Evaluación, distribución e impacto de contaminantes orgánicos prioritarios y emergentes en aguas costeras

Juan Ignacio Sánchez Avila

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EVALUACIÓN, DISTRIBUCIÓN E IMPACTO DE CONTAMINANTES ORGÁNICOS PRIORITARIOS Y EMERGENTES EN AGUAS COSTERAS

Memoria presentada para optar al grado de

Doctor por la Universidad de Barcelona

por

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Barcelona, junio 2012

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HACE CONSTAR:

que la presente memoria titulada **“Evaluación, Distribución e Impacto de Contaminantes Orgánicos Prioritarios y Emergentes en Aguas Costeras”** fue realizada por el Sr. **Juan Ignacio Sánchez Avila** en el Departamento de Química Ambiental del Instituto de Diagnóstico Ambiental y Estudios del Agua, adscrito al Consejo Superior de Investigaciones Científicas y bajo la tutoría de la Dra. Maria Teresa Galceran Huguet, Catedrática del Departamento de Química Analítica de la Universidad de Barcelona

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“La mayoría de los humanos son como las hojas que caen de los árboles, que vuelan y revolotean por el aire, vacilan y por último se precipitan al suelo. Otros, por el contrario, casi como estrellas siguen un camino fijo, ningún viento les alcanza, pues llevan en su interior su ley y su meta”
Siddharta Gautama

“El planeta ofrece cuanto el ser humano necesita, pero no cuanto el ser humano codicia”
Mahatma Gandhi.

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α-endsf	α-endosulfan
α-HCH	α-Hexaclorociclohexano
Ace	Acenafteno
Acy	Acenaftileno
ADI	“Acceptable daily intakes” o ingesta diaria aceptable
AEMA	Agencia Europea del Medio Ambiente
Ant	Antraceno
APs	Alquilfenoles
β-endsf	β-endosulfan
β-HCH	β-Hexaclorociclohexano
B(a)A	Benzo(a)antraceno
B(a)P	Benzo(a)pireno
B(b)F	Benzo(b)fluoranteno
B(ghi)P	Benzo(g,h,i)perileno
B(k)F	Benzo(k)fluoranteno
BBP	Benzilbutil ftalato
BDE 100	2,2’,4,4’,6-PentaBDE
BDE 153	2,2’,4,4’,5,5’-HexaBDE
BDE 154	2,2’,4,4’,5,6’-HexaBDE
BDE 183	2,2’,3,4,4’,5’,6-HeptaBDE
BDE 209	2,2’,3,4,4’,5’,6,6'-Deca BDE
BDE 28	2,4,4’-TriBDE
BDE 47	2,2’,4,4’-TetraBDE
BDE 99	2,2’,4,4’,5-PentaBDE
bdl	“Below detection limit” o inferior al límite de detección
BOE	Boletin Oficial del Estado
BPA	Bisfenol A
CE	Comunidad Europea
Chr	Criseno
cm	Centímetro
COMMPS	“Combined monitoring-based and modelling-based priority setting” o Sistema combinado de fijación de prioridades basado en mediciones y modelos
δ-HCH	δ-Hexaclorociclohexano
D(ah)A	Dibenzo(a,h)antraceno
DBO	Demanda bioquímica de oxígeno
DBP	Di-n-butil ftalato
DDT	Diclorodifeniltricloroetano
DEHA	Di (2-etilhexil) adipato
DEHP	Di (2-etilhexil) ftalato
DEP	Dietil ftalato
DMA	Directiva Marco del Agua
DMEM	Directiva marco sobre la estrategia marina
DMP	Dimetil ftalalato
DQO	Demanda química de oxígeno
dw	“Dry wet”o peso seco
EC_{50}	Concentración de exposición 50
EDAR	Estaciones depuradoras de aguas residuales
ESI	“Electrospray ionization” o ionización por electro-spray
eV	Electrovoltio
FAO	“Food and Agriculture Organization of the United Nations” u Organización de las Naciones Unidas para la alimentación y la agricultura.
FEADPT	Federación Española de Asociaciones de Puertos Deportivos y Pesqueros
Flr	Fluoranteno

Flu	Fluoreno
g	Gramo
GC	“Gas chromatography” o cromatografía de gases
h	Hora
ha	Hectáreas
hab	Habitantes
HCB	Hexaclorobenceno
HpCl	Heptacloro
HPLC	“High permormance liquid chromatography” o cromatografía de líquidos de alta resolución
I(cd)P	Indeno(1,2,3-c,d)pireno
IE	“Electron ionization” o ionización electrónica
km	Kilómetro
L	Litro
LC$_{50}$	Concentración letal 50
LLE	“Líquid-liquid extraction” o extracción líquido-líquido
log K$_{ow}$	Logaritmo del coeficiente de partición octanol-agua
m	Metro
MDL	“Methodological detection limit” o límite de detección metodológico
MEC	“Mesured environmental concentration” o concentración medida en el ambiente
mg	Miligramo
min	Minutos
mL	Mililitro
µL	Microlitro
mm Hg	Milímetros de mercurio
MS	“Mass spectrometry” o espectrometría de masas
MS/MS	Espectrometría de masas en tándem
Nap	Naftaleno
NCA	Normas de Calidad Ambiental
NCI	“Negative chemical ionization” o ionización química negativa
ng	Nanogramo
NOEC	“No-effect concentration” o concentración sin efecto
NP	4-nonilfenol (mezcla de isoméros)
NPEO$_1$	4-nonilfenol monoetoxilado
NPEO$_2$	4-nonilfenol dietoxilado
OCP	“Organochlorinated pesticide” o plaguicidas organoclorados
OMP	“Organic micropollutant” o microcontaminante orgánico
OP	4-tert-octilfenol
OSPAR	Referente al Convenio Oslo-Paris, sobre la Protección del Medio Marino del Atlántico Nordeste
PAHs	“Polycyclic aromatic hydrocarbons” o hidrocarburos policíclicos aromáticos
PBDE	“Polybromo diphenyl ethers” o éteres de polibromodifenilo
PCA	“Principal component analysis” o análisis de componentes principales
PCB 101	2,2’4,5,5’-Pentaclorobifenil
PCB 118	2,3’4,4’,5-Pentaclorobifenil
PCB 138	2,2’,3,4,4’,5 Hexaclorobifenil
PCB 153	2,2’4,4’5,5’ Hexaclorobifenil
PCB 180	2,2'3,4,4',5,5' Heptaclorobifenil
PCB 28	2,4,4’-Triclorobifenil
PCB 52	2,2’5,5'-Tetraclorobifenil
PCB	“Polychlorobiphenyls” o bifenilos policlorados o policlorobifenilos
PDMS	Polidimetilsiloxano
PEC	“Predicted envinronmental concentration” o concentración ambiental predicha

PEs	“Phthalate esters” o ésteres de ftalato
PFBS	“Perfluorobutane sulfonate” o sulfonato de perfluorobutano
PFC	“Perfluorochemicals” o compuestos perfluorados
PFHxS	“Perfluorohexane sulfonate” o sulfonato de perfluorohexano
PFNA	“Perfluorononanoic acid” o ácido perfluorononanóico
PFOA	“Perfluorooctanoic acid” o ácido perfluorooctanóico
PFOS	“Perfluorooctane sulfonate” o sulfonato de perfluorooctano
Phe	Fenantreno
Plast.	Plastificantes
PNEC	“Predicted No-Effect-Concentrations” o concentración sin efecto previsible
PNUMA	Programa de la Naciones Unidas para el Medio Ambiente
POPs	“Persistent organic pollutants” o contaminantes orgánicos persistentes
PPDB	“Pesticide Properties DataBase” o base de datos de propiedades de peticidas”
Pyr	Pireno
R^2	Coeficiente de determinación
REACH	“Register, evaluation, authorization of chemicals” o Registro, Evaluación, Autorización y Restricción de Sustancias Químicas
RQ	“Risk quotient” o cocientes de riesgo
RSD	“Relative standard deviation” o desviación estándar relativa
s	Segundo
SBSE	“Stir bar sorptive extraction” o extracción por adsorción en barras giratorias
SIM	“Selected ion monitoring” o monitorización por selección de ion
SPE	“Solid phase extraction” o extracción en fase sólida
SPME	“Solid phase microextraction” o microextracción en fase sólida
t	Tonelada
TGD	“Technical Guidance Document” o Documento de Orientación Técnica sobre Evaluación de Riesgos
UAE	“Ultrasonic assisted extraction” o extracción asistida por ultrasonido
UE	Unión Europea
US EPA	“United States Environmental Protection Agency” o Agencia de Protección Ambiental de Estados Unidos
ww	“Wet weight” o peso húmedo

CAPÍTULO 1: Introducción

Fotografía de la Costa Brava tomada por: Francisco Bono Blay

1.1 Introducción general

Las aguas costeras son aguas superficiales situadas hacia tierra desde una línea a una distancia de una milla náutica mar adentro, desde el punto más próximo de la línea de base que sirve para medir la anchura de las aguas territoriales y que se extienden, en su caso, hasta el límite exterior de las aguas de transición [1]. Representan la frontera que separa el mar abierto del área de desarrollo de actividades humanas y es por esto que son especialmente vulnerables ya que reciben impactos antropogénicos provenientes de actividades realizadas tierra adentro y de las que se realizan en el mar (Figura 1). Estas zonas de mar son un hábitat rico en arrecifes de corales, manglares y praderas de plantas marinas tales como las *Posidonias* [2; 3].

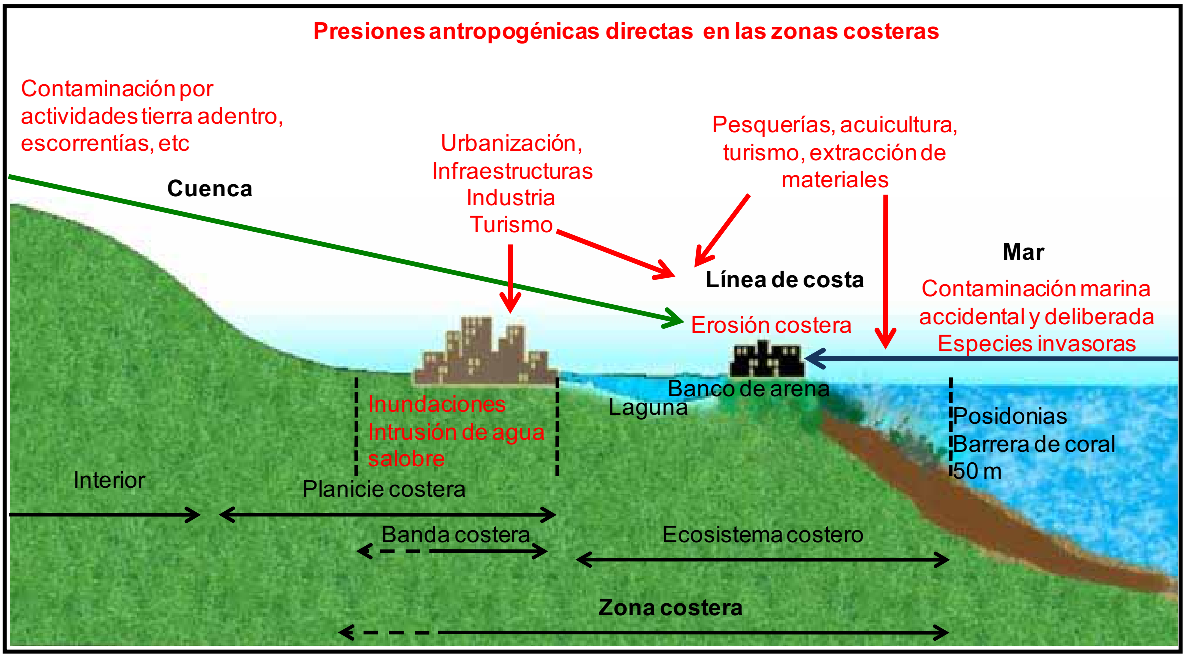

Figura 1. Presiones antropogénicas directas en las zonas costeras.
Fuente: Adaptación de PNUMA, 2009 [4]

Los principales factores antropogénicos que afectan las aguas costeras son el uso y explotación de recursos (p.ej. arena, petróleo, gas, pesca), desarrollo de infraestructuras, asentamientos humanos en las costas, industrialización, turismo, transporte marítimo, dragado de los mares, vertido de desperdicios (basura, sedimentos, sustancias peligrosas, derrames de petróleo), introducción de especies no autóctonas, entre otros [2; 3]. En la Tabla 1 se resumen estos factores antropogénicos y los efectos que pueden causar al medio ambiente costero.

Tabla 1. Relación de las actividades humanas y los problemas que ocasionan en las zonas costeras

Actividad humana	Agentes/consecuencias	Problema ocasionado en la zona costera
Urbanización y transporte	Cambios en los usos de la tierra (ej. puertos, aeropuertos); congestión del área natural a causa de caminos, vías y rutas aéreas; dragado de mares para construir puertos; derrames de aceites, combustibles; vertido de aguas residuales, basura; extracción de agua	Pérdida de hábitats y diversidad de especies; impacto visual; intrusión de agua de mar en los acuíferos; contaminación del agua; eutrofización; introducción de especies foráneas
Agricultura	Pérdida de tierras; uso de fertilizantes y plaguicidas; aumento de la población ganadera; extracción de agua; canalización de ríos	Pérdida de hábitats y diversidad de especies; contaminación del agua; eutrofización; reducción de entradas de agua fresca proveniente de los ríos
Turismo, recreación y caza	Desarrollo y cambios de usos de la tierra (ej. Construcción de campos de golf, hoteles, etc.); congestión del área natural a causa de caminos, vías y rutas aéreas; puertos y marinas; extracción de agua; vertido de aguas residuales y basura	Pérdida de hábitats y diversidad de especies; perturbación del medio; impacto visual; disminución del agua subterránea; intrusión de agua de mar en los acuíferos; contaminación del agua; eutrofización
Pesquerías y acuicultura	Construcción de puertos; instalaciones de procesamiento de pescados; artes de pesca; efluentes de granjas piscícolas	Sobrepesca; impacto en especies no-objetivo; basura, aceites y combustibles en las playas; contaminación del agua; eutrofización; introducción de especies foráneas; daño en el hábitat y cambio en las comunidades marinas
Industria (incluyendo la producción de energía)	Cambios en los usos de la tierra; construcción de plantas de generación de energía; extracción de recursos naturales; efluentes contaminados; agua de enfriamiento; molinos de energía eólica; embalses de ríos; presas de mareas	Pérdida de hábitats y diversidad de especies; contaminación del agua; eutrofización; contaminación térmica; impacto visual; reducción de entradas de agua fresca y sedimentos proveniente de los ríos; erosión costera

Fuente: De acuerdo con información de AEMA [5]

En los países Europeos la presión antropogénica en las zonas de costeras cada día es más elevada, ya que el litoral costero es el área de mayor desarrollo social, económico e industrial. Los 185,000 km de costas Europeas representan el 11% del territorio continental y en 2005 se estimaba que un 16% de la población (70 millones de hab.) se asentaba en estas zonas. Se calcula que en las costas europeas existen 280 ciudades que superan los 50,000 habitantes [3]. El Programa de Naciones Unidas para el Medio Ambiente, PNUMA (UNEP, por sus siglas en inglés) pronostica que en el año 2050 el 91% de las aguas costeras templadas y tropicales estarán fuertemente impactadas por el desarrollo humano [6; 7]. En la Figura 2 se muestra un mapa del impacto antropogénico de las zonas del litoral costero en el 2002 y una estimación para el año 2050. Puede apreciarse que la situación es más severa alrededor de Europa, la región Este de los Estados Unidos, el Este de China y el Suroeste de Asia.

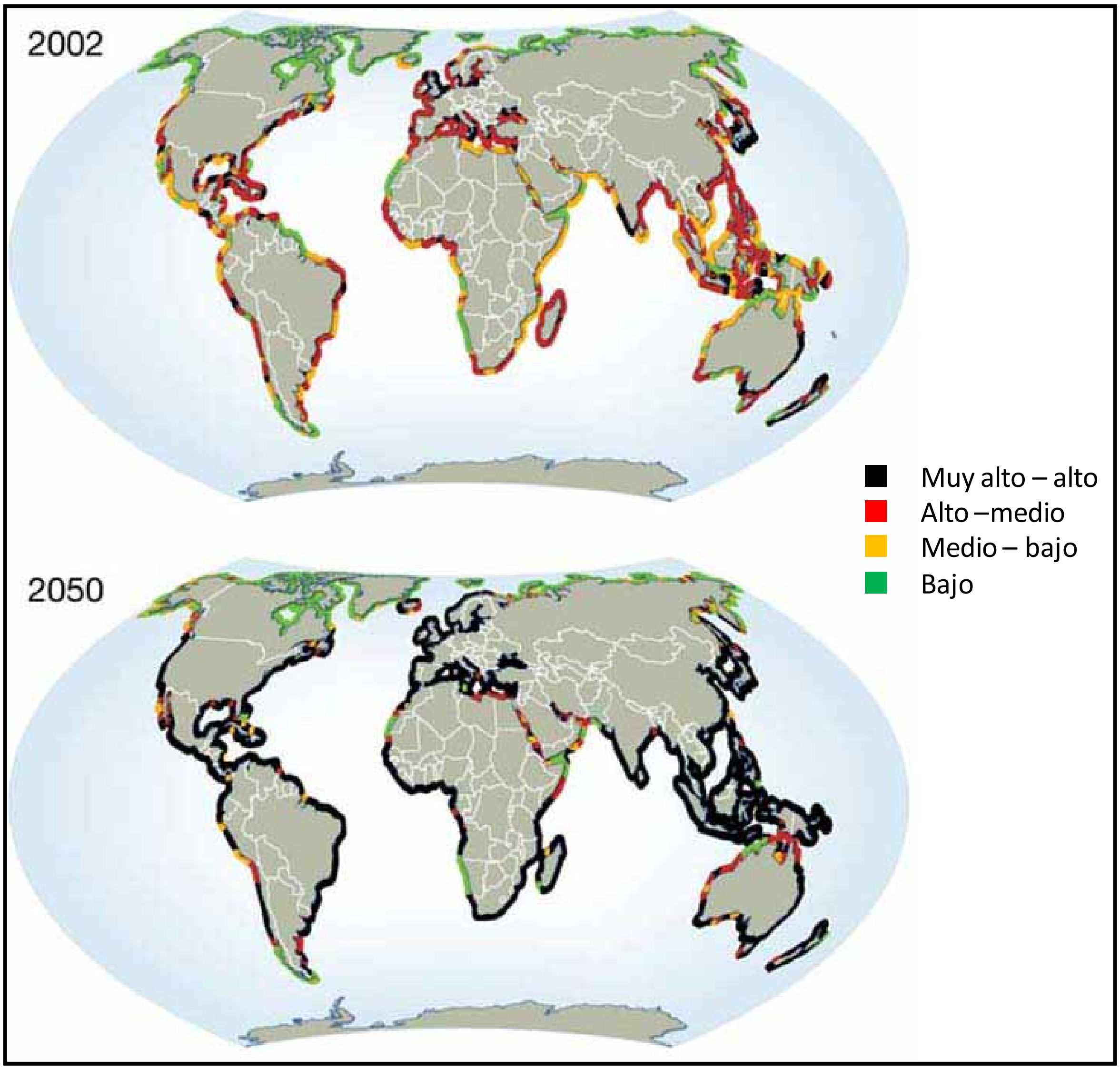

Figura 2. Distribución geográfica mundial del impacto antropogénico en las zonas costeras.
Fuente: Adaptación de PNUMA, 2006 [8]

1.1.1 Contaminación en las aguas costeras

La contaminación de las aguas costeras es uno de los efectos más frecuentes y severos ocasionados por las presiones antropogénicas en estas zonas [9; 10]. Además de poner en riesgo la salud humana y del ecosistema costero [6], la contaminación puede representar un obstáculo para la explotación de sus recursos (incluyendo la pesca) ya que al deteriorarse la calidad del agua de mar también se reducen los usos que se le puede dar [11]. En comparación con el mar abierto, los niveles de contaminación de las aguas costeras son sustancialmente superiores, ya que el efecto de dilución y autoregeneración es mucho menor, especialmente cuando el agua se encuentra estancada, o semi-encerrada como en el caso de los puertos y bahías [12].

En la literatura existen una gran cantidad de estudios referentes a la contaminación del agua en zonas costeras, enfocados principalmente a parámetros microbiológicos y/o fisicoquímicos entre los que se destacan el pH, temperatura, DBO (demanda bioquímica de oxígeno), DQO (demanda química de oxígeno), cantidad de nutrientes (fósforo y nitrógeno), biodiversidad, entre otros. En comparación, el número de estudios enfocados a la contaminación química son mucho menores [13].

Existen diferentes tipos de contaminantes en el medio marino y entre los más importantes se pueden mencionar a la contaminación por nutrientes, que es ocasionada por la presencia de materia orgánica y fertilizantes a base de nitrógeno y fósforo; la microbiana, ocasionada por microorganismos patógenos y la química ocasionada por la presencia de sustancias químicas como metales pesados y compuestos orgánicos [12]. La contaminación química por compuestos orgánicos, es el objeto de estudio en esta tesis.

La complejidad actual de la contaminación química tiene íntima relación con el elevado número de sustancias químicas liberadas en el ambiente de manera intencional o accidental. A diario se emplean sustancias químicas en productos de consumo, salud, servicio, alimentación y en un amplio número de tecnologías y procesos. Estos compuestos pueden ser derivados de sustancias de origen natural o formarse como subproductos de procesos naturales y antropogénicos o bien, ser producidos intencionalmente a partir de una síntesis química [14]. En 1981, se empleaban únicamente en la Unión Europea más de 100,000 diferentes sustancias químicas que en su gran mayoría son compuestos orgánicos. Esta cantidad aumenta año tras año y sólo se conocen una mínima parte de los efectos adversos que estos podrían causar en el en medio ambiente o en la salud humana [15; 16].

1.1.2 Principales fuentes de contaminación de las zonas costeras

La fuentes de contaminación de las aguas costeras pueden agruparse en dos principales categorías: la puntual y la difusa: Las fuentes puntuales descargan contaminantes en sitios específicos y son identificables y localizadas, discernibles, confinadas y discretas. Incluyen, pero no limitan, a cualquier tubería, zanja, canal, túnel, conducto, pozo, fisura discreta, contenedores, material rodante, operación concentrada de alimentación animal, buque o artefacto flotante, de donde los contaminantes son o pueden ser descargados. El flujo de contaminación de estas fuentes puede ser estimado y controlado por ejemplo, las descargas de aguas residuales y los cauces de ríos [17].

Las fuentes difusas o no puntuales, no se pueden localizar en un solo sitio ya que no tienen un punto específico de descarga. Provienen de infiltraciones, por medio del agua subterránea o como consecuencia de las lluvias y fenómenos de transporte atmosférico de contaminantes y la escorrentía superficial [18]. La fuente de esta contaminación puede ser a causa de una actividad reciente o pasada. Por su propia naturaleza, la gestión de la contaminación difusa es compleja y requiere un análisis cuidadoso y la comprensión de diversos procesos naturales y antropogénicos, como pueden ser las emisiones de transportes marítimos, intercambio de contaminantes por medio del aire, entre otras [19; 20]. Sin embargo, no siempre quedan claras las fronteras entre estas dos categorías, y en algunos casos es difícil distinguir si una fuente es puntual o difusa. En cualquier caso, la contaminación puntual es mucho más factible de identificar, gestionar y establecer medidas para su control [5; 17; 19].

Una de las fuentes más importantes de contaminación de las aguas costeras es la descarga directa de aguas residuales urbanas e industriales hacia los mares. Los sistemas de saneamiento de aguas residuales no resuelven por completo el problema de la contaminación. Las estaciones depuradoras de aguas residuales (EDAR) se diseñan y construyen para remover principalmente materia orgánica (medida como DBO o DQO). Sin embargo, éstas no logran eliminar por completo el complejo coctel de contaminantes contenido en los influentes. Sustancias químicas tales como plastificantes, pesticidas, hidrocarburos y combustibles, compuestos halogenadas, detergentes, surfactantes, etc., han sido detectados en los efluentes de las EDAR [12; 20]. El problema de la contaminación se agrava cuando no existen sistemas de saneamiento. De acuerdo con datos de la PNUMA, en el 2006 aproximadamente un 40% de las aguas residuales que se descargan en el mar Caspio reciben un tratamiento pre-descarga; en los mares y ríos de Europa Central, un 25%; en los mares de Latinoamérica y el Caribe, un 20% y en los mares de África y el Indo-Pacífico, únicamente de un 10 a un 20%. Por el contrario, en los países de Europa Occidental así como en el mar Báltico y en el Atlántico Norte el saneamiento de las aguas es mucho mayor, pues únicamente un 10-20% de las descargas no reciben tratamiento [7; 8] (Figura 3).

Figura 3. Estado del tratamiento de las aguas residuales que se descargan hacia el mar. ***Fuente: Según datos de PNUMA, 2006 [8].***

Los ríos son considerados también una fuente potencial de contaminantes de las aguas costeras. Al cruzar por las grandes ciudades y por zonas agrícolas pueden arrastrar en su cauce parte de la contaminación generada en las cercanías de su cuenca. Así mismo, los ríos son receptores de aguas residuales tratadas y no tratadas [21; 22]. En algunos ríos que confluyen hacia el mar se han detectado contaminantes como pesticidas [23; 24], surfactantes y detergentes [25; 26], plastificantes [27], retardantes de flama [28], entre otros. A este aporte de los ríos puede sumarse también la escorrentía de aguas desde áreas urbanas y agrícolas hacia las aguas costeras [5].

El incremento de estaciones de acuacultura en las zonas costeras también representa un impacto directo. En estas instalaciones se emplean una gran variedad de fármacos para prevenir infecciones, para la desinfección y terapia de especies cultivables y además otras sustancias que previenen el crecimiento no deseado de algas. La escorrentía de estas sustancias fuera de las instalaciones puede poner en riesgo otras especies no-objetivo en el medio ambiente costero. Se ha observado también una seria eutrofización de las aguas en torno a estas estaciones [5; 29]

El transporte marítimo de mercancías y pasajeros así como el recreativo tiene un impacto directo en el ambiente marino a través de descargas legales o ilegales de combustibles, aceites, desperdicios sólidos, aguas residuales y aguas de lastre. Las emisiones de los motores de los barcos y botes despiden hidrocarburos aromáticos y otros gases de combustión. Los productos químicos utilizados para evitar el crecimiento de algas en los cascos de los barcos pueden desprenderse y contaminar las aguas marinas. El transporte de petróleo y combustibles representa un riesgo de accidentes con serias repercusiones ambientales. Tal es el caso de la catástrofe ocasionada por el buque-tanque *Prestige* que en noviembre de 2002 derramó aproximadamente 50,000 toneladas de crudo pesado en las costas del Norte de España (mares Cantábrico y Gallego) [3; 10].

La basura (desperdicios sólidos) se considera una fuente moderna de contaminación de los mares. Pueden encontrarse desperdicios en el litoral costero o flotando en la superficie, en la columna de agua y en el lecho marinos. Además del daño por ingestión directa que pueden causar a la fauna marina, gran variedad desperdicios contienen sustancias tóxicas o con efectos carcinogénicos y/o mutagénicos que pueden ser liberadas en el agua de mar [10; 30]. La cantidad de desperdicios sólidos que se recolectan en las playas Europeas cada año (Tabla 2), es una muestra del riesgo que estos representan para las aguas costeras y en general para el medio ambiente marino.

Tabla 2. Cantidad de basura encontrada en las playas europeas.

Ubicación	Cantidad de basura	Composición
Mar del Norte (litoral Norte)	600 - 1400 [1]	Pequeñas piezas de plástico/poliestireno
Mar del Norte (litoral Sur)	200 - 600 [1]	
Mar Celta	600 - 800 [1]	
Bahía de Vizcaya y costas Ibéricas	100 - 300 [1]	
Mar Báltico	Alto: 700 - 1200 [1] Bajo: 6 - 16 [1]	Plásticos 30–60 %
Mediterráneo (litoral griego)	6.7 - 47.4 [2]	Plásticos: 56 % Metal: 17 % Vidrio: 11 %
Mar Negro	Plástico: 333–6250 [2] Vidrio: 222–1 455 [2]	

Fuente: Adaptación de AEMA, 2010 [10].

[1] piezas por cada 100 m de playa

[2] kg por cada km^2 de playa

El intercambio de contaminantes entre la atmósfera y las aguas superficiales es un fenómeno que afecta también la calidad de las aguas costeras. Existe un cierto grupo de contaminantes que son volátiles y que pueden alcanzar la atmósfera y mezclarse con ella por ejemplo, algunos hidrocarburos y pesticidas. Estos contaminantes se diluyen y transportan por el aire, siendo adsorbidos en las partículas sólidas de la atmósfera. Estas partículas se depositan posteriormente en el agua de mar o en los ríos o son acarreadas por las lluvias hacia las aguas superficiales [12].

1.1.3 Distribución, compartimentación y efectos de los contaminantes orgánicos en el medio marino

Una vez que los contaminantes orgánicos han alcanzado el medio marino, su comportamiento, destino (agua, aire, sedimentos, organismos vivos) y efectos dependerá de sus propiedades fisicoquímicas así como de factores propios del medio. Las propiedades químicas y físicas de los contaminantes dependen de su estructura molecular y la naturaleza de los átomos presentes en la molécula; distintas configuraciones de una misma molécula pueden hacer variar estas propiedades [18]. La solubilidad, hidrofobicidad (determinado por el coeficiente de partición octanol/agua, log K_{ow}) y la presión de vapor, juegan un papel muy importante para determinar el destino que tendrán los contaminantes en el medio marino [31; 32]. Al día de hoy, muchas de estas propiedades han sido evaluadas por medio de ensayos químicos, bioquímicos, físicos, o biológicos ya sea in-situ o a nivel laboratorio. Gran parte de estos valores experimentales se encuentran disponibles en diversas bases de datos como la “Base de datos interactiva de propiedades fisicoquímicas” editada por la compañía SRC [33] o como la PPDB, editada por la Universidad de Hertfordshire y el Proyecto FOOTPRINT de la CE en la que se agrupa únicamente propiedades de pesticidas [34]. Para aquellos contaminantes que no se han medido sus propiedades o sus valores no están disponibles, es posible realizar estimaciones de estas propiedades gracias a diferentes programas informáticos diseñados para este fin. Entre estos se puede mencionar al programa EPI Suite (por sus siglas en inglés: “Estimation Program Interface”) desarrollado por la Agencia de Protección Ambiental de Estados Unidos (“Environmental Protection Agency” o EPA) [35].

Los contaminantes orgánicos pueden encontrarse en el agua en diferentes estados. Los compuestos más polares pueden estar disueltos y transportarse por el agua de mar moviéndose gracias a las mareas y corrientes. Los compuestos más lipofílicos se unirán con la materia particulada rica en materia orgánica y puede sedimentar al fondo de los mares (Figura 4) dependiendo de sus propiedades fisicoquímicas [31; 32]. Así mismo pueden unirse con el material coloidal, las macromoléculas orgánicas y los microparticulados (principalmente de carbohidratos y péptidos) presentes en el agua de mar y formar suspensiones coloidales estables, permaneciendo así en la columna de agua [31; 36]. Los sedimentos pueden ser en muchos casos el destino final de los contaminantes en el medio marino y de esta manera pueden ser utilizados como una herramienta para monitorear la contaminación histórica de este entorno [28; 37]. A menudo, los contaminantes depositados en los sedimentos pueden resuspenderse y/o difundirse, retornando nuevamente a la columna de agua, lo cual incrementa su tiempo de residencia en ella y de esta manera volver a estar biodisponibles [38]. Los cambios en la concentración de sales en el agua de mar juega un papel muy importante en la solubilidad de los contaminantes y provoca una mayor asociación con el material particulado y/o sedimentos. Un aumento en la salinidad puede provocar una floculación del material disuelto generando una mayor cantidad de partículas capaces de retener a los contaminantes hidrofóbicos [39], fenómeno que ocurre en los estuarios (desembocaduras de los ríos en el mar) en donde se da un cambio salino brusco al mezclarse el agua de mar con el agua de río [40].

Muchos contaminantes orgánicos son transportados principalmente por el aire, recorriendo así grandes distancias y alejándose de la fuente de la que originalmente fueron emitidos [41; 42] y pueden posteriormente incorporarse al medio marino a través de la deposición seca o húmeda [43] (Figura 4). Este fenómeno es dependiente de la presión de vapor y solubilidad del compuesto así como temperatura y concentración en ambos medios [31]. Como la presión de vapor disminuye cuando se disminuye la temperatura, los contaminantes pueden ser atrapados en las aguas más frías y en las regiones polares [8; 44]. Los contaminantes atmosféricos también pueden ser incorporados en la microcapa de la superficie marina (Figura 4), que es rica en sustancias activas como lípidos y ácidos grasos hacia los que pueden tener afinidad y formar agregados estables [45].

Los organismos vivos pueden estar expuestos a la presencia de los contaminantes ya sea en la microcapa de la superficie, la columna de agua o los sedimentos marinos. Una vez acumulados, estos pueden ser metabolizados y/o excretados. Los contaminantes orgánicos con altos valores de K_{ow} pueden bioacumularse en los organismos marinos (generalmente en el tejido adiposo) y en algunos casos biomagnificarse a través de la cadena trófica [46; 47]. La bioacumulación de contaminantes orgánicos de origen antropogénico en la biota (plantas, plancton, crustáceos, moluscos, peces, mamíferos o aves) puede ser utilizada como otra herramienta para monitorear la calidad del medio marino.

Durante la incorporación y transporte de los contaminantes en el medio marino pueden sucederse fenómenos de degradación. La estabilidad molecular es un factor que determina el tiempo que un contaminante permanece en el ambiente y las distancias que puede recorrer [31]. La ruptura no está solamente determinada por la estabilidad del compuesto químico, sino también por factores ambientales como temperatura, nivel de radiación solar y pH [48-50]. Los microorganismos presentes en el medio juegan también un papel importante en la biodegradación de los contaminantes [51; 52]. Ciertos contaminantes tienen la capacidad de permanecer estables en el medio ambiente y de resistir a los procesos degradación (fotolítica, química o biológica), llegando a tener una vida media ($t_{1/2}$) de muchos años [18]. Lo anterior permite que permanezcan inalterados en los diferentes compartimientos ambientales durante largos períodos de tiempo, antes de ser degradados o rotos en sustancias menos peligrosas [18; 53]. Un contaminante se puede considerar persistente cuando su vida media en el agua es mayor de 2 meses; o si en suelos o sedimentos es mayor de 6 meses [54]. Mientras más persistente sea un contaminante, mayor es el riesgo que presenta para el medio ambiente, ya que sus efectos nocivos también estarán presentes durante mayor tiempo.

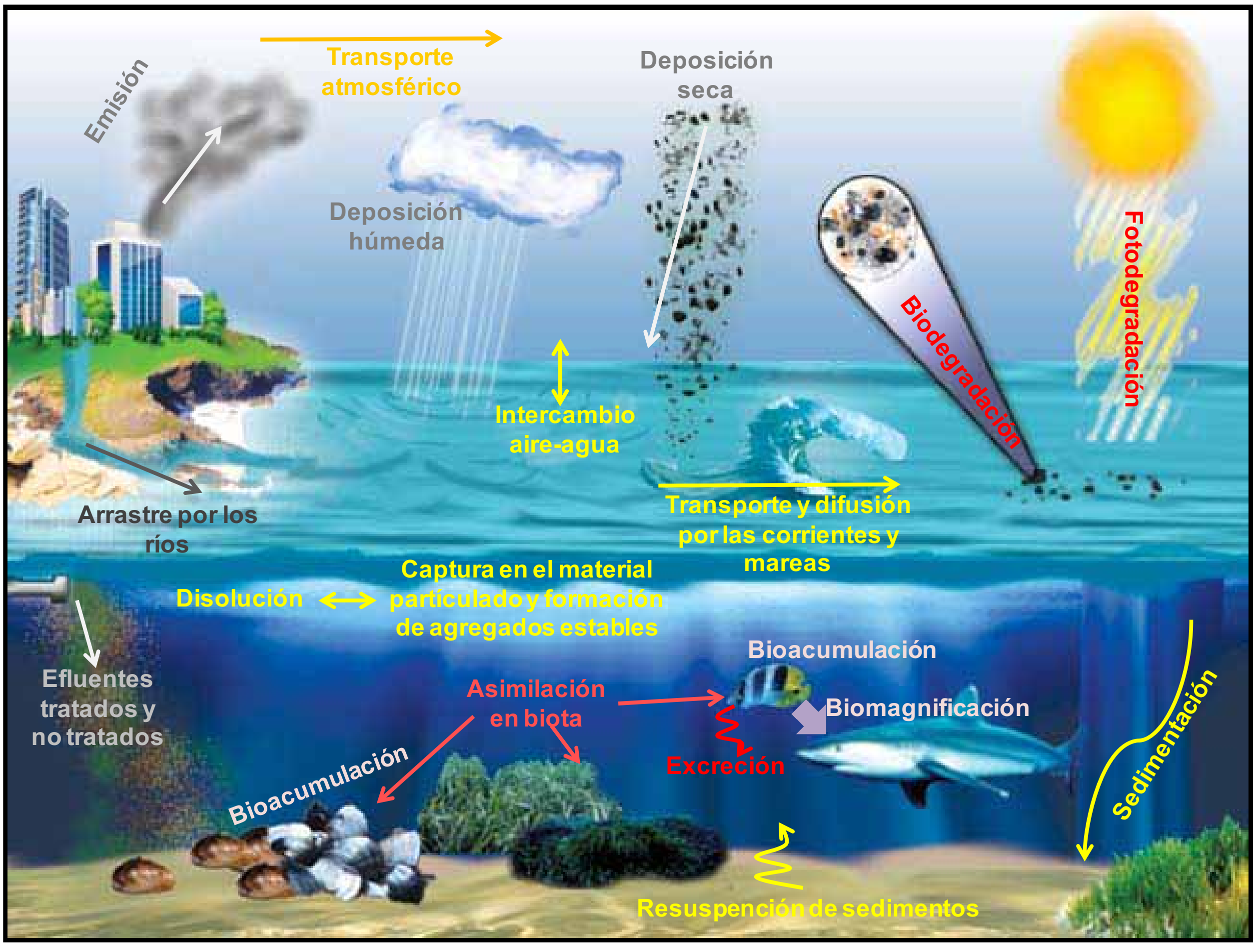

Figura 4. Distribución y compartimentación de los contaminantes orgánicos en el medio marino.

Una gran cantidad de sustancias pueden ser tóxicas, tanto para la flora y fauna marina, como también para el ser humano [55]. Entre los efectos tóxicos que los contaminantes orgánicos pueden ocasionar en el medio marino se pueden mencionar: daños en el sistema nervioso (neurotoxicidad) [56], en el sistema endocrino, y por consecuencia en la reproducción y desarrollo [57; 58], riesgo de cáncer y mutaciones [59; 60] hasta alteraciones a nivel del ecosistema [61].

1.2 Legislación ambiental del medio marino

A pesar de todos los esfuerzos y acciones emprendidas a nivel internacional, nacional y regional por proteger el medio ambiente marino y en especial el costero, aún quedan muchas lagunas en la legislación y en ocasiones, la legislación existente no es lo suficientemente clara. En Europa, las directivas son complejas debido especialmente a las excepciones, anexos e interconexiones varias entre ellas. Además de esto, antes de entrar en vigor deben de ser transpuestas en cada uno de los países miembros. A menudo la aprobación se hace con texto íntegro y añadiéndole prórrogas para adaptar la nueva ley al marco económico y legal nacional en vigor.

1.2.1 Convenios internacionales

Son diversos los convenios internacionales a los que España se ha suscrito para proteger el medio marino, a continuación se enumeran y describen los principales:

Convenio de Barcelona

En 1975, España y otros 15 países mediterráneos firmaron el “Plan de Acción para la Protección y el Desarrollo de la Cuenca del Mediterráneo” (PAM), junto con la CE y bajo la supervisión de PNUMA. Como marco jurídico del PAM, se adopta en 1976, el “Convenio para la Protección del Mar Mediterráneo Contra la Contaminación” o “**Convenio de Barcelona**”. En la conferencia de Barcelona de 1995 se enmienda el Convenio y pasa a denominarse “**Convenio para la Protección del Medio Marino y la Región Costera del Mediterráneo**”. Además se revisan los protocolos existentes y se adopta un nuevo PAM (la Resolución de Barcelona sobre medio ambiente y desarrollo sostenible en la cuenca del Mediterráneo). Su ámbito geográfico de aplicación son las aguas marinas e interiores del mar Mediterráneo, limitadas al oeste por el meridiano que pasa por el cabo Espartel (35° 47′ 14″ N, 5° 55′ 30″ O), y al este por los límites del estrecho de los Dardanelos (40° 13′ 0″ N, 26° 26′ 0″ E) entre los faros de Mehmetcik y Kumkale. Actualmente son 22 países los que han ratificado este nuevo PAM. Los protocolos, destinados a cumplir con el PAM [10; 62] son:

- Protocolo sobre la prevención de la contaminación causada por vertidos desde buques y aeronaves (“Protocolo de Inmersión o Dumping”). Enmendado en 1995 y aunque no ha entrado en vigor, España lo ratificó en 1999.
- Protocolo sobre cooperación para combatir la contaminación en situaciones de emergencia causadas por hidrocarburos y otras sustancias perjudiciales

(“Protocolo de Emergencia”). Sustituido en 2002, entró en vigor desde 2004 y fue ratificado por España en 2007.

- Protocolo sobre la protección del Mediterráneo contra la contaminación de origen marino y terrestre (“Protocolo COT”). Aprobado en 1996, fue ratificado por España en 1999 pero entró en vigor en 2008.
- Protocolo sobre áreas protegidas (Ginebra, 1982) que en 1995 se convierte en el Protocolo sobre Zonas Especialmente Protegidas y Diversidad Biológica en el Mediterráneo (“Protocolo ZEPIM”). Se encuentra en vigor desde 1999 aunque fue ratificado por España un año antes, en 1998.
- Protocolo para la protección del Mediterráneo contra la contaminación resultante de la exploración y explotación de la plataforma continental y del fondo del mar y su subsuelo (Madrid, 1994) (“Protocolo Offshore”). Aprobado en 1994, no ha entrado en vigor y no ha sido ratificado por España.
- Protocolo sobre la prevención de la contaminación del mar Mediterráneo por movimientos transfronterizos de desechos peligrosos y su eliminación. Aprobado en 1996, entró en vigor desde 2008 y no ha sido ratificado por España.
- El nuevo (“Protocolo GIZCM”) del Convenio de Barcelona, firmado por España y otras trece partes contratantes en 2008 y aprobado en 2010. El Protocolo establece los instrumentos al servicio de la gestión integrada de zonas costeras, analiza los riesgos que afectan a la zona costera y promueve la cooperación internacional. Es el primer instrumento jurídico vinculante en el mundo sobre gestión integrada de zonas costeras [10; 63]. Algunas medidas que se destacan son las siguientes:
  - Prohibición de construir en al menos los primeros 100 metros desde la línea de costa. Establecimiento de zonas protegidas, la libertad de acceso público al mar y control del desarrollo urbanístico en la costa.
  - Reducción al mínimo de los recursos naturales costeros e implantar una correcta gestión ambiental y promover códigos de buenas prácticas para las actividades desarrolladas en la costa.
  - Legislación, planificación y gestión.
  - Protección del patrimonio cultural de las zonas costeras, incluido el patrimonio cultural submarino.
  - Participación social y fomento de la sensibilización, formación, educación e investigación sobre la gestión integrada de zonas costeras.

Convenio OSPAR

Como resultado de la fusión del convenio de Oslo para la prevención de la contaminación marina provocada por vertidos desde buques y aeronaves, y el convenio de París para la prevención de la contaminación marina de origen terrestre, en 1992 se suscribe en París el “Convenio Sobre la Protección del Medio Marino del Atlántico Nordeste”, o **Convenio OSPAR**. Fue ratificado por España mediante su publicación en el BOE del 24 de junio de 1998. Además de España, lo firman 14 países más de la región que cubre el Atlántico Nordeste y la CE. Las partes contratantes de este convenio se reúnen cada año, y cada cinco años tiene lugar una reunión ministerial de las partes. OSPAR establece una serie de objetivos estratégicos en cuanto a diversidad biológica y ecosistemas; sustancias peligrosas; sustancias radiactivas; eutrofización y a industria del gas y del petróleo en alta mar [62; 64]. La aplicación del Convenio y sus Estrategias se lleva a cabo mediante la adopción de Decisiones (legalmente vinculantes), Recomendaciones (acciones que han de desarrollar las partes) y otros Acuerdos (otras cuestiones de importancia). El OSPAR cuenta con un programa conjunto de evaluación y seguimiento (Programa JAMP) que se encarga de todo lo relativo a mediciones de los cambios en el estado del medio marino, de los impactos de las actividades humanas, así como de evaluar la eficacia de las medidas de gestión. Algunos de los instrumentos más relevantes derivados del Convenio OSPAR son [62; 64]:

- Programa de vigilancia del medio marino o CEMP, por siglas en inglés (*Coordinated Environmental Monitoring Programme*). Estudia las tendencias temporales de la contaminación, realizando muestreos anuales y el estudio de la distribución espacial de la contaminación, cada 5 años, de los siguientes compuestos: mercurio, cadmio, plomo, PCBs, PAHs, TBTs, determinación de los efectos biológicos (Imposex), nutrientes. El programa establece si estas sustancias se deben analizar en agua, biota o sedimentos.
- Programa de monitoreo ambiental o CAMP, por siglas en inglés (*Comprehensive Atmospheric Monitoring Programme*) que tiene por objeto evaluar la entrada de 8 metales, lindano y compuestos nitrogenados en el área OSPAR a través de su deposición atmosférica.
- El programa sobre control de vertidos desde ríos y directos al mar o RID, por sus siglas en inglés (*Riverine Inputs and Direct Discharges*) obliga a cada Estado a informar sobre todos los vertidos industriales y urbanos que llegan al mar bien desde los ríos o de manera directa por vertidos en la costa. En los informes nacionales se incluye información sobre el volumen de vertido y las cargas totales vertidas de las siguientes sustancias: cadmio, mercurio, cobre, plomo, zinc,

lindano, PCBs, amonio, nitratos, fosfatos, nitrógeno total, fósforo total y sólidos en suspensión.

- España ha incluido al Parque Nacional de las Islas Atlánticas de Galicia y El Cachucho a la Red de Áreas Marinas Protegidas de la OSPAR.
- En 2002, OSPAR publicó la Lista Sustancias Químicas de Acción Prioritaria o "Lista OSPAR" donde se incluyen 42 substancias o grupos de substancias consideradas como prioritarias para su monitoreo en el medio ambiente y control para minimizar o eliminar su emisión.

Convenio de Estocolmo

El **Convenio de Estocolmo** sobre los contaminantes orgánicos persistentes[1] o POPs (por sus siglas en inglés, *Persistent Organic Pollutants*) proporciona un marco, basado en el principio de cautela, que persigue garantizar la eliminación segura y la disminución de la producción y el uso de estas sustancias nocivas para la salud humana y el medio ambiente. El convenio inicialmente afecta a doce POPs prioritarios, producidos deliberada y no deliberadamente. El objetivo a largo plazo es que este convenio abarque otras sustancias. Persigue limitar la contaminación por POPs y define las sustancias afectadas, así como las reglas de producción, importación y exportación de estas sustancias. Fue firmado en 2001 y entró en vigor en 2004. Los países firmantes se definen como las Partes entre ellos, España.

Los POPs prioritarios se encuentran listados en 3 Anexos. En el Anexo A se agrupan aquellas sustancias para las que las Partes tomen medidas para eliminar su producción y uso. En el Anexo B se incluyen aquellas sustancias para las que las Partes restrinjan su producción y uso, sólo para a una pequeña cantidad de propósitos bien definidos. En el Anexo C se listan aquellas sustancias para las que las Partes traten de reducir su emisión accidental y su vertido al medio ambiente. Las disposiciones del Convenio no se aplican a las cantidades de un producto químico destinado a la investigación en laboratorio

Para la aplicación del Convenio a escala internacional se han creado los tres organismos siguientes:

- Conferencia de las partes: Organismo principal, en el que participan todas las partes en el Convenio y, eventualmente, observadores. Fija las normas de los procedimientos de aplicación y es responsable de las decisiones importantes,

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[1] Los contaminantes orgánicos persistentes (POPs) son productos químicos que poseen ciertas propiedades tóxicas y que, contrariamente a otros contaminantes, son resistentes a la degradación. Los COP son especialmente perjudiciales para la salud humana y para el medio ambiente. Se bioacumulan, son transportados por el aire, el agua y las especies migratorias, y se acumulan en los ecosistemas terrestres y acuáticos.

como la inclusión de una nueva sustancia en el Convenio y el acuerdo de exenciones;
- Comité de examen de los contaminantes orgánicos persistentes: El Comité, compuesto de especialistas, tiene por función examinar las propuestas para incluir nuevas sustancias en el Convenio;
- Secretaría: Este organismo se encarga principalmente de las tareas administrativas.

En la 4a Reunión de la Conferencia de las Partes (4 al 8 de mayo del 2009) se modificaron los Anexos A, B y C del Convenio de Estocolmo con la finalidad de incluir 9 sustancias más en el listado original. A continuación se mencionan todas las sustancias incluidas hasta la fecha en el Convenio (originales y nuevos POPs), agrupadas en sus respectivos Anexos:

- Anexo A: aldrina, clordano, clordecona, dieldrina, eldrina, heptacloro, hexabromobifenil, éter de tetrabromodifenilo y éter de pentabromodifenilo (éter de pentabromodifenilo de calidad comercial), éter de hexabromodifenilo y éter de heptabromodifenilo (éter de octabromodifenilo de calidad comercial), hexaclorobenceno (HCB), α-hexaclorociclohexano (HCH), β-HCH, lindano (γ-HCH), mírex, pentaclorobenceno, los congéneres de bifenilos policlorados (PCBs), endosulfan técnico y sus isómeros relacionados y toxafeno.
- Anexo B: DDT y sulfonato de perfluorooctano (PFOS), sus sales y fluoruro de perfluorooctano sulfonilo (PFSO-F)
- Anexo C: Pentaclorobenceno, dibenzo-p-dioxinas (PCDD) y dibenzofuranos policlorados (PCDF), hexaclorobenceno (HCB) y PCBs.

Otros convenios

Se han suscrito otros convenios que tienen como finalidad promover el control efectivo de los vertidos de desechos y otras materias realizados desde buques al medio marino y la adopción de todas las medidas posibles para impedir su contaminación [62] :

- Convenio internacional sobre cooperación, preparación y lucha contra la contaminación por hidrocarburos (“Convenio OPRC”).
- Protocolo sobre sustancias nocivas y potencialmente peligrosas (“Protocolo HNS”).
- Convenio sobre la prevención de la contaminación desde los buques (“Convenio MARPOL”).

- Convenio sobre el control de los movimientos transfronterizos de los desechos peligrosos y su eliminación (“Convenio de Basilea”).
- Convenio sobre responsabilidad civil nacida de daños debidos a contaminación por los hidrocarburos para combustible de los buques (“Convenio Combustible”).
- Convenio relativo a la intervención en alta mar en caso de contaminación por hidrocarburos (“Convenio Intervención”).

1.2.2 Legislación en vigor

La Directiva Marco del Agua (DMA)

La **Directiva 2000/60/CE** del Parlamento Europeo y del Consejo [1], establece un marco comunitario de actuación en el ámbito de la política de aguas basada, de acuerdo a su filosofía, en un uso sostenible y respetuoso de los recursos, enfocada a conseguir como objetivos principales la racionalización en el uso, conservación y recuperación del recurso hídrico, el equilibrio entre el aprovechamiento y la sostenibilidad, ambiental y económicamente entendida de los ecosistemas continentales, estuarinos y litorales. La DMA pretende prevenir todo deterioro del estado de las masas de aguas continentales (superficiales y subterráneas) y costeras. Así mismo, para el año 2015 se alcanzar el “buen estado ecológico y químico” de todas las masas de agua naturales y el “buen potencial ecológico y el buen estado químico” de todas las masas de agua artificiales y muy modificadas (como pueden ser las aguas confinadas en los puertos o algunas masas de agua costeras sometidas a fuertes alteraciones hidromorfológicas). La DMA exige principalmente, realizar un estudio de las características de la demarcación, de las repercusiones de la actividad humana en el estado de las aguas y un análisis económico de los usos del agua (Art 5); la creación de un registro de zonas protegidas (Art 6); el establecimiento de programas de seguimiento del estado de las masas de agua (Art 8); la aplicación de la recuperación de los costes de los servicios relacionados con el agua (Art 9), el establecimiento de las medidas necesarias para asegurar la participación activa de todas las partes interesadas, etc.

Directiva de sustancias prioritarias

En el 2001, con la publicación de la primera modificación a la DMA en la **Decisión 2455/2001/CE** [65], se aprobó una primera lista de 33 sustancias prioritarias[1] o grupos de sustancias en el ámbito de la política de aguas (Tabla 3). También se contemplan

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[1] Sustancias prioritarias: Sustancias o grupo de sustancias que presentan un riesgo significativo para el medio acuático o a través de él.

medidas de selección para nuevas sustancias prioritarias, lo cual permite revisiones y adaptaciones de la primera lista lo cual debe realizarse como mínimo cada cuatro años. La categoría de prioritaria es establecida a través de evaluaciones de riesgo llevadas a cabo utilizando el sistema combinado de fijación de prioridades basado en mediciones y modelos, denominado COMMPS (combined monitoring-based and modelling-based priority setting). En la lista se incluyen sustancias peligrosas[1] (al incluirse en la lista son ahora “peligrosas prioritarias”) que provienen de acuerdos internacionales a los que la Comunidad Europea se encuentra suscrita, tales como el Convenio OSPAR. De acuerdo con la DMA y la Decisión 2455/2001/CE, deben interrumpirse o reducirse progresivamente la contaminación por vertido, emisión o pérdida de las sustancias peligrosas prioritarias, así que la selección de nuevas sustancias debe hacerse tomando en cuenta también el coste que supondría su eliminación y sus controles necesarios.

Tabla 3. Listado de los 33 contaminantes incluidos en el listado de sustancias prioritarias en el ámbito de la política de aguas (Decisión 2455/2001/CE).

	Contaminante o grupo de contaminantes prioritarios	Prioritario-peligroso
1.	Alacloro	
2.	Antraceno	X
3.	Atrazina	X
4.	Benceno	
5.	Difeniléteres bromados (sólo pentabromodifenileter) Difeniléteres bromados congéneres 28, 47, 99, 100, 153 y 154	X
6.	Cadmio y sus compuestos	X
7.	Cloroalcanos $C_{10\text{-}13}$	X
8.	Clorfenvinfós	
9.	Clorpirifós (Clorpirifós etil)	X
9a.	Plaguicidas de tipo ciclodieno: Aldrina, dieldrina, endrina, isodrina	
9b.	DDT total y 4,4’-DDT	
10.	1,2 dicloroetano	
11.	Diclorometano	
12.	Di(2-etilhexil)ftalato (DEHP)	X
13.	Diurón	X
14.	Endosulfán	X
15.	Fluoranteno	
16.	Hexaclorobenceno	X
17.	Hexaclorobutadieno	X
18.	Hexaclorociclohexano (lindano y sus isómeros α y β)	X
19.	Isoproturón	X
20.	Plomo y sus compuestos	X
21.	Mercurio y sus compuestos	X
22.	Naftaleno	X
23.	Níquel y sus compuestos	

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[1] Sustancias peligrosas: Sustancias o grupos de sustancias que son tóxicas, persistentes y pueden causar bioacumulación, así como otras sustancias o grupos de sustancias que entrañan un nivel de riesgo análogo, como los disruptores endócrinos.

Tabla 3…cont.

	Contaminante o grupo de contaminantes prioritarios	Prioritario-peligroso?
24.	Nonilfenol (4-Nonilfenol)	X
25.	Octilfenol (4-tert-octilfenol)	X
26.	Pentaclorobenceno	X
27.	Pentaclorofenol	X
28.	PAHs: benzo(a)pireno, benzo(b)fluoranteno, benzo(k)fluoranteno, benzo(g,h,i)perileno, indeno(1,2,3-cd)pireno	X
29.	Simazina	X
29a.	Tetracloro-etileno	
29b.	Tricloroetileno	
30.	Compuestos de tributilestaño (Catión de tributilestaño)	X
31.	Triclorobencenos	
32.	Triclorometano	
33.	Trifluralina	X

Reglamento sobre los contaminantes orgánicos persistentes

En el 2004 se publica el **Reglamento 850/2004/CE** [66], relativo a los POPs. Está dirigido a completar la legislación ya abundante de la UE relativa a los POPs que figuran en las listas del convenios internacionales suscritos por la Comunidad Europea, tales como el **Convenio de Estocolmo** (firmado en 2004) y el **Protocolo del Convenio sobre contaminación atmosférica transfronteriza a gran distancia provocada por contaminantes orgánicos persistentes** (firmado en 1979 y ratificado en 1998). El Reglamento se refiere de forma específica a la producción, comercialización, utilización, vertido y eliminación de las sustancias que son objeto de prohibiciones o limitaciones enumeradas en ambos acuerdos. Uno de sus objetivos es evitar cualquier hueco o incoherencia jurídica entre los textos comunitarios y los textos nacionales y favorecer una aplicación práctica más coherente. Con el fin de actualizar la lista de sustancias incluidas en la modificación del Convenio de Estocolmo del 2009, a este Reglamento se le han sumado dos más publicados en 2010, el 756/2010 [67] y el 757/2010 [68].

Directiva de Normas de Calidad Ambiental (NCA)

De la DMA se desprende la **Directiva 2008/105/CE** [9], publicada en 2008, que pretende regular la calidad química de las aguas superficiales de Europa, incluyendo a las aguas costeras. Esta Directiva define los límites de concentración o NCA (Normas de Calidad Ambiental) de los 33 contaminantes prioritarios mencionados en la Tabla 3 (Decisión 2455/2001/CE) en los diferentes tipos de cuerpos de agua. Exige que se realicen un inventario de emisiones, vertidos y pérdidas de las sustancias incluidas. Así mismo establece mediciones de NCA en biota o sedimentos para únicamente 3 compuestos. Esta Directiva se complementa con la Directiva 2009/90/EC [69] que establece las especificaciones técnicas del análisis químico y del seguimiento del estado de las aguas. Esta última determina que, en caso de no existir métodos analíticos normados, se deben emplear las mejores técnicas disponibles que no acarreen costes excesivos siempre siguiendo las prácticas de gestión de calidad aceptadas internacionalmente para asegura la comparabilidad de los resultados analíticos. Estas dos Directivas se transcriben a nivel español en el Real Decreto 60/2011 (21-01-2011) sobre las normas de calidad ambiental en el ámbito de la política de aguas [70]. El 31 de enero de 2012 la Comisión Europea presentó la Propuesta de Directiva 2011/0429 con lo que se pretende modificar las Directivas 2000/60/CE y 2008/105/CE en cuanto a las sustancias prioritarias en el ámbito de la política de aguas. En ésta propuesta se presenta modificaciones en algunos de los valores de NCA, e incluye un mayor número de sustancias que deberán analizarse en biota tales como peces y moluscos [71].

Directiva marco sobre la estrategia marina (DMEM)

En un intento por coordinar y unificar a nivel europeo toda la legislación existente referente al medio marino, en junio de 2008 se instauró la **Directiva 2008/56/CE** por la que se establece un marco de acción comunitaria para la política del medio marino. En ella exige que los Estados Miembros adopten las medidas necesarias para lograr o mantener un buen estado medioambiental del medio marino a más tardar en el año 2020 [72]. Así mismo establece las diferentes regiones y subregiones en las que se dividen las aguas marinas europeas:

- Mar Báltico
- Océano Atlántico Nororiental, que se divide en las subregiones:
  - el Mar del Norte
  - el Mar Céltico
  - el Golfo de Vizcaya y las Costas Ibéricas
  - en el Océano Atlántico, la región biogeográfica macaronésica, definida por las aguas que circundan las Azores, Madeira y las Islas Canarias
- Mar Mediterráneo, que se divide en las subregiones:
  - el Mediterráneo Occidental
  - el Mar Adriático
  - el Mar Jónico y el Mediterráneo Central
  - el Mar Egeo Oriental
- Mar Negro

La DMEM se aplica a las aguas, el lecho marino y el subsuelo situados más allá de la línea de base que sirve para medir la anchura de las aguas territoriales y que se extienden hasta el límite exterior de la zona en que cada Estado miembro ejerce derechos jurisdiccionales.

Las estrategias marinas constituyen un plan de acción que debe llevarse a cabo para cada demarcación marina, y que conlleva las siguientes acciones:

- Una evaluación inicial del estado ambiental actual y del impacto de las actividades humanas en el medio marino de cada demarcación marina (plazo: 15 de julio de 2012), que tendrá los siguientes elementos:
    - Un análisis de las características esenciales y del estado ambiental actual de la demarcación marina
    - Un análisis de los principales impactos y presiones que afectan al estado ambiental de la demarcación marina
    - Un análisis económico y social de la utilización del medio marino y del coste que supone el deterioro del mismo
- Definición del buen estado medioambiental de las aguas marinas. Plazo: 15 de julio de 2012
- Establecimiento de una serie de objetivos medioambientales e indicadores asociados. Plazo: 15 de julio de 2012
- Elaboración y aplicación de un programa de seguimiento. Plazo: 15 de julio de 2014
- Elaboración de un programa de medidas destinado a alcanzar o mantener el buen estado medioambiental. Plazo: año 2015
- Inicio del programa de medidas. Plazo: año 2016

La Directiva establece que los Estados miembros que compartan una región o subregión marina deben cooperar para las estrategias marinas sean coherentes y adopten un enfoque común. Esta cooperación se puede canalizar a través de los convenios marinos regionales, que en el caso de España se trataría del Convenio de Barcelona y el Convenio OSPAR.

Directiva de las aguas residuales

Como ya se mencionó previamente en la sección 1.1.2, una de las principales fuentes de contaminación de los mares y aguas costeras, son las descargas de aguas residuales. A nivel europeo existe la **Directiva 91/271/CE** [73] que provee medidas básicas acerca del tratamiento de aguas residuales exige la recolección de las aguas residuales a través de toda la región europea e implementa los controles y medidas mínimas orientadas a la reducción de nutrientes y sustancias consumidoras de oxígeno descargadas en las aguas frescas. Esto supondrá una reducción de la carga total de contaminantes que llegan a las aguas costeras.

Calidad de las aguas de baño

La **Directiva 2006/7/CE** relativa a la gestión de la calidad de las aguas de baño, está encaminada a proteger la salud de los bañistas que recurren a las zonas de aguas turísticas marítimas y continentales. Se enfoca principalmente a factores microbiológicos y establece el período y frecuencia en la que se deben hacer estas mediciones [74].

Reglamento REACH

Una legislación europea que no está relacionada directamente con las aguas costeras ni con el medio marino, pero que es una herramienta que supondrá tener un control estricto y un conocimiento detallado de los riesgos de las sustancias químicas, tanto para el medio ambiente como para la salud del ser humano es el **Reglamento 1907/2006/CE** o Reglamento de Registro, Evaluación, Autorización y Restricción de Sustancias Químicas (REACH) [15]. Esta regulación se creó con el fin de reducir el descontrol que ha habido durante años en la creación, producción y comercialización de sustancias químicas de las cuales, como ya se mencionó anteriormente (sección 1.1) se desconoce totalmente los efectos que pueden tener en el medio ambiente. Este Reglamento garantiza que los fabricantes, importadores y usuarios intermedios sólo fabricarán, comercializarán o usarán sustancias que no afecten negativamente a la salud humana o al medio ambiente. Uno de los resultados que hasta el día de hoy se han obtenido del REACH, es evidenciar que las actividades humanas han causado efectos significativos y severos en el medio ambiente costero y en general el marino [10; 72]. Los principales elementos que se contemplan en el REACH son [15]:

- Registro sistemático de todos los productos químicos, no sólo los nuevos sino también los existentes.
- Introducción de un procedimiento de autorización o de restricción en el caso de sustancias que generen especial preocupación.
- Responsabilidad de productores e importadores de evaluar los riesgos de sus productos.
- Mayor transparencia al facilitar el acceso de los ciudadanos a la información sobre las sustancias.
- Sustitución progresiva de las sustancias químicas más peligrosas cuando sean identificadas nuevas alternativas.

Acciones como las implantadas por el Reglamento REACH ayudarán a tener un mayor conocimiento de los riesgos que implicaría poner en el mercado “nuevas” sustancias químicas y además de que se irán incluyendo sustancias “ya existentes” que actualmente se comercializan y producen.

Ley de Agua Limpia

Aunque la Ley de Agua Limpia (*Clean Water Act*) no es una legislación europea, se considera conveniente mencionarla en esta sección. Ésta es la principal ley federal en los Estados Unidos que regula la contaminación del agua superficial. Establece los objetivos, y proporciona los instrumentos legales y económicos para reducir las descargas directas de contaminantes en los cuerpos de agua. Así mismo gestiona las directrices para el tratamiento de aguas residuales y aguas de escorrentía contaminadas [75]. Uno de los instrumentos más importantes que se pueden destacar de esta legislación son los Criterios de Calidad del Agua, homólogas a las NCA. Determina los niveles máximos de concentración para 120 contaminantes prioritarios y 47 no prioritarios en aguas dulces y saladas (valores críticos y agudos), con el fin de proteger la vida acuática. También se especifican los niveles máximos para la protección de la salud humana, en caso de que el agua o los organismos que se encuentran en el agua sean para consumo humano, basados en un riesgo de carcinogenicidad de 10^{-6} [76].

Como puede apreciarse, el número de sustancias legisladas y en especial las denominadas prioritarias es muy pequeño en comparación de la cantidad real de las que pueden contaminar y potencialmente causar efectos negativos en el medio ambiente y/o presentar un riesgo en la salud humana. A este último grupo de sustancias se denomina **contaminantes emergentes**. Estos se definen como los compuestos que no están regulados por alguna legislación referente al medio acuático, y se conoce poco o nada de sus efectos hacia la salud pública y a los ecosistemas. Los contaminantes emergentes no son necesariamente “sustancias nuevas” o de reciente creación y algunas de ellas se han detectado en el medio ambiente, y su presencia y efectos perjudiciales se hacen evidentes hasta el día de hoy [14]. Entre ellos se pueden incluir a un gran número de familias químicas incluyendo a los fármacos, drogas de abuso, productos de cuidado personal, esteroides y hormonas, compuestos perfluorados, retardantes de llama, aditivos industriales, agentes y aditivos para gasolina así como sus productos de transformación, entre otros [77]. Algunos de estos compuestos se producen y consumen en grandes cantidades y por consiguiente se introducen al ambiente continuamente, por lo que no necesariamente tienen que ser persistentes para causar efectos negativos [78].

1.3 Compuestos de estudio, su presencia y efectos adversos en el medio marino

Entre la gran diversidad química de sustancias prioritarias y emergentes, se eligieron 7 grupos de contaminantes para desarrollar el presente trabajo de tesis doctoral. Estos grupos son los hidrocarburos aromáticos policíclicos, los alquilfenoles, los plastificantes (ésteres de ftalatos y bisfenol A), los bifenilos policlorados, éteres de polibromodifenilo, plaguicidas organoclorados y compuestos perfluorados. A continuación se describen las principales características de cada familia.

1.3.1 Hidrocarburos aromáticos policíclicos

Los hidrocarburos policíclicos aromáticos ("polycyclic aromatic hydrocarbons" o PAHs) son un grupo de sustancias químicas orgánicas semivolátiles que consisten en 3 o más anillos de benceno fusionados, aunque en esta categoría también pueden incluirse algunos compuestos bicícilcos (con dos anillos de benceno) [79].

Las dos principales fuentes de PAHs en el medio ambiente son los combustibles fósiles, principalmente el petróleo crudo y la combustión incompleta de materia orgánica. Los PAHs son emitidos al aire desde procesos de combustión y dependiendo de la temperatura del aire pueden permanecer en forma gaseosa o ser adsorbidos en las partículas. En general todos los procesos térmicos que contengan carbono e hidrógeno pueden ser fuentes potenciales de PAHs. Las principales fuentes antropogénicas identificadas de estas sustancias hacia el aire son plantas termoeléctricas, incineradores de residuos sólidos, estufas, motores de vehículos, incluso de restaurantes de comida rápida [3; 80]. También pueden ser emitidas por fuentes naturales como los incendios forestales y los volcanes. Normalmente la formación de PAHs en los procesos térmicos está íntimamente relacionada con las condiciones del proceso, y la cantidad emitida de PAHs depende de la eficiencia de los sistemas de contención instalados en el sitio de emisión. Así mismo, pueden provenir de otras fuentes ya que se emplean como ingredientes activos de biocidas como el alquitrán, la creosota y productos similares utilizados para la conservación y protección de madera y además son componentes del asfalto [80].

La EPA recomienda el análisis de 16 PAHs entre su lista de sustancias prioritarias (Criterios de Calidad del Agua), aunque en la legislación europea (Decisión 2455/2001/CE), se incluyen solamente 6 PAHs como sustancias peligrosas-prioritarias y 2 como prioritarias que son los mismos compuestos que se incluyen en la Lista OSPAR

[81] (Tabla 3). Los PAHs son sustancias tóxicas, aunque los animales vertebrados (aves peces, mamíferos) pueden metabolizarlos. Sin embargo, algunos de los PAHs son compuestos carcinogénicos. En la Figura 5 se muestra la estructura química de los 16 PAHs estudiados en este trabajo (los recomendados por la EPA).

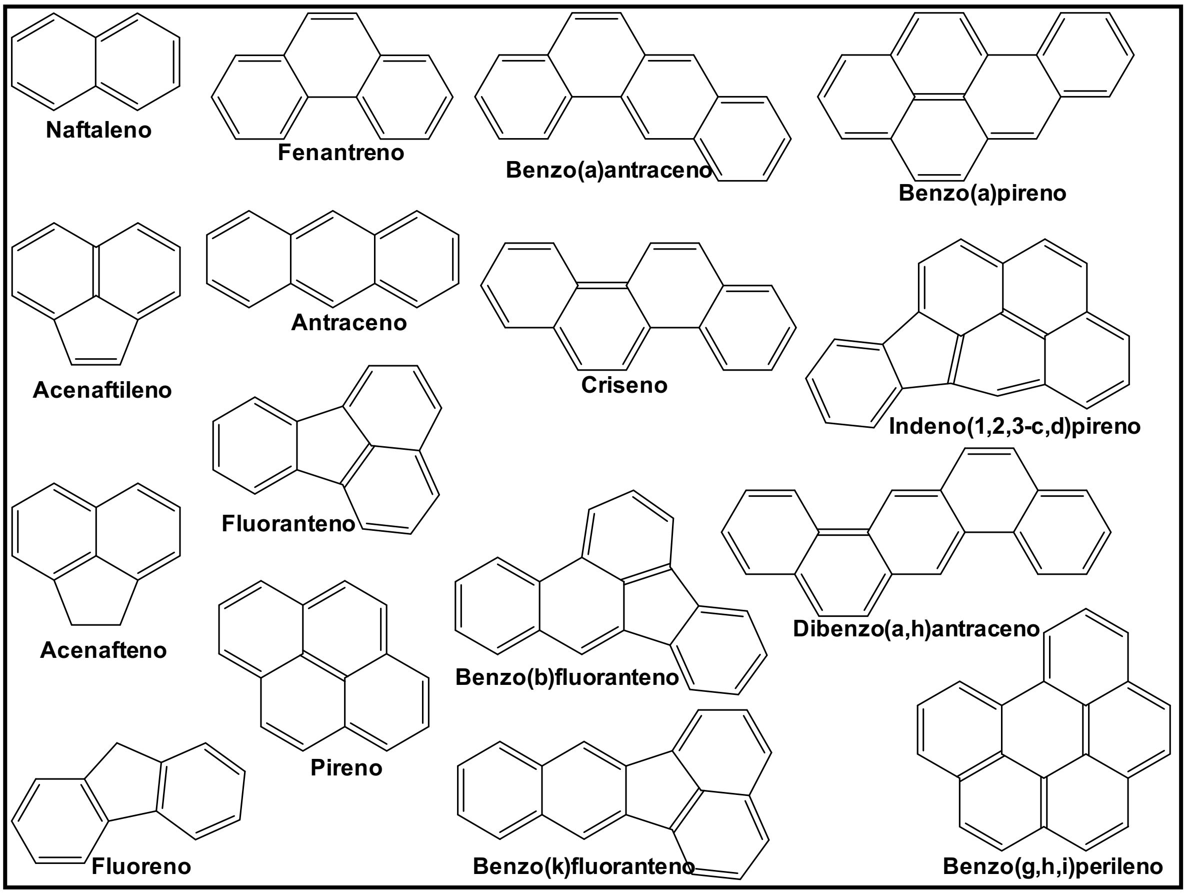

Figura 5. Estructura química de los 16 PAHs analizados

Se han detectado PAHs en aguas costeras y de mar abierto así como en otros compartimientos del medio marino (sedimentos, peces, moluscos) [82-85]. Las descargas de PAHs al agua pueden provenir de actividades industriales situadas en mar abierto o tierra adentro, así como de material de dragado. La disposición final sin control de residuos que contienen PAHs, como los provenientes de la industria de la construcción, tratamiento de maderas, etc., pueden ser fuentes difusas de contaminación de las aguas superficiales, marinas y subterráneas. Los PAHs son fotoquímica y biológicamente degradables. La vida media de los distintos compuestos varía considerablemente, desde semanas hasta años, dependiendo de características del medio que los rodean. Los PAHs adsorbidos en el material particulado y en los sedimentos se degradan en menor proporción y también son menos solubles y volátiles. Debido a su menor degradación, menor volatilidad y que son fuertemente adsorbidos por el particulado, los PAHs de alto peso molecular tienden a acumularse en los sedimentos [80]. Algunas de las propiedades fisicoquímicas y toxicológicas de los PAHs se indican en la Tabla 4.

1.3.2 Alquilfenoles

Los alquilfenoles (APs) son sustancias derivadas del fenol, que contienen uno o más grupos alquilos unidos al anillo aromático. Los APs etoxilados son surfactantes no-iónicos que tienen excelentes propiedades espumantes y son en general buenos detergentes humectantes, dispersantes y emulsionantes. Por todas estas propiedades, se encuentran hoy en día en todos los tipos de formulaciones de detergentes tanto industriales como domésticos, líquidos o en polvo, y en otras aplicaciones como plaguicidas y productos industriales. No producen iones en solución acuosa y por lo tanto son compatibles con los demás tipos de surfactantes y pueden integrarse en formulaciones complejas. Los nonilfenoles etoxilados (NPEOs) son los surfactantes más vendidos (80%). Los principales sectores industriales en donde se emplea estos productos son: limpieza industrial, institucional y doméstica (30% del uso en la UE), polimerización de emulsiones (12%), textiles (10%), síntesis química (9%) y pieles (8%) [86; 87]. Los octilfenoles etoxilados (OPEOs) se emplean secundariamente, pero pueden estar presentes como impurezas en las formulaciones de los NPEOs (max. 10%, típicamente entre 3 y 5%) [88].

Los términos nonilfenol (NP) y octilfenol (OP) representan un número muy grande de formas isoméricas de las fórmulas generales $C_8H_{17}C_6H_4(OH)$ y $C_9H_{19}C_6H_4(OH)$. Los grupos octil (C_8H_{17}) y nonil (C_9H_{19}) pueden estar ramificados de varias maneras o estar en forma de cadena lineal y a su vez, estar situados en la posición 2, 3 o 4 en el anillo de benceno. Las formas más comunes presentes ambientalmente como contaminantes suelen ser como diferentes isómeros de 4-nonilfenol y en el caso del octilfenol, en forma del 4-tert-octylphenol [88-90]. La mezcla isomérica de NP y el 4-tert-octylphenol están incluidos en la Lista OSPAR, en la Directiva de Sustancias Prioritarias y en las NCA [9; 65; 81]. En la Figura 6 se muestran las estructuras químicas de los alquilfenoles estudiados en este trabajo.

La peligrosidad del OP y el NP radica en que son sustancias lipofílicas que se acumulan en los fangos, sedimentos y biota. Así mismo el NP y los etoxilados de cadena corta son considerados disruptores endocrinos[1] [88; 89; 91; 92].

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[1] Disruptor endócrino o interruptor endócrino: Sustancia química capaz de alterar el equilibrio hormonal (o sistema endocrino) de animales, incluyendo a los humanos. Puede interrumpir algunos procesos fisiológicos controlados por hormonas, o generar una respuesta de mayor o menor intensidad que lo habitual. Pueden generar tumores cancerosos, defectos de nacimiento y otros desordenes del desarrollo o crecimiento.

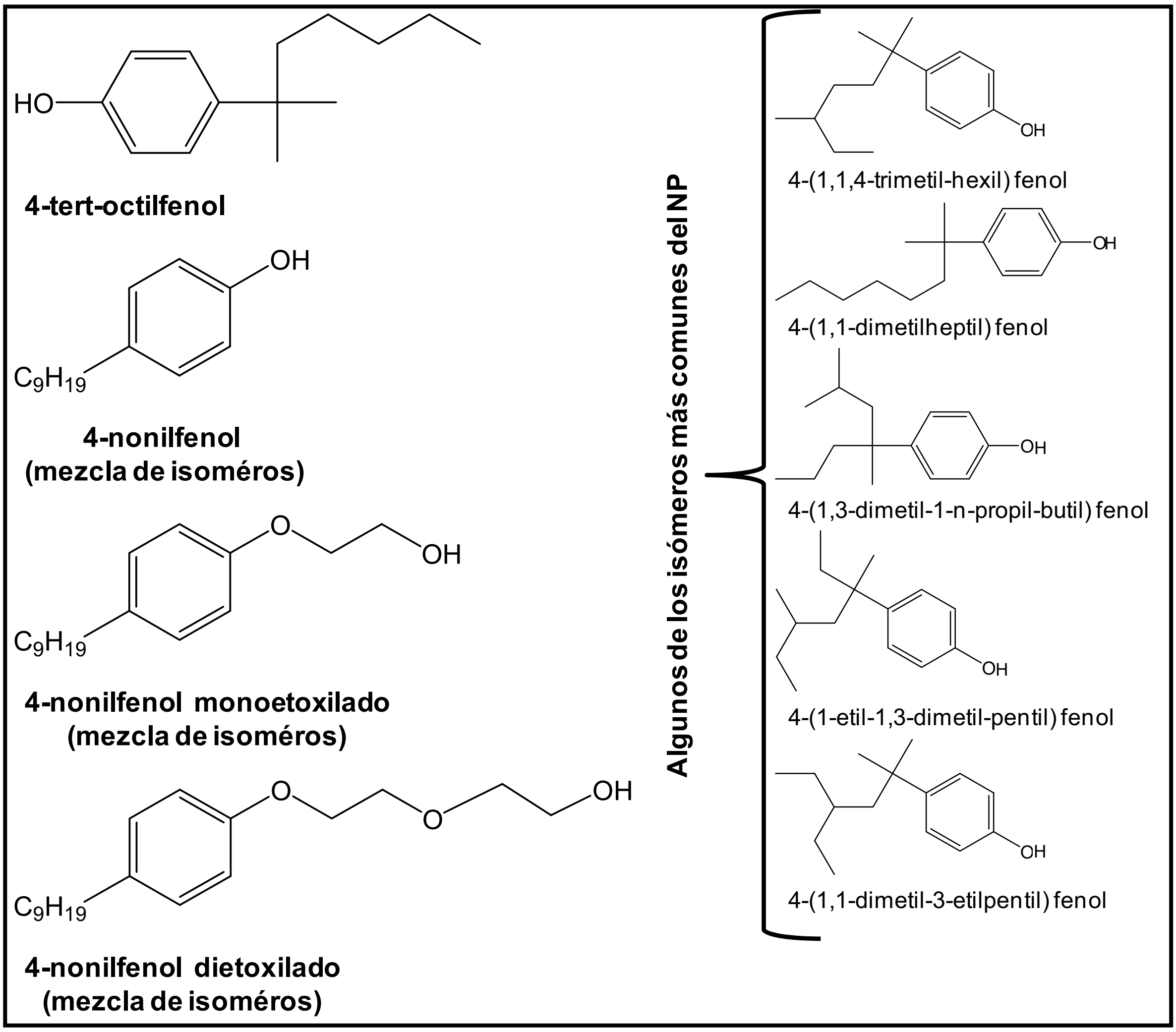


Figura 6. Estructura química de los 4 alquilfenoles estudiados

El NP únicamente se emplea como intermediario en la producción de otros compuestos químicos como los NPEOs, en cambio el 98% de la producción de OP puede usarse como tal, en la producción de resinas fenólicas. En Europa, la producción estimada de NPEOs en 1997 fue de 118,000 t y se utilizaron alrededor de 77,600 t. En el caso de OP, se produjeron 22,633 t y se utilizaron 22,854 t en el año 2001 [88; 89]. En 2003, se publicó la **Directiva 2003/53/EC** que limita la comercialización y el uso del NP y sus etoxilados restringiendo una cantidad máxima de 0.1% en ciertas formulaciones de uso industrial, doméstico y personal [93]. Generalmente los NPEOs tienen un intervalo de etoxilación de entre 6 a 12 monómeros y estos pueden degradarse rápidamente en el ambiente, perdiendo monómeros etoxilo (CH_3-CH_2-O-), pasando por $NPEO_2$ y $NPEO_1$ hasta llegar a NP, especialmente si las condiciones del medio son anaeróbicas [86; 89].

La presencia de alquilfenoles en el medio marino (agua, sedimentos, peces) ha sido reportada en diversos estudios [85; 87; 94]. Las principales fuentes de contaminación de los alquilfenoles son durante la fabricación y uso de los productos que los contienen.

Estos pueden llegar principalmente al agua a través de las descargas no tratadas de aguas residuales domésticas e industriales y también de los efluentes de las EDAR, ya que no son eliminados completamente en los procesos de depuración [21; 95]. Así mismo el empleo de fangos de depuradora como fertilizantes de suelos agrícolas, al lixiviar estas sustancias retenidas, puede contaminar los suelos y posteriormente el agua [89; 96]. En la Tabla 4 se enumeran las principales propiedades fisicoquímicas y toxicológicas de los 4 alquilfenoles estudiados.

1.3.3 Ftalatos

Los ftalatos o ésteres de ftalato ("phthalate esters" o PEs) son una familia de sustancias químicas industriales empleadas como suavizantes, adhesivos o disolventes en una gran variedad de procesos y productos industriales. Su principal uso es en la industria de los polímeros, como plastificante en el PVC y, en menor medida para la fabricación de artículos de consumo tales como selladores, pinturas, tintas de impresora, cosméticos, cubiertas de diferentes productos (automóviles, bobinas, telas cables, etc.) [97; 98].

Se estima que en 1998, se produjeron 71,000 t de di-n-butil ftalato (DBP), di-(2-etilhexil) ftalato (DEHP) y butilbenzil ftalato (BBP) en la UE y que en ese mismo año, se consumieron aproximadamente un total de 530,000 t. En el 1999, se prohibió en Europa la utilización de PVC que contuviera ciertos ftalatos para la fabricación de juguetes y artículos para niños menores de 3 años [99].

Los ftalatos pueden entrar al medio ambiente a través de pérdidas durante el proceso de manufactura y lixiviación de los productos finales, ya que estas sustancias no se encuentran unidas químicamente a la matriz polimérica [98; 100]. Esta migración puede darse durante la vida útil de los productos o durante la disposición final [101]. Las principales fuentes de contaminación son a través de aguas de lavado, por abrasión y arrastre desde los productos de uso doméstico, comercial o industrial que contienen PVC (pisos, paredes, tuberías, etc.) y posteriormente son descargadas directamente a los ríos o mares o hacia las plantas depuradoras [22; 102]. Los ftalatos pueden provenir también de la los residuos sólidos plásticos que son arrojados al mar o son arrastrados desde tierra adentro y que se encuentran formando grandes cúmulos flotantes en los mares o como micropartículas (microplásticos) que se desprenden de materiales más grandes, pudiendo así ser ingeridos por la biota marina [103].

El elevado uso histórico y actual de los ftalatos, así como el gran número de productos que los contienen (en especial al DEHP) ha hecho que su presencia se haya esparcido difusamente en el ambiente, inclusive en áreas marítimas remotas [98; 102; 104]. Únicamente el DEHP se encuentra incluido dentro de la Directiva de sustancias prioritarias y la de NCA; en la lista OSPAR se incluye además el DBP. Además del DEHP y DBP, en este trabajo se incluyen otros 4 compuestos considerados como contaminantes emergentes (Figura 7). Aunque la mayoría de estas sustancias se degradan con facilidad, incluirlas en este trabajo se justifica con que estas son descargadas continuamente y en grandes cantidades al medio acuático. Asimismo se ha evidenciado su toxicidad en diferentes estudios, relacionándose con daños en el hígado y los riñones [105]; se les atribuye propiedades de disrupción endocrina, afectando el metabolismo humano, de animales de laboratorio [106-108] y especies de mamíferos marinos y peces [22; 108; 109].

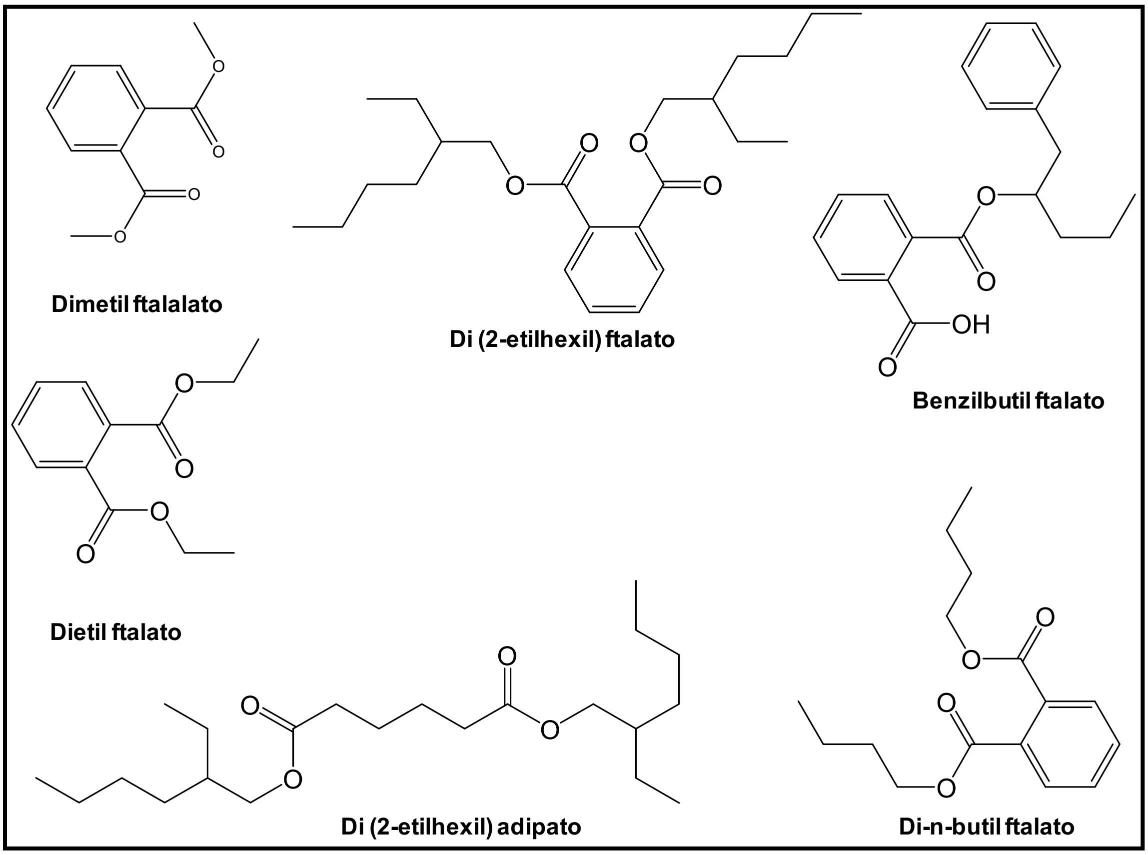


Figura 7. Estructura química de los 6 ftalatos estudiados

En la Tabla 4 se enumeran las principales propiedades fisicoquímicas y toxicológicas de los 6 ftalatos estudiados.

1.3.4 Bisfenol A

El bisfenol A (BPA) es el monómero empleado en la producción de policarbonato, polímero utilizado para la fabricación de biberones de bebes. Es el componente mayoritario de las resinas epóxicas como las usadas en los revestimientos de latas y selladores dentales; así mismo se emplea para fabricar poliestireno insaturado y retardantes de flama. También se utiliza como aditivo para el PVC, tintas de imprenta, y para el papel térmico, en pinturas en polvo, pulpa de papel, colorantes y encapsulación de partes eléctricas [110; 111]. El BPA es uno de los compuestos químicos con mayor producción a nivel mundial. En 2003 fue estimada en más de 2 millones t de las cuales el 72% se usó para plástico de policarbonato y un 21% para resinas epóxicas. En la Figura 8 se muestra la estructura química del bisfenol A.

El BPA en bajas dosis puede alterar la estructura química del cerebro y el comportamiento, el sistema inmunológico, la actividad enzimática, el sistema reproductivo de los machos y hembras de una gran variedad de animales incluyendo peces, ranas y mamíferos [112]. Así mismo es un disruptor endocrino y se ha demostrado en estudios con cultivos celulares, que puede estimular respuestas en células pancreáticas, crecimiento de tumores en células pituitarias, prostáticas y cáncer de pecho, entre otros [91; 113; 114].

El BPA es un contaminante emergente y a pesar de todos los estudios que evidencian sus efectos en el medio ambiente y la salud humana, no se ha incluido a esta sustancia en ninguna regulación Europea. Únicamente se menciona como sustancia de posible preocupación en el listado OSPAR. Se habla de que los estudios realizados por los fabricantes de BPA no han llegado a concluir que se trata de una sustancia con las características de persistencia, bioacumulación y toxicidad para poderla registrar como sustancia peligrosa, y estos mismos han desestimado los resultados obtenidos por la comunidad científica, ya que el coste de prohibirla y buscar un sustituto sería muy elevado [112].

Así como en el caso de los ftalatos, el BPA puede migrar desde los productos que lo contienen, ya sea durante su vida útil, como durante su disposición final. También pueden liberarse significativas cantidades al ambiente durante los procesos de manufactura [101]. La descarga de BPA por medio de efluentes domésticos e industriales después de un tratamiento en una EDAR, suele ser bajo debido a que los procesos biológicos degradan en gran medida este compuesto (generalmente más del 86%) [110; 115]. Sin embargo, si el BPA llega a alcanzar el medio marino, por medio de descargas no tratadas, la degradación en el agua de mar no se da con la misma intensidad que en las EDAR o los ríos, permaneciendo biodisponible en la columna de agua y/o en los sedimentos representando un riesgo para el ecosistema marino [48]. Ya que las cantidades de envases, bolsas y diversos productos plásticos encontrados flotando en el mar han aumentado drásticamente en los últimos años, la concentración de BPA en el medio marino también se ha dispersado e incrementado. Estos objetos plásticos no sólo representan un riesgo por liberar BPA en el agua, sino que también pueden ser ingeridos por los peces y mamíferos marinos, incrementando así el nivel de esta sustancia en los organismos [30; 97].

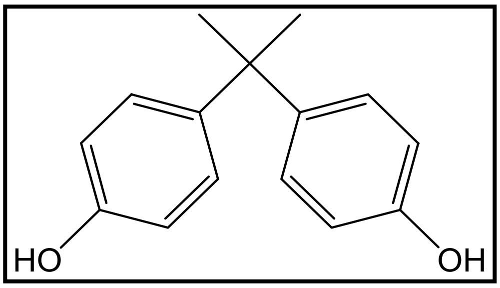


Figura 8. Estructura química del bisfenol A

En la Tabla 4 se enumeran algunas de las propiedades fisicoquímicas y toxicológicas del BPA.

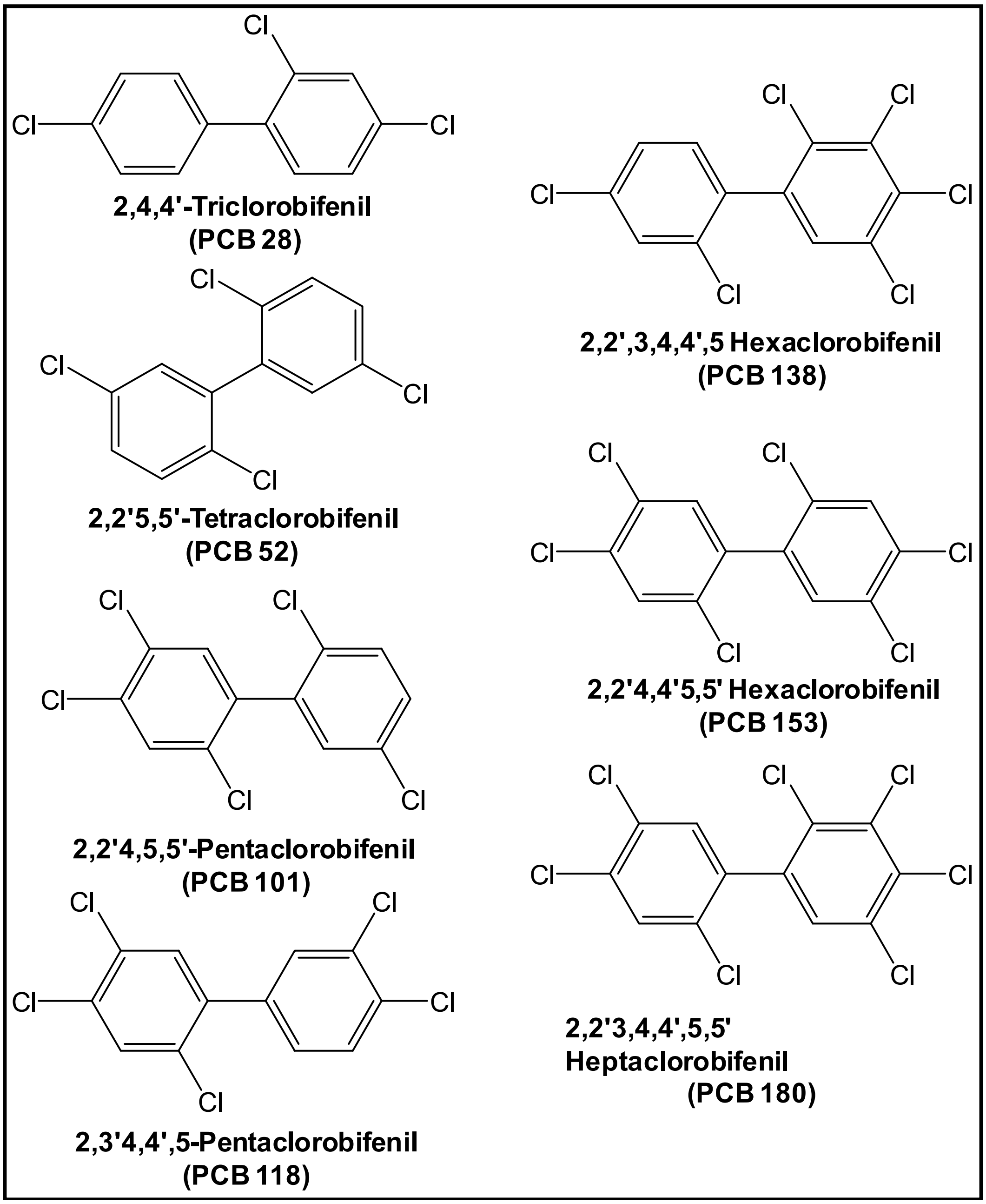
1.3.5 Bifenilos policlorados

Los bifenilos policlorados o policlorobifenilos (“polychlorobiphenyls” o PCBs) son un grupo de 209 congéneres producidos al introducir de uno a 9 átomos de cloro en un bifenilo. Su producción comercial abarcó desde los años treinta hasta mediados de los años ochentas y se calcula que en total se fabricaron más de un millón de toneladas a nivel mundial. Gracias a su extraordinaria estabilidad química y resistencia al calor, se emplearon en sistemas cerrados para enfriar transformadores dieléctricos, como fluidos de aislamiento en capacitadores y fluidos hidráulicos; también se han empleado en sistemas abiertos en aplicaciones como extensores de plaguicidas, selladores, papel para copia sin carbón, aceites industriales, pinturas, adhesivos, plásticos, productos ignífugos y para controlar el polvo en las carreteras. Desde 1970, se evidenció la persistencia en el medio ambiente de estas sustancias y a mediados de los años ochentas se prohibieron su fabricación en Europa y se restringieron sus usos [116; 117]. Están incluidos en la lista de POPs del Convenio de Estocolmo, por lo cual se incluyen también en el Reglamento 850/2004/EC. Históricamente se han comercializado diferentes mezclas de congéneres, entre ellos se pueden mencionar al Kanechlor (Japón), Aroclor (Estados Unidos y Reino Unido), Clophen (Alemaniay), Chlorofen (Polonia) y Phenoclor y Pyralène (Francia). El perfil de congéneres del Aroclor 1254 y 1260 se han identificado previamente como contaminantes globalmente distribuidos, especialmente en biota [118]. Se han realizado varios intentos por identificar marcadores (los congéneres mayoritarios) de las mezclas de PCB más empleadas y utilizarlos como trazadores de contaminación. Entre estos isómeros se puede mencionar al tri-CB 28, dominante en la composición del Kanechlor-300, Kanechlor-400, Aroclor 1016, Aroclor 1242 y Clophen A30; el tetra-CB 52 predominante en el Clophen A40; el penta-CB 101, dominante en la composición del Kanechlor-500 y Aroclor 1254; el penta CB 118, dominante en el Clophen A50; el hexa-CB 153, más abundante en el Clophen A60 y Clophen T64; el hepta-CB 180 en la composición del Kanechlor-600, Aroclor 1260 (junto con el hexa-CB 138), Aroclor 1262 y Chlorofen [119]. En la Figura 9 se muestran los 7 congéneres de PCB incluidos en esta tesis, los cuáles son los más representativos de las mezclas comerciales.

Los PCBs son altamente tóxicos ya que tienen propiedades de disrupción endócrina y pueden afectar negativamente a la reproducción y también al sistema inmunológico [91; 120]. Al ser hidrófobos, pueden bioconcentrarse preferentemente en tejidos grasos y biomagnificarse a través de la cadena trófica, especialmente en aves y mamíferos marinos [117; 120]. Su alta estabilidad química los hace ser persistentes y recalcitrantes. Pueden ser transportados grandes distancias, habiéndose ya esparcido por todo el globo, incluyendo sitios remotos y detectándose en todas las matrices ambientales (agua, aire, biota, sedimentos, suelos) [116; 117; 121-124].

A partir de su prohibición, se han buscado maneras eficientes de disponerlos. Estas medidas se publicaron en 1996 en la Directiva 96/59/EC acerca de la disposición final de policlorobifenilos y tetrabifenilos policlorados (PCB/PCT) [125].

Sus propiedades fisicoquímicas (baja solubilidad, alta hidrofobicidad) permiten preferentemente su acumulación en biota, material particulado y sedimentos [126]. Ya que no se han fabricado más desde los ochentas, las vías en las que estos contaminantes llegan al medio son a través del uso de productos que aun están en servicio que contienen estas sustancias como por ejemplo pérdidas de fluidos de los transformadores, de aislamiento, así como por medio de una ineficiente disposición final (lixiviación al agua y arrastre). Una fuente difusa más por la que actualmente se siguen detectando PCBs en el medio marino, es a través de la resuspensión de sedimentos contaminados [116; 123]. En la Tabla 4 se enumeran algunas de las propiedades fisicoquímicas y toxicológicas de los PCBs estudiados.



2,4,4'-Triclorobifenil (PCB 28)

2,2'5,5'-Tetraclorobifenil (PCB 52)

2,2'4,5,5'-Pentaclorobifenil (PCB 101)

2,3'4,4',5-Pentaclorobifenil (PCB 118)

2,2',3,4,4',5 Hexaclorobifenil (PCB 138)

2,2'4,4'5,5' Hexaclorobifenil (PCB 153)

2,2'3,4,4',5,5' Heptaclorobifenil (PCB 180)

Figura 9. Estructura química de los 7 PCB estudiados en este trabajo

1.3.6 Éteres de polibromodifenilo

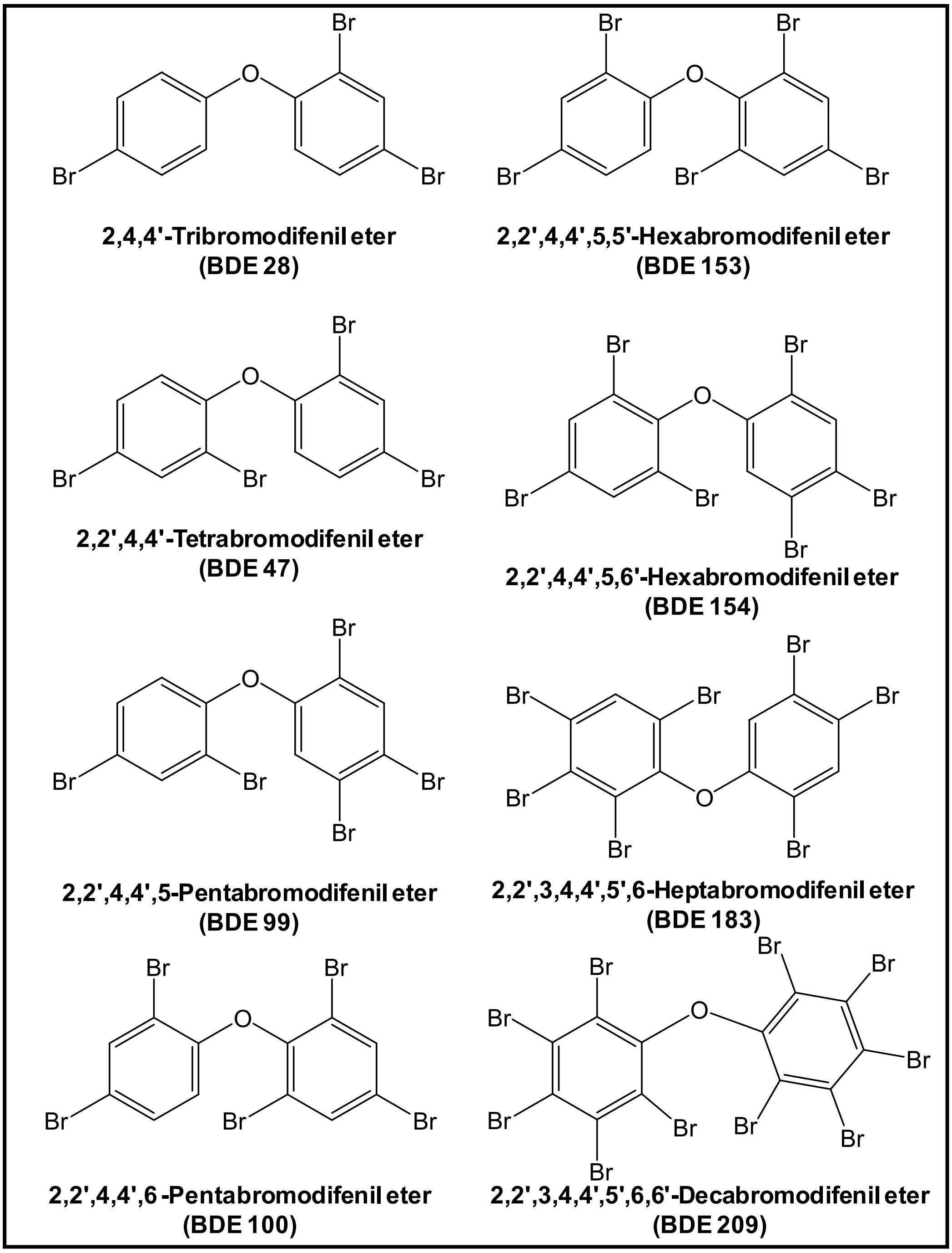
Los éteres de polibromodifenilo ("polybromo diphenyl ethers" o PBDEs) son un grupo de sustancias que se utilizan para retardar la combustión (retardantes de flama) en bienes y productos comerciales e industriales, especialmente en plásticos y espumas, resinas, aparatos electrónicos, materiales de construcción y textiles [127]. Por ejemplo, la espuma de poliuretano, que es ampliamente utilizada en los muebles tapizados, se trata con un 10-30% en peso de PBDE para reducir su flamabilidad [128]. Comercialmente se distribuyen tres productos, dos de los cuáles son mezclas de diversos congéneres. La primera de ellas llamada penta-BDE contiene 2,2',4,4'-tetraBDE (BDE-47), 2,2',4,4',5-

pentaBDE (BDE-99), 2,2’,4,4’,6-pentaBDE (BDE-100), 2,2’,4,4’,5,5’-hexaBDE (BDE-153) y 2,2’,4,4’,5,6’-tetraBDE (BDE-154), en una proporción aproximada de 9:12:2:1:1. La segunda, llamada octa-BDE contiene diversos congéneres de hexa a nona BDEs y, finalmente los deca-BDE está compuesta únicamente del decaBDE (BDE-209) [128; 129]. En 2001, la demanda mundial del mercado de PBDEs se estimó en 67,400 t, de las cuales el 83% correspondía a deca-BDE, después penta-BDE (11%) y finalmente octa-BDE (aprox. 6%) [130]. La Directiva de sustancias prioritarias prohibió la producción de penta-BDE en la Unión Europea, aunque aún se emplean productos que utilizaron en su fabricación esta mezcla de congéneres [66; 131]. En este trabajo se incluyeron 8 PBDEs considerando los legislados incluidos entre la formulación penta-BDE, como aquellos emergentes también disponibles en formulaciones comerciales (deca-BDE) así como los resultantes de la degradación de congéneres más bromados (Figura 10).

A pesar de los beneficios que proporcionan los PBDEs a la sociedad, diferentes estudios indican que estos migran desde los productos en los cuales se emplea, ingresando al medio ambiente y en el ser humano. Son ahora contaminantes globalmente distribuidos en todas las matrices ambientales (agua, aire, organismos marinos y terrestres, suelos) [42; 131-136] y sus valores parecen estar aumentando con el tiempo [130].

Los PBDEs son persistentes, se bioacumulan en la biota y se biomagnifican a través de la cadena alimenticia. En el medio marino se han detectado en peces, sedimentos en la columna de agua [42; 136; 137] e inclusive en focas, ballenas y marsopas cuyas concentraciones en muchos casos exceden más de 100 veces las detectadas en seres humanos [130]. Los efectos tóxicos de los PBDEs en animales son varios, pudiendo causar disrupción endocrina [138], desórdenes neurotóxicos, inmunotoxicidad, problemas en la reproducción y en algunos casos, cáncer [135; 139].

Las fuentes de contaminación más evidentes de los PBDEs hacia el medio marino son los efluentes industriales de sitios donde se utilizan estos compuestos (polímeros, plásticos, artículos electrónicos). Estos efluentes llegan a los ríos o se descargan directamente al mar [140]. Otras fuentes de emisión indirecta de PBDEs son los incineradores municipales, hospitalarios o de residuos peligrosos, las instalaciones de reciclado de plásticos y metales, los sitios de disposición final de residuos y artículos electrónicos así como los incendios [130]. Los compuestos altamente bromados son menos móviles en el ambiente ya que son menos solubles, menos volátiles y son adsorbidos principalmente en sedimentos. Por otro lado, los congéneres menos bromados son transportados más fácilmente por el agua y por el aire ya que son más solubles y al parecer son más bioacumulables [140]. Recientemente también se ha descubierto, que al igual que los ftalatos y el bisfenol A, estas sustancias se pueden desorber desde las pequeñas partículas y grandes objetos flotantes en la superficie marina [30]. El deca-BDE puede ser degradado en el ambiente transformándose en congéneres menos bromados, como los BDE 47 y BDE 99 que son los más ubicuos, lo cual a su vez explicaría la presencia de tri y tetra BDEs, que comúnmente no se encuentran como parte de las formulaciones comerciales [28; 42]. En la Tabla 4 se muestran algunas de las propiedades fisicoquímicas y toxicológicas de los PBDEs.



2,4,4'-Tribromodifenil eter (BDE 28)

2,2',4,4',5,5'-Hexabromodifenil eter (BDE 153)

2,2',4,4'-Tetrabromodifenil eter (BDE 47)

2,2',4,4',5,6'-Hexabromodifenil eter (BDE 154)

2,2',4,4',5-Pentabromodifenil eter (BDE 99)

2,2',3,4,4',5',6-Heptabromodifenil eter (BDE 183)

2,2',4,4',6 -Pentabromodifenil eter (BDE 100)

2,2',3,4,4',5',6,6'-Decabromodifenil eter (BDE 209)

Figura 10. Estructura química de los 8 PBDEs estudiados

1.3.7 Plaguicidas organoclorados

El termino plaguicida abarca un grupo muy amplio de sustancias de origen botánico, inorgánico o sintético empleados para matar o controlar insectos, malezas, enfermedades y otros organismos indeseables (plagas) [141]. A nivel mundial se comercializan más de 800 ingredientes activos en decenas de miles de formulaciones. Estos productos se usan extensamente en la agricultura, la salud pública, y en áreas domésticas y urbanas [142]. Dentro de los plaguicidas sintéticos se encuentran los que en su estructura química tienen átomos de cloro, denominados plaguicidas organoclorados (“organochlorinated pesticides” u OCPs). Estos se emplean principalmente como insecticidas. La mayoría de los insecticidas actúan directamente sobre el sistema nervioso o el metabolismo hormonal del animal y en particular, los organoclorados trastornan la cinética iónica de las bombas de sodio-potasio [143]. Dentro de éste grupo de plaguicidas se puede destacar al diclorodifeniltricloroetano (DDT), el lindano y el endosulfan (endsf). La gran mayoría de los OCPs están incluidos en la lista de sustancias incluidas en los anexos del Convenio de Estocolmo y en los Reglamentos Europeos 756/2010/EC y 757/2010/EC, por lo que se consideran sustancias prioritarias. Estos compuestos pueden ingresar al medio ambiente acuático a través de escorrentías agrícolas, descargas de efluentes tratados y no tratados, deposición atmosférica, etc. y pueden ser transferidos a través de las cadenas tróficas y finalmente bioacumulados en organismos acuáticos superiores [144; 145].

El DDT fue empleado indiscriminadamente durante la Segunda Guerra Mundial para proteger a los soldados y civiles contra la malaria y otras enfermedades transmitidas por insectos. Después de la guerra, se continuó usando para controlar enfermedades y se pulverizó en una gran variedad de sembradíos agrícolas, especialmente en el algodón. El DDT se continúa empleando en muchas regiones del mundo donde la malaria es un problema de salud pública. Este compuesto tiene alta estabilidad y persistencia ($t_{1/2} \approx$ 10-15 años en suelos, después de su aplicación), se bioacumula y biomagnifica y ya que posee una elevada transportabilidad, se encuentra globalmente distribuido en todas las matrices ambientales (agua, aire, organismos marinos y terrestres, suelos) [54; 142] y aún en regiones apartadas como el Ártico [146]. El término DDT se refiere al diclorodifeniltricloroetano, aunque se diferencian dos isómeros: el 4,4’-DDT ó 1,1’-(2,2,2-tricloroetilideno)-bis(4-clorobenceno) y el 2,4’-DDT ó 1-cloro-2[2,2,2-tricloro-1-(4-clorofenil)etil]benceno. Los productos comerciales de DDT consisten en una mezcla de isómeros, predominando el 4,4’-DDT (≈77.1%), con pequeñas cantidades de 2,4’-DDT (≈15%) y trazas de productos de degradación (Figura 11a). En el medio ambiente el DDT es degradado por diferentes procesos. Principalmente, bajo condiciones aeróbicas los microorganismos del suelo los degradan por medio de dehidroclorinación a 4,4’-DDE (1,1-dicloro-2,2-bis[p-clorofenilletileno) o a 2,4’-DDE (1,1-dicloro-2[o-clorofenil]-2[p-chlorofenil]-etileno), dependiendo del isómero de partida. Este proceso puede tardar muchos años en llevarse a cabo por completo. El DDE es un compuesto recalcitrante que no se degrada fácilmente en el ambiente. Bajo condiciones anaeróbicas y reductoras, los microorganismos y las porfirinas de hierro degradan el DDT en 4,4’-DDD (1,1-dicloro-2,2-bis[p-clorofenil]etano) o en 2,4’-DDD (1,1-dicloro-2[o-clorofenil]-2[p-clorofenil]etano) dependiendo del isómero de partida. Los microorganismos marinos también tienen la capacidad de degradar el DDT, principalmente a DDD. Sus elevados log K_{ow} (5.87-6.91) hacen que el DDT y sus productos de degradación tengan mayor afinidad a permanecer en los suelos y sedimentos, especialmente aquellos ricos en materia orgánica o bioacumulados en organismos. El DDT es altamente tóxico para los microorganismos, peces, anfibios y aves marinos y de agua dulce [147; 148]. El DDT se encuentra listado en el Anexo B del Convenio de Estocolmo [54], por lo que se considera un contaminante prioritario.

El lindano ha sido empleado como insecticida de amplio espectro en semillas, suelos, follaje, árboles y madera, así como tratamiento contra ectoparásitos en aplicaciones veterinarias y humanas. Su producción se ha reducido rápidamente en los últimos años. Lindano es el nombre común del isómero gama del hexaclorociclohexano (γ-HCH) [54]. De los 8 diferentes isómeros de HCH conocidos, el lindano es el que posee mayor actividad pesticida. Comercialmente se ha producido al lindano en formulaciones al 100% (99.9%), lo que resulta muy caro; otra alternativa de uso, que además resultaba más barata es una mezcla isomérica de HCHs que contiene generalmente un 55 a 80% de α-HCH, un 5 a 14% de β-HCH, un 8 a 15% de γ-HCH y de un 2 a 16% de δ-HCH (Figura 11b) [149]. Se calcula que por cada tonelada de lindano (al 100%) producida, se obtenían 8 t de subproductos no comerciales (el resto de isómeros) [150]. Las propiedades fisicoquímicas de los isómeros de HCH son diferentes, lo que permite que se compartimenten de distinta manera en el ambiente. El isómero α-HCH es más a fin a particionarse en el aire y es transportado largas distancias lo cual genera problemas ambientales en las regiones más frías y remotas del planeta; es carcinogénico tanto para el humano, como para animales y plantas. El isómero β-HCH domina en suelos, tejido animal y fluidos, además de ser más resistente a la hidrólisis y a la degradación en el medio ambiente [151]. El lindano es persistente, se bioaculuma fácilmente en la cadena trófica, se bioconcentra rápidamente y posee una elevada toxicidad (efectos en la reproducción, desarrollo y sistema inmunológico) [54]. El isómero δ-HCH es el más resistente a la degradación. Después de un largo período en el medio ambiente, los isómeros α- y γ-HCH son transformados en β-HCH. Una elevada concentración del isómero β-HCH sobre el α- y el γ-HCH es generalmente un indicativo de contaminación histórica [149; 151]. El lindano y los isómeros α- y β-HCH están listados dentro del Anexo A del Convenio de Estocolmo [54], por lo que se consideran sustancias prioritarias. Sin embargo, el lindado excepcionalmente se puede emplear como segundo tratamiento en el control de piojos y liendres de la cabeza.

El endosulfan ocurre en dos isómeros, el α- y β-endosulfan (Figura 11c). El endosulfan técnico es una mezcla en proporción 70:30 de los isómeros α- y β-endosulfan. Se emplea como acaricida e insecticida de amplio espectro en una amplia variedad de hortalizas, frutas, cereales y algodón, así como arbustos ornamentales, árboles, enredaderas y plantas ornamentales. Los mecanismos de acción del insecticida por contacto y por vía oral. El endosulfan tiene un potencial de bioacumulación relativamente alto en peces. La exposición al endosulfan genera riesgos crónicos y agudos para organismos terrestres y acuáticos. Se han reportado efectos en la reproducción y desarrollo de peces, y aves y mamíferos acuáticos. El endosulfan es un contaminante persistente y semivolátil, siendo el isómero β-endosulfan más persistente y el α-endosulfan más volátil. Su vida media en el agua se estima en 4 días, pero si las condiciones son anaeróbicas y/o con un pH bajo, la vida media podría alargarse. En el suelo, el isómero α-endosulfan desaparece más rápidamente que el β-endosulfan. Las vidas medias de los isómeros en suelos ácidos a neutros varía de 1 a 2 meses para el α- y de 3 a 9 meses para el β-endosulfan. Los principales productos de degradación son el sulfato de endosulfán (en el suelo) y el endosulfan diol (en el agua). Estos metabolitos pueden ser aún más tóxicos y persistentes que los compuestos parentales. El endosulfan ingresa al medio acuático principalmente por la disolución de los suelos contaminados, por escorrentía, por vaporización y/o adsorción en materia particulada y su posterior deposición. Este comportamiento permite que sea transportado por el agua, aire o sedimentos a regiones remotas del planeta [152; 153]. El endosulfan (mezcla técnica) se encuentra listado en el Anexo A del Convenio de Estocolmo [54], por lo que se considera un contaminante prioritario.

El hexaclorobenceno (HCB) es un fungicida empleado desde 1945 como tratamiento de semillas, y especialmente en el control de la caries del trigo. El HCB (Figura 11d) también es un subproducto de la manufactura de químicos industriales tales como el tetracloruro de carbono, percloroetileno, tricloroetano y el pentaclorobenceno. Así mismo se encuentra como impureza en muchas formulaciones de pesticidas incluyendo a la del pentaclorofenol y dicloram, entre otros. Fue empleado como disolvente y como intermediario y/o aditivo en la producción de PVC, fuegos pirotécnicos, municiones y tintas. Este compuesto es altamente insoluble en agua y semivolátil, por lo que puede ser transportado por medio de la atmósfera. Es muy resistente a la degradación y puede bioconcentrarse en el tejido adiposo. En el agua, puede adsorberse a la materia particulada y depositarse en los sedimentos. Se calcula que tiene una vida media de 2.7 a 6 años en el agua y atmósfera y más de 6 años en suelo/sedimentos. Se considera una sustancia con probable efecto carcinogénico y las principales rutas de exposición humana son a través de los peces, plantas y otros alimentos contaminados [54; 154]. Está incluido en el Anexo A del Convenio de Estocolmo, por lo que se considera un contaminante prioritario.

La aldrina, endrina, dieldrina y el heptacloro pertenecen al grupo de ciclodienos clorados (Figura 11e). La aldrina fue utilizada en la lucha contra los insectos del suelo, hormiga blanca, el gusano de la raíz del maíz, el gorgojo acuático del arroz y los saltamontes y se ha usado especialmente en grandes cantidades para cultivos de maíz y patata; también sirvió para proteger las estructuras de madera de las termitas. La dieldrina se utilizó en la agricultura en la lucha contra los insectos del suelo y contra varios insectos vectores de enfermedades, también se usó en la lucha contra las termitas y los barrenillos de la madera. La endrina fue usada desde los años cincuenta para el control de insectos en cultivos de algodón, arroz, caña de azúcar y maíz; también ha sido usado como rodenticida. El heptacloro es un insecticida no sistémico que actúa en el estómago y por contacto, utilizado fundamentalmente contra los insectos del suelo y las termitas. Se emplea en la lucha contra los insectos del algodón, los saltamontes y algunas plagas de cultivos, así como para combatir el paludismo y la malaria. Tanto la aldrina como la dieldrina fueron creados y comercializados a partir de 1948 y el heptacloro desde 1952 [155]. No son bioacumulables, sin embargo son altamente tóxicos para peces, aves y humanos. La aldrina es letal para los humanos en concentraciones alrededor de 83 mg kg^{-1} de peso corporal. La dieldrina además puede generar deformidades en embriones de ranas a niveles de unos cuantos µg g^{-1}. El heptacloro es considerado como carcinógeno. Estos compuestos tienen tiempos de vida elevados, por ejemplo la endrina puede persistir en el suelo/sedimentos hasta 12 años. Estos contaminantes son introducidos al medio acuático principalmente como el resto de pesticidas organoclorados, los suelos en donde fueron aplicados se disuelven y son arrastrados por escorrentía, por vaporización y/o adsorción en materia particulada y su posterior deposición [156; 157]. Estos 4 pesticidas están incluidos en el Anexo A del Convenio de Estocolmo.

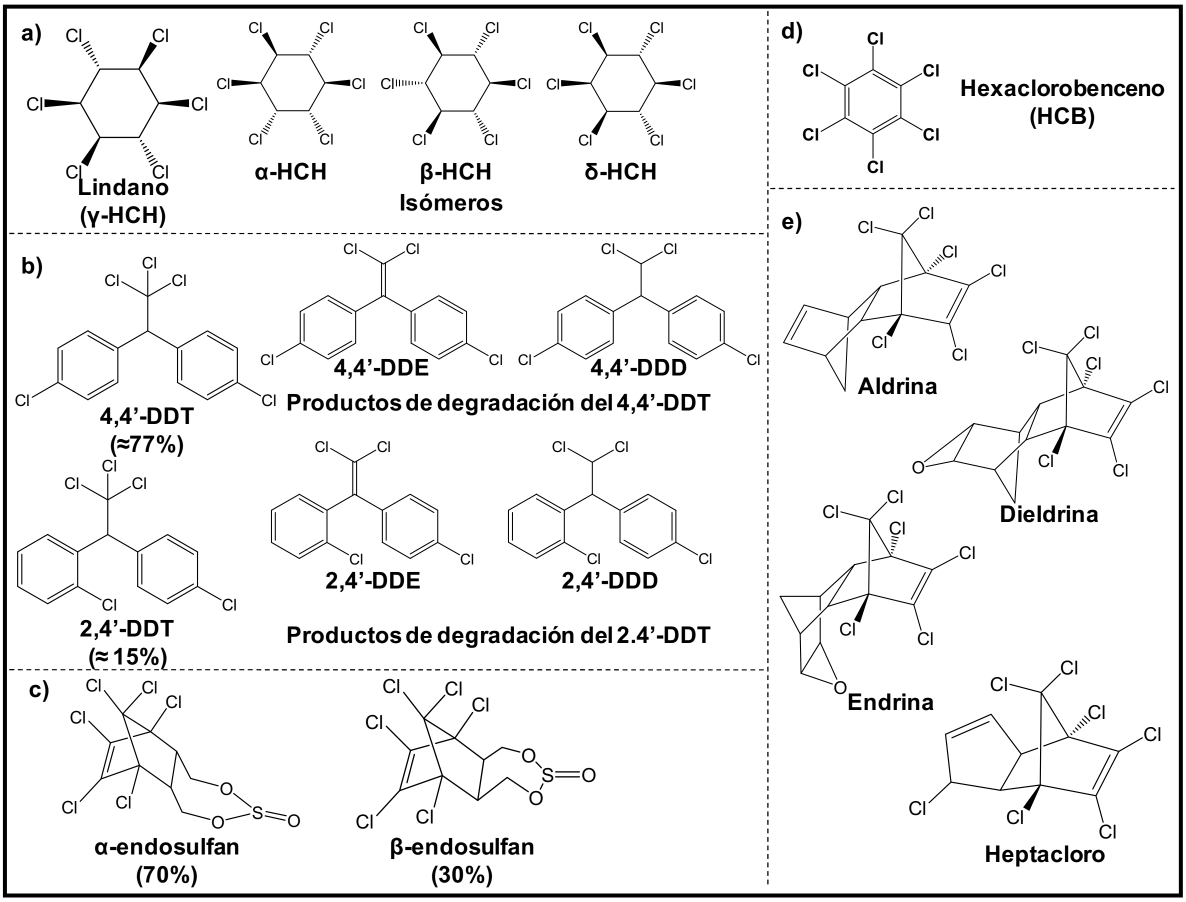


Figura 11. **Estructura química de los pesticidas estudiados y sus compuestos relacionados**

En la Tabla 4 se muestran algunas de las propiedades fisicoquímicas y toxicológicas de los OCPs.

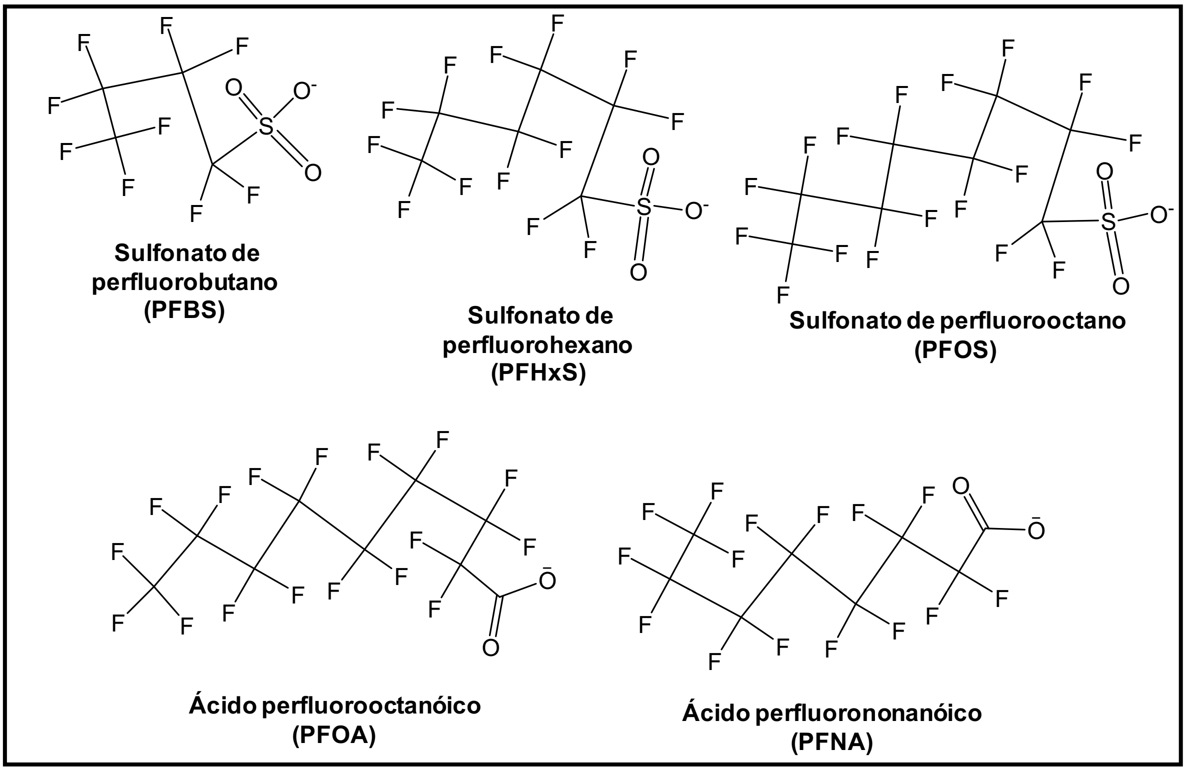
1.3.8 Compuestos perfluorados

Los compuestos perfluorados, o también llamados perfluoroquímicos o sustancias químicas polifluoradas ("perfluorochemicals" o PFCs), están compuestos por la cadena perfluorocarbonada con propiedades hidrofóbicas y oleofílicas, y en un extremo poseen un grupo funcional con carga (carboxilo o sulfonato), el cual es hidrofílico [158]. Esta combinación única de carácter hidrófobo y oleófobo permite que estas sustancias tengan uso como surfactantes. Son excelentes protectores de superficies gracias a su habilidad para repeler aceites, grasas y agua. Así mismo, reducen la tensión superficial en sistemas acuosos, lo que también hace que se empleen como agentes humectantes [159]. Algunas de las aplicaciones que se utilizan día a día son como protectores en papel, envases de cartón (para comida), tapices, pieles y textiles, en donde repelen grasas, humedad y suciedad. También son poco reactivos y ya que resisten también las altas temperaturas se emplean en la manufactura de fluoropolímeros para las cubiertas antiadherentes de sartenes, películas protectoras de ropa (para repeler agua y ayudar a que sean transpirables), en cintas aislantes para cables, en tubería resistente al fuego y agentes químicos y en las cintas para plomería. También se emplean en las espumas para extinguir incendios. En la industria se emplean en formulaciones en emulsificantes, humectantes y aditivos y cubiertas. El sulfonato de perfluorooctano (PFOS) ha sido el compuesto históricamente más utilizado a nivel mundial gracias a su excelente resistencia a los ácidos y a elevadas temperaturas [160].

Las mayores emisiones directas de PFCs al ambiente son a través de las descargas al aire y al agua durante los procesos industriales como la manufactura de perfluoroalquil carboxilatos y la manufactura y procesamiento de fluoropolímeros; también se pueden mencionar al uso de espumas contra incendios y a través de la lixiviación de productos de consumo e industriales que contienen PFCs. Entre las emisiones indirectas se encuentran las impurezas de PFCs en la fabricación de productos a base de perfluorooctilsulfonil fluoruro y la degradación de fluorotelómeros [160].

A partir de la inclusión del PFOS en el listado B del Convenio de Estocolmo (Conferencia de las Partes, mayo 2009), las grandes empresas productoras de este compuesto, tales como 3M y Dupont cesaron su producción. Este compuesto ha sido sustituido por otros PFCs con cadena más corta como el sulfonato de perfluorobutano (PFBS) [159; 160].

Las mismas propiedades de estabilidad que han hecho tan socorrido el uso de compuestos como el PFOS, producen un riesgo significativo para el medio ambiente, ya que pueden permanecer ahí indefinidamente. La elevada solubilidad de los PFCs hace que las masas de aguas sean el sumidero final de estas sustancias y que así mismo, las corrientes oceánicas sean excelentes medios de transporte [161-163]. Se ha demostrado que estas sustancias pueden ser transportadas fácilmente por el aire y por las corrientes oceánicas, siendo esta última, la principal vía de transporte hacia regiones remotas del planeta [159; 161]. Ya que estos compuestos existen principalmente en el agua como aniones, no se espera su volatilización en el aire. Sin embargo, debido a sus propiedades surfactantes, pueden formar micelas que son fácilmente transportables por el aire [164]. La presión de vapor del ácido perfluorooctanoico (PFOA) en forma neutra (0.017 mm Hg at 20 °C) indica que esta sustancia es altamente volátil, lo que permite también ser transportada por el aire [165]. Compuestos como el PFOS y PFOA han sido detectados en matrices ambientales (aguas superficiales y subterráneas, sedimentos y suelos) y biológicas (invertebrados, peces, anfibios, reptiles, aves, huevos de aves y mamíferos) así como en muestras de suero de la población general analizadas por toda la extensión del planeta. Otros como el sulfonato de perfluorohexano (PFHS) y el ácido perfluorononanoico (PFNA) también se encuentran globalmente distribuidos, pero en menor frecuencia. Las mayores concentraciones de PFCs en animales han sido detectadas en mamíferos predadores, como los osos polares, lo cual indica que estas sustancias son biomagnificadas a través de la cadena trófica [160; 166-169].



Sulfonato de perfluorobutano (PFBS)

Sulfonato de perfluorohexano (PFHxS)

Sulfonato de perfluorooctano (PFOS)

Ácido perfluorooctanóico (PFOA)

Ácido perfluorononanóico (PFNA)

Figura 12. Estructura química de los 5 compuestos perfluorados estudiados

En este trabajo se incluyen al compuesto prioritario PFOS y otros 4 PFCs emergentes (Figura 12). Algunas de sus propiedades fisicoquímicas y toxicológicas se muestran en la Tabla 4.

Tabla 4. Propiedades fisicoquímicas y toxicológicas de los compuestos estudiados

Compuesto	Acrónimo	No. CAS	Fórmula	Peso molecular	Log K_{ow}[a]	Solubilidad en agua 25 °C[a] (mg L^{-1})	Presión de vapor 25 °C[a] (mmHg)	Vida media agua[b] (dias)	Vida media suelo[b] (dias)	Vida media sedimentos[b] (dias)	Vida media aire[b] (dias)	Factor bioconcen.[b]	Valor crónico peces[b] (mg L^{-1})	Valor legislado NCA[c] (μg L^{-1})
Naftaleno	Nap	91-20-3	$C_{10}H_8$	128.2	3.30	3.1E+01	8.5E-02	38	75	340	0.8	70	1.0	2.4
Acenaftileno	Acy	208-96-8	$C_{12}H_8$	152.2	3.94	1.6E+01	6.7E-03	15	30	140	0.04	180	0.28	nl
Acenafteno	Ace	83-32-9	$C_{12}H_{10}$	154.2	3.92	3.9E+00	2.1E-03	38	75	340	0.2	180	0.18	nl
Fluoreno	Flu	86-73-7	$C_{13}H_{10}$	166.2	4.18	1.7E+00	6.0E-04	15	30	140	1.8	270	0.26	nl
Fenantreno	Phe	85-01-8	$C_{14}H_{10}$	178.2	4.46	1.2E+00	1.2E-04	60	120	540	1.2	1900	0.14	nl
Antraceno	Ant	120-12-7	$C_{14}H_{10}$	178.2	4.45	4.3E-02	6.5E-06	60	120	540	0.4	400	0.14*	0.4
Fluoranteno	Flr	206-44-0	$C_{16}H_{10}$	202.3	5.16	2.6E-01	9.2E-06	60	120	540	0.5	1200	0.05	1
Pireno	Pyr	129-00-0	$C_{16}H_{10}$	202.3	4.88	1.4E-01	4.5E-06	60	120	540	0.3	770	0.05	nl
Benzo(a)antraceno	B(a)A	56-55-3	$C_{18}H_{12}$	228.3	5.66	9.4E-03	2.1E-07	60	120	540	0.3	2900	0.019*	nl
Criseno	Chr	218-01-9	$C_{18}H_{12}$	228.3	5.81	2.0E-03	6.2E-09	60	120	540	0.3	3200	0.019*	nl
Benzo(b)fluoranteno	B(b)F	205-99-2	$C_{20}H_{12}$	252.3	5.78	1.5E-03	5.0E-07	60	120	540	0.9	3000	0.006*	Σ = 0.3
Benzo(k)fluoranteno	B(k)F	207-08-9	$C_{20}H_{12}$	252.3	6.11	8.0E-04	9.6E-10	60	120	540	0.3	5000	0.006*	
Benzo(a)pireno	B(a)P	50-32-8	$C_{20}H_{12}$	252.3	6.13	1.6E-03	5.5E-09	60	120	540	0.3	5100	0.006*	0.1
Indeno(1,2,3-c,d)pireno	I(cd)P	193-39-5	$C_{22}H_{12}$	276.3	6.70	1.9E-04	1.2E-10	60	120	540	0.3	12000	0.002*	Σ = 0.002
Benzo(g,h,i)perileno	B(ghi)P	191-24-2	$C_{22}H_{13}$	276.3	6.63	2.6E-04	9.5E-10	60	120	540	0.2	11000	0.002*	
Dibenzo(a,h)antraceno	D(ah)A	53-70-3	$C_{22}H_{14}$	278.4	6.75	2.5E-03	1.0E-10	60	120	540	0.3	6900	0.002*	nl
4-tert-octilfenol	OP	140-66-9	$C_{14}H_{22}O$	206.3	5.28	3.2E+01	4.8E-04	38	75	340	0.4	1400	0.006	nl
4-nonilfenol (mezcla de isoméros)	NP	84852-15-3	$C_{15}H_{24}O$	220.4	5.92	7.6E+00	9.4E-05	38	75	340	0.3	3700	0.006	2.0
4-nonilfenol monoetoxilado	$NPEO_1$	104-35-8	$C_{17}H_{28}O_2$	264.4	5.58	5.5E-01	1.8E-07	15	30	140	0.3	52	0.02	nl
4-nonilfenol dietoxilado	$NPEO_2$	20427-84-3	$C_{19}H_{32}O_3$	308.5	5.30	1.0E+00	9.1E-09	15	30	140	0.3	34	0.04	nl
Dimetil ftalalato	DMP	131-11-3	$C_{10}H_{10}O_4$	194.2	1.60	4.1E+02	3.1E-03	15	30	140	28	5	4.0	nl
Dietil ftalato	DEP	84-66-2	$C_{12}H_{14}O_4$	222.2	2.42	2.8E+02	2.1E-03	15	30	140	4.6	18	0.99	nl
Di (2-etilhexil) ftalato	DEHP	117-81-7	$C_{24}H_{38}O_4$	390.6	7.60	1.9E-02	1.4E-07	15	30	140	0.8	1700	n.e.	1.3
Benzilbutil ftalato	BBP	85-68-7	$C_{19}H_{20}O_4$	312.4	4.73	1.1E+00	8.2E-06	15	30	140	1.5	610	0.05	nl
Di-n-butil ftalato	DBP	84-74-2	$C_{16}H_{22}O_4$	278.3	4.50	2.7E+00	2.0E-05	9	17	78	1.8	430	0.06	nl
Di (2-etilhexil) adipato	DEHA	103-23-1	$C_{22}H_{42}O_4$	370.6	6.11	1.1E-01	8.5E-07	9	17	78	0.6	960	n.e.	nl
Bisfenol A	BPA	80-05-7	$C_{15}H_{16}O_2$	228.3	3.32	1.2E+02	2.9E-12	38	75	340	0.2	72	0.55	nl

Cont…Tabla 4.

Compuesto	Acrónimo	No. CAS	Fórmula	Peso molecular	Log K_{ow} ^{a}	Solubilidad en agua 25 °C ^{a} (mg L^{-1})	Presión de vapor 25 °C ^{a} (mmHg)	Vida media agua ^{b} (dias)	Vida media suelo ^{b} (dias)	Vida media sedimentos ^{b} (dias)	Vida media aire ^{b} (dias)	Factor bioconcen. ^{b}	Valor crónico peces ^{b} (mg L^{-1})	Valor legislado NCA ^{c} (µg L^{-1})
2,4,4’-Triclorobifenil	PCB 28	7012-37-5	$C_{12}H_7Cl_3$	257.5	5.62	2.7E-01	1.9E-04	60	120	540	13	9100	0.02	nl **
2,2’5,5'-Tetraclorobifenil	PCB 52	35693-99-3	$C_{12}H_6Cl_4$	292.0	6.09	1.5E-02	8.4E-06	180	360	1600	22	19000	0.01	nl **
2,2’4,5,5’-Pentaclorobifenil	PCB 101	37680-73-2	$C_{12}H_5Cl_5$	326.4	6.80	1.5E-02	2.5E-05	180	360	1600	50	55000	0.0015	nl **
2,3’4,4’,5-Pentaclorobifenil	PCB 118	31508-00-6	$C_{12}H_5Cl_5$	326.4	7.12	1.3E-02	9.0E-06	180	360	1600	50	45000	0.0015	nl **
2,2’,3,4,4’,5 Hexaclorobifenil	PCB 138	35065-28-2	$C_{12}H_4Cl_6$	360.9	7.44	1.5E-03	3.8E-06	180	360	1600	96	31000	0.0005	nl **
2,2’4,4’5,5’ Hexaclorobifenil	PCB 153	35065-27-1	$C_{12}H_4Cl_6$	360.9	7.75	9.5E-04	3.4E-06	180	360	1600	98	22000	0.0005	nl **
2,2'3,4,4',5,5' Heptaclorobifenil	PCB 180	35065-29-3	$C_{12}H_3Cl_7$	395.3	8.27	3.9E-03	9.8E-07	180	360	1600	150	12000	n.e.	nl **
2,4,4’-TriBDE	BDE 28	41318-75-6	$C_{12}H_7Br_3O$	406.9	5.88	2.6E-02	6.7E-07	60	120	540	11	3500	0.02	nl
2,2’,4,4’-TetraBDE	BDE 47	5436-43-1	$C_{12}H_6Br_4O$	485.8	6.77	1.5E-03	7.0E-08	180	360	1600	16	14000	0.003*	nl **
2,2’,4,4’,5-PentaBDE	BDE 99	60348-60-9	$C_{12}H_5Br_5O$	564.7	7.66	7.9E-05	3.1E-08	180	360	1600	16	14000	0.003*	nl **
2,2’,4,4’,6-PentaBDE	BDE 100	189084-64-8	$C_{12}H_5Br_5O$	564.7	7.66	1.1E-02	2.4E-08	180	360	1600	29	15000	0.00069*	nl **
2,2’,4,4’,5,5’-HexaBDE	BDE 153	68631-49-2	$C_{12}H_4Br_6O$	643.5	8.55	1.2E-04	1.6E-08	180	360	1600	71	2300	n.e.	nl **
2,2’,4,4’,5,6’-HexaBDE	BDE 154	207122-15-4	$C_{12}H_4Br_6O$	643.6	8.55	2.1E-03	2.8E-08	180	360	1600	42	2300	n.e.	nl **
2,2’,3,4,4’,5’,6-HeptaBDE	BDE 183	207122-16-5	$C_{12}H_3Br_7O$	722.5	9.44	3.9E-04	3.5E-09	360	360	1600	96	850	n.e.	nl **
2,2’,3,4,4’,5’,6,6'-Deca BDE	BDE 209	1163-19-5	$C_{12}Br_{10}O$	959.2	12.11	3.7E-04	4.7E-12	180	360	1600	460	42	n.e.	nl
α-Hexaclorociclohexano	α-HCH	319-84-6	$C_6H_6Cl_6$	290.8	3.80	2.0E+00	4.5E-05	180	360	1600	28	250	0.28	Σ = 0.04
β-Hexaclorociclohexano	β-HCH	319-85-7	$C_6H_6Cl_6$	290.8	3.78	2.4E-01	3.6E-07	180	360	1600	28	250	0.28	
δ-Hexaclorociclohexano	δ-HCH	319-86-8	$C_6H_6Cl_6$	290.8	4.14	1.0E+01	3.5E-05	180	360	1600	28	250	0.28	
γ-Hexaclorociclohexano	Lindano	58-89-9	$C_6H_6Cl_6$	290.8	3.72	7.3E+00	4.2E-05	180	360	1600	28	250	0.28	
α-endosulfan	α-endsf	959-98-8	$C_9H_6Cl_6O_3$	406.9	3.83	1.5E+00	6.0E-07	180	360	1600	2.0	160	0.8*	Σ =0.01
β-endosulfan	β-endsf	33213-65-9	$C_9H_6Cl_6O_3$	406.9	3.83	1.5E+00	1.3E-06	180	360	1600	2.0	160	0.8*	
2,4'-DDE	2,4’-DDE	3424-82-6	$C_{14}H_8Cl_4$	318.0	6.00	1.4E-01	6.2E-06	180	360	1600	1.1	4200	0.002	nl
2,4'-DDD	2,4’-DDD	53-19-0	$C_{14}H_{10}Cl_4$	320.0	5.87	1.0E-01	1.7E-05	180	360	1600	3.7	3500	0.013	nl
4,4'-DDE	4,4'-DDE	72-55-9	$C_{14}H_8Cl_4$	318.0	6.51	4.0E-02	6.0E-06	180	360	1600	1.1	9200	0.0017	Σ = 0.025
4,4'-DDD	4,4'-DDD	72-54-8	$C_{14}H_{10}Cl_4$	320.0	6.02	9.0E-02	1.7E-05	180	360	1600	3.7	4400	0.013	
2,4'-DDT	2,4'-DDT	789-02-6	$C_{14}H_9Cl_5$	354.5	6.79	8.5E-02	1.4E-06	180	360	1600	4.6	14000	0.002	
4,4'-DDT	4,4'-DDT	50-29-3	$C_{14}H_9Cl_5$	354.5	6.91	5.5E-03	7.5E-06	180	360	1600	4.6	17000	0.002	

Cont…Tabla 4

Compuesto	Acrónimo	No. CAS	Fórmula	Peso molecular	Log K_{ow} [a]	Solubilidad en agua 25 °C [a] (mg L^{-1})	Presión de vapor 25 °C [a] (mmHg)	Vida media agua [b] (dias)	Vida media suelo [b] (dias)	Vida media sedimentos [b] (dias)	Vida media aire [b] (dias)	Factor bioconcen. [b]	Valor crónico peces [b] (mg L^{-1})	Valor legislado NCA [c] (µg L^{-1})
Hexaclorobenceno	HCB	118-74-1	C_6Cl_6	284.8	5.73	6.2E-03	1.8E-05	180	360	1600	960	2800	0.012*	0.05
Aldrina	-	309-00-2	$C_{12}H_8Cl_6$	364.9	6.50	1.7E-02	1.2E-04	180	360	1600	0.046	9000	0.00033	Σ = 0.01
Dieldrina	-	60-57-1	$C_{12}H_8Cl_6O$	380.9	5.40	2.0E-01	5.9E-06	180	360	1600	1.8	1300	0.0017	
Endrina	-	72-20-8	$C_{12}H_8Cl_6O$	380.9	5.20	2.5E-01	3.0E-06	180	360	1600	1.8	1300	0.0017	
Heptacloro	HpCl	76-44-8	$C_{10}H_5Cl_7$	373.3	6.10	1.8E-01	4.0E-04	180	360	1600	0.046	4900	0.003	nl **
Sulfonato de perfluorobutano	PFBS	375-73-5	$C_4HF_9O_3S$	300.1	1.82	3.4E+02	5.2E-02	180	360	1600	120	3.2	110	nl
Sulfonato de perfluorohexano	PFHxS	355-46-4	$C_6HF_{13}O_3S$	400.1	3.16	6.2E+00	4.6E-03	180	360	1600	120	3.2	3.3*	nl
Sulfonato de perfluorooctano	PFOS	1763-23-1	$C_8HF_{17}O_3S$	500.1	4.49	1.0E-01	6.4E-03	180	360	1600	120	56	0.092*	nl
Ácido perfluorooctanóico	PFOA	335-67-1	$C_8HF_{15}O_2$	414.1	4.81	4.8E-01	1.5E-01	180	360	1600	120	31	0.073*	nl
Ácido perfluorononanóico	PFNA	375-95-1	$C_9HF_{17}O_2$	464.1	5.48	6.3E-02	8.3E-02	180	360	1600	31.0	56	0.012*	nl

[a] Fuente: Estimation Program Interface (EPI) Suite

[b] Estimado de http://www.pbtprofiler.net/Results.asp

[c] Según la Directiva 2008/105/EC, Anexo I, otras aguas superficiales

n.e.: No estimado

nl: No legislado según la Directiva 2008/105/EC

* Como el valor crónico es menor que la solubilidad, se estima que no habrá efecto crónico en la saturación

** Aunque no existe un valor de NCA para este compuetso, se encuentran dentro del listado del Convenio de Estocolmo

1.4 Métodos analíticos empleados para determinar los micro-contaminantes orgánicos en muestras del ambiente marino

Un microcontaminante orgánico es aquel que se encuentra presente en una matriz en concentraciones que van desde nanogramos (ng) hasta unos cuantos miligramos (mg) por cada litro o gramo, dependiendo si la matriz es líquida o sólida. Tal es el caso de los contaminantes orgánicos en las matrices del medio marino [58; 61]. Además, las diferentes matrices (ya sean aguas de mar, río o residuales, sedimentos o biota) están compuestas por una mezcla muy compleja de sustancias que pueden interferir en la identificación y cuantificación de los compuestos diana. Entre estas interferencias se pueden mencionar principalmente a la materia orgánica, lípidos, sales, metales, etc. Las metodologías a emplear deben tomar en cuenta desde la toma y conservación de muestras, su preparación (técnicas de extracción o preconcentración), separación analítica, detección, hasta el procesamiento de resultados. En este trabajo nos centraremos en el análisis de tres matrices ambientales, aguas (de mar y río y descargas de efluentes), sedimentos y biota (mejillones). En la Tabla 5 se enumeran las técnicas de preparación y la técnica de separación/análisis utilizadas en algunos estudios reportados en la literatura, así como los límites de detección del método y los porcentajes de recuperación alcanzados

1.4.1 Toma y conservación de las muestras

El muestreo es considerado una de las etapas más críticas en las determinaciones de contaminantes orgánicos en matrices ambientales. Es necesario reducir la incertidumbre derivada del muestreo, especialmente en matrices que no son homogéneas donde la distribución de los contaminantes varía, como por ejemplo, las aguas de mar o de río o las descargas de efluentes. Para reducir la incertidumbre y dependiendo del objetivo del estudio, se pueden realizar la toma de varias muestras de aguas superficiales (mínimo 2) en una misma área. En el caso de aguas residuales se prefiere una muestra compuesta a partir de varias muestras simples tomadas durante 24 h o una semana completa. También es aconsejable el emplear rutinariamente un blanco de campo, que consiste en llevar al sitio de muestreo agua desionizada y tratarla analíticamente como cualquier muestra [170]. Los blancos de sedimentos pueden recolectarse en sitios alejados de las fuentes de contaminación [171]. Durante el muestreo, también es indispensable evitar la contaminación de las muestras con otros compuestos presentes en el medio circundante, ajenos al estudio. Algunas recomendaciones prácticas son:

1. Para almacenar las muestras utilizar de preferencia, envases de vidrio de borosilicato color ámbar [171].
2. Lavar el material que contendrá la muestra con detergente Extran (Merck) y enjuagarlo repetidamente con agua desionizada, y finalmente con acetona [171]. Envolver el material con papel aluminio. Muflar el material a 450 ºC (cuando sea posible) durante 4 horas para eliminar cualquier rastro de contaminación por compuestos orgánicos que haya quedado retenido en el envase.
3. Tapar perfectamente los envases durante su transporte al sitio de muestreo, para evitar re-contaminación. Los tapones con aislante de politetrafluoroetileno (PTFE, comercialmente conocido como Teflón) son preferidos sobre los fabricados de plástico, principalmente porque los plásticos contienen PEs y BPA que pueden migrar a la muestra; además el PTFE posee una muy baja reactividad. Sin embargo, si se analizarán compuestos perfluorados, deberán de ser evitados todos los materiales que contenga PTFE o cualquier fluoropolímero, durante todo el procesamiento [172].
4. Para el muestreo de aguas de mar o río se pueden emplear botellas Niskin o similares que permita recolectar agua a una profundidad determinada. Para el muestreo de sedimentos se prefiere el empleo de dragas fabricados de acero inoxidable, por su baja reactividad, durabilidad y resistencia [171]. Es recomendable que en caso de utilizar vehículos como lanchas y barcos a motor para transportarse al sitio de muestreo, el motor se apague durante la toma de muestra para evitar la contaminación con PAHs y otras sustancias provenientes de la combustión de combustibles.
5. Una vez recolectadas las muestras, deben cerrarse los envases perfectamente, refrigerarse durante el transporte y evitar la luz directa para evitar degradación por fotólisis y/o acción microbiana, así como prevenir la volatilización de los compuestos de interés. Generalmente se recomienda acidificar (con ácido ascórbico, clorhídrico, o fosfórico) las muestras para inhibir el crecimiento microbiano y almacenarlas a 4 ºC, lo que permite mantenerlas por un máximo de 15 días. Sin embargo, compuestos como los analizados en este trabajo (PAHs y otros) requieren que los análisis se realicen 24 h después del muestreo (sin la adición de ácido) o 72 h (añadiendo ácido) [171].

1.4.2 Preparación: aislamiento y preconcentración

La etapa de preparación de la muestra es una de las que requiere más tiempo, cuidados y esfuerzos de todas las tareas analíticas. Las muestras ambientales requieren un tratamiento previo antes de realizar el análisis, en especial si la técnica que se empleará es cromatografía de gases. Este pretratamiento permite aislar y preconcentrar los contaminantes de la matriz ambiental primaria y eliminar las interferencias contenidas en ella. Se obtendrá con ello, una extracto selectivamente enriquecido con los contaminantes a estudiar con el fin de alcanzar los límites de detección instrumentales [173]. La necesidad de contar con técnicas de preparación de muestras lo más simples, rápidas, fiables, robustas y de ser posible, generando la mínima cantidad de residuos, ha llevado a desarrollar un gran número y variedad de técnicas.

La selección del método de aislamiento y preconcentración de microcontaminantes orgánicos adecuado dependerá de las propiedades fisicoquímicas de los analitos como por ejemplo, su volatilidad, polaridad, estabilidad y solubilidad. [174]. Además se debe de considerar también el factor económico. La gran mayoría de los contaminantes estudiados se consideran semivolátiles ya que sus presiones de vapor se encuentran entre 0.1 y 10^{-7} mm Hg (Tabla 4) y las polaridades varían en un intervalo muy amplio (log K_{ow} entre 1.6 a 12.1). En la literatura científica se pueden encontrar una gran variedad de metodologías analíticas (Tabla 5). Para este tipo de compuestos los métodos de aislamiento-preconcentración más utilizados en matrices acuosas son la extracción líquido-líquido (LLE), extracción en fase sólida (SPE), microextracción en fase sólida (MPE), extracción por adsorción en barras giratorias (SBSE), extracción por medio de sistemas micelares (CPE), entre otras. Para la extracción de muestras sólidas se encuentran la extracción Soxhlet, extracción asistida por ultrasonido (UAE), extracción asistida por microondas (MAE), extracción con líquidos presurizados (PLE), extracción acelerada con solventes (ASE), extracción por dispersión en matriz de fase sólida (MSPE), entre otras [170; 175; 176]. A continuación se mencionan algunas características de los métodos que han sido empleados en este trabajo de tesis:

- Extracción en fase sólida (“solid-phase extraction” o SPE). Esta técnica es el reemplazo de la clásica LLE. Se emplea para extraer contaminantes orgánicos semivolátiles o no volátiles de matrices acuosas. Consiste en adsorber los analitos en un sustrato específico (fase sólida), haciendo pasar la muestra por una micro-columna o cartuchos. Mediante el lavado del adsorbente previo a la elución de los analitos es posible descartar las sustancias provenientes de la matriz que causen interferencias y lograr una concentración de los analitos de 100 a 5000 veces. Gracias a esto, el método pude ser empleado para el aislamiento de trazas. Comercialmente existen cartuchos que contienen adsorbentes de diferentes tipos y tamaño de partícula, que permiten utilizar baja presión (vacío) para forzar la entrada de la muestra a través del adsorbente. El cartucho típico para extracción en fase sólida consiste en una columna corta (generalmente dispuestos en una jeringa abierta) que contiene un adsorbente con un tamaño de partícula nominal entre 50 a 60 µm, contenido entre un metal poroso o una membrana plástica. Los cartuchos son desechables. Se ha logrado diseñar los cartuchos para que se puedan extraer grandes volúmenes de muestras y que tengan tolerancia a la obstrucción por partículas y otros sólidos o agregados provenientes de la matriz. El proceso que se sigue en una extracción en fase sólida, empleando cualquier cartucho comercial es el siguiente: a) acondicionar el adsorbente, preparando el cartucho para la posterior interacción con la muestra; b) pasar la muestra para adsorber los analitos en el adsorbente; c) lavar el cartucho, para remover las impurezas de la matriz y d) eluir, para extraer los analitos con un disolvente selectivo. Como con ésta técnica es posible emplear disolventes apolares o polares, los extractos pueden ser inyectados en cromatografía de gases o de líquidos, según sea la naturaleza química de los contaminantes [170; 173].

- Extracción por adsorción con barras giratorias (“stir-bar sorptive extraction” o SBSE). El método se basa en la extracción por adsorción de los analitos en una cubierta polimérica adherida a una barra magnética giratoria. La extracción de los solutos de la fase acuosa en la cubierta de PDMS está controlada por el coeficiente de partición PDMS/W (aproximado al coeficiente de partición octalnol/agua, $\log K_{ow}$) de una masa de analito presente en un volumen conocido de muestra, de acuerdo a la ecuación: $\mathrm{K_{ow}} \gg \mathrm{K_{PDMS/W}} = \frac{\mathrm{C_{PDMS}}}{\mathrm{C_W}} = \frac{\mathrm{m_{PDMS}}}{\mathrm{m_W}} * \frac{\mathrm{V_W}}{\mathrm{V_{PDMS}}} = \frac{\mathrm{m_{PDMS}}}{\mathrm{m_W}}\beta$, donde $K_{PDMS/W}$ es el coeficiente de distribución entre el PDMS y el agua; C_{PDMS} y C_W son las concentraciones del soluto en la fase PDMS y el agua, respectivamente; m_{PDMS} y m_W son las masas del soluto en la fase PDMS y acuosa, respectivamente; y β es la relación de fases ($\beta = V_W / V_{PDMS}$) que representa el volumen de la cubierta de PDMS del “Twister” y el volumen de agua, respectivamente [44]. Una vez concentrados los contaminantes en la superficie polimérica, se procede a su desorción térmica en el cromatógrafo de gases. El método es ideal para compuestos apolares en muestras acuosas ya que su coeficiente de partición es mayor en la cubierta polimérica. Para contaminantes polares, será necesario añadir cloruro de sodio o metanol. El método debe optimizarse para obtener la mejor eficiencia de extracción. Los parámetros a optimizar son el tiempo de extracción, el volumen de muestra, la cantidad y tipo de la sustancia modificadora, el tiempo y temperatura de desorción y la temperatura del crio-enfoque en la cabeza de la columna cromatográfica. Al igual que la SPME, esta técnica no utiliza disolventes y se considera una técnica miniaturizada es decir, requiere un volumen muy bajo de muestra, lo que representa ventajas durante la toma, almacenamiento y procesamiento de muestras. Adicionalmente, este método presenta ventajas sobre la SPME, ya que la superficie de adsorbente empleado es mucho mayor y en consecuencia la sensibilidad del método es extremadamente superior. A diferencia de los cartuchos para SPE, las barras giratorias son reutilizables (tiempo de vida entre 20 y 50 análisis). También se pueden emplear alternativamente una desorción líquida, es decir empleando disolventes para extraer los analitos de la cubierta polimérica. Típicamente, la barra giratoria con el adsorbente se coloca en un vial con un disolvente apolar (hexano) y su posterior inyección en un cromatógrafo de gases o un disolvente polar (acetonitrilo o metanol) para inyección en cromatografía de líquidos. Sin embargo, los porcentajes de recuperación utilizando desorción líquida son muy inferiores a los obtenidos empleando desorción térmica [177; 178].

- Extracción asistida por ultrasonidos o sonicación (“ultrasonic assisted extraction” o UAE): Esta técnica emplea se emplea para extraer contaminantes orgánicos de matrices sólidas. Es rápida (15-60 min) y permite procesar cantidades pequeñas o grandes de muestra (1-30 g). La muestra es sumergida en un disolvente y empleando un baño de ultrasonidos, se generan burbujas microscópicas de vapor que posteriormente implosionan, produciendo fuertes ondas de choque en la muestra sólida. Gracias a estas ondas, el disolvente penetra en la muestra, extrayendo los analitos. Es una técnica muy económica. Es necesario filtrar el disolvente después de la extracción y en ocasiones realizar una limpieza o “clean-up” con generalmente cartuchos con un adsorbente de sílica o una mezcla de sílica gel-magnesio (comercialmente, Florisil) para eliminar las sustancias que pueden causar interferencias en el análisis, como por ejemplo las grasas y lípidos, que fueron extraídas junto con los analitos. [170; 175].

1.4.3 Separación analítica y detección

La determinación analítica requiere el uso de técnicas que proporcionen sensibilidad, selectividad, precisión y exactitud. Para cumplir con la legislación Europea [9] es necesario que el método permita detectar concentraciones iguales o por debajo de un 30% de los niveles especificados en los NCA. En caso de analizar contaminantes que no tengan un NCA, el límite de detección deberá ser lo más bajo posible, sin que esto requiera un costo excesivo [69]. En nuestro caso en particular, la meta fue alcanzar unos límites de detección lo más bajo posibles en comparación con los niveles estimados o publicados de toxicidad o legislados [179] para que de esta manera el monitoreo ambiental permita proteger a las especies más sensibles del ecosistema marino.

La cromatografía de gases (“gas chromatography” o GC) y la cromatografía de líquidos de alta resolución (“high permormance liquid chromatography” o HPLC) pueden ser acopladas con diferentes tipos de detectores para realizar la identificación y cuantificación de los compuestos de interés. La GC es una técnica de separación analítica con alta selectividad y resolución, buena precisión y exactitud, con intervalo dinámico de concentraciones y sensibilidad. Esta técnica está diseñada para el análisis de sustancias volátiles o semivolátiles y ha sido empleada satisfactoriamente para el análisis de PAHs, pesticidas, PCBs y PBDEs [180], PEs, APs y BPA [97; 181].

El acople de la GC con diferentes tipos de detectores, permite una adecuada identificación y cuantificación de los compuestos de interés. La elección de un de detector dependerá del tipo de sustancias a analizar. Detectores como el de ionización de flama (“flame-ionisation detector” o FID), de captura de electrones (“electroncapture detector” o ECD) y de foto-ionización (“photoionisation detector” o PIDs) han cedido el paso al de espectrometría de masas (“mass spectrometry” o MS) ya que ha demostrado una mayor selectividad, sensibilidad y versatilidad [182].

La técnica de ionización más empleada en GC-MS es la ionización electrónica (“electron ionization” o EI) a una energía de 70 eV. Los iones generados se detectan en el analizador, que puede ser de distintos tipos. Los cuadrupolos tienen la factibilidad de barrer (modo “scan”) un intervalo de masas (m/z) o de filtrar selectivamente una masa concreta (modo SIM o “selected ion monitoring”). Esto último aumenta la sensibilidad del método, pues discrimina interferencias que puedan afectar el análisis [180]. Existen otros tipos de ionización que por ser selectivos para determinados tipos de sustancias y no son útiles para el propósito de este trabajo. Entre ellos se puede mencionar a la ionización química (“chemical ionization” o CI) en modo positivo o negativo (“negative chemical ionization” o NCI). Esta última generalmente es empleada para compuestos halogenados.

Para conseguir una mayor sensibilidad y reducir las interferencias, se ha desarrollado un sistema de triple cuádruplo o MS en tándem (MS/MS). Consiste en dos analizadores cuadrupolares (Q1 y Q3) y una celda de colisión (Q2). Cada cuádruplo puede ser operado en modo SCAN o SIM. Las moléculas son fragmentadas ya sea por CI o EI y son filtradas selectivamente (SIM) en el primer cuadrupolo. En la celda de colisión los iones precursores son fragmentados gracias a un potencial eléctrico para generar nuevos fragmentos (ion producto). En el segundo cuadrupolo los iones producto son nuevamente seleccionados. La fragmentación de ion precursor a un ion producto se denomina transición [183].

De todos los contaminantes orgánicos considerados en este trabajo, los únicos que no es posible analizar por GC, son los PFCs ya que son poco volátiles y muy polares. Para analizar sustancias polares no volátiles o sustancias termolábiles es la cromatografía de líquidos de alta resolución (“high liquid cromatography” o HPLC) acoplada a MS/MS y ionización por electro-spray (“electrospray ionization” o ESI) [172; 176].

Tabla 5. Metodologías analíticas reportadas en la literatura, empleadas para el análisis de las familias de contaminantes prioritarios y emergentes considerados en este trabajo, en diferentes matrices del medio marino

Familia	Matriz	Técnica de preparación	Técnica de separación/análisis	Límite de detección del método *	Recuperación (RSD) en %	Fuente
PAHs	Agua de mar	SPE	HPLC-PDA	1.8-16 ng L^{-1}	86-102 (1-13)	[184]
		SBSE	GC-EI-MS	0.02-2.5 ng L^{-1}	89-112 (3-14)	[185]
	Agua de río	SPE	GC-EI-MS/MS	1.1-7.8 ng L^{-1}	67-92 (6-22)	[186]
		SPE	HPLC-UVD	5-58 ng L^{-1}	79-118 (2-5)	[187]
		SPE	HPLC-FLD	0.02-5 ng L^{-1}	86-94 (3-13)	[188]
	Efluente	SBSE	GC-EI-MS/MS	2-10 ng L^{-1}	62-108 (4-10)	[189]
		MAE-HS-SPME	GC-FID	30-1000 ng L^{-1}	89-103 (6-17)	[190]
		SPE	GC-EI-MS	0.4-263 ng L^{-1}	82-117 (3-27)	[191]
	Sedimentos	UAE	GC-EI-MS	0.1-2.0 ng g^{-1} dw	20-114 (2-16)	[192]
		UAE	GC-EI-MS	1.0-3.0 ng g^{-1} dw	70-119 (1-14)	[193]
		MAE	GC-EI-MS	0.5-11 ng g^{-1} dw	62-100 (0-35)	[194]
	Mejillones	PLE	GC-EI-MS	0.5-8.0 ng g^{-1} ww	66-121 (3-30)	[192]
		UAE	GC-EI-MS	6.0 -51 ng g^{-1} ww	55 -129 (4-30)	[195]
		MAE	GC-EI-MS	0.5-11ng g^{-1} ww	88-175 (0-16)	[196]
APs	Agua de mar	DLLME	HPLC-UV	86-119 ng L^{-1}	800-1600	[197]
		SBSE	GC-EI-MS	2.5-3.8 ng L^{-1}	100 - 117	[198]
		SPE	HPLC-ESI-MS/MS	0.12-0.14 ng L^{-1}	52-91	[199]
	Agua de río	SBSE	GC-EI-MS	0.2-10 ng L^{-1}	93-99 (5-15)	[200]
		SPE	HPLC-ESI-MS/MS	0.03 -0.2 ng L^{-1}	81-110	[201]
	Efluente	SPE	HPLC-ESI-MS/MS	90 - 440 ng L^{-1}	77-102 (7-13)	[202]
		SPE	GC-EI-MS	18-833 ng L^{-1}	61-107 (3-11)	[191]
	Sedimentos	ASE	HPLC-ESI-MS/MS	0.1-1.4 ng g^{-1} dw	67-110	[201]
		MAE	GC-EI-MS	100 ng g^{-1} dw	100 (7-30) NP	[194]
		SPE	HPLC-FD	30-60 ng g^{-1} dw	79-107 (5-20)	[203]
	Mejillones	MSPE	HPLC-FLD	10-30 ng g^{-1} ww	101-108 (1-10)	[204]
		UAE	HPLC-ESI-MS	5.0 ng g^{-1} ww	63-66 (11-14) NP	[205]
PEs	Agua de mar	SBSE	GC-EI-MS	0.3 - 19 ng L^{-1}	95 - 124	[198]
		UAE-ME	GC-EI-MS	28-133 ng L^{-1}	78-83 (2-11)	[206]
	Agua de río	SPE	GC-EI-MS	2-8 ng L^{-1}	72-104 (1-8)	[207]
	Efluentes	SPE	HPLC-MS/MS	19-79 ng L^{-1}	96-103 (3-5)	[208]
		SPE	GC-EI-MS	9.0-21 ng L^{-1}	89-108 (8-26)	[191]
		LLE	HPLC-FD	20 ng L^{-1}	87	[95]
	Sedimentos	Soxhlet	GC-EI-MS	0.09-0.6 ng g^{-1} dw	89-96	[209]
		MAE	GC-EI-MS	1-22 ng g^{-1} dw	95-100 (2-44)	[194]
		ASE	GC-EI-MS	6-11 ng g^{-1} dw	77-102 (5-12)	[109]
	Mejillones	SLE	GC-EI-MS	10-20 ng g^{-1} ww	70-90	[210]
BPA	Agua de mar	DLLME	HPLC-UV	700 ng L^{-1}	92.9-118 (3.1-11)	[197]
		SPE	LC-MS/MS	0.04 ng L^{-1}	80	[199]
	Agua de río	SPE	GC-EI-MS	10 ng L^{-1}	94.3 (6.3)	[211]
		SPE	HPLC-ESI-MS	2.0 ng L^{-1}	93 (<21)	[212]
	Efluentes	SPE	GC-EI-MS	150 ng L^{-1}	96-99 (4-4.2)	[181]
		SPE	HPLC-ESI-MS	20 ng L^{-1}	40 (<21)	[212]
		SPE	GC-EI-MS	54 ng L^{-1}	107 (26)	[191]

Cont…Tabla 5

Familia	Matriz	Técnica de preparación	Técnica de separación/análisis	Límite de detección del método *	Recuperación (RSD) en %	Fuente
PCBs	Agua de mar	SBSE	GC-EI-MS	0.4-10 ng L^{-1}	nm	[198]
		SBSE	GC-EI-MS	0.02-0.95 ng L^{-1}	89-118 (10-21)	[185]
	Agua de río	HS-SPME	GC-ITMS-MS	1.5-9.1 ng L^{-1}	nm	[213]
		SBSE	GC-EI-MS	0.05-0.1 ng L^{-1}	93-97	[214]
	Efluente	SLE	GC-ECD	0.3-1 ng L^{-1}	79-232 (6.3-24)	[215]
		SPE	GC-EI-MS	15-68 ng L^{-1}	88-124 (1-12)	[191]
		LLE	GC-ECD	1 ng L^{-1}	97-103 (10-11)	[95]
	Sedimentos	MAE	GC-EI-MS	0.4-1.0 ng g^{-1} dw	97-99 (1-17)	[194]
		MAE	GC-EI-MS	0.3-0.6 ng g^{-1} dw	86-109 (1-12)	[216]
		MAE	GC-ECD	0.008-0.016 ng g^{-1} dw	93-96	[217]
	Mejillones	UAE	GC-ECD	0.8-1.7 ng g^{-1} ww	57-142 (16-25)	[195]
		PLE	GC-EI-MS/MS	0.002-0.07 ng g^{-1}	90-106	[218]
PBDEs	Agua de mar	SBSE	GC-EI-MS	0.2-1. 0 ng L^{-1}	82 - 106	[219]
		SBSE	GC-EI-MS	0.1-2.9 ng L^{-1}	92-100 (8-17)	[185]
	Agua de río	CPE-UAE	GC-EI-MS	1.0-2.0 ng L^{-1}	97-108 (1.1-2.1)	[220]
		SPE	HPLC-NI- MS/MS	0.1-12 ng L^{-1}	78-92	[221]
	Efluente	SPE	GC-EI-MS	5.0-74 ng L^{-1}	103-114 (12-18)	[191]
		LLE	HPLC-NI- MS/MS	0.2-20 ng L^{-1}	43-47 (8-17)	[221]
		LLE	GC-EI-MS	2-5 ng L^{-1}	nm	[95]
	Sedimentos	SLE	GC-NCI-MS	0.007-0.171 ng g^{-1} dw	74-120 (13)	[28]
		SLE	GC-NCI-MS	0.01-0.04 ng g^{-1} dw	65.4-103.6 (<13)	[137]
		ASE	HRGC-HRMS	0.1-1.5 ng g^{-1} dw	nm	[131]
	Mejillones	SLE	GC-ECNI-MS	0.02-6.6 ng g^{-1} ww	67-80 (<15)	[135]
		PLE	HRGC-HRMS	1.4 to 2.8 ng g^{-1} ww	nm	[131]
OCPs	Agua de mar	SBSE	GC-EI-MS	1.3-5.4 ng L^{-1}	93.2-99.7 (3.7-7.1)	[222]
		SBSE	GC-EI-MS	0.26-14 ng L^{-1}	90-114 (8-18)	[185]
	Agua de río	SPE	GC-ECD	0.05-1.5 ng L^{-1}	76-91 (7.1-10.4)	[223]
		HS-SPME	GC-ITMS-MS	0.4-26.3 ng L^{-1}	nm	[213]
	Efluente	HS	HPLC-UVD	50-80 ng L^{-1}	87-101 (7-8)	[224]
	Sedimentos	MAE	GC-EI-MS	0.1-0.4 ng g^{-1}	73-117 (2-15)	[216]
		SPME	GC-EI-MS	0.04-0.1 ng g^{-1} dw	86-102 (3-14)	[225]
	Mejillones	SLE (Soxhlet)	GC-ECD	0.1-0.18 ng g^{-1} ww	nm	[226]
		SLE	GC-μECD	0.05 ng g^{-1} ww	81-108	[227]
PFCs	Agua de mar	SPE	HPLC-ESI-MS/MS	0.0006-3 ng L^{-1}	60-122	[228]
	Agua de río	SPE	HPLC-ESI-MS/MS	1.7-6.8 ng L^{-1}	91-114	[229]
	Sedimentos	UAE	HPLC-ESI-MS/MS	0.26-1 ng g^{-1} dw	81-109	[229]
	Mejillones	SPE	HPLC-ESI-MS/MS	1-10 ng g^{-1} ww	50-140	[169]

nm: No mencionado; dw: dry weight (peso seco); ww: wet weight (peso húmedo)

1.5 Zonas de estudio

1.5.1 Costa Catalana

Datos generales del Mar Mediterráneo

El mar Mediterráneo recibe el impacto antropogénico directo de 22 países. Entre los países europeos que se encuentran en su litoral se encuentran España, Turquía, Italia, Grecia, Eslovenia, Francia, Albania, Chipre, Croacia, Bosnia, Herzegovina, Serbia, Montenegro y Malta [10; 230]. En muchos aspectos, se le considera un gran lago ya que es un mar semi-cerrado que únicamente tiene flujo de agua fresca del Atlántico por el Estrecho de Gibraltar, con un largo período de recarga (aproximadamente de 90 años) así como un muy bajo nivel de mareas (micromareal) alcanzando cuando mucho 50 cm [230-232]. Este efecto reduce el potencial para la dilución y dispersión de los contaminantes disueltos y particulados. Es un mar bajo en nutrientes y con baja productividad pesquera. El régimen de temperaturas promedio anual mínimo es de unos 15º C llegando a elevarse hasta 21º C en el verano. Es uno de los mares más salados de Europa, con una salinidad promedio entre 3.62% y 3.9% [230; 231]. Su alta salinidad y temperaturas lo hacen unos de los 25 puntos calientes en diversidad biológica a nivel mundial [4].

La costa del mar Mediterráneo es una de las regiones más densamente pobladas de la Tierra. En el litoral costero habitan más de 150 millones de personas, llegandose a doblar este número en las temporadas turísticas veraniegas (220 millones de turistas, datos del 2008) [233]. Entre los sectores productivos asentados en el Mediterráneo se destacan la producción de energía, manufactura de metales, extracción de petróleo y gas, refinería de combustibles, industria química orgánica (entre otros, compuestos organoclorados), química inorgánica, curtido y acabado de pieles e industrias alimenticias. Cada año se producen en el Mediterráneo unos 60 accidentes marítimos por término medio, 15 de los cuales provocan vertidos de petróleo y productos químicos. Diariamente navegan por sus aguas 200 grandes buques petroleros, poniendo en riesgo el equilibrio de todo del ecosistema marino en esta zona [4; 233; 234].

Las principales fuentes puntuales de contaminación son los 69 ríos que desembocan en el, siendo el Ródano, el Po, el Ebro y el Nilo los mayores contribuyentes [4; 29]. Otras fuentes puntuales de contaminación son las descargas de aguas residuales. Cada año se producen en las costas del Mediterráneo, un aproximado de 3.8 billones m^3 de aguas residuales (2.5 millones m^3 producidos por turistas) y en el año 2004 se estimaba que de las 601 ciudades costeras del Mediterráneo con más de 10,000 hab, el 68% contaban con una EDAR para tratar sus aguas residuales de las cuales únicamente el 56% operaban con un tratamiento secundario y el resto con primario. A pesar de esto, se considera que diariamente se descargan 9 millones m^3 de aguas residuales no tratadas en las aguas costeras del Mediterráneo [233; 235; 236].

Costa Catalana

A. Descripción del litoral catalán

La costa Catalana (España), se encuentra al Noroeste del mar Mediterráneo y cuenta con 826.5 kilómetros de litoral de los cuales 213.4 km están modificados con muelles, diques y espigones y 1.2 km corresponden a desembocaduras de ríos. Al Norte, comienza en la Costa Brava, caracterizada por acantilados de pequeña altura y calas escondidas y largas playas a los extremos den las desembocaduras de los ríos Fluvià y la Muga. Luego sigue una larga línea de playas en la zona del Maresme, que solo se corta por los varios puertos comerciales y pesqueros. La comarca del Barcelonés se caracteriza por playas artificiales y el gran puerto comercial de Barcelona que se extiende a lo largo de más de nueve kilómetros. Luego en el macizo del Garraf se articulan destacables acantilados hasta Sitges donde comienzan nuevamente una zona de pequeñas playas y nuevo de numerosos puertos hasta llegar al del puerto de Tarragona. Éste es el segundo mayor puerto de Cataluña y se extiende por más de 5 kilómetros, antes de entrar en el Cabo de Salou. Las playas de esta zona son largas y toman el nombre de Costa Dorada en su vertiente turística. Hacia el Sur, la costa es de nuevo suave, y se caracteriza por una menor ocupación humana. El último gran accidente geográfico lo determina el Golfo de Sant Jordi y las tierras bajas del Delta del Ebro [46].

B. Clima

El clima en la costa de Cataluña a groso modo, es de tipo mediterráneo que se caracteriza por una pluviometría irregular y estacional, con inviernos húmedos y relativamente suaves y veranos muy secos y calurosos. Las temperaturas y la evapotranspiración potencial aumentan de norte a sur, inversamente a la pluviometría. En la Tabla 6 se resumen las características climáticas predominantes en la costa Catalana.

Los vientos dominantes en gran parte de la costa son de componente SO, salvo en la parte más septentrional, donde dominan los de componente N. Hay una fuerte variabilidad estacional, siendo los vientos del N mucho más frecuentes durante los meses invernales, y los vientos del SO más comunes durante el verano. En gran parte de la costa, durante los meses de verano el régimen de marinadas sopla del SO y NE durante el día. Los vientos de levante y siroco son en general poco frecuentes, pero se pueden presentar en otoño y primavera ocasionando fuertes temporales en toda la costa, acompañados de fuertes lluvias. El oleaje es, en general, el agente que actúa con más fuerza en el litoral catalán debido al escaso intervalo mareal del Mediterráneo. Se podrían diferenciar tres periodos de actuación de este agente: el período de calma o baja energía, de junio a septiembre, que se caracteriza por presentar las alturas de oleaje menores y períodos cortos; el período energético, de octubre hasta marzo, que está caracterizado por las mayores alturas de oleaje y periodos largos y finalmente hay un período de transición, de marzo a junio, en el que la altura del oleaje y el período decrecen [232].

Tabla 6. Generalidades climáticas en la costa de Cataluña

	Zona climática oceánica	Zona climática prelitoral	Zona climática litoral
Precipitación media anual (mm)	900-1000	600-1000	500-750
Régimen pluviométrico estacional	Equilibrado	Máx. otoño y primavera	Máximo otoño
Temperatura media anual (º C)	6-10	11-15	14.5-17
Variación térmica anual (º C)	13-15	15-18	14-15

Fuente: ACA [237]

La salinidad media anual en la costa Catalana varía entre 3.75 y 3.8%, a excepción de los sitios en que es menor debido a la presencia de un río, pudiendo disminuir hasta 3.65% [237].

C. Población y sectores productivos

Cataluña ocupa una superficie total de 32,107 km² y sus 12 comarcas costeras (Alt Empordà, Baix Camp, Baix Ebre, Baix Empordà, Baix Llobregat, Baix Penedès, Barcelonès, Garraf, Maresme, Montsià, Selva, Tarragonès) ocupan el 22.8% (7,319 km²). Según datos del 2010, en estas 12 comarcas habitan el 63% de la población de la Comunidad Autónoma (4,764,170 vs. 7,512,381), con una densidad de 651 hab. por cada km² [238].

Además del impacto que causa la elevada población regular en la Costa Catalana, esta área recibe anualmente una gran cantidad de turistas, en especial en la temporada de verano. En el año 2010 Cataluña recibió a 15,610,582 viajeros, colocándose en primer lugar a nivel español con el 19% del total. La densidad de oferta turística en la costa (en 2001) rondaba cerca de 186 plazas de establecimiento turístico por cada km² [239].

De acuerdo con los datos generados por el censo agrario del año 2009, la agricultura en Cataluña ocupa una superficie total de 1,147,532 ha, de las cuales el 70.9%

corresponden a cultivos de secano y el 29.1% restante a regadíos. De este total, 202,414 ha (18%) se encuentran en las 12 comarcas costeras. En relación a la ganadería, en Catalunya se registraron 18,640 explotaciones de las cuales el 19% se encuentran en las comarcas costeras, con un total de 13 millones de cabezas de ganado (26%) [240]. La pesca en Cataluña genera un total de 3,400 puestos de trabajo y de los 20 puertos pesqueros ubicados en el litoral se obtiene una producción anual de 35,000 t de pescado [241].

El sector industrial en Cataluña genera un valor añadido de 24,936 millones de euros (28% del PIB total de Cataluña) y 689,145 puestos de trabajo (27% del empleo). La actividad se concentra en los sistemas Baix Llobregat, Anoia, Garraf (con el 46% de la actividad total de Cataluña) y de sistema Besòs, Baix Maresme (26%) [237; 242]. En la Tabla 7 se muestra la distribución de las actividades industriales en las diferentes cuencas hidrológicas de Cataluña.

Tabla 7. Distribución de actividades industriales (%) en las cuencas hidrográficas de Cataluña en 2009

Actividad económica	Alto Llobregat	Alto Ter	Bajo Ebro, Montsià	Bajo Llobregat, Anoia, Garraf	Bajo Ter, Costa Brava Centro	Besòs, Bajo Maresme	Foix, Gaià, Francolí	Muga, Fluvià, Costa Brava Norte	Tordera, Costa Brava Sur, Alto Maresme	Segre	Noguera-Garona	Ebro
Alimentación, bebidas y tabaco	4	7	0.1	32	9	17	8	5	4	11	0.6	3
Textil, confección, cuero y calzado	7	5	0	36	3	33	3	2	8	3	0.3	1
Madera y corcho	5	4	0.3	20	21	15	9	6	5	10	1.2	4
Papel y artes gráficas	2	1	0.1	59	3	22	5	2	2	2	0.1	2
Química	2	1	0.1	52	2	28	9	1	3	1	0.1	1
Caucho y plástico	5	2	0.1	41	3	31	8	3	2	2	0.1	2
Otros minerales no metálicos	3	1	0.5	40	7	21	10	3	4	6	0.6	3
Metalurgia y productos metálicos	4	5	0.1	41	4	30	7	2	3	4	0.2	1
Maquinaria y equipo mecánicos	4	2	0	52	2	30	3	1	2	2	0.1	1
Equipamiento eléctrico, elect. y óptico	3	3	0.1	53	3	24	5	1	2	3	0.1	2
Material de transporte	3	0	0	60	2	22	5	1	3	1	0.1	1
Industrias manuf. diversas	4	5	0.5	40	4	26	6	2	3	3	0.3	7
Extractivas y ref. del petróleo	24	1	0.1	14	3	6	40	2	2	3	0.3	5
Total industrial	4	3	0.1	46	4	26	7	2	3	3	0.2	2

Fuente: Institut d'Estadística de Catalunya

D. Ríos de Cataluña que desembocan en las aguas costeras

Desde Cataluña se pueden identificar dos principales aportes de agua de río hacia el Mar Mediterráneo (Figura 13). El primero proviene del Río Ebro (cuenca del Ebro) que con un caudal promedio anual de 435 $m^3\ s^{-1}$ (medido en Tortosa). El segundo aporte es de casi 55-60 $m^3\ s^{-1}$ a través del caudal de un conjunto de pequeños ríos agrupados en las denominadas cuencas internas formadas por los ríos Llobregat, Ter, Muga, Fluvià, Tordera, Francolí, Besòs, Foix, Daró y Gaià, de los cuales, los últimos 3 no siempre aportan agua al mar ya que pueden estar modificados para el aprovechamiento en su cauce [243].

Figura 13. Ríos principales de Cataluña que desembocan en el Mediterráneo

E. Saneamiento

Cataluña cuenta con 319 sistemas de saneamiento o estaciones depuradoras de agua residual (EDAR), de los cuales 51 (16%) están ubicadas en poblaciones litorales y tratan las aguas residuales de más de la mitad de la población catalana conectada a los sistemas de saneamiento (58%). Esto representa el 78% de las aguas residuales que se generan en Cataluña. De estos sistemas de saneamiento litorales, 35 vierten sus efluentes directamente al mar y 16 lo hacen total o parcialmente, a cursos de agua fluviales, o se reutilizan después de un tratamiento terciario (datos del 2002). En la mayor parte de las EDAR litorales se realiza un tratamiento secundario y en algunos de ellos, un tratamiento terciario (en el litoral de Girona, un 25% de las aguas residuales se reutilizan para recargas de acuíferos y humedales, o para riego agrícola y de campos de golf). De los 35 sistemas que vierten sus aguas residuales al mar, la mayoría lo hace a través de emisarios submarinos, a excepción de 5 que abocan directamente en las aguas costeras (Portbou, Colera, Tossa de Mar, Garraf y San Carles de la Ràpita) [237; 244]. En el litoral catalán hay censados 94 emisarios submarinos, de los cuales 55 funcionan regularmente y 40 se utilizan exclusivamente en casos de emergencia, o bien están fuera de servicio. De los 55 emisarios en funcionamiento, 36 pertenecen a los sistemas de saneamiento urbanos y 18 son de titularidad privada (6 urbanos y 12 industriales) [237].

Por otra parte, se han identificado que de los 4,649 establecimientos industriales asentados en la comunidad autónoma (datos del 2003), el 71% descarga sus aguas residuales en un sistema de alcantarillado dirigido hacia una EDAR, mientras que el resto (1,326; 29%) lo hace directamente al mar o al cauce de un río [244], que finalmente podrían ser arrastradas hacia el mar.

F. Puertos

Los puertos representan una importante fuente de contaminación difusa de las aguas costeras. Cataluña es la tercera comunidad autónoma con mayor número de instalaciones náuticas y deportivas, después de Galicia y Baleares (datos de octubre del 2010). En Cataluña hay un total de 78 puertos, de los cuales 50 son deportivos, 20 son pesqueros, 5 son comerciales y 2 son industriales [245-247]. A través de los cinco puertos comerciales que existen en Cataluña (Palamós, Barcelona, Vilanova y la Geltrú, Tarragona y Sant Carles de la Ràpita) se mueve un tráfico de mercancías de casi de 32 millones de toneladas al año (datos del 2010); de este gran total el 98% del tráfico se realiza en los puertos de Barcelona (Figura 14) y Tarragona que son considerados dos de los más grandes e importantes de España [238]. En la Tabla 8 se enumeran los principales puertos del litoral catalán y las actividades que se realizan en ellos.

Los puertos pesqueros están distribuidos homogéneamente a lo largo de la costa catalana y en cuanto a su capacidad pesquera varían en un intervalo muy amplio: pueden albergar desde 14 embarcaciones (Torredembarra) hasta 204 (Arenys de Mar). El volumen de pesca en los puertos catalanes es considerable, y constituye el 15.8% de la totalidad del estado Español. Los puertos pesqueros con mayor volumen de pesca son (en orden de importancia decreciente) los puertos de Roses, Sant Carles de la Ràpita, Vilanova i la Geltrú, Tarragona y Barcelona [237; 238].

Por otra parte, el número de puertos deportivos en Cataluña es bastante elevado. En términos general, se puede decir que existe un puerto deportivo cada 10 km de costa. En 2010, se contabiliza un total de 30,406 amarres. Sin embargo, el tamaño o capacidad de estos puertos es muy variable, con un número mínimo de 60 amarres (puerto de Aiguablava, Begur) y un máximo de 5,000 (marina de Empuriabrava, en Castelló d'Empúries) [245; 247]. A esta elevada densidad de puertos, hay que sumarle las 3,492 embarcaciones que atracan en los fondeaderos que se encuentran dispersos por el litoral costero, llegándose a contabilizar 50 playas y/o calas, las cuales no cuentan con sistemas de recolección de aguas residuales, basureros y otros servicios disponibles en los puertos [245].

Tabla 8. Relación de los principales puertos en la costa de Cataluña

Puerto	Tipo
Provincia de Girona	
Port bou	B, C
Colera	C
Llançà	B, C
de la Selva	B, C
Roses	B, C
Canales Sta. Margarida	C
Marina Empuriabrava	C
Pere Pescador	C
La Escala	B, C
El Estartit	B, C
Aiguablava	B, C
Llafranc	B, C
Palamós	A, B, C
D'Aro	B, C
Sant Feliu Guíxols	B, C
La Cala Canyelles	C
Blanes	B, C
Provincia de Barcelona	
Arenys Mar	B, C
Mataró	B, C
Marina Premià	B, C
El Masnou	B, C
Marina Badalona	B, C
El Forum	B, C
Barcelona	A, B, C
Ginesta	B, C
El Garraf	C
Vallcarca	D
Aiguadolç	C
Vilanova i la Geltrú	A, B, C
Coma-Ruga	C
Provincia de Tarragona	
Segur de Calafell	C
Roda Barà	C
Torredembarra	B, C
Tarragona	A, B, C
Salou	C
Cambrils	B, C
Hospitalet-Vandellós	C
Calafat	C
Marina Sant Jordi	C
La Ametlla de Mar	B, C
La Ampolla	B, C
Deltebre	B
Sant Carles la Ràpita	A, B, C
Alcanar	D
Las Casas de Alcanar	B, C

A: Comercial; B: Pesquero; C: Deportivo; D Industrial
Fuente: Según datos de FEADPT [245] y Ports de la Generalitat [246]

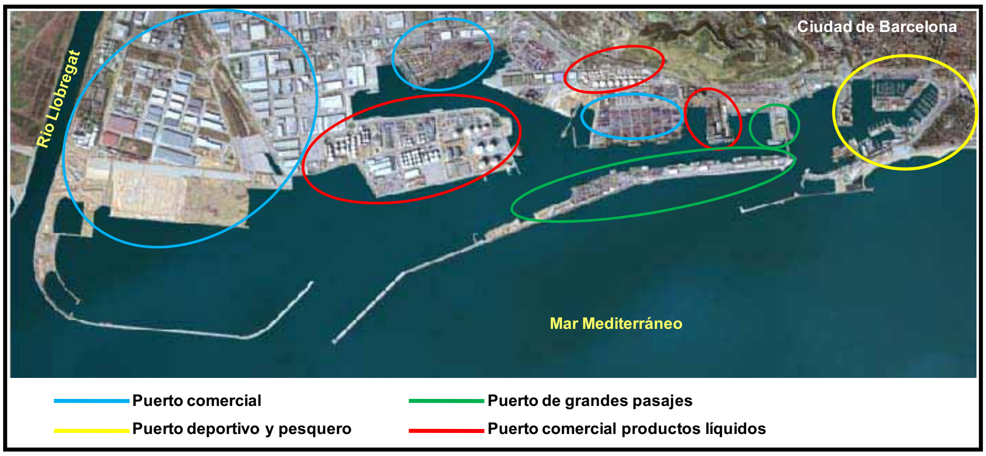


Figura 14. Distribución del Puerto de Barcelona a lo largo de sus 828.9 ha.
Fuente: Adaptado de Autoridad Portuaria de Barcelona [248]

1.5.2 Costa Cantábrica

Datos generales de la Costa Ibérica - Golfo de Vizcaya

La región marítima denominada Costa Ibérica - Golfo de Vizcaya se sitúa en el Océano Atlántico del Nordeste extendiéndose desde las costas de Francia, España y Portugal abarcando un área de 300,000 km^2. La morfología costera varía considerablemente, desde largas playas de arena del Golfo de Cádiz a un tramo rocoso casi continuo a lo largo del norte y noroeste de la Costa Ibérica así como humedales y rías [249]. Estas últimas son sistemas únicos que se encuentran en la Costa Ibérica. Son brazos de mar que se internan en la costa y que están sometidas a la acción de las mareas, con una circulación modulada del viento (tipo de estuario) y son lo que permite que la región tenga una alta productividad en especial, de mejillones. La morfología del fondo marino también es muy variable y una de las principales características de esta región, es una serie de cañones marinos extremadamente escalonados y profundos como los de Avilés y Lastres y Llanes ubicados a 7 km de la costa, alcanzando una profundidad de 4,500 m. El clima es característico de las latitudes templadas y está fuertemente influenciado por la afluencia de agua oceánica del Atlántico y la circulación de los vientos del Oeste, que vienen frecuentemente acompañados de sistemas de baja presión. En los meses de invierno pueden ocurrir grandes tormentas. La amplitud típica de las mareas es de 2.5 m y asociadas a las corrientes mareales que van de 0.1 a 1 m/s, pueden alcanzar los 6 m de altura [233; 250].

En la costa de la región se pueden encontrar industrias de varios tipos así como zonas agrícolas. Las principales actividades que se desarrollan en esta zona son la industria del metal, el turismo, pesca, acuacultura, transporte marítimo, extracción de grava y arena, generación de electricidad (eólica y mareal) [249]. Las principales amenazas al ecosistema costero de la zona son: la extracción y refinamiento de petróleo y combustibles; la eutrofización debido al alta cantidad de nutrientes de las descargas de aguas residuales urbanas e industriales; la agricultura, la industria química, la sobrepesca, el fondeo de barcos y botes, el turismo y los accidentes de barcos [250]. En este último punto se pueden mencionar al desastre del petrolero *Prestige* en 2002, provocando una masiva contaminación por hidrocarburos de la costa gallega en el Noroeste de España y el del petrolero *Erika* en 1999, que encalló en las costas de Bretaña [3; 250].

Costa del Mar Cantabrico: Asturias, Cantabria y País Vasco

A. Descripción del litoral Cantábrico

El Mar Cantábrico es el mar litoral de la Costa Ibérica que baña el norte de España y el suroeste de Francia; forma la parte sur del Golfo de Vizcaya. Se extiende desde la punta de Estaca de Bares, en la provincia de La Coruña, hasta la desembocadura del río Adur, en las costas del departamento francés de Las Landas, cerca de Bayona. Baña 800 kilómetros de costa compartida por las provincias de La Coruña y Lugo (Galicia), Asturias, Cantabria, Vizcaya y Guipúzcoa (País Vasco), y Labort, ya en Francia. Es un mar de transición entre los mares fríos del norte y los templados del trópico, lo que hace que sea ecotono (zona de transición donde se presentan muchas especies características de dos ecosistemas adyacentes) de especies vegetales y animales de aguas frías. La profundidad de las aguas costeras es esta zona puede alcanzar los 93.3 m. En la franja del Mar Cantábrico que abarca de la provincia de Asturias hasta el País Vasco, se distribuyen tramos de acantilados, playas y campos dunares, rías y estuarios.

B. Clima

El Norte de España es caracterizado por sus leves oscilaciones térmicas, repartición homogénea de las precipitaciones a lo largo del año y escasez relativa de heladas. Los fuertes vientos del noroeste que soplan sobre esta región litoral tienen su origen en las bajas presiones centradas sobre las islas británicas y el mar del Norte en combinación con el anticiclón de las Azores. La distancia recorrida por el viento y el mantenimiento de su dirección y velocidad constantes hacen que se generen olas que oscilan entre 2.5 y 3 metros de altura, lo que origina un mar muy agitado. En condiciones muy particulares, más propicias en los meses de abril-mayo y septiembre-octubre, los vientos del oeste pueden alcanzar magnitudes de galerna con olas que llegan a superar los 9 m de altura. A medida que nos aproximamos a la costa francesa las aguas van siendo más cálidas en verano (22-24 ºC) y más frías en invierno (10-12 ºC). Posee una salinidad media del 35%, aunque este dato varía mucho en función del régimen de lluvias imperante en la zona. Tiene una significativa amplitud de marea y es atravesado por la corriente del Golfo .

C. Población y sectores productivos

En las provincias de Asturias, Cantabria, Vizcaya y Guipúzcoa habitan 3,537,578 de personas (datos del 2010), siendo las ciudades costeras de Santander, Bilbao y Donostia-San Sebastian en donde se aglomera la mayor parte de la población [251]. El litoral correspondiente a Asturias es el menor poblado por la gran cantidad de accidentes geográficos que se presentan. En la franja de Cantabria-País Vasco se halla una importante proporción de los asentamientos urbanos de la región. La presencia de dichos núcleos urbanos supone una importante presión sobre el medio litoral costero [252; 253].

Según datos del 2002, en el País Vasco el sector servicios es un 60% de la actividad económica, mientras que el sector industrial abarca un 32% (concentrado principalmente en el área de Viscaya), el de la construcción un 7% y el agrario un 1% [254]. Según datos del 2007, en Cantabria el sector servicios corresponde a un 72%, el sector industrial (principalmente metales) un 18% (concentrado en las regiones costeras de El Astillero, Santoña, Marina de Cudeyo y Escalante) y el agrario aproximadamente un 2% [255]. En el caso de Asturias, los porcentajes económicos son similares, ocupando un 64% el sector de servicios, un 19% el industrial, un 12% el constructivo y un 6% el agrícola [256].

D. Estuarios

En Asturias los principales estuarios son los de Eo, Nalon, Sella y Villaviciosa [253]. En Cantabria se delimitan 15 estuarios, siendo los más importantes la Bahía de Santander, las Marismas de Santoña, las Marismas de S. Vicente de la Barquera y la Ria de Suances [252]. En el País Vasco se cuenta con 12 estuarios entre los que se pueden mencionar al estuario de la Ría de Bilbao, Urdaibai (considerado como zona protegida), Txingudi y Lea [254]. La mayoría de los estuarios en el Mar Cantábrico se hayan altamente afectados por presiones antropogénicas como las descargas de aguas residuales y las actividades derivadas de los puertos. En la Figura 15 se aprecian dos estuarios, uno en Cantabria y el otro en el País Vasco.

Figura 15. Imagen de dos estuarios. A la izquierda en Cantabria (Bahía de Santander) y a la derecha en el País Vasco (Oyarzun).
Fuente: Google Maps.

E. Saneamiento

En la comunidad de Asturias existen instaladas 21 EDAR, de las cuales 7 estan ubicadas en localidades costeras, con una capacidad de 394,000 habitantes equivalentes [253]. En el País Vasco la infraestructura de saneamiento es de 56 EDAR para dar servicio al 88% de la población, de las cuales se estima que la mitad de ellas (25) descargan en el litoral o en los estuarios [254]. Con respecto a la Comunidad Autónoma de Cantabria se han detectado 39 fuentes puntuales de contaminación al litoral costero de los cuales el 79% corresponden a descargas de aguas residuales urbanas, 8% a alivios de tormentas, 8% descargas provenientes de acuicultura y finalmente 5% a descargas de aguas residuales industriales [257]; en cuanto a los estuarios, se han contabilizado 220 puntos de vertido de aguas residuales, de los cuales el 64% proceden de núcleos urbanos (141), el 25% de origen industrial (56) y el 10% son vertidos de aguas pluviales o de alivios de tormenta (22) [252].

F. Puertos

En el País Vasco se han contabilizado 17 puertos, de los cuales 15 son puertos menores y los otros 2 son importantes centros de entrada y salida de productos petrolíferos, de mercancías generales, vehículos y bienes siderúrgicos. El tráfico de mercancias en el puerto de Bilbao ronda los 28,000,000 t anuales, cifra que le sitúa en el quinto puesto del ránking de movimientos del Estado español. Cuenta con las instalaciones para pequeñas embarcaciones, hasta grande buques petroleros que descargan en una planta de refinamiento. El Puerto de Pasajes, emplazado en una bahía se especializa en el tráfico de productos siderúrgicos y chatarras, papel y automóviles, con movimientos de mercancías que alcanzan los 6,000,000 t anuales [258].

En Cantabria, se contabilizan 9 puertos ubicados en los estuarios de San Vicente de la Barquera, ría de Suances, bahía de Santander y marismas de Santoña. En las aguas costeras únicamente hay 3 puertos ubicados en Comillas, Laredo y Castro Urdiales [252; 257]. De todos ellos, el más importante es el de Santander, manejando un tráfico de más de 5,000,000 t anuales (datos de marzo 2012); es un puerto de pasaje y comercial, destacando el manejo de cemento, biocarburantes, productos derivados del petróleo, aceites, aditivos, etc [259].

En Asturias hay una infraestructura de 21 puertos [245], siendo el más importante el Puerto de Gijon con un movimiento de 15,604,419 t en el 2010. Entre las mercancías que se trafican en este puerto se pueden mencionar el cemento, clínker, y abonos, gasóleo, gasolina, fuel, GLP, productos asfálticos y productos siderúrgicos [260]. El resto de puertos son mayoritariamente deportivos.

Tabla 9. Relación de los principales puertos en el litoral Cantábrico

Puerto	Tipo
País Vasco	
Elantxobe	B, C
Mundaka	C
Bermeo	A, B, C
Plentzia	C
Bilbao	A, B, C, D
Hondarribia	B, C
Donostia-San Sebastián	B, C
Orio	A, B, C
Getaria	B, C
Mutriku	B, C
Pasajes	A, B, C, D
Cantabria	
San Vicente	B, C
Suances	B, C
Requejada	A
Santander	A, B,
Colindres	B, C
Santoña	B, C
Asturias	
Aviles	A, B, C
Gijon	A, B, C

A: Comercial; B: Pesquero; C: Deportivo; D Industrial
Fuente: Según datos de FEADPT [231]

1.6 Justificación de la tesis

Las zonas costeras son las regiones más vulnerables de todo el medio marino, especialmente por su colindancia con las zonas de desarrollo humano tierra adentro. La escasez de estudios relacionados con la presencia, distribución e impacto de los contaminantes orgánicos en estas zonas marinas ha sido el detonante para la elección del tema de este trabajo. Es indispensable procurar la buena calidad de estas regiones y esto se logra como primer paso, realizando una vigilancia ambiental. Para ello se debe contar con métodos lo suficientemente reproducibles, exactos y precisos que incluyan un gran número de contaminantes, no solamente los que están legislados. Estos métodos deben tener la sensibilidad adecuada para determinar concentraciones muy bajas. Es indispensable la caracterización de las principales fuentes de contaminación y conocer la carga total de contaminantes liberados en las zonas costeras. Así mismo, es de gran importancia comparar los niveles de contaminación con los valores críticos de toxicidad para diferentes niveles de organismos del ecosistema marino y conocer así el grado de afectación que sufren. Esto se puede hacer por medio de modelos que miden el riesgo ambiental y aportan una calificación del estado químico de las aguas. El identificar a los contaminantes que causan efectos adversos y las zonas que se encuentran gravemente impactadas puede ser una herramienta clave para tomar las medidas necesarias y así mejorar la calidad de las aguas costeras.

En esta tesis en primer lugar se desarrollaron 3 metodologías analíticas multiresiduales utilizando la cromatografía de gases acolpada a espectrometría de masas para la identificación y cuantificación de microcontaminantes orgánicos semivolátiles de distintas familias y en diferentes matrices. La primera metodología basada en la SPE-GC-MS, se aplicó para identificar y evaluar el comportamiento de los contaminantes en las aguas residuales en una red de alcantarillado, hasta llegar a una EDAR, la cual es una de las principales fuentes de contaminación puntual de la Costa Catalana. Así mismo se evaluó la eficiencia de eliminación de estas sustancias y finalmente se estimó la cantidad de contaminantes que llegan al mar. La segunda metodología, basada en la SBSE-GC-MS se desarrolló para determinar los niveles de contaminantes orgánicos en la Costa Catalana y se compararon las concentraciones detectados con los valores legislados. La tercera metodología, basada en la SPE-GC-MS/MS pretendía mejorar los límites de detección de las anteriores técnicas, la capacidad de identificación y además se consideraron un mayor número de compuestos y matrices. Se empleó para determinar la presencia de OMPs en la Costa Catalana, estimar las fuentes de contaminación puntual (rios y efluentes de EDAR) y su carga, y con ello poder calcular el riesgo ambiental de esta zona costera. Así mismo la metodología basada en la SPE-GC-MS/MS se empleó también para analizar OMPs en agua de mar y estuarios, sedimentos y mejillones transplantados en el litoral Cantábrico. Por medio de los resultados obtenidos se determinaron los compuestos que ponen en riesgo la salud del medio ambiente marino en esta región y los sitios que sufren mayor afección. Además se realizó un monitoreo de una familia de contaminantes orgánicos polares, como son los PFCs.

Este trabajo cumple además con lo requerido por algunas normativas Europeaas como la Directiva Marco del Agua, la Directiva Marco sobre la Estrategia Marina y la Directiva de Normas de Calidad Ambiental las cuales exigen que los países miembros deben realizar una evaluación del estado químico de aguas, sedimentos y biota.

1.7 Objetivos

El objetivo principal de este trabajo de tesis fue incrementar el conocimiento acerca de la presencia, distribución, fuentes y el riesgo potencial que presentan los microcontaminantes orgánicos prioritarios y emergentes en el medio ambiente costero.

En este mismo contexto se pueden enumerar los siguientes objetivos específicos:

- Desarrollar metodologías analíticas sensibles y robustas basadas en la extracción en fase sólida (SPE), extracción por adsorción en barras giratorias (SBSE) y extracción asistida por ultrasonidos (UAE) y el análisis por cromatografía de gases acoplada a espectrometría de masas (GC-EI-MS) y masas en tándem (GC-EI-MS/MS) para la determinación de microcontaminantes orgánicos prioritarios y emergentes en agua de mar, aguas de río, aguas residuales, sedimentos y mejillones.
- Determinar la presencia de microcontaminantes orgánicos prioritarios y emergentes en zonas costeras de la Costa Catalana y del Mar Cantábrico.
- Identificar las fuentes de contaminación de las aguas costeras y estimar la carga de contaminantes hacia el mar.
- Determinar la acumulación de microcontaminantes orgánicos prioritarios y emergentes en sedimentos y mejillones.
- Evaluar el riesgo ambiental que supone la presencia de los contaminantes detectados en las aguas costeras del Mediterráneo Occidental y del Mar Cantábrico.

CAPÍTULO 2:

Determinación de microcontaminantes orgánicos prioritarios y emergentes en aguas y otras matrices de las zonas costeras

2.1 Introducción

En la literatura se destacan los estudios referentes a la presencia de PAHs, APs, PEs, BPA, PCBs y PBDEs en aguas de río y depuradoras en diferentes partes del mundo, pero la información disponible en las aguas costeras y en especial de la región de Cataluña es escasa. Estos contaminantes provienen de fuentes industriales y domésticas y se encuentran globalmente distribuidos. La mayoría de estudios utilizan metodologías analíticas enfocadas al análisis de una o dos familias químicas. Sin embargo son muy pocos los estudios que reporten metodologías para el análisis simultáneo de una batería de compuestos con amplios intervalos de propiedades fisicoquímicas (ver K_{ow}, Tabla 10).

Para realizar un adecuado programa de vigilancia ambiental es necesario contar con métodos optimizados y validados que aseguren la calidad de los resultados. En esta tesis se desarrollaron metodologías multiresiduales que contemplan un gran número de contaminantes de diferentes familias químicas y con diferentes intervalos de propiedades fisicoquímicas. Una gran ventaja de los métodos multiresiduales es la posibilidad de analizar varios compuestos en un único análisis, disminuyendo de esta manera los costos del análisis, el trabajo analítico y el tiempo invertido [261].

Los resultados científicos que se presenta en este Capítulo describen el desarrollo de tres metodologías analíticas para la determinación de microcontaminantes orgánicos semivolátiles prioritarios y emergentes en matrices tales como aguas de mar, efluentes de depuradora, aguas de río, sedimentos y mejillones. Estos métodos se basan en la SPE o SBSE para las marices acuosas o en la UAE para las matrices sólidas y el posterior análisis de los extractos por GC-EI-MS o GC-EI-MS/MS.

El Capítulo contiene una parte relacionada con la metodología experimental y un apartado donde se discuten los principales resultados obtenidos, según las muestras analizadas provenientes de zonas costeras catalanas.

Estos trabajos han dado 3 contribuciones científicas en revistas internacionales indexadas en el Índice de Citas Científicas o SCI (“Science Citation Index”).

2.2 Metodología experimental

El primer paso del protocolo analítico es el muestreo. Se recolectaron aguas residuales tomadas tanto de alcantarillas como del influente y efluente de las EDAR.

Para tomar las muestras de las alcantarillas se empleó una vara extensible con un vaso de precipitados previamente lavado en un extremo. En la Figura 16 se aprecian algunas imágenes del muestreo en una red de alcantarillado. Estas aguas se recolectaron en arquetas de la red de alcantarillado de la zona del Maresme. En esta zona se ubica una importante red industrial, destacando la fabricación de jabón y textiles, que se caracterizaron por sus aguas residuales coloreadas.

a) Apertura de la tapa de la alcantarilla

b) Aspecto de las aguas residuales dentro de los colectores

c) Toma de muestra

d) Trasvase de la muestra

Figura 16. Imágenes del muestreo en la red de alcantarillado

El influente y efluente de las EDARs se recolectaron empleando un muestreador automático que toma una alícuota cada hora para finalmente hacer una muestra compuesta de 24 h. En la Figura 17 se ilustra el muestreo de los influentes e influentes de las EDAR de Mataró y el equipo utilizado para recolectar las muestras compuestas.

Las muestras de agua de mar fueron recolectadas a unos 50 m de la costa ya sea a nado o desde un espigón o muelle (Figura 18a) utilizando siempre una botella de vidrio previamente lavada con acetona. Las muestras de agua de río fueron tomadas desde un puente, en el centro del cauce del río por medio de un dispotitivo formado de una estructura de acero que cubre una botella de vidrio, que se hace bajar hasta la profundidad deseada (Figura 18b). Las muestras de sedimento fueron colectadas con ayuda de una draga Van Veen de acero inoxidable (Figura 18c). En el caso de los mejillones se manejaron muestras trasplantadas en el fondo del mar con ayuda de buzos expertos (Figura 18d).

a) Fotografía del aspecto del influente de la EDAR de Mataró

b) Fotografía del aspecto del efluente de la EDAR de Mataró

c) Fotografía de panel de control del muestreador automático para el influente y efluente

d) Fotografía del interno del muestreador automático

Figura 17. a) Influente y b) efluente de la EDAR de Mataró y c) y d) equipo utilizado para realizar la recolecta de muestras compuestas

Una vez colectadas las muestras se almacenaron en botellas de vidrio color ámbar previamente lavadas y mufladas perfectamente cerradas y conservando en todo momento una cadena de frío (aprox. 4 ºC). Las muestras se procesaron en menos de 48 h después de su colecta para evitar su degradación. Los sedimentos se limpiaron retirando los residuos que pudieran contener, tales como plásticos, vidrios y piedras. Los mejillones se abrieron y se retiró el tejido de las conchas. Ambas matrices fueron liofilizadas, molidas, tamizadas y homogenizadas.

a) Muestreo de aguas de mar

A nado, ≈ 50 m de la costa

Desde un muelle o espigón

b) Muestreo de aguas de río

Desde un puente

c) Muestreo de sedimentos

Utilizando una draga

d) Muestreo de mejillones

Trasplante de los mejillones a cargo de buzos expertos

Figura 18. Imágenes de la toma de muestra de a) aguas de mar; b) aguas de río; c) sedimentos; d) mejillones trasplantados

A continuación se presenta el desarrollo de las 3 metodologías analíticas desarrolladas en este trabajo de tesis (Tabla 10).

Tabla 10. Metodologías analíticas desarrolladas para el análisis de los contaminantes orgánicos prioritarios y emergentes

	Metodología A	Metodología B	Metodología C **
No. de compuestos	42	49	51
Log K_{ow}	1.6 – 12.1	3.3 – 9.4	1.8 – 5.5
Contaminantes prioritarios *	24	38	33
Contaminantes emergentes	18	11	18
Cantidad de muestra por tipo de matriz	50 mL agua residual	100 mL agua de mar	1 L agua de mar 1 L agua de río 50 mL agua residual
Método de aislamiento/ preconcentración	SPE	SBSE	SPE
Tipo de adsorbente	Oasis HLB	PDMS (polidimetil-siloxano)	Oasis HLB
Elución	10 mL CH_2Cl_2/Hx (1:1) y 10 ml CH_2Cl_2/Ace (1:1)	Desorción térmica	Igual que en Metod. 1
Reconstitución	250 µL de AcEt	No se requiere	Aguas de mar y rio: 100 µL de AcEt Agua residual: 250 µL de AcEt
Separación analítica	GC	GC	GC
Modo de ionización	EI	EI	EI
Técnica de análisis	MS (modo SIM)	MS (modo SIM)	MS/MS (modo SRM)

CH_2Cl_2, diclorometano; Hx, hexano; Ace, acetona; AcEt, acetato de etilo

* De acuerdo con la Directiva 2008/105/EC, incluyendo además aquellas sustancias que encuentran restringidos por el Convenio de Estocolmo y el Reglamento 850/2004/EC

** Inicialmente se planeó analizar 53 contaminantes, pero al final de la optimización debido a problemas analíticos no se consideraron a los BDEs 183 y 209

La Metodología A se desarrolló para determinar 42 OMPs prioritarios y emergentes de aguas residuales. Para la optimización se utilizaron 50 mL de agua grado Mili-Q dopadas con los 42 analitos de interés y 8 patrones de recuperación (“surrogate standard”). Como técnica de aislamiento/preconcentración se empleó la SPE (Figura 19a).

Ya que el estudio de esta tesis se centra en el análisis de OMPs en aguas costeras, posteriormente se desarrolló una metodología para determinar 49 OMPs en aguas de mar (Metodología B). Para su optimización, se doparon con los 49 contaminantes aguas de mar artificial que consistían en 3.5% de NaCl disueltos en agua grado HPLC para emular la salinidad característica del Mar Mediterráneo. Como técnica de preconcentración/aislamiento se utilizó la SBSE (Figura 19b). A grandes rasgos, la SBSE tiene por ventaja sobre la SPE el empleo de pequeñas cantidades de muestra (≈100 mL), lo cual ahorra espacio durante su recolecta, transporte y almacenamiento. Además, es una técnica limpia ya que al no emplear disolventes, no se generan residuos peligrosos. Además es más económica ya que los “twisters” son reutilizables. Para análisis rutinarios de muestras, la SBSE es una técnica de aislamiento/preconcentración simple y ahorra tiempo al analista. La mayor desventaja de la SBSE es el alto costo del equipo necesario para desorber y crioenfocar la muestra en la columna de GC. Otra desventaja de la SBSE es que únicamente se comercializa un tipo de adsorbente (PDMS), en cambio para la SPE existe una gran variedad de tipos de cartuchos disponibles, a elegir dependiendo de la funcionalidad y selectividad que se desee.

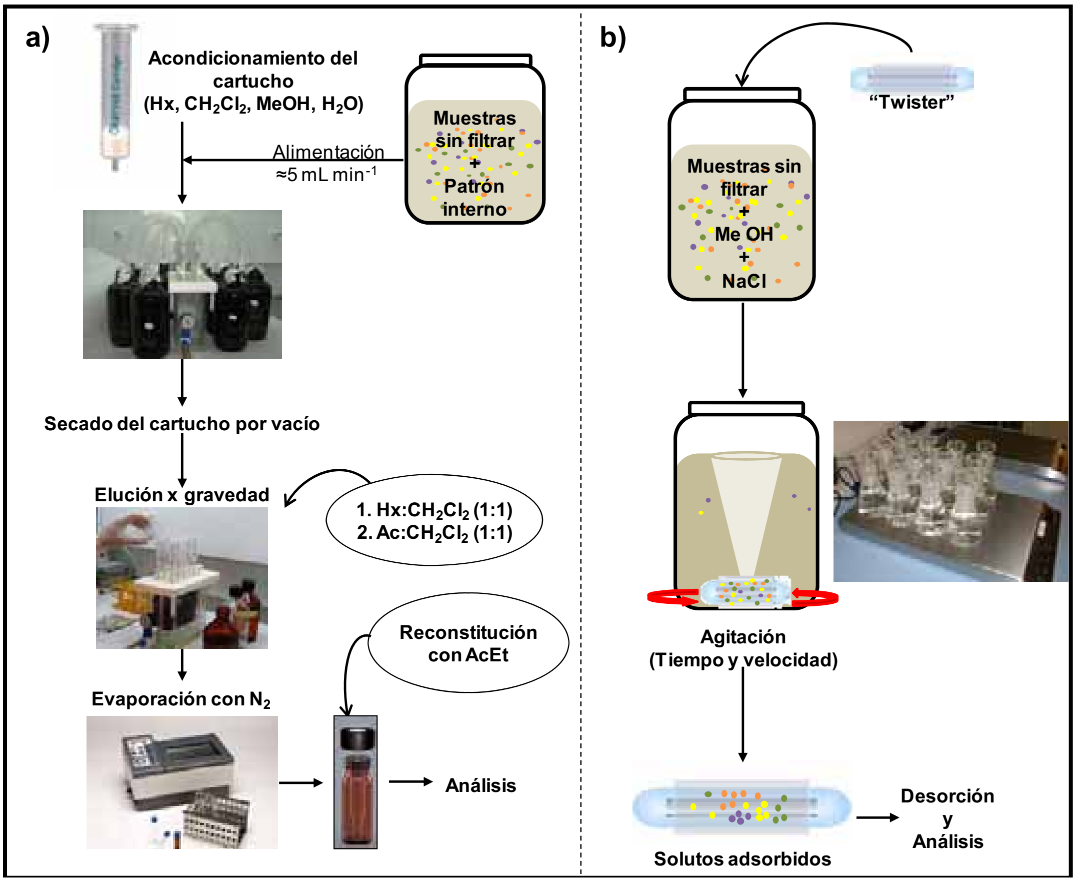


Figura 19. a) diagrama de la extracción en fase sólida (SPE) y b) por adsorción en barras agitadoras (SBSE)

Finalmente, ya que el realizar una vigilancia ambiental del medio marino no requiere únicamente del análisis de aguas de mar o de las descargas de aguas residuales que se vierten a las aguas costeras, se decidió desarrollar una metodología analítica que incluyó de manera integral el análisis de 53 OMPs en agua de mar, agua de río, efluentes de depuradora, además de sedimentos y mejillones (Metodología C). Los sedimentos fueron incluidos ya que representan un sumidero y/o destino final de gran parte de la contaminación del medio marino y los mejillones son ampliamente utilizados como organismos centinelas o bioindicadores. Como técnicas de preconcentración/aislamiento para las matrices acuosas se utilizó la SPE tal y como se optimizó en la Metodología A (Figura 19a) y para las matrices sólidas se utilizó una extracción asistida por ultrasonidos (UAE) y su posterior “clean-up” o limpieza con cartuchos de Florisil (Figura 20). Esta técnica es sencilla y económica, pero tiene el gran inconveniente de utilizar cantidades considerables de disolventes orgánicos. Para la optimización de la metodología se doparon con los 53 compuestos de estudio y 11 patrones de recuperación aguas de mar y de río recolectadas en zonas alejadas de fuentes de contaminación; también se doparon sedimentos del nacimiento del río Ebro y mejillones adquiridos en el supermercado. En todos los casos se determinaron las concentraciones de contaminantes en las matrices antes de dopar, para restar sus contribuciones.

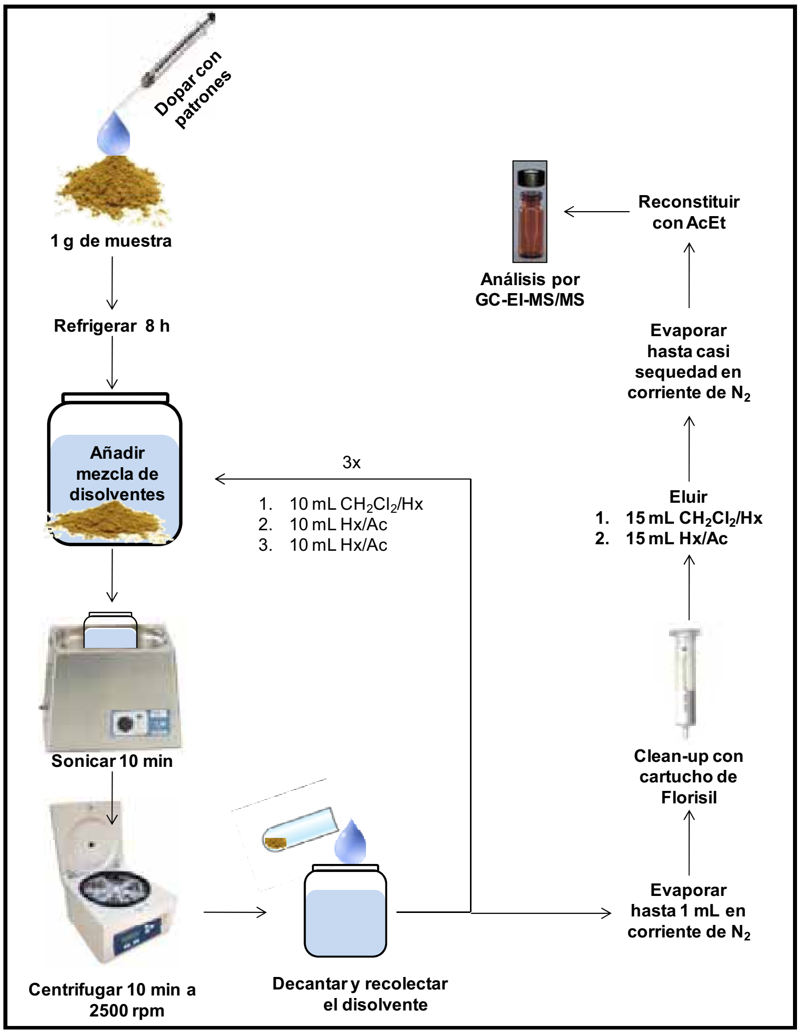


Figura 20. Diagrama de extracción asistida por ultrasonidos (UAE) empleado en las muestras de sedimentos y mejillones

En las tres metodologías se utilizó la GC como herramienta de separación analítica y en modo de impacto electrónico (EI). En la metodología A se empleó una columna cromatográfica de 15 m, mientras que en las otras 2, fue una columna de 30 m. El aumento de la longitud de la columna se debió a que al aumentar el número de compuestos se requería una mejor separación de los mismos, para evitar en lo posible colusiones entre los compuestos. Así mismo, con la columna de 30 m se aseguró la separación del 4,4'-DDD y 2,4'-DDT, lo que no sería posible con la de 15 m. Sin embargo, el empleo de la columna de 30 m imposibilitó la detección de los BDEs 183 y 209 que no eluyeron en la columna de 30 con el programa de temperaturas utilizado. Los compuestos que fueron más difíciles de optimizar cromatográficamente fueron el BPA y los NPEOs, que generalmente son analizados por HPLC o se derivatizan para aumentar su volatilidad y con ello su sensibilidad [262]. En este trabajo de tesis se tomó la decisión de no derivatizar y sacrificar la sensibilidad de estos compuestos como compromiso para así no afectar la respuesta del resto de analitos. En las Figuras 21, 22 y 23 se aprecian los cromatogramas de cada uno de los métodos desarrollados.

En las metodologías A y B se empleó el análisis por MS y en la metodología C se adaptaron las condiciones de separación cromatográfica pero utilizando en esta ocasión el análisis por MS en tándem. Con esta última técnica se alcanza una mayor sensibilidad y selectividad, reduciendo el efecto matríz. Esto es consecuencia de la monitorización del par de iones precursores o fragmentos característicos de cada compuesto aumentando con ello la relación señal/ruido. La mayor desventaja de la MS en tándem con respecto a la MS, es que para optimizar cada transición se requiere un mayor número de pasos, como por ejemplo una vez seleccionado el ion precursor hay que encontrar la energía de colisión adecuada para la formación de los iones producto. Además, el software es mucho más complejo que el que se utiliza en un GC-MS. Sin embargo, para aumentar la sensibilidad en las metodologías por GC-EI-MS el método de adquisición se trabajó en modo SIM utilizando los 3 iones más abundantes de cada analito y con el menor número de iones en cada ventana cromatográfica.

Durante el desarrollo de las 3 metodologías se analizaron blancos para restar la contribución que pueda provenir de la manipulación de las muestras y/o de los disolventes y materiales utilizados. En cada lote de análisis se incluyeron controles de calidad que consistían en agua grado HPLC dopadas con los analitos a una concentración conocida. Se manejaron curvas de calibración entre 5 a 9 valores de concentración, inyectándolas en el equipo en orden creciente de concentración. La linealidad fue aceptable en la gran mayoría de los casos (>0.993). Durante la secuencia analítica se intercalaron disolventes cada 4 muestras para reducir el efecto memoria que pueda quedar en el equipo. Así mismo se intercalaron cada 12 muestras un patrón de concentración conocida para controlar la sensibilidad del equipo a lo largo de la secuencia de inyección.

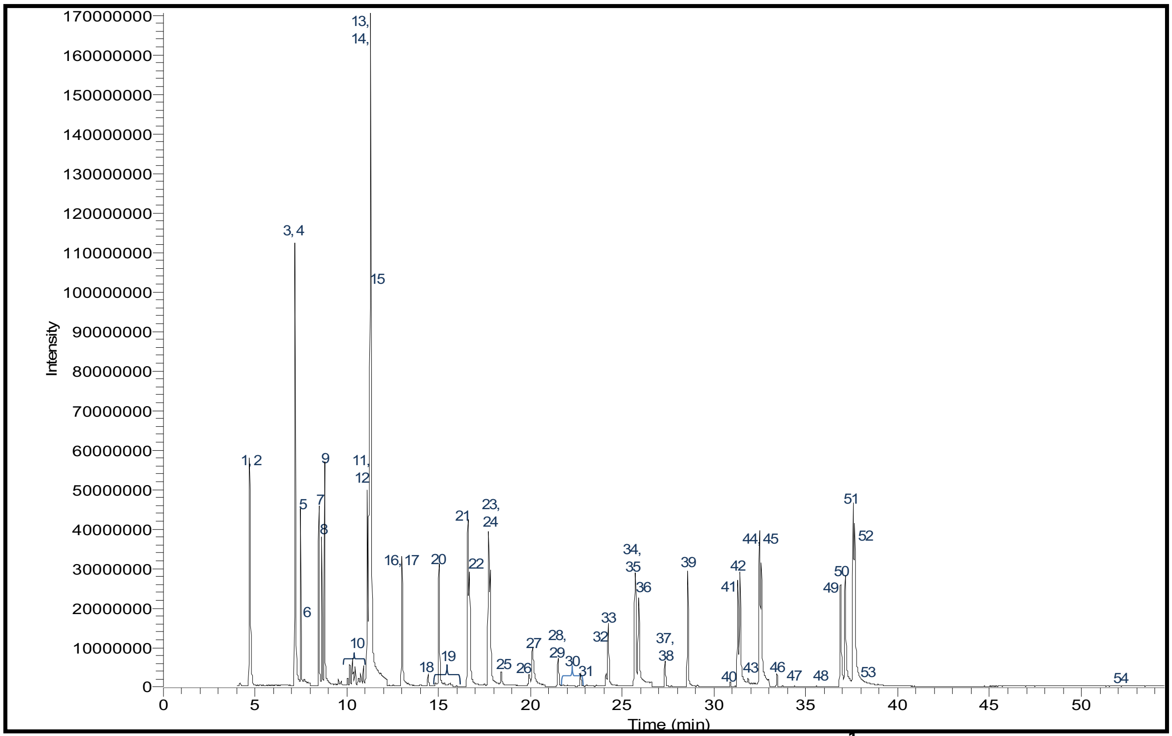


Figura 21. Cromatograma de GC-EI-MS de un patrón a 0.1 ng µL^{-1} de la mezcla de los contaminantes orgánicos analizados utilizando una columna cromatográfica de 15 m
1) Nap d_8; 2) Nap; 3) Acy d_8; 4) Acy; 5) DMP; 6) Ace; 7) Flu; 8) DEP; 9) OP; 10) NP; 11) Phe d_{10}; 12) Phe; 13) DPP D4; 14) Ant d_{10}; 15) Ant; 16) NP d_8; 17) PCB 28; 18) PCB 52; 19) NPEO$_1$; 20) DBP; 21) Flr d_{10}; 22) Flr; 23) Pyr d_{10}; 24) Pyr; 25) PCB 101; 26) BPA D$_{16}$; 27) BPA; 28) BDE 28; 29) PCB 118; 30) NPEO$_2$; 31) PCB 138; 32) PCB 153; 33) BBP; 34) B(a)A; 35) DEHA; 36) Chr; 37) BDE 47; 38) PCB 180; 39) DEHP; 40) BDE 100; 41) B(b)F; 42) B(k)F; 43) BDE 99; 44) B(a)P d_{12}; 45) B(a)P; 46) PCB 209; 47) BDE 154; 48) BDE 153; 49) I(cd)P; 50) D(ah)P; 51) B(ghi)P d_{12}; 52) B(ghi)P; 53) BDE 183; 54) BDE 209

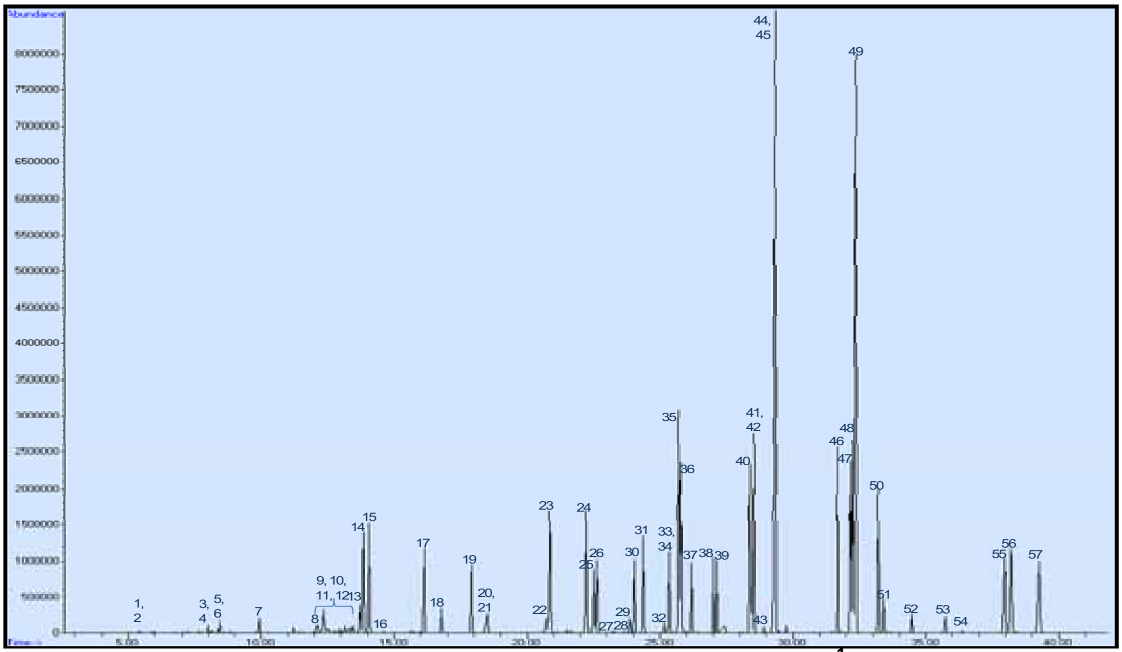


Figura 22. Cromatograma de GC-EI-MS de un patrón a 0.1 ng µL^{-1} de la mezcla de los contaminantes orgánicos analizados con una columna de 30 m
1) Nap d_8; 2) Nap; 3) Acy d_8; 4) Acy; 5) Ace d_{10}; 6) Ace; 7) Flu; 8) α-HCH; 9) NP; 10) HCB; 11) β-HCH; 12) Lindano; 13) Phe d_{10}; 14) Phe; 15) Ant; 16) δ-HCH; 17) PCB 28; 18) HpCl; 19) PCB 52; 20) PCB 65; 21) Aldrina; 22) HpCl-B; 23) Flr; 24) Pyr; 25) 2,4'-DDE; 26) PCB101; 27) α-endsf; 28); dieldrina; 29) 4,4'-DDE; 30) 2,4'-DDD; 31) endrina; 32) β-endsf; 33) PCB 118; 34) BDE 28; 35) 4,4'-DDD; 36) 2,4'-DDT; 37) PCB 138; 38) 4,4'-DDT; 39) PCB 153; 40) B(a)A; 41) Chr d_{12}; 42) Chr; 43) PCB 200; 44) PCB 180; 45) BDE 47; 46) BDE 100; 47) B(b)F; 48) B(k)F; 49) PBDE 99; 50) B(a)P; 51) Per-d_{12}; 52) BDE 154; 53) BDE 153; 54) BDE 183; 55) I(cd)P; 56) D(ah)P; 57) B(ghi)P

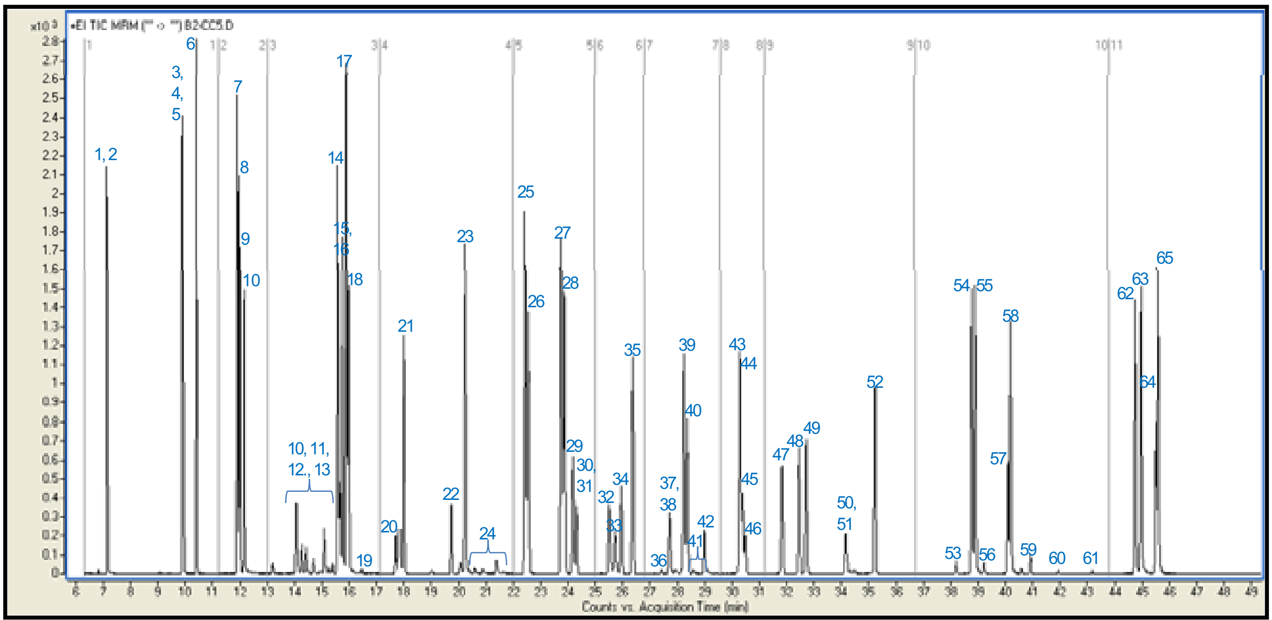


Figura 23. Cromatograma de GC-EI-MS/MS de un patrón a 0.1 ng µL^{-1} de la mezcla de OMPs
1) Nap d_8; 2) Nap; 3) DMP; 4) Acy d_8; 5) Acy; 6) Ace; 7) Flu; 8) DEP; 9) OP; 10) NP; 11) α-HCH; 12) β-HCH; 13) Lindano; 14) DPP D_4; 15) Phe d_{10}; 16) Phe; 17) Ant d_{10}; 18) Ant; 19) δ-HCH; 20) NP d_8; 21) PCB 28; 22) PCB 52; 23) DEHA; 24) NPEO$_1$; 25) Flr d_{10}; 26) Flr; 27) Pyr d_{10}; 28) Pyr; 29) 2,4'-DDE; 30) PCB 101; 31) α-endsf; 32) BPA d_{16}; 33) BPA; 34) 4,4'-DDE; 35) 2,4'-DDD; 36) β-endsf; 37) PCB 118; 38) BDE 28; 39) 4,4'-DDD; 40) 2,4'-DDT; 41) NPEO$_2$; 42) PCB 138; 43) 4,4'-DDT $^{13}C_{12}$; 44) 4,4'-DDT; 45) BBP; 46) PCB 153; 47) DEHP; 48) B(a)A; 49) Chr; 50) PCB 180; 51) BDE 47: 52) DBP; 53) BDE 100; 54) B(b)F; 55) B(k)F; 56) BDE 99; 57) B(a)P d_{12}; 58) B(a)P; 59) PCB 209; 60) BDE 154; 61) BDE 153; 62) I(cd)P; 63) D(ah)P; 64) B(ghi)P d_{12}; 65) B(ghi)P

2.3 Aplicación de los métodos desarrollados

La metodología A, SPE-GC-EI-MS, se aplicó para determinar OMPs prioritarios y emergentes en 13 muestras puntuales de aguas residuales urbanas, industriales o mixtas, tomadas a lo largo del recorrido de la red de alcantarillado de la Comarca del Maresme en Cataluña, así como 3 muestras compuestas (de 8 h) del influente y 3 del efluente de la EDAR de Mataró. Se realizó una estimación de la descarga diaria de contaminantes al Mar Mediterráneo y se calcularon los porcentajes de eliminación de los contaminantes analizados en la EDAR. Además se discutieron los datos con respecto a las implicaciones ambientales que tendrían estas descargas y finalmente se empleó el método para analizar agua subterránea de un pozo cercano a la red de tuberías para comprobar si existe alguna fuga que afecte la calidad de esta agua. Este trabajo se discute más adelante en el Artículo Científico 1 denominado **“Determination and occurrence of phthalates, alkylphenols, bisphenol A, PBDEs, PCBs and PAHs in an industrial sewage grid discharging to a Municipal Wastewater Treatment Plant”.**

La metodología B, SBSE-GC-EI-MS, se utilizó para determinar la carga de contaminantes orgánicos y prioritarios en 12 muestras de agua de mar recolectadas durante el mes de octubre del 2007 en la costa de Cataluña. Seis de estas muestras fueron colectadas en zonas de playa, 5 en puertos y la última en la zona de alimentación de la planta de desalinización del Llobregat. Las concentraciones detectadas fueron discutidas en relación a los valores legislados (NCA) en Europa o en Estados Unidos (EPA), así como su posible impacto ambiental. Este trabajo es presentado en el Artículo Científico 2 denominado **“Stir bar sorptive extraction-thermal desorption-gas chromatography-mass spectrometry: An effective tool for determining persistent organic pollutants and nonylphenol in coastal waters in compliance with existing Directives”.**

El desarrollo y validación de la metodología C consistente en SPE para muestras acuosas y UAE para muestras sólidas y análisis por GC-EI-MS/MS se detalla en el Artículo Científico 3 que lleva por título “**Development of a multi-residue method for the determination of organic micropollutants in water, sediment and mussels using gas chromatography-tandem mass spectrometry**.

ARTÍCULO CIENTÍFICO 1.

“Determination and occurrence of phthalates, alkylphenols, bisphenol A, PBDEs, PCBs and PAHs in an industrial sewage grid discharging to a Municipal Wastewater Treatment Plant”

Juan Sánchez-Avila, Jordi Bonet, Gemma Velasco, Silvia Lacorte.

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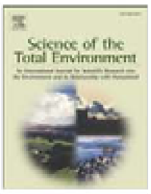

Determination and occurrence of phthalates, alkylphenols, bisphenol A, PBDEs, PCBs and PAHs in an industrial sewage grid discharging to a Municipal Wastewater Treatment Plant

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ABSTRACT

Industrial and urban discharges release organic contaminants which might affect the quality of receiving waters if not properly eliminated in Wastewater Treatment Plants (WWTP). This study is aimed to evaluate the source, transport and fate of contaminants of industrial origin in a sewage grid discharging to a WWTP and finally to the sea. The sampling network covered an industrial and urban area and wastewaters, influents and effluents of a WWTP were analyzed using a newly developed multiresidual method to capture a wide range contaminants (phthalates, alkylphenols, bisphenol A, PBDEs, PCBs and PAHs). Alkylphenols and phthalates followed by PAHs were the main compounds detected at levels between 0.01 to 698 μg l^{-1} in the sewage pipelines. At the WWTP influent they were detected at concentrations up to 345 μg l^{-1}. The contaminant load was eliminated in a 64–92% during the primary and secondary treatment of the plant. However, alkylphenols, phthalates bisphenol A and traces of PAHs were discharged with the effluent, producing a total net input of 825 g d^{-1} to the sea. The study of wastewaters herein proposed can be used to better predict the loads into WWTP to improve treatment conditions according to specific sewage inputs and to assess the risks associated with the continuous discharge of contaminants to receiving plants.

1. Introduction

Wastewaters receive the input of industrial and urban effluents and thus contain a cocktail of organic contaminants originated in raw materials and everyday products (Marttinen et al., 2003) which may be environmentally toxic or recalcitrant. Wastewaters are released from industries or urban areas in little pipelines which are merged in larger pipelines to finally be treated in Wastewater Treatment Plants (WWTPs), although sometimes they are discharged untreated to receiving waters. Several contaminants have been identified in wastewaters, such as alkylphenols (APs) (Vogelsang et al., 2006; Céspedes et al., 2005), phthalates (Céspedes et al., 2004; Fauser et al., 2003), phenols (Lacorte et al., 1999a), anilines (Lacorte et al., 1999b), polycyclic aromatic hydrocarbons (PAHs) (Vogelsang et al., 2006; Manoli and Samara, 1996), flame retardants (Richardson, 2008), among others. Unless removed by WWTPs, their presence in receiving waters is associated with toxicological effects at the μg l^{-1} levels, producing endocrine disruption in marine organisms (Porte et al., 2006), neurotoxicity (Tiffany-Castiglioni et al., 2006), and alterations at the ecosystem level (Chapman, 2004). For such reason, there is an increasing need to determine the source and occurrence of organic contaminants in wastewaters to estimate their inputs towards WWTPs, optimize treatment conditions in order to minimize the release of such toxic substances and finally ensure an effluent with a low contamination load.

Among the different families of pollutants, we have centered in those compounds that have high incidence in wastewaters and are being legislated in Europe. Directive 2008/105/EC (European Parliament and The Council, 2008) indicates hazardous compounds that should be monitored in surface waters, including diethylhexyl phthalate (DEHP), nonylphenol (NP), polychlorinated biphenyls (PCBs) or PAHs and Directive 2006/11/CE (European Parliament and The Council, 2006) establishes a list of compounds to be monitored in discharges and mentions some measures to protect the aquatic environment. This last Directive obliges the pertinent authority to draw up an inventory and authorize the discharges of certain hazardous substances into waters (seas and rivers). The substances of concern are grouped in List I that includes dangerous and hazardous compounds to be eliminated from discharges, including sewers and List II where dangerous substances should be diminished in discharges. Concerning this regulation, it is necessary to develop reliable and multiresidual methodologies to analyze a wide range of pollutants of different physico-chemical characteristics in wastewaters and discharges.

Among water types, wastewater analysis implies specific and sensitive methodologies to eliminate the great amount of organic

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matter, oils, metals, and faecal bacteria that may interfere with the analysis of target compounds. Several analytical methods have been developed to determine organic contaminants in wastewaters, and often they are directed to specific chemical families. Solid phase microextraction provides an efficient way to extract organic compounds without coextracting matrix interferences and has been used to determine phthalates and phenols in wastewaters, with great efficiency (Popp et al., 2000). Other methods imply solid phase matrix dispersion (Kočí et al., 2004) or stir bar sorptive extraction has been proven as an efficient way to recover several endocrine disrupters from wastewaters (Peñalver et al., 2003). Our study is based in the use of solid phase extraction (SPE) with Oasis 60 mg (3 cc) cartridges, which unlike other more specific methods have the capability to extract a wide range of contaminants of different physico-chemical properties in a single step.

The purpose of this study was (i) to optimize and validate an extraction and analytical method to determine priority organic contaminants (PAHs, PCBs, polybromodiphenyl ethers (PBDEs), APs, phthalates and bisphenol A (BPA)) in wastewaters considering both the dissolved and particulate phases, (ii) to study target compounds in wastewaters collected in a radial distribution of a sewage grid discharging into a municipal WWTP to estimate the source, transport, elimination and mass flow of contaminants to receiving seawater and (iii) to evaluate the potential lixiviation of industrial contaminants to groundwater. The area studied has important industrial activities such textiles (printing, dying, whitening, and finishing), production of dyes, paints, inks, plastics, polymers, detergents, soaps, essences, refination of greases and oils, metallic anodizing and coating, and other activities like automotive garages, fuel wholesale/retail. Also in this area important urban settlements exist.

2. Materials and methods

2.1. Chemicals and reagents

Sixteen Environmental Protection Agency (EPA) PAHs, six phthalates, seven PCBs, eight PBDEs, four APs and BPA were analyzed (Table 1). PAHs were purchased from AccuStandard (New Haven, CT, USA) as a solution mix at 1000 µg l^{-1} in methanol; phthalates were from Supelco (Bellefonte, PA, USA) as a mix solution at 500 µg l^{-1} in methanol; PCBs were from Dr. Ehrenstorfer (Augsburg, Germany) as a mix solution at 10 µg l^{-1} in iso-octane; PBDEs were from Cambridge Isotope Laboratories (Andover, MA, USA) as a mix solution at 10 µg l^{-1} in nonane; BPA was from Sigma-Aldrich (St. Louis, MO, USA) as a solid; nonylphenol (NP) was from Riedel-de Haën (Seelze, Germany) as a solid technical mixture of isomers; 4-nonylphenol-mono-ethoxylate

Table 1
Compounds studied, acronym, formula, and physicochemical and toxicological properties of the compounds considered in this study.

Compound	Acronym	Molecular formula	Log K_{ow}	Aquatic toxicity [a] (µg l^{-1})	Substance considered on List I or II [b]	Legislated level [c] (µg l^{-1})
Naphtalene	Nap	$C_{10}H_8$	3.3	2305	II	2.4
Acenaphtylene	Acy	$C_{12}H_8$	3.94	nf	II	nl
Acenaphthene	Ace	$C_{12}H_{10}$	3.92	1275	II	nl
Fluorene	Flu	$C_{13}H_{10}$	4.18	430	II	nl
Phenantrene	Phe	$C_{14}H_{10}$	4.46	383	II	nl
Anthracene	Ant	$C_{14}H_{10}$	4.45	95	II	0.4
Fluoranthene	Flr	$C_{16}H_{10}$	5.16	35	II	1
Pyrene	Pyr	$C_{16}H_{10}$	4.88	20	II	nl
Benzo[a]anthracene	B[a]A	$C_{18}H_{12}$	5.664	nf	II	nl
Crysene	Chr	$C_{18}H_{12}$	5.81	nf	II	nl
Benzo[b]fluoranthene	B[b]F	$C_{20}H_{12}$	5.78	nf	I	$\Sigma_{BbF+BkF}$
Benzo[k]fluoranthene	B[k]F	$C_{20}H_{12}$	6.11	nf	I	=0.03
Benzo[a]pyrene	B[a]P	$C_{20}H_{12}$	6.13	nf	I	0.1
Indeno[1,2,3-c,d]pyrene	I[cd]P	$C_{22}H_{12}$	6.7	nf	I	$\Sigma_{IcdP+BghiP}$
Benzo[g,h,i]perylene	B[ghi]P	$C_{22}H_{12}$	6.63	nf	I	=0.002
Dibenzo[a,h]anthracene	D[ah]A	$C_{22}H_{12}$	6.75	nf	I	nl
Dimethyl phthalate	DMP	$C_{10}H_{10}O_4$	1.6	45,900	nl	nl
Diethyl phthalate	DEP	$C_{12}H_{14}O_4$	2.42	86,000	nl	nl
Di-n-butyl phthalate	DBP	$C_{16}H_{22}O_4$	4.5	2990	nl	nl
Benzylbutyl phthalate	BBP	$C_{19}H_{20}O_4$	4.73	960	nl	nl
Di(2-ethylhexyl) adipate	DEHA	$C_{22}H_{42}O_4$	6.11	nf	nl	nl
Di(2-ethylhexyl) phthalate	DEHP	$C_{24}H_{38}O_4$	7.6	160	II	1.3
Bisphenol A	BPA	$C_{16}H_{15}O_2$	3.32	1000–20,000	II	nl
2,4,4′-Trichlorobiphenyl	PCB 28	$C_{12}H_7Cl_3$	5.62	nf	I	nl
2,2′5,5′-Tetrachlorobiphenyl	PCB 52	$C_{12}H_6Cl_4$	6.09	nf	I	nl
2,2′4,5,5′-Pentachlorobiphenyl	PCB 101	$C_{12}H_5Cl_5$	6.8	nf	I	nl
2,3′4,4′,5-Pentachlorobiphenyl	PCB 118	$C_{12}H_5Cl_5$	7.12	nf	I	nl
2,2′,3,4,4′,5 Hexachlorobiphenyl	PCB 138	$C_{12}H_4Cl_6$	7.44	nf	I	nl
2,2′4,4′5,5′ Hexachlorobiphenyl	PCB 153	$C_{12}H_4Cl_6$	7.75	nf	I	nl
2,2′3,4,4′,5,5′ Heptachlorobiphenyl	PCB 180	$C_{12}H_3Cl_7$	8.27	nf	I	nl
2,4,4′-TriBDE	BDE 28	$C_{12}H_7Br_3O$	5.88	nf	I	0.0005
2,2′,4,4′-TetraBDE	BDE 47	$C_{12}H_6Br_4O$	6.77	nf	I	0.0005
2,2′,4,4′,5-PentaBDE	BDE 99	$C_{12}H_5Br_5O$	7.66	nf	I	0.0005
2,2′,4,4′,6-PentaBDE	BDE 100	$C_{12}H_5Br_5O$	7.66	nf	I	0.0005
2,2′,4,4′,5,5′-HexaBDE	BDE 153	$C_{12}H_4Br_6O$	8.55	nf	I	0.0005
2,2′,4,4′,5,6′-HexaBDE	BDE 154	$C_{12}H_4Br_6O$	8.55	nf	I	0.0005
2,2′,3,4,4′,5′,6-HeptaBDE	BDE 183	$C_{12}H_3Br_7O$	9.44	nf	I	nl
DecaBDE	BDE 209	$C_{12}Br_{10}O$	12.11	nf	I	nl
Octylphenol	OP	$C_{14}H_{22}O$	5.28	90–140	II	0.1
Nonylphenol	NP	$C_{15}H_{24}O$	5.92	93–470	I	2
Nonylphenol monoethoxilathed	NP_1EO	$C_{17}H_{28}O_2$	5.58	100	I	nl
Nonylphenol diethoxilathed	NP_2EO	$C_{19}H_{32}O_3$	5.3	100	I	nl

nf.: Not found; nl: not legislated.

[a] EC50 – 48 h, *Daphnia magna*.

[b] Acording Directive 2006/11/EC.

[c] Acording Directive 2008/105/EC (Annex I, other surface waters).

(NP_1EO), 4-nonylphenol-di-ethoxylate (NP_2EO) and BPA were from Dr. Ehrenstorfer (Augsburg, Germany) as a solid and 4-tert-octylphenol (OP) from Supelco (Bellefonte, PA, USA) as a solid. Stock standard solutions (100 mg l^{-1}) of each compound were prepared in absolute methanol and stored in the dark at −20 °C until use, remaining stable for at least three months. These solutions were used to spike the water samples and draw the calibration curves.

The surrogate standards (used for analytes quantification) for PAHs was a mix solution at 2000 µg l^{-1} in methanol containing naphthalene d_8, acenaphthene d_{10}, phenanthene d_{10}, chrysene d_{12}, perylene d_{12} from Supelco (USA); phthalates surrogate standard was dipropylphthalate-3,4,5,6-d_4 from Riedel-de Haën (Seelze, Germany), purchased as a solid; PCBs and PBDEs surrogate standard was PCB 209 from Dr. Ehrenstorfer (Augsburg, Germany), used as a solution at 10 µg l^{-1} in iso-octane; alkylphenols surrogate standard was 4-n-nonylphenol-d_8 from Dr. Ehrenstorfer (Augsburg, Germany) as a solution at 100 µg l^{-1} in acetone; BPA surrogate was BPA-d_{16} from Sigma Aldrich (St. Louis, MO, USA) as a solid; anthracene d_{10} used as internal standard, from Dr. Ehrenstrofer as a solution of 10 µg l^{-1} in cyclohexane.

Solvents used were HPLC grade supplied by Merck (Darmstad, Germany). Different solid phase extraction (SPE) cartridges were evaluated: ENV+ (1 g, International Sorbent Technology, UK), Supelclean LC-18 (500 mg from Supelco, USA) and Oasis HLB (60 mg, from Waters, USA). Nitrogen for drying with 99.995% of purity was from Air Liquid (Barcelona, Spain).

2.2. Sample collection

Industrial, urban and mixed wastewater samples from the area of Maresme (Catalonia, Spain) were collected directly from sewers in 14 selected points of the sewage grid. Fig. 1 describes the distribution of each sampling point in the sewage grid discharging to the Mataró's WWTP, their fluxes and wastewater type. In this heavily industrialized area, wastewater is conduced to the WWTP by 3 branches (A, B and C). Pipe A1 and A2 collect wastewater from Vilassar de Mar and Cabrera de Mar and flow through branch A to the WWTP. Pipelines B1, B2, B3 and B4 collect wastewater from Dosrius and Orrius and merge in B5 with Argentona's wastewater. B7 collects Cabrera del Mar's wastewater and

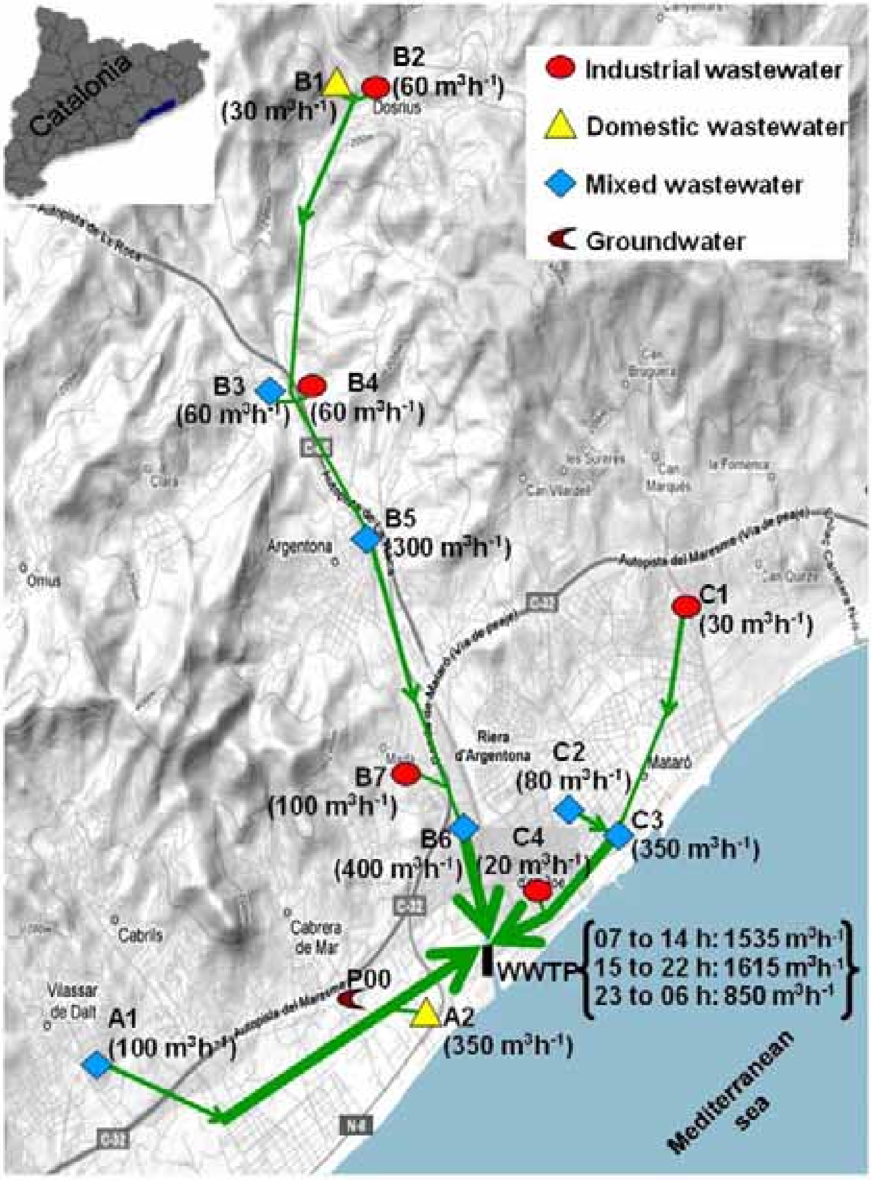
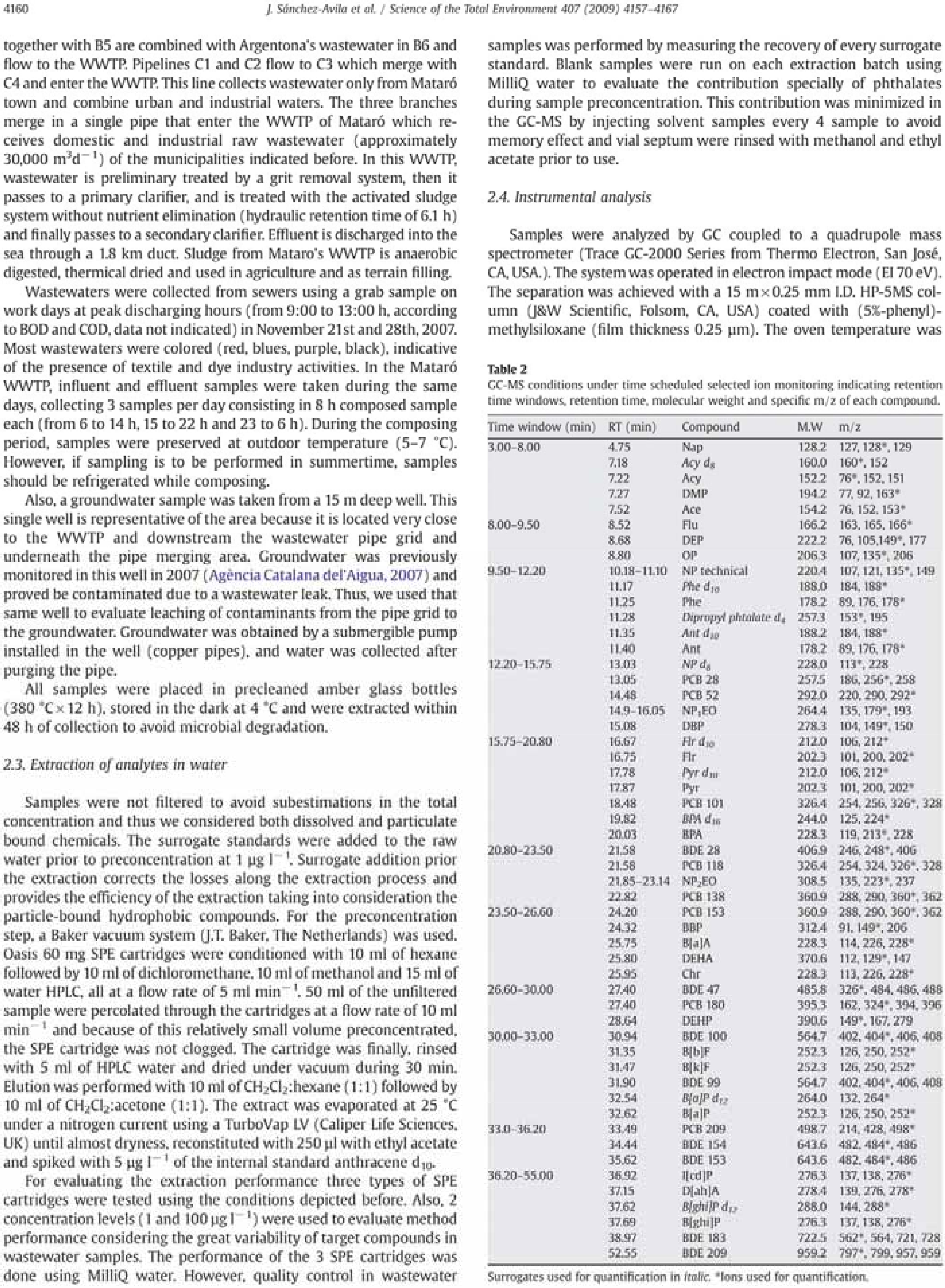


Fig. 1. Sampling points distribution in the piping net, fluxes ($m^3\ h^{-1}$) and water type at each point.



together with B5 are combined with Argentona's wastewater in B6 and flow to the WWTP. Pipelines C1 and C2 flow to C3 which merge with C4 and enter the WWTP. This line collects wastewater only from Mataró town and combine urban and industrial waters. The three branches merge in a single pipe that enter the WWTP of Mataró which receives domestic and industrial raw wastewater (approximately 30,000 m^3d^{-1}) of the municipalities indicated before. In this WWTP, wastewater is preliminary treated by a grit removal system, then it passes to a primary clarifier, and is treated with the activated sludge system without nutrient elimination (hydraulic retention time of 6.1 h) and finally passes to a secondary clarifier. Effluent is discharged into the sea through a 1.8 km duct. Sludge from Mataro's WWTP is anaerobic digested, thermical dried and used in agriculture and as terrain filling.

Wastewaters were collected from sewers using a grab sample on work days at peak discharging hours (from 9:00 to 13:00 h, according to BOD and COD, data not indicated) in November 21st and 28th, 2007. Most wastewaters were colored (red, blues, purple, black), indicative of the presence of textile and dye industry activities. In the Mataró WWTP, influent and effluent samples were taken during the same days, collecting 3 samples per day consisting in 8 h composed sample each (from 6 to 14 h, 15 to 22 h and 23 to 6 h). During the composing period, samples were preserved at outdoor temperature (5–7 °C). However, if sampling is to be performed in summertime, samples should be refrigerated while composing.

Also, a groundwater sample was taken from a 15 m deep well. This single well is representative of the area because it is located very close to the WWTP and downstream the wastewater pipe grid and underneath the pipe merging area. Groundwater was previously monitored in this well in 2007 (Agència Catalana del'Aigua, 2007) and proved be contaminated due to a wastewater leak. Thus, we used that same well to evaluate leaching of contaminants from the pipe grid to the groundwater. Groundwater was obtained by a submergible pump installed in the well (copper pipes), and water was collected after purging the pipe.

All samples were placed in precleaned amber glass bottles (380 °C × 12 h), stored in the dark at 4 °C and were extracted within 48 h of collection to avoid microbial degradation.

2.3. Extraction of analytes in water

Samples were not filtered to avoid subestimations in the total concentration and thus we considered both dissolved and particulate bound chemicals. The surrogate standards were added to the raw water prior to preconcentration at 1 $\mu g\ l^{-1}$. Surrogate addition prior the extraction corrects the losses along the extraction process and provides the efficiency of the extraction taking into consideration the particle-bound hydrophobic compounds. For the preconcentration step, a Baker vacuum system (J.T. Baker, The Netherlands) was used. Oasis 60 mg SPE cartridges were conditioned with 10 ml of hexane followed by 10 ml of dichloromethane, 10 ml of methanol and 15 ml of water HPLC, all at a flow rate of 5 ml min^{-1}. 50 ml of the unfiltered sample were percolated through the cartridges at a flow rate of 10 ml min^{-1} and because of this relatively small volume preconcentrated, the SPE cartridge was not clogged. The cartridge was finally, rinsed with 5 ml of HPLC water and dried under vacuum during 30 min. Elution was performed with 10 ml of CH_2Cl_2:hexane (1:1) followed by 10 ml of CH_2Cl_2:acetone (1:1). The extract was evaporated at 25 °C under a nitrogen current using a TurboVap LV (Caliper Life Sciences, UK) until almost dryness, reconstituted with 250 µl with ethyl acetate and spiked with 5 $\mu g\ l^{-1}$ of the internal standard anthracene d_{10}.

For evaluating the extraction performance three types of SPE cartridges were tested using the conditions depicted before. Also, 2 concentration levels (1 and 100 $\mu g\ l^{-1}$) were used to evaluate method performance considering the great variability of target compounds in wastewater samples. The performance of the 3 SPE cartridges was done using MilliQ water. However, quality control in wastewater samples was performed by measuring the recovery of every surrogate standard. Blank samples were run on each extraction batch using MilliQ water to evaluate the contribution specially of phthalates during sample preconcentration. This contribution was minimized in the GC-MS by injecting solvent samples every 4 sample to avoid memory effect and vial septum were rinsed with methanol and ethyl acetate prior to use.

2.4. Instrumental analysis

Samples were analyzed by GC coupled to a quadrupole mass spectrometer (Trace GC-2000 Series from Thermo Electron, San José, CA, USA.). The system was operated in electron impact mode (EI 70 eV). The separation was achieved with a 15 m × 0.25 mm I.D. HP-5MS column (J&W Scientific, Folsom, CA, USA) coated with (5%-phenyl)-methylsiloxane (film thickness 0.25 µm). The oven temperature was

Table 2
GC-MS conditions under time scheduled selected ion monitoring indicating retention time windows, retention time, molecular weight and specific m/z of each compound.

Time window (min)	RT (min)	Compound	M.W	m/z
3.00–8.00	4.75	Nap	128.2	127, 128*, 129
	7.18	*Acy* d_8	160.0	160*, 152
	7.22	Acy	152.2	76*, 152, 151
	7.27	DMP	194.2	77, 92, 163*
	7.52	Ace	154.2	76, 152, 153*
8.00–9.50	8.52	Flu	166.2	163, 165, 166*
	8.68	DEP	222.2	76, 105,149*, 177
	8.80	OP	206.3	107, 135*, 206
9.50–12.20	10.18–11.10	NP technical	220.4	107, 121, 135*, 149
	11.17	*Phe* d_{10}	188.0	184, 188*
	11.25	Phe	178.2	89, 176, 178*
	11.28	*Dipropyl phtalate* d_4	257.3	153*, 195
	11.35	*Ant* d_{10}	188.2	184, 188*
	11.40	Ant	178.2	89, 176, 178*
12.20–15.75	13.03	*NP* d_8	228.0	113*, 228
	13.05	PCB 28	257.5	186, 256*, 258
	14.48	PCB 52	292.0	220, 290, 292*
	14.9–16.05	NP_1EO	264.4	135, 179*, 193
	15.08	DBP	278.3	104, 149*, 150
15.75–20.80	16.67	*Flr* d_{10}	212.0	106, 212*
	16.75	Flr	202.3	101, 200, 202*
	17.78	*Pyr* d_{10}	212.0	106, 212*
	17.87	Pyr	202.3	101, 200, 202*
	18.48	PCB 101	326.4	254, 256, 326*, 328
	19.82	*BPA* d_{16}	244.0	125, 224*
	20.03	BPA	228.3	119, 213*, 228
20.80–23.50	21.58	BDE 28	406.9	246, 248*, 406
	21.58	PCB 118	326.4	254, 324, 326*, 328
	21.85–23.14	NP_2EO	308.5	135, 223*, 237
	22.82	PCB 138	360.9	288, 290, 360*, 362
23.50–26.60	24.20	PCB 153	360.9	288, 290, 360*, 362
	24.32	BBP	312.4	91, 149*, 206
	25.75	B[a]A	228.3	114, 226, 228*
	25.80	DEHA	370.6	112, 129*, 147
	25.95	Chr	228.3	113, 226, 228*
26.60–30.00	27.40	BDE 47	485.8	326*, 484, 486, 488
	27.40	PCB 180	395.3	162, 324*, 394, 396
	28.64	DEHP	390.6	149*, 167, 279
30.00–33.00	30.94	BDE 100	564.7	402, 404*, 406, 408
	31.35	B[b]F	252.3	126, 250, 252*
	31.47	B[k]F	252.3	126, 250, 252*
	31.90	BDE 99	564.7	402, 404*, 406, 408
	32.54	*B[a]P* d_{12}	264.0	132, 264*
	32.62	B[a]P	252.3	126, 250, 252*
33.0–36.20	33.49	PCB 209	498.7	214, 428, 498*
	34.44	BDE 154	643.6	482, 484*, 486
	35.62	BDE 153	643.6	482, 484*, 486
36.20–55.00	36.92	I[cd]P	276.3	137, 138, 276*
	37.15	D[ah]A	278.4	139, 276, 278*
	37.62	*B[ghi]P* d_{12}	288.0	144, 288*
	37.69	B[ghi]P	276.3	137, 138, 276*
	38.97	BDE 183	722.5	562*, 564, 721, 728
	52.55	BDE 209	959.2	797*, 799, 957, 959

Surrogates used for quantification in *italic*. *Ions used for quantification.

programmed from 70 °C (holding time 2 min) to 150 °C at 15 °C min^{-1}, to 200 °C at 3 °C min^{-1} and finally to 310 °C at 5 °C min^{-1}, keeping the final temperature for 9 min. Injection was performed in the splitless mode, keeping the split valve closed for 60 s. Helium was the carrier gas (50 ml min^{-1}). Injector, transfer line and ion source temperatures were 280 °C, 280 °C and 200 °C, respectively.

Peak detection and integration were carried out using Xcalibur software. For increased sensitivity and specificity, quantification was performed in time scheduled Selected Ion Monitoring (SIM) using the base peak and identification was done at three ions per compound (Table 2). Internal standard quantification was performed using the deuterated surrogate standards corresponding to each chemical family and PCB 209 was used to quantify both PCBs and PBDEs. The use of this compound as surrogate standard has been reported earlier (Samara et al., 2006) and its suitability has been proven. In our specific case, none of the main PCBs were detected in wastewater and thus, PCB 209 which is a minor PCB could be used. Calibrations curves were constructed for all target compounds over a concentration range of 0.05 to 10 mg l^{-1}.

3. Results and discussion

3.1. Performance of method

The optimization of the extraction method and chromatographic separation was necessary to obtain the best detection limits and recoveries for all target compound, specially when the method developed include a mixture of compounds with a wide range of physicochemical properties, polars and apolar substances with differences in K_{ow} from 1.6 to 12.11. GC-MS is an appropriated technique for determination of PAHs, PBDEs, phthalates, BPA and PCBs (Santos and Galceran, 2003) although APEOs are generally derivatized to increase their volatility and decrease their interaction with the stationary phase (Jeannot et al., 2002). We used GC-MS with a 15 m column to minimize the interaction of APEOs with the stationary phase and avoid BDE 209 degradation in the GC column (Fig. 2). The 42 target compounds and their surrogates could be resolved in 55 min (Fig. 2) although coelutions of two or more compounds appeared, such as PCB 52 and DBPH which coeluted with NP_1EO, BDE 28 with PCB 118, NP_2EO with PCB 138, and BDE 47 with PCB 180. Coeluted compounds could however be resolved by specific MS ions using the SIM mode given their different mass spectrum (Table 2).

Wastewater is a matrix containing a wide variety of interferences which might affect the detection of the target analytes. In addition, target analytes may be present in a wide range of concentrations in wastewater, so it is very important to ensure a high sensitivity, robustness and versatility of the method. Calibrations curves provided good linearity ($r^2 = 0.981–0.999$) for all compounds over a concentration range from 0.05 to 10 mg l^{-1}, except for Nap, Acy, DMP and DEHP (linear range between 0.05 to 7.5 mg l^{-1}). Under the acquisition conditions used, instrumental limits of detection (IDL) were between 0.08 and 15 pg, which ensured the low level detection of target compounds. We were interested in determining the total concentration of contaminants in the sample, not only the ones dissolved because that would produce a subestimation of the total concentration of analytes in water. Thus, samples were not filtered and the method developed using Oasis HLB was aimed to recover all compounds, from the lightest (Nap) to the heavier (BDE 209). The recoveries were efficient for most of them at the concentration target analytes are found in wastewater (from 1 to 100 μg l^{-1}). This was because we spiked the samples with surrogates prior to extraction to correct losses during the extraction process and that the solvent mix (Hexane/CH_2Cl_2 1:1 and Acetone/CH_2Cl_2 1:1) used in the method covered a wide range of polarity to extract the multianalytes retained in the solid phase sorbent and those ones adsorbed in the particulate matter. Commonly used solvents for this type of compounds in wastewaters are mixtures of hexane/CH_2Cl_2 (1:1) (Aparicio et al., 2007; Samara et al., 2006) or acetone/CH_2Cl_2 (1:1) (Cai et al., 2007; Busetti et al., 2006; Fauser et al., 2003), which yield acceptable recoveries. Initially, we tested hexane/CH_2Cl_2 and acetone/CH_2Cl_2 mixtures to recover target compounds in water spiked at 1 μg l^{-1} using 3 commercial SPE sorbents (ENV+, Supelclean LC-18 and Oasis

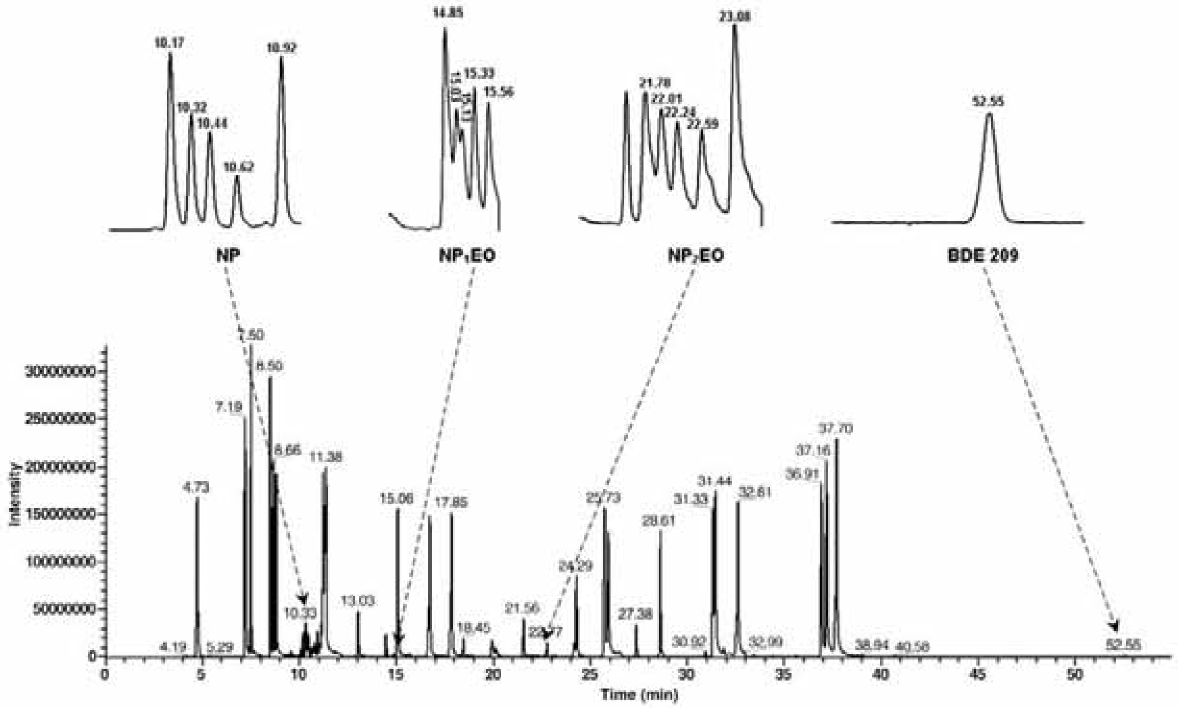
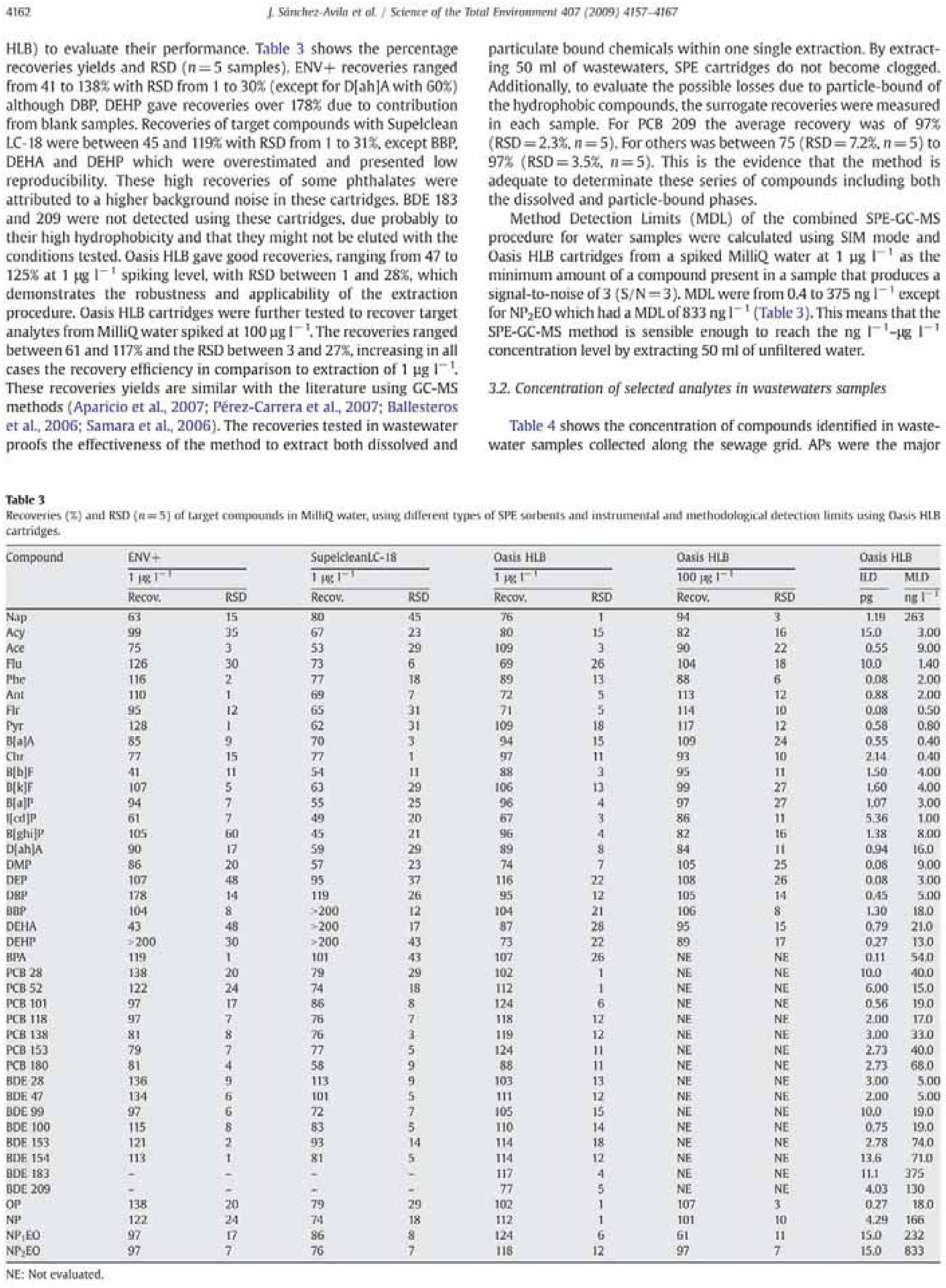


Fig. 2. SPE-GC-MS TIC chromatogram obtained for MilliQ water fortified with the target compounds at 1 μg l^{-1}.



HLB) to evaluate their performance. Table 3 shows the percentage recoveries yields and RSD ($n=5$ samples). ENV+ recoveries ranged from 41 to 138% with RSD from 1 to 30% (except for D[ah]A with 60%) although DBP, DEHP gave recoveries over 178% due to contribution from blank samples. Recoveries of target compounds with Supelclean LC-18 were between 45 and 119% with RSD from 1 to 31%, except BBP, DEHA and DEHP which were overestimated and presented low reproducibility. These high recoveries of some phthalates were attributed to a higher background noise in these cartridges. BDE 183 and 209 were not detected using these cartridges, due probably to their high hydrophobicity and that they might not be eluted with the conditions tested. Oasis HLB gave good recoveries, ranging from 47 to 125% at 1 μg l^{-1} spiking level, with RSD between 1 and 28%, which demonstrates the robustness and applicability of the extraction procedure. Oasis HLB cartridges were further tested to recover target analytes from MilliQ water spiked at 100 μg l^{-1}. The recoveries ranged between 61 and 117% and the RSD between 3 and 27%, increasing in all cases the recovery efficiency in comparison to extraction of 1 μg l^{-1}. These recoveries yields are similar with the literature using GC-MS methods (Aparicio et al., 2007; Pérez-Carrera et al., 2007; Ballesteros et al., 2006; Samara et al., 2006). The recoveries tested in wastewater proofs the effectiveness of the method to extract both dissolved and particulate bound chemicals within one single extraction. By extracting 50 ml of wastewaters, SPE cartridges do not become clogged. Additionally, to evaluate the possible losses due to particle-bound of the hydrophobic compounds, the surrogate recoveries were measured in each sample. For PCB 209 the average recovery was of 97% (RSD = 2.3%, $n=5$). For others was between 75 (RSD = 7.2%, $n=5$) to 97% (RSD = 3.5%, $n=5$). This is the evidence that the method is adequate to determinate these series of compounds including both the dissolved and particle-bound phases.

Method Detection Limits (MDL) of the combined SPE-GC-MS procedure for water samples were calculated using SIM mode and Oasis HLB cartridges from a spiked MilliQ water at 1 μg l^{-1} as the minimum amount of a compound present in a sample that produces a signal-to-noise of 3 (S/N = 3). MDL were from 0.4 to 375 ng l^{-1} except for NP_2EO which had a MDL of 833 ng l^{-1} (Table 3). This means that the SPE-GC-MS method is sensible enough to reach the ng l^{-1}–μg l^{-1} concentration level by extracting 50 ml of unfiltered water.

3.2. Concentration of selected analytes in wastewaters samples

Table 4 shows the concentration of compounds identified in wastewater samples collected along the sewage grid. APs were the major

Table 3

Recoveries (%) and RSD ($n=5$) of target compounds in MilliQ water, using different types of SPE sorbents and instrumental and methodological detection limits using Oasis HLB cartridges.

Compound	ENV+		SupelcleanLC-18		Oasis HLB		Oasis HLB		Oasis HLB	
	1 μg l^{-1}		1 μg l^{-1}		1 μg l^{-1}		100 μg l^{-1}		IID	MLD
	Recov.	RSD	Recov.	RSD	Recov.	RSD	Recov.	RSD	pg	ng l^{-1}
Nap	63	15	80	45	76	1	94	3	1.19	263
Acy	99	35	67	23	80	15	82	16	15.0	3.00
Ace	75	3	53	29	109	3	90	22	0.55	9.00
Flu	126	30	73	6	69	26	104	18	10.0	1.40
Phe	116	2	77	18	89	13	88	6	0.08	2.00
Ant	110	1	69	7	72	5	113	12	0.88	2.00
Flr	95	12	65	31	71	5	114	10	0.08	0.50
Pyr	128	1	62	31	109	18	117	12	0.58	0.80
B[a]A	85	9	70	3	94	15	109	24	0.55	0.40
Chr	77	15	77	1	97	11	93	10	2.14	0.40
B[b]F	41	11	54	11	88	3	95	11	1.50	4.00
B[k]F	107	5	63	29	106	13	99	27	1.60	4.00
B[a]P	94	7	55	25	96	4	97	27	1.07	3.00
I[cd]P	61	7	49	20	67	3	86	11	5.36	1.00
B[ghi]P	105	60	45	21	96	4	82	16	1.38	8.00
D[ah]A	90	17	59	29	89	8	84	11	0.94	16.0
DMP	86	20	57	23	74	7	105	25	0.08	9.00
DEP	107	48	95	37	116	22	108	26	0.08	3.00
DBP	178	14	119	26	95	12	105	14	0.45	5.00
BBP	104	8	>200	12	104	21	106	8	1.30	18.0
DEHA	43	48	>200	17	87	28	95	15	0.79	21.0
DEHP	>200	30	>200	43	73	22	89	17	0.27	13.0
BPA	119	1	101	43	107	26	NE	NE	0.11	54.0
PCB 28	138	20	79	29	102	1	NE	NE	10.0	40.0
PCB 52	122	24	74	18	112	1	NE	NE	6.00	15.0
PCB 101	97	17	86	8	124	6	NE	NE	0.56	19.0
PCB 118	97	7	76	7	118	12	NE	NE	2.00	17.0
PCB 138	81	8	76	3	119	12	NE	NE	3.00	33.0
PCB 153	79	7	77	5	124	11	NE	NE	2.73	40.0
PCB 180	81	4	58	9	88	11	NE	NE	2.73	68.0
BDE 28	136	9	113	9	103	13	NE	NE	3.00	5.00
BDE 47	134	6	101	5	111	12	NE	NE	2.00	5.00
BDE 99	97	6	72	7	105	15	NE	NE	10.0	19.0
BDE 100	115	8	83	5	110	14	NE	NE	0.75	19.0
BDE 153	121	2	93	14	114	18	NE	NE	2.78	74.0
BDE 154	113	1	81	5	114	12	NE	NE	13.6	71.0
BDE 183	–	–	–	–	117	4	NE	NE	11.1	375
BDE 209	–	–	–	–	77	5	NE	NE	4.03	130
OP	138	20	79	29	102	1	107	3	0.27	18.0
NP	122	24	74	18	112	1	101	10	4.29	166
NP_1EO	97	17	86	8	124	6	61	11	15.0	232
NP_2EO	97	7	76	7	118	12	97	7	15.0	833

NE: Not evaluated.

Table 4
Concentration of target compounds found in each sampling point (μg l^{-1}).

Compound	P00	A-1	A-2	B-1	B-2	B-3	B-4	B-5	B-6	B-7	C-1	C-2	C-3	C-4	Influent [a]	Effluent [a]
Nap	0.40	0.50	b.d.l.	b.d.l.	8.00	7.09	11.0	5.19	6.96	38.5	12.6	20.8	b.d.l.	0.87	4.50 ± 3.0	3.49 ± 1.8
Acy	b.d.l.	1.69	2.15	1.57	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	1.26	1.46	3.64	1.93	b.d.l.	5.05 ± 2.0	b.d.l.
Ace	b.d.l.	b.d.l.	b.d.l.	b.d.l.	1.00	b.d.l.	0.70	b.d.l.	b.d.l.	4.27	0.56	b.d.l.	b.d.l.	b.d.l.	0.03 ± 0.1	b.d.l.
Flu	0.12	0.45	0.27	0.22	1.77	2.80	0.22	1.42	2.33	13.3	0.87	0.69	0.19	0.17	1.15 ± 0.7	0.20 ± 0.1
Phe	b.d.l.	b.d.l.	b.d.l.	0.07	1.77	0.21	7.05	5.43	0.79	33.6	1.12	0.72	b.d.l.	b.d.l.	1.89 ± 2.4	b.d.l.
Ant	b.d.l.	b.d.l.	b.d.l.	b.d.l.	0.02	0.56	b.d.l.	1.86	1.98	1.33	0.18	b.d.l.	b.d.l.	b.d.l.	0.47 ± 1.0	b.d.l.
Flr	b.d.l.	0.05	0.09	0.08	3.28	0.30	0.04	0.42	0.36	0.79	0.86	0.35	0.12	0.12	0.27 ± 0.3	0.21 ± 0.2
Pyr	b.d.l.	b.d.l.	b.d.l.	0.05	12.4	1.74	b.d.l.	1.46	1.64	1.69	2.95	0.17	b.d.l.	b.d.l.	0.50 ± 0.7	0.007 ± 0.02
B[a]A	b.d.l.	b.d.l.	b.d.l.	b.d.l.	0.32	0.06	b.d.l.	0.11	0.64	b.d.l.	0.14	0.01	b.d.l.	0.02	0.05 ± 0.1	b.d.l.
Chr	b.d.l.	b.d.l.	b.d.l.	b.d.l.	2.89	0.56	b.d.l.	0.49	0.94	0.14	0.03	b.d.l.	b.d.l.	0.03	0.16 ± 0.1	b.d.l.
B[b]F	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	0.14 ± 0.1	b.d.l.
B[k]F	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	0.03 ± 0.06	b.d.l.
B[a]P	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	0.04 ± 0.01	b.d.l.
I[cd]P	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	0.009 ± 0.002	b.d.l.
Σ PAHs	**0.52**	**2.69**	**2.51**	**1.99**	**31.5**	**13.3**	**19.0**	**16.4**	**15.6**	**94.9**	**20.8**	**26.4**	**2.24**	**1.21**	**14.3**	**3.91**
DMP	0.12	0.32	1.12	0.45	2.90	1.37	0.15	1.76	0.95	0.74	7.05	0.28	4.94	0.17	0.60 ± 0.5	0.13 ± 0.02
DEP	b.d.l.	27.5	44.0	45.9	32.6	37.8	23.7	81.6	43.8	40.5	53.6	47.7	192	22.2	50.7 ± 19	49.8 ± 2.8
DBP	b.d.l.	b.d.l.	b.d.l.	b.d.l.	b.d.l.	28.7	b.d.l.	3.24	33.6	b.d.l.	b.d.l.	b.d.l.	16.9	b.d.l.	46.8 ± 15	b.d.l.
BBP	b.d.l.	b.d.l.	0.23	0.29	b.d.l.	0.13	b.d.l.	1.48	0.77	0.03	10.0	1.73	5.70	b.d.l.	0.67 ± 0.8	0.01 ± 0.03
DEHA	b.d.l.	1.28	1.79	3.00	1.70	1.27	1.48	1.41	1.40	1.59	1.98	2.41	24.1	1.30	6.47 ± 13	1.28 ± 0.5
DEHP	b.d.l.	9.88	20.2	19.5	35.7	15.5	12.8	43.0	258	8.00	36.4	19.6	287	7.47	47.9 ± 25	9.43 ± 8.4
Σ Phthalates	**0.12**	**39.0**	**67.3**	**69.1**	**72.9**	**84.8**	**38.1**	**133**	**339**	**50.9**	**109**	**71.7**	**531**	**31.1**	**153**	**60.7**
BPA	0.78	7.36	5.37	2.26	2.29	3.91	5.54	6.37	1.32	11.1	4.19	3.63	4.13	1.92	2.40 ± 0.7	0.62 ± 1.5
OP	b.d.l.	5.06	3.46	0.55	0.18	0.84	114	38.8	66.6	63.1	63.7	b.d.l.	176	b.d.l.	66.6 ± 64	53.8 ± 47
NP	b.d.l.	32.1	29.0	5.09	75.2	49.5	79.8	109	36.5	24.2	8.60	b.d.l.	193	43.2	102 ± 150	21.9 ± 32
NP_1EO	0.45	6.67	134	17.2	99.0	505	8.82	149	174	240	44.4	7.95	44.6	398	128 ± 58	47.7 ± 24
NP_2EO	b.d.l.	3.87	40.9	6.45	430	193	17.5	59.6	161	698	124	24.9	26.2	27.3	345 ± 75	12.6 ± 13
Σ APs	**0.45**	**47.7**	**207**	**29.3**	**604**	**748**	**220**	**356**	**438**	**1025**	**241**	**32.8**	**440**	**469**	**642**	**136**
Σ PBDEs	**b.d.l.**	**b.d.l.**	**b.d.l.**	**b.d.l.**	**b.d.l.**	**b.d.l.**	**b.d.l.**	**b.d.l.**	**b.d.l.**	**b.d.l.**	**b.d.l.**	**b.d.l.**	**b.d.l.**	**0.51**	**0.02**	**b.d.l.**

b.d.l.: Below detection limit.

[a] Averages and standard deviations ($n = 6$). Three sampling periods (6–14 h, 15–22 h and 23–6 h) in two sampling days.

compounds found, followed by phthalates, BPA and PAHs and finally PBDEs. No PCBs were detected. Table 5 compares the values detected in Mataro's WWTP influents with WWTP influents reported in other studies.

ΣAPs in industrial wastewaters (B2, B4, B7, C1, C4) ranged from 220 to 1025 μg l^{-1}, in mixed wastewaters (A1, B3, B5, B6, C2, C3) from 32.8 to 748 μg l^{-1}, and in domestic wastewaters (A2, B1) from 29.3 to 207 μg l^{-1}. Influents exhibited a concentration of 642 μg l^{-1}, much higher than in other WWTP influents of Catalonia where Céspedes et al. (2005) reported APs concentrations from 0.08 to 44.8 μg l^{-1}, considering only the dissolved fraction. Fauser et al. (2003) reported concentrations from 0.44 to 118 μg l^{-1} in a Danish WWTP influent. However, partitioning of OP and NP in the particulate phase of wastewaters has been identified thus indicating the need to monitor the particulate fraction to avoid subestimation of the total concentration (Céspedes et al., 2008). The elevated concentrations obtained in Maresme wastewaters evidence that alkylphenols-formuled products still are produced and used in industrial, domestic and agricultural formulations although the Directive 2003/53/EC (European Parliament and The Council, 2003) restricted their use in the EU at concentrations higher than 0.1%.

ΣPhthalate esters were found majority in mixed wastewaters at levels from 39 to 531 μg l^{-1}, in industrial wastewaters from 31.1 to 109 μg l^{-1}, in domestic wastewaters from to 67.3 to 69.1 μg l^{-1} and 153 μg l^{-1} in the influent. BPA ranged in industrial wastewaters from 1.92 to 11.1 μg l^{-1}, in mixed wastewaters from 1.32 to 7.36 μg l^{-1}, in domestic wastewaters from 2.26 to 5.37 μg l^{-1} and 2.40 μg l^{-1} in influent. In other Catalonian WWTP influents (domestic, industry and agriculture sources) concentrations from 0.07 to 14.1 μg l^{-1} were found

Table 5
Comparation of concentrations of pollutants in Mataro WWTP influent with WWTP influents reported in other studies.

Family compounds	Mataró	In other studies				
	Levels (μg l^{-1})	Levels (μg l^{-1})	Analytical method used	Dissolved (D) or particulate (P) phase	Area of study	Reference
Alkylphenols	66.2–345	0.08–44.8	HPLC-MS	D (filtered)	Llobregat (Catalonia)	Céspedes et al. (2005)
		0.10–17.5	HPLC-MS	D + P (filtered)	Ter (Catalonia)	Céspedes et al. (2008)
		0.44 –118	HPLC-MS	D + P (centrifugated)	Denmark (Roskilde)	Fauser et al. (2003)
		1.2–7.3	GC-MS	No phases, nor filtration or centrifugation was mentioned	Norway	Vogelsang et al. (2006)
Phthalate esters	0.60–50.7	3.33–14.08	HPLC-MS	D (filtered)	Llobregat (Catalonia)	Céspedes et al. (2005)
		0.07–35.4	HPLC-MS	D + P (centrifugated)	Denmark (Roskilde)	Fauser et al. (2003)
		0.18–188	GC-MS	D + P (not mentioned)	France (Paris)	Gasperi et al. (2008)
		1–122	GC-MS	No phases, nor filtration or centrifugation was mentioned	Finland	Marttinen et al. (2003)
		0.5–11	GC-MS	No phases, nor filtration or centrifugation was mentioned	Norway	Vogelsang et al. (2006)
BPA	2.40	0.09–6.98	HPLC-MS	D (filtered)	Llobregat (Catalonia)	Céspedes et al. (2005)
PAHs	0.009–5.05	0.2–5.2	GC-MS	D + P (No filtration or centrifugation was mentioned)	France (Paris)	Blanchard et al. (2004)
		3.56–4.62	HPLC-MS	D (filtered)	Italy (Venice)	Busetti et al. (2006)
		0.005–0.84	GC-MS	No phases, nor filtration or centrifugation was mentioned	Norway	Vogelsang et al. (2006)

(Céspedes et al., 2005). In Denmark levels from 0.07 to 35.4 µg l^{-1} were reported (Fauser et al., 2003); in a Paris sewer values were from 0.18 to 188 µg l^{-1} (Gasperi et al., 2008) and in Finland, from 1 to 122 µg l^{-1} (Marttinen et al., 2003). The large scale and widespread domestic and industrial use of phthalates and BPA including softeners in plastics, and additives in paints, glues and inks (Richardson, 2008; Aparicio et al., 2007) could explain the elevated amounts detected in these wastewaters, specially in those sampled directly from plastic and polymer industries discharges.

ΣPAHs in industrial wastewaters ranged from 1.21 to 94.9 µg l^{-1}, in mixed wastewaters from 2.69 to 26.4 µg l^{-1}, in domestic wastewaters from 1.99 to 2.51 µg l^{-1} and 14.3 µg l^{-1} in the WWTP influent. Values were higher compared to reported levels in WWTP influents of other countries. In a WWTP from Paris area, PAHs varied from 0.2 to 5.2 µg l^{-1} (Blanchard et al., 2004) and in an Italian WWTP from 3.56 to 4.62 µg l^{-1} (Busetti et al., 2006). The presence of PAHs in discharges can be originated by a wide range of sources like settled combustion, industrial processes or urban traffic (Busetti et al., 2006; Blanchard et al., 2004). In our case, lighter PAHs (Nap to Flr) were the most abundant compounds (Table 4), with Nap found at highest concentrations. Similar behavior was reported by Blanchard et al. (2004). Nap is used in many industrial processes as intermediate in the production of phthalic anhydre, Nap-sulfonate surfactants and in the preparation of synthetic dyes where hydrogenated Nap can be used as low volatility solvent (Preuss et al., 2003).

PBDEs, highly used as flame retardants, were only found in sample C4 and contained BDE 47 > BDE 183 > BDE 28 > BDE 100 = BDE 153 = BDE 154 > BDE 99 at a concentration between 0.03 and 0.14 µg l^{-1}. BDE 209 was found below quantification limit, although it is the main PBDE formulation used in Spain. PBDEs are likely trace constituents derived from products that contained the penta-BDE formulation. Even though penta-BDE has been removed from new products manufacturing in the EU, these products may have been in use for years, pre-dating bans (or from imported PBDE-containing products). The low concentration encountered was because PBDEs were diluted when merging with waters from the C pipeline. De Boer et al. (2003) reported high concentrations of PBDEs (including BDE 209) in a Dutch WWTP influent from textile industries, using a method based in Soxhlet extraction of particulate-matter. Given its elevated hydrophobicity, BDE 209 is strongly adsorbed to particulate matter (De Boer et al., 2003) and with the method used, concentrations of BDE 209 above 130 ng l^{-1} could only be detected.

Among all samples, B7 was the sampling site with the highest total concentration (1171 µg l^{-1}). This pipe line collects wastewater from an important industrial area with printing and textile activities and polymers, plastics and detergents production. Wastewater samples from this site were the most colored.

3.3. Fluxes of pollutants

Fig. 3 shows the fluxes of target compounds in pipelines (g h^{-1}). It was calculated by multiplying the concentration obtained by the estimated flux in m^3 h^{-1} (Fig. 1) at each sampling time. The pipes considered for the mass fluxes were those which directly discharge to the WWTP (A1–A2, B6, C3–C4) and considering the sampling period from 9 to 14 h (highest loads). The ΣPAHs of discharging pipelines was 8.38 g h^{-1} and the measured influent flow was 29.3 g h^{-1}. This increase of PAHs might be due to additional contamination from pumping lines of the WWTP. Contrarily, the flux of Σphthalates in the pipelines was 229 g h^{-1} and in the influent flow experimental values of 117 g h^{-1} were found. ΣAPs estimation from the pipelines was 416 g h^{-1} and in the influent was of 309 g h^{-1} and for BPA, 4.6 g h^{-1} in the pipeline and 3.8 g h^{-1} in the influent. Altogether, an average of 20% reduction was found during the transport of these contaminants through the pipeline to the WWTP influents. These variations in the concentrations were attributed to the several reactions that could occur during the transport of the pollutants in the sewage grid from the sampling points to the WWTP influent as volatilization, adsorption to particulate matter and degradation (Céspedes et al., 2008; Navarro et al., 2006).

3.4. Elimination in the WWTP and discharge to sea

One of the most important issues in relation to the discharge of WWTP effluents to receiving waters (lakes, rivers or sea) is the potential effect they might have upon flora and fauna. The APs daily removal efficiency in the Mataro's WWTP was of 80%. In a previous study, Céspedes et al. (2008) reported a removal efficiency of NP and

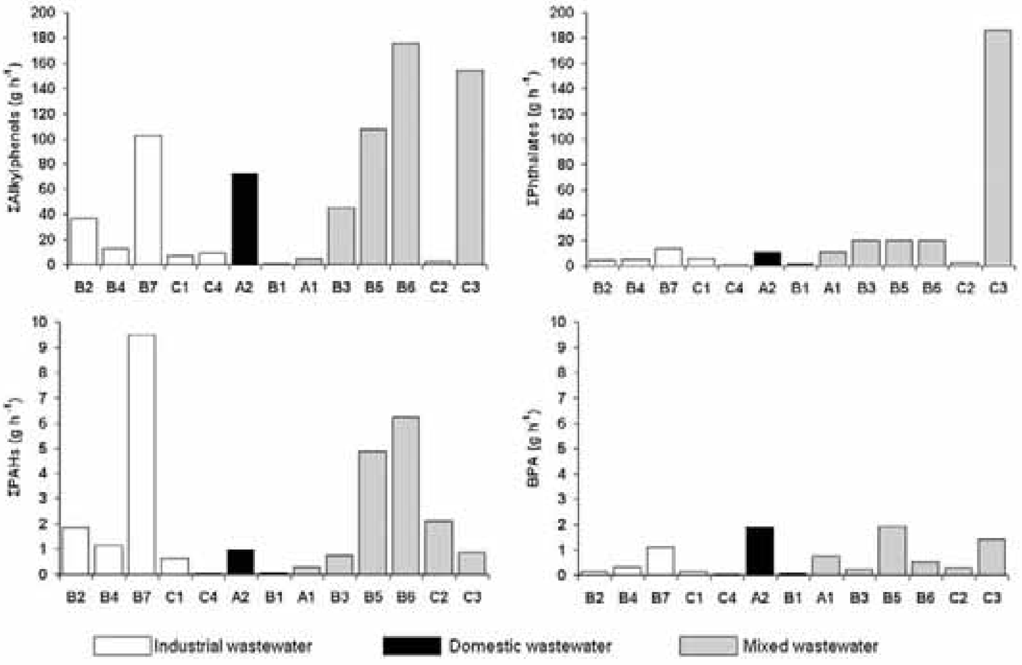


Fig. 3. Contaminants fluxes (g h^{-1}) at each sampling point through the sewage grid.

OP from 74 to 97% and for NPE$_{1-2}$O from 18 to 63% in 5 Catalonian WWTP, basically operated as Mataro's WWTP. In other studies from a Norwegian WWTPs, APs removal efficiencies varied from 60 to 90%, obtaining the best results in biological/chemical plants and the worst in chemical ones (Vogelsang et al., 2006). Although in most cases the removal efficiency is quite high, there is still a release of pollutants of potential high toxicity to the environment which might cause long term effects to the ecosystem. In our specific case, the estimated loads of target compounds into the NW Mediterranean Sea associated with the release of Mataró's WWTP effluent are shown in Fig. 4. OP was found in wastewaters influents at 66.6 µg l^{-1}, a concentration higher than NP. Generally, the presence of NP in wastewater predominates over OP when they are formed due to degradation of APEOs, where OP is found as an impurity (Céspedes et al., 2008; Tan et al., 2007; Céspedes et al., 2006). However, OP can be used as such in the production of phenolic resins, polymers, printing inks and pesticides formulations also water-based paints and electrical insulating varnishes (OSPAR Commission, 2006). The Mataro's wastewaters are highly influenced by textile, paints, inks, plastic, polymers, and metallic coating facilities where it is likely that OP based compounds are used. The little reduction of OP within the WWTP is due to their specific treatment conditions, which as in most cases, are not directed to eliminate this type of compounds. As a result of the treatment efficiency, a total amount of 573 g d^{-1} of APs is discharged to the sea, being OP a major constituent (250 g d^{-1}) followed by NP$_1$EO (189 g d^{-1}). These compounds are mainly discharged associated with the dissolved phase, since treatment eliminates most of the suspended solids that enter the WWTP.

Phthalates total daily removal efficiency in Mataro's WWTP was of 68%. Previous studies indicate that the removal efficiency for phthalates show wide variations depending on the type of treatment (Vogelsang et al., 2006; Fauser et al., 2003). However chemical and biological treatment eliminate from 50 to 60% in a Norwegian WWTP (Vogelsang et al., 2006). Considering the effluent flow rates from the Mataró's WWTP, the outflow of phthalates was estimated of 246 g d^{-1}, being DEP and DEHP the main contributors. The low removal of DEP and DEHP is basically attributed to DEP high solubility that may undergo treatment (Stales et al., 1997) while DEHP is inherently biodegradability under anaerobic conditions although its half life is estimated in 22 h (Fauser et al., 2003) versus the 6.1 h retention time in Mataró WWTP. Similar results were reported by Vogelsang et al. (2006), in WWTPs with similar retention times (6 h). In other Catalonian WWTP effluents, DEP and DEHP were detected in influents from 3.3 to 16.1 µg l^{-1} but no removal efficiency data was reported (Céspedes et al., 2006). BPA was detected at 2.4 µg l^{-1} in influents and the daily removal efficiency was of 68%, being the outflow of 3 g d^{-1}. In other Catalonian WWTP removal efficiencies from 30 to 90% were reported (Céspedes et al., 2006).

PAHs in influents varied from 0.009 to 5 µg l^{-1} but were the compounds released in lower amounts, indicating a high efficiency (92%) of the plant for removing these contaminants. Only Nap, Flu, Flr were discharged with the effluent producing a net amount of 5.6 g d^{-1} to the coastal waters. Although no studies were found for Catalan WWTP, 5 Norwegian WWTPs removal efficiencies ranged from 61 to 78% (chemical treatment) and 94 to 10% (chemical–biological treatment) and no removal was observed by mechanical treatment (Vogelsang et al., 2006).

The values encountered in Mataró's WWTP were similar in the 2 sampling dates performed (Table 3), indicating that although there are slight variations, the results do not vary considerably among sampling times and thus these estimates are preliminary to identify the potential environmental impact of wastewaters released to coastal areas.

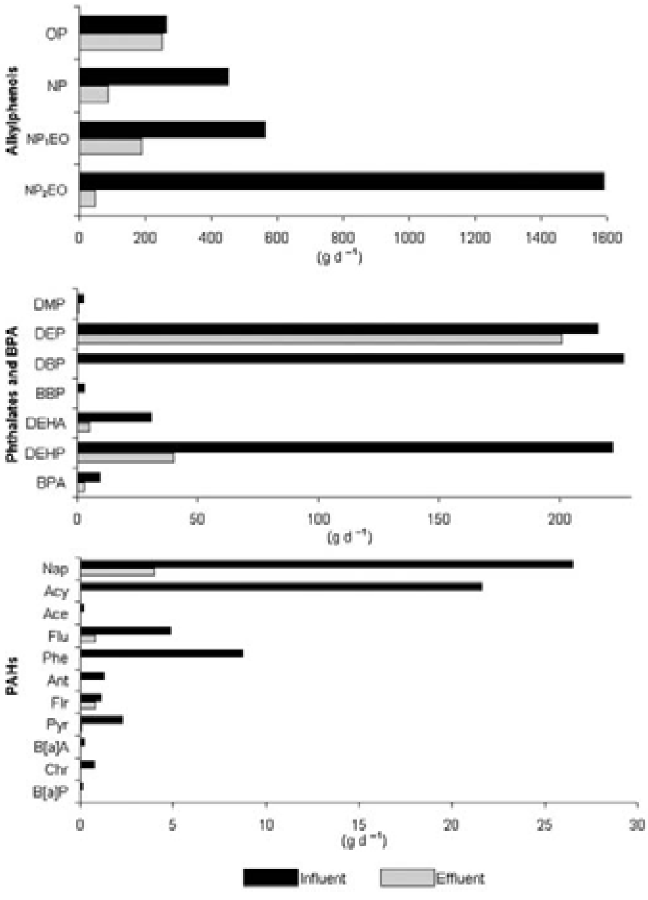


Fig. 4. Contaminant fluxes (g d^{-1}) in influent and effluent of Mataro's WWTP.

3.5. Environmental implications

Given the high temporal variations in wastewater composition as regards to the load of organic contaminants, the discharge levels to the sea will vary accordingly. This study contemplates the worst scenario which covers the sampling in a dry season to achieve discharges only from industrial and domestic sources without rain dilution and includes peak discharges during the morning. Within these conditions, the total amounts discharged daily to the sea are of high relevance. The main impact of contaminants is associated with the continuous input to the sea via discharge of WWTP, as occurs for PCBs and pesticides when discharged from the rivers (Gómez-Gutiérrez et al., 2006). The sampling was performed twice and we found little variation since target compounds were detected in both periods, indicating a relatively constant discharge of contaminants.

Target compounds studied are known as toxic for the environment, causing endocrine disruption (Zafra-Gómez et al., 2008; Tan et al., 2007; Céspedes et al., 2005) or mutagenic effects (Richardson, 2008; Busetti et al., 2006) in costal ecosystems. It has been shown that NP and OP have endocrine disruption with the recombinant yeast assay (RYA) at 79.2 ± 20 and 188 ± 84 µg l^{-1}, respectively (Céspedes et al., 2005) and have an acute toxicity measured with *Daphnia magna* with an EC50 of 93 to 470 µg l^{-1} and 90 to 140 µg l^{-1} while NP$_1$EO is less toxic, with EC50 of 2983 ± 395 µg l^{-1} (Table 1). Fish have also been used to evaluate the estrogenicity of NP and it has caused effects in rainbow trout (*Oncorhynchus mykiss*) at 20.3 mg l^{-1} and in medaka fish (*Oryzias latipes*) exposed to 0.1 mg l^{-1} NP (Soares et al., 2008). According to these values, some of the WWTP effluents are within this range of estrogenicity or toxicity. However, in this particular case the WWTP effluents are discharged to the sea and upon dilution therein the potential impact may be low and localized to the area of the outfall discharge. However, it should also be taken into consideration that NP and OP are highly hydrophobic (K_{ow} of 5.28 for OP and 5.92 for NP) and can be adsorbed and accumulated to suspended non-living

particulates and sediments (Navarro et al., 2009). In addition, many aquatic sediments are either oxygen-deficient or anaerobic, thus minimizing NP mineralization (Hale et al., 2000). No degradation of NP and OP accumulated in anoxic marine sediments was reported after 70 d of treatment (Ying and Kookana, 2003). This indicates that NP and OP might persist in sediments and become recalcitrant, inducing effect towards marine biota when bioavailable.

Phthalates are weakly estrogenic (Jobling et al., 1995) and have a estrogenicity effect with RYA between 2306 and 6960 μg l^{-1} (Céspedes et al., 2004) and an EC50 in *Daphnia magna* varying from 160 to 86,000 μg l^{-1}, levels much higher than those found in the WWTP effluent (Jobling et al., 1995). Phthalates are sparingly soluble, rapidly sorbed on particulate matter and their half-lives around 30 h, resulting in highly removal efficiency through microbial activity and sorption to sludge (Fauser et al., 2003). The impact to seawater is potentially low although one might consider that the huge amounts released daily could present some risk.

Among PAHs, Nap and Ant have higher concentration in effluents than their EC50 (48 h, *Daphnia magna*), but again, they are diluted when released to the sea. Despite the low level concentration of PAHs discharged by WWTP effluents, they contribute to the levels found in costal seawaters. These compounds were detected at coastal areas at a concentration of 246–1930 ng l^{-1} (Pérez-Carrera et al., 2007; Prieto et al., 2007) as a result of river inputs, WWTP effluents, marine activities and sporadically from petrochemical activities (Pérez-Carrera et al., 2007). Once dissolved in water, it is expected that they can be sorbed upon sediments and particulate matter (Busetti et al., 2006), algae (Hutchinson et al., 2003) or seaweeds (Porte et al., 2006), being then a source to filter-animals (mussels, oysters, clams, etc.) (Manoli and Samara, 1996) and might induce food chain biomagnification and possible harmful effects on biota and carcinogenicity on humans (Pérez et al., 2001).

Given the negative impact of industrial contaminants, EU Directives have started to take actions for environmental protection. As regards to Directive 2008/105/EC, for PAHs (List II, Directive 2006/11/EC aimed to diminish emissions), the maximum allowable concentration is of 2.4 μg l^{-1} for Nap, 0.4 μg l^{-1} for Ant and 1 μg l^{-1} for Flr. In effluent, Nap concentration was 3.49 μg l^{-1} which is over the limit. For phthalates, only DEHP is legislated in List II with maximum allowable concentration in discharges of 1.4 μg l^{-1}, which is exceed by an effluent concentration of 9.4 μg l^{-1}. For APs, NP maximum allowable concentration is 2 μg l^{-1} and for OP is 0.1 μg l^{-1}. NP and OP discharges dramatically exceed these limits (22 μg l^{-1} for NP and 54 μg l^{-1} for OP). NP was considerate in List I (Directive 2006/11/EC) as it is stated as hazardous in Directive 2008/105/EC, and also their ethoxylates because they can be degradated to NP. In addition, when Directive 2006/11/EC is adopted by the member states, authorities must establish measures to eliminate these substances from discharges.

3.6. *Lixiviation to groundwater*

Groundwater was analyzed to evaluate potential lixiviation of target compounds from the sewage grid due to pipe leakage. Table 4 (P00) indicates the levels of PAHs, APs, phthalates and BPA detected. ΣPAH concentration was of 0.52 μg l^{-1}, being Nap at 0.4 μg l^{-1} and Flu at 0.12 μg l^{-1} the main compounds detected. Nap and Flu correspond to more widespread appearance in wastewaters due to their higher solubility and higher leaching potential. For phthalates, only DMP was detected at a concentration of 0.12 μg l^{-1} while the plasticizer BPA was found at 0.78 μg l^{-1}. For APs, only NP$_1$EO was detected at a concentration of 0.45 μg l^{-1}. Previous studies reported the presence of NP, OP and BPA in groundwaters from industrial and agricultural origin at levels from 0.07 to 1.9 μg l^{-1}, due to leaching from in use products, basically pesticide formulations (Latorre et al., 2003). The levels encountered are of concern since they indicate contamination of the soil column. The proximity of the drainage pipes to the well and the high soil permeability due its composition (predominantly gravel with sands, clays and limestone) could enhance leaching to groundwaters. In addition, contamination of groundwaters may represent a potential health risk since many of them are intended for human consumption.

4. Conclusions

The multiresidual method developed to determine 42 priority contaminants in wastewaters of a heavily industrialized area permitted to identify alkylphenols, phthalates and PAHs at levels from 0.01 to 698 μg l^{-1}, while PBDEs were detected only once. It is shown that these compounds are transported through the sewage grid and diluted upon merging with different ducts, but there is little degradation. The WWTP effectively reduce the contaminant load in 65–80%. However, the alarm is there and considering that up to 800 g d^{-1} of compounds with toxic potential are discharged to the sea, actions should be undertaken to reduce or preferably eliminate the continuous high level input of contaminants to the sea by implementing tertiary treatments in this area where the incidence of industrial pollutants is high. In addition, it was found that these compounds leached to groundwaters and thus, groundwaters become highly vulnerable as regards to their use for domestic purposes. Thus, for a better wastewater management, it is needed to perform a more strict monitoring of contaminants through the sewage grid to better foresee the inputs of contaminants to WWTP and improve treatment conditions. This study is also intended to contribute to recent EU Directives aimed at reducing the discharge of hazardous compounds to the environment by proposing a relatively simple sampling and analytical protocol.

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ARTÍCULO CIENTÍFICO 2.

"Stir bar sorptive extraction-thermal desorption-gas chromatography-mass spectrometry: An effective tool for determining persistent organic pollutants and nonylphenol in coastal waters in compliance with existing Directives"

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Stir bar sorptive extraction-thermal desorption-gas chromatography–mass spectrometry: An effective tool for determining persistent organic pollutants and nonylphenol in coastal waters in compliance with existing Directives

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ABSTRACT

A multi-residual method based on stir bar sorptive extraction coupled with thermal desorption-gas chromatography–mass spectrometry (SBSE-TD-GC–MS) has been developed to measure 49 organic pollutants (organochlorine pesticides, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, polybrominated diphenyl ethers and nonylphenol) in seawater. Using 100 ml of water, the method exhibited good linearity, with recoveries between 86% and 118% and relative standard deviation between 2% and 24% for almost all compounds. The method was applied to determine target contaminants in Catalonian seawater, including coastal areas, ports and desalination plant feed water. Overall individual compound levels oscillated between 0.16 and 597 ng l^{-1}; PAHs and nonylpenol were the compounds found at the highest concentrations. The method provided LODs between 0.011 and 2.5 ng l^{-1}, lower than the Environmental Quality Standards (EQS) fixed by Directive 2008/105/EC. In compliance with the directive, this method can be used as a tool to survey target compounds and is aimed at protecting coastal ecosystems from chemical pollution.

1. Introduction

Chemical pollution poses a threat to the aquatic environment. Its effects include acute and chronic biotoxicity, accumulation in coastal and marine ecosystems, habitat and biodiversity loss, and threats to human health (European-Parliament-and-Council, 2008). The marine environment is, in many cases, the "ultimate sink" for large volumes of many pollutants (Pérez-Carrera et al., 2007). The production and emission of chemicals into coastal and marine environments usually derives from human settlements, resource use, and interventions such as infrastructural development and construction, agricultural activities, industrial developments, urbanization, tourism, etc. (Islam and Tanaka, 2004; Sánchez-Avila et al., 2009). As a result, a wide variety of organic compounds are present in coastal and marine ecosystems, including polycyclic aromatic hydrocarbons (PAHs) (Valavanidis et al., 2008), organochlorine pesticides (OCPs) and organophosphorus pesticides (OPPs), alkylphenols (APs) such as nonylphenol (NP) (Huertas et al., 2007; Islam and Tanaka, 2004), polychlorinated biphenyls (PCBs), and polybromodiphenyl ethers (PBDEs) (Pérez-Carrera et al., 2007), among others. The presence of these organic compounds is associated with toxicological effects at the ng–μg l^{-1} levels, producing endocrine disruption in marine organisms (Porte et al., 2006), neurotoxicity (Tiffany-Castiglioni et al., 2006), and alterations at the ecosystem level (Chapman, 2004).

The Water Framework Directive 2000/60/EC (European Parliament and Council, 2000) is one of the most important pieces of international legislation introduced for water protection in recent years. Following this Directive, the European Parliament and Council adopted the more recent Directive 2008/105/EC, establishing Environmental Quality Standards (EQS) for priority substances and certain other pollutants in inland surface waters and coastal waters. In addition, the United States Environmental Protection Agency (US-EPA) has recommended the Criterion Continuous Concentration (CCC) (2002) for water quality in order to ensure the protection of the most sensitive stages of saltwater organism life cycles from chronic exposure to priority and nonpriority pollutants. Pollutants need to be periodically monitored to ensure the protection of coastal ecosystems, which are highly vulnerable as a result of human activity.

Most of the levels imposed by these regulations (EQS or CCC) are on the order of ng l^{-1}; sensitive instrumental analytical methods are thus needed to meet legislative standards. GC–MS is an appropriate technique for the determination of PAHs, PBDEs, PCBs, APs, OCPs and DDTs at low concentration levels (Santos and Galc-

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eran, 2002). Since organic compounds are present at very low concentrations in seawater, it is necessary to use an additional, efficient preconcentration method prior to their analysis. Most modern approaches to sample pretreatment are oriented towards the minimization of solvent use, or, preferably, toward the elimination of solvent use (green chemistry), as well as toward the automation of the analysis, in order to improve the robustness of the entire analytical procedure (Prieto et al., 2007; Serôdio and Nogueira, 2004).

Stir bar sorptive extraction (SBSE) has been developed to extract volatile and semi-volatile compounds from water (David and Sandra, 2007). This technique is based on the sorption of apolar solutes present in aqueous samples onto a polydimethylsiloxane (PDMS) stir bar and is based on the principles of solid-phase microextraction (SPME), thus partitioning the analytes between the sample and an extracting phase. In short, a stir bar coated with PDMS is introduced into the sample. After a stirring period, the stir bar is removed, rinsed, dried and placed in an "injector tray". The compounds are thermo-desorbed and cryofocused in a programmable temperature vaporization (PTV) injector and then analyzed by GC–MS (David and Sandra, 2007; Pérez-Carrera et al., 2007; Prieto et al., 2007). SBSE presents advantages over liquid–liquid and solid-phase extraction because no solvent is used and small sample volumes can be analyzed since all preconcentrated analytes are desorbed. With respect to SPME, lower detection limits (sub-ng l^{-1} to ng l^{-1}), higher capacity, and better recoveries can be achieved since extraction is performed with a larger amount of PDMS. SBSE is also characterized by its high reproducibility due to minimum sample preparation and manipulation (David and Sandra, 2007).

SBSE has been successfully applied for the extraction of PAHs and PCBs (Pérez-Carrera et al., 2007; Prieto et al., 2007; Roy et al., 2005; Serôdio and Nogueira, 2004), PBDEs (Prieto et al., 2008), and NP (Prieto et al., 2007) from seawater and OCPs from fresh water (Baugros et al., 2008; León et al., 2006; Serôdio and Nogueira, 2004). However, the majority of these studies have been focused on a single specific family of compounds.

Thus, the objectives of the present study were (i) to develop and apply a multi-residue analytical method for the determination of trace concentrations of PAHs, PCBs, PBDEs, NP and OCP in seawater collected from coastal areas and ports in Catalonia and (ii) to study the advantages and disadvantages of the method as a tool to monitor organic pollutants in coastal waters in compliance with existing directives.

2. Experimental

2.1. Chemicals and reagents

NP, sixteen PAHs (regulated by US-EPA), seven PCBs, seven PBDEs and eighteen OCPs were analyzed (Table 1). NP was purchased from Riedel-de Haën (Seelze, Germany) as a solid technical mixture of isomers. PAHs were purchased from AccuStandard (New Haven, CT, USA) as a mix solution at 200 mg l^{-1} in methanol. PCBs (28, 52, 101, 118, 138, 153 and 180) were obtained from Dr. Ehrenstorfer (Augsburg, Germany) as a mixed solution at 10 mg l^{-1} in iso-octane. PBDEs (28, 47, 99, 100, 153, 154 and 183) were obtained from Cambridge Isotope Laboratories (Andover, MA, USA) as a mixed solution at 1 mg l^{-1} in nonane. Endosulfan (alfa and beta), aldrin, endrin, dieldrin and a technical mixture of hexachlorocyclohexanes (α, β, δ and γ) were purchased from Dr. Ehrenstorfer as solutions at 100 mg l^{-1} in methanol. Hexachlorobenzene as a solid and a mixed solution at 10 mg l^{-1} of DDT derivates were also obtained from Dr. Ehrenstorfer. A mixture of standard solutions (1–0.5 mg l^{-1}) containing all analytes was prepared in absolute methanol and stored in the dark at −20 °C until use, remaining stable for at least three months. Working standard solutions were prepared in methanol at 10, 1 and 0.01 ng μl^{-1}. These solutions were used to spike the unknown water samples and perform the calibration curves.

A mixed solution (at 2 mg l^{-1} in methanol) from Supelco (Bellefonte, USA) containing naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12} and perylene-d_{12} was used to provide surrogate standards for PAHs. PCBs, PBDEs and organochlorine pesticide surrogate standards were PCB 65 and PCB 200 purchased from Dr. Ehrenstorfer and used as a mixture solution at 10 µg l^{-1} in iso-octane. The nonylphenol surrogate standard used was 4-n-nonylphenol-d_8 (Dr. Ehrenstorfer) as a solution at 100 µg l^{-1} in acetone.

HPLC-grade solvents were obtained from Merck (Darmstadt, Germany). Nitrogen for drying with 99.995% of purity was obtained from Air Liquid (Barcelona, Spain). Sodium chloride (NaCl) with $\geqslant$99.5% of purity was obtained from J.T. Baker (Phillipsburg, NJ, USA).

Compound names and acronyms mentioned in this paper are summarized in Table 1.

2.2. Sample collection

Twelve subsurface (1 m depth) seawater samples from the Catalonian coast (NE Spain) were collected in October 2007 (Fig. 1). Six samples were obtained from the following coastal areas: Santa Pola (046395E, 4620218N), Sant Francesc (0483689E, 4613797N), Canet de Mar (0465024E, 4603734N), Tarragona (0354591E, 4552733N), Salou (0344651E, 4547905N) and Vilafortuny (0340402E, 454801N). Five additional samples were obtained from recreational and trade ports: Arenys de Mar (0463278E, 4602784N), Mataró (0453728E, 4597535N), Barcelona (0429963E, 4575194N), Tarragona (0349912E, 4549438N) and Salou (0342931E, 4548610N). Also, one additional sample was taken near the feed water for the El Prat del Llobregat desalination plant (0425582E, 4570514N). All these samples were placed in precleaned amber glass bottles (380 °C × 12 h) and stored in the dark at 4 °C. Extraction was performed with SBSE as described below on the same day as collection to avoid microbial degradation.

2.3. Extraction of analytes from seawater

In order to determine the total concentrations of the analytes in the samples, including both dissolved and particulate-bound chemicals, seawater samples were used directly, without preliminary filtration. Surrogate standards were added prior to preconcentration at 10 ng l^{-1}. Hundred milliliter of seawater and 10 ml of methanol (as organic modifier) were introduced into 100 ml Erlenmeyer flasks. The flasks were previously washed with bi-distilled water and rinsed with methanol. Extractions were carried out with stir bars coated with 20 mm length × 1.0 mm PDMS film thickness (the bars were previously conditioned for 4 h at 300 °C in a flow of helium and supplied by Gerstel GmbH (Mülheuim a/d Ruhr, Germany)). A 15 position magnetic stirrer (Gerstel) was used to perform agitation at 900 rpm over 24 h at room temperature in the dark. Afterward, the stir bars were removed with tweezers, rinsed with HPLC water, dried with a lint-free tissue and placed in a precleaned gas liner.

2.4. Instrumental analysis

Stir bars were thermally desorbed in a TD system (TDS2 from Gerstel) connected to a programmed-temperature vaporization (PTV) injector CIS-4 (Gerstel) by a heated transfer line at 300 °C. The PTV injector was installed in an Agilent 6890GC/5973MS system (Agilent Technologies, Palo Alto, CA, USA). TD was performed

Table 1
Compounds studied, acronym, water-octanol partition coefficients (Log $K_{o/w}$), legislated values and limits of detection (LOD).

Compound	Acronym	Log $K_{o/w}$	EQS[a] (ng l^{-1})	CCC[b] (ng l^{-1})	LOD (ng l^{-1})
Nonylphenol (isomer mixture)	NP	5.71	2000	1700	0.431
Naphthalene	Nap	3.30	2400	nm	1.25
Acenaphtylene	Acy	3.94	nl	nm	2.50
Acenaphthene	Ace	3.92	nl	nm	2.50
Fluorene	Flu	4.18	nl	nm	0.371
Phenantrene	Phe	4.46	nl	nm	0.091
Anthracene	Ant	4.45	400	nm	0.116
Fluoranthene	Flr	5.16	1000	nm	0.025
Pyrene	Pyr	4.88	nl	nm	0.036
Benzo[a]anthracene	B[a]A	5.66	nl	nm	1.04
Chrysene	Chr	5.81	nl	nm	0.098
Benzo[b]fluoranthene	B[b]F	5.78	Σ B[b]F + B[k]F	nm	0.017
Benzo[k]fluoranthene	B[k]F	6.11	=300	nm	0.074
Benzo[a]pyrene	B[a]P	6.13	100	nm	0.500
Indeno[1,2,3-c,d]pyrene	I[cd]P	6.70	Σ I[cd]P + B[ghi]P	nm	0.012
Benzo[g,h,i]perylene	B[ghi]P	6.63	=2	nm	0.221
Dibenzo[a,h]anthracene	D[ah]A	6.75	nl	nm	0.011
2,4,48-Trichlorobiphenyl	PCB 28	5.62	*	Sum of	0.095
2,2′5,5′-Tetrachlorobiphenyl	PCB 52	6.09	*	Congeners	0.063
2,2′4,5,5′-Pentachlorobiphenyl	PCB 101	6.80	*	=30.0	0.018
2,3′4,4′,5-Pentachlorobiphenyl	PCB 118	7.12	*		0.011
2,2′,3,4,4′,5 Hexachlorobiphenyl	PCB 138	7.44	*		0.035
2,2′4,4′5,5′ Hexachlorobiphenyl	PCB 153	7.75	*		0.019
2,2′3,4,4′,5,5′ Heptachlorobiphenyl	PCB 180	8.27	*		0.060
2,4,4′-TriBDE	BDE 28	5.88	0.2	nm	0.194
2,2′,4,4′-TetraBDE	BDE 47	6.77	0.2	nm	0.020
2,2′,4,4′,5-PentaBDE	BDE 99	8.20	0.2	nm	0.061
2,2′,4,4′,6-PentaBDE	BDE 100	8.00	0.2	nm	0.023
2,2′,4,4′,5,5′-HexaBDE	BDE 153	8.55	0.2	nm	0.021
2,2′,4,4′,5,6′-HexaBDE	BDE 154	8.55	0.2	nm	0.011
2,2′,3,4,4′,5′,6-HeptaBDE	BDE 183	9.44	nl	nm	0.086
Hexachlorobenzene	HCB	5.73	50	nm	0.275
Alfa-hexachlorocyclohexane	α-HCH	3.80	Σ α + β + γ + δ isomers	nm	18.3
Beta-hexachlorocyclohexane	β-HCH	3.78	=40	nm	37.5
Gamma-hexachlorocyclohexane	Lindane	3.72		160	25.4
Delta-hexachlorocyclohexane	δ-HCH	4.14		nm	19.7
Alfa-endosulfan	α-endosulfan	3.83	Σ α + β-endosulfan	8.70	1.47
Beta-endosulfan	β-endosulfan	3.83	=10	8.70	0.581
Aldrin	–	6.50	Σ aldrin + dieldrin	130	0.332
Dieldrin	–	5.40	=10	1.90	0.330
Endrin	–	5.20	*	23.0	0.274
Heptachlor	–	6.10	*	3.60	0.042
Heptachlor epoxide B	–	4.98	*	3.60	0.024
o, p′-Dichlorodiphenyldichloroethane	2,4′-DDD	5.87	nl	nm	0.387
o, p′-Dichlorodiphenyldichloroethylene	2,4′-DDE	6.00	nl	nm	0.042
o, p′-Dichlorodiphenyltrichloroethane	2,4′-DDT	6.79	Σ 2,4′-DDT + 4,4′-DDD	nm	0.026
p, p′-Dichlorodiphenyldichloroethane	4,4′-DDD	6.02	+4,4′-DDE + 4,4′-DDT	nm	0.014
p, p′-Dichlorodiphenyldichloroethylene	4,4′-DDE	6.51	=25	nm	0.136
p, p′-Dichlorodiphenyltrichloroethane	4,4′-DDT	6.91	10	1	0.026

nl: Not EQS available.
nm: Not mentioned.

[a] Environmental quality standard for other surface waters, according Directive 2008/105/EC.

[b] Criterion Continuous Concentration (US-EPA, 2002). Estimate of the highest concentration of a toxic substance in salt water to which an aquatic community can be exposed indefinitely without unacceptable effect.

* Not EQS available, but restricted or banned their use by Directive 79/117/EC and Regulation (EC) No. 850/2004.

from 15 °C (holding time 0.8 min), then increased at 60 °C min^{-1} up to 280 °C for 7 min (desorption parameters). A helium flow of 50 ml min^{-1} was used. The PTV injector temperature was held at 8 °C (cryofocusing temperature) for 0.1 min and then increased to 325 °C at 10 °C s^{-1}, then held for 7 min. An Agilent HP-5MS capillary column was used (30 m × 0.25 mm i.d. × 0.25 µm, 5% phenyl methyl siloxane film thickness). The oven temperature was programmed to increase from 70 (holding time 2 min) to 150 °C at 25 °C min^{-1}, then to 200 °C at 3 °C min^{-1}, and finally to 280 °C at 8 °C min^{-1}. The oven was then held constant for 10 min. Transfer line and ion source temperatures were 280 and 230 °C, respectively.

Peak detection and integration were carried out using MSD ChemStation (Agilent) software. For increased sensitivity and specificity, quantification was performed in time-scheduled Selected Ion Monitoring (SIM) using three or four ions per compound (Table 2). Internal standard quantification was performed using deuterated compounds from the PAH family, and PCB 65 and PCB 200 were used to quantify organochlorine pesticides, PCBs and PBDEs. The ion mass program used for quantification is detailed in Table 2.

2.5. Quality control

Spiked 100 ml of HPLC water samples with 0.5, 1, 2.5, 5, 10, 50, 100, 250 and 500 ng l^{-1} of all the target compounds were preconcentrated with SBSE and thermally desorbed to construct the calibration curve. The method was optimized using synthetic seawater (a mixture of 35 g of NaCl dissolved in 1000 ml of HPLC water). For-

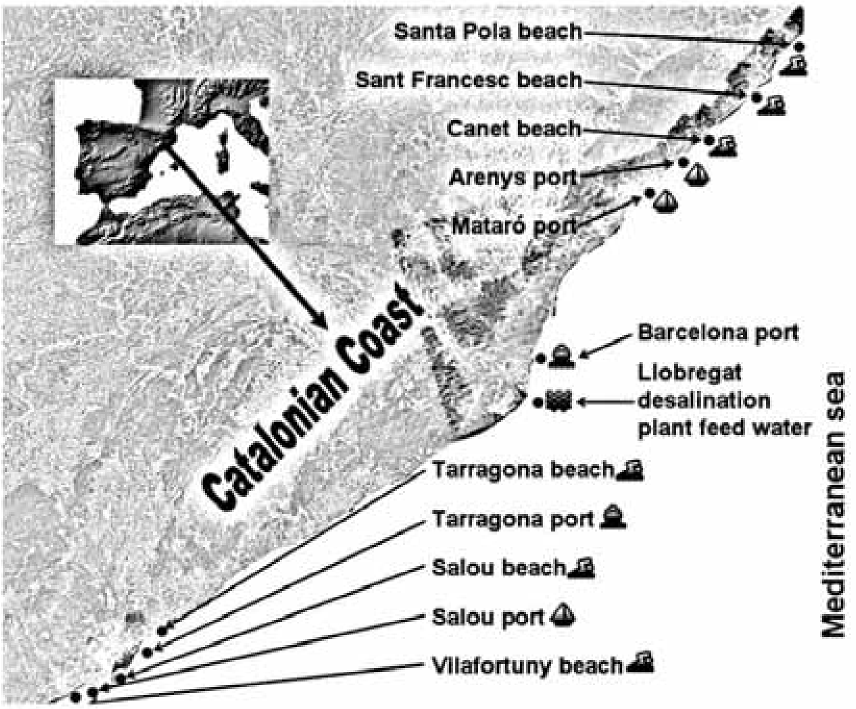

Fig. 1. Sampling points in the Catalonian coast.

tified artificial seawater samples with 2.5 ng l^{-1} of target compounds were used to calculate percentage recoveries in quintuplicate. Blank analyses were performed at laboratory conditions using HPLC water. Blank concentration levels were below the limit of detection (LODs); therefore, no further blank subtraction was necessary.

3. Results and discussion

3.1. Performance of the SBSE-GC–MS method

The method was developed and tested with coastal waters in an attempt to propose a monitoring method to meet current legislative targets regarding organic pollutants. For practical reasons, the miniaturization of conventional sampling methods is desirable; if small sample volumes can be gathered and further analyzed, the burden of transporting large volumes of seawater is diminished. However, contaminants in seawater are present at very low concentrations as a result of dilution effects. Analytical methods must, therefore, be optimized to detect sub-ng l^{-1} concentrations. In addition, seawater is a sample matrix containing a wide range of possible interferences (organic matter, plankton, particulate material, etc.), which might affect the detection of target analytes at ultra-trace levels. Thus, the optimization of quality parameters associated with the analytical method is essential for ensuring low detection limits, high recoveries, versatility and the robustness of the method, especially when a mixture of compounds with a wide range of physico-chemical properties and polar and apolar substances with differences in log $K_{o/w}$ from 3.30 to 9.44 are analyzed. Previous studies have reported the optimization of SBSE for PAHs, PCBs, PBDEs, NP and OC pesticides, and this information was used to develop the multi-residual methodology applied in the present study (León et al., 2003; Llorca-Porcel et al., 2006; Pérez-Carrera et al., 2007; Prieto et al., 2008). The analytical parameters optimized in those studies are presented in Table 3. The following sections describe the quality parameters obtained in the present study.

3.1.1. Mass spectrometric conditions

SIM conditions were optimized so that each compound was monitored at three characteristic ions (two for the surrogate standards). The base peak was used for quantification, while the entire mass spectral pattern was used for unequivocal compound confirmation. Table 2 reports the retention time of each target compound and the three ions monitored. The chromatographic separation developed allowed the resolution of all analytes in 46 min (Fig. 2). A coelution of the NP isomers with HCB appeared but was then efficiently resolved by their specific MS ions (Table 2). Given the high selectivity of the detector in resolving multi-analytes, MS is recommended for the unequivocal trace analysis of multi-analytes in seawater where confirmation and quantification can be performed with a single analysis.

3.1.2. Selection of extraction conditions

Extraction efficiency is a fundamental parameter in multi-residue analysis using SBSE-GC–MS. The recommended volume is 100 ml (Pérez-Carrera et al., 2007; Prieto et al., 2007). Polar solutes with $K_{o/w} < 3.3$ normally result in very low recoveries in SBSE extractions. Salting out is a technique usually employed in SBSE extraction to achieve higher polar solute recoveries; NaCl is added to reduce the solutes' water solubility and increase the partitioning coefficient between PDMS fiber and water. However, the addition of NaCl has been shown to reduce the extraction of apolar compounds (David and Sandra, 2007; Huertas et al., 2007; León et al., 2003). In other methods developed with seawater, NaCl was not added in order to avoid reducing the extraction efficiency of more apolar compounds like PAHs and PBDEs (the ionic strength of the aqueous media is increased by NaCl addition) (Huertas et al., 2007; Prieto et al., 2008; Roy et al., 2005; Serôdio and Nogueira, 2004). On the contrary, several authors (León et al., 2003; Llorca-Porcel et al., 2006; Pérez-Carrera et al., 2007; Prieto et al., 2008)

Table 2
GC–MS conditions under time-scheduled selected ion monitoring indicating retention time (Rt) of each compound and specific *m*/*z* of each compound.

Rt (min)	Compounds	Selected ions (*m*/*z*)			
5.39	*Nap d_8*	136.0	108.0		
5.43	Nap	128.1	127.1	129.1	102.1
8.00	*Acy d_8*	160.0	80.0		
8.08	Acy	152.1	151.1	153.1	76.0
8.37	*Ace d_{10}*	164.0	162.0	160.0	
8.45	Ace	153.9	152.9	151.9	
9.92	Flu	166.1	167.1	82.4	
12.07	α-HCH	180.9	218.9	182.9	111.0
12.10–13.20	NP (isomer mix)	134.9	149.0	106.9	
12.35	HCB	283.7	285.7	248.7	250.7
13.24	β-HCH	182.9	218.9	180.9	
13.45	Lindane	180.9	182.9	218.9	
13.72	*Phe d_{10}*	188.0	187.0	0.0	
13.82	Phe	178.1	176.1	152.1	89.0
14.04	Ant	178.1	176.1	152.1	89.0
14.79	δ-HCH	180.9	218.9	182.9	109.0
16.12	PCB 28	255.8	258.0	185.9	
16.77	Heptachlor	271.8	100.0	273.8	236.8
17.93	PCB 52	291.8	289.8	219.8	221.8
18.44	*PCB 65*	291.7	289.7	219.8	221.7
18.49	Aldrin	262.9	66.1	260.9	292.9
20.71	Heptachlor epox. B	352.8	81.0	354.8	262.8
20.84	Flr	202.1	200.1	203.1	101.0
22.21	Pyr	202.1	200.0	203.1	
22.50	2,4′-DDE	245.8	247.8	317.8	175.9
22.62	PCB 101	325.7	327.7	253.8	255.8
22.63	α-Endosulfan	194.9	206.9	240.9	264.9
23.85	Dieldrin	262.9	79.1	276.9	236.8
24.02	4,4′-DDE	246.0	317.9	176.0	248.0
24.36	2,4′-DDD	234.9	236.8	164.9	
24.76	Endrin	262.9	81.0	244.9	316.9
25.16	β-Endosulfan	194.9	206.9	236.9	264.8
25.34	PCB 118	325.7	327.7	253.8	255.8
25.37	BDE 28	245.8	247.8	405.6	407.7
25.70	4,4′-DDD	235.0	237.0	165.0	199.0
25.78	2,4′-DDT	234.8	236.8	164.9	
26.19	PCB 138	359.7	361.7	289.7	291.8
27.00	4,4′-DDT	235.0	237.0	165.0	199.0
27.13	PCB 153	359.7	361.7	289.8	291.7
28.36	B[a]A	228.1	226.1	229.1	
28.42	*Chr d_{12}*	240.0			
28.51	Chr	228.1	226.1	229.1	
28.90	*PCB 200*	429.6	427.6	357.7	287.7
29.22	PCB 180	393.7	395.7	323.7	325.8
29.29	BDE 47	485.7	487.7	325.8	327.8
31.67	BDE 100	403.7	563.6	405.7	565.6
32.15	B[b]F	252.1	250.1	253.1	
32.23	B[k]F	252.1	250.1	253.1	
32.32	BDE 99	403.7	563.6	405.7	565.6
33.18	B[a]P	252.1	250.1	253.1	126.0
33.41	*Perylene d_{12}*	264.2	260.1	0.0	0.0
34.45	BDE 154	483.6	643.5	485.6	645.5
35.71	BDE 153	483.6	643.5	485.6	645.5
36.36	BDE 183	483.6	647.4	485.6	645.4
37.92	I[cd]P	276.1	274.1	277.1	138.0
38.17	D[ah]A	278.1	276.1	279.1	139.0
39.20	B[ghi]P	276.1	274.1	277.1	138.0

Surrogates used for quantification in *italic*.
Target ion used for quantification, in first position.

have stated that for more hydrophobic compounds (i.e., PAHs and PBDEs), SBSE will lose its substantial efficiency because these analytes are adsorbed onto the glass walls and other surfaces, causing analyte loss and the reduction of recovery yields. Instead, SBSE methods developed for these types of compounds involved the addition of methanol in amounts ranging from 5% to 20%, to reduce adsorption phenomena and avoid analyte loss (León et al., 2003; Prieto et al., 2007, 2008). Methanol addition is also helpful for liberating colloidal and particle-associated contaminants (King et al., 2004). Dissolved and particulate-bound chemicals could thus be extracted and further examined. In this study, the addition of 10% methanol was chosen as a compromise between the physico-chemical properties of target compounds and the natural NaCl content of the seawater used. In regard to extract time, some studies have reported that with extraction times of up to 12 h no significant improvement in method sensitivity was achieved (Huertas et al., 2007; Prieto et al., 2007). However, other studies have stated that an extraction time of 14 h offers a good compromise between sensitivity and practicability (León et al., 2003, 2006; Pérez-Carrera et al., 2007). In our case, a stirring time of 24 h at 900 rpm speed was finally selected as optimal for increasing method sensitivity and achieving sorption equilibria for all analytes. Fig. 3 shows the recovery yields and RSD ($n = 5$ samples) of each compound calculated with 2.5 ng l^{-1} of target compounds diluted in synthetic seawater. Recoveries ranged from 86% to 118%. Recoveries exceeding 100% with an absence of components in the blank were not unusual, but fell within the 70–120% error accepted for use of mass spectrometric-based methods. Relative standard deviation (RSD) values were acceptable and ranged between 2% and 24%, except for δ-HCH (71%, RSD = 14%). These results are similar to those obtained in studies using methods validated with 100 ml natural or artificial seawater (Huertas et al., 2007; Pérez-Carrera et al., 2007; Prieto et al., 2007, 2008).

3.1.3. Selection of desorption conditions

TD is affected mainly by desorption time and temperature, flux and cryofocusing temperature (Huertas et al., 2007; León et al., 2003; Llorca-Porcel et al., 2006; Pérez-Carrera et al., 2007; Prieto et al., 2007; Roy et al., 2005). Several authors have evaluated different desorption temperatures and fixed their optimal values at either 280 or at 300 °C (Table 3). Nevertheless, 280 °C was preferred in the current study, because siloxane bleeding from the PDMS coating is significantly lower at this temperature (León et al., 2003).

Stir bar suppliers recommend desorption times from 4 to 8 min at a desorption flow of 50 ml min^{-1}. In some of the cases summarized in Table 3, the values were established as a compromise because time (5–10 min) and flow (50–100 ml min^{-1}) had no significant effect on some of the analytes or had a positive effect on others (León et al., 2003, 2006; Pérez-Carrera et al., 2007; Prieto et al., 2008). Prieto et al. (2007) used multiple linear regressions to optimize desorption parameters and showed that long desorption times and desorption flows lower than 70 ml min^{-1} yielded the best signals for most of the analytes. In accordance with the factors described above, a flux of 50 ml min^{-1} and desorption time of 7 min were finally selected for the method developed in this work.

A wide range of cryofocusing temperatures (−150 to 60 °C) were initially evaluated by some authors and are summarized in Table 3. Temperatures below 0 °C caused difficulties as a result of nitrogen consumption, water condensation, and frosting in the injector port (León et al., 2003). Volatile analytes exhibit losses at 40 °C, and no differences arose between 0 and 20 °C (León et al., 2003; Prieto et al., 2008). For practical reasons, a temperature of 8 °C was selected for the method herein developed; good analytical responses were obtained.

3.1.4. Linear range and sensitivity

SBSE-GC–MS is a relatively recently proposed extraction technique differing from other more traditional extraction methods in that calibration is performed against spiked water samples. In SBSE-GC–MS, all extracted analytes are simultaneously determined, avoiding a multi-step extraction, minimizing sample losses, and allowing LODs value to be diminished. SBSE's most significant disadvantage, however, is that only low volumes (e.g., 100 ml) are recommended for extraction. It is therefore important to ensure 100% recoveries and to use highly sensitive MS acquisition conditions. The calibration curves obtained in the present study showed

Table 3
SBSE and TDU parameters and compounds studied in other published studies.

SBSE parameters			TDU parameters				Compounds studied and matrix	References
NaCl (%)	MeOH (%)	Time (h)	Cryofocus (°C)	Temp. (°C)	Time (min)	Flux (ml min^{-1})		
10	0	12	30	280	7	nm	PAHs, OCPs, APs in estuarine and seawater	Huertas et al. (2007)
20	0	14	20	280	6	75	PAHs, OCPs, OPPs in ground, tap and surface water	León et al. (2003, 2006)
30	20	12	−50	300	10	23	PAHs, NP, PBDEs, PCBs, and others in estuarine and seawater	Prieto et al. (2007)
10	0	14	20	280	7	75	PAHs, OCPs, PCBs, OPPs in seawater	Pérez-Carrera et al. (2007)
0	5	1	40	320	3	150	PAHs, OCPs, OPPs, APs, herbicides and others in MilliQ water	Serôdio and Nogueira (2004)
0	5	1	nm	280	1	50	PAHs in seawater	Roy et al. (2005)
0	40		20	300	10	50	PBDEs and other flame retardants in seawater	Prieto et al. (2008)
0	20	25	20	280	6	75	PBDEs in surface water	Llorca-Porcel et al. (2006)

nm: Not mentioned.

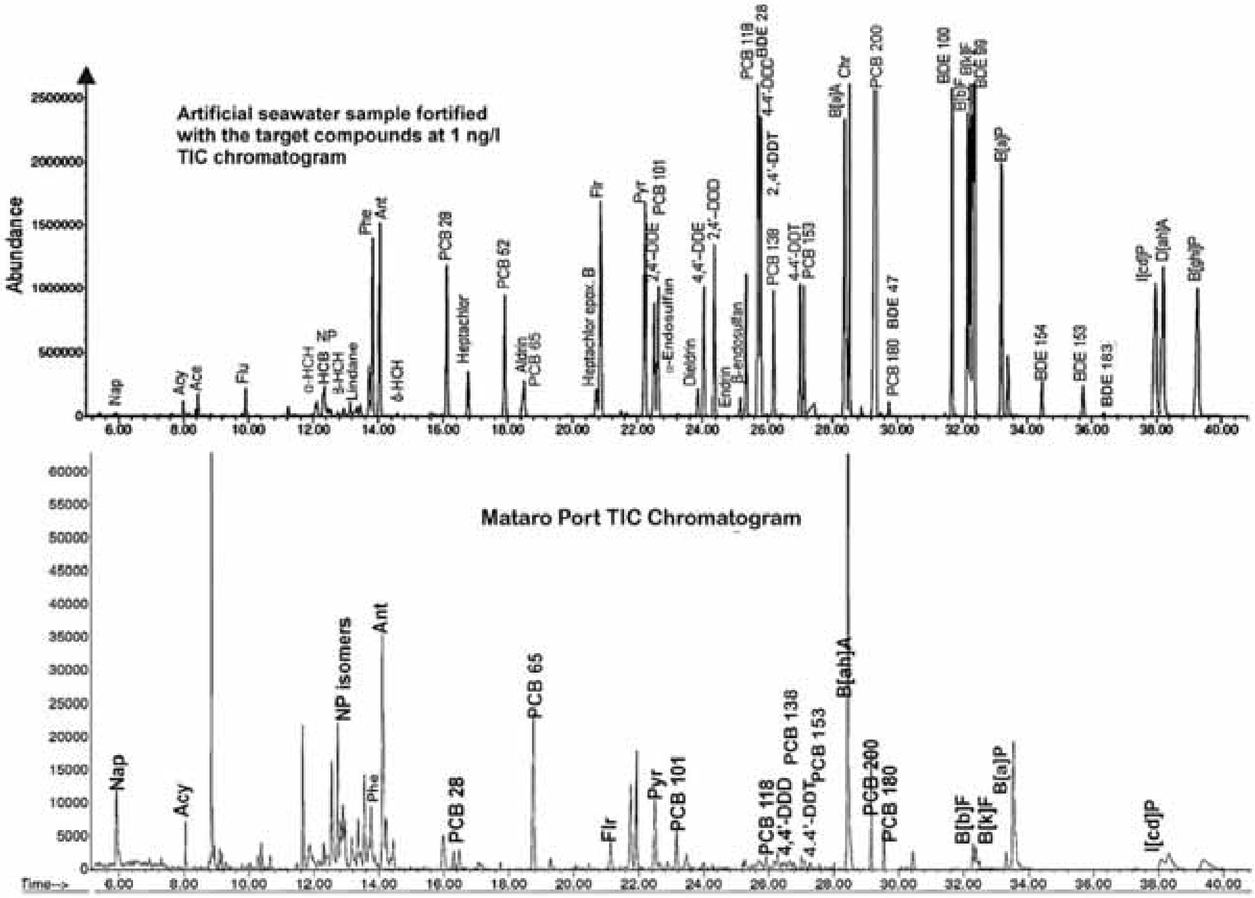

Fig. 2. SBSE-TD-GC–MS TIC chromatograms obtained for a synthetic seawater sample fortified with the target compounds at 1 ng l^{-1} and for a real sample.

good linearity responses ($r^2 = 0.988–0.999$) for almost all compounds over the concentration range of 0.5–500 ng l^{-1}, except for endrin and dieldrin (concentration range of 1.0–250 ng l^{-1}). Detection limits were calculated using three times the signal-to-noise value at the lowest point of the calibration curve. Under the selected acquisition conditions, LODs were between 0.011 and 2.5 ng l^{-1} (Table 1), except for α, β, γ and δ HCH (18.3–37.5 ng l^{-1}). Thus, the LODs obtained did ensure the low level detection of target compounds in the sub-ng l^{-1} range; this was in agreement with LOD values obtained in previous studies (León et al., 2006; Popp et al., 2003; Prieto et al., 2007). Overall, these LODs were 1 to <5000 times lower than their EQS and their CCC (Table 1). Even for PBDEs where EQS are as low as 0.2 ng l^{-1}, their LODs varied from 0.011 to 0.194 ng l^{-1}. For the compounds under consideration, the developed method met the requirements of European and US-EPA regulations.

3.2. Occurrence of selected pollutants in Catalonian coastal seawaters

The applicability of the method was tested by analyzing open coastal waters and port waters. Table 4 shows the compounds de-

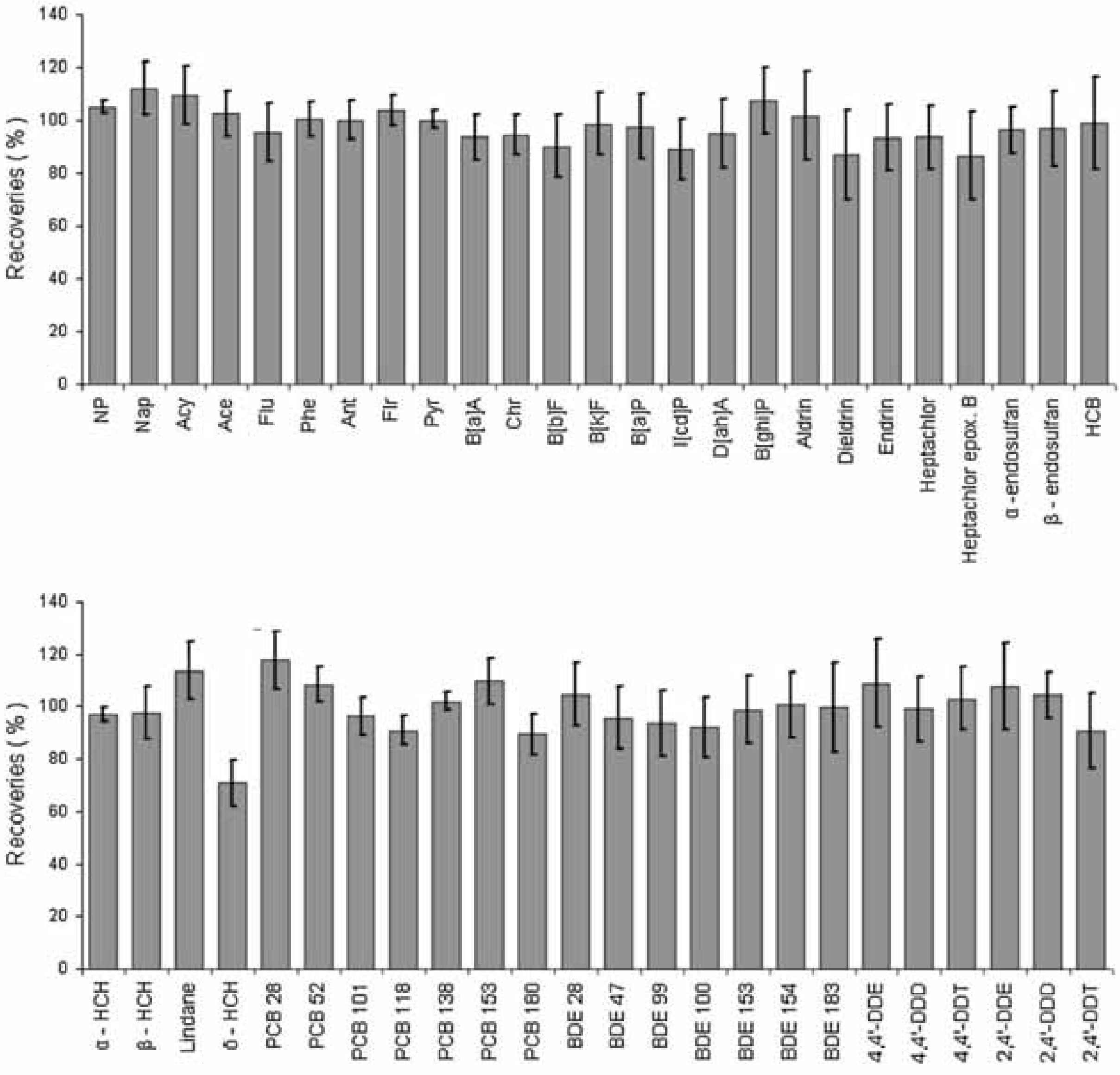

Fig. 3. Mean recoveries (%) and standard deviation ($n = 5$) of 2.5 ng l^{-1} of the compounds studied in synthetic seawater.

tected in each sample. PAHs were the major identified compounds, followed by NP, PCBs, and finally by OCPs. No PBDEs were detected.

Individual PAHs were detected in all samples. The sum of concentrations of PAHs (ΣPAHs) in ports ranged from 132 to 441 ng l^{-1} and in coastal waters from 38.0 to 139 ng l^{-1}. In the desalination plant feed water, the levels were determined to be 86.2 ng l^{-1}. Four to ten PAHs were detected in each sample, with Nap, Flr, Pyr and Phe being the most frequent. Baumard et al. (1998) observed that these same compounds were the PAHs with the highest concentrations in mussels sampled in the Mediterranean sea (in areas near Catalonia and France). The high concentrations of these PAHs in ports were attributed to the presence of maritime transports using petroleum-based products and combustibles and also to fuel combustion (Valavanidis et al., 2008). Their presence in coastal waters was attributed to the intense recreational and touristic boat activities which have a high concurrence along the Catalan coast. Similar individual PAHs levels were detected in coastal waters from France (Saint-Anne and Moulin Blanc) with concentrations from 0.08 to 14.6 ng l^{-1} (Roy et al., 2005). Concentrations were also similar in other regions of the Mediterranean Sea (Saronikus Gulf, Greece), at 20–160 ng l^{-1} (Valavanidis et al., 2008). On the Spanish Atlantic coast, concentrations from 0.4 to 168 ng l^{-1} (Getxo, Basque Country) (Prieto et al., 2007) and from 3 to 389 ng l^{-1} (Cadiz Bay) (Pérez-Carrera et al., 2007) were detected.

NP was detected in 10 out of 12 samples. NP in the desalination plant feed water was 597 ng l^{-1}; in ports it ranged from 71.0 to 529 ng l^{-1} and in coastal waters from 22.8 to 235 ng l^{-1}. The high concentration in the desalination plant feed water could have been caused by the discharge of wastewater treatment plant effluents and river discharges (WWTP) the Barcelona airport. Concentrations of <30 ng l^{-1} were detected in German estuaries and from 150 to 4100 ng l^{-1} in Spanish ocean water (Soares et al., 2008). The presence of NP in coastal waters is caused mainly by the direct discharge of Aps' polyethoxylates, which are used in a large variety of industrial and domestic applications (i.e., detergents, plastics, pesticides formulation) (Soares et al., 2008). NP is not completely eliminated by WWTPs, and it is released with effluent waters to rivers or to the sea (Céspedes et al., 2006, 2008). Recently, the dis-

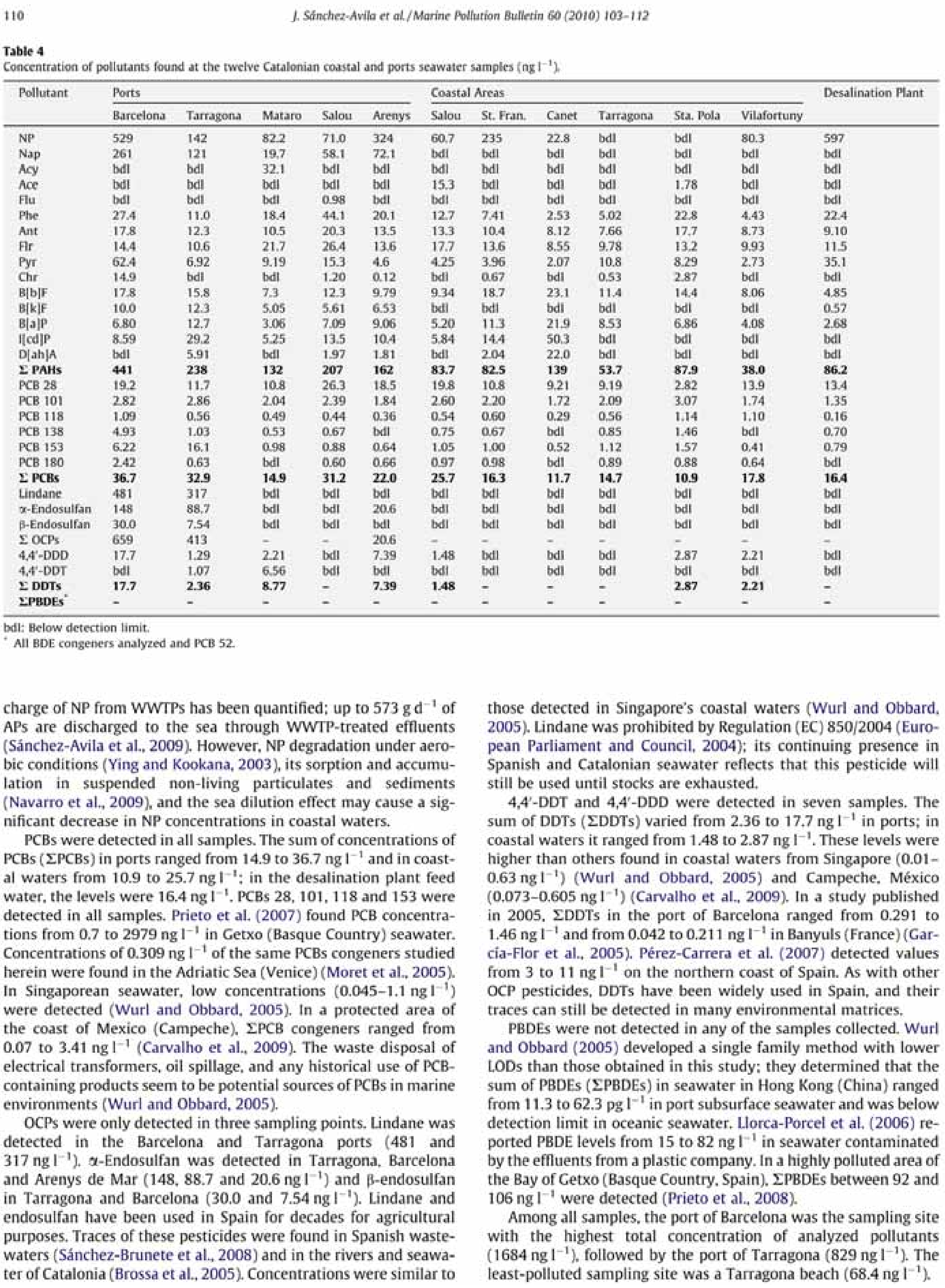

Table 4
Concentration of pollutants found at the twelve Catalonian coastal and ports seawater samples (ng l^{-1}).

Pollutant	Ports					Coastal Areas						Desalination Plant
	Barcelona	Tarragona	Mataro	Salou	Arenys	Salou	St. Fran.	Canet	Tarragona	Sta. Pola	Vilafortuny	
NP	529	142	82.2	71.0	324	60.7	235	22.8	bdl	bdl	80.3	597
Nap	261	121	19.7	58.1	72.1	bdl	bdl	bdl	bdl	bdl	bdl	bdl
Acy	bdl	bdl	32.1	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
Ace	bdl	bdl	bdl	bdl	bdl	15.3	bdl	bdl	bdl	1.78	bdl	bdl
Flu	bdl	bdl	bdl	0.98	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
Phe	27.4	11.0	18.4	44.1	20.1	12.7	7.41	2.53	5.02	22.8	4.43	22.4
Ant	17.8	12.3	10.5	20.3	13.5	13.3	10.4	8.12	7.66	17.7	8.73	9.10
Flr	14.4	10.6	21.7	26.4	13.6	17.7	13.6	8.55	9.78	13.2	9.93	11.5
Pyr	62.4	6.92	9.19	15.3	4.6	4.25	3.96	2.07	10.8	8.29	2.73	35.1
Chr	14.9	bdl	bdl	1.20	0.12	bdl	0.67	bdl	0.53	2.87	bdl	bdl
B[b]F	17.8	15.8	7.3	12.3	9.79	9.34	18.7	23.1	11.4	14.4	8.06	4.85
B[k]F	10.0	12.3	5.05	5.61	6.53	bdl	bdl	bdl	bdl	bdl	bdl	0.57
B[a]P	6.80	12.7	3.06	7.09	9.06	5.20	11.3	21.9	8.53	6.86	4.08	2.68
I[cd]P	8.59	29.2	5.25	13.5	10.4	5.84	14.4	50.3	bdl	bdl	bdl	bdl
D[ah]A	bdl	5.91	bdl	1.97	1.81	bdl	2.04	22.0	bdl	bdl	bdl	bdl
Σ PAHs	**441**	**238**	**132**	**207**	**162**	**83.7**	**82.5**	**139**	**53.7**	**87.9**	**38.0**	**86.2**
PCB 28	19.2	11.7	10.8	26.3	18.5	19.8	10.8	9.21	9.19	2.82	13.9	13.4
PCB 101	2.82	2.86	2.04	2.39	1.84	2.60	2.20	1.72	2.09	3.07	1.74	1.35
PCB 118	1.09	0.56	0.49	0.44	0.36	0.54	0.60	0.29	0.56	1.14	1.10	0.16
PCB 138	4.93	1.03	0.53	0.67	bdl	0.75	0.67	bdl	0.85	1.46	bdl	0.70
PCB 153	6.22	16.1	0.98	0.88	0.64	1.05	1.00	0.52	1.12	1.57	0.41	0.79
PCB 180	2.42	0.63	bdl	0.60	0.66	0.97	0.98	bdl	0.89	0.88	0.64	bdl
Σ PCBs	**36.7**	**32.9**	**14.9**	**31.2**	**22.0**	**25.7**	**16.3**	**11.7**	**14.7**	**10.9**	**17.8**	**16.4**
Lindane	481	317	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
α-Endosulfan	148	88.7	bdl	bdl	20.6	bdl	bdl	bdl	bdl	bdl	bdl	bdl
β-Endosulfan	30.0	7.54	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
Σ OCPs	659	413	–	–	20.6	–	–	–	–	–	–	–
4,4′-DDD	17.7	1.29	2.21	bdl	7.39	1.48	bdl	bdl	bdl	2.87	2.21	bdl
4,4′-DDT	bdl	1.07	6.56	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
Σ DDTs	**17.7**	**2.36**	**8.77**	**–**	**7.39**	**1.48**	**–**	**–**	**–**	**2.87**	**2.21**	**–**
ΣPBDEs[*]	–	–	–	–	–	–	–	–	–	–	–	–

bdl: Below detection limit.
[*] All BDE congeners analyzed and PCB 52.

charge of NP from WWTPs has been quantified; up to 573 g d^{-1} of APs are discharged to the sea through WWTP-treated effluents (Sánchez-Avila et al., 2009). However, NP degradation under aerobic conditions (Ying and Kookana, 2003), its sorption and accumulation in suspended non-living particulates and sediments (Navarro et al., 2009), and the sea dilution effect may cause a significant decrease in NP concentrations in coastal waters.

PCBs were detected in all samples. The sum of concentrations of PCBs (ΣPCBs) in ports ranged from 14.9 to 36.7 ng l^{-1} and in coastal waters from 10.9 to 25.7 ng l^{-1}; in the desalination plant feed water, the levels were 16.4 ng l^{-1}. PCBs 28, 101, 118 and 153 were detected in all samples. Prieto et al. (2007) found PCB concentrations from 0.7 to 2979 ng l^{-1} in Getxo (Basque Country) seawater. Concentrations of 0.309 ng l^{-1} of the same PCBs congeners studied herein were found in the Adriatic Sea (Venice) (Moret et al., 2005). In Singaporean seawater, low concentrations (0.045–1.1 ng l^{-1}) were detected (Wurl and Obbard, 2005). In a protected area of the coast of Mexico (Campeche), ΣPCB congeners ranged from 0.07 to 3.41 ng l^{-1} (Carvalho et al., 2009). The waste disposal of electrical transformers, oil spillage, and any historical use of PCB-containing products seem to be potential sources of PCBs in marine environments (Wurl and Obbard, 2005).

OCPs were only detected in three sampling points. Lindane was detected in the Barcelona and Tarragona ports (481 and 317 ng l^{-1}). α-Endosulfan was detected in Tarragona, Barcelona and Arenys de Mar (148, 88.7 and 20.6 ng l^{-1}) and β-endosulfan in Tarragona and Barcelona (30.0 and 7.54 ng l^{-1}). Lindane and endosulfan have been used in Spain for decades for agricultural purposes. Traces of these pesticides were found in Spanish wastewaters (Sánchez-Brunete et al., 2008) and in the rivers and seawater of Catalonia (Brossa et al., 2005). Concentrations were similar to those detected in Singapore's coastal waters (Wurl and Obbard, 2005). Lindane was prohibited by Regulation (EC) 850/2004 (European Parliament and Council, 2004); its continuing presence in Spanish and Catalonian seawater reflects that this pesticide will still be used until stocks are exhausted.

4,4′-DDT and 4,4′-DDD were detected in seven samples. The sum of DDTs (ΣDDTs) varied from 2.36 to 17.7 ng l^{-1} in ports; in coastal waters it ranged from 1.48 to 2.87 ng l^{-1}. These levels were higher than others found in coastal waters from Singapore (0.01–0.63 ng l^{-1}) (Wurl and Obbard, 2005) and Campeche, México (0.073–0.605 ng l^{-1}) (Carvalho et al., 2009). In a study published in 2005, ΣDDTs in the port of Barcelona ranged from 0.291 to 1.46 ng l^{-1} and from 0.042 to 0.211 ng l^{-1} in Banyuls (France) (García-Flor et al., 2005). Pérez-Carrera et al. (2007) detected values from 3 to 11 ng l^{-1} on the northern coast of Spain. As with other OCP pesticides, DDTs have been widely used in Spain, and their traces can still be detected in many environmental matrices.

PBDEs were not detected in any of the samples collected. Wurl and Obbard (2005) developed a single family method with lower LODs than those obtained in this study; they determined that the sum of PBDEs (ΣPBDEs) in seawater in Hong Kong (China) ranged from 11.3 to 62.3 pg l^{-1} in port subsurface seawater and was below detection limit in oceanic seawater. Llorca-Porcel et al. (2006) reported PBDE levels from 15 to 82 ng l^{-1} in seawater contaminated by the effluents from a plastic company. In a highly polluted area of the Bay of Getxo (Basque Country, Spain), ΣPBDEs between 92 and 106 ng l^{-1} were detected (Prieto et al., 2008).

Among all samples, the port of Barcelona was the sampling site with the highest total concentration of analyzed pollutants (1684 ng l^{-1}), followed by the port of Tarragona (829 ng l^{-1}). The least-polluted sampling site was a Tarragona beach (68.4 ng l^{-1}).

3.3. Environmental and legislative implications

PAHs levels found in Catalonian coastal waters were much lower than their EQS (Table 1). Once dissolved in water, it is presumed that PAHs can be sorbed upon sediments (Eljarrat et al., 2001) and particulate matter (Busetti et al., 2006), algae (Hutchinson et al., 2003), or seaweed (Porte et al., 2006), thus becoming available to filter-animals (mussels, oysters, clams, etc.) (Manoli and Samara, 1996), which might induce food chain biomagnification and possible harmful effects on biota and carcinogenic effects (Baumard et al., 1998).

Levels of NP found in Catalonian coastal waters were lower than their CCC and EQS values (Table 1). However, NP has been found at levels from 0.008 to 1.05 mg kg^{-1} in sediments from a Catalonian port (Petrovic et al., 2002), where it could be bioaccumulated by algae and harm fish and marine biota as a result of its endocrine disruptor effects (Soares et al., 2008).

Although no EQS were mentioned for PCBs (Table 1), these substances were banned by Regulation (EC) No. 850/2004 (European Parliament and Council, 2004), except in items that were already in use at the time. CCC values (Table 1) were exceeded in all sampling points except Canet and Santa Pola Beach. It is well-known that PCBs possess poor water solubility and tend to accumulate in sediments and in the fatty tissues of fish, shellfish and seals. Dissolved and particulated-bound concentrations should be continuously monitored since PCBs may cause potential damage to animals (Kot-Wasik et al., 2004). The US-EPA (2002) recommends against human consumption of organisms from waters with ΣPCB concentrations higher than 0.064 ng l^{-1}, a value based on the bioaccumulation of these substances in fish and a carcinogenicity of 10^{-6} risk. This ΣPCB limit was exceeded in all samples (10.9–36.7 ng l^{-1}).

Regarding OCPs, the sum concentrations of α and β-endosulfan exceeded EQS in the ports at Barcelona, Tarragona and Arenys de Mar (178, 96.3 and 20.6 vs. 10 ng l^{-1}). CCC values (8.70 ng l^{-1}) were also exceeded at all three sample points. Lindane concentrations in the ports at Barcelona and Tarragona were higher than their CCC value (481 and 317 ng l^{-1} vs. 160 ng l^{-1}). The three OCPs detected (see Section 3.4) are highly persistent in the environment and potentially harmful to wildlife, have endocrine disruptor effects (Brossa et al., 2005) and could cause cancer in humans (Sánchez-Brunete et al., 2008).

With respect to DDTs, EQS and CCC values were close to but did not exceed the concentrations detected (Table 1). DDT is considered highly persistent and toxic and exhibits long biological half-life and high lipophilicity (García-Flor et al., 2005). The US-EPA (2002) recommends against human consumption of organisms from waters with a concentration higher than 0.220 ng l^{-1} of 4,4′-DDT and 0.310 ng l^{-1} of 4,4′-DDD, values based on bioaccumulation of these substances in fish and a carcinogenicity of 10^{-6} risk. These limits were exceeded in the ports at Barcelona, Tarragona, Mataró, Arenys de Mar and the beaches at Salou, Santa Pola and Vilafortuny. In general, Catalonian coastal waters could be considered of standard quality according to the EQS for the substances analyzed, except for some specific analytes, particularly in the ports sampled.

4. Conclusions

The multi-residual method developed based on SBSE-TD-GC–MS allowed the ultra-trace identification of 49 compounds in seawater. PAHs, PCBs, OCPs and DDTs were detected at levels from 0.16 to 597 ng l^{-1} in coastal water and ports from the Catalonian coast. No PBDE congeners were detected. All measured concentrations were generally below the Environmental Quality Standards established by the EU Directive 2008/105/EC. The SBSE-TD-GC–MS method developed here has proven to be useful in screening persistent organic pollutants and nonylphenol with a sensitivity of ng l^{-1} levels. Its high reliability, reproducibility, and robustness ensure the viability of its use for the routine multi-residual analysis of toxic substances in seawater following recent European Union legislation standards and US-EPA recommendations for seawater contaminants. Furthermore, since only small volumes of water are necessary, sampling and extraction procedures (generally performed from a boat) can be greatly simplified. Furthermore, the proposed method is more environmentally friendly than other, more traditional methods because only minimal amounts of toxic organic solvents are used.

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ARTÍCULO CIENTÍFICO 3.

“Development of a multi-residue method for the determination of organic micropollutants in water, sediment and mussels using gas chromatography-tandem mass spectrometry”

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Development of a multi-residue method for the determination of organic micropollutants in water, sediment and mussels using gas chromatography–tandem mass spectrometry

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This study describes the development of a multiresidue method based on gas chromatography–electron ionization-tandem mass spectrometry (GC–EI-MS/MS) for the detection of sixteen polycyclic aromatic hydrocarbons (PAHs), five phthalate esters (PEs), seven polychlorinated biphenyls (PCBs), six polybrominated diphenyl ethers (PBDEs), six alkylphenols (APs), three organochlorined pesticides and their isomers or degradation products (OCPs) and bisphenol A in seawater, river water, wastewater treatment plant (WWTP) effluents, sediments and mussels. Solid phase extraction (SPE) was used for the extraction of target analytes in aqueous samples, and ultrasound assisted extraction for solid samples. GC–EI-MS/MS acquisition conditions in selected reaction monitoring (SRM) using two transitions per compound were optimized. In this way, quantification and unequivocal identification of organic micropollutants were performed in compliance with the Decision 2002/657/EC. Good linearity responses with coefficients of determination higher than 0.99 were obtained. Methodological detection limits (MDLs) in seawater ranged from 0.1 to 6 ng L^{-1}; in river water from 0.1 to 4.8 ng L^{-1}; in WWTP effluents from 1 to 75 ng L^{-1}; in sediments from 1 to 150 ng g^{-1} and in mussels from 1 to 125 ng g^{-1}. MDLs and recovery yields were compared with other published methods and similarities or even improvements were achieved. The optimized method was applied to analyze five samples from each matrix collected in coastal areas, showing its potential use for marine pollution monitoring.

1. Introduction

The increasing worldwide pollution of coastal and open seawater with thousands of industrial and natural chemical compounds is one of the key environmental problems facing humanity [1]. A wide variety of organic micropollutants have been identified in coastal and marine ecosystems, including phthalate esters (PE), bisphenol A (BPA), polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polybromodiphenyl ethers (PBDEs) and alkylphenols (APs), among others [2–6]. Studies done in several parts of the world identified the rivers and wastewater treatment plant (WWTP) discharges as the main contributors of such pollutants to the marine environment [7–12]. Pollutants entering into the marine environment are distributed in water, suspended particles or accumulated in sediment and in biota, depending on their physicochemical properties [13–16]. Although most of these compounds are present at the ng to μg L^{-1} concentrations range [2,3,5], they trigger endocrine disruption in marine organisms [17], neurotoxicity [18], and alterations at the ecosystem level [19].

Monitoring and biomonitoring programs have been going on for several decades [20–22] and outline strategies and guidelines to assess micropollutants to protect the marine environment [6,23]. Still there are lacks in the monitoring strategies: most of the monitoring projects focus on just a single group of chemically related pollutants and all relevant compartments within a study area are not always investigated simultaneously [6,12]. There is a need of sensitive monitoring methods allowing complete extraction and analysis of a wide range of organic contaminants from whole (without filtering) water samples and from other matrices [24]. Only when the levels of pollutants in various compartments and the main sources of pollution have been identified, the environmental quality and the anthropogenic impact can be fully assessed [9,12].

The low concentrations of micropollutants in the marine environment in addition to the complexity of those matrices are two major difficulties associated with the detection and quantification of pollutants. For legislative [1] and toxicological reasons, sensitive and specific analytical procedures are required to detect concentrations that can be injurious to aquatic life [6,15]. Gas

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chromatography coupled to tandem mass spectrometry (GC–MS/MS) is a relatively new analytical technique which provides reliability and sensitivity for the trace analysis of micropollutants in the environment [25–27]. The use of MS/MS has been considered as a valuable approach which minimizes or even removes matrix interferences by selecting adequate precursor and product ions. The use of two stages of mass analysis in MS/MS, offers the possibility of applying selected reaction monitoring (SRM), one of the most selective and sensitive approaches for quantification and confirmation, especially in trace analysis where normally there is high chemical noise [28,29]. At the same time, an efficient extraction and cleanup method is necessary to avoid poor ionization, background noise or damages in the GC system [30]. A typical advantage of these analytical procedures is that they have been designed as multiresidue approaches [6]. Multiresidue methods permit the analysis of a wide range of pollutants with varying physico-chemical parameters in a single run. In terms of cost and time, in front specific chemical families using different methodologies is beneficial because they can "capture" all the chemical information of a sample [22].

The aim of the present study was to optimize a multiresidue analytical method for the determination of trace concentrations of PE, BPA, PAHs, OCPs, PCBs, PBDEs, and APs in seawater, river water, WWTP effluents and also in sediments and mussels using GC–MS/MS and to demonstrate its performance by analyzing environmental samples. This study represents the first approach which will enable the routine determination of organic micropollutants in environmental matrices with an attempt to improve marine environment monitoring strategies.

2. Materials and methods

2.1. Reagents and materials

Sixteen PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene, and benzo(g,h,i)perylene), five phthalate esters (dimethyl phthalate, diethyl phthalate, butyl benzyl phthalate, di-2-ethylhexyl phthalate, and di-2-ethylhexyl adipate), seven PCBs (PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, and PCB 180), eight PBDEs (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, BDE 183, and BDE 209), four APs (octylphenol, nonylphenol branched, nonylphenol monoethoxylated, and nonylphenol diethoxylated), three OCPs and its isomers or degradation products (2,4′-DDE, 4,4′-DDE, 2,4′-DDD, 4,4′-DDD, 2,4′-DDT, 4,4′-DDT; α, β, γ and δ-hexachlorocyclohexanes; α and β endosulfans) and bisphenol A were analyzed (Table 1). A solution mix of PAHs in methanol at 1000 ng μL^{-1} was purchased from AccuStandard (New Haven, CT, USA); a solution mix of phthalate esters in methanol at 500 ng μL^{-1} was from Supelco (Bellefonte, PA, USA); a solution mix of PCBs in iso-octane at 10 ng μL^{-1} was from Dr. Ehrenstorfer (Augsburg, Germany); a solution mix of PBDEs in nonane at 10 ng μL^{-1} was from Cambridge Isotope Laboratories (Andover, MA, USA); a technical mixture of α and β endosulfan in methanol at 100 ng μL^{-1}, a technical mixture of hexachlorocyclohexanes (α, β, δ and γ) in methanol at 100 ng μL^{-1} and a solution mix of DDT derivates in cyclohexane at 10 ng μL^{-1} were from Dr. Ehrenstorfer; a solid technical mixture of 4-nonylphenol isomers was from Riedel-de Haën (Seelze, Germany); 4-nonylphenol-mono-ethoxylate, 4-nonylphenol-di-ethoxylate and bisphenol A were from Dr. Ehrenstorfer (Augsburg, Germany) as a solid and 4-tert-octylphenol from Supelco (Bellefonte, PA, USA) as a solid. Standard solutions (0.5 and 1 ng μL^{-1}) of each compound were prepared in ethyl acetate and stored in the dark at −20 °C until use. These solutions were used to spike the water samples and draw the calibration curves.

The surrogate standard mix containing acenaphthylene d_8, benzo(a)pyrene d_{12}, benzo(g,h,i)perylene d_{12}, fluoranthene d_{10}, naphthalene d_8, phenanthrene d_{10}, and pyrene d_{10} at 200 ng μL^{-1} in methanol was purchased from Supelco (Bellefonte, USA). A mixture solution of PCB 209 at 10 ng μL^{-1} in iso-octane was from Dr. Ehrenstorfer. A solution of 4-n-nonylphenol-d_8 at 100 ng μL^{-1} in acetone was from Dr. Ehrenstorfer. Bisphenol A-d_{16} was from Supelco.

Solvents used were HPLC grade supplied by Merck (Darmstad, Germany). Oasis HLB solid phase extraction (SPE) cartridges (200 mg) and Florisil (500 mg) cartridges were from Waters (USA). Nitrogen for drying with 99.995% of purity was from Air Liquid (Barcelona, Spain).

2.2. Sample preparation

2.2.1. Water extraction

Unfiltered water samples (1000 mL of sea or river waters or 250 mL of WWTP effluent) were analyzed to avoid sub-estimations in the total concentration and thus, both dissolved and particulate bound chemicals were considered. The surrogate standards were added to the raw water prior to preconcentration at 10 ng L^{-1} in sea or river waters and at 100 ng L^{-1} in WWTP effluents. For the preconcentration step, a Baker vacuum system (J.T. Baker, The Netherlands) was used. Oasis HLB (200 mg) SPE cartridges were conditioned with 10 mL of hexane followed by 10 mL of dichloromethane, 10 mL of methanol and 15 mL of ultrapure water, all at a flow rate of 5 mL min^{-1}. Samples were percolated through the cartridges at a flow rate of 10 mL min^{-1}. The cartridge was finally rinsed with 5 mL × 3 of ultrapure water and dried under vacuum during 30 min. Elution was performed with 10 mL of dichloromethane/hexane (1:1, v/v) followed by 10 mL of dichloromethane/acetone (1:1, v/v). The extracts were evaporated at 25 °C under nitrogen current using a TurboVap LV (Caliper Life Sciences, UK) until almost dryness. Ultrapure, sea and river water extracts were reconstituted with 100 μL of ethyl acetate and WWTP effluent extracts with 250 μL. Extracts were spiked with 0.1 ng μL^{-1} of the internal standard anthracene d_{10}.

2.2.2. Extraction of mussels and sediments

One gram of freeze-dried and homogenized mussel samples (*Mytilus galloprovincialis*) were spiked with the surrogate standards to get a final concentration of 200 ng g^{-1}. One gram of freeze-dried and homogenized <120 μm particle sediment samples were weighted and the surrogate standards were added to a final concentration of 50 ng g^{-1}. Samples were homogenized and kept at 4 °C overnight and subsequently liquid–solid extracted by sonication (10 min) using different solvents: method 1 extraction was performed with 2× 10 mL of dichloromethane/hexane and 1× 10 mL of dichloromethane/acetone; method 2 with 2× 10 mL dichloromethane/hexane and 1× 10 mL of hexane/acetone; method 3 with 1× 10 mL of dichloromethane/hexane and 2× 10 mL of hexane/acetone. After each extraction step, samples were centrifuged for 10 min at 2500 rpm. Extracts were combined and concentrated to approximately 1 mL under a nitrogen current using a TurboVap LV at 25 °C. Extracts were subsequently cleaned up using Florisil (5 g) SPE cartridges, previously conditioned with 20 mL of hexane/dichloromethane (1:1, v/v) and 20 mL of hexane/acetone (1:1, v/v). The sample extract was eluted with 15 mL of hexane/dichloromethane (1:1, v/v) and 15 mL of hexane/acetone (1:1, v/v). The eluent was evaporated until almost dryness under a nitrogen current at room temperature and reconstituted with ethyl acetate to a final volume of 200 μL for mussels and 500 μL

Table 1
GC–MS/MS method parameters.

Time segment (min)	Retention time (min)	Target compound	Mol. weight	Identification transition (*Q*)	*Q* Col. Energy (eV)	Confirmation transition (*q*)	q Col. Energy (eV)	*q*/*Q* ratio (%)
06.0–11.2	7.18	Naphthalene	128	128 > 102	20	128 > 77	45	19.7
	9.90	Dimethyl phthalate	194	163 > 77	25	163 > 133	10	25.8
	9.92	*Acenaphthylene D_8*	160	160 > 158	35	160 > 132	30	99.0
	9.95	Acenaphthylene	152	152 > 126	25	152 > 150	35	97.9
	10.41	Acenaphthene	154	154 > 153	15	154 > 152	35	41.4
11.2–13.0	11.89	Fluorene	166	166 > 165	20	166 > 163	55	7.5
	11.98	Diethyl phthalate	222	149 > 65	25	149 > 121	10	52.9
	12.16	Octylphenol	206	135 > 107	15	135 > 77	30	78.9
13.0–17.1	14.04	α-hexachlorocyclohexane	291	181 > 145	15	219 > 183	15	24.1
	15.16	β-hexachlorocyclohexane	291	181 > 145	15	219 > 183	15	35.0
	15.38	γ-hexachlorocyclohexane	291	181 > 145	15	219 > 183	15	24.7
	15.56	*Dipropyl phthalate D_4*	254	153 > 69	20	153 > 125	10	42.4
	15.65	*Phenanthrene D_{10}*	188	188 > 184	45	188 > 160	45	84.3
	15.76	Phenanthrene	178	178 > 152	30	178 > 176	45	39.3
	15.88	*Anthracene D_{10}*	188	188 > 184	45	188 > 160	45	80.9
	15.96	Anthracene	178	178 > 176	50	178 > 152	25	46.7
	16.48	δ-hexachlorocyclohexane	291	181 > 145	15	219 > 183	15	26.7
	14.0–15.3	Nonylphenol branched	220	135 > 107	10	135 > 77	35	22.7
17.1–22.0	17.72	*Nonylphenol D_8*	228	113 > 81	25	113 > 54	45	32.2
	17.98	PCB 28	258	256 > 186	30	258 > 151	50	24.1
	19.70	PCB 52	292	292 > 220	45	292 > 222	45	73.2
	20.20	Di-2-ethylhexyl phthalate	391	149 > 65	25	149 > 121	10	50.7
	20.0–21.5	Nonylphenol monoethoxylated	264	179 > 135	5	179 > 107	25	58.3
22.0–25.0	22.40	*Fluoranthene D_{10}*	212	212 > 208	45	212 > 210	30	77.7
	22.54	Fluoranthene	202	202 > 200	45	202 > 150	55	19.5
	23.74	*Pyrene D_{10}*	212	212 > 208	45	212 > 210	30	84.2
	23.85	Pyrene	202	202 > 200	45	202 > 150	55	17.7
	24.14	2,4′-DDE	318	246 > 176	45	318 > 248	45	8.3
	24.28	PCB 101	326	326 > 256	45	326 > 254	45	82.3
	24.1–24.3	α-endosulfan	407	241 > 206	10	195 > 159	5	29.7
	25.54	*Bisphenol A D_{16}*	244	224 > 97	30	224 > 125	20	79.6
25.0–26.8	25.86	Bisphenol A	228	213 > 119	20	228 > 213	5	50.0
	25.92	4,4′-DDE	318	246 > 176	45	318 > 248	20	47.6
	26.32	2,4′-DDD	320	235 > 165	25	237 > 165	20	65.5
26.8–29.6	27.39	β-endosulfan	407	241 > 206	10	195 > 159	5	85.4
	27.6–29.1	Nonylphenol diethoxylated	309	223 > 135	5	223 > 107	25	42.9
	27.67	PCB 118	326	326 > 254	50	326 > 256	50	85.9
	27.71	BDE 28	407	409 > 249	45	407 > 247	15	1.7
	28.21	4,4′-DDD	318	235 > 165	25	237 > 165	20	65.7
	28.33	2,4′-DDT	355	235 > 165	25	237 > 165	20	47.1
	28.95	PCB 138	361	360 > 290	45	360 > 288	50	33.3
29.6–31.2	30.24	*4,4′-DDT $^{13}C_{12}$*	355	247 > 177	25	247 > 212	20	6.3
	30.25	4,4′-DDT	355	235 > 165	20	237 > 165	20	64.5
	30.35	Butyl benzyl phthalate	312	149 > 65	25	149 > 121	10	51.4
	30.45	PCB 153	361	360 > 290	45	360 > 288	45	47.8
31.2–36.7	31.79	Di-2-ethylhexyl adipate	371	129 > 55	15	129 > 101	5	75.9
	32.43	Benz(a)anthracene	228	228 > 226	45	228 > 224	55	34.5
	32.68	Chrysene	228	228 > 226	45	228 > 224	55	28.8
	34.11	PCB 180	395	394 > 324	45	394 > 322	45	33.3
	34.17	BDE 47	486	486 > 326	15	488 > 328	55	2.8
36.7–43.8	38.20	BDE 100	565	404 > 297	25	566 > 406	20	71.1
	38.74	Benzo(b)fluoranthene	252	252 > 250	45	250 > 248	50	10.0
	38.86	Benzo(k)fluoranthene	252	252 > 250	45	250 > 248	45	9.6
	39.17	BDE 99	565	404 > 297	45	566 > 406	20	47.5
36.7–43.8	40.01	*Benzo[a]pyrene D_{12}*	254	264 > 260	45	264 > 236	40	26.6
	40.16	Benzo(a)pyrene	252	252 > 250	45	252 > 224	55	13.1
	40.87	*PCB 209*	499	498 > 428	30	498 > 426	30	59.2
	41.89	BDE 154	644	484 > 324	45	646 > 486	30	82.8
	43.13	BDE 153	644	646 > 486	30	484 > 324	50	46.8
43.8–56.0	44.72	Indeno(1,2,3-cd)pyrene	276	276 > 274	45	276 > 272	55	10.2
	44.92	Dibenzo(a,h)anthracene	278	278 > 276	40	278 > 274	55	24.3
	45.46	*Benzo[g,h,i]perylene D_{12}*	288	288 > 284	40	288 > 286	40	39.2
	45.55	Benzo(g,h,i)perylene	276	276 > 274	50	276 > 272	55	9.1
	–	BDE 183[a]	722	561 > 402	45	563 > 404	45	–
	–	BDE 209[a]	959	799 > 640	50	801 > 642	55	–

Surrogates in cursive.

[a] Not included in the SRM method.

Table 2
GC–EI-MS/MS instrumental quality parameters of the 51 pollutants considered in the developed methodology (coefficient of determination, points used, IDL and instrumental reproducibility).

Target analyte	R^2	Points used	Lineal range	(ng μL^{-1})	IDL (pg inj.)	Instrumental reprod.[a] (RSD, %)
Naphthalene	0.9995	9	0.010	1.0	0.7	12
Acenaphthylene	0.9984	9	0.010	1.0	0.8	7
Acenaphthene	0.9990	9	0.010	1.0	1.1	9
Fluorene	0.9986	9	0.010	1.0	0.3	8
Phenanthrene	0.9992	9	0.010	1.0	1.1	3
Anthracene	0.9992	9	0.010	1.0	1.5	4
Fluoranthene	0.9994	9	0.010	1.0	1.2	3
Pyrene	0.9994	9	0.010	1.0	1.2	4
Benz(a)anthracene	0.9997	9	0.010	1.0	0.8	11
Chrysene	0.9993	9	0.010	1.0	0.8	9
Benzo(b)fluoranthene	0.9993	9	0.010	1.0	0.7	10
Benzo(k)fluoranthene	0.9998	9	0.010	1.0	0.7	8
Benzo(a)pyrene	0.9994	9	0.010	1.0	0.8	7
Indeno(1,2,3-cd)pyrene	0.9992	9	0.010	1.0	0.6	8
Dibenzo(a,h)anthracene	0.9988	9	0.010	1.0	4.4	8
Benzo(g,h,i)perylene	0.9993	9	0.010	1.0	0.3	12
Dimethyl phthalate	0.9991	9	0.010	1.0	0.8	10
Diethyl phthalate	0.9910	8	0.025	1.0	0.8	9
Di-2-ethylhexyl phthalate	0.9932	8	0.025	1.0	2.6	10
Butyl benzyl phthalate	0.9972	9	0.010	1.0	1.6	7
Di-2-ethylhexyl adipate	0.9943	9	0.010	1.0	0.4	5
Bisphenol A	0.9974	9	0.010	1.0	2.1	8
Octylphenol	0.9943	9	0.010	1.0	1.1	7
Nonylphenol branched	0.9958	9	0.010	1.0	3.7	8
Nonylphenol monoethoxylated	0.9972	9	0.010	1.0	5.0	9
Nonylphenol diethoxylated	0.9985	9	0.010	1.0	5.5	11
BDE 28	0.9987	5	0.010	0.1	1.5	9
BDE 47	0.9993	5	0.010	0.1	2.5	10
BDE 99	0.9942	5	0.010	0.1	6.5	9
BDE 100	0.9999	5	0.010	0.1	5.1	7
BDE 153	0.9934	5	0.010	0.1	6.4	4
BDE 154	0.9954	5	0.010	0.1	10	13
PCB 28	0.9989	9	0.010	1.0	0.4	7
PCB 52	0.9992	9	0.010	1.0	1.9	6
PCB 101	0.9990	9	0.010	1.0	2.5	7
PCB 118	0.9989	9	0.010	1.0	3.2	11
PCB 138	0.9991	9	0.010	1.0	2.6	9
PCB 153	0.9986	9	0.010	1.0	2.9	10
PCB 180	0.9992	9	0.010	1.0	2.6	7
2,4′-DDE	0.9981	9	0.010	1.0	0.7	7
4,4′-DDE	0.9988	9	0.010	1.0	1.1	4
2,4′-DDD	0.9980	9	0.010	1.0	0.6	8
4,4′-DDD	0.9973	9	0.010	1.0	0.6	6
2,4′-DDT	0.9968	9	0.010	1.0	0.9	11
4,4′-DDT	0.9964	9	0.010	1.0	0.9	8
α-Hexachlorocyclohexane	0.9998	8	0.010	1.0	2.0	8
β-Hexachlorocyclohexane	0.9994	8	0.010	1.0	20	11
γ-Hexachlorocyclohexane	0.9989	8	0.010	1.0	17	8
δ-Hexachlorocyclohexane	0.9996	8	0.010	1.0	13	13
α-Endosulfan	0.9986	9	0.010	1.0	1.8	10
β-Endosulfan	0.9970	9	0.010	1.0	6.5	10

[a] Analysis performed in 3 different days.

for sediments. Internal standard anthracene d_{10} was added at a concentration of 1 ng μL^{-1}.

2.2.3. GC–EI-MS/MS conditions

Analysis was performed using an Agilent 7890A GC System (Agilent Technologies, Palo Alto, CA, USA) interfaced to a 7000A triple quadrupole mass spectrometer system (Agilent, USA) in EI (+70 eV). Two μL of extracts were injected using an Agilent 7683A autosampler. Injector port temperature was 290 °C. Injection was performed in splitless mode, keeping the split valve closed for 1.5 min and a purge flow of 30 mL min^{-1}. Initially, a 15 m HP-5MS capillary column was used to detect BDE 209. However, PCBs and PAHs coeluted and hindered compound identification, and therefore a 30 m capillary column was used (30 m × 0.25 mm i.d. × 0.25 μm, 5% phenyl methyl siloxane, Agilent HP-5MS) and these compounds could not be included in the multiresidue method. A constant flow of helium at 1.6 mL min^{-1} was used as the carrier gas. The oven temperature was programmed from 70 °C (holding time 4 min) to 150 °C at 25 °C min^{-1}, to 225 °C at 3 °C min^{-1} and finally to 310 °C at 5 °C min^{-1}, keeping the final temperature for 10 min. Transfer line and ionization source temperatures were 290 °C and 250 °C, respectively. Nitrogen was used as collision gas at 1.5 mL min^{-1}. For increased sensitivity and specificity, peak detection and quantification were performed in selected reaction monitoring (SRM). Individual SRM conditions were experimentally developed for each compound and precursor and product ions, collision energies and other parameters were developed (Table 1). The target compounds were positively identified by comparison of their retention times and two SRM transitions to the standard solutions. A MassHunter WorkStation Acquisition Software rev. B.02.01 (Agilent Technologies) was used for data acquisition and automatic integration and quantification of the results.

Table 3

Method validation parameters for the 51 studied compounds at each matrix.

Target pollutant	Ultrapure water ($n=5$)[a]			Seawater ($n=5$)[a]			River water ($n=5$)[a]			WWTP effluent ($n=5$)[b]			Mussels ($n=4$)[c]			Sediments ($n=4$)[d]		
	Recov. (%)	RSD (%)	MDL (ng L^{-1})	Recov. (%)	RSD (%)	MDL (ng L^{-1})	Recov. (%)	RSD (%)	MDL (ng L^{-1})	Recov. (%)	RSD (%)	MDL (ng L^{-1})	Recov. (%)	RSD (%)	MDL (ng g^{-1})	Recov. (%)	RSD (%)	MDL (ng g^{-1})
Naphthalene	100	26	0.4	84	3	0.3	96	32	0.2	99	16	8	69	31	12	70	18	19
Acenaphthylene	85	1	0.8	113	4	0.2	108	11	0.7	101	8	18	94	10	19	83	2	19
Acenaphthene	101	9	1	116	9	1	113	11	0.5	120	12	9	93	13	7	86	11	17
Fluorene	111	12	0.7	107	13	0.9	109	11	0.6	116	6	4	94	2	7	103	13	8
Phenanthrene	93	18	0.5	97	8	0.7	109	7	0.1	100	12	6	90	1	18	104	3	7
Anthracene	103	14	0.7	102	8	1	107	5	0.3	103	17	10	92	1	26	104	4	13
Fluoranthene	99	16	1	107	9	0.9	99	11	0.3	103	2	10	94	8	20	97	8	8
Pyrene	103	14	0.9	106	6	0.7	104	10	0.3	113	9	8	99	9	14	106	5	4
Benz(a)anthracene	105	17	1	100	5	0.5	88	3	0.3	91	1	15	104	8	31	93	8	2
Chrysene	109	14	0.6	115	4	0.8	90	10	0.3	92	0	10	86	13	24	83	14	1
Benzo(b)fluoranthene	108	11	1	87	9	1	84	6	0.4	94	5	10	106	5	18	90	6	2
Benzo(k)fluoranthene	101	12	1.0	93	11	1	76	6	0.4	90	0	10	90	13	11	86	10	1
Benzo(a)pyrene	99	10	1	75	8	2	79	14	0.5	90	3	11	98	12	21	85	6	23
Indeno(1,2,3-cd)pyrene	81	11	0.9	70	13	0.9	89	9	0.2	69	13	6	109	8	41	107	4	5
Dibenzo(a,h)anthracene	100	14	0.8	88	9	1.0	90	7	0.6	97	6	16	109	6	58	82	6	48
Benzo(g,h,i)perylene	98	17	0.9	82	13	0.8	79	5	0.2	83	2	11	103	10	14	84	15	8
Dimethyl phthalate	117	7	2	126	6	2	109	8	0.7	113	11	24	81	2	10	114	9	6
Diethyl phthalate	104	2	0.4	88	9	0.5	116	27	0.2	139	2	12	103	3	4	81	18	6
Di-2-ethylhexyl phthalate	119	19	0.3	151	4	0.3	83	2	0.1	147	12	5	115	6	16	89	8	11
Butyl benzyl phthalate	115	13	0.6	117	16	0.9	110	17	0.4	86	9	9	91	14	18	109	4	4
Di-2-ethylhexyl adipate	103	20	0.7	107	8	2	120	4	0.4	111	11	38	87	5	27	97	10	6
Bisphenol A	104	17	1	96	10	2	101	15	1	111	4	6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Octylphenol	98	7	0.2	106	14	0.3	96	8	0.5	118	13	7	119	8	26	84	11	38
Nonylphenol branched	108	7	1	108	6	0.9	108	18	1	97	12	28	91	12	83	107	10	48
Nonylphenol monoethoxylated	107	29	1	95	12	1	119	17	1	106	6	55	103	19	125	93	2	150
Nonylphenol diethoxylated	108	18	2	90	6	4	71	1	2	67	22	42	85	5	110	105	2	103
BDE 28	99	7	0.6	70	19	0.9	90	6	0.4	87	4	15	90	9	3	104	9	11
BDE 47	95	20	0.6	102	22	1	102	20	0.5	78	21	27	94	12	0.9	95	4	24
BDE 99	75	11	0.7	91	21	3	100	8	0.5	114	16	44	96	12	1.2	100	5	5
BDE 100	73	9	2	98	22	4	76	8	3	100	15	75	87	11	3	88	2	8
BDE 153	70	13	1	102	12	2	73	12	2	83	6	36	82	10	2	84	7	44
BDE 154	80	17	2	108	2	6	102	4	5	101	10	75	99	9	5	101	12	25
PCB 28	107	9	0.2	86	15	0.1	76	7		86	9	1	81	5	4	81	13	3
PCB 52	78	2	0.8	101	15	0.8	107	2	0.3	113	17	27	81	2	13	86	12	13
PCB 101	102	17	0.5	90	1	0.4	94	0	0.3	116	4	9	87	3	16	93	7	13
PCB 118	95	8	0.7	79	17	0.9	78	14	0.3	92	9	20	78	10	13	102	6	5
PCB 138	113	7	1	79	9	1	80	17	0.6	89	10	22	79	5	19	103	8	8
PCB 153	110	8	0.9	87	17	1	73	18	0.3	86	12	27	77	5	12	105	7	5
PCB 180	99	15	0.6	92	3	2	83	10	0.4	83	9	22	80	10	6	92	8	2
2,4′-DDE	74	3	0.3	96	9	0.3	94	21	0.4	87	17	5	89	3	32	90	4	2
4,4′-DDE	87	2	1	90	11	1	89	7	0.5	78	19	7	89	5	21	109	5	3
2,4′-DDD	88	13	1	88	20	2	86	17	0.1	74	0	7	96	3	20	99	9	27

Table 3 (*Continued*)

Target pollutant	Ultrapure water ($n = 5$)[a]			Seawater ($n = 5$)[a]			River water ($n = 5$)[a]			WWTP effluent ($n = 5$)[b]			Mussels ($n = 4$)[c]			Sediments ($n = 4$)[d]		
	Recov. (%)	RSD (%)	MDL (ng L^{-1})	Recov. (%)	RSD (%)	MDL (ng L^{-1})	Recov. (%)	RSD (%)	MDL (ng L^{-1})	Recov. (%)	RSD (%)	MDL (ng L^{-1})	Recov. (%)	RSD (%)	MDL (ng g^{-1})	Recov. (%)	RSD (%)	MDL (ng g^{-1})
4,4'-DDD	108	7	0.7	79	7	1	90	7	0.4	87	16	8	96	12	17	98	5	4
2,4'-DDT	100	12	1	99	7	1	86	15	0.6	74	9	11	85	13	15	102	1	5
4,4'-DDT	108	9	0.3	94	5	1	111	11	0.5	79	11	8	98	5	4	98	5	24
α-Hexachlorocyclohexane	94	6	0.2	83	5	0.2	77	9	0.7	82	14	11	78	8	33	96	13	3
β-Hexachlorocyclohexane	103	7	0.7	93	15	4	70	4	0.7	114	20	17	101	1	14	84	9	24
γ-Hexachlorocyclohexane	111	4	2	95	10	1	82	6	0.2	84	14	9	78	2	11	82	1	73
δ-Hexachlorocyclohexane	108	8	2	106	10	4	73	3	0.5	106	1	56	86	4	12	100	15	59
α-Endosulfan	108	9	1	105	6	1	117	5	0.7	95	7	14	97	3	7	97	3	34
β-Endosulfan	112	20	3	99	18	3	94	3	0.9	82	2	44	94	11	71	94	11	39

n.a., not analyzed.

[a] Fortified at 10 ng L^{-1} of target compounds.

[b] Fortified at 250 ng L^{-1} of target compounds.

[c] Fortified at 200 ng L^{-1} of target compounds.

[d] Fortified at 100 ng L^{-1} of target compounds.

2.3. Analytical performance

The validation criterion applied to assess the performance of the developed method was based on Commission Decision 2002/657/EC [31] and Taverniers et al. [32]. Quantification was performed using the internal standard method. A 9 points calibration curve was analyzed in triplicate at 0.010, 0.025, 0.050, 0.075, 0.10, 0.25, 0.50, 0.75 and 1.0 ng μL^{-1} for all target compounds except for the PBDEs (0.010–0.10 ng μL^{-1}). Linearity was assumed when the regression coefficient was greater than 0.99 with RSD lower than 15%, value established according to the concentration range used in the calibration curve. For instrumental repeatability, 6 determinations in different days (2 concentrations/3 replicates each) were performed. The instrumental detection limits (IDLs) were calculated by a signal-to-noise ratio of three, using the lowest concentration standard [31,32].

The accuracy of the method was assessed by means of recovery experiment, analyzing 4 types of water (ultrapure, sea, river and WWTP effluent), mussels and sediments. The samples were spiked with all 53 target compounds at 10 ng L^{-1} for ultrapure, river and sea waters, 250 ng L^{-1} for WWTP effluents, 200 ng g^{-1} for mussels and 100 ng g^{-1} for sediments. The precision of the method was determined in terms of relative standard deviation (RSD, %) from the recovery experiments ($n \geq 3$). In accordance with method validation criteria [31,32] for concentrations ≤ 1 μg L^{-1}, good recoveries are considered from 40 to 120% with RSD $\leq$ 30% and for concentrations upto 1000 ng g^{-1}, good recoveries are considered from 80 to 110% with RSD $\leq$ 16. Sample blanks ($n \geq 3$) were rigorously analyzed to eliminate any external source of contamination and to evaluate phthalate contribution in the extraction procedures. Methodological detection limits (MDL) were calculated at a signal-to-noise ratio of five using the spiked matrices.

3. Results and discussion

3.1. Optimization of the GC–MS/MS parameters

The process of product ion in MS/MS involves the isolation of a single parent ion and then the dissociation of the precursor ion into characteristic product ions. First, individual standards were injected in GC/MS in the full-scan mode (50–1500 m/z mass range). The ion with the highest abundances for each analyte was selected as the base peak. Precursor ions were then subjected to different collision energy voltages (between 5 and 60 eV) to generate the subsequent product ions. The two most abundant fragments were chosen. SRM transitions of precursor ions fragmenting into product ions at specific collision energy values were defined for each target compound. In line with the Decision 2002/657/EC [31], the use of two transitions (one precursor with two product ions or two precursors with one product ion) confirms positive findings by SRM. Briefly, the Directive implements the concept of identification points (IPs) according to which in the MS/MS determination 1.0 IP is earned from a precursor ion and 1.5 IPs are earned from a product ion. The unequivocal confirmation of compound identity requires at least 3 and 4 identification points for legislated and banned substances, respectively. This criterion was originally defined for the determination of organic contaminants in animal products, although it is being increasingly accepted in the analysis of environmental and food matrices [28,29]. In this study, two product ions resulting from fragmentation of one precursor ion (4 IPs) or two product ions each resulting from two different precursor ions (5 IPs) were monitored. As shown in Table 1, a total of 95 different SRM transitions were monitored by GC–EI-MS/MS to cover all target pollutants and the surrogate standards. For each target compound, the transition with the highest intensity was selected as the quantitative transition (Q) and the other one as the confirmation transition (q). The relative intensity ratios expressed as percentage ($q/Q \times 100$) were calculated from the most concentrated calibration standard using the MassHunter Workstation Software (Table 1). The ($q/Q \times 100$) ratio was established as an additional identification parameter. According to the Decision 202/657/EC [31] a tolerance of ±20% was considered when ($q/Q \times 100$) was >50%; of ±25% when was between 20 and 50%; of ±30% when was between 10 and 20% and of ±50% when was <10%. This criterion was accomplished for all analyzed compounds, even for the less concentrated calibration standards.

The high stability of PAHs makes them difficult to fragment in EI conditions which leads to the formation of the molecular ion ($M^{+\bullet}$). The main advantage of the MS/MS technique is the possible fragmentation of PAHs in the collision cell, leading, under drastic conditions (high energy voltage applied to the collision cell: 30–60 eV) to the formation of specific product ions, such as $[M-H]^+$ (for example acenaphthene, 154 > 153), $[M-2H]^+$ (for example fluoranthene, 202 > 200), $[M-C_2H_2]^+$ (for example naphthalene, 128 > 102) and in some cases $[M-C_4H_2-2H]^+$ (for example pyrene, 252 > 200) and $[M-4H]^+$ (for example chrysene, 228 > 224) [33]. These ions were used for the SRM acquisition conditions.

The GC–EI-MS/MS spectra for PEs were obtained by selecting the base peak at m/z 163 $[M-OCH_3]^+$ for dimethyl phthalate and m/z 149 $[C_9H_9O_2]^+$ for all the other PEs as the characteristic precursor ion. GC–EI-MS/MS spectra of all PEs except dimethyl phthalate exhibit product ions (m/z 121, 93 and 65) corresponding to the consecutive elimination of CO groups. Dimethyl phthalate showed an abundant ion at m/z 133 that corresponds to the loss of formaldehyde [34]. Transitions from parent to product ions include the above mentioned ions (Table 1).

The most intense peak of the isotopic molecular cluster, generally $[M]^+$ or $[M+2]^+$, was chosen as PCBs precursor ion. Congeners of the same degree of chlorination (homologue group) gave a similar full scan spectrum. Product ions were formed by the loss of chlorine atoms [35,36], which were used as SRM transitions.

Precursor ions for nonylphenol isomers and ethoxylates in GC–EI-MS/MS fragmentation were produced by benzylic cleavages $[M-C_6H_{13}]^+$ forming m/z 135 for nonylphenol isomers, m/z 179 for monoethoxylated isomers, and m/z 223 for diethoxylated isomers [37,38]. For octylphenol the same process occurred with the formation of m/z 135 $[M-C_5H_{11}]^+$. Product ions were formed by m/z 107 $[C_7H_7O]^+$ and m/z 77 $[C_6H_5]^+$.

The precursor ions for PBDEs were $[M]^+$ and $[M+2]^+$ followed by $[M-2Br]^+$. Fragmentation of the precursor ion was congener dependent: for tri-BDE (BDE 28) and tetra-BDE (BDE 47) characteristic product ions were $[M-2Br]^+$ when the parent ion was $[M]^+$ as well as $[M-2Br+2]^+$ when the parent ion was $[M+2]^+$; for penta-BDE (BDEs 99 and 100) the fragmentation of parent ions $[M-2Br]^+$ produced a product ion $[M-COBr]^+$ and the fragmentation of $[M+2]^+$ produce $[M-2Br+2]^+$; in the case of hexa-BDEs (BDEs 153 and 154), hepta-BDE (BDE 183) and deca-BDE (BDE 209), product ions were $[M-4Br]^+$ when the parent was $[M-2Br]^+$ and the product ion $[M-2Br+2]^+$ when the parent was $[M+2]^+$ [39,40]. During the optimization step, signals obtained for BDE 183 and 209 were very low, even with a standard concentration of 1 ng μL^{-1} and by SRM, they were not detected since they suffer degradation and signal suppression with a 30 m column length. For the analysis of those compounds a 5–15 m column length is required which minimize column residence times [41,42]. In this study, the use of a shorter column was unsuitable for the analysis of PCBs and PAHs and as compromise BDEs 183 and 209 were excluded from the multiresidue developed method. Consequently, the multiresidual method only considered 51 compounds.

OCPs are easily fragmented in the EI source. The molecular ion or/and the dechlorinated molecular ion were the predominant ions produced by EI [35]. The precursor ions for 2,4′ and 4,4′-DDD were the m/z 235 $[M-CHCl_2]^+$ and m/z 237 $[M-CHCl_2+2]^+$; for 2,4′ and 4,4′-DDE, the m/z 246 $[M-2Cl]^+$ and m/z 318 $[M]^+$; for 2,4′ and 4,4′-DDT, m/z 235 $[M-CCl_3]^+$ and m/z 237 $[M-CCl_3+2]^+$. Precursor ions for α, β, γ, and δ-hexachlorocyclohexanes were m/z 291 $[M-2HCl-Cl]^+$ and m/z 219 $[M-HCl_2+2]^+$. For α and β endosulfan precursor ions were chosen from the most intense peak of higher mass, in this case m/z 241 and m/z 195 [43]. The product ions for all the OCPs were formed by successive dechlorination from the corresponding precursor ion [30,35,36] (Table 1).

GC parameters such as injector temperature (250, 280 and 290 °C), flow (1.5–2 mL min^{-1}) and temperature gradients were optimized for a better resolution and elution of the chromatographic peaks. The optimized GC parameters were those mentioned in Section 2.3. Although the acquisition software MassHunter Workstation allows up to 90 SRM transitions per time segment, a practical way to obtain well shaped peaks and good sensitivity is setting more retention time windows with a few SRM transitions [28]. After an exhaustive examination of the distribution of the peaks on the total ion chromatogram, it was divided into 12 time segments with not more than 20 SRM transitions in each. Table 1 report the retention time of each target compound and the time segments used. With the conditions developed all analytes were resolved in 46 min. Although some chromatographic coelution appeared (nonylphenol branched co-eluted with α, β, and γ-hexachlorocyclohexane; nonylphenol monoethoxylated with PCB 28, PCB 52 and di-2-ethylhexyl phthalate; α-endosulfan with PCB 101; nonylphenol diethoxylated with 4,4′-DDE, 2,4′-DDT and PCB 138), they were efficiently resolved by their specific MS/MS transitions. Given the high selectivity of the triple quadrupole detector to resolve multianalytes, SRM is recommended for the unequivocal multiresidue trace analysis of environmental matrices where high selectivity and sensitivity are needed.

Good linearity responses were obtained over the concentrations range tested for all target pollutants (except for diethyl phthalate and di-2-ethylhexyl phthalate, 0.025–1.0 ng μL^{-1}). The coefficients of determination (R^2) ranged from 0.9910 (diethyl phthalate) to 0.9999 (BDE 100). Results are shown in Table 2. IDLs were in the range of 0.3–20 pg injected (Table 2) which ensured the low level detection of target compounds. Inter-day instrumental precision ranged from 3 to 13% (RSD, $n = 6$) and the response was considered repeatable given the 15% limit, in accordance with the method validation criteria [31,32].

3.2. GC–MS/MS performance using environmental samples

Surrogate standards were added prior the extraction in order to correct for possible losses along the overall extraction process and/or instrumental deviations. In supplementary data (Table S1) a list of the target compounds with each corresponding internal standard is presented. Fig. 1 shows the recovery yields of the surrogates used.

3.3. Water

The extraction efficiency of target analytes from the spiked ultrapure, river, seawater and wastewater was performed using Oasis HLB cartridges and eluting with dichloromethane/hexane (1:1, v/v) and dichloromethane/acetone (1:1, v/v) as is described by Sánchez-Avila et al. [8] (Section 2.2.1). Blank analysis revealed the presence of phthalates, naphthalene, acenaphthene, fluorene and bisphenol A below their MDL in ultrapure water. The accuracy of the method, evaluated in terms of recovery yields (%), precision as the RSD (%, $n \geq 3$) and the MDL for each matrix are

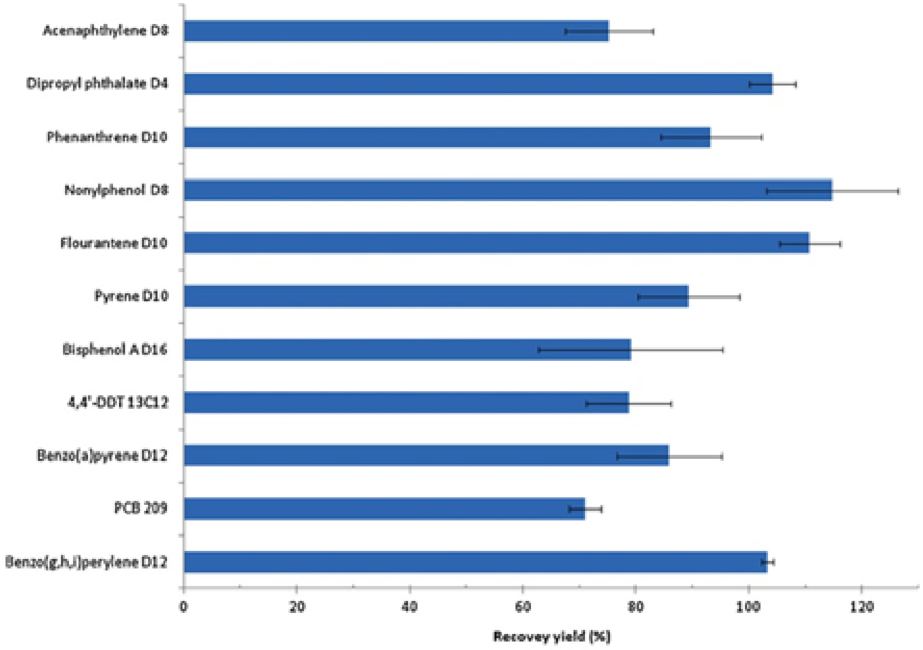

Fig. 1. Recovery yields of the surrogate standards used in the extraction of water samples ($n = 10$).

shown in Table 3. Contribution of phthalates and nonylphenol monoethoxylated in river, seawater and WWTP effluents were subtracted from the spiked sample. Most of the recoveries accomplished the range established (40–120% with RSD ≤ 30%) except some phthalates due to its contribution during both the extraction and analytical steps. No compound was recovered below 60%. Phthalates and alkylphenols are ubiquitously present in the laboratory atmosphere, materials and instruments [44,45] which slightly overestimate the amount extracted. Regarding the precision of the method, the RSD range criterion was accomplished for almost all compounds except for naphthalene (32%) in river water. MDL for ultrapure water ranged from 0.16 to 2.50 ng L^{-1}; for seawater, from 0.12 to 6.00 ng L^{-1}; for river water, from 0.11 to 4.84 ng L^{-1} and for WWTP effluent, from 1.30 to 75 ng L^{-1} (Table 3). Recoveries obtained for river and sea water were similar to the values for ultrapure water. This fact indicates that interferences in these two types of waters were minimal. Despite the high complexity of the WWTP effluents, MDL values were only 2–10 folds higher than those obtained for ultra pure water which proves a selectively low signal enhancement due to matrix contribution.

3.4. Sediments and mussels

Ultrasound assisted extraction is a simple, fast and robust technique and has been previously used successfully for extractions of solid matrices (sediment, insect, mussel, fish, and sludge, among others) for multiresidual analyses of organic pollutants [15,45,46].

An important step for solid–liquid extraction was the selection of an extraction solvent that provides reasonable recovery yields for multi-target compounds. In mussels, preliminary tests using different solvent combinations were performed. Method 1 was based on the methodology used for water extraction and methods 2 and 3 were used with the aim to reduce the use of dichloromethane as much as possible. Overall, recoveries for method 1 ranged from 33 to 110%; for method 2, from 11 to 164% and for method 3, from 69 to 119%. Due to the improvement of the recoveries and also the reduction in the use of dichloromethane method 3 was selected for the solid–liquid extraction of the target analytes in sediments and mussels.

In laboratory blank samples, dimethyl phthalate, nonylphenol and nonylphenol monoethoxylated were detected below MDL. The contribution of diethyl phthalate and di-2-ethylhexyl adipate detected in sediment and mussels was subtracted from the spiked samples for the determination of the accuracy. The accuracy of the method for sediments spiked at 100 ng g^{-1} and mussels at 200 ng g^{-1} ($n \geq 3$) is indicated in Table 3. Most of the recoveries for the target compounds accomplished the range established (80–100%, with RSD ≤ 16%) although some compounds were recovered at low percentages (Table 3). Regarding to the precision of the method, the RSD range criterion was accomplished for almost all compounds except for naphthalene (31%) and nonylphenol monoethoxylated (19%) in mussels and naphthalene (18%) and diethyl phthalate (18%) in sediments. MDL for sediments ranged from 1 to 150 ng g^{-1} and for mussels from 0.9 to 125 ng g^{-1} (Table 3).

3.5. Comparison to previously reported methods

In the literature a wide variety of analytical methodologies for the determination of organic pollutants in environmental matrices are available. Recent trends are directed to use or develop multiresidue methods to obtain maximum information from a sample and reduce time and cost of analysis [22,29,47]. In these methods, validation is of great importance to ensure the quality and performance of the method. One of the main problems in environmental analysis still relies in the matrices used for quality control evaluation. The great majority of the MDLs and recoveries reported by other authors were calculated using Milli-Q, distilled or ultrapure water, so matrix effect could not be assessed and compared with the results obtained herein. Table S2 (supplementary material) presents a compilation of the MDLs and recovery yields from water, sediments or biota samples, according to the open bibliography. For PAHs, MDLs were similar to those previously reported for the analysis of seawater using GC–EI-MS [2] and river water using HPLC–FLD [48]; for WWTP effluents, lower MDLs were achieved

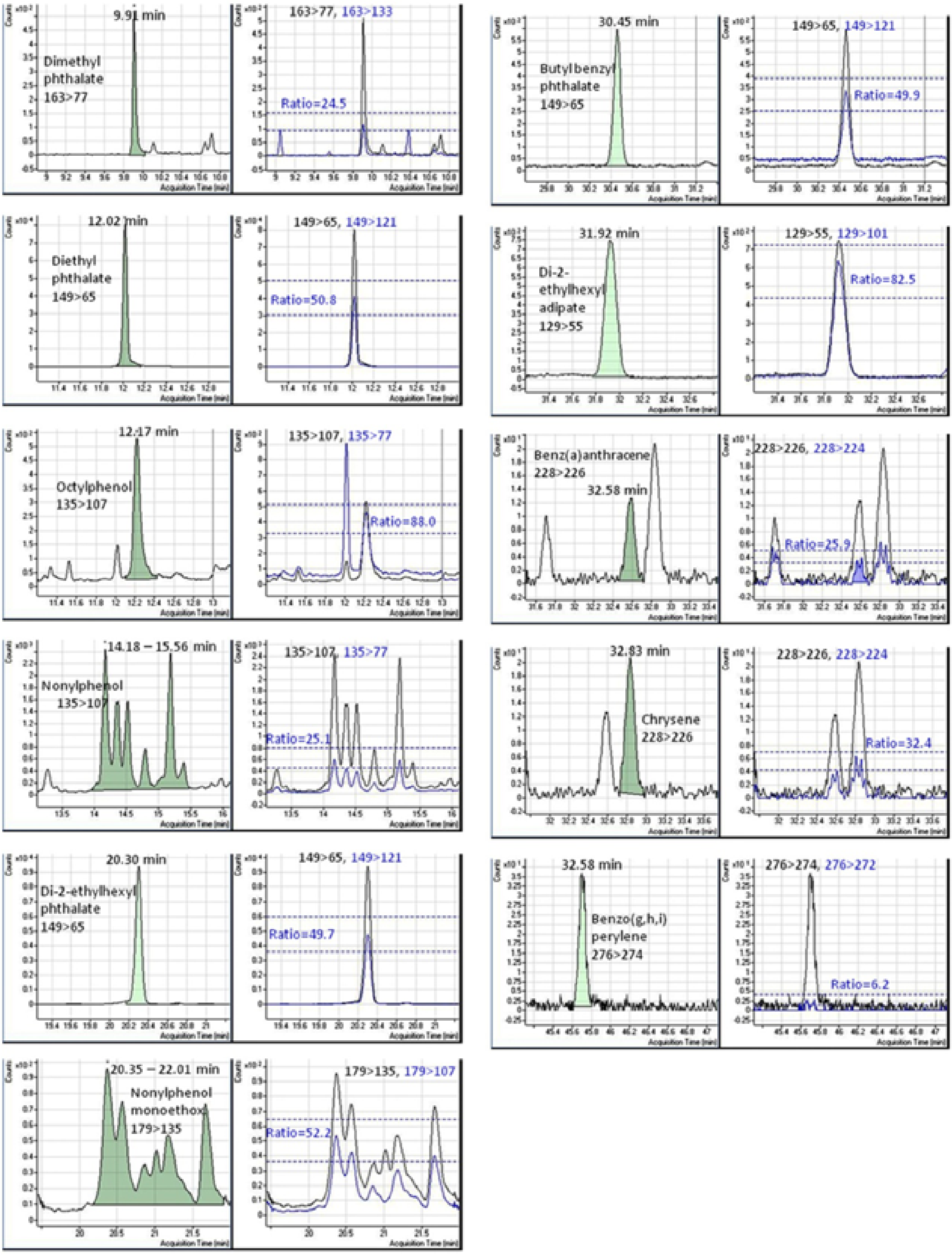

Fig. 2. Seawater SRM chromatograms of the Q (left) and q transitions with the ratio ($q/Q \times 100$) (right) from detected target pollutants. Obtained from MassHunter quantification software.

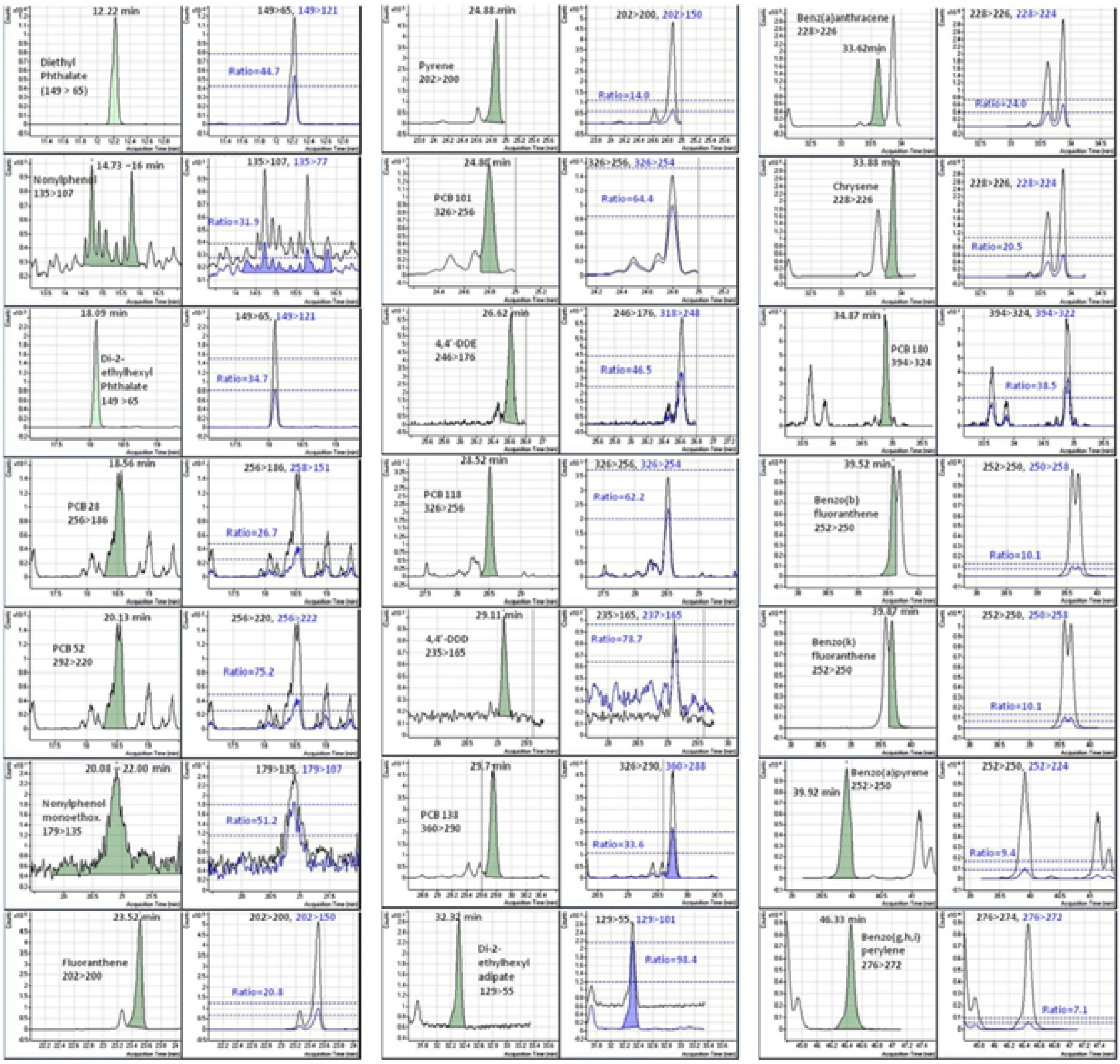

Fig. 3. Mussel SRM chromatograms of the Q (left) and q transitions with the ratio ($q/Q \times 100$) (right) from detected target pollutants. Obtained from MassHunter quantification software.

using GCxGC–TOF–MS [47]; for sediments and mussels, Martinez et al. [49] achieved ten times lower MDLs using GC–EI-MS for the analysis of PAHs as a single chemical family. For PEs, ten times lower MDLs were achieved in this study compared with previously reported methods for the analysis of seawater using GC–EI-MS [50]; for river water, one hundred times lower than using HPLC–UV [51]; for WWTP effluents, three times lower than using HPLC–MS/MS [52]; for sediments, four times lower MDLs were obtanied using GC–EI-MS of a single chemical family [53]; for mussels similar MDLs were achieved using GC–EI-MS [54]. For bisphenol A, two to three times lower MDLs were achieved in this study compared with other reported methods using HPLC–ESI-MS for river water and WWTP effluents [55]; for seawater, 50 times lower MDLs were achieved using HPLC–ESI-MS/MS [56]. For PBDEs, similar MDLs were obtained in this study compared with other reported method for river water and WWTP effluents using HPLC–NI–APPI–MS/MS [57]; for sediments and mussels, five to one thousand times lower MDLs were reported in other studies using GC–NCI–MS due to the high sensitivity of NCI for brominated compounds [58,59]. For PCBs similar MDLs were achieved in this study compared with previously reported studies for seawater using GC–EI-MS [2], river water using GC–EI-MS [60] and WWTP effluents using GC–ECD [61]; for sediments and mussels, 250–2000 times lower MDLs were obtained in previously reported methods using GC–ECD due to the high sensitivity of ECD for organohalogenated compounds without distinction [58,62]. For OCPs, similar MDLs were obtained in this study compared with previously reported studies for seawater [2] and sediments [63] using GC–EI-MS and for river water and WWTP effluents using GCxGC–TOF–MS [47]; for mussels 40 times lower MDLs were obtained in other study using GC–ECD [64]. Recovery yields were comparable in all matrices with previously reported methods, and even improved as in the case of PEs in river water and mussels. Compared to other methods, GC–MS/MS offers sensitivity and selectivity, with the additional advantage that sample interferences are eliminated, despite the environmental matrix. In addition, although MDLs were higher than those reported for PBDEs and PCBs using ECD or NCI–MS, identification capabilities are enormously enhanced by using SRM with two transitions per compound and permit quantification based on isotope dilution (ID) [58,59].

3.6. Application to real samples

The optimized GC–EI-MS/MS method was applied to the multiresidue analysis of 51 compounds in sea and river water, WWTP effluents, sediments and mussels collected in NE Spain. The concentrations of the target pollutants detected in the samples are presented in Table 4. The matrix effect was reduced or even eliminated despite the type of sample, as evidenced in the SRM chromatograms for seawater (Fig. 2) and mussel (Fig. 3). The MassHunter quantification software provides in the SRM chromatograms, the peaks from the Q and the q transitions and shows with dot lines the ($q/Q \times 100$) acceptation range, according to Directive 2002/657/EC [31]. Confirmation of positives was achieved by the RT, the two SRM transitions and also the real ($q/Q \times 100$) ratios were compared with the experimental ($q/Q \times 100$) ratios.

Five samples from each matrix were analyzed. Phthalates, alkylphenols, bisphenol A and some PAHs were detected in all matrices. Di-2-ethylhexyl phthalate and 4-nonylphenol were the most ubiquitous compounds followed by butyl benzyl phthalate, fluoranthene and pyrene. All of them are pollutants derived from industrial and urban activities [65,66]. PCBs, PBDEs and OCPs were detected only in samples collected in river water and occasionally in seawater. The analysis of river waters and especially, of coastal waters where compounds are expected at low concentration, gives evidence of the high sensitivity of the method for the analysis of a wide range of priority micropollutants. PAHs, PEs, APs, BPA, PCBs, PBDEs and OCPs detected in WWTP effluents, sediments or mussels show the performance of the method to determine samples with high interfering compounds. The widespread distribution of pollutants such as APs, PEs and PAHs in coastal environment makes quite clear the urgent need to establish monitoring programs to control the presence of organic contaminants. In this sense, the methodology herein developed constitutes a new tool for the survey, distribution and partitioning of pollutants in the water-sediment-biota system.

4. Conclusions

The potential of GC–EI-MS/MS for the multiresidue determination of 51 organic micropollutants in environmental matrices (sea and river water, WWTP effluents sediments and mussels) has been proven. The excellent sensitivity and selectivity of the triple quadrupole allow the confirmation of the presence of a given compound by two SRM transitions and the use of the ($q/Q \times 100$) ratio, according to European Directives. The entire methodology developed can be used to assess the levels of pollutants in the environmental matrices in an attempt to enhance the marine ecosystem monitoring strategies.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.07.056.

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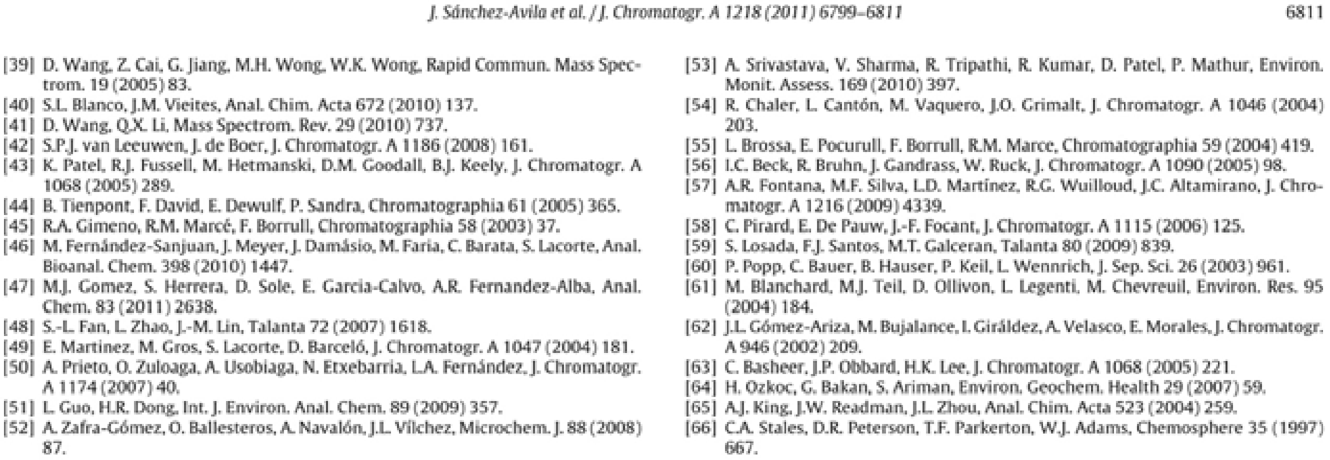

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Supplementary Data

Table S1

List of the target compounds with each corresponding internal standard

Surrogate	Target compound
4,4'-DDT $^{13}C_{12}$	2,4'-DDE
	4,4'-DDE
	2,4'-DDD
	4,4'-DDD
	2,4'-DDT
	4,4'-DDT
Acenaphthylene d_8	Acenaphthylene
	Acenaphthene
	Fluorene
Benzo[a]pyrene d_{12}	Benzo(b)fluoranthene
	Benzo(k)fluoranthene
	Benzo(a)pyrene
Benzo[g,h,i]perylene d_{12}	Indeno(1,2,3-cd)pyrene
	Dibenzo(a,h)anthracene
	Benzo(g,h,i)perylene
Bisphenol A d_{16}	Bisphenol A
Dipropyl phthalate d_4	Dimethyl phthalate
	Diethyl phthalate
	Di-2-ethylhexyl phthalate
	Butyl benzyl phthalate
	Di-2-ethylhexyl adipate
Fluoranthene d_{10}	Fluoranthene
Naphthalene d_8	Naphthalene
Nonylphenol d_8	Octylphenol
	Nonylphenol branched
	Nonylphenol monoethoxylated
	Nonylphenol diethoxylated
PCB 209	PCB 28
	PCB 52
	PCB 101
	PCB 118
	PCB 138
	PCB 153
	PCB 180
	BDE 28
	BDE 47
	BDE 99
	BDE 100
	BDE 153
	BDE 154

Nota:

La información contenida en la “**Table S2**” forma parte de la Tabla 5, dentro del cuerpo de este trabajo de tesis.

2.4 Discusión de los resultados

Las tres metodologías desarrolladas permitieron la identificación inequívoca de 24, 38 y 33 contaminantes prioritarios y entre 11 y 18 contaminantes emergentes en diferentes matrices relacionadas con el medio marino. Las 3 demostraron su versatilidad ya que fue posible determinar simultáneamente contaminantes con un amplio intervalo de propiedades fisicoquímicas como es el log K_{ow}. Los métodos fueron sensibles (ver MDLs en Tabla 11) precisos (variabilidad <20% en la mayoría de los casos) y permitieron una elevada recuperación de los analitos (69-120%).

Tabla 11. Prestaciones de las metodologías desarrolladas

	Metodología A SPE-GC-EI-MS	Metodología B SBSE-GC-EI-MS	Metodología C SPE ó UAE-GC-EI-MS/MS
Límites de detección del método (MDL)*	Agua residual: 0.4-833	Agua de mar: 0.01-38	Agua de mar: 0.1-6.0 Agua de río: 0.1-4.8 Agua residual: 1-75 Sedimentos: 1-150 Mejillones: 0.9-125
Recuperación (RSD) en %	Agua residual: 69-124	Agua de mar: 86-118	Agua de mar: 70-126 Agua de río: 71-117 Agua residual: 69-120 Sedimentos: 70-114 Mejillones: 69-115
Variabilidad (RSD) en %	Agua residual: 1-26	Agua de mar: 2-24	Agua de mar: 1-20 Agua de río: 1-21 Agua residual: 0.3-27 Sedimentos: 1-18 Mejillones: 1-31

* MDL para aguas de mar, río y efluentes de depuradora en ng L^{-1}; para sedimentos en ng g^{-1} dw; para mejillones en ng g^{-1} ww.

La sensibilidad lograda al utilizar la SBSE-GC-EI-MS en aguas de mar para la mayoría de los compuestos fue 10 veces superior a la que se alcanzó con SPE-GC-EI-MS/MS (Tabla 11). Esta alta sensibilidad está relacionada con que en la SBSE, no se manejan extractos, sino que toda la carga de contaminantes aislada en el adsorbente es inyectada (desorbida) en el equipo; en cambio en la SPE se genera un extracto, del cual únicamente se inyectaron unos 2 µL. La sensibilidad alcanzada con el método SBSE-GC-EI-MS fue suficientemente alta como para cumplir con la exigencia de la Directiva 2009/90/CE [69]. Esta Directiva establece que los límites de detección de los métodos empleados para realizar la vigilancia ambiental de las aguas, debe ser un 30% menor del valor de las NCA o algún nivel de calidad ambiental relevante (en este caso si no se tiene una NCA, se comparan con los valores de toxicidad crónica para peces, ver Tabla 4). Con la técnica SPE-GC-EI-MS/MS se destaca que para los congéneres de PBDEs la sensibilidad es 10 veces más baja de lo requerido. Generalmente para los PBDEs y otros compuestos halogenados, la técnica de detección por excelencia es la GC-NCI-MS, sin embargo ésta no es apta para el análisis del resto de los analitos. Además al tratarse de metodologías multiresiduales es necesario llegar a un compromiso con la finalidad de obtener buenas recuperaciones y una sensibilidad aceptable para el resto de los compuestos, en vez de conseguir las óptimas condiciones para cada uno de ellos. Sin embargo, en la Propuesta de Directiva 2011/0429 [71] donde se marcan nuevos valores de NCA, algunas de las concentraciones máximas son más bajas, pero en el caso de los congéneres de PBDEs aumenta a 14 ng L^{-1}. En caso de autorizarse estos nuevos valores de NCA, ambos métodos multiresiduales cumplirán al 100% con la exigencia de las Directivas Europeas. Respecto a los MDLs para las aguas de río y residuales, sedimentos y mejillones, éstos son lo suficientemente bajos como para determinar niveles de concentración ambientalmente relevantes. Este método sería adecuado para llevar a cabo el programa de seguimiento de contaminantes requerido por la DMEM que tiene como plazo para realizarse el 15 de julio de 2014.

La utilización de patrones de recuperación en las tres metodologías fue de vital importancia, pues durante el aislamiento/preconcentración se manipulan constantemente las muestras. Se pueden dar perdidas de los analitos más volátiles especialmente en la evaporación del disolvente (metodologías A y C).

Los estudios realizados en la red de alcantarillado del Maresme demostraron que las EDAR tienen una gran capacidad de eliminar contaminantes orgánicos, sin embargo los porcentajes que quedan remanentes se liberan al mar. Únicamente en la EDAR de Mataró se demostró que se vierten 800 g por día, considerando sólo los 42 contaminantes estudiados. Estos vertidos generan una pluma de contaminación desde la salida del emisario y una dispersión de los contaminantes, tal como se demostró en un estudio realizado a posteriori. Para ello, se recolectaron 5 muestras de las aguas costeras (a 1 m de la superficie) cercanas a la descarga del emisario submarino de la EDAR de Mataró cuya salida se encuentra a 2 km de la costa. Una de las muestra se recolectó en la superficie justo en la salida del emisario (EM-0), otra a 500 m (EM-1) y una más a 1000 m hacia la costa (EM-2). Las otras dos se tomaron a 500 (EM-3) y 1000 m (EM-4) desde la salida del emisario, mar adentro (Figura 24). Las muestras de agua de mar fueron analizadas empleando SPE-GC-MS/MS. En la superficie, justo a la salida del emisario (EM-0), la ΣPAHs (71 ng L^{-1}) y la ΣPlast. (1197 ng L^{-1}) fue mayor que en el resto de sitios (Figura 24). Conforme se aleja del emisario, hacia mar adentro, estas concentraciones fueron disminuyendo (ΣPAHs: EM-3, 24 ng L^{-1} y EM-4, 8.5 ng L^{-1}; ΣPlast: EM-3, 133 ng L^{-1} y EM-4, 88 ng L^{-1}) lo que indica una efecto de dilución. Al acercarse a la costa en EM-1, la ΣPAHs (26 ng L^{-1}) y la ΣPlast (220 ng L^{-1}) disminuyó, sin embargo en EM-2, nuevamente volvió a incrementarse (ΣPAHs, 42 ng L^{-1} y ΣPlast, 644 ng L^{-1}). El intenso tráfico marítimo de la zona, la cercanía del Puerto de Mataró y la existencia de otros emisarios industriales no controlados son factores no descartables que contribuyen a este incremento de concentraciones cerca de la costa. Esto explicaría también el porqué la ΣAPs fue mayor en EM-1 (10 ng L^{-1}) que a la salida del emisario (EM-0, 5.5 ng L^{-1}) y que únicamente se hayan detectado congéneres de PCBs (PCB 52, 101, 118, 138, 153 y 180) en EM-2 (ΣPCBs, 9.8 ng L^{-1}).

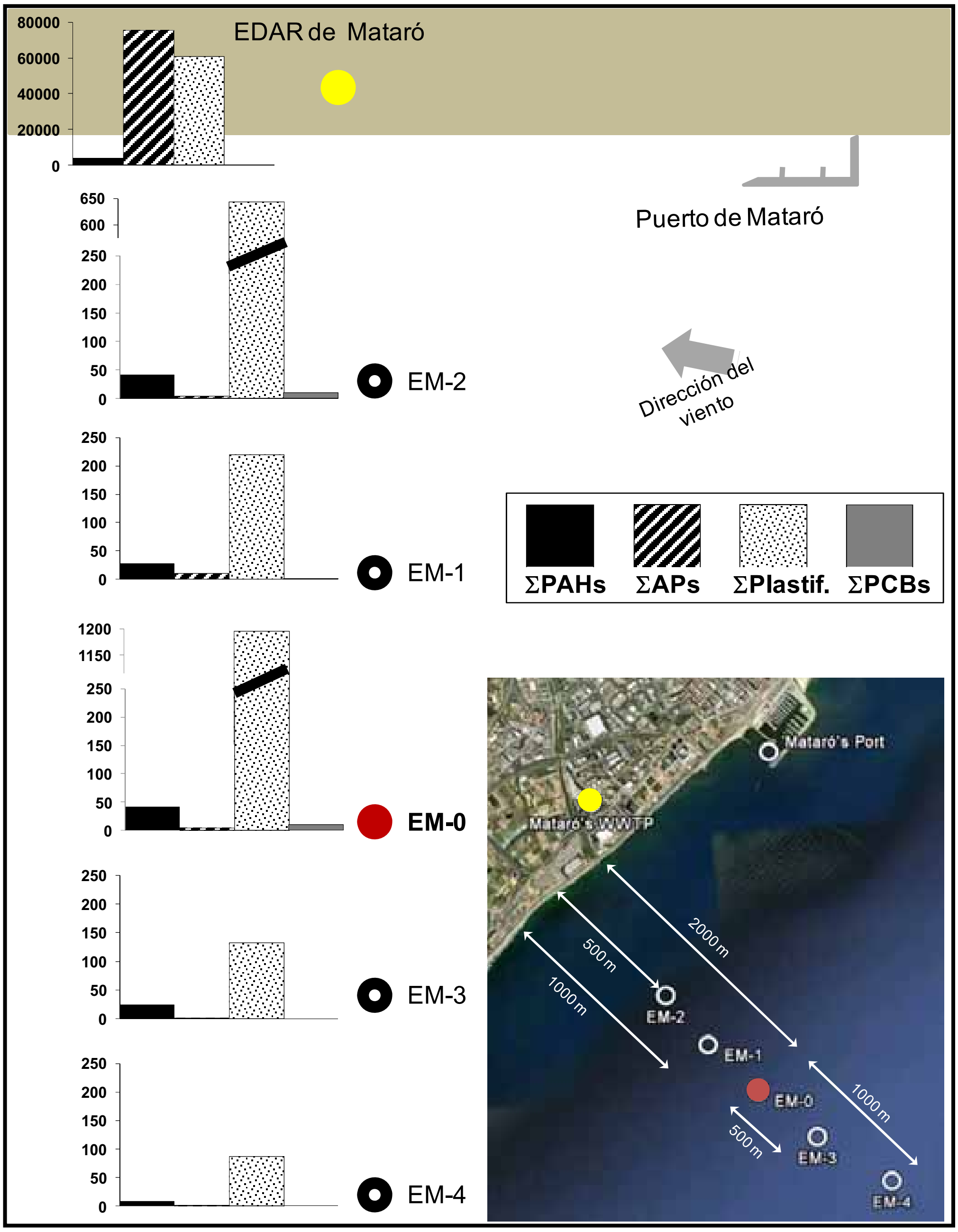

Figura 24. Resultados de la determinación de OMPs en aguas costeras cercanas a la salida del emisario de la EDAR de Mataró (en ng L^{-1})

Si se comparan los niveles de OMPs en los efluentes de la EDAR de Mataró y de la salida del emisario, se puede observar que hay una rápida dispersión en las aguas de la superficie de la salida del emisario. Así mismo se observa que la pluma de contaminación en torno de la boca del emisario tiende a la dilución. Por tanto, los emisarios submarinos representan una fuente de contaminación de OMPs en las zonas costeras. Estos vertidos, conjuntamente con los aportados por otras actividades (marítimas, descargas, etc.) contribuyen a la propagación de contaminantes a niveles de ng L^{-1} a lo largo de la costa.

La detección de contaminantes a niveles traza en las muestras reales de agua de mar hacen patente la sensibilidad y selectividad de los métodos desarrollados. El análisis de las muestras de agua colectadas en puertos y playas de la costa Catalana sentó un precedente de los niveles de contaminación por OMPs en esta región (0.16 a 597 ng L^{-1}), aún en zonas donde no se esperaría encontrar estos compuestos como es el caso de la alimentación de una planta de desalinización. Estos niveles son similares a los detectados en otras regiones del mundo con moderada-intensa actividad industrial y turística y asentamientos urbanos en la costa, tal como se muestra en la Tabla 12.

Tabla 12. Comparación de concentraciones de OMPs detectados en el muestreo piloto realizado en la costa Catalana con otros sitios alrededor del mundo

Familia química	Concentración en ng L^{-1}	Sitio de muestreo	Referencia
PAHs individuales	**0.53 –261**	**Cataluña, España**	**Artículo 2**
	0.08 – 14.6	Saint-Anne y Moulin Blanc, Francia	[263]
	20.0 – 160	Golfo de Saronikus, Grecia	[84]
	0.40 – 168	País Vasco, España	[198]
	3.0 – 389	Bahía de Cádiz, España	[264]
NP	**22.8 – 597**	**Cataluña, España**	**Artículo 2**
	<30.0	Estuarios alemanes	[265]
	150 – 4100	País Vasco, España	[198]
ΣPCBs	**14.9 – 36.7**	**Cataluña, España**	**Artículo 2**
	3884 – 4313	País Vasco, España	[198]
	0.05 – 1.8	Singapur	[266]
	0.07 – 3.4	Campeche, México	[267]
Lindano y endsf	**7.5 – 481**	**Cataluña, España**	**Artículo 2**
	1600	Cataluña, España	[268]
	0.0004 – 0.027	Singapur	[266]
ΣDDTs	**1.48 – 17.7**	**Cataluña, España**	**Artículo 2**
	<6.0 – 35.0	Cataluña, España	[268]
	0.01 – 0.63	Singapur	[266]
	0.07 – 0.61	Campeche, México	[267]
	0.29 – 1.46	Puerto de Barcelona, España	[269]
	0.04 – 0.21	Bañuls, Francia	[269]
	3.0 – 11	Costa Atlántica española	[264]

La detección de PAHs, APs, PEs, PCBs, PBDEs y OCPs en sedimentos y mejillones colectados en las costas ha demostrado la ubiquidad de estos compuestos en los diferentes compartimientos ambientales. La elevada concentración de compuestos derivados de actividades industriales y urbanas como el DEHP, NP, BBP, Flr y Pyr en el ambiente costero refleja la urgente necesidad de establecer programas de monitoreo con la finalidad de proteger la calidad de sus aguas.

Para finalizar, es necesario destacar que las metodologías desarrolladas son idóneas para realizar estudios de vigilancia de los contaminantes orgánicos prioritarios y emergentes en las aguas costeras de cualquier región. Un posterior desarrollo de metodologías multiresiduales con un mayor número de contaminantes sería de gran utilidad en estos estudios de vigilancia. El análisis de aguas residuales y de río permitirá estimar las principales fuentes de contaminación y los sedimentos y mejillones, aportarán datos de la compartimentación de los contaminantes.

CAPÍTULO 3:
Vigilancia y estimación del riesgo ambiental en zonas costeras

3.1 Introducción

3.1.1 Campañas de muestreo

Como se ha descrito extensamente en el Capítulo 1, las zonas costeras están sometidas a un gran número de presiones antropogénicas provocando la contaminación de sus aguas y afectando la calidad del ecosistema marino. Con el fin de proteger la salud ambiental en estas zonas vulnerables es necesario realizar una monitorización química que tenga en cuenta la legislación vigente así como la toxicidad de los contaminantes. Con la finalidad de ampliar el escaso conocimiento del comportamiento, distribución e impacto de los contaminantes orgánicos en aguas costeras, se diseñaron dos campañas de muestreo en dos regiones costeras. Estas zonas se caracterizan por su un elevado desarrollo urbano, una importante actividad industrial así como un elevado tráfico marítimo.

En la primera campaña se consideró como objetivo el litoral NE del País, específicamente en las aguas costeras de Cataluña, en el Mar Mediterráneo. En la segunda campaña, se monitoreó el litoral N de España, en las aguas costeras de las Provincias de Asturias, Cantabria y País Vasco, en el Mar Cantábrico. En la Tabla 13 se enumeran los diferentes tipos de matrices muestreadas en cada campaña. Las muestras se recolectaron y procesaron utilizando las herramientas descritas en el Capítulo 2.

Tabla 13. Resumen de las muestras de agua recolectadas en cada campaña

Concepto	Costa Catalana		Costa Cantábrica
Sitios de muestreo *	60		13
Destinadas al análisis de	51 OMPs	5 PFCs	51 OMPs
Tipo de muestra			
Aguas de mar (costa y puertos) superficial	39	38	6
Aguas de mar (costa) a diferentes profundidades	-	-	12
Aguas de transición (bocas de río o estuarios)	7	7	15
Aguas de río	6	6	-
Aguas residuales (efluente)	8	8	6
Sedimentos	-		10
Mejillones	-		12

* Considerando costeros, estuarinos, cauces de ríos y EDAR

En primavera del 2009 se recolectaron aguas en 46 sitios costeros de Cataluña, diferenciando 22 zonas de costa, 17 puertos y 7 bocas de los ríos. Además se colectaron muestras de los 6 principales ríos que desembocan en el Mar Mediterráneo (de N a S, Muga, Fluvià, Ter, Besòs, Llobregat y Ebro). Inicialmente se había decidido incluir un mayor número de ríos, tales como el Francolí, pero al desembocar dentro de las instalaciones del Puerto de Tarragona, fue imposible acceder al sitio. Otros dos ríos que en un principio se habían considerado fueron el Foix y el Tordera cuyo caudal era muy escaso en esta temporada y por ello se descartaron del estudio. También se recolectaron muestras compuestas (24 h) de los efluentes de las 8 principales EDAR que descargan en el Mar Mediterráneo por medio de emisarios submarinos. Cada sitio se muestreó una sola vez. Cabe mencionar que las muestras se recolectaron por duplicado, destinados a diferentes tipos de análisis. La primera réplica se utilizó para la determinación de 51 contaminantes semivolátiles y la segunda para determinar PFCs. Para el muestreo de las replicas destinadas al análisis de PFCs se tuvo como cuidado adicional, el evitar el contacto con cualquier material que pudiera contener polímeros perfluorados como el PTFE o teflón para no "contaminar" las muestras.

La hidrografía costera en la zona de Cataluña es muy diferente a la de la zona del Mar Cantábrico. En el Mar Cantábrico se pueden encontrar estuarios con una amplitud y profundidad considerable como para ser navegables desde el mar. Esto ha sido aprovechado para instalar puertos comerciales, pesqueros e industriales. Estas regiones costeras son aún más vulnerables ya que en muchos casos las corrientes no son lo suficientemente fuertes como para dispersar la continua descarga de contaminantes. En otoño del 2009 se recolectaron muestras en 3 puntos del Mar Cantábrico, que son de especial interés porque se situaron justo en la superficie donde se realiza la descarga de emisarios submarinos, vertidos industriales o escorrentías. Así mismo, en estos 3 puntos se tomaron muestras en el fondo, lo más cercano a la salida del emisario y a 15 m de profundidad. Se recolectaron también muestras de agua de estuario en 8 sitios, diferenciando 2 zonas supuestamente no contaminadas, alejadas de fuentes de contaminación y con la categoría de Parque Natural (San Vicente) o Reserva de la Biósfera (Urdabai); 3 aledañas a puertos y finalmente 3 afectadas por descargas de aguas residuales y fuentes difusas. Se recolectaron muestras compuestas de efluentes de 2 EDAR. Adicionalmente para conocer la compartimentación de los contaminantes en el ecosistema marino se muestrearon sedimentos de los 11 puntos de muestreo, aunque en uno de ellos no fue posible recolectarlos pues el fondo se encontraba cubierto de chatarra y restos de escombros. También se trasplantaron mejillones cebra (*Mytilus galloprovincialis)*. Estos mejillones eran originarios de las rías gallegas tomados de una misma batea con el fin de que presenten características morfológicas y actividad metabólica semejante (4.9-7.2 cm de longitud). De aquí, los mejillones se dividieron en dos partes, acondicionado una de ellas para sitios de baja salinidad (1.7%) y la otra para sitios con salinidad estándar. Se tomaron muestras representativas de mejillones de los 2 acondicionamientos (t_0) antes de ser trasplantados en los sitios de muestreo. A los 21 días se recogieron las unidades trasplantadas (t_{21}) para determinar la bioacumulación de contaminantes. Únicamente pudieron analizarse los mejillones de 10 sitios, ya que una unidad fue arrastrada por las fuertes corrientes causadas por una tormenta. Durante la recolecta a t_{21} se tomaron muestras de aguas de mar o estuario para tener análisis de cada sitio por duplicado.

3.1.2 Análisis de las muestras

Todas las muestras recolectadas en ambas campañas fueron analizadas de acuerdo al protocolo analítico descrito en el Artículo 3, “Development of a multi-residue method for the determination of organic micropollutants in water, sediment and mussels using gas chromatography–tandem mass spectrometry”. Para la determinación de PFCs en aguas costeras de la Costa Catalana se utilizó una preconcentración por SPE, con diferencia de que se extremaron precauciones para que todo el material empleado no contuviera PTFE y otros polímeros perfluorados y como técnica de separación y análisis se empleó la cromatografía de líquidos acoplada a espectrometría de masas en tándem con ionización por electro-espray (Tabla 14). La metodología fue adaptada de Taniyasu et al., 2005 [172].

Tabla 14. Metodología analítica para el análisis de los PFCs

No. de compuestos	5
Intervalo de log K_{ow}	1.8 – 5.5 [a]
Volumen de muestra	50 mL, agua residual 1 L, agua de río 1L, agua de mar
Método de aislamiento/ preconcentración	SPE
Tipo de adsorbente	Oasis HLB
Elución	10 mL de metanol
Reconstitución	Aguas de mar y rio: 200 µL de acetonitrilo/ agua HPLC (30:70) Agua residual: 500 µL de acetonitrilo/ agua HPLC (30:70)
Separación analítica	HPLC
Modo de ionización	ESI –
Técnica de análisis	MS/MS (modo SRM)

[a] Este valor es estimado, pues debido a sus propiedades tanto hidrofilicas - lipofilicas, experimentalmente se forma una emulsión que no permite su medición

3.1.3 Herramientas quimiométricas

Los datos obtenidos del análisis de contaminantes de las aguas de mar de la Costa Catalana (SPE-GC-MS/MS) fueron tratados quimiométricamente con el fin de obtener información sobre las principales fuentes de contaminación. Se utilizó para ello el Análisis de Componentes Principales o PCA (“principal component analysis”). La matriz original de datos consistió en 46 sitios de muestreo agrupados por tipo de muestra (efluentes de EDAR, agua de río, agua de mar en la costa, agua de mar en el puerto, agua de mar en la boca de río) y 37 contaminantes (se excluyeron aquellos que el 10% o menos estuvieran debajo del límite de detección). Para aplicar la herramienta de PCA es necesario que no existan datos vacios, por lo que los valores inferiores al límite de detección fueron rellenados con la mitad de éste.

El PCA es una técnica que permite reducir y simplificar el número de variables del sistema de datos mediante la formación de combinaciones lineales de las variables originales (en este caso, las concentraciones de los contaminantes analizados). Se forman nuevas variables ortogonales no correlacionadas entre sí que se denominan componentes principales (PCs). La nueva matriz se descompone en “scores”, que describe la composición de las muestras según las nuevas variables y en “loadings”, que describe la contribución de cada analito a los PCs. La información relevante del conjunto de datos se encuentra concentrada en los primeros PCs, explicando así el primero de ellos, el mayor porcentaje de varianza [270; 271]. Matemáticamente se puede expresar con la ecuación $X = T \bullet P^T + E$. Donde X es la matriz de datos originales, T es la matriz de scores, P^T es la matriz de loadings y E la matriz de los valores residuales. El número de PCs a trabajar se elige según la varianza total explicada. Cuantos menos PCs se consideren, más sencillo será el análisis. Por otro lado cuanto mayor sea el número de PCs, mayor será la varianza total acumulada y el análisis será más exacto.

Con cada PC fue posible identificar una posible fuente de contaminación. Gráficamente se representó una matriz de scores en 2 ejes, ilustrando los patrones de contaminación dominantes existentes entre las muestras y permitiendo localizar aquellas con fuentes de contaminación en común.

Como los datos ambientales no siempre son de la misma magnitud, fue necesario pretratar los datos para evitar que una variable domine a la otra. Ya que no existe un tratamiento ideal, fue necesario realizar diferentes pruebas y combinaciones de las mismas hasta visualizar de manera adecuada la información extraída del PCA. El pretratamiento aplicado a los datos consistió en una transformación logarítmica y un centrado de las medias.

3.1.4 Estimación del riesgo

Reportar únicamente las concentraciones de los contaminantes orgánicos detectados en campañas de monitoreo no sería de gran relevancia si no se estudia el riesgo que ocasionan. Los datos químicos pueden transformarse a concentraciones toxicológicamente relevantes y permiten estimar el riesgo que presentan en el ambiente. Es por ello que uno de los objetivos de este trabajo de tesis fue evaluar el riesgo que representan los contaminantes orgánicos en las aguas costeras teniendo en cuenta las dinámicas de cada zona de muestreo.

Como bien se mencionó en los Capítulos anteriores, los compuestos de estudio fueron elegidos porque existe constancia de su potencial riesgo al medio marino. Muchos de ellos pueden presentar toxicidad para las especies más sensibles aún a concentraciones muy bajas. Así mismo, algunos de los contaminantes poseen también propiedades carcinogénicas, como el caso de I(cd)P, B(ghi)P y D(ah)A [59; 79; 272]. Para determinar la toxicidad de un contaminante se realizan estudios exponiendo a un organismo a concentraciones determinadas del contaminante por un período específico de tiempo y en condiciones determinadas. Generalmente la toxicidad puede expresarse como la concentración de exposición (“effective concentration”) que induzca una cierta respuesta en el 50% de la población de estudio. Estas respuestas suelen ser tan diversas como por ejemplo, afectaciones en el crecimiento, comportamiento, reproducción, entre otros. Los valores de EC_{50} van acompañados de la especie en la que se realizó el estudio, el tiempo de exposición y la respuesta [273]. Existe otro indicador o punto final, de la toxicidad que se refiere a la concentración letal (LC o “lethal concentration”) que afectaría al 50% de la población de estudio (LC_{50}), en 24-96 h (toxicidad aguda), el cual es más empleado en estudios del medio acuático. También existen otros puntos finales como la concentración sin efecto o NOEC (“No-effect concentration”) [274]. El Documento de Orientación Técnica sobre Evaluación de Riesgos o TGD (“Technical Guidance Document”) [273] recomienda el uso de NOEC o en otro caso la concentración sin efecto previsible o PNEC (“Predicted No-Effect-Concentrations”) que se refiere a la concentración en la que no se esperarán que ocurran efectos inaceptables. Ésta calcula empleando resultados de toxicidad aguda en especies de laboratorio (generalmente LC_{50}) dividido entre un factor de seguridad que dependerá de la cantidad de resultados disponibles (Tabla 15). Los valores de toxicidad pueden encontrarse en bases de datos como la ECOTOX [275]. Cuando no se conozcan estos valores es posible utilizar modelos de estimación, como el programa ECOSAR de la EPA [35].

Tabla 15. Punto final (indicador) de la concentración aguda de 51 contaminantes de estudio en 3 organismos representativos de la cadena trófica marina y factor de seguridad empleado en la estimación del riesgo.

Compuesto	Peces			Algas			Crustáceos misidáceos			Factor de seguridad
		Especie	mg L^{-1}		Especie	mg L^{-1}		Especie	mg L^{-1}	
Nap	NOEC	*Oncorhynchus kisutch*	1.8	NOEC	*Scenedesmus subspicatus*	4.15	EC50	*Artemia salina*	2.11	10
Acy	LC50	*	2.31	LC50	*	1.917	LC50	*	0.582	1000
Ace	NOEC	*Cyprinodon variegatus*	0.52	EC50	*Skeletonema costatum*	0.5	LC50	*Americamysis bahia*	0.19	50
Flu	LC50	*Oncorhynchus mykiss*	0.82	NOEC	*Pseudokirchneriella subcapitata*	1.67	LC50	*Artemia salina*	3	50
Phe	NOEC	*Oncorhynchus mykiss*	0.032	NOEC	*Scenedesmus subspicatus*	2.74	LC50	*Americamysis bahia*	0.003	50
Ant	LC50	*	1.187	NOEC	*Scenedesmus subspicatus*	0.003	LC50	*Americamysis bahia*	0.535	100
Flr	LC50	*Oncorhynchus mykiss*	0.091	NOEC	*Phaeodactylum tricornutum*	0.08	NOEC	*Americamysis bahia*	0.011	50
Pyr	NOEC	*Fundulus heteroclitus*	0.015	NOEC	*Scenedesmus subspicatus*	1.44	LC50	*Americamysis bahia*	0.025	50
B(a)A	LC50	*	0.14	EC50	*	0.292	LC50	*	0.011	1000
Chr	LC50	*	0.14	EC50	*	0.292	LC50	*Artemia salina*	3	1000
B(b)F	LC50	*	0.047	EC50	*	0.138	LC50	*	0.003	1000
B(k)F	NOEC	*Platichthys flesus*	0.01	EC50	*	0.138	LC50	*	0.003	100
B(a)P	NOEC	*Fundulus heteroclitus*	0.007	NOEC	*Scenedesmus subspicatus*	0.25	LC50	*	0.003	100
I(cd)P	LC50	*	0.016	EC50	*	0.065	LC50	*	0.0005	1000
B(ghi)P	LC50	*	0.016	EC50	*	0.065	LC50	*	0.0005	1000
D(ah)A	NOEC	*Platichthys flesus*	0.01	EC50	*	0.065	LC50	*	0.0006	50
OP	NOEC	*Salmo salar*	0.01	EC50	*	0.381	LC50	*Americamysis bahia*	0.048	50
NP	NOEC	*Salmo salar*	0.074	EC50	*	0.164	NOEC	*Americamysis bahia*	0.016	10
NP$_1$EO	LC50	*	0.145	EC50	*	0.312	LC50	*	0.011	1000
NP$_2$EO	LC50	*	0.296	EC50	*	0.541	LC50	*	0.028	1000
BPA	NOEC	*Oncorhynchus mykiss*	0.2	NOEC	*Scenedesmus subspicatus*	250	LC50	*Americamysis bahia*	1.2	10
DMP	NOEC	*Cyprinodon variegatus*	3.2	NOEC	*Pseudokirchneriella subcapitata*	10	NOEC	*Americamysis bahia*	22.2	10
DEP	NOEC	*Oncorhynchus mykiss*	3.8	NOEC	*Pseudokirchneriella subcapitata*	3.65	NOEC	*Americamysis bahia*	0.26	10
DEHP	NOEC	*Oncorhynchus mykiss*	0.502	EC50	*	0.008	NOEC	*Americamysis bahia*	34.5	10
BBP	NOEC	*Oncorhynchus mykiss*	0.48	NOEC	*Pseudokirchneriella subcapitata*	0.1	NOEC	*Americamysis bahia*	0.32	10
DEHA	LC50	*Oncorhynchus mykiss*	0.78	LC50	*Pseudokirchneriella subcapitata*	0.78	LC50	*	0.927	50
2,4’-DDE	LC50	*	0.028	EC50	*	0.273	LC50	*	0.007	1000
4,4’-DDE	LC50	*Oncorhynchus mykiss*	0.032	EC50	*	0.273	LC50	*	0.007	100
2,4’-DDD	LC50	*	0.096	EC50	*	0.246	LC50	*	0.006	100
4,4’-DDD	LC50	*Oncorhynchus mykiss*	0.07	EC50	*	0.246	LC50	*	0.006	50
2,4'-DDT	NOEC	*Oryzias latipes*	0.0005	EC50	*	0.061	LC50	*	5.39E-04	10
4,4’-DDT	NOEC	*Gambusia affinis*	0.01	LC50	*Scenedesmus quadricauda*	8.5	LC50	*Americamysis bahia*	4.50E-04	10

Tabla 15 Tabla 15**…Cont.**

Compuesto	Peces			Algas			Crustáceos misidáceos			Factor de seguridad
		Especie	mg L^{-1}		Especie	mg L^{-1}		Especie	mg L^{-1}	
α-HCH	EC50	*Oryzias latipes*	0.032	NOEC	*Scenedesmus subspicatus*	1.25	LC50	*Americamysis bahia*	0.006	10
β-HCH	NOEC	*Oryzias latipes*	0.032	NOEC	*Scenedesmus subspicatus*	1.25	LC50	*Americamysis bahia*	0.006	10
δ-HCH	NOEC	*Oryzias latipes*	0.03	NOEC	*Scenedesmus subspicatus*	1.25	LC50	*Americamysis bahia*	0.006	10
Lindane	NOEC	*Oncorhynchus tshawytscha*	0.032	NOEC	*Scenedesmus subspicatus*	1.25	LC50	*Americamysis bahia*	0.006	10
α-endsf	LC50	*Oncorhynchus mykiss*	0.0006	EC50	*	83.024	LC50	*	6.719	100
β-endsd	LC50	*Oncorhynchus mykiss*	0.0037	EC50	*	83.024	LC50	*	6.719	100
PCB 28	LC50	*	0.112	EC50	*	0.258	LC50	*	0.008	1000
PCB 52	LC50	*	0.035	EC50	*	0.115	LC50	*	0.002	1000
PCB 101	LC50	*	0.01	EC50	*	0.051	LC50	*	2.99E-04	1000
PCB 118	LC50	*	0.01	EC50	*	0.051	LC50	*	2.99E-04	1000
PCB 138	LC50	*	0.003	EC50	*	0.022	LC50	*	5.65E-05	1000
PCB 153	NOEC	*Oncorhynchus mykiss*	0.051	EC50	*	0.022	LC50	*	5.65E-05	100
PCB 180	LC50	*	0.0009	EC50	*	0.01	LC50	*	1.06E-05	1000
BDE 28	LC50	*	0.121	EC50	*	0.31	EC50	*Acartia tonsa*	0.013	100
BDE 47	NOEC	*Fundulus heteroclitus*	0.05	EC50	*	0.102	LC50	*Palaemonetes pugio*	2.36E-02	50
BDE 99	LC50	*	0.05	EC50	*	0.033	EC50	*Acartia tonsa*	4.20E-03	100
BDE 100	LC50	*	0.05	EC50	*	0.033	EC51	*Acartia tonsa*	1.20E-03	100
BDE 153	LC50	*	0.0009	EC50	*	0.01	LC50	*	7.98E-06	1000
BDE 154	LC50	*	0.0009	EC50	*	0.01	LC50	*	7.98E-06	1000

El estudio de vigilancia ambiental de 51 contaminantes orgánicos en las aguas costeras de Cataluña se complementó con una estimación del riesgo utilizando cocientes de riesgo o RQs (“Risk quotient”). Para calcular el RQ se necesita conocer la concentración ambiental predicha o PEC (“predicted envinronmental concentration”) o en su caso la concentración medida en el ambiente o MEC (“mesured environmental concentration”) de un contaminante, divida entre su respectivo PNEC. Matemáticamente se puede expresar como:

$$RQ=\frac{MEC}{PNEC} \text{ ó } RQ=\frac{MEC}{\left(LC_{50}/_{f}\right)}$$

Donde f, es el factor de seguridad. Los RQ individuales de cada contaminante, en cada sitio de muestreo fueron sumados para obtener el RQ en ese punto de muestreo. El TGD recomienda establecer RQs para tres niveles representativos del medio acuático, es decir en organismos tales como peces, algas y crustáceos planctónicos (*Daphnia magna*). Como la *Daphnia magna* es un organismo de agua dulce, se decidió emplear datos de un organismo de agua salada como los crustáceos misidáceos (*Artemia salina* o *Americamysis bahia*) [274]. Finalmente, los resultados de la estimación del riesgo fueron representados en un mapa para observar las zonas en las que existe un mayor riesgo para las especies evaluadas.

Por otra parte, también se evaluó el riesgo de los contaminantes en las aguas costeras del Mar Cantábrico, pero esta vez utilizando una metodología diferente y más completa que tuviera en consideración no sólo la toxicidad al medio, sino también factores como bioacumulación y posibles afectaciones al ser humano. Para ello se siguió el procedimiento Combinado de Fijación de Prioridades Basado en Mediciones y Modelos o COMMPS (“Combined Monitoring-based and Modelling-based Priority Setting Scheme”) el cual ha sido utilizado en tareas tan relevantes como el establecimiento de la lista de sustancias prioritarias y prioritarias peligrosas en el ámbito de la política de aguas [65]. Este procedimiento sólo se puede aplicar si se tienen varias réplicas para cada sitio de muestreo. Para que una sustancia sea tomada en cuenta en el procedimiento COMMPS debe ser detectada en al menos 5 sitios; serán excluidas todas aquellas en que el porcentaje de positivos sea menor al 10%. Los valores inferiores al límite de detección (bdl) serán remplazados por la mitad del correspondiente MDL [276]. En este sentido, se consideraron 36 contaminantes de los 51 estudiados. El modelo cuantifica el riesgo asociado a la exposición de una sustancia química haciendo uso de las concentraciones de programas de vigilancia ambiental, obteniendo primero el índice denominado Índice de Exposición (“Exposure Index”). Por otro lado se modelan los efectos directos e indirectos que podrían ocasionar los contaminantes en organismos acuáticos, para ello se usan valores de la toxicidad (PNEC) y bioacumulación. En el modelo se consideran también posibles efectos indirectos en el ser humano, asignando un valor de acuerdo a las frases de riesgo (R-phrases) designadas por la Directiva 2001/59/CE [277]. Estas R-phrases pueden obtenerse de los envases y los certificados de calidad de cada compuesto. De los efectos de toxicidad al medio y al ser humano se obtiene el segundo índice llamado Índice de Efectos ("Effect Index"). Finalmente el modelo establece una jerarquía de las sustancias químicas de acuerdo a un índice de prioridad del riesgo ("Priority index"). Estos índices se grafican y puede observarse el compuesto o compuestos cuya presencia y concentración representen un problema para las zonas de la costa en esta región. Por último para la región costera y estuarina de Asturias-Cantabria-País Vasco, se calculó un índice de riesgo por sitio de muestreo, contemplando el riesgo de exposición y de efecto obtenidos en el procedimiento COMMPS para una representación geográfica del riesgo [276].

3.2 Desarrollo experimental

La vigilancia ambiental de PFCs en la costa catalana y la estimación de fuentes de contaminación se describen en el Artículo Científico 4 denominado **"Spatial distribution and sources of perfluorochemicals in the NW Mediterranean coastal waters (Catalonia, Spain)"**.

La vigilancia ambiental, estimación de las fuentes y evaluación del riesgo ambiental en la costa catalana se discuten en el Artículo Científico 5 titulado **"Organic micropollutants in coastal waters from NW Mediterranean Sea, Spain: Distribution, sources and potential risk"**.

Finalmente, la vigilancia ambiental, estimación de las fuentes y evaluación del riesgo ambiental en la costa Cantábrica se discuten en el Artículo Científico 6 que lleva por título **"Sources, fluxes and risk of organic micropollutants to the Cantabrian Sea (Spain)"**

ARTÍCULO CIENTÍFICO 4.

"Spatial distribution and sources of perfluorochemicals in the NW Mediterranean coastal waters (Catalonia, Spain)"

Juan Sánchez-Avila, Johan Meyer, Silvia Lacorte.

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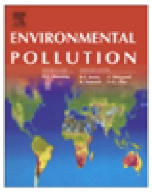

Spatial distribution and sources of perfluorochemicals in the NW Mediterranean coastal waters (Catalonia, Spain)

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River waters and wastewater treatment plant effluents are the main sources of perfluorochemicals to NW Mediterranean Sea.

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ABSTRACT

This study provides the first evidence of the sources and loads of perfluorochemicals (PFCs) to the NW Mediterranean Sea. Five PFCs were analyzed in 45 seawater samples collected along the Catalan coast. Total PFCs ranged from 0.07 to 13.0 ng/l, being the levels higher in ports than in coastal waters. To determine the sources of PFCs, 8 wastewater treatment plants (WWTPs) effluents and 6 rivers discharging to the sea were also analyzed. WWTP effluents contained total PFCs levels ranging from 3.47 to 132 ng/l but due to the relatively low discharge flows, they contributed to 34.7 g/d to the sea. Total PFCs in rivers ranged from 2.24 to 21.9 ng/l and were the principal PFCs contributors to the sea. Overall, a total load of 190 g/d of PFCs are discharged to the NW Mediterranean coast. The effects and risk of PFCs discharges to the Mediterranean basin are discussed.

1. Introduction

Concern about the presence of perfluorinated chemicals (PFCs) is growing because they are globally distributed, environmentally persistent, bioaccumulative and potentially harmful to organisms (Hekster et al., 2003). PFCs have been used in a wide array of applications, but they are mainly known for being components of polytetrafluoroethylene (PTFE) and for their use as water repellents and in consumer products such as pans, carpets, detergents and flame retardants (Hekster et al., 2003; OSPAR, 2006). These compounds are introduced in the environment from the release of in-use products, from industrial or waste water treatment (WWTP) effluents (Bossi et al., 2008; Clara et al., 2009; Lin et al., 2009; Schultz et al., 2006), run-off, accidental spills or by the release of non treated discharges (Paul et al., 2009). PFCs are transported via surface waters to the sea. Therefore, seas and oceans become a major reservoir of PFCs as well as the most important medium for their distribution (Taniyasu et al., 2005; Yamashita et al., 2008). So far PFCs have been detected at levels ranging from 0.03 to 730 ng/l in coastal areas of Hong Kong, China, Korea, Japan (So et al., 2004; Taniyasu et al., 2005), Norway (Ahrens et al., 2009a) and Germany (Ahrens et al., 2010). PFCs are diluted in open seawaters but because of their persistance, PFCs have been identified in the Pacific and North and Mid Atlantic Oceans at the 0.2–439 pg/l level, 100–1000 times lower than in coastal waters (Yamashita et al., 2005, 2008). They have also been detected at very low concentrations in remote sites without anthropogenical activities, like Antarctica seawater (5.1 to 22.6 pg/l) (Wei et al., 2007). Once in the aquatic ecosystem, they are accumulated in biota and magnify in the food chain (Kannan et al., 2005). PFCs concentrations have been reported in fish (<2–315 ng/g ww) (Llorca et al., 2009; Nania et al., 2009; Sinclair et al., 2006), in several aquatic birds (<12–1780 ng/g ww) (Kannan et al., 2001; Meyer et al., 2009) and in mammals such as Greenland polar bears (1285 ng/g ww) (Bossi et al., 2005), and dolphins and whales from the Italian Coast of the Mediterrenenan Sea (Kannan et al., 2002). The effects they may cause to aquatic organisms is still unclear but perfluorooctanoate sulfonate (PFOS) may cause tumors, immunotoxic, reproductive and/or developmental effects (Beach et al., 2006).

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The Mediterranean Sea is an enclosed basin which receives the pollution impact of 21 bordering countries, and 69 river run-off and effluent discharges have been identified as the main culprit of pollution. Specifically, the NW Mediterranean is a highly urbanized and industrialized area with textile, tanneries, recycled paper and chemical industries as main sectors. This area receives also the discharge waters of 6 main rivers, being the Ebro the most important river in Spain discharging to the Mediterranean. In previous studies it has been observed that river waters and WWTP effluents are the main contributors of polycyclic aromatic hydrocarbons (PAHs), alkylphenols (APs), plasticizers and other contaminants to the sea (Navarro et al., 2010; Sánchez-Avila et al., 2009). These sources have been identified as the main cause of seawater pollution of the area, where ng/L concentration of several organic contaminants have been detected in coastal waters from Catalonia (Sánchez-Avila et al., 2010). However, the presence, sources and fate of PFCs in the NW Mediterranean area are still unrevealed and therefore, the environmental risk cannot be assessed.

The objective of the present study was to evaluate the occurrence and mass flow of PFCs to the NW Mediterranean seawater (Catalan coast), considering rivers and WWTP effluents discharging to the sea as pollution sources and seawater as receptor. The inputs of PFCs in coastal waters were estimated according to the levels of PFCs detected in river waters and WWTP effluents discharging to the sea via submarine emissaries and their specific fluxes. The study area (400 km of coast) is highly industrialized and urbanized and the impact of industrial facilities and urban settlements on PFCs release was also evaluated. The five PFCs studied were perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA) and perfluorononanoate (PFNA).

2. Materials and methods

2.1. *Sampling sites and sample collection*

The study area comprised the NW Mediterranean (Fig. 1), from Roses (42.1544° N, 3.1807° E) to Castellón (39.9697° N, 0.0140° E). Table 1 reports the details about the sample locations, the type of waters sampled, the coordinates and the characteristics of each site. Sampling was carried out in spring 2009 (specific dates indicated in Table 1). Forty five seawaters were sampled which corresponded to 29 coastal waters (C1 to C29, of which C01, C02, C03, C13, C18, C23 and C24 correspond to seawater collected at the mouth of a river) and 16 ports (P1 to P16). These sampling locations comprised all municipalities with more than 5000 inhabitants and some had important industrial activities (Table 1). In addition, all WWTP (W1 to W8) treated effluents discharging in this coastline via submarine sea emissaries were analyzed. Finally, the main 6 rivers (R1 to R6) discharging to the NW Mediterranean were sampled. All water samples were collected in 1 l clean amber glass bottles previously washed with water and Extran (Merck, Darmastadt, Germany), rinsed with distilled water and methanol and baked at 450 °C to eliminate organic matter. PTFE materials and any suspect fluoropolymer containing material were avoided during the sampling and analytical steps. To avoid sample manipulation and prevent any cause of external contamination during sampling and extraction, waters were transported, stored and analyzed in the same bottle used for sampling. For river and port water collection, bottles were placed in a drag for a better accessibility to the samples. Grab seawater samples were collected by an expert swimmer at 50–100 m from the coast line and at 1–2 m deep. Port seawaters grab-samples were collected near the port mouth at 1–2 m deep. Rivers grab-samples were collected in the middle of the river flow at 1 m depth at the mouth of the river (3–10 km inland). The WWTP samples were made of 24 h composite aliquots taken from the effluent.

2.2. *Chemicals*

Individual PFBS, PFHxS, PFOS, PFOA, PFNA and the surrogate standards ^{13}C-PFOS and ^{13}C-PFOA at 50 μg/ml were purchased from Wellington Laboratories (Ontario, Canada). Methanol, acetonitrile (SupraSolv grade) and HPLC water (LiChrosolv grade) were supplied by Merck (Darmastadt, Germany). Oasis® HLB 6 cc (200 g) extraction cartridges were supplied by Waters (Massachusetts, USA).

2.3. *Solid phase extraction*

Waters were pre-concentrated by Solid Phase Extraction (SPE) without preliminary filtration to obtain the total concentration of PFCs in water considering both the particulate and dissolved phases. Prior to preconcentration, samples were spiked with 10 ng of ^{13}C-PFOS and ^{13}C-PFOA as surrogates standards. SPE was carried out with Oasis® HLB 6 cc (200 mg) extraction cartridges, similar as described elsewhere (Taniyasu et al., 2005). Cartridges were conditioned with 5 ml of methanol and 5 ml of HPLC water. Cartridges were loaded with 1 l of seawater or 1 l of river water or 50 ml of WWTP effluent at approximately 5 drops per second. Cartridges were then washed with 10 ml 10% methanol solution in HPLC water. After vacuum drying, 10 ml of methanol was used as extraction solvent. Extracts were dried under a gentle N_2 current. Sea and river water extracts were reconstituted with 200 μl of a 30/70 v/v acetonitrile/HPLC water solution, and WWTP effluent extracts with 500 μl of the same solution.

2.4. *Instrumental analysis*

Analysis were performed using an ACQUITY UPLC™ (Ultra Performance Liquid Chromatography) System from Waters (Milford, MA, USA) interfaced to a triple quadrupole mass spectrometer AQUITY TQD (Waters, USA) equipped with an electrospray ionization (ESI) interface. Analysis were performed in negative ion mode. An Acquity UPLC BEH C18 column (50 mm × 2.1 mm, 1.7 μm particle size Waters, USA) was used as mobile phase residue trap to remove any contamination from the mobile phases. The chromatographic column was a LiChroCART HPLC RP-18e column (125 mm × 2 mm × 5 μm particle size, Merck, Germany) and the flow rate was set at 0.4 ml/min. The column was heated at 50 °C and 10 μl of extract was analyzed. The mobile phase consisted in 2 mM ammonium acetate in water (solvent A) and acetonitrile (solvent B) and was delivered at a flow rate of 0.4 ml/min. Gradient elution program started from 30% of solvent B, held for 0.5 min and increased to 90% B in 4.5 min; held for 0.1 min and then increased to 100% B in 0.1 min and held for 0.9 min. Initial conditions were gathered in 1 min and finally the system was stabilized for 3 min. The total run time was 6 min with an equilibration time of 5 min between injections. Ions were acquired in Multiple Reaction Monitoring (MRM). The optimal MRM conditions used for each compound are presented in Supplementary Material (Table S1).

2.5. *Quality control and quantification*

Percent recoveries were determined using HPLC water free of PFCs spiked with 0.4 ng/l of target compounds. Accuracy and precision were determined by analyzing this spiked HPLC water samples ($n = 5$) and were expressed as mean recovery and relative standard deviation (RSD). Values are presented in Table 2. Percentage recoveries and RSD for surrogate standards in HPLC water were 102% ± 8 for ^{13}C-PFOS and 101% ± 12 for ^{13}C-PFOA, respectively; in seawater was 83% ± 17 and 108% ± 21; in river water was 82% ± 11 and 80% ± 25; in WWTP effluent was 92% ± 16 and 83% ± 23.

Quantification was performed by the internal standard method. A 9 points calibration curve was constructed at 0.01, 0.05, 0.1, 0.5, 1.0, 2.0, 5.0, 10 and 20 ng/ml with good linearity over this concentration range ($R^2 > 0.9979$). The limits of detection and quantification of the analytical method (Table 2) were calculated with the minimum concentration of analyte that produced a signal-to-noise ratio (S/N) of 3:1 and 6:1, respectively.

Sample blanks were rigorously performed to eliminate any external source of contamination. Blank samples were below detection limits except for PFOA, where blank values were below quantification limit. The ion chromatogram of the five target compounds and the two surrogate standards is shown in Supplementary Material (Fig. S1). The optimized conditions permitted to determine ultra low ng/l level concentration of PFCs in the different water matrices studied (WWTP effluents, river and seawater).

3. Results and discussion

3.1. *Occurrence of PFCs in effluents from municipal WWTPs*

The PFCs concentrations in effluent water from eight municipal WWTP discharging to NW Mediterranean Sea (in Catalonia) are summarized in Table 3. All target compounds were detected in all WWTP effluent samples. PFOS (2.79–72.1 ng/l) and PFOA (3.47–61.9 ng/l) were the two major PFCs (Table 3), followed by PFHxS (5.80–25.3 ng/l), PFNA (1.87–14.1 ng/l) and finally by PFBS (0.35–2.03 ng/l). The highest ΣPFCs concentration (considering the sum of the five PFCs analyzed) was found in Tarragona WWTP effluent (W07)(132 ng/l) followed by Prat de Llobregat WWTP effluent (W06) (76.0 ng/l), close to Barcelona. Tarragona and Prat de

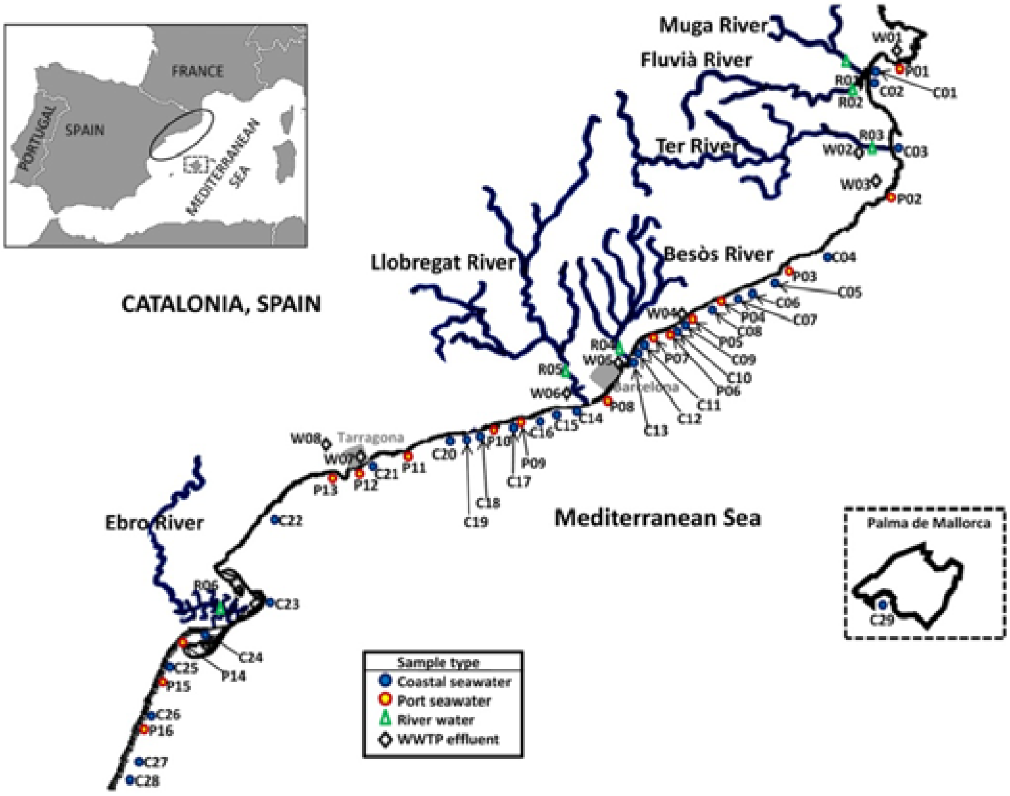

Fig. 1. Sampling locations for seawater, river water and WWTPs effluents along the Catalan coast.

LLobregat WWTP receive domestic sewage waters from large cities (143 884 and 1 092 573 inhabitants, respectively) as well as industrial wastewaters. The ΣPFCs concentrations in the other six WWTPs evaluated were lower (3.47–17.6 ng/l) and had a different pattern distribution (Table 3). The PFC distinct distributions are attributed to the different treatment processes in each plant and the wastewater origin (Sinclair and Kannan, 2006). The studied WWTP operate with flocculation followed by secondary aerobic digestion (secondary treatment) and in Roses, Palamòs and Prat de Llobregat, with tertiary treatment (Table 1).

PFCs have been reported in WWTP effluents from other countries. Since PFOS and PFOA are the most ubiquitous and the highest contributors of the ΣPFCs, they are the main compounds mostly analyzed in wastewaters. PFOS and PFOA values detected in the present study were higher than those reported in six Danish WWTP effluents (<1.5–18.1 ng/l and 8.7–24.4 ng/l, for PFOS and PFOA respectively) (Bossi et al., 2008), similar to ten WWTP effluents from non specified USA cities (1.1–130 ng/l and 2.5–97 ng/l) (Schultz et al., 2006), to WWTP effluents from Georgia and Kentucky WWTP (USA) (1.8–28 ng/l and 6.7–183 ng/l) (Loganathan et al., 2007), to nine from Germany (<0.06–82.2 ng/l and 12.3–77.6 ng/l) (Ahrens et al., 2009b) and six from Canada (10–210 ng/l and 6–55 ng/l) (Furdui et al., 2008). Values were lower than 21 WWTP effluents from Austria (4–340 ng/l and 10–220 ng/l) (Clara et al., 2008) and six from the New York State, USA (3–68 ng/l and 58–1050 ng/l) (Sinclair and Kannan, 2006). The results show that PFCs undergo physico-chemical and biological treatment processes and therefore, WWTP effluents become an important source of PFCs to receiving waters.

3.2. Occurrence of PFCs in rivers

Rivers are the main vehicle for the transport and mobilization of contaminants and are the main drivers of contaminants to the sea. Rivers, especially Mediterranean rivers which have a strong seasonal regime and scarce flows (Table 1), receive the input of treated or untreated sewage effluents and urban discharges and agricultural run-off. Consequently, rivers become a sink of wastewater, as observed when comparing the similar fluxes of St. Adrià del Besòs and Prat de Llobregat WWTP with Besòs and Llobregat river flows. We were particularly interested in determining the levels of PFCs at the river mouths, with an attempt to estimate the mass loads of these compounds to the sea. The PFCs concentrations in the six principal rivers converging to the Catalan Sea are shown in Table 3. All river samples contained at least one PFC. PFOA (0.79–9.63 ng/l) and PFOS (1.09–9.56 ng/l) were the two major PFCs. PFNA (0.23–1.62 ng/l), PFBS (0.09–0.88 ng/l) and PFHxS (0.09–0.64 ng/l) were detected at trace concentrations. The highest ΣPFCs concentration (considering the sum of the five PFCs analyzed) was found in the Llobregat River (R05, 21.9 ng/l) followed by the Besòs River (R04, 16.6 ng/l) and the Ter River (R03, 16.3 ng/l). The Llobregat (R05) and the Besòs River (R04) surround the Barcelona town and thus receive the direct discharge of Barcelona and upstream WWTPs.

PFOA has been identified previously as the major industrial contaminant present in some European Rivers (detected in the 97% of samples) (Loos et al., 2009). PFOS and PFOA values determined in this study were similar to those reported for the Paraiba do Sul River, Brazil (<0.1–1.32 ng/l and <0.09–1.22 ng/l, for PFOS and PFOA respectively) (Quinete et al., 2009), for the Ganges River, India

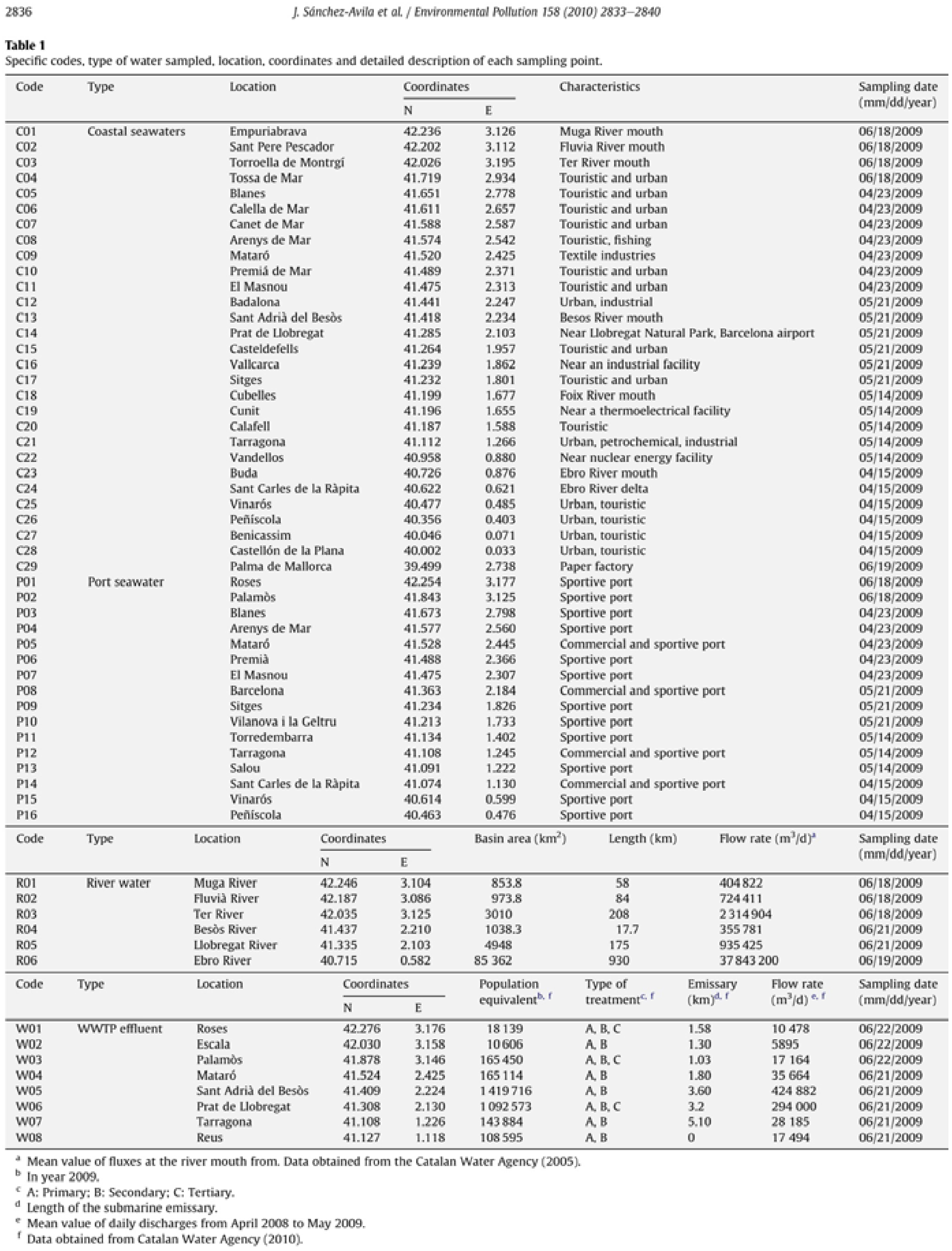

Table 1
Specific codes, type of water sampled, location, coordinates and detailed description of each sampling point.

Code	Type	Location	Coordinates		Characteristics	Sampling date (mm/dd/year)
			N	E		
C01	Coastal seawaters	Empuriabrava	42.236	3.126	Muga River mouth	06/18/2009
C02		Sant Pere Pescador	42.202	3.112	Fluvia River mouth	06/18/2009
C03		Torroella de Montrgí	42.026	3.195	Ter River mouth	06/18/2009
C04		Tossa de Mar	41.719	2.934	Touristic and urban	06/18/2009
C05		Blanes	41.651	2.778	Touristic and urban	04/23/2009
C06		Calella de Mar	41.611	2.657	Touristic and urban	04/23/2009
C07		Canet de Mar	41.588	2.587	Touristic and urban	04/23/2009
C08		Arenys de Mar	41.574	2.542	Touristic, fishing	04/23/2009
C09		Mataró	41.520	2.425	Textile industries	04/23/2009
C10		Premiá de Mar	41.489	2.371	Touristic and urban	04/23/2009
C11		El Masnou	41.475	2.313	Touristic and urban	04/23/2009
C12		Badalona	41.441	2.247	Urban, industrial	05/21/2009
C13		Sant Adrià del Besòs	41.418	2.234	Besos River mouth	05/21/2009
C14		Prat de Llobregat	41.285	2.103	Near Llobregat Natural Park, Barcelona airport	05/21/2009
C15		Casteldefells	41.264	1.957	Touristic and urban	05/21/2009
C16		Vallcarca	41.239	1.862	Near an industrial facility	05/21/2009
C17		Sitges	41.232	1.801	Touristic and urban	05/21/2009
C18		Cubelles	41.199	1.677	Foix River mouth	05/14/2009
C19		Cunit	41.196	1.655	Near a thermoelectrical facility	05/14/2009
C20		Calafell	41.187	1.588	Touristic	05/14/2009
C21		Tarragona	41.112	1.266	Urban, petrochemical, industrial	05/14/2009
C22		Vandellos	40.958	0.880	Near nuclear energy facility	05/14/2009
C23		Buda	40.726	0.876	Ebro River mouth	04/15/2009
C24		Sant Carles de la Ràpita	40.622	0.621	Ebro River delta	04/15/2009
C25		Vinarós	40.477	0.485	Urban, touristic	04/15/2009
C26		Peñíscola	40.356	0.403	Urban, touristic	04/15/2009
C27		Benicassim	40.046	0.071	Urban, touristic	04/15/2009
C28		Castellón de la Plana	40.002	0.033	Urban, touristic	04/15/2009
C29		Palma de Mallorca	39.499	2.738	Paper factory	06/19/2009
P01	Port seawater	Roses	42.254	3.177	Sportive port	06/18/2009
P02		Palamòs	41.843	3.125	Sportive port	06/18/2009
P03		Blanes	41.673	2.798	Sportive port	04/23/2009
P04		Arenys de Mar	41.577	2.560	Sportive port	04/23/2009
P05		Mataró	41.528	2.445	Commercial and sportive port	04/23/2009
P06		Premià	41.488	2.366	Sportive port	04/23/2009
P07		El Masnou	41.475	2.307	Sportive port	04/23/2009
P08		Barcelona	41.363	2.184	Commercial and sportive port	05/21/2009
P09		Sitges	41.234	1.826	Sportive port	05/21/2009
P10		Vilanova i la Geltru	41.213	1.733	Sportive port	05/21/2009
P11		Torredembarra	41.134	1.402	Sportive port	05/14/2009
P12		Tarragona	41.108	1.245	Commercial and sportive port	05/14/2009
P13		Salou	41.091	1.222	Sportive port	05/14/2009
P14		Sant Carles de la Ràpita	41.074	1.130	Commercial and sportive port	04/15/2009
P15		Vinarós	40.614	0.599	Sportive port	04/15/2009
P16		Peñíscola	40.463	0.476	Sportive port	04/15/2009

Code	Type	Location	Coordinates		Basin area (km²)	Length (km)	Flow rate (m³/d)[a]	Sampling date (mm/dd/year)
			N	E				
R01	River water	Muga River	42.246	3.104	853.8	58	404 822	06/18/2009
R02		Fluvià River	42.187	3.086	973.8	84	724 411	06/18/2009
R03		Ter River	42.035	3.125	3010	208	2 314 904	06/18/2009
R04		Besòs River	41.437	2.210	1038.3	17.7	355 781	06/21/2009
R05		Llobregat River	41.335	2.103	4948	175	935 425	06/21/2009
R06		Ebro River	40.715	0.582	85 362	930	37 843 200	06/19/2009

Code	Type	Location	Coordinates		Population equivalent[b, f]	Type of treatment[c, f]	Emissary (km)[d, f]	Flow rate (m³/d)[e, f]	Sampling date (mm/dd/year)
			N	E					
W01	WWTP effluent	Roses	42.276	3.176	18 139	A, B, C	1.58	10 478	06/22/2009
W02		Escala	42.030	3.158	10 606	A, B	1.30	5895	06/22/2009
W03		Palamòs	41.878	3.146	165 450	A, B, C	1.03	17 164	06/22/2009
W04		Mataró	41.524	2.425	165 114	A, B	1.80	35 664	06/21/2009
W05		Sant Adrià del Besòs	41.409	2.224	1 419 716	A, B	3.60	424 882	06/21/2009
W06		Prat de Llobregat	41.308	2.130	1 092 573	A, B, C	3.2	294 000	06/21/2009
W07		Tarragona	41.108	1.226	143 884	A, B	5.10	28 185	06/21/2009
W08		Reus	41.127	1.118	108 595	A, B	0	17 494	06/21/2009

[a] Mean value of fluxes at the river mouth from. Data obtained from the Catalan Water Agency (2005).
[b] In year 2009.
[c] A: Primary; B: Secondary; C: Tertiary.
[d] Length of the submarine emissary.
[e] Mean value of daily discharges from April 2008 to May 2009.
[f] Data obtained from Catalan Water Agency (2010).

Table 2
Methodological detection limit (LOD) and quantification limit (LOQ) and mean recoveries of the target compounds.

Compound	LOD (ng/L)	LOQ (ng/L)	Mean recoveries	
			Recovery (%)	RSD (%)
PFBS	0.04	0.07	71	5
PFHxS	0.01	0.03	91	7
PFOS	0.01	0.03	108	8
PFOA	0.04	0.08	86	10
PFNA	0.03	0.06	85	2

Table 3
PFCs concentrations determined in seawater, river water and WWTP effluents from the different samples collected in Catalonia.

Sample location		PFBS (ng/l)	PFHxS (ng/l)	PFOS (ng/l)	PFOA (ng/l)	PFNA (ng/l)	ΣPFCs (ng/l)
Coastal seawater	C01	0.12	<0.03	0.90	0.68	0.32	2.02
	C02	<0.07	0.05	0.73	0.78	0.24	1.79
	C03	0.24	0.21	1.61	1.42	0.23	3.71
	C04	<0.07	<0.03	<0.03	<0.08	0.15	0.15
	C05	<0.07	<0.03	<0.03	<0.08	0.08	0.08
	C06	<0.07	<0.03	<0.03	0.13	<0.06	0.13
	C07	<0.07	<0.03	0.14	<0.08	0.07	0.21
	C08	<0.07	<0.03	<0.03	0.23	<0.06	0.23
	C09	<0.07	0.04	0.10	0.15	0.25	0.54
	C10	<0.07	<0.03	<0.03	0.15	<0.06	0.15
	C11	<0.07	<0.03	<0.03	0.07	<0.06	0.07
	C12	<0.07	<0.03	<0.03	<0.08	0.22	0.22
	C13	<0.07	0.06	0.07	1.07	0.84	2.03
	C14	<0.07	<0.03	0.15	<0.08	0.24	0.38
	C15	<0.07	<0.03	0.08	0.08	<0.06	0.15
	C16	<0.07	0.05	0.07	<0.08	0.24	0.36
	C17	<0.07	0.04	<0.03	0.15	0.14	0.33
	C18	<0.07	<0.03	0.14	0.35	0.17	0.66
	C19	<0.07	<0.03	0.17	0.32	0.11	0.59
	C20	<0.07	<0.03	<0.03	0.17	<0.06	0.17
	C21	<0.07	0.47	1.48	0.47	0.16	2.57
	C22	<0.07	0.07	0.57	0.64	0.193	1.47
	C23	<0.07	<0.03	3.93	0.25	<0.06	4.19
	C24	0.07	0.04	2.26	1.86	0.77	4.99
	C25	<0.07	<0.03	<0.03	0.11	<0.06	0.11
	C26	<0.07	<0.03	<0.03	0.09	<0.06	0.09
	C27	<0.07	0.07	0.06	<0.08	0.17	0.30
	C28	<0.07	<0.03	0.05	0.19	0.09	0.33
	C29	<0.07	<0.03	0.09	0.64	0.49	1.23
Port seawater	P01	<0.07	<0.03	<0.03	0.55	<0.06	0.55
	P02	<0.07	0.04	0.09	1.14	0.17	1.43
	P03	0.11	0.06	1.86	0.52	0.34	2.87
	P04	<0.07	0.09	0.18	0.44	0.19	0.90
	P05	0.14	<0.03	0.38	0.38	0.22	1.13
	P06	0.17	<0.03	0.39	0.53	0.23	1.33
	P07	0.07	<0.03	0.26	0.50	0.17	1.01
	P08	0.08	0.98	8.38	2.25	1.35	13.0
	P09	0.23	0.06	0.59	1.01	0.29	2.18
	P10	<0.07	0.06	0.38	0.83	0.24	1.50
	P11	<0.07	0.04	0.35	0.39	0.25	1.03
	P12	<0.07	2.09	7.07	1.54	0.24	10.9
	P13	<0.07	0.07	0.63	0.90	0.30	1.91
	P14	<0.07	0.05	0.45	0.87	0.23	1.62
	P15	<0.07	0.05	0.19	1.70	0.17	2.10
	P16	<0.07	<0.03	0.21	1.52	0.23	1.96
River water	R01	0.67	0.16	1.40	6.17	1.25	9.62
	R02	0.09	0.09	1.41	1.26	0.23	3.07
	R03	0.59	0.60	9.56	4.83	0.69	16.3
	R04	0.74	<0.03	7.70	8.12	<0.06	16.6
	R05	0.88	0.64	9.13	9.63	1.62	21.9
	R06	<0.07	0.12	1.09	0.79	0.23	2.24
WWTP effluent	W01	<0.07	<0.03	<0.03	3.47	<0.06	3.47
	W02	<0.07	<0.03	5.41	5.05	<0.06	10.5
	W03	0.92	<0.03	2.79	8.88	1.87	14.5
	W04	0.35	<0.03	3.30	9.39	2.50	15.5
	W05	0.40	<0.03	8.23	6.84	2.12	17.6
	W06	<0.07	<0.03	14.1	61.9	<0.06	76.0
	W07	2.03	25.3	72.1	18.1	14.1	132
	W08	0.45	5.80	3.67	6.54	<0.06	16.5

(<0.04–1.81 ng/l and <0.04–0.648 ng/l) (Yeung et al., 2009), for the Danube River, Austria (<4.0–35 ng/l and <1.0–19 ng/l) (Clara et al., 2009), for the Elbe River, Germany (0.6–2.9 ng/l and 2.8–9.6 ng/l) (Ahrens et al., 2009b), for the Ontario Lake Tributaries, Canada (2.6–22.9 ng/l and 1.4–38.1 ng/l) (Furdui et al., 2008), for the Niagara River, USA (3.3–6.7 ng/l and 18–22 ng/l) and for the Hudson River, USA (1.5–3.4 ng/l and 22–173 ng/l) (Sinclair et al., 2006). Higher values were found in Kyoto River, Japan (4.1–10 ng/l and 7.9–110 ng/l) (Senthilkumar et al., 2007). The highest PFOS and PFOA values in rivers were observed for the Po River, Italy (1–25 ng/l and 1–1270 ng/l) (Loos et al., 2008) and the Scheldt River, Belgium and The Netherlands (110–154 ng/l and 73–88 ng/l) (Loos et al., 2009). Recently, Ericson et al. (2008) reported concentrations of 1.59–2.47 ng/l of PFOS and 1.45–1.90 ng/l of PFOA in the Ebro River, Spain. Those values are similar to the obtained herein (PFOS, 1.09 ng/l and PFOA 0.792 ng/l) in the same river (R06).

3.3. Occurrence of PFCs in seawater from coastal areas and ports

PFCs arriving seawater undergo a dilution process and spread along the oceanic current (Yamashita et al., 2005). In our study, PFCs were detected in all coastal seawater samples at the low ng/l concentration (Table 3). PFOA was the most ubiquitous compound (76%) followed by PFNA (69%), PFOS (62%), PFHxS (34%), and finally by PFBS (10%). PFOS (0.05–3.93 ng/l) was found at overall highest concentrations (Table 3), followed by PFOA (0.07–1.86 ng/l), PFNA (0.071–0.84 ng/l), PFHxS (0.04–0.47 ng/l) and finally PFBS (0.07–0.24 ng/l). The total PFCs concentration in coastal seawaters ranged from 0.07 ng/l in El Masnou (C18) to 4.99 ng/l in San Carles de la Rapita (C24). Fig. 2 shows a comparison of the total PFCs concentration in the 45 seawater sampled and data is organized in 3 groups depending on the sampling zone: (i) samples collected in urban areas, which are spread along the coast; (ii) seawaters sampled just at the outflow of the rivers and (iii) samples collected in ports. Concentrations in urban areas < river mouths < ports.

The Catalan coast can be considered as a highly urbanized area, where the population range from 10 606 to 1 419 716 inhabitants (Table 1). In general, thresholds ΣPFC concentrations (considering the sum of the five PFCs analyzed) were found throughout coastal waters (average of 0.449 ng/l), with exception of Tarragona (C21, 2.57 ng/l) due to the proximity of a petrochemical complex, Vandellos (C22, 1.47 ng/l), close to a nuclear-electrical facility and Palma de Mallorca bay (C29, 1.23 ng/l) which receive treated or untreated industrial effluents. The ubiquitous presence of PFCs along the coast is an indicator of their widespread distribution and give evidence on the risk this may pose to the overall area.

River mouths were identified as a hot spot as regards to PFC pollution since samples collected at the outflow of the rivers had a significant higher concentration (*t*-test one side, $p = 0.003$, $p \leq 0.05$) than coastal seawaters from urbanized areas (0.66–4.99 ng/l vs. 0.07–2.57 ng/l) and indicate that these coastal micro-ecosystems can be highly impacted by river pollution. The Mediterranean is an enclosed basin where waters have a limited exchange rate and thus, pollution plumes can be difficult to mobilize and disperse, with the negative effects this can cause to the coastal ecosystems. Specifically, sites with high impact were St. Carles de la Rapita (C24, 4.99 ng/l) and Buda Beach (C23, 4.19 ng/l), both corresponding to the Ebro River mouth and Torroella de Montgri (C03, 3.71 ng/l) sampled near the Ter River mouth. These areas are highly agricultural and the presence of these compounds

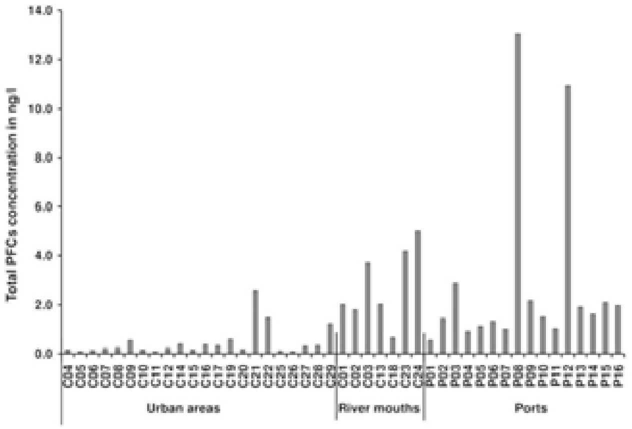

Fig. 2. ΣPFCs concentration (sum of 5 PFCs analyzed) detected in Catalonia seawater, first ordered by type (urban areas, river mouths and ports) and then from North to South.

are attributed to the discharge of WWTP effluents as well as run-off from agricultural fields. PFCs are used as additives in many pesticide mixtures.

Port seawaters contained PFCs at levels higher than coastal waters in urbanized areas. In ports, PFOA was present in all samples (100%), followed by PFOS and PFNA (94%), PFHxS (69%) and finally by PFBS (38%). PFOS (0.09–8.38 ng/l) was found at overall highest concentrations followed by PFOA (0.38–2.25 ng/l), PFHxS (0.04–2.09 ng/l), PFNA (0.17–1.35 ng/l) and finally by PFBS (0.07–0.23 ng/l). The ΣPFCs concentration in port seawaters ranged from 0.55 ng/l at Roses port (P01) to 13.0 ng/l at Barcelona port (P08) or 10.9 ng/l in Tarragona port (P12). Roses port is situated at the North of the Catalan coast and is a small municipality with tourism as the main activity, while Barcelona together with Tarragona are the largest ports in the NW Mediterranean coast with an important commercial traffic and associated industrial activities. The use of paints, carpets and treatments for water and grease repellence (Paul et al., 2009) for ship and dock protection could contribute to the relatively high presence of PFCs in port seawater. Shipping and boat maintenance are carried out in these ports and can also be responsible for the release of PFCs to surrounding waters.

Only a few studies report PFCs concentrations in coastal zones. Compared with Catalonia coastal water, similar PFOS and PFOA concentrations were found in the Norwegian coast (n.d. and 0.07–0.35 ng/l, for PFOS and PFOA respectively), in the open North Sea (n.d. to 0.07 ng/l and 0.02–0.07 ng/l), in the Baltic Sea (n.d. to 0.38 ng/l and 0.25–4.55 ng/l) (Ahrens et al., 2010), in the Guanabara Bay, Brazil (0.40–0.92 ng/l and 0.70–3.25 ng/l) (Quinete et al., 2009) and in the German Bight (0.69–3.95 ng/l and 2.67–7.83 ng/l) (Ahrens et al., 2009a). Relatively high values were found in South Korea coastal areas (0.04–3.10 ng/l and 1.3–11.0 ng/l) (So et al., 2004), in Tokyo Bay, Japan (13.7 ng/l and 34.8 ng/l) (Taniyasu et al., 2005) and in the western Korea coast (4.11–450 ng/l and 2.95–68.6 ng/l) (Naile et al., 2010). The highest reported values were detected in Kyeonngi Bay, Korea (730 ng/l and 320 ng/l) (So et al., 2004) and in Tomakomai Bay, Japan (2880 ng/l and 62.4 ng/l) (Taniyasu et al., 2005). The type of coast (enclosed bays, coastal areas) as well as the anthropogenic pressure may influence the release of PFCs to the sea. However, all these studies reflect that PFCs have become a relatively new emerging contaminant of seawater ecosystems worldwide and thus, attention should be given to identify and evaluate the main sources of pollution, the bioaccumulation potential and the impact these compounds may pose to marine ecosystems.

3.4. Mass flow of PFCs to the sea

In our study, rivers and submarine WWTP emissaries have been identified as the main sources of PFCs to seawater, although runoff and discharge of untreated urban or industrial waters to the NW Mediterranean cannot be neglected but since precise data is not available, this contribution was not taken into consideration. For calculating the PFCs mass flow to the sea, the levels of PFCs in rivers and WWTP effluents discharging to the sea via submarine emissaries were considered (Table 3) as well as the effluents discharges and river water flows (Table 1). The inputs to the sea are calculated according to:

$$\text{PFC discharge} = \text{PFC concentration} \times \text{water flow}$$

Fig. 3 shows the daily ΣPFCs inputs to the NW Mediterranean Sea. Although ΣPFC concentrations in WWTP effluent through marine emissaries were drastically higher than in rivers, their flows were much lower, so their inputs resulted in some cases several hundred-fold lower than the calculated in rivers. Effluents discharges to the marine environment coming from the eight WWTP studied represent 34.7 g/d of ΣPFCs, being Prat de Llobregat and Sant Adrià del Besòs the highest contributors with 22.4 and 7.48 g/d, respectively. Lower mass flows were reported in WWTP effluents from the USA, such as Georgia (0.52–7.62 g/day) or Kentucky (2.22–3.11 g/day considering the sum of 8 PFCs) (Loganathan et al., 2007). However, similar effluent discharges were reported for two WWTP from New York, USA (approximately 27.6 and 8.58 g/day, considering 8 PFCs) (Sinclair and Kannan, 2006).

On the other side, rivers were the main cause of PFC discharge to the sea. In the case of the Ebro river (R06), although the concentration of total PFC was rather low (2.24 ng/l), its contribution to the marine environment was the highest (84.6 g/d) due to its elevated flow. Those high inputs could explain the high presence of PFCs in seawaters from Buda (C23) and Sant Carles de la Ràpita (C24), taken at the Ebro River mouth proximity. The six Catalan rivers studied represent a total PFCs discharge of 154 g/d. Other studies report PFC river inputs to the sea of similar or higher values. The Elbe River PFCs contribution to North Sea was approximately two times higher (312 g/day) (Ahrens et al., 2009b). Ganges river, had similar PFCs values to reported herein and due to it high flux, it discharges approximately 518 g each day to the Bay of Bengal, India (Yeung et al., 2009). The Po River discharge is significantly higher, with an estimated discharge of 7.2 kg/d to the Adriatic sea (Loos et al., 2008). McLachlan et al. (2007) estimated a global PFCs flux of 39 kg/d (considering only PFOA) considering the 14 major rivers in Europe (excluding any Catalonian river).

3.5. Environmental risks associated to PFCs discharges

The use of PFC containing products has lead to the wide dispersion in the environment, and so far potential effects are still unknown. To protect the environment and human health, some actions are being taken in several countries by own initiative or by the recent restriction in the use of some compounds. From a production point of view, only PFOS is being regulated. In the European Union, Directive 2006/122/EC (European-Parliament-and-Council, 2006) has been adopted to restrict the marketing and use of PFOS in new products, establishing maximum concentrations depending on it use. To date, no official new information has been reported on details of uses and safer alternative substances or technologies for PFCs. 3 M Company the major PFC producer, in 2003 replaced the PFOS-based substances by PFBS (OSPAR, 2006).

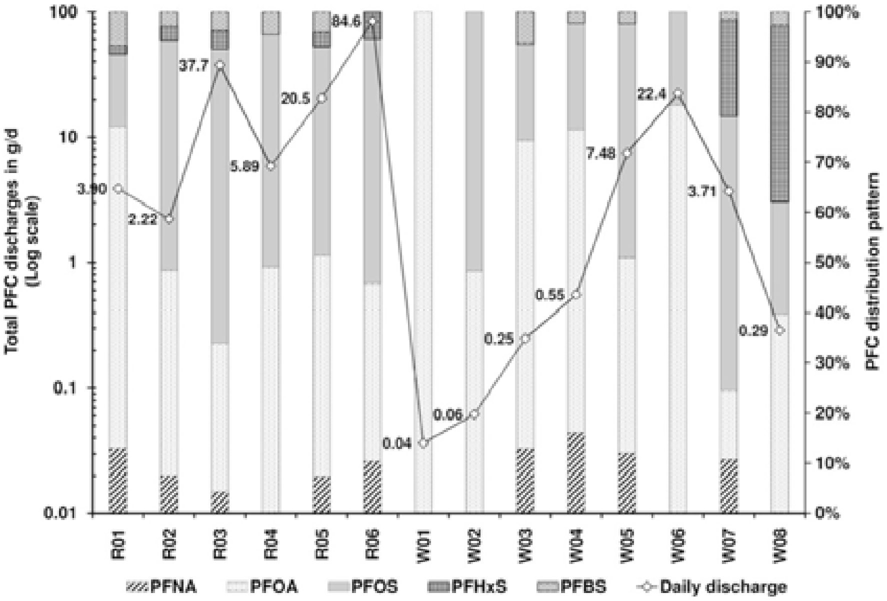

Fig. 3. ΣPFCs (sum of 5 PFCs analyzed) daily discharges from Catalonia rivers and WWTP effluents into NW Mediterranean Sea and PFCs distribution (in percentage) at each site (discharges presented in Log scale).

From an environmental point of view, PFOS has been added to Annex B (Parties must take measures to restrict the production and use of the chemicals) of the Stockholm Convention on Persistent Organic Pollutants (POPs) in 2009 (Stockholm-Convention-Secretariat, 2009). Also it was included in the OSPAR (Oslo-Paris Convention for the Protection of the Marine Environment of the North-East Atlantic) list of chemicals for priority action (OSPAR, 2007).

PFCs in the aquatic environment are not legislated in the European Community neither by the Environmental Protection Agency (EPA). Nevertheless, with the aim to establish water quality values, several studies report recommended maximum values to protect the most sensitive aquatic species. Multiple approaches are available to derive quality values and the most used are the Great Lakes Initiative guidelines by EPA. Those values are calculated using reported acute and subacute toxicity values of PFOS and PFOA to aquatic invertebrates, fish, plants and birds (Giesy et al., 2010). Hekster et al. (2003) calculated an indicative maximum permissible concentration (iMPC) in surface water of 5 μg/l for PFOS and 300 μg/l for PFOA. Recently, Giesy et al. (2010) calculated criteria maximum concentrations (CMC) for the most sensitive aquatic species (like *Daphnia magna*) for PFOS (21 μg/l) and PFOA (25 mg/l). The concentrations of PFOS and PFOA detected in this study were 100-1000 times lower than the CMC and iMPC, but still there are a lot of uncertainties about the toxicities at low concentration levels.

Although the PFCs concentrations reported in Catalan rivers (2.24–21.9 ng/l) and coastal waters (0.07–4.99 ng/l) are low, there is a strong evidence proving the bioaccumulation properties of some PFCs, specially perfluoroalkyl sulfonates with more than seven fluorinated carbons (Conder et al., 2008). Llorca et al. (2009) detected the presence of PFOS (8.24 ng/g dw) and PFOA (2.5 ng/g dw) in swordfish and Fernández-Sanjuan et al. (in press) observed PFOS and PFOA at levels of 0.2–1.4 ng/g ww in sardines and anchovy fish from Mediterranean Sea. Nania et al. (2009) found PFOS (<2–43 ng/g) and PFOA (<1.5–40 ng/g) in benthonic fishes from the Mediterranean Sea. Accumulation of PFCs in fish represent the source of PFCs to higher trophic level organisms and a potential risk for the marine ecosystem as a whole.

4. Conclusions

This study provides the first evidence on the sources, geographical distribution and mass flows of PFCs to the NW Mediterranean Sea. PFOS and PFOA were the main compounds detected in port and coastal seawaters, at concentrations from 0.05 to 8.38 ng/l and 0.38 to 2.25 ng/l, depending on the proximity of river outflows or submarine emissaries. Rivers contained PFCs at concentrations of 2.24–21.9 ng/l, and were identified as the major transport route of PFCs to the sea while WWTPs effluents, with relatively higher PFC levels (3.37–132 ng/l), represented a lower contribution to the sea due to their lower flows. It has been estimated that 190 g of PFCs are discharged each day to the NW Mediterranean Sea, considering only rivers and treated effluents while it is known that in the study area, many municipalities discharge untreated sewage waters directly to the sea. Therefore, higher values can be expected. Although PFCs are diluted in seawater, the continuous discharge of PFCs to the sea may represent an emerging risk which may limit the use of coastal waters for human activity with serious implications in the long term. In the recent years, coastal waters have become vulnerable areas because of their intermediate position between open seas and human activities. The increase in population and industrial pressure along rivers and coastal areas pose an important threat on those coastal ecosystems since contaminants such as PFCs are accumulated in biota and become a source to other organisms, including humans. Conservation of the water quality in coastal regions is a "must" and starts with the reliable understanding on the sources and pathways of contaminants to the sea, which will contribute to the improvement and settlement of management actions to avoid anthropogenic organic pollution end up in oceans.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.envpol.2010.06.022.

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Table S1
Retention time, precursor and product ions for the compounds analyzed.

Compound	Acronym	Retention time (min)	Transition (m/z)	Cone Voltage (V)	Collision energy (V)	Dwell time
Perfluorobutane sulfonate	PFBS	2.68	299 > 80, 99	45	29	0.20
^{13}C - Perfluorooctanoic acid labeled	M-PFOA	3.23	417 > 372	17	10	0.05
Perfluorooctanoic acid	PFOA	3.24	413 > 369	17	10	0.05
Perfluorohexane sulfonate	PFHxS	3.54	399 > 80, 99	45	35	0.05
Perfluorononaoic acid	PFNA	3.57	463 > 419	16	11	0.05
Perfluorooctane sulfonate	PFOS	4.25	499 > 80, 99	45	40	0.05
^{13}C - Perfluorooctane sulfonate labeled	M-PFOS	4.26	503 > 80, 99	45	40	0.05

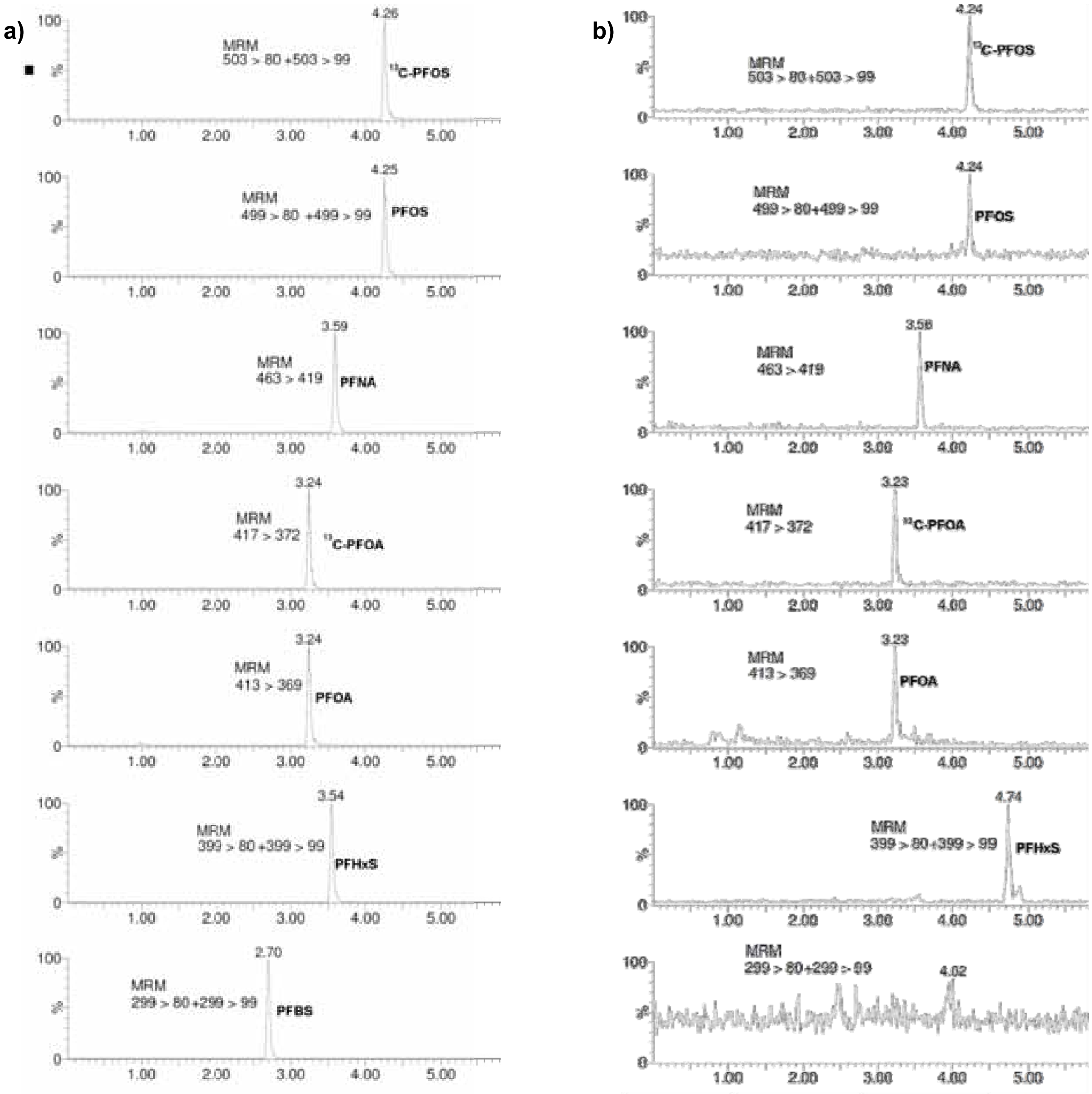

Fig. S1. Ion chromatogram of the perfluorochemicals (PFCs) of (a) the surrogate standars at 1.0 ng/ml and (b) C29 seawater sample.

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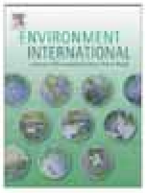

Organic micropollutants in coastal waters from NW Mediterranean Sea: Sources distribution and potential risk

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ABSTRACT

This study provides a first estimation on the sources, distribution and risk of organic micropollutants (OMPs) in coastal waters from NW Mediterranean Sea. Polycyclic aromatic hydrocarbons, polychlorinated biphenyls, organochlorinated pesticides, polybrominated diphenyl ethers, phthalates and alkylphenols were analyzed by solid phase extraction and gas chromatography coupled to tandem mass spectrometry (SPE–GC–EI-MS/MS). River waters and wastewater treatment plant effluents discharging to the sea were identified as the main sources of OMPs to coastal waters, with an estimated input amount of around of 25,800 g d^{-1}. The concentration of ΣOMPs in coastal areas ranged from 17.4 to 8442 ng L^{-1}, and was the highest in port waters, followed by coastal and river mouth seawaters. A summarized overview of the patterns and sources of OMP contamination on the investigated coastal sea waters of NW Mediterranean Sea, as well as of their geographical distribution was obtained by Principal Component Analysis of the complete data set after its adequate pretreatment. Alkylphenols, bisphenol A and phthalates were the main contributors to ΣOMPs and produced an estimated significant pollution risk for fish, algae and the sensitive mysid shrimp organisms in seawater samples. The combination of GC-MS/MS, chemometrics and risk analysis is proven to be useful for a better control and management of OMP discharges.

1. Introduction

Chemical pollution in marine waters presents a threat to the aquatic environment with effects such as acute and chronic toxicity to aquatic organisms, accumulation in the ecosystem and losses of habitats and biodiversity, as well as threats to human health (2008). However, the impact of organic micropollutants (OMPs), such as phthalate esters, pesticides and other endocrine-disrupting compounds in the marine environment is poorly studied (Gimeno et al., 2004). Among those, coastal waters have become vulnerable areas because of their intermediate position between open seas and human activities (Nellemann and Corcoran, 2006; Sánchez-Avila et al., 2010a). The increase in population and industrial pressure along rivers and coastal areas poses an important threat on those coastal ecosystems since OMPs are accumulated in sediments and biota and become a source to other organisms. A wide variety of OMPs are present in coastal and marine ecosystems, including polycyclic aromatic hydrocarbons (PAHs) (Valavanidis et al., 2008), plasticizers such as phthalate esters (PEs) and bisphenol A (BPA) (Fromme et al., 2002), organochlorine pesticides (OCPs), alkylphenols (APs) (Sánchez-Avila et al., 2010b), polychlorinated biphenyls (PCBs), and polybromodiphenyl ethers (PBDEs) (Pérez-Carrera et al., 2007), among others. OMPs are daily discharged to seawater principally via wastewater treatment plant effluents (WWTP) and also river inputs which can transport pollutants from inland activities (Céspedes et al., 2006; Gómez-Gutiérrez et al., 2007a; Sánchez-Avila et al., 2009).

The low concentrations of micropollutants in the marine environment in addition to the complexity of those matrices are two major difficulties associated with the detection and quantification of OMPs. The progress on the development of new and more powerful analytical methods has made available their determination in rivers, seawater and wastewater at concentrations of ng L^{-1} (Sánchez-Avila et al., 2011). In addition, multiresidue methods permit the analysis of a wide range of pollutants with varying physicochemical parameters in a single run which in terms of cost and time, in front of family-related compound using different methodologies, is beneficial because they can "capture" all the chemical information of a sample (Lacorte et al., 2000). However, routine monitoring and reporting of abiotic and biotic concentrations of OMP are of limited use, unless such data can be related directly to the assessment of public health and ecological risk (Wu et al., 2008). Ecological risk assessment can be estimated under diverse methodologies, such as the combined monitoring-based and modeling-based priority setting (COMMPS) procedure, which is applicable when there exists a large monitoring database (Teixido et al., 2010). The tool used herein for assessing the ecological risk involves a comparison between environmental concentrations (either predicted, PEC, or measured, MEC) with predicted no effect concentrations (PNEC), denominated Risk Quotients (RQs)

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(Sanderson et al., 2004; Wu et al., 2008). PEC values are calculated using several models taking into consideration application rates, persistence, leaching, sorption and compound bioaccumulation or directly from monitoring data while PNEC values are usually calculated on the basis of critical concentrations, e.g. EC_{50}, LC_{50} (50% effect concentration or 50% lethal concentration, respectively) and NOEC (non-observed effect concentration). Monitoring data must be compared with toxicological data and concentrations of contaminants below adverse effects thresholds are of little environmental concern (except, perhaps, for identifying the source of contamination).

The Catalan Coast is a highly urbanized region of the NW Mediterranean Sea with important industrial and agricultural activities. Coastal waters receive the continuous input from treated and untreated WWTP discharges, run-off and river waters. This coastal zone has been studied previously and several OMPs have been detected, suggesting that anthropogenic activities have an impact on coastal water quality, sediments and biota. For example, DDT and PCBs (Bayona et al., 1991; Gómez-Gutiérrez et al., 2007b; Scarpato et al., 2010) and PAHs (Baumard et al., 1999; Galgani et al., 2011) were identified in sediments, mussels and also in fish tissues (Albaiges et al., 1987). Those findings reveal the Catalan coast area as "hot spot" area for pollutants. However very few data has been reported on the OMP concentration in the seawater and the risk this may pose to the coastal ecosystem.

The objective of the present study was to evaluate the occurrence and mass flow of 51 target OMPs (PAHs, PEs, BPA, APs, OCPs, PCBs and PBDEs) to the NW Mediterranean sea (Catalan coast), considering rivers and WWTP effluents discharging to the sea as pollution sources and seawater as receptor. The inputs of OMPs in coastal waters were estimated according to the levels of target pollutants detected in river waters and WWTP effluents discharging to the sea via submarine emissaries and their specific fluxes. Principal Component Analysis (PCA) was applied to the experimental data set in order to identify main sources of OMP contamination and their distribution. Finally, RQs were estimated using fish, algae and mysid shrimp as reference organisms to assess the environmental risk of OMPs in the NW Mediterranean coastal waters.

2. Materials and methods

2.1. Study area and sample collection

The study area comprised the NW Mediterranean Sea (Table 1), from Roses in Catalonia, Spain (42.1544° N, 3.1807° E) to Castellón in Valencia, Spain (39.9697° N, 0.0140° E). Table 1 reports the details about the sample locations, the type of waters sampled, the coordinates and the characteristics of each site. Sampling was carried out in spring–summer 2009 (from March to July). Forty six seawaters were sampled which corresponded to 22 coastal waters (C1 to C22), 7 seawaters collected at the mouth of a river (M01 to M07) and 17 ports (P1 to P17). These sampling locations comprised all municipalities with more than 5000 inhabitants and some had important industrial activities (Table 1). In addition, all WWTP (W1 to W8) treated effluents discharging in this coastline via submarine sea emissaries were analyzed. Finally, the main 6 rivers (R1 to R6) discharging to the NW Mediterranean were sampled. It is important to remark that rivers were sampled in low flow conditions which represent the "worst case scenario". Originally the sampling covered more Catalan rivers which could not be sampled because they were dry in this period. All water samples were collected in 1 L clean amber glass bottles previously washed with water and Extran (Merck, Darmastadt, Germany), rinsed with distilled water and acetone and baked at 450 °C to eliminate organic matter. To avoid sample manipulation and prevent any cause of external contamination during sampling and extraction, waters were transported, stored and analyzed in the same bottle used for sampling. Transport and storage were done maintaining samples at 4 °C. All samples were extracted within 48 h of collection to avoid degradation of target compounds. For river and port water collection, bottles were placed in a drag for a better accessibility to the samples. Grab seawater (coastal and river mouths) samples were collected by an expert swimmer at 50 to 100 m from the coast line and at 1 to 2 m deep. Port seawater grab-samples were collected near the port mouth at 1 to 2 m deep. River grab-samples were collected in the middle of the river flow at 1 m depth at the mouth of the river (3-10 km inland). The WWTP samples were made of 24 h composite aliquots taken from the effluent.

2.2. Extraction and gas chromatography coupled to mass spectrometry analysis

The analytical procedure was based on a previously reported validated method based on solid phase extraction Oasis 200 mg cartridges and gas chromatography coupled to tandem mass spectrometry for the determination of 51 OMPs (16 PAHs, 4 APs, BPA, 5 PEs, 7 PCBs, 6 PBDEs, 12 OCPs (including isomers or degradation products) in sea, river waters and WWTP effluents (Sánchez-Avila et al., 2011). The optimized conditions permitted to determine ultra low ng L^{-1} level concentration of the OMPs in the different water matrices studied.

Unfiltered water samples (1000 mL of sea or river waters or 250 mL of WWTP effluent) were analyzed to avoid sub-estimations in the total concentration and thus, both dissolved and particulate bound chemicals were considered. The surrogate standards (acenaphthylene d_8, benzo(a)pyrene d_{12}, benzo(g,h,i)perylene d_{12}, fluoranthene d_{10}, phenanthrene d_{10}, pyrene d_{10} PCB 209, 4-n-nonylphenol-d_8 and bisphenol A-d_{16}) were added prior to preconcentration at 10 ng L^{-1} in sea or river waters and at 100 ng L^{-1} in WWTP effluents. After extraction, samples were analyzed using an Agilent 7890A GC System (Agilent Technologies, Palo Alto, CA, USA) interfaced to a 7000A triple quadrupole mass spectrometer system (Agilent, USA) in EI (+70 eV). Two µL of each extract was injected using an Agilent 7683A autosampler. The use of two transitions (one precursor with two product ions or two precursors with one product ion) confirms positive findings by Selected Reaction Monitoring (SRM) according to the European legislation (Commission of the European Communities, 2002). Quantification was performed using the internal standard method. A 9 point calibration curve was analyzed in duplicate from 0.010 to 1.0 ng μL^{-1} for all target compounds except for PBDEs (0.010 to 0.10 ng μL^{-1}). Good linearity responses were obtained over the concentration range tested for all target pollutants. The coefficients of determination (R^2) obtained were higher than 0.9910. The methodological detection limits (MDL) and recovery yields are described in Sánchez-Avila et al. (2011).

2.3. Statistical analysis

Statistical significance of results was determined by the one-way analysis of variance (one-way ANOVA).

2.4. Principal Component Analysis

To establish relationships between sources and seawater pollution, a multivariate exploratory data analysis study was performed using Principal Component Analysis (PCA) (Jolliffe, 2002). PCA is a multivariate technique which looks for a reduced set of new orthogonal variables or principal components, which explain maximum data variance and allow for an easier data interpretation of the correlations existing among the original variables and samples (Tauler, 2000). PCA is applied to the matrix of experimental data whose rows are the samples and whose columns are the variables or analyzed OMPs. The set of samples was subdivided in five different groups according to water type: WWTP effluents, river water, coastal water, river mouth seawater and port seawaters. Different data pretreatments were applied to the experimental data matrix like autoscaling (mean centring and dividing by their standard deviation)

Table 1
Specific codes, type of water sampled, location, coordinates and detailed description of each sampling point.

Code	Type	Location	Coordinates		Characteristics
			N	E	
C01	Coastal area seawaters	Tossa de Mar	41.719	2.934	Touristic and urban
C02		Blanes	41.651	2.778	Touristic and urban
C03		Calella de Mar	41.611	2.657	Touristic and urban
C04		Canet de Mar	41.588	2.587	Touristic and urban
C05		Arenys de Mar	41.574	2.542	Touristic, fishing
C06		Mataró	41.52	2.425	Textile industries
C07		Premiá de Mar	41.489	2.371	Touristic and urban
C08		El Masnou	41.475	2.313	Touristic and urban
C09		Badalona	41.441	2.247	Urban, industrial
C10		Casteldefells	41.264	1.957	Touristic and urban
C11		Vallcarca	41.239	1.862	Near an industrial facility
C12		Sitges	41.232	1.801	Touristic and urban
C13		Cubelles	41.199	1.677	Foix River mouth (dry in the sampled period)
C14		Cunit	41.196	1.655	Near a thermoelectrical facility
C15		Calafell	41.187	1.588	Touristic
C16		Tarragona	41.112	1.266	Urban, petrochemical, industrial
C17		Vandellos	40.958	0.88	Near nuclear energy facility
C18		Vinarós	40.477	0.485	Urban, touristic
C19		Peñíscola	40.356	0.403	Urban, touristic
C20		Benicassim	40.046	0.071	Urban, touristic
C21		Castellón de la Plana	40.002	0.033	Urban, touristic
C22		Palma de Mallorca	39.499	2.738	Paper factory
M01	River mouth seawaters	Muga River mouth	42.236	3.126	–
M02		Fluvia River mouth	42.202	3.112	–
M03		Ter River mouth	42.026	3.195	–
M04		Besos River mouth	41.418	2.234	–
M05		Llobregat River mouth	41.285	2.103	–
M06		Ebro River mouth	40.726	0.876	–
M07		Ebro River mouth	40.622	0.621	–
P01	Port seawater	Roses	42.254	3.177	Sportive port
P02		Palamós	41.843	3.125	Sportive port
P03		Blanes	41.673	2.798	Sportive port
P04		Arenys de Mar	41.577	2.56	Sportive port
P05		Mataró	41.528	2.445	Commercial and sportive port
P06		Premià	41.488	2.366	Sportive port
P07		El Masnou	41.475	2.307	Sportive port
P08		Barcelona	41.363	2.184	Commercial and sportive port
P09		Sitges	41.234	1.826	Sportive port
P10		Vilanova i la Geltru	41.213	1.733	Sportive port
P11		Torredembarra	41.134	1.402	Sportive port
P12		Tarragona	41.108	1.245	Commercial and sportive port
P13		Salou	41.091	1.222	Sportive port
P14		Sant Carles de la Ràpita	41.074	1.13	Commercial and sportive port
P15		Vinarós	40.614	0.599	Sportive port
P16		Peñíscola	40.463	0.476	Sportive port
P17		Castellón de la Plana	39.97	0.014	Commercial and sportive port

Code	Type	Location	Coordinates		Basin area (km^2)	Length (km)	Flow rate (m^3 d^{-1}) [a]
			N	E			
R01	River water	Muga River	42.246	3.104	853.8	58	846,720
R02		Fluvià River	42.187	3.086	973.8	84	447,552
R03		Ter River	42.035	3.125	3010	208	1,257,120
R04		Besòs River	41.437	2.21	1038.3	17.7	407,808
R05		Llobregat River	41.335	2.103	4948	175	1,442,880
R06		Ebro River	40.715	0.582	85,362	930	37,584,000

Code	Type	Location	Coordinates		Population equivalent [b,c]	Type of treatment [c,d]	Emissary (km) [c,e]	Flow rate (m^3 d^{-1}) [c,f]
			N	E				
W01	WWTP effluent	Roses	42.276	3.176	20,418	A, B, C	1.58	12,000
W02		Escala	42.03	3.158	11,355	A, B	1.3	6000
W03		Palamós	41.878	3.146	51,673	A, B, C	1.03	25,000
W04		Mataró	41.524	2.425	177,818	A, B	1.8	30,000
W05		Sant Adrià del Besòs	41.409	2.224	1,444,844	A, B	3.6	400,000
W06		Prat de Llobregat	41.308	2.13	1,092,573	A, B, C	3.2	300,000
W07		Tarragona	41.108	1.226	177,818	A, B	5.1	30,000
W08		Reus	41.127	1.118	108,595	A, B	0	18,000

[a] Mean value of fluxes at the river mouth (last 5 years). Data obtained from the Catalan Water Agency (2011a).
[b] In year 2009.
[c] Data obtained from Catalan Water Agency (2011b).
[d] A: Primary; B: Secondary; C: Tertiary.
[e] Length of the submarine emissary.
[f] Mean value of daily discharges from April 2008 to May 2009.

all the variables for all the five data groups together in the same data matrix; autoscaling the variables for each of the five groups separately and then joining them together to form a new data matrix; using the logarithm (in basis 10, $\log_{10}$) of all data values and then mean centring. In this particular study, the best combination of data pretreatments resulted to be the combination of $\log_{10}$ and mean centring, since it allowed for a better interpretation of the principal components and for a better separation of sample groups. Log_{10} pre-treatment adjusted the different scales of the measured variables (OMPs), and reduced the variation between small and large values; mean centring on the other hand, changed the data origin from zero to the mean value. In this way the contribution of the different variables to the total variance is made similar, regardless of their changes in the original scale of concentrations and of their average (offset) contribution. The remaining part gives information about what distinguished the different analyzed samples. Each principal component (PC) is interpreted by means of the loadings (linear combination of the original variables) which describe the composition of the possible contamination sources and by the coefficients or scores, whose numerical values give information about the contribution of these sources (described by the principal components) to the samples. Principal components are ordered sequentially according to the amount of explained data variance. PCA analysis was performed with the *MATLAB 7* (The Mathworks, Inc, Natick, MA, USA) computer environment and *PLS Toolbox 3.5* (Eigenvector Research Inc., Manson, WA, USA).

2.5. Mass flow of organic micropollutants to the sea

The eight WWTP discharges through submarine emissaries and the six rivers studied were previously identified as a source of pollution of perfluorochemicals to the NW Mediterranean coastal waters, (Sánchez-Avila et al., 2010a). To calculate the OMP mass flow to the sea, the concentrations detected in rivers and WWTP effluents (Table 2) were considered as well as the effluent discharges and river water flows (Table 1). The inputs to the sea were calculated according to:

$$Mass\,flow\left(g\,d^{-1}\right) = \left[Concentration\ of\ pollutants\left(g\,m^{-3}\right)\right] \times \left[flux\ of\ water\left(m^{3}\,d^{-1}\right)\right].$$

2.6. Seawater risk assessment

Seawater risk for the detected OMPs was assessed on the basis of the risk quotient (RQ or hazard quotient, HQ) on non target organisms, as described by several authors (Ginebreda et al., 2010; Sanderson et al., 2004; Santos et al., 2007; Vryzas et al., 2009). In order to cover different thropic levels of the aquatic ecosystems, RQs were estimated for three seawater organisms: fish, algae and mysid shrimp and were calculated from the measured environmental concentration (MEC) of each organic micropollutant. The chronic toxicity usually expressed as PNEC or NOEC was used for each compound. When PNEC neither NOEC were not reported in the literature, EC_{50} or LC_{50} values from standard ecotoxicological tests were used (in all cases, the lowest value reported was chosen to set up the worst case scenario), eventually after correction by an assessment factor. At present, there is no precise guidance on what assessment factors are appropriate, and these are generally applied on a case-by-case basis, taking into account the nature of the concern identified and the available data. In the cases where at least one short term assay at one trophic level is available, an assessment factor of 100 is used. When data from one long term assay with either fish or zooplankton is available, an assessment factor of 50 was used (Vryzas et al., 2009). In the particular case where EC_{50} or LC_{50} was not available from the literature, it was estimated using the software program ECOSAR (Ecological Structure Activity Relationships) from the U.S. Environmental Protection Agency. A detailed description of the ecotoxicological values for each pollutant used for PNEC calculation is presented in the Supplementary Material (Table S1). RQs obtained were characterized using the maximum probable risks for ecological effect guideline established by Marcus et al. (2010) as follows:

- $RQ < 1.0$, indicates no significant risk;
- $1.0 \leq RQ < 10$, indicates a small potential for adverse effects;
- $10 \leq RQ < 100$, indicates significant potential for adverse effects; and
- $RQ \geq 100$, indicates that adverse effects should be expected.

3. Results and discussion

3.1. Mass flow and pollution profile of organic micropollutants to the sea

The eight WWTP evaluated are the most important as regards to habitant-equivalent and all of them discharge directly to the sea via marine emissaries (Table 1). In Table 2, the concentration ranges from each pollutant and sampling site is shown. In Fig. 1 the profile of pollutants discharged (see columns, in percentage) and the estimated daily total OMP inputs (see dots graphic, in $g\,d^{-1}$) to the NW Mediterranean Sea by the 8 WWTP, is shown. Although total OMP concentrations in WWTP effluent were drastically larger than that from rivers, mass inputs resulted to be several fold lower than those calculated for rivers due to the lower flows. WWTP effluents from Sant Adrià del Besòs (W05) and Prat de Llobregat (W06) were the highest contributors to total organic micropollutant discharges with 3602 and 3981 $g\,d^{-1}$ (Fig. 1), respectively and correspond to plants that receive large amounts of industrial and urban discharges from the biggest cities from Catalonia. The eight WWTP studied discharge 8800 $g\,d^{-1}$ of ΣOMPs via submarine emissaries to the NW Mediterranean Sea. In WWTP effluents, APs were the major compounds detected, followed by plasticizers (PEs and BPA), PAHs and finally by OCPs. No PCBs and PBDEs were detected (Fig. 1). Even though the elimination of APs in the WWTPs usually is more than 95% (Céspedes et al., 2008; Loos et al., 2007), the results evidenced that alkylphenol-formulated products still are produced and used in industrial, domestic and agricultural formulations although Directive 2003/53/EC (European Parliament and Council, 2003) restricted their use in the EU at concentrations lower than 0.1%. The high concentrations of PEs and BPA found in WWTP effluents is a result of their large scale and widespread domestic and industrial use, including softeners in plastics, and additives in paints, glues and inks (Fromme et al., 2002). Although the reduction in the concentration of BPA and PEs in the WWTP systems is elevated (68–84%) (Céspedes et al., 2005; Sánchez-Avila et al., 2009), they undergo treatment due to the high solubility of those compounds and the high concentrations found in the influents (Mohapatra et al., 2010). BPA is an endocrine disrupting compound and is being considered for inclusion in Directive 2008/105/CE relating to environmental quality standards (European Parliament and Council, 2008). Compared to other studies, the low presence of PAHs in WWTP effluents from Catalonia was a consequence of the high capacity of biological treatments (Table 1) that can remove up to 98% of those compounds (Vogelsang et al., 2006). With respect to the detected OCPs (α-endsf and lindane), their presence in the WWTP effluents reflects that despite their prohibition in the European Union by Regulation (EC) 850/2004 (European Parliament and Council, 2004) they may be still in use, probably until stocks are exhausted (Martí et al., 2011).

Rivers are the main vehicle for the transport and mobilization of contaminants and are the main drivers of contaminants to the sea (Gómez-Gutiérrez et al., 2006; Sánchez-Avila et al., 2010a). Mediterranean rivers have strong seasonal regime and scarce flows (Table 1). They receive the input of treated or untreated sewage effluents and urban discharges and agricultural run-off, becoming a sink of contaminants. This fact can be observed if the fluxes of St. Adrià del Besòs and

Table 2
Summary data for the different water samples analyzed, indicating number of samples with concentration over the MDL, minimum, maximum and mean of target pollutants.

Pollutant Family	Compound	Coastal seawater (n = 22)				Port seawater (n = 17)				River mouth seawater (n = 7)				River water (n = 6)				WWTP effluent (n = 8)			
		Det.	Range		Mean	Det.	Range		Mean	Det.	Range		Mean	Det.	Range		Mean	Det.	Range		Mean
			Min	Max			Min	Max			Min	Max			Min	Max			Min	Max	
			ng L^{-1}	ng L^{-1}	ng L^{-1}		ng L^{-1}	ng L^{-1}	ng L^{-1}		ng L^{-1}	ng L^{-1}	ng L^{-1}		ng L^{-1}	ng L^{-1}	ng L^{-1}		ng L^{-1}	ng L^{-1}	ng L^{-1}
PAHs	Nap	4	1.4	60	19	16	0.8	66	7.4	–	bdl	bdl	–	6	0.9	13	4.1	8	56	227	151
	Acy	3	3.5	5.4	4.1	6	2.5	5.2	3.7	1	4.1	4.1	4.1	4	2.2	5.6	3.6	–	bdl	bdl	–
	Ace	1	3.4	3.4	3.4	4	1.9	2.8	2.3	–	bdl	bdl	–	1	2.3	2.3	2.3	6	170	261	187
	Flu	1	9.0	9.0	9.0	9	2.0	22	5.2	1	3.4	3.4	3.4	3	2.0	2.9	2.3	–	bdl	bdl	–
	Phe	9	0.7	2.7	1.3	12	0.7	3.4	1.7	5	0.8	2.4	1.5	5	0.8	2.8	1.4	8	16	31	22
	Ant	2	1.5	2.9	2.2	5	1.3	4.9	2.9	1	3.2	3.2	3.2	3	0.4	1.0	0.7	4	20	35	25
	Flr	2	0.9	1.4	1.1	16	1.1	17	3.6	–	bdl	bdl	–	6	1.3	2.1	1.6	–	bdl	bdl	–
	Pyr	3	0.7	1.3	1.1	15	1.5	18	3.3	3	0.7	0.8	0.8	6	1.5	2.5	1.9	6	128	134	131
	B(a)A	–	bdl	bdl	–	16	2.1	23	4.7	–	bdl	bdl	–	5	2.0	2.8	2.3	–	bdl	bdl	–
	Chr	5	0.8	1.5	1.1	16	1.7	20	4.3	1	0.8	0.8	0.8	5	1.7	2.9	2.2	–	bdl	bdl	–
	B(b)F	–	bdl	bdl	–	3	1.5	14	6.0	–	bdl	bdl	–	4	1.0	2.1	1.5	–	bdl	bdl	–
	B(k)F	–	bdl	bdl	–	2	1.3	10	5.9	–	bdl	bdl	–	4	0.8	2.4	1.4	–	bdl	bdl	–
	B(a)P	–	bdl	bdl	–	1	15	15	15	–	bdl	bdl	–	2	2.1	2.3	2.2	–	bdl	bdl	–
	I(cd)P	10	1.7	2.5	2.0	11	1.8	25	4.3	4	1.7	2.1	2.0	4	1.8	3.9	2.7	–	bdl	bdl	–
	D(ah)A	8	1.5	1.8	1.6	9	1.5	2.0	1.7	1	1.6	1.6	1.6	3	1.6	3.7	2.8	–	bdl	bdl	–
	B(ghi)P	–	bdl	bdl	–	12	1.2	20	3.2	–	bdl	bdl	–	3	1.2	3.2	2.3	–	bdl	bdl	–
APs	OP	6	1.4	13	4.7	16	2.7	198	20	2	33	145	89	6	3.6	41	13	8	255	825	395
	NP	12	1.2	153	52	16	25	689	237	2	3.1	73	38	6	16	114	61	8	594	2060	1021
	NP EO_1	15	1.9	108	34	16	6.5	275	64	5	1.3	194	49	6	4.5	36	18	7	125	1750	833
	NP EO_2	14	4.0	88	26	16	6.0	177	47	5	4.8	66	22	6	8.4	34	16	6	490	991	684
Plast.	BPA	20	2.3	102	18	17	5.3	350	57	7	3	77	22	6	8.4	64	20	7	380	1999	762
	DMP	2	2.8	142	73	5	3.9	12	6.9	1	5.2	5.2	5.2	2	2.5	5.5	4.0	–	bdl	bdl	–
	DEP	2	24	483	253	14	24	871	265	2	70	162	116	6	52	284	127	8	29	3206	1786
	DEHP	20	31	617	145	16	64	5965	762	7	22	209	92	6	122	4983	1193	8	1723	9223	3722
	BBP	19	1.3	104	19	16	3.5	804	128	7	3	75	16	6	4.8	23	11	–	bdl	bdl	–
	DEHA	11	2.1	304	48	16	3.0	271	94	3	47	93	73	6	1.9	149	35	–	bdl	bdl	–
OCPs	2,4′-DDE	–	bdl	bdl	–	–	bdl	bdl	–	–	bdl	bdl	–	1	1.0	1.0	1.0	–	bdl	bdl	–
	4,4′-DDE	–	bdl	bdl	–	–	bdl	bdl	–	–	bdl	bdl	–	2	1.6	5.7	3.6	–	bdl	bdl	–
	2,4′-DDD	–	bdl	bdl	–	–	bdl	bdl	–	–	bdl	bdl	–	3	1.4	8.6	4.0	–	bdl	bdl	–
	4,4′-DDD	–	bdl	bdl	–	–	bdl	bdl	–	1	2.3	2.3	2.3	3	2.1	39	21	–	bdl	bdl	–
	2,4′-DDT	–	bdl	bdl	–	–	bdl	bdl	–	–	bdl	bdl	–	1	31	31	31	–	bdl	bdl	–
	4,4′-DDT	–	bdl	bdl	–	–	bdl	bdl	–	–	bdl	bdl	–	1	13	13	13	–	bdl	bdl	–
	α-HCH	–	bdl	bdl	–	–	bdl	bdl	–	2	0.5	0.9	0.7	–	bdl	bdl	–	–	bdl	bdl	–
	Lindane	–	bdl	bdl	–	1	1.4	1.4	1.4	2	4.5	16	10	4	1.4	32	9.6	2	115	197	156
	α-Endsf	–	bdl	bdl	–	2	2.9	3.0	2.9	1	3.0	3.0	3.0	–	bdl	bdl	–	5	71	326	201
	β-Endsf	–	bdl	bdl	–	1	14	14	14	–	bdl	bdl	–	–	bdl	bdl	–	–	bdl	bdl	–
PCBs	PCB 28	2	0.2	0.3	0.2	2	0.1	0.2	0.2	1	0.2	0.2	0.2	4	1.3	19	7.4	–	bdl	bdl	–
	PCB 52	6	0.9	3.2	1.8	10	0.8	30	4.9	5	1.0	2.0	1.5	4	1.0	4.8	3.5	–	bdl	bdl	–
	PCB 101	–	bdl	bdl	–	5	0.7	1.0	0.8	1	1.0	1.0	1.0	4	0.7	6.7	4.6	–	bdl	bdl	–
	PCB 118	1	1.1	1.1	1.1	2	1.2	1.5	1.3	1	1.1	1.1	1.1	3	4.2	6.4	5.2	–	bdl	bdl	–
	PCB 138	–	bdl	bdl	–	1	1.3	1.3	1.3	–	bdl	bdl	–	3	2.1	6.0	3.8	–	bdl	bdl	–
	PCB 153	–	bdl	bdl	–	2	1.6	1.6	1.6	–	bdl	bdl	–	3	3.5	6.8	4.8	–	bdl	bdl	–
	PCB 180	–	bdl	bdl	–	–	bdl	bdl	–	–	bdl	bdl	–	2	2.5	6.0	4.2	–	bdl	bdl	–
PBDEs	BDE 28	–	bdl	bdl	–	1	19	19	19	–	bdl	bdl	–	2	1.7	3.1	2.4	1	59	59	59
	BDE 47	–	bdl	bdl	–	1	4.2	4.2	4.2	–	bdl	bdl	–	1	1.1	1.1	1.1	–	bdl	bdl	–
	BDE 99	–	bdl	bdl	–	–	bdl	bdl	–	–	bdl	bdl	–	1	0.6	0.6	0.6	–	bdl	bdl	–

bdl.: Below the MDL.
β-HCH, δ-HCH, BDE 100, BDE 153 and BDE 154 were not detected.

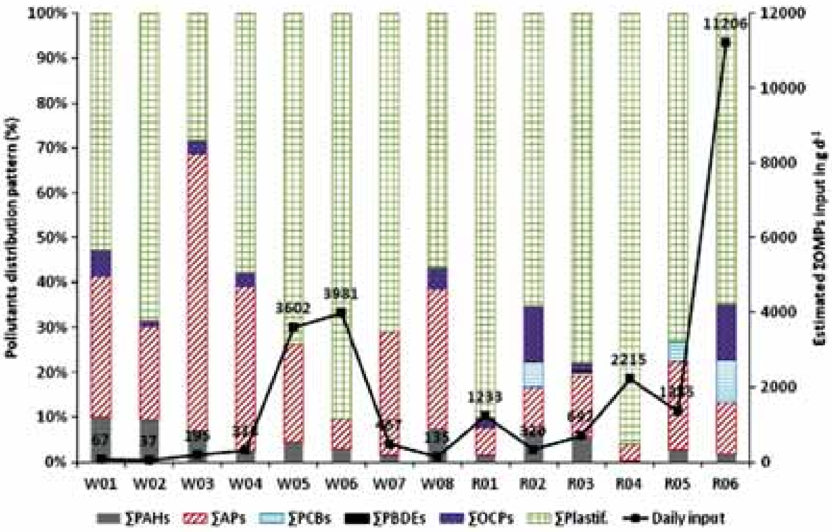

Fig. 1. Estimated daily total OMP inputs to the NW Mediterranean Sea (right axis, in g d^{-1}) and pollutants distribution pattern discharged (left axis, in percentage).

Prat de Llobregat WWTP are compared with Besòs and Llobregat river flows (Table 1). As a result, rivers were the main cause of OMP discharge to the sea. In Fig. 1 the profile of pollutants discharged and the ΣOMP input by the 6 principal rivers converging to the Catalan Sea is shown. In the case of the Ebro River (R06), although the concentration of ΣOMPs was the lowest (see Table 2, 298 ng L^{-1}), its contribution to the marine environment was the highest (11,206 g d^{-1}) due to its much higher flow (Fig. 1). The six Catalan rivers studied herein represent a ΣOMP discharge of 17,030 g d^{-1}. This continuous discharge of pollutants represents a risk to the marine environment, especially from pollutants with endocrine disruption effects and bioaccumulative potential. APs and BPA were the most ubiquitous compounds followed by PEs, PAHs, PCBs, OCPs and finally PBDEs. The presence of APs and PEs in rivers is caused principally by WWTP discharges, industrial activities and also run off where water easily elutes and swept physically bound plasticisers to rivers (Fromme et al., 2002). PAHs could reach rivers principally from urban run-off, municipal and industrial effluents, oil spillage or leakage and atmospheric deposition (Patrolecco et al., 2010). The major current sources of PCBs to rivers include sewage treatment works, and combined sanitary overflows, although re-suspension of contaminated bed sediments and direct urban runoff may contribute to the PCB load to rivers (Meharg et al., 2003; Pham and Proulx, 1997). The high presence of DDE and DDD compared to DDT in Fluvià and Ebro River (ratio DDE + DDD/DDT > 1) evidenced a historic use of this pesticide, as evidenced in previous studies (Lacorte et al., 2006).

The comparison of our results with other previously reported is rather difficult because the same target pollutants were not analyzed and the total discharges are not analogous. However, Ahel et al. (1994) reported ΣAP discharges of 3.8 and 12,000 g d^{-1} from 2 WWTP from Germany; Pham and Proulx (1997) estimated 703 g d^{-1} of ΣPAHs + PCBs discharging from a Canadian WWTP. Dargnat et al. (2009) reported ΣPEs fluxes from 68 to 161 g d^{-1}. A previous study, reports a total discharge from Mataró's WWTP (W04) of 864 g d^{-1}, considering similar target pollutants, slightly higher than the results herein presented. In comparison with our results, Rhône River (flux of 1.7–4.8 × 10^{+08} m^3 d^{-1}) discharges approximately 38,000 g d^{-1} only for ΣPAHs (Sicre et al., 2008).

3.2. Occurrence of organic micropollutants in seawater

The Catalan coast is characterized by the settlement of intensive industry in special chemical and petrochemical facilities and also receives run-off from agricultural activities (fruits, vegetables, greenhouse, and wines, among others) and urban treated or untreated discharges. These activities contribute to coastal contamination through direct or indirect discharges. The Catalan Water Agency (*"Agencia Catalana de l'Aigua"*) is the administrative body in charge of monitoring seawater quality along the Catalan coast. In a monitoring study carried out through 2007–2010 (Table 3), PAHs, PCBs, PBDEs DEHP and NP were identified at concentrations of ng L^{-1} levels in 9 sites (Catalan Water Agency, 2011c).

The 46 seawater samples analyzed were categorized in 3 groups depending on the sampling type: seawater sampled in ports (P01–P17); seawater sampled in a river mouth (M01- M07) and finally seawater sampled in coastal areas (C01–C22) (Table 1). The OMP concentrations in the 46 seawater samples are shown in Table 2. BPA was the most ubiquitous compound followed by PEs, APs, PAHs, PCBs, OCPs and finally PBDEs.

Plasticizers were detected in all 46 seawater samples. Single PE and BPA concentrations (see Table 2) ranged from 1.3 to 5965 ng L^{-1} (DEHP > DEP > BBP > BPA > DEHA > DMP), being BPA and DEHP the most ubiquitous compounds. ΣPEs ranged from 4.5 (Vinarós C18) to 7393 ng L^{-1} (Barcelona Port, P08) and were significantly different ($p = 0.02 < 0.05$) among the sampling sites, indicating geographical differences (river mouths < coastal areas < ports). Similar ΣPE concentrations were detected in the Spanish coastal areas and ports from Basque Country (199–2638 ng L^{-1}) and higher values were reported in Valencia (250–20,000 ng L^{-1}) (Martí et al., 2011). In open waters from the North Sea and the coastal area of the Kiel Bight, low ΣPEs were found (60–206 ng L^{-1}) (Ehrhardt and Derenbach, 1980; Xie et al., 2005). No studies were found reporting BPA concentrations in Spanish seawaters. In Halifax Harbour, Canada, concentrations of ~2.6 ng L^{-1} were found (Robinson et al., 2009) and similar concentrations to this study were reported from Singapore coastal seawater (40–190 ng L^{-1}) (Basheer and Lee, 2004). Plasticizers (BPA and PEs) are leached to aquatic environment via rivers and urban runoff. Also, plastics are dumped directly to the ocean through fishery and maritime activities (Hirai et al., 2011). The highest values detected in ports were a consequence of the intensive use and discharges and spills of plastic-based products.

APs were detected in 39 seawater samples. Single AP concentrations (see Table 2) ranged from 1.2 to 689 ng L^{-1} (NP > $NPEO_1$ > OP > $NPEO_2$), being $NPEO_1$ the most ubiquitous compound. ΣAPs were significantly different ($p = 0.0002 < 0.05$) among the 3 seawater types (river mouth < coastal areas < ports), ranging from 2.9 (Calella de Mar, C03) to 757 ng L^{-1} (Mataró Port, P05). Similar ΣAPs were

Table 3
Historical data of some OMPs in the NW Mediterranean Sea (concentrations in ng L^{-1}).

Sampling point	Sampling period (n)	ΣPAH range (Average)	DEHP range (Average)	NP range (Average)	ΣOCPs range (Average)	ΣBDE range (Average)	Source
Arenys de Mar	2007–2009 (4)	<0.5–9.1 (9.1)	148–239 (193)	<1–9.1 (9.7)	<0.2–1.3 (0.6)	<0.01–0.3 (0.3)	Catalan Water Agency (2011c)
(C05)	2009 (1)	12.7	171	98	bdl	bdl	This work
Blanes	2010 (3)	<0.5	429–1015 (744)	<1	<0.2–0.2 (0.2)	<0.01–0.1 (0.1)	Catalan Water Agency (2011c)
(C02)	2009 (1)	30	527	258	1.4	bdl	This work
Besos river mouth (M04)	2007–2010 (28)	<0.5–35 (10)	167–12,835 (1913)	<1–409 (76)	<0.2–8.1 (1.4)	<0.01–1.1 (0.4)	Catalan Water Agency (2011c)
	2009 (1)	5.3	126	bdl	17	bdl	This work
Barcelona Port	2007–2010 (32)	<0.5–261 (22)	140 –1237 (462)	<1–719 (169)	<0.2 –2.0 (0.4)	<0.01–0.8 (0.3)	Catalan Water Agency (2011c)
(P08)	2009 (1)	694	216	5965	bdl	23	This work
Llobregat river mouth (M05)	2007–2010 (45)	<0.5–35 (16)	<120–32,474 (2935)	<1–158 (60)	<0.2–6.8 (0.7)	<0.01–6.4 (0.5)	Catalan Water Agency (2011c)
	2009 (1)	2.1	50	bdl	bdl	bdl	This work
Tarragona Port	2007–2010 (22)	<0.5–35 (14)	348–13,748 (2045)	<1–56 (29)	<0.2–0.5 (0.1)	<0.01–1.0 (0.4)	Catalan Water Agency (2011c)
(P12)	2009 (1)	30	703	144	bdl	bdl	This work
Salou Port	2007–2009 (4)	<0.5	<120–303 (303)	<1	<0.2–0.4 (0.2)	<0.01–0.4 (0.3)	Catalan Water Agency (2011c)
(P13)	2009 (1)	25	165	192	bdl	bdl	This work
Ebro river mouth (M06)	2007–2010 (42)	<0.5–8.3 (2.3)	127–11,051 (852)	<1–108 (62)	<0.2–16 (2.0)	<0.01–0.9 (0.3)	Catalan Water Agency (2011c)
	2009 (1)	2.5	209	3.1	bdl	bdl	This work
Ebro river mouth (M07)	2007–2010 (53)	<0.5–70 (35)	136–32,855 (2284)	<1–102 (62)	<0.2–1.9 (0.3)	<0.01–0.7 (0.3)	Catalan Water Agency (2011c)
	2009 (1)	0.9	61	73	bdl	bdl	This work

n = Number of samples.
bdl = below detection limit.

detected in the Spanish coastal area from the Basque Country's (74–717 ng L^{-1}) (Prieto et al., 2007). High ΣAPs were found in the Israeli's Mediterranean coastal waters (900–2600 ng L^{-1}) (Zoller, 2006; Zoller et al., 2004), in Dutch estuaries (40–1963 ng L^{-1}) (Jonkers et al., 2003) and in Thessaloniki's coastal areas and harbor (489–2097 ng L^{-1}) (Arditsoglou and Voutsa, 2008). The AP presence in seawater is attributed principally to direct discharges of WWTP and rivers (Céspedes et al., 2006) and other diffuse sources such as the use of detergents, and other alkylphenol-based products in maritime activities (Soares et al., 2008).

PAHs were detected in 39 seawater samples. Single PAH concentrations (see Table 2) ranged from 0.7 to 65 ng L^{-1} (Nap > I(cd)P > B(a)A > Flu > B(ghi)P > Chr > Pyr > Flr > B(a)P > B(b)F > B(k)F > Acy > Ant > Phe > Ace > D(ah)A), being Phe the most ubiquitous compound. ΣPAHs were significantly different ($p = 0.04 < 0.05$) among the 3 seawater sample types (river mouths < coastal areas < ports) ranging from 0.9 (Ebro River mouth, M07) to 178 ng L^{-1} (Barcelona Port, P08). Higher ΣPAHs were reported in the Spanish coast from the Basque Country (215–246 ng L^{-1}) (Prieto et al., 2007) and in the Cadiz Bay (476–1939 - ng L^{-1}) (Pérez-Carrera et al., 2007). Similar ΣPAHs were reported in the Greek Mediterranean sea (113 to 459 ng L^{-1}) (Valavanidis et al., 2008). The presence of PAHs in seawater is attributed to petroleum contamination (spillage of oil from ships, maritime transport accidents), fallout from air pollution (combustion of fuels), and terrestrial runoff (municipal sewage discharges, industrial waste water) (Valavanidis et al., 2008).

PCBs were detected in 22 seawater samples. Single PCB concentrations (see Table 2) ranged from 0.1 to 30 ng L^{-1} (PCB 52 > PCB 153 > PCB 118 > PCB 138 > PCB 101 > PCB 28), being PCB 52 the most ubiquitous compound. PCB 180 was not detected. ΣPCBs were significantly similar ($p = 0.1 < 0.05$) among the 3 seawater sample types, ranging from 0.8 (Blanes, P03) to 30 ng L^{-1} (Barcelona, P08). More than 100-fold ΣPCB concentrations (3884–4314 ng L^{-1}) were detected in Basque Country's coastal areas (Spain) (Prieto et al., 2007). Similar ΣPCB concentrations were found in the Gulf of Mexico (0.07–3.4 ng L^{-1}) (Carvalho et al., 2009) and in Singapore coastal areas (0.05–1.80 ng L^{-1}) (Wurl and Obbard, 2005). The waste disposal of electrical transformers, oil spillage, and any historical use of PCB containing products is reported as potential sources of PCBs in marine environments (Wurl and Obbard, 2005).

OCPs were detected in 6 samples. OCP concentrations (see Table 2) varied from 0.5 to 16 ng L^{-1} (Lindane > β-endsf > α-endsf > 4,4′-DDD > α-HCH), being lindane and α-endsf the most ubiquitous compounds. ΣOCP concentrations were significantly different ($p = 0.04 < 0.05$) among sampling sites (ports < river mouths), ranging from 1.4 (Blanes Port, P03) to 17 ng L^{-1} (Besòs River mouth, M04) indicating rivers and ports as a source of OCPs to coastal areas. These values are somewhat lower than those reported by Catalan Water Agency (Table 3) (mean ΣOCPs of 0.1–0.7 ng L^{-1}). In Spain, similar OCP values including HCHs, DDT and endsf were found in Valencia coastal areas (1.9–8.3 ng L^{-1}) (Martí et al., 2011) and in Cadiz Bay (3–11 ng L^{-1}) (Pérez-Carrera et al., 2007). Lower OCP levels were found in coastal waters from Singapore (0.01–0.63 ng L^{-1}) (Wurl and Obbard, 2005) and the Gulf of México (0.07–0.6 ng L^{-1}) (Carvalho et al., 2009). DDTs, endsf and lindane have been widely used in Spain, and their traces are still detected in many environmental matrices (Hildebrandt et al., 2008; Lacorte et al., 2006).

PBDEs (BDE 28 and 47) were only detected in one port sample (Barcelona, P08) with ΣPBDE concentration of 23 ng L^{-1}, higher than mean ΣPBDE values reported by the Catalan Water Agency (Table 3) in coastal waters (0.1–0.5 ng L^{-1}) and lower than reported in Basque Country's coastal areas (92–106 ng L^{-1}) (Prieto et al., 2008). PBDEs, are used mainly as flame retardants in furniture, electronic devices, including TV sets and personal computers, automotive parts, textiles and thermoplastics, etc. and can be released during their production, use and disposal and reach the marine environment (Prieto et al., 2008).

To summarize, the ΣOMPs were significantly different ($p = 0.03 < 0.05$) among sampling sites, being the highest in ports, ranging from 83 (Palamòs, P02) to 8443 ng L^{-1} (Barcelona, P08), followed by coastal areas, ranging from 17 (Vinarós, C18) to 1036 ng L^{-1} (Palma de Mallorca, C22) and the lowest values were detected in river mouths, ranging from 160 (Fluvià River mouth, M02) to 607 ng L^{-1} (Besòs River mouth, M04). Barcelona port is among the biggest port in the Mediterranean Sea, and the daily commercial and tourist ship activities could explain the high OMP values detected. In C22 (Palma de Mallorca), the OMP presence was related to paper and textile factories and urban discharges near the sampling site. And finally, according to the results reported in Section 3.2, elevated OMP concentrations were found in Besòs River mouth because it is one of the most polluted rivers since it flows through a very industrialized and urbanized area.

Table 3 reports a 3 year sampling results (2007–2010) for some legislated OMPs, realized by Catalan Water Agency. Monitoring campaigns were performed only for the legislated pollutants with the aim to accomplish the Water Framework (European Parliament and Council, 2000). In 9 coastal areas monitored by the Catalan Water Agency, DEHP was the most ubiquitous compound (detected on 91% of the samples), followed by OCPs (43%), PBDEs (30%), NP (24%) and

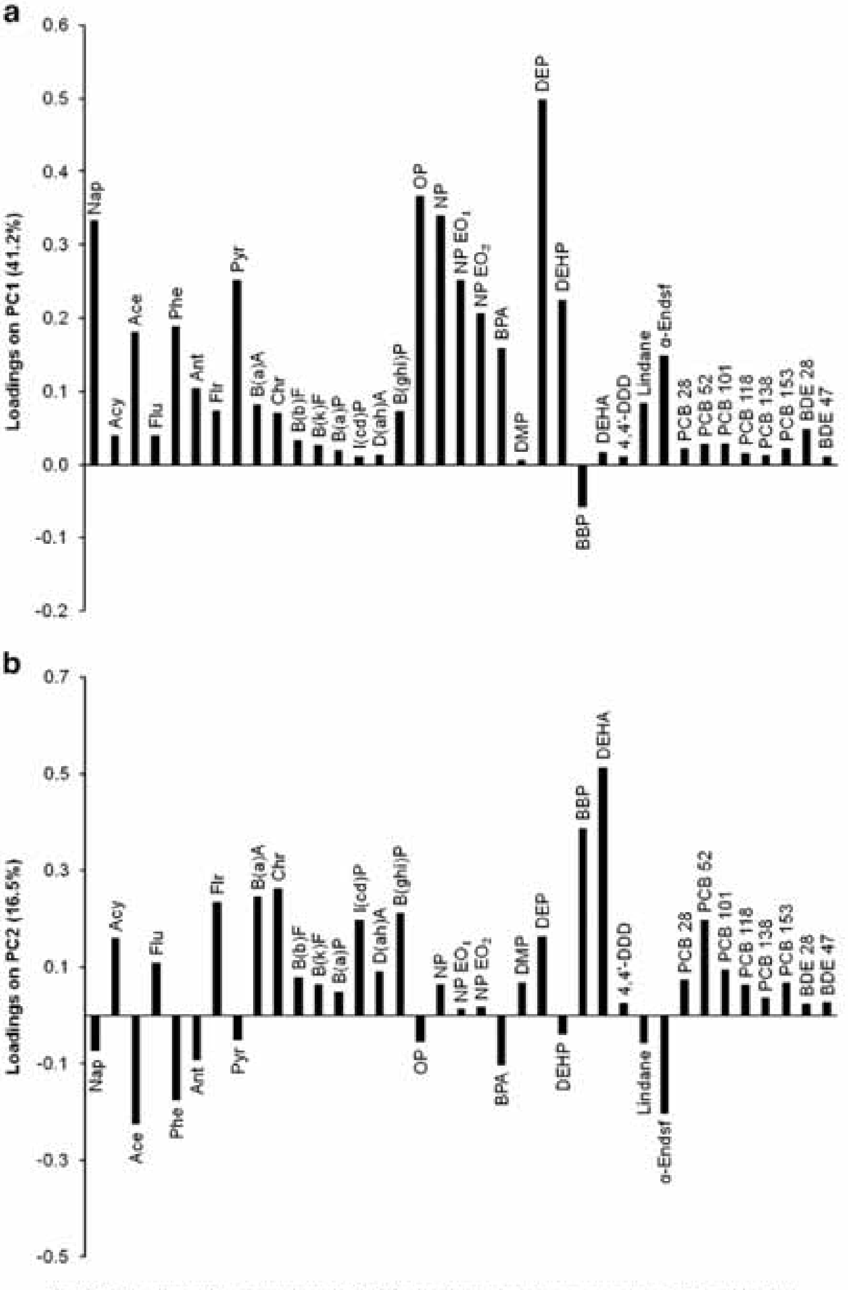

Fig. 2. PCA loadings of the investigated OMPs for the four principal components: a) PC1 and b) PC2.

finally PAHs (6%). DEHP ranged from <120 to 32,855 ng L^{-1}; NP, from <1 to 719 ng L^{-1}; ΣPAH range from <0.5 to 261 ng L^{-1}; ΣOCPs, from <0.2 to 16 ng L^{-1} and ΣPBDEs, from <0.01 to 6.0 ng L^{-1}. Although our results were obtained from one of the sampling campaigns performed in "the worst case scenario" conditions, there exists a concordance with the data reported by the Catalan Water Agency (2011c). Even seawater is in constant move, only few variations between results (specially in PAHs) were observed. This shows that the results reported herein were representative of the state of Catalan coast, and were not a result of a sporadic pollution episode.

3.3. Sources and distribution of OMPs according to Principal Component Analysis

PCA analysis was used to provide a summarized and graphical overview of the main possible sources of OMPs in NW Mediterranean sea coastal waters. The main goal of this study was to elucidate similarities and differences between the studied water bodies with respect to the main OMP sources. Among the 51 pollutants analyzed, 37 were finally considered for PCA. Values such as "below the detection limit" were set to half their limit. Compounds not detected and with only one apparition, were discarded.

PCA of the $\log_{10}$ and mean centered pre-treated data matrix explained 69.8% of the variance using 4 PCs.

Component 1 (PC1) explained 41.2% of the total variance, and described a generalized background contamination source loaded by all investigated OMP (Fig. 2a), with special high contribution of DEP, OP, NP, Nap, Pyr, $NPEO_1$, $NPEO_2$, DEHP and BPA. This profile provides evidence on the prevalence of plasticizers and alkylphenols in WWTP effluents and rivers as main contamination source in the geographical region under study, as identified in a previous study (Sánchez-Avila et al., 2009).

Component 2 (PC2) explained 16.5% of the total variance, and it describes the contrast between different type of combustion contamination sources (Fig. 2b). On one of them, the contribution of DEHA, BBP, DEP and some heavy PAHs as Flr, B(a)A, I(cd)P, B(ghi)P is specially significant (high positive loadings). DEHA, BBP and DEP are plasticizers used in the manufacture of PVC and other plastics and are among the most predominant phthalates in household waste and landfill (Huang et al., 2008). On the other hand, the presence of high molecular PAHs is related to vehicle emissions and industrial pyrolysis (Manoli and Samara, 1996). The other contamination source derives from low molecular PAHs (Ace, Phe, Nap, Ant and Pyr), generally related with petroleum-derived residues or coal combustion and wood stoves. α-endsf and lindane were also detected with relatively large negative loadings in PC2 (Fig. 2b), together also with OP, BPA and DEHP. Therefore, this second PC helps to distinguish samples with high contribution of BBP and DEHA and between high and low molecular PAHs (including also lindane and α-endsf).

Component 3 (PC3) explained 6.9% of the total variance, and it is significantly loaded by the contribution of DEP (especially on some specific samples, like sample C16, in Tarragona). PC3 has also relatively large negative loadings for $NPEO_1$, $NPEO_2$, OP, DEHA, BBP, I(cd)P and BPA, which describes a different possible source of these compounds compared to DEP. Finally, component 4 (PC4) explained only 5.2% of the total variance, and it describes a contamination pattern with relatively higher loadings for OCPs and PCBs. Although PCBs are banned since 1985 (European Environment Agency, 2011), they still remain in sediments and water samples (Moret et al., 2005) due to their historic use in PCB-containing products. This forth component loading profile is interpreted as "historical past pollution" source from substances whose use is banned at present.

Fig. 3 shows PC1 vs. PC2 score plot. In this figure, three sample groups are distinguished. A first group (bottom right, in red) includes only WWTP effluent samples, which according to PCA loadings, contains a generalized high pollution level, with high contributions of APs, PEs and BPA, resulting from industrial and urban discharges (positive PC1 loadings), and with low molecular PAHs (negative PC2 loadings) too. In a second group (upper, right) river and port seawater samples and sample C16 (Tarragona) were grouped, evidencing again a generalized high pollution level containing APs and plasticizers. These sites are again impacted by industrial and urban discharges (towards the right of the plot, with higher positive PC1 loadings) and also receiving a high influence from heavy PAHs released from ships and boats and industrial processes (towards the upper part of the plot, with higher PC2 loadings). In a third group, coastal (except C16, Tarragona) and river mouth seawater samples were grouped, in which general pollution had low impact (low negative PC1 loadings) but with some samples containing OCPs and light PAHs (negative PC2 loadings), compounds that are present at threshold concentrations either from direct inputs although they can also be transported by the air (Cardellicchio et al., 2007). PC1 vs PC2 score plot evidences therefore the differences between coastal and river mouth seawater samples on one side (on the left of the plot) and more contaminated port, river and WWTP samples on the other side (on the right of the plot).

3.4. Environmental risk assessment of the NE Spain coast's seawater

Analytical measurements of the concentrations of organic pollutants in the marine environment per se is of limited use, unless such data can be clearly related to their potential biological and ecological effects via a risk assessment-based approach (Wu et al., 2008). In order to estimate the potential adverse effects of the OMP levels detected in this monitoring on seawater environment, Technical Guidance Document on Risk Assessment (TGD) (European Chemicals Bureau, 2003) requires the assessment of three representative trophic levels of the ecosystem. In our case, three seawater organisms were chosen: algae, mysid shrimps and fish. The chronic toxicity values reported in bibliography depend on the type of toxicity test conducted, the duration of the exposure test, the level of organization considered (community-level vs individual responses) and, particularly, to on-site-specific environmental factors (Gómez-Gutiérrez et al., 2007a). NOEC was available for some compounds and when not, EC_{50} or LC_{50} with an assessment factor of 50 or 100 was used (Table S1). In the case that more than one value was found, the lowest value was the one taken in consideration to set up the worst scenario possible (García-Galán et al., 2011) and also by considering that the monitoring data were obtained in low flow river conditions when the pollutants arrive to the sea less diluted. When no chronic toxicity values were available, EC_{50} was estimated by the software program ECOSAR (US

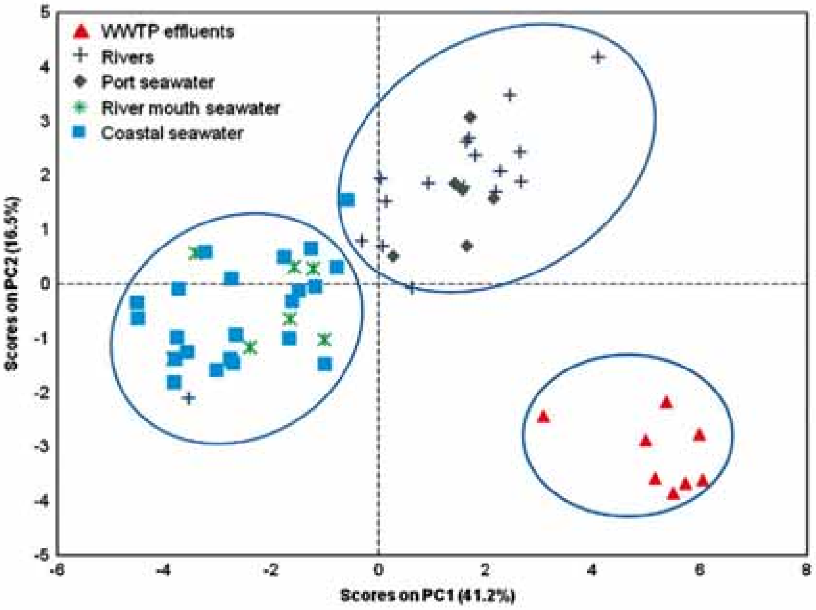

Fig. 3. PCA scores of the different investigated samples: PC1 vs PC2; (circles indicate possible sample groups according to their origin).

Environmental Proteccion Agency, 2011) and divided by an assessment factor of 1000 (Sanderson et al., 2003). In this work, RQ values were calculated for the sampled NW Mediterranean coastal waters. Joint effects of such mixtures, in particular of mixtures of chemicals with a similar mode of action, are often concentration additive (Van Loon et al., 1997). Therefore, since more than one compound were present, their possible joint effects were considered (Ginebreda et al., 2010; Santos et al., 2007) and finally a sum of individual RQs was obtained per sample site. RQ results for the three evaluated organisms are illustrated in Fig. 4 (for details, see Table S1 in supplementary material). From the 46 seawaters evaluated, no risk was found for fish and algae in 38 sites. Small potential adverse effects were found (1 ≤ RQ < 10) for fish in six sites and for algae in eight sites. Significant potential adverse effects for fish were obtained for Barcelona Port, P08 (Fig. 4a).

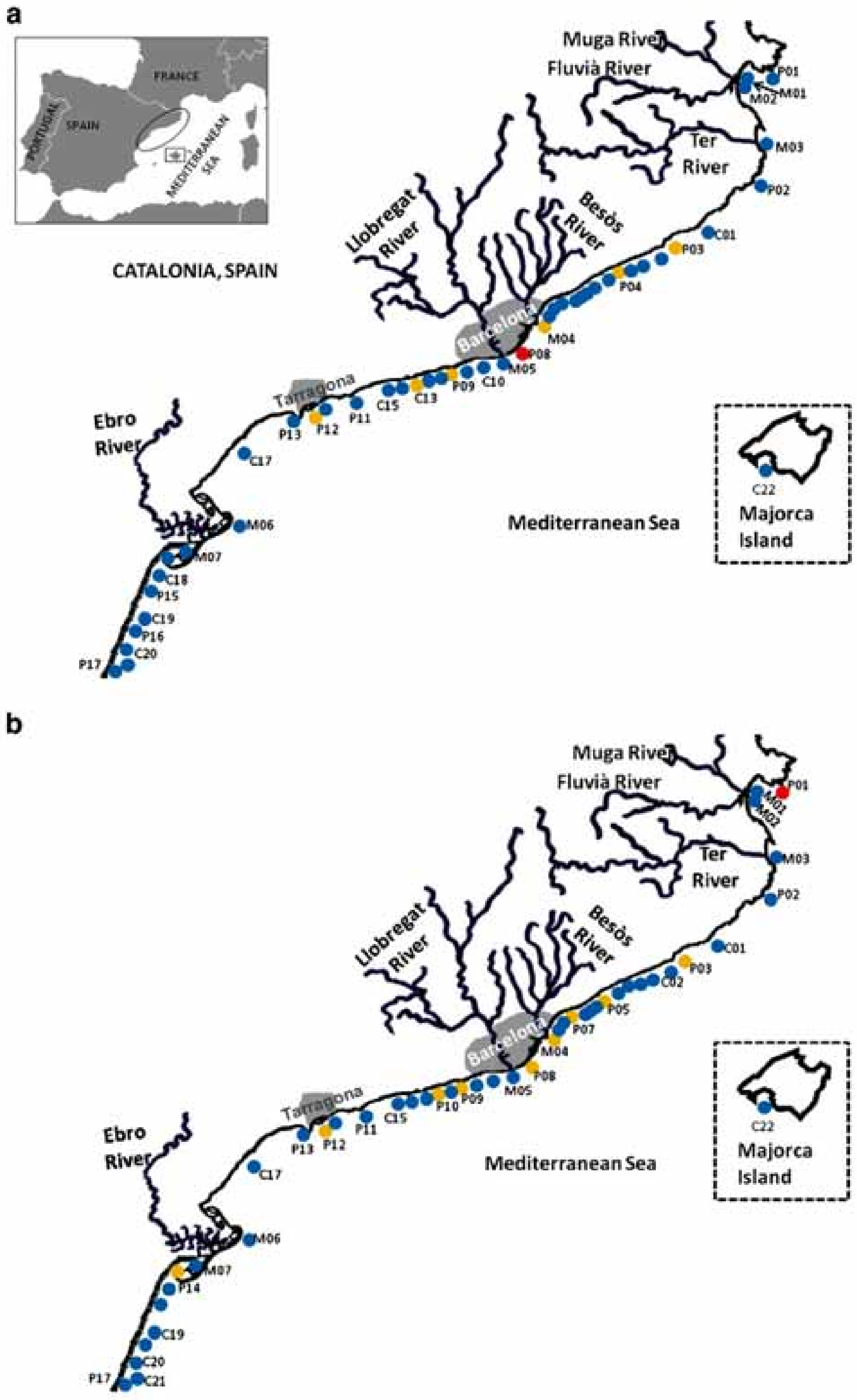

Fig. 4. RQs estimated for three seawater organisms in the NE Spanish Coast, considering the impact of the OMPs detected: a) Fish; b) algae; c) Mysid shrimp.

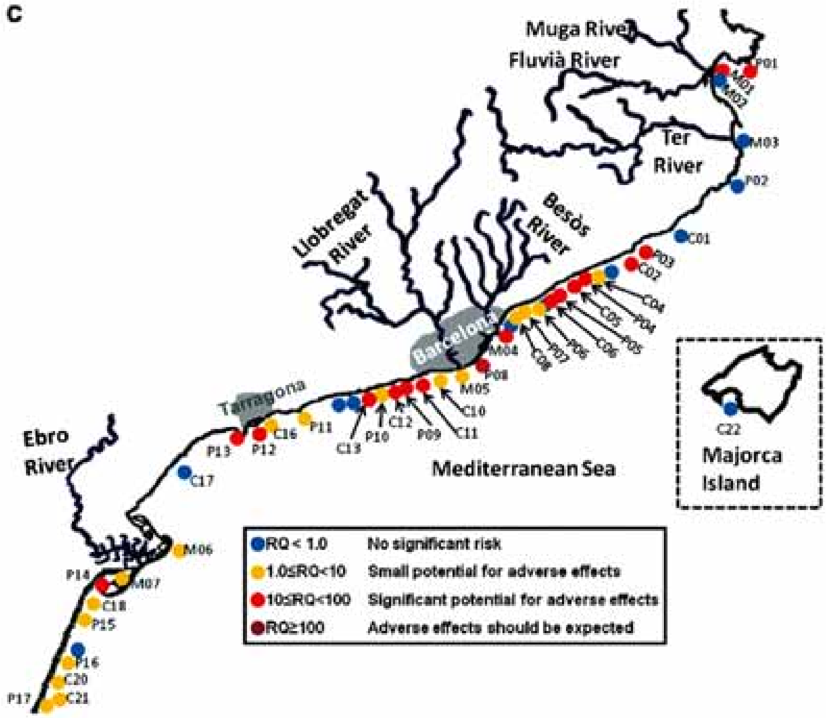

Fig. 4 (*continued*).

Among the organisms evaluated, results evidenced that in the NW Mediterranean Coast, mysid shrimp is the most sensitive organisms affected by OMP. In 24% of the seawater samples, no significant risk was estimated (Fig. 4c); in 39% of the samples, small potential adverse effects were found; in 35% of the samples, significant potential for adverse effects and in only one sample from Barcelona Port (P08), clear adverse effects may occur. Although the major risk would be expected for the most polluted site, C22 (Palma de Majorca), its RQ value was below 1 for the three evaluated organisms. This is a consequence of the fact that RQ calculation involves the toxicological effect of a substance on a particular specie (according to its physico-chemical and toxicological properties) and the specific synergies with other OMPs. Whereas some chemicals cause toxicological effects already at very low concentrations, others do not. In the case of sample C22, its pollution is mostly produced by high concentrations of phthalates and alkylphenols, which are much lower than their chronic toxicity values at the mg L^{-1} level (more than 2-fold higher than the actual experimentally detected concentrations). On the other side, samples with traces of PCBs and PBDEs (with chronic toxicity values in ng-mg L^{-1} levels), gave RQ values generally >1 (like P08, P09, C11 and C16 samples). The results obtained agree with a previously reported study where Barcelona area presented the highest risk in the NW Mediterranean region, considering PCBs and DDTs in sediments core samples (Gómez-Gutiérrez et al., 2007a). Fig. 4 shows the pollution risk distribution over the Catalan coast and these results can be then used as a tool for environmental policies implementation at a regional scale. It is necessary further on-site toxicological data to clarify the degree in which adverse effects towards the marine ecological community will be effectively produced in the studied zones and if synergistic effects among pollutants can occur (Graumann et al., 1999). Consequently, the RQ values calculated in this study are intended for management purposes and as benchmark levels for identifying the areas of the NW Mediterranean that can be of special concern in relation to seawater contamination by OMPs.

4. Conclusions

This study was intended to determine the impact of 51 OMPs in NW Mediterranean Coastal water samples and to identify their pollution sources. 49 OMPs were detected in ports, coastal areas and river mouth seawater samples. The most ubiquitous compounds were bisphenol A (96% of the samples), di-2-ethylhexyl phthalate (93%) and butyl benzyl phthalate (91%). Rivers and WWTP effluents discharging to the sea were identified as the main sources of OMPs to coastal seawater, although runoff and direct discharge of untreated urban or industrial waters to the NW Mediterranean Sea cannot be neglected. Since not enough precise data for the later was available, this contribution was not considered in the total final balance. The estimated loading of OMPs from the obtained concentrations to the NW Mediterranean Sea, via the 6 rivers and 8 WWTP analyzed was of $25{,}800\ \text{g d}^{-1}$. Ebro river being the major contributor due to its high water flux. Principal Component Analysis allowed the summarized description of the main contamination sources on the investigated samples and their differentiation according to the sample origin and geographical distribution. According to these different contamination sources, three main sample groups were distinguished, one group of WWTP effluents containing the highest levels of contaminants, another one with river and port seawater samples, and finally, a third one with coastal and river mouth seawater samples which were the least polluted. Environmental risk assessment indicates that concentrations detected in the seawater samples represent a potential risk for sensitive marine organisms like the mysid shrimp. The need for coastal water monitoring programs to be implemented in the area for a better management and control of the OMP discharges to coastal seawater, with the final goal of minimizing their negative long term effects on the environment is confirmed. Finally, it should be mentioned that the Mediterranean Sea is an enclosed basin highly affected by industrial and urban discharges of 22 countries, and thus, the uncontrolled discharge can become a serious problem in the long term.

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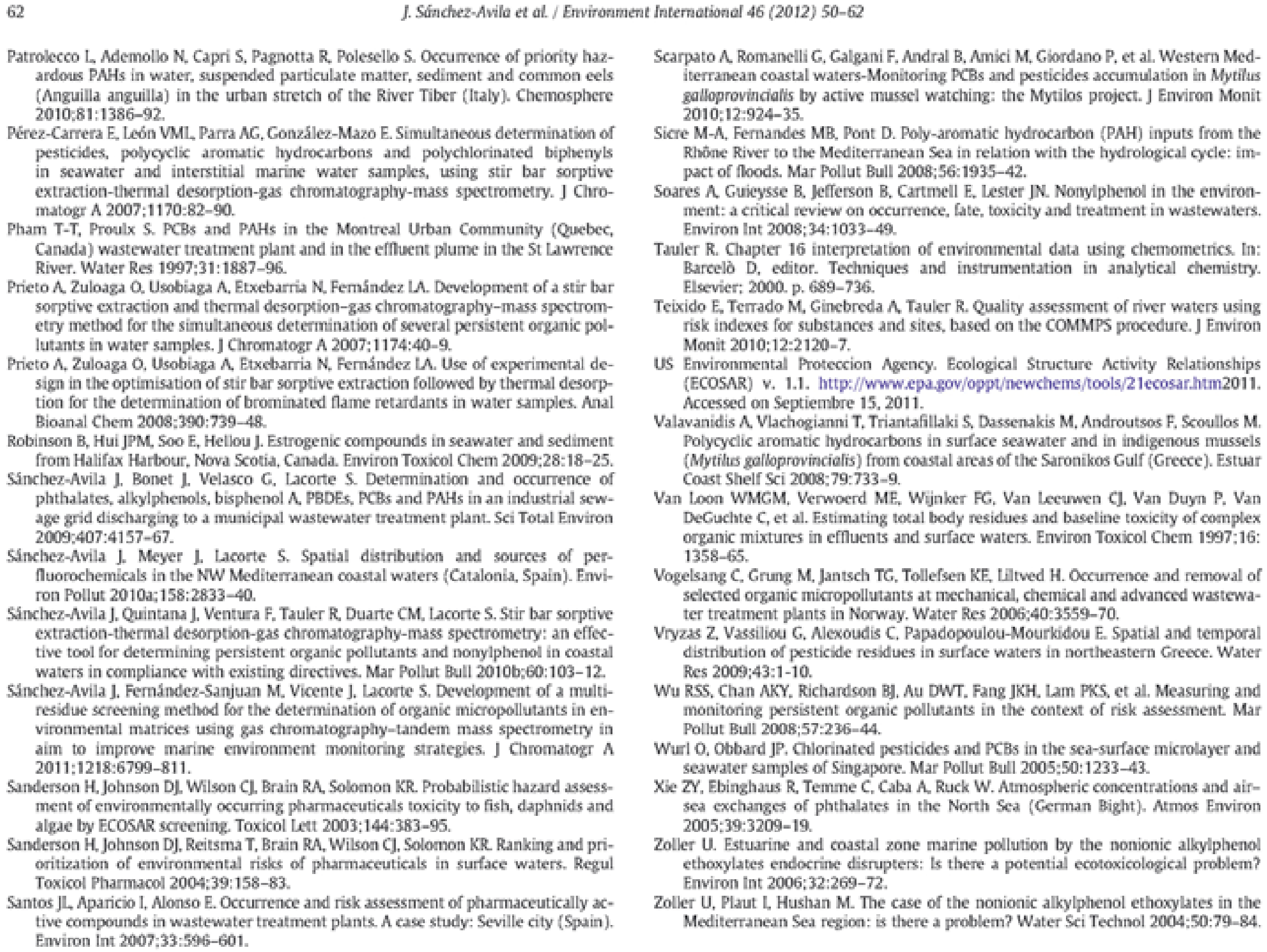

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Nota:
La información contenida en el Material Suplementario (**Tabla S1**) de este artículo se utilizó para construir la Tabla 14 en el cuerpo de esta tesis.

ARTÍCULO CIENTÍFICO 6.

“Sources, fluxes and risk of organic micropollutants to the Cantabrian Sea (Spain)”

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Sources, fluxes and risk of organic micropollutants to the Cantabrian Sea (Spain)

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ABSTRACT

The sources, distribution and risk of 51 organic micropollutants (OMPs) was studied by analyzing coastal seawater, sediments and caged mussels from the Cantabrian Sea (NW Spain). Gas chromatography coupled to tandem mass spectrometry (GC-MS) was used to determine polycyclic aromatic hydrocarbons, polychlorinated biphenyls, organochlorinated pesticides, polybrominated diphenyl ethers, phthalates esters, bisphenol A and alkylphenols. The sum of OMPs concentration in coastal/estuarine waters varied from 74 to 61147 ng L^{-1} and wastewater treatment plant effluents were identified as the main sources of contamination discharging up to 15321 g d^{-1} (44333 ng L^{-1}). Contaminants were accumulated in sediments which contained from 517 to 59317 ng g^{-1} (dry weight) and bioaccumulation in caged mussels exposed for 21 days from 101 to 6620 ng g^{-1} (wet weight). A summarized overview of the patterns and sources of OMPs on the investigated coastal/estuarine waters and sediments evidenced that wastewater discharges, combustion and industrial activities contribute significantly to the widespread of pollutants along the Cantabrian Sea. Chemical results were combined with the "Combined Monitoring-based and Modelling-based Priority Setting Scheme" COMMPS procedure for risk assessment analysis. From an exposure point of view, the most relevant substances were alkylphenols, whereas the priority substance risk index ranked nonylphenol, benzo(g,h,i)perylene and benzo(a)anthracene as the most concerning compounds. Finally, the chemical status of the different sampling locations was estimated using site risk indexes. Those indexes can be conveniently displayed in quality geographical maps and are considered a valuable tool for the environmental management and risk assessment of the region under study.

KEYWORDS

Organic micropollutants; coastal and estuarine seawater, sediments and mussels; pollution sources; COMMPS procedure; site risk indexes

1. Introduction

Industrial, municipal and treated or untreated wastewater discharges through smokestack, pipe, ditch, tunnel, or conduit are identified as point source pollution to coastal areas. Point source pollution can contribute in the form of oxygen-depleting nutrients (Enell and Fejes, 1995; Howarth et al., 2002; Kennish, 2002) and pathogens that cause serious health hazards to the environment (Walter et al., 1989). In addition, these point sources can significantly contribute to coastal water pollution in the form of toxic organic contaminants (Gasperi et al., 2008; Robles-Molina et al., 2010; Sanchez-Avila et al., 2009) and heavy metals (Al-Muzaini et al., 1999; Bubb and Lester, 1991; Mendiguchía et al., 2007). The magnitude of the pressure (industry, population, agricultural run-off) and vulnerability of receiving water (geographic, climatic and hydrology, dilution capacity, protection of the area, existing monitoring surveys) are the main factors that explain the impact of the discharges. Other activities such as shipping, motor boats and port activities (Deniz and Kilic, 2010; Mestres et al., 2010), oil spillages (Bayoumi and Ghalwash, 1999), ballast water discharges and litter (European Environment Agency, 1995) also affect coastal pollution.

The Cantabrian Sea is located in the Northern Spain, comprising the Autonomous Communities of Asturias, Cantabria and The Basque Country. This region is characterized by a mild weather, strongly influenced by oceanic water affluence from the Atlantic Ocean and the West winds circulation accompanied by low pressure systems (European Environment Agency, 2002; UNEP, 2009). Approximately 1.25 million persons inhabit in the coastal municipalities from the 3 Autonomous Communities (Instituto Nacional de Estadística, 2006). This coastal area is highly industrialized with textiles, food, chemical, petro-chemical, mining, automotive industry, metals transformation, waste management facilities, ports and dredging activities (Basque Country Water Agency, 2006; Cantabric Environment Country Planning and Accommodation Council, 2011). This region was revealed as a hot spot area for perfluorinated compounds in estuarine water, sediments and mussels (Gómez et al., 2011); PAHs, PCBs, PEs, butyltins, and metals in mussels (Bartolomé et al., 2010); and PAHs in mussels (Soriano et al., 2007), sediment and fish (Puy-Azurmendi et al., 2010).

Current strategies to monitor the impact of Organic Micro Pollutants (OMPs) in coastal system combine the monitoring of water-sediment-biota compartments to provide comprehensive information on the presence and fate of OMPs (Beiras et al., 2003). OMPs are adsorbed to particulate matter and are easily sedimented, and sediments become a reservoir of contaminants (Rudolf, 1991). On the other hand, OMPs in water or resuspension from sediments produce the accumulation to organisms (Baumard et al., 1998a). Among others, mussels (*Mytilus* sp.) have been widely used as bioindicators of contamination (Baumard et al., 1999; Fernandez-Sanjuan et al., 2010; Valavanidis et al., 2008). It has a wide distribution, abundance, has a sedentary behaviour, and a pronounced ability to accumulate organic compounds. Filter-feeding mussels process large volumes of sea water (approximately 2 dm^3 per hour), thereby accumulating organic compounds in concentrations two to five orders of magnitude greater than those in the ambient sea water (Fossato and Canzonier, 1976; Goldberg, 1986; Stegeman and Teal, 1973). In comparison to high trophic levels organisms such as fish and marine mammals, the enzyme systems metabolizing organic contaminants in mussels exhibit rather a low activity (Boon et al., 1989; Stegeman and Lech, 1991). The pattern of organic pollutant concentrations detected in mussels could therefore resemble their pattern in the ambient water to a good approximation (Potrykus et al., 2003). Recently, the European Commission (2011) proposed the inclusion of biota monitoring such as mussels, within the environmental quality standards (EQS) established by the Directive 2008/105/EC (European Parliament and Council, 2008). Also, this proposal incorporates some modifications in the EQS for inland surface waters and other surface waters (considering the coastal waters).

The objective of the present study was to evaluate the sources of OMPs (such as WWTP effluents, and submarine emissaries and industrial discharges) to estuarine coastal waters from the Cantabrian Sea and to assess the risk, considering the concentration of OMPs, their specific toxicity, the frequency of detection and the fluxes released towards coastal areas. Chemical analyses combined with risk assessment were used to discern the impact of OMPs.

2. Materials and methods

2.1. Study area and sample collection

Waters, sediments and transplanted mussels were sampled in estuarine and coastal areas from Asturias, Cantabria and Basque Country (North Spain). These areas receive mainly the discharges of treated or untreated urban wastewaters via emissaries, run-off of industrial wastes and port activities. Table 1 describes the matrices sampled in each site, their coordinates and the principal pressures/impacts affecting each site. Sites were grouped in 3 categories according the principal pressures: (i) 5 coastal/estuarine waters with WWTP discharges through submarine emissaries (VM, PÑ, BE) and uncontrolled discharges (SV, UR); (ii) 6 estuarine waters with port/harbour activities (PA, PP, PL and PS) or industrial effluents (I1, I2) and (iii) 2 WWTP effluents (WG, WS). Coastal and estuarine waters were collected at 1 m deep and surface sediments at the sea bed. All samples were collected in 1 L clean amber glass bottles. To avoid sample manipulation and prevent any cause of external contamination during sampling and extraction, samples were transported, stored and analyzed in the same bottle used for sampling. After collection, samples were stored at 4º C until transport to the main laboratory and waters were processed within one week while sediments were frozen, freeze dried and sieved through 120 µm. Sediment in PP (see Table 1) was not possible to sample, because the seabed was covered with rubble and scrap iron.

Table 1.
Specific codes, location, type of matrixes sampled, coordinates and detailed description of each sampling point.

Type	Site	Matrixes sampled	Coordinates UTM		Pressures/impacts
			X	Y	
Coastal areas and estuarine waters with low pressures/impacts identified	VM	Virgen del Mar Coastal water Sediment Mussels	428421	4816444	Outfall discharge from a WWTP via submarine emissary
	PÑ	Peñarubia Coastal water Sediment Mussels	287593	4827957	Outfall discharge from a WWTP via submarine emissary
	BE	Berria Coastal water Sediment Mussels	463064	4815080	Outfall discharge from a WWTP via submarine emissary
	SV	San Vicente de la Barqueira Estuarine water Sediment Mussels	386987	4804122	Diffuse contamination; dredging; fishing harbour; recreational activities
	UR	Urdaibai Estuarine water Sediment Mussels	525765	4804626	Diffuse contamination; CSOs; dredging; shipyards; fishing harbour; recreational activities
Estuarine waters with high pressures/impacts identified	PP	Pasaia Harbour Estuarine water Mussels	588073	4796829	CSOs; Industrial/harbour activities (i.e. metallurgical loading/unloading); fishing harbour; diffuse contamination
	PA	Arriluce Harbour Estuarine water Sediment Mussels	498639	4798825	Marina harbour, boat maintenance, petrol station spills
	PS	Santander Harbour Estuarine water Sediment Mussels	433024	4810293	Industrial/harbour activities; CSOs
	PL	Plenzia Estuarine water Sediment Mussels	504644	4805045	Diffuse contamination, CSOs, recreational activities
	FE	Industry discharge Surface water Sediment Mussels	432922	4806381	Industrial effluents (storm drainage, cooling system, factory treatment plant); CSOs; historical contamination (i.e. heavy metals)
	GA	WWTP discharge Surface water Sediment Mussels	501724	4794781	Effluent discharges from WG
WWTP effluent	WG	WWTP 1effluent			Receives urban and industrial wastewater. Secondary/tertiary treatment; 1000000 inhabitants (flow ~ 4 m^3 s^{-1})
	WS	WWTP 2 effluent			Receives urban and industrial

CSO: Combined sewer overflows

Transplanted mussels were placed in estuaries and coastal waters receiving urban or industrial discharges and in ports for a period of 3 weeks. First, 40 to 50 mm length mussels were collected from mussel crops from the Galician Rías (NW Spain), cleaned from epiphytes and caged in polyethylene experimental units. Experimental units were acclimated in an open circuit aquarium for 7 days. Before placing the mussels in each study site, 10 individuals from 4 experimental units were collected to quantify the contamination levels before the exposure (t_0). The experimental units were anchored onto fixed structures at 30 m depth (BE, VM and PÑ) while in the other stations were held between 1 – 4 m of depth. After 21 days of exposure (t_{21}), 10 additional mussels were collected, except from PÑ where the experimental unit was lost because of a storm. Once collected, 10 mussels were ground to a homogenized mix, freeze dried, homogenized again and kept in a glass jar at -20º C until extraction.

Sampling was carried out in October 2009 (t_0) and repeated in November 2009 (t_{21}). Sea sampling was performed by professional scuba-divers.

Table 2
Target compounds analyzed with their chemical family, CAS number and abbreviatio

Chemical family	Target compound	CAS No.	Abbreviation
Polyaromatic hydrocarbons (PAHs)	Naphtalene	91-20-3	Nap
	Acenaphtylene	208-96-8	Acy
	Acenaphthene	83-32-9	Ace
	Fluorene	86-73-7	Flu
	Phenantrene	85-01-8	Phe
	Anthracene	120-12-7	Ant
	Fluoranthene	206-44-0	Flr
	Pyrene	129-00-0	Pyr
	Benzo(a)anthracene	56-55-3	B(a)A
	Chrysene	218-01-9	Chr
	Benzo(b)fluoranthene	205-99-2	B(b)F
	Benzo(k)fluoranthene	207-08-9	B(k)F
	Benzo(a)pyrene	50-32-8	B(a)P
	Indeno(1,2,3-c,d)pyrene	193-39-5	I(cd)P
	Benzo(g,h,i)perylene	191-24-2	D(ah)A
	Dibenz(a,h)anthracene	53-70-3	B(ghi)P
Plasticizers (PEs + BPA)	Dimethyl phthalate	131-11-3	DMP
	Diethyl phthalate	84-66-2	DEP
	Bis(2-ethylhexyl) phthalate	117-81-7	DEHP
	Benzylbutyl phthalate	85-68-7	BBP
	Bis(2-ethylhexyl) adipate	103-23-1	DEHA
	Bisphenol A	80-05-7	BPA
Alkylphenols (APs)	4-tert-octhylphenol	140-66-9	OP
	4-Nonylphenol (isomer mixture)	84852-15-3	NP
	4-Nonylphenol monoethoxylate	104-35-8	$NPEO_1$
	4-Nonylphenol diethoxylate	20427-84-3	$NPEO_2$
Polychlorobiphenyls (PCBs)	2,4,4'-Trichlorobiphenyl	7012-37-5	PCB 28
	2,2'5,5'-Tetrachlorobiphenyl	35693-99-3	PCB 52
	2,2'4,5,5'-Pentachlorobiphenyl	37680-73-2	PCB 101
	2,3'4,4',5-Pentachlorobiphenyl	31508-00-6	PCB 118
	2,2',3,4,4',5- Hexachlorobiphenyl	35065-28-2	PCB 138
	2,2'4,4'5,5'-Hexachlorobiphenyl	35065-27-1	PCB 153
	2,2'3,4,4',5,5'-Heptachlorobiphenyl	35065-29-3	PCB 180
Organochlorine pesticides (OCPs)	2,4'-DDE	3424-82-6	2,4'-DDE
	4,4'-DDE	72-55-9	4,4'-DDE
	2,4'-DDD	53-19-0	2,4'-DDD
	4,4'-DDD	72-54-8	4,4'-DDD
	2,4'-DDT	789-02-6	2,4'-DDT
	4,4'-DDT	50-29-3	4,4'-DDT
	α-Hexachlorocyclohexane	319-84-6	α-HCH
	β-Hexachlorocyclohexane	319-85-7	β-HCH
	δ-Hexachlorocyclohexane	319-86-8	γ-HCH
	γ-Hexachlorocyclohexane	58-89-9	δ-HCH
	α-endosulfan	959-98-8	α-endsf
	β-endosulfan	33213-65-9	β-endsf
Polybromobiphenyl ethers (PBDEs)	2,4,4'-TriBDE	41318-75-6	BDE 28
	2,2',4,4'-TetraBDE	5436-43-1	BDE 47
	2,2',4,4',5-PentaBDE	60348-60-9	BDE 99
	2,2',4,4',6-PentaBDE	189084-64-8	BDE 100
	2,2',4,4',5,5'-HexaBDE	68631-49-2	BDE 153
	2,2',4,4',5,6'-HexaBDE	207122-15-4	BDE 154

2.2. *Analytical procedure*

Fifty one target compounds, summarized in Table 2, were analyzed in water, sediment and mussels samples as detailed in Sánchez-Avila, et al. (2011). Briefly, unfiltered water samples (1000 mL of coastal/estuarine/surface waters or 250 mL of WWTP effluent) was spiked with the surrogate standards (acenaphthylene d_8, benzo(a)pyrene d_{12}, benzo(g,h,i)perylene d_{12}, fluoranthene d_{10}, phenanthrene d_{10}, pyrene d_{10} PCB 209, 4-n-nonylphenol-d_8 and bisphenol A-d_{16}) at 10 ng L^{-1} in seawaters and at 100 ng L^{-1} in WWTP effluents and were solid phase extracted using Oasis HLB 200 mg cartridges. Extracts were reconstituted with 100 µL of ethyl acetate and spiked with 0.1 ng μL^{-1} of the internal standard anthracene d_{10}.

One gram of homogenized mussels (*Mytilus galloprovincialis*) or homogenized sediments (< 120 µm fraction) were spiked with the surrogate standards at 200 ng g^{-1}. Samples were liquid-solid extracted by sonication (10 min) using 1 x 10 mL of dichloromethane/hexane and 2 x 10 mL of hexane/acetone (in triplicate). After extraction, samples were centrifuged for 10 min at 2500 rpm. Extracts were combined and concentrated to approximately 1 mL under a nitrogen current using a TurboVap LV at 25°C. Extracts were subsequently purified using Florisil (5 g) SPE cartridges (Waters, MA, USA), previously conditioned with 20 mL of hexane/dichloromethane (1:1 v/v) and 20 mL of hexane/acetone (1:1 v/v). The sample extract was eluted with 15 mL of hexane/dichloromethane (1:1 v/v) and 15 mL of hexane/acetone (1:1 v/v). The eluent was evaporated until almost dryness under a nitrogen current at room temperature and reconstituted with ethyl acetate to a final volume of 200 µL for mussels and 500 µL for sediments. Internal standard anthracene d_{10} was added at a concentration of 1 ng μL^{-1}.

Extracts were analyzed using an Agilent 7890A GC System (Agilent Technologies, Palo Alto, CA, USA) interfaced to a 7000A triple quadrupole mass spectrometer system (Agilent, USA) in electron ionization (EI, +70 eV). Two µL of each extract were injected using an Agilent 7683A autosampler, following the conditions optimized previously by Sánchez-Avila, et al. (2011). The use of two transitions (one precursor with two product ions or two precursors with one product ion) confirms positive findings by Selected Reaction Monitoring (SRM) according to European legislation (European Commision, 2002). Quantification was performed using the internal standard method. A 9 points calibration curve was analyzed in duplicate from 0.010 to 1.0 ng μL^{-1} for all target compounds except for the PBDEs (0.010 to 0.10 ng μL^{-1}). Good linearity responses were obtained over the concentrations range tested for all target pollutants. The coefficients of determination (R^2) obtained were higher than 0.991. The methodological detection limits (MDL) and recovery yields are described in Sánchez-Avila et al. (2011).

2.3. *Mass flow of organic micropollutants to the sea*

For calculate the OMPs mass flow to the sea, the concentrations detected in WWTP effluents (Table 3) were considered as well as the effluents discharges flows (Table 1). The pollutant inputs (*PI*, in g d^{-1}) to the sea were calculated according to (1):

$$PI_{ij} = C_i * f_j \qquad (1)$$

Where C_i is the concentration (in g m^{-3}) of the pollutant i and f_j is the effluent discharge flow (in m^3 d^{-1}) of the WWTP j.

2.4. *Substance priority risk index*

The COMMPS (Combined Monitoring-based and Modelling-based Priority Setting Scheme) procedure establishes a ranking of chemical substances according to a risk priority index, generated using four descriptors: (i) the 90th percentile of the observed concentrations in the analyzed seawaters, (ii) the predicted no effect concentration (PNEC) of the target compounds, (iii) an index for the indirect aquatic effect score (EFS_i) and (iv) an index for the human health score (EFS_h) based on R-phrases (Risk phrases) for chemical substances (Lerche et al., 2002). In this study, this procedure has been applied to the 51 OMPs analyzed in the seawater samples, as described by Teixidó et al. (2010) and according to the European Technical Guidance Document on Risk Assessment (European Chemicals Bureau, 2003). In agreement with the description of requirements for inclusion in the COMMPS procedure, from the 51 compounds initially measured, only 35 were finally included in the data set for subsequent analysis. The risk priority index for a particular substance *i*, *I_prior*, is obtained as the product of a substance's exposure index, *I_exp*, and its corresponding effect index, *I_eff* (2). The details about the calculation of each index is described in Teixidó et al. (2010).

$$I\_prior_i = I\_exp_i * I\_eff_i \qquad (2)$$

2.5. *Site pollution risk index*

Teixidó et al. (2010) proposed a new procedure to compute a site pollution risk index based in the COMMPS procedure. It uses similar criteria to evaluate the potential risk of different substances present in a specific sampling site and, subsequently, takes all these substances into account to calculate a new score for the priority index, being characteristic for each particular site. The selection criteria to define the organic compounds to be included in the calculation of the site pollution risk index was the percentage of positive values (above detection limit) encountered. Substances were excluded if the total number of positive findings of mean values was less than 15%. In this way, 35 compounds were finally selected to be included in the evaluation of the site pollution risk index, corresponding to those more frequently detected.

3. Results

3.1. Occurrence and pollution profile of OMPs in water samples

The Cantabrian Sea (North Spain) coastal areas receive the impact of OMPs generated in in-land activities. Because of discharges from WWTP effluents, submarine emissaries, uncontrolled emissaries, harbour activities and industrial effluent, coastal and estuarine waters contained individual OMPs at levels of 0.1 to 35583 ng L^{-1}. In Table 3, the concentration averages from each pollutant and sampling site are summarized. In coastal/estuarine waters, result of two samplings were averaged (except for PS, 1 sampling) and in WWTP, from 3 samplings. APs, plasticizers (PEs and BPA) and PAHs were the most ubiquitous compounds, detected in the 100% of the water samples, followed by OCPs (69%), PCBs (54%) and finally PBDEs (38%).

3.1.1. Occurrence and pollution profile of OMPs in WWTP effluents.

When chemicals arrive to WWTPs, the secondary/tertiary treatments can remove a 68 – 98% of the OMPs burden (Céspedes et al., 2006; Clara et al., 2010). Still, WWTP effluents can be a potential source of contaminants to surface and coastal waters (Sanchez-Avila et al., 2009). Quantification of the loads of contaminants from WWTP effluents is a way to determine the risk to receiving waters. OMPs identified in the 2 WWTP effluents studied are reported in Table 3. Considering the ΣOMPs of 44333 and 32975 ng L^{-1} in WG and WS, respectively, a daily OMPs flow of was estimated of 15321 (WG) and 4558 g d^{-1} (WS). Plasticizers followed by APs were the main contributors of ΣOMPs in WWTP effluents. ΣPlasticizers in WG and WS were of 29235 and 17370 ng L^{-1} and a daily discharge (using eq. 1) of 10103 and 3782 g d^{-1} was estimated to receiving waters. DMP, DEP and DEHP and BPA were found at high concentration, attributed to their widespread use in the fabrication of resins, plasticizers, and flame retardants among others, in household and industrial products (Staples et al., 1998; Staples et al., 1997). On the other hand, ΣAPs mean concentrations in WWTP effluents were 14576 and 15255 ng L^{-1} and was estimated a daily output (using eq. 1) of 5037 g d^{-1} (WG) and 3321 g d^{-1} (WS). The high levels detected in effluents is consequence of the high utilization of APs in detergents, softeners and other industrial and household products (Soares et al., 2008).

Although secondary/tertiary treatments can remove ≈89% of the total PAH burden within a WWTP (Charalabaki et al., 2005; Manoli and Samara, 2008), PAHs were detected in all effluents. ΣPAHs in WG and WS were of 275 and 199 ng L^{-1} and was estimated a daily output (using eq. 1) of 95 and 43 g d^{-1}, respectively, to receiving waters. The main contributors are indicated in Figure 3. Pollutant input sources to the WWTP are domestic and industrial wastes, runoff waters and also, street sweeping waters that collect atmospheric dry deposits, dusts from asphalt erosion, residues from petroleum products and various trash (Blanchard et al., 2001).

ΣOCPs concentrations in WWTP effluents were 66 and 85 ng L^{-1} and was estimated a daily output (using eq. 1) of 23 (WG) and 19 g d^{-1} (WS) to receiving waters. In a WWTP, persistent organic pollutants, such as OCPs, can derive as a component of urban or agricultural runoff or drainage into the sewerage system, including wet and dry deposition from the atmosphere, and via the contribution of industrial discharges. During secondary/tertiary treatments up to 74 - 89% of the total OCP burden is removed, depending on the OCP (Katsoyiannis and Samara, 2004).

ΣPCBs concentrations in WWTP effluents were 121 and 51 ng L^{-1} and was estimated a daily output (using eq. 1) of 42 (WG) and 11 g d^{-1} (WS) to receiving waters. Even PCBs used in new products was banned by European Legacy (European Council, 1996; European Environment Agency, 2011), there still exist products containing those chemicals. PCBs are introduced in WWTP by many pathways, such as car traffic, industrial waste, incinerators, and domestic heating via both atmospheric transport and local activity (Blanchard et al., 2001; European Environment Agency, 2011). During secondary/tertiary treatments up to 65 - 81% of the total PCB burden is removed (Blanchard et al., 2004; Katsoyiannis and Samara, 2004).

The least detected compounds were PBDEs (Table 3). ΣPBDEs concentrations in WWTP effluents were 60 and 15 ng L^{-1} and was estimated a daily output (using eq. 1) of 21 (WG) and 3.0 g d^{-1} (WS) to receiving waters. PBDEs have been used as flame retardants since the early 1970s. Areas of use include a variety of consumer and commercial products, such as furniture, electronic devices, including TV sets and personal computers, automotive parts, textiles and thermoplastics. PBDEs can be released from these products during production, use and disposal and, as a consequence, these compounds are now ubiquitous in the environment (Prieto et al., 2008). Once in WWTP, PBDEs burden is removed with an efficiency of 90 – 99% during secondary/tertiary treatment (Clarke et al., 2010; Vogelsang et al., 2006). Because they are additives mixed into polymers and are not chemically bound to the plastic or textiles, they can be easily released into the environment (Renner, 2000).

3.1.2. OMPs in estuaries and coastal waters.

The presence of OMPs in the Cantabrian coastal/estuarine waters is directly related with the anthropogenic activities carried out in the area and water management strategies. Although seawater has a high dilution capacity, the continuous release of contaminants can pose a severe risk in the long term, as the dilution capacity may be lower than the loads of contaminants. Submarine emissaries can be a source of OMPs to coastal waters. These emissaries release their effluents in 2-3 km from the coast, approximated at 30 m deep and therefore, are potential contributors to coastal water pollution. The concentration of OMPs in surface seawater sampled in the of the mouth of the emissary was between 0.4 to 42 ng L^{-1}, with PEs being the main contributors, followed by APs and PAHs, which were found at similar concentrations. PCBs, PBDEs and OCPs were not detected. ΣOMPs were of 47, 50 and 112 ng L^{-1} in VM, PÑ and BE. If these concentrations are compared to WWTP effluents where the ΣOMP were of 44334 and 32974 ng L^{-1} in WG and WS, it is easily observed that pollutants are rapidly dispersed in seawater after release from emissaries. Dilution rates can be roughly estimated of 100-1000 times. Finally, considering the fluxes of 1.6, 0.7 and 5.1 m^3 s^{-1} from VM, PÑ and BE emissaries, the OMP load to coastal areas is of 1419, 1995 and 9358 g d^{-1}.

On the contrary, SV and UR are areas which are considered as unpolluted and with little anthropogenic impact. In fact, the Urdaibai estuary is a natural region and a Biosphere Reserve of Biscay, Basque Country formed by the mouth of the river Oka. Urdaibai covers an area of 220 km^2 with some 45,000 inhabitants mainly from Bermeo and Gernika. The economy is based on metallurgy, fishing, farming and forestry and tourism. On the other hand, San Vicente de la Barquera located in Cantabria (Spain) has a population of 4532 inhabitants and has a surface of 41.5 km^2 and belongs (in 80%) to the Natural Park of Oyambre, a protected area due to its high ecological and landscape value. However, the ΣOMPs were of 211 and 515 ng L^{-1} in UR and SV, with APs, PE and PAHs as main contributors. The higher levels compared to VM, PÑ and BE are attributed to non-treated discharges to coastal waters. Therefore, these areas can be considered as impacted taking into consideration their high protection status.

On the other hand, estuaries with port settlements, industrial effluents or with WWTP discharges contained ΣOMPs from 1174 to 122289 ng L^{-1} and therefore, it can be considered that maritime activities and waste releases are main sources of contamination. This is the case of GA estuary, the most polluted, which receives the direct and continued discharge of WG and also non-point sources or unidentified discharges of untreated domestic and industrial wastewaters. AP, followed by PAHs and PE were the main contributors (Table 3), whereas PCBs, OCs and PBDEs were sporadically detected and at low ng L^{-1} concentration. The ΣAPs concentrations detected in coastal and estuary sites from Cantabrian Sea were similar to other Spanish regions as in coastal waters from Catalonia, Spain (3 – 694 ng L^{-1}) (Sánchez-Avila et al., 2012), Portugal (only NP: 600 – 30000 ng L^{-1}) (Azevedo et al., 2001), in Dutch estuaries (40 – 1963 ng L^{-1}) (Jonkers et al., 2003), in Thessaloniki's coastal areas and harbours (489 – 2097 ng L^{-1}) (Arditsoglou and Voutsa, 2008) and in Masan Bay, Korea (40.8 – 36000 ng L^{-1}) (Li et al., 2008). Among APs, NP and OP are the only regulated by Directive 2008/105/EC. According to the maximum allowable concentration (MAC) established for other surface waters, GA exceeded the limit of 2000 ng L^{-1} for NP (5999 ng L^{-1}). On the other hand, the presence of PEs and BPA in the Cantabrian coastal and estuarine waters is a clear indicator of industrial pollution sources (treated/untreated discharges), leaching from dumps (Fromme et al., 2002), urban waste pathways (Chaler et al., 2004) and the extensive shipping traffic (Antizar-Ladislao, 2009). The ΣPlasticizers concentrations detected in Cantabrian coastal/estuarine waters were similar to those in coastal waters from Catalonia (15 – 1028 ng L^{-1}) (Sánchez-Avila et al., 2012) and the Basque Country (74 – 703 ng L^{-1}) (Prieto et al., 2007), both in Spain. Our results were lower than in Thessaloniki's, Greek estuaries (489 – 2097 ng L^{-1}) (Arditsoglou and Voutsa, 2008) and similar to Dutch estuaries (40-1963 ng $L^{-}1$) (Jonkers et al., 2003). Among plasticisers, the annual average (AA) concentration allowed for DEHP in other surface waters is 1300 ng L^{-1}, which was not exceeded by any coastal or estuarine water sample.

PAHs in coastal and estuarine waters are related with petroleum contamination (spillage of oil from ships, maritime transport accidents), fallout from air pollution (combustion of fuels), and terrestrial runoff (municipal sewage discharges, industrial waste water) (Valavanidis et al., 2008). The high concentrations detected in estuarine waters impacted by harbour settlements and industrial run-off are directly related to the important shipping and industrial activities developed near those sampling points. Compared with other Spanish regions, ΣPAHs concentrations detected in Cantabrian coastal/estuarine waters were higher than those reported in Catalonia (0.8 – 83 ng L^{-1}) (Sánchez-Avila et al., 2012) and were comparable to other harbours and bays from the Basque Country and Cadiz (215 – 1939 ng L^{-1}) (Pérez-Carrera et al., 2007; Prieto et al., 2007). In other worldwide studies, the levels were similar than those reported in the Greek Mediterranean sea (113 – 459 ng L^{-1}) (Valavanidis et al., 2008), Singapore coast (88 – 1473 ng L^{-1}) (Basheer et al., 2003), polluted estuaries from China (139 – 1718 ng L^{-1}) (Men et al., 2009) and Argentina (nd – > 4000 ng L^{-1}) (Arias et al., 2009). Among PAHs, new MAC are established in the Proposal 2011/0429 for other surface waters (European Commision, 2011): B(a)P, 27 ng L^{-1}; B(b)F, 17 ng L^{-1}; B(ghi)P, 0.82 ng L^{-1}. In FE, the B(b)F limit was exceeded (57 ng L^{-1}) and all the samples except VM, exceeded the B(ghi)P limit (see Table 3).

ΣOCPs concentrations in estuaries impacted by ports and industrial activities ranged from 0.9 to 1313 ng L^{-1}. DDT metabolites were detected only in FE and could be associated to the historical use of this pesticide. Lindane (γ-HCH) was the predominant OCP detected in PA, and corresponds to previous studies where it was associated to an incident in an old pesticide factory (Concha-Graña et al., 2006). ΣOCPs concentrations in Cantabrian coastal areas were comparable to seawater from Singapore (1.6 – 19 ng L^{-1}) (Basheer et al., 2003), from Bering and Chukchi Seas, China (ΣDDTs: 0.14 – 0.26 ng L^{-1}) (Strachan et al., 2001) and in the North Sea (0.1 to 3.5 ng L^{-1}) (Ilyina et al., 2006). In the Dailao River estuary, China, higher ΣOCPs concentrations were reported (ΣHCH: 67-311 ng L^{-1}; ΣDDTs: 2.0-427 ng L^{-1}) (Tan et al., 2009). Among the analyzed OCPs, the MAC in other surface waters established by Directive 2008/105/EC was exceeded only for HCH isomers (Σα- β- γ- δ-HCH: 20 ng L^{-1}) in PA (1313 ng L^{-1}, γ and β-HCH), GA (113 ng L^{-1}, γ-HCH) and PL (54 ng L^{-1}, γ-HCH).

ΣPCBs concentrations in high impacted estuaries ranged from 2.9 to 25 ng L^{-1}. The presence of PCB in estuarine seawaters (GA, PS, PA, FE, PP) is related with the important industrial and shipping activities, the waste disposal of electrical transformers, oil spillage, and any historical use of PCB containing products (Wurl and Obbard, 2005). In Spanish-French Mediterranean coast similar levels were detected (0.08 – 43 ng L^{-1}) (García-Flor et al., 2005). Similar ΣPCBs concentrations are reported in the SW and NE Singapore's coastal waters (0.22 – 20.41 ng L^{-1}) (Basheer et al., 2003) and lower concentrations in the Houston Ship Channel, USA (0.49 – 8.5 ng L^{-1}) (Howell et al., 2011) and Bering and Chukchi Seas (0.6 – 1.0 ng L^{-1}) (Strachan et al., 2001).

Finally, PBDEs were seldom detected. The most ubiquitous congeners were BDEs 28, 47 and 99. ΣPBDEs in estuaries impacted by ports and industrial activities ranged from 1.9 to 5.1 ng L^{-1}. These concentrations were lower than other polluted areas from the Basque country, Spain (92 – 108 ng L^{-1}) (Prieto et al., 2008) and the industrialized Zhujiang River Estuary, China (9 – 128 ng L^{-1}) (Luo et al., 2008; Wang et al., 2011). For PBDEs congeners, the MAC in other surface waters established by Proposal 2011/0429 (ΣBDEs 28, 47, 99, 100, 153, 154 of 14 ng L^{-1}) was not exceeded in any sample.

Figure 1 shows the ΣOMPs in the seawaters analyzed. Among all the samples sites, GA estuary seawater was the most polluted site with a ΣOMPs mean concentration of 61147 ng L^{-1}, followed by PA (6294 ng L^{-1}), PP (5456 ng L^{-1}), FE (3125 ng L^{-1}), PL (2400 ng L^{-1}), PS (1173 ng L^{-1}), BE (776 ng L^{-1}), UR (258 ng L^{-1}), SV (106 ng L^{-1}), PÑ (84 ng L^{-1}) and finally VM (74 ng L^{-1}).

Table 3.
Summary data for the different water samples analyzed, indicating number of samples analyzed, mean of target pollutants and standard deviation.

		Coastal and estuarine water with low impacts/pressures					Estuarine water with high impacts/pressures						WWTP effluent	
		VM	PÑ	BE	SV	UR	PA	PP	PL	PS	FE	GA	WG	WS
Chemical family	Compound	Average (n=2)	Average (n=2)	Average (n=2)	Average (n=2)	Average (n=2)	Average (n=2)	Average (n=2)	Average (n=2)	(n=1)	Average (n=2)	Average (n=2)	Average (n=3)	Average (n=3)
PAHs	Nap	bdl	bdl	bdl	bdl	4.7 ±6	bdl	2.7 ±3	9.3 ±13	bdl	bdl	17 ±6	16 ±8.3	46 ±48
	Acy	bdl	1.0 ±0.9	bdl	bdl	3.1 ±2	68 ±70	1.1 ±1	2.4 ±0.1	0.3	20 ±11	8.2 ±0.9	10 ±2	47 ±62
	Ace	1.1 ±0.5	bdl	1.3 ±0.6	1.1 ±0.4	bdl	7.4 ±1	bdl	1.2 ±0.5	16	6.3 ±0.5	32 ±14	26 ±19	bdl
	Flu	7.1 ±3	37 ±50	4.3 ±5	28 ±22	1.2 ±0.4	45 ±27	bdl	1.6 ±0.1	15	33 ±7	35 ±18	22 ±22	21 ±19
	Phe	1.7 ±1	0.6 ±0.5	bdl	1.4 ±0.4	0.7 ±0.7	2.0 ±1	61.5 ±14	8.5 ±0.8	93	34 ±0.6	13 ±5	4.2 ±2	bdl
	Ant	0.6 ±0.1	0.5 ±0.1	0.6 ±0.0	0.7 ±0.1	0.8 ±0.3	3.9 ±2	10.4 ±1.8	8.0 ±0.9	27	41 ±7	6.3 ±0.8	34 ±10	bdl
	Flr	0.4 ±0.1	0.6 ±0.1	0.4 ±0.1	0.7 ±0.3	2.3 ±3	9.5 ±3	38.6 ±14	20.2 ±1.3	42	641 ±269	27 ±10	6.7 ±2	bdl
	Pyr	bdl	bdl	0.4 ±0.1	0.5 ±0.02	1.7 ±2	6.7 ±4	39.4 ±19	19.6 ±5.6	37	280 ±95	20 ±9	27 ±23	7.7 ±13
	B(a)A	2.0 ±0.1	1.8 ±0.0	1.1 ±0.9	1.8 ±0.1	2.7 ±1	2.8 ±1	24.7 ±2.7	1.8 ±0.6	10	118 ±36	5.3 ±3	32 ±0.3	32 ±0.3
	Chr	0.4 ±0.1	0.4 ±0.0	bdl	0.4 ±0.001	1.6 ±2	6.3 ±4	19.6 ±0.3	1.0 ±0.2	14	168 ±45	6.2 ±4	9.1 ±7	6.1 ±11
	B(b)F	1.3 ±0.9	1.8 ±0.0	1.7 ±0.1	1.8 ±0.2	3.1 ±2	3.3 ±2	2.7 ±0.5	2.4 ±0.2	12	57 ±77	5.0 ±3	10 ±8	bdl
	B(k)F	1.4 ±1.0	1.9 ±0.1	1.2 ±0.8	1.9 ±0.2	2.3 ±2	2.9 ±1	2.5 ±0.5	2.3 ±0.1	7.2	70 ±22	3.9 ±2	18 ±12	bdl
	B(a)P	1.1 ±0.4	1.1 ±0.4	bdl	1.3 ±0.1	2.6 ±2	2.6 ±2	1.9 ±0.3	1.9 ±0.3	8.9	63 ±21	3.2 ±2	44 ±33	21 ±21
	I(cd)P	bdl	1.2 ±1.2	bdl	1.3 ±1	3.1 ±2	3.1 ±2	2.7 ±0.6	2.5 ±0.3	7.2	13 ±18	3.9 ±2	4.9 ±3	5.4 ±5
	D(ah)A	bdl	bdl	bdl	bdl	1.0 ±0.2	0.9 ±0.1	bdl	bdl	3.2	26 ±14	1.2 ±0.5	7.0 ±7	bdl
	B(ghi)P	bdl	1.2 ±0.04	1.1 ±0.1	1.3 ±0.3	2.4 ±2	2.7 ±2	2.3 ±0.6	1.8 ±0.4	12	73 ±38	3.9 ±3	4.9 ±5	13 ±13
	Σ PAHS	**17**	**49**	**12**	**42**	**33**	**167**	**210**	**65**	**304**	**1642**	**192**	**275**	**199**
Plastif.	DMP	bdl	1.0 ±0.1	bdl	bdl	1.0 ±0.3	1.6 ±1	12 ±13	1.6 ±1	bdl	0.9 ±0.1	5.5 ±0.9	15232 ±12922	2887 ±3917
	DEP	2.8 ±4	bdl	630 ±186	bdl	bdl	bdl	bdl	41 ±58	bdl	319 ±108	1090 ±36	6533 ±3219	8096 ±6224
	DEHP	16 ±6	4.2 ±6	29 ±7	18 ±8	4.0 ±5	88 ±20	133 ±49	21 ±23	269	52 ±4	89 ±41	6025 ±3189	3977 ±3243
	BBP	10 ±0.8	1.8 ±0.9	42 ±39	2.4 ±2	3.1 ±3	9.5 ±5	28 ±14	2.2 ±1	25	8.1 ±9	30 ±19	706 ±159	598 ±343
	DEHA	21 ±28	bdl	2.1 ±2	0.7 ±0.02	12 ±16	11 ±0.02	35 ±28	9.6 ±13	bdl	35 ±23	bdl	36 ±24	60 ±21
	BPA	2.3 ±0.8	2.3 ±0.7	1.6 ±0.2	2.0 ±0.4	4.0 ±3	13 ±8	341 ±432	15 ±18	310	210 ±281	50 ±56	703 ±275	1751 ±620
	Σ Plast.	**52**	**9.3**	**705**	**23**	**24**	**122**	**549**	**91**	**603**	**625**	**1264**	**29235**	**17370**
APs	OP	0.8 ±0.1	0.6 ±0.2	13 ±6	4.5 ±2	16 ±19	182 ±38	39 ±55	113 ±80	8.9	7.2 ±5	740 ±590	3991 ±678	6624 ±965
	NP	1.9 ±0.5	4.3 ±3	28 ±16	18 ±10	20 ±26	744 ±44	580 ±101	365 ±131	81	127 ±73	5999 ±1122	5788 ±4114	7098 ±6216
	NP EO1	2.0 ±1	13 ±17	6.8 ±5	8.1 ±10	14 ±18	804 ±557	819 ±372	294 ±229	64	171 ±100	17224 ±1016	1666 ±171	735 ±40
	NP EO2	bdl	8.1 ±6	9.7 ±7	9.6 ±7	150 ±192	2952 ±717	3248 ±1129	1377 ±368	101	537 ±168	35583 ±19807	3130 ±61	798 ±66
	Σ APs	**4.6**	**26**	**57**	**40**	**200**	**4682**	**4687**	**2170**	**255**	**842**	**59547**	**14576**	**15255**
PCBs	PCB 28	bdl	bdl	bdl	bdl	bdl	1.0 ±0.6	1.2 ±0.3	0.1 ±0.04	1.5	0.6 ±0.2	6.0 ±4	16.3 ±9	bdl
	PCB 52	bdl	bdl	bdl	bdl	bdl	0.5 ±0.3	0.7 ±0.1	bdl	0.7	5.0 ±1.4	2.8 ±2.0	16 ±17	9.4 ±4
	PCB 101	bdl	bdl	bdl	bdl	bdl	1.1 ±0.9	0.5 ±0.1	bdl	1.0	0.6 ±0.4	3.2 ±3.0	14 ±10	1.9 ±0.2
	PCB 118	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	1.3	bdl	bdl	27 ±22	12 ±12
	PCB 138	bdl	bdl	bdl	bdl	bdl	2.9 ±3	bdl	bdl	1.5	2.1 ±0.7	5.7 ±6	27 ±2	10 ±10
	PCB 153	bdl	bdl	bdl	bdl	bdl	2.3 ±1.6	0.5 ±0.1	bdl	1.6	1.0 ±0.2	4.5 ±4	8.0 ±13	7.2 ±12
	PCB 180	bdl	bdl	bdl	bdl	bdl	1.9 ±2	bdl	bdl	bdl	0.5 ±0.1	3.2 ±2	12 ±6	10.7 ±9
	Σ PCBs	**—**	**—**	**—**	**—**	**—**	**9.7**	**2.9**	**—**	**7.7**	**9.9**	**25**	**121**	**51**
OCPs	2,4'-DDE	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
	4,4-DDE	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
	2,4'-DDD	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.3 ±0.1	bdl	bdl	27 ±30
	4,4'-DDD	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.6 ±0.1	bdl	bdl	16 ±18
	2,4'-DDT	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
	4,4'-DDT	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
	α-HCH	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
	β-HCH	bdl	bdl	bdl	bdl	bdl	0.9 ±0.1	bdl	bdl	bdl	bdl	bdl	bdl	bdl
	γ-HCH	bdl	bdl	bdl	bdl	bdl	1312 ±90	7.0 ±3	54 ±3	bdl	bdl	113 ±50	31 ±27	bdl
	δ-HCH	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
	α-endsf	bdl	bdl	1.2 ±0.2	bdl	bdl	bdl	bdl	bdl	bdl	bdl	1.3 ±0.3	36 ±21	42 ±8
	β-endsf	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	2.3 ±1	bdl	bdl
	Σ OCPs	**—**	**0.0**	**1.2**	**—**	**—**	**1313**	**7.0**	**54**	**—**	**0.9**	**117**	**66**	**85**
PBDEs	BDE 28	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.8	0.7 ±0.1	1.0 ±0.5	21 ±12	4.0 ±2
	BDE 47	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	1.1	3.4 ±1	0.9 ±0.2	20 ±12	3.1 ±3
	BDE 99	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.9	1.0 ±0.4	0.8 ±0.1	1.6 ±3	1.6 ±3
	BDE 100	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	2.5 ±1	2.9 ±5
	BDE 153	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	7.8 ±13	3.1 ±3
	BDE 154	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	7.8 ±14	bdl
	Σ PBDEs	**—**	**—**	**—**	**—**	**—**	**—**	**—**	**—**	**2.7**	**5.1**	**2.7**	**60**	**15**

bdl: Below detection limit
2,4'-DDE, 4,4'-DDE, 2,4'-DDT, 4,4'-DDT, α-HCH and δ-HCH were no detected

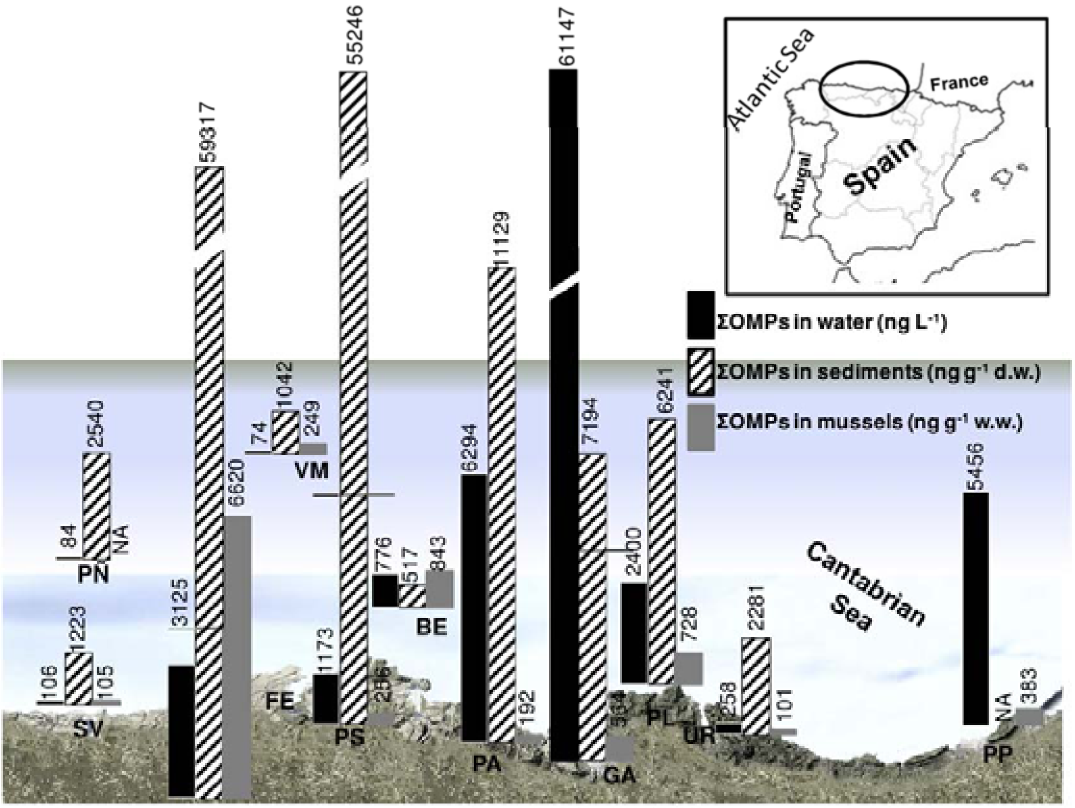

Fig. 1. Total OMPs (ΣOMPs) in the sea and estuarine waters, sediments and mussels and their geographic distribution along the Cantabrian Sea.

3.2. Accumulation of OMP in sediment samples

Table 4 shows the concentration of target analytes in sediment samples. Individual OMPs concentrations ranged from 3 to 12199 ng g^{-1} dry weight (dw). PAHs and PEs were the most ubiquitous compounds (100% of detection), followed by PCBs (80%), APs (60%) and finally OCPs (40%). No PBDEs were detected.

PAHs were detected in all samples being Flu, Phe, Ant, Flr, Pyr, B(a)A, Chr and B(b)F+B(k)F the predominant compounds. ΣPAHs concentrations ranged from 248 (VM) to 58713 ng g^{-1} dw (FE). The highest concentrations were detected in sediments from PÑ, underneath a submarine emissary. The concentrations in decreasing order were as follows: FE >PS >PA >GA >PÑ >UR >SV >PL >BE >VM. Sediments ΣPAHs levels in coastal/estuarine areas were similar to those measured in Barcelona harbour (8400 ng g^{-1} dw) (Baumard et al., 1998b) and previously, in Cantabrian estuaries (520 – 4900 ng g^{-1}) (Antizar-Ladislao, 2009). Levels were similar to other polluted sites around the world, where shipping traffic and industrial activities are developed, as in the Gulf of Rijeka, Croatia (213 – 695 ng g^{-1}) (Bihari et al., 2007), Mar Piccolo of Taranto, Italy (380 to 12750 ng g^{-1} dw) (Cardellicchio et al., 2007), Saronikus Gulf , Greece (1288 – 1759 ng g^{-1} dw) (Valavanidis et al., 2008) and Oder and Kiel harbours in the Baltic Sea (30 – 30100 ng g^{-1} dw) (Baumard et al., 1999). PAHs were used as fingers prints of pyrolitic or petrogenic origin of pollution. Molecular indices based on Phe/Ant and Flr/Pyr were used as described in previous studies (Yunker et al., 2002). In Figure 2, the origins of PAHs according the Ant/(Phe+Ant) and Flr/(Flr+Pyr) ratios is shown. Based on this crossplot, all the sediment samples fall predominantly on the combustion area. According to Flr/(Flr+Pyr) ratio, BE and SV sediments' (ratio <0.4) were in the petrogenic area; VM and PL (ratio >0.4 and <0.5) fall in the region characteristic of liquid fossil fuel (vehicle and crude oil) combustion; the rest of the sediments (ratio >0.5), fall in the area of grass/wood/coal combustion. The two ratios together indicated for BE and SV mixed combustion (primary vehicle emissions) and petroleum sources (crude oil products and fuel spilling); for VM and PL the sources were mainly urban and heavy duty traffic; for the rest of the sediments, the behaviour is the same as observed in urban sediments, typically related with heavy duty traffic, agricultural biomass burning, industrial combustion processes and house heating in the winter due to the cold temperatures (Yunker et al., 2002).

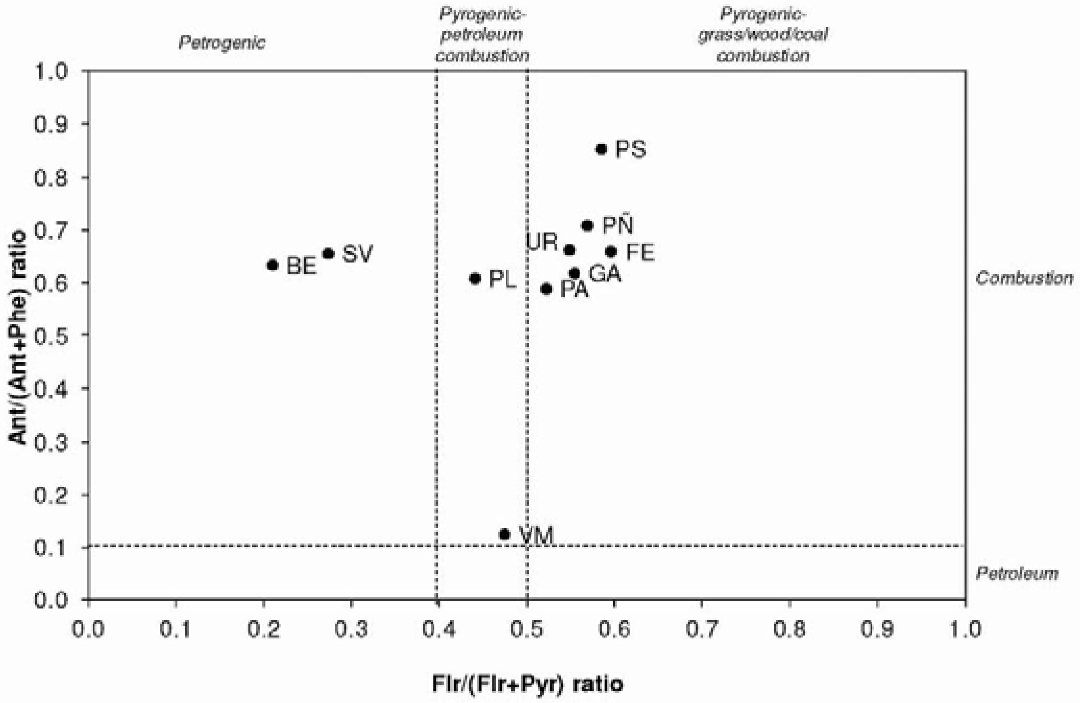

Fig 2. PAHs origin according to the Flr/(Flr+Pyr) vs. Ant/(Ant+Pyr) ratios from the sediments from the Cantabrian Sea.

Regarding to plasticizers, BPA was not analyzed and DMP was not detected in sediments. The low accumulation of DMP could be explained by their high solubility (410 mg L^{-1}) and low K_{ow} (1.60) being more affinity to remain in water or by their significant degradation processes occurring soon after sedimentation (Chaler et al., 2004). PEs were detected in all samples and DEHP was the most ubiquitous compound. ΣPEs ranged from 83 (PA) to 5799 ng g^{-1} dw (PL). Concentrations in decreasing order were as follows: PL >PS >GA >UR >VM >SV >PÑ >FE >BE >PA. Results agreed with previous studies for sediments from Cantabrian and Basque Country estuaries (200 – 3000 ng g^{-1} dw) (Antizar-Ladislao, 2009; Chaler et al., 2004). Also, similar levels were reported in Netherlands marine sediments (1.3 – 7600 ng g^{-1} dw) (Klamer et al., 2005; Vethaak et al., 2005). Higher levels were reported in Ontario Harbor (mean, 29700 ng g^{-1} dw) (McDowell and Metcalfe, 2001). There was no apparent relationship between concentrations in sediments and vicinity of likely sources (e.g., harbours, sewage discharges, shipping traffic and industrial zones), indicating that PEs in sediments were not the best indicators of industrial or urban wastes (Chaler et al., 2004).

PCBs were detected in 8 samples. ΣPCBs ranged from 25 to (VM) to 437 ng g^{-1} dw (GA), being PCB 28 and 138 the predominant congeners. Concentrations in decreasing order were as follows: GA >PA >FE >PS >UR >PÑ >PL >VM. In the Cantabrian estuaries, lower values were previously reported (2.9 to 37 ng g^{-1} dw) (Antizar-Ladislao, 2009). In Catalonia coastal areas, lower levels were detected (7.2 – 44 ng g^{-1} dw) even near submarine emissaries (7.1 – 18 ng g^{-1} dw) (Castells et al., 2008). Also, lower results were detected in the Dutch North Sea (0.3 – 27.2 ng g^{-1} dw) (Klamer et al., 2005) and higher concentrations were reported for the Mar Piccolo of Taranto, Italy (2 to 1684 ng g^{-1} d.w) (Cardellicchio et al., 2007). Although no PCBs were detected in waters from VM and PÑ, those compounds could persist in sediments for years, indicating an historical industrial pollution. The presence of PCBs is related with industrial/urban sources (Meharg et al., 2003), and the highest PCBs levels were detected in GA, PA, FE and PS where important industrial activities are developed or either are receptors of sewage discharges.

APs were detected in 6 sediment samples and NP was the most ubiquitous compound. $NPEO_1$ and $NPEO_2$ were below the detection limit in all samples since those substances are degradated to NP in anaerobic conditions. ΣAPs ranged from 79 to (FE) to 592 ng g^{-1} dw (PS). Concentrations in decreasing order were as follows: PS >GA >VM >PA >FE. In the Cadiz Bay, Spain, similar concentrations were reported (50 – 1164 ng g^{-1} dw) (Lara-Martín et al., 2006). Also, similar concentrations were reported in sediments from Masan Bay, Korea (mean of 248 ng g^{-1} dw) (Hong et al., 2009) and higher levels were detected in the northeast coastal environment in China, (8.8 – 1000 ng g^{-1} dw) (Wang et al., 2010) and in a coastal marsh from Canada (190 – 4260 ng g^{-1} dw) (Mayer et al., 2007). NP is a hydrophobic compound with a log K_{ow} value of 4.48 and low solubility in water, therefore it partitions favourably to organic matter and has low mobility, limiting its capacity for spreading in the aqueous phase of soil and sediments. In sediments it has an estimated half-life of more than 60 years (Soares et al., 2008).

OCPs were detected in 4 estuarine sediments, being 4,4'-DDE the most ubiquitous compound. ΣOCPs ranged from 56 (PS) to 1471 ng g^{-1} dw (PA). Concentrations in decreasing order were as follows: PA >GA >FE >PS. Lower concentrations were detected in coastal areas from Portugal with similar OCP profile (0.08 – 26 ng g^{-1}dw) (Carvalho et al., 2009b) and Philippines (ΣDDT, mean of 9.5 ± 2.4 ng g^{-1}) (Carvalho et al., 2009a). Similar concentrations were reported for North Bohai Sea, China (ΣHCH: <1965 ng g^{-1} and ΣDDT <93 ng g^{-1}) (Hu et al., 2010). The concentrations found in sediments were explained by the historical use of those pesticides. DDT undergoes slow degradation to DDE and DDD in the natural environment by chemical and biological processes (Hu et al., 2010). 2,4'-DDT neither 4,4'-DDT were not found, indicating a historical use of this pesticide. Regarding to HCHs isomers, commercial formulations generally are used either as the technical HCH mixture (α formulation dominated by the α-isomer) or as lindane (a formulation containing almost 100% of the γ-isomer) (Chrysikou et al., 2008). After a long period of weathering, α- and γ-HCH can be transformed into β-HCH (Hu et al., 2010). According to our results, the predominance of the isomers α and γ, indicates a recently use of HCH pesticides.

Figure 1 shows the ΣOMPs in the sediments analyzed. Among all the samples sites, FE estuary sediment was the most polluted sample with a ΣOMPs mean concentration of 59317 ng g^{-1}, followed by PS (55246 ng g^{-1} dw), PA (11129 ng g^{-1} dw), GA (7194 ng g^{-1} dw), PL (6214 ng g^{-1} dw), PÑ (2540 ng g^{-1} dw), UR (2281 ng g^{-1} dw), SV (1223 ng g^{-1} dw), VM (1042 ng g^{-1} dw) and finally BE (517 ng g^{-1} dw).

3.3. *Accumulation of OMP in mussels*

Table 4 shows the concentration of the target analytes in mussel samples. Individual OMPs concentrations ranged from 1 to 1577 ng g^{-1} wet weight (ww). PAHs and PCBs were the most widespread compounds (100% of detection), followed by APs (90%), OCPs (60%) and finally by plasticisers (30%). No PBDEs were detected. OMPs levels in t_0 mussel were below the detection limits, except for some PAHs and PEs. After an exposure of 21 days (t_{21}), transplanted mussels accumulated OMPs, with Flu. Flr, Pyr B(a)P, NP and PCB 28 the most bioaccumulative compounds.

In t_{21} mussels, PAHs were detected in all sites, being Flr and Pyr the most ubiquitous. ΣPAHs concentrations oscillated between 19 (UR) and 6566 ng g^{-1} ww (FE). Concentrations in decreasing order were as follows: FE >GA >PS >BE >PP >PA >VM >PL >SV >UR. Similar values in all samples, except for FE were detected in *Mytilus galloprovincialis* from the Spanish and French Mediterranean Sea (25 – 337 ng g^{-1} ww) (Baumard et al., 1998a; Bouzas et al., 2011) and from the Gulf of Rijeka, Croatia (49 – 134 ng g^{-1} ww) (Bihari et al., 2007). For FE, similar high values were detected in mussels from Western Baltic Sea polluted harbours (up to 3880 ng g^{-1} ww) (Baumard et al., 1999). In all the mussels analyzed, tetra-PAHs (with 4 rings) were the predominant accumulated PAHs. This ability of mussels to accumulate high molecular weight compounds was evidenced previously by Baumard et al. (1998a). According to the new EQS for biota established by the Proposal 2011/0429, the sum of concentrations of B(a)P, B(b)F, B(k)F, B(ghi)P and I(cd)P in molluscs should not exceed 10 ng g^{-1} ww. This limit was surpassed in PA (22 ng g^{-1} ww), PP (33 ng g^{-1} ww), PS (40 ng g^{-1} ww), GA (68 ng g^{-1} ww) and more than 200 times in FE (2340 ng g^{-1} ww).

APs were detected in t_{21} mussels from 9 sites, being NP the most ubiquitous compound. ΣAPs concentrations ranged from 18 (PA and PL) to 103 ng g^{-1} ww (PP). Concentrations in decreasing order were as follows: PP >UR >SV >GA >PS >VM >FE >PL ≈PA. Similar concentrations were reported for mussels from Valencia, Spain coasts (<7 -120 ng g^{-1} ww) (Bouzas et al., 2011) and from Masan Bay, Korea (considering only NP, 51 – 290 ng g^{-1} dw) (Li et al., 2008). More than 10 times higher levels were reported for benthic invertebrates from a coastal marsh in Canada (6670 – 13400 ng g^{-1} dw) (Mayer et al., 2007). NP is highly lipophylic and may consequently be accumulated by aquatic organisms, particularly bivalve molluscs (Ahel et al., 1993). It was previously noticed that APs, especially (NP and NPEs) are able to mimic the action of endogenous estrogens by binding estrogenic receptors (Crisp et al., 1998; Porte et al., 2006; Soares et al., 2008). Although the risks that NP and NPEOs pose to marine species is well documented, no EQS in biota are reported for APs.

PCB 28 was detected in all t_{21} mussels. ΣPCBs concentrations ranged from 1.0 (SV and PS) to 160 ng g^{-1} ww (PG). Concentrations in decreasing order were as follows: GA >PP >PA >FE >PL >VM >BE >UR >SV ≈ PS. Our results agree with a previous surveillance study realized in the NW Spanish coast during 1991 to 2009 and 2002 to 2004 (*Mytilus galloprovincialis*: 1.0 – 196 ng g^{-1} ww) (Bartolomé et al., 2010; Bellas et al., 2011) with similar PCB profiles. Lower ΣPCBs concentrations were reported for *Mytilus galloprovincialis* from the Western Mediterranean Coast (6 – 17 ng g^{-1} ww) (Sole et al., 1994) and *Mytilus edulis* from Greenland (0.03 – 0.1 ng g^{-1} ww) (Cleemann et al., 2000). The hexachlorinated congeners, PCB 138 and PCB 153, dominated the profiles in mussels from industrial areas as a consequence of their use in commercial mixtures (Bartolomé et al., 2010). EQS for biota only was considered for fish and for the dioxin-like PCB congeners (PCB 77, 118, 114, 105, 126, 156, 169 and 189). In our case, only the dioxin-like PCB 118 was detected in PP and PG mussels.

Among OCPs, only the DDT degradation products, 4,4'-DDE and 4,4'-DDD were found in 6 t_{21} mussels sites. ΣOCPs concentrations oscillated between 4.2 (PS) and 25 ng g^{-1} ww (PG). Concentrations in decreasing order were as follows: GA >PP >FE >UR >SV >PS. Higher concentrations were reported for *Mytilus galloprovincialis* from the Western Spanish Mediterranean Coast (ΣDDTs and lindane: 1.1 – 64 ng g^{-1} ww) (Sole et al., 1994). Lower levels were reported for *Mytilus edulis* from Greenland (ΣDDTs: 0.2 – 0.8 ng g^{-1} ww) (Cleemann et al., 2000) and higher concentrations were reported for *Mytilus edulis* from the Nordic Seas (ΣDDTs+HCHs: 238 – 3480 ng g^{-1} lipid weight) (Gustavson and Jonsson, 1999) and from Bohai coast, China (ΣDDTs: 4.5 – 129 ng g^{-1} ww) (Yang et al., 2004). As OCPs are lipophylic and capable of being biomagnified, they could pose a serious threat to upper trophic levels of aquatic communities (Farre et al., 2002; Garcia et al., 2000) and potentially to humans (Gray et al., 2001). Those pollutants generate growing danger to human health and a vast number of marine organisms, such as, adverse effects on endocrine system (Crisp et al., 1998; Long et al., 1995), cancers and chronic-toxic effects (Dich et al., 1997; Krieger et al., 1994). Despite all these known effects, no EQS are established or proposed for biota in European legislation. ΣPEs concentrations oscillated between 98 (VM) and 658 ng g^{-1} ww (PL) in T_{21} mussels and DEP and DEHP were the only compounds detected in PL >BE >VM V. Similar values were previously reported for oysters from Urdaibai estuary (<10 – 2300 ng g^{-1} ww) (Chaler et al., 2004) and more than 50-fold higher values were reported for native mussels from Bilbao Estuary (1301 – 27664 ng g^{-1} ww) (Bartolomé et al., 2010). PEs have been shown to be carcinogenic and estrogenic and pose in risk the health of human and animals (Staples et al., 1997).

Figure 1 shows the ΣOMPs in the mussels analyzed. Transplanted t_{21} mussels, from FE estuary were the ones with the highest ΣOMPs bioaccumulation (6620ng g^{-1} ww), followed by BE (843 ng g^{-1} ww), PL (728 ng g^{-1} ww), GA (564 ng g^{-1} ww), PP (383 ng g^{-1} ww), PS (256 ng g^{-1} ww), VM (249 ng g^{-1} ww), PA (192 ng g^{-1} ww), SV (105 ng g^{-1} ww) and finally UR (101 ng g^{-1} ww).

Table 4.
Summary data for the target pollutants detected in mussel and sediment samples.

	Mussels (in ng g^{-1} wet weight)										**Sediments (in ng g^{-1} dry weight)**									
	Coastal and estuarine water with WWTP discharges thought submarine emissaries				**Estuarine water with port/harbor activities or industrial effluents**						**Coastal and estuarine water with WWTP discharges thought submarine emissaries**					**Estuarine water with port/harbor activities or industrial effluents**				
Compound	**VM**	**BE**	**SV**	**UR**	**PA**	**PP**	**PL**	**PS**	**FE**	**GA**	**VM**	**PN**	**BE**	**SV**	**UR**	**PA**	**PL**	**PS**	**FE**	**GA**
Nap	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	31	46	22	25	31	43	bdl	750	53	86
Acy	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	4.2	bdl	bdl	59	bdl	bdl	121	287	bdl	1733	100	107
Ace	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	23	68	bdl	bdl	28	91	bdl	6980	91	62
Flu	3.9	3.3	bdl	1.5	bdl	bdl	3.8	2.5	1.7	1.8	28	140	19	23	96	216	38	8922	182	109
Phe	5.9	6.7	5.7	4.8	bdl	bdl	bdl	18	13	6.2	3.0	266	35	76	122	362	135	4975	492	175
Ant	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	21	108	20	40	62	253	87	843	253	108
Flr	56	109	18	7.4	58	90	22	62	1577	98	32	344	41	120	272	1610	26	9744	5479	677
Pyr	35	78	11	5.3	47	71	13	52	1252	99	36	261	155	319	225	1478	33	6932	3723	547
B(a)A	bdl	bdl	bdl	bdl	bdl	6.5	bdl	15	647	24	7.0	131	11	41	91	614	18	2026	2661	243
Chr	6.2	6.8	bdl	bdl	11	6.2	bdl	22	609	38	21	156	17	48	111	708	21	1962	6288	293
B(b)F+B(k)F	3.9	3.7	bdl	bdl	5.7	22	bdl	bdl	190	22	22	133	17	46	114	789	21	2001	8695	326
B(a)P	5.5	bdl	bdl	bdl	9.2	6.1	4.1	24	1361	29	23	126	bdl	46	107	719	bdl	1811	10102	314
I(cd)P	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	13	bdl	bdl	15	bdl	bdl	16	120	bdl	319	929	47
D(ah)A	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	123	bdl	bdl	186	bdl	56	134	1267	bdl	2064	12199	514
B(ghi)P	bdl	bdl	2.9	bdl	7.1	5.1	bdl	16	775	17	bdl	84	11	28	73	616	bdl	1104	7464	254
Σ PAHs	**116**	**208**	**38**	**19**	**137**	**207**	**42**	**210**	**6566**	**335**	**248**	**2123**	**348**	**868**	**1601**	**9174**	**380**	**52166**	**58713**	**3863**
DEP	bdl	576	bdl	bdl	bdl	bdl	603	bdl	bdl	bdl	422	174	bdl	311	532	bdl	5781	984	139	971
DEHP	98	57	bdl	bdl	bdl	bdl	55	bdl	bdl	bdl	32	52	170	36	69	8.6	18	418	48	256
BBP	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	53	8.2	15
DEHA	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	52	40	bdl	8.0	10	75	bdl	825	bdl	58
Σ PEs	**98**	**633**	—	—	—	—	**658**	—	—	—	**505**	**266**	**170**	**355**	**611**	**83**	**5799**	**2281**	**195**	**1301**
OP	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	6.9	38	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	39
NP	32	bdl	22	32	18	58	18	16	20	37	225	94	bdl	bdl	bdl	109	bdl	592	79	303
NP EO_1	bdl	bdl	38	41	bdl	45	bdl	24	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
Σ APs	**32**	—	**60**	**73**	**18**	**103**	**18**	**41**	**20**	**44**	**263**	**94**	—	—	—	**109**	—	**592**	**79**	**342**
PCB 28	2.8	2.5	1.0	1.2	2.3	3.3	2.1	1.0	1.9	12	11	11	bdl	bdl	11	23	10	21	16	41
PCB 52	bdl	bdl	bdl	bdl	bdl	8.8	bdl	bdl	bdl	8.3	bdl	bdl	bdl	bdl	bdl	13	bdl	bdl	bdl	19
PCB 101	bdl	bdl	bdl	bdl	5.8	10	bdl	bdl	4.1	19	bdl	bdl	bdl	bdl	bdl	29	bdl	21	19	40
PCB 118	bdl	bdl	bdl	bdl	bdl	7.3	bdl	bdl	bdl	4.5	bdl	bdl	bdl	bdl	14	21	bdl	20	17	23
PCB 138	bdl	bdl	bdl	bdl	23	22	5.2	bdl	11	54	14	17	bdl	bdl	17	83	12	34	50	118
PCB 153	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	43	bdl	19	bdl	bdl	19	66	14	33	42	100
PCB 180	bdl	bdl	bdl	bdl	6.0	3.8	1.7	bdl	2.3	20	bdl	10	bdl	bdl	9.4	59	bdl	23	35	96
Σ PCBs	**2.8**	**2.5**	**1.0**	**1.2**	**37**	**55**	**8.9**	**1.0**	**19**	**160**	**25**	**56**	—	—	**70**	**293**	**36**	**150**	**179**	**437**
2,4'-DDE	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	7.9	bdl	16
4,4'-DDE	bdl	bdl	5.9	8.3	bdl	12	bdl	4.2	11	15	bdl	bdl	bdl	bdl	bdl	376	bdl	48	140	178
2,4'-DDD	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	4.9	bdl	bdl	bdl	bdl	bdl	314	bdl	bdl	bdl	234
4,4'-DDD	bdl	bdl	bdl	bdl	bdl	5.7	bdl	bdl	3.9	5.4	bdl	bdl	bdl	bdl	bdl	773	bdl	bdl	bdl	713
α-HCH	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	7.9	bdl	bdl	11	15
β-HCH	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	26
γ-HCH	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	69
Σ OCPs	—	—	**5.9**	**8.3**	—	**18**	—	**4.2**	**15**	**25**	—	—	—	—	—	**1471**	—	**56**	**151**	**1251**

bdl: Below detection limit
DMP, NP EO_2, 2,4'-DDT, 4,4'-DDT, α-endsf, β-ends, δ-HCH and all PBDEs were no detected.

3.4. *Effect, exposure and priority risk indexes*

For assessing the potential risks OMPS could produce in the Cantabrian sea coastal environment, two Risk indexes based on the adapted European COMMPS procedure (Teixido et al., 2010) were estimated for sea and estuary waters. The risk associated with the exposure of any of the studied organic pollutants (Priority and exposure risk indexes) and the level of risk in each sampling site (Site pollution risk index) were evaluated. In Figure 3, the behaviour of the different investigated compounds regarding their calculated exposure (I_exp) and effect index (I_eff) values is shown. Those indexes were finally combined into a characteristic Priority Index (I_prio). In Figure 3, the detection frequencies of the target OMPs in coastal and estuarine waters are plotted in decreasing order. According to the rules established in the COMMPS procedure (European Chemicals Bureau, 2003; Teixido et al., 2010), the 37 compounds with an incidence in coastal and estuarine waters >15%, were selected for the estimation of the risk indexes.

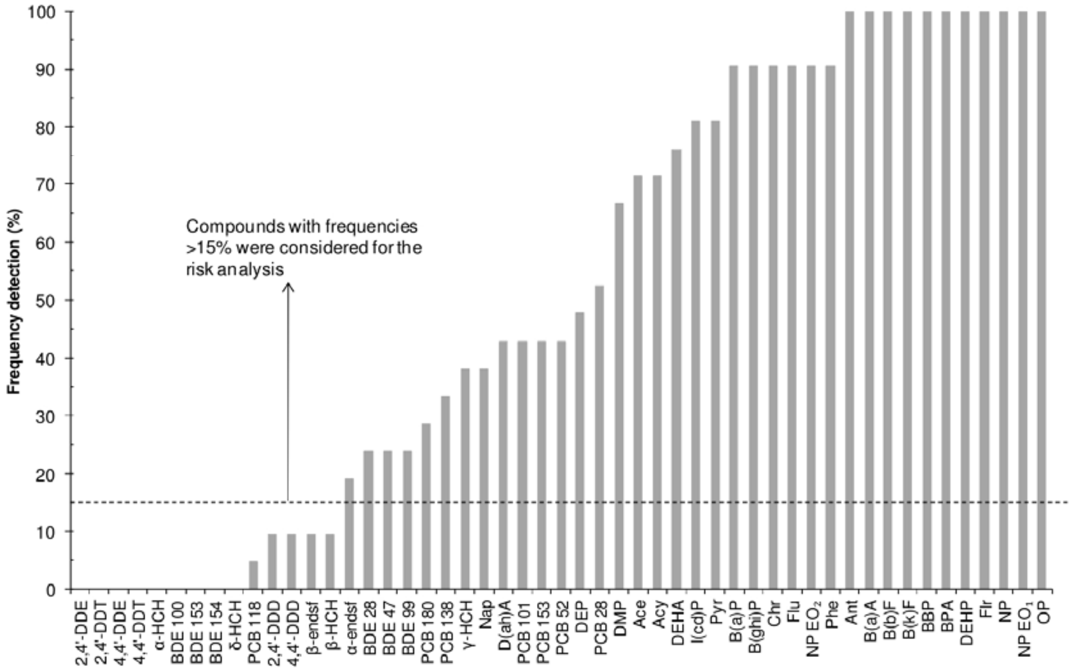

Fig 3. Detection frequency (in %) of the analyzed compounds in the Cantabrian coast/estuarine waters

Figure 4 plots the estimated exposure, effect and priority indexes for the 37 considered compounds. In general, a higher exposure index indicates a higher occurrence in a higher number of locations and at higher concentrations. The exposure indexes estimated in sea and estuary waters from Cantabrian Sea ranged from 2.9 to 8.0 (average, 4.8) being similar to those estimated for rivers from Catalonia, Spain (average, 4.4) (Teixido et al., 2010). Families that showed higher exposure indexes were APs and plasticisers, the most widespread compounds with the highest concentrations (averages: AP, 7.1 and plasticisers, 5.6) followed by PAHs (average, 4.4), OCPs (average, 4.4), PCBs (average, 3.3) and finally the less ubiquitous and with lowest concentrations PBDEs (average 2.8). $NPEO_2$, $NPEO_1$ and NP were the first 3 ranked compounds.

The effect index gives an indication of the danger of a particular chemical compound for humans and aquatic organisms, taking into consideration its persistence, toxicity and bioaccumulation capacity. By chemical families, the highest values corresponded to PCBs (average 8.9), followed by PAHs (average 8.5), PBDEs (average, 7.7), OCPs (average 7.4), APs (average 6.1) and finally plasticisers (average 5.8). B(ghi)P, D(ah)A and B(k)F were the first 3 ranked.

Among the studied compounds and according to the priority index, NP was the substance with the highest value. NP had a high exposure and high effect indexes, indicating that unfortunately, this endocrine disruptor compound is highly widespread along the Cantabrian Sea. B(ghi)P, the second ranked compound, had high exposure, but a lower effect index compared with NP. PCBs and high molecular PAHs (with 5 or 6 rings) had high effect index but with a rather low detection in Cantabrian coastal/estuarine waters.

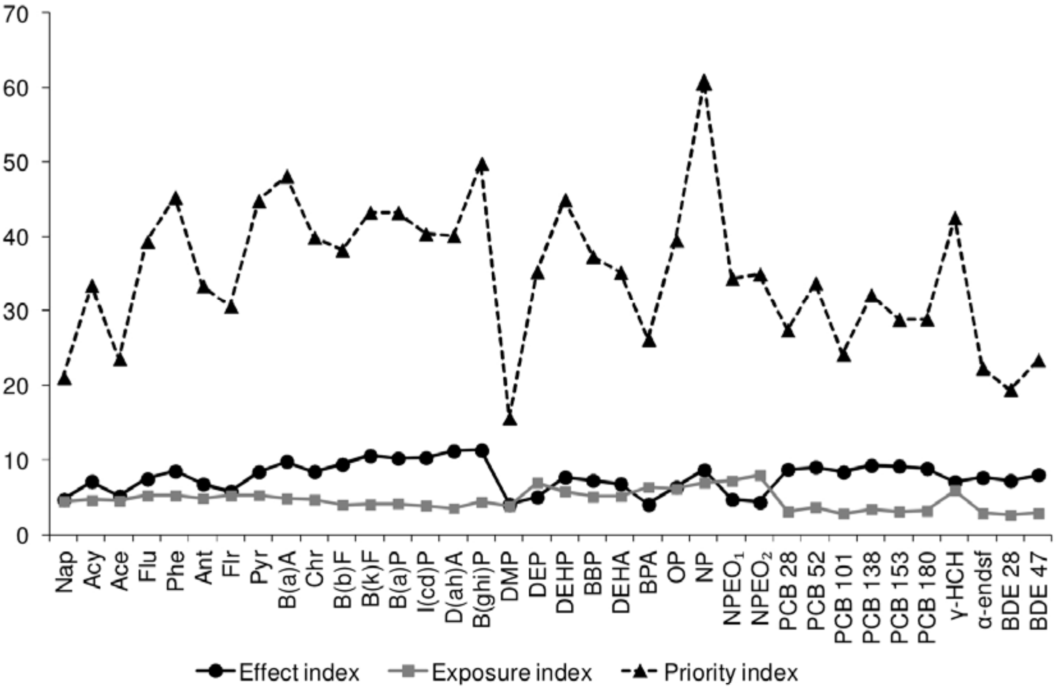

Fig. 4. Results of the effect and exposure indexes calculated using the COMMPS procedure and the priority index for the target OMPs

3.5. Site pollution risk index

Site pollution risk index was calculated for the coastal and estuarine waters using a robust methodology proposed by Teixidó et al. (2010). Since target compounds were detected in the 11 sites with a frequency higher than 20%, all the sites were included in the site pollution risk estimation. Figure 5 shows the distribution of the site pollution index over the Cantabrian Sea. The index values were normalized and divided in 5 categories depending on its range of pollution (being 0 de lowest level and 1 the upper one) and displayed with a different colour pattern in a map. Results showed that the high impacted areas, with industrial activities and/or ports-ship traffic and/or effluents and sewers discharges had higher risk indexes. Results evidenced that FE and GA, were the most impacted sites. Following, port activities, WWTP discharges, industrial and urban discharges and historical contamination due to metal works could explain this pollution distribution. The third ranked sites were PA and PS, with important ship traffic, sewers overflow, recreational activities and other diffuse sources, which contribute significantly to the pollution of this area. The less risk areas were the coastal sites of BE, PÑ, VM and the estuarine SV and suggest that submarine emissaries or uncontrolled discharges to open seas do not have a high contribution on risk coefficients. Probably, the effect of sea currents are important in the dilution of the pollutants' burden. These results show the distribution of the pollution risk over the study geographical area (Fig. 5), and can be used as a tool for the implementation of the different environmental policies at a regional scale.

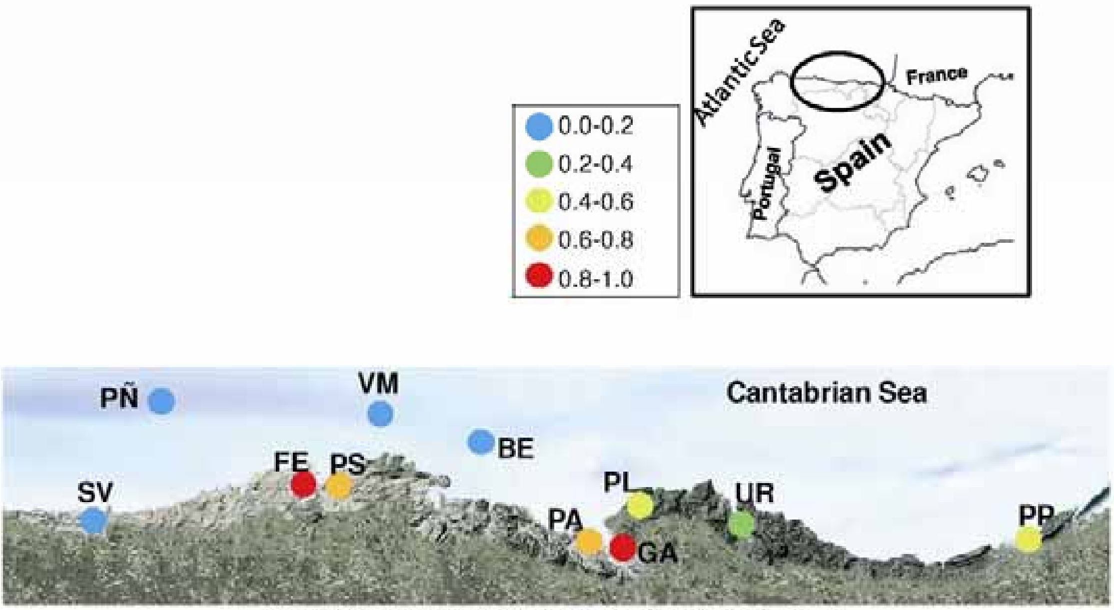

Fig 5. Site pollution risk index in the Cantabrian Sea area

3.6. *Conclusions*

The analysis of the 51 target OMPs in waters and sediments from coastal areas and estuaries from the Cantabrian Sea evidenced the widespread pollution along this area caused by point source pollution due to WWTP effluents, industrial discharges and port activities. APs, PE and combustion sources (according to PAHs ratios in sediments) were identified as main pollutants to the sea. Specifically, WWTP effluents discharge daily to receiving waters 19880 g OMPs while submarine emissaries discharge 9675 g d^{-1}. In waters, the levels of NP, B(b)F, B(ghi)P and HCH isomers in some sites exceeded the EQS established by Directive 2008/105/EC and the Proposal 2011/0429. On the other hand, caged mussels were used as bioindicators of pollution and revealed that accumulation occurred within 21 d of exposure with some PAHs, NP and PCB 28 as main accumulative compounds. Finally, sediment samples were good indicators of pollution and showed high OMPs loads in areas with harbor and industrial effluents. The OMPs levels in sea and estuarine waters and sediments were generally similar to other polluted areas in Spain and around the world. Chemical analyzes were reinforced by the COMMPS risk procedure, taking into account not only the pollutant burden, but also toxicological data. The use of the site pollution specific risk index allows for fast visualization and a comparison of the more environmentally stressed coastal/estuarine location areas. The proposed index can be easily used to determine the risk of OMPs in coastal areas, once OMPs releases through emissaries, ports or industrial effluents are identified and long term pollution sources.

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3.3 Discusión de los resultados

Las dos campañas de muestreo desarrolladas en las costas de las provincias de Cataluña y de Asturias-Cantabria-País Vasco permitieron recolectar un total de 125 muestras en aguas costeras y de transición (estuarios). Estos dos programas han permitido incrementar el conocimiento de la presencia de contaminantes orgánicos prioritarios y emergentes en las zonas costeras. En todas las muestras, sin excepción, se detectó al menos uno de 42 OMPs. Nueve compuestos no se detectaron (bdl) en ninguna muestra (2,4'-DDE, 4,4'-DDE, 2,4'-DDT, 4,4'-DDT, β-HCH, δ-HCH, BDE 100, BDE 153 y BDE 154). El compuesto más ubicuo fue el BPA (96%) seguido de DEHP (95%) y BBP (93%). Los OMPs se detectaron en un amplio intervalo de concentraciones, desde 0.14 (PCB 28) hasta 35,583 ng L^{-1} (NPEO$_2$). La vigilancia ambiental en aguas de las costas y estuarios permitió divisar incumplimientos de la NCA para la suma de I(cd)P y B(ghi)P (2 ng L^{-1}) en 23 sitios, la suma de B(b)F y B(k)F (30 ng L^{-1}) en 1, NP (2000 ng L^{-1}) en 1, BDE 28 (2.0 ng L^{-1}) en 1, BDE 47 (2.0 ng L^{-1}) en 2 y la suma de HCHs (40 ng L^{-1}) en 2. La Figura 25 muestra el intervalo de concentraciones de ΣOMPs en las dos zonas de estudio. Los compuestos detectados en mayor concentración fueron los APs y plastificantes y, en menor concentración, los PBDEs.

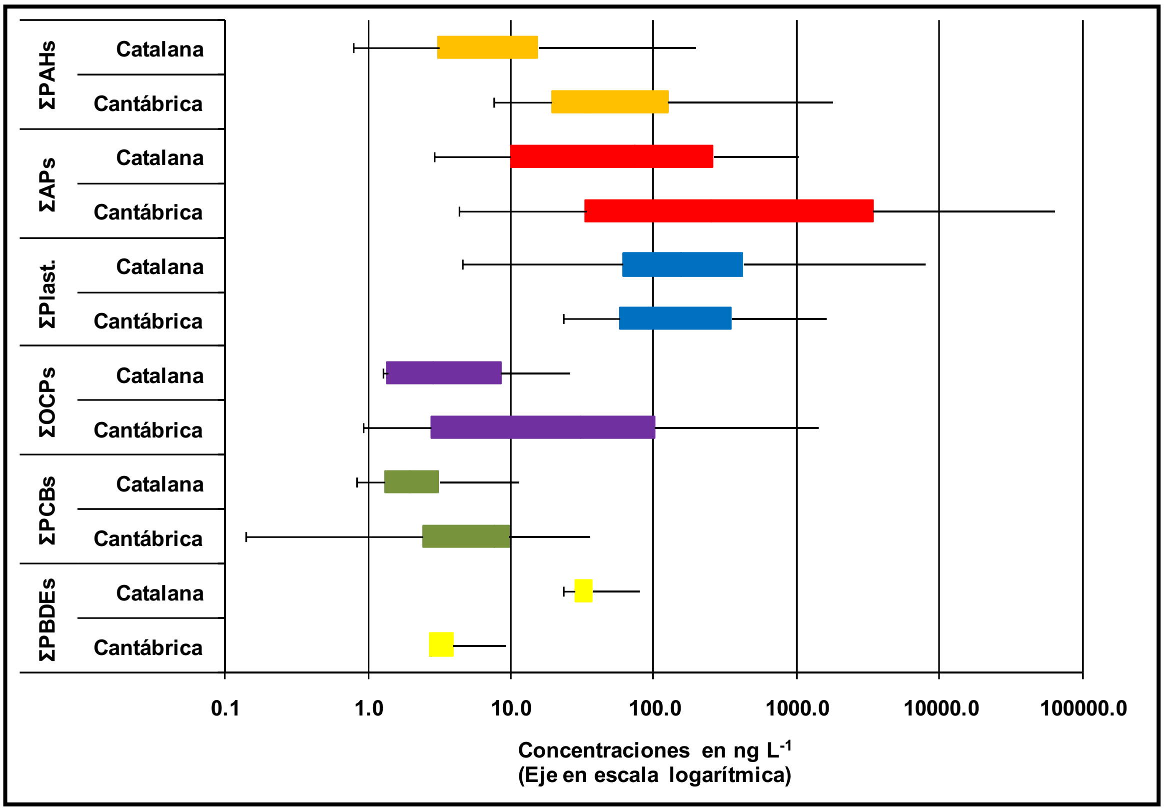

Figura 25. Gráfica de cajas de las concentraciones de OMPs detectados en las aguas costeras en la costa Catalana y estuarios y costas en el mar Cantábrico

Los 5 sitios de muestreo con mayores niveles de contaminación, expresados como ΣOMPs (mencionados en orden decreciente) fueron el estuario cerca del vertido de la EDAR de Galindo (País Vasco, 61147 ng L^{-1}), el Puerto de Barcelona (Cataluña, 8443 ng L^{-1}), el Puerto de Arriluce (País Vasco, 6294 ng L^{-1}), el Puerto de Pasaia (País Vasco, 5268 ng L^{-1}) y el estuario cerca del vertido industrial de la compañía Ferroatlántica (Cantabria, 3125 ng L^{-1}). Los 5 sitios con menores niveles de contaminación (mencionados en orden creciente) fueron la costa de Vinarós (Cataluña, 17 ng L^{-1}), la costa de Peñíscola (Valencia, 32 ng L^{-1}), la costa de Calella (Cataluña, 51 ng L^{-1}), la costa de Virgen del Mar (Cantabria, 74 ng L^{-1}) y el Puerto de Palamós (Cataluña, 83 ng L^{-1}). En general, las concentraciones detectadas en los estuarios y costas del Mar Cantábrico fueron superiores a las de la zona Catalana. Esto está relacionado con que los estuarios son zonas confinadas y además refleja la elevada actividad industrial y portuaria desarrollada.

En la Figura 26 se muestran los intervalos de concentración de los OMPs en los efluentes de las EDAR evaluadas en ambas campañas. Los OMPs individuales variaron en un intervalo entre 1.6 (BDE 99) y 15,232 ng L^{-1} (DMP). Los compuestos más ubicuos fueron Nap, OP, NP, DEP y DEHP (100%). En ninguno de los efluentes se detectó la presencia de 2,4'-DDE, 4,4'-DDE, 2,4'-DDT, 4,4'-DDT, α-HCH, β-HCH, δ-HCH y β-endos. La EDAR con una concentración de OMPs más elevada en el efluente fue la de Galindo (País Vasco, 44333 ng L^{-1}), posteriormente la de San Román (Cantabria, 32957 ng L^{-1}) y en tercera posición la de Tarragona (Cataluña, 15552 ng L^{-1}). En las EDAR de Cataluña se detectaron entre 7 y 14 contaminantes, en cambio en las de Cantabria entre 33 y 41. En estas últimas se detectaron PAHs de elevado peso molecular así como PCBs, PBDEs y trazas de OCPs, contaminantes que están íntimamente relacionados con descargas provenientes de industrias, procesos de combustión y otras actividades agrícolas. Así como sucedió en el caso de las aguas de mar y de transición, las concentraciones en los efluentes de las dos EDAR recolectadas del Norte de España, son mayores que las de las EDAR de Cataluña, en especial los APs y plastificantes.

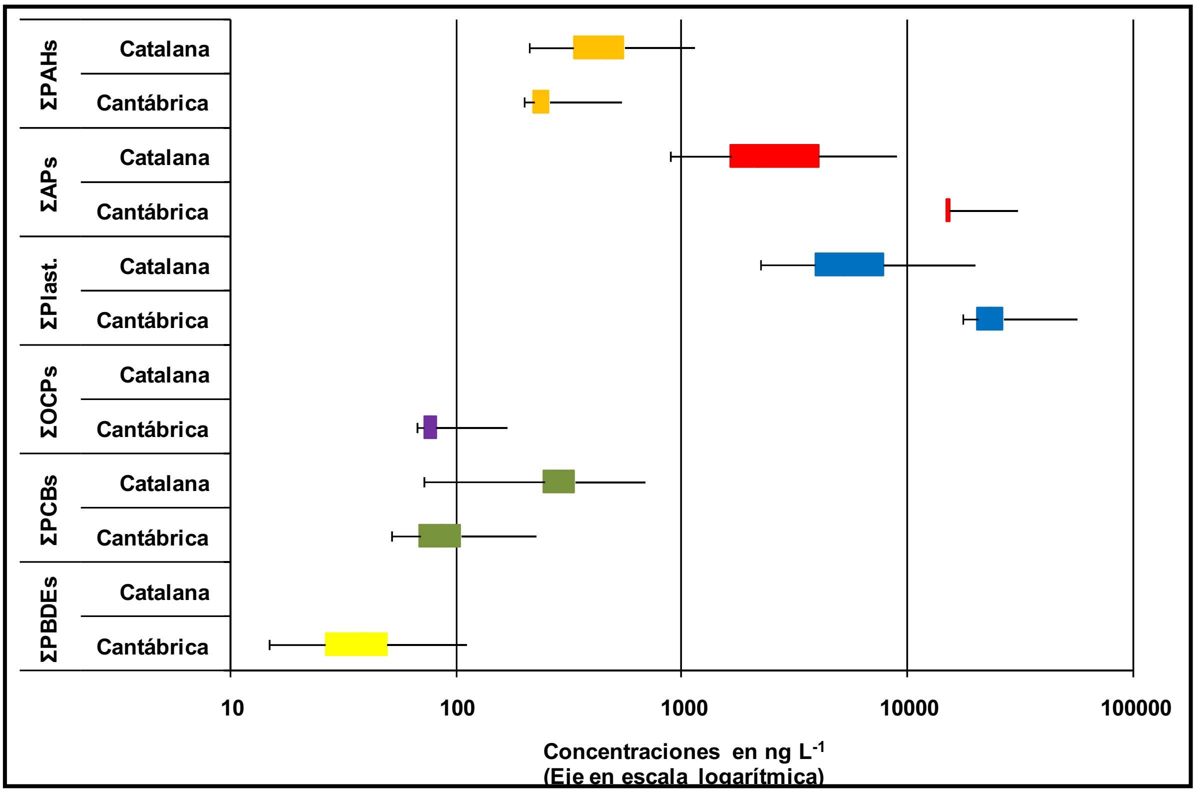

Figura 26. Gráfica de cajas de las concentraciones de OMPs detectados en los efluentes de las 8 EDAR Catalanas y las 2 EDAR del Mar Cantábrico

El comportamiento en la columna de agua, de los OMPs vertidos desde emisarios submarinos se puede apreciar en un estudio realizado a posteriori. Se tomaron aguas de mar a diferentes profundidades a partir de la boca de 3 emisarios submarinos de Asturias-Cantabria y País Vasco. Los tres emisarios muestreados se sitúan aproximadamente a 2 km de la costa, uno en Virgen del Mar (VM), el segundo en Peñarrubia (PÑ) y el tercero en Berria (BE). En la Figura 27 se muestran los perfiles de concentración de OMPs en la columna de agua, en el fondo (boca del emisario), a media profundidad y en la superficie. Las concentraciones de APs y plastificantes en la boca de los emisarios se encuentran entre los 100 y 1000 ng L^{-1} y a medida que se asciende a la superficie las concentraciones se diluyen, disminuyendo un orden de magnitud.

La elevada-media solubilidad de los APs y plastificantes permite que estas sustancias sean transportadas por las corrientes [92; 97] y aunque pueden sufrir degradación, la descarga de estas sustancias es continua pudiendo ocasionar efectos a largo plazo. Los PBDEs y PCBs fueron encontrados sólo a la salida del emisario. Estos son menos solubles y tienen mayor valor de K_{ow}, por lo que tienden a ser secuestrados por la materia particulada y depositarse en los sedimentos. El perfil de los PAHs es completamente diferente, ya que a las concentraciones a la salida del emisario se diluyen a media profundidad, pero en la superficie vuelven a aumentar. Esto es a consecuencia de que estas sustancias provienen principalmente de los procesos de combustión, son transportados por la atmósfera y en regiones donde el tráfico marítimo es intenso, parece haber una recarga de estos contaminantes [278]. Por esta razón se puede concluir que la presencia de PAHs en la superficie de las aguas costeras en esta región no es totalmente atribuida a las descargas de los emisarios, sino que también existen otras fuentes como el transporte atmosférico.

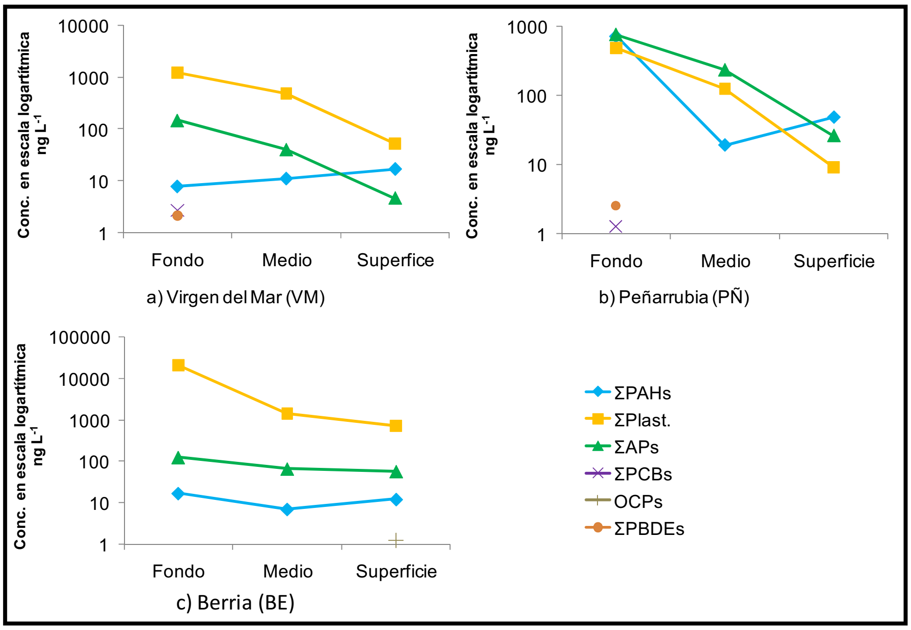

Figura 27. Perfil de concentraciones de OMPs en la columna de agua de mar, a partir de la salida de los emisarios submarinos de EDARs

Los resultados de PCA para las aguas de Cataluña demostraron que las principales fuentes de contaminación en las aguas costeras son las descargas de las EDAR y los ríos, pero además existen otras fuentes importantes que contribuyen al deterioro de la calidad química de estas vulnerables zonas. Entre ellas se pudieron destacar procesos de combustión ya sea de origen industrial, doméstico o proveniente de actividades portuarias. Este mismo comportamiento se pudo apreciar al analizar los sedimentos de la costa Cantábrica, en donde los contaminantes más ubicuos fueron los PEs y PAHs de elevado peso molecular. En ambas regiones se observa además una marcada influencia del uso histórico de pesticidas al detectarse en los ríos Catalanes y sedimentos cantábricos elevadas concentraciones de productos de degradación de DDT (DDE y DDD) y otros OCPs también restringidos en la CE tales como lindano y α-endsf.

Los mejillones trasplantados después de 21 días de exposición bioacumularon en los sitios con mayor actividad industrial y portuaria, contaminantes con potencial carcinogénico como PAHs de elevado peso molecular tales como I(cd)P, D(ah)A, B(ghi)P, Chr, B(b)F y B(k)F, así como los congéneres PCB 28, 52, 101, 118, 138 y 180 y metabolitos de DDT. A pesar de conocerse los efectos tóxicos del DDT y otros OCPs, no existen NCA para biota en la legislación europea. Aunque estos mejillones trasplantados no serán ingeridos, se ha hecho una comparación con base en niveles aceptables de ingesta diaria (ADI o "acceptabled daily intakes") de DDT propuestos por la FAO-UNEP, que corresponde a 5000 ng kg^{-1} d^{-1}. Para una persona de 70 kg, la ADI sería de 350000 ng d^{-1} [279]. Si se estima un consumo de 1 kg de estos mejillones, una persona estaría ingiriendo una dosis de DDT entre 4200 a 25000 ng g^{-1}, valor por debajo de la ADI. Sin embargo, en Canadá la ADI de DDT es de 400 ng g^{-1} [279]. Para una persona de 70 kg, correspondería a 24000 ng g^{-1}, valor que se excedería únicamente en los trasplantados en Galindo (25000 ng g^{-1}). Para el DEHP se encontraron también valores de ADI, que corresponde a 50000 ng kg^{-1} peso corporal [280]. Para una persona de 70 kg, consumiendo 1 kg de mejillones trasplantados en los sitios del Cantábrico, la ingesta de DEHP estaría más de 500 veces por debajo del ADI (3897 – 6831 ng g^{-1}). Los mejillones también bioacumularon otros OMPs con propiedades de disrupción endocrina como el NP. Esto puede representar problemas tanto a nivel ecosistema como de salud humana.

Por otro lado, el análisis de 5 PFCs en las aguas costeras, puertos, ríos y efluentes de EDAR de Cataluña representó el primer estudio de la presencia de estos contaminantes en esta región costera (Figura 28). Si se comparan las concentraciones detectadas con los resultados reportados en otras partes del mundo, se hace evidente la ubiquidad de estas sustancias, en especial de PFOS y PFOA, tal y como se muestra en la Figura 29.

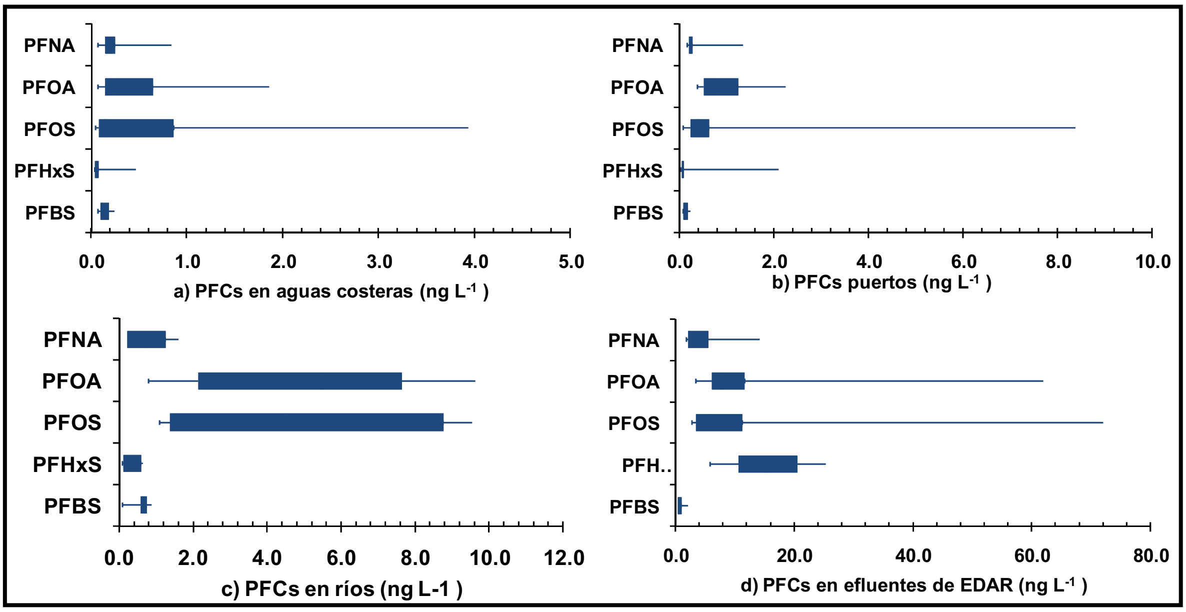

Figura 28. Gráfica de cajas de las concentraciones de PFCs detectados en las aguas costeras, puertos, ríos y efluentes de EDAR en Cataluña

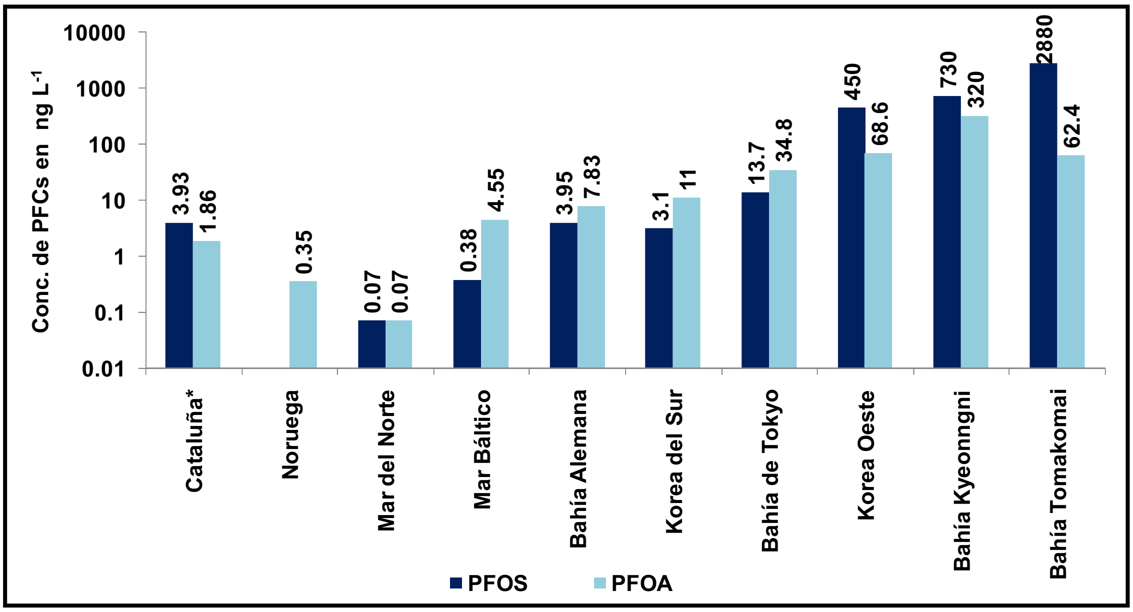

Figura 29. Concentración (eje en escala logarítmica) de PFOS y PFOA en aguas de mar de diferentes regiones del mundo (* estudio actual)

La metodología basada en RQs y el procedimiento COMMPS demostraron su capacidad para estimar el riesgo ocasionado por la presencia de OMPs prioritarios y emergentes en las regiones evaluadas. La integración de datos de toxicidad, bioacumulación y efectos en la salud humana en el procedimiento COMMPS hace de ésta metodología una herramienta más exacta y confiable para definir el impacto de la contaminación. En la región litoral del Cantábrico la sustancia que representó un mayor riesgo para el ambiente costero, tanto por su presencia como por sus efectos fue el compuesto prioritario NP. El hecho de contar con sólo una réplica por sitio en la campaña de muestreo en la Costa Catalana no permitió la aplicación del procedimiento COMMPS. Sin embargo, para fines de un estudio inicial, la estimación del riesgo por medio de RQs ha sido adecuada, pues se apreciaron las zonas con mayor impacto y en las cuales se debe realizar una vigilancia más exhaustiva, con un monitoreo más frecuente y así poder adoptar medidas para la remediación de la contaminación. Cabe mencionar que como trabajo adicional se realizó una nueva estimación del riesgo para las aguas costeras de Cataluña sumando a los resultados reportados los RQs calculados a partir de la vigilancia ambiental de PFCs. Sin embargo, como las concentraciones detectadas de PFCs se encuentran varias veces por debajo de los valores de toxicidad, los RQs son tan pequeños que no cambian sus valores.

Para reforzar los hallazgos en cuanto al riesgo ambiental, se recomienda realizar análisis toxicológicos in-situ para clarificar los efectos sinergísticos o de competición que puedan producirse en el medio.

CONCLUSIONES GENERALES

Los estudios llevados a cabo en la presente tesis han permitido llegar a las siguientes conclusiones:

1. Las metodologías multiresiduales desarrolladas permitieron el análisis de 51 contaminantes orgánicos semivolátiles de las familias de PAHs, APs, PCBs, PBDES, OCPs, PEs y BPA y 5 compuestos orgánicos polares de la familia de los PFCs en matrices ambientales relacionadas con las aguas costeras, tales como aguas de mar, aguas residuales, aguas de río, sedimentos y mejillones.

2. Las metodologías multiresiduales mostraron un excelente rendimiento (entre 69 y 120%) y una sensibilidad óptima (límites de detección entre 0.01 a 38 ng L^{-1} para aguas de mar) para ser utilizadas como herramientas en la vigilancia ambiental de contaminantes orgánicos prioritarios y emergentes.

3. Los estudios de vigilancia ambiental llevados a cabo permitieron identificar 47 contaminantes orgánicos en aguas costeras españolas, en el litoral Catalán y en litoral de Asturias-Cantabria-País Vasco, siendo las concentraciones detectadas mayores en estuarios y puertos que en costas y bocas de ríos. Las concentraciones detectadas se encontraron por debajo de los Niveles de Calidad Ambiental establecidos por la Directiva 105/2008/CE, excepto en algunos sitios para I(cd)P, B(ghi)P, B(b)F, B(k)F, NP, BDE 28, BDE 47 y HCHs.

4. Las descargas de efluentes y ríos fueron identificadas como las principales fuentes de contaminación de las aguas costeras. Es evidente que existen otras más como las actividades marítimas y otras escorrentías agrícolas, urbanas e industriales que al no estar identificadas, no fue posible estimar su aporte.

5. La contaminación descargada a través de los emisarios submarinos de las plantas depuradoras se diluye tanto de manera horizontal como vertical, esparciendo los contaminantes por las aguas costeras. A corto plazo esta contaminación no representa un riesgo para estas zonas, pero si podría serlo a un largo término.

6. Además de distribuirse en la columna de agua, los contaminantes orgánicos prioritarios y emergentes estudiados se acumulan en sedimentos, pudiendo ser por tanto disponibles para organismos bentónicos como los mejillones.

7. Los mejillones son buenos indicadores de la contaminación ambiental y permiten determinar la biodisponibilidad de los contaminantes. La bioacumulación de contaminantes carcinogénicos y disruptores endócrinos en mejillones

trasplantados en zonas con elevado impacto antropogénico podría representar un riesgo para otras especies de organismos expuestos a los elevados niveles de concentración.

8. El análisis de riesgo demostró que las zonas potencialmente más afectadas por los contaminantes orgánicos son los puertos y sitios cercanos a las descargas de EDAR y ríos.

9. Los datos de la distribución geográfica de los contaminantes que se obtienen del análisis de riesgo pueden ser aplicados en programas de gestión y tomar medidas pertinentes para alcanzar una calidad adecuada de las aguas costeras.

10. Es recomendable ampliar el número de muestras en las regiones que parecen tener un índice de riesgo mayor, con la finalidad de determinar si el riesgo se mantiene a través del tiempo.

11. Es recomendable el abarcar otras regiones costeras aún no estudiadas y con ello determinar el o los contaminantes cuya presencia represente un elevado riesgo para el ambiente costero y con ello recomendar su inclusión en la lista de sustancias prioritarias en el ámbito de la política de aguas.

12. Es recomendable incrementar el número de contaminantes contemplados en las metodologías multiresiduales, sin sacrificar su sensibilidad y eficiencia, y aplicarlas en la vigilancia ambiental de las costas, con el fin de ampliar el conocimiento de la presencia de contaminantes en las zona costeras.

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