPLC BEH C18 (100 mm x 2.1 mm, 1,7 µm) column from Waters ((Waters Corporation, Milford, Ma, USA) with a pre-column of the same material (5 mm x 2.1 mm, 1,7 µm) in an Acquity ultra-high performance liquid chromatographic system (UHPLC) (Waters) coupled to a Q-Exactive Orbitrap mass analyser (Thermo Scientific, Waltham, Ma, USA). Electrospray ionization, operating in ESI+ and ESI- ionization modes, was used, as for the target analysis. Each sample was analysed twice with different acquisition modes: Data Dependent Acquisition (DDA), where the 5 most intense ions from the low energy scan (4 eV) were selected and fragmented again at high collision energy (40 eV) to acquire MS/MS (excluding already selected ions for the next 30 seconds), and Data Independent Acquisition (DIA) using a ramp of 10-40 eV of collision energy (CE) for the high energy function.

QA/QC

Several steps were followed to ensure the quality of the results, which can be consulted elsewhere (Sunyer-Caldú et al., 2021). Briefly, 5 procedural blanks were prepared with HPLC-grade water, underwent the extraction procedure, and any peak present in the samples below a threshold of three times its intensity in the blanks was discarded. Matrix-matched calibration curves and isotopic dilution were used to minimize matrix effects in the quantification by target analysis. Analytical standards, reagent blank samples, and quality control solutions were included in each batch together with the study samples.

Suspect screening workflow

Data processing of the samples started with the transformation of the DIA files from proprietary (*.raw) format to generic (*.mzML) with ProteoWizard v. 3.0.2 (Kessner et al., 2008). Then, low and high-energy functions were split up and uploaded to the Norman Digital Sample Freezing Platform (Alygizakis et al., 2019). Then, compounds included in the database S13 EUCOSMETICS (complete list of 3246 compounds available in spreadsheet) (von der Ohe and Aalizadeh, 2020) from The NORMAN Suspect List Exchange-(NORMAN-SLE) (Taha et al., 2022) were searched in our samples. Note that the suspect list contains compounds that, although not cosmetic, are used in some of these products (e.g. some pharmaceuticals). The criteria for identifying the compounds were based on mass accuracy, isotopic pattern, predicted LC retention time (Aalizadeh et al., 2021, 2019), and evaluation of the MS/MS fragmentation. For the LC retention time index plausibility, a mix of control standards was injected in every 35 samples (Aalizadeh et al., 2019).

Tentative identification of compounds was verified or discarded manually by comparing the DDA data acquired for each tentatively assigned chemical with the available spectra in online databases (namely massBank, mzCloud, and PubChem (MoNA, 2022; Pubchem, 2022; Thermo Scientific, 2022). The detailed workflow used for the suspect screening is shown in **Figure 1**.

Statistical analysis of data

Individual correlations were tested among all the compounds with the Pearson test (95% of confidence). For the suspect screening, the peak areas were used to study the correlations instead of the quantified concentration. RStudio open software, v. 1.2.5001 (2019) Rstudio, Inc. (the USA) was used to carry out the variable correlation analysis and to generate the heatmaps using the ggplot2 package (Wickman, 2016).

Results

Determination of UVFs and PBs. Target analysis

Table S3 lists the concentrations of the UVFs and PBs measured in cord blood. A summary of the method's limits of detection (MLOD) and quantification (MLOQ), detection frequencies, range, and average values for the target compounds is shown in **Table 2**.

Table 2. Limits of detection and quantification, frequencies of detection, range, and average values of the target compounds.

	BP3	BP1	BP2	4HB	DHB	DHMB
MLOD (ng/mL)	0.01	0.03	0.04	0.04	0.02	0.04
MLOQ (ng/mL)	0.05	0.1	0.1	0.1	0.06	0.2
DF (%)	17	3	6	1	0	0
Range (ng/mL)	8.9-22.9	6.9-7.0	2.0-53.3	7.5	<MLOD	<MLOD
Average* (ng/mL)	12.7	6.9	14.9	7.5	-	-
	AVO	**BP4 (-)**	**BePB (-)**	**BuPB (-)**	**PrPB (-)**	**MePB (-)**
MLOD (ng/mL)	0.03	0.03	0.02	0.02	0.03	0.05
MLOQ (ng/mL)	0.1	0.1	0.08	0.08	0.09	0.2
DF (%)	14	4	0	0	1	6
Range (ng/mL)	8.9-23.9	1.1-5.3	<MLOD	<MLOD	5.2	5.2-10.5
Average* (ng/mL)	12.4	0.1	-	5.2	7.3	3.3

MLOD: limit of detection of the method; MLOQ: limit of quantification of the method; DF: detection frequency; *Average calculated with only positive samples

In 24 samples UVFs and/or PBs were detected. In the other 45 samples, none of the target compounds were found. The ranges and detection frequencies showed high variability, for instance, BP2 ranged from 2.0 to 53.3 ng/mL.

BP3, BP1, BP2, 4HB, BP4, AVO, PrPB, and, MePB were detected in at least one sample of the cohort, being 4HB and PrPB only found in one of them. In contrast, 4DHB, DHMB, BePB, and BuPB were not detected. The chemical most frequently detected was BP3 (17%), followed by AVO (14%). The highest concentration was found for BP2 (53.3 ng/mL), followed by AVO (23.9 ng/mL) and BP3 (22.9 ng/mL). However, BP3 had the highest average concentration (2.2 ng/mL), followed by AVO (1.8 ng/mL), BP2 (0.9 ng/mL), and MePB (0.4 ng/mL).

Suspect screening

From the 3,426 chemicals included in the suspect list used, 1,620 peaks gave a tentative positive finding according to their accurate mass. After applying additional filters, including (I) a plausible chromatographic retention time index (RTI), (II) the presence of at least 2 MS/MS fragments with a correct mass accuracy, (III) subtraction of the chemicals present in the blanks, and (IV) removal of the potentially endogenous chemicals that are intrinsically present in human blood (they are included in the suspect list due to their use in PCPs), only 64 features remained. Then, the experimental MS/MS spectra of these 64 features were compared with those available in online spectral databases (MassBank, mzCloud, Pubchem (MoNA, 2022; Pubchem, 2022; Thermo Scientific, 2022). After this constrained selection, 21 chemicals from diverse families (e.g. UVFs, surfactants, hair dyeing products, preservatives, analgesics, or plasticizers) were tentatively identified. Ten of these compounds were further confirmed at Level 1 (Schymanski et al., 2014) by purchasing the corresponding reference standard, while the other 8 were discarded (**Table S4**), and 3 remained tentatively identified at Level 2a or 3.

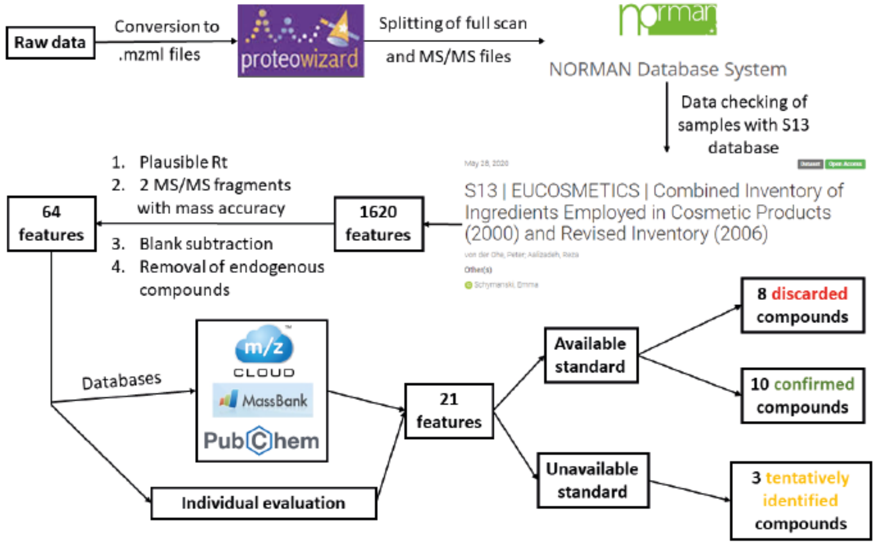


Figure 1. Suspect screening workflow.

The confirmed and tentatively identified chemicals are shown in **Table 3,** along with their confidence levels, chemical structure, detection frequency, and common use.

Among the tentatively identified compounds, UVF and two preservatives were found (umbelliferone (UMB), 8-hydroxyquinoline (HCH), and 1,2-benzisothiazolin-3-one (TBH), respectively). Three compounds (namely 12-hydroxy-2-ethylhexylester-octadecanoic acid (OCT), N-Methyl-2-pyrrolidone (MPY), and 2-[2-(4-nonylphenoxy) ethoxy] ethanol (NEE)) have multiple applications, but they are mainly used as rheology modifiers of the PCPs (e.g. surfactants, emollients, etc.). Also, four pharmaceuticals or TPs of them were found (erythromycin (ERY), 4-aminoantipyrine (4-AAP), 4-methylaminoantipyrine (4-MAP), 4-dimethylaminoantipyrine (4-DMP)), probably due to their direct consumption. Interestingly, the last three are derivatives from the same parent compound (metamizole) and were also found in the same samples. Finally, two plasticizers were also found (Diisononyl adipate (ADI) and 2,2'-Methylenebis (4-methyl-6-tert-butylphenol (MBM)).

Some chemicals such as ERY (Bulska et al., 2015) or UMB have been previously detected in cord blood (Wang et al., 2021), while other compounds, namely 4-AAP, 4-MAP, 4-DMP, ADI, MPY, BTH, NEE, BZX, HCH, MBM, and OCT have never been reported in this biofluid. Some of these chemicals, however, have been found in humans' bloodstream as the three metamizole metabolites (4-APP, 4-MAP, and 4-DMP) (Carretero et al., 1995; Ojha et al., 2009). Nevertheless, the rest of the confirmed compounds have not been reported in human blood. The compound most frequently found through suspect screening was 4-APP and 4-MAP (20.3%), both metabolites of metamizole, a widely used non-steroid anti-inflammatory pharmaceutical, and were found simultaneously in the same samples. The plasticizer MBM (13%) and the UVF UMB (8.7%) were also observed.

Table 3. Information on the 13 compounds tentatively identified or confirmed in decreasing order of detection frequency, with their abbreviation, structure, detection frequency, and common use.

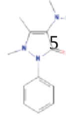
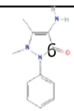
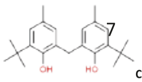
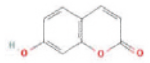
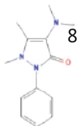
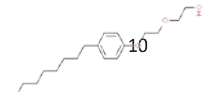
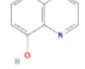
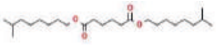
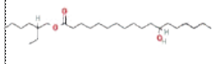
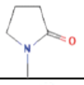
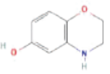
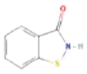
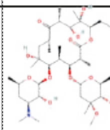
Compound (Acronym)	Confidence level	Frequency (%)	Additional evidences for the identification	Common use	Structure
4-Methylaminoantipyrine (4-MAP)	Level 2a	20.3	· Presence of characteristic fragments m/z: 187.0867 [$C_{11} H_{11} N_2 O$]; 159.0918 [$C_{10} H_{11} N_2$]; 146.0599 [$C_9 H_8 N O$]; 125.0709 [$C_6 H_9 N_2 O$]; 97.0761 [$C_5 H_9 N_2$]; 70.0652 [$C_4 H_8 N$]; 56.0498 [$C_3 H_6 N$] · Plausible r_T according to the QSRR model (2.91) · Rt and MS/MS spectra plausible with the confirmed compound 4-AAP · Detected in the exact same samples as the confirmed compound 4-APP (parent) · Similarity with mzCloud [Creference7208#T11397#c#2636018]	**Pharmaceutical TP:** · Analgesic · Anti-inflammatory · Antipyretic	 5 $C_{12} H_{15} N_3 O [M-H]^+$
4-Aminoantipyrine (4-AAP)	Level 1	20.3	· Presence of characteristic fragments m/z: 187.0868 [$C_{11} H_{11} N_2 O$]; 159.0918 [$C_{10} H_{11} N_2$]; 146.0599 [$C_9 H_8 N O$]; 94.0653 [$C_6 H_8 N$]; 83.0604 [$C_4 H_7 N_2$]; 56.0498 [$C_3 H_6 N$] · Similarity with mzCloud [Creference1100#T1843#c#209083] · CONFIRMED with reference standard	**Pharmaceutical TP:** · Analgesic · Anti-inflammatory · Antipyretic	 6 $C_{11} H_{13} N_3 O [M-H]+$
2,2'-Methylenebis(4-methyl 6-tert-butylphenol) (MBM)	Level 1	13	· Presence of characteristic fragments m/z: 164.1159 [$C_{10}\ {}^{13}C\ H_{15} O$]; 163.1126 [$C_{11} H_{15} O$] · Similarity with mzCloud [Creference1138#T1896#c#211685] · CONFIRMED with reference standard	**PCP/Plastizier:** · Plastizier · Antioxidant · Fragance	 7 $C_{23} H_{32} O_2 [M-H]^-$
Umbelliferone (UMB)	Level 1	8.7	· Presence of characteristic fragments m/z: 135.0440 [$C_8 H_7 O_2$]; 133.0282 [C8 H5 O2]; 95.0489 [$C_6 H_7 O$]; 92.0254 [$C_6 H_4 O$]; 79.0543 [$C_6 H_7$] · CONFIRMED with reference standard	**Personal care product:** · Sunscreen agent · Brightener for textiles	 $C_9 H_6 O_3 [M-H]^+$
4-Dimethylaminoantipyrine (4-DMP)	Level 2a	4.3	· Presence of characteristic fragments m/z: 159.0919 [$C_{10} H_{11} N_2$]; 113.1074 [$C_6 H_{13} N_2$] 111.0918 [$C_6 H_{11} N_2$]; 98.0841 [$C_5 H_{10} N_2$]; 84.0806 [$C_5 H_{10} N$]; 72.0808 [$C_4 H_{10} N$]; 58.0654 [$C_3 H_8 N$] · Plausible r_T according to the QSRR model (3.04) · Rt and MS/MS spectra plausible with the confirmed compound 4-AAP and 4-MAP · In the samples (3) that was detected, 4-AAP and 4-MAP had already been detected · Similarity with mzCloud [Creference980#T1667#c#199018]	**Pharmaceutical TP:** · Analgesic · Anti-inflammatory · Antipyretic	 8 9 $C_{13} H_{17} N_3 O [M-H]^+$
2-[2-(4-Nonylphenoxy) ethoxy]ethanol (NEE)	Level 1	4.3	· Presence of characteristic fragments m/z: 213.1493 [C12 H21 O3]; 59.0137 [C2 H3 O2]; · CONFIRMED with reference standard	**Personal care product:** · Emulsifying · Surfactant · Cleansing	 10 $C_{19} H_{32} O_3 [M-H]^-$

Table 3. (continued)

8-Hydroxychinolin (HCH)	Level 1	2.9	· Presence of characteristic fragment m/z: 115.0430 [C8 H5 N] · Similarity with MassBank of North America [record MoNA038139] · CONFIRMED with reference standard	**Personal care product:** · Fungicide · Stabilizer	17  $C_9 H_7 N O [M-H]^-$
Diisononyl adipate (ADI)	Level 1	2.9	· Presence of characteristic fragments m/z: 147.0650 [$C_6 H_{11} O_4$]; 129.0544 [$C_6 H_9 O_3$] 101.0596 [$C_5 H_9 O_2$]; 85.1011 [$C_6 H_{13}$]; 71.0855 [$C_5 H_{11}$]; 57.0701 [$C_4 H_9$] · Similarity with mzCloud [Creference3197#T5205#c#615531] · CONFIRMED with reference standard	**PCP/Plastizier:** · Plastizier · Surfactant · Emollient	 $C_{24} H_{46} O_4 [M-H]^+$
12-Hydroxy-2-ethylhexyl ester-octadecanoic acid (OCT)	Level 3	1.4	· Presence of characteristic fragments m/z: 292.2732 [$C_{26} H_{49} O_2$]; 283.2652 [$C_{18} H_{35} O_2$]; 255.2331 [$C_{16} H_{31} O_2$]; 125.0977 [$C_8 H_{14} O$]; 111.0816 [$C_7 H_{12} O$] · Plausible r_T in NI (9.51) according to the QSRR model	**Personal care product:** · Body lotion/wash · Emollient · Fragance	 $C_{26} H_{52} O_3 [M-H]^-$
N-Methyl-2-pyrrolidone (MPY)	Level 1	1.4	· Presence of characteristic fragments m/z: 82.0652 [$C_5 H_8 N$]; 69.0335 [$C_4 H_5 O$]; 58.0290 [$C_2 H_4 NO$] · Similarity with mzCloud [Creference2810#T3983#c#401122] · CONFIRMED with reference standard	**Personal care product:** · Surfactant · Industrial product	 18 $C_5 H_9 NO [M-H]^+$
3,4-Dihydro-2H-1,4-Benzoxazin-6-ol (BZX)	Level 1	1.4	· Presence of characteristic fragments m/z: 134.0250 [$C_7 H_4 O_2 N$]; 108.0458 [$C_6 H_6 N O$]; 107.0380 [$C_6 H_5 N O$] · CONFIRMED with reference standard	**Personal care product:** · Hair dyeing · Hair colorant	 $C_8 H_9 N O_2 [M-H]^-$
1,2-Benzisothiazolin-3-one (BTH)	Level 1	1.4	· Presence of characteristic fragments m/z: 134.0060 [$C_7 H_4 N S$]; 109.0107 [$C_6 H_5 S$]; 105.0336 [$C_7 H_5 O$] · Similarity with mzCloud [Creference998#T4100#c#416564] · CONFIRMED with reference standard	**Personal care product:** · Antioxidant · Antimicrobial · Preservative	 19 $C_7 H_5 N O S [M-H]^+$
Erythromycin (ERY)	Level 1	1.4	· Presence of characteristic fragments m/z: 158.1177 [$C_8 H_{16} N O_2$]; 116.1071 [$C_5 H_{10} N O_2$]; 98.0965 [$C_6 H_{12} N$]; 83.0492 [$C_5 H_7 O$]; 72.0809 [$C_4 H_{10} O$] · Similarity with mzCloud [Creference2533#T3836#c#383816] · CONFIRMED with reference standard	**Pharmaceutical:** · Antibiotic	 $C_{37} H_{67} N O_{13} [M-H]^+$

Correlations among compounds

The Pearson test was applied to identify correlations among all the compounds. In the target analysis, positive correlations were found between BP2 and 4HB (1), BP3 and AVO (0.72), BP1 and BP2 (0.69), BP1 and 4HB (0.69), and MePB and PrPB (0.68) (**Figure 2**). In the suspect screening, positive correlations were observed between 4-AAP and 4-MAP (0.82), MPY and 4-DMP (0.80), OCT and 4-MAP (0.80), 4-AAP and OCT (0.62), and ADI and HCH (0.58) (**Figure 3**).

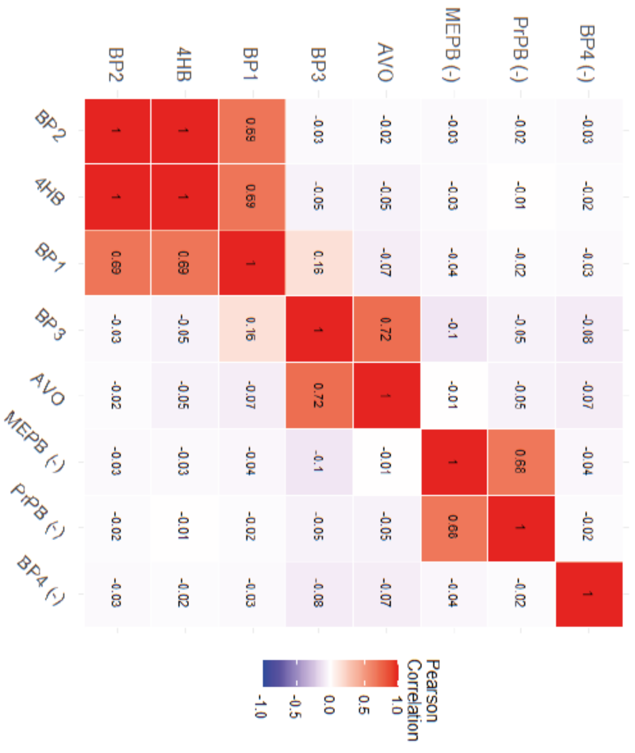


Figure 2. Heatmap of Pearson's correlation values for the selected compounds in target analysis.

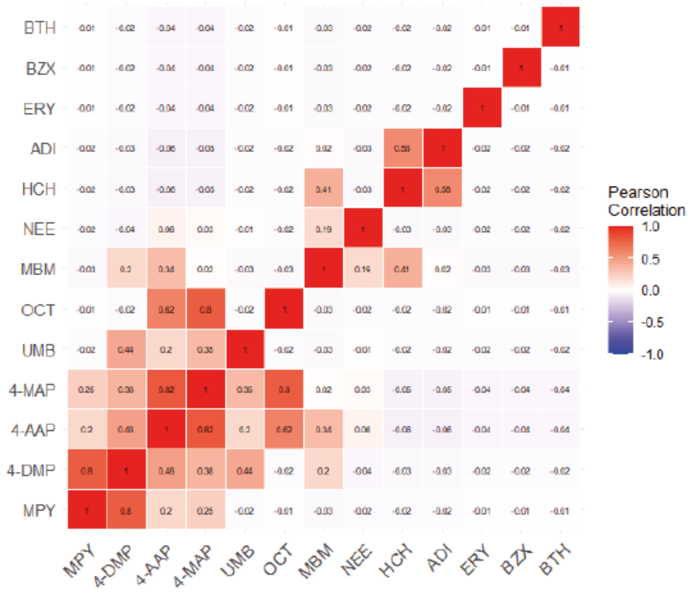


Figure 3.

Heatmap of the Pearson's correlation values for the found compounds in the suspect screening.

Discussion

Exposure pathways

The available information on the human bioaccumulation of UVFs and PBs, is scarce, particularly about mother-fetus and mother-child transfer. In the few studies reporting them in matrices related to the fetus, it has been demonstrated that these compounds can cross the placenta and reach the cord blood. Furthermore, they have also been detected in humans' urine, serum, breast milk, amniotic fluid, or placental tissue (Azzouz et al., 2016; Gao et al., 2015; Gil-Solsona et al., 2021; Kolatorova Sosvorova et al., 2018; Li et al., 2018; Mínguez-Alarcón et al., 2019; Philippat et al., 2013; Song et al., 2020; Towers et al., 2015; Vela-Soria et al., 2014, 2017; Wang et al., 2021; Zhao et al., 2019). Concentrations of the target chemicals measured in the present study were very similar to those reported in a few available cord blood studies.

Zhang et al. (2013) and Krause et al. (2018) analysed BP3, 4HB, BP1, DHMB, and BP2 in very small cohorts (n=22), detecting only the first three substances at levels ranging from 0.12 to 2.93 ng/mL. Song et al. (2020) analysed BP3, 4HB, BP1, DHMB, and BP2 in a bigger cohort (n=97), detecting all of them at levels in the range of 0.01 - 34.9 ng/mL. Finally, (Kolatorova Sosvorova et al., 2018) analysed MePB and PrPB in 27 cord blood samples, detecting both in the 0.46-4.14 ng/mL range.

Although the exposure pathways of the fetus to these xenobiotics are still unknown, we can establish different hypotheses for each detected compound. For example, BP3 is the most used UVF worldwide (Downs et al., 2022) and was the most frequently detected in the cohort (17.4%). The primary exposure for the mother to this chemical can be through dermal adsorption. There are a wide variety of products containing BP3, which in Spain is allowed in formulations up to 6%. In comparison, others such as AVO or BP4 are limited to 4% (European Parliament and the Council of the European Union, 2009), and it is used as light stabilizer or photodegradation inhibitor agent (Suzuki et al., 2005). Additional exposure can result from ingesting contaminated food or water and by air inhalation. The significant presence of this compound in daily-life products would explain its ubiquitous occurrence. Placental transfer of chemicals is influenced by their physicochemical properties (e.g. octanol-water partition constant (log Kow) and molecular weight) (Zhang et al., 2020). BP3 has a high log Kow (3.79), which suggests that the placental barrier should retain it. However, the high correlation between maternal blood and cord blood levels observed in previous studies (Krause et al., 2018; Song et al., 2020) indicates that despite the fact it may be partially retained in the placenta, BP3 reaches the cord blood and subsequently, the fetus.

There are studies reporting higher concentrations of BP3 in cord blood (56 ng/mL) than in a mother's bloodstream and higher or similar detection frequencies (18.9-55 %) (Krause et al., 2018; Song et al., 2020; Zhang et al., 2013), which led us to assume that BP3 can easily cross the placental barrier.

BP1 is the major metabolite of BP3 (Valle-Sistac et al., 2016) and displays greater estrogenic activity than its parent compound (Kawamura et al., 2005; Takatori et al., 2003). It has been reported at higher concentrations (52 ng/mL) in cord blood (Song et al., 2020), but it has also been found in urine and placenta (Krause et al., 2018; Zhang et al., 2013). From the other metabolites of BP3, namely 4DHB, 4HB, and DHMB (Kasichayanula et al., 2005), only 4HB in one sample was found. 4HB has more estrogenic potential than BP3 (Morohoshi et al., 2005; Suzuki et al., 2005) and has been detected in urine (Krause et al., 2018), placenta (Valle-Sistac et al., 2016), and quite frequently in cord blood (Frederiksen et al., 2017; Zhang et al., 2013). However, no correlation between 4HB and BP3 has been reported, as concluded in our study. A few works have targeted DHMB in the placenta (Valle-Sistac et al., 2016) or blood (Zhang et al., 2013), but it was not detected, despite it being detected at low concentrations in cord blood (Song et al., 2020). Some authors have reported that BP3 and BP1 are excreted in urine as glucuronide conjugates (Calafat et al., 2008). However, due to the lower log Kow of BP3 metabolites compared to that of BP3 (**Table 1**), they might be easily excreted without reaching cord blood. Notwithstanding, further studies should be performed to confirm this hypothesis.

BP2 is a benzophenone derivative with more estrogenic activity than that displayed by BP3 (Kawamura et al., 2005). It is commonly used in PCPs formulations and food container plastics. Given its widespread use, its presence in cord blood would be most likely related to the direct application of PCPs on the skin and subsequent dermal absorption.

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Other studies reported BP2 occurrence in urine (Bae et al., 2016) and placenta (Valle-Sistac et al., 2016; Vela-Soria et al., 2011), but it was detected neither in maternal blood nor in cord blood (Krause et al., 2018; Zhang et al., 2013) before.

AVO is not a usually monitored UVF, but it is commonly used as a substitute for BP3 in the formulation of sunscreens, body lotions, etc., likely due to their similar chemical structures. It was included in our study, and surprisingly it was the second most frequently detected UVF (15%), just after BP3 (17%). There is a lack of information relative to this compound in human matrices, and to the best of the authors' knowledge, this is the first time it has been detected in cord blood. Considering the high concentrations found along with its antiestrogenic potential (Hong et al., 2016), obesogenic disrupting activity (Ahn et al., 2019), and mutagenic activity (ECHA EUROPE, 2022a), AVO emerges as an interesting chemical to be monitored in further studies.

BP4, present only in 3 samples, has been previously reported in the placenta (Valle-Sistac et al., 2016) and cord blood (Song et al., 2020) at 0.25-5.41 ng/g and 0.02-0.13 ng/mL, respectively. The lower detection frequency and concentration of BP4 would be explained by its high polarity (log Kow=0,88), which leads it to a lower tendency to bioaccumulate in organic compartments, being quickly excreted. Moreover, BP4 has a molecular structure originating more steric effects than other benzophenones, probably hindering its crossing through organic barriers such as the placenta.

Additional literature reporting UVFs toxicity in animals shows that these chemicals affect pubertal development and thyroid and reproductive functions (Kim and Choi, 2014; Krause et al., 2012; Schlumpf et al., 2008). Exposure of zebrafish to BP3 caused interference with normal sex development (Kinnberg et al., 2015), and in rats, it increased the uterus size (Schlumpf et al., 2001).

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Within the PBs family, MePB was the most detected compound (5.8%) and with the highest concentration value (10.5 ng/mL). The other PB detected was PrPB in only one sample. BuPB and BePB were not detected. This result is in accordance with those reported in plasma (Kolatorova Sosvorova et al., 2017), cord blood (Kolatorova Sosvorova et al., 2018; Pycke et al., 2015), and placenta (Valle-Sistac et al., 2016). In all of them, MePB was the most detected paraben, with concentration levels very similar to the ones obtained in this study (from 0.06 to 319 ng/mL). Other authors also reported PrPB as the second most detected paraben in a similar concentration range (0.17 - 64.7 ng/mL). BuPB and BePB were not detected in any of these studies, as in the present work. The four PBs selected in this study, especially MePB, are extensively used in the cosmetic and pharmaceutical industries for gels, shampoos, lotions, drugs, or toothpaste (Shivashankara et al., 2013), which makes easier its consumption through the ingestion, explaining its presence in cord blood.

Studies reporting PBs toxicity in animals show that these compounds induce adverse effects on sperm production and testosterone levels (Boberg et al., 2010) or altered gonadal hormone signalling or metabolism (Braun et al., 2014).

Extended suspect screening of PCPs

An extended screening analysis was performed to expand the chemical domain and obtain a more comprehensive picture of the presence of PCPs in the evaluated cohort, taking advantage of the power of HRMS. This approach allowed us to identify 13 additional chemicals, 10 confirmed and 3 tentatively identified.

Information and potential pathway identification about each confirmed compound are explained thereupon. An interesting case was the confirmation of 4-AAP in 14 samples. This compound is the main metabolite of metamizole, an analgesic, anti-inflammatory, and antipyretic, which use during pregnancy is discouraged because it can produce agranulocytosis (Dathe et al., 2017). Two other metamizole metabolites, 4-MAP and 4-DMP, were also found, being 4-MAP in the same 14 samples and 4-DMP only in 3. The high detection frequency of these compounds in the cohort suggests that pregnant women could have consumed them and that their metabolites had a great facility to cross the placental barrier and reach the fetus' environment.

BZX, detected in 1.4% of the samples and used as a colourant in dyes, was the most unexpected compound detected, as hair dyeing is not recommended during pregnancy because of the potential toxicity of its components (Manjunatha et al., 2020). Although it was only detected in one sample, it is evidence of its capacity to cross the placental barrier. Other chemicals, such as BTH or HCH, were detected in 2.9% and 1.4% of the samples, respectively, and are antimicrobials usually used as preservatives in cosmetics, surfactants such as NEE (4.3%), or plasticizers such as ADI and MBM (2.9% and 13%, respectively), widely used in food and drink packaging, were also present in cord blood.

Other chemicals identified in the suspect screening have not been reported as toxic, like ERY, UMB, or MYP, found in 1.4%, 8.7%, and 1.4% of the samples, respectively. The antibiotic ERY was only detected in one sample due to its consumption (facultative prescription) by the mother before delivery. ERY use is not discouraged during pregnancy and is often prescribed when the waters break early (Romøren et al., 2012). The UV filter UMB (also known as 7-hydroxycoumarin) was already reported in cord blood (Wang et al., 2021).

MPY is often used in manufacturing pigments, cosmetics, and pharmaceuticals, among other products, as a skin penetration enhancer (Åkesson and Jönsson, 2000). MPY, found in one single sample, was included in an exposure study in pregnant rats, showing no abnormal development in the vital organs (Lee et al., 1987) despite high concentrations; it showed lethargy, respiratory difficulty, and excessive mortality in the mothers.

Some confirmed compounds through the suspect screening also present associated health risks. For example, metamizole presents a risk of agranulocytosis, so its use as a pharmaceutical is not recommended during pregnancy (Gualde and Malinvaud, 1982), NEE, BTH, and BZX are very toxic to aquatic life and present acute toxicity when swallowed (ECHA EUROPE, 2022b, 2022c, 2022d), UMB has been associated with glucose intolerance (Sim et al., 2014), and MPY, HCH, and MBM have been demonstrated to have reproductive toxicity and may damage the unborn child (Europe, 2022; European Union, 2017; Solomon et al., 1995). In addition, it is important to highlight that some of the compounds found in the suspect screening do not have reported studies about their potential toxicity or adverse effects, probably because their occurrence data is also scarce.

Correlations among compounds

In the target analysis, a strong positive correlation (Pearson's test) was found between BP2 and 4HB (0.99), easily explained as both are phase I metabolites of BP3 so that they could be generated in similar ratios after BP3 degradation. The same explanation applies to the correlations BP1- BP2 (0.69) and BP1- 4HB (0.69) (Figure 2). Similar results were obtained by Wang and Kannan (2013) in urine, where they found a correlation among 4HB, BP2, BP1, and DHMB.

The second highest correlation found was between BP3 and AVO (0.72). It could be explained as the mothers that use PCPs where both BP3 and AVO are present in the formulation (as both are the most UVFs used worldwide and are commonly used together (Geoffrey et al., 2019) have similar values in the cord blood. The last correlation was between MePB and PrPB (0.68), probably because both are the most common preservatives in many daily-use products.

In the suspect screening, the highest correlation value was found between 4-AAP and 4-MAP (0.82) (Figure 3), as expected since 4-MAP is a metabolite of 4-AAP, and both were found in the same samples. Other compounds, such as MPY and OCT, were only detected in one sample, meaning that probably their correlation with 4-DMP (0.80), 4-MAP (0.80), and 4-AAP (0.62). This correlation is not fully understood, but it could be just a coincidence because they occur in the only sample where MPY and OCT were detected.

A quite significant correlation was observed between ADI and HCH (0.58). Considering that ADI is a plasticizer and HCH a stabilizer, both used in cosmetics, it is probable that both were used together in personal care and hygiene products.

Key findings and study limitations

The present work evidence that some UVFs and PBs can reach the human blood and cross the placental barrier, as concentrations of BP3, BP1, BP2, AVO, BP4, MePB, and PrPB were found in umbilical cord blood. The compounds measured at the highest concentrations were BP3 (2.21 ng/mL) and AVO (1.80 ng/mL) in good agreement with their predominant use in products containing UV filters. To the best of the author's knowledge, this is the first study reporting cord blood concentrations of the obesogenic UV filter AVO. The most frequently detected and predominant paraben was MePB, in agreement with its wide use.

Our findings also demonstrate that not only frequently used PCPs are capable of reaching the fetus's environment. By applying complementary suspect screening, the chemicals 4-AAP, 4-MAP, 4-DMP, ADI, MPY, BTH, NEE, BZX, HCH, MBM, and OCT's were determined for the first time in umbilical cord blood. These outcomes document that many compounds can reach the fetus during this critical period of development and that many of them were unexpected, such as the metamizole metabolites (which use is discouraged during pregnancy), the 4-AAP, 4-MAP, and 4-DMP.

Despite the well-documented results, this study is based on a limited number of samples. The relatively small population size, consisting of a cohort of 69 cord blood samples, provides for the first time cord blood plasma levels of certain chemicals included in personal and hygiene products of daily use. The reported concentrations may be considered a reference for the background levels of benzophenone-type UVFs and PBs in cord blood pregnant women related to current lifestyle and environmental contamination exposure. In light of the results, further research is guaranteed to unravel the role of PCPs in fetal growth and the mechanisms of action behind it.

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References

Aalizadeh, R., Alygizakis, N.A., Schymanski, E.L., Krauss, M., Schulze, T., Ibáñez, M., McEachran, A.D., Chao, A., Williams, A.J., Gago-Ferrero, P., Covaci, A., Moschet, C., Young, T.M., Hollender, J., Slobodnik, J., Thomaidis, N.S., 2021. Development and Application of Liquid Chromatographic Retention Time Indices in HRMS-Based Suspect and Nontarget Screening. Anal. Chem. 93, 11601–11611. https://doi.org/10.1021/acs.analchem.1c02348

Aalizadeh, R., Nika, M.C., Thomaidis, N.S., 2019. Development and application of retention time prediction models in the suspect and non-target screening of emerging contaminants. J. Hazard. Mater. 363, 277–285. https://doi.org/10.1016/j.jhazmat.2018.09.047

Agawin, N.S.R., Sunyer-Caldú, A., Díaz-Cruz, M.S., Frank-Comas, A., García-Márquez, M.G., Tovar-Sánchez, A., 2022. Mediterranean seagrass Posidonia oceanica accumulates sunscreen UV filters. Mar. Pollut. Bull. 176. https://doi.org/10.1016/j.marpolbul.2022.113417

Ahn, S., An, S., Lee, M., Lee, E., Pyo, J.J., Kim, J.H., Ki, M.W., Jin, S.H., Ha, J., Noh, M., 2019. A long-wave UVA filter avobenzone induces obesogenic phenotypes in normal human epidermal keratinocytes and mesenchymal stem cells. Arch. Toxicol. 93, 1903–1915. https://doi.org/10.1007/s00204-019-02462-1

Åkesson, B., Jönsson, B.A.G., 2000. Biological monitoring of N-methyl-2-pyrrolidone using 5-hydroxy-N-methyl- 2-pyrrolidone in plasma and urine as the biomarker. Scand. J. Work. Environ. Heal. 26, 213–218. https://doi.org/10.5271/sjweh.534

Alygizakis, N.A., Oswald, P., Thomaidis, N.S., Schymanski, E.L., Aalizadeh, R., Schulze, T., Oswaldova, M., Slobodnik, J., 2019. NORMAN digital sample freezing platform: A European virtual platform to exchange liquid chromatography high resolution-mass spectrometry data and screen suspects in "digitally frozen" environmental samples. TrAC - Trends Anal. Chem. 115, 129–137. https://doi.org/10.1016/j.trac.2019.04.008

Azzouz, A., Rascón, A.J., Ballesteros, E., 2016. Simultaneous determination of parabens, alkylphenols, phenylphenols, bisphenol A and triclosan in human urine, blood and breast milk by continuous solid-phase extraction and gas chromatography-mass spectrometry. J. Pharm. Biomed. Anal. 119, 16–26. https://doi.org/10.1016/j.jpba.2015.11.024

Bae, J., Kim, S., Kannan, K., Buck Louis, G.M., 2016. Couples' urinary concentrations of benzophenone-type ultraviolet filters and the secondary sex ratio. Sci. Total Environ. 543, 28–36. https://doi.org/10.1016/j.scitotenv.2015.11.019

Balmer, M.E., Buser, H.-R., Müller, M.D., Poiger, T., 2005. Occurrence of Some Organic UV Filters in Wastewater, in Surface Waters, and in Fish from Swiss Lakes. Environ. Sci. Technol. 39, 953–962. https://doi.org/10.1021/es040055r

Boberg, J., Taxvig, C., Christiansen, S., Hass, U., 2010. Possible endocrine disrupting effects of parabens and their metabolites. Reprod. Toxicol. 30, 301–312. https://doi.org/https://doi.org/10.1016/j.reprotox.2010.03.011

Braun, J.M., Just, A.C., Williams, P.L., Smith, K.W., Calafat, A.M., Hauser, R., 2014. Personal care product use and urinary phthalate metabolite and paraben concentrations during pregnancy among women from a fertility clinic. J. Expo. Sci. Environ. Epidemiol. 24, 459–466. https://doi.org/10.1038/jes.2013.69

Bulska, M., Szcześniak, P., Pięta-Dolińska, A., Oszukowski, P., Orszulak-Michalak, D., 2015. The placental transfer of erythromycin in human pregnancies with group B streptococcal infection. Ginekol. Pol. 86, 33–39. https://doi.org/10.17772/gp/1896

Calafat, A.M., Wong, L.Y., Ye, X., Reidy, J.A., Needham, L.L., 2008. Concentrations of the sunscreen agent benzophenone-3 in residents of the United States: National Health and Nutrition Examination Survey 2003-2004. Environ. Health Perspect. 116, 893–897. https://doi.org/10.1289/ehp.11269

Carretero, I., Vadillo, J.M., Laserna, J.J., 1995. Determination of antipyrine metabolites in human plasma by solid-phase extraction and micellar liquid chromatography. Analyst 120, 1729–1732. https://doi.org/10.1039/an9952001729

ChemSpider | Search and share chemistry [WWW Document], 2022.

Dathe, K., Padberg, S., Hultzsch, S., Meister, R., Beck, E., 2017. Metamizole use during first trimester — A prospective observational cohort study on pregnancy outcome 1197–1204. https://doi.org/10.1002/pds.4277

Díaz-Cruz, M.S., Molins-Delgado, D., Serra-Roig, M.P., Kalogianni, E., Skoulikidis, N.T., Barceló, D., 2019. Personal care products reconnaissance in EVROTAS river (Greece): Water-sediment partition and bioaccumulation in fish. Sci. Total Environ. 651, 3079–3089. https://doi.org/10.1016/j.scitotenv.2018.10.008

Downs, C.A., Bishop, E., Diaz-Cruz, M.S., Haghshenas, S.A., Stien, D., Rodrigues, A.M.S., Woodley, C.M., Sunyer-Caldú, A., Doust, S.N., Espero, W., Ward, G., Farhangmehr, A., Tabatabaee Samimi, S.M., Risk, M.J., Lebaron, P., DiNardo, J.C., 2022. Oxybenzone contamination from sunscreen pollution and its ecological threat to Hanauma Bay, Oahu, Hawaii, U.S.A. Chemosphere 291. https://doi.org/10.1016/j.chemosphere.2021.132880

ECHA EUROPE, 2022a. Summary of classification and labellling of avobenzone [WWW Document]. URL https://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/discli/details/213397

ECHA EUROPE, 2022b. Summary of Classification and Labelling of 1,2-benzisothiazolin-3-one [WWW Document]. On-line. URL https://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/discli/details/53588

ECHA EUROPE, 2022c. Summary of Classification and Labelling of nonylphenol ethoxylated. On-line.

ECHA EUROPE, 2022d. Summary of Classification and Labelling of 3,4-dihydro-2H-1,4-benzoxazin-6-ol. On-line.

Europe, E., 2022. Substance infocard 6,6’-di-tert-butyl-2,2’-methylenedi-p-cresol.

European Parliament and the Council of the European Union, 2009. Regulation (EC) No 1223/2009 of the european parliament and of the council of 30 November 2009 on cosmetic products.

European Union, 2017. ▶B REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL.

Fenni, F., Sunyer-Caldú, A., Ben, H., Diaz-cruz, M.S., 2022. Contaminants of emerging concern in marine areas : First evidence of UV filters and paraben preservatives in seawater and sediment on the eastern coast of Tunisia ☆309. https://doi.org/10.1016/j.envpol.2022.119749

Fleming, T.P., Watkins, A.J., Velazquez, M.A., Mathers, J.C., Prentice, A.M., Stephenson, J., Barker, M., Saffery, R., Yajnik, C.S., Eckert, J.J., Hanson, M.A., Forrester, T., Gluckman, P.D., Godfrey, K.M., 2018. Origins of lifetime health around the time of conception: causes and consequences. Lancet 391, 1842–1852. https://doi.org/10.1016/S0140-6736(18)30312-X

Frederiksen, H., Nielsen, O., Skakkebaek, N.E., Juul, A., Andersson, A.M., 2017. UV filters analyzed by isotope diluted TurboFlow-LC–MS/MS in urine from Danish children and adolescents. Int. J. Hyg. Environ. Health 220, 244–253. https://doi.org/10.1016/j.ijheh.2016.08.005

Gago-Ferrero, P., Díaz-Cruz, M.S., Barceló, D., 2013. Multi-residue method for trace level determination of UV filters in fish based on pressurized liquid extraction and liquid chromatography-quadrupole-linear ion trap-mass spectrometry. J. Chromatogr. A 1286, 93–101. https://doi.org/10.1016/j.chroma.2013.02.056

Gao, C.J., Liu, L.Y., Ma, W.L., Zhu, N.Z., Jiang, L., Li, Y.F., Kannan, K., 2015. Benzonphenone-type UV filters in urine of Chinese young adults: Concentration, source and exposure. Environ. Pollut. 203, 1–6. https://doi.org/10.1016/j.envpol.2015.03.036

Geoffrey, K., Mwangi, A.N., Maru, S.M., 2019. Sunscreen products: Rationale for use, formulation development and regulatory considerations. Saudi Pharm. J. 27, 1009–1018. https://doi.org/10.1016/j.jsps.2019.08.003

Gil-Solsona, R., Nika, M.C., Bustamante, M., Villanueva, C.M., Foraster, M., Cosin-Tomás, M., Alygizakis, N., Gómez-Roig, M.D., Llurba-Olive, E., Sunyer, J., Thomaidis, N.S., Dadvand, P., Gago-Ferrero, P., 2021. The Potential of Sewage Sludge to Predict and Evaluate the Human Chemical Exposome. Environ. Sci. Technol. Lett. 8, 1077–1084. https://doi.org/10.1021/acs.estlett.1c00848

Gualde, N., Malinvaud, G., 1982. In vitro generation of cytotoxic lymphocytes during noramidopyrine-induced agranulocytosis. Clin. Immunol. Immunopathol. 24, 220–226. https://doi.org/10.1016/0090-1229(82)90233-1

Hong, H., Rua, D., Sakkiah, S., Selvaraj, C., 2016. Consensus Modeling for Prediction of Estrogenic Activity of Ingredients Commonly Used in Sunscreen Products. Enironmental Res. Public Heal. https://doi.org/10.3390/ijerph13100958

In, S.-J., Kim, S.-H., Go, R.-E., Hwang, K.-A., Choi, K.-C., 2015. Benzophenone-1 and Nonylphenol Stimulated MCF-7 Breast Cancer Growth by Regulating Cell Cycle and Metastasis-Related Genes Via an Estrogen Receptor α-Dependent Pathway. J. Toxicol. Environ. Heal. Part A 78, 492–505. https://doi.org/10.1080/15287394.2015.1010464

Kasichayanula, S., House, J.D., Wang, T., Gu, X., 2005. Simultaneous analysis of insect repellent DEET, sunscreen oxybenzone and five relevant metabolites by reversed-phase HPLC with UV detection: application to an in vivo study in a piglet model. J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci. 822, 271–277. https://doi.org/10.1016/j.jchromb.2005.06.015

Kawamura, Y., Mutsuga, M., Kato, T., Iida, M., Tanamoto, K., 2005. Estrogenic and Anti-Androgenic Activities of Benzophenones in Human Estrogen and Androgen Receptor Mediated Mammalian Reporter Gene Assays. J. Heal. Sci. 51, 48–54. https://doi.org/10.1248/jhs.51.48

Kessner, D., Chambers, M., Burke, R., Agus, D., Mallick, P., 2008. ProteoWizard: Open source software for rapid proteomics tools development. Bioinformatics 24, 2534–2536. https://doi.org/10.1093/bioinformatics/btn323

Kim, S., Choi, K., 2014. Occurrences, toxicities, and ecological risks of benzophenone-3, a common component of organic sunscreen products: A mini-review. Environ. Int. 70, 143–157. https://doi.org/https://doi.org/10.1016/j.envint.2014.05.015

Kinnberg, K.L., Petersen, G.I., Albrektsen, M., Minghlani, M., Awad, S.M., Holbech, B.F., Green, J.W., Bjerregaard, P., Holbech, H., 2015. Endocrine-disrupting effect of the ultraviolet filter benzophenone-3 in zebrafish, Danio rerio. Environ. Toxicol. Chem. 34, 2833–2840. https://doi.org/10.1002/etc.3129

Kolatorova Sosvorova, L., Chlupacova, T., Vitku, J., Vlk, M., Heracek, J., Starka, L., Saman, D., Simkova, M., Hampl, R., 2017. Determination of selected bisphenols, parabens and estrogens in human plasma using LC-MS/MS. Talanta 174, 21–28. https://doi.org/10.1016/j.talanta.2017.05.070

Kolatorova Sosvorova, L., Vitku, J., Hampl, R., Adamcova, K., Skodova, T., Simkova, M., Parizek, A., Starka, L., Duskova, M., 2018. Exposure to bisphenols and parabens during pregnancy and relations to steroid changes. Environ. Res. 163, 115–122. https://doi.org/10.1016/j.envres.2018.01.031

Krause, M., Frederiksen, H., Sundberg, K., Jørgensen, F.S., Jensen, L.N., Nørgaard, P., Jørgensen, C., Ertberg, P., Juul, A., Drzewiecki, K.T., Skakkebaek, N.E., Andersson, A.M., 2018. Presence of benzophenones commonly used as UV filters and absorbers in paired maternal and fetal samples. Environ. Int. 110, 51–60. https://doi.org/10.1016/j.envint.2017.10.005

Krause, M., Klit, A., Blomberg Jensen, M., Søeborg, T., Frederiksen, H., Schlumpf, M., Lichtensteiger, W., Skakkebaek, N.E., Drzewiecki, K.T., 2012. Sunscreens: are they beneficial for health? An overview of endocrine disrupting properties of UV-filters. Int. J. Androl. 35, 424–436. https://doi.org/10.1111/j.1365-2605.2012.01280.x

Lee, K.P., Chromey, N.C., Culik, R., Barnes, J.R., Schneider, P.W., 1987. Toxicity of N-methyl-2-pyrrolidone (NMP): teratogenic, subchronic, and two-year inhalation studies. Fundam. Appl. Toxicol. Off. J. Soc. Toxicol. 9, 222–235. https://doi.org/10.1016/0272-0590(87)90045-5

Leppert, B., Strunz, S., Seiwert, B., Schlittenbauer, L., Schlichting, R., Pfeiffer, C., Röder, S., Bauer, M., Borte, M., Stangl, G.I., Schöneberg, T., Schulz, A., Karkossa, I., Rolle-Kampczyk, U.E., Thürmann, L., von Bergen, M., Escher, B.I., Junge, K.M., Reemtsma, T., Lehmann, I., Polte, T., 2020. Maternal paraben exposure triggers childhood overweight development. Nat. Commun. 11, 1–12. https://doi.org/10.1038/s41467-019-14202-1

Li, X., Sun, H., Yao, Y., Zhao, Z., Qin, X., Duan, Y., Wang, L., 2018. Distribution of Phthalate Metabolites between Paired Maternal-Fetal Samples. Environ. Sci. Technol. 52, 6626–6635. https://doi.org/10.1021/acs.est.8b00838

Manjunatha, B., Han, L., Kundapur, R.R., Liu, K., Lee, S.J., 2020. Herbul black henna (hair dye) causes cardiovascular defects in zebrafish (Danio rerio) embryo model. Environ. Sci. Pollut. Res. 27, 14150–14159. https://doi.org/10.1007/s11356-020-07762-z

Matta, M.K., Zusterzeel, R., Pilli, N.R., Patel, V., Volpe, D.A., Florian, J., Oh, L., Bashaw, E., Zineh, I., Sanabria, C., Kemp, S., Godfrey, A., Adah, S., Coelho, S., Wang, J., Furlong, L.-A., Ganley, C., Michele, T., Strauss, D.G., 2019. Effect of Sunscreen Application Under Maximal Use Conditions on Plasma Concentration of Sunscreen Active Ingredients: A Randomized Clinical Trial. JAMA 321, 2082–2091. https://doi.org/10.1001/jama.2019.5586

Meeker, J.D., Yang, T., Ye, X., Calafat, A.M., Hauser, R., 2011. Urinary concentrations of parabens and serum hormone levels, semen quality parameters, and sperm DNA damage. Environ. Health Perspect. 119, 252–257. https://doi.org/10.1289/ehp.1002238

Mínguez-Alarcón, L., Chiu, Y.H., Nassan, F.L., Williams, P.L., Petrozza, J., Ford, J.B., Calafat, A.M., Hauser, R., Chavarro, J.E., 2019. Urinary concentrations of benzophenone-3 and reproductive outcomes among women undergoing infertility treatment with assisted reproductive technologies. Sci. Total Environ. 678, 390–398. https://doi.org/10.1016/j.scitotenv.2019.04.452

Molins-Delgado, D., Olmo-Campos, M. del M., Valeta-Juan, G., Pleguezuelos-Hernández, V., Barceló, D., Díaz-Cruz, M.S., 2018. Determination of UV filters in human breast milk using turbulent flow chromatography and babies' daily intake estimation. Environ. Res. 161, 532–539. https://doi.org/10.1016/j.envres.2017.11.033

MoNA, 2022. MassBank of North America.

Morohoshi, K., Yamamoto, H., Kamata, R., Shiraishi, F., Koda, T., Morita, M., 2005. Estrogenic activity of 37 components of commercial sunscreen lotions evaluated by in vitro assays. Toxicol. Vitr. 19, 457–469. https://doi.org/https://doi.org/10.1016/j.tiv.2005.01.004

Ojha, A., Rathod, R., Padh, H., 2009. Quantification of 4-methylaminoantipyrine, the active metabolite of dipyrone, in human plasma. Bioanalysis 1, 293–298. https://doi.org/10.4155/bio.09.26

Phiboonchaiyanan, P.P., Busaranon, K., Ninsontia, C., Chanvorachote, P., 2017. Benzophenone-3 increases metastasis potential in lung cancer cells via epithelial to mesenchymal transition. Cell Biol. Toxicol. 33, 251–261. https://doi.org/10.1007/s10565-016-9368-3

Philippat, C., Wolff, M.S., Calafat, A.M., Ye, X., Bausell, R., Meadows, M., Stone, J., Slama, R., Engel, S.M., 2013. Prenatal exposure to environmental phenols: Concentrations in amniotic fluid and variability in urinary concentrations during pregnancy. Environ. Health Perspect. 121, 1225–1231. https://doi.org/10.1289/ehp.1206335

Pubchem, 2022. PubChem [WWW Document]. URL https://pubchem.ncbi.nlm.nih.gov/

Pycke, B.F.G., Geer, L.A., Dalloul, M., Abulafia, O., Halden, R.U., 2015. Maternal and fetal exposure to parabens in a multiethnic urban U.S. population. Environ. Int. 84, 193–200. https://doi.org/10.1016/j.envint.2015.08.012

Reimann, B., Vrijens, K., Roels, H.A., Wang, C., Cosemans, C., Van Overmeire, I., Nawrot, T.S., Plusquin, M., 2021. In utero exposure to parabens and early childhood BMI z-scores – Associations between placental ethyl paraben, longitudinal BMI trajectories and cord blood metabolic biomarkers. Environ. Int. 157, 106845. https://doi.org/10.1016/j.envint.2021.106845

Romøren, M., Lindbæk, M., Nordeng, H., 2012. Pregnancy outcome after gestational exposure to erythromycin – a study from Norway. https://doi.org/10.1111/j.1365-2125.2012.04286.x

Sang, Z., Leung, K.S.-Y., 2016. Environmental occurrence and ecological risk assessment of organic UV filters in marine organisms from Hong Kong coastal waters. Sci. Total Environ. 566–567, 489–498. https://doi.org/https://doi.org/10.1016/j.scitotenv.2016.05.120

Schlumpf, M., Cotton, B., Conscience, M., Haller, V., Steinmann, B., Lichtensteiger, W., 2001. In vitro and in vivo estrogenicity of UV screens. Environ. Health Perspect. 109, 239–244. https://doi.org/10.1289/ehp.01109239

Schlumpf, M., Durrer, S., Faass, O., Ehnes, C., Fuetsch, M., Gaille, C., Henseler, M., Hofkamp, L., Maerkel, K., Reolon, S., Timms, B., Tresguerres, J.A.F., Lichtensteiger, W., 2008. Developmental toxicity of UV filters and environmental exposure: a review. Int. J. Androl. 31, 144–151. https://doi.org/10.1111/j.1365-2605.2007.00856.x

Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P., Hollender, J., 2014. Identifying small molecules via high resolution mass spectrometry: Communicating confidence. Environ. Sci. Technol. 48, 2097–2098. https://doi.org/10.1021/es5002105

Shivashankara, A.R., Haniadka, R., Fayad, R., Palatty, P.L., Arora, R., Baliga, M.S., 2013. Chapter 42 - Hepatoprotective Effects of Zingiber officinale Roscoe (Ginger): A Review, in: Watson, R.R., Preedy, V.R.B.T.-B.F. as D.I. for L. and G.D. (Eds.), . Academic Press, San Diego, pp. 657–671. https://doi.org/https://doi.org/10.1016/B978-0-12-397154-8.00034-8

Sim, M.-O., Ham, J.R., Lee, H.-I., Seo, K.-I., Lee, M.-K., 2014. Long-term supplementation of umbelliferone and 4-methylumbelliferone alleviates high-fat diet induced hypertriglyceridemia and hyperglycemia in mice. Chem. Biol. Interact. 216, 9–16. https://doi.org/10.1016/j.cbi.2014.03.003

Solomon, H.M., Burgess, B.A., Kennedy, G.L.J., Staples, R.E., 1995. 1-Methyl-2-pyrrolidone (NMP): reproductive and developmental toxicity study by inhalation in the rat. Drug Chem. Toxicol. 18, 271–293. https://doi.org/10.3109/01480549509014324

Song, S., He, Y., Huang, Y., Huang, X., Guo, Y., Zhu, H., Kannan, K., Zhang, T., 2020. Occurrence and transfer of benzophenone-type ultraviolet filters from the pregnant women to fetuses. Sci. Total Environ. 726, 138503. https://doi.org/10.1016/j.scitotenv.2020.138503

Sunyer-Caldú, A., Peiró, A., Díaz, M., Ibáñez, L., Gago-Ferrero, P., Silvia Diaz-Cruz, M., 2021. Development of a sensitive analytical method for the simultaneous analysis of Benzophenone-type UV filters and paraben preservatives in umbilical cord blood. MethodsX 8. https://doi.org/10.1016/j.mex.2021.101307

Suzuki, T., Kitamura, S., Khota, R., Sugihara, K., Fujimoto, N., Ohta, S., 2005. Estrogenic and antiandrogenic activities of 17 benzophenone derivatives used as UV stabilizers and sunscreens. Toxicol. Appl. Pharmacol. 203, 9–17. https://doi.org/10.1016/j.taap.2004.07.005

Taha, H.M., Aalizadeh, R., Alygizakis, N., et. al., 2022. The NORMAN Suspect List Exchange (NORMAN-SLE): Facilitating European and Worldwide Collaboration on Suspect Screening in High Resolution Mass Spectrometry. Environ. Sci. Eur. https://doi.org/https://doi.org/10.21203/rs.3.rs-1902466/v1

Takatori, S., Kitagawa, Y., Oda, H., Miwa, G., Nishikawa, J.I., Nishihara, T., Nakazawa, H., Hori, S., 2003. Estrogenicity of metabolites of benzophenone derivatives examined by a yeast two-hybrid assay. J. Heal. Sci. 49, 91–98. https://doi.org/10.1248/jhs.49.91

Thermo Scientific, 2022. mzCloud [WWW Document]. URL https://www.mzcloud.org/

Towers, C. V., Terry, P.D., Lewis, D., Howard, B., Chambers, W., Armistead, C., Weitz, B., Porter, S., Borman, C.J., Kennedy, R.C.M., Chen, J., 2015. Transplacental passage of antimicrobial paraben preservatives. J. Expo. Sci. Environ. Epidemiol. 25, 604–607. https://doi.org/10.1038/jes.2015.27

Valle-Sistac, J., Molins-Delgado, D., Díaz, M., Ibáñez, L., Barceló, D., Silvia Díaz-Cruz, M., 2016. Determination of parabens and benzophenone-type UV filters in human placenta: First description of the existence of benzyl paraben and benzophenone-4. Environ. Int. 88, 243–249. https://doi.org/10.1016/j.envint.2015.12.034

Vela-Soria, F., Gallardo-Torres, M.E., Ballesteros, O., Díaz, C., Pérez, J., Navalón, A., Fernández, M.F., Olea, N., 2017. Assessment of parabens and ultraviolet filters in human placenta tissue by ultrasound-assisted extraction and ultra-high performance liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1487, 153–161. https://doi.org/10.1016/j.chroma.2017.01.041

Vela-Soria, F., Jiménez-Díaz, I., Rodríguez-Gómez, R., Zafra-Gómez, A., Ballesteros, O., Navalón, A., Vílchez, J.L., Fernández, M.F., Olea, N., 2011. Determination of benzophenones in human placental tissue samples by liquid chromatography-tandem mass spectrometry. Talanta 85, 1848–1855. https://doi.org/10.1016/j.talanta.2011.07.030

Vela-Soria, F., Rodríguez, I., Ballesteros, O., Zafra-Gómez, A., Ballesteros, L., Cela, R., Navalón, A., 2014. Simplified matrix solid phase dispersion procedure for the determination of parabens and benzophenone-ultraviolet filters in human placental tissue samples. J. Chromatogr. A 1371, 39–47. https://doi.org/10.1016/j.chroma.2014.10.063

von der Ohe, P., Aalizadeh, R., 2020. S13 | EUCOSMETICS | Combined Inventory of Ingredients Employed in Cosmetic Products (2000) and Revised Inventory (2006). https://doi.org/10.5281/ZENODO.3959386

Wang, A., Abrahamsson, D.P., Jiang, T., Wang, M., Morello-Frosch, R., Park, J.S., Sirota, M., Woodruff, T.J., 2021. Suspect Screening, Prioritization, and Confirmation of Environmental Chemicals in Maternal-Newborn Pairs from San Francisco. Environ. Sci. Technol. 55, 5037–5049. https://doi.org/10.1021/acs.est.0c05984

Wang, L., Kannan, K., 2013. Characteristic profiles of benzonphenone-3 and its derivatives in urine of children and adults from the United States and China. Environ. Sci. Technol. 47, 12532–12538. https://doi.org/10.1021/es4032908

Wang, S.Q., Balagula, Y., Osterwalder, U., 2010. Photoprotection: a Review of the Current and Future Technologies. Dermatol. Ther. 23, 31–47. https://doi.org/10.1111/j.1529-8019.2009.01289.x

Wickman, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Zhang, B., He, Y., Zhu, H., Huang, X., Bai, X., Kannan, K., Zhang, T., 2020. Concentrations of bisphenol A and its alternatives in paired maternal–fetal urine, serum and amniotic fluid from an e-waste dismantling area in China. Environ. Int. 136, 105407. https://doi.org/10.1016/j.envint.2019.105407

Zhang, T., Sun, H., Qin, X., Wu, Q., Zhang, Y., Ma, J., Kannan, K., 2013. Benzophenone-type UV filters in urine and blood from children, adults, and pregnant women in China: Partitioning between blood and urine as well as maternal and fetal cord blood. Sci. Total Environ. 461–462, 49–55. https://doi.org/10.1016/j.scitotenv.2013.04.074

Zhao, H., Wei, D., Li, M., Du, Y., 2013. Substituent contribution to the genotoxicity of benzophenone-type UV filters. Ecotoxicol. Environ. Saf. 95, 241–246. https://doi.org/10.1016/j.ecoenv.2013.05.036

Zhao, X., Qiu, W., Zheng, Y., Xiong, J., Gao, C., Hu, S., 2019. Occurrence , distribution , bioaccumulation , and ecological risk of bisphenol analogues , parabens and their metabolites in the Pearl River Estuary , South. Ecotoxicol. Environ. Saf. 180, 43–52. https://doi.org/10.1016/j.ecoenv.2019.04.083

6.2.2 Publication #14 Supplementary information

Target and suspect screening of UV filters and parabens personal care products (PCPs) in human cord blood: prenatal exposure after mother-foetus transfer

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Submitted to Environment International

Section S1. Chemicals and reagents.
Tables. (Tables S1-S4).

Section S1. Chemicals and reagents

The UVFs benzophenone-3 (BP3), benzophenone-1 (BP1), benzophenone-4 (BP4), 4-hydroxybenzophenone (4HB), 4,4’-Dihydroxybenzophenone (4DHB), avobenzone (AVO) and the PBs methyl paraben (MePB), propyl paraben (PrPB), benzyl paraben (BePB) and butyl paraben (BuPB) were purchased from Sigma Aldrich (Darmstadt, Germany). Benzophenone-2 (BP2) and 2,2’-Dihydroxy-4-methoxybenzophenone (DHMB) were obtained from Merck (Darmstadt, Germany).

The isotopically labeled compounds used were 2-hydroxy-4-methoxybenzophenone-2′,3′,4′,5′,6′-d5 (BP3-d_5), benzyl paraben-d4 (BePB-d_4) and 5-(2,5-dimethylphenoxy)-2,2-bis(trideuteriomethyl)pentanoic acid (Gemfibrozil-d_6) were from CDN isotopes (Quebec, Canada).

Water and methanol (MeOH) of high-performance liquid chromatography (HPLC) grade were obtained from J.T. Backer (Deventer, The Netherlands) and the nitrogen (99.995% purity) was supplied by Air Liquide (Barcelona, Spain). Formic acid (FA) and ammonium acetate ($AcNH_4$) were from Merck (Darmstadt, Germany). For the extraction process tert-butyl ether (MTBE), sodium chloride (NaCl), sodium hydrogen carbonate ($NaHCO_3$) from Sigma Aldrich (Darmstadt, Germany) and ammonium formate (NH_4HCO_2) from Fisher Scientific (Fair Lawn, NJ, USA) were used.

Table S1. Standard compounds name and SMILES purchased from Sigma-Aldrich for identity confirmation.

Compound	SMILES
1,2-Benzisothiazolin-3-one	c12c(cccc2)s[nH]c1=O
Amiloxate	COc1ccc(C=CC(=O)OCCC(C)C)cc1
Benzoic acid, 2-hydroxy-, 2-(2-hydroxypropoxy)-1-methylethyl ester	CC(O)COCC(C)OC(=O)c1ccccc1O
Isopropyl methoxycinnamate	COc1ccc(C=CC(=O)OC(C)C)cc1
Capryoyl salicylic acid	CCCCCCCC(=O)Oc1ccccc1C(O)=O
7-hydroxycoumarin (umbelliferone)	Oc1ccc2ccc(=O)oc2c1
4-aminoantipyrine	c(ccc1N(N(C=2C)C)C(=O)C2N)cc1
Dodecamethylcyclohexasiloxane	C[Si]1(O[Si](O[Si](O[Si](O[Si](O[Si](O1)(C)C)(C)C)(C)C)(C)C)(C)C)C
N-butylbenzenesulfonamide	CCCCNS(=O)(=O)C1=CC=CC=C1
Erythromycin	CCC1C(C(C(C(=O)C(CC(C(C(C(C(C(=O)O1)C)OC2CC(C(C(O2)C)O)(C)OC)C)OC3C(C(CC(O3)C)N(C)C)O)(C)O)C)C)O)(C)O
Diisononyl adipate	CC(C)CCCCCCOC(=O)CCCCC(=O)OCCCCCCC(C)C
N-Methyl-2-pyrrolidone	CN1CCCC1=O
2-[2-(4-Nonylphenoxy)ethoxy]ethanol	CCCCCCCCCc1ccc(OCCOCCO)cc1
Dodecylbenzenesulfonic acid	CCCCCCCCCCCCc1ccccc1S(O)(=O)=O
3,4-dihydro-2H-1,4-Benzoxazin-6-ol	Oc1ccc2OCCNc2c1
2-Ethylhexyl salicylate	CCCCC(CC)COC(=O)c1ccccc1O
8-Hydroxychinolin	C1=CC2=C(C(=C1)O)N=CC=C2
2,2'-Methylenebis(4-methyl-6-tert-butylphenol)	C1=C(C=C(C(=C1C(C)(C)C)O)CC2=CC(=CC(=C2O)C(C)(C)C)C)C

Table S2. Biological and anthropometrical parameters of the pairs mother-newborns.

Delivery: (0=spontaneuous, 1=induced); Parity: (0=primiparous, 1=pregnant before); Gender: (F= female, M=male)
Smoking: (Y=Yes, N=No); Gestational diabetes: (Y=Yes, N=No); Pre-gestacional diabetes: (Y=Yes, N=No)

Sample number	Mother age	Delivery	Gestational age (weeks)	Gender	Weight (g)	Length (cm)	Cranial perimeter (cm)	Parity	Smoking	Gestational diabetes	Pre-gestacional diabetes
1	33	0	38.3	F	3605	50	36.0	1	N	N	N
2	31	0	39.0	F	2930	49	36.0	0	N	N	N
3	30	1	41.0	M	3550	52	36.0	1	N	N	N
4	27	0	39.0	M	3730	51	35.0	0	Y	N	N
5	37	0	41.0	F	3590	51	34.0	1	N	N	N
6	30	0	41.2	F	3515	50	35.0	1	N	N	N
7	29	1	36.0	M	3140	48	35.0	1	N	N	Y
8	30	1	37.2	M	3200	49	35.0	1	N	N	N
9	34	0	41.4	M	3460	49	35.0	0	N	Y	N
10	31	0	39.6	M	3305	49	34.0	0	N	N	N
11	34	0	39.3	F	2970	49	34.0	1	N	N	N
12	31	0	38.3	F	2500	49	32.0	0	N	N	N
13	34	0	39.3	F	2500	46	32.0	1	N	N	N
14	32	1	40.2	M	3400	50	36.0	1	N	N	N
15	29	0	39.2	F	3970	50	33.0	0	N	N	N
16	25	1	40.0	F	3400	52	36.0	0	N	N	N
17	25	0	40.2	F	3420	49	34.0	0	N	N	N
18	28	0	40.3	M	3580	50	36.0	1	N	N	N
19	20	0	41.0	F	3620	51	35.0	0	N	N	N
20	26	1	41.4	F	4010	51	36.0	0	N	N	N
21	32	0	38.0	M	3020	49	35.0	0	N	N	N
22	27	0	40.2	M	3915	53	36.5	1	N	N	N
23	32	1	38.2	F	2840	48	34.0	1	N	N	N
24	32	1	37.0	M	2630	48	33.0	1	N	N	N
25	31	0	39.4	F	3080	47	34.0	0	N	N	N
26	33	1	37.2	M	2880	48	32.0	0	N	N	N
27	27	0	39.6	F	3380	51	34.0	0	Y	N	N
28	26	0	38.0	F	3370	49	34.5	1	N	N	N
29	38	0	38.2	F	3100	49	35.0	1	N	Y	N
30	27	0	39.6	F	3100	50	35.0	0	Y	N	N
31	29	1	39.1	M	4050	51	34.0	0	N	N	N
32	39	0	38.5	F	3775	51	35.0	1	N	N	N
33	28	0	41.0	F	3100	49	34.0	0	N	N	N
34	29	0	37.3	M	3370	52	36.0	1	N	N	N

Table S2. Continued.

35	34	0	40.0	F	2880	48	34.0	0	N	N	N
36	36	1	37.2	F	2300	45	31.0	0	N	N	N
37	43	0	38.0	F	3200	48	36.0	1	N	N	N
38	31	1	40.0	M	4560	52	36.5	1	Y	Y	N
39	25	0	41.4	F	3720	52	35.0	0	N	N	N
40	28	0	38.5	M	3350	51	35.0	0	N	N	N
41	34	0	38.0	F	2960	49	34.0	1	N	N	N
42	26	0	39.3	M	3180	50	34.5	0	N	N	N
43	25	0	40.6	F	2900	47	32.0	1	N	N	N
44	32	0	40.0	F	3880	48	Not available	0	N	N	N
45	27	0	39.2	F	3130	48	34.0	0	N	N	N
46	24	1	39.0	M	3400	48	34.0	0	N	N	N
47	34	0	38.0	M	3510	50	34.5	1	N	Y	N
48	26	0	41.0	F	3600	51	36.0	0	N	N	N
49	31	0	40.3	F	3925	52	35.0	1	N	N	N
50	32	1	39.0	F	3770	50	34.0	0	N	Y	N
51	39	0	39.5	F	2980	48	33.0	0	N	N	N
52	35	0	39.0	M	3605	52	36.5	1	N	Y	N
53	26	0	39.6	F	3995	51	35.0	0	N	N	N
54	32	0	39.3	F	3285	50	34.5	0	N	N	N
55	31	1	40.2	M	3280	48	35.0	0	N	N	N
56	28	0	41.5	M	3340	50	36.5	0	N	N	N
57	31	0	41.0	M	3770	51	35.5	0	N	N	N
58	37	0	39.0	F	3390	49	35.0	1	N	N	N
59	43	1	39.4	F	3570	50	36.0	1	N	N	N
60	35	0	39.3	M	3575	49	35.0	0	N	N	N
61	32	0	40.0	F	3260	50	30.0	0	N	N	N
62	21	0	41.0	F	2670	48	34.0	0	N	N	N
63	29	0	40.5	F	3065	48	34.5	0	N	N	N
64	23	0	38.4	M	3240	51	35.0	0	N	N	N
65	27	0	40.3	M	3095	52	33.0	1	N	N	N
66	26	0	41.3	F	3140	49	34.0	0	N	N	N
67	37	0	39.0	M	3530	50	34.5	1	Y	N	N
68	29	0	39.0	M	3355	50	35.0	0	N	N	N
69	31	0	39.0	M	4050	53	37.0	1	N	N	N

Table S3. Concentrations of target compounds, the total sum by the families, and the total sum, with range, frequency of detection and average values.

Sample Name	BP3	BP1	BP2	4HB	DHB	DHMB	AVO	BePB (-)	BuPB (-)	PrPB (-)	MEPB (-)	BP4 (-)	∑UVFs	∑PBs	∑TOTAL
1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4	**19.1**	n.d.	n.d.	n.d.	n.d.	n.d.	**15.4**	n.d.	n.d.	n.d.	n.d.	n.d.	**34.5**	n.d.	**34.5**
5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**8.5**	n.d.	n.d.	**8.5**	**8.5**
12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
13	n.d.	n.d.	**2.1**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**2.1**	n.d.	**2.1**
14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**5.3**	**5.3**	n.d.	**5.3**
15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
16	n.d.	**6.9**	**53.3**	**7.5**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**67.7**	n.d.	**67.7**
17	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**3.6**	**3.6**	n.d.	**3.6**
18	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
19	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**1.1**	**1.1**	n.d.	**1.1**
20	**22.9**	n.d.	**2.4**	n.d.	n.d.	n.d.	**23.9**	n.d.	n.d.	n.d.	n.d.	n.d.	**49.2**	n.d.	**49.2**
21	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**9.1**	n.d.	n.d.	n.d.	n.d.	n.d.	**9.1**	n.d.	**9.1**
23	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
24	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**10.1**	n.d.	n.d.	n.d.	n.d.	n.d.	**10.1**	n.d.	**10.1**
25	**13.6**	n.d.	n.d.	n.d.	n.d.	n.d.	**10.1**	n.d.	n.d.	n.d.	n.d.	n.d.	**23.7**	n.d.	**23.7**
26	**9.0**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**9**	n.d.	**9**
27	**9.4**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**9**	n.d.	**9.4**
28	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
29	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
31	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
32	**8.9**	n.d.	n.d.	n.d.	n.d.	n.d.	**10.5**	n.d.	n.d.	n.d.	n.d.	n.d.	**19.4**	n.d.	**19.4**
33	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
34	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
35	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
36	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**9.5**	n.d.	n.d.	n.d.	**5.2**	n.d.	**9.5**	**5.2**	**14.7**
37	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
38	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
39	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
40	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table S3. Continued.

41	**9.8**	n.d.	n.d.	n.d.	n.d.	n.d.	**17.5**	n.d.	n.d.	n.d.	n.d.	n.d.	**27.3**	n.d.	**27.3**
42	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
43	**9.9**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**9.9**	n.d.	9.9
44	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
45	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**5.2**	n.d.	n.d.	**5.2**	**5.2**
46	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
47	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
48	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
49	**12.6**	n.d.	n.d.	n.d.	n.d.	n.d.	**9.2**	n.d.	n.d.	n.d.	n.d.	n.d.	**21.8**	n.d.	**21.8**
50	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
51	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
52	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
53	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
54	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
55	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
56	n.d.	n.d.	**2.0**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**2**	n.d.	**2**
57	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**5.2**	**10.5**	n.d.	n.d.	**15.7**	**15.7**
58	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
59	**14.0**	n.d.	n.d.	n.d.	n.d.	n.d.	**9.0**	n.d.	n.d.	n.d.	n.d.	n.d.	**23**	n.d.	**23**
60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
61	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
62	**13.6**	**7.0**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**20.6**	n.d.	**20.6**
63	**9.8**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**9.8**	n.d.	**9.8**
64	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
65	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
66	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
67	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
68	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
69	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Values are in ppb (ng/mL); n.d.: not detected; ∑UVFs: total sum of ultraviolet filters;
∑PBs: total sum of parabens; ∑Total: total sum of each sample

Table S4. List of false positives identified after the identity confirmation process,CAS# and molecular formula.

Compound	CAS number	Molecular formula
Amiloxate	71617-10-2	$C_{15} H_{20} O_3$
Benzoic acid, 2-hydroxy-, 2-(2-hydroxypropoxy)-1-methylethyl ester	68683-31-8	$C_{13} H_{18} O_5$
Isopropyl methoxycinnamate	5466-76-2	$C_{13} H_{16} O_3$
Capryoyl salicylic acid	78418-01-6	$C_{15} H_{20} O_4$
Dodecylbenzenesulfonic acid	27176-87-0	$C_{18} H_{30} O_3 S$
2-Ethylhexyl salicylate	118-60-5	$C_{15} H_{22} O_3$
Dodecamethylcyclohexasiloxane	540-97-6	$C_{12} H_{36} O_6 Si_6$
N-butylbenzenesulfonamide	3622-84-2	$C_{10} H_{15} N O_2 S$

6.3 Discussion

The works included in this chapter provide novel data on the bioaccumulation and risk of PCPs in the human body. Publications #4 and #11 demonstrate that the risk assessment is useful for estimating the potential adverse effects of compounds of interest. Meanwhile, Publications #13 and #14 contribute to broadening the knowledge about the human exposome in a critical development period, demonstrating that specific PCPs can reach the human blood and cross the placental barrier. As in previous chapters, a general comparison and discussion of the results are included in this section.

Daily intake estimation (EDI) and risk assessment

For the samples included in this thesis representing food for human consumption, i.e. fish and vegetables, the daily intake of CECs via ingestion was estimated. The risk quotients (RQ) of the compounds found at the highest concentrations are listed in **Table 6.2**. In general, SCY was the compound with the highest RQ in all the crops and fish, showing a similar bioaccumulation and risk pattern, even though the matrices and sampling locations differed. The highest RQ of SCY was notorious in fish and carrots. Among crops, carrots presented the highest hazard index (HI, the sum of all RQ), followed by tomatoes and lettuces. This HI order agrees with the sum of CECs' concentrations uptaken by each crop. However, in lettuce and tomatoes, the highest RQ corresponded to DCF.

Overall, the estimated risk quotients shown in the table indicate that the concentrations found in the fish and crops posed no risk to human health since all estimated values were far from one. Even though this estimation shows that it is safe to consume the fish and, especially, the crops irrigated with waste and reclaimed water, it has to be considered that some ADI values were not available in the literature.

BP4, for example, was found at considerable concentrations in tomatoes (68.7 ng/g average), but the RQ could not be calculated. In addition, using the target analysis approach, only some CECs and TPs were included in the study, giving a partial picture of the CECs' occurrence in the crops. It means some undetected contaminants could change the reported RQ values, depending on the concentrations. Furthermore, the RQs are estimations, and although they are useful as qualitative indicators, human biomonitoring is mandatory to know the actual exposure to these contaminants.

Table 6.2. Estimated daily intake (EDI), acceptable daily intake (ADI) and risk quotients (RQ) for the compounds found at highest concentrations in fish, lettuces, tomatoes, and carrots. BW: Body weight

Fish			
	4HB	EtPABA	SCY
EDI (µg/kg*BW*day)	1.8E-02	4.1E-03	7.0E-02
ADI (µg/kg*BW*day)	N.A.	N.A.	70.35
RQ	N.A.	N.A.	0.001

Lettuces						
	SCY	DCF	ATL	CBZ	OXL	BZT
EDI (µg/kg*BW*day)	3.8E-04	3.1E-04	2.4E-05	3.6E-06	3.4E-05	6.1E-05
ADI (µg/kg*BW*day)	6.3	1.4	2.7	2.9	2.5	1.5
RQ	6.0E-05	2.2E-04	8.9E-06	1.2E-06	1.3E-05	4.1E-05

Tomatoes						
	SCY	DCF	CBZ	OXL	BZT	NPX
EDI (µg/kg*BW*day)	1.9E-03	4.6E-03	1.6E-04	1.1E-04	7.8E-06	2.1E-04
ADI (µg/kg*BW*day)	6.3	1.4	2.9	2.5	1.5	7.1
RQ	3.0E-04	3.3E-03	5.4E-05	4.4E-05	5.2E-06	3.0E-05

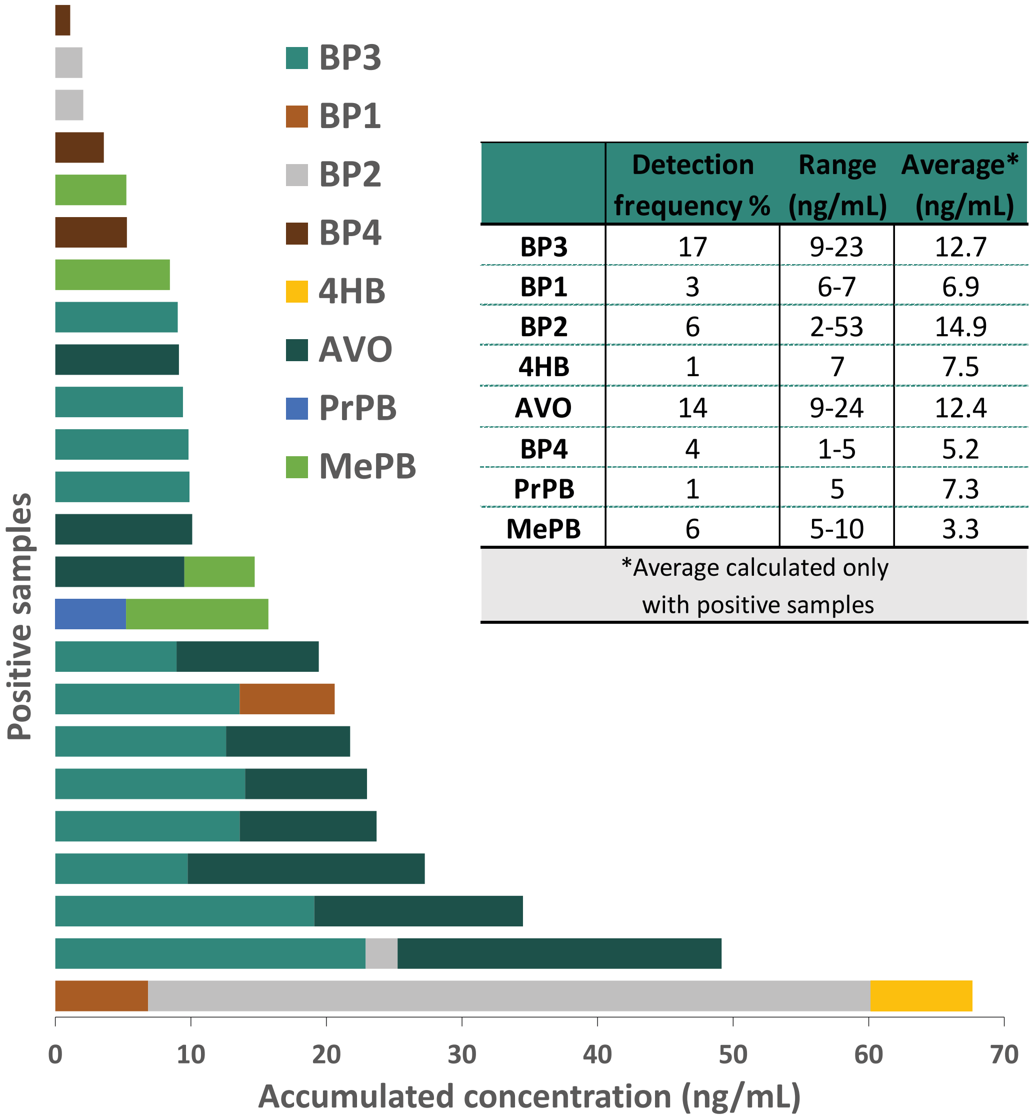
Carrots				
	SCY	DCF	ATL	OXL
EDI (µg/kg*BW*day)	2.7E-02	9.8E-04	1.2E-03	8.0E-05
ADI (µg/kg*BW*day)	6.3	1.4	2.7	2.5
RQ	4.31E-03	7.0E-04	4.4E-04	3.2E-05

Human exposure to CECs

As explained in the introduction of this chapter, the daily intake and risk assessment are based on estimations that can serve as guide values. The best approach to assess the fate of CECs is by monitoring them in humans. Publications #13 and #14 demonstrate that CECs, in general, and PCPs, in particular, are present in the human body and can reach pregnant women's foetuses. **Figure 6.3** summarises the results of the target analysis obtained in the positive samples (left) and the range, average and frequencies of detection of the UVFs and PBs analysed (right). A total of 11 samples (16% of the cohort of 69 individuals) present accumulated values higher than 10 ng/mL in the umbilical cord blood. These values are concerning since they demonstrate that these compounds (most endocrine disruptors) can reach the foetus at concentrations up to 60 ng/mL. Curiously, a pattern was observed for some of the positive samples; BP3 (light green in the figure) and AVO (dark green in the figure) were found together at very similar concentrations. Even the detection frequencies, concentration ranges and average concentrations are nearly identical. This could be expected since both UVFs are commonly used together and at similar concentrations in many PCPs' formulations (Geoffrey et al., 2019), and both present high logk_{ow} values (>3.5), showing a similar tendency to bioaccumulate.

Relevance of non-target strategies

The majority of studies focus on the most frequently used and persistent compounds because they are more likely to be present in the samples, mainly using target strategies. An exception are target methodologies focusing on specific families of compounds (e.g. phthalates), where metabolites are usually included. In the target study of Publication #14, 8 of the 12 target compounds (8 benzophenones derivatives and 4 PBs) were at least detected in one of the cord blood samples, with detection frequencies ranging between 1.4 and 17.4 %. In order to expand the chemical domain and obtain a more holistic picture of PCPs exposure, an additional suspect screening of 3,426 PCPs was performed. It included a combined inventory of ingredients employed in cosmetic and personal care products. Of those 3,426 compounds, 13 additional compounds were tentatively identified, and 10 could finally be confirmed with their standard.



	Detection frequency %	Range (ng/mL)	Average* (ng/mL)
BP3	17	9-23	12.7
BP1	3	6-7	6.9
BP2	6	2-53	14.9
4HB	1	7	7.5
AVO	14	9-24	12.4
BP4	4	1-5	5.2
PrPB	1	5	7.3
MePB	6	5-10	3.3

*Average calculated only with positive samples

Figure 6.3. PCPs' accumulated concentrations in positive samples (ng/mL) (left) and detection frequency, range and average of the target PCPs (right).

The detection frequencies in the suspect screening ranged between 1.4 and 20.3 %. This shows that some of the identified compounds were present in more samples than BP3, the most frequently detected target compound. To the author's knowledge, this is the first evidence of the presence of eleven of these thirteen compounds in cord blood. The presence of some CECs, such as erythromycin (ERY), an antibiotic often prescribed when the waters break early (Romøren et al., 2012), is evident. But other CECs' occurrence, such as 3,4-Dihydro-2H-1,4-Benzoxazin-6-ol (BZX), a hair dyeing, was much more complicated to predict and would not be selected *a priori* for target analysis. The information available for most of these compounds is very limited, especially regarding toxicological information. Some of them (such as N-Methyl-2-pyrrolidone (MPY)) display reproductive toxicity and may damage the foetus. These effects are especially concerning for compounds present in the foetus' blood while developing.

Therefore, the complementary use of target and non-target strategies is key to obtaining an overall picture of the contaminants present. These two works may be considered a preliminary and integrated study to provide the required analytical methodology and base concentration levels of benzophenone-type UVFs and PBs in human cord blood. We expect this will help further the determination of other PCPs in human umbilical cord blood in new studies. In addition, further research is guaranteed to assess the long-term effects of the determined PCPs in the development of the foetus and throughout the life of the child.

6.4 References

Alygizakis, N.A., Oswald, P., Thomaidis, N.S., Schymanski, E.L., Aalizadeh, R., Schulze, T., Oswaldova, M., Slobodnik, J., 2019. NORMAN digital sample freezing platform: A European virtual platform to exchange liquid chromatography high resolution-mass spectrometry data and screen suspects in “digitally frozen” environmental samples. TrAC - Trends Anal. Chem. 115, 129–137. https://doi.org/10.1016/j.trac.2019.04.008

Amin, M.M., Ebrahim, K., Poursafa, P., 2017. Development of a dispersive liquid–liquid microextraction (DLLME) method coupled with GC/MS as a simple and valid method for simultaneous determination of phthalate metabolites in plasma. Int. J. Environ. Anal. Chem. 97, 1362–1377. https://doi.org/10.1080/03067319.2017.1422497

Baker, B.H., Wu, H., Laue, H.E., Boivin, A., Gillet, V., Langlois, M.-F., Bellenger, J.-P., Baccarelli, A.A., Takser, L., 2020. Methylparaben in meconium and risk of maternal thyroid dysfunction, adverse birth outcomes, and Attention-Deficit Hyperactivity Disorder (ADHD). Environ. Int. 139, 105716. https://doi.org/10.1016/j.envint.2020.105716

Casas, L., Fernández, M.F., Llop, S., Guxens, M., Ballester, F., Olea, N., Basterrechea, M., Santa, L., Rodríguez, M., Riaño, I., 2011. Urinary concentrations of phthalates and phenols in a population of Spanish pregnant women and children. Environ. Int. 37, 858–866. https://doi.org/10.1016/j.envint.2011.02.012

Dualde, P., Pardo, O., Corpas-burgos, F., Kuligowski, J., 2020. Biomonitoring of parabens in human milk and estimated daily intake for breastfed infants. Chemosphere 240. https://doi.org/10.1016/j.chemosphere.2019.124829

Fent, K., Weston, A.A., Caminada, D., 2006. Ecotoxicology of human pharmaceuticals 76, 122–159. https://doi.org/10.1016/j.aquatox.2005.09.009

Fernández, M.F., Mustieles, V., Suárez, B., Reina-Pérez, I., Olivas-Martinez, A., Vela-Soria, F., 2021. Determination of bisphenols , parabens , and benzophenones in placenta by dispersive liquid-liquid microextraction and gas chromatography-tandem mass spectrometry. Chemosphere 274. https://doi.org/10.1016/j.chemosphere.2021.129707

Fisher, M., Macpherson, S., Braun, J.M., Hauser, R., Walker, M., Feeley, M., Mallick, R., Be, R., 2017. Paraben concentrations in maternal urine and breast milk and its association with personal care product use. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.6b04302

Frederiksen, H., Jensen, T.K., Jørgensen, N., Kyhl, H.B., Husby, S., Skakkebæk, N.E., Main, K.M., Juul, A., Andersson, A., 2013. Human urinary excretion of non-persistent environmental chemicals: an overview of Danish data collected between 2006 and 2012. https://doi.org/10.1530/REP-13-0522

Frederiksen, H., Krause, M., Andersson, A., Jørgensen, N., Rehfeld, A., 2021. UV filters in matched seminal fluid , urine , and serum samples from young men 3, 345–355. https://doi.org/10.1038/s41370-020-0209-3

Geer, L.A., Pycke, B.F.G., Waxenbaum, J., Sherer, D.M., Abulafia, O., Halden, R.U., 2017. Association of birth outcomes with fetal exposure to parabens, triclosan and triclocarban in an immigrant population in Brooklyn, New York. J. Hazard. Mater. 323, 177–183. https://doi.org/10.1016/j.jhazmat.2016.03.028

Geoffrey, K., Mwangi, A.N., Maru, S.M., 2019. Sunscreen products: Rationale for use, formulation development and regulatory considerations. Saudi Pharm. J. 27, 1009–1018. https://doi.org/10.1016/j.jsps.2019.08.003

Gil-Solsona, R., Nika, M.C., Bustamante, M., Villanueva, C.M., Foraster, M., Cosin-Tomás, M., Alygizakis, N., Gómez-Roig, M.D., Llurba-Olive, E., Sunyer, J., Thomaidis, N.S., Dadvand, P., Gago-Ferrero, P., 2021. The potential of sewage sludge to predict and evaluate the human chemical exposome. Environ. Sci. Technol. Lett. 8, 1077–1084. https://doi.org/10.1021/acs.estlett.1c00848

Gustafsson, Å., Bergman, Å., Weiss, J.M., 2022. Estimated daily intake of per- and polyfluoroalkyl substances related to different particle size fractions of house dust. Chemosphere 303, 135061. https://doi.org/10.1016/j.chemosphere.2022.135061

Hond, E. Den, Paulussen, M., Geens, T., Bruckers, L., Baeyens, W., David, F., Dumont, E., Loots, I., Morrens, B., Nemery, B., Bellevaux, D., Nelen, V., Schoeters, G., Larebeke, N. Van, Covaci, A., 2013. Biomarkers of human exposure to personal care products: Results from the Flemish Environment and Health Study (FLEHS 2007 – 2011). Sci. Total Environ. 463–464, 102–110. https://doi.org/10.1016/j.scitotenv.2013.05.087

Kim, S., Choi, K., 2014. Occurrences, toxicities, and ecological risks of benzophenone-3, a common component of organic sunscreen products: A mini-review. Environ. Int. 70, 143–157. https://doi.org/https://doi.org/10.1016/j.envint.2014.05.015

Kolatorova Sosvorova, L., Vitku, J., Hampl, R., Adamcova, K., Skodova, T., Simkova, M., Parizek, A., Starka, L., Duskova, M., 2018. Exposure to bisphenols and parabens during pregnancy and relations to steroid changes. Environ. Res. 163, 115–122. https://doi.org/10.1016/j.envres.2018.01.031

Krause, M., Frederiksen, H., Sundberg, K., Jørgensen, F.S., Jensen, L.N., Nørgaard, P., Jørgensen, C., Ertberg, P., Juul, A., Drzewiecki, K.T., Skakkebaek, N.E., Andersson, A.M., 2018. Presence of benzophenones commonly used as UV filters and absorbers in paired maternal and fetal samples. Environ. Int. 110, 51–60. https://doi.org/10.1016/j.envint.2017.10.005

Li, N., Ho, W., Shiu, R., Wu, S., Tsang, E.P.K., Ying, G., 2019. Ultra violet filters in the urine of preschool children and drinking water 133. https://doi.org/10.1016/j.envint.2019.105246

Meeker, J.D., Yang, T., Ye, X., Calafat, A.M., Hauser, R., 2011. Urinary concentrations of parabens and serum hormone levels, semen quality parameters, and sperm DNA damage. Environ. Health Perspect. 119, 252–257. https://doi.org/10.1289/ehp.1002238

Molins-Delgado, D., Olmo-Campos, M. del M., Valeta-Juan, G., Pleguezuelos-Hernández, V., Barceló, D., Díaz-Cruz, M.S., 2018. Determination of UV filters in human breast milk using turbulent flow chromatography and babies’ daily intake estimation. Environ. Res. 161, 532–539. https://doi.org/10.1016/j.envres.2017.11.033

Pachkowski, B., Post, G.B., Stern, A.H., 2019. The derivation of a Reference Dose (RD) for perfluorooctane sulfonate (PFOS) based on immune suppression. Environ. Res. 171, 452–469. https://doi.org/10.1016/j.envres.2018.08.004

Park, N., Hee, Y., Choi, K., Lee, E., Jee, Y., Hoan, J., Kho, Y., 2019. Parabens in breast milk and possible sources of exposure among lactating women in Korea. Environ. Pollut. 255, 113142. https://doi.org/10.1016/j.envpol.2019.113142

Reimann, B., Vrijens, K., Roels, H.A., Wang, C., Cosemans, C., Van Overmeire, I., Nawrot, T.S., Plusquin, M., 2021. In utero exposure to parabens and early childhood BMI z-scores – Associations between placental ethyl paraben, longitudinal BMI trajectories and cord blood metabolic biomarkers. Environ. Int. 157, 106845. https://doi.org/10.1016/j.envint.2021.106845

Romøren, M., Lindbæk, M., Nordeng, H., 2012. Pregnancy outcome after gestational exposure to erythromycin – a study from Norway. https://doi.org/10.1111/j.1365-2125.2012.04286.x

Smolders, R., Schramm, K., Nickmilder, M., 2009. Applicability of non-invasively collected matrices for human biomonitoring 10, 1–10. https://doi.org/10.1186/1476-069X-8-8

Song, S., He, Y., Huang, Y., Huang, X., Guo, Y., Zhu, H., Kannan, K., Zhang, T., 2020. Occurrence and transfer of benzophenone-type ultraviolet filters from the pregnant women to fetuses. Sci. Total Environ. 726, 138503. https://doi.org/10.1016/j.scitotenv.2020.138503

Taha, H.M., Aalizadeh, R., Alygizakis, N., et. al., 2022. The NORMAN Suspect List Exchange (NORMAN-SLE): Facilitating European and Worldwide Collaboration on Suspect Screening in High Resolution Mass Spectrometry. Environ. Sci. Eur. https://doi.org/https://doi.org/10.21203/rs.3.rs-1902466/v1

Uldbjerg, C.S., Lim, Y., Gregersen, L.S., Br, E. V, Krause, M., Frederiksen, H., Andersson, A., 2022. Presence of parabens , phenols and phthalates in paired maternal serum, urine and amniotic fluid 158. https://doi.org/10.1016/j.envint.2021.106987

Valle-Sistac, J., Molins-Delgado, D., Díaz, M., Ibáñez, L., Barceló, D., Silvia Díaz-Cruz, M., 2016. Determination of parabens and benzophenone-type UV filters in human placenta: First description of the existence of benzyl paraben and benzophenone-4. Environ. Int. 88, 243–249. https://doi.org/10.1016/j.envint.2015.12.034

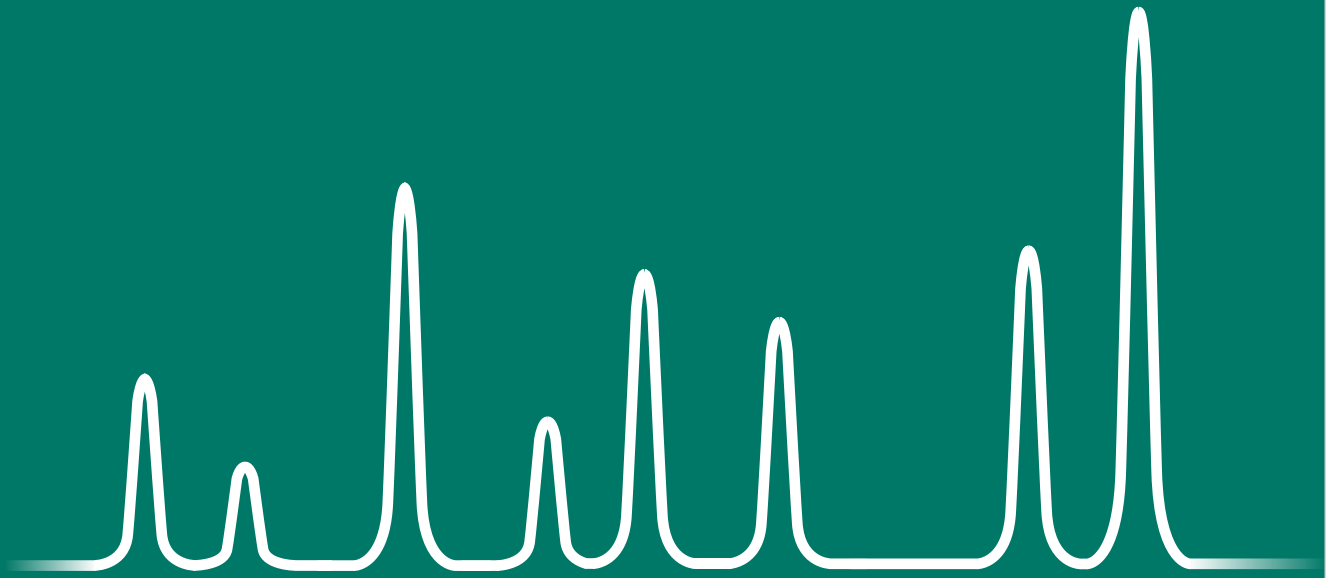
Vela-Soria, F., Gallardo-Torres, M.E., Ballesteros, O., Díaz, C., Pérez, J., Navalón, A., Fernández, M.F., Olea, N., 2017. Assessment of parabens and ultraviolet filters in human placenta tissue by ultrasound-assisted extraction and ultra-high performance liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1487, 153–161. https://doi.org/10.1016/j.chroma.2017.01.041

von der Ohe, P., Aalizadeh, R., 2020. S13 | EUCOSMETICS | Combined Inventory of Ingredients Employed in Cosmetic Products (2000) and Revised Inventory (2006). https://doi.org/10.5281/ZENODO.3959386

Wang, A., Abrahamsson, D.P., Jiang, T., Wang, M., Morello-Frosch, R., Park, J.S., Sirota, M., Woodruff, T.J., 2021. Suspect screening, prioritization, and confirmation of environmental chemicals in maternal-newborn pairs from San Francisco. Environ. Sci. Technol. 55, 5037–5049. https://doi.org/10.1021/acs.est.0c05984

Wang, J., Pan, L., Wu, S., Lu, L., Xu, Y., Zhu, Y., Guo, M., Zhuang, S., 2016. Recent advances on endocrine disrupting effects of UV filters. Int. J. Environ. Res. Public Health 13, 1–11. https://doi.org/10.3390/ijerph13080782

Zhang, T., Sun, H., Qin, X., Wu, Q., Zhang, Y., Ma, J., Kannan, K., 2013. Benzophenone-type UV filters in urine and blood from children, adults, and pregnant women in China: Partitioning between blood and urine as well as maternal and fetal cord blood. Sci. Total Environ. 461–462, 49–55. https://doi.org/10.1016/j.scitotenv.2013.04.074



Chapter 7

Conclusions

7. Conclusions

The wide range of CECs and the diverse physicochemical properties they present suppose a challenge from an analytical point of view. Furthermore, to correctly assess their occurrence and fate, very diverse samples of origin and nature need to be analysed. It supposes developing, validating, and applying robust analytical methodologies specific and sensitive enough to these samples, as has been done in this thesis. Despite the consequent workload, investment and complexity, **novel results on CECs' occurrence in environmental compartments have been obtained, the evaluation of new removal techniques has been carried out, the feasibility of reusing water for agriculture has been assessed, and, finally, human exposure to selected CECs has been evaluated**. From all this, the following conclusions have been drawn:

· UVFs and PBs, are ubiquitous in aquatic environmental compartments. UVFs and PBs occurrence is reported for the first time in seagrass and non-studied areas such as the Madhia coast in Tunisia. However, their bioaccumulation trends and mechanisms behind are different among environmental matrices. Therefore, using the contaminants' lipophilic properties is not enough to reliably predict their occurrence in different environmental samples.

· UVFs and PBs temporal trends were evaluated in seagrass, showing that these contaminants were already present in the marine environment 20 years ago on the Mallorca Island coastline. The trends varied among the locations, but the ones with higher anthropogenic impact correlated with the levels of the contaminants, demonstrating the strong effect of big cities to close environmental ecosystems. Furthermore, the contamination levels seem to increase in the last few years.

· A lab-scale and two pilot wastewater treatment technologies, algal ponds and SAT with reactive barriers, have been evaluated through the continuous monitoring of CECs along the processes. It showed that real-scale technologies are better suited for testing under real conditions as integrate factors that cannot be mimicked at laboratory scale.

· The combination of different removal techniques can enhance the CECs removal rates. It was shown by the outstanding performance of combining reactive barriers with a SAT system after secondary wastewater treatment. However, actual elimination techniques are still far from a complete CECs removal from wastewater, and further studies are needed in this direction.

· A new analytical methodology based on QuEChERS extraction proved useful for routine analysis of CECs in different crops. The type of crop (e.g. fruit, root, or leaf) and the cultivation variables ((e.g. irrigation water quality, soil composition, or irrigation system) can condition the final uptake of contaminants, showing different accumulation trends. Carrots were the crop with higher uptaken values, followed by tomatoes and lettuce. Besides, using reclaimed water by sprinkling irrigation in clayey soil was generally the best cultivation condition to lower crop uptake.

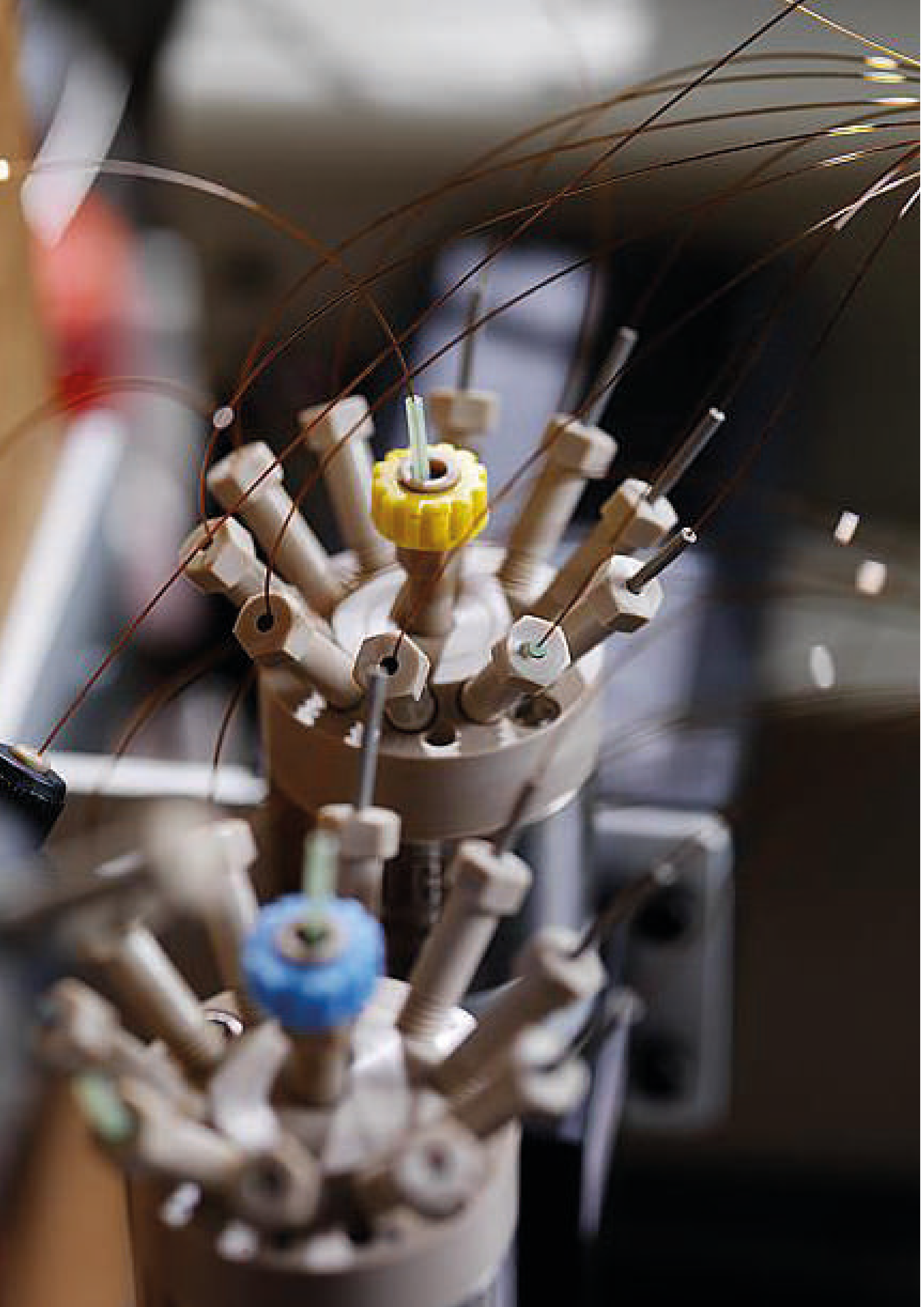
· The formation of TPs during the uptake processes is probable. Complex transformations are impossible to predict and include in target analysis approaches, making the use of suspect (non-target) approaches a complementary and valuable tool.

· The RQ are a valuable tool to estimate the risk of the detected contaminants to human health, but it should be used as a qualitative parameter since it is an estimation. The best procedure to evaluate human exposure to CECs is the analysis of human samples, despite the complexity it involves.

· PCPs' capacity to bioaccumulate in pregnant women has been proved. The ability of these contaminants to cross the placental barrier constitutes prenatal exposure to the foetus in critical development stages. BP3's and MePB's extensive use in formulations of daily-use products agrees with the detection frequencies found in the umbilical cord blood. Besides, the sunscreen AVO is reported for the first time in cord blood.

· An additional suspect screening of the cord blood samples has been crucial to tentatively identify 13 PCPs, 10 finally confirmed with the respective standard. Eleven of these PCPs are reported for the first time in cord blood. It demonstrates that not only frequently used PCPs reach the human blood and cross the placental barrier with unknown effects.

Overall, from all the work reported in this thesis, it can be concluded that CECs are extensively present in the aquatic environment, basically because WWTPs are not able to remove them completely from wastewater. Due to its persistence, CECs can be uptaken by crops when irrigated with reclaimed water and, ultimately, bioaccumulated in the human body but posing no risk at the determined values. Their occurrence in the umbilical cord and the associated capacity to cross the placental barrier is especially concerning. These facts, and the lack of toxicological information available, make them contaminants of great interest that require exhaustive monitoring at all levels.



Annex

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