ENVIRONMENTAL RISK ASSESSMENT BY MICROPLASTICS POLLUTION ON CATALONIA COASTAL AREAS

Nora Exposito Lorenzo

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Environmental Risk Assessment by Microplastics pollution on Catalonia coastal areas

DOCTORAL THESIS

UNIVERSITAT ROVIRA i VIRGILI

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FAIG CONSTAR que aquest treball, titulat "**Environmental Risk Assessment by Microplastics pollution on Catalonia coastal areas**", que presenta .**Nora Exposito Lorenzo**.per a l'obtenció del títol de Doctor, ha estat realitzat sota la meva direcció al Departament d'Enginyeria Quimica d'aquesta universitat.

HAGO CONSTAR que el presente trabajo, titulado "**Environmental Risk Assessment by Microplastics pollution on Catalonia coastal areas**", que presenta **Nora Exposito Lorenzo**.para la obtención del título de Doctor, ha sido realizado bajo mi dirección en el Departamento de Ingeniería Química de esta universidad.

I STATE that the present study, entitled "**Environmental Risk Assessment by Microplastics pollution on Catalonia coastal areas**.", presented by **Nora Exposito Lorenzo** for the award of the degree of Doctor, has been carried out under my supervision at the Department of Chemical Engineering of this university.

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Abbreviations

MPs: Microplastics

OECD: Organisation for Economic Co-operation and Development

UNEP: United Nations Environment Programme

FAO: Food and Agriculture Organization of the United Nations

ECHA: European Chemical Agency

EFSA: European Food and Safe Authority

EP: European Parliament

WWTP: Waste Water Treatment Plant

PES: Polyester

PA: Polyamide

PP: Polypropylene

PE: Polyethylene

PVC: Polyvinylchloride

EPS: Expanded polystyrene

PET: Polyethylene terephthalate

PS: Polystyrene

PMMA: Polymethyl methacrylate

PU-PUR: Polyurethane

ABS: Acrylonitrile butadiene styrene

PC: Polycarbonate

PAN: Polyacryilonitile

EVA/EVOH: Ethylene-vinyl acetate/Ethylene vinyl alcohol

SEAC: Committee for Socio-economic Assessment

RAC: Risk Assessment Committee

REACH: Registration, Evaluation, Authorization and Restriction of Chemicals regulation

FTIR: Fourier-transform infrared spectroscopy

GENCAT: Generalitat de Cataluña

Contents

Summary

Currently Micoplastics (MPs) definition considers physical and chemical defining properties beside size and origin as some authors have stablished. They are defined as "any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1 μm to 5 mm, of either primary (intentionally manufactured microbeads and fibres) or secondary origin (mesoplastics breakdown in fragments), which are insoluble in water". MPs are persistence in the environment because the majority of mesoplastics have life-time between hundreds to thousands of years and have been ubiquitous in marine sediment, surface seawater and sand, water column, deep sea floor, aquatic organism, freshwater, atmosphere, and soil. The Mediterranean Sea receives approx. 100,000 tons of plastics per year, 50% of which are from terrestrial contributions from cities near the coast and 30% come from river discharges, these plastic discharges can become Microplastics (MPs) due to fragmentation, increasing MPs loads. Similarly, river discharges receiving high and constant volumes of treated wastewater (although they present a high removal efficiency) combined with macro and mesoplastics fragmentation and inputs of agricultural and urbane runoff increases MPs load in rivers mouth. In addition, tourism increase human pressure in summer season by multiplying the population in coastal areas by several times. For this reason, Mediterranean Sea currently has been proposed as the sixth greatest accumulation zone for marine litter and the most affected regarding to MPs (243,853 plastic pieces/km^2, 82% of them in microplastic form). According to a recent study that MPs pollution was modelled in the Mediterranean Sea, one of the most contaminated areas (seawater and bottom sediments) is the Catalan Sea, between Balearic Island and Catalonia (NE, Spain). MPs can be ingested by low trophic suspension, filter and deposit feeders, detritivores, and planktivorous. MPs ingested may be excreted either as waste or retained/translocated into tissues, making trophic transfer in food chain. During the process of ingestion, the physical components of MPs can make harmful effects to the organisms. MPs may present a physical hazard by: nutrient dilution, internal abrasion, clogging feeding appendages or the digestive system; decrease the feeding activity and stimulus, mobility and weight, reduction on the growth of microalgae, significant reduction in the chlorophyll content and photosynthetic efficiency, decreased growth rates, lower steroid hormone levels, impairment of the reproductive system and even mortality. Other effects include oxidative stress, alteration in enzyme production and metabolism, and embedment in tissues. Intake of different MPs shapes (fragments, beads, fibres, films) by molluscs have been demonstrated, in turn, those contaminants present in water are absorbed in the MPs, which are then transferred to aquatic organisms. On the other hand, according to EFSA (2016), the processing and packaging of seafood can be another source of contamination for MPs. Bivalves are responsible for a large part of human exposure to MPs because they filter a large volume of seawater, being able to accumulate MPs from the environment. In relation to human exposure through consumption of

aquatic species, it will depend on the part of which is consumed. It has been observed that most MPs are retained in the digestive tract and the gills. Microplastic less than 150 μm in diameter have the potential to translocate into human tissues, trigger a localized immune response. Animal studies and "*in vitro*" assays in human cells lines have shown possible adverse effects from exposure to Nano and MPs (oxidative stress and structural damages, reproductive endocrine disruption system in fishes, as well as toxicity in gastrointestinal, liver and neuronal system). Gene expression alteration and genotoxicity, as well as changes in gut microbiota -with seafood being a major medium of exposure- have been also observed. In recent years, the increasing awareness of the population regarding plastic marine pollution and the conflict between the two main economic sectors in Catalonia coast region, industry and tourism, emphasise the importance of this topic for the region. Due to the potential risk posed by MPs to the marine environment and humans, there is a great interest among the scientific community to develop standardized methods of analysis to quantify and characterize the levels of microplastic contamination in coastal areas. Given the biological richness and concentration of aquaculture and fisheries economic activities in the Mediterranean Sea, the effects of plastic pollution on marine and human life are expected to be particularly frequent in this plastic accumulation region. To contribute to knowledge about MPs hazards in environment, the general objective of this Thesis is to provide scientific knowledge on the origin, levels, dynamics and impact of MPs on coastal ecosystems in order to improve their management and preservation of marine environment. In addition, provide tools for legislation, and ultimately contribute to knowledge to prevention and mitigation pollution measures**.** The results will help the policy makers and regulators take sound decisions that protect the environment as: deeper polymer research and development, changes in waste management, chemical legislation review about plastic additives and verified current overconsumption of plastic products to control their production, use, disposal, and recycling. The Catalan coast is analyzed as a case study because MPs pollution might to affect the good ecology state of regions as Ebro's Delta or get worse the deficient (very degraded) ecology state localized in main ports. The specific objectives included: to optimize the methodology of MPs extraction in environmental samples waters, marine sediments, beach sands and edible molluscs of Catalan coast as a contribution to global standardization methodology, to obtain integrated view of the concentration, composition and distribution of MPs in coastal environment and possible sources and to assess human exposure of MPs by autochthonous shellfish ingestion, to characterize the environmental risk by MPs pollution in Catalonia coastal areas.

Among the main results are Methods of analysis of MPs, with different sequential steps, including visual sorting, density separation, alkaline hydrolysis, oxidation (Fenton/hydrogen peroxide), surfactant use, enzymatic hydrolysis, fluorescence with Nile Red staining and spectroscopic techniques (FTIR/RAMAN). These methods have shown to be effective for the extraction, quantification, classification and polymer characterization of MPs in seawater, subtidal marine

sediments, sandy beach sediments and molluscs with recovery rates spanning from 60 to 100 %. MPs were ubiquitous and exhibited different distribution pattern in environmental matrices (seawater surface, beaches and marine sediments) on Catalonia coast possibly by their physical-chemical properties and interaction with environmental and climate parameters. Towards to southern zones in Catalonia coast, it seems the seawater, subtidal sediments and beaches with high pollution level increases. High levels of MPs pollution (danger) according PLI index was observed in superficial seawater of Tarragona harbour area. Currently there is no threat or risk by MPs pollution >80µm sizes in seawater surface (upper pelagic compartment) on Tarragona coast according RCR and RI index because concentration in the upper pelagic compartment remains below the safe concentration. All seawater surface sites are composited by polymers with high hazard score. MPs ≥0.5 mm in subtidal marine sediments on Tarragona coast not showed threat or risk by MPs pollution according RCR and PLI index, but in Tarragona harbour (industrial zone) sediments high pollution levels were observed. MPs <0.5 mm cause pollution risks according RDR, PLI and RI indexes in marine sediments ubicated in: Miracle beach north, Pineda beach north, Pineda beach south, Cambrils-Cavet beach and Hospitalet beach of Tarragona centre-south zone. Almost all sites of marine sediments showed high hazard polymer index risk, composited by polymers with high hazard score as PVC and ABS. According PLI index estimated with MPs <0.5 mm abundance, more polluted beaches from Catalonia coast are ubicate in South Barcelona, Tarragona and South Catalonia zones. Zone less polluted resulted North Catalonia and North Barcelona. Highest PLI values were observed in La Pineda beach. In majority Tarragona city coast beaches and subtidal sediments sites showed the same PLI and hazard level.

Consumption of molluscs is as an important route of exposure to MPs for the population of Catalonia coast. The daily intake of MPs through molluscs consumption was estimated to be 22.2, 20.4 and 9.67 MPs, for adults, elderly and pregnant women, respectively. The mean annual MPs (≥ 20µm) consumption for the adult population was estimated in 8,103 MPs, with 95th percentile of 19,418 MPs/year. Spain annual intakes by MPs concentrations were higher than obtained for other worldwide zones due mainly to higher MPs concentration in different molluscs groups of Catalonia coast and relatively higher consumption for Spain population. The bivalve depuration process did not remove MPs from the organisms. Only changes in their morphology, size and composition were observed. The time of depuration could be a key parameter.

1.-Introduction

1.1 Plastic production and waste generation

The global generation of plastic has shown a continuous growth for more than 50 years, in 2002 the production was 204 million tonnes while in 2019 was 367 million tonnes, without including the production of recycled plastics (PlasticsEurope, 2015; PlasticsEurope, 2021). Thus, increase in production was observed mainly in packaging, with a useful life less than 5 years, from 39.6 to 40.5%, and electrical and electronics from 5.6 to 6.2 % (PlasticsEurope, 2015; PlasticsEurope, 2021). It should be noted, however, that these data do not include the production of synthetic fibres used in the textile sector, which was estimated to be 59 million tonnes in 2015 (Geyer et al., 2017).

The total production of plastic from the 1950s to 2015 is estimated at 7.8 billion tonnes, half of which has been produced only in the last 13 years (Geyer et al., 2017). About 2.5 billion tonnes are currently in use, while 4.9 billion tonnes have become waste, 800 million of them have been incinerated and the rest distributed between landfills and the environment (Figure 1). The global flow of plastic materials is far to be circular, it is estimated that only 600 million tonnes have been recycled and of these recycled plastics, once used, only 10% are recycled again (Geyer et al., 2017). In addition, using worldwide data on solid waste management, population density, and economic status, Jambeck et al. (2015) estimated that the mass of land-based plastic waste entering to oceans annually were around 8 million tonnes ranging from 4.8 to 12.7 million tonnes.

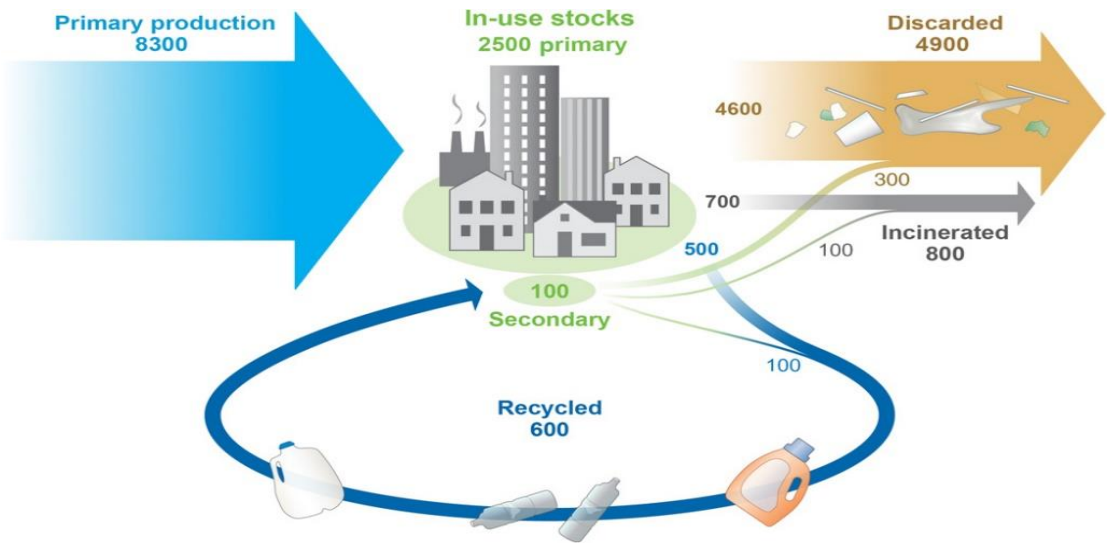


Figure 1.1 Overall production, use and distribution of plastic polymers, synthetic fibres and plastic additives, from 1950 to 2015 (in millions of tonnes), extracted from Geyer et al., 2017 in Science Advances.

Nowadays, in Europe, the recycling rate is increasing but still the residues are disposed to landfill, Thus, considering the 29 million of tonnes post-consumer waste collected in 2020, 34.6% were recycled, while a 42% were incinerated with energy recovery and finally 23.4% were landfilled (PlasticsEurope, 2021). According OECD Global database, estimated global leakage to the environment (terrestrial and aquatic) was 22 million tonnes in 2019, the vast majority (19.4 million tonnes) are macroplastics, recognisable items such as beverage bottles, and most (82%) found their way into the natural environment as a result of inadequate collection and disposal (OECD, 2022). Microplastics (MPs), also make up a sizeable share of total leakage (12%), largely reaching the environment (by OECD member countries) through wear to tyres and road markings, as well as the accidental loss of plastic pellets and washing of synthetic textile fibres.

According also to OECD, in 2019, 6.1 million tonnes of plastic waste leaked into aquatic environments (lakes, rivers, and ocean), 109 Mt of plastics are estimated to have accumulated in rivers globally to date, with 1.7 Mt flowing into the ocean. This means that they will accumulate year by year and continue to flow through rivers into seas and oceans for decades.

Table 1.1 depicts production of common larger plastics and main uses from 2017 to 2021.

Table 1.1 Relative global demand for, and main uses of, common plastics from 2017 to 2020.

Polymer	Density (g/cm^3)	Production (%)	Products
PP	0.83-0.92	19.3[a]-19.7[b]%	Food packaging, sweet and snack wrappers, hinged caps, microwave containers, pipes, automotive parts, bank notes, etc.
LDPE/LLDPE	0.91-0.94	17.4[b]-17.5[a]%	Reusable bags, trays, and containers, agricultural film, food packaging film, etc.
HDPE/MDPE	0.94-0.97	12.3[a]-12.9[b]%	Toys, milk bottles, shampoo bottles, pipes, houseware, etc
PVC	1.16-1.58	9.6[b]-10.2[a]%	Window frames, profiles, floor and wall covering, pipes, cable insulation, garden hoses, inflatable pools, etc
PS/EPS	1.04-1.1	6.1[b]-6.6[a]%	Eyeglass frames, plastic cups, egg trays, packaging, building insulation, etc.
PET	0.96-1.45	7.4[a]-8.4[b]%	Bottles for water, soft drinks, juices, cleaners, etc.
PUR	1.2	7.7[a]-7.8[b] %	Building insulation, pillows and mattresses, insulating foams for fridges, etc
Others	-	18.1[b]-19[a]%	Hubcaps (ABS), optical fibres (PBT), eyeglasses lenses, roofing sheets (PC), touch-screens (PMMA), cable coating in telecommunications (PTFE), many applications in aerospace, medical implants, surgical devices, membranes, valves and seals, protective coatings, phenolic resins, epoxide resins, melamine resins, urea resins and others
SOURCE: PlasticsEurope Market Research Group (PEMRG) and Conversion Market & Strategy GmbH, a (2017), b (2020). In: PlasticsEurope (2018), PlasticsEurope (2021), An et al. (2020), and Sun et al. (2020). PP: polypropylene, LDPE: low-density polyethylene, LLDPE: linear low-density polyethylene, MDPE: medium density polyethylene, HDPE: high density polyethylene, PVC: polyvinyl			

chloride, PS: polystyrene, EPS: expanded polystyrene, PET: polyethylene terephthalate, PUR: polyurethane.

Finally, in 2050, there will be more plastic than fish in the sea, if current trends persist, 12 billion tonnes of plastic litter in landfills and the environment will accumulate according to a report by the Food and Agriculture Organization of the United Nations (FAO) and UN Environment Report (UNEP, 2018).

1.2 Biodegradability of Plastics

One of the main characteristics of plastic materials is their high durability. Most of the monomers used to synthesize plastics come from petroleum and none of the most commonly used polymers are really biodegradable. According to polymer experts, all plastics that are discharged into the oceans remain non-mineralized, whole or fragmented, and some of them reach diameters of less than 20 µm (Andrady et al., 2011; Thompson et al., 2004; Thompson et al., 2005). This fact makes plastic waste ubiquitous in the environment, to the point that they have been suggested as geological indicators of the proposed new era, the Anthropocene (Zalasiewicz et al., 2016).

The reason of the long persistence of plastics is that most polymers found in the environment, such as polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC), and polyethylene terephthalate (PET), have a very long carbon backbone structure (molecular weight of 500,000 to 3 or 6 million grams per mole) that provides hydrophobicity (lipophilia) and make them resistant to hydrolysis, oxidation and enzymatic degradation. Moreover, plastic additives make them more toxic and therefore more persistent. Thus, microorganisms cannot assimilate or mineralize these polymers, allowing them to accumulate in the environment (Kyrikou and Briassoulis, 2007). Some estimations indicate that, depending on the type of polymer, the lifetime of some plastics in the absence of oxygen and light (in the deep sea, for example) can be hundreds or even thousands of years (Barnes et al., 2009; Gasperi et al., 2018; GESAMP, 2016; Ivleva et al., 2017).

Several studies refer to the (bio)degradation of plastics, a term that is sometimes confused with biodeterioration and/or biofragmentation. It is important to clarify that for a compound, in this case a polymeric material, to be considered biodegradable, it must be able to undergo several processes or stages, which can be summarized as follows (Lucas et al., 2008):

1. (Bio)deterioration: this is the fragmentation of the plastic into smaller pieces due to both abiotic (mechanical action, light, temperature) and biotic factors (often due to

colonization by biofilms). This stage usually modifies some of the mechanical, physical and chemical properties of the plastics, such as colour, flexibility, etc.

2. Depolymerization (or biofragmentation): consists of the progressive breakdown of polymer molecules by reducing their molecular weight and thus generating oligomers, dimers and monomers. This process is usually caused by the action of catalytic agents (enzymes, free radicals) produced by microorganisms.

3. Assimilation and mineralization: the molecules generated in the previous step cross the cell membranes and, once inside the cytoplasm, they are incorporated into the cell metabolism to produce energy, biomass, storage vesicles and numerous primary and secondary metabolites that will later be mineralized (CO_2, CH_4, N_2, CH_4, H_2O, salts...) and excreted.

These processes are usually more effective or crucial when biotic and abiotic factors act synergistically. It is important to point out that the degradation process can stop at any of these steps and the complete mineralization of the most commonly used polymers (PE, PP, PS, PVC, PET) has not been demonstrated in fully conclusive studies.
Plastic degradation (biodeterioration and/or biofragmentation) in the environment mainly changes their physical and chemical properties, such as colour, surface morphological, crystallinity, particles size, and density (Guo and Wang, 2019), as shown in figure 1.2.

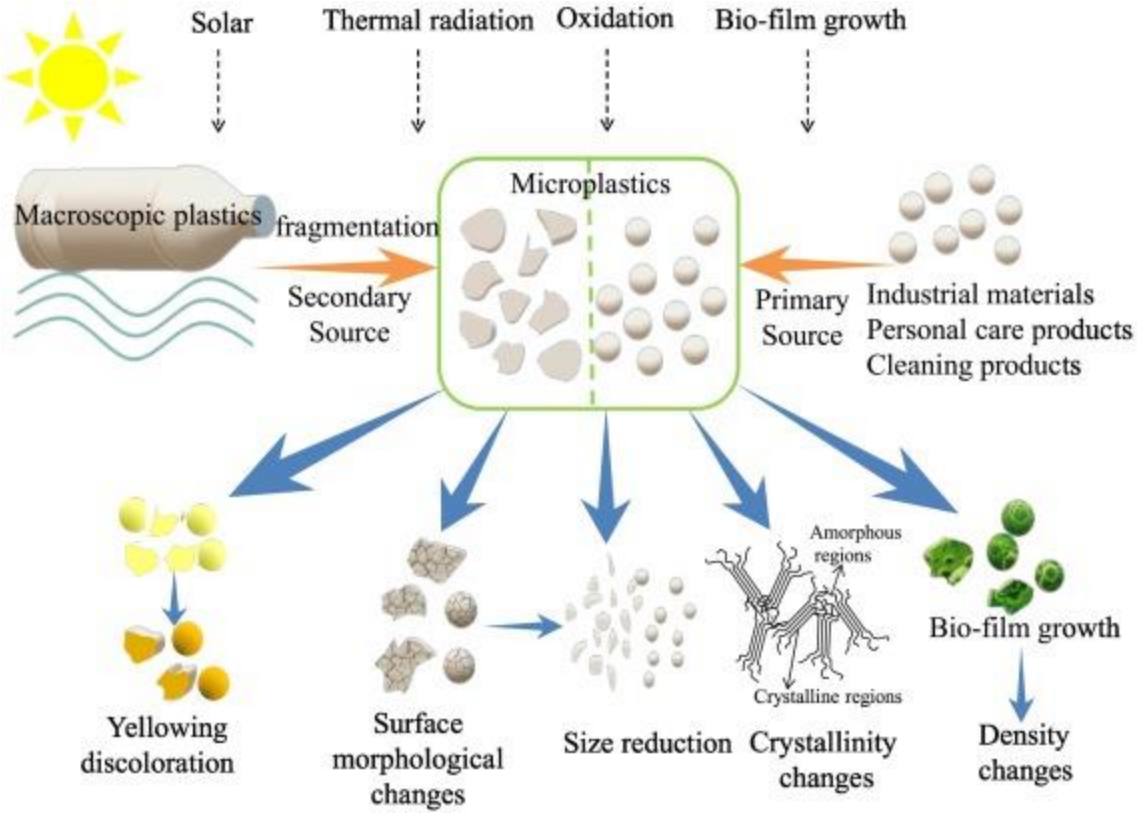


Figure 1.2. Properties changes of microplastics after degradation extracted from Guo and Wang (2019).

1.3 Microplastics Definition

The definition of Microplastics (MPs) is wide, covering small particles less than 5mm of synthetic polymer that resist the (bio)degradation, can persist for long periods of time and tends to accumulate (ECHA, 2019; EFSA, 2016). Moreover, MPs are also defined as "any synthetic solid particle or polymeric matrix, with regular or irregular shape with a size ranging from 1 μm to 5 mm, of either primary (intentionally manufactured microbeads and fibres) or secondary origin (mesoplastics breakdown in fragments), which are insoluble in water" (Frias and Nash, 2019; Verschoor, 2015).

Finally, MPs is complex mixture of synthetic polymers material with differently shaped referred to fragments, fibres, spheroids, granules, pellets, flakes, films or beads often containing harmful additives a proportion of which could transfer to biological fluids and tissues. (EFSA, 2016; GESAMP, 2015; GESAMP, 2019; Fromme et al., 2014; Linares et al., 2015; Lithner et al., 2011).

1.4 Microplastics types, sources and inputs

Primary microplastics:

Particles directly released in the environment as small particles, represent between 15-31% of MPs in the oceans. Main sources of primary MPs are laundering of synthetic clothes (35%) and abrasion of tires through driving (28%), both classified as unintentionally MPs. Other type of primary MPs are intentionally MPs that are added in consumers products, such as personal care products, like microbeads in facial scrubs that count as 2% of primary MPs (An et al., 2020; ECHA, 2019, European Parliament, 2018;).

Primary MPs comprises large number of origin and applications such as plastic pellets for industrial uses, microbeads of personal care products, such as exfoliating pearls, and cleaning products, paint resin, synthetic fibres released during laundry into wastewater, plastic running tracks in schools, artificial turf, rubber road in cities, vehicle tire wear (An et al., 2020; EFSA, 2016).

Most used synthetic material for textile applications is polyester (70%), followed by polyamides (nylon), rayon and other semi-synthetic fibres (Geyer et al., 2017). It is estimated that EU consumers discard about 5.8 million tonnes of textiles annually about 11 kg per person of which roughly two thirds are synthetic (Beasley and Georgeson, 2014). Between 200,000 and 500,000 tonnes of microplastic fibres from textiles enter the marine environment each year (Ellen MacArthur Foundation, 2017; Sherrington, 2016).

Microfibres have been estimated to comprise up to 35% of primary MPs in marine environments, a major proportion of MPs on coastal shorelines and to persist for decades in soils treated with sludge from wastewater treatment plants (Cristaldi et al., 2020; Henry et al., 2019; Yang et al., 2021).

Secondary microplastics:

Secondary sources of MPs are the large plastic particles fragments or items which have not been properly disposed (Pettipas et al., 2016). Over time, MPs are gradually formed by the fragmentation of these plastic structures under the action of physical, biological, and chemical processes (weathering) such as UV light irradiation aging (photo-oxidation, dominant cause of degradation), thermo-oxidative degradation, and hydrolytic-oxidation, biological crushing (microorganisms as *Micrococcus sp.* can slowly degrade surface plastic polymers), and by waves mechanical action (Celina et al., 2013; Cole et al., 2011; GESAMP, 2015; Raziyafathima et al.,

2016). Secondary MPs account for 69-81% of MPs found in the oceans (European Parliament, 2018)

Secondary MPs at the same time can break down into small fragments, creating nanoplastics, plastic particles with sizes below 100 or 1000 nm, depending on the definition adopted (EFSA, 2016). Larger plastics involve mainly municipal debris as: plastic bags, plastic bottle (mainly used as disposable containers for liquids or solids, such as beverages, pickles, honey, dried fruit, edible oils, and agricultural veterinary drugs), disposable plastic tableware (lunch boxes, plates, saucers, straws, knives, forks, spoons, cups, bowls), plastic packaging. Other sources are fishing wastes (nets, buoys, floating boxes, fishing rods, fish tank) and farming film. In addition, the automotive, construction, aerospace, and medical industries also consume significant amounts of plastic in the following products: hinged covers, hub caps and other automobile parts, touch screens, pipes and fittings, foundations, roofs, floors, panels, roads, insulation, cable jackets, window frames, profiles, flooring, wall coverings, building insulation, valves, seals, among others (Table 1.1) (An et al., 2020).

Secondary MPs can input to the ocean from breakdown of mesoplastics exposed to environmental factors in land, or due breakdown of larger plastics transported and deposited by river (Simon-Sanchez et al., 2019).

Overall, the use of MPs in products that result in release to the environment are not adequately controlled. In general, it is estimated that the EU uses 145,000 tonnes of MPs and 42,000 tonnes of MPs end up in the environment each year. The largest source of pollution is the granular fill material used in artificial turf pitches, with emissions of up to 16,000 tonnes. In addition, it is estimated that around 176,000 tonnes of secondary MPs are formed and released into European surface waters every year (ECHA, 2021).

1.5 Microplastics in environment

Microplastics have been found in air, seawater, freshwater and terrestrial ecosystems. Specific features of MPs, such as density, morphologies, and an extremely long life, making a significant contribution to the inter-environmental compartments transportation, with ecological and human health risks (Ahmad et al., 2020).

Land act as the first dumping site for plastics. These enter into environment through direct littering, improper waste management, accidentally loss during the disposal process and industrial spillages (Horton and Dixon, 2018). The use of sewage sludge as fertilizer in agriculture also introduces a significant number of MPs (Nizzetto et al., 2016). Urban and agricultural runoff and

wastewater discharges are the main source of MPs inputs in freshwater and coastal areas. Once it enters into an environment, MP particles undergo transportation by different mechanisms, which depends on the type of particle and environmental characteristics and conditions (Horton and Dixon, 2018).

The effluents of wastewater treatment plant (WWTP) are an important source of MPs in environment and contribute ~5% of the total environmental MPs. In Europe, up to 520,000 tons of MPs per year are released from WWTPs into the freshwater bodies (Alimi et al., 2018; Carr et al., 2016; Horton et al., 2017; Park et al., 2020; Sol et al., 2020; Xu et al., 2019). Although WWTP does not target MPs specifically, there is a consensus that WWTPs remove at least between 90% and 99% of the MPs. However, WWTP effluent is still a significant source of MPs released into the environment due to the large volumes discharged (Liu et al., 2021; Talvitie et al., 2017). Emissions of up to $1.83 \cdot 10^{10}$ MPs/day from a single, medium-sized (30,000–50,000 m^3/day) WWTP were established, however MPs loads vary considerably depending on each WWTP characteristics such as, capacity, raw wastewater MPs concentration and MPs removal efficiency (Freeman et al., 2020; Leslie et al., 2017).

Polyester (PES) and polyamide (PA) microfibers and polyethylene (PE) particles are typically the most abundant MPs in secondary and tertiary effluents. A contributing factor to the dominance of fibres in effluents is their long thin shape, which allows them to pass through even fine-grained filters (Browne et al., 2011; Lares et al., 2018; Murphy et al., 2016; Vollertsen and Hansen, 2017; Ziajahromi et al., 2017). In addition, a large portion of the MPs removed from WWTP may be retained in the sludge ranging from 4.2 to 15.4 MP/g (Mahon et al., 2017). Sun et al., (2019), found sewage sludge with concentration between 1,500 to 170,000 MPs/kg dry weight representing synthetic fibres between 63 and 80% of the MPs found. Nizzetto et al., (2016) estimated that Spanish agricultural soils could receive around 100,000 tonne of MPs per year as a result of the application of sewage sludge. The detection of synthetic fibres in the soil has even been proposed as an indicator of the amount of biosolids contribution (Zubris and Richards, 2005).

Rivers are considered one of the most important pathways for plastic transport from land to ocean, due to high content of MPs fibres and fragments in WWTP discharges and runoff from biosolids application (Lebreton et al., 2017; Leslie et al., 2017).

Some researchers have indicated that the atmospheric deposition is also potential source of MPs in the aquatic environment (Bläsing and Amelung, 2018; Free et al., 2014). MPs (mainly fibres) may be blown out from surfaces of poorly managed landfills or streets and stay in the air and

travel long distances until fallout. Dris et al., (2016), based on a 1-year and a 6-month monitoring period, found atmospheric fallout between 2 and 355 particles/m^2/day mostly fibres, in a study in urban and suburban Paris areas. The fibres ranged from 200 to 600 µm, 29% were plastic, with the majority constituting by cellulosic or natural origin. In a lately study also in dense urban area of Paris, Dris et al. (2017), found outdoor MPs concentrations were significantly lower ranging between 0.3 and 1.5 fibres/m^3 compared with indoor concentration ranged from 1.0 to 60 fibres/m^3.

MPs may be generated from various sources, have various pathways and be transported between different environmental media (e.g., freshwater, marine water, sediments, and soils). Figure 1.3, shows a summary of entry paths and transport routes of MPs in the environment.

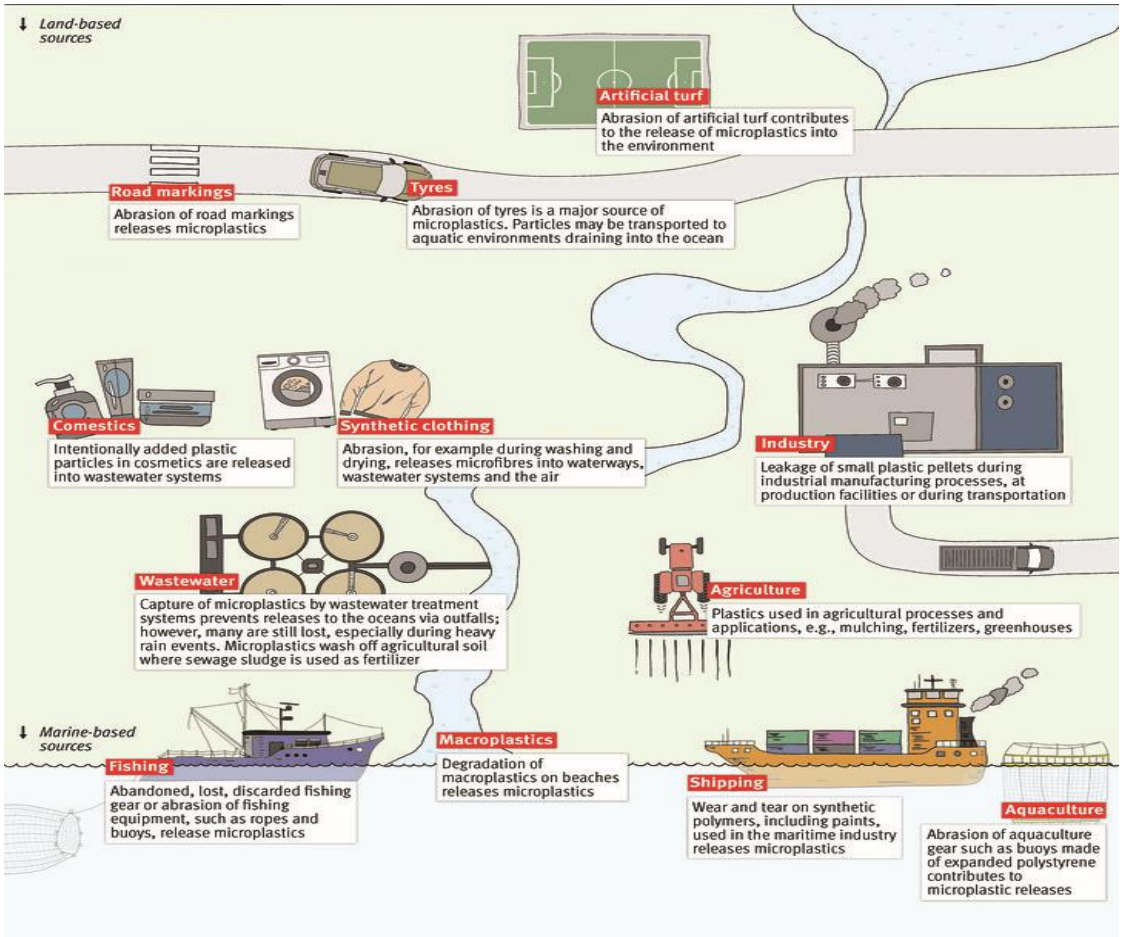


Figure 1.3. Summary of entry paths and transport routes of MP in the environment. Source Waldschäger et al., (2020). Illustration by GRID-ARENDAL, UNEP (2021)

1.6 Microplastics Fate and Transport

The physical and chemical properties (shape, density, chemical composition) of MPs particles as well as area of water surface, depth, prevailing wind, surface current have a substantial effect on their transport and retention in aquatic systems. Floating MPs are transported along rivers and

into oceans, by adventive transport, the intermediate size class of MP may be preferentially transported downstream (Besseling et al., 2017; Fischer et al., 2016). Once they enter the ocean, MP particles can travel great distances and be spread rapidly by water current, winds, turbulence according their density.

In general, the larger the size, the easier it will be the drift in the uppermost layer. Hence, MPs are less affected by stoke drift resulted from waves and superficial winds so that they are more likely to be carried offshore (Isobe et al., 2014). Additionally, turbulence in the upper-water layer can vertically mix buoyant MPs and undergo vertical transportation in the water column (van Sebille et al., 2015). Reisser et al. (2015) found that MPs with lower rise velocities are more susceptible to vertical transport thus MPs with higher density are more likely to retain in sediments and be transported to deeper sediments layers.

Reisser et al., (2015) pointed out that vertical mixing might affect the size distribution of plastics floating at the surface because smaller plastics are more susceptible to vertical transport. Besides the accumulate in oceanic gyres and shallow water sediments, deep-sea sediments are a potential sink of MPs. Woodall et al., (2014) found that fibres are up to four times of magnitude more abundant (per unit volume) in sediments than surface seawater in the Atlantic Ocean, the Mediterranean Sea, and the Indian Ocean.

Continuous weathering processes can strongly affect the fate of plastic debris in the aquatic environment. Fragmentation into smaller particles change the condition of the MPs and their hydrodynamic behaviours (Barnes et al., 2009; Lambert and Wagner, 2016; Ter Halle et al., 2016). The MP density does not remain constant, the media can influence the interactions between particles and organic material/organisms, with changes in buoyancy or sinking and transference between different environmental compartments (Ahmad et al., 2020). Thus hetero-aggregation and biofouling increases the weight of particles, affecting resilience and accelerating their sinking on bottom sediments (Andrady et al., 2011; Lagarde et al., 2016; Lobelle et al., 2011; Wright et al., 2013)

MPs sinks can be temporary or permanent. Possible sinks for MPs include sedimentation, shore deposition, and ingestion by organisms, these sinks, however, can be dynamic (Law et al., 2010). MPs can be trapped in sediments over a long time but affected by wave action, currents or bioturbation or other disturbances, the trapped MPs may be more readily suspended from bottom sediments than larger ones because their smaller size and lower density compared to natural sediments (Kershaw and Rochman, 2015). MPs ingested by organisms may either be excreted and to change MPs particles density.

1.7 Microplastics in Mediterranean Sea

Mediterranean Sea is a semi-enclosed sea considered as one of the potential plastic accumulation zones. Is considered as the sixth greatest accumulation zone for marine litter: this sea holds only 1% of the world's waters, but concentrates between 5-10 % (7%) of all global MPs, with an estimates of MPs surface similar to those of the 5 subtropical gyres (convergence areas) (Cózar et al., 2015; Lebreton et al., 2012; Liubartseva et al., 2018; van Sibylle et al., 2015). Woodall et al. (2014) reported MPs pollution in sediments from deep cores.

Environmental pressures as densely populated areas (around 10% of the global coastal population) with intense industrial, touristic and human activity, Atlantic MPs inputs (by Strait of Gibraltar), inadequate wastes policies in some countries, important discharges of big rivers as Rhone, Nile, Ebro, Po, intense maritime and fishing activities, few outlets and marine currents patterns cause a high concentration of MPs in Mediterranean Sea (Mistri et al., 2017; Suaria et al., 2016).

According 2D Lagrangian model developed by Liubartseva et al. (2018), plastic particles were found piled up substantially on coastlines and the sea bottom in Mediterranean Sea. A big amount of plastic litter was found in the Catalan Sea, being Barcelona among cities with the major plastic debris contributing in the Mediterranean Sea. They concluded that terrestrial sources of every Mediterranean country are responsible for the Mediterranean plastic pollution. In Mediterranean surface waters, estimations of the load of surface plastic ranging approximately between 1000 to 3000 estimated by Cózar et al. (2015) to 23,150 tonnes estimated by Liubartseva et al. (2018), dominated by a range of millimetre-sized plastic fragments (Eriksen et al., 2014; Guven et al., 2017, Suaria et al., 2016).

In that context, it has recently been estimated that the Mediterranean Sea receives between 150 and 610 thousand tonnes of plastics each year, 6% of which are MPs (Boucher et al., 2020). According to Liubartseva et al. (2018), more than 50% of plastics come from their own coastal cities terrestrial inputs. A recent modelling study has suggested that some Mediterranean Protected Areas are under high risk of being affected by MPs and the actual protection levels are not effective against this type of pollution (Soto-Navarro et al., 2021). Simon-Sanchez et al. (2019) estimated that the Ebro surface water represents an input of $2.14 \cdot 10^9$ MPs/year to the Mediterranean Sea. MPs in suspension in the river water column have the potential to be discharged into the sea combined with others riverine suspended particulates, thus the high MPs enrichment observed in marine sediments proposed to the seabed as a sink of MPs from the land. (Leslie et al., 2017).

MPs has been found in a wide range of concentrations in seawater surface, sediments and sands in Mediterranean Sea (Cincinelli et al., 2019; Constant et al., 2019; Filgueiras et al., 2019). MPs quantification on sandy beaches along the coastline has been carried out in a number of locations with average concentration ranging from 22±23.2 MPs/kg in Spain (Granada) to 2433±2000 MPs/kg in Lebanon (Tripoli) (Godoy et al., 2020; Kazour et al., 2019). Exceptional high concentrations were found in Tunisian coast sandy area (enclosed Lagoon of Bizerte) with values between 3,000 and 18,000 MPs/kg (Abidli et al., 2017).

Regarding submerged sediments, in western Mediterranean Sea levels ranged from 0.43 to 4 MPs/kg at 71.5 m depth in Italy and from 240 to 900 MPs/kg at 10 m depth in Spain (Balearics Islands) (Alomar et al., 2016; Mistri et al., 2020). While in other Mediterranean Sea zones, Vianello et al., (2013), reported higher values in a coastal lagoon in Venice, located near an industrial zone ranging from 672 to 2175 items/kg dry sediment (average 1445±458 items/kg dry sediment) at <1±0.5 m.

According MPs in the surface of the sediments, the lowest concentration have been found ranging from 0 MP/m^2 to 75 MPs/m^2 in Italy (Central Adriatic Sea) at 7-142 m depth (Mistri et al., 2017) while, the highest concentrations of 1.9 million MPs/m^2 were found in Italy (Tyrrhenian Sea) at 600-900 m depth with more than 70% being fibres with sizes from 0.1 to 1 mm. (Kane et al, 2020).

In Mediterranean Sea surface water values were reported between 0.10±0.04 MPs/m^3 found in Italy (west coast of Sardinia offshore) and 453±335 MPs/m^3 in Bizerte Lagoon, Tunez (Wakkaf et al., 2020). Although the highest concentration in open sea were found in Lebanese coast (6.7 MPs/m^3) and in Israel coast (7.68±2.38 particles/m^3). In general, the Mediterranean Sea have an average of 0.15-0.31 MPs/m^3 and 1.25 ± 1.62 MPs/m^2 in the northwestern-central regions. (Cózar et al., 2015; de Lucia et al., 2014; Fossi et al., 2016; Kazour et al., 2019; Suaria et al., 2016; van der Hal et al., 2017; Wakkaf et al., 2020).

MP sizes were typically in the range: 0.3–5 mm in surface water, 1–2 mm on sand beaches and in sediment (Papadimitriu and Allison, 2022). Most studies identified polymer type, with the three polymers most frequently reported being polyethylene (PE), polypropylene (PP) and polystyrene (PS), consistent with the polymers most commonly used in packaging, for take-away food containers and thermal insulation (Chen et al., 2021). According de Haan et al. (2019) polymers such as PE (54.5%), PP (16.5%) polyester (9.7 %), were the most found MPs in the Mediterranean Sea. On beaches, polyester was the most frequently reported polymer and nylon fibres were most commonly reported in water samples (Papadimitriu and Allison, 2022).

1.8 Microplastics effects in Environment

The persistence and the potential for adverse effects or bioaccumulation of MPs is a cause of concern. Once released, they can be extremely durable and persistent in the environment, lasting thousands of years, and practically impossible to remove. Currently it is not possible to determine the impact of such long-term exposure on the environment (ECHA, 2021; Lusher et al., 2014).

Carbery et al., (2018) reports that over 690 marine species have been affected by MPs, these particles have been found in the digestive tract and gills of marine organisms from different trophic levels (Thiele et al., 2021; Su et al., 2018). Due to their small size, MPs and nanoplastics (even smaller particles that are created from the further degradation of MPs), may be readily intentional ingested (due to their resemblance to a natural food source) or nonselective ingested and thereby enter the food chain (ECHA, 2021; Lusher 2017).

MPs can be ingested by low trophic suspension, filter and deposit feeders, detritivores, and planktivorous. MPs ingested may be excreted either as waste or retained/translocated into tissues, making trophic transfer in food chain. During the process of ingestion, the physical components of MPs can make harmful effects to the organisms. MPs may present a physical hazard by: nutrient dilution, internal abrasion, clogging feeding appendages or the digestive system; decrease the feeding activity and stimulus, mobility and weight, reduction on the growth of microalgae, significant reduction in the chlorophyll content and photosynthetic efficiency, decreased growth rates, lower steroid hormone levels, impairment of the reproductive system and even mortality (Besseling et al., 2013; Besseling et al., 2014; Hartmann et al., 2017; Lei et al., 2018; Rehse et al., 2016; Rist et al., 2017; Wright et al., 2013). Other effects include oxidative stress, alteration in enzyme production and metabolism, and embedment in tissues (Lei et al., 2018; Welden and Cowie, 2016).

Particles with a high capacity to accumulate in organisms (as small particles) and translocate into tissues are expected to have a stronger physical impact. Shape also plays an important role since irregular, sharp fragments are more likely to cause damage than round, smooth particles. Fibres are more likely to accumulate in the digestive system. The capacity of individual species to egest MPs is also considered as an important factor because this process will determine how long an organism is exposed to the particles (Rist et al., 2017; Wright et al., 2013)

More importantly, after ingestion, MPs may transfer and release toxic chemicals (Rochman et al., 2013; Wardrop et al., 2016), hence, their toxic effects may be more severe with chemical injury

compared to physical damage (Lithner et al., 2011; Rochman et al., 2013). Some MPs contain hazardous chemicals as additives (4%) during their formation (e.g., bisphenol A, phthalates, terephthalates, alkanedioates, trialkyl phosphates, polybrominated diphenyl ethers (PBDEs)), and siloxanes. In addition, MPs can interact with other environmental pollutants by acting as carriers and potentially modulating their toxicity. MPs absorb/release several harmful/toxic chemicals already present in the water body, (due the large surface-volume ratio by surface porous, rough, and irregular) concentrating them several folds (6 orders of magnitude), as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), dichloro diphenyl trichloroethane (DDT), perfluoroalkyl substances (PFASs) and heavy metals (Capriotti et al., 2021; EFSA, 2016; Karkanorachaki et al., 2018; Llorca et al., 2014; Standmüller et al., 2002; Thiemann et al., 2021; Zhang et al., 2015)

Concentrations of up to 2,750 ng/g of PCBs and 24,000 ng/g of PAHs have been found in microplastic deposited at beaches (EFSA, 2016). Capriotti et al., (2021), screened MPs collected in the Adriatic Sea for PCBs, PAHs, DDTs and a small number of organophosphate pesticides, they observed a clear difference between inshore and offshore samples, thus, inshore MPs were contaminated by ΣPCB concentration of 65.67ng/g-plastic compared to 37.78 ng/g-plastic at offshore sites. Some of the additives in MPs are mild estrogens and could act as hormonal disruptors. Other pollutants such as PCBs are known to bio-accumulate along the food chain and can act as neurotoxins or can exhibit genotoxicity (Schantz, et al., 1996). Karapanagioti et al., (2011), reported adsorbed chemicals on pellets collected from beaches with median PCB concentrations (PCB66–206) in the range from 66 to 270,000pg/g plastic resin pellet, being pellets collected from beaches near known pollution sources with 1–2 orders of magnitude concentration higher than pellets from remote beaches.

Studies have been reported pathogenics bacteria over MPs and the extension of colonization depend on MPs shape associated with an increased risk of transmitting diseases and toxic chemicals (Gundogdu et al., 2022; Lusher et al., 2017; Zettler et al., 2013).

It has been observed that the hydrophobicity (number of non-polar functional groups) of the contaminants and MPs composition is the most important factor that facilitates their concentration in the particles but others physical and chemical properties of MPs and environmental parameters influence their sorption activity as: crystallinity and density, plastic type, polarity and functional groups exposed, size, shape, degree of ageing/weathering, environment salinity and pH. (Andrady, 2011; Guo and Wang, 2019). The concentrations of HCHs and DDTs found on MPs are lower than PAHs and PCBs in global environments. The aged MPs have higher sorption capacities of pollutants than the virgin ones. Thus, PE typically sorbs more organic chemicals

than other kinds of MPs, and aged MPs to sorb more pollutants than virgin ones e.g., the nonpolar perfuorooctanesulfonamide partitions more readily onto nonpolar PE, whereas some polar antibiotics sorb readily onto the relatively polar plastic, polyacrylamide (PA) (Guo and Wang et al., 2019).

The accumulated pollutants (especially heavy metals and chemicals additives) transfer out into surrounding environment, with possible negative impact on organisms (Fonseca et al. 2017; Hartman et al., 2017; WHO, 2019). Uptake of contaminated MPs by aquatic organisms provides a feasible way for introduction of these hazardous substances into the aquatic food web and bring risks to marine organisms of different trophic levels (Wu et al., 2019). Thus, adverse effects include oxidative damage (Li et al., 2020a), neurotoxicity (Avio et al., 2015), organ pathology (Rochman et al., 2013), metabolic abnormalities (Oliveira et al., 2013), and mortality (Browne et al., 2013). However, the evidences of MPs ingestion accelerate the transfer of the associated toxicants to aquatic biota it is not clear if the exposure by MPs is higher than other exposure pathways (Koelmans et al., 2016).

MPs have been detected in predatory animals, as aquatic predators tend to choose their prey purposively; bioaccumulation is likely to be an important pathway for MPs and associated compound ingestion by upper trophic levels (Wu et al., 2019). In addition, experimental evidences indicates that MPs have the potential to be transferred between trophic levels. Farrell and Nelson (2013), demonstrated that trophic transfer occurs between mussels and crabs. Similarly, trophic transfer of contaminants, e.g., persistent organic pollutants (POPs), has been reported its biomagnification (EFSA, 2016). However, the absorption rate of the MPs by fish is generally considered low, although the bioavailability depends on the physical and chemical properties of the MPs ingested (Gundogdu et al., 2022). Other authors as Walkinshaw et al. (2020), found that MPs do not biomagnifies, and that organisms at lower trophic levels are more likely to have a greater risk of microplastic contamination than apex predators. However, organisms higher up the food chain may not contain as many MPs per gram body weight, risks are still present from contaminant transfer and associated chronic effects.

1.9 Microplastics in food

Seafood contamination by MPs have recently been highlighted as an emerging concern for global food security (Dawson et al., 2021). Due consumption of marine organisms without gut removals (shrimp, bivalves, dry and small pelagic fishes) or tissues with MPs accumulated (fish fillets), MPs and associated compounds can be transferred to human with an increased risk of transmitting diseases and toxic chemicals (Gündogdu et al., 2022).

All commercially important organisms were shown to contain MPs. The higher average number of particles found per fish was up to 9.9±8.1 MPs/individual (Anastasopoulou et al., 2018). Filgueiras et al., (2020), observed a high percentage of anchovy and sardines (87%) with MPs in the Alboran Sea (Spain) with average value of 1.92±0.95 and 1.77±1.42 MPs/individual, respectively. In shrimp from North Sea (Channel area and Southern part) an average of 0.75 MPs/g was found (Devriese et al., 2015), while, in 66% of shrimp (*Aristeus antennatus)* from Sardinia region showed an average value of 1.66±0.10 MPs/individual (Cau et al., 2019). Regarding bivalves, the average number of particles range from 0.2 to 4 MPs/g with a maximum levels of $8.83 \cdot 10^4$ MPs/g for particles smaller than 3 μm. High levels of MPs were found in 86% of oysters (*Scutiger spinosus*) from the Levantine Basin (Eastern Mediterranean zone) with values of 7.2±1.4 MPs/individual while 46% of mussels (*Mytilus* spp.) showed an average value of 1.9±0.2 MPs/individual (Carbery et al., 2018; Digka et al., 2018; ECHA, 2021; EFSA, 2016; Ferrante et al., 2022; Kazour et al., 2019; Leslie and Depledge, 2020; Rist et al., 2018b). Big fishes are eviscerated before its consumption however, MPs can also be found in muscular tissue. Some authors have shown that MPs can move into organs and tissues easily (Cappello et al., 2021; Ding et al., 2018; Lusher et al., 2017). Ferrante et al. (2022) found maximum MPs concentrations of $9.04 \cdot 10^4$ MPs/g for particles smaller than 3 μm in fish fillets.

Fibres were the most frequent morphology reported across all sample types and in general MPs sized from 0.5 to 1.5 mm (Papadimitriu and Allison, 2022). Polyethylene (PE) was the most commonly reported polymer, with smaller amounts of PS and PP (Papadimitriu and Allison, 2022). In a recent study, Piyawardhana et al. (2022) highlighted the occurrence of microplastic (PE, PET, PS, PVC and PP) in commercial dried fishes widely consumed across Asian countries.

Other food and beverages such as, table salt, beer, honey, sugar, fruits and vegetables, bottle water and tap water are also polluted with MPs and can contribute significantly to MPs intake (EFSA, 2021; Iñiguez et al., 2017; Oliveri Conti et al., 2020; Smith et al., 2018; Zuccarello et al., 2019).

1.10 Microplastics effects on human Health

Although the amount of MP contamination in the aquatic ecosystem is predicted to increase dramatically in the near future, the adverse effects on human health of this pollution are still not fully understood (Gündogdu et al., 2022). Ingestion and inhalation are considered routes for human exposure to MPs evidenced by MPs presence in stool and lung tissues from autopsies (Amato-Lourenço et al., 2021; Liebmann et al., 2018). In the case of food consumption, the annual intake of MPs for a person is estimated to be between 39,000 and 52,000 particles (Cox et al., 2019). According EFSA (2016), only MPs smaller than 150 μm may translocate across the

mammalian gut epithelium causing systemic exposure; the absorption of these MPs is expected to be limited (≤0.3%) while only the smallest fraction (size <1.5 μm) may penetrate deeply into organs. MPs bigger than 150 μm are not absorbed and effects on the immune system and inflammation of the gut are to be expected. Particles with size bigger than 10 μm could be taken by specialized cells such as macrophages via phagocytosis and can be present in the liver and other remote organs such as the spleen and lymph nodes in rats (Tomazic-Ezic et al., 2001).

The gastrointestinal transit time of particles with sizes 1-2 mm, was investigated in 13 healthy volunteers after ingestion of MPs using a test meal (McIntyre et al., 1997). The researchers noted a marked acceleration in gastrointestinal passage time attributed to mechanical activation of mucosal receptors of the intestine (increased upper gut sectretions) after stimulation of enteric nerves. The effect would be worst in the case of inflamed gastrointestinal mucosal diseases, such as peptic ulcer, ulcerative colitis, and Crohn's disease (Gündogdu et al., 2022). The gastrointestinal tract has been identified as a possible target organ during experimental research in rats and mice. Lu et al., (2018) observed a decrease of mucin secretion in the gut, induction of gut microbiota dysbiosis, and hepatic lipid metabolism disorder in mice after oral exposure to polystyrene MPs (0.5 and 50 μm) for 35 days at concentrations of 100 and 1,000 μg/L in drinking water. By contrast, Stock et al., (2019), found that most of MPs were excreted by faecal excretion following 28-day oral exposure and very small number of MPs was found in the intestines. In other remote organs such as the kidneys, spleen, or liver the MPs were not found. Zheng et al., (2021) reported that polystyrene (5 μm) caused an increase of intestinal permeability with acute colitis and lipid disorders in the liver of mice at a concentration of 500 μg/L in drinking water for 28-day exposure, Finally, Li et al., (2020b) found in mice exposed with polyethylene MPs (10-150 μm) at concentrations 6, 60, and 600 μg/day for 5-week oral via, induction of minor intestinal inflammation, as well as, increased the secretion of IL-1α in the serum.

In vitro experiments with different cell-culture models have been conducted to investigate the toxicity of Nano- and MPs particles. Schirinzi et al., (2017), found potential cytotoxic effects of micro- and NP particles in terms of oxidative stress using cerebral and epithelial human cells. Similarly, Liu et al., (2020), using a Caco-2 monolayer cell culture model, PS (polystyrene) -MPs (100 nm and 5 μm) at concentrations of 1 and 10 μg/mL caused cytotoxicity, disorders to transport function and increased proinflammation. By contrast, Busch et al., (2021) did not find acute toxicity of PS (50nm) or PVC-MPs (<50 μm) in a model of the healthy intestine but observed an increase in the release of IL-1β and a loss of epithelial cells during active inflammatory. Smaller PS-MPs (460nm-100μm) are potential immunostimulants that induce pro-inflammatory cytokines in a concentration and size-dependent manner (Hwang et al., 2020). Çobanoğlu et al. (2021) used particles of sizes ranging 10 to 45 μm of PE-MP (>70% polyethylene and <30%

additives), with concentrations between 25 -500 μg/mL at 48 hours of exposure and found genotoxic and cytotoxic effects (induction of elevated micronucleus, nucleoplasmic bridge, and nuclear bud frequencies) on the human peripheral lymphocyte cell-culture model. Similarly, Stock et al., (2019) observed cytotoxicity by PS-MPs (1-10 μm) on Caco-2-based cell-cultures only in much higher concentrations as 10^8 particles/mL. By contrast, not cytotoxicity on Caco-2 cells were observed with PS particles (50 and 200 nm) at concentrations between 15 and 250 μg/mL for up to 120 min of exposure (Abdelkhaliq et al., 2018).

Several toxic chemicals are associated with MPs, such as additives, which can increase the toxicity of MPs and enhance their accumulation and biomagnification in food chains (Carbery et al., 2018; Leslie and Depledge, 2020; Rist et al., 2018a). In that way, Deng et al., (2018) found that co-exposure of PE-MPs (0.5-1.0 μm) and organophosphorus flame retardants (OPFRs) induced greater oxidative stress, neurotoxicity and enhanced disruption of amino acids as well as energy metabolism in mice than OPFR alone, after 90 days exposure. Rubin and Zucker (2022), evaluated the sorption and desorption potential of Triclosan (TCS, antibacterial and fungicide chemical compound) and polystyrene microbeads according the hydrogen interactions. Surface functionality of the microbeads highly increased their adsorption capacity of TCS, from 2.3 mg TCS for non-functionalized microbeads to 4.6 mg and 6.1 mg TCS per gram of microbeads for amino- and carboxyl-functionalized MPs. TCS was desorbed from both functionalized and non-functionalized MPs (dependent on the matrix complexity and protein content as well as microbead functionality) when changing from environmental conditions to cellular conditions. Toxicity tests suggested that while low concentrations of TCS and MPs (separately) have minor toxic effect toward Caco-2 cells, TCS-MPs at similar concentrations have an order of magnitude higher toxicity than pristine MPs and demonstrated that co-presence of these environmental contaminants poses risks to the environment and human health.

1.11 Microplastics restrictions

The first step the EU about MPs restrictions is to reduce the harmful impact of intentionally-added MPs pollution on nature and people. In January 2019, ECHA's Risk Assessment Committee (RAC) supported the proposed restriction for the intentional use of MPs (due they will inevitably be released to the environment with fertilizer, cleaning and laundry products, cosmetics, products used in the chemical, oil and gas industry, filler products among others) and further recommended stricter criteria for biodegradable polymers. The Committee for Socio-economic Assessment (SEAC) adopted its final opinion in December 2020. The final validation of the European Parliament is scheduled for the first half of 2022 and transition periods will be granted, depending

on the type of product (ECHA, 2019). The use of MPs in many products will be restricted under REACH (Registration, Evaluation, Authorization and Restriction of Chemicals regulation) and the proposal would prevent by nearly 400,000 tonnes from 2030 to 2050 of MPs releases with a cost of €19.1 billions (Scientific committees-ECHA, 2019).

However, this new regulation does not apply to products that releases MPs during use or disposal (secondary MPs). It is estimated that the restriction of single-use plastics will contribute to the reduction of the generation of MPs of secondary origin. The European Union has issued the Directive (EU) 2019/904 (on the reduction of the impact of certain products on the environment), commonly known as the Single-Use Plastic Directive (European Parliament, 2019). The main products referred in the Directive are food containers, cups for beverages, cutlery, plates, straws, cotton bud sticks, cosmetics and personal care products. Plastics items subject to regulation represent 50% of the EU marine litter (European Commission, 2021, European Commission, 2019)

Previously, others restrictions were stablished to reduce plastics bags wastes. The Directive (EU) 2015/720 of the European parliament stablished the reduction of the consumption of plastic bags, inspired by the success of removing plastic bags from supermarkets in designed in two phases in 2015 (European Parliament, 2015). However, it did not include light bags for vegetables and fruits. Directive (EU) 2018/852 aims to increase and improve packaging waste recycling measures to move towards a circular economy in Europe, and establishes that, by the end of 2025, a minimum of 65% by weight of all packaging waste will be recycled.

The impact on the apparel and textiles industry will probably be limited in the short-term. Producers, brands and retailers can take steps to reduce MPs shedding in apparel and textiles. The OECD reports that changing the design and manufacture of textiles and garments can decrease shedding rates by up to 80-90%.

In parallel, in the “European Green Deal” and new “circular economy action plan” (CEAP), the European Commission announced a new initiative to address the unintentional release of MPs in the environment. It aims to develop labelling, standardization, certification and regulatory measures on unintentional release of MPs, including measures to increase the capture of MPs at all relevant stages of products’ lifecycle. Further, develop and harmonize methods for measuring unintentionally released of MPs, especially from tyres and textiles, and delivering harmonized data on MPs concentrations in seawater (European Commission, 2020).

1.12 Spain case restrictions

Following the objective of a 15% reduction in the generation of waste compared with the year from 2010 to 2020, in Catalonia region was established the Law 5/2017 for the obligation for companies to charge for plastic bags with handles, framed in the program Prevention and Management of Waste and Resources 2013 to 2020 (PRECAT20) of Waste Agency of Catalonia (Generalitat de Catalunya, 2017; Generalitat de Catalunya, 2020a; Generalitat de Catalunya, 2020b). After that, the Royal Decree 293/2018, on the reduction of the consumption of plastic bags is established, by charging for the supply of plastic bags (restricting plastic bags dispersion in the environment) and, in turn, the Registry of Producers is created (GENCAT, 2022). This decree is applied only to light bags, which are usually single-use bags that generate more waste. A proposal in Spain, that limits for the first time the use of single-use plastics in certain commercial areas is the Law on Waste and Contaminated Soils, the purpose of the future law on waste and contaminated soils is to incorporate into the Spanish legal system the modifications that were introduced by Directive (EU) 2018/851, as well as review the current regulations on waste and contaminated soils approved in Law 22/2011, in order to comply with the new objectives established in the new European waste directives that make up the Circular Economy Package, as well as with the new objectives derived from the single-use plastics directive that arise from the "European Strategy for the plastic in a circular economy" (Plastigaur, 2021)

This law, imposing restrictions on the sale of single-use plastics, for example in the food area. In addition, it promotes the separate collection of plastic bottles, and imposes a tax of 0.45 € per kilogram on non-reusable plastic containers. This law will come into force in 2023 (MITECO, 2021).

References

Abdelkhaliq, A., van der Zande, M., Punt, A., Helsdingen, R., Boeren, S., Vervoort, J.J.M., Rietjens, I.M.C.M., Bouwmeester, H., 2018. Impact of nanoparticle surface functionalization on the protein corona and cellular adhesion, uptake and transport. J. Nanobiotechnology 16, 70.

Abidli, S., Toumi, H., Lahbib, Y., El Menif, T.N., 2017. The first evaluation of microplastics in sediments from the complex lagoon-channel of Bizerte (Northern Tunisia). Water Air Soil Pollut. 228, 262.

Ahmad, M., Li, J.-L., Wang, P.-D., Hozzein, W.N., Li, W.-J., 2020. Environmental perspectives of microplastic pollution in the aquatic environment: A review. Mar. Life Sci. Technol. 2(4), 414-430.

Alimi, O.S., Budarz, J.F., Hernandez, L.M.,Tufenkji, N., 2018. Microplastics and nanoplastics in aquatic environments: Aggregation, deposition, and enhanced contaminant transport. Environ. Sci. Technol. 52(4), 1704-1724.

Alomar, C., Estarellas, F., Deudero, S., 2016. Microplastics in the Mediterranean Sea: Deposition in coastal shallow sediments, spatial variation and preferential grain size. Mar. Environ. Res. 115, 1-10.

Amato-Lourenço, L.F., Carvalho-Oliveira, R., Júnior, G.R., dos Santos Galvão, L., Ando, R.A., Mauad, T., 2021. Presence of airborne microplastics in human lung tissue. J. Hazard. Mater. 416, 126124.

An, L., Liu, Q., Deng, Y., Wu, W., Gao, Y., Wei, L., 2020. Sources of microplastic in the environment. In: He, D, Luo, Y. (eds.), Microplastics in terrestrial environments: Emerging contaminants and major challenges. The Handbook of Environmental Chemistry (Vol. 95), Springer Cham, pp. 143-159.

Anastasopoulou, A, Viršek, M.K., Varezić, D.B., Digka, N., Fortibuoni, T., Koren, Š., Mandić, M., Mytilineou, C., Pešić, A., Ronchi, F., Šiljić, J., Torre, M., Tsangaris, C., Tutman, P., 2018. Assessment on marine litter ingested by fish in the Adriatic and NE Ionian Sea macro-region (Mediterranean). Mar Pollut Bull. 133, 841-51.

Andrady, A.L., 2011. Microplastics in the marine environment, Mar. Pollut. Bull. 62(8), 1596-1605.

Avio, C.G., Gorbi, S., Milan, M., Benedetti, M., Fattorini, D., d'Errico, G., Pauletto, M., Bargelloni, L., Regoli, F., 2015. Pollutant's bioavailability and toxicological risk from microplastics to marine mussels. Environ Pollut. 198, 211-22.

Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Phil. Trans. R. Soc. B. 364, 1985-1998.

Beasley, J., Georgeson, R., Arditi, S., Barzak, P., 2014. Advancing resource efficiency in Europe: Indicators and waste policy scenarios to deliver a resource efficient and sustainable Europe. European Environmental Bureau, Brussel. Last accessed: July 2022. [Online] https://eeb.org/wp-content/uploads/2019/05/Advancing-Resource-Efficiency-in-Europe-2014.pdf

Besseling, E., Quik, J.T.K., Sun, M., Koelmans, A.A., 2017. Fate of nano- and microplastic in freshwater systems: A modeling study. Environ. Pollut. 220, 540-548.

Besseling, E., Wegner, A., Foekema, E.M., Van den Heuvel-Greve, M.J., Koelmans, A.A. 2013. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.). Environ. Sci. Technol. 47(1), 593-600.

Besseling, E., Wang, B., Lürling, M., Koelmans, A.A., 2014. Nanoplastic affects growth of *S. obliquus* and reproduction of *D. magna*. Environ. Sci. Technol. 48, 12336-12343.

Bläsing, M., Amelung, W., 2018. Plastics in soil: Analytical methods and possible sources. Sci. Total Environ. 612, 422-435.

Boucher, J., Bilard G., 2020. The Mediterranean: Mare plasticum. Gland, Switzerland: IUCN (International Union for Conservation of Nature), x+62 p.

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines woldwide: Sources and sinks. Environ. Sci. Technol. 45(21), 9175-9179.

Browne, M.A., Niven, S.J., Galloway, T.S., Rowland, S.J., Thompson, R.C., 2013. Microplastic moves pollutants and additives to worms, reducing functions linked to health and biodiversity. Curr. Biol., 23(23), 2388-2392

Busch, M., Bredeck, G., Kämpfer, A.A.M., Schins, R.P.F., 2021. Investigations of acute effects of polystyrene and polyvinyl chloride micro- and nanoplastics in an advanced in vitro triple culture model of the healthy and inflamed intestine. Environ. Res. 193, 110536.

Cappello, T., De Marco, G., Oliveri Conti, G., Giannetto, A., Ferrante, M., Mauceri, A., Maisano, M., 2021. Time-dependent metabolic disorders induced by short-term exposure to polystyrene microplastics in the Mediterranean mussel *Mytilus galloprovincialis*. Ecotoxicol. Environ. Saf. 209, 111780.

Capriotti, M., Cocci, P., Bracchetti, L., Cottone, E., Scandiffio, R., Caprioli, G., Sagratini, G., Mosconi, G., Bovolin, P., Palermo, F.A., 2021. Microplastics and their associated organic pollutants from the coastal waters of the central Adriatic Sea (Italy): Investigation of adipogenic effects *in vitro*. Chemosphere 263, 128090.

Carbery, M., O'Connor, W., Palanisami, T., 2018. Trophic transfer of microplastics and mixed contaminants in the marine food web and implications for human health. Environ. Int. 115, 400-409.

Carr, S.A., Liu, J., Tesoro, A.G., 2016. Transport and fate of microplastic particles in wastewater treatment plants. Water Res. 91, 174-182.

Cau, A., Avio, C.G, Dessì, C., Follesa, M.C., Moccia, D., Regoli, F., Pusceddu, A., 2019. Microplastics in the crustaceans *Nephrops norvegicus* and *Aristeus antennatus*: Flagship species for deep-sea environments? Environ Pollut. 255, 113107.

Celina, M.C., 2013. Review of polymer oxidation and its relationship with materials performance and lifetime prediction. Polym. Degrad. Stab. 98(12), 2419-2429.

Chen, Y., Awasthi, A.K., Wei, F., Tan, Q., Li, J., 2021. Single-use plastics: Production, usage, disposal, and adverse impacts. Sci. Total Environ. 752, 141772.

Cincinelli, A., Martellini, T., Guerranti, C., Scopetani, C., Chelazzi, D., Giarrizzo, T., 2019. A potpourri of microplastics in the sea surface and water column of the Mediterranean Sea. TrAC-Trend. Anal. Chem. 110, 321-326.

Çobanoğlu, H., Belivermiş, M., Sıkdokur, E., Kılıç, Ö., Çayır, A., 2021. Genotoxic and cytotoxic effects of polyethylene microplastics on human peripheral blood lymphocytes. Chemosphere 272, 129805.

Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. 62(12), 2588-2597.

Constant, M., Kerhervé, P., Mino-Vercellio-Verollet, M., Dumontier, M., Sànchez Vidal, A., Canals, M., Heussner, S., 2019. Beached microplastics in the Northwestern Mediterranean Sea. Mar. Pollut. Bull. 142, 263-273.

Cox, K.D., Covernton, G.A., Davies, H.L., Dower, J.F., Juanes, F., Dudas, S.E., 2019. Human consumption of microplastics. Environ. Sci. Technol. 53(12), 7068-7074.

Cózar, A., Sanz-Martín, M., Martí, E., Gonzalez-Gordillo, J.I., Ubeda, B., Gálvez, J.A., Irigoien, X., Duarte, C.M., 2015. Plastic accumulation in the Mediterranean Sea. PLoS One. 10(4), e0121762.

Cristaldi, A., Fiore, M., Zuccarello, P., Oliveri Conti, G., Grasso, A., Nicolosi, I., Copat, C., Ferrante, M., 2020. Efficiency of wastewater treatment plants (WWTPs) for microplastic removal: a systematic review. Int. J. Environ. Res. Public Health 17(21), 8014.

Dawson, A.L., Santana, M.F.M., Miller, M.E., Kroon, F.J., 2021. Relevance and reliability of evidence for microplastic contamination in seafood: A critical review using Australian consumption patterns as a case study. Environ. Pollut. 276, 116684.

de Haan, W.P., Sanchez-Vidal, A., Canals, M., 2019. Floating microplastics and aggregate formation in the Western Mediterranean Sea. Mar. Pollut. Bull. 140, 523-535.

de Lucia, G.A., Caliani, I., Marra, S., Camedda, A., Coppa, S., Alcaro, L., Campani, T., Giannetti, M., Coppola, D., Cicero, A.M., Panti, C., Baini, M., Guerranti, C., Marsili, L., Massaro, G., Fossi, M.C., Matiddi, M., 2014. Amount and distribution of neustonic micro-plastic off the western Sardinian coast (Central-Western Mediterranean Sea). Mar. Environ. Res. 100, 10-16.

Deng, Y., Zhang, Y., Qiao, R., Bonilla, M.M., Yang, X., Ren, H., Lemos, B., 2018. Evidence that microplastics aggravate the toxicity of organophosphorus flame retardants in mice (*Mus musculus*). J. Hazard. Mater. 357,348-354.

Devriese, L.I., van der Meulen, M.D., Maes, T., Bekaert, K., Paul-Pont, I., Frère, L., Robbens, J., Vethaak, A.D., 2015. Microplastic contamination in brown shrimp (*Crangon crangon*, Linnaeus 1758) from coastal waters of the Southern North Sea and Channel area. Mar. Pollut. Bull. 98 (1-2), 179-187.

Digka, N., Tsangaris, C., Torre, M., Anastasopoulou, A., Zeri, C., 2018. Microplastics in mussels and fish from the Northern Ionian Sea. Mar Pollut Bull. 135, 30-40.

Ding, J., Zhang, S., Razanajatovo, R.M., Zou, H., Zhu, W., 2018. Accumulation, tissue distribution, and biochemical effects of polystyrene microplastics in the freshwater fish red tilapia (*Oreochromis niloticus*). Environ. Pollut. 238, 1-9.

Dris, R., Gasperi, J., Mirande, C., Mandin, C., Guerrouache, M., Langlois, V., Tassin, B., 2017. A first overview of textile fibers, including microplastics, in indoor and outdoor environments. Environ. Pollut. 221, 453-458.

Dris, R., Gasperi, J., Saad, M., Mirande, C., Tassin, B., 2016. Synthetic fibers in atmospheric fallout: A source of microplastics in the environment? Mar. Pollut. Bull. 104, 290-293.

European Commission, 2019. Single use plastics. Last accessed: July 2022. [Online] https://environment.ec.europa.eu/topics/plastics/single-use-plastics_en

European Commission, 2020. Microplastics. Last accessed: July 2022. [Online] https://environment.ec.europa.eu/topics/plastics/microplastics_en

European Commission, Directorate-General for Environment, 2021. Turning the tide on single-use plastics, Available from: Publications Office. Last accessed: July 2022. [Online] https://data.europa.eu/doi/10.2779/417522

ECHA, European Chemical Agency, 2019. ECHA proposes to restrict intentionally added microplastics. Last accessed: July 2022. [Online] https://echa.europa.eu/es/-/echa-proposes-to-restrict-intentionally-added-microplastics

ECHA, European Chemical Agency, 2021. Microplastics. Last accessed: July 2022. [Online] https://echa.europa.eu/hot-topics/microplastics

EFSA, European Food and Safe Authority, 2016. Statement on the presence of microplastics and nanoplastics in food, with particular focus on seafood. EFSA Journal 14(6), 4501, 30 pp.

EFSA, European Food and Safe Authority, 2021. Scientific Colloquium 25: A coordinated approach to assess the human health risks of micro- and nanoplastics in food. Last accessed: July 2022. [Online] https://www.efsa.europa.eu/en/events/event/update-scientific-colloquium-25-coordinated-approach-assess-human-health

Ellen MacArthur Foundation, 2017. Circular fashion a new textiles economy: redesigning fashion´s future. Last accessed: July 2022. [Online] https://ellenmacarthurfoundation.org/a-new-textiles-economy

EP, European Parliament, 2015. Directive (EU) 2015/720 of the European Parliament and of the Council of 29 April 2015 amending Directive 94/62/EC as regards reducing the consumption of lightweight plastic carrier bags. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32015L0720

EP, European Parliament, 2018. Microplastics: sources, effects and solutions. Last accessed: July 2022. [Online] https://www.europarl.europa.eu/news/en/headlines/society/20181116STO19217/microplastics-sources-effects-and-solutions

EP, European Parliament, 2019. Directive (EU) 2019/904 of the European Parliament and of the Council of 5 June 2019 on the reduction of the impact of certain plastic products on the environment. Last accessed: July 2022. [Online] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32019L0904

Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G., Reisser, J., 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLoS One 9(1), e111913.

Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). Environ. Pollut. 177, 1-3.

Ferrante, M., Pietro, Z., Allegui, C., Maria, F., Antonio, C., Pulvirenti, E., Favara, C., Chiara, C., Grasso, A., Omayma, M., Conti Gea, O., Banni, M., 2022. Microplastics in fillets of Mediterranean seafood: A risk assessment study. Environ. Res. 204, 112247.

Filgueiras, A.V., Gago, J., Campillo, J.A., Leon, V.M., 2019. Microplastic distribution in surface sediments along the Spanish Mediterranean continental shelf. Environ. Sci. Pollut. Res. 26, 21264-21273.

Filgueiras, A.V., Preciado, I, Cartón, A, Gago, J., 2020. Microplastic ingestion by pelagic and benthic fish and diet composition: A case study in the NW Iberian shelf. Mar Pollut Bull. 160, 111623.

Fischer, E.K., 2016. Microplastic pollution in lakes and lake shoreline sediments: A case study on Lake Bolsena and Lake Chiusi (central Italy). Environ. Pollut. 13, 648-657.

Fonseca, M.M.A., Gamarro, E.G., Toppe, J., Bahri, T., Barg, U., 2017. The impact of microplastics on food safety: The case of fishery and aquaculture products. FAO Aquaculture Newsletter 57, 43-45.

Fossi, M.C., Marsili, L., Baini, M., Giannetti, M., Coppola, D., Guerranti, C., Caliani, I., Minutoli, R., Lauriano, G., Finoia, M.G., Rubegni, F., Panigada, S., Bérubé, M., Urbán Ramírez, J., Panti, C., 2016. Fin whales and microplastics: The Mediterranean Sea and the Sea of Cortez scenarios, Environ. Pollut. 209, 68-78.

Free, C.M., Jensen, O.P., Mason, S.A., Eriksen, M., Williamson, N.J., Boldgiv, B., 2014. High-levels of microplastic pollution in a large, remote, mountain lake, Mar. Pollut. Bull. 85(1), 156-163.

Freeman, S., Booth, A.M., Sabbah, I., Tiller, R., Dierking, J., Katja, K., Rotter, A., Ben-David, E., Javidpour, J., Angel, D.L., 2020. Between source and sea: The role of wastewater treatment in reducing marine microplastics. J. Environ. Manage. 266, 110642.

Frias, J.P.G.L., Nash, R., 2019. Microplastics: Finding a consensus on the definition. Mar. Pollut. Bull. 138, 145-147.

Fromme, H., Lahrz, T., Kraft, M., Fembacher, L., Mach, C., Dietrich, S., Burkardt, R., Völkel, W., Göen, T., 2014. Organophosphate flame retardants and plasticizers in the air and dust in German daycare centers and human biomonitoring in visiting children (LUPE 3). Environ. Int. 71, 158-163.

Gasperi, J., Wright, S.L., Dris, R., Collard, F., Mandin, C., Guerrouache, M., Langlois, V., Kelly, F.J., Tassin, B., 2018. Microplastics in air: are we breathing it in? Curr. Opin. Environ. Sci. Health 1, 1-5.

GENCAT, Agència de Residus de Catalunya-ARC. Last accessed: July 2022. https://residus.gencat.cat/es/ambits_dactuacio/recollida_selectiva/projecte_ceres

Generalitat de Catalunya, 2017. Law 5/2017 of fiscal, administrative, financial, and public sector measures and creation and regulation of taxes on large commercial establishments, on stays in tourist establishments, on radiotoxic elements, on packaged sugary drinks, and on carbon dioxide emissions. Last accessed: July 2022. [Online] http://portaldogc.gencat.cat/utilsEADOP/PDF/7340/1600738.pdf

Generalitat de Catalunya, Waste Agency of Catalunya, 2020a. Executive summary of the General program prevention and management of waste and resources of Catalonia 2013-2020. Last accessed: July 2022. [Online] https://residus.gencat.cat/web/.content/home/ambits_dactuacio/planificacio/precat20_resum_executiu_en.pdf

Generalitat de Catalunya, Waste Agency of Catalunya, 2020b. General program of prevention and management program of waste and resources of Catalonia 2013-2020. Last accessed: July 2022. [Online]

http://www20.gencat.cat/docs/arc/Home/Ambits%20dactuacio/Planificacio/PROCAT20/PRECAT20.pdf

GESAMP, 2015. Sources, fate and effects of microplastics in the marine environment: a global assessment. In: Kershaw, P.J. (Ed.), IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, London: International Maritime Organization. Rep. Stud. No. 90, p. 96.

GESAMP, 2016. Sources, fate and effects of microplastics in the marine environment: part two of a global assessment. In: Kershaw, P.J., Rochman, C.M. (Eds.), IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, London: International Maritime Organization. Rep. Stud. No. 93, p. 220.

GESAMP, 2019. Guidelines or the monitoring and assessment of plastic litter and microplastics in the ocean. In: Kershaw, P.J., Turra, A., Galgani, F. (Ed.), IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP/ISA Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, London: International Maritime Organization. Rep. Stud No. 99, p. 130.

Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 3(7), e170078.

Godoy, V., Prata, J.C., Blázquez, G., Almendros, A.I., Duarte, A.C., Rocha-Santos, T., Calero, M. and Martín-Lara, M.A., 2020. Effects of distance to the sea and geomorphological characteristics on the quantity and distribution of microplastics in beach sediments of Granada (Spain). Sci. Total Environ. 746, 142023.

Gündogdu, S., Rathod, N., Hassoun, A., Jamroz, E., Kulawik, P., Gokbulut, C., Aït-Kaddour, A., Özogul, F., 2022. The impact of nano/micro-plastics toxicity on seafood quality and human health: facts and gaps. Crit. Rev. Food Sci. Nutr., 1-19.

Guo, X., Wang, J., 2019. The chemical behaviors of microplastics in marine environment: A review. Mar. Pollut. Bull. 142, 1-14.

Guven, O., Gokdag, K., Jovanovic, B., Kideys, A.E., 2017. Microplastic litter composition of the Turkish territorial waters of the Mediterranean Sea, and its occurrence in the gastrointestinal tract of fish. Environ. Pollut. 223, 286-294.

Hartmann, N.B., Rist, S., Bodin, J., Jensen, L.Hs., Schmidt, S.N., Mayer, P., Meibom, A., Baun, A., 2017. Microplastics as vectors for environmental contaminants: Exploring sorption, desorption, and transfer to biota. Integr. Environ. Assess. Manag. 13(3), 488-493.

Henry, B., Laitala, K., Klepp, G.I., 2019. Microfibres from apparel and home textiles: Prospects for including microplastics in environmental sustainability assessment. Sci. Total Environ. 652, 483-494.

Horton, A.A., Dixon, S.J., 2018. Microplastics: an introduction to environmental transport processes. WIREs Water 5(2), e1268.

Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C., 2017. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. Sci. Total Environ. 586, 127-141.

Hwang, J., Choi, D., Han, S., Jung, S.Y., Choi, J., Hong, J., 2020. Potential toxicity of polystyrene microplastic particles. Sci. Rep. 10, 7391.

Iñiguez, M.E., Conesa, J.A., Fullana, A., 2017. Microplastics in Spanish table salt. Sci. Rep. 7, 8620.

Isobe, A., Kubo, K., Tamura, Y., Kako, S., Nakashima, E., Fujii, N., 2014. Selective transport of microplastics and mesoplastics by drifting in coastal waters. Mar. Pollut. Bull. 89(1-2), 324-330.

Ivleva, N.P., Wiesheu, A.C., Niessner, R., 2017. Microplastic in aquatic ecosystems. Angew. Chem. Int. Ed. 56(7), 1720-1739.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science 347(6223), 768-771.

Kane, I.A., Clare, M.A., Miramontes, E., Wogelius, R., Rothwell, J.J., Garreau, P., Pohl, F., 2020. Seafloor microplastic hotspots controlled by deep-sea circulation. Science 368(6495), 1140-1145.

Karapanagioti, H.K., Endo, S., Ogata Y., Takada, H., 2011. Difuse pollution by persistent organic pollutants as measured in plastic pellets sampled from various beaches in Greece. Mar. Pollut. Bull. 62(2), 312-317.

Karkanorachaki, K., Kiparissis, S., Kalogerakis, G.C., Yiantzi, E., Psillakis, E., Kalogerakis, N., 2018. Plastic pellets, meso- and microplastics on the coastline of Northern Crete: Distribution and organic pollution. Mar. Pollut. Bull. 133, 578-589.

Kazour, M., Jema, S., Issa, C., Khalaf, G., Amara, R., 2019. Microplastics pollution along the Lebanese Coast (Eastern Mediterranean Basin): Occurrence in surface water, sediments and biota samples. Sci. Total Environ. 696, 133933.

Koelmans, A.A., Bakir, A. Burton, G.A., Janssen, C.R., 2016. Microplastic as a vector for chemicals in the aquatic environment: Critical review and model-supported re-interpretation of empirical studies. Environ. Sci. Technol. 50(7), 3315-3326.

Kyrikou, I., Briassoulis, D., 2007. Biodegradation of agricultural plastic films: A critical review. J. Polym. Environ. 15, 125–150.

Lagarde, F., Oliviera, O., Zanella, M., Daniel, P., Hiard, S., Caruso, A., 2016. Microplastic interactions with freshwater microalgae: Hetero-aggregation and changes in plastic density appear strongly dependent on polymer type. Environ. Pollut. 215, 331-339.

Lambert, S., Wagner, M., 2016. Characterization of nanoplastics during the degradation of polystyrene. Chemosphere, 145, 265-268.

Lares, M., Ncibi, M.C., Sillanpää, M., Sillanpää, M., 2018. Occurrence, identification and removal of microplastic particles and fibers in conventional activated sludge process and advanced MBR technology. Water Res. 133, 236-246.

Law, K.L., Morét-Ferguson, S., Maximenko, N.A., Proskurowski, G., Peacock, E.E., Hafner, J., Reddy, C.M., 2010. Plastic accumulation in the North Atlantic subtropical gyre. Science 329 (5996), 1185-1188.

Lebreton, L.C.M., Greer, S.D., Borrero, J.C., 2012. Numerical modelling of floating debris in the world's oceans. Mar. Pollut. Bull. 64(3), 653-661.

Lebreton, L.C.M., van der Zwet, J., Damsteeg, J.-W., Slat, B., Andrady, A., Reisser, J., 2017. River plastic emissions to the world' s oceans. Nat. Commun. 8, 15611.

Lei, L., Wu, S., Lu, S., Liu, M., Song, Y., Fu, Z., Shi, H., Raley-Susman, K.M., He, D., 2018. Microplastic particles cause intestinal damage and other adverse effects in zebrafish *Danio rerio* and nematode *Caenorhabditis elegans*. Sci. Total Environ. 619-620,1-8.

Leslie, H.A., Brandsma, S.H., van Velzen, M.J.M., Vethaak, A.D., 2017. Microplastics en route: Field measurements in the Dutch river delta and Amsterdam canals, wastewater treatment plants, North Sea sediments and biota. Environ. Int. 101, 133-142.

Leslie, H.A., Depledge, M.H., 2020. Where is the evidence that human exposure to microplastics is safe? Environ. Int. 142, 105807.

Li, B., Ding, Y., Cheng, X., Sheng, D., Xu, Z., Rong, Q., Wu, Y., Zhao, H., Ji, X., Zhang, Y., 2020a. Polyethylene microplastics affect the distribution of gut microbiota and inflammation development in mice. Chemosphere 244, 125492.

Li, X., Hu, J., Qiu, R., Zhang, X., Chen, Y., He, D., 2020b. Joint toxic effects of polystyrene nanoparticles and organochlorine pesticides (chlordane and hexachlorocyclohexane) on *Caenorhabditis elegans*. Environ. Sci. Nano. 7(10), 3062-3073.

Liebmann, B., Köppel, S., Königshofer, P., Bucsics, T., Reiberger, T., Schwabl, P., 2018. Assessment of microplastic concentrations in human stool: Final results of a prospective study. Poster Presentation at Nano and Microplastics in Technical and Freshwater Systems– Microplastics 10. Environment Agency Austria.

Linares, V., Bellés, M., Domingo, J.L., 2015. Human exposure to PBDE and critical evaluation of health hazards. Arch. Toxicol. 89(3), 335-356.

Lithner, D., Larsson, Å., Dave, G., 2011. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Sci. Total Environ. 409(18), 3309-3324.

Liu, S., Wu, X., Gu, W., Yu, J., Wu, B., 2020. Influence of the digestive process on intestinal toxicity of polystyrene microplastics as determined by *in vitro* Caco-2 models. Chemosphere 256, 127204.

Liu, W., Zhang, J., Liu, H., Guo, X., Zhang, X., Yao, X., Cao, Z., Zhang, T., 2021. A review of the removal of microplastics in global wastewater treatment plants: Characteristics and mechanisms. Environ. Int. 146, 106277.

Liubartseva, S., Coppini, G., Lecci, R., Clementi, E., 2018. Tracking plastics in the Mediterranean: 2D Lagrangian model. Mar. Pollut. Bull. 129(1), 151-162.

Llorca, M., Farré, M., Karapanagioti, H.K., Barceló D., 2014. Levels and fate of perfluoroalkyl substances in beached plastic pellets and sediments collected from Greece. Mar. Pollut. Bull. 87(1-2), 286-291.

Lobelle, D., Cunliffe, M., 2011. Early microbial biofilm formation on marine plastic debris. Mar. Pollut. Bull., 62(1), 197-200.

Lu, L., Wan, Z., Luo, T., Fu, Z., Jin, Y., 2018. Polystyrene microplastics induce gut microbiota dysbiosis and hepatic lipid metabolism disorder in mice. Sci. Total Environ. 631-632, 449-458.

Lucas, N., Bienaime, C., Belloy, C., Queneudec, M., Silvestre, F., Nava-Saucedo, J.-E. 2008. Polymer biodegradation: Mechanisms and estimation techniques – A review. Chemosphere 73(4), 429-442.

Lusher, A., Bråte, I.L.N., Hurley, R., Iversen, K., Olsen, M., 2017. Testing of methodology for measuring microplastics in blue mussels (*Mytilus* Spp) and sediments, and recommendations for future monitoring of microplastics (R & D project). Oslo: Norwegian Institute for Water Research. . Last accessed: July 2022. [Online] https://niva.brage.unit.no/niva-xmlui/bitstream/handle/11250/2470297/7209-2017.pdf?sequence=1&isAllowed=y

Lusher, A.L., Burke, A., O'Connor, I., Officer, R., 2014. Microplastic pollution in the Northeast Atlantic Ocean: validated and opportunistic sampling. Mar. Pollut. Bull., 88(1-2), 325-333.

Mahon, A.M., O'Connell, B., Healy, M.G., O'Connor, I., Officer, R., Nash, R., Morrison, L., 2017. Microplastics in sewage sludge: Effects of treatment. Environ. Sci. Technol. 51(2), 810-818.

McIntyre, A., Vincent, R.M., Perkins, A.C., Spiller, R.C., 1997. Effect of bran, ispaghula, and inert plastic particles on gastric emptying and small bowel transit in humans: The role of physical factors. Gut. 40(2), 223-227.

Mistri, M., Infantini, V., Scoponi, M., Granata, T., Moruzzi, L., Massara, F., De Donati, M., Munari, C., 2017. Small plastic debris in sediments from the central Adriatic Sea: types, occurrence and distribution. Mar. Pollut. Bull. 124(1), 435-440.

Mistri, M., Scoponi, M., Granata, T., Moruzzi, L., Massara, F., Munari, C., 2020. Types, occurrence and distribution of microplastics in sediments from the northern Tyrrhenian Sea. Mar. Pollut. Bull. 153, 111016.

MITECO, Ministry for Ecological Transition and Demographic Challenge, 2021. « La Ley de Residuos y Suelos Contaminados para una Economía Circular entra en la recta final de su tramitación parlamentaria ». Last accessed: July 2022. [Online] https://www.miteco.gob.es/es/prensa/211223ndpleyderesiduos_tcm30-534543.pdf

Murphy, F., Ewins, C., Carbonnier, F., Quinn, B., 2016. Wastewater treatment works (WwTW) as a source of microplastics in the aquatic environment. Environ. Sci. Technol. 50(11), 5800-5808.

Nizzetto, L., Futter, M., Langaas, S., 2016. Are agricultural soils dumps for microplastics of urban origin? Environ. Sci. Technol. 50(20), 10777-10779.

OECD, Organisation for Economic Co-operation and Development, 2022. Plastic leakage and greenhouse gas emissions are increasing. Last accessed: July 2022. [Online] https://www.oecd.org/environment/plastics/increased-plastic-leakage-and-greenhouse-gas-emissions.htm

Oliveira, M., Ribeiro, A., Hylland, K., Guilhermino, L., 2013. Single and combined effects of microplastics and pyrene on juveniles (0+ group) of the common goby *Pomatoschistus microps* (Teleostei, Gobiidae), Ecol. Indic. 34, 641-647.

Oliveri Conti, G., Ferrante, M., Banni, M., Favara, C., Nicolosi, I., Cristaldi, A., Fiore, M., Zuccarello, P., 2020. Micro- and nano-plastics in edible fruit and vegetables. The first diet risks assessment for the general population. Environ. Res. 187, 109677.

Papadimitriu, M., Allinson, G., 2022. Microplastics in the Mediterranean marine environment: a combined bibliometric and systematic analysis to identify current trends and challenges. Microplastics and Nanoplastics 2, 8.

Park, T.-J., Lee, S.-H., Lee, M.-S., Lee, J.-K., Park, J.-H., Zoh, K.-D., 2020. Distributions of microplastics in surface water, fish, and sediment in the vicinity of a sewage treatment plant. Water, 12(12), 3333.

Pettipas, S., Bernier, M., Walker, T.R., 2016. A Canadian policy framework to mitigate plastic marine pollution. Mar. Policy, 68, 117-122.

Piyawardhana, N., Weerathunga, V., Chen, H.-S., Guo, L., Huang, P.-J., Ranatunga, R.R.M.K.P., Hung, C.-C., 2022. Occurrence of microplastics in commercial marine dried fish in Asian countries. J. Hazard. Mater. 423(Part B), 127093.

PlasticsEurope, 2015. Plastics - the Facts 2014/2015: An analysis of European lastests plastics production, demand and waste data. Brussels: Association of Plastic Manufacturers. Last accessed: July 2022. [Online] https://plasticseurope.org/wp-content/uploads/2021/10/2014-Plastics-the-facts.pdf

PlasticsEurope, 2018. Plastics - the Facts 2018. An analysis of European plastics production, demand and waste data. Brussels: Association of Plastic Manufacturers. Last accessed: July 2022. [Online] https://plasticseurope.org/wp-content/uploads/2021/10/2018-Plastics-the-facts.pdf

PlasticsEurope, 2021. Plastics - the facts 2021. An analysis of European plastics production, demand and waste data for 2019-2020. Plastics Europe. Brussels: Association of Plastic Manufacturers. Last accessed: July 2022. [Online] https://plasticseurope.org/wp-content/uploads/2021/12/Plastics-the-Facts-2021-web-final.pdf

Plastigaur, 2021. « Actualización sobre el impuesto al plástico ». Last accessed: July 2022. [Online] https://www.plastigaur.com/el-impuesto-al-plastico-en-detalle-actualizado-dic-2021/

Raziyafathima, M., Praseetha, P.K., Rimal, I.R.S., 2016. Microbial degradation of plastic waste: a review. J. Pharm. Chem. Biol. Sci., 4(2), 231-242.

Rehse, S., Kloas, W., Zarfl, C., 2016. Short-term exposure with high concentrations of pristine microplastic particles leads to immobilisation of *Daphnia magna*. Chemosphere 153, 91-99.

Reisser, J., Slat, B., Noble, K., du Plessis, K., Epp, M., Proietti, M., de Sonneville, J., Becker, T., Pattiaratchi, C., 2015. The vertical distribution of buoyant plastics at sea: An observational study in the North Atlantic Gyre. Biogeosciences, 12(4), 1249-1256.

Rist, S., Almroth, B.C., Hartmann, N.B., Karlsson, T.M., 2018a. A critical perspective on early communications concerning human health aspects of microplastics. Sci. Total Env. 626, 720-726.

Rist, S., Baun, A., Hartmann, N.B., 2017. Ingestion of micro- and nanoplastics in *Daphnia magna* - quantification of body burdens and assessment of feeding rates and reproduction. Environ. Pollut. 228, 398-407.

Rist, S., Hartmann, N.B., 2018b. Aquatic Ecotoxicity of Micro plastics and Nanoplastics: Lessons Learned from Engineered Nanomaterials. In: Wagner M., Lambert S. (eds) Freshwater Microplastics. The Handbook of Environmental Chemistry, vol 58. Springer, Cham.

Rochman, C.M., Manzano, C., Hentschel, B.T., Simonich, S.L.M., Hoh, E., 2013. Polystyrene plastic: A source and sink for polycyclic aromatic hydrocarbons in the marine environment. Environ. Sci. Technol. 47(24), 13976-13984.

Rubin, A.E., Zucker, I., 2022. Interactions of microplastics and organic compounds in aquatic environments: A case study of augmented joint toxicity. Chemosphere 289, 133212.

Schantz, S.L., Seo, B.W., Moshtaghian, J., Peterson, R.E., Moore, R.W., 1996. Effects of gestational and lactational exposure to TCDD or coplanar PCBs on spatial learning. Neurotoxicol. Teratol. 18(3), 305-313.

Schirinzi, G.F., Pérez-Pomeda, I., Sanchís, J., Rossini, C., Farré, M., Barceló, D., 2017. Cytotoxic effects of commonly used nanomaterials and microplastics on cerebral and epithelial human cells. Environ. Res., 159, 579-587.

Sherrington, C., 2016. Plastics in the marine environment. Available from: Eunomia. Last accessed: July 2022. [Online] https://www.eunomia.co.uk/reports-tools/plastics-in-the-marine-environment/#:~:text=94%25%20of%20the%20plastic%20that,little%20actually%20ends%20up%20there.

Simon-Sánchez, L., Grelaud, M., Garcia-Orellana, J., Ziveri, P., 2019. River Deltas as hotspots of microplastic accumulation: The case study of the Ebro River (NW Mediterranean). Sci. Total Environ. 687, 1186-1196.

Smith, M., Love, D.C., Rochman, C.M., Neff, R.A., 2018. Microplastics in seafood and the implications for human health. Curr. Environ. Health Rep. 5(3), 375-386.

Sol, D., Laca, A., Laca, A., Díaz, M., 2020. Approaching the environmental problem of microplastics: Importance of WWTP treatments. Sci. Total Environ. 740, 140016.

Soto-Navarro, J., Jordá, G., Compa, M., Alomar, C., Fossi, M.C., Deudero, S., 2021. Impact of the marine litter pollution on the Mediterranean biodiversity: A risk assessment study with focus on the marine protected areas. Mar. Pollut. Bull. 165, 112169.

Stock, V., Bohmert, L., Lisicki, E., Block, R., Cara-Carmona, J., Pack, L.K., Selb, R., Lichtenstein, D., Voss, L., Henderson, C.J., Zabinsky, E., Sieg, H., Braeuning, A., Lampen, A., 2019. Uptake and effects of orally ingested polystyrene microplastic particles *in vitro* and *in vivo*. Arch. Toxicol. 93(7),1817-1833.

Su, L., Cai, H., Kolandhasamy, P., Wu, C., Rochman, C.M., Shi, H., 2018. Using the Asian clam as an indicator of microplastic pollution in freshwater ecosystems. Environ. Pollut. 234, 347-355.

Suaria, G., Avio, C.G., Mineo, A., Lattin, G.L., Magaldi, M.G., Belmonte, G., Moore, C.J., Regoli, F., Aliani, S., 2016. The Mediterranean plastic soup: Synthetic polymers in Mediterranean surface waters. Sci. Rep. 6, 37551.

Sun, J., Dai, X., Wang, Q., van Loosdrecht, M.C.M., Ni, B.-J., 2019. Microplastics in wastewater treatment plants: Detection, occurrence and removal. Water Res. 152, 21-37.

Talvitie, J., Mikola, A., Koistinen, A., Setälä, O., 2017. Solutions to microplastic pollution - Removal of microplastics from wastewater effluent with advanced wastewater treatment technologies. Water Res. 15(123), 401-407.

Ter Halle, A., Ladirat, L., Gendre, X., Goudouneche, D., Pusineri, C. Routaboul, C., Tenailleau, C., Duployer, B., Perez, E., 2016. Understanding the fragmentation pattern of marine plastic debris. Environ. Sci. Technol. 50, 5668-5675.

Thiele, C.J., Hudson, M.D., Russell, A.E., Saluveer, M., Sidaoui-Haddad, G., 2021. Microplastics in fish and fishmeal: An emerging environmental challenge? Sci. Rep. 11, 2045.

Thiemann, T., 2021. Isolation of Phthalates and Terephthalates from Plant Material-Natural Products or Contaminants? Open Chem. J., 8, 1-36.

Thompson, R.C., Olson, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at sea: Where is all the plastic? Science, 304(5672), 838.

Thompson, R., Moore, C., Andrady, A., Gregory, M., Takada, H., Weisberg, S., 2005. New directions in plastic debris. Science. 310(5751), 1117.

Tomazic-Jezic, V.J., Merritt, K., Umbreit. T.H., 2001. Significance of the type and the size of biomaterial particles on phagocytosis and tissue distribution. J. Biomed. Mater. Res. 55(4), 523-529.

UNEP, United Nations Environment Programme, 2018. The state of plastics: World Environment Day Outlook 2018. Last accessed: July 2022. [Online] https://www.unep.org/resources/report/state-plastics-world-environment-day-outlook-2018

UNEP, GRID-Arendal, Basel Conventions, 2021. Drowning in plastics: Marine litter and plastic waste vital graphics. Switzerland: UNEP. 77 p.

van der Hal, N., Ariel, A., Angel, D.L., 2017. Exceptionally high abundances of microplastics in the oligotrophic Israeli Mediterranean coastal waters. Mar. Pollut. Bull., 116(1-2), 151-155.

van Sebille, E., Wilcox, C., Lebreto, L., Maximenko, N., Hardesty, D.B., van Franeker, J.A., Eriksen, M., Siegel, D., Galgani, F., Law, K.L., 2015. A global inventory of small floating plastic debris. Environ. Res. Lett. 10, 124006.

Verschoor, A.J., 2015. Towards a definition of microplastics considerations for the specification of physico-chemical properties. RIVM Letter Report 2015-0116. National Institute for Public Health and the Environment, The Netherlands, p. 38. Last accessed: July 2022. [Online] https://www.rivm.nl/bibliotheek/rapporten/2015-0116.pdf

Vianello, A., Boldrin, A., Guerriero, P., Moschino, V., Rella, R., Sturaro, A., Da Ros, L., 2013. Microplastic particles in sediments of Lagoon of Venice, Italy: First observations on occurrence, spatial patterns and identification. Estuarine, Coastal and Shelf Science, 130, 54-61.

Vollertsen, J., Hansen, A.A., 2017. Microplastic in Danish wastewater: Sources, occurrences and fate. Environmental Project Report 1906. p. 56. Available from: The Danish Environmental Protection Agency. Last accessed: July 2022. [Online] https://www2.mst.dk/Udgiv/publications/2017/03/978-87-93529-44-1.pdf

Wakkaf, T., El Zrelli, R., Kedzierski, M., Balti, R., Shaiek, M., Mansour, L., Tlig-Zouari, S., Bruzaud, S., Rabaoui, L., 2020. Characterization of microplastics in the surface waters of an urban lagoon (Bizerte lagoon, Southern Mediterranean Sea): Composition, density, distribution, and influence of environmental factors. Mar. Pollut. Bull., 160, 111625.

Waldschläger, K., Lechthaler, S., Stauch, G., Schüttrumpf, H., 2020. The way of microplastic through the environment: Application of the source-pathway-receptor model (review). Sci. Total Environ., 713, 136584.

Walkinshaw, C., Lindeque, P.K., Thompson, R., Tolhurst, T., Cole, M. 2020. Microplastics and seafood: lower trophic organisms at highest risk of contamination. Ecotoxicol. Environ. Saf. 190,110066.

Wardrop, P., Shimeta, J., Nugegoda, D., Morrison, P.D., Miranda, A., Tang, M., Clarke, B.O, 2016. Chemical pollutants sorbed to ingested microbeads from personal care products accumulate in fish. Environ. Sci. Technol. 50(7), 4037-4044.

Welden, N.A.C., Cowie, P.R., 2016. Long-term microplastic retention causes reduced body condition in the langoustine, *Nephrops norvegicus*. Environ. Pollut. 218, 895-900.

WHO, World Health Organization, 2019. Call for experts on human health risks from exposure to microplastic. Last accessed: July 2022. [Online] https://www.who.int/news-room/articles-detail/call-for-experts-on-human-health-risks-from-exposure-to-micropalstic

Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy, B.E., Thompson R.C., 2014. The deep sea is a major sink for microplastic debris. R. Soc. Open Sci. 1(4), 140317.

Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: A review. Environ. Pollut., 178, 483-492.

Wu, P., Huang, J., Zheng, Y., Yang, Y., Zhang, Y., He, F., Chen, H., Quan, G., Yan, J., Li, T., Gao, B., 2019. Environmental occurrences, fate, and impacts of microplastics. Ecotoxicol. Environ. Saf. 184, 109612.

Xu, X., Jian, Y., Xue, Y., Hou, Q., Wang, L.P., 2019. Microplastics in the wastewater treatment plants (WWTPs): Occurrence and removal. Chemosphere 235, 1089-1096.

Yang, L., Zhang, Y., Kang, S., Wang, Z., Wu, C., 2021. Microplastics in soil: A review on methods, occurrence, sources, and potential risk. Sci. Total Environ, 780, 146546.

Zettler, E.R., Mincer, T.J., Amaral-Zettler, L.A., 2013. Life in the "plastisphere": Microbial communities on plastic marine debris. Environ. Sci. Technol. 47(13), 7137-7146.

Zhang, W., Ma, X., Zhang, Z., Wang, Y., Wang, J., Wang, J., Ma, D., 2015. Persistent organic pollutants carried on plastic resin pellets from two beaches in China. Mar. Pollut. Bull., 99(1-2), 28-34.

Zheng, H., Wang, J., Wei, X., Chang, L., Liu, S., 2021. Proinflammatory properties and lipid disturbance of polystyrene microplastics in the livers of mice with acute colitis. Sci. Total Environ. 750, 143085.

Ziajahromi, S., Neale, P.A., Rintoul, L., Leusch, F.D.L., 2017. Wastewater treatment plants as a pathway for microplastics: Development of a new approach to sample wastewater-based microplastics. Water Res. 112, 93-99.

Zubris, K.A.V., Richards, B.K., 2005. Synthetic fibers as an indicator of land application of sludge. Environ. Pollut., 138(2), 201-211.

Zuccarello, P., Ferrante, M., Cristaldi, A., Copat, C., Grasso, A., Sangregorio, D., Fiore, M., Oliveri Conti, G., 2019. Exposure to microplastics (<10 μm) associated to plastic bottles mineral water consumption: the first quantitative study. Water Res. 157, 365-371.

2. Objetives

Due to the potential risk posed by MPs to the marine environment and humans, there is a great interest among the scientific community to develop standardized methods of analysis to quantify and characterize the levels of microplastic contamination in coastal areas. Given the biological richness and concentration of aquaculture and fisheries economic activities in the Mediterranean Sea, the effects of plastic pollution on marine and human life are expected to be particularly frequent in this plastic accumulation region.

To contribute to knowledge about MPs hazards in environment, **the general objective of this Thesis is to provide scientific knowledge on the origin, levels, dynamics and impact of MPs on coastal ecosystems in order to improve their management and preservation of marine environment. In addition, provide tools for legislation, and ultimately contribute to knowledge to prevention and mitigation pollution measures.** The results will help the policy makers and regulators take sound decisions that protect the environment as: deeper polymer research and development, changes in waste management, chemical legislation review about plastic additives and verified current overconsumption of plastic products to control their production, use, disposal, and recycling.

The Catalan coast will be analyzed as a case study. Specifically, in the Catalan Coast the MPs might to affect the good ecology state of regions as Ebro's Delta or get worse the deficient (very degraded) ecology state localized in main ports (Agencia Catalana del Agua 2007-2012 report, https://aca.gencat.cat/).

The project has the following specific objectives:

- To optimize the methodology of MPs extraction in environmental samples waters, marine sediments, beach sands and edible molluscs of Catalan coast as a contribution to global standardization methodology.

- To obtain integrated view of the concentration, composition and distribution of MPs in coastal environment and possible sources.

- To assess human exposure of MPs by autochthonous shellfish ingestion.

- To characterize the environmental risk by MPs pollution in Catalonia coastal areas.

3.-Materials and Methods

Analysis of MPs from environmental samples requires procedures as extraction (separation and purification), identification, quantification and polymeric composition. First, larger particles ≥0.5 mm are removed with tweezers and their identification and quantification is often performed by visual approaches (visual sorting) (Hidalgo-Ruz et al., 2012; Rocha-Santos & Duarte, 2017; Sturm et al., 2021). The particles were extracted by size and shape under stereoscopic microscope from 8 to 80 X magnification in LEICA DMS 1000 and LEICA MZ10 equipped with a FLEXACAM C1 and confirmed small particles shape in an optical microscope Olympus CX41. Particles were extracted following the criteria: fibres associated with the sediment fragments, weathered fibres, pellets or granules with colour and homogeneous surface, colored fragments without strips or striations, fragments not break to preasure, pellets or transparent fragments non-irisdescent, non-crystalline. These were grouped according their density measured through the immersion of particles in three different solutions: water, sodium chloride NaCl and zinc chloride with density 1 g/mL, 1.2 g/mL and 1.8 g/mL respectively (figure 2.3). The particles density range is determined according its buoyancy in each solution. Particles without buoyancy in zinc chloride $ZnCl_2$ was not considered as "microplastics".

In order to analyse the smaller plastic fraction (<0.5 mm), MPs particles were extracted from sand and sediments by density separation using zinc chloride ($ZnCl_2$, 1.8 g/ml) one of the most common techniques in the extraction of MPs (Rocha-Santos & Duarte, 2017). The density separation is based on density differences of plastics (0.8-1.6 g/cm^3) and sediment (2.7-3 g/cm^3) (Hidalgo-Ruz et al., 2012; Rocha-Santos & Duarte, 2017). The separation the plastic particles from sand grains is through sample agitation, after mixing, the relatively low-density particles remain in buoyancy and are collected by supernatant extraction (figure 2.3 and 2.4)

According to Prata et al., (2019), the plastic density is dependent on polymer type, additive concentration, and even adsorbed substances and organisms. For seawater and biota, density separation step is applied for mineral from no mineral particles separation in order to cleaning filters for MPs identification. The $ZnCl_2$ solution collected can be reused by filtering previously through nitrocellulose Millipore 47mmØ, 0.45µm pore size filter.

For both fractions is needed to remove organic matter for MPs identification and quantification, biological material is often confused with plastics (e.g., darker algae fragments, wood), leading to overestimation of environmental concentrations and increasing the number of particles subjected to further analysis (Prata et al., 2019). The organic matter removal was carried out with chemically, enzymatic or both procedures without destroying any MPs particle present (damage or discolour). Potassium hydroxide (good digestion of organic matter and recovery of plastics),

hydrogen peroxide (with little to no degradation of polymers), wet peroxide solutions (Fenton's solution: Hydrogen peroxide/iron sulfate, $H_2O_2/FeSO_4$ acidified) and enzymes (less likely to induce damage to MPs) as protease, lipase, cellulase and chitinase were used for digestion processes (Sancataldo et al., 2020).

Information regarding the size, shape, surface morphology and some elemental composition (non-routine) of plastic particles were achieved by using stereoscopic, optical and scanning electron microscopy plus energy-dispersive X-ray (ESEM-EDS).

For polymeric composition analysis of MPs spectroscopic techniques were used, Raman and FTIR offered reliable and fast analysis of polymer identification. The resulting IR spectrum reveals similar information as Raman spectrum, the sample been characterized and identified on the basis of the chemical structure and by comparison with known reference spectra or by knowledge of the chemical bond stretching in spectral regions. A cheaper and fast screening approach was also used for MPs identification based on selective fluorescent staining using the lipophilic fluorescent dye Nile Red (NR) proposed by Maes et al. (2017), due lipophilic properties of fluorescent dye NR, tends to adsorb on the surface of nonpolar materials such as plastics (Sturm et al., 2021). Being a lipophilic dye, some dyed non plastic organic substances may show fluorescence, but always weaker than MPs; therefore, studies recommend the use of this technique in conjunction with the digestion of organic matter through digestion with 30-35% Hydrogen peroxide (H_2O_2) (Sturm et al., 2021; Tagg et al., 2015). Even though biogenic material, such as algae, seaweed, wood, feathers and mollusc shells are not stained. Furthermore, due the ability of NR to change of colour as the polarity of the solvent changes (i.e., solvatochromic nature), a categorization of plastic nature it is possible (Erni-Cassola et al., 2017).

Briefly, MPs extracted with density separation were filtered on PC (Polycarbonate) filter 10 µm pore size and 25mm Ø, followed by an organic matter removal step, the solution was filtered in the same PC filter and cleaned with ultrapure water (distilled water filtered through 0.45 µm nitrocellulose pore size) and observed under stereoscopic microscope to ensure organic matter complete removal and iron particles precipitated. It is recommended to use a few drops of nitric acid (HNO_3) very diluted solution (0.02 M) and wash the PC filter to prevent iron precipitating from Fenton's reagent on it.

The filter containing the sample was stained with 2-3 drops of NR 5-10 mg/L (solution preserved in 25 mL vials in the dark), of Nile Red (NR) methanol solution after 10-30 min of incubation. The PC filter is placed on Sedgwick-Rafter camera and observed under an optical fluorescence microscope Zeiss Axioskop 40 FL 4-10x/0.25 (objetives 4x y 10x, 40-100x total magnification) (at different wavelengths. For MPs identification, plastics and no plastics products (commonly

found on the market and nature) identified with spectroscopic techniques were after stained with Nile Red, the emission fluorescence and intensity were recorded for excitation wavelength from 362±12-546±12, and an identification key was stablished (Table 3.1).

Under the fluorescence microscope, the filter stained with the NR solution is excited at different wavelengths (362±12nm, 450-490 nm, 546±12 nm) with emission in different colours light (yellow-green: 492-587nm, red/orange: 597-700 nm, and blue: 455-492 nm, respectively). The identification of MPs was carried according to the table 2.1, depending on the nature of the polymer the fluorescence intensity measure qualitatively is registered for each wavelength (high intensity ✓✓✓ (3), medium intensity ✓✓ (2), low intensity ✓ (1). The sizes were measure using the Sedgwick-Rafter gridding combined with 0.1 mm high precision eyepiece micrometer. The MPs registered were only classified in "fibres" or "particles" avoiding mistakes in morphology classification by fluorescence interferences.

Table 3.1. Emission colours and intensity of different polymers after staining with Nile Red methanolic solution

Plastic polymer	RED/ORANGE	GREEN	YELLOW	BLUE
PP	✓	✓✓✓	✖	✓
PE (HDPE, LDPE)	✓	✓✓✓	✖	✓
PS (PS, EPS)	✓✓✓	✖	✓✓✓	✖
PU	✓✓✓	✖	✓✓✓	✖
PET	✓	✖	✓	✓✓
PVC	✓✓✓	✖	✖	✖
PMA	✓✓	✓✓	✖	✖
PVAc	✓✓✓	✓✓✓		✓✓✓
PES	✓✓	✖	✓✓	✓✓
PA	✓✓	✓	✖	✓✓
Cotton (natural cellulose)	✖	✖	✖	✖
Wood	✓	✖	✖	✖
Chitin	✓	✖	✖	✖
Treated Chitin/wood	✖	✖	✖	✖
Wheel rubber	✖	✖	✖	✖

Optimal methodologies were developed adapting the previous processes sequence and parameters developed previously by Claessens et al., (2013); Coppock et al., (2017); Dehaut et al., (2016); Hidalgo-Ruz et al., (2012); Hurley et al., (2018); Löder et al., (2017); Lusher et al., (2014); Maes et al., (2017); Mai et al., (2018); Masura et al., (2015); Munno et al., (2018), Nuelle et al., (2014), and to specific Catalonia Coast samples requirements for turbidity and organic matter removal. The methodology proposed offers faster and practicable procedures for MPs extraction from sand, sediment, seawater, biota samples with usage of gently reagents or enzymes to obtain clear filters for MPs measures. The MPs pre-selection based in buoyancy in visual sorting step guided and

supported the spectroscopy analysis for sand and sediment samples as well as changes of H_2O_2:$FeSO_4$ relation in Fenton's reagents for O.M. removals and turbidity avoid filter yellowish coloration. Nile Red fluorescent technique was used after a density-based extraction, digestion and filtration steps.

The general scheme for MPs quantification and identification in sand, sediment and seawater samples is shown in figure 3.1 and 3.2. The optimized methodologies for environmental samples are shown from figure 3.3 to 3.7. Still is under research the agitation and sonication effect on MPs fragmentation. Total process for seawater samples MPs extraction from 0.05 to 5 mm size takes maximum 8 days, for sand and sediment MPs extraction from 0.02 to 5 mm takes 7 days for complete organic matter removals and 3 days applying only Nile Red fluorescence technique. Finally, for biota, MPs extraction from 0.02 to 5 mm fulfilling all phases and density separation steps, takes 17 days. The phases were divided in four: phase 1 **KS**: Potasium Hidroxide (KOH) and Sodium dodecyl sulfate (SDS), phase 2: **E+H** enzymes and hydrogen peroxide, phase 3: **F-Ch**, Fenton processes and Chitinase enzyme., phase 4: **DS**, density separation.

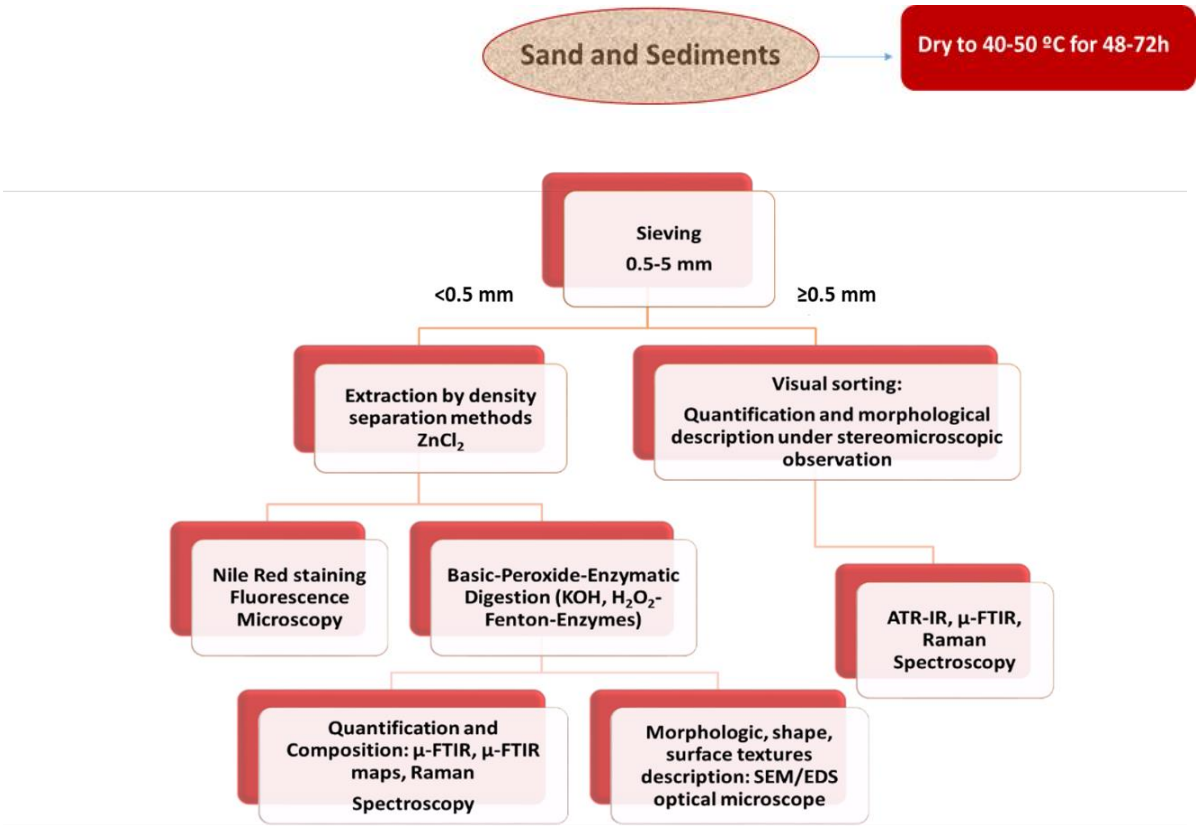


Figure 3.1. General scheme for microplastics extraction and identification in sand and sediment samples.

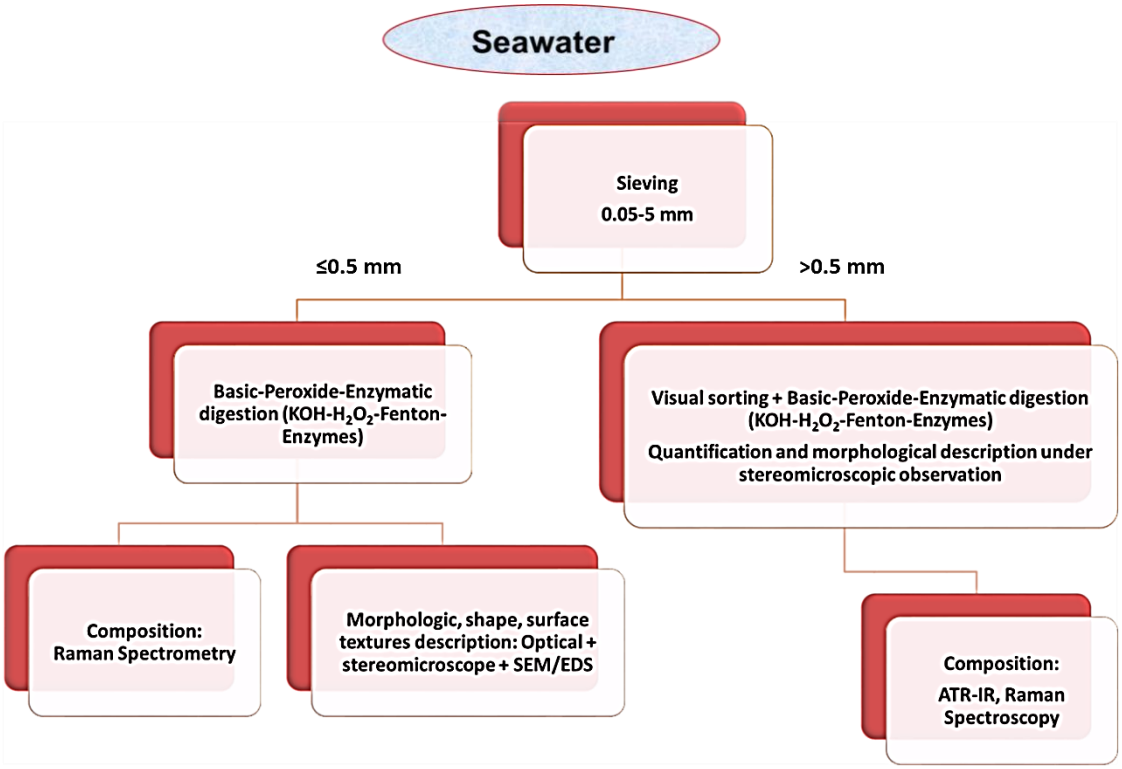
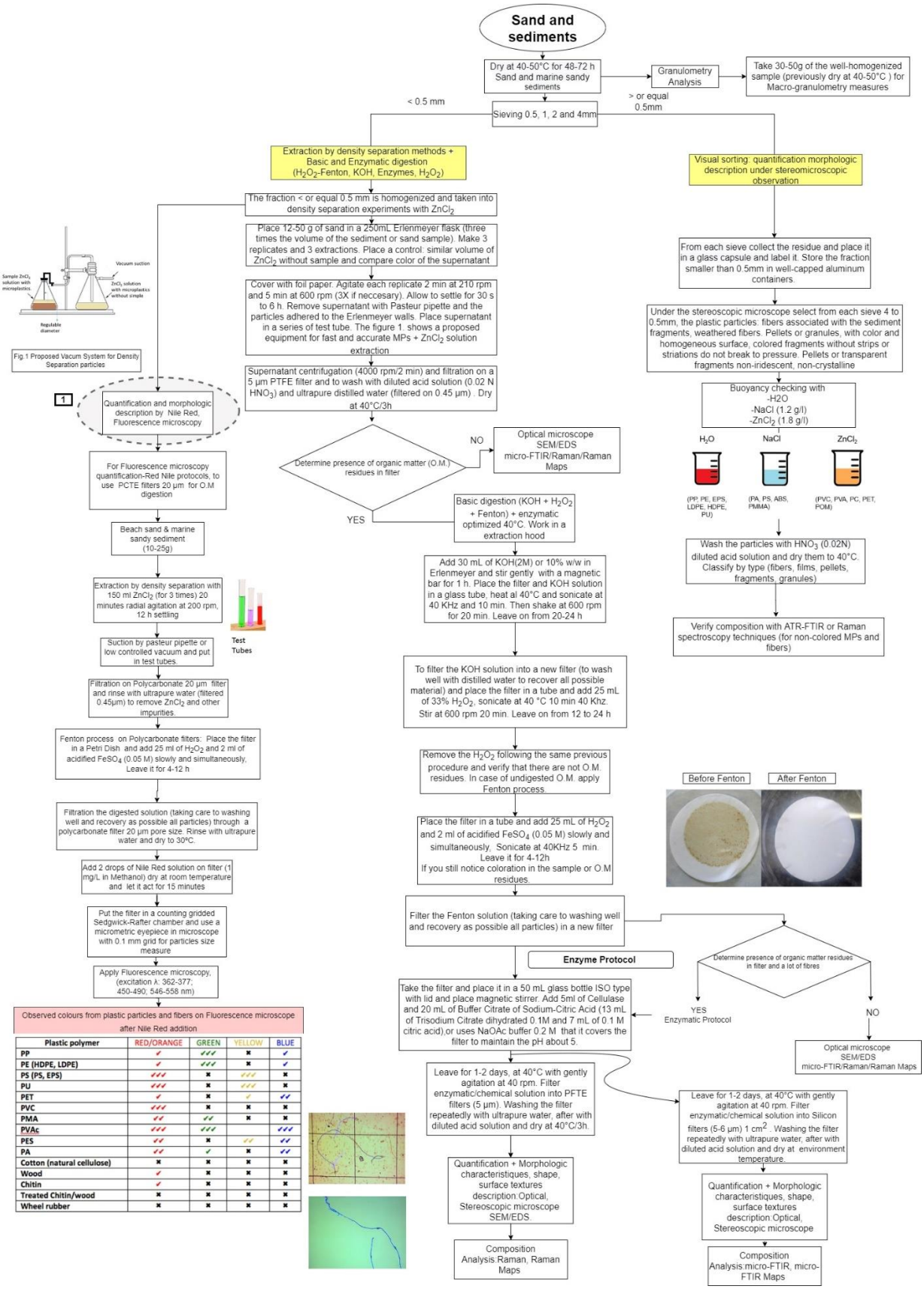
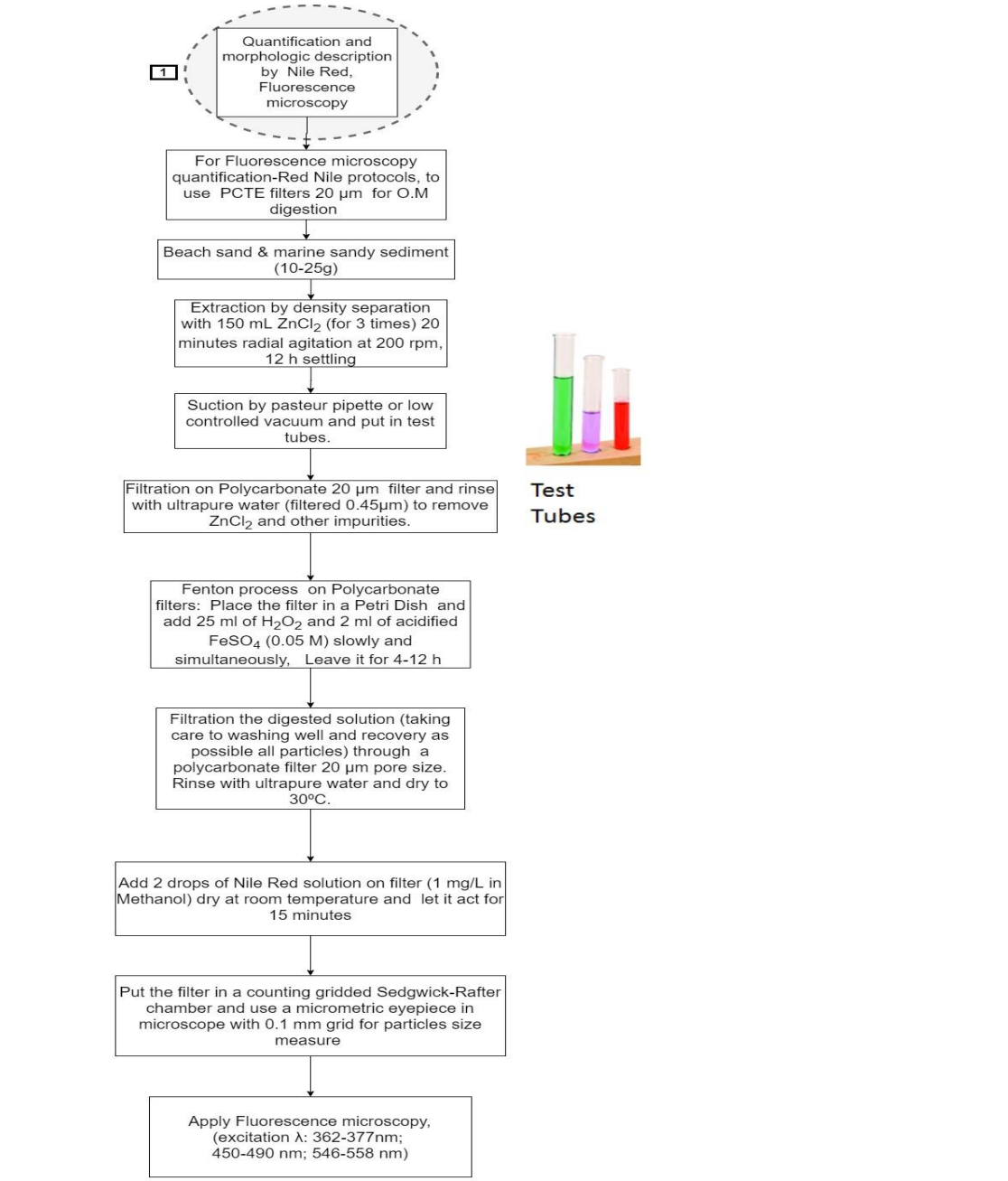


Figure 3.2. General scheme for microplastics extraction and identification in sand and sediment samples.



Plastic polymer	RED/ORANGE	GREEN	YELLOW	BLUE
PP	✓	✓✓✓	✖	✓
PE (HDPE, LDPE)	✓	✓✓✓	✖	✓
PS (PS, EPS)	✓✓✓	✖	✓✓✓	✖
PU	✓✓✓	✖	✓✓✓	✖
PET	✓	✖	✓	✓✓
PVC	✓✓✓	✖	✖	✖
PMA	✓✓	✓✓	✖	✖
PVAc	✓✓✓	✓✓✓		✓✓✓
PES	✓✓	✖	✓✓	✓✓
PA	✓✓	✓	✖	✓✓
Cotton (natural cellulose)	✖	✖	✖	✖
Wood	✓	✖	✖	✖
Chitin	✓	✖	✖	✖
Treated Chitin/wood	✖	✖	✖	✖
Wheel rubber	✖	✖	✖	✖

Figure 3.3. Methodology for microplastics extraction and identification in sand and sediment samples.



Observed colours from plastic particles and fibers on Fluorescence microscope after Nile Red addition

Plastic polymer	RED/ORANGE	GREEN	YELLOW	BLUE
PP	✔	✔✔✔	✖	✔
PE (HDPE, LDPE)	✔	✔✔✔	✖	✔
PS (PS, EPS)	✔✔✔	✖	✔✔✔	✖
PU	✔✔✔	✖	✔✔✔	✖
PET	✔	✖	✔	✔✔
PVC	✔✔✔	✖	✖	✖
PMA	✔✔	✔✔	✖	✖
PVAc	✔✔✔	✔✔✔		✔✔✔
PES	✔✔	✖	✔✔	✔✔
PA	✔✔	✔	✖	✔✔
Cotton (natural cellulose)	✖	✖	✖	✖
Wood	✔	✖	✖	✖
Chitin	✔	✖	✖	✖
Treated Chitin/wood	✖	✖	✖	✖
Wheel rubber	✖	✖	✖	✖

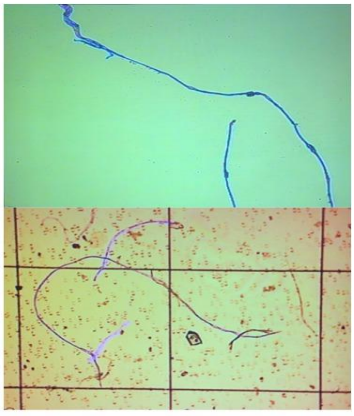


Figure 3.4. Methodology for microplastics extraction and identification in sand and sediment samples (Nile Red fluorescence methodology description)

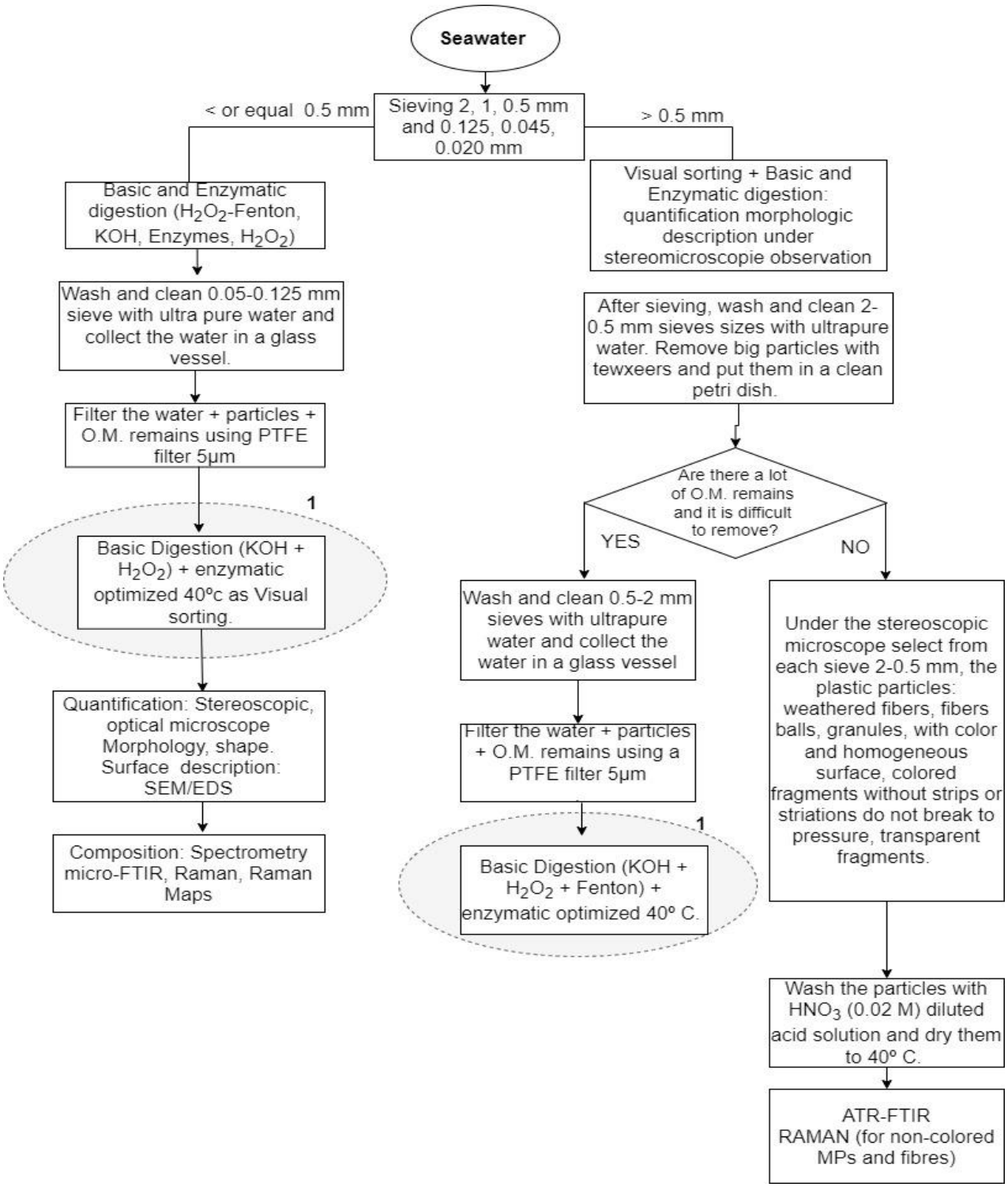


Figure 3.5. Methodology for microplastics extraction and identification in seawater samples

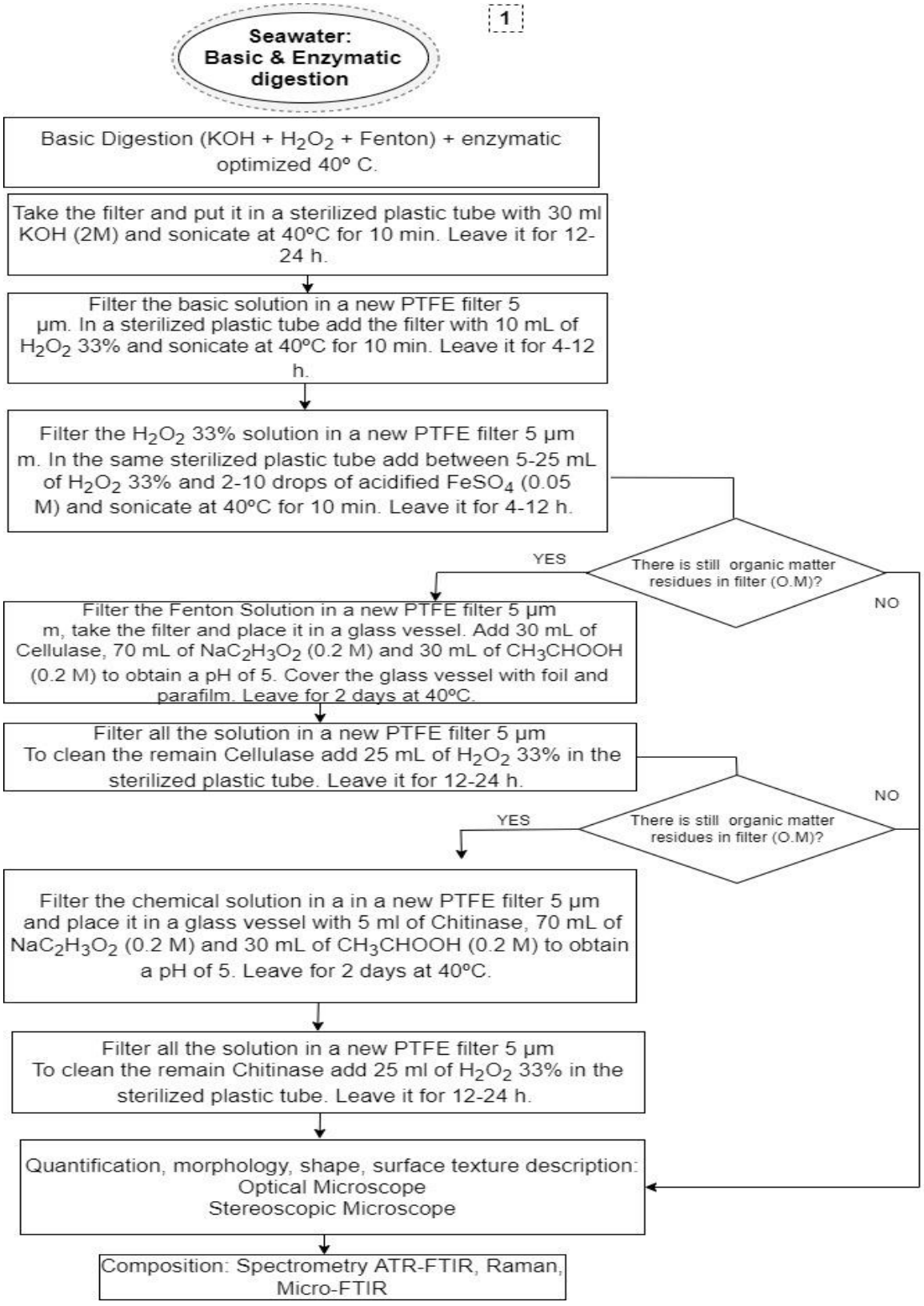


Figure 3.6. Methodology for microplastics extraction and identification in seawater samples (Basic & Enzymatic digestion methodology description)

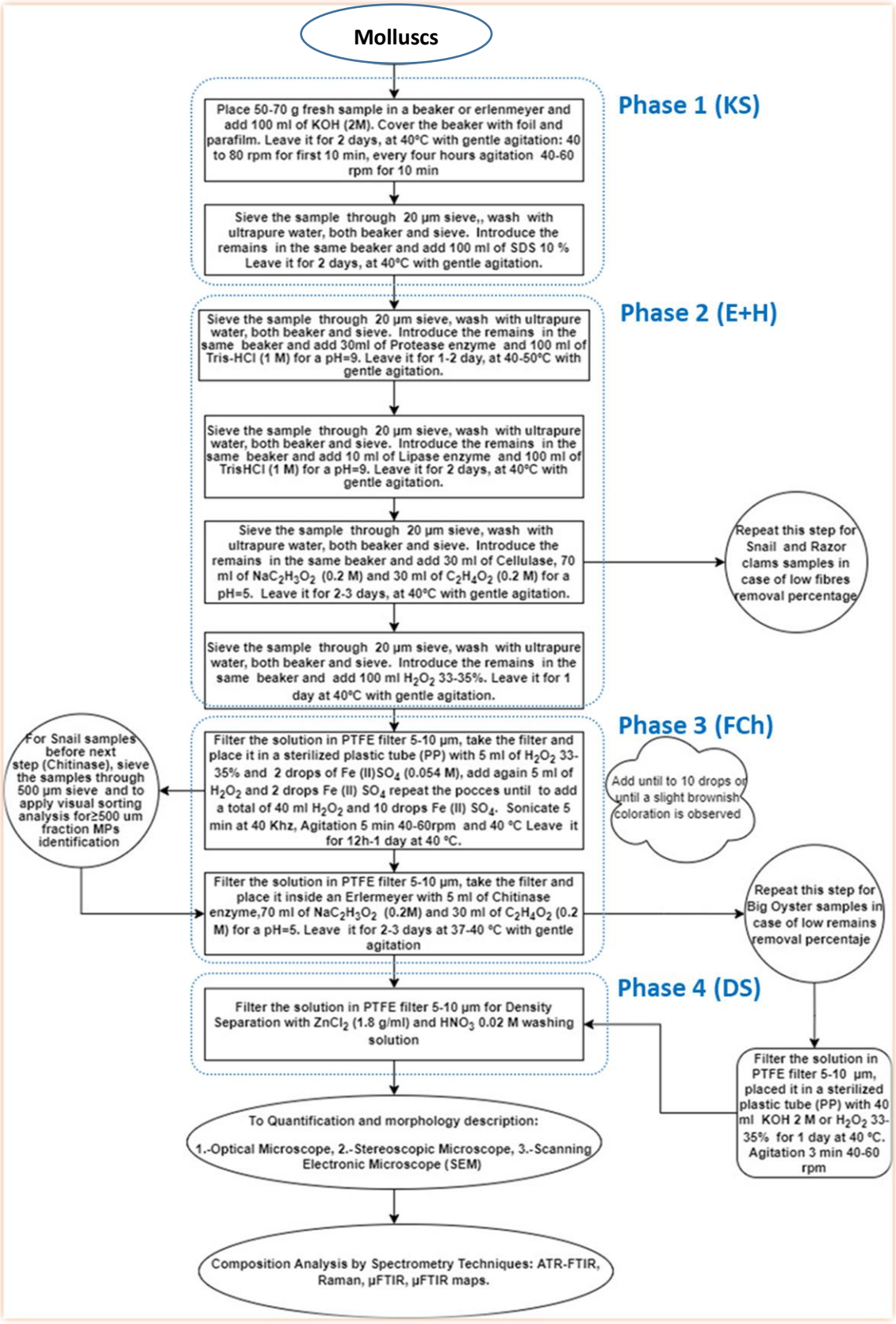


Figure 3.7. Methodology for microplastics extraction and identification in molluscs samples by phases

The Spectroscopic techniques, are based in vibrational spectroscopy. The vibrational energy levels are unique to each molecule; the resulting IR and Raman spectrum provide a fingerprint of a particular molecule (Larkin, 2017). An advantage of these methods is that they are non-destructive and are suitable to identify the smaller MP fraction directly on filter in Raman case. Raman techniques present lower water interference, a higher sensitivity to non-polar functional groups; it is able to measure the wavenumber range below 650 cm-1 (Strungaru et al., 2019). Aliphatic and aromatic compounds, C=C compounds are well detectable in Raman while aliphatic compounds and polyesters are well detectable in IR (Käppler et al., 2016).

Vibrational spectroscopy is concerned with the detection of transitions between energy levels in molecules that result from stretching and bending vibrations of the interatomic bonds. The vibrational frequencies are shown to be characteristic of particular functional groups in molecules. At room temperature, most molecules exist in their ground vibrational states and in order to excite them to higher vibrational states energy must be absorbed. The Raman and IR techniques are used to detect changes in vibrational energy states, but they differ in the manner in which interactions with the exciting radiation occurs (Campbell et al., 2000).

Fourier-transform infrared (FTIR) Spectroscopy

In IR spectroscopy, the excitable vibrations depend on the composition and molecular structure of a substance and are wavelength specific. When molecular vibrations result in a change in the bond dipole moment, because of change in the electron distribution in the bond, it is possible to stimulate transitions between energy levels by interaction with electromagnetic radiation of an appropriate frequency. When the vibrating dipole is in phase with the electric vector of the incident radiation, the vibrations are enhanced and there is transfer of energy from the incident radiation to the molecule. The energy of the IR radiation that excites a specific vibration will -depending on the wavelength- be absorbed to a certain amount, which enables the measurement of characteristic IR spectra (Campbell et al., 2000; Käppler et al., 2017).

Plastic polymers possess highly specific IR spectra with distinct band patterns. Molecular vibrations, which are Raman inactive are IR active and vice versa (Löder and Gerdts, 2015; Renner et al., 2016; Ribeiro-Claro et al., 2017; Shim et al., 2017). The size limited is about 10-20 µm.

For larger particles ≥0.5 mm is applied attenuated total reflection (ATR), reflectance and transmission mode. The Infrared Spectrophotometer with Attenuated Total Plugin Reflectance (ATR-IR) has a diamond on which the sample is placed. The light IR passes through the glass, is absorbed by the sample, and is reflected back to generate a spectrum. The instrument itself

performs a 16-scan overlay; each scan provides a spectrum. The final spectra are a spectrum with the most repeated peaks. The range and spectral resolution can be defined based on analysis time and quality desired. Spectral range 4000 to 230 cm-1 and spectral resolution 4 cm-1.
For MPs composition analysis, match spectra major or equal than 60% of similarity with the reference spectra were accepted for particles and major or equal than 40% for fibres Omnic Picta Software was used for selecting spectra libraries for spectra comparison. The libraries selected were HR Nicolet Sampler Library, Hummel Polymer Sample Library, Polymer Laminate Films, Wizard Library. All of them are specific libraries of polymers; however, these libraries do not take in account to environmental samples weathered or ageing and an additional library was created with a collection of spectra

Spectra unidentified were compared with two own libraries elaborated with more common plastics in the market and weathered fragments, films and fibres from environmental samples applying the same criteria for reference spectra acceptance. Also, items were analyzed according to their characteristic's absorption band of each polymer chemical grouping bonds according to Campbell et al. (2000) and Coates (2000), for frequencies ranging mostly from 3650-500 cm-1, 1480-1430 cm-1 for C-C aromatic ring stretching, 1790-1700 cm-1 for double binding C-O stretching, 2980-2780 cm-1 for C-H stretching of aliphatic and 3150-3030 cm-1 of aromatics. For aliphatic organohalogen, natural and synthetic cellulose identification, frequencies from 1150 to 550 cm-1, 1200-1000 cm-1, 1500-1200 cm-1 (C-H and C-O-H bending modes), 2900-2820 cm-1 (C-H stretching modes) and from 3600 to 3000 cm-1 (O-H stretching modes) were also evaluated. The rejected items were counted as the definitive unidentified category.

For identifying the smaller MPs fraction from environmental samples, the FTIR coupled with a microscope, called micro-FTIR mapping has to be applied. Micro-FTIR (µ-FTIR), is applied for smaller particles <0.5 mm and fibres with extremely time-consuming when targeting the whole sample filter surface at a high spatial resolution because it uses only a single detector element. IR technique have a wide spectral coverage, far, mid and near infrared in sections (broken), resolution bands not are so narrow (Löder and Gerdts, 2015; Renner et al., 2016; Ribeiro et al., 2016; Shim et al., 2017; Strungaru et al., 2019). For µ-FTIR maps, an area of 9 mm^2 was stablished randomly on silicius filters (without any interference and with accurate IR spectra generation), in that area a spectrum was made every 25 µm with a total of more of 15000 spectra for analysis with Omnic Picta Software and libraries selected as HR Nicolet Sampler Library, Hummel Polymer Sample Library, Polymer Laminate Films, Wizard Library. The µ-FTIR maps were obtained by reflexion, four (4) scans accumulations, spectral resolution 4 cm-1 and spectral range of 4000-715 cm-1. Spectra unidentified with Omnic Picta Software were identified as above mentioned.

Another, µ-FTIR maps type is made on calcium fluoride (CaF_2) slides into a 1 cm^2 selected area and placing fibers, fragments, film, pellets and foam in files one beside another for sccannig and analysis. Usually, the µ-FTIR data are transformed to false-color images on the basis of integration on specific spectral regions, such as 1480-1430 cm^{-1} (C-H bending, aromatic ring stretching), 1790-1700 cm^{-1} (C-O stretching) or 2980-2780 cm^{-1} (C-H stretching), followed by an on-screen analysis by selecting bright areas manually and comparing the spectra to references or available databases (Strungaru et al., 2019).

Raman Spectroscopy

The Raman spectroscopy consist in the Identification of plastic through monochromatic laser source irradiation. The laser depends on the system used, thus available laser wavelengths usually range between 500 and 800 nm. The interaction of the laser light with the molecules and atoms of the sample (vibrational, rotational, and other low-frequency interactions) results in differences in the frequency of the backscattered light when compared to the irradiating laser Löder and Gerdts, 2015; Renner et al., 2016; Ribeiro-Claro et al., 2017; Shim et al., 2017). Thus, the Raman principle is the detection of light scattered inelastic by molecules interacting with incident monochromatic radiation. When electromagnetic radiation interacts with matter a certain fraction of the incident fraction is scattered elastically, (Rayleigh scattering) such that its frequency remains unchanged, but a very small fraction of the radiation will interact inelastically and is scattered with a different frequency (Raman scattering). The differences between the wavelengths of scattered and incident radiation arise due to induced transitions of the vibrational states of molecules (Campbell et al., 2000). Raman activity involves changes in polarizability of the bonds but no all vibrations are detectable (Käppler et al., 2017).

Figure 3.8 shows the energy diagrams of Stokes, Rayleigh, and anti-Stokes Raman scattering. According Lupoi et al., (2015), photons from the excitation source collide with the molecule, promoting the molecule to a short-lived virtual state, whereby it instantaneously emits energy and relaxes back to the lowest vibrational state with identical frequency to the incident light (Rayleigh), has a net increase in energy (Stokes), or has lost energy (anti-Stokes).

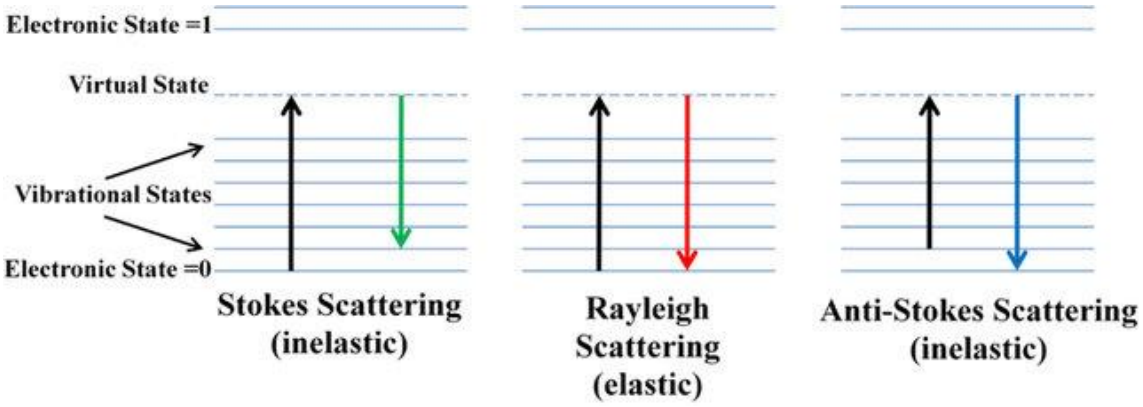


Figure 3.8. Energy diagrams of Stokes, Rayleigh, and anti-Stokes Raman scattering (from Lupoi, 2012).

Raman can detect particles down to 1-2 µm in size. Further, Raman technique have better resolution than IR, wider continuous spectral coverage and bands are narrower (Löder and Gerdts, 2015; Renner et al., 2016; Ribeiro-Claro et al., 2017; Shim et al. 2017). Raman measurements can provide additional information about contained organic or inorganic fillers, pigments or dyes, which are not available by micro-FTIR (Imhof et al., 2016). Coupling with microscopy, (confocal laser-scanning microscopy), for localization of polymer particles in filters

If Raman microscopy is combined with Raman spectral imaging it is possible to generate spatial chemical images based on the Raman spectra of the sample. The drawbacks are the interference by fluorescence excited through laser focus on residues of biological origin, biofilms and inorganic material avoiding the generation of interpretable Raman spectra. (Löder and Gerdts, 2015; Renner et al., 2016; Ribeiro-Claro et al., 2017; Shim et al., 2017; Strungaru et al., 2019)

3.1 Blanks design

Sand and marine sandy sediment blanks were applied for each used method. One blank while sieving and two blanks were applied for each sample on visual sorting methodology for airborne contamination. One blank for density separation for comparing supernatant colour. A procedural blank (with the same reagents volume that the samples) for basic-enzymatic digestion procedure and fluorescent analysis was applied. The blanks values, for each method were then subtracted from the partial particle counting.

For seawater samples, particles deposition was determined for two months with an estimated rate of 0.04 fibers/cm^2/day. This rate was applied for the total area filtered in a day. An additional blank was applied for basic-enzymatic digestion for each sample. The blanks values, for each method were then subtracted from the partial particle counting

For biota, blanks were applied during the samples processing time consisting in two open cleaned Petri dish to capture of airborne fibres. Procedural blank, were run with every set of samples in digestion processes fulfilling the methodology step by step and by phases. Finally, blanks were applied during visual sorting and MPs recognition (opened cleaned Petri dish). Additional blanks in lyophilisation processes were applied one for every set of sub-samples. Results were corrected by fibres and particles contaminations

3.2 Prevention and assessment of samples contamination

The sieving, filtration and washing steps for samples proccesing, as well as some basic-enzymatic procedures were made into fume hood and laminar flow cabinet. Glassware and metallic ware were used wherever possible instead plastic ware. All equipment, glassware and metallic ware was thoroughly rinsed with first alcohol 96% and then ultrapure water before usage. Distillate water and chemical solutions were filtered through 0.45 µm filters before usage (for fibres removals). Samples, subsamples, filters glassware and metallic ware was covered with aluminium paper for plastic contamination minimization via air exposure and store inside fume hood. All laboratory counterstops were cleaned with alcohol 96% and fibre-free napkins. Clean cotton laboratory coats and latex gloves were used during all processes. The samples were covered during filtration, digestion and visual identification.

3.3 Time and Recovery rates

The recovery rates for all methodologies developed were estimated using virgin plastics spherical PE particles spanning from 53-63 µm, 125-150 µm, 250-300 µm and 450-500 µm, with density 1.02-1.06 g/cm^3, purchased from the company Cospheric Inc, USA. The sand, sediment and seawater samples were spiked with PE spheres at the different sieve sizes, and subjected to the whole procedure. The biota samples were spiked into soft wet tissues and subjected to the whole procedure. For sediments and sands samples the recovery rate was between 85 and 100%, for seawater samples between 60 and 97% and for biota samples between 60 and 99% being 53-63 µm spheres those showed the lesser values of recovery rates.

References

Campbell, D., Pethrick, R.A., White, J.R., 2000. Polymer characterization: Physical techniques (2nd Edition). CRC Press, Stanley Thornes (Publishers)LTD. United Kingdom 481 pp.

Claessens, M., Meester, S.D., Landuyt, L.V., Clerck, K.D., Janssen, C.R., 2011. Occurrence and distribution of microplastics in marine sediments along the Belgian coast. Mar. Pollut. Bull. 62(10), 2199-2204.

Claessens, M., Van Cauwenberghe, L., Vandegehuchte, M.B., Janssen, C.R., 2013. New techniques for the detection of microplastics in sediments and field collected organisms. Mar. Pollut. Bull., 70(1-2), 227-233.

Coates, J., 2000. Interpretation of infrared spectra, A practical approach in encyclopedia of analytical chemistry (10815-10837 pp.). In: Meyers, R.A. (Ed.) John Wiley & Sons Ltd,

Coppock, R.L., Cole, M., Lindeque, P.K., Queirós, A.M., Galloway, T.S., 2017. A small-scale, portable method for extracting microplastics from marine sediments. Environ. Pollut. 230, 829-837.

Dehaut, A., Cassone, A.L., Frère, L., Hermabessiere, L., Himber, C., Rinnert, E., Rivière, G., Lambert, C., Soudant, P., Huvet, A., Duflos, G., Paul-Pont, I., 2016. Microplastics in seafood: Benchmark protocol for their extraction and characterization. Environ. Pollut. 215, 223–233.

Erni-Cassola, G., Gibson, M. I., Thompson, R. C., & Christie-Oleza, J. A., 2017. Lost, but found with Nile Red: a novel method for detecting and quantifying small microplastics (1 mm to 20 μm) in environmental samples. Environ. Sci. Technol. 51(23),13641-13648.

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: A review of the methods used for identification and quantification. Environ. Sci. Technol. 46, 3060-3075.

Hurley, R.R., Lusher, A.L., Olsen, M., Nizzetto, L., 2018. Validation of a method for extracting microplastics from complex, organic-rich, environmental matrices. Environ. Sci. Technol. 52(13), 7409-7417.

Imhof, H.K., Laforsch, C., Wiesheu, A.C., Schmid, J., Anger, P.M., Niessner, R., Ivleva, N.P., 2016. Pigments and plastic in limnetic ecosystems: a qualitative and quantitative study on microparticles of different size classes. Water Res. 98, 64-74.

Käppler, A., Fischer, D., Oberbeckmann, S., Schernewski, G., Labrenz, M., Eichhorn, K.-J., Voit, B., 2016. Analysis of environmental microplastics by vibrational microspectroscopy: FTIR, Raman or both? Anal. Bioanal. Chem. 408(29), 8377-8391.

Larkin, P., 2017. Infrared and Raman spectroscopy: Principles and spectral interpretation (2nd edition), Elsevier, p.p. 286.

Löder, M.G.J., Gerdts, G., 2015. Methodology used for the detection and identification of microplastics - A critical appraisal (p.p. 201-227). In: Bergmann, M., Gutow, L., Klages, M. (eds.), Marine Anthropogenic Litter, Springer Cham, p.p. XVIII, 447.

Lupoi, J.S., Gjersing, E., Davis, M.F., 2015. Evaluating lignocellulosic biomass, its derivatives, and downstream products with Raman spectroscopy. Front Bioeng. Biotechnol. 3, 50.

Lupoi, J.S., Smith, E.A., 2012. Characterization of woody and herbaceous biomasses lignin composition with 1064 nm dispersive multichannel Raman spectroscopy. Appl. Spectrosc. 66(8), 903-910.

Lusher, A.L., Burke, A., O'Connor, I., Officer, R., 2014. Microplastic pollution in the Northeast Atlantic Ocean: Validated and opportunistic sampling. Mar. Pollut. Bull. 88(1-2), 325-333.

Maes, T., Jessop, R., Wellner, N., Haupt, K., Mayes, A.G., 2017. A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. Sci. Rep. 7, 44501.

Mai, L., Bao, L.-J., Shi, L., Wong, C.S., Zeng, E.Y., 2018. A review of methods for measuring microplastics in aquatic environaments. Environ. Sci. Pollut. Res. 25, 11319-11332.

Masura, J., Baker, J., Foster, G. and Arthur, C., 2015. Laboratory methods for the analysis of microplastics in the marine environment: recommendations for quantifying synthetic particles in waters and sediments. Siver Spring, MD, NOAA Marine Debris Division, 31pp. (NOAA Technical Memorandum NOS-OR&R-48).

Munno, K., Helm, P.A., Jackson, D.A., Rochman, C., Sims, A., 2018. Impacts of temperature and selected chemical digestion methods on microplastic particles. Environ. Toxicol. Chem. 37, 91-98.

Nuelle, M.-T., Dekiff, J.H., Remy, D., Fries, E., 2014. A new analytical approach for monitoring microplastics in marine sediments. Environ. Pollut. 184, 161-169.

Prata, J.C.; da Costa, J.P.; Duarte, A.C.; Rocha-Santos, T.,2019. Methods for sampling and detection of microplastics in water and sediment: A critical review. TrAC Trends Anal. Chem., 110, 150–159.

Renner, G., Schmidt, T.C., Schram, J., 2017. Chapter 4: Characterization and quantification of microplastics by infrared spectroscopy (p.p. 67-118). In: Rocha-Santos, T.A.P., Duarte. A.C., Characterization and Analysis of Microplastics. Comprehensive Analytical Chemistry Handbook (Vol. 75). Elsevier, p.p. 286.

Ribeiro-Claro, P., Nolasco, M.M., Araújo, C., 2017. Chapter 5: Characterization of microplastics by Raman spectroscopy (p.p. 119-151). In: Rocha-Santos, T.A.P., Duarte. A.C., Characterization and Analysis of Microplastics. Comprehensive Analytical Chemistry Handbook (Vol. 75). Elsevier, p.p. 286.

Rocha-Santos, T.A., Duarte, A.C., (Eds), 2017. Characterization and analysis of microplastics (1st Ed.) Elsevier, 286 pp.

Sancataldo, G., Avellone, G., Vetri, V., 2020. Nile Red lifetime reveals microplastic identity. Environ. Sci. Process Impacts, 22(11), 2266-2275.

Shim, W.J., Hong, S.H., Eo, E. S., 2017. Identification methods in microplastic analysis: A review Anal. Methods, 9, 1384-1391.

Strungaru, S.-A., Jijie, R., Nicoara, M., Plavan, G., Faggio, C., 2019. Micro- (nano) plastics in freshwater ecosystems: abundance, toxicological impact and quantification methodology. Trends Analyt. Chem. 110, 116-128.

Sturm, M. T., Horn, H., Schuhen, K., 2021. The potential of fluorescent dyes-comparative study of Nile red and three derivatives for the detection of microplastics. Analytical and Bioanalytical Chemistry, 413(4), 1059-1071.

Tagg, A.S., Sapp, M., Harrison, J.P., Ojeda, J.J., 2015. Identification and quantification of microplastics in wastewater using focal plane array-based reflectance micro-FT-IR imaging. Analytical chemistry, 87(12), 6032-6040.

CHAPTER 1-MICROSPLASTICS IN COASTAL AREAS

1.-Sub-chapter 1.1 Microplastics in marine environmental compartments

Microplastics levels, size, morphology and composition in marine water, sediments and sand beaches. Case study of Tarragona coast (western Mediterranean).

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Abstract

Mediterranean Sea has been proposed as the sixth greatest accumulation zone for marine litter and the most affected regarding to MPs. Tarragona (Catalonia, NE Spain) coastal region suffers high pressure due to highly urbanization of coastline, tourism, industrial harbour and petrochemical/plastic manufactures complex activities. In recent years, the conflict between the plastic marine pollution and the two main economic sectors in Tarragona region: industry and tourism emphasise the importance of this topic for the region. The present study aims to quantify and characterize in size, morphology and composition of the MPs spanning from 500 to 5,000 µm, in sandy beaches, marine sediments and from 80 to 5,000 µm in seawaters of Tarragona (Catalonia, NE Spain) coastal region (western Mediterranean). MPs mean abundance were 1.3 items/m^3 in surface seawater, 32.4 items/kg in marine sediments, and 10.7 items/kg in sandy beaches. Polyester fibres were dominant MPs in bottom sediments and seawater while polyethylene and polypropylene fragments were the main MPs in beaches. The fibres balls associated with bottom sediments, organic remains and plankton were abundant, masking the real quantity of fibres in each reservoir. The abundance by volume of seawater MPs was higher to those found in oceanic areas and similar to other areas of Mediterranean Sea with high MPs accumulation, corroborating the Western Mediterranean Sea basin as a region of MPs accumulation. MPs composition and abundance suggest the input of numerous land-base-sources, WWTP effluents discharges, watercourses, and emissaries as the most important. As an integrative point of view (water, sand beach and sediments) of MPs marine pollution study in western Mediterranean Sea, the dynamics of MPs pollution in Tarragona coast were characterized by seawater as the media that receive and facilitates MPs dispersion and fragmentation, the shoreline acts as an intermediate reservoir with constant weathering and active exchange with seawater surface and the sediments acts as a significant sink for medium MPs sizes.It was necessary to adapting developed protocols and guidelines for MPs analysis to obtain harmonized and comparable results.

Key words: Microplastics, plastic pollution, sea, sediment, sand beach.

1.1.1 Introduction

Currently Micoplastics (MPs) definition consider physical and chemical defining properties beside size and origin as some authors have stablished (Arthur et al., 2009; GESAMP, 2016; Savoca et al., 2019; Thompson et al., 2004). They are defined as "any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1 μm to 5 mm, of either primary (intentionally manufactured microbeads and fibres) or secondary origin (mesoplastics breakdown in fragments), which are insoluble in water" (Frias and Nash, 2019; Verschoor, 2015).

MPs are persistence in the environment because the majority of mesoplastics have life-time between hundreds to thousands of years (Barnes et al., 2009; Gasperi et al., 2018; GESAMP, 2016; Ivleva et al., 2017) and have been ubiquitous in marine environment (Andrady, 2011; Barboza and Gimenez, 2015; Deforges et al., 2014; Shim et al., 2018; Wright et al., 2013), marine sediment, surface seawater and sand (Alomar et al., 2016; Cincinelli et al., 2019; Constant et al., 2019; de Haan et al., 2019; Zhou et al., 2018), water column (Baini et al., 2016), deep sea floor, aquatic organism (Chae et al., 2017; Sanchez-Vidal et al., 2018), freshwater (Simón-Sanchez et al., 2018; Strungaru et al., 2019), atmosphere (Gasperi et al., 2018), and soil (Nizzeto et al., 2016).

In one hand, primary MPs in the environment are coming mainly from the direct release of MPs-containing products such as pre-production pellets (used to manufacture plastic products), plastic micro- and nano-beads as additive in personal care products and textile fibres entering into the aquatic systems by wastewater discharge (Sundt et al., 2014). It should take into account that synthetic textiles release MPs (as fibres) to the environment during washing as they cannot be completely removed in wastewater treatment plants (Sun et al., 2019). Some authors (Allen et al., 2019; Dris et al., 2015; 2016; 2017) also pointed out the importance of the atmospheric deposition of fibres not only in urban areas but also in remote sites. On the other hand, secondary microplastics are formed by weathering (ocean current dynamics, UV radiation, wave's action, abrasion, biofouling, bacterial colonization) and fragmentation of mismanaged large plastic presents in the seawater (Carbery et al., 2018; GESAMP, 2015; Lebreton et al., 2017; Wu et al., 2019). Primary and secondary MPs from continental sources enters to the ocean largely through storm-water, agricultural and urban runoff, wastewater discharges, flowing into watercourses or directly discharged into coastal waters for industrial spillage or during pellets transport (Cozar et al., 2014). These MPs due to their small size and similarity with marine plankton, can be ingested by invertebrates, bivalves, and fish entering in the food web (Wright et al., 2013). Human exposure to MPs comes mainly form the ingestion route, through contaminated food in the environment or during food preparation, packaging and drinking water although the air inhalation cannot be neglected (Chang et al., 2020; Gasperi et al., 2018, Oliveira et al., 2019). There is a

lack of knowledge regarding the effects in humans (Yong et al., 2020), however, these MPs could act as carriers of marine pollution (heavy metals and hydrophobic organic compounds), chemical additives incorporated in manufacture, even pathogen and/or invasive microorganisms. MPs and associated compounds can bioaccumulate through the food webs including humans with possible risks to health due to the fast desorption rates found in experimental physiological gut conditions (Ashton et al., 2010; Bakir et al., 2014; Carbery et al., 2018; GESAMP, 2015; Masó et al., 2003; Ogata et al., 2009; Picó and Barceló, 2019; Wright et al., 2013).

Mediterranean Sea has a high human pressure, both urban and industrial, and the fact that it is almost a close sea with 90-year renewal period and due to plastics persists for periods exceding 100 years, aggravates the environmental impact or plastics pollution (Jambeck et al., 2015; Mack et al., 2019; Pinna et al., 2019). In addition, tourism increase human pressure in summer season by multiplying the population in coastal areas by several times. For this reason, Mediterranean Sea currently has been proposed as the sixth greatest accumulation zone for marine litter and the most affected regarding to MPs (243,853 plastic pieces/km^2, 82% of them in microplastic form) (Alomar et al., 2016; Constant et al., 2019; Cozar et al., 2015; de Lucia et al., 2014; Fossi et al., 2016; Suaria, et al., 2016).

According to a recent study (Liubartseva et al., 2018) that MPs pollution was modelled in the Mediterranean Sea, one of the most contaminated areas (seawater and bottom sediments) is the Catalan Sea, between Balearic Island and Catalonia (NE, Spain). Inside that area, it is found Tarragona coastal region with high pressure by tourism, industrial harbour and petrochemical and plastic manufactures complex. In recent years, the increasing awareness of the population regarding plastic marine pollution and the conflict between the two main economic sectors in Tarragona region, industry and tourism, emphasise the importance of this topic for the region (Rovira et al., 2018).

The present study aims to quantify and characterize in size, morphology and composition of the MPs ranging from 500 to 5,000 µm, present in sandy beaches and marine sediments, and from 80 to 5,000 µm in surface seawaters of Tarragona (Catalonia, NE Spain) coastal region (western Mediterranean), impacted by urban, tourism and industrial activities. To our knowledge, this is the first attempt to study MPs marine pollution in an integrated way (water, sand beach and marine sediments) in western Mediterranean Sea as a contribution to global MPs levels report and assessing the distribution of MPs in marine environment for ecological risk assessment.

1.1.2. Materials and Methods

1.1.2.1 Study area

Tarragona, a crossroads between the axis of the Mediterranean and the Ebro mouth, is located around 100 km at south from Barcelona metropolitan area. The coasts of Tarragona are located according to the FAO classification on the western Mediterranean. Tarragona city has 131,094 inhabitants and is the centre of 30 km radius area, with more than 580,000 residents. In the area, there is the most important petrochemical cluster in southern Europe. As confirmed by the Tarragona Chemical Business Association (AEQT), the total production of the Tarragona petrochemical sector in 2019 was 19,516,493 tones, the sixth highest total production in the last 15 years, supplying the most demanding sector as rubber and plastic transformation industry (14 %). Tarragona harbour is the fifth most important port in Spain and it is also an important stop for the transit of tourism cruise ships.

Tarragona Sea have surface current flows mainly from the northward most of the year. Inside the harbour there is the Francolí river (838 km^2 watershed area) mouth with a water flow of approximately 1.18 m^3/s (Figure 1). Twelve biological wastewater treatment plant (WWTP) effluents discharges in its watershed with flow between 100 to 7,200 m^3/day. Inside to Tarragona harbour exists an important discharge of a big WWTP with flow about 24,000 m^3/d and at 4 km to the south, in front of La Pineda beach, there is other WWTP effluents with a flow of 47,500 m^3/day.

The contribution of MPs due to the wind transport from urban areas to seawater surface and sand MPs abundance was not considered, because winds towards land with low-speed prevailing during the sampling period.

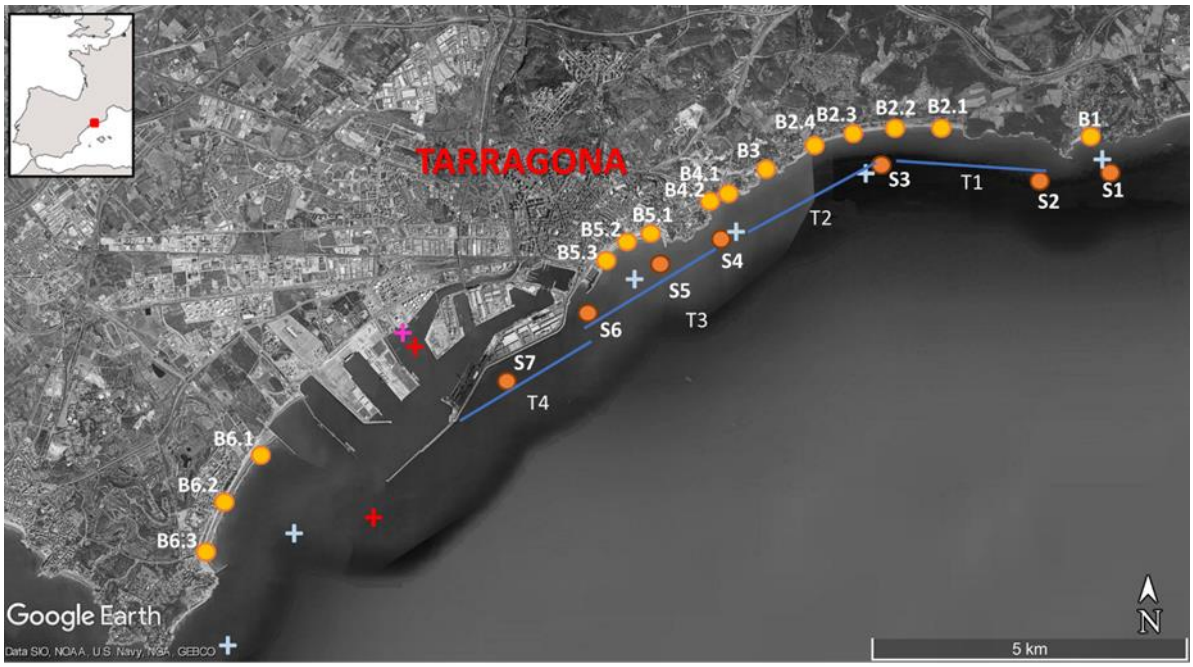

Figure 1. Sampling sites localization on Tarragona Coast (Catalonia, Spain). Sandy beaches in yellow (B); marine sediments marked in orange (S), Superficial marine water transects marked in blue (T). Red crosses are WWTP discharges, pink cross is Francolí river discharges and blue crosses are runoff discharges emissaries.

1.1.2.2 Sampling

Sand, sediment and water samples were collected between September and October 2018. During this period, the geostrophic current was mostly from North to South and South to North with 50% and 33% of the time, respectively, and a current velocity ranged from 0 to 0.5 m/s. The wind velocity ranged from 0.92 to 9.1 m/s (1.41 m/s to 4.29 m/s on September 10th) and the Beaufort scale from 1 (small waves without scum) to 3 (medium waves). Because the wind velocity values not were greater than 5 m/s, it was not necessary to correct experimental values do to turbulent downward fluxes (de Haan et al., 2019; Reisser et al., 2015). Transects for surface water and sampling points for beach sand and sediments are showed at Figure 1 and Table S1 (Supplementary Information).

a.- Surface seawater

Four seawater surface samples from Tarragona coast (from La Mora to Tarragona harbour) were collected along shoreline using 80 µm mesh neuston net, circular opening 24 cm wide, 1 m long, horizontal transects between 746 to 1017 m were towed at a speed of 1.5–2 knots. The tows were separated from shoreline between 500 and 924 m and in front of sand beach sampling points. Average volume filtered was 45.2 m^3.

b.- Marine sediments

Seven sediment composite samples were collected approximately at the start and end of each transect of seawater samples. Two divers descended between 15 and 17 m depth and collected several subsamples in an area of 25 m^2 of top 5 cm sediment layer with a metal spoon and glass bottle. Average weight collected was 600 g. The bottom was characterized by sandy patches next to *Posidonia oceanica* and *Cymodocea* seagrass meadows.

c.- Beach sands

The deposits of debris lines on the Mediterranean beaches depend mainly on the storm surges due to atmospheric conditions such as: pressure, wind and wave height (Constant et al 2019). Six different beaches were selected according of anthropogenic activities conditions as industrial pressure, tourism, urbanism, runoff, river mouth and wastewater treatment effluents discharges. The beaches are mainly low to moderate slope (5– 45º), fine sand and are located alongshore 19 km of Tarragona city (Figure 1). For the determination of MPs in beaches, 14 samples were collected from six beaches. These beaches are from north to south: La Mora and Sabinosa, characterized by low tourism and urbanism pressure activities, Arrabasada and Llarga with tourism pressure and runoff influence, Miracle with tourism, urbanism, and runoff influence and La Pineda with industrial, tourism, river mouth, runoff and wastewater effluents discharges influences.

As the MSFD- Guidance on Monitoring of Marine Litter in European Seas guidance document (European Commission, 2013) and NOAA Marine Debris Program (Masura et al., 2015) recommends, samples were collected randomly in the intertidal zone, which is influenced by waves dynamic with interchangeable material between sand and seawater, into a 100 m section or transect. At intertidal zone, at upper 5 cm, the five subsamples were collected randomly into a square 40 cm x 40 cm (0.16 m^2 area) with Kubiena boxes of 125 cm^3 (fixed volume) and stored in aluminium boxes covered to avoid contamination. Average weight of sand beach collected was 1.5 kg.

Due to supralittoral zone of beaches in Tarragona coast are cleaned periodically with sand renewal (especially in autumn and spring), only the intertidal area was considered as the most representative area for the study of the MPs transport from sand to water.

1.1.2.3 Sample treatment

Regarding MPs analysis methodology, until now there are not exist standardized protocols instead considerable efforts showed by scientific community (Besley et al., 2017; Coppock et al., 2017;

Löder et al., 2017; Mai et al., 2018; Masura et al., 2015; Munno et al., 2018; Nuelle et al., 2014; Prata et al., 2019; Wagner et al., 2017).

In present study, a fast and practicable methodology for MPs extraction were applied adapting the processes sequence and parameters developed to specific samples requirements improving processes for turbidity and organic matter removal with less usage of expensive reagents and plastic materials to obtain clear filters for accurate MPs measures

a.- Surface seawater

The seawater samples retained in the cod-end of the net were carefully rinsed. The residues content of the cod-end was finally transferred to a sample container, stored frozen, and kept in the dark until further analysis (Bergmann et al., 2015)

The seawater samples were sieved through a column of clean sieves (4, 2, and 1 mm; and 500, 125, and 45 μm) inside a fume hood. The sieves >500 μm (4, 2, and 1 mm) were cleaned with ultrapure water. The big particles and fibres of organic matter were removed with tweezer; the big possible plastic particles and fibers were placed it in a covered petri dish and the residue was filtered on Teflon filter (PTFE 5 μm pore size). The sieves 500, 125 and 45 μm residues were filtered on Teflon filter.

Particles and fibres ≥500 μm without organic matter were selected with the stereoscopy microscope from 4 to 62 X magnification (LEICA DMS 1000). The possible plastic particles were: weathered fibres, fibres balls, granules with colour and homogeneous surface, coloured fragments without strips or striation, fragments that do not break by applying light pressure with tweezers, transparent fragments, films, pellets, and spheres. These particles were analysed individually for microplastic composition (see 2.4 Section)

Particles and fibres above 500 μm that contained a large amount of organic matter were filtered on Teflon filter 5 μm pore size filters.For organic matter removals, the PTFE 5 μm pore size filters were treated as follows: Alkali digestion (KOH 2M) followed by a Fenton process (H_2O_2 and $FeSO_4$) at 40 ºC and combined with enzymatic digestion (cellulase) with sonication extraction and agitation steps were applied (Karami et al., 2017; Löder et al., 2017; Mai et al., 2018; Munno et al., 2018; Prata et al., 2019; Shokri, 2018). Next, plastic particles and fibres were observed using the stereoscopy microscope (LEICA DMS 1000).

b.- Sand and Sediment

Sand beach and sediment samples were dried at 40 °C during 48 h and weighed; a small part was collected for granulometry analysis. Samples were sieved through a column of clean sieves (4, 2, 1 mm and 500 μm) placed in a mechanical shaker. Particle <500 μm were stored in well-capped

aluminium containers for future analysis. Particle ≥500 μm were analysed with visual sorting procedure: quantification and morphologic description under stereomicroscopic observation from 8 to 62 X magnification. The possible plastic particles from 4 to 0.5 mm were: fibres associated with sand or sediment, white and coloured fragments, film weathered without strips or striations, plastics that do not break to pressure and pellets or transparent fragments non-iridescent, non-crystalline (Hidalgo-Ruz et al., 2012). The buoyancy of these particles was checked testing on tree solutions: H_2O, saturated NaCl (density 1.2 g/L), and saturated $ZnCl_2$ (density 1.8 g/L).

The criteria to reject or accept the particle as a plastic was based to individual plastic density and its buoyancy in each solution; they were grouped according the buoyancy solutions. The particle with non-buoyancy in none of solutions it was considered inorganic particle (Löder et al., 2017; Mai et al., 2018; Munno et al., 2018; Prata et al., 2019):

1.1.2.4 Quality control

To avoid sample contamination a closed and isolated site (fume hood) was established in the lab for samples processing, wearing laboratory cotton coats, using glass or steel materials and tools rinsed with 70% ethanol and after with ultrapure (MilliQ® water filtered on 0.45 μm pore size filter (GF/F Whatman, 47 mm diameter, nitrocellulose), and covered in aluminium foils. All reagents were made with ultrapure water, stored in glass bottle and filtered (<0.43 μm pore size) before their use. Samples and subsamples were covered with aluminium paper for plastic contamination minimization through air deposition. For sand and sediment analysis two blanks were applied for each sample analysis: in sieving and visual sorting methodology. The work place particles deposition rate was determined for two months with a value of 0.04 fibres/cm^2/day. This rate was applied for the total filter area filtered in a day by sample. One blank for morphology examination on stereoscopy and optical microscope was located as an open petri dish. One blank for sample in basic—enzymatic digestion procedure was established for each sample. The blanks values, for each method were then subtracted from the total particle counting and correcting for contamination due to deposit particle and fibres from laboratory environment.

1.1.2.5 Microplastic (size, morphology and polymeric composition) characterization

Microplastics were classified based on their polymeric composition, size, and morphology as: fibre (including filaments and fishing line), films (items with a two-dimensional shape), fragments (items with a three-dimensional shape), and pellets (solid spheres; solids foam spheres).

In surface seawater, a total of 34 fibres and 90 particles (fragments, pellets, films) of each size fraction-until 45 μm fraction, were examined to determine their polymeric composition with a confirmation percentage of 25% and 100%, respectively. Particles size > 0.5 mm were analysed

with a Thermo Scientific NICOLET ATR-FTIR spectrometer with OMNIC™ Paradigm Software, with an Attenuated Total Reflectance (ATR) adapter; the measurements were performed in reflection mode in the range of 400–3600 cm−1, with 50 scans at a resolution of 10 cm−1. The background was done before analysis and every 20 samples. By contrast, particles between 0.08 and 0.5 mm, filtered on PTFE filter, were examined totally with spectroscope-microscope Raman Renishaw 20X, 50 X objective magnification microscope-, 785 nm edge, laser state 1%, 10%, 50% intensity, bands from 25 cm^{-1}, 1200l/mm. The spectra polymer obtained with Raman spectroscopic technique were performed with WIRE 5.3 Software, the spectra were corrected for baseline displacement.

For sand samples a total of 14 fibres and 196 particles (fragments, pellets, films) of each size fraction until the 4 mm fraction were examined to determine their polymeric composition with a confirmation percentage of 39 % and 98%, respectively. For sediment, samples a total of 78 fibers and 46 particles (fragments, pellets, films) of each size fraction-until the 4 mm fraction-were examine with a confirmation percentage of 44 % and 100% respectively.

Sand and sediment Particles >0.5 mm were analysed with a Thermo Scientific NICOLET ATR-FTIR spectrometer with OMNIC™ Paradigm Software, with an Attenuated Total Reflectance (ATR) adapter; the measurements were performed in reflection mode in the range of 400–3600 cm^{-1}, with 50 scans at a resolution of 10 cm^{-1}. The background was done before analysis and every 20 samples.

The fibres found in sand and sediment samples, due to their morphology (small ratio volumes/length) and fragments equal to 0.5 mm, were analysed with a microscope IR-(μFTIR) spectrometer following a mapping procedure with a Thermo Scientific NICOLET iN10 with OMNIC™ Specta, and MCT detector opening 25 μm, accumulations 1 scans, spectral resolutions 16 cm-1, spectral range 4000-715 cm^{-1}, ultrafast mapping 4x4 mm. The spectra not were corrected for light-reflectance penetration and baseline displacement. Kubelka-Munk or Kramers-Kronig corrections were not applied.

To identification of each polymer spectra obtained with ATR-FTIR and μFTIR techniques were performed comparing the unknown spectra with OMIC software libraries database, only match spectra with major or equal than 75% of similarity with reference spectra were accepted, the rejected items were counted as the temporary unidentified category. The unidentified spectra were also identified comparing with BIO-RAD IR spectral databases, matches, major or equal than 75% of similarity, were accepted (Supplementary information Figure S1).

For temporary unidentified spectra with libraries, other criteria for spectral analysis were to applied:

a.- The identification of the IR spectra was supported by comparing spectra with a library of own elaboration taking into account weathered particles analysed, only the spectra whose peaks were 60-70% or more coincident with the peaks of the reference spectra were identified.

b.- The rejected items were analysed according their characteristics absorption band or vibration modes of each polymer chemical grouping bonds for frequencies ranging mostly from 3200-1250 cm^{-1}(Primpke et al., 2018): 1480-1430 cm^{-1} for C-C aromatic ring stretching, 1790-1700 $cm^{-1}$for double binding C-O stretching, 2980-2780 cm^{-1} for C-H stretching of aliphatic and 3150-3030 cm^{-1} of aromatics. For aliphatic organohalogen, natural and synthetic cellulose identification, additional frequencies from 1150 to 550 cm^{-1} and from 3600 to 3000 cm^{-1} were evaluated. The rejected items were counted as the definitive unidentified category.

The Raman spectra polymer were identified comparing the spectra with WIRE 5.3 polymer libraries database. Only match spectra with major or equal than 75% of similarity with reference spectra were accepted, the rejected items were counted as the temporary unidentified category. The identification of the Raman temporary unidentified spectra was supported by comparing spectra with a library of own elaboration taking into account the main plastics products on the market, only the spectra whose peaks were 60-70% or more coincident with the peaks of the reference spectra were identified. The rejected items were also analysed according their characteristics absorption band, (Supplentary information Figure S2-S4).

1.1.3. Results and Discussion

1.1.3.1 Superficial seawater

Levels of MPs on surface seawater in Tarragona coastal area and their characterization of size and morphology are summarized in Table 1. Microplastics (from 80 µm to 5 mm) were analysed in four transects in Tarragona coastal surface waters. Mean MP levels was 1.3 MPs/m^3 ranging from 0.4 to 3.0 MPs/m^3. Highest levels were found in the transect T4 close to the Tarragona harbour and lowest values in T3 between Arrabasada and Miracle Beach. Almost all MPs were below 2 mm constituted by fragments (between 29% and 46%) and fibres (between 54% and 63%).

MPs from 4-5 mm only was found in Transect 1. Excepting for Transect 2, approaching Tarragona Harbour (North-South directions) there was a decrease of MPs spanning from 80 µm to 0.5 mm and an increase of MPs size spanning from 1 to 2 mm. According morphology there were a decreased of fragments and films proportion to 29 and 7%, respectively; conversely, fibre proportion lightly increased to 63 %.

The Transect 4 presented higher proportion of MPs size 1 to 2 mm, increasing fibres proportion and lower proportion of pellets. In Transect 2 between Llarga and Arrabasada, there was higher proportion of MPs size spanning 80 µm to 0.5 mm and a slightly higher proportion of fibres (54%) than fragments (46 %).

Regarding the general composition of these MPs (Figure 2), the majority of the fragments, pellets and films were made of polyethylene (46%) and polypropylene (22%) moreover other materials such as polyvinyl chlorine (1%), polystyrene (4%), polyurethane (4%), polyethylene terephthalate (8%), polycarbonate (1%), acrylonitrile-butadiene-styrene copolymer (1%), and polymethylmethacrylate (6%). Taking into account fibres, almost all were made of polyester (polyethylene terephthalate) (53%) and polyurethane (spandex or elastane) (23%). Other fibres found were made of polyamide (3%) and polyethylene (3%). The composition made of all fragments, pellets and films were analysed. By contrast, only 25% of fibres were analysed due to their identification difficulty. For particle groups as pellets, fragments and films, the non-identified MPs were very low (7%), and respect fibre group was 18%. From the 18 % of non-identified fibres, all of them were synthetic but were not clearly identify as particular polymer.

Respect to MPs composition, the main polymers found from fragments were low density polymers as polyethylene and polypropylene, followed by polyethylene terephthalate, polystyrene, polymethylmethacrylate and polyurethane, related with the thermoplastics more demanded by European countries mainly for packaging uses, construction and building, and others (furniture, electrical apparatus) (PlasticsEurope, 2013, 2017, 2019) (Suplementary information Figure S5a-c). From 2012 to 2018 the European plastics demand increased up to 51.2 million tonnes (7.6 % in Spain) with 40 % for packaging uses with about 50 % demand for specific materials as polypropylene and polyethylene (low, medium or high density), 7.9 % for polyurethane and 7.7 % for polyethylene terephthalate and 6.4 % for polystyrene (PlasticsEurope 2013, 2017,2019).

These results are in accordance with other studies in Western and Central Mediterranean regions and Northern Catalonia coast (de Hann et al., 2019; Suaria et al., 2016). Polyethylene and polypropylene, are high-demanded polymers and with low density. So, they are frequently found as dominating floating polymers (Hidalgo-Ruz et al., 2012; Prata et al., 2018). Other polymers found were high-density polymers as polyvinylchloride, polymethylmethacrylate and polycarbonate. Probably are related with local hydrodynamics processes (local upwelling) that keep those particles in buoyance. According to de Hann et al. (2019), the presence of heavy density MPs floating in seawater surface is related with the aggregation with floating organic matter (phytoplankton, algae, inorganic suspended) keeping MPs in buoyancy (Figure 3A).

The MPs abundance in seawater in Tarragona coastal area was not homogenous. There were high density zones alternated with areas of low abundance, focusing the high abundances near Tarragona harbour area. As it has been shown in Figure 1, many sources from land-based inputs influence this area, such as wastewater treatment plants discharges, watercourse inflow (Francolí River), surface runoff, emissary discharges and harbour activities. The accumulation effect of MPs here was magnified by intersection of Port structure of southward littoral marine surface currents carrying MPs from seawater upstream.

The general seawater MPs morphology was characterized by textile synthetic fibres (present in ordinary clothes, sport clothes, socks, swimsuits). The higher abundance was observed in Tarragona Port influenced by wastewater treatment discharges, confirming that WWTP does not eliminate completely the fibres from washing machine and the fibres probably comes from direct discharges WWTP. The contribution of fibres to seawater via surface runoff can be also considered (Dris et al., 2015).

Table 1. Levels and characteristics (size and morphology) of MPs in Tarragona costal area.

			% Size category (mm)					% Morphology type			
	n	**Levels**	**5-4**	**4-2**	**2-1**	**1-0.5**	**0.5-0.08**	**Frg**	**Pel**	**Fil**	**Fib**
Surface water		items/m^3									
Transect 1	1	0.8	11	0	0	52	37	44	0	0	56
Transect 2	1	1.0	0	0	0	23	77	46	0	0	54
Transect 3	1	0.4	0	0	37	26	37	32	0	10	58
Transect 4	1	3.0	0	0	57	16	27	29	1	7	63
Sediment		items/kg									
Punta de la Mora	1	32	0	56	0	44	NA	56	0	0	44
Cala de Roca Plana	1	5.6	0	0	33	67	NA	0	0	0	100
Llarga Center	1	22	0	50	7	43	NA	36	0	0	64
Miracle-Arrabasada	1	41	0	12	38	50	NA	11	4	31	54
Miracle Center	1	5.5	25	0	0	75	NA	0	0	0	100
Harbour (marina)	1	32	0	17	55	28	NA	17	0	17	66
Harbour (industrial)	1	89	0	22	22	56	NA	24	0	4	72
Sand Beach		items/kg									
La Mora	1	0.7	0	0	100	0	NA	0	0	100	0
Savinosa	1	1.3	0	0	100	0	NA	0	0	0	100
Llarga	4	1.1±1.9 [0-3.9]	0	29	0	71	NA	43	0	14	43
Arrabasada	2	5.3±1.2 [4.4-6.1]	5	18	24	53	NA	29	0	12	59
Miracle (Sept.)	3	2.0±0.7 [1.4-2.7]	0	56	22	22	NA	22	34	22	22
Miracle (June)	4	6.9±8.1 [0-15]	0	59	41	0	NA	36	30	11	23
Pineda	3	42±19 [21-54]	2	13	59	26	NA	76	16	1	7

Microplastic levels (mean ± standard deviation) and [Range] in water expressed as in items/m^3; in sediments and sand beaches expressed as items/kg_{dw}. NA: Not analysed. Frg: Fragment. Pel: Pellet. Fil: Film. Fib: Fibres

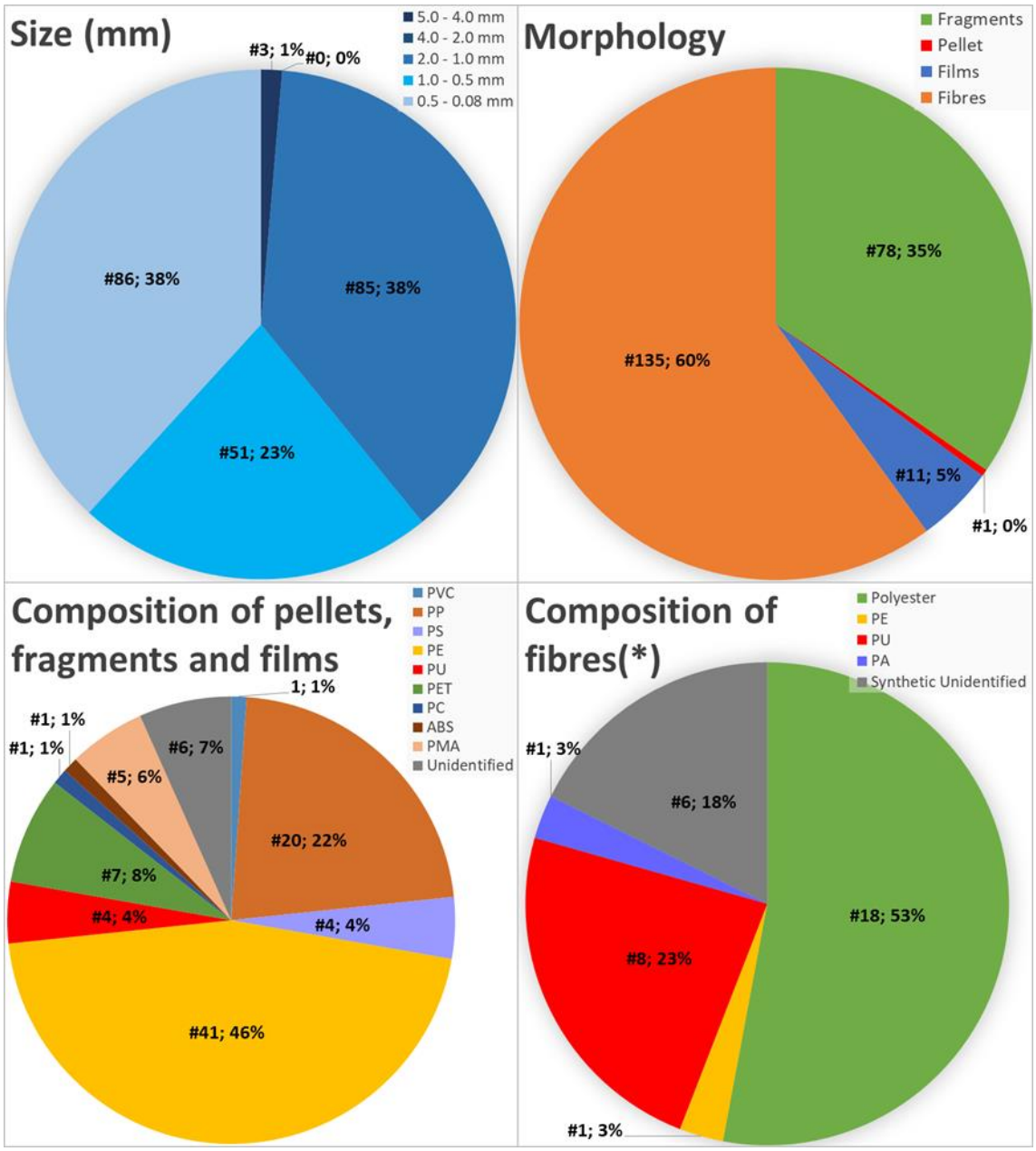

Figure 2. Size distribution (from 5 mm to 80 µm), morphology (fragments, pellets, films and fibres), and composition of MPs in surface seawaters in Mediterranean costal area of Tarragona. Result expressed as (#) number of MPs; percentage (%). *Composition analysed in the 25% of the fibres PVC: polyvinylchloride, PP: Polypropylene; PS: Polystyrene; PE: Polyethylene; PU: Polyurethane; PET: Poly(ethyleneterephthalate); PC: Polycarbonate: ABS: Acrylonitrile-butadiene-styrene copolymer; PMA: Poly(methylmethacrylate); and PA polyamide.

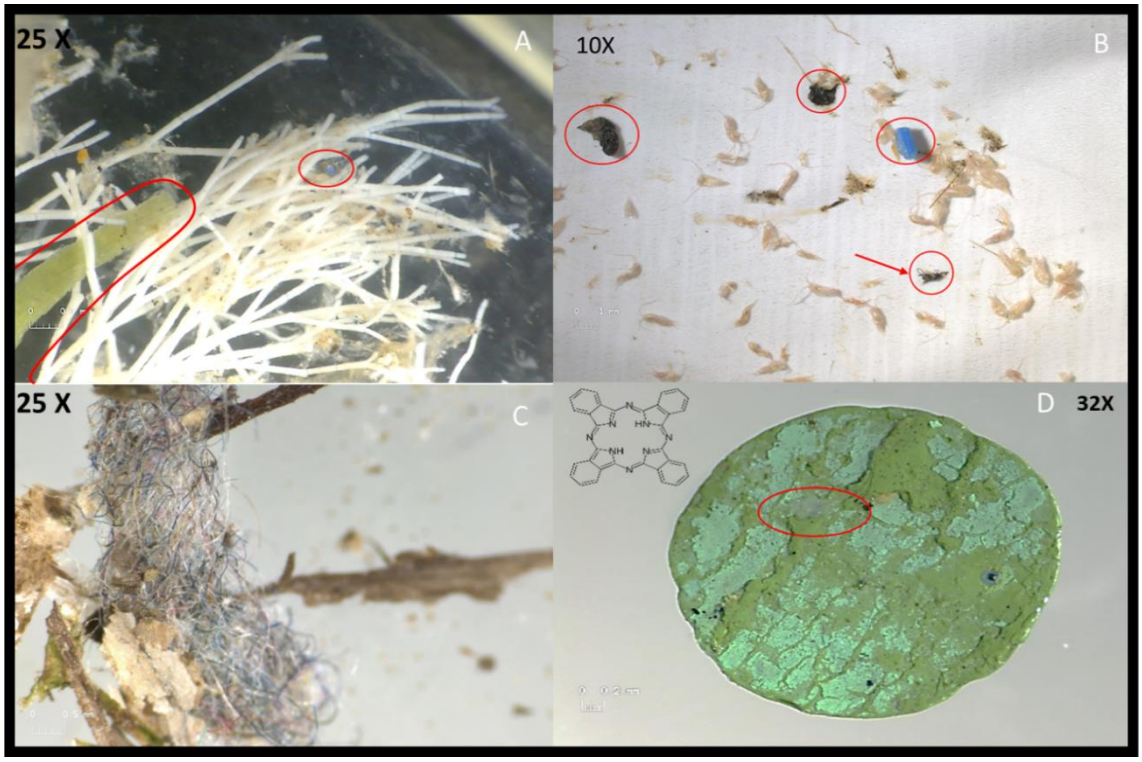

Figure 3. Microplastics associated to environmental samples. A: microplastics associated with algae aggregations. B: microplastics associated with plankton. C: fibres ball associated with marine sediments and algae organic remains. D: microplastic weathered with biofilm attached.

Microplastics has been shown could bypass the WWTP (depending on the treatment units employed) entering into the aquatic water bodies and finally accumulated in the environment (Carr et al., 2016; Murphy et al., 2016). These authors have reported wastewater effluents contain synthetic fibres, such as polyester and nylon. The number of fibres shed into raw wastewater by a single garment during the washing process has been >1,900 fibres per wash, garment. The release of fibres from polyester, polyester-cotton blend and acrylic fabrics has been counted as high as 730,000 fibres from acrylic clothing for every 6 kg of laundry (Browne, 2011; Napper and Thompson, 2016).

In general, the WWTPs with tertiary treatment processes had a lower MPs concentration (0-51 particle/L) in the effluent than those with only primary or secondary treatment processes (9·10-4 - 447 particle/L). However, studies also showed the tertiary treatment in some WWTPs did not further decrease the MPs concentration in the effluent (Mason et al., 2016). The total MPs discharges from WWTPs were still considerably high (even if there are low abundances), as most of these facilities process millions of litres of wastewater every day. The estimated average diary efflux (based on annual efflux and effluent concentration), was 2·106 particle/day, corresponding to 1.37·105 m3/day; about 9-110 kg of micro-fibres are discharged in WWTPs effluent daily

(Hartline et al., 2016; Sun et al., 2019). Based on these reported values, the study area of Tarragona coastal receive approximately 1.3·106 particle/day from WWTP effluents. This area is affected by three principals WWTP localized in the coastal zone: Tarragona, Vila-seca and Altafulla; the latter located upstream of the sampling area with indirect influence on it by current from the northern.

Because of the lack of standardized procedures for sampling, separation, identification and size measurement, it is difficult to compare MPs concentrations among different studies. Nonetheless, a comparison of the existing data on the abundance of floating MPs in the Mediterranean Sea, and other seas and oceans has been carried out to compare the data obtained in the present study (Table 2).

Average abundance in Tarragona coast respect to filtered volume was 1.30±0.98 items/m3. In comparison with Mediterranean regions, this value was higher than average results obtained for Western and Central Mediterranean regions but lower than, Eastern Mediterranean regions. These regions in Western and Central Mediterranean (Ligurian, Sardinian, Tyrrenean, and Sicily Sea, Po Rhone and Tet delta, and Cartagena coast) with lower values than Tarragona coast are characterized by river discharges, high industrial, touristic and urban activities, and Atlantic currents influence. Many of these areas showed high fibres proportion with polyester as dominant composition.

Regarding the composition of fragments, these regions are also dominated by the most abundant polymers (polyethylene and polypropylene). It is important to highlight that the average MPs values on the Tarragona coast were greater than values from Otrasto Sea Strain in Central Mediterranean Sea (Adriatic Sea) and North Catalan Coast in Western Mediterranean Sea, both areas with industrial-urban pressure and high flow river discharges. The polymeric composition of fragments from the North Catalan Coast was similar to those found in Tarragona Coast, possibly due to similar consumption patterns, socio-economic status and waste management characteristics in Catalonia. It should be taken into account that Tarragona surface seawater MPs mean levels (sized from 0.5 to 5 mm) was 0.81±0.70, higher than values from some Western and Central Mediterranean regions (Table 2).

Table 2. Microplastics levels in surface water of Mediterranean Sea and other oceans of the world.

Autor	Year	Region*	Ubication	MPs/m^3	Sampling	Size
Present study	2018	wM-B	La Mora-Llarga Tarragona City	0.80	Nn	80 µm-5 mm
			Llarga-Arrabasada-Tarragona city	1.03		
			Arrabasada -Miracle Tarragona city	0.41		
			Tarragona Harbour-Tarragona city	2.96		
			Tarragona Coast mean	1.30±0.98		
Fossi et al. 2012	2011	wM-S	Ligurian sea mean	0.94±2.55	SnWP2	200 µm-5 mm
		wM-S	Sardinian Sea mean	0.13±0.27		
Fossi et al. 2016	2013	wM-S	Ligurian sea mean	0.49±1.66	SnWP2	200 µm-5 mm
		wM-S	Sardinia Sea mean	0.24±0.43		
Panti et al. 2015	2012/13	wM-S	Norwest Sardinia sea mean	0.17±0.32	SnWP3	200 µm-5 mm
de Lucia 2014	2012/13	wM-S	Central west-Sardinia Sea mean	0.15±0.11	Mt	500 µm-5 mm
Van der Hal et al. 2017	2017	eM-L	Israel coast	7.68±2.38	Nn	300 µm-5 mm
Kazour et al. 2019	2018	eM-L	Lebanese coast	4.3±2.2	Mt	200 µm-5 mm
de lucia 2018	2017	wM-S	Italian minors islands -Elba	0.33±0.02	Mt	333µm-5mm
		wM-S	Italian minors islands -Ventotene	0.26±0.28		
		wM-S	Italian minors islands -Ischia	0.71±0.20		
		wM-S	Italian minors islands -Eolie	0.29±0.26		
		cM-A	Italian minors islands -Tremiti	0.13±0.04		
		cM-A	Po Delta	0.64±0.33		
		wM-S	Tevere Delta	0.57±0.22		
Constant et al. 2018	2016	wM-Ly	Rhone delta	0.19	Mt	300 µm-5 mm
		wM-Ly	Tet delta	0.18		
de hann et al. 2019	2015	wM-B	Northern Catalonia coast mean	0.73±0.77	Mt	335 µm-5 mm
		wM-B	Cartagena coast mean	0.27±0.17		
		wM-B	Campo Dalias Coast mean	0.31±0.40		
Baini et al. 2018	2013/14	wM-S	North Tyrrenean Sea (0.5 km) Winter	0.05±0.05	Mt	330 µm-5 mm
		wM-S	North Tyrrenean Sea (0.5 km) Spring	0.09±0.10		
		wM-S	North Tyrrenean Sea (0.5-20 km) Winter	0.29±0.46		
		wM-S	North Tyrrenean Sea (0.5-20 km) Spring	0.26±0.26		
Suaria et al. 2016	2013	wM-B	Menorca West Coast	2.85±3.26	Nn	700 µm-5 mm
		cM-A	Otrasto Sea Strain	0.74±1.75		
		wM-S	Sicily Sea West Coast	0.66±0.62		
		wM-S	Tunisia and Algeria coast	0.54±0.62		
Liu et al. 2020	2016	nwP	Jiaozhou Bay	0.095	Nn	500 µm-5 mm
Moore et al. (2002)	2000/01	ecP	California inshore (mouth of the San Gabriel River)	7.25	Mt	333 µm-5 mm
			California offshore	2.23		
Goldstein et al. (2012)	1999/10	ecP	North Pacific Gyre mean ten years record	0.43	Mt	333 µm-5 mm
Ramírez-Alvarez et al. 2020	2016/17	ecP	Todos Santos Bay (Mexico)	[0.01-0.70]	Mt	333 µm-5 mm
Mu et al. 2019	2017	nwP	North-western Pacific Ocean	0.03±0.02	Mt	333 µm-5 mm
		nwP	Bering Sea	0.09±0.09		
		Ar	Chukchi sea	0.23±0.07		
McEachern et al. 2019	2017	wcAt	Tampa Bay, Florida	4.5±2.3	Nn	330 µm-5 mm
Garcia et al. 2020	2010	swAt	Equatorial Brazilian coast	0.14 ± 0.11	Nn	120µm -5 mm
Lusher et al. 2015	2014	neat	Artic, south of Svalbard	0.34±0.31	Mt	333 µm-5 mm
Lacerda et al. 2019	2017	An-At	Antarctic peninsula	0.009±0.005	Mt	330 µm-5 mm
Aliabad et al. 2019	-	wIn	Chabahar Bay, Iran	0.49 ± 0.43	Nn	333 µm-5 mm

Mean standard ± deviation. [Range]. Nn: Neuston net; Mt: Manta trawl; SnWP2: Standart Vertical Plankton Net WP2; SnWP3: Standart net WP3; *Regions acoording FAO Major fishing areas. WM: west Mediterranean sea (subdividded in B: Balearic; S: Sardinia; and Ly: Lyon gulf); CM: Cental Mediterranenan sea (subdivided in A: adriatic and I: Ionian); EM: Eastern Mediterranean sea (subdivided in Ag: Aegean and L: Levant); BS: Black sea (subdivided in BS: Black sea; M: Marmara sea and Az: Azov sea);nwP: northwest Pacific; ecP: Estern cental Pacific; wcAT: west central Atlantic; swAt: south-west Atlantic; neAt: northeast Atlantic; An-At: Atlantic-Antarctic; wIn: Western Indian ocean

Conversely, Tarragona coast average values were lower than values from others Mediterranean regions such as west Menorca, Israel and Lebanese coast. The East Mediterranean regions are characterized as large accumulations zones due to enclose morphology, the presence of Lagmuir circulation, Nile River discharges and high terrestrial runoff. Other areas with higher MPs abundances were in Pacific Ocean, the California inshore (river mouth) and offshore, and in the Atlantic Ocean, Tampa Bay. Moreover, only Tarragona harbour area values were up to seven-fold higher than North Pacific Gyre and Todos Santos Bay (Mexico) values. This means that despite considering the Pacific Ocean to be a hot spot for MPs, with a highest MPs abundance, there is high variability, possibly due to high energy waterflow patterns, dilution and dispersion phenomenon (MP abundances have been observed to decrease with depth). In this sense, the values are not constant and it is possible strong variation in a temporal (from one season to another) and spatial scale.

According to Liubartseva et al., (2018), high inputs of plastics from rivers and population areas from Barcelona City and its vicinities ranked Catalan Sea as one of the most polluted areas by plastics in the Mediterranean Sea. However, de Haan et al., (2019), found relative low MPs abundance values. The big difference found between the Northern region of Catalonia and the coasts of Tarragona is that the transect of Northern Catalonia samples were collected 4 km away from the coast while in our study the collection was just up to 1 km. one characteristic of Tarragona coast is that there are directs inputs of numerous land-based sources and less influence of high energetic level of oceanographic processes, resulting in high MPs abundances. Meanwhile, North Catalonia MPs values is influenced by the main geostrophic northern current what occasionally meanders over the littoral favouring MP higher dispersion rates and transport away from the coast. Moreover, results are also influenced by MPs transported from Ligurian Sea and Lyon Gulf in northern current.

Present results do not agree with those of Baini et al. (2018) that reported very low values, up to 0.5 km from the coast of North Tyrrenean Sea in summer and winter. However, results are in agreement with Pedrotti et al., (2016). These authors observed that the relative abundance of small fragments (<2 mm) was greater within the 1 km coastal strip. That suggest there is not only a rapid fragmentation along the coast (probably related to weathering mesoplastics on the beaches and incorporate to seawater by waves action) but also a contribution of land-based small size MPs (runoff, outfalls and treated wastewater). Furthermore, it is important the contribution of macroplastics and mesoplastics by land-based source that can be potentially fragmented on the seawater surface.

We have observed that like- synthetic fibres ball associated with plankton and other MPs fragments mask the real values of MPs abundance. Due to the difficulty of quantifying the real number of fibres within the ball, we count it as a unit fibre. (Figure 3B). The nature of organic

aggregates entrapping MPs favors decreasing sinking MPs rates with low efficiency in carbon export to the deep sea, leading MPs availability to fish and invertebrates confusing MPs-bearing aggregates with food sources (Long et al., 2015; Lusher et al., 2016). Conversely, the MPs aggregates containing exo-polymers particles enhances the particle-MPs flocculation with higher sinking rate and later deposit to deeper sea levels (Passow, 2002).

1.1.3.2 Marine Sediments

Regarding subtidal sediments, levels of MPs sized from 0.5 to 5mm ranged from 5.5 to 89 MPs/kg_{dw} with a mean value of 32 MPs/kg_{dw}. The highest levels were found in the sediments samples collected around industrial harbour (89 MPs/kg_{dw}) followed by a nearby sampling area, Arrabasada-Miracle beaches, (41 MPs/kg_{dw}) influenced by tourism and urbanism activities) and La Mora (32 MPs/kg_{dw}). S1, S3, S4, S6 and S7 shown the highest MPs levels and are located very close to emissaries for runoff watercourses and specially wastewater effluent discharges and Francolí river mouth in Port area (Figure 1). Taking into account all the samples the majority of MPs in sediments were between 1 mm and 0.5 mm (49%); sizes of 2-1 mm and 4-2 mm were 25% each (Figure 4). Most MPs were fibres (64%) followed by fragments (25%) and films (10%).

Regarding the composition, fragment, pellets and films were made of polyethylene (55%), polyvinyl (11%) and phenolic resins (9%). Other micro-sized materials found were made of polystyrene (4%), polyethylene terephthalate (4%), and even chemical additives (2%) without polymeric composition recorded (Suplementary information Figure S6a-b). Regarding fibres, polyester (61%), polyamide (29%), polyacrylonitrile (7%) and polypropylene (3%). All fragments, pellets and films found were analysed and 15% were synthetic but unable to define particular polymers composition. One-third (34%) of the fibres were identified as synthetic polymers.

To assess whether the abundances of MPs in subtidal sediments are closely related to their grain size, inorganic and organic composition, data from the local reports (DARP report, 2018), was consulted. All the stations studied were very fine grain (0.1-0.5 mm), with increasing the organic matter concentration (0.31-2.65%), sulphur (0.01-0.08%). Metals and metalloids, were below EPA sediment screening benchmarks (except for Hg and Pb), along the Tarragona coast from La Mora to Tarragona harbour, with highest abundances in harbour area. There was no clear trend between sediment grain size and MP abundance in Tarragona coastal sediments, however significant correlations were found between the presence of MPs and the total organic carbon ($r= 0.91$; $p=0.002$), the anthropic contamination of Hg ($r= 0.83$; $p=0.010$) and Pb ($r= 0.88$; $p=0.005$) in analysed sediments.

All stations showed high fibres proportion excepting for Punta de la Mora where it was found higher fragments proportions. It has been observed a lot of like-synthetic fibres balls associated with sediments grains and organic matter (seagrass, calcareous and algae remains) more than observed in seawater samples. These like-synthetic fibres balls were abundant in areas with high MPs abundances with the disadvantage of masking real values of fibres and total MPs abundance (these fibres balls were counted as a single fibre item) (Figure 3C).

A significant fraction of the 78 confirmed fibres, about 51 were identified as cellulose without differentiating whether they were synthetic or not due to unclear spectra resolution. The cellulosic fibres were excluded for total MP count resulting in average cellulose fibres abundances in Tarragona sediments approximately 14.4±6.90 fibres/kgdw. In fragments, pellets and films, the dominating composition was low density polyethylene (0.91-0.94 g/cm^3 according Pilato, 2013), followed by higher density polymers (1.2-1.45 g/cm^3) polyvinyl>Phenolic resins>polyethylene terephthalate). The proportion of higher density polymers was higher than in seawater composition. In case of fibres, the dominating composition was high proportion of higher density polymers (1.05-2.3 g/cm^3) as Polyester>polyamide> polyacrylonitrile

It is important to highlight the observation of MPs fragments with signs of weathering as bioturbation, biofilm growth, abrasion, and bacterial colonization, degrading the plastic material and exposing chemical additives such as phthalate, an endocrine disruptor, toxic for aquatic life (Harris et al., 1997; Ye et al., 2014). The primary and secondary MPs exposure to environmental conditions or weathering will change their properties such as surface morphological size, crystallinity, colours (yellowing discoloration) and densities, which may influence their physical and chemical actions in environments (Guo and Wang, 2019; Zhou et al., 2018).

The change of MPs characteristics and biological colonization/impact may influence on its aggregations capability. The MPs and organic matter set in “aggregates” may provide a transport mechanism of fragments, films, pellets and fibres to the deep stratus in the sea more likely in photic-sediments. Even the aggregation could be the main processes for fibres balls formation. Once on the bottom, MPs are expected to continue fragmenting by weathering (interaction with sediments, eventual currents in shallows deep, and benthic organism ingestion) processes until they reach to nano-size stage but in a slow time scale (because it is an environment with a lower incidence of UV light, low abrasion action, and low mechanical wave interaction). Other probably mechanism to transport low density MPs from surface to bottom is through MPs ingestion by biota, faecal pellets expulsion, new aggregations formations, and finally deposition in the bottom (Cole et al., 2016). In summary, marine hetero-aggregations, biofouling, faecal deposition and local hydrodynamics suggest being the possible causes of the presence of low-density MPs and synthetic-like fibres balls in the bottom.

The abundances of MPs in this study are comparable to those observed worldwide despite the wide range of MP sizes analysed and the different existing techniques for sediment analysis. Based in the worldwide data showed in Table 3, comparing same regions in Western Mediterranean–Balearic Sea, in entire Spanish coast, only two studies reported greater average MP abundances in deeper water (43-154 m), with similar sizes and composition to this study. The hot spots were observed in sediments samples close to densely populated areas i.e., Barcelona coast, conversely the lower values were found in Mallorca Island. Probably the depth influences the MPs abundances. Alomar et al., (2016) found in Mallorca shallow depths (8-10 m) values as high as to 897.35 ± 103.31 MPs/kg_{dw} in pristine areas far-away from densely populated areas. According mean values from shallow depth areas from Poland coast inshore and South Portugal, the results of this study were higher but compared with Central and Western Mediterranean regions as Telaščica bay (Croatia), Stromboli Island, Lipari Island, *Cymodocea* Bottoms and Amphioxus sand, conversely the values were lower.

The Tarragona coast MP abundance in sediments were lower than the Northwest Pacific regions including South Yellow Sea –China, Tokyo Bay Channel and Central Mediterranean regions as Venice lagoon. These areas (with values higher than thousands) are characterized by high abundance of urbanized and industrialized activities. The values found close to Tarragona harbour area demonstrate the harbour areas (with commercial and industrial activities) are zones with high MPs abundances. The values observed in our study, although lower, agrees with Belgium harbour and South African Durban Bay Harbour values, 166.7±92.1 and 1600 items/kg_{dw}, respectively (Table 3). Considering other Ocean Atlantic areas, Tarragona Industrial harbour showed similar abundance values and composition (higher fibres proportion) as UK Coast (Plymouth).

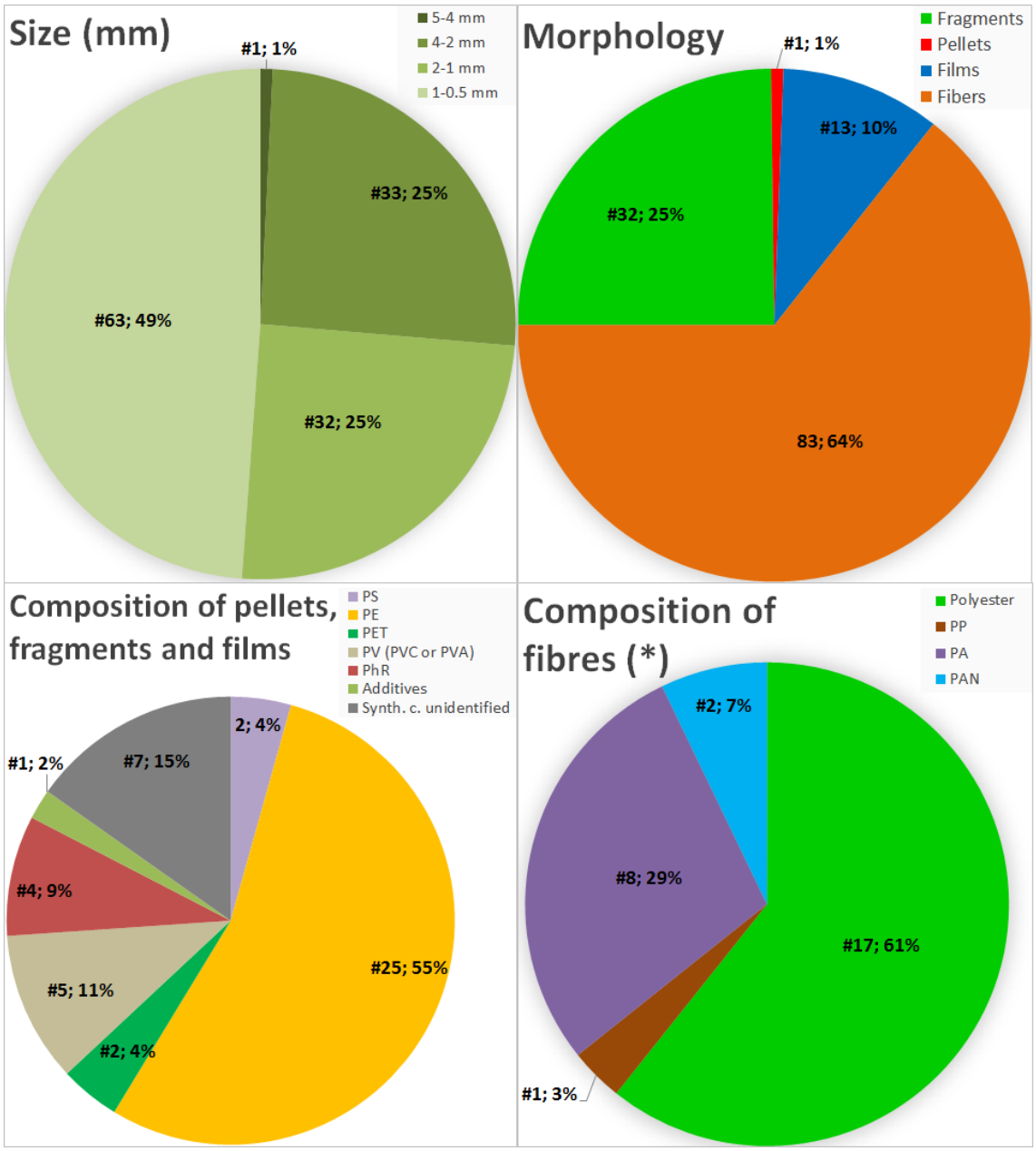

Figure 4. Size distribution (from 5 mm to 0.5 mm), morphology (fragments, pellets, films and fibres), and composition of MPs in marine sediments in Mediterranean costal area of Tarragona. Result expressed as (#) number of MPs; percentage (%). (*) Composition analysed in the 34% of the fibres considered as synthetics, excluding cellulose fibres. PP: Polypropylene; PS: Polystyrene; PE: Polyethylene; PET: Poly(ethyleneterephthalate); PV (PVC or PVA): Polyvinyl including Polyvinilchloride or polyvinyil alcohol; PhR: Phenolic resin; PC: Polycarbonate; PAN: Polyacryilonitile; Synth. c. unidentified: synthetic coloured unidentified; and PA polyamide.

Table 3. Microplastics levels in marine sediments of Mediterranean Sea and oceans of the world.

Autor	Year	Region	Ubication	MPs /kg dw	Deep (m)	Size
Present study	2018	wM-B	La Mora	31.7	13	0.5-5 mm
			Cala de Roca Plana	5.6	14.4	
			Llarga Centro	21.6	15.3	
			Miracle-Arrabasada	40.5	14	
			Miracle Center	5.5	15	
			Harbour (marina)	31.7	14	
			Harbour (industrial)	88.6	17	
			Tarragona Coast Mean	32.4±28.4	13-17	
Bayo et al. 2019	2017/18	wM-B	Mar menor	53.1±7.6	-	
Filgueiras et al. 2019	2014/15	wM-B	Spanish mediteranean coast	113±89	43-154	0.5-5 mm (mainly fibres)
Blaškovića et al 2017	2015	cM-A	Telaščica bay (Croatia)	194±117	3.0-15	0.063-5 mm
Vianello et al 2013	2013	cM-A	Venice Lagoon (Italy)	1445±458	<1±0.5	0.030-0.5 mm
Fastelli et al 2016	2015	wM-S.	Stromboli Island	151±34	3.0-15	0.063-5 mm
			Lipari Island	679±346		
Renzi et al 2018	2016	cM-A. Caorle coast	Cymodocea Bottoms	170±95	3.5	0.4-5 mm
			Amphioxus sand+ Mäerl Bottoms	199±115	14.8	
			Amphioxus sand Bottoms	233±133	17.5	
Claessens et al., 2011	2011	neAt	Belgium Harbour mean	167±92.1**	sumerged	0.038–1 mm
			Belgium Continental shelf	97.2±18.6**		
			Belgium coast mean	91.9±21.9**		
			Belgium offshore mean	105±9.9**		
Graca et al 2017	2014	neAt-Baltic Sea	Poland Coast. inshore	21.8±5.4	11.1-18	0.1–5.0 mm
			Poland Coast. offshore	1.5±2.1	70-106	
Frias et al 2016	2013	neAt	South Portugal. mean	8.2±9.2	18.7-19.4	>0.001-5 mm
Thompson et al., 2004	2004	neAt	UK coast (Plymouth)	86.0*	Subtidal	
Zhang et al. 2020	2018	nwP	Western Pacific Ocean	240±291	4601-5732	<1 mm
Matsuguma et al 2017	2012	nwP	Tokyo Bay Channel	1845	5	0.315-5 mm
		wIn	South Africa-Durban bay Harbour	1600	3	
Wang et al 2019	2017	nwP	South Yellow Sea -China	1765	< 20	0.05-5 mm
			South Yellow Sea -China	2135±1020	20-40	

Results expressed in Mean standard ± deviation of number of MP per kilogram of dry weight. * Original unit (# fibres/ 50 mL) converted using an average sediment density of 1600 kg /m^3 (Fettweis et al., 2007) and 1.25 as average wet sediment/dry sediment ratio.**not fibers included.*Regions according FAO Major fishing areas. wM: west Mediterranean sea (subdivided in B: Balearic; S: Sardinia; and Ly: Lyon gulf); cM: Central Mediterranean sea (subdivided in A: Adriatic and I: Ionian); eM: Eastern Mediterranean sea (subdivided in Ag: Aegean and L: Levant); BS: Black sea (subdivided in BS: Black sea; M: Marmara sea and Az: Azov sea);nwP: northwest Pacific; ecP: Eastern central Pacific; wcAT: west central Atlantic; swAt: south-west Atlantic; neAt: northeast Atlantic; An-At: Atlantic-Antarctic; wIn: Western Indian ocean

1.1.3.3 Beach sand

Sand of beaches (intertidal zone) presented a mean MPs level of 10.7 items/kg_{dw} with range between 0.7 MPs/kg_{dw} in La Mora beach and 43 MPs/kg_{dw} in La Pineda beach (Table 1). As it was observed with sediments and superficial seawaters, the sand samples located close to the harbour presented the highest levels of MPs. In general, the main size category was those comprised between 2 and 1 mm, although the dominant size varied depending on the sampling point. Fragments were the principal MP morphology (68%), especially in La Pineda beach, followed by pellet (15%) and fibres (13%). Fragments, pellets and films were composed almost exclusively of polyethylene (47%), polypropylene (36%), polystyrene (6%), polyethylene terephthalate (6%), and chemical additives (3%) without polymeric composition recorded (Figure 5). However, fibres were composed of polyester (45%), polyamide (33%) and synthetic cellulose polymers (22%).

All fragments, pellets and films found were analysed and only 2% could not be identified. Meanwhile, only one third (29%) of the fibres were identified as synthetic polymers and all of them identified (Figure 5). MPs morphology values of Tarragona coast were influenced mostly by La Pineda and Arrabasada beach covering both of them much as the values: 97% of the total fragments, 91% of the pellets, 56% of the film and 77 % of the fibres.

The results in beach sand confirm the MPs pollution on the three environment components is ubiquitous. All sampling beach showed MPs, unlike seawater and sediments samples, sandy beach samples showed an important percentage of larger MPs ranging size of 4 to 2 mm, and high proportion of pellets (primary MPs) and fragments.

Isobe et al., (2014) found that relatively large mesoplastics with greater buoyancy are likely to be carried onshore (by drift of stokes, currents and wind) faster than MPs. However, Liutbarkeva et al., (2018) found the stokes drift in the Catalan Sea is relatively low; therefore, the higher proportion of MPs spanning from 2 to 4 mm in the intertidal zone is due to the presence of mesoplastics on the Tarragona beaches carried to onshore due to wind induced-waves and surface currents favoured by beach orientation. These mesoplastics will be fragmented in shoreline by the mechanical action of the waves and immediately will form large MPs that can be incorporated back into the seawater and to continue its fragmentation there.

The high direct touristic pressure in La Pineda beach, its morphology, nearby Francolí river discharges, runoff, numerous emissaries and wastewater effluents discharges combined with dominant southward littoral drift currents and local hydrodynamics, causing high quantity MPs arrive to beach. This effect is intensified by the storm events accumulating large deposits of MPs and mesoplastics on hide tide line that can be fragmented and transported to intertidal zone due

to touristic activity and rainfalls. The pellets as primary MPs found on the beach, especially in La Pineda, possibly comes from accidental spillage into industrial drainage and discharged by emissaries to the sea or through accidental spillage by transportation due to the proximity to industrial activity and dispersed by terrestrial runoff.

Despite observing lower fibres number compared with water and sediment, like-synthetic ball fibers were again found in El Miracle and Arrabasada. The beach sand fibres abundance values, probably were underestimated by the presence of this type of aggregations (counted as a unit), in many cases these balls were mixed or associated with inorganic and organic material from the surrounding environment.

The composition of MPs in intertidal zone from Tarragona coast beaches was similar to subtidal sediments MPs composition, with higher proportion of most common and low-density polymers (polyethylene and polypropylene) for fragments meanwhile for fibres were polyester and polyamide. Other polymers such as synthetic cellulose in fibres and silicone in fragments were also found. Although the fragments composition from seawater and beaches were similar, the fibres were different because low density polyurethane was observed in seawater.

Fragments with signs of weathering exposing chemical additives such as Erucyl-Erucamide and Tris (2.4-diterbutyl-phenyl) phosphite were also observed. This demonstrate that MP in the intertidal zone can experiment weathering processes due to intense UV radiation, abrasive sand interaction, mechanical forces such as hydraulic shear forces, photo-oxidative degradation by UV radiation, thermal oxidation and salinity (Figure 3D).

A significant fraction (6 out 36 confirmed fibres), were identified as cellulose without differentiating whether it is synthetic or not due to unclear spectra resolution; the cellulosic fibres were excluded for total MPs count. Synthetic cellulose was considered in this study as manufactured fibres from regenerated cellulose (cellulose chemically modified), with lower density than cellulose not chemically modified (natural fibre) 1.44 g/cm3 and 1.50 g/cm3 respectively, e.g. rayon variety: viscose, modal, Lyocell, cupromonium rayon and cellulose acetate (Rana et al., 2014; Sanchez-Vidal et al., 2018). Cellulose acetate is a regenerated as well as a modified cellulosic fibre unlike viscose and cuprammonium rayon which are pure regenerated cellulosic fibres (Rana et al., 2014). Because the chemical composition and properties of the natural cellulose is significantly modified during the manufacturing process for rayon, this type of fibres has been included in studies reporting man-made synthetic fibres. Environmental impact of these fibres has been assessed using lifecycle assessment (Rana et al., 2014).

According this analysis rayon have different characteristics than natural cellulose, with more impacts in human toxicity and freshwater aquatic ecotoxicity despite Lyocell fibres is the only kind rayon completely biodegradable.

The spatial distribution of MPs at small scale on the beaches was heterogeneous changing also temporally from summer to autumn. It was also found different composition and abundance in June than September. In June, (beginning touristic activities) the abundance was higher, dominated by fragments from 2-4 mm composite by five different polymers (polystyrene (32%), polyethylene (32%), polypropylene (26%), Poly(methylmethacrylate) (6%), Poly (ethylene-vinyl acetate)-PEVA (3%), meanwhile fibres composite were polystyrene (20%) and synthetic cellulose (80%). Conversely in September (after touristic activities), the abundance was lower, with similar fragments and fibres proportion; fibres composition was dominated only by synthetic cellulose (100%) meanwhile fragments composition varied with high proportion polyethylene (57%) followed by similar proportion of Poly(methylmethacrylate) (14%), polypropylene (14%) and polystyrene (14%).

The differences in abundance and composition according to the sampling month may be due to the characteristics of the tourist activity in June (summer) and September (autumn). In summer, there are a large number of people on the beach contributing with MPs inputs and their transport from supralitoral and high tide line to intertidal zone. According to this hypothesis, at beginning summer, MPs should fill the beaches intertidal zone. In autumn, it was not observed, probably by low touristic activity and temporal storms causing renewal of polluted intertidal sand (moving particles to supralittoral zone again).

Comparing MPs abundances of sand beaches from Tarragona coast with values from other Mediterranean Sea regions and oceanic beaches, it was observed that average Tarragona coast values were lower than most of studies shown in Table 4. Beaches abundance values of the Atlantic, Indian and Pacific Oceans and the others Mediterranean regions were much higher than those found in our study, with plastic sizes from 0.034 to 5 mm. Studies carried out exclusively in the intertidal zone of Belgium coast (Groenendijk, Koksijde-Bad, Knokke) and Slovenia coast (zones nearby harbour and touristic relevance), reported values also higher than the Tarragona coast values (highlighting that the studies done in Belgium included a wide size range).

The average Tarragona coast values were only similar to values from Atlantic zones as UK coast (Plymouth), Belgium coastal line-De Panne (low tide), Belgium Coastal low tide line mean and in between Greece (Northern Crete) values. These zones are densely populated with high pressure by touristic activities. Tarragona sand beach levels values were only higher than Germany-Nordesney Island high and low tide line, Germany Rostock Coast intertidal zones considering only particles and Baltic Sea, Russia (Vistula Spit) on high tide line. The Germany zones were

characterized by low anthropogenic influence and Vistula Spit influenced by windy events combined with recreation, fishing, and navigation activities.

Specifically, La Pineda MPs abundance values were similar to other oceanic beach zones as Netherlands Coast –Ijmuiden low tide line (near to urbanized zone), India Dhanushkodi low tide line (touristic and fishering activities), Hauts-de-France region from high, low and intertidal line (combination of protected area, low flow river discharges and industrialized zone). La Pineda beach values were higher than Belgium Coastal line mean including two tide lines (low and high tide line), Baltic Sea, Russia (Cape Tarán) high tide line, all of these areas densely populated. Conversely, la Pineda values were lower than Atlantic beaches (high tide line) located in Lithuania-Klaipéda (Baltic Sea), France-Normandy, Portugal-Madeira; the first area characterized by being a harbour city with a lagoon outlet that receive river flows discharges with industrial and agricultural pollution and the last two areas by tourist activities.

Regarding other studies with similar MP sizes focused in western Mediterranean regions made in high tide line and showed in Table 4, La Pineda MP abundances values were about four-fold lower than other densely populated and industrialized zones near Tarragona (as Barcelona beach, Catalonia Coast-Spain), and other densely populated zones from Spanish coast as Denia. With respect to other studies carried out on beaches of the central and eastern Mediterranean Sea (at the high tide line), la Pineda values were from two to thirsty fold lower than those found in densely populated areas as Israel -Tel Aviv, Turkey-Dikili, Italy-Sicily beaches and Lido di Dante zone (Italy); the latter area located in the Adriatic Sea and near important rivers mouth as Po River mouth.

The MPs morphology in most of the studies mentioned above and showed in Table 4, were mainly fibres, in agreement with composition found in Arrabasada and Savinosa beaches and contrary to the dominant morphology found in La Pineda beach where they were mainly made up of fragments. Regarding the composition of the MPs in these studies, it was similar to that of Tarragona coast beaches with common plastic polymers as polypropylene, polyethylene, polystyrene and polyethylene terephthalate.

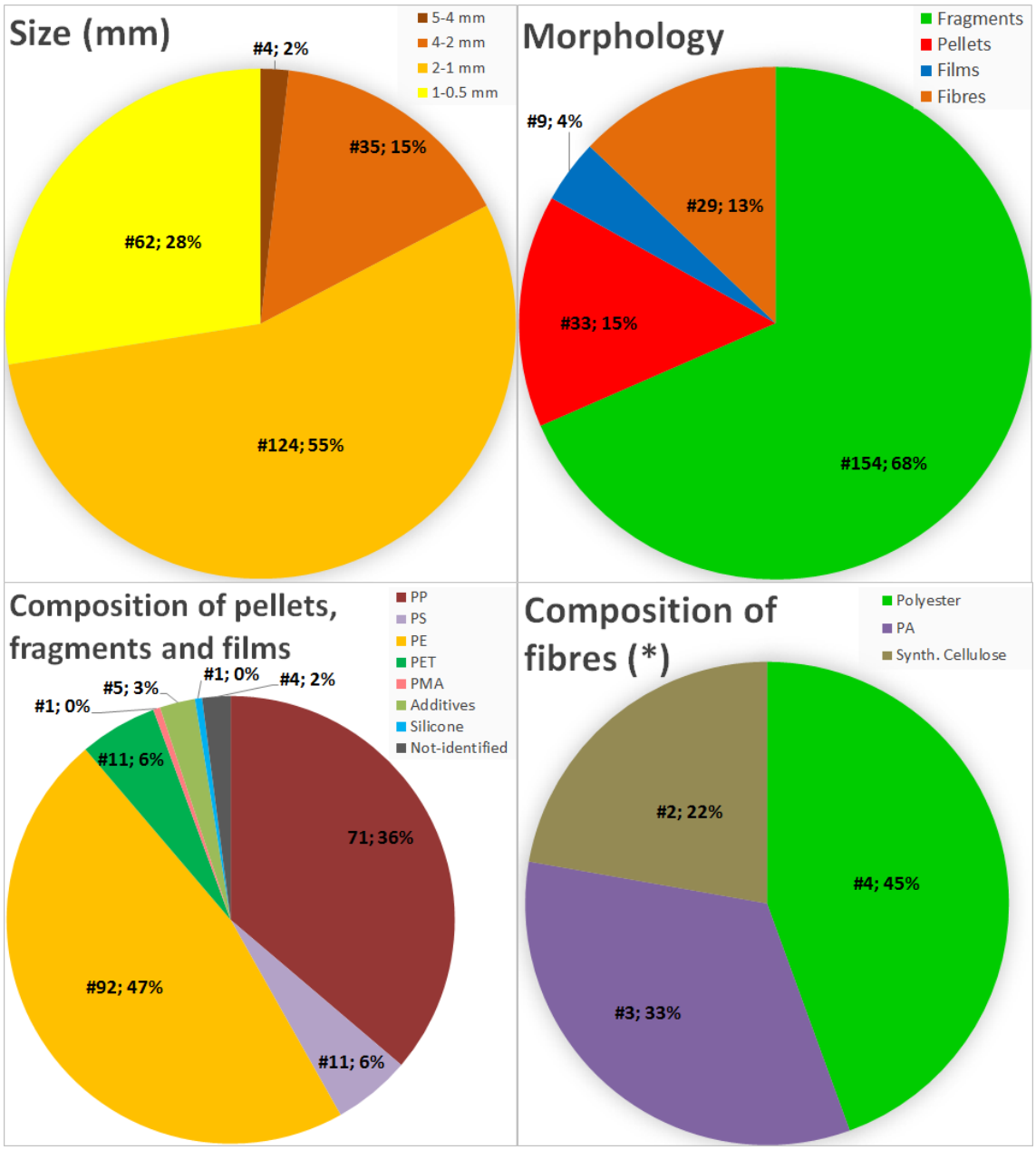

Figure 5. Size distribution (from 5 mm to 0.5 mm), morphology (fragments, pellets, films and fibres), and composition of MPs in sand beaches in Mediterranean costal area of Tarragona. Result expressed as (#) number of MPs; percentage (%). (*) Composition analysed in the 30% of the fibres considered as synthetics, excluding cellulose fibres. PP: Polypropylene; PS: Polystyrene; PE: Polyethylene; PET: Poly(ethyleneterephthalate); PC: Polycarbonate; PMA: Poly(methylmethacrylate); Synth. Cellulose: synthetic cellulose; and PA polyamide.

Table 4. Microplastics levels (items/kg_{dw}) in sand of beaches of Mediterranean Sea and oceans of the world.

Author	Year	Region**	Ubication	Mps/kg_{dw}	Zone	Size
This study	2018	wM-B	Tarragona-La Mora	0.7	I	0.5-5 mm
			Tarragona-Savinosa	1.3		
			Tarragona-Llarga	1.1±1.9		
			Tarragona-Arrabasada	5.3±1.2		
			Tarragona-Miracle (September)	2.0±0.7		
			Tarragona-Miracle (June)	6.9±8.1		
			Tarragona-Pineda	42±19		
			Tarragona Mean	10.7±18		
Thompson et al 2004	2004	neAt	UK coast (Plymouth)	8.00*	H	
Claessens et al., 2011	2011	neAt	Belgium, Groenendijk	156.2	H	0.038-5 mm
				77.9	I	
			Belgium, Koksijde-Bad	95.9	H	
				103.1	I	
			Belgium , Knokke	124.2	H	
				100	I	
Van Cauwenberghe et al 2013	2010/11	neAt	Belgium coastal line-De Panne	20.4	H	<1 mm
				7.2	L	
			Belgium Coastal Line-Zwin	18.2	H	
				12	L	
			Belgium Coastal line mean	17.6	H	
				9.2	L	
Laglbauer et al 2014	2012	cM-A	Slovenia Coast	178±133	I	0.25-5 mm
Piñon-Colin, et al 2018	2015/16	ecP	California Gulf and Ocean mean	135±92	H	0.034-5mm
			California gulf Mean	76±12		
			Ocean pacific coastline mean	179±50		
Piperagkas et al., 2019	2015/16	eM-Ag	Greece- Northern Crete	22±11 5.5±5.0	Dry I	0.042-5mm
Karlsson et al.2017	2014	neAt	Netherlands Coast -Ijmuiden	48±55	L	< 5 mm
Constant et al 2019	2016	wM-Ly	France-Têt river discharge. South	58±53	Dry, H, L	0.063-5mm
			France-Têt river discharge North	166±205		
Yu et al 2016	2015	nwP	Bohai Sea, China -Xingcheng	163.3	I	< 5 mm
Dekiff et al 2014	2011	neAt	Germany , Nordesney Island	1.7±0.4	H, I	< 1 mm
Stolte et al 2015	2014	neAt	Germany, Rostock Coast)	[42-532] (fibers)*	I	< 1 mm
				[3.8-5.5] (particles)*		
Esiukova, 2017	2015/16	neAt	Baltic sea, Russia (Vistula Spit)	2.1±1.6	H	0.5-5 mm
			Baltic sea, Russia (Cape Tarán)	36.2±58.6		
Tiwari et al 2019	2017	In	India-Mumbai	220±50	L	0.036-5 mm
			India-Tuticorin	181±60		
			India-Dhanushkodi	45 ±12		
Doyen et al 2019	2017	neAt	Hauts-de-France region	46.5±17.3	H, I, L	< 5mm
Lots et al 2017	2015/17	neAt	Lithuania -Klaipéda	700±296	H	0.3-3mm
		neAt	France-Normandy	156±29		
		neAt	Portugal-Madeira	92±15		
		cM-A	Bosnia	76±13		
		eM-L	Israel -Tel Aviv	168±16		
		cM-A	Italy -Lido di Dante,	1512±187		
		eM-Ag	Turkey-Dikili,	248±47		
		cM-I	Italy-Sicily	160±31		
		wM-B	Spain-Barcelona	148±23		
		wM-B	Spain-Denia	156±29		

Results expressed in Mean standard ± deviation or [Range] of number of MP per kilogram of dry weight. * Original unit (# fibres/ 50 mL) converted using an average sediment density of 1600 kg /m3 (Fettweis et al., 2007) and 1.25 as average wet sediment/dry sediment ratio. **Regions acoording FAO Major fishing areas. wM: west Mediterranean sea (subdivided in B: Balearic; S: Sardinia; and Ly: Lyon gulf); cM: Central Mediterranean sea (subdivided in A: Adriatic and I: Ionian); eM: Eastern Mediterranean sea (subdivided in Ag: Aegean and L: Levant); BS: Black sea (subdivided in BS: Black sea; M: Marmara sea and Az: Azov sea);nwP: northwest Pacific; ecP: Eastern central Pacific; wcAT: west central Atlantic; swAt: south-west Atlantic; neAt: northeast Atlantic; An-At: Atlantic-Antarctic; In: Indian ocean

1.1.4 General Discussion

The developed protocols for MPs analysis have drawbacks and advantages and it was necessary to adapting them for different environmental samples requirements. In visual sorting, the MPs pre-selection based in buoyancy, guided and supported the spectroscopy analysis, changes of H_2O_2:$FeSO_4$ relation in Fenton procedures removed organic matter and turbidity avoiding filter yellowish coloration.

In this study MPs pollution in western Mediterranean coastal environments was determined. Sandy beaches, marine sediment and superficial seawater from Tarragona coast showed MPs of different sizes, morphology, composition and significant mean values differences in average MP density in each one compartment. Seawater near the coast <1km, were around 0.0013 items/kg (0.0013 items/L), in beach sands around 10.7 items /kg ($7^{*}\cdot10^{3}$ higher than seawater values) and marine sediments around 32.4 items/kg ($2^{*}\cdot10^{4}$ higher than seawater values).

Considering the MPs pollution dynamics in Tarragona coast, the seawater is the media that receive and facilitates MPs transport. The shoreline acts as an intermediate reservoir with constant weathering and active exchange with seawater. Sediments acts as a significant sink for MPs from seawater, with high residence times.

Despite the differences observed in abundance values for sand, sediment and water, three polymers were common in the three compartments: polyethylene, polystyrene, and polyethylene terephthalate. Microplastics fragment and fibres composition always was dominated by polyethylene and polyethylene terephthalate (polyester), respectively. Polypropylene polymer was identified in beaches and seawater, with higher proportion in beaches. However polyvinyl polymers were detected in water and marine sediments with higher proportion in this last compartment. As many authors have observed, in Tarragona coast, MPs after entry or formed (by fragmentation) into the seawater can be distributed in the marine media according their densities alone, in hetero-aggregations or by suffering biofouling, and transported by main and superficial currents, wind (wave height) and by biota ingestion (invertebrates and fish) (Cozar et al., 2014; GESAMP, 2015; Wu et al., 2019). The growth of bio-films on MPs, increase its density and affects its sedimentation velocity through water column.

The number of secondary MPs will increase exponentially as the size becomes smaller. In steady state, the abundance–size distribution should follow a power law, with a scaling exponent equal to the spatial dimension of the plastic objects (Cozar et al., 2014). According to this assumption, the size distribution of MPs in seawater samples with high proportion of fragments 1 to 2 mm and 0.08 to 0.5 mm maybe follow a continuous and very fast fragmentation to high millimetric scale to medium millimetric scale and micrometric scale.

On Tarragona coast, there is a high degree of spatial variability in the MPs abundance values for samples of seawater, sediments and sands. The proximity to urban and industrial areas, rivers discharges, wastewater discharges, runoff, watercourses discharges (emissaries) together with the geostrophic southward currents (typical of the Catalan coast), coastal drift, predominant winds, coastal shoreline morphology and the presence of anthropic structures (Port seawall) that modify the surface currents direction and change the normal hydrodynamics in the coastal zone, causes high MPs accumulation in harbor area and La Pineda beach. In turn, the variability of MPs abundances in seawater was influenced by circulation and mixing processes (thermohaline gradients) of superficial strata.

Rivers receive MPs and mesoplastics from different sources (sewer overflows, wastewater effluents discharges, soil runoffs, air dusts, precipitation and touristic activity waste disposal) and carry them to the ocean. Only plastics particles with lower or equal density than freshwater density, arrive to the seas (Lots et al., 2017). In the case of the Francolí River, this can be an important land-based MP source to Tarragona coast together with small watercourses fluxes and runoffs, due to the large number of populated areas and wastewater discharges effluents located on its watershed.

Mansui et al., (2015) modelled the effects of circulation on plastic accumulation in the Mediterranean, which finding in the western basin some specific gyres could retain and export floating objects and redistribute them after a shift in the large-scale circulation considering this area as one the most energetic regions. Liutbarkeva et al., (2018) found high spatio-temporal variability in sea-surface plastic concentrations without any stable long-term accumulations, specifically in the Catalan Sea. The North Current tends to weaken, which could result in plastic debris being trapped in the vicinity of its origin with substantial accumulation of plastics on coastlines and the sea bottom. These authors concluded that plastic pollution in that area is caused primarily by its own terrestrial sources.

The MP accumulation observed on a small scale on Tarragona coastline, is mainly due to the land-based sources of the city (densely populated), river mouths, wastewater treatment plants, runoff and outfalls of watercourses. Other factors influencing the accumulation of MPs on Tarragona coast is also related to direct inputs from sources to short distance from de coast. The quantity and type of MPs will depend mainly of the degree of waste management in Francolí watershed and Tarragona city, WWTP depuration efficiency and hydrodynamics conditions on the coast.

Comparing MPs abundance in seawater (by volume filtered) with other studies, it has been observed higher values of MPs abundances on the coast of Tarragona than those found in oceanic areas, and similar abundances values to other areas of Mediterranean Sea with high accumulation

of MPs. This corroborate that the entire Mediterranean Sea basin (despite the variability of circulation in the basin that alters its spatial distribution), as a large region of MPs accumulation.

Microplastics hetero aggregations have the potential to be ingested by a wide range of marine organisms, including zooplankton, invertebrates, and fish depending on its size and morphology, in seawater surface and bottom sediments. The immediate possible toxicological effects of MPs involve physical blockage, entanglement, abrasion, loss of mobility in energy expenditure, absent stimulus for feeding, fish respiratory system block. Other long-term effects observed in laboratory studies may involve alterations in the gene regulation, immune response, behavioral alterations and embryonic development of fishes, as well as, histological damage, alteration of cellular oxidative balance and abnormal larval development in mussels (Andrady, 2011; Besseling et al., 2013; Lehtiniemi et al., 2018; Limonta et al., 2019; Paul-Pont, et al., 2016; Ravit et al., 2017; Rist et al., 2018; Rist et al., 2019; Ross et al., 2015).

These toxicological effects of MPs could have a possible synergistic interaction between MPs and absorbed micro-pollutants/microbial pathogens; pollutants adsorbed on MPs are polychlorinated biphenyls (PCBs), HCH's and many others compounds included into 78% of priority pollutants (Guo et al., 2019; Rochman et al., 2013). MPs also can act as a sink of pollutants, highlighting the high quantity and large surface-to-volume ratio in marine environment (Rodrigues et al., 2019).

On the Tarragona coast, there were found MPs on sediments and sands with weathering signals with its surface modified with pores and roughness evidenced by the growth of microorganisms on the surfaces. These MPs can induce its high affinity for persistent organic pollutants and heavy metals present in sediments or seawater, particularly in the Port area where high concentrations of lead and mercury were found.

Other signs of MPs weathering and fragmentation on Tarragona coast is the polymers additives exposition, loss of MPs structures and surface exposure. All this was evidenced in our samples by the presence of specifically additives spectra in the identification of MPs with FTIR. At molecular level, various degradation mechanisms exist and the domination of one mechanism over the others often depends on the polymer type. Most important mechanisms are chain scission (breaking the chemical bonds of the polymer molecule or depolymerisation) and chain stripping (the side atoms/groups attached to polymer chain are released) (Lithner et al., 2011)

Polymers additives are chemical compounds added to improve the polymers performance (e.g. during shaping of the polymer, through injection moulding, extrusion, blow moulding, vacuum moulding, etc.). They are responsible for certain characteristics in plastics like flexibility, strength, ageing resistance and color, enhancing the final functional properties of a plastic product. Additives are not usually bound to the polymer matrix, are of low molecular weight, and

may be present in large amounts. They often account for the major leaching and emissions of chemical substances from plastic materials (Hahladakis et al., 2018; Lithner et al, 2011) and most of them are classified as toxic, carcinogenic, or endocrine disruptors (Rochman, 2013).

The plastic additives found in the samples collected in the sand of La Pineda beach were Erucil-Erucamida and Tris (2,4-diterbutyl-phenyl) phosphite. Erucyl-Erucamide is a slip compound responsible for significantly reducing the surface coefficient of friction of a polymer, also used to enhance the polymer with antistatic properties, enable better mould release, reduced melt viscosity, and anti-sticking properties. Tris (2.4-diterbutyl-phenyl) phosphite delay the overall oxidative degradation of plastics when exposed to ultraviolet (UV) light (Bhunia et al., 2013). Some studies showed high migrations capacity of these additives in aqueous and oily solutions and drinking water at 40ºC after short-term storage (Cooper and Tice, 1995; Gao et al., 2011; Li et al., 2016)

The only plastics additives found in sediment samples (Tarragona Port) were phthalates. They are plasticizers used for improving the flexibility, durability and stretch-ability of polymeric films, improvement impact resistance in the final plastic film (Bhunia et al., 2013). The ingestion of plastics containing these additives by marine organisms can be of great concern because some of them have the ability to accumulate. Even if plastics are not ingested, the additives contained in MPs are sources of exposure. Brominated diphenyl ethers (PBDE), phthalates, nonylphenols (NP), bisphenol A (BPA) and antioxidants are some of the most common plastic additives found in marine environments. Exposure studies based on leaching processes conducted on a wide range of polymers and target organisms confirmed toxicity of plastics additives. Leaching of additives by plastic ingestion was more relevant for species with longer gut retention times, such as fish, and was also occasionally be relevant for seas worms. (Koelmans et al., 2013; Koelmans et al., 2014; Hermabessiere et al., 2017; Rochman et al., 2013; Teuten et al., 2009).

The risk of exposure to polymeric additives is due not only to migration or leakage into the surrounding environment and possible toxicity to aquatic organisms, but also because the affinity of other contaminants for MPs (enhanced by hydrophobic/polar groups of the exposed additives). To estimate the real risk associated to MPs effects of exposure to these particles it is important to collect environmental data with accurate MPs measures, and thus to obtain deeply knowledge about the negative impact of MPs on the local aquatic biota (GESAMP, 2015). To assess an accurate environmental risk, it is necessary to collect data about: a) the number of particles, b) the size distribution, c) shape, d) surface properties, e) polymer composition (and its additives) and density of the particles. However, until now, the environmental risk assessment of MPs in the marine environment has been carried out based on assumptions from the data available in

literature and extrapolations. The data obtained of integration on Tarragona coast environment might be very useful for a precisely Environmental Risk Assessment by MPs in the area.

This study also provides background information on the occurrence of MPs in three integrated environmental components such as sand, seawater and sediments on Tarragona coastal area. This information will be used for the implementation of the Marine Strategy Framework Directive in accordance to the European Union directive that commits the EU Member States to take action aimed at achieving the "Good Environmental status" (GES) of the EU's marine environment by 2020". In particular, descriptor 10 focuses on the emerging problem of marine litter and its effects on the marine environment and biota.

1.1.5. Conclusions

In present work, MP abundance, size, morphology and composition were determined in sandy beaches, sediments and superficial seawater in Tarragona costal area. Microplastics mean abundance were 1.3 MPs/m^3 in surface sea water, 32.4 items/kg marine sediments, and 10.7 items/kg in sand of beaches. The abundance by volume of seawater MPs was higher to those found in oceanic areas and similar to other areas of Mediterranean Sea with high MPs accumulation, corroborating the Western Mediterranean Sea basin as a region of MPs accumulation. Fibres were the dominant MPs morphology in bottom sediments and seawater while fragments were mainly dominant in beaches. By Fibres probably comes from effluents from WWTP and urban runoff (emissaries discharges). Fibres balls associated with bottom sediments with organic debris and plankton were abundant, masking the real amounts of fibres in each reservoir. Taking into account composition, the highly demanded polymers (polyethylene and polypropylene) were the most abundant for fragments and polyester (polyethylene terephthalate) for fibres. In order to harmonize results and address specific problems such as fibres balls, it is necessary to adapt protocols and guidelines developed for MPs analysis. It was necessary to adapting developed protocols and guidelines for MPs analysis in order to harmonize results and deal with specific problems such as fibres balls.

The size range 4-5 mm was the lowest proportion among the three environmental compartments; the proportion of 2-4 mm was present only in bottom sediments and sandy beach. The proportion of 1-2 mm size was higher in the sandy beaches and seawater (sand proportion higher than in seawater). The size range 0.5-1 mm was most abundant in bottom sediments than seawater, suggesting a selective deposition (size-selective loss processes) on this size to sediments. The high proportion of 1-2 mm observed in seawater and sandy beach suggests seawater is the media that receive and facilitates MPs dispersion, the shoreline acts as an intermediate reservoir with constant weathering and active exchange with seawater surface and the sediments acts as a significant sink for MPs 0.5-1 mm from seawater. The level of MPs fragments size below 0.5 mm

in seawater suggests active fragmentation on surface by weathering action (mechanical forces, photo-oxidation and temperature).

The composition and abundance of the MPs observed, suggests that the main contribution comes from numerous land base-sources (through streams, terrestrial runoff, watercourse emissaries, port activities and WWTP effluents discharges). Fisheries and marine transport were less important sources of MPs on Tarragona coast.

Microplastics fate analysis is of fundamental importance to determine the nature of pollution and effects on the coastal environment. Three-dimensional numerical simulations taking account the sinking and beaching dynamic processes, seawater density, velocity wind-driven currents and stokes drift will help us to understand the microplastic residence times and the fluxes between compartments. Future studies are needed to assess spatial and temporary trends in MPs abundance on Catalan coastal environment.

References

Aliabad, M.K., Nassiri, M., Kor, K., 2019. Microplastics in the surface seawaters of Chabahar Bay, Gulf of Oman (Makran Coasts). Mar. Pollut. Bull. 143, 125-133.

Alomar, C., Estarellas, F., Deudero, S., 2016. Microplastics in the Mediterranean Sea: Deposition in coastal shallow sediments, spatial variation and preferential grain size. Mar. Environ. Res. 115, 1-10.

Allen, S., Allen, D., Phoenix, V.R., Le Roux, G., Durántez Jiménez, P., Simonneau, A., Binet, S., Galop, D., 2019. Atmospheric transport and deposition of microplastics in a remote mountain catchment. Nat. Geosci., 12, 339-344.

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62,1596-1605.

Ashton, K., Holmes, L., Turner, A., 2010. Association of metals with plastic production pellets in the marine environment. Mar. Pollut. Bull. 60, 2050-2055.

Arthur, C., Baker, J., Bamford, H. (Eds), 2009. Proceedings of the international research workshop on the occurrence, effects, and fate of microplastic marine debris. NOAA Technical Memorandum NOS-OR&R-30.

Baini, M., Fossi, M.C., Galli, M., Caliani, I., Campani, T., Finoia, M.G., Panti, C., 2018. Abundance and characterization of microplastics in the coastal waters of Tuscany (Italy): The application of the MSFD monitoring protocol in the Mediterranean Sea. Mar. Pollut. Bull. 133, 543-552.

Bakir, A., Rowland, S.J., Thompson, R.C., 2014. Enhanced desorption of persistent organic pollutants from microplastics under simulated physiological conditions. Environ. Pollut., 185, 1623.

Barboza, L.G, Gimenez, B.C., 2015. Microplastics in the marine environment: Current trends and future perspectives. Mar. Pollut. Bull. 97, 5-12.

Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. J. Polym. Environ. 15, 1985-1998.

Bayo, J., Rojo, D., Olmos, S., 2019. Abundance, morphology and chemical composition of microplastics in sand and sediments from a protected coastal area: The Mar Menor lagoon (SE Spain). Environ. Pollut. 252, 1357-1366.

Bergmann, M., Gutow, L., Klages, M. (Eds), 2015. Marine anthropogenic litter. Springer Cham, XVIII, 447 pp.

Besley, A., Vijver, M.G., Behrens, P., Bosker, T.A., 2017. A standardized method for sampling and extraction methods for quantifying microplastics in beach sand. Mar. Pollut. Bull. 114, 77-83.

Besseling, E., Wegner, A., Foekema, E.M., van den Heuvel-Greve, M.J., Koelmans, A.A., 2013. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.). Environ. Sci. Technol. 47(1), 593-600.

Bhunia, K., Sablani, S.S., Tang, J., Rasco, B., 2013. Migration of chemical compounds from packaging polymers during microwave, conventional heat treatment, and storage. Compr. Rev. Food Sci. Food Saf. 12, 523-545.

Blašković, A., Fastelli, P., Čižmek, H., Guerranti, C., Renzi, M. 2017. Plastic litter in sediments from the Croatian marine protected area of the natural park of Telaščica bay (Adriatic Sea). Mar. Pollut. Bull. 114(1), 583-586.

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines woldwide: Sources and sinks. Environ. Sci. Technol. 45(21), 9175-9179.

Carbery, M., O'Connor, W., Palanisami, T. 2018. Trophic transfer of microplastics and mixed contaminants in the marine food web and implications for human health. Environ. Int. 115, 400-409.

Carr, S.A., Liu, J., Tesoro, A.G., 2016. Transport and fate of microplastic particles in wastewater treatment plants. Water Res. 91, 174-182.

Chae, Y., An, Y.J., 2017. Effects of micro- and nanoplastics on aquatic ecosystems: Current research trends and perspectives. Mar. Pollut. Bull. 124(2), 624-632.

Chang, X., Xue, Y., Li, J., Zou, L., Tang, M., 2020. Potential health impact of environmental micro- and nanoplastics pollution. J. Appl. Toxicol. 40(1), 4-15.

Cincinelli, A., Martellini, T., Guerranti, C., Scopetani, C, Chelazzi, D., Giarrizzo, T., 2019. A potpourri of microplastics in the sea surface and water column of the Mediterranean Sea. Trends Analyt. Chem. 110, 321-326.

Claessens, M., Meester, S.D., Landuyt, L.V., Clerck, K.D., Janssen, C.R., 2011. Occurrence and distribution of microplastics in marine sediments along the Belgian coast. Mar. Pollut. Bull. 62(10), 2199-2204.

Cole, M., Lindeque, P.K., Fileman, E., Clark, J., Lewis, C., Halsband, C., Galloway, T.S., 2016. Microplastics alter the properties and sinking rates of zooplankton faecal pellets. Environ. Sci. Technol., 50(6), 3239-3246.

Constant, M., Kerhervé, P., Mino-Vercellio-Verollet, M., Dumontier, M., Sànchez Vidal, A., Canals, M., Heussner, S., 2019. Beached microplastics in the Northwestern Mediterranean Sea. Mar. Pollut. Bull. 142, 263-273.

Constant, M., Philippe, K., Sola, J., Sanchez-Vidal, A., Canals, M., Heussner, S., 2018. Floating microplastics in the Northwestern Mediterranean Sea: Temporal and spatial heterogeneities (pp. 9-15). In: Cocca, M., Di Pace, E., Errico, M.E., Gentile, G., Montarsolo, A., Mossotti, R., Proceedings of the International Conference on Microplastic Pollution in the Mediterranean Sea. Springer Cham.

Cooper, I., Tice, P.A., 1995. Migration studies on fatty acid amide slip additives from plastics into food simulants. Food Addit. Contam. 12(2), 235-244.

Coppock, R.L., Cole, M., Lindeque, P.K., Queirós, A.M., Galloway, T.S., 2017. A small-scale, portable method for extracting microplastics from marine sediments. Environ. Pollut. 230, 829-837.

Cózar A, Echevarría F, González-Gordillo JI, Irigoien X, Ubeda B, Hernández-León S, Palma AT, Navarro S, García-de-Lomas J, Ruiz A, Fernández-de-Puelles ML, Duarte CM, 2014. Plastic debris in the open ocean. Proc Natl Acad Sci U S A. 111(28):10239-44.

Cozar, A., Sanz-Martín, M., Martí, E., González-Gordillo, J.I., Ubeda, B., Gálvez, J.A., Irigoien, X., Duarte, C.M., 2015. Plastic accumulation in the Mediterranean Sea. PLoS One 10, 1-12.

DARP, Departament d'Acció Climàtica, Alimentació i Agenda Rural, 2018. Caracterització de les Biocenosis de Fanerógames marines al Municipi de Tarragona. Resolució ARP/619/2019. TecnATox – Fundació URV-(FEMP), 79 pp.

de Haan, W.P., Sanchez-Vidal, A., Canals, M., 2019. Floating microplastics and aggregate formation in the Western Mediterranean Sea. Mar. Pollut. Bull. 140, 523-535.

de Lucia, G.A., Vianello, A., Camedda, A., Vani, D., Tomassetti, P., Coppa, S., Palazzo, L., Amici, M., Romanelli, G., Zampetti, G., Cicero, A.M., Carpentieri, S., Di Vito, S., Matiddi, M., 2018. Sea water contamination in the Vicinity of the Italian minor islands caused by microplastic pollution. Water 10, 1108.

de Lucia, G.A., Caliani, I., Marra, S., Camedda, A., Coppa, S., Alcaro, L., Campani, T., Giannetti, M., Coppola, D., Cicero, A.M., Panti, C., Baini, M., Guerranti, C., Marsili, L., Massaro, G., Fossi, M.C., Matiddi, M., 2014. Amount and distribution of neustonic micro-plastic off the Western Sardinian coast (Central-Western Mediterranean Sea). Mar. Environ. Res. 100, 10-16.

Dekiff, J.H., Remy, D., Klasmeier, J., Fries, E. 2014. Occurrence and spatial distribution of microplastics in sediments from Norderney. Environ. Pollut. 186, 248-256.

Desforges, J.-P.W., Galbraith, M., Dangerfield, N., Ross, P.S., 2014. Widespread distribution of microplastics in subsurface seawater in the NE Pacific Ocean. Mar. Pollut. Bull. 79, 94-99.

Dris, R., Gasperi, J., Mirande, C., Mandin, C., Guerrouache, M., Langlois, V., Tassin, B., 2017. A first overview of textile fibers, including microplastics, in indoor and outdoor environaments. Environ. Pollut. 221, 453-458.

Dris, R., Gasperi, J., Saad, M., Mirande, C., Tassin, B., 2016. Synthetic fibers in atmospheric fallout: A source of microplastics in the environment? Mar. Pollut. Bull. 104, 290-293.

Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N., Tassin, B., 2015. Microplastic contamination in an urban area: A case study in Greater Paris. Environ. Chem. 12, 592-599.

Doyen, P., Hermabessiere, L., Dehaut, A., Himber, C., Decodts, M., Degraeve, T., Delord, L., Gaboriaud, M., Moné, P., Sacco, J., Tavernier, E., Grard, T., Duflos, G., 2019. Occurrence and identification of microplastics in beach sediments from the Hauts-de-France region. Environ. Sci. Pollut. Res. 26, 28010-28021.

Esiukova, E., 2017. Plastic pollution on the Baltic beaches of Kaliningrad region, Russia. Mar. Pollut. Bull. 114, 1072-1080.

Fastelli, P., Blašković, A., Bernardi, G., Romeo, T., Čižmek, H., Andaloro, F., Russo, G.F., Guerranti, C., Renzi, M., 2016. Plastic litter in sediments from a marine area likely to become protected (Aeolian Archipelago's islands, Tyrrhenian Sea). Mar. Pollut. Bull. 113, 526-529.

Fettweis, M., Du Four, I., Zeelmaekers, E., Baeteman, C., Francken, F., Houziaux, J.-S., Mathys, M., Nechad, B., Pison, V., Vandenberghe, N., Van den Eynde, D., Van Lancker, V., Wartel, S., 2007. Mud origin, characterisation and human activities (MOCHA). Final Scientific Report. Belgian Science Policy Office, Brusels. 59pp

Filgueiras, A.V., Gago, J., Campillo, J.A., León, V.M., 2019. Microplastic distribution in surface sediments along the Spanish Mediterranean continental shelf. Environ. Sci. Pollut. Res. 26, 21264-21273.

Fossi, M.C., Marsili, L., Baini, M., Giannetti, M., Coppola, D., Guerranti, C., Caliani, I., Minutoli, R., Lauriano, G., Finoia, M.G., Rubegni, F., Panigada, S., Bérubé, M., Urbán Ramírez, J., Panti, C., 2016. Fin whales and microplastics: The Mediterranean Sea and the Sea of Cortez scenarios. Environ. Pollut. 209, 68-78.

Fossi, M.C., Panti, C., Guerranti, C., Coppola, D., Giannetti, M., Marsili, L., Minutoli, R., 2012. Are baleen whales exposed to the threat of microplastics? A case study of the Mediterranean fin whale (*Balaenoptera physalus*). Mar. Pollut. Bull. 64, 2374-2379.

Frias, J.P.G.L., Gago, J., Otero, V., Sobral, P., 2016. Microplastics in coastal sediments from Southern Portuguese shelf waters. Mar. Environ. Res. 114, 24-30.

Frias, J.P.G.L., Nash, R., 2019. Microplastics: Finding a consensus on the definition. Mar. Pollut. Bull. 138, 145–147.

Gao, Y., Gu, Y., Wei, Y., 2011. Determination of polymer additives-antioxidants and ultraviolet (UV) absorbers by high-performance liquid chromatography coupled with UV photodiode array detection in food simulants. J. Agric. Food Chem. 59(24), 12982-12989.

Garcia, T.M., Campos, C.C., Mota, E.M.T., Santos, N.M.O., Campelo, R.P.D.S., Prado, L.C.G., Melo Junior, M., Soares, M.D.O., 2020. Microplastics in subsurface waters of the western equatorial Atlantic (Brazil). Mar. Pollut. Bull. 150, 110705.

Gasperi, J., Wright, S.L., Dris, R., Collard, F., Mandin, C., Guerrouache, M., Langlois, V., Kelly, F.J., Tassin, B., 2018. Microplastics in air: Are we breathing it in? Curr. Opin. Environ. Sci. Health 1, 1-5.

GESAMP, 2015. Sources, fate and effects of microplastics in the marine environment: a global assessment. In: Kershaw, P.J. (Ed.), (IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, London: International Maritime Organization. Rep. Stud.GESAMP No. 90, p. 96.

GESAMP, 2016. Sources, fate and effects of microplastics in the marine environment: part two of a global assessment. In: Kershaw, P.J., Rochman, C.M. (Eds.), IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, London: International Maritime Organization. Rep. Stud. No. 93, p. 220.

Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 3(7), e1700782.

Goldstein, M.C., Rosenberg, M., Cheng, L., 2012. Increased oceanic microplastic debris enhances oviposition in an endemic pelagic insect. Biol. Lett. 8, 817-820.

Graca, B., Szewc, K., Zakrzewska, D., Dołęga, A., Szczerbowska-Boruchowska, M., 2017. Sources and fate of microplastics in marine and beach sediments of the Southern Baltic Sea - A preliminary study. Environ. Sci. Pollut. Res. 24, 7650-7661.

Guo, X., Wang, J., 2019. The chemical behaviors of microplastics in marine environment: A review. Mar. Pollut. Bull. 14, 21-14.

Hahladakis, J.N., Velis, C.A., Weber, R., Iacovidou, E., Purnell, P., 2018. An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. J. Hazard. Mater. 344, 179-199.

Harris, C.A., Henttu, P., Parker, M.G., Sumpter, J.P., 1997. The estrogenic potential of phthalate esters *in vitro*. Environ. Health Perspect. 105(8), 802-811.

Hartline, N.L., Bruce, N.J., Karba, S.N., Ruff, E.O., Sonar, S.U., Holden, P.A., 2016 Microfiber masses recovered from conventional machine washing of new or aged garments. Environ. Sci. Technol. 50(21), 11532-11538.

Hermabessiere, L., Dehaut, A., Paul-Pont, I., 2017. Occurrence and effects of plastic additives on marine environments and organisms: A review. Chemosphere 182, 781-793.

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: A review of the methods used for identification and quantification. Environ. Sci. Technol. 46, 3060-3075.

Paul-Pont, I., Lacroix, C., González Fernández, C., Hégaret, H., Lambert, C., Le Goïc, N., Frère, L., Cassone, A.L., Sussarellu, R., Fabioux, C., Guyomarch, J., Albentosa, M., Huvet, A.,

Soudant, P., 2016. Exposure of marine mussels Mytilus spp. to polystyrene microplastics: Toxicity and influence on fluoranthene bioaccumulation. Environ. Pollut. 216, 724-737.

Ivar do Soul, J.A., Spengler, Â., Costa, M.F., 2009. Here, there and everywhere: Small plastic fragments and pellets on beaches of Fernando de Noronha (Equatorial Western Atlantic). Mar. Pollut. Bull. 58(8), 1236-1238.

Ivleva, N.P., Wiesheu, A.C., Niessner, R., 2017. Microplastic in aquatic ecosystems. Angew. Chem. Int. Ed. 56(7), 1720-1739.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science 347(6223), 768-771.

Karami, A., Golieskardi, A., Choo, C.K., Romano, N., Ho, Y.B., Salamatinia, B. 2017. A high-performance protocol for extraction of microplastics in fish. Sci. Total Environ. 578, 485-494.

Karlsson, T.M., Vethaak, A.D., Almroth, B.C., Ariese, F., van Velzen, M., Hassellöv, M., Leslie, H.A., 2017. Screening for microplastics in sediment, water, marine invertebrates and fish: Method development and microplastic accumulation. Mar. Pollut. Bull. 122(1-2), 403-408.

Kazour, M., Jemaa, S., Issa, C., Khalaf, G., Amara, R., 2019. Microplastics pollution along the Lebanese coast (Eastern Mediterranean Basin): Occurrence in surface water, sediments and biota samples. Sci. Total Environ. 696, 133933.

Koelmans, A.A., Besseling, E., Wegner, A., Foekema, E.M., 2013. Plastic as a carrier of POPs to aquatic organisms: a model analysis. Environ. Sci. Technol. 47(14), 7812-7820 [published correction appears in Environ. Sci. Technol. 47(15), 8992-3].

Koelmans, A.A., Besseling, E., Foekema, E.M., 2014. Leaching of plastic additives to marine organisms. Environ. Pollut. 187, 49-54.

Kukulka, T., Proskurowski, G., Morét-Ferguson, S., Meyer, D.W., Law, K.L., 2012. The effect of wind mixing on the vertical distribution of buoyant plastic debris. Geophys. Res. Lett. 39, L07601.

Lacerda, A.L.F., Rodrigues, L.S., van Sebille, E., Rodrigues, F.L., Ribeiro, L., Secchi, E.R., Kessler, F., Proietti, M.C., 2019. Plastics in sea surface waters around the Antarctic Peninsula. Sci. Rep. 9, 3977.

Laglbauer, B.J.L., Franco-Santos, R.M., Andreu-Cazenave, M., Brunelli, L., Papadatou, M., Palatinus, A., Grego, M., Deprez, T., 2014. Macrodebris and microplastics from beaches in Slovenia. Mar. Pollut. Bull. 89, 356-366.

Lebreton, L.C.M., Van Der Zwet, J., Damsteeg, J.-W., Slat, B., Andrady, A., Reisser, J., 2017. River plastic emissions to the world's oceans. Nat. Commun. 8, 1-10.

Lehtiniemi, M., Hartikainen, S., Näkki, P., Engström-Öst, J., Koistinen, A., Setälä, O., 2018. Size matters more than shape: Ingestion of primary and secondary microplastics by small predators. Food Webs 17, e00097.

Limonta, G., Mancia, A., Benkhalqui, A., Bertolucci, C., Abelli, L., Fossi, M.C., Panti, C., 2019. Microplastics induce transcriptional changes, immune response and behavioral alterations in adult zebrafish. Sci. Rep. 9, 15775.

Lithner, D., Larsson, A., Dave, G., 2011. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Sci. Total Environ. 409(18), 3309-3324.

Li, C., Xu, J., Chen, D., Xiao, Y., 2016. Detection of phthalates migration from disposable tablewares to drinking water using hexafluoroisopropanol-induced catanionic surfactant coacervate extraction. J. Pharm. Anal. 6(5), 292-299.

Liu, T., Zhao, Y., Zhu, M., Liang, J., Zheng, S., Sun, X., 2020. Seasonal variation of micro- and meso-plastics in the seawater of Jiaozhou Bay, the Yellow Sea, Mar. Pollut. Bull. 152, 110922.

Liubartseva, S., Coppini, G., Lecci, R., Clementi, E., 2018. Tracking plastics in the Mediterranean: 2D Lagrangian model. Mar. Pollut. Bull. 129, 151-162.

Löder, M.G.J., Imhof, H.K., Ladehoff, M., Löschel, L.A., Lorenz, C., Mintenig, S., Piehl, S., Primpke, S., Schrank, I., Laforsch, C., Gerdts, G., 2017. Enzymatic purification of microplastics in environmental samples. Environ. Sci. Technol. 51, 14283-14292.

Long, M., Moriceau, B., Gallinari, M., Lambert, C., Huvet, A., Raffray, J., Soudant, P., 2015. Interactions between microplastics and phytoplankton aggregates: impact on their respective fates. Mar. Chem. 175, 39-46.

Lots, F.A.E., Behrens, P., Vijver, M.G., Horton, A.A., Bosker, T., 2017. A large-scale investigation of microplastic contamination: Abundance and characteristics of microplastics in European beach sediment. Mar. Pollut. Bull. 123, 219-226.

Lusher, A.L., Tirelli, V., O'Connor, I., Officer, R., 2015. Microplastics in Arctic polar waters: The first reported values of particles in surface and sub-surface samples. Sci. Rep. 5, 14947.

Lusher, A.L., O'Donnell, C., Officer, R., O'Connor, I., 2016. Microplastic interactions with North Atlantic mesopelagic fish. ICES J. Mar. Sci. 73, 250-262.

Mack, L., Andersen, H.E., Beklioğlu, M., Bucak, T., Couture, R.-M., Cremona, F., Ferreira, M.T., Hutchins, M.G., Mischke, U., Molina-Navarro, E., Rankinen, K., Venohr, M., Birk, S., 2019. The future depends on what we do today – Projecting Europe's surface water quality into three different future scenarios. Sci. Total Environ. 668, 470-484.

Mai, L., Bao, L.-J., Shi, L., Wong, C.S., Zeng, E.Y., 2018. A review of methods for measuring microplastics in aquatic environaments. Environ. Sci. Pollut. Res. 25, 11319-11332.

Masó, M., Garcés, E., Pagès, F., Camp, J., 2003. Drifting plastic debris as a potential vector for dispersing Harmful Algal Bloom (HAB) species. Sci. Mar., 67(1), 107-111.

Mason, S.A., Garneau, D., Sutton, R., Chu, Y., Ehmann, K., Barnes, J., Fink, P., Papazissimos, D., Rogers, D.L., 2016. Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent. Environ. Pollut. 218, 1045-1054.

Masura, J., Baker, J., Foster, G., Arthur, C., Herring, C. (Ed), 2015. Laboratory methods for the analysis of microplastics in the marine environment: Recommendations for quantifying synthetic particles in waters and sediments. NOAA Technical Memorandum NOS-OR&R-48, pp. iii, 31.

Matsuguma, Y., Takada, H., Kumata, H., Kanke, H., Sakurai, S., Suzuki, T., Itoh, M., Okazaki, Y., Boonyatumanond, R., Zakaria, M.P., Weerts, S., Newman, B., 2017. Microplastics in sediment cores from Asia and Africa as indicators of temporal trends in plastic pollution. Arch. Environ. Contam. Toxicol. 73, 230-239.

McEachern, K., Alegria, H., Kalagher, A.L., Hansen, C., Morrison, S., Hastings, D., 2019. Microplastics in Tampa Bay, Florida: Abundance and variability in estuarine waters and sediments. Mar. Pollut. Bull. 148, 97-106.

Moore, C.J., Moore, S.L., Weisberg, S.B., Lattin, G.L., Zellers, A.F., 2002. A comparison of neustonic plastic and zooplankton abundance in southern California's coastal waters. Mar. Pollut. Bull. 44, 1035-1038.

Mu, J., Zhang, S., Qu, L., Jin, F., Fang, C., Ma, X., Zhang, W., Wang, J., 2019. Microplastics abundance and characteristics in surface waters from the Northwest Pacific, the Bering Sea, and the Chukchi Sea. Mar. Pollut. Bull. 143, 58-65.

Munno, K., Helm, P.A., Jackson, D.A., Rochman, C., Sims, A., 2018. Impacts of temperature and selected chemical digestion methods on microplastic particles. Environ. Toxicol. Chem. 37, 91-98.

Murphy, F., Ewins, C., Carbonnier, F., Quinn, B., 2016. Wastewater treatment works (WwTW) as a source of microplastics in the aquatic environment. Environ. Sci.Technol. 50(11), 5800-5808.

Napper, I.E., Thompson, R.C., 2016. Release of synthetic microplastic plastic fibres from domestic washing machines: effects of fabric type and washing conditions. Mar. Pollut. Bull. 112(1), 39-45.

Nizzetto, L., Bussi, G., Futter, M.N., Butterfield, D., Whitehead, P.G., 2016. A theoretical assessment of microplastic transport in river catchments and their retention by soils and river sediments. Environ. Sci. Process. Impacts 18(8), 1050-1059.

Nuelle, M.-T., Dekiff, J.H., Remy, D., Fries, E., 2014. A new analytical approach for monitoring microplastics in marine sediments. Environ. Pollut. 184, 161-169.

Ogata, Y., Takada, H., Mizukawa, K., Hirai, H., Iwasa, S., Endo, S., Mato, Y., Saha, M., Okuda, K., Nakashima, A., Murakami, M., Zurcher, N., Booyatumanondo, R., Pauzi, M., Quang, L., Gordon, M., Miguez, C., Suzuki, S., Moore, C., Karapanagioti, H.K., Weerts, S., Mcclurg, T., Burres, E., Smith, W., Van Velkenburg, M., Selby, J., Lang, R.C., Laursen, D., Danner, B., Stewardson, N., Thompson, R.C., 2009. International pellet watch: Global monitoring of persistent organic pollutants (POPs) in coastal waters. 1. Initial phase data on PCBs, DDTs, and HCHs. Mar. Pollut. Bull. 58, 1437-1446.

Oliveira, M., Almeida, M., Miguel, I., 2019. A micro(nano)plastic boomerang tale: A never ending story? Trends Analyt. Chem. 112, 196-200.

Panti, C., Giannetti, M., Baini, M., Rubegni, F., Minutoli, R., Fossi, M.C., 2015. Occurrence, relative abundance and spatial distribution of microplastics and zooplankton NW of Sardinia in the Pelagos Sanctuary Protected Area, Mediterranean Sea. Environ. Chem. 12, 618-626.

Passow, U., 2002. Transparent exopolymer particles (TEP) in aquatic environments. Prog. Oceanogr. 55, 287-333.

Pedrotti, M.L., Petit, S., Elineau, A., Bruzaud, S., Crebassa, J.C., Dumontet, B., Martí, E., Gorsky, G., Cózar, A., 2016. Changes in the floating plastic pollution of the mediterranean sea in relation to the distance to land. PLoS One 11, 1-14.

Picó, Y., Barceló, D., 2019. Analysis and prevention of microplastics pollution in water: Current perspectives and future directions. ACS Omega 4, 6709-6719.

Pilato, L., 2013. Phenolic resins: 100 Years and still going strong. React. Funct. Polym. 73, 270-277.

Pinna, M.S., Bacchetta, G., Cogoni, D., Fenu, G., 2019. Is vegetation an indicator for evaluating the impact of tourism on the conservation status of Mediterranean coastal dunes? Sci. Total Environ. 674, 255-263.

Piñon-Colin, T.D.J., Rodriguez-Jimenez, R., Pastrana-Corral, M.A., Rogel-Hernandez, E., Wakida, F.T., 2018. Microplastics on sandy beaches of the Baja California Peninsula, Mexico. Mar. Pollut. Bull. 131, 63-71.

Piperagkas, O., Papageorgiou, N., Karakassis, I., 2019. Qualitative and quantitative assessment of microplastics in three sandy Mediterranean beaches, including different methodological approaches. Estuar. Coast. Shelf Sci. 219, 169-175.

PlasticsEurope. Plastics - The Facts 2013. An analysis of European plastics production, demand and waste data for 2012. Brussels: Association of Plastic Manufacturers. Last accessed: September 2022. [Online] https://plasticseurope.org/wp-content/uploads/2021/10/2013-Plastics-the-facts.pdf

PlasticsEurope. Plastics - The Facts 2017. An analysis of European plastics production, demand and waste data for 2016. Brussels: Association of Plastic Manufacturers. Last accessed: September 2022. [Online] https://plasticseurope.org/wp-content/uploads/2021/10/2017-Plastics-the-facts.pdf

PlasticsEurope. Plastics - The Facts 2019. An analysis of European plastics production, demand and waste data for 2018. Brussels: Association of Plastic Manufacturers. Last accessed: September 2022. [Online] https://plasticseurope.org/wp-content/uploads/2021/10/2019-Plastics-the-facts.pdf

Prata, J.C., da Costa, J.P., Duarte, A.C., Rocha-Santos, T., 2019. Methods for sampling and detection of microplastics in water and sediment: A critical review. Trends Analyt. Chem. 110, 150-159.

Primpke, S., Wirth, M., Lorenz, C., Gerdts, G., 2018. Reference database design for the automated analysis of microplastic samples based on Fourier transform infrared (FTIR) spectroscopy. Anal. Bioanal. Chem 410, 5131-5141.

Ramírez-Álvarez, N., Rios Mendoza, L.M., Macías-Zamora, J.V., Oregel-Vázquez, L., Alvarez-Aguilar, A., Hernández-Guzmán, F.A., Sánchez-Osorio, J.L., Moore, C.J., Silva-Jiménez, H., Navarro-Olache, L.F., 2020. Microplastics: Sources and distribution in surface waters and sediments of Todos Santos Bay, Mexico. Sci. Total Environ. 703, 134838.

Rana, S., Pichandi, S., Shama P., Fangueiro, R., 2014. Regenerated cellulosic fibers and their implications on sustainability (pp. 239-276). In: Muthu, S.S. (Ed.), Roadmap to sustainable textiles and clothing, textile science and clothing technology. Springer.

Ravit, B., Cooper, K., Moreno, G., Buckley, B., Yang, I., Deshpande, A., Meola, S., Jones, D., Hsieh., A., 2017. Microplastics in urban New Jersey freshwaters: distribution, chemical identification, and biological affects. AIMS Environ. Sci. 4(6), 809-826.

Reisser, J., Slat, B., Noble, K., Du Plessis, K., Epp, M., Proietti, M., De Sonneville, J., Becker, T., Pattiaratchi, C., 2015. The vertical distribution of buoyant plastics at sea: An observational study in the North Atlantic Gyre. Biogeosciences 12, 1249-1256.

Renzi, M., Blašković, A., Fastelli, P., Marcelli, M., Guerranti, C., Cannas, S., Barone, L., Massara, F., 2018. Is the microplastic selective according to the habitat? Records in amphioxus sands, Mäerl bed habitats and *Cymodocea nodosa* habitats. Mar. Pollut. Bull. 130, 179-183.

Rist, S., Carney Almrothb, B., Hartmann, N.B., Karlsson, T.M., 2018. Critical perspective on early communications concerning human health aspects of microplastics. Sci. Total Environ. 626, 720-7262.

Rist, S., Baun, A., Almeda, R., Hartmann, N.B., 2019. Ingestion and effects of micro- and nanoplastics in blue mussel (*Mytilus edulis*) larvae. Mar. Pollut. Bull. 140, 423-430.

Rochman, C., Hoh, E., Kurobe, T., Teh., S.J., 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Sci. Rep. 3, 3263.

Rodrigues, J.P., Duarte, A.C., Santos-Echeandía, J., Rocha-Santos, T., 2019. Significance of interactions between microplastics and POPs in the marine environment: A critical overview. Trends Analyt. Chem. 11, 252-260.

Ross, P.S., Morales-Caselles, C., 2015. Out of sight, but no longer out of mind: microplastics as a global pollutant. Integr. Environ. Assess. Manag. 11(4), 719-728.

Rovira, J., Schuhmacher, M., Nadal, M., Domingo, J.L., 2018. Contamination by coal dust in the neighborhood of the Tarragona Harbor (Catalonia, Spain): A preliminary study. Open Atmos. Sci. J. 12, 14-20.

Sanchez-Vidal, A., Thompson, R.C., Canals, M., de Haan, W.P., 2018. The imprint of microfibres in southern European deep seas. PloS One 13(11), 1-12.

Savoca, S., Capillo, G., Mancuso, M., Bottari, T., Crupi, R., Branca, C., Romano, V., Faggio, C., D'Angelo, G., Spanò, N., 2019. Microplastics occurrence in the Tyrrhenian waters and in the gastrointestinal tract of two congener species of seabreams. Environ. Toxicol. Pharmacol. 67, 35-41.

Shokri, A., 2018. Application of Sono-photo Fenton process for degradation of phenol derivatives in petrochemical wastewater using full factorial design of experiment. Int. J. Ind. Chem. 9, 295-303.

Shim, W.J., Hong, S.H., Eo, S., 2018. Chapter 1 - Marine Microplastics: Abundance, Distribution, and Composition. In: Zeng, E.Y. (Ed.) Microplastic contamination in aquatic environments: An emerging matter of environmental urgency, Elsevier, p 26.

Simon-sánchez, L., Grelaud, M., Garcia-orellana, J., Ziveri, P., 2019. River Deltas as hotspots of microplastic accumulation: The case study of the Ebro River (NW Mediterranean). Sci. Total Environ. 687, 1186-1196.

Stolte, A., Forster, S., Gerdts, G., Schubert, H., 2015. Microplastic abundances in beach sediments along the German Baltic coast. Mar. Pollut. Bull. 99, 216-229.

Strungaru, S.-A., Jijie, R., Nicoara, M., Plavan, G., Faggio, C., 2019. Micro- (nano) plastics in freshwater ecosystems: abundance, toxicological impact and quantification methodology. Trends Analyt. Chem. 110, 116-128.

Suaria, G., Avio, C.G., Mineo, A., Lattin, G.L., Magaldi, M.G., Belmonte, G., Moore, C.J., Regoli, F., Aliani, S., 2016. The Mediterranean plastic soup: Synthetic polymers in Mediterranean surface waters. Sci. Rep. 6, 37551.

Sun, J., Dai, X., Wang, Q., van Loosdrecht, M.C.M., Ni, B.-J., 2019. Microplastics in wastewater treatment plants: Detection, occurrence and removal. Water Res. 152, 21-37.

Sundt, P, Schulze, P.E, Syversen, F., 2014. Sources of microplastic- pollution to the marine environment. Project report. Norwegian Environment Agency, 108. Last accessed: September 2022. [Online] https://www.miljodirektoratet.no/globalassets/publikasjoner/M321/M321.pdf

Teuten, E.L., Saquing, J.M., Knappe, D.R., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Philos. Trans. R. Soc. Lond. B Biol. Sci. 364(1526), 2027-2045.

Thompson, R.C., Olson, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at sea: Where is all the plastic? Science, 304(5672), 838.

Tiwari, M., Rathod, T.D., Ajmal, P.Y., Bhangare, R.C., Sahu, S.K., 2019. Distribution and characterization of microplastics in beach sand from three different Indian coastal environaments. Mar. Pollut. Bull. 140, 262-273.

Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M.B., Mees, J., Janssen, C.R., 2013. Assessment of marine debris on the Belgian Continental. Shelf. Mar. Pollut. Bull. 73, 161-169.

Van der Hal, N., Ariel, A., Angel, D.L., 2017. Exceptionally high abundances of microplastics in the oligotrophic Israeli Mediterranean coastal waters. Mar. Pollut. Bull. 116, 151-155.

Vianello, A., Boldrin, A., Guerriero, P., Moschino, V., Rella, R., Sturaro, A., Da Ros, L. 2013. Microplastic particles in sediments of Lagoon of Venice, Italy: First observations on occurrence, spatial patterns and identification. Estuar. Coast. Shelf Sci. 130, 54-61.

Verschoor, A.J., 2015. Towards a definition of microplastics considerations for the specification of physico-chemical properties RIVM Letter report. National Institute for Public Health and the Environment**.** The Netherlands, p. 38.

Wagner, J., Wang, Z.-M., Ghosal, S., Rochman, C., Gassel, M., Wall, S., 2017. Novel method for the extraction and identification of microplastics in ocean trawl and fish gut matrices. Anal. Methods 9, 1479-1490.

Wang, J., Wang, M., Ru, S., Liu, X., 2019. High levels of microplastic pollution in the sediments and benthic organisms of the South Yellow Sea, China. Sci. Total Environ. 651, 1661-1669.

Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: A review. Environ. Pollut. 178, 483-492.

Wu, P., Huang, J., Zheng, Y., Yang, Y., Zhang, Y., He, F., Chen, H., Quan, G., Yan, J., Li, T., Gao, B., 2019. Environmental occurrences, fate, and impacts of microplastics. Ecotoxicol. Environ. Saf. 184, 109612.

Ye, T., Kang, M., Huang, Q., Fang, C., Chen, Y., Shen, H., Dong, S., 2014. Exposure to DEHP and MEHP from hatching to adulthood causes reproductive dysfunction and endocrine disruption in marine medaka (*Oryzias melastigma*). Aquat. Toxicol. 146, 115-126.

Yong, C.Q.Y., Valiyaveetill, S., Tang, B.L., 2020. Toxicity of microplastics and nanoplastics in mammalian systems. Int. J. Environ. Res. Public Health 17(5), 1509.

Yu, X., Peng, J., Wang, J., Wang, K., Bao, S., 2016. Occurrence of microplastics in the beach sand of the Chinese inner sea: The Bohai Sea. Environ Pollut. 214, 722-730.

Zhang, D., Liu, X., Huang, W., Li, J., Wang, C., Zhang, D., Zhang, C., 2020. Microplastic pollution in deep-sea sediments and organisms of the Western Pacific Ocean. Environ. Pollut. 259, 113948.

Zhou, Q., Zhang, H., Fu, C., Zhou, Y., Dai, Z., Li, Y., Tu, C., Luo, Y., 2018. The distribution and morphology of microplastics in coastal soils adjacent to the Bohai Sea and the Yellow Sea. Geoderma 322, 201-208.

2.-Sub-chapter 1.2. Microplastics in subtidal marine sediments

Due to the influence of industrial, commercial and tourist activities on the Tarragona coast, the concentration of microplastics (MPs) in submerged marine sediments was determined for MPs ranging from sizes from 50 µm to 5 mm. In addition, two methodologies, µFTIR and fluorescence, were used for quantification. This study will supply the basic data for risk assessment by MPs in some zones of Tarragona coast and hot spots establishment of MPs pollution in Catalonia coast.

1.2.1 Materials and Methods

1.2.1.1 Sampling

Six (n=6) samples were collected in July 2020 from an area distributed along Tarragona coast and located to southern-central area of Catalonia (NE, Spain). The points selected presented different characteristics: Miracle beach (**Tg N** and **Tg S)** for urban and touristic activities, and submarine emissaries; Pineda beach (**Pin N** and **Pin S)** for river receiving WWTP effluents discharges, submarine emissaries, harbor and industrial activities, and urban runoff, Cambrils (**Cam**) and Hospitalet del Infant **(Hos)** for mostly touristic activity and urban runoff (figure 1).

Figure 1. Sampling points along Tarragona coast (yellow dots), Middle-Southern Zone

Marine sediments samples were collected according Chapter 1.1 description. Briefly, Samples were collected 400 m from the coast and a depth between 5–16 m (Table1). Two scuba divers descended to the bottom and collected five subsamples randomly in an area of 25 m^2 of top 5 cm sediment layer with a metal spoon and glass bottle and refrigerated at −20 °C prior to analysis. Average weight collected was between 300 and 1300 g. Sandy patches next to Posidonia oceanic

seagrass meadows (abundant near Hospitalet) and *Cymodocea* seagrass meadows abundant in Middle Tarragona coast characterized the bottom.

1.2.1.2 Physical Chemical Parameters

All samples were dried at 40ºC for 48h and weighed. For granulometry analysis, 30 g of sample was sieved through a column of sieves on a metal shaker and the weight of each sieve rest was registered. The sample remaining was sieved and fractions of material greater and less than 0.5 mm were stored in separate and labeled glass bottles.

Table 1. Submarine sediment sampling points along Tarragona coast

Marine sediments			
	Coordinates	**Depth (m)**	**Place**
Tg N	N41° 06´ 38.78´´; E1° 16´ 18.40´´	16	Miracle Beach North (Punta Grossa)
Tg S	N41° 06´ 13.55´´; E1° 15´ 27.17´´	14	Miracle Beach South (Final beach section)
Pin N	N41° 04´ 15.99´´; E1° 11´ 21´´	11	Pineda Beach North
Pin S	N41° 04´ 06´´; E1° 11´ 17.97´´	9	Pineda Beach South
Cam	N41° 02´ 56.32´´; E1° 05´ 6.39´´	4.9	Cambrils Beach (Cavet Beach)
Hos	N40° 58´.41.23´´; E0° 55´ 55.39´´	9.4	Hospitalet Beach

Sediment granulometry was determined based on quantifying the distribution of particles of a substrate according to their size (USDA Scale), the different sizes of sediment were separated by mechanical sieving using 4 mm, 2 mm, 1 mm, 0.5 mm, 0.25 mm, 0.1 mm sieves. Fine fractions (silt and clay) were separated by the Robinson pipette method, based on sedimentation.

Furthermore, chemical analysis were carried out as Total Carbon (Total C), Nitrogen (N) and Sulfur (S) (Tiessen & Moir, 2000a,b); Total Organic Carbon (TOC) (Tiessen & Moir, 2000a; U.S. EPA, 2002); Carbonates (as equivalent calcium carbonate, $CaCO_3$) was calculated by difference between Total C and TOC to obtain inorganic carbon; Organic Matter concentration (O.M) (Nelson & Sommers, 1996); Total Phosphorus (TP) through acid extraction and analysis with ICP-OES. In addition, metals and metalloids were analysed using US EPA 3051 method with concentrated nitric acid and hydrogen peroxide for extractions and ICP-OES for chemical analysis.

1.2.1.3 Microplastics extraction, identification and quantification

For these samples, MPs extractions and identification procedures were applied for MPs from 50 µm to 5 mm. Visual sorting was applied for ≥0.5 mm and density separation, for <0.5 mm (see M&M sand and sediments methodology). Controls or blanks were applied for sieving, visual sorting and density separation processes both for the fluorescence (for fibres and particles deposition and resuspension for comparing supernatant color) and for µFTIR methodologies. Blanks results obtained in each method were subtracted from the counting according morphology.

1.2.1.4 Microplastics quantification

The procedure for MPs quantification by Red Nile staining plus fluorescence methodology was applied as described in Materials and Methods section. For MPs composition, particles isolated by visual sorting were analyzed by ATR-IR while particles <0.5 mm and all fibres were analized by µ-FTIR maps how it is described in Materials and Methods section.

1.2.2. Results and Discussion

1.2.2.1 Physical and chemical parameters

According granulometry analysis (Table 1), marine sediments from El Miracle and la Pineda beach (**Tg N, Tg S, Pin N and Pin S**) presented between 90 and 94 % of the grain size ranging between 0.25 and 0.1 mm and in consequence classified as fine sand sediments. On the other hand, in the southern sampling points, **Cam** showed a 50% of medium sand-grain size (0.25-0.5 mm) and **Hos** showed coarser sediment with 38% between 0.5-1 mm grain size and 6% of gravel (>2 mm) formed mainly by the accumulation of remains of carbonated and siliceous shells. It seems that towards southern zones the sediments tend to be coarser.

Table 1. Granulometry sediments samples according USDA scale.

Sample	USDA Scale						
	Graves >2 mm (%)	Very coarse sand 1-2 mm (%)	Coarse sand 0.5-1 mm (%)	Medium Sand 0.25-0.5 mm (%)	Fine Sand 0.1-0.25 mm (%)	Very fine sand 0.05-0.1 mm (%)	Silt and Clay < 0.05 mm (%)
Tg N	0.0	0.31	0.24	2.85	92.96	3.54	0.10
Tg S	0.1	0.17	0.37	8.85	89.07	1.34	0.10
Pin N	0.03	0.10	0.17	4.84	93.89	0.87	0.10
Pin S	0.6	0.23	0.33	5.34	91.09	2.34	0.07
Cam	0.07	0.57	5.26	50.07	43.80	0.20	0.03
Hos	5.64	24.28	38.23	12.44	16.04	3.10	0.27

Chemical parameters analyzed are shown in Table 2. The presence of carbonates in sediments influence pH values (basic) and can promote the precipitation of nutrients as phosphorus and heavy metals. Sediments presented high proportions of calcium carbonate $CaCO_3$ (calcareous sediments), low concentrations of nutrients (N, P) and sulfur (S), without accumulations of O.M humified or linked to the mineral matrix.

Table 2. Chemical parameters of marine sediment from Tarragona coast

Sample	Total C (%)	TOC (%)	O.M 550ºC (%)	Total N (%)	Total S (%)	Inorganic C (%)	$CaCO_3$ (%)	Total P (%)	C/N	C/P
Tg N	4.52	0.15	1.89	0.02	0.159	4.37	36.4	0.023	8	6
Tg S	4.11	0.4	1.51	0.005	0.048	3.71	30.9	0.006	80	71
Pin N	3.46	0.15	1.45	0.01	0.082	3.32	27.6	0.051	15	3
Pin S	3.84	0.14	1.73	0.005	0.089	3.7	30.8	0.027	28	5
Cam	1.13	0.15	2.35	0.01	0.024	0.98	8.2	0.037	15	4
Hos	4.1	0.24	1.35	0.005	0.63	3.86	32.1	0.048	48	5

The values of total metals and metalloids concentration in mg/kg and benchmarks are showed in Table 3. Aluminum and iron registered the highest values. Both are part of the structure of silicates, iron oxides and hydroxides that are is very abundant in soils and sediment. These metals have no set benchmarks stablished by U.S. EPA because they are not considered pollutants.

Arsenic exceeded the reference values set by U.S. EPA at 7.24 mg/kg in two samples out six (**Tg N** and **Tg S)**. It is important to highlight that arsenic is a metalloid that naturally (geological background levels) is present at relatively high concentrations in soils and sediments of Catalan coast and river basin (GENCAT, 2009; Roig et al., 2013). Regarding mercury, there are not benchmarks established by U.S.EPA, in replacement, benchmark is adapted from Leipe et al. (2013). They observed pre-industrial levels of the Holocene in Baltic Sea sediments values between 0.02 and 0.05 mg/kg. According to this, only **Pin N** sampling point slightly exceeded this value, which is not expected to pose a risk to ecosystems because mercury analyzed refers to the total content and that the fraction of bioavailable mercury is most likely much lower (de Castro-Català et al., 2016).

In relation to the rest of elements as cadmium, copper, chromium, cobalt, nickel, lead and zinc, any of the sediments analyzed exceed the EPA benchmarks, which would indicate that risks by these elements to benthic communities is low. For vanadium, the EPA has not benchmarked, however values reported by El-Moselhy (2006), were up to 40.58 mg/kg, the average value of Mediterranean sediments. Nor sediment samples exceed this value either.

Table 3. Total metals and metalloids (mg/kg dw) levels in sediments. a: U.S. EPA benchmarks for the protection of marine aquatic ecosystems. a: U.S. EPA (2014), b: Reference values for Hg according to Leipe et al. (2013).

	Tg N	Tg S	Pin N	Pin S	Cam	Hos	Values from EPA (a)	Value from Leipe et al., (2013) (b)
As	7.62	8.34	6.77	7.06	6.87	3.57	7.24	-
Cd	0.09	0.08	0.08	0.08	0.04	0.07	0.68	-
Cr	3.35	2.66	2.66	3.74	6.74	1.5	52.3	-
Cu	2.1	1.8	2.28	2.82	3.88	1.81	18.7	-
Co	1.5	1.04	1.48	1.65	2.7	0.76	50*	-
Hg	0.041	0.005	0.067	0.003	0.03	0.005	-	0.05
Ni	7.2	5.92	6.57	7.18	1.82	4.73	15.9	
Pb	3.52	2.88	3.26	3.47	6.74	4.02	30.2	
V	7.05	5.44	6.37	6.96	20.96	4.44	-	
Zn	25.99	15.73	18.96	23.23	40.21	16.72	124	
Al	2068	1600	2358	2611	5816	2440	-	
Fe	4222	3615	4687	5225	9315	2425	-	
* Protection value of freshwater ecosystems recommended by U.S. EPA								

1.2.2.2 Microplastics extraction, quantification and identification results

Marine sediment sampled were sieved and divided by fractions: bigger or equal than 0.5 mm (≥0.5 mm) and smaller than 0.5 mm (<0.5 mm). MPs levels are showed in figure 2. In general, MPs ≥0.5 mm concentration in all sites of Tarragona coast was lower with values ranging from 2 to 13 MPs/kg dry sediment, **Tg N** and **Pin N** were the points with highest values (figure 4). The average value of 6.67±3.72 was lower comparing with subchapter 1.1

According of morphology, the MPs for each sampling point were classify in only two categories: fibres and particles. Particles included fragments and films. This categorization was made for comparing MPs concentration values in both fractions higher and less to 0.5 mm. In present study, predominant morphology was fibres. For MPs ≥0.5 mm, the morphology dominant were fibres (53%) followed by fragments (28%) and films (19%) with sizes between 2 and 5 mm (83 %) (figure 3). Sizes between 1-0.5 mm not were found.

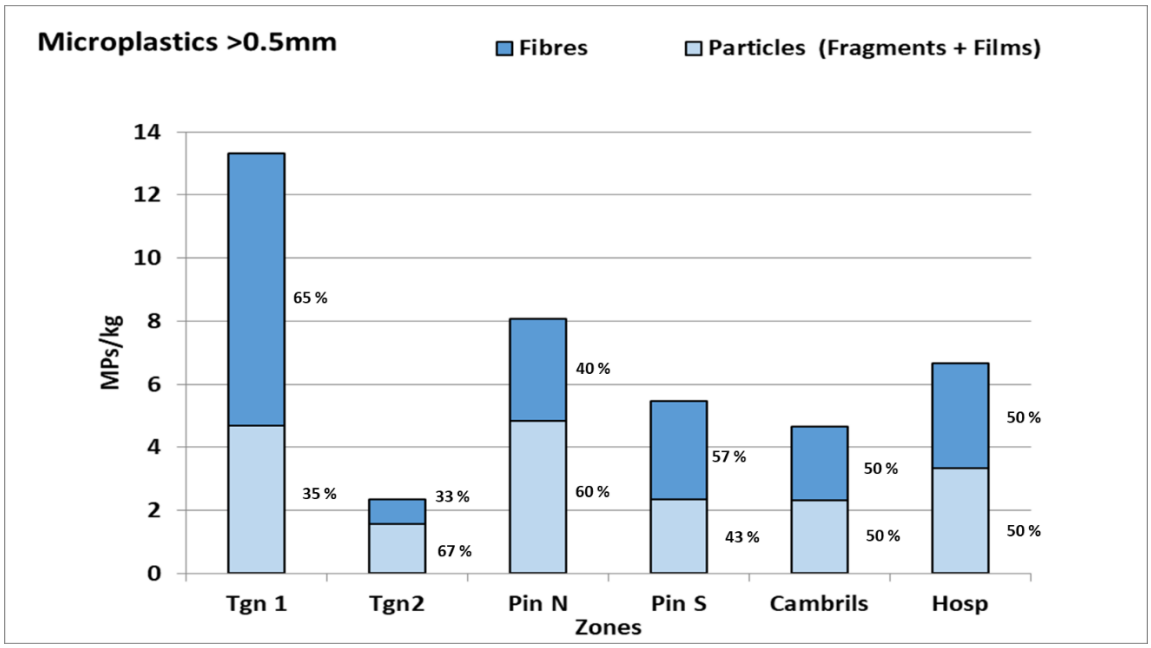

Figure 2. Microplastics concentration in marine sediments from Tarragona central southern zone for sizes ≥0.5 mm.

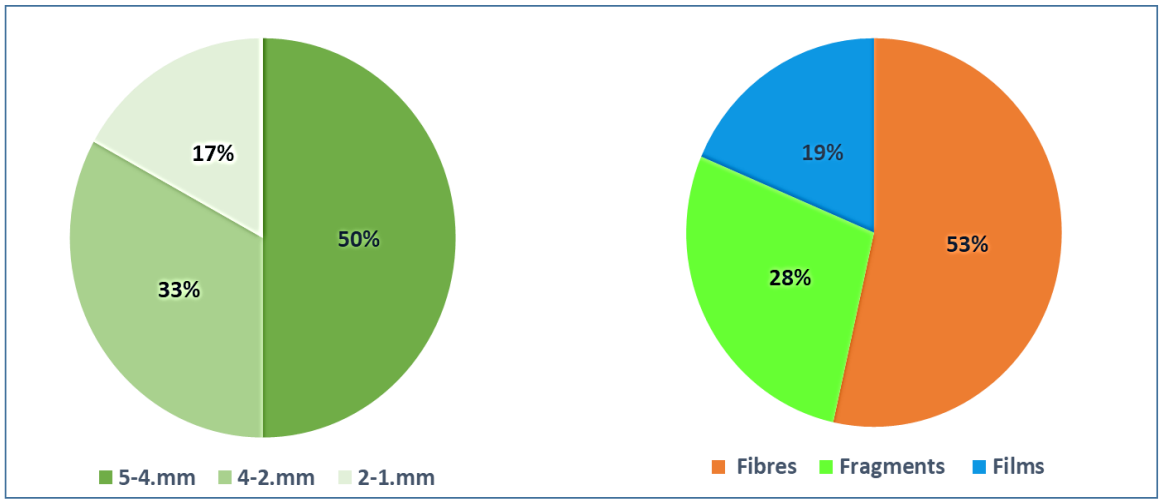

Figure 3. Microplastics morphology and sizes in marine sediment from Tarragona central southern zone ≥0.5 mm.

Quantification by Infrared Methods

For <0.5 mm fraction, MPs levels were higher than ≥0.5 mm MPs levels. MPs below 0.5 mm present concentrations ranging from 316 to 2106 MPs/kg with a size from 60 to 800 µm (figure 4). It is important to highlight that the size particles were reported from 50 µm because the spectra match values were higher or equal 60% at this size. Below this size spectra and particle image appeared confuse or not enough clear to maps interpretation in Omnic Picta Software.

The highest value was found in **Cam** (2106 MPs/kg) followed by **Pin N** (1595 MPs/kg). High proportion of fibres were found in **Cam** (70 %), by contrast particles dominated in **Pin N** point

with 60 % while fibres obtained a 40 %. The lowest <0.5 mm MPs values was found in **Tg S** (318 MPs/kg) with 100 % fibres.

The fibers were 202 ± 190 µm size and particles 114 ± 98.3 µm with a size range from 50 to 800 and 50 to 500, respectively. The most frequently found size was 100 µm for fibres and 60 µm for particles. For fibres, the 85 % was comprised between 50 to 350 µm and regarding particles 84 % of them ranged between 50 and 150 µm (figure 5).

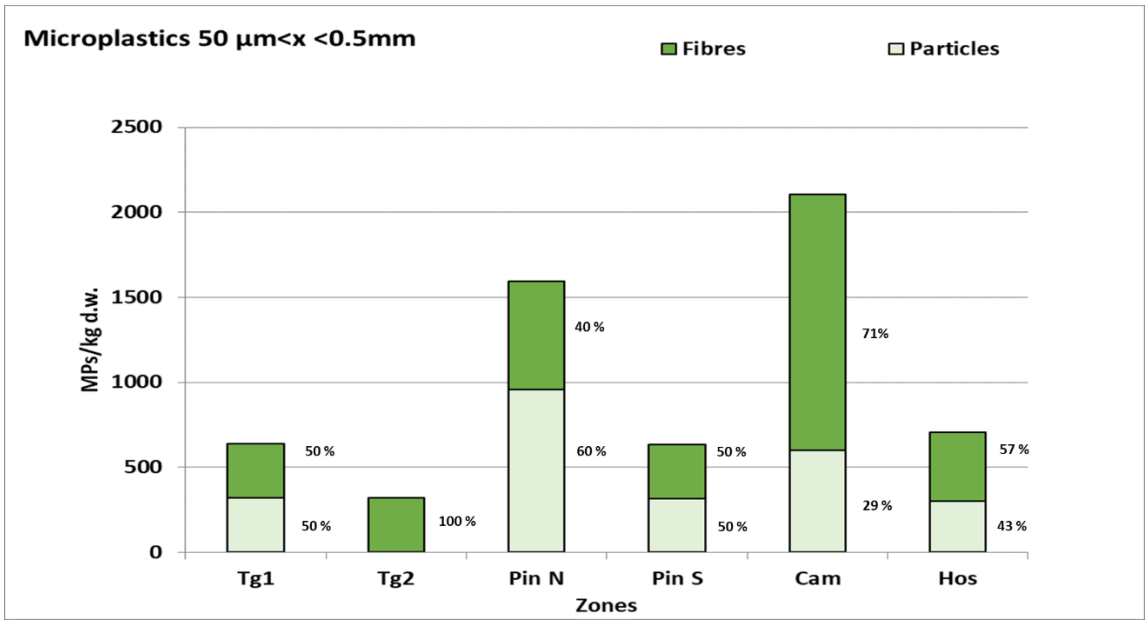

Figure 4. Microplastics concentration in marine sediment from Tarragona central southern zone for sizes 50 µm<x<0.5 mm with µFTIR methodology.

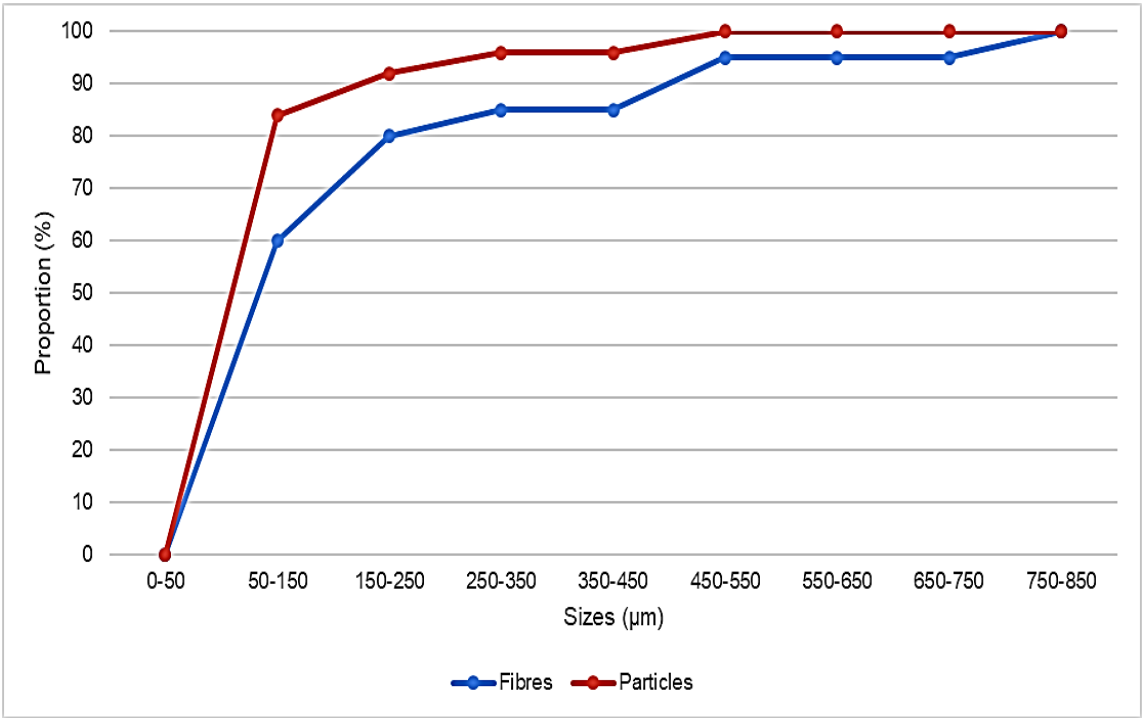

Figure 5. Proportion of MPs according size range in Tarragona central southern zone by µFTIR methodology

In case **Cam**, this zone is influenced by touristic activities, WWTP discharges effluents influence from the north (Cap of Salou), direct WWTP discharges, urban runoff, directly little stream discharges (ACA, 2022; GENCAT 2022). These anthropogenic pressures combined with shallow sampling depth (-5 m), bottom type, seagrass presence (*P. oceanica*) could be influencing the high values observed in **Cam**. Description of antropogenic sources in every sampled site is detailed in supplementary information.

These findings suggest that Tarragona sediments have the potential to accumulate MPs as Zalasiewicz et al., (2016) findings, but without a homogeneous distribution. The MPs presence in these shallow bottoms probably depend mostly of point discharge distance and type of source. However other parameters affect also MPs accumulation in sediment such as sedimentation dynamics (composition, size and aggregation), surface currents direction, wind velocity, depth, bottom type and coastline morphology (accumulating in areas with limited removal due to natural coast morphology, breakwaters or harbours) (de Hann et al., 2021; Liubartseva et al., 2018; Martin-Lara et al., 2021; Zambianchi et al., 2017).

Liubartseva et al., (2018) with the use of stochastic dispersion models (two-dimensional Lagrangian framework and Monte Carlo techniques) found there are low probabilities plastics accumulations occur at long term on the surface by contrast the accumulation of plastic is located largely in the coastline and marine sediments, being coastline an area of greatest accumulation. Moreover, MPs in the sediment samples could dragged from intertidal zone from mesoplastics

fragmentation or coming from seawater column through mesoplastics fragmentation. Mesoplastics washed ashore on beaches degrade into MPs and by waves action could spread in coastal waters, which with near-shore obstacles could get trapping in sediments by their high density and low-energy environment (Isobe et al., 2014). On the other hand, mesoplastics sedimented from seawater column could be fractured in seabed by rolling and boucing action and particles derived remain in sediments or resuspended (specially from 1-4 mm size) (Harris et al., 2020). Mesoplastics fragmentation could occur also in surface and denser fragment go to the bottom by vertical setting**.** In either case, the mesoplastics fragmentation and suspension of secondary MPs in seawater combined with grade of ageing, biofouling, and aggregation modify their density and shape causing MPs sink and accumulation in the sediment. (Chae et al., 2015; Cole et al., 2011; Cózar et al., 2014; Long et al., 2015). Thus, MPs down to a certain size (<200 μm), have low residence time in the surface ocean because ageing processes with organic matter aggregation accompanied by biofouling lead to further draw-down from surface and accumulate into the deep layers and bottom (Alomar et al., 2016; Enders et al., 2015)

In case fibres, with high buoyancy due high surface to volume ratio, fibres aggregation with each other and with suspended sediments, combined with its density contributed to their high concentration observed in mostly Tarragona area (Li et al., 2019) (figure 6).

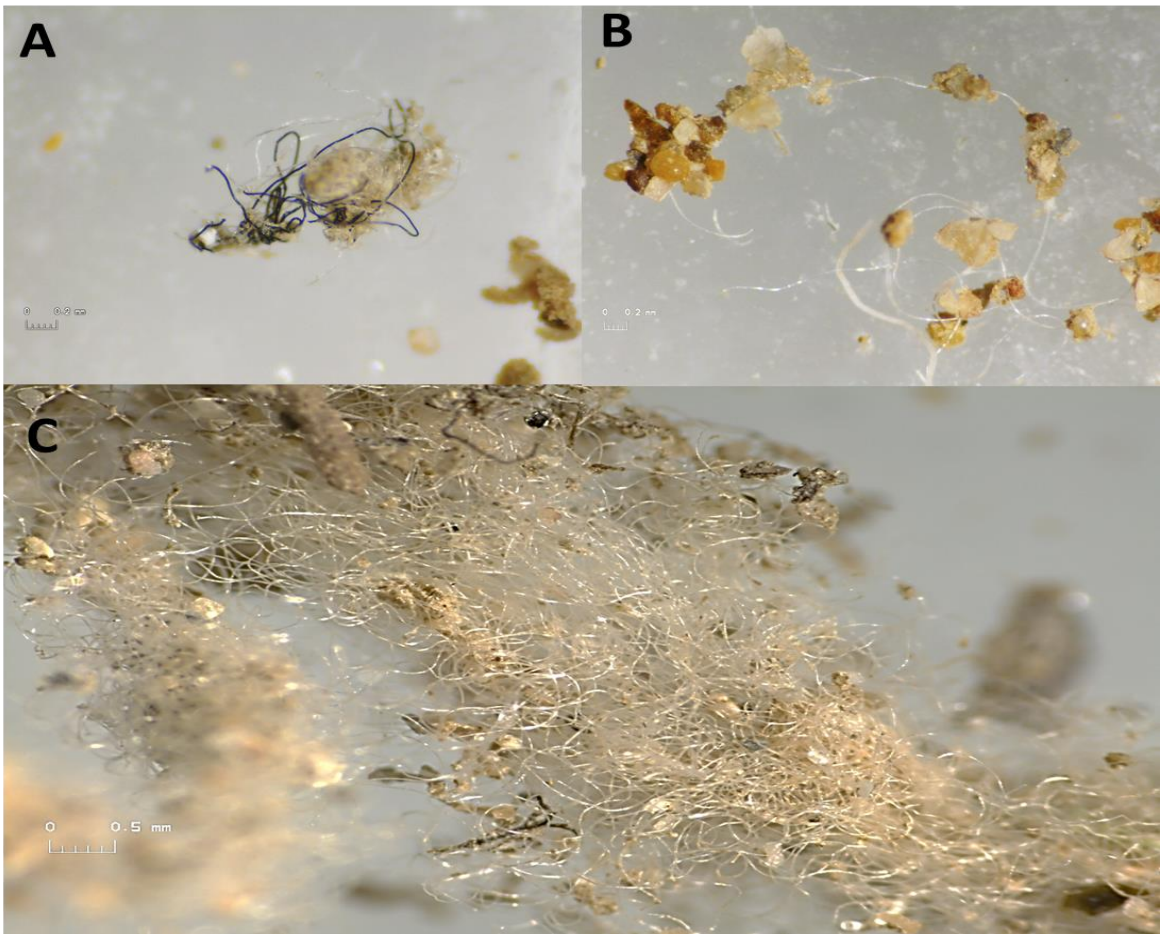

Figure 6. Fibres associated each other and with organic and inorganic materials in marine sediments of Tarragona coast. Stereoscopy microscope images: A and B, 40 X magnification. C, 20 X magnification.

The average value found here of 999±692 MPs/kg (61 % fibres and 29% particles) was a little bit higher compared with Alomar et al., (2016), studies of 900±100 MPs/kg in Spain Mediterranean coast (Balearic Islands, wester Mediterranean. The values found by Filgueiras et al., (2019), in Spanish Mediterranean continental shelf coast, were also lesser than values of this study, thus the values ranged from 45.9±23.9 to 280.3±164.9 MPs/kg d.w. at 43 to 154 water depth and distances between 1.87 to 11.03 km away from shoreline. In Barcelona, this study found 132.7 ± 67.8 MPs/kg. Dahl et al., (2021), found values as high as 3819 MPs/kg d.w. at 4.8 m water depth and from 0-1 cm sediment surface in Almeria (SE Spain).

In addition, compared with other zones, in Mediterranean Sea, the values found in this study remain still higher, thus, the average values found by Abidli et al, (2018) up to 461.25±29.74 MPs/kg in Lagoon Bizerte (Tunisian coast) with fibres predominance. This location is reportedly heavily polluted by agricultural, aquaculture and industrial wastewaters. Cannas et al., (2017), reported concentrations at Tyrrhenian Sea with a maximum of 1069 MPs/kg d.w. (fibres > 80%).

By in contrast, Vianello et al. (2013), reported similar values than found in present study in subtidal sediments of a coastal lagoon in Venice, located near an industrial zone from 672 to 2175 items/kg dry sediment. However, compared with values from Easter Mediterranean Sea, our values were lower than thus found by Kazour et al. (2019) with an average concentration in Lebanese coast of 2433 ± 2000 MPs/kg d.w. using also $ZnCl_2$ as a solution for density separation processes.

In case sediments from other worldwide zones, e.g., Haave et al., (2019), found very high concentrations almost up to 70 times higher than the value of this study. In an urbanized fjord in Norway (discharge sites for wastewater and deposition in deep regions) with average concentrations ranging from 190 to 77,000 MPs/kg d.w. with sizes 95 % between 11 to 100 µm.

Quantification by Fluorescence Methods

MPs concentration in marine sediments measured by fluorescence method is showed in figure 7. It is important to highlight that the size particles were reported from 50 µm for comparison with µFTIR methodology, and better fluorescence intensity appreciation. Only were considered the particles and fibres fluorescence with high intensity ✓✓✓ (3) in wavelength colour filters applied due to high organic matter content and low organic matter removal efficiency.

Excepted by Miracle zone **(Tg S)**, the concentration values observed in **Tg N**, **Pin N**, **Pin S**, **Cam** and **Hos** were lesser than obtained by µ-FTIR maps ranging from 304 to 1322 MPs/kg. Similar to µ-FTIR methodology, by fluorescence the highest value was found in **Cam** site. In case of **Pin N,** the concentration was 5 times lower, it seems that fluorescence methods promote the fibres identification due to the large proportion of fibres found. However lower values and with the exception of **Pin N**, the values showed the same trend as µ-FTIR methodology.

The fibres were predominant in all zones ranging from 60 to 100%. The fibers were 508 ± 466 µm size and particles 92 ± 38 µm with a size range from 60 to 2000 µm and 60 to 200 µm respectively. The frequently size for fibres was 200 µm, while for particles was 80 µm lightly higher than sizes found by µFTIR methodology. For fibres, the 85 % was comprised between 50 to 950 µm and for particles, 91 % was between 50 and 150 µm similar than µFTIR methodology results (figure 8).

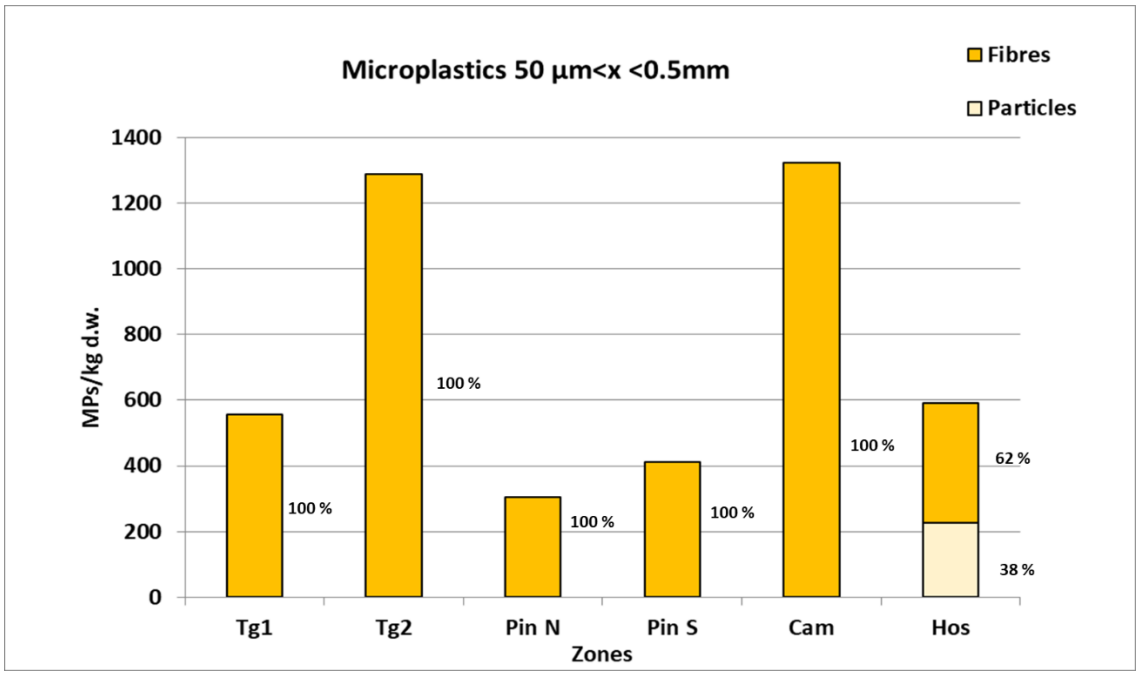

Figure 7. Microplastics concentration in marine sediment from Tarragona central southern zone for sizes 50 µm<x<0.5 mm with fluorescent methodology

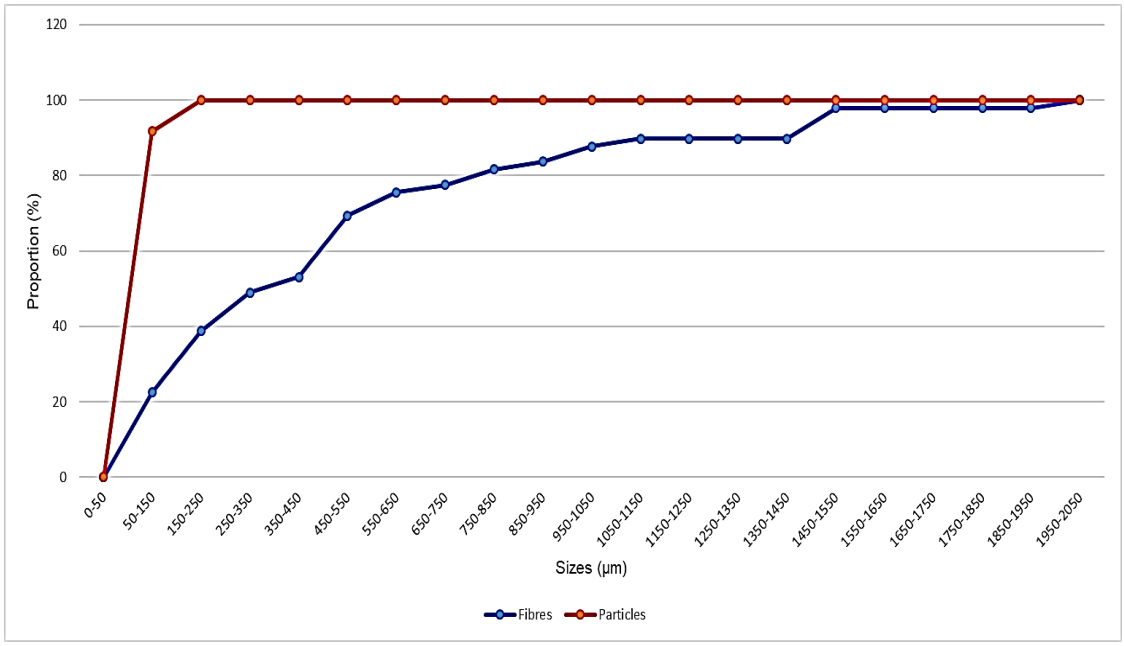

Figure 8. Proportion of MPs according size range in Tarragona central southern zone by Fluorescence methodology

Instead, fluorescence microscopy is a simple, fast and economical technique for MPs identification compared with spectroscopic techniques. However, the polymers identification majority depend on the interpretation of fluorescence type and intensities (qualitative scale) perceived by the technician as well as optical microscope used, (because there is not consensus

on a quantitative fluorescence intensity of each polymer) obtaining in some cases false positive or negative (Sancataldo et al., 2020).

Other factors that possibly contributed to the values differences were: different sizes and shape of filters for MPs identification and quantification affecting the particles distribution on filters as well as controls type applied. In consequence, for reliable results is recommended checking fluorescent microscopy with spectroscopy for MPs analysis (figure 9). It is important to highlight, both techniques are not efficient for identification of weathered MPs. For identification of weathered and high degraded MPs is needed the construction of autochthonous fluorescence library supported by spectra recognition of environmental MPs weathered (Sancataldo et al., 2020; Silva et al., 2018).

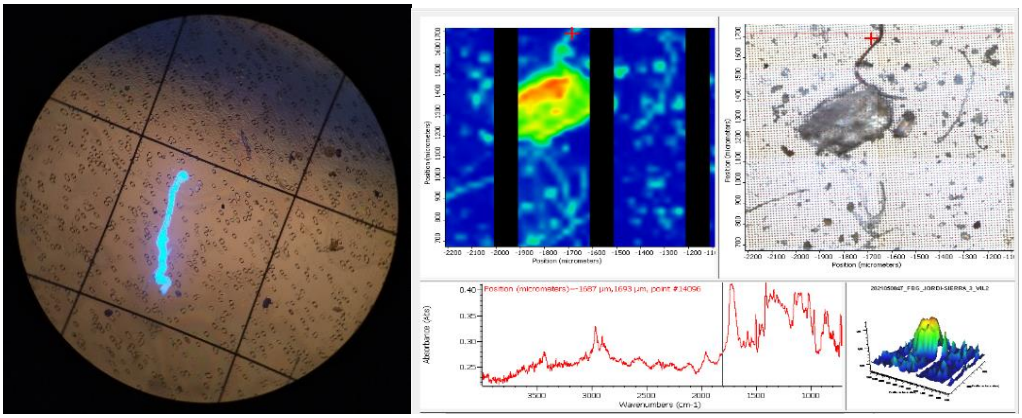

Figure 9. Fluorescence image of a polyester fibre, µ-FTIR map and IR spectrum.

1.2.2.3 Microplastics composition

Regarding MPs composition, the polymers composition for both sediment fractions ≥0.5 mm and <0.5 mm were grouped and showed in figure 10. Were identified eight (8) plastic polymers: polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS), polyvinyl chloride (PVC), polyamide (PA), poly-propylene-ethylene copolymer (PP-PE copolymer), acrylonitrile butadiene styrene (ABS) (figure 9). The sum of proportion of less dense (compared with seawater,1.02-1.03 g/cm^3), polymers as PE (0.89-0.98 g/cm^3), PP (0.85-0.92g/cm^3), PP-PE copolymer (0.90 g/cm^3), was higher than denser ones in **Tg N**, **Tg S** and **Pin N** with a total proportion of 50-53% while in **Pin S** was equal with 40% (Crow polymer database, 2022; Enders et al., 2015). By in contrast, the sum of denser polymers proportion as PET (1.38-1.41 g/cm^3), PS (1.04-1.06 g/cm^3, PVC (1.38-1.41 g/cm^3), PA (1.12-1.15 g/cm^3) and ABS (1.03-1.14 g/cm^3), was high in **Cam** and **Hos** zones with proportion of 50-57% (Crow polymer database, 2022; Enders et al., 2015).

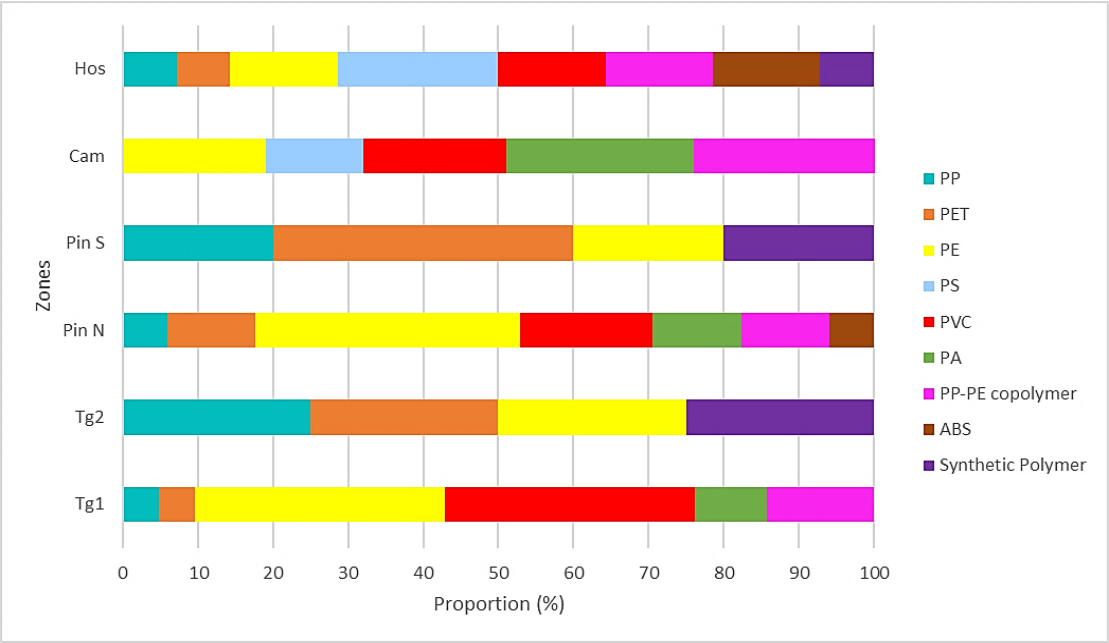

Figure 10. Microplastics composition in marine sediments from Tarragona centre-southern zone analysed with µ-FTIR.

The Pineda beach in front of sediment zone collection specifically in supralittoral zone (surface sand zone) have been found a big quantity of PE virgin pellets about 5 mm and density of 9 pellets/m^2 (Good Karma Projects, 2022) probably comes from chemical industrial-urban run-off and port activities. These findings combined with MPs inputs from other anthropogenic sources, influenced the morphology, quantity and composition of MPs in this zone.

It was observed in two sampling points as **Cam, Hos** high percentage of denser MPs as PET (7-17%), PS (13-21%), ABS (14%), PA (25%) and PVC (14-19%). However, **Pin N** showed high value of less dense with 35 % of PE while in **Tg N** although with high value of less dense polymers due to PE value proportion (33%) also showed the highest PVC proportion with 33%. In **Tg S** less dense polymer dominated with values for both PE and PP of 50% (25 % each one) and finally for **Pin S** was found high PET proportion (40%)

In summary, presence of denser MPs in sediments could be explained by their weight promoting settling faster than less density MPs, but the presence of latter ones it is possible by particles and fibres aggregation with changes in their density. Thus, aggregation combined with biofilm colonization promoted the PE and PP particles settle down faster than particles without interaction with natural particles and biota. The velocity of settlement will depend on surface currents intensity, size and water column-mixing grade.

According, Schmidtmann et al., (2020), fate of MPs in aquatic system can be strongly influenced by iron hydroxides and high ionic strength that promotes particles sedimentation. However, PE particles with sizes about 1 mm had less interaction and aggregation with suspended solids (<10

µm) due overwhelming buoyancy. It is possible that small fragments (<0.5 mm) of PE can be colonized (biofouling) and to aggregated with marine phytoplankton (dinoflagellates and diatoms) and buoyant inorganics particles in seawater surface, sinking faster (Grego et al., 2022; Long et al., 2017).

1.2.2.4 Potential MPs effects on biota in Tarragona marine sediments

Tarragona Coast shows MPs accumulation in marine sediments, making them available to benthic communities even more by sediment resuspension through storms action (Habib et al., 2021). Exposure studies demonstrated that benthic invertebrates feed directly on MPs, mistaking them for prey or selectively feed MPs in place of food, with possible MPs transfer by consumption both fecal pellets or entire organism affecting different trophic levels (Lusher et al., 2015). MPs accumulation (ingestion vs egestion), composition and size could be directly related to their toxic effects (Xiong et al., 2019).

Adverse effects in local benthic organisms could be due to MPs itself (disrupting feeding and digestion, weight loss, reduction growth and impairment on life history, time dependent oxidative stress). MPs not only affect directly to benthic organisms, but also indirectly affect them by changing the structure of their sedimentary habitats, impairing their food resources (Zhang et al., 2020). Moreover, organic persistent pollutants and pathogen microorganisms attached or absorbed in/to MPs could be released once ingested (Besseling et al., 2013; Green et al., 2016; Hartman et al., 2017; Li et al., 2022; Missawi et al.,2020; Redondo-Hasselerham et al., 2018; Silva et al., 2019; Wang et al., 2021; Wright et al., 2013). Although, Koelmans et al., (2016) concluded the role of MPs as vectors for to organisms is considered minor in comparison to that of natural exposure pathways. Finally, Plastics additives are found in marine biota and have been shown to cause endocrine disorders (Koelmans et al., 2014). They can affect reproduction, impair development and to induce genetic aberrations in environmentally relevant exposures (Crain et al., 2007; Galloway, 2015; Oehlmann et al., 2009; Rani et al., 2015).

There is also evidence NPs derived from MPs fragmentation adversely could affect seagrass growth (by leaf loss rate, oxidative stress, impair seagrass photosynthetic machinery) being MPs and products of fragmentation a novel threat (stressors) to seagrass ecosystems (supplier of ecological services) (Menicagli et al., 2022).

1.2.3 Conclusions

MPs distribution on marine sediment on Tarragona coast seems by patching. Concentration of MPs ≥0.5 mm tends to be low, and for <0.5 mm tends to be high specially in front of Pineda beach, **Pin N** and Cambrils **Cam**, Cavet beach.

There was no relationship between MPs high concentration and sand grain size or physicochemical parameters. Eight (8) different plastic polymers were found, being PE was common polymer in all zones while high density polymers proportion tend to be high specially for PVC, PA and PET.

The values of MPs concentration obtained with fluorescent methodology were smaller than with µFTIR in almost all sites but following the same trend. Fluorescence methodology enhances fibres identification in marine submerged sediment samples.

References

Abidli, S., Antunes, J.C., Ferreira, J.L., Lahbib, Y., Sobral, P., El Menif, T.N., 2018. Microplastics in sediments from the littoral zone of the north Tunisian coast (Mediterranean Sea). Estuar. Coast Shelf Sci. 205, 1-9.

ACA, Agencia Catalana del Agua, 2022. Wastewater treatment plants. Last accessed: August 2022. https://aca.gencat.cat/es/laigua/infraestructures/estacions-depuradores-daigua-residual/

Alomar, C., Estarellas, F., Deudero, S., 2016. icroplastics in the Mediterranean Sea: Deposition in coastal shallow sediments, spatial variation and preferential grain size. Mar. Environ. Res. 115, 1-10.

Besseling, E., Wegner, A., Foekema, E.M., van den Heuvel-Greve, M.J., Koelmans, A.A., 2013. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.). Environ. Sci. Technol. 47(1), 593-600.

Campbell, D., Pethrick, R.A., White, J.R., 2000. Polymer characterization: Physical techniques (2nd Edition). CRC Press, Stanley Thornes (Publishers)LTD. United Kingdom 481 pp.

Cannas, S., Fastelli. P., Guerranti, C., Renzi, M., 2017. Plastic litter in sediments from the coasts of south Tuscany (Tyrrhenian Sea). Mar. Pollut. Bull. 119, 372-375.

Chae, D.-H., Kim, I.-S, Kim, S.-K., Song, Y.K., Shim, W.J., 2015. Abundance and distribution characteristics of microplastics in surface seawaters of the Incheon/Kyeonggi coastal region. Arch. Environ. Contam. Toxicol. 69, 269-278.

Claessens, M., Meester, S.D., Landuyt, L.V., Clerck, K.D., Janssen, C.R., 2011. Occurrence and distribution of microplastics in marine sediments along the Belgian coast. Mar. Pollut. Bull. 62(10), 2199-2204.

Cluzard, M., Kazmiruk, T.N., Kazmiruk, V.D., Bendell, L.I., 2015. Intertidal concentrations of microplastics and their influence on ammonium cycling as related to the shellfish industry. Arch. Environ. Contam. Toxicol. 69, 310-319.

Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: A review. Mar. Pollut. Bull. 62(12), 2588-2597.

Cózar, A., Echevarría, F., González-Gordillo, J.I., Irigoien, X., Úbeda, B., Hernández-León, S., Palma, A.T., Navarro, S., García-de-Lomas, J., Ruiz, A., Fernández-de-Puelles, M.L., Duarte, C.M., 2014. Plastic debris in the open ocean. PNAS 111(28), 10239-10244.

Crain, D.A., Eriksen, M., Iguchi, T., Jobling, S., Laufer, H., Leblanc, G.A., Guillette, L.J., 2007. An ecological assessment of bisphenol-A: Evidence from comparative biology. Reprod. Toxicol. 24(2), 225-239.

Crow polymer database, 2022. Polyacrylic acid and super absorbents. Last accessed: August 2022. https://polymerdatabase.com/Polymer%20Brands/PAAs.html

Dahl, M., Bergman, S., Björk, M., Diaz-Almela, E., Granberg, M., Gullström, M., Leiva-Dueñas, C., Magnusson, K., Marco-Méndez, C., Piñeiro-Juncal, N., Mateo, M.A., 2021. A temporal record of microplastic pollution in Mediterranean seagrass soils. Environ. Pollut. 273, 116451.

de Castro-Catala, N., Kuzmanovic, M., Roig, N., Sierra, J., Ginebreda, A., Barceló, D., Pérez, S., Petrovic, M., Picó, Y., Schuhmacher, M., Muñoz, I., 2016. Ecotoxicity of sediments in rivers: Invertebrate community, toxicity bioassays and the toxic unit approach as complementary assessment tools. Sci. Total Environ. 540, 297-306.

de Haan, W.P., Uviedo, O., Ballesteros, M., Canales, I., Curto, X., Guart, M., Higueras, S., Molina, A., Sanchez-Vidal, A., The Surfing for Science Group, 2022. Floating microplastic loads in the nearshore revealed through citizen science. Environ. Res. Lett. 17(4), 045018.

El-Moselhy, K.M. 2006. Distribution of vanadium in bottom sediments from the marine coastal area of the Egyptian seas. Egypt. J. Aquat. Res. 32(1), 12-21.

Enders, K., Lenz, R., Stedmon, C.A., Nielsen, T.G., 2015. Abundance, size and polymer composition of marine microplastics ≥10 mm in the Atlantic Ocean and their modeled vertical distribution. Mar. Pollut. Bull. 100(1), 70-81.

Erni-Cassola, G., Gibson, M. I., Thompson, R. C., & Christie-Oleza, J. A. (2017). Lost, but found with Nile Red: a novel method for detecting and quantifying small microplastics (1 mm to 20 μm) in environmental samples. Environ. Sci. Technol. 51(23),13641-13648.

EU, European Union, 2008. European Union Risk Assessment Report. Bis(2-ethylhexyl) phthalate (DEHP). Institute for Health and Consumer Protection, European Chemicals Bureau. Luxembourg: Office for Official Publications of the European Communities.588 pp

Filgueiras, A.V., Gago, J., Campillo, J.A., León, V.M., 2019. Microplastic distribution in surface sediments along the Spanish Mediterranean continental shelf. Environ. Sci. Pollut. Res. 26, 21264-21273.

Frias, J.P.G.L., Gago, J., Otero, V., Sobral, P., 2016. Microplastics in coastal sediments from Southern Portuguese shelf waters. Mar. Environ. Res. 114, 24-30.

Galloway, T.S., 2015. Micro- and nano-plastics and human health (pp. 343–366). In: Bergmann, M., Gutow, L., Klages, M. (Eds) Marine Anthropogenic Litter. Springer Cham.

GENCAT, 2009. Nivells genèrics de referència dels elements traça en sòls a Catalunya per a la protecció de la salut humana, Generalitat de Catalunya. https://residus.gencat.cat/es/ambits_dactuacio/sols_contaminats/nivells_generics_de_referencia_ngr Last accessed: August 2022

GENCAT, 2022. https://aca.gencat.cat/es/laigua/el-medi-hidric-a-catalunya/aiguees-de-bany/. Last accesed August 2022.

Good Karma Projects, 2022. https://goodkarmaprojects.org/2022/05/05/good-karma-day-voluntarios/ Last accessed: August 2022

Green, D.S., Boots, B., Sigwart, J., Jiang, S., Rocha, C., 2016. Effects of conventional and biodegradable microplastics on a marine ecosystem engineer (*Arenicola marina*) and sediment nutrient cycling. Environ. Pollut. 208 (Part B), 426-434.

Grego, M., Virsek, M.K., Bajt, O., 2022. Microplastics in seawater and sediments distribution and transport (pp. 31-73). In: Bonanno, G., Orlando-Bonaca, M., Plastic pollution and marine conservation: Approaches to protect biodiversity and marine life. Academic Press.

Haave, M., Lorenz, C., Primpke, S., Gerdts, G., 2019. Different stories told by small and large microplastics in sediment - first report of microplastic concentrations in an urban recipient in Norway. Mar. Pollut. Bull. 141, 501-513.

Habib, R., Thiemann, T., 2021. Microplastic in Commercial Fish in the Mediterranean Sea, the Red Sea and the Arabian Gulf. Part 1: The Mediterranean Sea. J. Water Resour. Prot. 13(8), 563-587.

Harris, P.T.,2020. The fate of microplastic in marine sedimentary environments: A review and synthesis. Mar. Pollut. Bull. 158, 111398 .

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. Environ. Sci. Tech. 46(6), 3060-3075.

Kazour, M., Jemaa, S., Issa, C., Khalaf, G., Amara, R., 2019. Microplastics pollution along the Lebanese coast (Eastern Mediterranean Basin): Occurrence in surface water, sediments and biota samples. Sci. Total Environ. 696, 133933.

Koelmans, A.A., Bakir, A., Burton, G.A., Janssen, C.R., 2016. Microplastic as a vector for

chemicals in the aquatic environment: Critical review and model-supported reinterpretation of empirical studies. Environ. Sci. Technol. 50(7), 3315-3326.

Koelmans, A.A., Besseling, E., Foekema, E.M., 2014. Leaching of plastic additives to marine organisms. Environ. Pollut. 187, 49-54.

Leipe, T., Moros, M., Kotilainen, A., Vallius, H., Kabel, K., Endler, M., Kowalski. N., 2013. Mercury in Baltic Sea sediments - Natural background and anthropogenic impact. Geochemistry, 73(3), 249-259.

Li, Y., Wang, X., Fu, W., Xia, X., Liu, C., Min, J., Zhang, W., Crittenden, J.C., 2019. Interactions between nano/micro plastics and suspended sediment in water: Implications on aggregation and settling. Water Res. 161, 486-495.

Li, Z., Chang, X., Hu, M., Fang, J.K.-H., Skolova, I.M., Huang, W., Xu, E.G., Wang, Y., 2022. Is microplastic an oxidative stressor? Evidence from a meta-analysis on bivalves. J. Hazard. Mater. 423(Part B), 127211.

Long, M., Moriceau, B., Gallinari, M., Lambert, C., Huvet, A., Raffray, J., Soudant, P., 2015. Interactions between microplastics and phytoplankton aggregates: Impact on their respective fates. Mar. Chem. 175, 39-46.

Long, M., Paul-Pont, I., Hègaret, H., Moriceau, B., Lambert, C., Huvet, A., Soudant, P., 2017. Interactions between polystyrene microplastics and marine phytoplankton lead to species-specific hetero-aggregation. Environ. Pollut. 228, 454-463.

Lusher, A.L., Tirelli, V., O'Connor, I., Officer, R., 2015. Microplastics in Arctic polar waters: The first reported values of particles in surface and sub-surface samples. Sci. Rep. 5, 14947.

Martín-Lara, M.A., Godoy, V., Quesada, L., Lozano, E.J., Calero, M., 2021. Environmental status of marine plastic pollution in Spain. Mar. Pollut. Bull. 170, 112677.

Menicagli, V., Ruffini Castiglione, M., Balestri, E., Giorgetti, L., Bottega, S., Sorce, C., Spanò, C., Lardicci, C., 2022. Early evidence of the impacts of microplastic and nanoplastic pollution on the growth and physiology of the seagrass *Cymodocea nodosa*. Science of The Total Environment. 838(3), 156514.

Missawi, O., Bousserrhine, N., Belbekhouche, S., Zitouni, N., Alphonse, V., Boughattas, I., Banni, M., 2020. Abundance and distribution of small microplastics (≤ 3 μm) in sediments and seaworms from the Southern Mediterranean coasts and characterisation of their potential harmful efects. Environ. Pollut. 263(Part A), 114634.

Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon, and organic matter (pp. 961-1010). In: Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T., Sumner, M.E. (Eds), Methods of soil analysis: Part 3 chemical methods, SSSAJ.

Oehlmann, J., Schulte-Oehlmann, U., Kloas, W., Jagnytsch, O., Lutz, I., Kusk, K.O., Wollenberger, L., Santos, E.M., Paull, G.C., Van Look, K.J.W., Tyler, C.R., 2009. A critical analysis of the biological impacts of plasticizers on wildlife. Philos. Trans. R. Soc. Lond. B Biol. Sci. 364(1526), 2047-62.

Rani, M., Shim, W.J., Han, G.M., Jang, M., Al-Odaini, N.A., Song, Y.K., Hong, S.H., 2015. Qualitative analysis of additives in plastic marine debris and its new products. Arch. Environ. Contam. Toxicol. 69(3), 352-366.

Redondo-Hasselerharm, P.E., Falahudin, D., Peeters, E.T.H.M., Koelmans, A.A., 2018. Microplastic effect thresholds for freshwater benthic macroinvertebrates. Environ. Sci. Technol. 52(4), 2278-2286.

Roig, N., Sierra, J., Ortiz, J.D., Merseburger, G., Schuhmacher, M., Domingo, J.L., Nadal, M., 2013. Integrated study of metal behavior in Mediterranean stream ecosystems: A case study. J. Hazard. Mater. 263(Part 1), 122-130.

Sancataldo, G., Avellone, G., Vetri, V., 2020. Nile Red lifetime reveals microplastic identity. Environ. Sci. Process Impacts, 22(11), 2266-2275.

Schmidtmann, J., Papastavrou, G., Helfricht, N., Peiffer, S., 2020. Heteroaggregation of micro-polystyrene in the presence of amorphous iron hydroxide (ferrihydrite), EGU General

Assembly 2020. Last accessed: August 2022. https://meetingorganizer.copernicus.org/EGU2020/EGU2020-14509.html

Silva, A.B., Bastos, A.S., Justino, C.I.L., da Costa, J.P., Duarte, A.C., Rocha-Santos, T.A.P., 2018. Microplastics in the environment: Challenges in analytical chemistry - A review. Anal. Chim. Acta 1017, 1-19.

Silva, A.B., Bastos, A.S., Justino, C.I.L., da Costa, J.P., Duarte, A.C., Rocha-Santos, T.A.P., 2018. Microplastics in the environment: Challenges in analytical chemistry - A review. Anal. Chim. Acta. 1017, 1-19.

Silva, C.J.M., Silva, A.L.P., Gravato, C., Pestana, J.L.T., 2019. Ingestion of small-sized and irregularly shaped polyethylene microplastics affect *Chironomus riparius* life-history traits. Sci. Total Environ. 672, 862-868.

Sturm, M. T., Horn, H., Schuhen, K., 2021. The potential of fluorescent dyes—Comparative study of Nile red and three derivatives for the detection of microplastics. Analytical and Bioanalytical Chemistry, 413(4), 1059-1071.

Tagg, A.S., Sapp, M., Harrison, J.P., Ojeda, J.J., 2015. Identification and quantification of microplastics in wastewater using focal plane array-based reflectance micro-FT-IR imaging. Analytical chemistry, 87(12), 6032-6040.

Tiessen, H., Moir, J.O. (2000a). Total and organic carbon. A: Soil sampling and methods of soil analysis. Carter, MR. Canadian Society of Soil Science.

Tiessen, H., Moir, J.O. (2000b). Total nitrogen. A: Soil sampling and methods of soil analysis. Carter, MR. Canadian Society of Soil Science.

U.S. EPA. 2014. Marine Sediment Screening Benchmarks. U.S. Environmental Protection Agency. A Marine Sediment Screening Benchmarks | US EPA. Last accessed: August 2022

U.S. EPA., United States Environmental Protection Agency, 2002. Methods for the determination of total organic carbon (TOC) in soils and sediments. NCEA-C- 1282 EMASC-001.

Vianello, A., Boldrin, A., Guerriero, P., Moschino, V., Rella, R., Sturaro, A., Da Ros, L. 2013. Microplastic particles in sediments of Lagoon of Venice, Italy: First observations on occurrence, spatial patterns and identification. Estuar. Coast. Shelf Sci. 130, 54-61.

Wang, J., Guo, X., Xue, J., 2021. Biofilm-developed microplastics as vectors of pollutants in aquatic environments. Environ. Sci. Technol. 55(19), 12780-12790.

Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy, B.E., Thompson R.C., 2014. The deep sea is a major sink for microplastic debris. R. Soc. Open Sci. 1(4), 140317.

Xiong, X., Tu, Y., Chen, X., Jiang, X., Shi, H., Wu, C., Elser, J.J., 2019. Ingestion and egestion of polyethylene microplastics by goldfish (*Carassius auratus*): influence of color and morphological features. Heliyon 5(12), e03063.

Zalasiewicz, J., Waters, C.N., Ivar do Sul, J.A., Corcoran, P.L., Barnosky, A.D., Cearreta, A., Edgeworth, M., Gałuszka, A., Jeandel, C., Leinfelder, R., McNeill, J.R., Steffen, W., Summerhayes, C., Wagreich, M., Williams, M., Wolfe, A.P., Yonan, Y., 2016. The geological cycle of plastics and their use as a stratigraphic indicator of the Anthropocene. Anthropocene 13, 4-17.

Zambianchi E., Trani M., Falco P., 2017. Lagrangian transport of marine litter in the Mediterranean Sea. Front. Environ. Sci. 5, 5.

Zhang, B., Wu, D., Yang, X., Teng, J., Liu, Y., Zhang, C., Zhao, J., Yin, X., You, L., Liu, Y., Wang, Q., 2019. Microplastic pollution in the surface sediments collected from Sishili Bay, North Yellow Sea, China. Mar. Pollut. Bull. 141, 9-15.

Zhang, D., Liu, X., Huang, W., Li, J., Wang, C., Zhang, D., Zhang, C., 2020. Microplastic pollution in deep-sea sediments and organisms of the Western Pacific Ocean. Environ. Pollut. 259, 113948.

3.-Sub-chapter 1.3. Microplastics in intertidal sandy beach sediments

Microplastics levels along Catalonia coast (western Mediterranean): Approach for development of a fast and low-cost methodology for coastal hot spots establishment and mitigation measures application.

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Abstract

The accumulation of plastic is largely located in the coastline and marine sediments, with the coastline being the areas with the highest accumulation according to stochastics models. One of area with high accumulation is the coast of Catalonia. Considering beaches as MPs accumulation zones and their environmental risks by interaction with aquatic organism, still there are knowledge gaps regarding MPs coastal distribution in Spain. Thus, beaches studies in north-western Mediterranean zone have been made in spatial scale either local as Granada Coast zone, Mar Menor Lagoon and Ebro Delta, punctual in Barcelona and Denia city beaches or at continental scale along Spanish coast with only six beaches studied. It is needed a study in regional scale including a lot of beaches in one of the urban, industrialized and more touristic zone as Catalonia coast. MPs quantification and identification regularly involve time-consuming techniques taking in account visual identification followed by confirmatory, as spectroscopic techniques. It is needed to apply fast and easy method to MPs monitoring and correctives measures application as fluorescence staining a quantification method that provides a fast, convenient, and cheap way to quantify MPs. The aim of this work is to demonstrate the existence of MPs pollution on Catalonia coast beaches, analysing MPs distribution and the relationships with beaches and environmental features. Through a fast quantitative method, MPs are quantified by fractions using Fluorescence microscopy with Nile red dye staining and visual sorting combined with spectroscopic techniques, as a first attempt to stablish baseline levels for hot spots definition and enhancing environmental legislation as well as for mitigation measures application. The study took place along the beaches of Catalonia, a coastal segment approximately of 580 km long between north of Creus Cape and south of Ebro Delta. Seventy intertidal sand samples (n=70) were collected from 43 beaches, a square area of 40 cm x 40 cm was marked randomly and five random subsamples were taken inside from the top 5 cm using Kubiena boxes with fixed volume of 125 cm^3 to ensure the same sand volume collection in all beaches. Two fractions, equal or larger than 0.5 mm and smaller than 0.5 mm, were stored in separate clean glass jars. To extract the microplastics from sands, two procedures were used. For ≥0.5 mm fraction, visual sorting was used for MPs extraction and for <0.5 mm fraction, density separation was used. Prior to microplastics extraction, a sand grain size analysis was performed. Microplastics were quantified using Fluorescence (Red Nile staining) methodology. After fluorescence analysis, a subset of samples (n=23) was chosen randomly and analysed with µ-FTIR techniques for methodology comparison and composition analysis. Variability of MPs larger than 0.5 mm concentration was high being the overall mean level was 7.2±11.7 MPs/kg of dry sand ranging from <0.55 to 56.8 MPs/kg, the morphology proportion of ≥0.5 mm MPs from entire Catalonia coast was 60% of fragments followed by 15% of films, 14% fibres, 10% pellets and 3% foam. The size predominant was the range 2-1 mmm with 42 %. The zones which presented highest >0.5 mm MPs concentrations were South Barcelona and Tarragona. The beaches with the highest abundance in South Barcelona were Cala de Vallcarca (CAV) with 31 MPs/kg and Platja Far de Sant Cristòfol-Vilanova (VIL 01) with 25 MPs/kg. In case of Tarragona coast, La Pineda beach (PIN 1 and PIN 3) showed the highest values with more than 50 MPs/kg. Concentration analyzed for <0.5 mm, of MPs from 60 to 500 µm ranged from 0 to 2013 MPs/kg of dry sand considered some beaches as La Pineda and Bahia del Fangar N as possible hot spots, the fibres were predominant in the majority of beaches studied. Regarding particles (fragments, films, pellets and foam) composition, for both fractions majority polymers were PE, PP, PET and PS. PVC appeared abundant only in particles MPs<0.5 mm fraction. For fibres, the majority composition was polyester, polyamide, PP and PAN. Direct or through river WWTP discharges, industrial port activities, urban and agriculture runoff combined with fine size grain sand, wind direction opposite to beach orientation (facing to) as well as local currents coming at the same direction to beach orientation are important factor for MPs accumulation. Fluorescence staining methodology provided good approximations regarding MPs quantification as long as organic matter be totally removed and taking in account fibres and particles ≥60 µm. For accurate results, the fluorescence must be quantified depending on the Nile Red properties and behaviour in different solvent, temperature and wavelength excitation. For fast hot spot establishment, it is recommended an exploratory MPs concentration with Nile Red staining, confirmation the results with spectroscopy techniques (FTIR) and continuous monitoring with Nile Red for saving time and cost in mitigation application measures.

Key words: Microplastics, µFTIR, Red Nile, sandy beach.

1.3.1 Introduction

The Mediterranean Sea receives approx. 100,000 tons of plastics per year, 50% of which are from terrestrial contributions from cities near the coast and 30% come from river discharges, these plastic discharges can become Microplastics (MPs) due to fragmentation, increasing MPs loads. Similarly, river discharges receiving high and constant volumes of treated wastewater (although they present a high removal efficiency) combined with macro and mesoplastics fragmentation and inputs of agricultural and urbane runoff increases MPs load in rivers mouth (Bergmann et al., 2015; de Lucia et al., 2014; Liubartseva et al., 2018).

Therefore, the Mediterranean Sea has been proposed as one of the regions most impacted by MPs in the world considered an area of accumulation by its semi-enclosed nature accumulating about 7% of estimated marine load plastic (Cozar et al., 2015). The reported MP concentrations are comparable to those found in the convergence zone of ocean gyres, pointing to this basin as one of the world's greatest plastic accumulation areas (Simón-Sanchez et al., 2022). The distribution of plastic and MPs within 10-15 km of the coastline depend on variability of surface flow, coastal dynamics, and morphology as well as the distance from the coast of the discharge point (Zambianchi et al., 2017, Liubartseva et al., 2018). Liubartseva et al., (2018), determined that in the Mediterranean Sea, the accumulation of plastic is largely located in the coastline and marine sediments, with the coastline being the areas with the highest accumulation according to stochastics models. One of area with high accumulation is the coast of Catalonia (Exposito et al. 2021).

Kaandorp et al., (2020), estimated the floating plastic budget of the Mediterranean Sea, 49-63% of these inputs end up beaching. Beaches are highly dynamic and changing natural environments; sediment size/composition and slope are key geomorphological elements for characterizing the beaches as they appear interrelated with the prevailing wave climate, overall tidal range, sediment supply and state of tides (Cabezas-Rabadán et al., 2021, Harris, 2020). According to sediments dynamics, beaches can be considered as a net sediment sink (i.e., a depositional sedimentary environment), net erosional (retreating coast) or is otherwise in equilibrium in terms of sea level, sediment supply and sediment removal processes, thus, these parameters can affect pollutants distribution especially persistent and low biodegradability as MPs in beaches (Harris, 2020).

Waves broken line zone have potential MPs formation from mesoplastics fragmentation and therefore has interchangeable material between seawater and sand. This exchange may be an indicator of the MPs present in the seawater and the degree of pollution of the area (Rocha-Santos & Duarte, 2017). Several studies have reported moderate to high levels of marine litter on Mediterranean beaches (Constant et al., 2019; Kaberi et al., 2013; Lots et al., 2017; Munari et al., 2017) including on the southwestern zone (Abidli et al., 2017; Grini et al., 2022; Tata et al., 2020) and according Simón-Sanchez et al., 2022, the presence of plastics in beaches cannot be

neglected, considered them as transitional environments between terrestrial and marine systems acting simultaneously as pathways and dynamic storage of MPs pollutants

From terrestrial-seawater dynamics in beaches, MPs could be suspended in seawater and be available to ingestion (mistaken for food by active selection or filter feeding strategies) or interaction with aquatic organisms (Savoca et al., 2019), affecting aquatic organisms directly due to their morphology, size and indirectly by associated toxic compounds (Huerta et al., 2018; Rist et al., 2018). Thus, MPs promote morphological changes and growth reduction in algae (Mao et al., 2018), mortality in zooplankton (Aljaibachi and Callaghan, 2018), energy expenditure in mussels (Détree and Gallarde-Escarate, 2018), and negative effects on swimming performance in fishes (Barboza et al., 2018). Regarding the consumption of MPs, Compa et al., (2018), analyzed samples of planktivorous fishes as *Sardina pilchardus* and *Engraulis encrasicolus* of four points on the Catalan coast (three in Catalonia North and one in Catalonia Central zone), and found polyester (PES) fibres in all intestinal tract analyzed.

Considering beaches as MPs accumulation zones and their environmental risks by interaction with aquatic organism, still there are knowledge gaps regarding MPs coastal distribution in Spain. Thus, beaches studies in north-western Mediterranean zone have been made in spatial scale either local as Granada Coast zone, Mar Menor Lagoon and Ebro Delta, punctual in Barcelona and Denia city beaches or at continental scale along Spanish coast with only six beaches studied (Bayo et al., 2019; CEDEX, 2019; Godoy et al., 2020; Lots et al., 2017; Simón-Sanchez et al., 2019). It is needed a study in regional scale including a lot of beaches in one of the urban, industrialized and more touristic zone as Catalonia coast.

MPs quantification and identification regularly involve time-consuming techniques taking in account visual identification followed by confirmatory, as spectroscopic techniques. It is needed to apply fast and easy method to MPs monitoring and correctives measures application. The fluorescence staining and quantification method provides a fast, convenient, and cheap way to quantify MPs. In this scenario, Nile Red (NR) staining would be useful to spot hidden MPs and help for further spectroscopic analysis. A combination of fluorescence microscopy after NR staining followed by Fourier-transform infrared spectroscopy (FTIR) confirmation would reduce the likelihood of missing MPs in the identification of field samples, as well as the time needed to check every plastic-like particle by spectroscopy (Erni-Cassola et al., 2017; Maes et al., 2017; Prata et al., 2019). Furthermore, NR has the advantages of high adsorption to plastics and good affinity for various polymers it is possible unknown newly synthetized polymers not included in the FTIR spectrum library can be identified (Karami et al., 2017).

However, this method has several limitations e.g., the co-staining of natural organic material as natural lipids, therefore, organic matter should be eliminated (by oxidation) to make this method as an efficient alternative. Some refractory organic matter can be effectively stained with NR but could not be identified with microscopy and spectroscopy (Shim et al., 2016; Stanton et al., 2019).

MPs in beach sediments have been quantified with Nile Red with concentrations ranging from 1.7 to 881.3 MPs/kg in beaches of Baltic Sea, Arabian Sea, Bengal Bay, Granada beaches-Spain, and punctual zones of Spanish Coast (CEDEX, 2019; Godoy et al., 2020; Hengstmann et al., 2018; Tiwari et al.,2019) and it is possible in beaches sediment with relatively low organic matter concentration the NR methodology could offers best results.

The aim of this work is to demonstrate the existence of MPs pollution on Catalonia coast beaches, analysing MPs distribution and the relationships with beaches and environmental features. Through a fast quantitative method, MPs are quantified by fractions using Fluorescence microscopy with Nile red dye staining and visual sorting combined with spectroscopic techniques, as a first attempt to stablish baseline levels for hot spots definition and enhancing environmental legislation as well as for mitigation measures application. Furthermore, this may contribute supporting the studies carried out at a national level in accordance with the Marine Strategy Framework Directive on Marine Strategy (2008/56/EC), for European Regional Seas and the UNEP/MAP Barcelona Convention for the Mediterranean Sea (https://www.unep.org/unepmap/) (González-Fernández and Hanke, 2020).

1.3.2. Materials and Methods

1.3.2.1 Study area

Coastline of Catalonia (NE Spain) is a densely populated coast, with 10,000 inhabitants per kilometre, and highly urbanized in a 59% of the coastal strip (Generalitat de Catalunya, 2019). The study took place along the beaches of Catalonia, a coastal segment approximately of 580 km long between north of Creus Cape and south of Ebro Delta. The types of coasts and their extensions are as follows: cliffs, 208 km; low coast, 52 km; beaches, 280 km; ports and maritime structures, 40 km. More information regarding Catalan coast, meteorology and sand characteristics were reported in Supplementary materials (Annexes).

1.3.2.2 Sampling

According to the guidance document MSFD- Guidance on Monitoring of Marine Litter in European Seas (European Commission, 2013), the NOAA Marine Debris Program (Masura et al., 2015) and modified from Frias et al., (2018), samples were collected randomly in the intertidal zone, as it is the zone of influence of wave dynamics and potentially interchangeable zone between seawater and sand. Beaches are a deposition point for floating plastics (density lower than seawater-most synthetic polymers) deposited by waves and are also capable of accumulating sinking (by biofouling and particle adherence) plastic particles (Hidalgo-Ruz et al., 2012; Woodall et al., 2014).

Seventy intertidal sand samples (n=70) were collected from 43 beaches along the Catalonia (NE Spain) coast (figure 1). Characteristics regarding touristic pressure, anthropogenic pressures, rivers discharges, pluvial and WWTP emissaries, shoreline morphology, slope, facing and orientation of each beach sampled were noted. Geographically samples are divided from north to south in 5 zones: North Catalonia, North Barcelona, South Barcelona, Tarragona and South Catalonia. The samples were collected between September and October 2018.

Transects were stablished according beach length parallel to shoreline from one to four transects. More replicates were collected in beaches near from Tarragona coast due to its proximity to the laboratory where the samples would be processed and the need to transport them as fast as possible to avoid contamination. For the rest of the beaches in Catalonia North, Barcelona North, Barcelona South and Catalonia South one transects by beach was stablished. Samples were chosen taking in to account beaches or part of them near to chemical industrial complex, ports, main rivers flow, agriculture and aquaculture runoff.

For samples collection, a square area of 40 cm x 40 cm was marked randomly and five random subsamples were taken inside from the top 5 cm using Kubiena boxes with fixed volume of 125 cm^3 to ensure the same sand volume collection in all beaches. Samples were stored in covered aluminium boxes to avoid airborne fibres contamination (figure 2). Plastic materials were avoided and cotton clothes were worn.

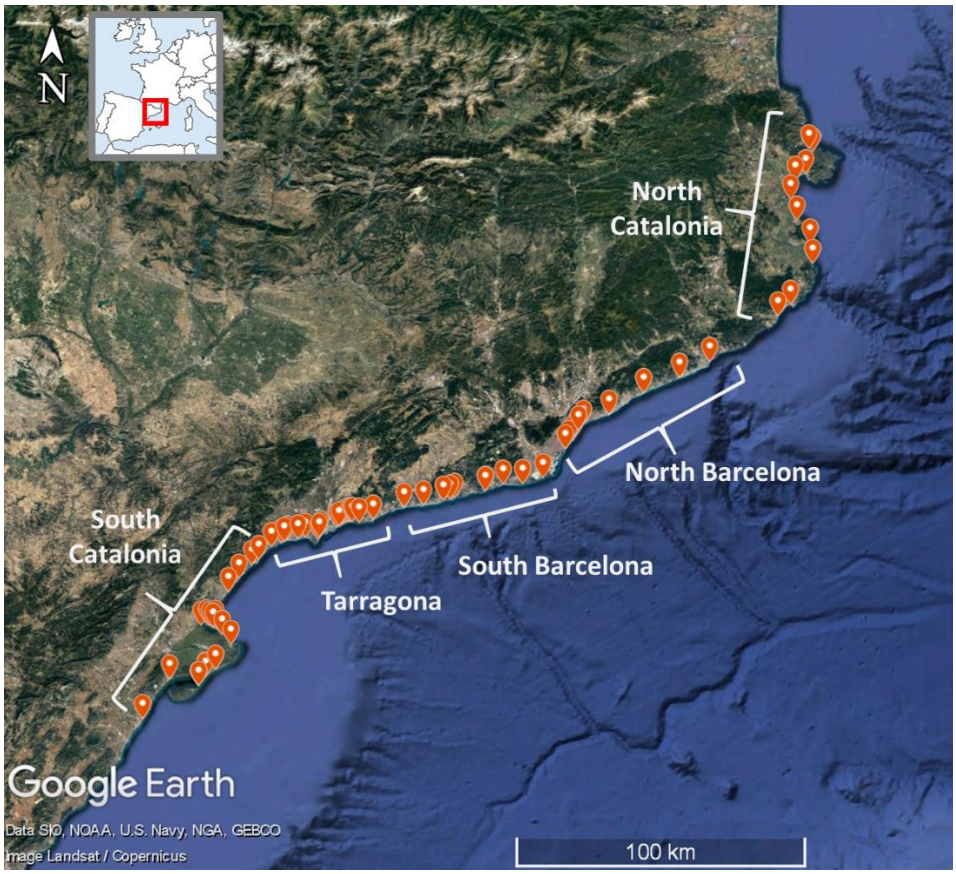

Figure 1. Catalonia coast map with the locations of sampling points.

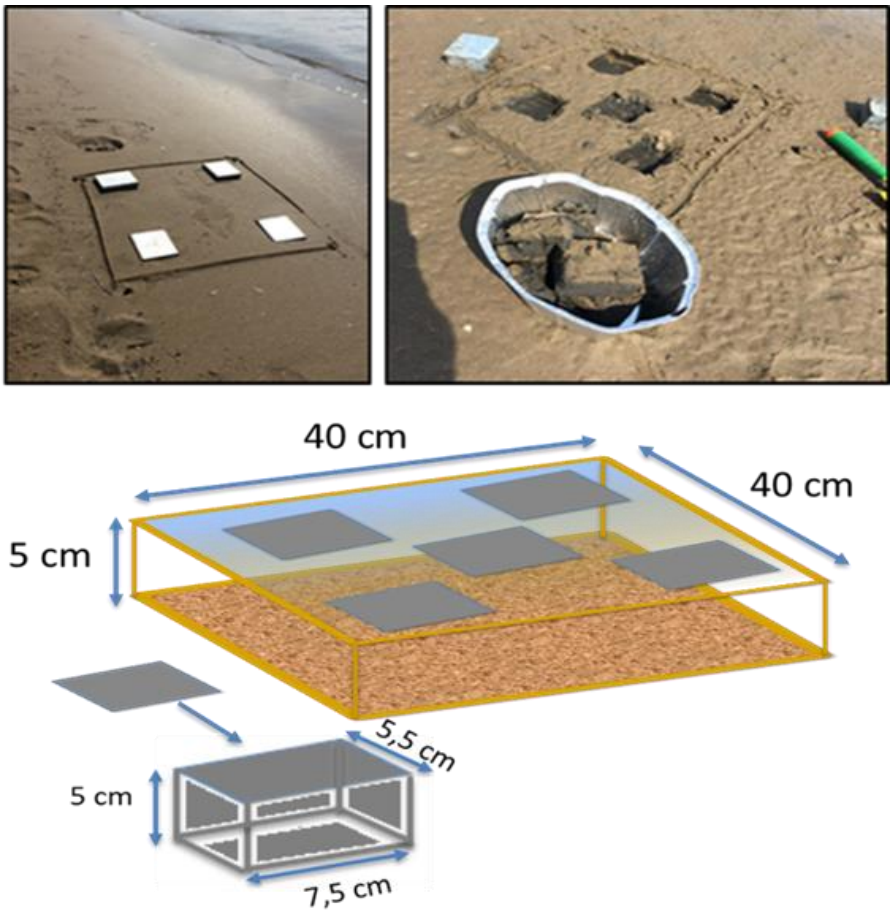

Figure 2. Intertidal sampling and Kubiena boxes description.

1.3.2.3 Sand grain size analysis

Prior to sand grain size analysis, the samples were dried at 40-50°C for 48h and weighed. Then, 30 g of sample was sieved through a column of sieves (5, 2, 1, 0.5, 0.25, 0.1, 0.05 and 0.02 mm) with a shaker and the sample weight for each sieve size was noted for particle size distribution analysis.

1.3.2.4 Microplastics extraction

Total dry sample was sieve and passed through a 0.5, 1, and 2-mm sieve. Two fractions, equal or larger than 0.5 mm and smaller than 0.5 mm, were stored in separate clean glass jars. To extract the microplastics from sands, two procedures were used. For ≥0.5 mm fraction, visual sorting was used for MPs extraction and for <0.5 mm fraction, density separation was used.

Visual sorting

Both, the morphology and size of the microplastics ≥0.5 mm were analysed. The retained materials suspected to be plastic were sorted by size, colour and shape, since plastic identification can be performed visually (Hidalgo-Ruz et al., 2012; Rocha-Santos & Duarte, 2017; Sturm et al.,

2021). Particles were recognized according to several criteria such as: no cellular structure is visible, but do not break easily, colour, no stretch marks (Hidalgo-Ruz et al., 2012).
However, the error of visual identification can range from 20 to 70% (Sturm et al., 2021), as small fragments can easily be mistaken for scales, shells or glass. Therefore, after isolating the particles suspected to be plastic with tweezers and a stereoscopic microscope LEICA MZ10 equipped with a FLEXACAM C1 (8-80x magnification), they were immersed in a battery of test tubes with solutions of different density as H_2O, NaCl, $ZnCl_2$ solution (density 1, 1.2 and 1.8 g/mL respectively); only the floating particles were collected. Subsequently the particles were cleaned and their composition was determined by IR spectroscopic techniques.

Density separation and organic matter removal

Recent studies indicate that density separation is particularly useful for separating denser inorganic particles from MPs (Prata et al., 2019). From 12.5 to 30 g of well-homogenised sample <0.5 mm fraction was weighed into a 250 mL Erlenmeyer; 150 mL of filtered $ZnCl_2$ solution (filtered on nitrocellulose membrane with pore size of 0.45 μm) of density 1.8 g/cm^3 was added. The samples were shaken on an orbital shaker for 30 min at 150 rpm, allowed to stand for 24h, waiting for the sediment to settle to the bottom, while the lower density particles than $ZnCl_2$ solution floated to the surface of the solution. At the first the supernatant was then extracted with a glass Pasteur pipette and the rest of supernatant was vacuum filtered on a 20 μm pore size transparent polycarbonate membrane filters (PCTE, 25mm Ø, 20 μm pore size, Whatman). A second extraction was performed for 24 h. To remove organic matter from the sample, as it can result in false positives, the polycarbonate filters with the filtered material were subjected to Fenton oxidation (H_2O_2 (33%) + $FeSO_4$ (0.05M)) in appropriate amounts to avoid temperature reactions above 50°C and thus not affect the MPs structure present in the samples.
Recovery test were performed using three replicates of sand (12.5 g), previously maintained 550°C for 3h in a muffle furnace and homogenized in an axial shaker for 1h. Fluorescent-labelled MPs of different sizes (450-500 μm, 250-300 μm, 125-150 μm and 53-63 μm) were spiked. The recovery efficiencies obtained after two sequential extractions for the 450-500 and 250-300 μm sizes were 100%, and 85 and 80% for the 125-150 μm and 53-63 μm sizes, respectively.

1.3.2.5 Microplastics analysis

Once plastic have been extracted, the ≥0.5 mm fraction was analysed with Attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR) while the <0.5 mm fraction was analysed by fluorescence technique. After fluorescence analysis, a subset of samples (n=23) was chosen randomly and analysed with μ-FTIR techniques for methodology comparison and composition analysis.

Microplastic analysis by Fluorescence techniques

For fluorescence analysis, it is necessary to stain the polycarbonate filter containing the sample with 2 drops of Nile Red, 5 mg/L solution in methanol. Nile Red (NR) is a fluorescent dye that due to its lipophilic properties tends to adsorb on the surface of non-polar materials such as plastics (Sturm et al., 2021), allowing in situ staining of samples. Under a fluorescence microscope, Zeiss Axioskop 40 FL 10x/0,25, objetives 4x y 10x (40-100 magnification) the stained sample is excited at different wavelengths; thus, NR solution was excited at different wavelengths as: 362±12nm, 450-490 nm, 546±12 nm with emission in different colours light yellow-green: 492-587nm, red/orange: 597-700 nm, and blue: 455-492 nm, respectively. Due a lipophilic dye, some dyed organic substances may show fluorescence and interfere with microplastics analysis, but always weaker than microplastics; therefore, studies recommend the use of this technique in conjunction with the digestion of organic matter (Sturm et al., 2021; Tagg et al., 2015). Particles that fluoresced in red/orange and in any other colour (green, yellow and/or blue) with qualitative intensity between 2 (✓✓) and 3 (✓✓✓) were considered as plastic. Median intensity as 2 (✓✓) was considered due fluorescence characteristics of weathered particles (possible weak fluorescence) (Table 1, figure 3).

For simplification in quantification, the morphologic classification was divided only in two groups: “particles” and “fibres”. Particles groups included fragments, foam, pellets and films. The size was estimated using the Sedgwick-Rafter gridding combined with 0.1 mm high precision eyepiece micrometer.

Table 1. Emission colours and intensity of different polymers after staining with Nile Red methanolic solution.

Plastic polymer	RED/ORANGE	GREEN	YELLOW	BLUE
PP	✓	✓✓✓	✗	✓
PE (HDPE, LDPE)	✓	✓✓✓	✗	✓
PS (PS, EPS)	✓✓✓	✗	✓✓✓	✗
PU	✓✓✓	✗	✓✓✓	✗
PET	✓	✗	✓	✓✓
PVC	✓✓✓	✗	✗	✗
PMA	✓✓	✓✓	✗	✗
PVAc	✓✓✓	✓✓✓	✗	✓✓✓
PES	✓✓	✗	✓✓	✓✓
PA	✓✓	✓	✗	✓✓
Cotton (natural cellulose)	✗	✗	✗	✗
Wood	✓	✗	✗	✗
Chitin	✓	✗	✗	✗
Treated Chitin/wood	✗	✗	✗	✗
Wheel rubber	✗	✗	✗	✗

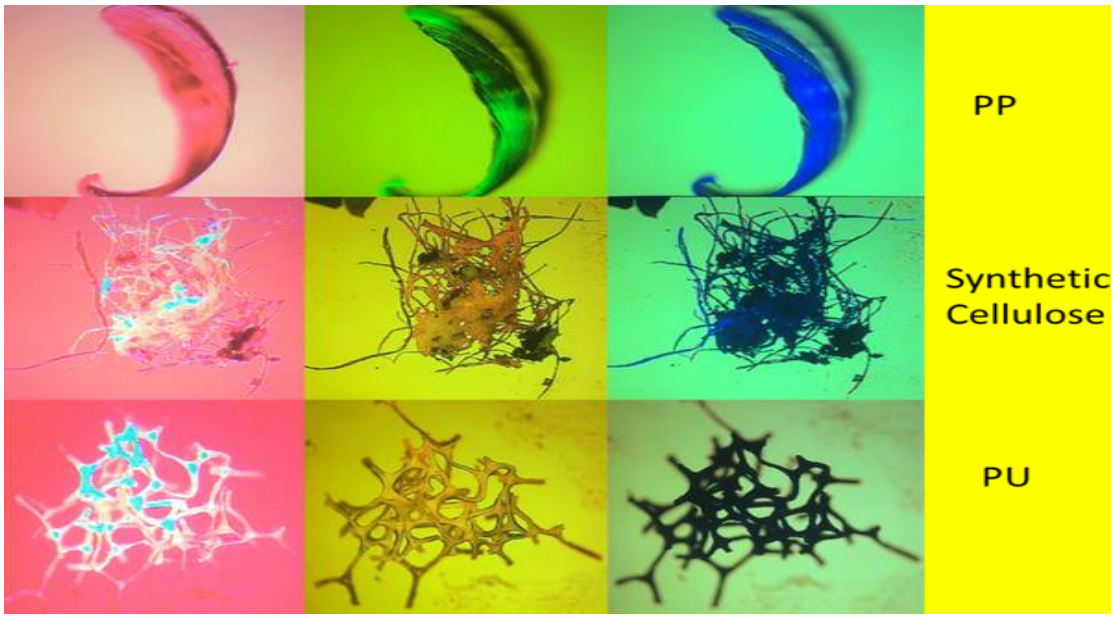

Figure 3. Fluorescence imagen of common polymers: Polypropylene (PP), synthetic cellulose (Rayon), Polyurethane (PU).

Microplastic analysis by Infrared techniques

Particle's size greater or equal than 0.5 mm were analysed by infrared spectroscopy with a Thermo Scientific GladiATR Highest Performance NICOLET Diamond ATR-FTIR spectrometer with OMNIC™ Paradigm Software. The measurements were performed in reflection mode in the range of 400–4000 cm^{-1} with 16 scans at a spectral resolution of 4 cm^{-1}. The background was done before analysis and every 6 samples.

For particles less than 0.5 mm, MPs retained and stained in the polycarbonate filter were extracted with ultrapure water in an ultrasonic bath at 40ºC for 30 minutes. The supernatant was then filtered through a reusable 10x10 mm and 5-6 µm pore size silicon filter (Macroporus silicon membrane, 904675-W25). The filter was examined for sample homogeneity verification, a square of 3x3 mm over homogeneous filters zone were chosen randomly and analysed with an ultrafast mapping microscope Thermo Scientific Nicolet™ iN™10 with MCT detector with pixel aperture 25x25 µm in transmission mode, in the range of 715-4000 cm^{-1} with 4 scans at a spectral resolution of 4 cm^{-1}.

Similarly, to fluorescence technique, for spectroscopy it was made the same simplification when µ-FTIR maps were analysed. Two groups were established: "particles" and "fibres". Particles groups included fragments, foam, pellets and films.

1.3.2.6 Blanks

To prevent MPs contamination extraction was performed in a laminar flow hood, only glass material was used and all material was cleaned first with ethanol 70% and washed with filtered deionised or ultrapure water (distillate water filtered on 0.45 µm pore size nitrocellulose

membrane). Laboratory cotton coats and blue nitrile gloves were worn. To discard contamination with MPs airborne particles a blank sample was performed during sieving of each sample and procedural blanks were applied during extraction steps every 5-6 samples for twelve blanks.
On sieving blanks were not found any fibres, fragments or films. In case of <0.5 mm fraction fluorescence (polycarbonate filters) and µ-FTIR (silicon filters) blank procedures. On average MPs obtained were 2.6±2.0 particles and 1.2±1.2 fibres for fluorescence, and 2.2 ± 1.5 MPs particles and 0 fibres for the infrared method. The value of each blank was subtracted to the samples.

1.3.2.7 Statistics

In order to compare the differences between groups geographical areas, at the first a Kruskal-Wallis test was applied and then the Mann-Whitney U test to compare pairs of samples with significant level $p<0.05$. A difference was considered as statistically significant when the probability was lower than 0.05 ($p <0.05$). Spearman correlations (no parametric measure for no lineal relation) (ρ) were performed for Fluorescence and Spectroscopy methodology comparison (range differences) ($p=0.000$) in MPs concentration determination due data did not follow a normal distribution. Wilson's T-statistic method was applied to observe the differences between these both methodologies with $p<0.05$ significant level. For relate beach facing to (orientation), beach morphological shoreline, slope to seawater and close anthropogenic pressures (number and type) with MPs parameters (concentration, size and morphology), an ANOVA test for data following a parametric distribution (variance homogeneity), or the Kruskal Wallis tests for non-parametric data (not variance homogeneity), were used to assess differences between groups. IBM SPSS Statistics v.28 software was used.

1.3.3. Results and Discussion

MPs concentration and composition were analysed by means of fluorescence and infrared spectroscopy techniques. Both methods have been compared and the MPs concentration, size and morphology type have been studied in relation to the geographical location of each beach and their relationship with the sand grain size distribution, beach morphological shoreline, facing to (orientation), slope to seawater, and close anthropogenic pressures (number and type).

1.3.3.1 Samples, meteorological parameters, and sand characteristics

Catalonia coast is a sedimentary coast with heterogeneous sand-grain size distribution. The coast is mainly composed by medium and fine sandy beaches together with some coarse, very coarse-

coarse sand, coarse-fine sand and gravel ones. Thus 39% of beaches analysed were fine sand type, 27% medium sand, 20 % coarse sand, 10 % very coarse sand, 1% very fine gravel and 7% of sand types mixture. In general terms, northern beaches had medium to coarse grain size while south Catalonia and Tarragona zones had a fine grain size. Sand-grain size distribution and meterological condition and characteristics of each sampling point are summarized in supplementary information (Annexes).

1.3.3.2 Microplastics ≥0.5mm

For the analysis of MPs larger than 0.5 mm (500-5000 μm), the visual sorting technique was used and approximately an average of 1.52 ± 0.16 kg of sand per sampling point was analysed (Table 2). Using this technique, 819 particles larger than 0.5 mm were isolated, of which the composition of 671 particles were identified as plastic polymer by ATR-IR spectroscopy.
The results of this study indicate that MPs have been detected in almost all sediment samples from the five zones of Catalonia Coast: North Catalonia, North Barcelona, South Barcelona, Tarragona and South Catalonia. Around the 11% of samples (n=8) MPs larger than 0.5 mm was not observed (Table 2). Variability of MPs larger than 0.5 mm concentration was high being the overall mean level was 7.2±11.7 MPs/kg of dry sand ranging from <0.55 to 56.8 MPs/kg (Figure 4).

The zones which presented highest >0.5 mm MPs concentrations were South Barcelona and Tarragona with 9.6±10.6 and 12.0 ±17.5 MPs/kg, respectively. Other zones, south Catalonia, north Barcelona, and North Catalonia, present >0.5 mm MPs levels of 5.8±8.7, 2.8±1.8, and 2.0±0.7 MPs/kg, respectively. Levels found in North Catalonia zone (2.0±0.7 MPs/kg) was significantly ($p<0.05$, $p= 0.013$-0.045) lower than those found in the South-Barcelona (9.6±10.6 MPs/kg) and Tarragona (12.0 ±17.5 MPs/kg) zones. It means that Catalonia North zone tent to be a zone with low MPs≥0.5 mm concentration

The beaches with the highest abundance in South Barcelona were Cala de Vallcarca (CAV) with 31 MPs/kg and Platja Far de Sant Cristòfol-Vilanova (VIL 01) with 25 MPs/kg. In case of Tarragona coast, La Pineda beach (PIN 1 and PIN 3) showed the highest values with more than 50 MPs/kg.

In general, 29 beaches showed predominance of sizes from 2 to 1 mm manly in North Catalonia, Tarragona and South Catalonia zones while 28 beaches showed sizes from 4 to 2 mm mainly in North and South Barcelona.

In summary, the morphology proportion of ≥0.5 mm MPs from entire Catalonia coast was 60% of fragments followed by 15% of films, 14% fibres, 10% pellets and 3% foam. The size predominant was the range 2-1 mmm with 42 % (Figure 5). Despite the pellets proportion was low, it was found with relative high concentrations in beaches such as: Pineda beach (PIN 1, 2 and 3) and Miracle beach (MIR2) with a pellet level of 2.9, 3.9, 14 and 3.6 pellets/kg, respectively, both beaches with a contribution arround 25%. These two beaches, 5 sampling points, are close to Tarragona petrochemical complex where several plastic manufactures are located. Similarly, beaches near river discharges as Sant Adrià del Besòs (STA) in Besòs river discharge, Bahia del Fangar Faro (BF-F) in Ebro River Delta, and El Prat (EPLL) in Llobregat river discharge presented a level of 2.4, 1.8 and 1.7 pellets/kg, respectively. The high proportion of pellets found, especially at La Pineda beach (PIN 1, 2, and 3), could be due to the proximity to Tarragona petrochemical complex where several plastic manufactures are located. Due to spills, transport of products and non-efficient waste management pellets could be realesed to environment and arrive to coastal areas. Marine superficial currents and wind may distribute these large quantities of pellets and deposit them in shoreline. Most of the pellets identified by ATR-FTIR were polyethylene (PE) and polypropylene (PP), low dense polymers that can be easily dispersed by winds and currents.

In relation to general composition of >0.5 mm MPs, principals polymers detected were PE with 56.5%; followed by polypropylene (PP) (23.7%), polystyrene (PS) (10.3%), polyethylene terephthalate (PET) (4.1%), and polyamide (PA) (1.3%). Other polymers detected with a proportion below 1% were polyurethane (PU), poly (ethylene-vinyl acetate) (EVA), polyvinylchloride (PVC) and other polymers (polycarbonate, acrylonitrile-butadiene-styrene copolymer (ABS), poly (methyl methacrylate), alkyd resin and acrylate polymers) (Figure 5).

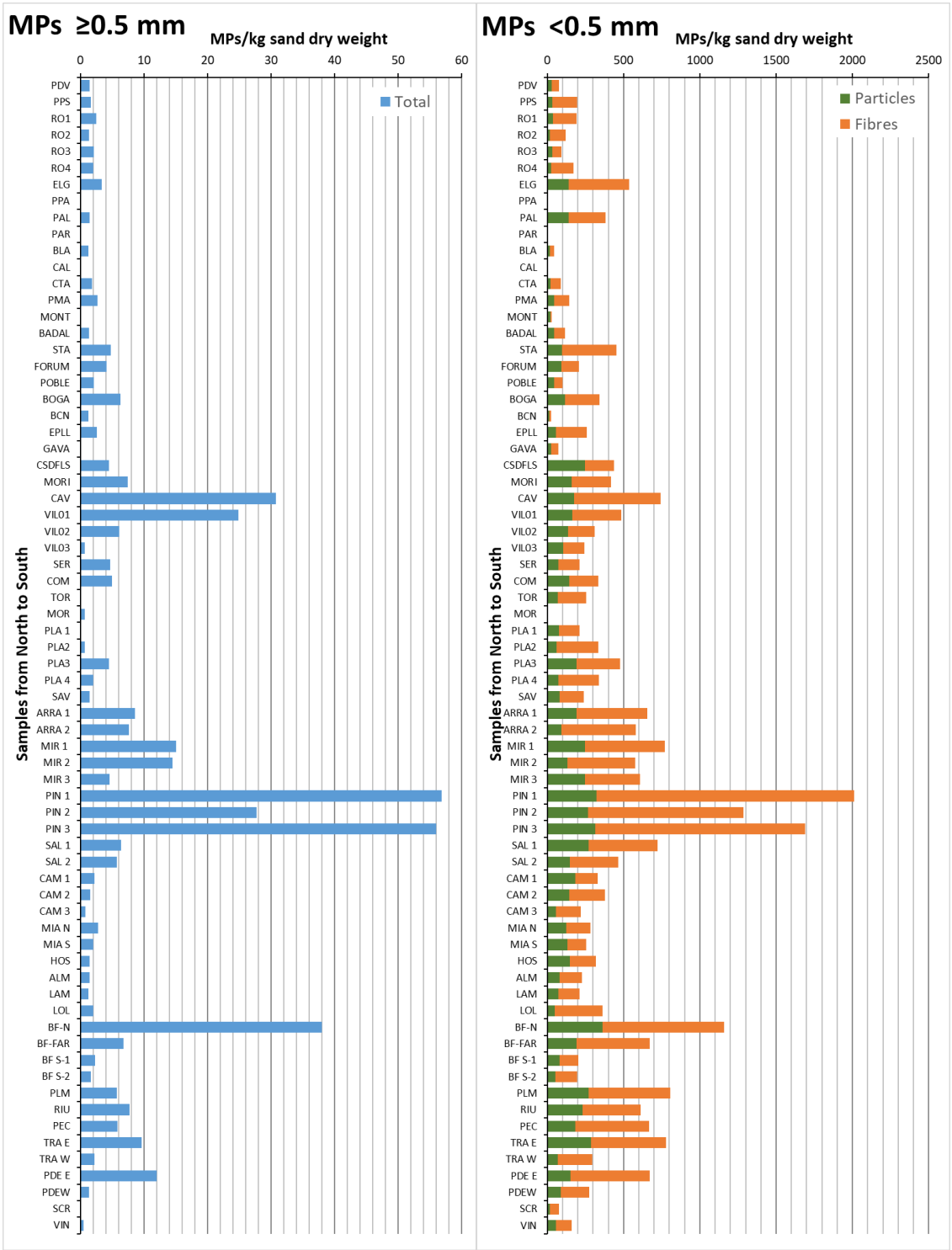

Figure 4. MPs in sand samples (n=70) collected from Catalan beaches.

Table 2. Weight of beach sand sampled, number of MPs larger than 500 μm isolated (Num.), concentration of MPs (Conc.), size distribution and morphology type (fragments, pellets, film, fibres and foams).

Sand Beach	Weight (kg)	Nº MP	Level (MP/kg)	% Size category (mm)				% Morphology type				
				5−4	4−2	2−1	1-0.5	Frag.	Pellet	Film	Fib	Foam
PDV	2.16	3	1.39	33	0	33	33	33	0	0	67	0
PPS	1.84	3	1.63	0	33	67	0	100	0	0	0	0
RO1	1.61	4	2.48	25	25	25	25	50	25	25	0	0
RO2	1.52	2	1.31	0	0	100	0	0	0	100	0	0
RO3	1.41	3	2.12	0	0	100	0	0	0	0	100	0
RO4	1.49	3	2.01	0	100	0	0	33	33	33	0	0
ELG	1.50	5	3.33	40	0	40	20	100	0	0	0	0
*PPA	1.64	0	<0.60	0	0	0	0	0	0	0	0	0
PAL	1.40	2	1.43	0	50	0	50	50	0	0	0	50
*PAR	1.72	0	<0.58	0	0	0	0	0	0	0	0	0
N Catalonia			**2.0±0.7**									
BLA	1.64	2	1.22	50	50	0	0	50	0	50	0	0
CAL	1.60	0	<0.62	0	0	0	0	0	0	0	0	0
CTA	1.63	3	1.84	0	100	0	0	100	0	0	0	0
PMA	1.50	4	2.67	25	50	25	0	50	0	0	50	0
MONT	1.55	0	<0.64	0	0	0	0	0	0	0	0	0
BADAL	1.53	2	1.31	50	50	0	0	50	0	50	0	0
STA	1.69	8	4.73	0	38	25	38	38	50	13	0	0
FORUM	1.45	6	4.14	33	33	33	0	33	0	0	50	17
POBLE	1.42	3	2.11	0	0	100	0	100	0	0	0	0
BOGA	1.59	10	6.28	40	10	50	0	40	10	40	10	0
BCN	1.64	2	1.22	50	50	0	0	50	0	50	0	0
N BCN			**2.8±1.8**									
EPLL	1.16	3	2.58	33	67	0	0	33	67	0	0	0
*GAVA	1.50	0	<0.7	0	0	0	0	0	0	0	0	0
CSDFLS	1.34	6	4.48	17	17	67	0	0	0	0	100	0
MORi	1.49	11	7.4	9	9	55	27	91	9	0	0	0
CAV	1.59	49	30.76	22	45	33	0	73	2	18	2	4
VIL 01	1.45	36	24.81	36	53	11	0	61	0	28	11	0
VIL 02	1.64	10	6.11	50	20	30	0	40	10	10	0	40
VIL 03	1.59	1	0.63	100	0	0	0	0	0	100	0	0
SER	1.07	5	4.67	40	60	0	0	80	0	20	0	0
COM	1.42	7	4.94	57	14	29	0	43	0	29	29	0
S BCN			**9.6±10.6**									
*TOR	1.82	0	<0.55	0	0	0	0	0	0	0	0	0
MOR	1.49	1	0.7	0	0	100	0	0	0	100	0	0
*PLA 1	1.50	0	<0.6	0	0	0	0	0	0	0	0	0
PLA 2	1.53	1	0.7	0	0	0	100	0	0	0	100	0
PLA 3	1.56	7	4.5	0	0	14	86	43	0	14	43	0
PLA 4	1.50	3	2.0	0	33	33	33	33	0	0	66	0
SAV	1.48	2	1.4	0	0	100	0	0	0	0	100	0
ARRA 1	1.62	14	8.6	7	21	29	43	36	0	14	50	0
ARRA 2	1.57	12	7.6	0	0	50	50	33	0	0	67	0
MIR 1	1.46	22	15.0	0	59	41	0	64	14	5	18	0
MIR 2	1.65	24	14.5	0	50	50	0	46	25	17	13	0
MIR 3	1.53	7	4.6	0	57	14	29	29	29	14	29	0
PIN 1	1.62	92	56.8	0	10	66	24	90	5	0	4	0
PIN 2	1.33	37	27.7	8	11	70	11	76	14	5	5	0
PIN 3	1.43	80	56.0	0	16	51	33	64	25	1	10	0
SAL 1	1.40	9	6.4	0	12	22	66	22	33	12	33	0
SAL 2	1.41	8	5.7	25	38	25	13	0	25	63	0	13
CAM 1	1.38	3	2.2	67	0	33	0	67	0	0	33	0
CAM 2	1.30	2	1.5	0	100	0	0	100	0	0	0	0
CAM 3	1.33	1	0.8	0	100	0	0	100	0	0	0	0
TGN			**12.0±17.5**									
MIA N	1.40	4	2.8	0	25	25	50	0	0	25	50	25
MIA S	1.51	3	2.0	100	0	0	0	0	0	0	100	0
HOS	1.44	2	1.4	0	0	100	0	0	0	0	100	0
ALM	1.40	2	1.4	100	0	0	0	0	50	0	50	0
LAM	1.64	2	1.2	100	0	0	0	0	50	0	0	50
LOL	1.51	3	2.0	67	33	0	0	67	0	33	0	0
BF N	1.37	52	38.0	27	21	42	10	63	0	35	0	2
BF FAR	1.61	11	6.8	36	45	9	9	36	27	0	18	18
BF S 1	1.29	3	2.3	0	33	67	0	67	0	33	0	0
BF S 2	1.27	2	1.6	0	50	50	0	50	0	50	0	0

PLM	1.58	9	5.7	78	11	11	0	11	0	44	33	11
RIU	1.55	12	7.7	33	8	42	17	8	0	50	42	0
PEC	1.55	9	5.8	11	22	44	22	60	0	0	40	0
TRA E	1.67	16	9.6	13	50	38	0	13	6	81	0	0
TRA W	1.37	3	2.2	0	33	33	33	0	0	0	100	0
PDE E	1.50	18	12.0	11	44	33	11	83	0	0	0	17
PDE W	1.53	2	1.3	0	100	0	0	0	0	0	100	0
*SCR	1.44	0	<0.69	0	0	0	0	0	0	0	0	0
VIN	1.93	1	0.5	0	0	100	0	0	0	0	100	0
SCatalonia			**5.8±8.7**									
Total (n=70)			**7.2±11.7**									

Zones: N Catalonia: North Catalonia; N BCN: North Barcelona SBCN: South Barcelona; TGN: Tarragona; S Catalonia: South Catalonia;
***Not were included in average calculation.**

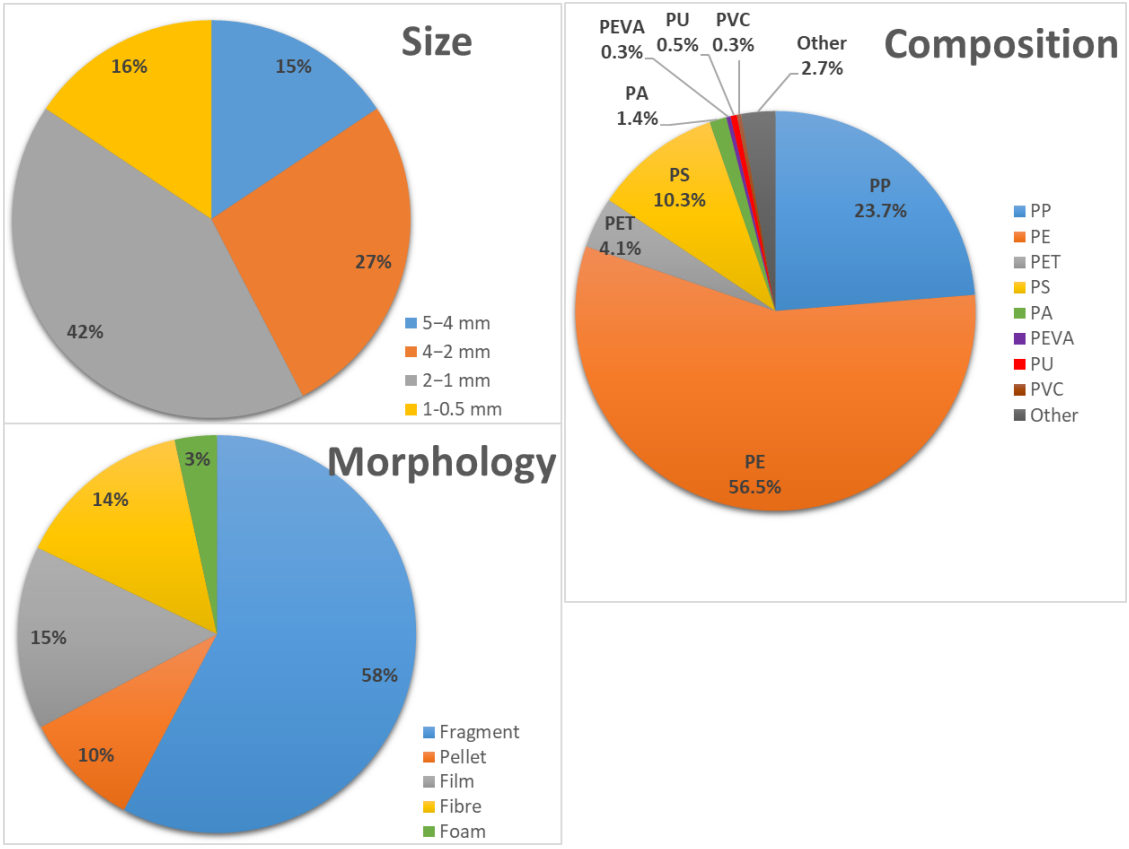

Figure 5. Morphology, size distribution and composition of MP≥500 µm in sand beaches. Results expressed as percentage. PE: Polyethylene; PP: Polypropylene; PS: Polystyrene; PET: Polyethylene terephthalate; PA: polyamide; PU: Polyurethane; EVA: Ethylene-vinyl acetate; PVC: Polyvinyl chloride; Other polymers found were Polycarbonate, Acrylonitrile-butadiene – styrene copolymer, Poly (methyl methacrylate), alkyd resin and acrylate polymers.

1.3.3.3 Microplastics <0.5 mm

In general, <0.5 mm MPs were found in almost all samples analysed and the concentration level varied considerably from one beach to another and even between samples from the same beach as illustrated in figure 4, indicating MPs extensive dispersion. In 6 % of samples (n=4) from four different beaches, <0.5mm MPs were not detected using fluorescence methodology.

For the analysis of MPs smaller than 0.5 mm, density separation was used to isolate the plastics and two different techniques were used to quantify them: staining with Nile Red and subsequent analysis by fluorescence microscopy; and quantification and identification of the polymers by µ-FTIR Spectroscopy maps for a subset of 23 samples randomly selected. Results are shown in table

5. It was only reported particles from 60 to 500 µm size and fibres >60 µm because better intensity and colour fluorescence appreciation was observed combined with a better spectra resolution in µ-FTIR maps. This range size was closed to recommended by Frias et al., (2018), to sample MPs in sediments based on the currently available methodologies, the lower size limit for monitoring purposes is 100 µm

Fluorescence techniques

Concentration analyzed for <0.5 mm MPs were higher than levels ≥0.5 mm MPs. These values ranged from tens or hundreds to thousands of MPs/kg of dry sand in some beaches considered them as possible hot spots. Furthermore, the fibres were predominant in the majority of beaches studied for <0.5 mm MPs.

The variability was also large and in general, it was observed that the average concentration in Catalonia coast was 115±90 MPs/kg and 274±292 MPs/kg for fibres and particles, respectively, and total MPs level of 388±367 MPs/kg. These data indicate that 70% of the MPs of the beaches correspond to fibres according to this analysis methodology. Zones with the highest concentration values were Tarragona and South Catalonia zone with average particles concentration of 158±95 and 139±94 MPs/kg dry sand, respectively, and fibres levels of 449±430 and 294±203 MPs/kg, respectively (Table 5). However, values obtained for South Barcelona zone were similar than those obtained in South Catalonia 128±65 MPs/kg and 244±140 MPs/kg for particles and fibres respectively. For Catalonia and Barcelona North zones, the values were significant ($p<0.05$, $p=0.000$-0.04) lower than the other zones ranging from 45±51 and 46±39 MPs/kg for particles and 131±121 and 95±108 MPs/kg for fibres respectively.

Therefore, it is considered for the north zone of Catalonia specifically from Barcelona to Cap de Creus as zones with lower concentration of particles and specifically Barcelona North zone tent to be a zone with low fibres concentration for sizes ≥ 60 µm.

Similarly, to ≥ 0.5 mm, in <0.5 mm MPs fraction the beaches in South Barcelona Tarragona coast and South Catalonia zone with high concentration were Cala de Vallcarca beach (CAV), La Pineda beach (PIN 1, PIN 2, PIN 3) and Bahía del Fangar North (BF-N) respectively. The Pineda beach showed values from 1226 to 2013 MPs/kg dry sand and from 79 to 84% of fibres while Bahía del Fangar North showed values of 1159 MPs/kg with 69% of fibres and Cala de Vallcarca beach (CAV) with 742 MPs/kg and 76 % fibres. However, looking in detail, it can be seen that samples exposed to seafront and east part of Ebro Delta in Catalonia Southern zone have higher values than samples in west side, which is protected from strong waves.

Table 5. Concentrations of MP (Particles and fibres) smaller than 500 µm in beach sands according fluorescence and infrared techniques, particles and fibres mean size, standard deviation (SD) and median values.

Sand Beach	Fluorescence (MP/kg)		Infrared (MP/kg)		Particles Size (µm)			Fibres Size (µm)		
	Part.	Fib.	Part.	Fib.	Mean	SD	Median	Mean	SD	Median
PDV	30	47	-	-	82	16	70	589	422	500
PPS	33	161	38	76	133	81	100	638	376	500
RO1	38	152	-	-	137	65	80	504	412	400
RO2	16	104	-	-	130	114	80	756	394	750
RO3	32	62	43	92	90	30	90	594	769	400
RO4	25	148	-	-	80	8	80	276	420	60
ELG	138	398	289	446	83	16	75	470	416	400
*PPA	0	0	-	-	0	0	0	0	0	0
PAL	139	242	102	359	165	181	100	592	291	500
*PAR	0	0	-	-	0	0	0	0	0	0
N Catalonia	**45±51**	**131±121**	**118±118**	**243±187**						
BLA	17	27	-	-	95	61	80	636	275	500
*CAL	0	0	-	-	0	0	0	0	0	0
CTA	20	68	-	-	120	60	100	1480	2204	1000
PMA	43	99	61	68	109	43	100	881	487	800
MONT	19	9	-	-	108	45	100	807	244	950
BADAL	45	72	-	-	122	88	100	311	151	250
STA	95	359	122	320	93	55	90	690	190	600
FORUM	93	114	101	151	116	71	100	745	427	700
POBLE	45	57	-	-	88	26	80	280	84	300
BOGA	117	225	-	-	109	84	80	665	352	600
BCN	14	11	-	-	108	37	80	936	1051	600
N BCN	**46±39**	**95±108**	**95±31**	**180±128**						
EPLL	55	205	-	-	85	12	75	286	265	400
GAVA	25	48	-	-	90	20	90	633	153	600
CSDFLS	245	193	-	-	142	77	120	579	500	450
MORI	160	258	240	320	113	62	95	373	206	300
CAV	177	565	283	424	94	44	80	416	531	160
VIL 01	165	319	130	390	95	18	100	794	1241	350
VIL 02	134	175	-	-	81	9	80	850	583	700
VIL 03	104	138	-	-	103	25	100	484	395	300
SER	71	142	-	-	89	8	80	309	120	275
COM	142	192	160	320	90	17	100	960	966	1000
S BCN	**128±65**	**224±140**	**203±71**	**364±52**						
TOR	68	185	-	-	169	132	100	659	664	600
MOR	0	0	-	-	0	0	0	0	0	0
PLA 1	75	138	-	-	103	51	100	450	129	400
PLA 2	61	273	-	-	100	20	100	350	172	300
PLA 3	191	287	157	315	180	215	90	288	195	400
PLA 4	74	264	-	-	98	12	100	700	346	700
SAV	79	159	-	-	88	11	90	803	992	992
ARRA 1	192	464	276	395	150	126	100	93	12	100
ARRA 2	94	487	-	-	90	24	90	429	150	500
MIR 1	248	521	-	-	91	10	100	760	1258	200
MIR 2	132	443	-	-	108	50	90	243	204	150
MIR 3	247	359	480	320	91	15	100	721	658	700
PIN 1	323	1690	-	-	94	16	90	1144	556	500
PIN 2	265	1021	-	-	100	18	100	110	17	120
PIN 3	314	1375	526	947	136	138	100	1043	444	900
SAL 1	271	450	-	-	134	69	100	601	330	500
SAL 2	146	320	-	-	122	58	100	609	359	600
CAM 1	184	147	175	233	147	72	110	960	1187	600
CAM 2	145	234	-	-	135	97	100	601	473	600
CAM 3	58	160	-	-	136	118	100	745	520	550
TGN	**158±95**	**449±430**	**323±171**	**442±288**						
MIA N	124	159	-	-	84	13	80	880	712	800
MIA S	131	124	65	196	136	104	100	350	229	300
HOS	146	173	156	160	132	65	100	872	1263	400
ALM	79	149	-	-	110	50	100	800	705	550
LAM	71	139	142	284	117	39	100	1167	828	1000
LOL	47	314	-	-	122	71	100	489	380	400
BF N	363	796	318	637	113	43	100	589	401	400
BF FAR	192	479	-	-	135	67	100	780	438	1000
BF S 1	79	126	-	-	98	38	80	568	337	500
BF S 2	54	142	-	-	118	120	80	745	461	600

PLM	269	538	160	640	95	28	80	463	438	300
RIU	232	380	-	-	131	78	100	1600	1457	1000
PEC	183	483	-	-	146	176	100	995	1426	500
TRA E	288	489	232	320	129	50	100	527	356	500
TRA W	70	225	180	158	110	39	100	729	829	400
PDE E	151	519	-	-	96	51	80	486	262	450
PDE W	88	186	-	-	102	42	80	1179	1443	450
SCR	16	59	-	-	119	61	100	675	427	800
VIN	55	103	-	-	133	124	80	695	521	500
S Catalonia	**139±94**	**294±203**	**179±79**	**342±211**						
Total (n=70)	**115±90**	**274±292**	**193±126**	**329±204**						

Zones: N Catalonia: North Catalonia; N BCN: North Barcelona SBCN: South Barcelona; TGN: Tarragona; S Catalonia: South Catalonia; * MPs are not detected

The mean size of isolated particles ranged from 80±8 to 180±215 µm, whereas fibres tend to be larger, with a mean length ranging from 93±12 to 1600±1457 µm with median length variation from 60 to 1000 µm considering all selected Catalan beaches. In Figure 6 and 7 depicted that the 90% of mean and median of particles sizes were below or equal to 140 µm, while the 80% of fibres were below 870 µm. Regarding to the MPs size analysed, in general it can be concluded that more than 50% of the mean size of particles found in intertidal zone were smaller or equal than 110 µm. Godoy et al., (2020), found similar results with more than 50% of particles size less to 100 µm in Granada beaches. Comparing with worldwide regions, this study MPs sizes <0.5 mm were within Claessens et al., (2011), size range from 38 to 1mm found in Belgium beaches at three lines high tide, intertidal and broken line. Similarly, Hengstmann et al., (2018), found a range from 63 to 630 µm in in three lines of Isle of Rügen in Baltic Sea. These small sizes observed in intertidal zone of beaches can move to seawater through wave action in storms events and remain in suspension and translate to offshore according wave/current energy (Enders et al., 2015). According, Isobe et al., (2014), through a numerical model, suggested that mesoplastics are selectively conveyed onshore by a combination of Stokes drift and terminal velocity (physical processes), dependent on fragment sizes. It was suggested that mesoplastics washed ashore on beaches degrade into microplastics, and that the microplastics, which are free from near-shore trapping, are thereafter spread offshore in coastal waters.

These MPs of small sizes, are more suitable substrate for absorption of persistent, bio accumulative and toxic pollutants and thus more harmful for the marine biota where they can be released and concentrated in the tissues of organisms (Barnes et al., 2009; Mato et al., 2001), since these microplastics are mistaken for food.

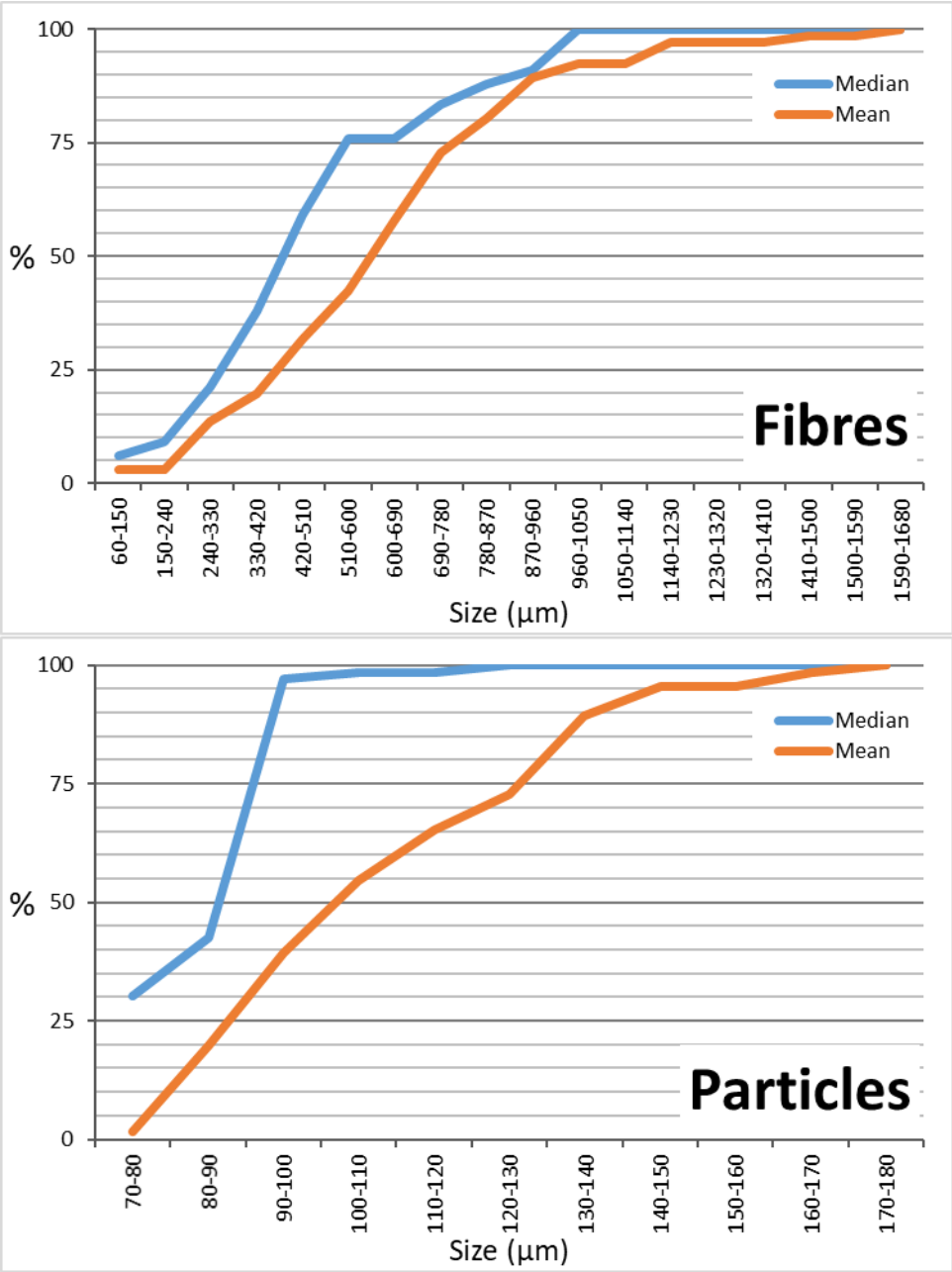

Figure 6. Mean and median size distribution values of fibres and particles MPs<0.5 mm fraction determined by Fluorescence methodology

Spectroscopic techniques

One of the objectives of this study was to compare the two proposed methodologies, fluorescence with spectroscopic techniques for feasibility evaluation of fluorescence techniques for hot spots establishment. The results obtained by µ-FTIR spectroscopic techniques are shown in Table 5.

It is important to highlight that, in this study; synthetic cellulose was not considered a plastic polymer. Cellulose can be of natural origin (e.g., cotton) and can also be of semi-synthetic origin (rayon, viscose, cellophane, nitrocellulose…). The µ-FTIR microscopy technique usually not

differentiate accurately both types of cellulose from environmental due probably weathering processes that fibres experimented. Since these fibres often undergo structural changes (seawater weathering) during their transport in the environment or even during the sample digestion processes to remove organic matter (oxidation with hydrogen peroxide) associated with. Due this uncertainty, both natural and synthetic cellulose, were not take into account in present work.

Similarly, to some results obtained with fluorescence methodology, the results obtained with µ-FTIR also range from tens to hundreds MPs/kg of sand in some beaches. In general, the average of MPs concentration was 522±307 MPs/kg of dry sand, with 193±126 MPs/kg particles and 329±203 fibres.

It is important to highlight that for both methodologies, the fibres were the predominant morphology caused probably by sieving action and small size of fibres diameters promoting their filtering through the sieves with accumulation in fraction <0.5 mm. According, Phuong et al., (2021), the dry sieving method may not be effective in retaining fibres. Regarding composition of particles MPs between 60 and 500 µm in size analysed were identified polymers as PE (48%), PP (23%), PET (7%), PVC (7%), and PS (6%). Other polymers such as PU, alkyd resin, EVA, Polyisobutane (PIB) and Poly (methyl methacrylate) present contributions below 2.5% (figure 8). Regarding fibres, the polymers found were Polyester PES (37%) followed by Polyamide (PA) (24%); PP (23%), Polyacrylonitrile (PAN) (8%) and PE (6%) (figure 7).

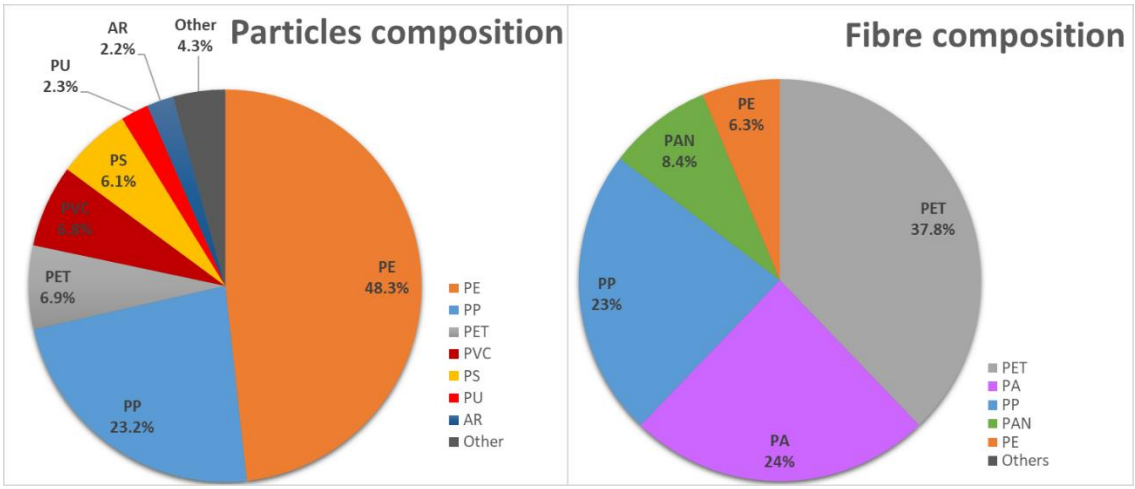

Figure 7. Composition of the MPs≤500 µm (particles 60-500 µm and fibres >60 µm) in sand beaches. Results were expressed as percentage. PE: Polyethylene; PP: Polypropylene; PET: Polyethylene terephthalate; PVC: Polyvinyl chloride; PS: Polystyrene; PU: Polyurethane; AR: Alkyd resinPA: Polyamide; PAN: Polyacrylonitrile; Other: Acrylonitrile-butadiene –styrene copolymer, and acrylate polymers; EVA: Ethylene-vinyl acetate; PIB: Polyisobutane; PTFE: Polytetrafluorehtylene; PMM: Poly(methyl methacrylate);

It is noted that much of the polymer composition observed in MPs ≥0.5 mm was observed for MPS< 0.5 mm possibly derived from fragmentation of bigger particles. Thus, majority polymers

as PE, PP, PET, PS, were in similar proportion in both fractions. Similarly, minority polymers as Alkyd resin, Acrylate polymers and EVA were in low proportion. PA was associated with fibres and PVC was found in higher proportion in only MPs<0.5 fractions, it means, possibly PVC is deposited in intertidal zone by waves or from backshore fragmented previously.

It is possible that large microplastics and mesoplastics arrive to intertidal zone from accumulation zones of beach (backshore) by touristic activity and continuous drags from high tide line. In addition, inputs of sea waves enhance fragmentation by mechanical forces action (due to increased friction and abrasion in sand), photolysis and thermo-oxidation producing smaller MPs particles less to 0.5 mm and in consequence increasing their abundancy. In case of fibres, due their high length/wide relation have high surface and low settling velocity and remain buoyant on seawater and potentially can be deposited on intertidal zone. Once deposited by mechanical action and length is associated with sediment, staying attached with it a long time according Khatmullina and Isachenko, (2017).

On the other hand, fibres once deposited on a beach may become incorporated into the sediment matrix also through pore-water migration. The vertical movement of small fibres (occurring as silt or clay sized particles) through porous beach sediments via pore water has been suggested by earlier studies (e.g., Hidalgo-Ruz et al., 2012). In a similar way, Rusch et al., (2000) explained vertical profiles of particulate organic matter (POM) within permeable intertidal sediments to be a result of vertical transport via pore water. If fibres, rather than fragments, are preferentially trapped within porous beach sands through pore-water migration, this could explain their abundance.

Comparison between Red Nile plus fluorescence and spectroscopic techniques

Microplastics smaller 0.5 mm analyzed by both methods, µFTIR and Red Nile plus florescence, presented significant correlations for particles (ρ=0.790, $p<0.001$) and for fibres (ρ=0.903, $p<0.001$). Levels of MPs analysed using both methodologies did not present significant differences for particles ($p=0.446$) and fibres ($p=0.909$). In figure 8, it is shown, the equation with relationship between both methodologies, an exponential equation resulted as the best fit with a R^2 around 0.9.

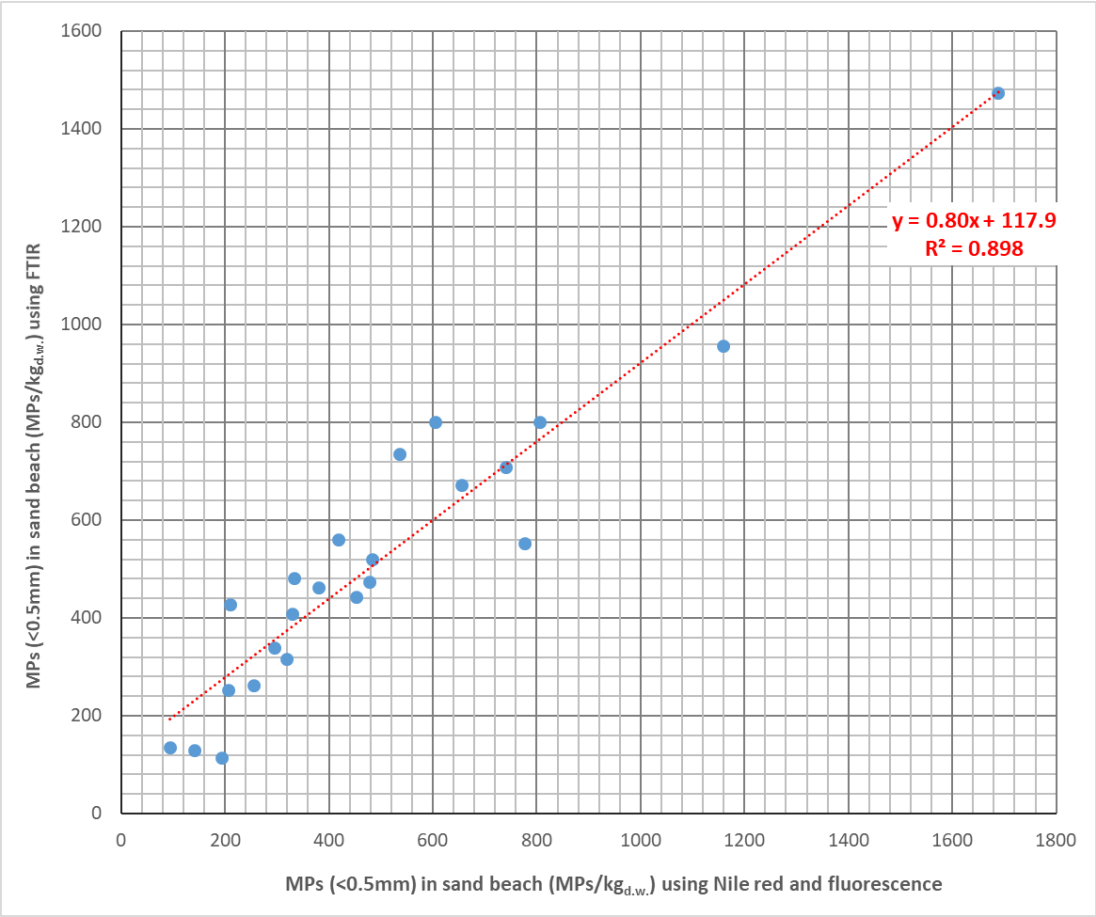

Figure 8. Levels of microplastic smaller tahn 0.5 mm in the samples (n=23) analyzed using Nile red plus fluorescence and FTIR.

In figures 9 and 10, it is shown fluorescent particles and fibres as well as µ-FTIR maps respectively.

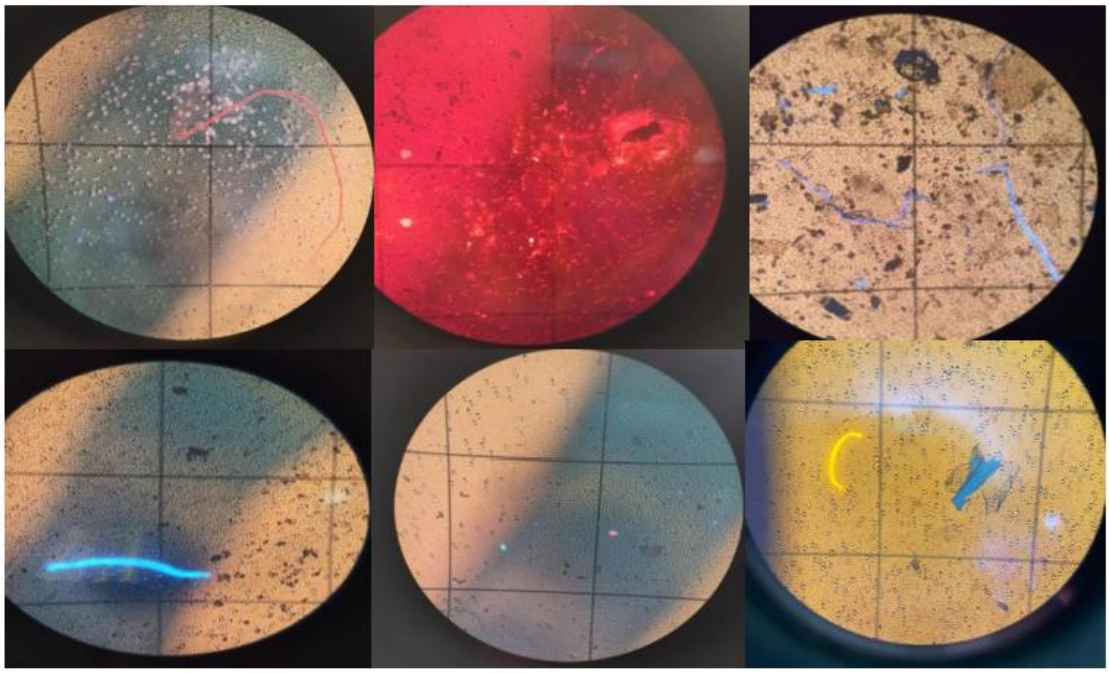

Figure 9 Fluorescence image for fibres and particles in MPs<0.5mm fraction

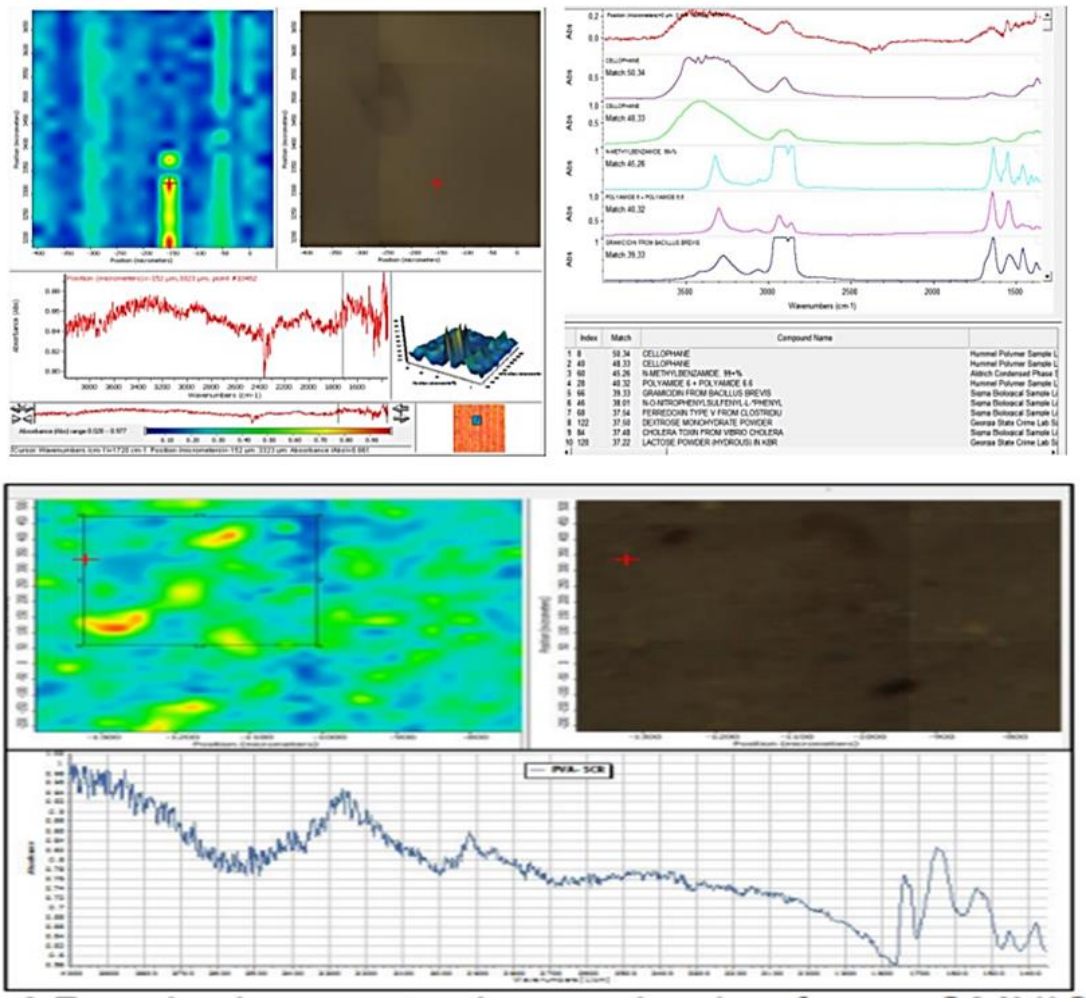

Figure 10. µFTIR maps particles and spectra in OMNIC PICTA Software for MPs<0.5mm fraction

The here presented Nile Red plus fluorescence technique use resulted a good, simply and fast approximation about MPs quantification for MPs<0.5 mm fraction in sandy beaches indicated by good correlations with FTIR values. However, researcher have found a maximum 100% overestimation of MP particles and not recommend this methodology alone for quantifying MPs in field samples (Shim et al., 2016; Stanton et al., 2019). For false positives elimination, in this study, is proposed an exhaustive register of Nile red's ability to identify different plastic and natural patterns types combined with MPs weathered from environmental samples, thus the fluorescence should be quantified for not to use subjective criteria. Furthermore, for high representativeness, fluorescence quantification should take in account: autofluorescence, as well as, detecting and registering changes in fluorescence intensity regarding dye concentration, solvent type, and temperature because staining effects depends on these factors (Hengstmann et al., 2018; Shim et al., 2016). In this way, it is obtained a "colour quantification" or "fingerprint database" for each wavelength filter (blue, green, red and UV) of a specific fluorescent microscope.

On the other hand, comparing with others worldwide studies, average values obtained for Catalonia and Barcelona North zone were similar to the average values from seventeen (17) studies in beaches of 200 MPs/kg reported by Harris et al., (2020). However, the other zones as Barcelona Southern, Tarragona and Catalonia Southern zone the average concentration were greater than 200 MPs/kg indicating possibly these zones are considered as hot spots for MPs pollution.

At the Mediterranean area currents arise, partly from the circulation created by the differences in temperature and salinity (thermohaline circulation) that flows from the northern (Gulf of Lion-France) to the southern in western Mediterranean Sea zone The Gulf of Lions itself is in fact a semi-circular continental shelf, so that most of the northern current flows as a major vein along the upper part of the continental slope by entire the Spanish coast to the border between the Alboran and the Algerian Sea, (CoCoNet Portal, 2022; El-Geziry and Bryden , 2010).The main permanent path from north to southern could be the responsible of the accumulation of MPs preferentially toward Catalonia Southern zone.

Comparison between anthropogenic pressures, beach morphology and meteorological parameters with MPs concentration.

In order to know what parameter is influencing MPs accumulation and distribution along Catalonia coast MPs concentration for both fractions, size, morphology type, sand grain and size distribution were related with beach morphological shoreline, facing to (orientation), slope to seawater, beach type, close anthropogenic pressures (number and type), appling a one-way ANOVA/Kruskal Wallis tests (with variance homogeneity previously assessed).

Parameters as: morphological shoreline and facing to (orientation), were stablished according criteria showed in tables from Table S3, S4 (supplementary information, Annexes), modified from López-Úbeda (2016), and GENCAT, (2022a), slope to seawater, beach type, close anthropogenic pressures (number and type) within a radius of 7-12 km were taken from GENCAT, (2022b), Table S5 to Table S9.

Finally, meteorological data referred to one day before the sampling date, however, due to duality in the data regarding current surface direction (coming from), wind direction (towards) and velocity, these parameters were not included in statistical analysis.

Significant differences between sand grain size and MPs concentration were observed, beaches with fine sand grain size presented MPs above 0.5 mm levels significant ($p<0.05$) higher than very coarse sand beaches. For MPs smaller than 0.5 mm there was significant differences between

concentration for fine and coarse sand ($p<0.05$, $p=0.03$) and fine and very coarse sand grain ($p<0.001$-0.012). Thus, beaches with fine sand showed higher concentration (575±460 MPs/kg) than coarse sand (251±209 MPs/kg) and in turn with very coarse sand (118±150 MPs/kg). In the same line, Maes et al. (2017) found a negative relationship between sediment grain size and the number of MPs in seabed sediments. It seems therefore that MPs accumulation could have marked pattern, thus MPs accumulation tends to increase towards to the south of the Catalonia coast. One of the factors as marine currents, which in this area of the Mediterranean Sea, predominate from north to the southern could promote the distribution and accumulation of MPs toward to the southern, and the other factors could be the fine sand grain size present mainly in southern Catalonia. Fine sand grain size enhances the retention of MPs particles and fibres. The MPs, fibres and particles can be deposited in this zone by touristic activity and by transportation from berm (backshore) and high tide line (previously deposited) or by water (buoyant particles and fibres deposited when waves hit the sand) and accumulate toward intertidal zone with a constant exchange of materials from water to sand and vice-versa . Bigger and small particles and fibres can accumulate in first layers if sand by fine grain size sand retain them.

Regarding beach morphology, no significant ($p>0.05$) differences were found between MPs levels of both sizes (≥0.5 mm or <0.5 mm) in enclosed, semi enclosed and open beaches. However, for MPs ≥ 0.5 mm, significant ($p<0.001$) higher levels of MPs fragments proportions were detected in enclosed beaches compared with open beaches. It is important to highlight that although there were not significant differences between open and enclosed regarding MPs<0.5 mm concentration, high MPs levels were found in enclosed beaches.

The anthropogenic pressures are defined as the principal sources of MePs (mesoplasticos) and MPs to seawater and includes mainly river discharges into ocean, direct or indirect WWTP discharges and poor waste management in coastal zones. One of the important sources are the river and little streams receiving runoff from roads, agricultural plastics, and from land fertilized with WWTP sludge. As well as, direct urban runoff (with tyre abrasion fragments and secondary microplastics) and WWTP discharges with microfibers for washing, personal care microspheres combined or not with urban runoff (Sun et al., 2019; UN Environment-Programme, 2020). Finally, poor waste management refers to non-controlled spillage, mismanagement of collection and classification of garbage that potentially are dragged or dumped into the sea.
No significant ($p>0.05$) differences were noted in MPs levels and morphology for both sizes regarding the presence of anthropogenic pressure (WWTP discharges, runoff, little streams, ports….), meteorological parameters (winds and currents) and characteristic of beaches (beach orientation or beach surroundings: urban, rural, and industrial, shoreline beach morphology). Touristic activity is common for all beaches of Catalonia Coast maybe the intensity and efficiency

of garbage management differs between beaches but it is difficult to measure this parameter in short term.

The findings of this study were in line to Bayo et al., (2019) in Mar Menor Lagoon (Spain) zone findings, thus, they found a higher concentration of MPs in urban beaches than in natural or semi-natural beaches, but without significant statistical differences.

Coastal areas have a complex hydrodynamic and interactions with river plumes, structures and currents, playing a significant role in microplastic distribution. Tables were elaborated with anthropogenic pressures, slope and shoreline morphology for beaches that showed low and high MPs concentrations (>150 MPs/kg) for<0.5 mm by each region (Annexes) and related with current and wind direction as well as facing to. Some factors influencing MPs distribution of those beaches could be:

1.-The proximity of emission sources and type: shallow WWTP discharges and wide distribution of plume, industrial port activities and big river mouth receiving WWTPs, urban and agriculture runoff.

2.- Touristic pressure

3.-Wind and local seawater surface currents direction (meteorological favourable parameters). Wind direction opposite to beach orientation (facing to) as well as local currents coming at the same direction to beach orientation are important factor for MPs accumulation.

4.-Beach shoreline morphologies, enclosed or semi-enclosed beaches combined with meteorological favoured parameter, anthropogenic pressures (proximity to big river discharges) and median-fine sand grain size could influence in MPs accumulation.

5.- Principal current in Catalonia coast from North toward Southern and its interaction with coast structures or geographical formations could be also influencing the transport and redistribution of MPs present faraway to the coast (e.g. deep WWTP discharges offshore or river big discharges plumes as Ebro or Besos Delta). Thus, floating plastic debris tends to be carried over vast distances by wind and surface currents and to settle or accumulate on shorelines (Eriksen et al., 2014; Galgani et al., 2015).

6.-Combination of favourable conditions and anthropogenic pressures in a large term

7.-Bathymetry and bottom characteristics of beaches, thus the presence of Posidonia seagrass dampens the strong effect of waves and currents, preventing coastal erosion and promoting

sedimentation and the retention of suspended solids, it is an effective protector against storms (Almela and Marbá, 2009).

8.-Heterogeneity of the coastline sedimentation and terrain slope along beach

Beaches are dynamic systems with ever-changing conditions. Results here reported indicate that distribution of MPs in Catalonia Coast is heterogeneous and vary spatially. In addition, it is probable also, temporal variation occurs with meteorological parameters, touristic activity and discharges plumes variation with seasonal periods. Constant et al., (2019) suggested that the spatial distribution of MPs (quantity) on Mediterranean beaches (which are microtidal coasts), can exhibit high heterogeneity at small scales and can change within a short time span (one month), further MPs concentrations depend on the sampling strategy.

Comparison between other Mediterranean Sea studies

MPs concentration are compared with results from other Mediterranean Sea regions. Catalonia coast as a zone with extensive and heterogeneous MPs distribution but with low pollution levels. The majority of these studies were realized in high tide line or backshore where the accumulation of microplastics and mesoplastics (potential origin of microplastics by fragmentation) is high and according with Galgani et al., (2015), MPs and mesoplastics often escape of clean-up operations. However, it was observed in intertidal zone of Slovenia and Algeria high concentrations, about tenfold of average found in this study.

The maximum MPs concentration obtained in present study was higher than those reported for different regions of Mediterranean Sea and Spain coast instead these values were from high tide line or backshore samples (Table 6). While average values were close to values found in Ebro Delta (Spain), Toscana (Italy), or Algeria (Cannas et al., 2017; Guerranti et al., 2017; Simon-Sanchez et al 2019; Tata et al., 2020). The high values of this study could be due to the extraction method, with $ZnCl_2$ versus NaCl. Most of the studies reviewed used NaCl (density 1.2 g/mL) for separation solution, which has certain limitations to separate the densest polymers (PET, PVC, etc). By contrast the solution of $ZnCl_2$ (density 1.8 g/mL) used in this study allows the separation of all types of polymers.

Other study performed in beaches in the Lyon Gulf, near to Catalonia North zone (Constant et al., 2019), found an average level ranging from 58±53 to165±205 MPs/kg dry sand with fibres predominance. These values were similar to those obtained in nearest beaches in present study, as PDV (Platja de la Vall -Cap de Creus) and El Port de la Selva (PPS), with values between 77 and 194 MP/kg However MPs presented different composition with PS as the most found polymer. Lots et al., (2017) found 148 ± 23 MPs/kg in Barcelona city beaches. Calculating the mean of the four beaches analysed in Barcelona region in present study, turned out in 169 ± 137

MPs/kg considering the MPs size range of 60 to 500 µm. In Catalonia southern zone, in Ebro Delta, Simon-Sanchez et al., (2019) found an average value of 422 ± 119 MP/kg with wide size range of <50-5000 µm. These results were similar than the reported in present work (576 ± 302 MP/kg and 661± 272 MP/kg according fluorescence and µ-FTIR methodologies, respectively). However, Simon-Sanchez et al., (2019) studied the high tide line and in these areas where the accumulation is high and the frequency of exchange with seawater is low compared with intertidal zone which can undergo great daily variations in terms of accumulation of MPs

According to Godoy et al., (2020), who also used fluorescence and FTIR as methodologies for MPs quantification, MPs concentration in Granada (Spain) beaches (22±23-45±25 MP/kg) were low. These could be due to sediment characteristics, which provide a gravel texture, a substrate very porous, not compact and easily for particle removal by waves' action. These difficult the MPs particles settling and remaining sometime in the beach and in consequence are not suitable for MPs retentions

Some researchers have stated that not only the eastern Mediterranean coasts present high MPs concentration. In Southern Mediterranean coasts, MPs were found at high concentrations. Thus, in Annaba Gulf- Algeria the concentration was 649±184 MPs/kg with fibre predominance (Tata et al., 2020). Furthermore, Grini et al., (2022), found for northern Algeria in intertidal zone concentrations ranging from 1.85±0.64 to 123±85 MPs/kg, for MPs sizes between 1-5 mm. Finally, in beaches of Lagoon Bizerte (Tunisia), MPs reach levels up to 18000 MPs/kg dry sand, the highest recorded value in Mediterranean regions.

Finally, and comparing with other worldwide zones, Claessens et al., (2011) found in intertidal line of three Belgium beaches a MPs concentration average ranging from 78 ±9.9 to 102±37 MPs/kg dry sand with fibres predominance, similar than the results obtained in present study.

In general, the most commonly found polymers in Mediterranean were PE, PP, PS, PET, PA, A: acrylic, PU, PMM and PVC: polyvinyl chloride. In present study in Catalonia beaches, polymers such as EVA, polyacrylate (PAC), PAN, polycarbonate (PC) and ABS were also found in low rates. By in contrast, in Turkey, Argelia and Valencia studies others polymers such as styrene-butadiene-styrene copolymer (SBS), ethylene propylene diene (EPD): and polyvinylidene dichloride (PVCD), Ethylene propylene, butyl Branham, Ethylene vinyl alcohol and polytetrafluoroethylene were. According production PE and PP account up to 50% of the production and most used polymers followed by PVC with 9.6% used in pipes and cable isolation, PET (8.4%) used in bottles, PU (7.8%) used in isolation foams, PS (6.1%) for packaging (PlascticEurope, 2021). According fibres composition, polyester (PES) was the most used fibre accounting for 52% of the global fibre market in 2020, followed by polyamide (PA) (nylon) with 5 % and acrylics 1.6 % (Preferred Fiber&Materials Market Report 2021; Britannica Science, 2022).

It is important highlight the variability of the methods used between studies makes it difficult to provide a reliable comparison on the level of pollution, morphology and the size range distribution of MPs from the references. Harmonization of protocols for monitoring microplastics in general sediments (sampling, extraction, identification, analytical methods and size fractions) is strongly recommended to ensure consistency of results. Thus, it is important taking in account the needed of methodologies for fast pollution evaluation and timely action for its mitigation or remediation.

Table 6. MPs abundance in different beaches from the Mediterranean area. An average sediment density of 1600 kg m−3 was used as per Kaberi et al., 2013 to convert units of volume or area to kg (16kg x 3cm thickness). NA: not analysed; NR: analysed but not reported; PE: Polyethylene; PP: Polypropylene; PS: Polystyrene; PET: Polyethylene terephthalate; PA: polyamide; PES: polyester; A: acrylic; PU: Polyurethane; EVA: Ethylene-vinyl acetate; PVC: Polyvinyl chloride BB: butyl Branham; EP: Ethylene propylene; CTA: Cellulose triacetate, PMM: poly(methyl methacrylate); PAC: polyacrylate; SBS: styrene-butadiene-styrene copolymer; EPD: ethylene propylene diene; PVC: polyvinyl chloride; PVCD: polyvinylidene dichloride; CEL: cellulose; PAM: polyacrylamide; PU: polyurethane, PVF: polyvinyl fluoride; EVOH: Ethylene vinyl alcohol; PTFE: polytetrafluoroethylene; PC: polycarbonate; AR: alkyd resin; PIB: polyisobutane * only pellets were analyzed.

Location	Sampled area	Separation method	Extractant, density solution	Size range analyzed (mm)	Identification method	Average concentration (MPs/kg d.w)	% fibres	Polymers	Reference
Kea island, Greece	Backshore	Sieving	-	1-4	Visual+IR	7.8±8.6 5.7± 7.0	NA	Pellet: 82%PE, 11%PP, 7%PET Fragments :71%PE, 24% PP, 5%PS	Kaberi et al 2013
Gulf of Trieste, Slovenia	Intertidal Backshore	Flotation + sieving	NaCl, 1.2 g/mL	0.25-5 <5	Visual	178 (backshore) 170 (intertidal)	85	NA	Laglbauer et al., 2014
South Tuscany, Italy	Shore	Sieving + flotation	NaCl, 1.2 g/mL	<5	Visual	402±100	NR	NA	Cannas et al., 2017
Maremma regional park, Italy	Shore	Sieving + flotation	NaCl, 1.2 g/mL	0.8-5	Visual	409±357	NR	NA	Guerranti et al., 2017
NW Adriatic sea, Italy	Backshore	Visual	-	1-5	Visual + FTIR	6-des	10	38% PE, 34% PP, 12% PA, 9% PS, 4% PET, 2% PVC, <1% PU	Munari et al., 2017
Cyprus	High tide line	Sieving	-	<5	Visual	21±23	NA	NR	Duncan et al., 2018
Crete, Greece	Backshore superficial	Flotation + peroxyde	NaCl, 1.2 g/mL	<0.05-5 0.5-5	Visual + FTIR	21±23	60	NR	Piperagkas et al., 2019
Ebro Delta, Spain	High tide line	Flotation	NaCl, 1.2 g/mL	1-5	Visual+ FTIR	422 ± 119	89	24% PA, 16% PE, 12% PMM, 12% PES, 8% PP, 4% PAC	Simon-Sanchez et al 2019
Salamina island, Greece	Backshore	Flotation	NaCl, 1.2 g/mL	0.3-5	Visual + FTIR	8±10	NA	Pellets + fragments PE, PS	Tziourrou et al., 2019
Datça peninsula, Turkey	High tide line	Sieving+ flotation+ Peroxide	NaCl, 1.2 g/mL	1-5	Visual + FTIR	1154±700	13	58% PE, 28% PS, 18% SBS, 12% PP, 12% EPD, 4% PVCD	Yabanli et al., 2019
Bizerte Lagoon , Tunisia	-	Flotation	NaCl, 1.40 g/mL	0.8-5	Visual	7960±6840	82-91	-	Abidli et al., 2017
Granada, Spain	Intertidal, High tide line Backshore	Flotation	NaCl, 1.2 g/mL	<0.1-5	Visual +Fluorescence+ FTIR	22±23-45±25	NA	Fragments, Microspheres PA, PP, PE, PET	Godoy et al., 2020
Tuscany, Italy	Backshore	Flotation	NaCl, 1,034g/mL	1-5	Visual + RAMAN	2.2±0.5 12±9.9	NA	76% PS, 15% PE, 5% PP, 4% others	Merlino et al., 2020
Gulf of Annaba, Algeria	Backshore	Flotation + sieving	NaCl, 1.2 g/mL	0.8-5	Visual + FTIR	405±246	57-83	48% PE, 16% PP, 14% PET, 9 %PS, 7% BB, 3% EP, 3% CTA	Tata et al., 2020

Iskenderun bay, Turkey	High tide line	Flotation+ sieving	Lithium methatungstate, 1,6 g/mL	0.3-5	Visual + FTIR	4.4-9.9*	NA	Pellets: 66% PE, 22%PP, 9% EPD	Gündogdu et al., 2022

Table 6 (continuation). MPs abundance in different beaches from the Mediterranean area. An average sediment density of 1600 kg m−3 was used as per Kaberi et al., 2013 to convert units of volume or area to kg (16kg x 3cm thickness). NA: not analysed; NR: analysed but not reported; PE: Polyethylene; PP: Polypropylene; PS: Polystyrene; PET: Polyethylene terephthalate; PA: polyamide; PES: polyester; A: acrylic; PU: Polyurethane; EVA: Ethylene-vinyl acetate; PVC: Polyvinyl chloride BB: butyl Branham; EP: Ethylene propylene; CTA: Cellulose triacetate, PMM: poly(methyl methacrylate); PAC: polyacrylate; SBS: styrene-butadiene-styrene copolymer; EPD: ethylene propylene diene; PVC: polyvinyl chloride; PVCD: polyvinylidene dichloride; CEL: cellulose; PAM: polyacrylamide; PU: polyurethane, PVF: polyvinyl fluoride; EVOH: Ethylene vinyl alcohol; PTFE: polytetrafluoroethylene; PC: polycarbonate; AR: alkyd resin; PIB: polyisobutane * only pellets were analyzed.

Location	Sampled area	Separation method	Extractant, density solution	Size range analyzed (mm)	Identification method	Average concentration (MPs/kg d.w)	% fibres	Polymers	Reference
Barcelona, Spain	High tide line	Flotation	NaCl, 1.2 g/mL	0.3-5	Visual + Raman	148 ± 23	98	PES, PP, PE, Dyes: Mortoperm blue, Hostaperm blue, Neozapon blue, Drimaren navy blue, Drimaren brilliant green, cobalt Phthalocyanine, Indigo	Lots et al., 2017
Cassis, France						124 ± 36			
Lido di Dante, Italy						1512± 187			
Dikili, Turkey						248±47			
Pilion, Greece						232±93			
Tel Aviv, Israel						168±16			
San Mauro Italy						76±13			
Bosnia						84±12			
Creta island, Greece	High tide line Backshore	Sieving	-	1-4	Visual	0.3-96	NA	NA Only pellets & fragments	Karkanorachaki et al., 2018
Lyon Gulf , France	Low, mid and upper line	Sieving + Flotation	NaCl, 1.2 g/mL	0.06-5	Visual + IR	165±205	59	21%PS, 20%PES, 20%A, 12%PE, 10%PP, <5% other polymers	Constant et al., 2019
Tarragona, Spain	Intertidal	Sieving	-	0.5-5	Visual + FTIR	11±18	13	Particles: 47% PE, 36% PP, 6% PET, 6% PS, 3% Additives, 1% PAM, 0.5% Silicone, "% others Fibres: 45% PES, 33% PA, 22% synthetic cellulose	Expósito et al., 2021
Valencia, Spain	High tide line	Flotation	NaCl, 1.2 g/mL	<0.2-5	Visual + FTIR	58±14	68	42% PE, 14% Rubber, 10% Latex, 9% PP, 5% CEL, 4% EVOH, 4% HDPE, 3% PMM, 3% PAM, 2%PU, 1%PS, 1% PES, 1% PVF	Novillo-Sanjuan et al., 2021
Catalan coast, Spain	Intertidal	Sieving	-	0.5-5	Visual+ FTIR	7.2±11.7	28	56% PE, 24% PP, 10% PS, 4% PET, 1% PA, 1 % (PU, EVA, PVC), <1% (PC, ABS, PMM, AR, A)	Present study
Catalan coast, Spain		Sieving + Flotation + Fenton	$ZnCl_2$, 1.8g/mL	0.06-5	Flurorescence	388 ± 367	70	**Particles**: 48% PE, 23% PP, 7% PET, 7% PVC, 6% PS, 2% PU, 2%	Present study

Catalan coast, Spain					µFTIR	522 ± 307 (Both particles and fibres)	63	AR, 1% EVA, 1% PIB, 1% PTFE, 1% PMM, 1% other **Fibres**: 38% PES, 24% PA, 23% PP, 8% PAN, 6% PE	

1.3.4 Conclusions

This study represents the first assessment of MPs pollution in beach sediments along entire Catalonia coastline (>500km). MPs were present in almost all sampling points with a high variability between beaches and within beaches sampling sites. The highest MPs pollution is registred in southern part of Catalonia (South Barcelona, Tarragona and South Catalonia zones). These data are useful to define baselines for MPs in the Western Mediterranean region. MPs hotspot (pellets and fibres) were detected in beaches close to petrochemical industry and with high anthropogenic pressure and low water removal.

Fibres predominated in fractions MPs<0.5 mm and fragments in MPs≥0.5 mm. Pellets, although in low proportion, were abundant in beaches near to industrialized areas in Tarragona zone (Pineda beach) and near river discharges.

Regarding particles (fragments, films, pellets and foam) composition, for both fractions majority polymers were PE, PP, PET and PS. PVC appeared abundant only in particles MPs<0.5 mm fraction. For fibres, the majority composition was polyester, polyamide, PP and PAN. All of these polymers are common constituents of packaging, bags, bottles, caps, pipes, cable coating and textiles (clothing and furniture) products.

Shallow WWTP discharges, wide distribution of plume, direct or through river WWTP discharges, industrial port activities, urban and agriculture runoff combined with fine size grain sand, wind direction opposite to beach orientation (facing to) as well as local currents coming at the same direction to beach orientation are important factor for MPs accumulation.

Fluorescence staining methodology provided good approximations regarding MPs quantification as long as organic matter be totally removed and taking in account fibres and particles ≥60 μm. For accurate results, the fluorescence must be quantified depending on the Nile Red properties and behaviour in different solvent, temperature and wavelength excitation. For fast hot spot establishment, it is recommended an exploratory MPs concentration with Nile Red staining, confirmation the results with spectroscopy techniques (FTIR) and continuous monitoring with Nile Red for saving time and cost in mitigation application measures.

Further research is recommended to confirm the influence of local marine currents and wind direction on the transport and accumulation of MPs from near and far sources as well as coastal sedimentation dynamics evaluation in order to identify MPs pattern distribution along the beaches of Catalonia coast.

References

Abidli, S., Toumi, H., Lahbib, Y., El Menif, N.T., 2017. The first evaluation of microplastics in sediments from the complex lagoon-channelof Bizerte (Northern Tunisia). Water Air Soil Pollut. 228, 262.

Aljaibachi, R, Callaghan, A., 2018. Impact of polystyrene microplastics on Daphnia magna mortality and reproduction in relation to food availability. PeerJ. 6, e4601.

Almela, E., Marbá, N., 2009. *Posidonia oceanica*. 1120 praderas *P. oceanica*. Gobierno de España, Ministerio de Medio Ambiente y Medio Rural y Marino. 129 pp

Barboza, L.G.A., Dick Vethaak, A., Lavorante, B.R.B.O., Lundebye, A.K., Guilhermino, L., 2018. Marine microplastic debris: An emerging issue for food security, food safety and human health. Mar Pollut Bull. 133, 336-348.

Bayo, J., Rojo, D., Olmos, S., 2019. Abundance, morphology and chemical composition of microplastics in sand and sediments from a protected coastal area: The Mar Menor lagoon (SE Spain). Environ. Pollut. 252, 1357-1366.

Bergmann, M., Gutow, L., Klages, M. (Eds), 2015. Marine anthropogenic litter. Springer Cham, XVIII, 447 pp

Britannica Science, 2022 https://www.britannica.com/science/polyacrylonitrile

Cabezas-Rabadán, Carlos, Josep E. Pardo-Pascual, and Jesus Palomar-Vázquez. 2021. Characterizing the Relationship between the sediment grain size and the shoreline variability defined from sentinel-2 derived shorelines. Remote Sens. 13(14), 2829.

Cannas, S., Fastelli. P., Guerranti, C., Renzi, M., 2017. Plastic litter in sediments from the coasts of south Tuscany (Tyrrhenian Sea). Mar. Pollut. Bull. 119, 372-375.

CEDEX, 2019. Microparticle Monitoring Program on Beaches (BM-6). Technical Report for the Ministry for the Ecological Transition and the Demographic Challenge, pp. 211.

Claessens, M., Meester, S.D., Landuyt, L.V., Clerck, K.D., Janssen, C.R., 2011. Occurrence and distribution of microplastics in marine sediments along the Belgian coast. Mar. Pollut. Bull. 62(10), 2199-2204.

CoCoNet Portal 2022,

Compa, M.,Ventero, A., Iglesias, M., Deudero, S., 2018. Ingestion of microplastics and natural fibres in *Sardina pilchardus* (Walbaum, 1792) and *Engraulis encrasicolus* (Linnaeus, 1758) along the Spanish Mediterranean coast. Mar. Pollut. Bull. 128, 89-96.

Constant, M., Kerhervé, P., Mino-Vercellio-Verollet, M., Dumontier, M., Sànchez Vidal, A., Canals, M., Heussner, S., 2019. Beached microplastics in the Northwestern Mediterranean Sea. Mar. Pollut. Bull. 142, 263–273

Cozar, A., Sanz-Martín, M., Martí, E., González-Gordillo, J.I., Ubeda, B., Gálvez, Á., Irigoien, J., Duarte, C.M., 2015. Plastic accumulation in the Mediterranean Sea. PLoS One 10, 1–12

de Lucia, G.A., Caliani, I., Marra, S., Camedda, A., Coppa, S., Alcaro, L., Campani, T., Giannetti, M., Coppola, D., Cicero, A.M., Panti, C., Baini, M., Guerranti, C., Marsili, L., Massaro, G., Fossi, M.C., Matiddi, M., 2014. Amount and distribution of neustonic micro-plastic off the Western Sardinian coast (Central-Western Mediterranean Sea). Mar. Environ. Res. 100, 10-16.

Détrée, C., Gallardo-Escárate, C., 2018. Single and repetitive microplastics exposures induce immune system modulation and homeostasis alteration in the edible mussel Mytilus galloprovincialis. Fish Shellfish Immunol. 83, 52-60.

Duncan, E.M., Arrowsmith, J., Bain, C., Broderick, A.C., Lee, J., Metcalfe, K., Pikesley, S.K., Snape, R.T.E., van Sebille, E., Godley, B.J., 2018. The true depth of the Mediterranean plastic problem: Extreme microplastic pollution on marine turtle nesting beaches in Cyprus. Mar. Pollut. Bull. 136, 334-340.

El-Geziry, T.M., Bryden, I.G., 2010. The circulation pattern in the Mediterranean Sea: issues for modeller consideration. J. Oper. Oceanogr. 3(2), 39-46.

Enders, K., Lenz, R., Stedmon, C.A., Nielsen, T.G., 2015. Abundance, size and polymer composition of marine microplastics ≥10 mm in the Atlantic Ocean and their modeled vertical distribution. Mar. Pollut. Bull. 100(1), 70-81.

Enders, K., Lenz, R., Stedmon, C.A., Nielsen, T.G., 2015. Abundance, size and polymer

composition of marine microplastics ≥10μm in the Atlantic Ocean and their modelled vertical distribution. Mar. Pollut. Bull. 100(1), 70-81.

Eriksen, M., Lebreton, H.S. Carson, M. Thiel, C.J. Moore, J.C. Borerro, F. Galgani, P.G. Ryan, J. Reisser, 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLoS One 9(12), e111913.

Erni-Cassola, G., Gibson, M. I., Thompson, R. C., & Christie-Oleza, J. A. ,2017. Lost, but found with Nile Red: a novel method for detecting and quantifying small microplastics (1 mm to 20 μm) in environmental samples. Environ. Sci. Technol. 51(23),13641-13648.

Expósito, N., Rovira, J., Sierra, J., Folch, J., Schuhmacher, M., 2021. Microplastics levels, size, morphology and composition in marine water, sediments and sand beaches. Case study of Tarragona coast (western Mediterranean). Sci Total Environ. 786, 147453.

Filgueiras, A.V., Gago, J., Campillo, J.A., León, V.M., 2019. Microplastic distribution in surface sediments along the Spanish Mediterranean continental shelf. Environ. Sci. Pollut. Res. 26(21), 21264-21273.

Frias, J., Pagter, E., Nash, R., O'Connor, I., Carretero, O., Filgueiras, A., Viñas, L., Gago, J. Antunes, J., Bessa, F., et al., 2018. Standardised protocol for monitoring microplastics in sediments. JPI-Oceans BASEMAN project. 23 p.

Galgani, F., Hanke, G., Maes, T., 2015. Global distribution, composition and abundance of marine litter (29-56 pp.). In: Bergmann, M., Gutow, L., Klages, M. (Eds). Marine anthropogenic litter. Springer, Cham.

Gencat, 2022a.. https://acanet.gencat.cat/geco/platges/perfilszonabany.asp. Last accesed September 2022

Gencat, 2022b. https://aca.gencat.cat/es/laigua/el-medi-hidric-a-catalunya/aiguees-de-bany/. Last accesed September 2022

Godoy, V., Prata, J.C., Blázquez, G., Almendros, A.I., Duarte, A.C., Rocha-Santos, T., Calero, M. and Martín-Lara, M.A., 2020. Effects of distance to the sea and geomorphological characteristics on the quantity and distribution of microplastics in beach sediments of Granada (Spain). Sci. Total Environ. 746, 142023.

González-Fernández, D., Hanke, G., 2020. Monitoring Approaches for Marine Litter in the European Sea Basins. In: Stock, F., Reifferscheid, G., Brennholt, N., Kostianaia, E. (eds) Plastics in the Aquatic Environment - Part II. The Handbook of Environmental Chemistry, vol 112. Springer, Cham, pp. 139-156.

Grini, H., Metallaoui, S., González-Fernández, D., Bensouilah, M., 2022. First evidence of plastic pollution in beach sediments of the Skikda coast (northeast of Algeria). Mar. Pollut. Bull. 181, 113831.

Guerranti, C., Cannas, S., Scopetani, C., Fastelli, P., Cincinelli, A., Renzi, M., 2017. Plastic litter in aquatic environments of Maremma Regional Park (Tyrrhenian Sea, Italy): Contribution by the Ombrone river and levels in marine sediments. Mar. Pollut. Bull. 117(1-2), 366-370.

Gündoğdu, S., Ayat, B., Aydoğan, B., Çevik, C., Karaca, S., 2022. Hydrometeorological assessments of the transport of microplastic pellets in the Eastern Mediterranean. Sci. Total Environ. 823, 153676.

Harris, P.T., 2020. The fate of microplastic in marine sedimentary environments: A review and synthesis. Mar. Pollut. Bull. 158, 111398 .

Hengstmann, E., Tamminga, M., Vom Bruch, C., Fischer, E.K., 2018. Microplastic in beach sediments of the Isle of Rügen (Baltic Sea)-Implementing a novel glass elutriation column. Mar. Pollut. Bull. 126, 263-274.

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: A review of the methods used for identification and quantification. Environ. Sci. Technol. 46, 3060-3075..

http://gismarblack.bo.ismar.cnr.it:8080/mokaApp/apps/COCOV3H5/index.html?null Last accesed September 2022

https://textileexchange.org/app/uploads/2021/08/Textile-Exchange_Preferred-Fiber-and-Materials-Market-Report_2021.pdf . Last accesed December 2022

https://www.britannica.com/science/polyacrylonitrile

Huerta Lwanga, E., Thapa, B., Yang, X., Gertsen, H., Salánki, T., Geissen, V., Garbeva, P., 2017.

Decay of low-density polyethylene by bacteria extracted from earthworm's guts: A potential for soil restoration. Sci. Total Environ. 624, 753-757.

Isobe, A., Kubo, K., Tamura, Y., Kako, S., Nakashima, E., Fujii, N., 2014. Selective transport of microplastics and mesoplastics by drifting in coastal waters. Mar. Pollut. Bull. 89(1-2), 324-330.

Kaandorp, M.L.A., Dijkstra, H.A., Van Sebille, E., 2020. Closing the mediterranean marine floating plastic mass budget: inverse modelling of sources and sinks. Environ. Sci. Technol. 54(19), 11980-11989.

Kaberi, H., Zeri, C., Mousdis, G., Papadopoulos, A., Streftaris, N., 2013. Microplastics along the shoreline of a Greek island (Kea isl., Aegean Sea): types and densities in relation to beach orientation, characteristics and proximity to sources Proc. 4th Int. Conf. Environ. Manag. Eng. Plan. Econ. SECOTOX Conf. Mykonos island, Greece, pp. 197-202.

Karami, A., Golieskardi, A., Choo, C.K., Romano, N., Ho, Y.B., Salamatinia, B., 2017. A high-performance protocol for extraction of microplastics in fish. Sci. Total. Environ. 578, 485-494.

Karkanorachaki, K., Kiparissis, S., Kalogerakis, G.C., Yiantzi, E., Psillakis, E., Kalogerakis, N., 2018. Plastic pellets, meso- and microplastics on the coastline of Northern Crete: Distribution and organic pollution. Mar. Pollut. Bull. 133, 578-589.

Khatmullina, L., Isachenko, I., 2017. Settling velocity of microplastic particles of regular shapes. Mar. Pollut. Bull. 114(2), 871-880.

Laglbauer, B.J.L., Franco-Santos, R.M., Andreu-Cazenave, M., Brunelli, L., Papadatou, M., Palatinus, A., Grego, M., Deprez, T., 2014. Macrodebris and microplastics from beaches in Slovenia. Mar. Pollut. Bull. 89, 356-366

Liubartseva, S., Coppini, G., Lecci, R., Clementi, E., 2018. Tracking plastics in the Mediterranean: 2D Lagrangian model. Mar. Pollut. Bull. 129(1), 151-162.

Lots, F.A.E., Behrens, P., Vijver, M.G., Horton, A.A., Bosker, T, 2017. A large-scale investigation of microplastic contamination: abundance and characteristics of microplastics in European beach sediment. Mar. Pollut. Bull. 123(1-2), 219-226.

Maes, T., Jessop, R., Wellner, N., Haupt, K., Mayes, A.G., 2017. A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. Sci. Rep. 7, 44501.

Mao, Y., Ai, H., Chen, Y., Zhang, Z., Zeng, P., Kang, L., Li, W., Gu, W., He, Q., Li, H., 2018. Phytoplankton response to polystyrene microplastics: Perspective from an entire growth period. Chemosphere 208, 59-68.

Masura, J., Baker, J., Foster, G. and Arthur, C., 2015. Laboratory methods for the analysis of microplastics in the marine environment: recommendations for quantifying synthetic particles in waters and sediments. Siver Spring, MD, NOAA Marine Debris Division, 31pp. (NOAA Technical Memorandum NOS-OR&R-48).

Merlino, S., Locritani, M., Bernardi, G., Como, C., Legnaioli, S., Palleschi, V., Abbate, M., 2020. Spatial and temporal distribution of chemically characterized microplastics within the protected area of Pelagos Sanctuary (NW Mediterranean Sea): Focus on natural and urban beaches. Water 12(12) 3389.

Munari, C., Scoponi, M., Mistri, M., 2017. Plastic debris in the Mediterranean Sea: types, occurrence and distribution along Adriatic shorelines. Waste Manage. 67, 385-391.

Naji, A., Esmaili, Z., Mason, S.A., Dick Vethaak, A., 2017. The occurrence of microplastic contamination in littoral sediments of the Persian Gulf, Iran. Environ. Sci. Pollut. Res. 24, 20459-20468.

Novillo, O., Raga, J.A., Tomás, J., 2021. Microdebris in three Spanish Mediterranean beaches located at a sporadic loggerhead turtles' (Caretta caretta) nesting area. Reg. Stud. Mar. Sci. 49, 102116.

Piperagkas, O., Papageorgiou, N., Karakassis, I., 2019. Qualitative and quantitative assessment of microplastics in three sandy Mediterranean beaches, including different methodological approaches. Estuar. Coast. Shelf Sci. 219, 169-175.

Plastics Europe 2021, Market Research Group (PEMRG) and Conversio Market & Strategy GmbH. Demand estimations do not include recycled plastics.

Prata, J.C.; da Costa, J.P.; Duarte, A.C.; Rocha-Santos, T.,2019. Methods for sampling and detection of microplastics in water and sediment: A critical review. TrAC Trends Anal. Chem., 110, 150–159.

Preferred Fiber&Materials Market Report 2021.

Rist, S., Hartmann, N.B., 2018b. Aquatic Ecotoxicity of Micro plastics and Nanoplastics: Lessons Learned from Engineered Nanomaterials. In: Wagner M., Lambert S. (eds) Freshwater Microplastics. The Handbook of Environmental Chemistry, vol 58. Springer, Cham.

Rocha-Santos, T.A., Duarte, A.C., (Eds), 2017. Characterization and analysis of microplastics (1st Ed.) Elsevier, 286 pp.

Rusch, A., Huettel, M., Forster, S., 2000. Particulate organic matter in permeable marine sands—Dynamics in time and depth. Estuar. Coast. Shelf Sci. 51(4), 399-414.

Savoca, S., Capillo, G., Mancuso, M., Bottari, T., Crupi, R., Branca, C., Romano, V., Faggio, C., D'Angelo, G., Spanò, N., 2019. Microplastics occurrence in the Tyrrhenian waters and in the gastrointestinal tract of two congener species of seabreams. Environ. Toxicol. Pharmacol. 67, 35-41.

Shim, W.J., Song, Y.K., Hong, S.H., Jang, M., 2016. Identification and quantification of microplastics using Nile red staining. Mar. Pollut. Bull. 113(1-2), 469-476.

Simon-Sánchez, L., Grelaud, M., Franci, M., Ziveri, P., 2022. Are research methods shaping our understanding of microplastic pollution? A literature review on the seawater and sediment bodies of the Mediterranean Sea. Environ. Pollut. 292, 118275.

Simon-Sánchez, L., Grelaud, M., Garcia-Orellana, J., Ziveri, P., 2019. River Deltas as hotspots of microplastic accumulation: The case study of the Ebro River (NW Mediterranean). Sci. Total Environ. 687, 1186-1196.

Stanton, T., Johnson, M., Nathanail, P., Gomes, R.L., Needham, T., Burson, A., 2019. Exploring the efficacy of Nile red in microplastic quantification: A costaining approach. Environ. Sci. Technol. Lett. 6(10), 606-611.

Sturm, M. T., Horn, H., Schuhen, K., 2021. The potential of fluorescent dyes-comparative study of Nile red and three derivatives for the detection of microplastics. Analytical and Bioanalytical Chemistry, 413(4), 1059-1071.

Tata, T., Belabed, B.E., Bououdina, M., Bellucci, S., 2020. Occurrence and characterization of surface sediment microplastics and litter from North African coasts of Mediterranean Sea: preliminary research and first evidence. Sci. Total Environ. 713, 136664.

Tiwari, M., Rathod, T.D., Ajmal, P.Y., Bhangare, R.C., Sahu, S.K., 2019. Distribution and characterization of microplastics in beach sand from three different Indian coastal environments. Mar. Pollut. Bull. 140, 262-273.

Tziourrou, P., Megalovasilis, P., Tsounia, M., Karapanagioti, H.K., 2019. Characteristics of microplastics on two beaches affected by different land uses in Salamina Island in Saronikos Gulf, east Mediterranean. Mar. Pollut. Bull. 149, 110531.

Ubeda, L.I., 2016. Clasificación morfológica de las playas y modelado del perfil tansversal en Valencia, Alicante, Murcia. TESIS Universidad de Alicante. 170 pp

UN Environment Programn (UNEP), 2020. Water Pollution by Plastics and Microplastics: A review of thecnical solutions from source to sea. IWMI. 112 pp.

Wang, Z., Su, B., Xu, X., Di, D., Huang, H., Mei, K., Dahlgren, R.A., Zhang, M., Shang, X., 2018. Preferential accumulation of small (< 300 μm) microplastics in the sediments of a coastal plain river network in eastern China. Water Res. 144, 393-401.

Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy, B.E., Thompson R.C., 2014. The deep sea is a major sink for microplastic debris. R. Soc. Open Sci. 1(4), 140317.

Yabanli, M., Yozukmaz, A., Şener, İ., Tokul Ölmez, Ö.T., 2019. Microplastic pollution at the intersection of the Aegean and Mediterranean Seas: A study of the Datça Peninsula (Turkey). . Mar. Pollut. Bull. 145. 47-55.

Zambianchi, E., Trani, M., Falco, P., 2017. Lagrangian transport of marine litter in the Mediterranean Sea. Front. Environ. Sci. 5, 5.

Zhao, S., Zhu, L., Li, D., 2016. Microscopic anthropogenic litter in terrestrial birds from Shanghai, China: Not only plastics but also natural fibers. Sci. Total Environ. 550, 1110-

1115.

CHAPTER 2-MICROSPLASTICS IN BIOTA

Levels of microplastics and their characteristics in molluscs from North-West Mediterranean Sea: Human intake

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Abstract

Microplastics (MPs) are accessible for organisms with active filter feeding strategies, as are many marine molluscs, which live attached or semi-buried in sediments. In the present study, MPs (from 0.02 to 5 mm) concentration, morphology, and composition were determined in consumed mollusc species of the Catalan coast (NW Mediterranean Sea). Microplastic concentrations, morphologic characteristics and composition were studied according to species, catchment zones and depuration condition. Finally, human intake of MPs through molluscs' consumption was determined. More than 2300 individuals were analysed, being 1460 MPs extracted and their size, and polymeric composition registered. Big oysters and mussels showed the highest MPs concentration by individual, with levels of 22.8±14.4 and 18.6±23.0 MPs/individual, respectively. Mean annual MPs (≥20μm) consumption for adult population was estimated in 8,103 MPs/year, with a 95th percentile of 19,418 MPs/year. It suggests that the consumption of molluscs is an important route of MPs exposure for the Catalan population.

Keywords: Plastic pollution, mussels, seafood, human exposure, Catalonia (Spain)

2.1 Introduction

Microplastics (MPs) are ubiquitous in all environmental marine compartments (Expósito et al., 2021; Farady et al., 2019). Due to their small size, they are accessible for organisms with active filter feeding strategies as many marine molluscs, which live attached or semi-buried in sediments (Carbery et al., 2018; Xiong et al., 2019; Zhao et al., 2018). After active water filtration, plastic, as well as natural particles (inorganic and organic), are trapped in the mollusc's gill filaments according to their pore size. When an amount of food load is reached, a pre-ingestion selection is made, with labial palps selecting or rejecting part of total filtered particles. Digestion occurs in digestive glandule or in intestine for smallest or largest particles, respectively (Ward et al., 2019a). Intake of different MPs shapes (fragments, beads, fibres, films) by molluscs have been demonstrated (Li et al., 2016; Wegner et al., 2012). In turn, those contaminants present in water are absorbed in the MPs, which are then transferred to aquatic organisms (Kolandhasamy et al., 2018; Li et al 2019). On the other hand, according to EFSA (2016), the processing and packaging of seafood can be another source of contamination for MPs.

Bivalves are responsible for a large part of human exposure to MPs because they filter a large volume of seawater, being able to accumulate MPs from the environment (Cho et al., 2019). In relation to human exposure through consumption of aquatic species, it will depend on the part of which is consumed. It has been observed that most MPs are retained in the digestive tract and the gills (Carbery et al., 2018).

Microplastic less than 150 μm in diameter have the potential to translocate into human tissues, trigger a localized immune response (EFSA, 2016; Prata et al., 2020; Wright and Kelly, 2017). Animal studies and "*in vitro*" assays in human cells lines have shown possible adverse effects from exposure to nano and MPs (oxidative stress and structural damages, reproductive endocrine disruption system in fishes, as well as toxicity in gastrointestinal, liver and neuronal system) (Chang et al., 2020; Prüst et al.,2020; Wang et al., 2019a; 2019b). Gene expression alteration and genotoxicity, as well as changes in gut microbiota -with seafood being a major medium of exposure- have been also observed (van Raamsdonk et al., 2020; Wang et al., 2020). Although the knowledge regarding toxicity of MPs in humans is still limited, it has been established that the toxicity is primarily influenced by exposure concentration, particle components, adsorbed contaminants, organs involved, and individual susceptibility (Rahman et al., 2021). For this reason, to date neither safety threshold, nor tolerable daily intake, have been established and the risk to humans from dietary exposure must be still assessed.

On the other hand, in recent years studies on MPs in a wide range of marine molluscs, including commercial and wild bivalves, have been conducted (Abidli et al 2019; Ding et al.,2020; Ding et al.,2021; Li et al., 2015; Reguera et al., 2019; Rochman et al., 2015; Van Cauwenberghe and

Janssen, 2014; Vandermeersch et al., 2015; Ward et al., 2019b). However, only few surveys were representative of wide groups of autochthonous molluscs.

Few studies have evaluated the Estimated Daily Intake (EDI) by autochthonous bivalves and own national surveys about molluscs population consumption. Cho et al., (2019), estimated the annual dietary intake of MPs (MPs/year/person) by Korean population via shellfish consumption based on a national's examination surveys about ingestion rates and MPs analysis of the top four autochthonous commercial bivalve species. Cox et al., (2019), calculated EDI (MPs/day/person) for seafood (fishes and shellfish) combined with others food items including inhalation, focusing on the American caloric intake in relation to their recommended daily intake and the average of number of microplastic particles in food obtained from others fourteen studies.

Danopoulos et al., (2020), estimated EDI as MPs/person/year combining global consumption estimates (FAO, 2020) with the data from outcomes of the statistical summary of MPs in the global seafood samples and conclude that seafood is a major verified vector for human exposure to MPs. Ferrante et al., (2022), considered EDI (MPs/day/kg body weight) for adults and children of authoctonous mussel *Mytilus galloprovincialis* and fishes separately for MPs less to 3 µm from south coast of Mediterranean Sea based on global consumption estimates.

There are also few studies regarding MPs levels in molluscs from the Mediterranean Sea, and especially from the NW Mediterranean coastal area. Catalonia (NE Spain) is an important producer and consumer of molluscs. For that reason, the current investigation was focused on MPs concentrations estimations of commercially relevant molluscs from the Catalan coast (NW Mediterranean Sea). The present study is a first step for estimating the MPs intakes through seafood consumption in Spain. Our study was aimed at: i) to develop an to optimize a methodology for MPs extraction; ii) to determine the concentration, morphology, and composition of MPs, in various highly consumed molluscs' species; iii) to compare MPs concentration and characteristics between species, catchment zones and depuration condition, and finally iv) to establish MPs intakes through molluscs consumption by the Catalan population.

2.2 Materials and Methods

Since the existing protocols for the determination of MPs in molluscs were not adequate, we developed a new protocol, which was adapted to the requirements of the current study. The present protocol was adapted from those reported by various authors (Catarino et al., 2018; Cho et al., 2019; Courtene-Jones et al., 2017; Fang et al., 2019; Jahan et al., 2019; Karami et al., 2017; Li et al., 2015, 2018; Masura et al., 2015; Xu et al., 2020; Wu et al., 2020). The optimized methodology comprises higher sample weight and individuals' number, whole organism treatment, and the use of enzymes for the digestion of specific substrates.

2.2.1 Sampling

Six molluscs' species were selected among those most widely and frequently consumed by the Catalan population. Wedge clams, Donax trunculus (WC), marine snail, Bolinus brandaris (S), razor clams Ensis siliqua (RC), fine clams Tapes decussatus (FC), big oyster Crassostrea gigas (BO) and mussels Mytilus galloprovincialis (M) were included in this survey. Molluscs' species were sampled in October-November 2017 from biggest anglers' cooperatives along the Catalan coast (377 km). The Catalan areas selected for the study were the following: Barcelona (Central), Girona (North) and Tarragona (South). Different sea zones of catchment of North West Mediterranean (FAO 37-1) (GENCAT, 2021) and depuration grade in molluscs' samples were considered (Table 1; Figure 1), (see supplementary materials for coordinates, environmental MPs concentration and anthropogenic activities associated in each sub-zones Table A2).

Table 1. Molluscs samples description

Code	Name	Specie	Depuration	Catchment Zone	Habitat	Weight (g_{ww}/individual)	Nº samples (Total individuals)
WCnd(S)	Wedge clams	*Donax trunculus*	No	South	Suspension/filter feeder Infaunal	0.263	2 (383)
WCd(S)			48 h	South		0.263	2 (387)
WC(N)			NR	North		0.306	2 (373)
WC(C)			NR	Central		0.219	2 (489)
S(S)	Snails	*Bolinus brandaris*	NR	south	Carnivorus, Depredator Epifaunal	3.53	4 (68)
S(C)			NR	Central		5.42	5 (55)
RCnd(S)	Razor clams	*Ensis siliqua*	No	South	Suspension/filter feeder Infaunal	5.11	6 (59)
FCd(S)	Fine Clams	*Tapes decussatus*	<24 h	South	Suspension/filter feeder Infaunal	2.09	3 (74)
BOnd(S)	Big Oyster	*Crassostrea gigas*	No	South	Suspension/filter feeder Epifaunal	12.3	4 (19)
BOd(S)			>72 h	South		9.78	5 (28)
Md(S)	Mussel	*Mytilus galloprovincialis*	48 h	South	Suspension/filter feeder Epifaunal	2.37	4 (88)
Mnd(S)			No	South		3.37	4 (62)
Mnd(N)			NO	North		2.90	11 (223)

NR: Not reported

Figure 1. Sampling areas and molluscs sampled in the Catalan coast (Spain). WC: wedge clams, BO: big oyster, S: snail, RC: razor clams, M: mussels, FC: fine clams.

2.2.2 Samples processing

Between 2 and 4 kg of each sample type of molluscs were purchased and acquired by donation fisherman cooperatives. The samples were wrapped in aluminium foils and kept refrigerated during the transport to the laboratory. Subsequently, samples were rinsed with ultrapure water (distillate water filtrate to 0.45 µm through filters), being external byssus threads removed. For representability, more than 50 individuals were analysed for each sample of mussels, razor clams, fine clams, wedge clams, and snail according to depuration conditions and catchment zones (Dehaut et al., 2018). In turn, up to 28 individuals were analysed for oysters. A number of soft tissues sub-samples (50-70 g) were prepared for analysis, being these sub-samples lyophilized (Telstar Cryodos-50/80) during a period of 48 h in a clean and fibre free beaker. All sub-samples were kept at -20 °C until analysis.

2.2.3 Microplastics extractions

A general protocol for MPs ≥ 20µm extraction in 50-70 g sub-samples of soft tissues was partially -or completely- applied, depending on molluscs sample characteristics. The removal of organic and inorganic matter from the sample was carried out by three phases and an additional density separation step (Figure A1, supplementary materials). The first phase required 4 days for complete procedure, while the second one 8 days, and the third one 4 days, with a total of 16 days. The density separation step took one additional day.

The first phase (**KS**) is a combination of alkaline hydrolysis (KOH 2M) (**K**) and surfactants (SDS 10% (**S**) at 40ºC with agitation. The second phase (**E+H**), consisted in enzymatic hydrolysis with protease, lipases, and celluloses (**E**), followed by an oxidation with hydrogen peroxide 33-35 % (**H**), at 40ºC and gently agitation. The third step (**F+Ch**) involved wet peroxide oxidation (Fenton processes, oxidation in presence of a iron Fe (II) catalyst to digest labile organic matter) (**F**), and enzymatic hydrolysis with Chitinase (**Ch**) at 40ºC and agitation. Finally, in the case of presence of inorganic matter, density separation (**DS**) with $ZnCl_2$ solution (1.8 g/ml) was carried out (for chemical volume and filtration steps details see supplementary materials figures A1 and A2).

Each phase should be applied based on the amount of organic matter removed in the previous phase through decision steps. Once the organic matter removal has been achieved (based on interference grade for easy particles detection and identification), the process stops. The sample is then ready for density separation steps, or for identification, thereby it is not necessary to complete all phases. The application of alkaline, enzymatic hydrolysis and Fenton processes in phases and sequential steps offer an effective MPs extraction and their different morphologies at less time with less airborne fibres contamination, avoiding significant damages on surface of particles, and reducing costs for chemical reagents. The percentage of removal of organic matter obtained with the application of the three phases is up to 98%.

The chemical reagents and filters were the following: absolute ethanol (Scharlau, >99,9%, Barcelona-Spain), potassium hydroxide (Sigma-Aldrich, 99,9%, Madrid-Spain), hydrogen peroxide stabilized (PamReac AppliChem ITW Reagents 33% w/v Barcelona-Spain), hydrogen peroxide aqueous solution stabilized (Thermo-Scientific Acros 33-35% w/v, Madrid-Spain), zinc chloride (Acros Organics, >98,0%, Madrid-Spain), zinc chloride anhydrous (Sigma-Aldrich, ≥98 %, Madrid-Spain), SDS (PamReac AppliChem ITW Reagents, 99%, Barcelona-Spain), iron (II) sulphate heptahydrate (Sigma-Aldrich, >99,0%, Madrid-Spain), nitric acid (Scharlau, 2N, Barcelona-Spain), sodium acetate anhydrous (Scharlau, 99%, Barcelona-Spain), acetic acid (Scharlau, 96 %, Barcelona-Spain), Tris-HCl (Scharlau, 99 %, Barcelona-Spain), PTFE 5, 10 µm pore size filters (Sartorius, Madrid-Spain). The enzymes used were Chitinase 1000 U/ml, Celullase TXL >1000 U/ml, Lipase FE-01 >18000 U/ml, Protease A-01 > 1000 U/ml provide by ASA Spezialenzyme GmbH (Wolfenbüttel-Germany).

2.2.4 Microplastics quantification and identification

All particles suspected of being MPs were totally quantified on PTFE filters of each subsample using both, a stereoscopic (LEICA MZ10 coupled to FLEXACAM C1) camera, and an optical microscope (Olympus CX41). MPs were classified according to the morphology as fibres (including filaments and fishing line), films (particles with a two-dimensional shape), fragments (particles with a three-dimensional shape), and pellets (solid spheres; solids foam spheres). Some particles suspected of being MPs on the PTFE filter were photographed and marked under a stereomicroscope (LEICA MZ10) equipped with a FLEXACAM C1 digital camera.

MPs particles were measured by its longest axis and categorized into nine size categories (0.02-0.05 mm; 0.05-0.125 mm; 0.125-0.25 mm; 0.25-0.5 mm; 0.5-1 mm; 1-2 mm; 2-3 mm; 3-4 mm; 4-5 mm). For MPs measurement, the PTFE filters (47 mm) were divided into ten columns (3.5 mm wide). For each column, all particles of each morphology type, colour, thickness, and size were picked varying the magnification from 8 to 80 X. The biggest and medium particles were placed on a clean PTFE filter, and the smaller ones (<0.5 mm) into a square of 1 cm^2 area on calcium fluoride (CaF_2) slide. For smaller MPs and abundant particles ≤50µm, the original filters were divided in four quadrants. In each section, four fields from 40 to 80X magnification were randomly chosen, being particles of each morphology and sizes measured. The CaF_2 slide and filter were photographed and the particles length were measured with ImageJ software (Schneider et al., 2012) (See supplementary material, Figure A3a)

Polymeric composition analysis was carried out for all photographed and measured particles. Fragments and films between 0.5 to 5 mm for each subsample were grouped and placed on clean PTFE filters. Fibres, fragments and films from 0.02 to 0.5 mm were arranged on calcium fluoride (CaF_2) slides. Particles size larger than 0.5 mm were analysed by infrared spectroscopy technologies with a Perkin Elmer Frontier instrument with a Spectrum Software and an

Attenuated Total Reflectance (ATR) accessory. The instrument has a DTGS detector, Glowbar source and CsI beam splitter. The spectral range analysed was 4000 to 230 cm^{-1} with 4 cm^{-1} accumulations and 4 cm^{-1} of spectral resolution. The background was done before analysis every 6 samples. By contrast, the identification particles smaller than 0.5 mm on a CaF_2 slide was carried out with a μ-FTIR microscope Thermo Nicolet iN10 MX, with Omnic Picta Software. The instrument has a MCT array-imaging detector in transmission mode. Linear array detector measures two lines of 8 pixels (16 pixels at a time). Each pixel has an aperture of 25 x 25 microns. IR spectra were recorded with 4 accumulations and 4 cm^{-1} of spectral resolution (See supplementary material, Figure A3b y c). The spectral range was measured from 4000 to 715 cm^{-1}.

For the identification of each polymer, the spectra obtained with the ATR-FTIR and μFTIR techniques were performed, being the unknown spectra compared with OMIC software libraries database. It includes HR Nicolet Sampler Library, Hummel Polymer Sample Library, Polymer Laminate Films, Wizard Library, and an own library with more than 80 IR spectra (see Annexes). Only match spectra with major -or equal- than 75% of similarity with reference spectra were accepted. The rejected items were counted as the temporary unidentified category. The unidentified spectra were also identified comparing with BIO-RAD IR from University of Barcelona (Barcelona, Spain) spectral databases. The matches of similarity major or equal than 75% were accepted.

For white and transparent films and fragments >0.5 mm, PTFE filter, were examined with spectroscope-microscope Raman Renishaw 20X, 50 X objective magnification microscope-, 785 nm edge, laser state 1%, 10%, 50% intensity, bands from 25 cm-1, 1200l/mm. The spectra polymer obtained with Raman spectroscopic technique was performed with WIRE 5.3 Software, being the spectra corrected for baseline displacement. The Raman spectra polymer were identified comparing the spectra with WIRE 5.3 polymer libraries database. Only match spectra with major -or equal. than 75% of similarity with the reference spectra were accepted- The spectres with similarities below 75% were compared with a library of own elaboration taking into account the main plastics products on the market. Only the spectra whose peaks were 60-70% or more coincident with the peaks of the reference spectra were identified and designated according coincident reference polymer name. The rejected items were also analysed according to their characteristics absorption band. A total of 1460 particles were measured and analysed by spectroscopic methods (see supplementary materials for particle number analysed by molluscs group, Table A3).

2.2.5 Quality control and recovery rates

To prevent contamination, distilled water (MilliQ® water), and all solutions prepared, were filtered before the use through sterilized nitrocellulose membrane filters GF/F 0.45 μm (Whatman

47 mm diameter, Maidstone, United Kingdom), and stored in glass bottles. All glassware, sieves and fine tools as scalpels, and stainless-steel tweezers, were washed with ultrapure water (distilled water filtrate through 0.45 µm sterilized membrane filters) and alcohol 70% after washing. All materials were wrapped in aluminium foil and stored in a clean fume hood. All laboratory countertops were cleaned with alcohol 96% and fibre-free napkins. Clean cotton laboratory coats and latex gloves were used during all processes. All glass material were immediately covered to reduce MPs airborne contamination in extraction and lyophilisation procedures. Samples processing and digestion procedures were conducted in clean fume hood and laminar flow cabinet. Blanks were used in every step of samples processing and digestion. Five and 10 cleaned Petri dish were placed during the samples processing and during MPs visual sorting, respectively, in order to calculate the deposition of airborne fibres. Eleven procedural blanks, with the same reagents volume that the samples, were run with every set of samples in digestion processes fulfilling the methodology, step by step and by phases. Finally, three blanks in lyophilisation processes were applied; one for every set of sub-samples. The results were corrected by fibres and particles contaminations. After processing and preparations of sub-samples, lyophilisation procedures and visual sorting of MPs analysis, airborne fibres and particles contamination, were not detected in any blank. However, after the digestion process fibres were found in 91% of the controls. The fibres in controls assigned to every set were classified by colour, being subtracted from the total quantity found in the respective subsamples. The numbers of fibres in controls ranged from 3 to 17 and the colours were the following: blue, yellow, red, black, grey, green, and transparent.

To ensure methodology effectiveness in the MPs extraction from the samples, recovery rates were calculated in parallel with samples analysis. Samples of three molluscs were randomly chosen and spiked with coloured virgin plastic spherical particles of polyethylene (PE) spanning from 53 to 500 µm diameter with density 1.02-1.06 g/cm^3 (purchased from Cospheric Inc,, California, USA). Subsamples around 50-55 g of soft tissues of molluscs were dissected for spiking. The tissues were carefully cut and inoculated for different parts with coloured PE mix spheres ranging from 53-500 µm. The recovery rates were 60% for 53-63 µm, 60 % for 125-150 µm, 74% for 250-300 µm, and 99 % for 425-500 µm. Neither damages by agitation (mechanic forces), nor chemical attacks, were found in spheres (Figure A4, Supplementary materials).

2.2.6 Dietary Exposure

The dietary intake of MPs from mussels, oysters and clams by the Catalan population was estimated using a deterministic method by applying the following equation:

$$DI_t = \sum C_f \times X_{f,t}$$

where DI_t is the dietary intake of MPs of population group t (MPs/day), C_f is the concentration of the MPs in each samples group *f* (mussels, oyster or clams) (MPs/$g_{w.w}$); and $X_{t,f}$ is the mean consumption of samples group *f* (mussels, oyster or clams) by population group t (g/day). Three different population groups were considered: adults, elderly, and pregnant women. The mean consumption was obtained from the EFSA consumption data, taking account only effective consumers surveyed (EFSA, 2021). The mean consumption of each population group was normalized by dividing the dietary intake by the mean body weight of each population group, which was established in 77 kg for adults, 70.5 kg for elderly and 65 kg for pregnant women (Gonzalez et al., 2019).

2.2.7 Data analysis

The MPs concentration in molluscs samples was presented as MPs particles per individual (MPs/individual), per gram of wet weight fresh tissue (MPs/$g_{w.w}$), and per gram of dry weight tissue (MPs/$g_{d.w}$). Data were analysed using the SPSS 24.0. Normality tests were carried out on samples grouped by species with Shapiro-Wilk normality test (n <50). Non-parametric Kruskal–Wallis test followed by multiple comparisons was carried out to asses MPs concentrations and morphology differences. A non-parametric Mann-Whitney U test was also conducted in order to determine the difference in MPs concentrations and morphology between pairs of molluscs' species. The levels of statistical significance were established at $p < 0.05$.

Regarding the size of the MPs Kolmogorov-Smirnov Test were carried out for samples grouped by species to check normality data distribution (for samples n>50; and significance levels p>0.05). Results indicate that in general MPs' sizes follow a normal distribution with p between 0.109 and 0.200. Levene and ANOVA tests with Tukey post hoc ($p < 0.05$) for independent samples were applied to assess MPs sizes differences between molluscs' species. Hypotheses were contrasted with a significance level of 0.05.

2.3 Results and Discussion

2.3.1 Samples processing and analysis

Despite the effort of the scientific community for developing methodologies for MPs extraction and analysis from seafood, to date there is not a standardized protocol. The methodologies developed in the current study have advantages such as gentle enzymatic and chemical reagents (for MPs integrity protection). A large number of samples (2310 individual organisms) were analyzed and a single protocol was applied to all of them.

The recovery percentages were probably influenced by diameter of 20 µm pore size sieve of 20 cm, as well as the morphology and type of MPs patterns (rounded, smooth surface and virgin). The MPs spiked inside the fresh tissues were probably not wrapped and attached by the fresh

tissue like the environmental MPs. Until now, there are not defined standardized methods and materials to determine the recovery percentages for MPs extraction. A change in sieve size to smaller diameters would lead to a higher recovery of MPs, especially the smallest ones.

2.3.2 Abundance of microplastics in molluscs

MPs were detected in all samples of analysed molluscs. Mean MPs levels ranged from 0.32±0.27 to 32.0±13.38 MPs/individual and from 0.54±0.43 to 8.17±10.0 MPs/$g_{w.w.}$ (Table 2). Big oysters, razor clams, and mussels showed the highest concentrations of MPs by individuals with wide range of concentrations from 2.65 to 99.9 MPs/individual. Specifically, in non-depurated big oysters (BOnd(S)), razor clams RCnd (S)), and mussels from north (Mnd(N)), MPs levels ranged from 12.5 to 42.5 MPs/individual, from 2.65 to 37.1 MPs/individual, and from 4.27 to 99.9 MPs/individual, respectively. The lowest MPs concentrations were found in wedge clams from north (WC(N)), ranging from 0.12 to 0.51 MPs/individual. Based on wet weight, the ranges were again wider in non-depurated mussels from north (Mnd(N)), with values ranging between 0.68 and 34.4 MPs/$g_{w.w.}$, followed by mussels depurated from south (Md(S)), ranging from 0.68 to 9.65 MPs/$g_{w.w.}$, and fine clams depurated (FCd(S)), ranging from 1.66 to 10.5 MPs/$g_{w.w.}$. The lowest MPs concentration (wet weight) was found in marine snails from south (S(S)), with levels between 0.19 and 1.06 MPs/$g_{w.w.}$ (Table 2).

The average MPs concentrations by wet weight, by dry weight, and by individual, according to the groups of molluscs are depicted in Figure 2. Big oysters and mussels showed the highest concentrations: 22.8±14.4 and 18.6±23.0 MPs/individual, respectively. Mussels and fine clams showed the highest level by wet weight with values of 6.47±7.95 and 4.97±4.78 MPs/$g_{w.w.}$, respectively. The lowest concentration by individual was found in wedge clams (0.49±0.23 MPs/individual), and the lowest concentration by wet weight was observed in snails (0.94±0.62 MPs/$g_{w.w.}$). Regarding MPs concentrations by dry weight, values ranged from 3.11±3.11 to 32.7±27.8 MPs/$g_{d.w.}$, for snails and fine clams, respectively.

Figure 2 shows large significant differences between species when MPs concentrations are expressed either by wet weight, or by individuals. Wedge clams presented significant lower levels ($p<0.05$) (0.12 MPs/individual) than the remaining species studied. In addition, snails registered significant ($p<0.05$) lower MPs levels per individual (4.6 MP/individual) than mussels and big oysters (18.6 and 22.8 MPs/individual, respectively). Regarding levels by wet weight, mussels showed significant ($p<0.05$) higher levels than wedge clams, snails, and big oysters, while fine clams and wedge clams presented significant ($p<0.05$) higher levels than snails.

Table 2: Microplastic levels (by wet weight and by individual) in molluscs samples.

Code*	n samples (total individuals analyzed)	Wet weight (MPs/$g_{w.w.}$)				Individual (MPs/individual)			
		Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
WCnd(S)	2 (383)	1.99	0.82	1.41	2.57	0.52	0.22	0.37	0.68
WCd(S)	2 (387)	2.56	1.18	1.72	3.40	0.67	0.31	0.45	0.89
WC(N)	2 (373)	1.04	0.89	0.40	1.67	0.32	0.27	0.12	0.51
WC(C)	2 (489)	2.09	0.51	1.74	2.45	0.46	0.11	0.38	0.54
S(S)	4 (68)	0.54	0.43	0.19	1.06	1.92	1.50	0.67	3.74
S(C)	5 (55)	1.26	0.58	0.59	2.16	6.80	3.17	3.20	11.7
RCnd(S)	6 (59)	2.45	2.59	0.52	7.26	12.5	13.2	2.65	37.1
FCd(S)	3 (74)	4.97	4.78	1.66	10.5	10.4	9.99	3.47	21.9
BOnd(S)	4 (19)	2.60	1.09	1.01	3.45	32.1	13.4	12.5	42.5
BOd(S)	5 (28)	1.58	1.16	0.15	2.85	15.5	11.3	1.49	27.9
Md(S)	4 (88)	4.78	3.82	0.68	9.65	11.3	9.05	1.60	22.9
Mnd(S)	4 (62)	3.50	2.17	1.60	6.62	11.8	7.33	5.41	22.3
Mnd(N)	11 (223)	8.17	10.0	1.47	34.4	23.7	29.0	4.27	99.9

SD: standard deviation. *Code: spice in capital letter: WC: wedge clams, S: snail, RC: razor clams, FC: fine clams, M: mussels, and BO: big oyster. nd: not depurated, d: depurated; between brackets Catchment areas: (S): South, (N): north and (C): central.

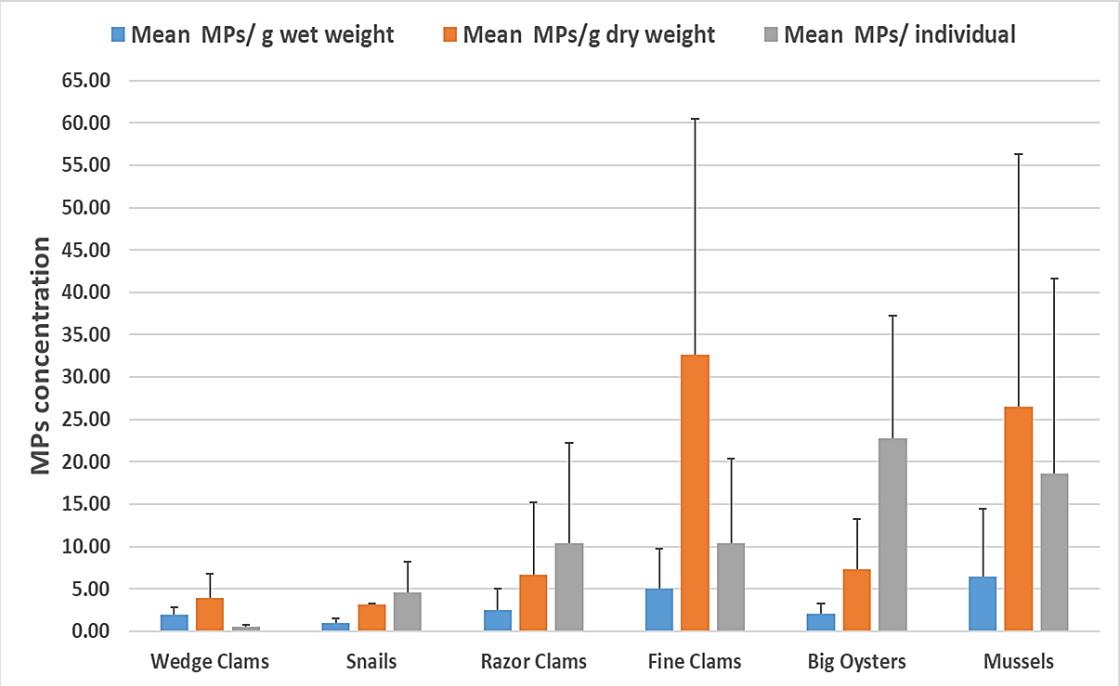

Figure 2. MPs concentration by molluscs groups. Bars indicates standard deviations

2.3.3. Microplastics size

The sizes of 1460 MPs found in the mollusc samples were analysed. In general, terms, a half of the total particles measured was found in the sizes between 0.02 and 0.5 mm, while 74% of them were less than 1 mm (Figure 3). The sizes of particles found in the present study were higher than those found by Cho et al. (2019) and Li et al. (2015, 2016), in bivalves from Korean and China markets and their coastal areas. Cho et al. (2019) reported particles sizes smaller than 300 µm, accounting the 65% of total of MPs. Similarly, Li et al. (2015, 2016), found that MPs sizes below 250 µm constituted up to 84% of the total MPs in commercial bivalves, and 79% in coastal mussels.

With respect to species, similar profiles were detected for clams (wedge, fine and razor) and snails, with differences in the distribution of accumulated MP of less than 10%. Oysters showed a higher concentration for sizes between 0.02 and 0.125 mm (although non-significant: $p>0.05$). Only mussels showed a significant ($p<0.001$) higher concentration in fractions from 0.02 to 1 mm. This difference was due to the fact that samples collected in the north of Catalonia presented a high number of MPs, between 0.02 and 0.125 mm, which counted around 50% of MPs. Excluding those samples, the mussel size profile was similar to that of the oyster.

2.3.4. Microplastics morphology

MPs morphology in each sample of molluscs collected from Catalan coast is depicted in Figure 4. Fibres, fragments and films were detected in molluscs samples with overall mean proportions of 74%, 13% and 13%, respectively. For all samples, the most predominant type of MPs were fibres, with percentages ranging between 50 and 92%, excepting the non-depurated Northern Mussels (Mnd(N)), in which the fragments represented 64% of the total MPs, followed by fibres,34%.

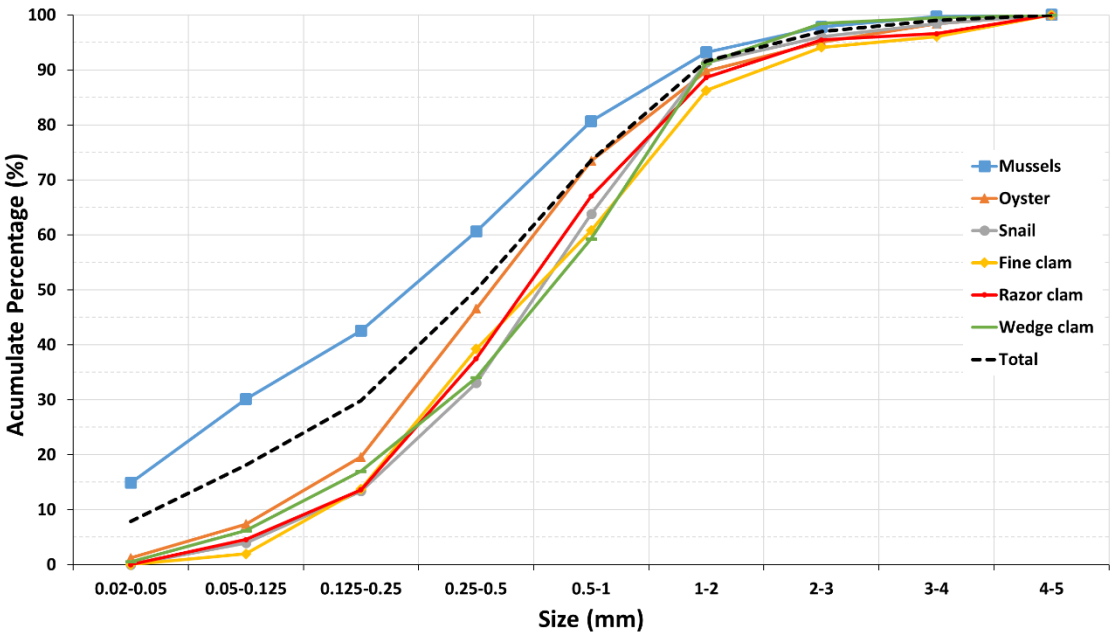

Figure 3. Microplastics accumulated size distribution globally and according to molluscs' species

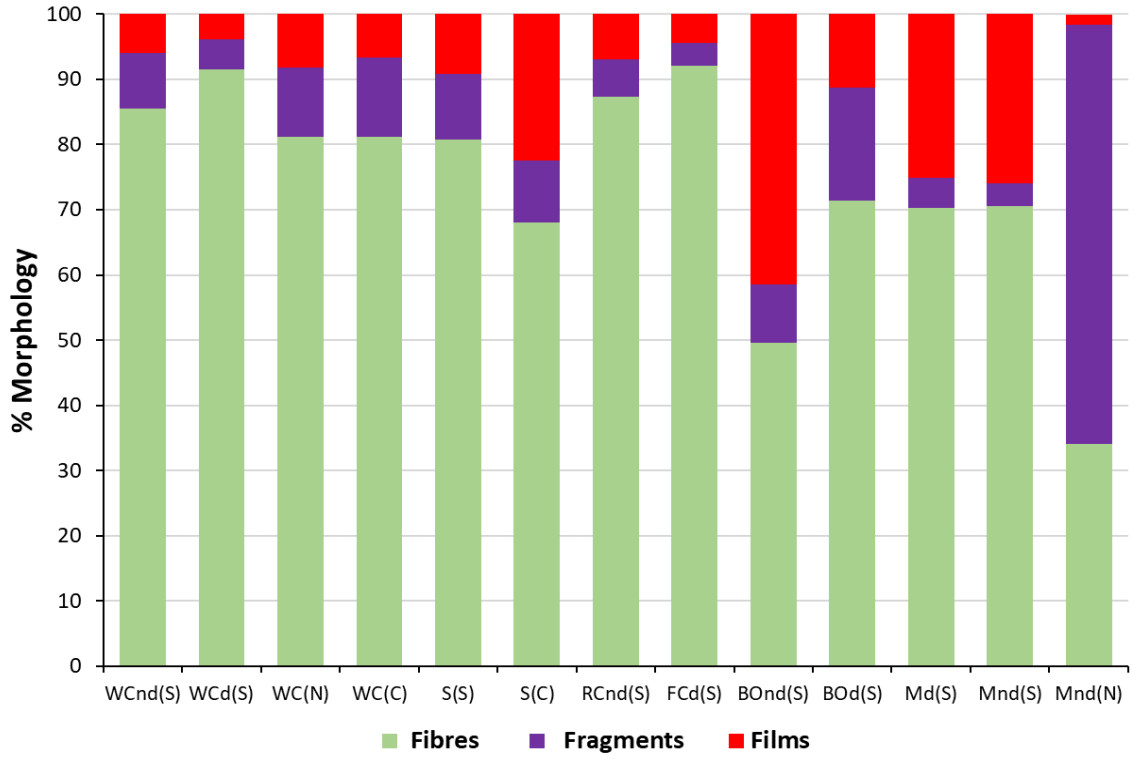

Figure 4. Morphologic types of microplastics in molluscs samples

These samples (Mnd(N)) presented a higher content of small MPs (section 3.3) in the form of fragments. Interestingly, two PE pellets were found in two subsamples of non-depurated Northern mussel sample. This meant 0.04% of the total MPs of that sample.

Comparing molluscs samples, differences according to species, catchment zone and depuration condition were observed. According to species, wedge clams showed significant ($p<0.05$) differences with snails regarding fibres and fragments concentration (g wet weight) and also in the percentages of fibres and film. Wedge clams showed also significant ($p<0.05$) differences with fine clams in relation to film concentration (g wet weight) and fragment percentage. Similarly, snails showed also significant differences with fine clams. Mussels showed significant ($p<0.05$) differences with snails, razor clams, and oysters with respect to fibres, fragments concentration, and films percentages. Razor clams, fine clams and oysters did not show significant ($p>0.05$) differences between them, regarding the MPs morphology.

Based on the catchment zones, snails and mussels showed significant ($p<0.05$) differences in films, fragment, and fibres concentrations, as well as their percentages. Snails from central S(C) and south S(S) zones had average films concentrations of 0.28 ± 0.15 and 0.05 ± 0.03 MPs/$g_{w.w.}$, respectively. In contrast, the percentage of fibres was lower in the central (65±13%) than in the south (79± 5%) zone. Regarding mussels, fragments were significantly ($p<0.01$) higher in the north (5.64±8.98 MPs/$g_{w.w.}$) than in the south (0.17±0.08 MPs/g $_{w.w}$). For wedge clams, no

significant ($p>0.05$) differences in morphology proportion between organism from north, south and central catchment zones were observed.

Regarding depuration conditions, wedge clams did not present significant ($p>0.05$) differences in MPs morphology. However, oysters showed significant ($p<0.05$) higher levels of films in non-depurated (1.01 ± 0.77 MPs/$g_{w.w.}$) than in depurated (0.18 ± 0.37 MPs/$g_{w.w.}$). That means a significant ($p<0.05$) reduction from 42% to 11% of the total MPs. By contrast, mussels from south (S) showed similar concentrations of films, fragments, and fibres between depurated, Md (S), and non-depurated Mnd (S)

The predominance of MPs in the form of fibres in molluscs is related to their presence in environment fibres. It tends to predominate in seawater and marine sediments in many regions of the world, including the Mediterranean Sea (de Haan et al., 2019; Expósito et al., 2021; Filgueiras et al., 2019; Lots et al., 2017; Reineccius et al., 2020; Sanchez-Vidal et al., 2018; Suaria et al., 2020). In addition, these results are in agreement with those of Ward et al. (2019a), who found long microfibres in mussels and oysters, although mussels ingest fibres regardless of their length.

2.3.5. Microplastic composition

A total of 1460 particles were analysed for polymeric composition by spectral techniques. Twenty-one different polymers types were identified in the samples of molluscs. The most prevalent polymer was polyethylene (PE), with a contribution of 54%, followed by polyester (PES), and synthetic cellulose (rayon or viscose), with percentages of 17% and 12%, respectively. Other detected polymers were polyvinylidene fluoride (PVDF) (4%); polypropylene (PP) (3%); polyamide (PA) (2.4%); polyacrylonitrile (PAN) (2.2%); polycarbonate (PC) (0.5 %); polyurethane (PU) (0.3%) and polystyrene (PS) (0.3%). Other polymers were detected at lower rates (≤0.2%): expanded polystyrene (EPS), ethylene vinyl alcohol/ethylene vinyl acetate (EVOH/EVA), polyethylene terephthalate (PET), poly(ethylene-co-vinyl acetate) (PEVA), poly(vinyl alcohol) (PVA), polymethyl methacrylate (PMMA), polystyrene acrylate ester, PE-vinylchloride, polyacrylate, polyvinyl chloride (PVC) and PP-PE diene. Around 3% of man-made polymers, could not be properly identified, being classified as “synthetic polymers”. (Figure A5, A6, A7a-e Supplementary materials)

Only five polymers (polyethylene, polyester, synthetic cellulose, polyamide polypropylene, polyvinylidene fluoride) were detected in all species at different percentages, contributing in more than 85% of the total MPs (Figure 5). The polymer PVDF was frequently detected in oysters of the Catalonia coast, from 17 to 26%, lower values than the percentages reported by Ding et al. (2020) in oysters from China, where PVDF was frequently detected, accounting for 68%. The polymer PVDF was also found in razor clams, snails, and mussels from the Catalonia coast, but at low proportions (between 1.5 and 6%).

Differences in polymer abundance observed between the species of molluscs could be due to the culture methods and the habitat/feeding characteristics of each species. PES fibres were found at high proportions in clams. PES presents higher density (1.26-1.34 g/cm^3) than seawater (1.02 g/cm^3) and could to form hetero-aggregates with organic and inorganic particles (Wheeler and Lower, 2021), being heavier and deposited on sediment and available to organisms which inhabits in marine bottoms such clams. In mussels cultured in the upper layer of the water column, the polymer PE was found at high quantity due to its low-density (0.92-0.94 g/cm^3) and small particles sizes accessible to mussels' organism. Similarly, Cho et al. (2019), found PES relatively abundant in species grown in the deeper layer of the water column, such as scallops and intertidal sediment as Manila clam. High proportions of PE were observed in all samples of molluscs. It might be due to its massive production and consumption (Plastics Europe, 2013, 2017, 2019, 2021), as well as its high buoyancy and transportability at seawater based on its low specific density (Cincinelli et al., 2017). The polymer composition predominant of fibres from molluscs' samples was polyester, followed by synthetic cellulose -which is expected to originate from textiles- reflecting the global production of these artificial fibres (The Fiber Year, 2019).

With respect to the catchment area, a greater diversity of polymers was observed in the southern and central areas (17 polymers) in relation to the north (8 polymers). PE was the predominant polymer found in organisms from northern zone, with 84% of the total MPs. In contrast, in the central and south areas, MPs composition profile was very similar, with PE, PES, PP, and synthetic cellulose, PVDF (polyvinylidene fluoride), PAN and PA counting 90% -or even more- of the total MPs.

In the current survey, synthetic cellulose was considered as MP. According to Remy et al. (2015) and Halstead et al. (2018), although these polymers can be rapidly degraded, their intakes can cause adverse biological effects. Yadav and Hakkarainen (2021) reported that the degree of acetylation in cellulose acetate polymer reduces its degradation potential. In spite of this, the methodology followed in the current study, in which the removal of organic matter was carried out through combined alkaline hydrolysis, enzymatic and Fenton processes, the non-degradation of these fibres could be attributable to the large amounts of non-biodegradable compounds that they contain.

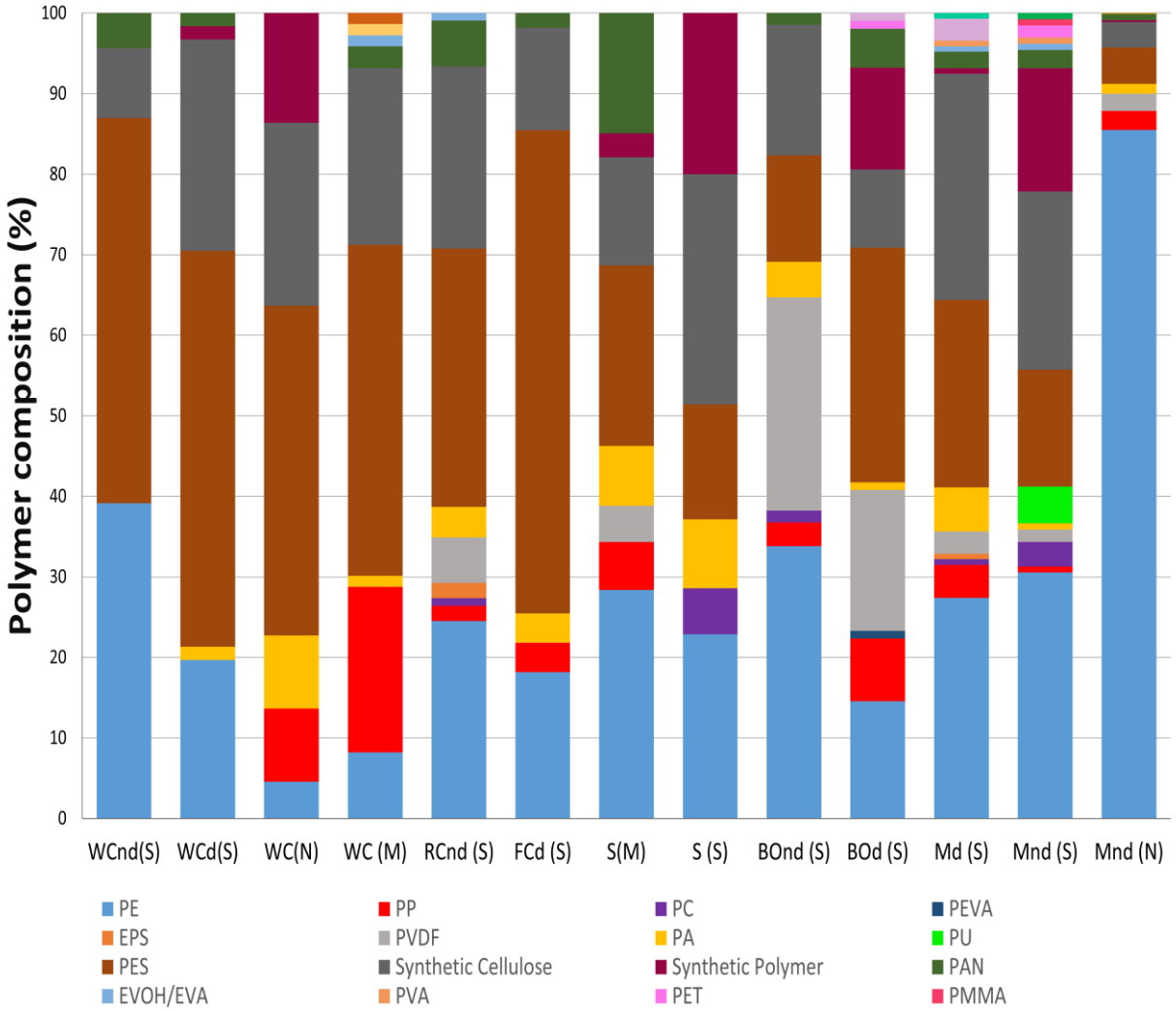

Figure 5. Microplastics composition in molluscs

2.3.6. Comparison of microplastic concentration, morphology, and sizes between molluscs' samples.

It is probably that MPs filter efficiency depends on bivalve size, mechanism to particles selection, and environment conditions. The molluscs evaluated in the present survey had different feeding habits and habitats (i.e., sediment or water column) (Table 1). Bivalves (clams, oysters and molluscs) are filter feeder of large seawater volumes and ingest the suspended MPs from water column (mussels and oysters), or interface water-sediment (wedge, razor and fine clams) (Li et al., 2015).

Wedge clams, inhabits on sandy beaches, where it forms extensive dense beds living buried in soft bottoms (FAO, 2022). This species showed the lowest MPs concentration by individual, which is due to its low ability to ingest large MPs. According to Ding et al., (2020), features of

MPs ingested by shellfish were related to the biometric parameters of shellfish. Fine (*Tapes decussatus*) and razor clam (*Ensis siliqua*) also belong to clams group, which lives buried in soft sand or mud bottoms, where they digging into different depths (infaunal) (FAO, 2022). These species did not show differences in the concentration of MPs nor with the oyster (*Crassostrea* gigas), which is not buried (epifaunal) and stay attached in strong substrate by cementing one of the valves (FAO, 2022). In the current investigation, oysters were cultivated, combining at the first on-off bottom culture, and after suspended culture and harvesting (trays culture), considering it as water-dwelling in adult phase in the Ebro Delta. By localisation habitat, the MPs found in clams seem to come from to bottom resuspension. Ward et al., (2019a) reported abundance of MPs (especially fibres) in clams, being more similar to sediment than to water, a fact that has been also reported by other authors (Ding et al., 2020; Su et al., 2018). It seems that fine clams have lower excretion rates, which makes difficult to excrete microfibres and microfragments. (Keisling et al., 2020).

The snails showed lower MPs concentration (wet weight). Gastropod species such as *Bolinus brandaris* are demersal organism inhabitants sandy-muddy (detrital) and soft bottoms with carnivorous predators' habits. MPs can be indirectly ingested from prey already containing MPs particles (Ablidi et al., 2019). These species showed significant lower concentration (wet weight) compared with clams. It suggests that the presence of MPs in the area of the selected prey was low, or their MPs excretion capacity was high.

Mussels are called "true surface dwellers" and the organisms here analysed came from suspended culture attached to the substratum, by threads or "byssus" (Piarulli et al., 2020; FAO, 2022). Mussels showed higher MPs concentration (wet weight) compared to other species as wedge clams, snails and oysters (razor clam showed only differences in fragments concentrations). It indicates that the habitats or location in water column-sediment environment, and the shell size, have an influence on the accumulation of MPs in the organisms. In this same line, Ding et al., (2020), found that MPs intake of water-dwelling shellfish was significantly different from that of sediment-dwelling shellfish. Mussels trap a higher number of MPs due to their size, high pumping and filtration rates (9.3 ± 1.7 L/day) (Catarino et al., 2018; Rist et al., 2019). According to Ward et al. (2019a), the particles trapped in the gill filaments are translocated after a selection process to the digestive glandule and intestine. Mussels are exposed to the sea-surface microlayer, where small particles and most of the high buoyancy particles -including MPs- are periodically resuspended from sediments and sand by currents and waves to water column. Intake of different plastic particle sizes (30 nm to several millimetres) and shapes (fragments, beads, fibres and films) have been observed in mussels (Li J et al., 2016 cited by Rist et al., 2019; Wegner et al., 2012). In turn, Kolandhasamy et al., (2018), showed the adherence and ingestion as the mainly process of MPs accumulation in mussels. MPs uptake is observed in multiple organs of mussels, including foot and mantle, being the intestine, the organ containing the highest level of MPs. Another organ

containing MPs is the byssus, but merged instead of adhered (Li et al., 2019). According to bivalves feeding habits, gills may also be another organ that may contain MPs. In the case of the oysters, in this study is a water dwelling as mussels, and the differences regarding MPs concentration were probably due to ingestion-egestion-rejection differences between species and MPs pollution of cultivation zone.

Regional differences were observed between snails (from the central and south zones) and mussels (from the north and southern zones) regarding MPs morphology and size, which was probably due to the different MP pollution in these zones of the Catalan coast.

MPs burden from the northern zone of the Catalonia coast is influenced by a marine current that transports MPs from the northern Mediterranean Sea (France). WWTP effluents, as well as surface runoff of urban soils and discharges of mismanaged plastic, already weathered from industrialized and densely populated coastal areas (Constant et al., 2019; de Haan et al., 2019) -such as Barcelona and Badalona- are responsible for these high levels of pollution (Andrady, 2011; Cózar et al., 2015; Jambeck et al., 2015). The central area of the Catalonia coast receives influence of MPs, and mismanaged plastics discharged by the Rivers Llobregat and Besòs (both basins receptors of WWTP effluents from urban and industrialized zones, agriculture run off) into the Mediterranean coast, and distributed by principal current and local hydrodynamics to shoreline (Dalmau-Soler et al., 2021; Schirinzi et al., 2020). Finally, the southern zone is highly influenced by the Ebro River and the Ebro Delta River activities. The Ebro River discharges affect surrounding environments as estuarine sediments, sandy beaches (northern edge,) and seawater surface, which is evidenced by MPs higher concentration in three environment matrices, being the fibres the predominant morphology with presence of secondary MPs (fragments and films). Furthermore, agriculture activities in the Ebro Delta (intensive rice production) could promote the release of MPs accumulated in the soil (mainly fibres) matrix enriched with biosolids to the aquatic environment (Simon-Sánchez et al., 2019; Sun et al., 2019). Fishing and aquaculture activities in the Ebro Delta could be also other potential sources of MPs. Both activities use materials made of fibres and polymers synthetic (nylon and polyethylene) as ropes, nets, floats and mesh (Andrady, 2011; Covernton et al., 2019).

MPs sizes in mussels were significantly smaller than those in clams, oysters and snails. Ding et al. (2020) also found that sizes of MPs in the sediment-dwelling shellfish were significantly larger than those in the water-dwelling shellfish species. In turn, Qu et al. (2018) reported a positive quantitative correlation between abundance of plastic particles in two species of mussels and its surrounding waters, with more incidence of smaller MPs intake. The size of particles partially determines preferential rejection or ingestion. Thus, particles up to 200-300 μm are efficiently captured and ingested, and small particles are only efficiently captured if they are incorporated into aggregates, or are highly agglomerated (Van Cauwenberghe et al., 2013; Ward et al., 2019a). There is a maximum limit of particle size that can be ingested, which is probably in the range of

600–900 μm. However, the particle selection is never 100% efficient, and therefore, fibres can be ingested by various bivalve species (Ward et al., 2019a). In some cases, bivalves simply find and engulf the particulate matter. In fact, as other invertebrates, the size of the oral opening in bivalves is not fixed and can stretch. For example, it has been reported that oysters ingested larvae of nine different invertebrate species, measuring between 100 and >500 μm in length, at efficiencies of about 80% (Ward et al., 2019a).

In this study, between 60-85% of the MPs in bivalves had sizes between 20 μm and 1 mm. This means that most MPs were probably ingested. Microplastics from 2 to 5 mm accounted only an 8%, thus, its presence in bivalves could be due to oral stretching and not rejection for a special condition of organism, which led to engulf plastic particles (e.g., starving), as well as an inefficient particle selection combined with adherence in others body parts. Specifically for mussels, mussels not depurated from the North zone showed up to 85% of MPs with a size below 1 mm, while mussels from the South (depurated and not depurated) showed up to 80% of MPs for the same range size (<1 mm) stating that MPs could be mostly ingested.

The depuration processes effects on MPs concentration were not observed in wedge clams from southern, mussels from southern and northern, and oysters from southern. Significant differences in MPs morphology were only observed in oysters, and MPs morphology and sizes in mussels. Furthermore, differences in specific polymers were observed between non-depurated and depurated organisms, although the number of different polymers was similar. These results are attributable to individual MPs ingestion-egestion dynamics and differences in MPs pollution along the Catalan coast. The depuration time was perhaps not long enough for the efficient removal of MPs from the organisms. Depuration times were 48 h for wedge clams and mussels and >72 h for oysters. Nevertheless, van Cauwenberghe and Janssen (2014) found significant differences in depurated and non-depurated oysters and mussels with a time of 72 h. According to Ward et al. (2019a), after 168 h (7 days) of depuration, only residual amounts of plastic particles were found in bivalve tissues. Birnstiel et al., (2019) reported that a depuration time of 93 h was quite effective in removing blue fibres in wild and farmer mussels. However, mussels still presented high abundance of MPs even after depuration, suggesting that depuration process was not sufficiently efficient for completing MPs removals. The depuration processes in bivalves could be influenced by the digestion processes of plastic particles in the organisms. Zhao et al., (2018), observed that the residence time of MPs in clams, mussels, and oysters in the hind gut is of the order of hours, while the residence time in the digestive diverticula is of the order of days. Furthermore, it is probably that bivalves' contamination by MPs occurs after depuration during packaging and processing of live depurated organisms (EFSA, 2016).

2.3.7 Comparison of microplastic concentration in molluscs with others similar studies

Microplastic levels comparison among different studies it is not an easy task due to lack of a standardized analysis, the MPs size considered in each study, as well as the identification techniques used (Ding et al., 2020). Sampling area and cultivation techniques can further influence the MPs concentration, morphology and composition. Notwithstanding, the concentrations reported by different studies are an indicative of the MP contamination levels in different regions and type of molluscs (wild, commercial, or farmed).

Table 3 summarizes the MPs levels found in the present study, which were compared with those recently reported in the scientific literature. The highest MPs values by individuals was observed in wild mussels (*Mytilus edulis*) from Nova Scotia (Halifax-Harbor), Canada, with levels ranging between 34 and 178 MPs/individuals (Mathalon and Hill, 2014). However, the levels of MPs by individuals of molluscs purchased from markets of Catalonia coast tend to be higher than molluscs from Iran, China, South Korea, Italy, Greece, France, UK, Norway and Canada (British Columbia) (Brate et al., 2018; Cho et al., 2019; Covernton et al., 2019; Digka et al., 2018; Ding et al., 2018, 2020; Li et al., 2015; Li et al., 2018; Naji et al., 2018; Phuong et al., 2018; Piarulli et al., 2020).

Morphology distribution and sizes range of MPs in molluscs from the Catalonia coast were similar than those found in China, South Korea, Iran, North Sea, Canada, the Atlantic coast of Spain, and Tunisia (Abidli et al., 2019; Cho et al., 2019; Covernton et al., 2019; Ding et al., 2018, 2020; Leslie et al., 2017; Naji et al., 2018; Reguera et al., 2019). Predominant MPs sizes found in the current survey (between 125 µm and 1 mm) were higher than sizes reported in Belgium, French, UK, the Dutch coastline and North Sea (Digka et al., 2018; Li et al., 2018; Murphy et al., 2018; van Cauwenberghe and Janssen, 2014, 2015). However, size range here found was in range with those reported in Atlantic French and Norway coast (Bråte et al., 2018; Phuong et al., 2018;).

According to the MPs composition, Catalonia molluscs presented the same polymers found in molluscs from China, South Korea, UK, coastal lagoons of North Adriatic, Tunisia and Canada (Abidli et al.,2019; Cho et al., 2019; Covernton et al., 2019; Ding et al., 2020; Li et al., 2018; Piarulli et al., 2020). Predominant polymers found in the current investigation (PE and PES) were the same than those reported in South Korea, United Kingdom and the Adriatic Sea (Cho et al., 2019; Li et al., 2018; Piarulli et al., 2020). It is important to remark that the EVA polymer was only found here and in the coastline of Norway (Bråte et al., 2018).

The levels of MPs in fresh tissues of molluscs purchased from markets of Catalonia tend to be higher than wild ones from other coastal zones of Spain. Also, higher than wild, commercial and farmed molluscs from other Mediterranean Sea coasts (Tunisian and Greece), as well as other regions worldwide as China, South Korea, United Kingdom, France, Belgium, Netherlands, Norway and North Sea (Abidli et al., 2019; Brate et al., 2018; Cho et al., 2019; Digka et al., 2018; Ding et al., 2018, 2020; Li et al., 2015; Li et al., 2018; Phuong et al., 2018; Reguera et al., 2019;

Van Cauwenberghe and Janssen, 2014; Van Cauwenberghe et al., 2015; Zhang et al., 2022). The MPs concentration and plastics pollution sources in marine environment of molluscs catchment zones of worldwide regions is specified in Table A4 (supplementary materials).

However, the average levels here found were lower than those found in other regions of Italy (natural stock and mariculture from Tyrrhenian Sea, Ligurian Sea, Central Adriatic Sea), Iran and Canada coastline (Vancouver Island coast) (Murphy, 2018; Naji et al., 2018; Renzi et al., 2018)

Regarding MPs concentration per dry weight (g.d.w), in general, values found here were lower (from 3.11±0.12 to 32.67±27.78 MPs/g.d. w) than found in North Sea by Karlson et al., (2017) (37 MPs/g.d. w) and Leslie et al., (2017) (20-105 MPs/g.d. w). However, for *C. gigas* the values were higher of 7.30 ±5.93 MPs/g.d. w compared with obtained by Converton et al., (2019) on *C. gigas* from Canada (British Columbia).

The variation of MPs concentration and composition in molluscs was related to the difference in regional MPs pollution status, differences in ingestion-egestion-rejection capacity by species, and the influence of differences in analytical methods. For a reliable comparison of results for the monitoring of MPs contamination in edible marine organisms, standardized and optimized methods are clearly necessary.

Table. 3. Summary of global investigation on microplastics in shellfish samples.

Location	Species	Morphology	Size (µm)	MPs/g wet weight	MPs/individual	Predominant Composition	Reference
Commercial molluscs from Catalonia coast (Spain)	*Mytilus galloprovincialis, Tapes decussatus, Crassostrea gigas, Bolinus brandaris, Ensis siliqua, Donax trunculus*	Fib, Frg, Fil	20-5000	Range: 0.19-34.43 (Mean range 0.54-8.17)	Range:0.12-99.85 (Mean range 0.32-32.1)	PE (4-85%), PES (4-60%), synthetic cellulose (3-29 %), PVDF (0-27%), PP (0-20%), PAN (0-14%), PA (0-9%), PC, PU, PS (0-6%)"	This study
Commercial shellfish from Qingdao and Xiamen (China)	*Mytilus galloprovincialis, Perna viridis, Ruditapes philippinarum, Crassostrea gigas, Sinonovacula constricta, Scapharca subcrenata, Meretrix lusoria, and Busycon canaliculatu*	Fib, Frg, Fil	10–5000	0.8–4.4 (digestive system)	1.2–6.0 (in digestive system)	Rayon (42-44 %), Chlorinated Polyethylene (12-14%), PET (5-16 %) PVC (7-10%), PVDF (24%)	Ding et al., 2020
Fishery market of Shanghai (China)	*Scapharca subcrenata; Tegillarca granosa; Mytilus galloprovincialis; Patinopecten yessoensis; Alectryonella plicatula; Sinonovacula constricta; Ruditapes philippinarum; Meretrix lusoria; Cyclina sinensis* (Bivalves)	Fib, Frg, Pell	5–5000	2.1–10.5	4.3–57.2	-	Li et al., 2015
Comercial bivalves from Qingdao (China)	*Chlamys farreri, Mytilus galloprovincialis*	Fib, Frg	25–5000	2.0–12.8 (digestive system)	1.9–19.4 (digestive system)	-	Ding et al., 2018
Commercial molluscs from Seoul, Gwangju, and Busan (South Korea)	*Mytilus edulis; Patinopecten yessoensis; Tapes philippinarum; Crassostrea gigas* (Bivalves)	Fib, Frg, Fil	43–4720	0.15±0.20	0.97±0.74	Frg: PE (24%), PP (23%), PS (22%), PEVA (4%), PET (2%), PU (2%), acrylic (2%) Fib: PES (82%),PP (6%), acrylic (6%) , nylon (3%).	Cho et al., 2019
Wild molluscs of Persian Gulf (Iran)	*Amiantis umbonella; Amiantis purpuratus; Pinctada radiata; Cerithidea cingulata; Thais mutabilis*	Fib, Fil, Frg, Pell	10–5000	0.2–21.0 (mean)	3.7–17.7 (mean)	-	Naji et al., 2018
Supermarket from Brittany, France Farm in Germany from the North Sea	*Crassostrea gigas* *Mytilus edulis* (depurated and not depurated)	Frg	>25	0.47±0.16 (*C. gigas* nd) 0.35±0.05 (*C.gigas* d 72 h) 0.36±0.07 (*M. edulis* nd) 0.24±0.07 (*M. edulis* d 72 h)	NA	-	Van Cauwenberghe and Janssen, 2014
France, Belgium, and Netherland coast	*Mytilus edulis*	Frg	20–90	0.2±0.3	NA	-	Van Cauwenberghe et al. 2015
Italian natural stocks and mariculture plants	*Mytilus galloprovincialis*	Fib	750–6000	4.4-11.4 (mean)	3.6-12.4 (mean)	-	Renzi et al.,2018
Ionian Sea (Greece)	*Mytilus galloprovincialis*	Frg, Fib	40–737 55–620	Wild: 5.3±0.5 Farmed: 2.5±0.3	Wild: 1.7±0.2 Farmed: 2.0±0.2	PE 75%, PP 12.5% and PTFE 12.5%	Digka et al., 2018
Loire Estuary, Atlantic coast (France)	*Crassostrea gigas*	Frg, Fib	20–1300	0.18±0.16	2.10±1.71	PE 47%, PP 25%, ABS 15 %	Phuong et al., 2018
United Kingdom coast	*Mytilus edulis*	Fib, Frg, sphere, Fil	8-4700	0.7–2.9	1.1–6.4	PES 43 %, Rayon 26 %, Cellulose 14 %	Li et al., 2018
Norway coast	*Mytilus spp.*	Fib, Frg, Foa, Pell	70–3870	0.97±2.61 (range: 0-7.9)	1.5±2.3 (range: 0-6.9)	Cellophane 64%, “parking lot tar” and EVA 19%, PET	Bråte et al., 2018

						9.9%, Acrylic 2.9%, PP 1.2%, PE 1%	
Coastal lagoons on the North Adriatic (Italy)	*Cerastoderma glaucum, Mytilus galloprovincialis, Limecola balthica, Scrobicularia plana*	Fib, Frg, Foa	60–3000	NA	Range:0.01±0.01 to 0.25±0.12	PES 98%, LDPE 1.5%, PP 0.25%, PAN 0.25%	Piarulli et al., 2020
Comercial molluscs of Bizerte Lagoon (Tunisia)	*Mytilus galloprovinciali, Ruditapes decussatus, Crassostrea gigas, Bolinus brandaris*	Fib, Frg, Fil	50–5000	0.70±0.11 to 1.48±0.02	NA	Fib: PP 100% Frg: PP 60%, PE 40% Fil: PE 50%, PP 50%	Abidli et al., 2019
Farmed and wild mussels of Halifax Harbor (Canada)	*Mytilus edulis (farmed and wild)*	Fib	>0.8 µm	-	Wild: 34-126 Farmed: 75-178	-	Mathalon and Hill, 2014
Vancouver island coast (Canada)	*Crassostrea gigas* *Mytilus edulis*	Fib, Frg, Pell	<530	*C. gigas* (Wild): 77±126 *C. gigas* (Farmed): 213±154 *M. edulis* (Wild): 138±202 *M. edulis* (Farmed): 259±114	NA	-	Murphy, 2018
Wild mussels of Ria of Vigo and Cantabrian Sea, Atlantic coast (Spain)	*Mytilus spp*	Fib, Frg, Pell	20-5000	Ria of Vigo: 1.59±1.28 Cantabrian Sea: 2.55±2.80	Ria of Vigo 2.19±1.57 Cantabrian Sea 2.81±2.80	-	Reguera et al., 2019
Farmed and wild from Jiaozhou Bay (China)	*Ruditapes philippinarum* *Crassostrea gigas (wild and farm)*	Fib, Frg	500-8201	*C.gigas* : 0.92±0.80 *R. philippinarum* 1.51±1.27 Range: 0.16 - 12.09	*C.gigas*: 2.34±1.80 *R.philippinarum*: 2.00±1.99 Range: 1-9	Cellophane 48%, PET 21 %	Zhang et al., 2022
North Sea (Netherlands)	*Mytilus edulis*	Fib, Frg	30–2000	37 MPs/$g_{d.w.}$	NA	-	Karlson et al., 2017
North Sea (Netherlands)	*Littorina littorea; Mytilus edulis; Cassostrea gigas*	Fib, Pell, Fil	10–5000	20–105 MPs/$g_{d.w.}$	NA	-	Leslie et al., 2017
British Columbia coast (Canada)	*Crassostrea gigas*	Fibre, Frg, Pell	10–5000	0.04±0.06 MPs/ $g_{d.w.}$	0.22±0.28	Fib: Polyester, Nylon	Covernton et al., 2019

NA: Not assessed. Fib: Fibres, Frg: Fragments, Film: Fil, Pell: pellets or spheres; Foa: Foam; nd: no depurated; d: depurated

2.3.8 Estimated microplastic daily intake through consumption of molluscs

Taking into account the levels of MPs detected in depurated bivalves (mussels, clams and oysters) and the consumption of molluscs by the Spanish population (adults, elderly and pregnant women) from EFSA consumption data (taking account only effective consumers surveyed) (EFSA, 2021), the daily and yearly MPs intake by that population through molluscs' consumption was estimated (Table 4).

The MPs intake through consumption of molluscs was 22.2, 20.4 and 9.67 MPs/day for adult, elderly and pregnant women, respectively. In turn, the adult annual consumption of MPs was set at 8,103, with a 95th percentile of 19,418 MPs/year. Mussels were the main contributor to the intake of MPs, with more than 60% of the total intake. It is important to highlight that these values are obtained from the population who are certainly effective molluscs consumers, but they do not represent the total population surveyed; that is to say, consumers and non-consumers.

The current results are 40-fold higher than those found in Korean population through shellfish consumption (212 MPs/person/year) (Cho et al., 2019). Both, consumption rates and MPs present in shellfish, were lower than those of our survey. The intake of MPs by a Chinese population (Zhang et al., 2022) was found to be 1270 MPs/year, which is between 6 and 7 times lower than that of the current study. Danopoulos et al, (2020), estimated the intake of MPs/year combining global consumption estimates by the FAO/UN (Food and Agriculture Organization of the United Nations) with data from outcomes of applying meta-analysis of the levels of MPs contamination in seafood (mussels, crustaceans, fish and echinoderms). It was concluded that seafood is a major verified vector for human exposure to MPs. According to the results of Danopoulos et al. (2020) (6,306 MPs/person/year), the intake for adults was a little bit smaller than this study mean value. By contrast, in Italy Ferrante et al. (2022) reported an adult MPs yearly intake through mussels' consumption of 5,709,330 MPs/year (15,642 MPs/day). However, in that study MPs sizes analysed ranged between 1.8 and 2.5 µm. In accordance with the results of the present study, Van Cauwenberghe and Janssen (2014) estimated an European annual MPs dietary intake of 11,000 MPs/year with data of MPs concentration found in mussels and oyster from France and Germany. In Northern Tunisia, Abidli et al. (2019) estimated a dietary intake of MPs through the consumption of bivalves and gastropods between 23 and 40 MPs/year for the general population, and between 2,556 and 4,920 MPs/year from local fishermen.

Table 4. MPs daily and yearly intake (MPs/day, MPs/year, and MPs/kg/day) through consumption of depurated molluscs by Spanish consumers.

	Adults	Elderly	Pregnant women
Mollusc consumption (g/day) [a]			
Clams	1.14 (2.10)	1.20 (2.22)	0.60 (0.60)
Mussels	2.89 (8.95)	3.02 (10.5)	1.40 (4.20)
Oysters	1.69 (NR)	NR	NR
MPs consumption (MPs/day)			
Clams	5.67 (10.4)	5.96 (11.0)	2.98 (2.98)
Mussels	13.8 (42.8)	14.4 (50.2)	6.69 (20.1)
Oysters	2.67 (NR)	NC	NC
Total	22.2 (53.2)	20.4 (61.2)	9.67 (23.1)
MPs consumption (MPs/year)			
Total	8,103 (19,418)	7,446 (22,338)	3,530 (8,431)
MPs consumption (MPs/kg/day)			
Total	0.287 (0.690)	0.290 (0.868)	0.148 (0.353)

NR: Not reported; NC: Not calculated. Results expressed as mean (95th percentile).
[a](EFSA; 2021)

2.4 Conclusions

In the present study, a method of analysis of MPs, with different sequential steps, including alkaline hydrolysis, surfactant use, oxidation, enzymatic hydrolysis, density separation and spectroscopic techniques (IR/RAMAN), have been proposed. It has shown to be effective for the extraction, quantification and chemical characterization of MPs in molluscs. All analyzed samples of commercial molluscs from the Catalan coast, contained MPs. Intra- and inter-species variability was high due to the different feeding strategies of each species, the location in the sediment/water column zone, the shell size, as well as the specific local MPs pollution. Microplastics size and shape could affect the ingestion or egestion rate of each organism. Wedge clams and snails showed the lowest concentration of MPs: 0.49±0.23 MP/individual and 0.94±0.62 MP/$g_{w.w}$, respectively. On the other hand, big oysters and mussels showed the highest concentration per individual (22.8±14.4 and 18.6±23.0 MPs/individual, respectively), while mussels showed the highest (wet weight) level (6.47±7.95 MPs/$g_{w.w}$). In general, fibres were the predominant morphology. Of the total particles measured (1460), a 74% showed sizes smaller than 1 mm, whereas 20% were between 0.02 and 0.150 mm, a critical range for passage through the human intestinal barrier. PES polymer, synthetic cellulose, PVDF and PA, were abundant in depurated organisms, with PE in non-depurated organisms. A similar number of polymers for both conditions were observed. In terms of localization, PE was the predominant polymer in the organisms from the Northern zone, while PES, PP, PAN and PA were mainly found in the organisms from the Central zone. Finally, synthetic cellulose, PVDF and the remaining MPs (PC, PMMA, PVC, PET, PVA, PEVA, EVOH/EVA, PS and EPS) were predominant in the organisms from the Southern coast of Catalonia. The bivalve depuration process did not remove MPs from the organisms. Only changes in their morphology, size and composition were observed. The time of depuration could be a key parameter. Regarding human exposure, the daily intake of MPs

through molluscs consumption was estimated to be 22.2, 20.4 and 9.67 MPs, for adults, elderly and pregnant women, respectively. The mean annual MPs (≥ 20μm) consumption for the adult population was estimated in 8,103 MPs, with 95th percentile of 19,418 MPs/year. It suggests that consumption of molluscs is as an important route of exposure to MPs for the population of Catalonia. Anyway, the potential human health risks are still unknown. Therefore, further investigations are clearly required.

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References

Abidli, S., Toumi, H., Lahbib, Y., El Menif N., 2017. The First Evaluation of Microplastics in Sediments from the Complex Lagoon-Channel of Bizerte (Northern Tunisia). Water Air Soil Pollut. 228, 262.

Abidli, S., Antunes, J.C., Ferreira,J.L., Lahbib, Y., Sobral, P., El Menif, N., 2018. Microplastics in sediments from the littoral zone of the north Tunisian coast (Mediterranean Sea). Estuar. Coast Shelf Sci. 205,1-9.

Abidli, S., Lahbib, Y., Trigui El Menif, N., 2019. Microplastics in commercial molluscs from the lagoon of Bizerte (Northern Tunisia). Mar. Pollut. Bull. 142, 243–252.

Agencia Catalana del Agua. 2022. Available from: https://aca.gencat.cat/es/inici/index.html. Last accessed March 2022

Andrady, A.L., 2011. Microplastics in the marine environment. Mar Pollut Bull. 62, 1596–1605.

Birnstiel, S., Soares-Gomes, A., da Gama, B.A.P., 2019. Depuration reduces microplastic content in wild and farmed mussels. Mar. Pollut. Bull. 140, 241–247.

Booth, A., Kubowicz, S., Beegle-Krause, C.J., Skancke, J., Nordam, T., Landsem, E., Throne-Holst, M., Jahren, S., 2017. Microplastic in global and Norwegian marine environments: Distributions, degradation mechanisms and transport. Sintef. Ocean A.S.1-147 pp

Bråte, I.L.N., Hurley, R., Iversen, K., Beyer, J., Thomas, K.V., Steindal, C.C., Green, N.W., Olsen, M., Lusher, A., 2018. Mytilus spp. as sentinels for monitoring microplastic pollution in Norwegian coastal waters: a qualitative and quantitative study. Environ. Pollut. 243, 383–393.

Campbell, D., Pethrick, R.A., White, J.R., 2000. Polymer characterization: Physical techniques (2nd Edition). CRC Press, Stanley Thornes (Publishers)LTD. United Kingdom 481 pp.

Carbery, M., O'Connor, W., Palanisami, T., 2018. Trophic transfer of microplastics and mixed contaminants in the marine food web and implications for human health. Environ. Int. 115, 400–409.

Catarino, A.I., Macchia, V., Sanderson, W.G., Thompson, R.C., Henry, T.B., 2018. Low levels of microplastics (MP) in wild mussels indicate that MP ingestion by humans is minimal compared to exposure via household fibres fallout during a meal. Environ. Pollut. 237, 675–684.

Chang, X., Xue, Y., Li, J., Zou, L., Tang, M., 2020. Potential health impact of environmental micro- and nanoplastics pollution. J. Appl. Toxicol. 40, 4–15.

Cho, Y., Shim, W.J., Jang, M., Han, G.M., Hong, S.H., 2019. Abundance and characteristics of microplastics in market bivalves from South Korea. Environ. Pollut. 245, 1107–1116.

Cincinelli, A., Scopetani C., Chelazzi, D., Lombardini, E., Martellini, T., Katsoyiannis, A., Fossi, M.C., Corsolini, S., 2017. Microplastic in the surface waters of the Ross Sea (Antarctica): Occurrence, distribution and characterization by FTIR. Chemosphere 175, 391-400.

Constant, M., Kerhervé, P., Mino-Vercellio-Verollet, M., Dumontier, M., Sànchez Vidal, A., Canals, M., Heussner, S., 2019. Beached microplastics in the Northwestern Mediterranean Sea. Mar. Pollut. Bull. 142, 263–273.

Courtene-Jones, W., Quinn, B., Murphy, F., Gary, S.F., Narayanaswamy, B.E., 2017. Optimisation of enzymatic digestion and validation of specimen preservation methods for the analysis of ingested microplastics. Anal. Methods-U.K. 9, 1437–1445.

Covernton, G.A., Collicutt, B., Gurney-Smith, H.J., Pearce, C.M., Dower, J.F., Ross, P.S., Dudas, S.E., 2019. Microplastics in bivalves and their habitat in relation to shellfish aquaculture proximity in coastal British Columbia, Canada. Aquac. Environ. Interact. 11, 357–374.

Cózar, A., Sanz-Martín, M., Martí, E., González-Gordillo, J.I., Ubeda, B., Gálvez, Á., Irigoien, J., Duarte, C.M., 2015. Plastic accumulation in the Mediterranean Sea. PLoS One 10, 1–12.

Dalmau-Soler, J., Ballesteros-Cano, R., Boleda, M.R., Paraira, M., Ferrer, N. & Lacorte, S., (2021), Microplastics from headwaters to tap water: presence and removal in a drinking water treatment plant in Barcelona Metropolitan area (Catalonia, NE Spain). Environ. Sci. Pollut. Res. 28, 59462–59472.

Danopoulos, E., Jenner, L.C., Twiddy, M., Rotchell, J.M., 2020. Microplastic contamination of seafood intended for human consumption: a systematic review and metaanalysis. Environ. Health. Perspect. 128, 126002.

de Haan, W.P., Sanchez-Vidal, A., Canals, M., 2019. Floating microplastics and aggregate formation in the Western Mediterranean Sea. Mar. Pollut. Bull. 140, 523–535.

de Haan, W.P., Uviedo, O., Ballesteros, M., Canales, I., Curto, X., Guart, M., Higueras, S., Molina, A., Sanchez-Vidal, A., Surfing for Science Group3.,2022. Floating microplastic loads in the nearshore revealed through citizen science. Environ. Res. Lett. 17, 045018.

Dehaut, A., Cassone, A.L., Frère, L., Hermabessiere, L., Himber, C., Rinnert, E., Rivière, G., Lambert, C., Soudant, P., Huvet, A., Duflos, G., Paul-Pont, I., 2016. Microplastics in seafood: Benchmark protocol for their extraction and characterization. Environ. Pollut. 215, 223–233.

Digka, N., Tsangaris, C., Torre, M., Anastasopoulou, A., Zeri, C., 2018. Microplastics in mussels and fish from the northern Ionian Sea. Mar. Pollut. Bull. 135, 30–40.

Ding, J., Li, J., Sun, C., He, C., Jiang, F., Gao, F., Zheng, L., 2018. Separation and identification of microplastics in digestive system of bivalves. Chin. J. Anal. Chem. 46, 690–697.

Ding, J., Li, J., Sun, C., Jiang, F., He, C., Zhang, M., Ju, P., Xiangyu, N., 2020. An examination of the occurrence and potential risks of microplastics across various shellfish. Sci. Total Environ. 739, 139887.

Ding, J., Sun, C., He, C., Li, J., Ju, P., Li, F., 2021. Microplastics in four bivalve species and basis for using bivalves as bioindicators of microplastic pollution. Sci. Total Environ. 782, 146830.

EFSA (European and Food Safety and Authority), 2016. Presence of microplastics and nanoplastics in food, with particular focus on seafood. EFSA Journal; 14, e04501.

EFSA, (European Food Safety Authority), 2021. Food consumption data. Available from: https://www.efsa.europa.eu/en/data-report/food-consumption-data. Last accessed: February 2022.

Expósito, N., Rovira, J., Sierra, J., Folch, J., Schuhmacher, M., 2021. Microplastics levels, size, morphology and composition in marine water, sediments and sand beaches. Case study of Tarragona coast (western Mediterranean). Sci. Total Environ. 786, 147453.

Fang, C., Zheng, R., Chen, H., Hong, F., Lin, L., Lin, H., Guo, H., Bailey, C., Segner, H., Mu, J., Bo, J., 2019. Comparison of microplastic contamination in fish and bivalves from two major cities in Fujian province, China and the implications for human health. Aquaculture 512, 734322.

FAO, Food and Agriculture Organization of the United Nations). 2022. Cultured Aquatic Species Information Programme (CASIP). Fisheries and Aquaculture Division. Rome. Available from: https://www.fao.org/fishery/en/collection/culturedspecies. Last accessed: March 2022.

FAO (Food and Agriculture Organization of the United Nations). 2020. Fishery Statistical Collections; Consumption of Fish and Fishery Products. FAOSTAT -Food Balance Sheets [Online]. Rome, Italy. Available from: https://www.fao.org/fishery/statistics/global-consumption/en. Last accessed 22 October 2020.

Farady, S.E., 2019. Microplastics as a new, ubiquitous pollutant: strategies to anticipate management and advise seafood consumers. Mar. Pol. 104, 103–107.

Ferrante, M., Pietro, Z., Allegui, C., Maria, F., Antonio, C., Pulvirenti, E., Favara, C., Chiara, C., Grasso, A., Omayma, M., Gea, O.C., Banni, M., 2022. Microplastics in fillets of Mediterranean seafood. A risk assessment study. Environ. Res. 204, 112247.

Filgueiras, A.V., Gago, J., Campillo, J.A., León, V.M., 2019. Microplastic distribution in surface sediments along the Spanish Mediterranean continental shelf. Environ. Sci. Pollut. Res. 26, 21264–21273.

Galli, M.,Tepsich, P., Baini, M., Panti, C., Rosso, M., Vafeiadou, A., Pantelidou, M.,Moulins, A., Fossi, M.C., 2022. Microplastic abundance and biodiversity richness overlap: Identification of sensitive areas in the Western Ionian Sea. Mar. Pollut. Bull.177, 113550

GENCAT, Generalitat de Catalunya. Departament d'Acció Climàtica, Alimentació i Agenda Rural. 2021. Shellfish production zones in Catalonia. Available from: http://agricultura.gencat.cat/ca/detalls/Article/Zones-de-produccio-marisquera. Last accessed December 2021

GENCAT, Generalitat de Catalunya. 2022. Territori i Urbanisme. Available from: https://territori.gencat.cat/ca/06_territori_i_urbanisme/. Last accessed March 2022

Gonzalez, N., Calderon, J., Rubies, A., Timoner, I., Castell, V., Domingo, J.L., Nadal, M.,2019. Dietary intake of arsenic, cadmium, mercury and lead by the population of Catalonia, Spain: Analysis of the temporal trend. Food Chem. Toxicol. 132:110721

Halstead, J.E., Smith, J.A., Carter, E.A., Lay, P.A., Johnston, E.L., 2018. Assessment tools for microplastics and natural fibres ingested by fish in an urbanised estuary. Environ. Pollut. 234, 552–561.

Jahan, S., Strezov, V., Weldekidan, H., Kumar, R., Kan, T., Sarkodie, S.A., He, J., Dastjerdi, B., Wilson, S.P., 2019. Interrelationship of microplastic pollution in sediments and oysters in a seaport environment of the eastern coast of Australia. Sci. Total Environ. 695, 133924.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science 347 (80), 768–771.

Karami, A., Golieskardi, A., Choo, C.K., Romano, N., Ho, Y.B., Salamatinia, B., 2017. A high-performance protocol for extraction of microplastics in fish. Sci. Total Environ. 578, 485e494.

Keisling, C., Harris, R.D., Blaze, J., Coffin, J., Byers, J.E., 2020. Low concentrations and low spatial variability of marine microplastics in oysters (Crassostrea virginica) in a rural Georgia estuary. Mar. Pollut. Bull. 150, 110672.

Kolandhasamy, P., Su, L., Li, J., Qu, X., Jabeen, K., Shi, H., 2018. Adherence of microplastics to soft tissue of mussels: a novel way to uptake microplastics beyond ingestion. Sci. Total Environ. 610–611, 635–640.

Leslie, H.A., Brandsma, S.H., van Velzen, M.J.M., Vethaak, A.D., 2017. Microplastics en route: field measurements in the Dutch river delta and Amsterdam canals, wastewater treatment plants, North Sea sediments and biota. Environ. Int. 101, 133–142.

Li, W.C., Tse, H.F., Fok, L., 2016. Plastic waste in the marine environment: A review of sources, occurrence and effects. Sci. Total Environ. 566–567, 333–349.

Li, J., Qu, X., Su, L., Zhang, W., Yang, D., Kolandhasamy, P., Li, D., Shi, H., 2016. Microplastics in mussels along the coastal waters of China. Environ. Pollut. 214, 177–184

Li, J., Green, C., Reynolds, A., Shi, H., Rotchell, J.M., 2018. Microplastics in mussels sampled from coastal waters and supermarkets in the United Kingdom. Environ. Pollut. 241, 35–44.

Li, J., Yang, D., Li, L., Jabeen, K., Shi, H., 2015. Microplastics in commercial bivalves from China. Environ. Pollut. 207, 190–195.

Li, Q., Sun, C., Wang, Y., Cai, H., Li, L., Li, J., Shi, H., 2019. Fusion of microplastics into the mussel byssus. Environ. Pollut. 252, 420–426.

Lots, F.A.E., Behrens, P., Vijver, M.G., Horton, A.A., Bosker, T., 2017. A large-scale investigation of microplastic contamination: abundance and characteristics of microplastics in European beach sediment. Mar. Pollut. Bull. 123, 219–226.

Masura, J., Baker, J., Foster, G. and Arthur, C., 2015. Laboratory methods for the analysis of microplastics in the marine environment: recommendations for quantifying synthetic particles in waters and sediments. Siver Spring, MD, NOAA Marine Debris Division, 31pp. (NOAA Technical Memorandum NOS-OR&R-48).

Mathalon, A., Hill, P., 2014. Microplastic fibres in the intertidal ecosystem surrounding Halifax Harbor, Nova Scotia. Mar. Pollut. Bull. 81, 69–79.

Murphy, C.L., 2018. A comparison of microplastics in farmed and wild shellfish near Vancouver Island and potential implications for contaminant transfer to humans. Available from ProQuest Dissertations Publishing. Retrieved from: https://search. proquest.com/docview/2027207899?accountid¼11528.Last accessed March 2022.

Naji, A., Nuri, M., Vethaak, A.D., 2018. Microplastics contamination in molluscs from the northern part of the Persian Gulf. Environ. Pollut. 235, 113–120.

Phuong, N.N., Poirier, L., Pham, Q.T., Lagarde, F., Zalouk-Vergnoux, A., 2018. Factors influencing the microplastic contamination of bivalves from the French Atlantic coast: location, season and/or mode of life?. Mar. Pollut. Bull. 129, 664–674.

Piarulli, S., Vanhove, B., Comandini, P., Scapinello, S., Moens, T., Vrielinck, H., Sciutto, G., Prati, S., Mazzeo, R., Booth, A.M., Van Colen, C., Airoldi, L., 2020. Do different habits affect microplastics contents in organisms? A trait-based analysis on salt marsh species. Mar. Pollut. Bull. 153, 110983.

PlasticsEurope, 2013. Plastics - the facts 2013. An analysis of European plastics production, demand and waste data for 2012. Plastics Europe. Association of Plastic Manufacturers, Brussels.

PlasticsEurope, 2017. Plastics - the facts 2017. An analysis of European plastics production, demand and waste data for 2016. Plastics Europe. Association of Plastic Manufacturers, Brussels.

PlasticsEurope, 2019. Plastics - the facts 2019. An analysis of European plastics production, demand and waste data for 2018. Plastics Europe. Association of Plastic Manufacturers, Brussels.

PlasticsEurope, 2021. Plastics - the facts 2021. An analysis of European plastics production, demand and waste data for 2019-2020. Plastics Europe. Association of Plastic Manufacturers, Brussels

Prata, J.C., da Costa, J.P., Lopes, I., Duarte, A.C., Rocha-Santos, T., 2020. Environmental exposure to microplastics: an overview on possible human health effects. Sci. Total Environ. 702, 134455.

Prüst, M., Meijer, J., Westerink, R.H.S., 2020. The plastic brain:neurotoxicity of micro-and nanoplastics. Part. Fibre Toxicol. 17, 24.

Qiu, Q.X., Peng, J.P., Yu, X.B., Chen, F., Wang, J., Dong, F., 2015. Occurrence of microplastics in the coastal marine environment: first observation on sediment of China. Mar. Pollut. Bull. 98 (1-2), 274-280

Qu, X., Su, L., Li, H., Liang, M., and Shi, H., 2018. Assessing the relationship between the abundance and properties of microplastics in water and in mussels. Sci. Total Environ. 621, 679–686.

Rahman, A., Sarkar, A., Yadav, O.P., Achari, G., Slobodnik, J., 2021. Potential human health risks due to environmental exposure to nano- and microplastics and knowledge gaps: a scoping review. Sci. Total Environ. 757, 143872.

Reguera, P., Viñas, L. and Gago, J., 2019, Microplastics in wild mussels (*Mytilus spp.*) from the north coast of Spain. Sci. Mar. 83, 337–347.

Reineccius, J., Appelt, J.S., Hinrichs, T., Kaiser, D., Stern, J., Prien, R.D., Waniek, J.J., 2020. Abundance and characteristics of microfibres detected in sediment trap material from the deep subtropical North Atlantic Ocean. Sci. Total Environ. 738, 140354.

Remy, F., Collard, F., Gilbert, B., Compère, P., Eppe, G., Lepoint, G., 2015. When microplastic is not plastic: the ingestion of artificial cellulose fibres by macrofauna living in seagrass macrophytodetritus. Environ. Sci. Technol. 49, 11158–11166.

Renzi, M., Guerranti, C., Blašković, A., 2018. Microplastic contents from maricultured and natural mussels. Mar. Pollut. Bull. 131, 248–251.

Rist, S., Steensgaard, I. M., Guven, O., Nielsen, T. G., Jensen, L. H., Møller, L. F. & Hartmann, N. B., 2019. The fate of microplastics during uptake and depuration phases in a blue mussel exposure system. Environ. Toxicol. Chem. 38, 99-105.

Rochman, C.M., Tahir, A., Williams, S.L., Baxa, D.V., Lam, R., Miller, J.T., Teh, F.C., Werorilangi, S., Teh, S.J., 2015. Anthropogenic debris in seafood: plastic debris and fibres from textiles in fish and bivalves sold for human consumption. Sci. Rep. 5, 1-10.

Sanchez-Vidal, A., Thompson, R.C., Canals, M., de Haan, W.P., 2018. The imprint of microfibres in southern European deep seas. PLoS One 13, 1–12.

Simon-Sánchez, L., Grelaud, M., Garcia-orellana, J., Ziveri, P., 2019. River Deltas as hotspots of microplastic accumulation: the case study of the Ebro River (NW Mediterranean). Sci. Total Environ. 687, 1186–1196.

Schirinzi, G.F., Köck-Schulmeyer, M., Cabrera, M., González-Fernández, D., Hanke, G., Farré, M., Barceló, D.,2020. Riverine anthropogenic litter load to the Mediterranean Sea near the metropolitan area of Barcelona, Spain. Sci Total Environ.714,136807.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675.

Su, L., Cai, H., Kolandhasamy, P., Wu, C., Rochman, C.M., Shi, H., 2018. Using the Asian clam as an indicator of microplastic pollution in freshwater ecosystems. Environ. Pollut. 234, 347–355.

Suaria, G., Musso, M., Achtypi, A., Bassotto, D., Aliani, S., 2020. Textile Fibres in Mediterranean Surface Waters: Abundance and Composition. In book: Proceedings of the 2nd International Conference on Microplastic Pollution in the Mediterranean Sea Publisher. Springer Nature Switzerland AG 2020 M. Cocca et al. (Eds.): ICMPMS 2019, SPWA, pp. 62–66,

Sun, J., Dai, X., Wang, Q., van Loosdrecht, M. C. M., Bing-Jie, N., 2019. Microplastics in wastewater treatment plants: detection, occurrence and removal. Water Res. 152, 21–37.

Tagg, A.S., Sapp, M., Harrison, J.P., Ojeda, J.J., 2015. Identification and quantification of microplastics in wastewater using focal plane array-based reflectance micro-FT-IR imaging. Analytical chemistry, 87(12), 6032-6040.

Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M.B., Janssen, C.R., 2015. Microplastics are taken up by mussels (Mytilus edulis) and lugworms (Arenicola marina) living in natural habitats. Environ. Pollut. 199, 10–17.

Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human consumption. Environ. Pollut. 193, 65–70.

van Raamsdonk, L.W.D., van der Zande, M., Koelmans, A. A., Hoogenboom, R. L.A.P, Peters, R.J.B., Groot, M.J., Peijnenburg, A.A.C.M., Wessepoel, Y.J.A., 2020. Current insights in to monitoring, bioaccumulation, and potential health effects of Microplastics present in the food chain. Foods 9, 72.

Vandermeersch, G., Van Cauwenberghe, L., Janssen, C.R., Marques, A., Granby, K., Fait, G., Kotterman, M.J.J., Diogène, J., Bekaert, K., Robbens, J., Devriese, L., 2015. A critical view on microplastic quantification in aquatic organisms. Environ. Res. 143, 46–55.

Wang J., Li Y., Lu L., Zheng M., Zhang X., Tian H., Wang W., Ru, S., 2019a. Polystyrene microplastics cause tissue damages, sex-specific reproductive disruption and transgenerational effects in marine medaka (*Oryzias melastigma*). Environ. Pollut. 254, 113024.

Wang W., Gao, H., Jin, S., Li, R., Na, G., 2019b. The ecotoxicological effects of microplastics on aquatic food web from primary producer to human: a review. Ecotoxicol. Environ. Saf. 173, 110–117.

Wang Y.L., Lee, Y. H., Chiu, I. J., Lin, Y. F., Chiu, H. W., 2020. Potent impact of plastic nanomaterials and micromaterials on the food chain and human health. Int. J. Mol. Sci. 21, 1727.

Ward, J.E., Zhao, S., Holohan, B.A., Mladinich, K.M., Griffin, T.W., Wozniak, J., Shumway, S.E., 2019a. Selective Ingestion and Egestion of Plastic Particles by the Blue Mussel (Mytilus edulis) and Eastern Oyster (*Crassostrea virginica*): Implications for Using Bivalves as Bioindicators of Microplastic Pollution. Environ. Sci. Technol. 53, 8776–8784.

Ward, J.E., Rosa, M., Shumway, S.E., 2019b. Capture, ingestion, and egestion of microplastics by suspension-feeding bivalves: a 40-year history. Anthropocene Coasts 2, 19-29.

Wegner, A., Besseling, E., Foekema, E.M., Kamermans, P., Koelmans, A.A., 2012. Effects of nanopolystyrene on the feeding behavior of the blue mussel (*Mytilus edulis*.). Environ. Toxicol. Chem. 31, 2490–2497.

Wheeler, R. M.; Lower, S. K., 2021. Statistical Thermodynamic Description of Heteroaggregation between Anthropogenic Particulate Matter and Natural Particles in Aquatic Environments. ACS Earth Space Chem 5(4),980–989

Wright, S. L., Kelly, F. J., 2017. Plastic and Human Health: A Micro Issue?. Environ. Sci. Technol. 51, 6634– 6647.

Wu, F., Wang, Y., Leung, J.Y., Huang, W., Zeng, J., Tang, Y., Tang, Y., Chen, J., Shi, A., Yu, X., Xu, X., Zhang, H., Cao, L., 2020. Accumulation of microplastics in typical commercial aquatic species: a case study at a productive aquaculture site in China. Sci. Total Environ. 708, 135432.

Xiong, X., Tu, Y., Chen, X., Jiang, X., Shi, H., Wu, C., Elser, J.J., 2019. Ingestion and egestion of polyethylene microplastics by goldfish (*Carassius auratus*): influence of color and morphological features. Heliyon 5, e03063.

Xu, X., Wong, C.Y., Tam, N.F.Y., Lo, H.S., Cheung, S.G., 2020. Microplastics in invertebrates on soft shores in Hong Kong: Influence of habitat, taxa and feeding mode. Sci. Total Environ. 715, 136999.

Yadav, N., Hakkarainen, M., 2021. Degradable or not? Cellulose acetate as a model for complicated interplay between structure, environment and degradation. Chemosphere 265, 128731.

Zhang, K., Liang, J., Liu, T., 2022. Abundance and characteristics of microplastics in shellfish from Jiaozhou Bay, China. J. Ocean. Limnol. 40, 163–172

Zheng, Y. F., Li, J. X., Cao, W., Liu, X. H., Jiang, F. H., Ding, J. F., Yin, X. F., Sun, C. J., 2019. Distribution characteristics of microplastics in the seawater and sediment: a case study in Jiaozhou Bay, China. Sci. Total Environ. 674, 27-35.

Zhao, S.Y., Zhu, L.X., Wang, T., Li, D.J., 2014. Suspended microplastics in the surface water of the Yangtze Estuary system, China: first observations on occurrence, distribution. Mar. Pollut. Bull. 86 (1-2), 562e568

Zhao, S., Ward, J.E., Danley, M., Mincer, T.J., 2018. Field-based evidence for microplastic in marine aggregates and mussels: implications for trophic transfer. Environ. Sci. Technol. 52, 11038–11048.

CHAPTER 3-DISCUSSION

3.1.-Microplastics in Catalonia coastal Areas-Environmental Risk Assessment

Ecological risk assessment is a process that evaluates the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to one or more stressors (U.S. EPA, 1992). The process is used to systematically evaluate and organize data, information, assumptions, and uncertainties in order to help understand and predict the relationships between stressors and ecological effects in a way that is useful for environmental decision making. An assessment may involve chemical, physical, or biological stressors, and one stressor or many stressors may be considered (U.S. EPA, 1998).

Ecological risk assessments are developed within a risk management context to evaluate human-induced changes that are considered undesirable. Ecological risk assessments can be used to predict the likelihood of future adverse effects (prospective) or evaluate the likelihood that effects are caused by past exposure to stressors (retrospective). In many cases, both approaches are included in a single risk assessment. (U.S. EPA, 1998). Ecological risk assessments inform an environmental damage assessment in which loss of ecological services (e.g., flood protection, fishable waters) will be valued monetarily.

Environmental Risk Assessment (ERA) is a process that evaluates the likelihood or probability that adverse effects may occur to environmental values, as a result of human activities (i.e., a formal procedure for identifying and estimating the risk of environmental damage). The ERA procedure is triggered prior to a significant decision affecting the environment. It can be broken into three broad stages: preparation, involving collecting and examining relevant background information, and establishing the focus for the assessment; conducting the assessment; and, interpreting, reporting and applying results of the assessment (Ministry of Environment, Lands and Parks of Canada, 2000)

Risk assessment generally comprises several sequential steps: (step 1) problem formulation, a critical first step, including hazard identification; (step 2) hazard characterisation that examines potential hazards and their magnitude; (step 3) exposure characterisation that estimates levels and likelihood of exposure; and (step 4) integrative risk characterisation in which the magnitude of consequences and the likelihood of occurrence are integrated. Risk characterisation (step 4) may identify risks that require management measures (Arpaia, 2013).

Finally, an evaluation of the overall risk of) (step 5) shall be made taking into account the results of the ERA and associated levels of uncertainty, the weight of evidence and the risk management strategies proposed in the receiving environment(s). The ERA is conducted starting with step 1 and moving to step 5; step 2 and 3 can however be carried out in parallel (Arpaia, 2013). In figure 1, shows the six steps of the environmental risk assessment (ERA) according to Directive 2001/18/EC (EC, 2001) and Regulation (EC) No. 1829/2003.

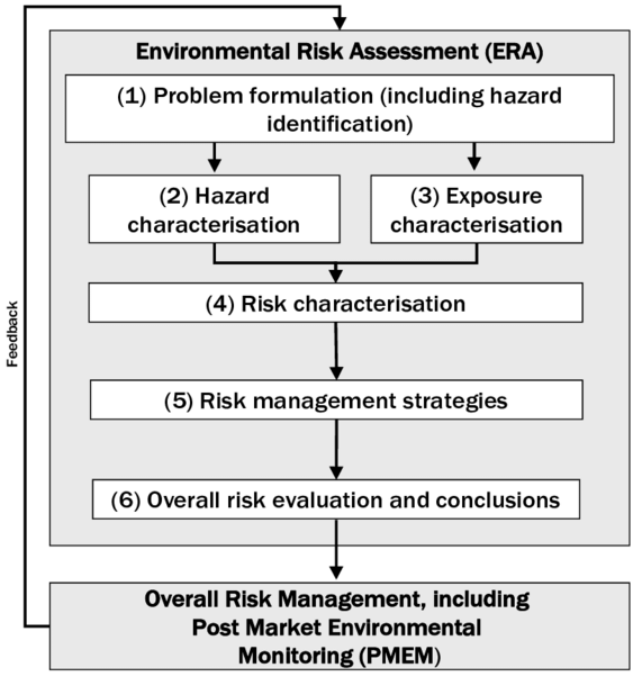


Figure 1: Six steps of the environmental risk assessment (ERA) and the relationship to overall risk management, including post-market environmental monitoring. Taken from Arpaia (2013).

Ecological risk assessment process is based on two major elements: characterization of effects and characterization of exposure. These provide the focus for conducting the three phases of risk assessment: problem formulation, analysis, and risk characterization (U.S EPA,1998),

Each step in an ERA is detailed below according to the guidelines U.S EPA (1998):

1.-In problem formulation, the purpose for the assessment is articulated, the problem is defined, and a plan for analyzing and characterizing risk is determined. Three principal criteria are used to select ecological values that may be appropriate for assessment endpoints (survival, growth, and reproduction of fish, aquatic invertebrates, and algae): (1) ecological relevance (any level of organization as individual, population, community, ecosystem, landscape)., (2) susceptibility to known or potential stressors, and (3) relevance to management goals.

2.-Analysis is a process that examines the two primary components of risk, exposure and effects, and their relationships between each other and ecosystem characteristics providing the necessary data for determining or predicting ecological responses to stressors under exposure conditions of interest.

3.-For effects characterization, ecotoxicological hazard assessments for individual chemicals are routinely conducted on the basis of laboratory data from standardized tests using organisms from major trophic levels (primary producers, primary and secondary consumers). According to the European chemicals legislation REACH this information is condensed into a Predicted No Effect Concentration (PNEC) for the considered ecosystem. The PNEC is derived by selecting the most sensitive biotest (representing the most sensitive trophic level) and applying an appropriate assessment factor (AF), which accounts for intra- and interlaboratory variation of the data, biological variance, short-term to long-term extrapolation and laboratory to field extrapolation (ECHA, 2008).

4.-Risk characterization is the final phase of ecological risk assessment and is the culmination of the planning, problem formulation, and analysis of predicted or observed adverse ecological effects related to the assessment endpoints. Completing risk characterization allows risk assessors to clarify the relationships between stressors, effects, and ecological entities and to reach conclusions regarding the occurrence of exposure and the adversity of existing or anticipated effects.

3.1.1 Risk Assessment of MPs present in Tarragona coast.

The impacts of MPs on the marine environment are classified in direct impacts due MPs structure (shape and surface characteristics) and indirect impacts associated with the chemicals present **or attached to** MPs, taking into account MPs role as a vector of organic pollutants (Bakir et al., 2017; Everaert et al., 2018; Koelmans et al., 2016; Ziccardi et al., 2016) and potential toxicological damages; (Hartman et al., 2017; Lohmann, 2017).

Currently, there is not standardized approach to systematically assess the environmental risk of MPs (Liu et al., 2022), approaches with different indicators, taking in account MPs concentration, polymer types or MPs toxicity (based in a specie sensitivity distribution-SSD) in order to better understand the risk of MPs in the aquatic environment are applied.

Burns and Boxall (2018), were the first to systematically assess the possible risk of MPs in water columns and sediments of both freshwater and marine environments with calculation of predicted no-effect concentrations values (PNEC). The aggregated data (combined marine and freshwater)

suggested that MPs pose no immediate threat to the environment, but no risk assessment specific to one compartment or the other was performed in this study.

The risk assessment based in PNEC values were performed in Tarragona marine environment taking into account seawater surface and sediments matrix separately as opposed to Burns and Boxall (2018), and in base to exposure assessment solely on in situ measured environmental concentrations.

Other index or approaches used for potential threat assessment of MPs to marine environment, included different indicators MPs concentration in environment compared with the lowest concentration reported, polymer types or in combination in order to better understand the risk of MPs in Tarragona coast.

3.1.1.1 Risk assessment in seawater

For seawater risk assessment three index were applied. At the first, risks by MPs composition in seawater was evaluated in a polymer risk index (H) that evaluate hazardous effect of polymer composition regarding a hazard score approach. The hazard index was calculated based on the model described by Lithner et al., (2011); Fang et al., (2019), and Xu et al., (2018) and adapted by Ding et al., (2020), and Liu et al., (2022):

$$H = \sum Pn \; x \; Sn \qquad \text{eq. 1}$$

Where Pn is the percent or proportion of MPs polymer types collected at each sampling area on seawater and Sn is the score of each plastic polymers which was stablished by Lithner et al., (2011), according hazard classification of monomers and additives. Regarding to composition analysis for each sampling zone, the hazard score for polymer type calculated by Lithner et al. (2011) and health hazard description is shown in Table 1 and Table 2 respectively.

Lithner et al., (2011) identified and compiled health hazards classification of chemicals used in thermoplastic and thermosetting polymers production. A hazard ranking model was developed based on the hazard classes and categories specified in the EU classification and labelling (CLP) regulation, Annex VI, which is based on the UN Globally Harmonized System (classifications including the 1st Adaptation to Technical Progress have been used, European Commission, 2009). These classifications reflect the intrinsic hazardous properties of a substance or mixture and do not consider exposure. Substances not found in Annex VI were also searched in the European chemical Substances Information System (ESIS) database.

The polymers were ranked based on monomers and additives hazard classification (table 2.3.1). Five range levels of hazards as 1-10, 10-100, 100-1000, 1000-10000, >10000 has been used with a factor of 10 (which makes the system very rough) for health hazard criteria. Hazard grades with corresponding statements were assigned for each hazard level from I to V (table 1.1.1.2). The index H was compared with hazard levels from 1 to >10000 and corresponding hazard grade showed in table 3.

Table 1 Hazard score for monomers and additives of polymers detected in microplastics in seawater, assigned by Lithner et al. (2011).

Polymer	Abbreviation	Monomers	Chemical Additives	Score (Hazard grade[a])
Polyvinyl chloride	PVC	Vinyl chloride	Benzyl butyl phthalate (BBP)	**10551 (V)**
Polypropylene	PP	Propylene		**1 (I)**
Polyethylene terephthalate	PET	Ethylenglicol Terephthalic acid Dimethyl terephthalate		**4 (II)**
Polyethylene	PE	Ethylene		**11 (II)**
Polystyrene	PS	Styrene	Pentane	**30 (II)**
Expanded Polystyrene	EPS	Styrene	Pentane	**44 (III)**
Polyacrylonitrile	PAN	Acrylonitrile		**11521 (V)**
Polyacrylic acid	PAA	Acrylic acid		**230 (III)**
Polymethyl methacrylate	PMMA	Methyl methacrylate		**1021 (IV)**
Polyamide 6,6 (Nylon 6,6)	PA	Adipic acid Hexamethylenediamine		**63 (III)**
Polyurethane, polyether based flexible foam	PUR	Propylene oxide Toluene-diisocyanate		**13884 (V)**
Polycarbonate	PC	Bisphenol A Phosgene		**1177 (IV)**
Acrylonitrile-butadiene-styrene terpolymer	ABS	Styrene Acrylonitrile 1,3-butadiene		**6552 (V)**
Polybutylene terephthalate	PBT	Dimethyl terephthalate		–
Polyvinylidene fluoride	PVDF	Vinylidene fluoride		–
Polytetrafluoroethylene	PTFE	Tetrafluoroethylene		–
Polyvinyl alcohol	PVA	Vinyl alcohol		–
Synthetic Cellulose o Rayon	–	–		–

[a] The value for the score of each polymer is taken from Lithner et al. (2011).
– represents that its hazard score cannot be determined.

Table 2 Health hazard description for hazard level and grade

Less hazardous					**Most hazardous**
Hazard level	**<10**	**10 – 100**	**100 – 1000**	**1000 – 10000**	**>10000**
Risk category (hazard grade)	I	II	III	IV	V
Hazard statements	No health hazard	**1**.-Acute toxicity (cat. 4 — oral) **2**.-Specific target organ toxicity — single exposure (cat. 3)	**1**.-Carcinogenicity (cat. 2) **2**.-Reproductive toxicity (cat. 2; lact.) **3**.-Acute toxicity (cat. 3 — oral) **4**.-Specific target organ toxicity — single exposure (cat. 2) **5**.-Specific target organ toxicity — repeated exposure (cat. 2)	**1.**-Germ cell mutagenicity (cat. 2) **2**.-Acute toxicity (cat. 1; 2 — oral) **3**.-Specific target organ toxicity — single exposure (cat. 1) **4.**-Specific target organ toxicity — repeated exposure (cat. 1)	**1**.-Carcinogenicity (cat. 1A; 1B) **2**.-Germ cell mutagenicity (cat. 1A; 1B) **3**.-Reproductive toxicity** (cat. 1A; 1B) **4**.-Persistent, bioaccum., toxic/very persistent, very bioaccum. ***

Adapted from Lithner et al. (2011) and Ding et al. (2020)
Reproductive toxicity: May damage **fertility and the unborn child (**d**evelopment); suspected of damaging fertility and the unborn child
*** Non incorpored into the CLP regulation taken from Ding et al., (2020)
Carcinogenetic Category 1A: Known to have carcinogenic potential for humans.
Carcinogenetic Category 1B: Presumed human carcinogens
Carcinogenetic Category 2: Suspected human carcinogens
Germ cell mutagenicity Category 1A: Chemicals known to induce heritable mutations in germ cells of humans
Germ cell mutagenicity Category 1B: Chemicals regarded as if they induce heritable mutations in germ cells of humans
Germ cell mutagenicity Category 2: Chemicals, which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans
Reproductive toxicity Category 1A: Known human reproductive toxicants,
Reproductive toxicity Category 1B: Presumed human reproductive toxicants - largely based on animal studies.
Reproductive toxicity Category 2; lact: Suspected human reproductive toxicant - Evidence from animal and/or human studies is limited/ Effects on via lactation
Acute Toxicity Category 1: Fatal if is swallowed, LD50 5 mg/kg body weight
Acute Toxicity Category 2: Fatal if is swallowed, LD50 50 mg/kg body weight
Acute Toxicity Category 3: Toxic if is swallowed, LD50 300 mg/kg body weight
Acute Toxicity Category 4: Harmful if is swallowed, LD50 2000 mg/kg body weight
Specific target organ toxicity — single exposure Category 1: Substances that have produced significant toxicity in humans, or that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to produce significant toxicity in humans following single exposure C≤ 300 mg/kg body weight. (The guidance value C is usually bigger than NOAEL and more like **LOAEL**)
Specific target organ toxicity — single exposure Category 2: Substances that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to be harmful to human health following single exposure. 2000≥C>300 mg/kg body weight.
Specific target organ toxicity — single exposure Category 3: Transient target organ effects. C guidance values do not apply
Specific target organ toxicity — repeated exposure Category 1: Reliable evidence on the substance or mixture (including bridging) of an adverse effect on specific organ/systems or systemic toxicity in humans or animals. May be named for specific organ/system. C≤ 10 mg/kg bw/d
Specific target organ toxicity — repeated exposure Category 2: Evidence on the substance or mixture (including bridging) of an adverse effect on specific organ/systems or systemic toxicity from animal studies or humans. May be named for specific organ/system. C ≤ 100 mg/kg bw/d

The results of the polymer risk index H of each sampling sites for seawater surface are shown in Figure 2. By evaluation the risk values and according to the hazard level criteria showed in table 3, all sites showed risks values >100 with hazard level between III and IV, it means effects as: suspected human carcinogens and reproductive toxicant via lactation, induce heritable mutations in the germ cells of humans, fatal if is swallowed, potential to be harmful to human health following single exposure, evidence on (including bridging) of an adverse effect on specific organ/systems substance or mixture

The highest value was found in T1 site La Mora-Llarga beach of 4485 with PVC and PU composition which have both of them the highest hazard scores of 10551 and 13884 and abundance of 28 and 11%. The monomers related with these polymers are vinyl chloride for PVC, propylene oxide and toluene-diisocyanate for PUR. The lesser value was found in T3 site Arrabasada-Miracle of 140 with PU composition and abundance of 14%. For T2 site Llarga-Arrabasada the risk value was 148457 with presence of PU, PMMA and PC with abundance of 9, 22 and 4% respectively. The monomers related with PC and PMMA were bisphenol A (1177 – IV) and methyl methacrylate (1021-IV) respectively.

Despite in T4 site were found the higher concentration values, the risk was not the highest with value of 797because the presence of PU and ABS was in low percentage, 5 and 2% respectively. The ABS polymer (6552 -V), have the monomers styrene, acrylonitrile and1,3-butadiene.

Although PE, PET; PP were the most frequently types of MPs occurring in seawater of Tarragona coast with abundance from 9 to 52%, the polymers are not considered as hazardous polymer because their low hazard score of 11, 4 and 1 respectively.

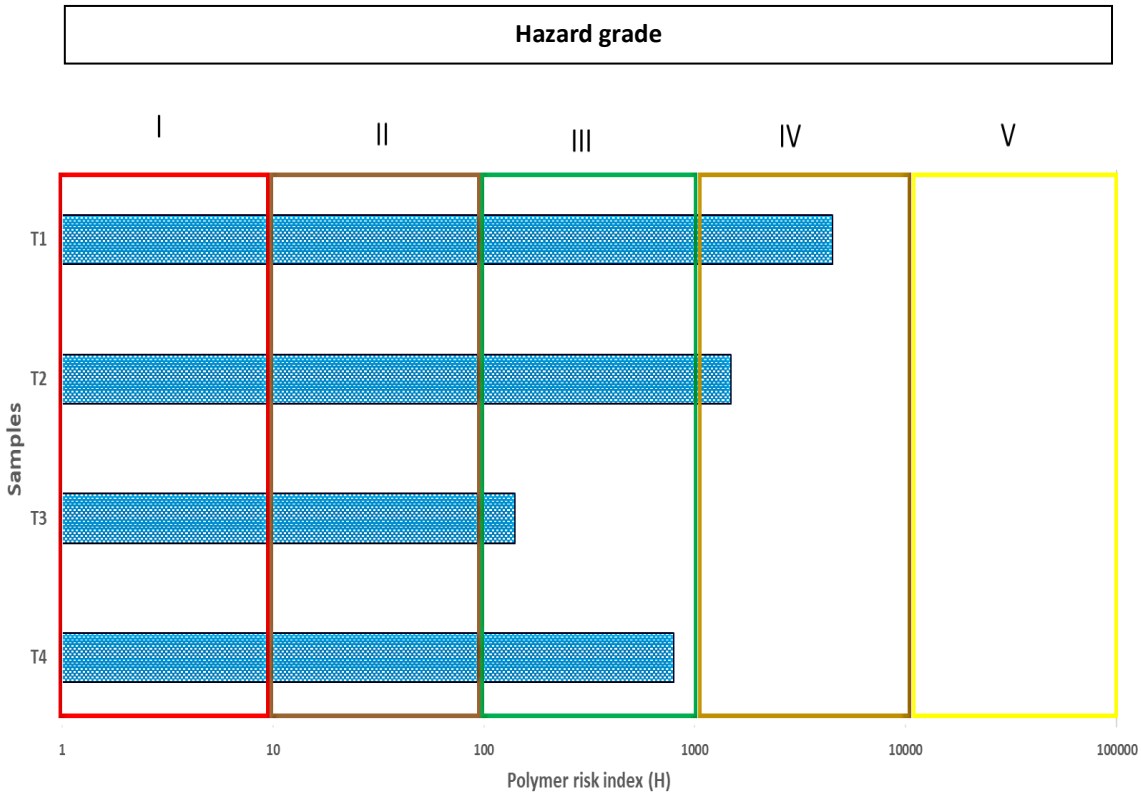


Figure 2. Hazard polymer index (H) for seawater on Tarragona North-Centre zone. T1: La Mora-Llarga beach, T2: Llarga-Arrabassada, T3: Arrabassada -Miracle, T4: Tarragona harbour

Table 3. HI hazard level criteria

HI	Hazard Level
<10	I
10-100	II
100-1000	III
1000-10000	IV
>10000	V

a.-Pollution index

Beside MPs polymer, MPs concentration is also an important index to evaluate the MP risk. Thus, Pollution load index (PLI) was applied related to MPs concentration to assess the degree of pollution levels in Tarragona coast seawater according (Tomlinson et al., 1980). The PLI model was set up according to the relationship between PLI and MPs concentration (Ci) at each sampling site and as follows:

$$PLIsampling\ site = \sqrt{\frac{Ci}{Coi}} \qquad \text{eq. 2}$$

$$PLIcoastal\ zone = \sqrt[n]{PLI1 \times PLI2 \times PLI3 \times \ldots . PLIn} \qquad \text{eq. 3}$$

Where **Ci** represent the concentration of MPs in environment samples, **Coi** is the minimal MPs concentration at each sampling site but as it is not known, reference values will be taken from another studies. Thus, according Isobe et al., (2014), a value of 0.005 MPs/m^3 (0.000005 MPs/L) is considered as the lowest value found in seawater for "small plastics fragments". In addition, were chosen the lowest value found in Mediterranean Sea and worldwide area for PLI comparison. The lowest value for Mediterranean Sea was 0.10 ± 0.04 MPs/m^3 (0.0001±0.00004 MPs/L) in West coast of Sardinia towards shoreline (de Lucia et al., 2014), with sizes from 0.5-5 mm. While for worldwide, lowest value was 0.0032 MPs/m^3 (0.0000032 MPs/L) found in Ross Sea in Antartica at 5 m depth below surface, sizes >60 µm to 5 mm and with fragments predominance (Cincinelli et al., 2017).

b.-Risk Index

Another index, as the potential ecological risk index (RI) method not only considers the environment concentrations but also the composition and proportion of pollutants (Hakanson, 1980; Peng et al., 2018; Yin et al., 2021). Calculated as follows:

$$C_f^i = \frac{C^i}{C_t^i} \qquad \text{eq. 4}$$

$$T_r^i = \frac{Pi}{C^i} \times Si \qquad \text{eq. 5}$$

$$E_r^i = T_r^i \times C_f^i \qquad \text{eq. 6}$$

$$RI = \sum_{i=1}^{n} E_r^i \qquad \text{eq.7}$$

C_f^i is the pollution index of MPs, C^i is the pollution concentration of MPs and C_t^i is the reference value estimated through mathematical model by Everaert et al., (2018) Pi is the concentration of specific MPs polymer i, Si is the hazard index of polymers according to Leithner et al. (2011), T_r^i is an ecotoxicity response factor, which is the sum of the percentage of plastic polymer i and the product of the polymer hazard index, E_r^i is the potential ecological risk index , and n is the number of types of MPs in the sample.

In this study, the reference value C_t^i is referred to PNEC obtained from a specie sensitivity distribution (SSD) model method for marine species developed by Everaert et al., (2018). This

method enables predicted ecologically safe thresholds for cases in which chronic ecotoxicity data are sufficient. The specie sensitivity distribution (SSD) model assessed chronic toxicity data points for MPs in marine biota related with various endpoints. Chronic not observed effect concentrations (NOEC) and chronic lowest observed effect concentration (LOEC) were inferred according to European Union (EU) legislation (EU, 2006). If several chronic NOEC or LOEC for different toxicological endpoints were available for a single species, the lowest value was used. LOEC values were converted to NOEC by dividing them by 2 (OECD, 1995). the particles size used were from 0.05 to 316 µm, the composition was PVC, PS, HDPE, PET and PE, the most sensitive endpoints were growth, metabolic rate, reproduction, abundance and biomass, filtration and respiration rates, reproduction and life span, antioxidant capacity and DNA damage, energy balance and gametogenesis, mortality, ingestion rate, feeding and fertility and finally the biological groups chosen were Ochrophyta (algae), Mollusca, Arthropoda, and Echinodermata. Fourteen species were studied.

The collected toxicity data was fit to a log-normal distribution and then the HC5 value based on the SSD was determined. The normal distribution was fitted to the log-transformed effect data according Aldenberg and Jaworska (2000), and the predicted no effect concentration (PNEC) was calculated by HC5 with a value of 33.3 MPs/L (95 % confidence interval: 0.36-13943 MPs/L). Given the limited number of taxonomic groups representing only a few feeding strategies and trophic levels and the current lack of standard test methods the highest assessing factor (AF) i.e 5 was used on the HC5 (Jung et al., 2021), resulting in a predict no effect concentration PNEC for pelagic environment of 6.65 MPs/L or 6650 MPs/m3.

However, according to Jung et al., (2021), studies from Everaert et al., (2018) not considered environmentally relevant shapes and the size range was narrow including sizes below 20 µm difficult to extract and identify in environmental samples by conventional and currents methodology. Jung et al., (2021) conducted an aquatic ecological risk assessment for non-spherical (fragment and fibres) MPs in the size range from 20 to 300 µm for marine environment using a species sensitivity distribution (SSD) approach with 11 chronic toxicity data points covering 8 taxonomic classes (fish, crustacean, algae, echinoderm, mollusc, macrophyte, coral and amphipod) for fragment and fibres MPs in the size range 20-300 µm with ecologically relevant endpoints (i.e mortality, growth, development, reproduction and population growth) and taking in account no observed effect concentration (NOEC), lowest observed effect concentration (LOEC) and 10% effective concentration (EC_{10}). The derived HC_5 was 59.8 MPs/L (95% confidence interval CI: 13.7-261.6 MPs/L) and then the PNEC was estimated to be 12.0 MPs/L (95% confidence interval CI: 2.7-52.3 MPs/L) by dividing the derived HC_5 value by an assessment factor (AF) of 5.

Lately, Everaert et al., (2020), updated the PNEC values for floating MPs in seawater using also a SSD probabilistic model based in effect data of PE, PP and PS polymers but with wide size range from 1 µm to 5 mm (1-100 µm most frequently), wider range compared with Everaert et al., (2018) wide size range. The used 23 different species effect threshold concentration data from eight phyla, endpoints with clear links to population dynamics as growth, mortality, reproduction, biomass and NOEC values.

The resulting median unacceptable level (Predicted No-Effect Concentration, PNEC), was 1.21 $*10^5$ MPs/ m^3 or 121000 MPs/L (95% CI: 7.99 $*10^3$ MPs/m^3 -1.49 $*10^6$ MPs/m^3) Compared with values above mentioned, this value is very high.

Despite, update PNEC range values, only were chosen median PNEC values derived by Everaert et al., (2018) and Jung et al., (2021) for RCR and RI estimation, instead media PNEC derived by Everaert et al., (2020), for avoid underestimating of RCR and RI due to the selection of very high values of PNEC.

c.-Risk characterization ratio (RCR)

In addition, the risk assessment was also identified by comparing the measured environmental concentration (MEC) of MPs and PNEC. A risk characterization ratio is calculated as the radio of the PEC and the PNEC.

$$RCR = \frac{PEC}{PNEC} \qquad \text{eq. 8}$$

When this RCR is <1 no immediate risk for the environment is discerned, as environmental concentrations are lower than the concentration below which adverse effects will most likely not occur (i.e., the PNEC). Increasing environmental concentrations will subsequently result in the increase of the RCR. Values of RCR>1, indicates that environmental concentrations are exceeding the safe concentration defined by the PNEC, and it is concluded that a risk to the environment cannot be excluded. Similarly, to RI index, for RCR estimation were selected PNEC values from Everaert et al., (2018) and Jung et al., (2021).

In table 4, it´s shown MPs pollution index values for seawater surface sites monitored and their MPs concentration, taking account PNEC derived by Everaert et al., (2018) and Jung et al., (2021) and lowest MPs concentration for Mediterranean Sea, Antartic Peninsule and Japan Sea. In table 5 it`s shown the risk assessment criteria for PLI and RI index.

Table 4. MPs pollution index in surface seawater

Zone	MEC (MPs/L)	RCR MEC/PNEC (Jung et al., 2021)	RCR MEC/PNEC (Everaert et al., 2018)	PLI based on lower Mediterranean Sea values (de Lucia et al., 2014)	PLI based on Ross Sea (Antartica) values (Cincinelli et al., 2017)	PLI based on Japan Sea values (Isobe et al., 2014)	RI (Everaert et al., 2018)
La Mora-Llarga beach (T1)	0.0008	6.67E-05	1.20E-04	2.8	15.8	12.6	0.54 (0.3) *
Llarga-Arrabassada (T2)	0.00103	8.58E-05	1.55E-04	3.2	17.9	14.4	0.20 (0.11) *
Arrabassada - Miracle (T3)	0.00041	3.42E-05	6.15E-05	2.0	11.3	9.05	0.12 (0.07) *
Tarragona Harbour (T4)	0.00296	2.47E-04	4.44E-04	5.4	30.4	24.3	0.35 (0.2) *
Tarragona North-Centre zone	-		-	3.2	17.7	14.1	-

(*) values estimated with PNEC derived by Jung et al., (2021)

Table 5. Risk assessment criteria for MPs pollution

PLI*	Hazard Level	RI*	Risk level
<10	I	<150	Minor
-	-	150-300	Medium
10-20	II	300-600	High
20-30	III	600-1200	Danger
>30	IV	>1200	Extremely danger

*Based on hazard level criteria proposed by Yin et al., (2021)

Risk assessment results through risk characterization ratio (RCR) method suggested that all monitored sites on seawater surface of north-center zone of Tarragona coast were far lower than 1 because MPs concentration were far lower than both PNEC values proposed by Everaert et al., (2018) and Jung et al., (2021). It means that there is a negligible risk to marine biota by MPs pollution with sizes ≥80μm-5mm based only in MPs structure and not focused on the potential role of MPs as chemical vectors. Instead, lower ratios found in Tarragona north-centre zones, the zone Tarragona harbour (T4) presented the highest values.

It was foreseen assuming the currents rate of MPs concentration increasing in seawater year by year since 2018, no eminent threat of microplastic pollution beyond to 2118 (Figure 3) taking account only PNEC derivation proposed by Everaert et al., (2108) of 6.65 MPs/L because is the lowest value currently reported. However, Tarragona harbor site (T4) presented the value nearest to reach to PNEC values for next 60 and 100 years.

The values of RCR obtained here can be underestimated because if MPs of small sizes <80 µm with different morphologies and composition are also considered (because mostly of the ecotoxicological assays taking in account monodisperse particles, narrow sizes range, small sizes and spheroidal morphology) the RCR could be near or higher than the current value estimated by Everaert et al, (2018).

By in contrast, if the value of Everaert et al., (2020) is taken into account, RCR values greater than 1 may not be achieved at this area and RCR values remain still low.

In addition, if mesoplastic and MPs are discharged to coastal zones continuously without sustainable alternatives for plastics usage, wastes management improvement and science-based policy guidelines on safe concentrations are not urgently applied in combination with increase of rate of mesoplastics discharges and fragmentation will cause an exponential increase of MPs concentration in seawater surface on Tarragona coast reaching to concentrations higher than PNEC in short term.

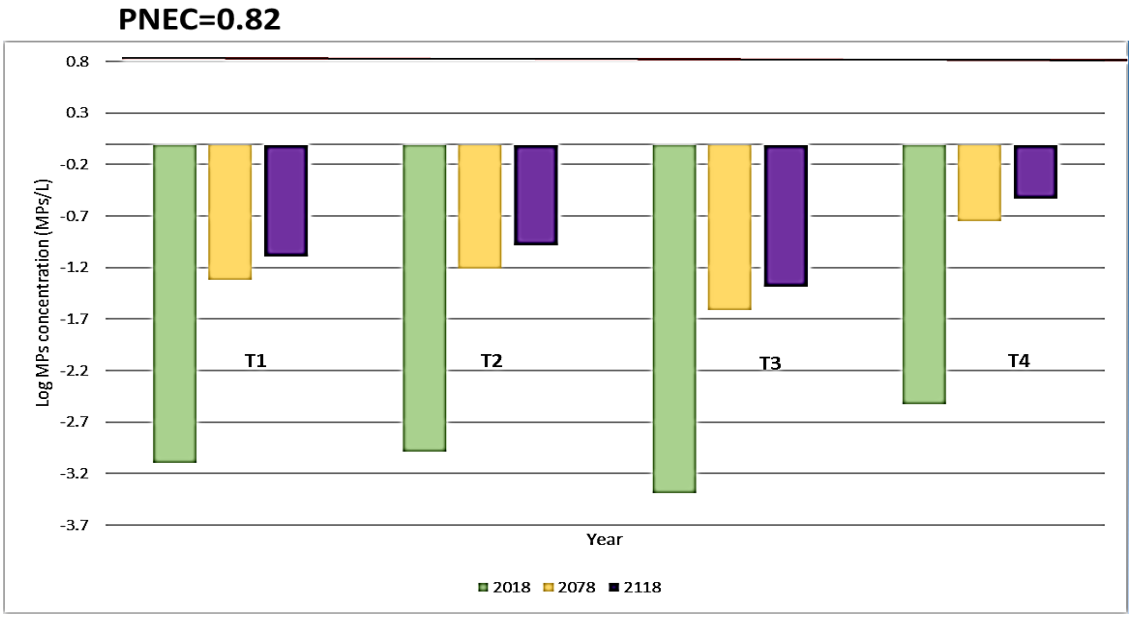


Figure 3. Present and future expected (60 and 100 years) of environmental MPs concentration in seawater surface on Tarragona north-centre zone. PNEC and concentration values were expressed in log (10) for better representation. T1: La Mora-Llarga beach, T2: Llarga-Arrabassada, T3: Arrabassada -Miracle, T4: Tarragona harbour

According to calculated PLI, risk degree at each sampling sites given in table 5 were in risk category I or low polluted (minor) with values <10 according to lowest Mediterranean Sea values, similarly for entire Tarragona north-centre coast, the risk category also was in risk category I. By contrast, considering Japan Sea values, T1 and T2 were found in risk category II or high polluted, T3 remained in same category (I) and T4 or Tarragona harbour increased of category from I to III

or danger. Likewise, entire Tarragona coast zone also increased in category from I to II or high polluted.

With Antartica values, risk degree increased from I to category II (10-20) for T3 site become high polluted while for T1 and T2 the category remained in level II. Similarly, T4 remained in category III (20-30) or danger and finally entire Tarragona coast zone in category II or high polluted.

It is important to highlight, that PLI index values depend on the reference value assigned, thus using Mediterranean Sea values, Tarragona coast sites remain in category I with minor pollution levels, while if it is compared with Japan Sea and Antartica values, a hotspot is observed in T4 with danger levels although entire Tarragona coastal zone remain in category II or high polluted.

The RI values of all sampling points are also shown in table 4 all sites were far lesser than 150 with minor risk indicating the potential risk by MPs concentration and polymer composition is low. The maximum value calculated with PNEC derivated by Everaert et al, (2018) and Jung et al., (2021) was in T1 La Mora-Llarga beach of 0.54 and 0.3 respectively by presence of PVC and PU in polymer composition with 39%. The minimum value was found in T3 Arrabasada-Miracle of 0.12 and 0.07 respectively by presence of PU with 14 %. The average of entire Tarragona north-centre zones was 0.3 and 0.17

It is observed that there is an area as T1, which have low values of RCR and MPs pollution load index (PLI) but have high risk for MPs polymer composition with the highest hazard risk polymer index H value and RI. By contrast, MPs exhibited high concentration in Tarragona harbour (T4) followed by Llarga-Arrabasada zone (T2), the first one zone influenced by harbour, touristic and urban activities and the second one by runoff discharges emissaries in the entire transect but the lowest values of H index were found in T4.

The high values of MPs concentration and pollution load index (PLI) observed in T4 and T2 zones proving anthropogenic activities play a critical role on the plastic pollution in Tarragona coast. MPs pollution is caused by both the concentration of MPs and the hazardous polymers as an integrative view and is necessary to be considered to comprehensively evaluate the ecological risk caused by MPs.

Distribution of MPs on Tarragona coastal areas depend on local complex hydrodynamic process and interactions with tides, river plumes and currents, with tendency MPs transport from the coasts to the offshore in the absence of trapping zones (Zhang et al., 2020). Furthermore mesoplastics (incorporated by river mouths and effluents dischargues into the sea) are selectively

conveyed onshore by coastal physical processes (Isobe et al., 2014), thus dependent on fragment sizes, mesoplastics washed ashore on beaches degrade into MPs, and after MPs, are able to spread depending on close areas with civil structures (groynes) or with converging surface currents that could cause accumulation in patches.

3.1.1.2 Risk assessment in sediments

a.- Hazar polymer index (H)

For sediments risk assessment, basically the same three index applied for seawater samples, were also applied. The hazard polymer index (H) was calculated for both fractions <0.5 mm and ≥0.5 mm using equation 1 described above. According to composition analysis for each sampling zone, the hazard score for polymer type calculated by Lithner et al. (2011) is shown in table 1 (the health hazard description is taken from table 2).

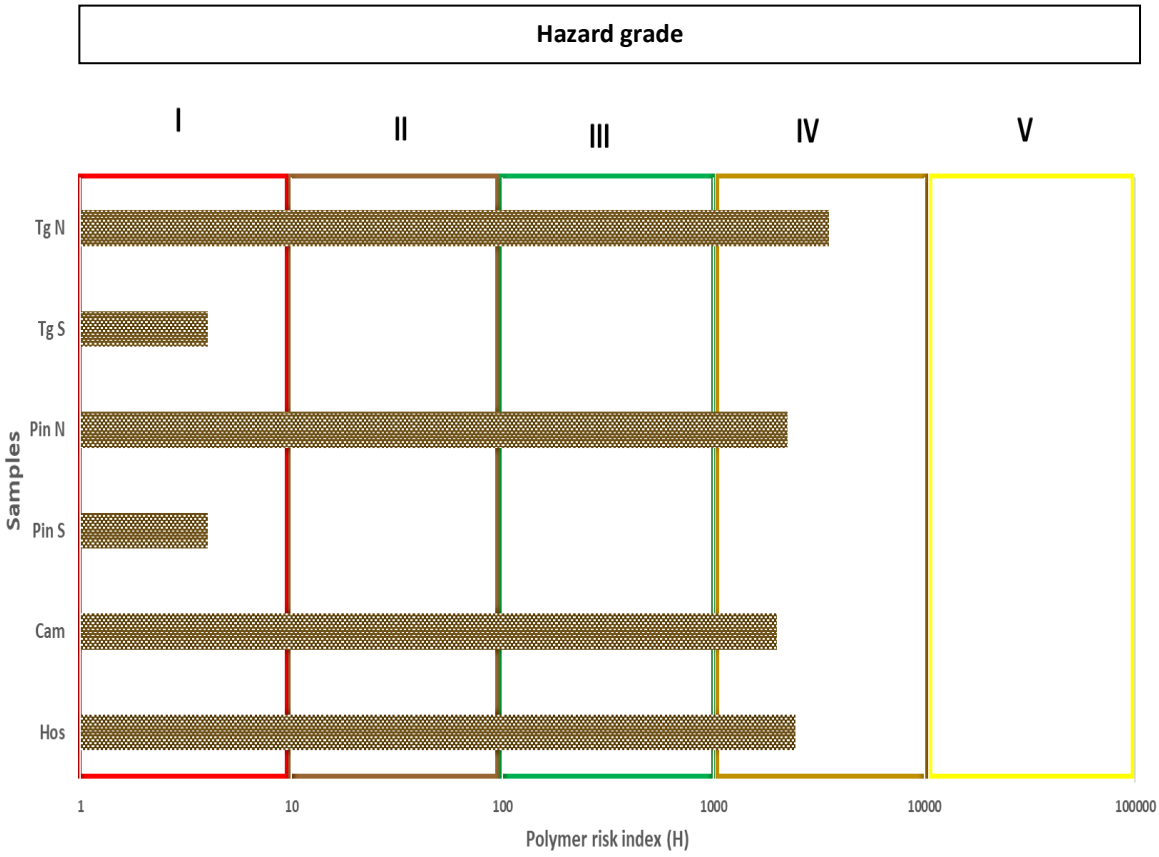


Figure 4. Hazard polymer index (H) for marine sediments on Tarragona southern-centre zone. Tg N: Miracle beach north (Punta Grossa), Tg S: Miracle beach south (Final beach section), Pin N: Pineda beach north, Pin S: Pineda beach south, Cam: Cambrils beach (Cavet Beach), Hos: Hospitalet beach

The results of the hazard polymer risk index H of each sampling sites for marine sediments surface are shown in Figure 4. According to the risk values observed and the hazard level criteria showed

in table 3, from six sites evaluated, four showed risks values higher than 1000 with hazard level of IV, it means that the MPs polymers composition have potential to induce heritable mutations in the germ cells of humans, fatal if is swallowed, potential to produce significant toxicity in humans following single exposure with reliable evidence on the substance or mixture of an adverse effect on specific organ/systems or systemic toxicity in humans or animals.

The highest value was found in **Tg N** site Miracle beach north of 3527 with PVC (10551-V) composition which have and abundance of 33%. The lesser value was found in **Pin S** site Pineda beach south and **Tg S** site Miracle beach southern zone of 4 with PET (4-II) composition of 40% and 25 % respectively and hazard level I, it means the polymers found have no health hazard.

For **Cam** site where was found the highest MPs concentration value, the polymer risk index H was 1999 with PVC composition of 19%. That value was followed by 2259 in **Pin N** site with PVC and ABS (6552-V) composition of 16 and 8% respectively and 2452 in **Hos** with PVC and ABS composition both with 14%.

Although PE had a high proportion in polymer composition of **Tg N** and **Pin N** (35 and 33% respectively) that polymer is not considered as hazardous polymer because its low hazard score of 11.

b.-Risk characterization ratio (RCR) ≥0.5 mm and <0.5 mm fractions

A risk characterization ratio was calculated as the radio of the PEC and the PNEC (equation 8). Since the toxicity data of MPs on sediment-dwelling organisms were insufficient for construction of SSD model due to lack of reliable long-term concentration –response effect data at environmentally relevant MPs concentration and in addition of a sufficient data on a wider range of organisms representing different feeding strategies and more trophic levels (Everaert et al., 2018). Therefore, the predicted PNEC in sediments was not provided by this method. Based on long-term effect data of Van Cauwenberghe et al., (2015), Everaert et al., (2018) applied an assessment factor (AF) of 1000 for NOEC of the most sensitive endpoint (EU, 2006) of *Arenicola marina* metabolic rate, $5.4x10^5$ MPs/kg of polystyrene (PS) microspheres 10-90 µm resulting a PNEC for marine sediments (PNEC benthic) of 540 MPs/kg of sediment. This PNEC value was chosen for MPs risk characterization ratio in marine sediments. At least, the range of particles size found in marine sediments spanning from of 50-500µm, mostly between 50 to 250 µm partially matches the range used by Van Cauwenberghe et al., (2015).

This conservative AF was chosen because there still very little data on the toxicity of MPs in marine sediments. Thus, for PNECsediment, the lowest NOEC/EC10 value for sediment living organisms is divided by assessment factors 10~100 (Chemsafetypro, 2022).

It is important to highlight that reference values used as PNECbenthic and PNECseawater did not consider a wide range of particles sizes or fibres being this morphology the most frequently found in environment and even also believed to exert higher toxicity in comparison to others MPs shapes (Qiao et al., 2019; Stienbarger et al., 2021). On the other hand, the reference value must have similar sizes and morphology proportion that samples values but not were found data that met all the requirements, thus these values were the best available so far.

c.-Pollution index ≥0.5 mm and <0.5 mm fractions

Pollution load index (PLI) for sediment samples was estimated using equation 2, for **Coi** minimum MPs concentration was used the lowest value observed in Mediterranean Sea by Mistri et al., (2020) of 0.43 MPs/kg at 53 m depth (sandy bottoms) in offshore zone between Gulf of Follonica (Tuscany) and Elba Island (Piombino channel-Italy) with sizes from 1 to 5 mm and morphology mostly represented by fibres (filaments). Not lower values were found for other worldwide zones.

d.-Risk Index (RI) <0.5 mm

RI index was estimated using equation 7 (for Tarragona Southern-centre zone), assuming MPs concentration in sediments in that zone was mainly <0.5 mm because MPs concentration for ≥0.5 mm fraction was practically lower in comparison. For C_t^i reference value was chosen the same value of PNEC for risk characterization ratio estimation for marine sediments ≥ 0.5 mm based on long-term effect data of Van Cauwenberghe et al., (2015), and calculated with an AF of 1000 for NOEC of the most sensitive endpoint (EU, 2006) of *Arenicola marina* metabolic rate, resulting a PNEC for marine sediments ($PNEC_{benthic}$) of 540 MPs/kg of sediment. This value was chosen for both RI index estimation and MPs risk characterization ratio in marine sediments.

In table 6, it is shown the values of risk characterization ratio (RCR), RI, and PLI for marine sediments both fractions ≥0.5 mm and <0.5 mm. For ≥0.5 mm all sites showed values lesser than 1. It means as environmental concentrations are much lower than the concentration below which adverse effects will most likely not occur or PNEC proposed by Everaert et al., (2018).

Table 6. MPs pollution index in marine sediment for both fraction ≥0.5 mm and <0.5 mm

Zone	MEC ≥ 0.5 mm (MPs/kg d.w)	MEC/PNEC ≥0.5 mm	PLI based in Mediterranean Sea values (Mistri et al., 2020) for ≥0.5 mm	MEC <0.5 mm (MPs/kg d.w)	MEC/PNEC <0.5 mm	PLI based in Mediterranean Sea values (Mistri et al., 2020) for <0.5 mm	RI
La Mora	32	0.059	8.6				
Cala de Roca Plana	6	0.010	3.6				
Llarga Centro	22	0.040	7.1				
Miracle-Arrabasada	41	0.075	9.7				
Miracle North (Tg N)	13	0.024	5.5	**637**	**1.18**	**38.5**	**3980**
Miracle Center	6	0.010	3.6				
Miracle South (Tg S)	2	0.004	2.1	318	0.59	27.1	2.25
Harbour (marina)	32	0.059	8.6				
Harbour (industrial)	89	0.164	14.4				
Pineda North (Pin N)	8	0.015	4.3	**1595**	**2.95**	**60.9**	**6390**
Pineda South (Pin S)	5	0.009	3.4	**633**	**1.17**	**38.3**	4.49
Cambrils (Cam)	5	0.009	3.4	**2106**	**3.90**	**69.9**	**7466**
Hospitalet (Hos)	7	0.013	4.0	**706**	**1.30**	**40.5**	**3068**

Tarragona North-Centre zone	-	-	7.1				
Tarragona Centre-South zone	-	-	3.7			43.6	

Likewise, in table 6 for marine sediments fractions <0.5 mm, risk characterization ratio (RCR) values show only one of the monitored sites **Tg S** (Miracle beach southern) not posed immediate risk to benthic marine biota because the environmental concentration is below to PNEC (RCR<1) which adverse effects will most likely no occur. While the rest of sites **Tg N**, **Pin N**, **Pin S**, **Cam**, **Hos** appeared with high risk with RCR>1 indicating environmental concentrations are exceeding the safe concentration defined by PNEC and the risk to the environment cannot be excluded. The higher values were found in **Cam** and **Pin N** with values much higher than 1.

The results of risks for marine sediments <0.5 mm fraction was quite different than marine sediment fraction ≥0.5 mm and seawater surface regarding PLI index. The PLI, calculated showed minor risk (<10) for almost all sites for MPs concentration ≥0.5 mm (table 6), while for Harbour (Industrial) zone the risk category was II (10-20) or high polluted. Furthermore, both Tarragona North-Centre zone and Tarragona Centre-Southern zone were found in category I or minor risk. By contrast, PLI values for <0.5 mm almost all sites showed risk category IV or extremely danger with values >30. Similarly, for entire Tarragona centre-southern coast the risk category was IV. However, **Tg S** site showed a risk category III (20-30) or danger.
It is important to highlight that reference or minimun values used for PLI index calculation in seawater surface and marine sediments samples were the lowest MPs values available in similar zones because there no were found previous baseline microplastics studies in Tarragona coast. On the other hand, the reference value must have similar sizes and morphology proportion that samples values but not were found data that met all the requirements.

Opposite to seawater surface, the RI values of four of six sampling points of marine sediments fraction <0.5 mm shown in table 6 as **Tg N**, **Pin N**, **Cam** y **Hos** were far higher than 1200, being extremely danger level indicating the potential risk by MPs concentration and polymer composition is very high. While in **Pin S** and **Tg S** was found values much lower than 150 with minor risks. The maximum values calculated were in **Cam** and **Pin N** of 7466 and 6390 by presence of **PVC** around a 19 % for both sites and **ABA** with 6% in **Pin N**. The minimum value was found in **Tg S** by presence of **PET** (with 25 %. Similar results were evidenced with hazard polymer index H. The average of entire Tarragona centre-southern zones for marine sediment fraction <0.5 mm was 3485 with extremely danger risk level. Although the benchmark values and formulas used for PLI and RI index are quite different, the two indices at majority of sampling points reflect similar trends being **Pin N** and **Cam** the sites that presented the highest values.

Based on the scores of three indexes, the sites which presents high values of RI, RCR and PLI and therefore higher risks for environment were four from six as **Tg N**, **Pin N, Cam**, **Hos**. The

highest values of these indexes were found in **Pin N** and **Cam** which presented high MPs concentration. Furthermore, also hazard polymer index (H) was higher for these two sites therefore both sites are considered as a hot spot for MPs pollution

Pin N site with high MPs concentration is undoubtedly one of the most polluted sites due continuous land base (urban and industrial sources) MPs inputs. However, was in **Cam** site where was found the highest MPs concentration despite having less influence from land sources than in **Pin N**. According Liu et al., (2022), it is possible that MPs could be transported from highly polluted site to other areas and promote their accumulation by MPs through interaction and association as well as coastal hydrodynamic conditions.

The Species Sensitivity Distribution (SSD) method was used in this study to assess the ecological risks of MPs on aquatic environment with PNEC derived by Everaert et al., (2018) and Jung et al., (2021). However, the useful data provided by SSD methods for environmental risks stimations, SSD methodology present still limited toxicity data of aquatic organisms (specially in marine sediments) made it impossible to derive a reliable water quality criterion (WQC) for chemical compounds and difficult to implement an accurately ecological risk assessment (Wu et al., 2022).

In order to obtain a realistic risk assessment, the European Chemicals Agency (ECHA) suggests integrating quantifiable uncertainty and variability into the risk assessment paradigm through probability distributions (ECHA, 2012). According Everaert et al., (2020), a probabilistic risk assessment combines a probability distribution for in situ concentrations with a probability distribution for ecotoxicological data (Solomon et al., 2000). As such, a probabilistic risk assessment makes maximal use of the variability in the data and results for the studied environmental compartment.

The probabilistic risk distribution (PRD) is calculated as the logarithmic (log10) difference between the distribution of MPs concentrations and the distribution of effect concentrations. Environmental risks are to be expected if log10 PRD >0.

The probabilistic approach is a useful tool that could be used for comparing in situ MPs concentrations with the corresponding unacceptable levels not only for seawater surface but for marine sediments.

3.1.2 Microplastics and Aquatic Life

The number of MPs particles increases exponentially with decreasing particle size (Song et al., 2014), thus increasing environmental concentrations enhance the probability of organism

interacting with MPs (Everaert et al., 2018). In addition, small sizes MPs particles are available for ingestion by a wide array of marine biota and once ingested, this synthetic polymer does not undergo any enzymatic degradation via a metabolic pathway as no enzyme is available to degrade the same into simpler non-toxic chemical compounds (de Witte et al., 2014; Gola et al., 2021; Van Cauwenberghe and Janssen, 2014). Jeong et al., (2016), found MPs effects increase with size decrease. They observed that anti-oxidant related enzymes and mitogen-activated protein kinases signalling pathways were significally activated in marine rotifers in response to MPs exposure in a size-dependent way.

According to Gray and Weinstein (2017), Harry and Carrington, (2020); Van Cauwenberghe et al., (2015), and Van Cauwenberghe and Janssen (2014), MPs smaller than 300 µm are favourably ingest by invertebrates as shrimps and bivalves. Furthermore, the particle toxicity of MPs is not only affected by their size but by its shape, it means the toxicity of fragments and fibres is higher than spherical shape MPs. Thus, Ziajahromi et al., (2017) showed the acute median lethal concentration were up to 9 times lower than those spherical MPs in freshwater zooplankton *Ceriodaphnia dubia*.

The negative impacts on MPs-exposed species can be grouped into 2 types of effects: physical and chemical (metabolic and physiologic). Principal effects are: disminution in the feeding of aquatic organisms, energy transfer, benthic-pelagic coupling, water clarity (clarification rate) effects on growth (reduction in the carbon biomass), energy efficiency (rapid consumption of lipids) and fertility (small-sized eggs, bad ovocyte quality, reduce sperm motility and hatchings), feeding habits, spawning, inflammation, increase in enzyme activity (superoxide dismutase and catalase), metabolic pathways alteration reduction in photosynthetic activity along with the oxidative stress (Cole et al., 2015; Gola et al., 2021; Galloway and Lewis, 2016; Harry and Carrington, 2020; Issac & Kandasubramanian, 2021; Lu et al., 2016; Sussarellu et al., 2016).

In seawater surface MPs sizes found were mainly from 80 µm to 2 mm included particles less to 300 µm that is potentially ingested by epipelagic biota. Furthermore, high prevalence of no espherical MPs as fibres, fragments, and films, becomes MPs pollution in seawater as a threat to aquatic organisms. Although environmetal relevant exposure concentrations in Tarragona coast are very far from of corresponding unacceptable levels in seawater surface (PNECseawater), currently the risk by MPs pollution is inexistent. However, if very small >1 to 80 µm MPs are quantified, the situation could get worse and it is possible environmetal relevant exposure concentrations is closer to PNEC value. However, so far there is not standardized reliable and rapid quantification methodology for very small MPs sizes

Furthermore, if the entry input rate of MPs to seawater increases in coming years, at the same size proportions (80 µm-2 mm) with additional (exponential) fragmentation rate and increasing toward smallest micrometer-sized, it is possible aquatic organisms of coast of Tarragona could be in risk by MPs pollution in the middle or short term, specially in zones with higher levels of MPs.

Mehinto et al., (2022), using SSD approach established four toxicity threshold levels for food dilutions in particles sizes ranging from 1 to 5000 µm or tissue traslocation in particles ranging from 1 to 83 µm. The levels and measures for each threshold are described in table 7. Thus, it is probably that in Tarragona coast the threshold values for food dilution and tissue traslocation be reached currently or in a short term (if the MPs inputs and the fragmentation rate are increased)

Table 7. Proposed microplastics toxicity thresholds for food dilution and tissue translocation.

Threshold	Toxicity thresholds for food dilution relevant for particles sizes between 1-5000 µm Particles/L (95% CI)	Toxicity thresholds for tissue translocation dilution relevant for particles sizes between 1-83 µm Particles/L (95% CI)
1.-"Investigative monitoring"	0.3*	60*
2.- "Discharge monitoring"	3 (0.3 to 66)	312 (57-4680)
3.- "Management planning"	5 (0.4 to 219)	890 (118 to 19,000)
4.-"Source control measures"	34 (3 to 859)	4110 (493 to 69,100)

* Threshold 1 is the lower 95% CI of the HC5 calculated for Threshold 2, therefore confidence intervals could not be reported for this threshold

Because there are no standardized methodologies and equipment necessary for reliable microplastics quantification <20 µm, it is not possible to determine at what threshold the seawater concentration corresponding to and as well as to define measures that should be applied for Tarragona coast MPs pollution mitigation.

Regarding, benthonic communities, chronic exposure to MPs (particles) ranging from 1-1300 µm diameter size, caused intestinal injury, energy reserves decrease, growth and food uptake affectation and oxidative stress, decreases in weight by reduction in feeding activity, inflammation in nematode, benthic lugworms, Ascidians and sea snail larvae. (Besseling et al., 2013; Lo & Chan, 2018; Messinetti et al., 2019; Shang et al., 2020; Wright et al., 2013; Yu et al., 2020). Sizes higher than 100 µm can affect the health (respiration rate) and behaviour (burrowing activity) of lugworms in high concentrations on sandy sediments (Green et al., 2016).

Given the current MPs concentration (<0.5 fraction) in marine sediments, it is likely that, local organisms are already at risk, because these concentrations exceed the PNECbenthic values above

which adverse ecological effects are likely to occur. Pineda north beach, and Cambrils (Cavet beach) can be considered as sites where to carry out mitigation measures (hot spots).

Althought, some ecotoxicity assays with benthic organisms were made with higher concentrations in sediments (7.4 %) at 5 cm layer, it is possible that not homogeneous and by patches MPs distribution on subtidal marine sediment surface in Tarragona coast promote zones of very high concentrations. Due to fibres and particles sizes range 50-800 µm found partially overlap with the size range that produce effects in benthic lugworms (130-1500 µm) and potentially ingested by them evidenced by MPs presents in their faeces casts between 35-1000 µm (Van Cauwenberghe et al., 2015), it is possible some benthic groups are being affected. Moreover, if the small ones <50µm MPs could be quantify, it is possible that MPs effect includes a greater amplitude of benthic groups (including larvae) and the risk will be even greater for the evaluated sites.

Similarly, to seawater, if the rate of MPs input to marine sediment were to increase in the coming years, the risk would increase and could be of concern also for other sites as Miracle and Hospitalet beaches.

Finally, to date, it is not known how MPs exposure interacts with other global change stressors (e.g., high temperature) and species diversity. According to Rockstrom et al. (2009), an equal number of MPs in an oligotrophic open ocean with low biomass and low biodiversity may result in less impact on the ecosystem than in more diverse and biomass-rich upwelling zones or reef structures.

To mitigate and prevent the potential impacts of MPs on sediments in coastal zones research on in-depth, about MPs occurrence and fate, in urban water (runoff and wastewater efluents and afluents) is needed as well as removal of MPs in the urban water management systems for sanitation of coastal zones receiving these discharges.

Others recommendation por mitigation strategies application and sources controls could include:

- Exploring new MPs removal tecnologies in WWTP (
- Education, outreach and awareness
- Source identification
- Increasing monitoring by reporting additional case studies
- Development of bioplastics (biodegradable) to replace Single Use Plastics (SUPs).
- Because, MPs can release toxics additives, there is an obvious and increasing need for innovation to develop safer materials, non-toxic alternatives on the market, it means that harmful substances should be replaced with different chemicals (not compound of similar

structure and potential to harm). Thus, there is need to be systems in place to avoid such regrettable chemical substitutions.

Finally, future ecotoxicological work should urgently explore the contribution of MPs pollution to the multiple stressor factors in marine ecosystems

3.1.4 Microplastics and persistant compounds associated

MPs also can accumulate harmful pollutants from the surroundings thereby acting as transport vectors; and simultaneously can leach out chemicals (Issac & Kandasubramanian, 2021). Given the variety of additives used in plastic products and their detection in MPs debris collected in surveys, it is expected that they will be found in environment matrices as seawater and sediment (Hermabessiere et al., 2017). Plastic additives in MPs are detected at concentrations up to six orders of magnitude higher than in the surrounding water (Rochman et al., 2015).

The risk of toxicity by MPs is due not only to the MPs themselves but also to the hazardous substances they contain (dyes, heavy metals, organic chemical compounds) that can potentially leak (due to the action of gastric surfactants, pH, and temperature) (Gola et al., 2021) (Setälä et al., 2014). However, the extent to which these substances are transferred into food chains is unknown (Granek et al., 2020).

Thus, Besseling et al., (2017) and Browne et al., (2013) showed an increases chronic susceptibility of marine worms to PCB´s when exposed to MPs (PE) size range 10–180 μm as well as accumulation of compounds likes nonylphenol, PBDE, and triclosan inside the tissues (at smaller sizes, could transfer chemicals into the tissues directly, without the need for gastric desorption) from polyvinyl chloride (PVC) particles size 230 μm (presorbed with pollutants) with effects as impaired immune response along with physiological stress (feeding affectation) and mortality.

Thereby, the MPs ingestion can transfer pollutants and additives to their tissues at concentrations sufficient to disrupt ecophysiological functions linked to health and biodiversity, it is possible that benthic organisms of Tarragona coast could be affected by MPs pollution not only by their structure but persistant associated compound that involve pollutants adhered and additives

From six sites monitored, four showed PVC and two sites ABS (heavy polymers) in MPs composition. On the other hand, Pineda north and Cambrils beach not only showed higher concentrations (<0.5 mm) but also proportions of these polymers between 6 and 19%, considering these two sites as sources of contamination by MPs not only by concentration but by composition

3.1.4.1 Microplastics and additives of concern

Regarding chemicals migration or leakeages, PVC polymers have "Trojan-Horse" because can release toxic additives or monomers as dibenzodioxins and dibenzofurans by e.g., weathered or heated under the sunlight after exposing to organisms (Bhatt et al., 2021; Ma et al., 2020). Many of these additivies can persist for a long time and travel long distances within MPs matrix with environment and human health concern (accumulation in fat tissues, liver, kidney, brain and spleen, proteins binding, endocrine disruptors) (Stockholm Convention, 2015).

The initial concentration of the chemical substance present in the plastic, the thickness, crystallinity and the surface structure of the plastic are all factors that influence the migration rate (Hahladakis et al., 2018). Potential release studies of brominated flame retardants (BFRs) (Kim et al., 2006), phthalates (Rijk and Ehlert, 2001), bisphenol-A (Brede et al., 2003) showed in general that released concentrations were lower than the established legal limit values; however, in some occasions, these were considerably higher.

Some Flame retardants as brominated flame retardants (BFRs: HBB, decaBDE as decabro-modiphenylethane-DBDPE, HBCD) and PFOS/PFOA (used for surface treatment on carpets and textiles) where listed in Annexs of Stockholm Convention from 2004-2017 for elimination (Stockholm Convention, 2015). Regarding plasticizers, the European Union has restricted some phthalates as since 1999, The European Parliament targets DEHP plasticizer in recycled PVC, and DEHP has gradually been replaced by diisononyl phthalate (DiNP), diisodecyl phthalate (DiDP) and di (2-Propyl Heptyl) phthalate (DPHP), which represented 57% of plasticiser consumption in Europe in 2015.

Some companies have voluntarily removed them from their products and advertise them as "phthalate-free". (EP, 2016). Bisphenol A (BPA) can also be used as plasticiser/antioxidant or in other polymers as PP, PE, PVC may also represent a threat to the environment (most pervasive). Although BPA has been banned in children's products, it is still used in many water bottles and plastic containers and in the epoxy resins that protect canned foods from contamination (Peretz et al., 2014; Sajiki & Yonekubo, 2003).

Despite the restrictions currently imposed on these substances, these still present in MPs derived from mesoplastics and macroplastics elaborated before 2000's. Moreover, substances of proven high concern still being incorporated into plastics matrix. Due MPs persistent nature, these substances remain incorporated in plastic structure long time and could be release into the environment through continuos fragmentation up to nanometrics scale.

According The Regional Activity Centre for Sustainable Consumption and Production (SCP/RAC), regional centre of both the Stockholm Convention on Persistent Organic Pollutants (SCRC-Spain) and the Barcelona Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean in a report of 2020, currently there is still a wide range of toxic chemicals used as plastic or polymer additives – for example, chemicals that have not yet been subject to international controls (such as many endocrine-disrupting chemicals) or recognised POPs which are allowed under exemptions.

Recent initiatives, such as the mapping exercise carried out by the European Chemicals Agency (ECHA) can serve as a first step towards this effort. Initiative by ECHA (2018-2019) and industry for mapping plastic additives resulted in a list of over 418 functional additives or pigments used in plastics, including information on the polymers they are most commonly found in and the typical concentration ranges. The mapping considered only substances registered under REACH at above 100 tonnes per year, and focused on plasticisers, flame retardants, pigments, antioxidants, antistatic agents, nucleating agents and various types of stabilisers. From the total, 58% of the substances were not under regulatory scrutiny under REACH or CLP (Classification, Labelling and Packaging-CLP; Regulation EC No 1272/2008) and 11% of the substances remained without any information on their function. It means that Members State has evaluated or will evaluate them over the coming years (ECHA, 2018a; ECHA, 2018b).

The list is called the Community rolling action plan (CoRAP) substance evaluation. For each substance, it shows hows the evaluating Member State, the year (or the planned) of evaluation and a short description of the concern which led to it being placed on the list. For each substance the hazard classification & labelling as well as properties of concern have been described (ECHA, 2019; ECHA, 2022).

Thus, additives type, status definition, propierties of concern, plastic type associated found in seawater and marine sediment compartments on Tarragona coast, their function in plastic and % in polymer matrix are listed in table 8.

Table 8. Community rolling action plan (CoRAP) substance evaluation for plastics additives, harmonised classification and labelling (CLH).

Status definition	CoRAP (Sustances Evaluation) Properties of concern	Plastic type	Plastic additive	Function in plastic	(%)
Conclude	Potential endocrine disruptor Harmful to aquatic life with long lasting effects	Polyolefin-I; PUR; Polyolefin-II; PVC (soft); ABS; PVC (rigid); PMMA; PC; (E)PS	Octabenzone (2-hydroxy-4-n-octyloxybenzophenone)	Light stabilizers: Material protectants, Slip promoter	0.2 - 5.0
Officially recognised in the EU	Toxic to Reproduction (Harmonised C&L)	Polyolefin-I; PVC (soft); PVC (rigid)	Dibutyltin dilaurate	Heat stabilisers: Material protectants	3
Conclude	Suspected carcinogenic and/or mutagenic and/or reprotoxic properties (according to CLP harmonized), Potential endocrine disruptor.				
Conclude for p-Cresol only	Suspected carcinogenic and/or mutagenic and/or reprotoxic properties (according to CLP harmonized), Potential endocrine disruptor.	PVC (soft), PVC (rigid)	Triphenyl phosphite	Heat stabilizer	3
Officially recognised in the EU	Toxic to Reproduction (Harmonised C&L, Candidate list of SVHCs).	Polyolefins I y II; PA	3,3',3",5,5',5"-hexa-tert-butyl-α,α',α"-(mesitylene-2,4,6-triyl)tri-p-cresol	Antioxidants: Material protectants, Other stabilazer	0.5 - 2.5
Officially recognised in the EU	Endocrine Disrupting (Candidate list of SVHCs)	PUR; PVC (soft); ABS; (E)PS	Di-2-ethylhexyl phthalate (DEHP)	Plasticiser: Additives for processability	2.0 - 35.0
Broad agreement	Toxic to Reproduction (100% of REACH registrations				
Conclusion under preparation	Suspected PBT/vPvB, Exposure of environment				
Ongoing	Potential endocrine disruptor	PUR; PVC (soft)	1,1'-(isopropylidene) bis[3,5-dibromo-4-(2,3-dibromo-2-methylpropoxy) benzene]	Flame retardants: Physical-chemical property improvers	15
Officially recognised in the EU	Toxic to Reproduction (Harmonised C&L, Candidate list of SVHCs- Substances of Very High Concern).	PVC (soft), PUR	Bis(2-propylheptyl) phthalate (DPHP)	Plasticiser: Additives for processability	10.0 - 35.0

Broad agreement	Toxic to Reproduction (100% of REACH registrations).	PVC	Decanedioic acid, 1,10-bis (2-ethylhexyl) ester	Plasticiser: Additives for processability	10.0 - 35.0
Officially recognised in the EU	Endocrine Disrupting (Candidate list of SVHCs)				
Officially recognised in the EU	Toxic to Reproduction (Harmonised C&L, Candidate list of SVHCs- Substances of Very High Concern).				
Broad agreement	Toxic to Reproduction (100% of REACH registrations).	PUR; PVC (soft)	Dibutyl phthalate (DBP)	Plasticiser: Additives for processability	10.0 - 35.0
Officially recognised in the EU	Endocrine Disrupting (Candidate list of SVHCs)				

The plastic additives substances of high concern described in table 8 present in the current and future polymers matrix of MPs could have impacts on environmental health. For instance, Brominated flame retardants (BFRs), including polybrominated diphenyl ethers (PBDEs) and brominated biphenyls (PBBs) have been eliminated and replaced by other FRs, existing products containing toxic or potentially toxic chemical will remain in use for decades, and new products containing these and similar chemicals that will permeate in the environment (Hennebert, 2021).

Thus, flame retardans as 2,2',6,6'-tetrabromo-4,4'-isopropylidenediphenol (TBBPA) and 1,1'-(ethane-1,2-diyl) bis[pentabromobenzene] (EBP) (presents in Polyolefins, PU, PVC between 5-35 %), are proposed as follow up for Suspected Reprotoxic, Potential endocrine disruptor, Suspected PBT/vPvB (Potentially Persistent, Bioaccumulative and Toxic/very Persistent and very Bioaccumulative). The TBBPA which is normally chemically bound to the polymer different to the other brominated flame retardants compounds of lately attention as 1,2-bis (2,4,6-tribromophenoxy) ethane (BTBPE) which is not chemically bound to the polymer matrix, and they can leach into the surrounding environment (Engler et al., 2012; Meeker et al., 2009).

The BTBPE is Under assessment as Persistent, Bioaccumulative and Toxic compound and included in list as a candidate of Substance of very high concern (SVHC) (ECHA, 2023).

Regarding phthalates, the table 8, shows information about status definition for propierties concern for DEHP as Endocrine Disrupting (Officially Recognised) similar state conditions than “chemical cousin” of phthalates with similar structure as Dibutyl phthalate (DBP) (Fantke et al., 2015; Weber et al., 2018). Both compounds DEHP and DBP are candidates for status definition of Broad agreement as Toxic to Reproduction (100% of REACH registrations), and Candidate list of SVHCs (Substances of Very High Concern).

Bis(2-propylheptyl) phthalate (DPHP) another “chemical cousin” of phthalates is Officially recognised as Toxic to Reproduction corroboring that potential substitute compounds can also be toxic (Fantke et al., 2015; Weber et al., 2018).

In summary, exists a large number of chemical compounds considered as harmful associated to MPs found in seawater and marine sediment of Tarragona coast. Despite the low concentration of plastics additives released it can be considered the marine environment on Tarragona coast can be in danger highlinting that the guideline values do not take into account the low levels at which endocrine-disrupting chemicals may have an effect (Hahladakis et al., 2018) nor do they consider the toxicity of mixtures.

3.1.5 Pollution Load Index in Catalonia beaches

Similarly than marine sediments, Pollution Load Index (PLI) was estimated to assess the degree of pollution levels in Catalonia coast beaches. Using equation number 2 and 3 (Tomlinson et al., 1980), the PLI model was set up according MPs concentration in each fraction and for beach compared with Coi, the minimal MPs concentration that at this time it was assumed the lowest concentration ever found in beach samples. Thus, according Korez et al., (2019), low values were reported by nine sampling sites in Slovenia beaches ranging from 0.5±0.5 to 1±0.8 MPs/kg with morphology comprising fragments, fibres, films and foams and sizes from >100 µm to 5 mm. The lowest value of 0.5±0.5 was taken for PLI estimations. In addition, for values indetermined of MPs concentration in MPs>0.5 mm fraction, these values were substituted by minimal value of 0.5. For MPs<0.5 mm fraction, only values from fluorescence methodology were taken into account because showed MPs concentration in entire Catalonia coastal zones.

It is shown for entire region of Catalonia coast the hazard level for MPs≥0.5 mm pollution was within level I to exception PIN 1 and PIN 3 sites; it means PLI<10 or minor pollution (Figure 5, 6, 7). In La Pineda beach (PIN 1 and PIN 3), the hazard level reached to category II or high polluted.

By in contrast when MPs concentration increase in MPs<0.5 mm fraction, the PLI increases to >30 or level IV extremely danger. Thus, in North Catalonia and Barcelona zone beaches with level IV were Els Griells- L'estartit-**ELG** and Sant Adrià del Besòs-**STA** respectively (Figure 5). The rest of sites were in category between II and III or high polluted and danger.

For South Barcelona zone two beaches were in level IV (>30) as Cala de Vallcarca-**CAV**, and Platja Far de Sant Cristòfol-**VIL 01** (Figure 6), the rest of sites were in category II and III.

Towards to southern zones, it seems the beaches with high pollution level increases, thus in Tarragona and South Catalonia coast, nine and seven beaches respectively presented levels IV (Figure 6 and 7) as: l'Arrabasada (**ARRA 1**, **ARRA 2**), Miracle (**MIR 1**, **MIR 2**, **MIR 3**). La Pineda (**PIN 1**, **PIN 2**, **PIN 3**) and Salou (**SAL 1** and **SAL 2**) beaches in Tarragona zone, and finally, Bahía del Fangar (**BF-N**, **BF-FAR**), La Marquesa (**PLM**), Riumar (**RIU**), Los Eucaliptos (**PEC**), Trabucador (**TRA E**), Alfacs Bay (Playa del Delta-**PDE E**) in South Catalonia zone.

Only five sites were in category I or minor pollution for MPs≥0.5 mm fraction as **PPA**, and **PAR** (North Catalonia), **CAL** and **MONT**, (North Barcelona), **TOR** (Tarragona) with MPs concentration <1 MPs/kg. Similarly for MPs<0.5 mm **PPA**, **PAR**, **CAL** and **MOR** (Tarragona)

with category I showed values of 0 MPs/kg. Only **BLA** and **BCN** (North Barcelona) showed values for MPs concentration >1 and category I.

Comparing PLI values from Cambrils beach (**CAM 1, CAM 2, CAM 3)** sites and Cambrils subtidal sediments (**Cam**) it is observed higher values of PLI in subtidal sediments (>30) with hazard level IV than in beaches with level III (20-30) due high concentrations of fibres and particles in marine sediments (2106 MPs/kg d.w) compared with values found in beaches (218-379 MPs/kg d.w.). Similarly, in Hospitalet sites PLI in beaches (**HOS**) were lesser between 20-30 with hazard level III while PLI in sediments (**Hos**) was >30 or hazard level IV. This shows that in these areas, the MPs distribution probably is not homogeneous and there is not correspondence between subtidal sediments and sand beaches samples.

By in contrast, only in majority Tarragona city coast beaches (**MIR 01**, **MIR 02**, **MIR 03**, **PIN 1**, **PIN 2**, **PIN 3**) and subtidal sediments (**Tg N**, **Tg S**, **Pin N**, **Pin S**) sites showed the same hazard level. Thus, all beaches showed hazard level IV as in sediments with the exception of Tg S site with hazard level of III or danger.

For entire zones, in Catalonia coast as South Barcelona Tarragona and South Catalonia zone were considered more polluted zones with hazard level of III (danger) and PLI values between 20- 30 for MPs<0.5 mm (Figure 7). Zone less polluted resulted North Catalonia and North Barcelona zone with hazard level of II or PLI values <20 clasified as only high polluted. Regarding MPs≥0.5 mm, all zones were in category I or minor pollution.

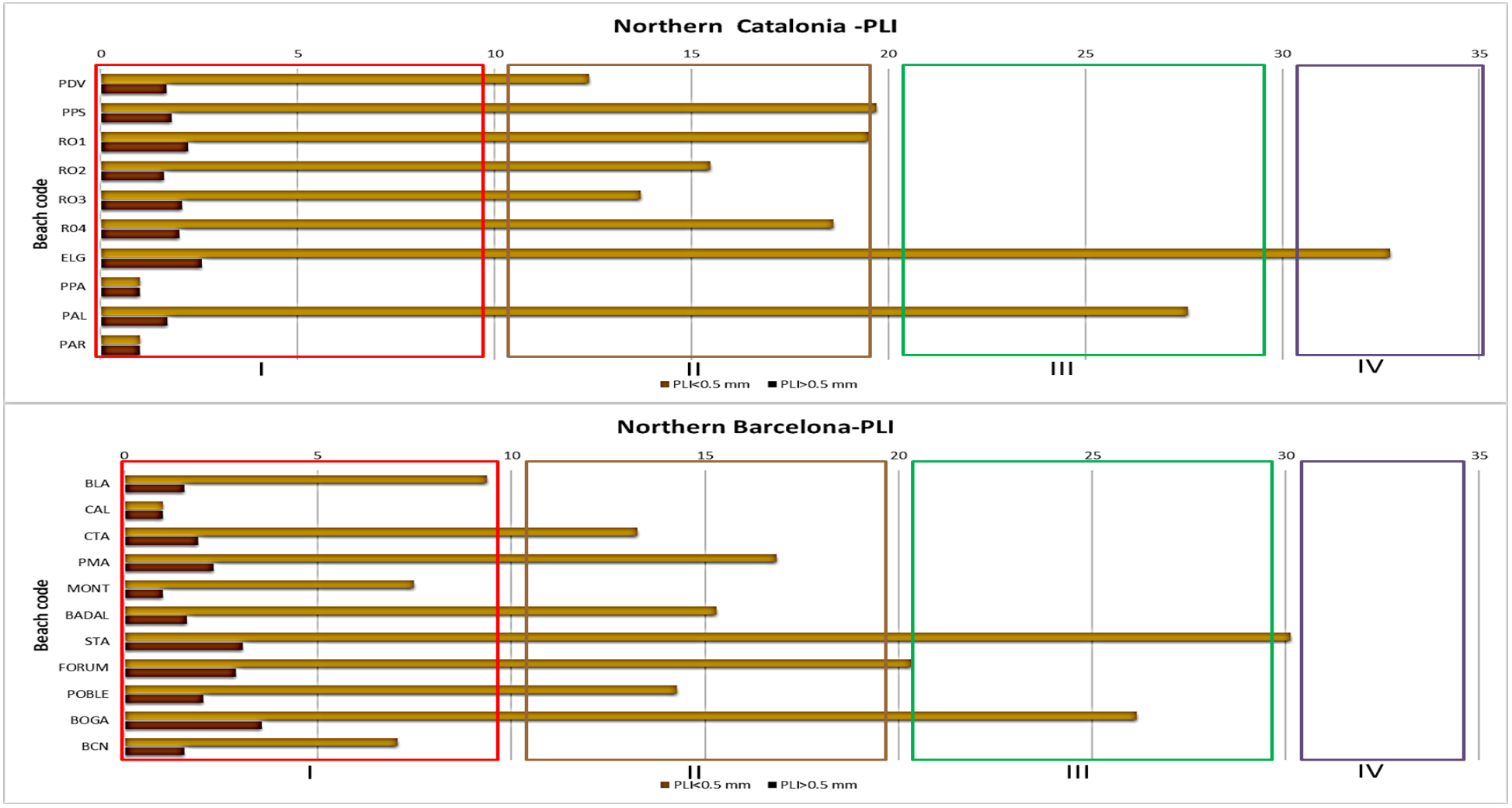


Figure 5. Pollution Load Index (PLI) for beaches on Catalonia and Barcelona North zone

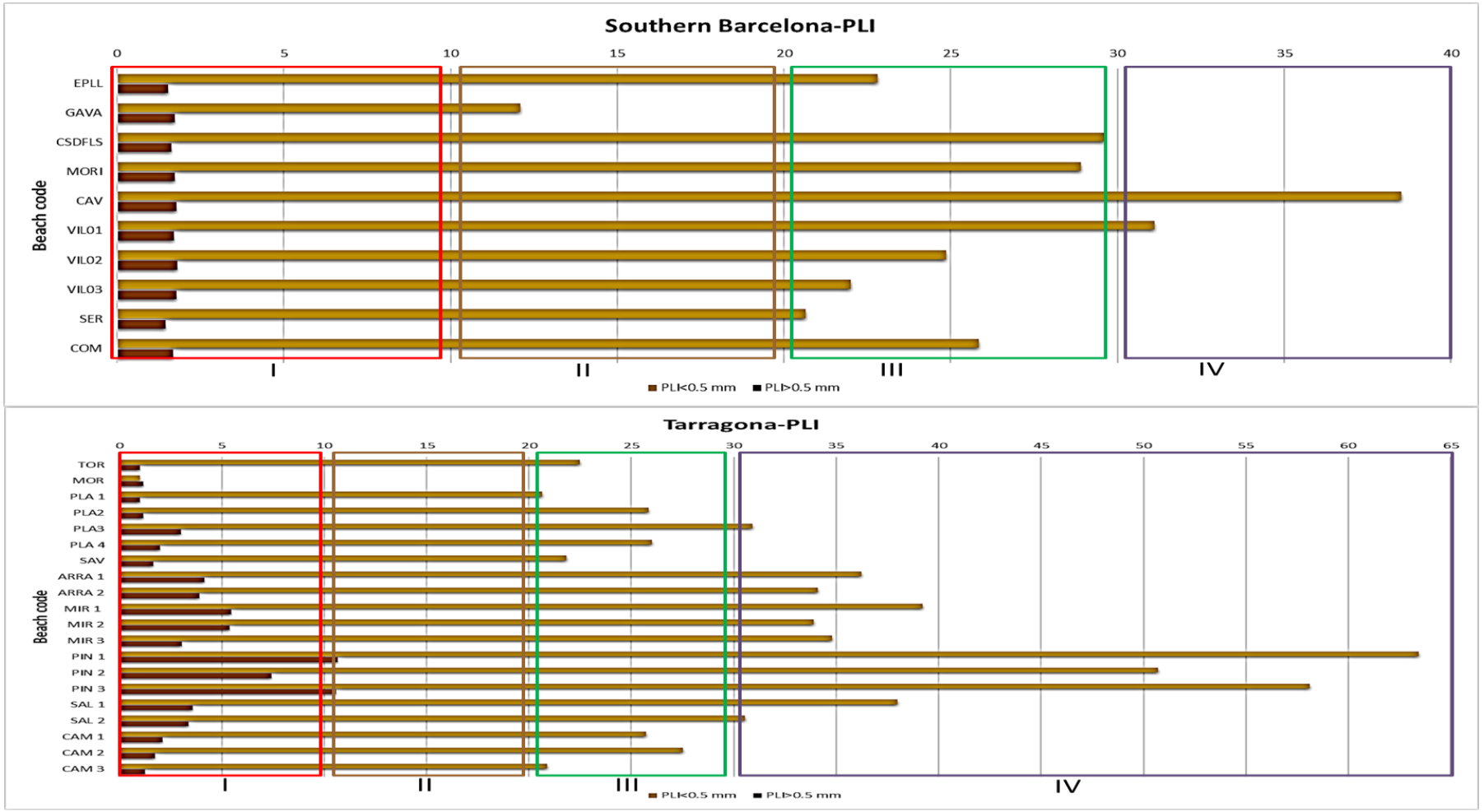


Figure 6. Pollution Load Index (PLI) for beaches on Barcelona South and Tarragona zone

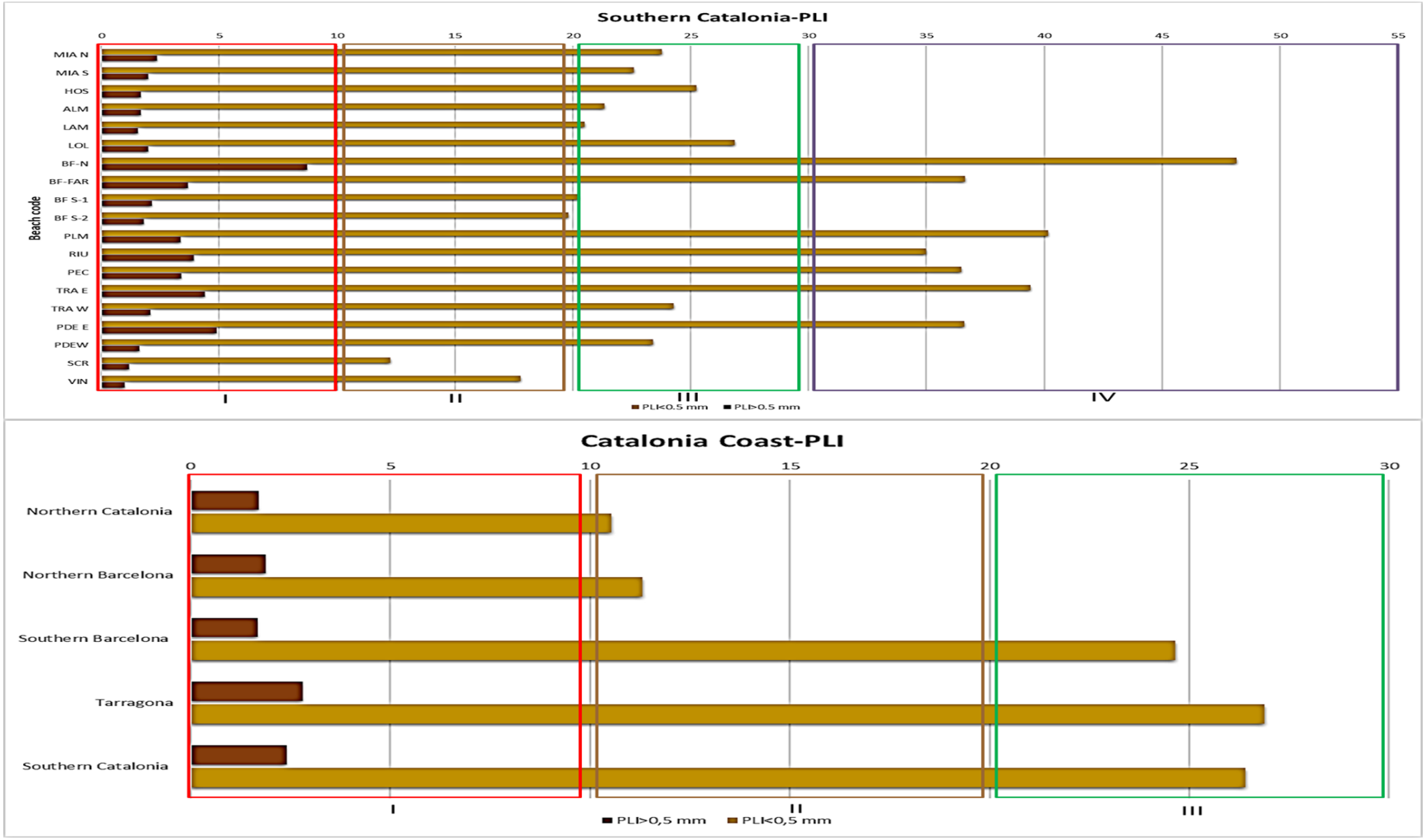


Figure 7. Pollution Load Index (PLI) for beaches on Catalonia South zone and summary of PLI on entire Catalonia coast.

3.1.6 Discussion Summary

MPs were ubiquitous and exhibited different distribution pattern in environmental matrices (seawater surface, beaches and marine sediments) on Catalonia coast possibly by their physical-chemical properties and interaction with environmental and climate parameters.

Currently there is no threat or risk by MPs pollution >80µm sizes in seawater surface (upper pelagic compartiment) on Tarragona coast according RCR index and RI index. However, zones presented high levels of MPs pollution (danger) according PLI index as Tarragona harbour T4 (PLI= 30). All seawater surface sites showed high hazard polymer index risk, H> 100 and grade between III and IV, composited by polymers with high hazard score as PVC, PU, PC, PMMA and ABS, due to their monomers and/or usual additives. The exposure concentration in the upper pelagic compartiment remains below (0.0008-0.0029 MPs/L) the safe concentration (6.65 MPs/L) currently and up to 2118 assuming a non exponential increase of MPs concentration in seawater surface on north-centre Tarragona coast. However, this does not necessarily exclude that aquatic ecosystem could be at risk for MPs in the future considering MPs small sizes <80 µm and the fast-increasing trend of MPs inputs in coastal zones.

Similar to seawater surface, marine sediments ≥0.5 mm on Tarragona coast not showed threat or risk by MPs pollution (RCR: 0.004-0.164 and PLI 2.1-9.7), but in Tarragona harbour (industrial zone) sediments high pollution levels were observed (PLI=14.4).The risk assessment results showed that MPs cause pollution risks according RDR, PLI and RI indexes in marine sediments fractions <0.5 mm of: Miracle beach north (Tg N), Pineda beach north (Pin N), Pineda beach south (PIN S), Cambrils-Cavet beach (Cam) and Hospitalet beach (Hos) of Tarragona centre-south zone (RCR:1.2-3.9; PLI: 38.5-69.9; RI:3980-7466). To exception Pin S and Tg S, all sites of marine sediments showed high hazard polymer index risk, H> 1000 and grade IV, composited by polymers with high hazard score as PVC and ABS.

According PLI index by zones in beaches from Catalonia coast, more polluted zones were considered South Barcelona, Tarragona and South Catalonia zones with PLI values between 20-60 and hazard level of III or danger with hot spots that reach hazard level of IV. Pollution indexes that consider polymer types combined with MPs concentration as the Risk Index are necessary and useful to comprehensively evaluate the ecological risk caused by MPs.

3.2. - Microplastics in Spanish people diet, contribution by seafood consumption and human health risk implications.

3.2.1 Comparison of MPs annual dietary intake between countries

For a comparative purpose, MPs annual dietary intake of selected molluscs studied in the present thesis were compared (tableb 9) using MPs levels detected in molluscs of several studies and the dietary intake of those molluscs obtained from FAOSTAT data (2009-2013) (FAOSTAT, 2021). MPs annual dietary ingestion for Spain were higher than those registered for South Korea, Tunisia, France and Italy due mainly to higher MPs concentration in Catalan molluscs combined with the relatively high consumption of these organisms by Spanish population. However, annual MPs ingestion throught mollusc consumption in Spain was lower than in China and Canada due to their high MPs concentrations in molluscs.

Finally, the average MPs dietary intake throught molluscs in Catalonia Coast (depurated and not depurated) calculated with FAOSTAT data of 7874 MPs/year were in the range of average dietary intake for all groups ages calculated with EFSA, (2021) (observed in Chapter 2), from 3531 to 8092 MPs/year. The maximun dietary intake estimated with FAOSTAT data using the range average of MPs concentration in molluscs varied from 2050 to 14104 MPs/year and was lower than 95th percentile value for all groups data calculated with EFSA (2021), that ranged from 8416 to 22347 MPs/year.

Table 9. Comparison of Estimated annual dietary intake of microplastics (MPs/year) via molluscs consumption in various countries with this study using average FAOSTAT data (2009-2013)

Average range concentration in molluscs (a) **(MPs/g w.w)**	**References molluscs MPs concentration (MPs/g wet weight)**	**Country**	**Statistical average molluscs consumption (kg/p*year) FAOSTAT Reference (b)**	**Dietary intake of MPs via molluscs consumption (MPs/year)**
3.60±5.35 (total molluscs) 3.40±3.03 (total molluscs depurated) Range average 0.94-6.47	This study	Spain (Catalonia Coast)	2.18±0.285	Average total molluscs: 7874 Average total molluscs depurated: 7416 Range average: 2050-14104
0.15 ±0.20 (0.07-0.34)	Cho et al., 2019	South Korea	3.48±0.34	522 244-1183
2.1-10.5	Li et al., 2015	China	2.77±0.18	5810-29050

0.18-0.23	Phuong et al., 2018a	France	2.42±0.23	436-557
0.05-0.12	Bonello et al., 2018	Italy	1.44±0.11	72-172
0.79-1.48	Abidli et al., 2019	Tunisia	0.027±0.006	21-39
89-259	Murphy, 2018	Canada	1.13±0.05	100867-292670

Shellfish supply data (2009-2013) obtained from the Food and Agriculture Organization Corporate Statistical Database (FAOSTAT) (FAOSTAT, 2021). a.-The mean concentration of microplastics measured in market bivalves for various countries (referenced). b.- The supply data based on soft tissue was derived from the data based on whole body (including shell and soft tissue) provided by FAOSTAT by applying the mean weight ratio of soft tissue to whole body (1:3) suggested by Cho et al., 2019.

3.2.2. Microplastics risk assessment (potential human health risk)

MPs also contain potentially hazardous chemicals such as monomers and additives. Some of them are Bisphenol A (BPA) considered as endocrine disrupting chemical due estradiol-like activity (Talsness et al., 2009), phthalates and brominated flame retardants (polybrominated diphenyl ethers (PBDEs) are also endocrine disrupting chemicals (Hahladakis et al., 2018; Talsness et al., 2009). The potential human health effects are mainly, but not only, related to interference with normal reproductive development and function (Hauser and Calafat, 2005)

Currently, there is no standardized approach to systematic assessment the potential environmental risk of MPs, monomers and additives exposure. As a consequent, it was applied an approach taking polymer types into consideration in order to better understand the risk of MPs composition in the human health.

According to previous studies (Ding et al., 2020; Fang et al., 2019; Lithner et al., 2011; Xu et al., 2018) and applying the polymer risk index H showed in Section 3.1.1 (equation 1), the potential risk of MPs composition in commercial Catalan molluscs was assessed. In this case, Pn is the percentage or proportion of MPs polymer types detected in soft tissue of each mollusc species, and Sn is the hazard score taken from Lithner et al., (2011), for the type of polymer the MPs are made of. The polymer types of MPs and the corresponding hazard scores of plastic polymers stablished by Lithner et al., (2011) are showed in table 1.

Hazard grades with corresponding statements assigned for each hazard level from I to V is shown in table 2. The index H was compared with hazard levels from 1 to >10000 and corresponding hazard grade showed in table 3. The figure 8 shows the polymer risk index (H) per sample caused by MPs composition. Samples as snail from south S(S), mussels not depurated from north Mnd (N) and wedge clams from north, WC (N) showed low risk index with hazard grade between I and II (<100) with implications in human health from not health hazard effects to acute toxicity (harmful if is swallowed) and transient target organ effects.

Snails from middle zone S(M), showed the highest H value with grade IV (1000-10000) including effects as heritable mutations in the germ cells of humans. Furthermore, potential to produce significant toxicity in humans following single exposure and finally, reliable evidence on the substance or mixture (including bridging) of an adverse effect on specific organ/systems.

The rest of samples of different species, zones catchment and depuration condition showed a hazard grade of III (100-1000) and additional implications as: suspected human carcinogens and reproductive toxicant via lactation, toxic if swallowed, potential to be harmful to human health following single exposure, evidence on (including bridging) of an adverse effect on specific organ/systems substance or mixture.

It is important to highlight that depuration processes in molluscs do not decrease the grade of polymer risk index.

The high percentages of polymer PAN in these samples and PVC presence (only in wedge clams from middle) with high hazard scores 11,521 (V) and 10,551 (V) respectively caused that samples reached to a high polymer risk index. Specifically due to "Trojan-Horse" effect, PVC could release toxic additives and release carcinogenic monomers (e.g. dibenzodioxins and dibenzofurans) by e.g., weathered or heated under the sunlight after exposing to humans (Bhatt et al., 2021; Ma et al., 2020). PVC, categorized as a "very high hazard" polymer, might release monomers and intrinsic plastic additives when entering the marine environment.

Similarly, Ding et al., (2020), found shellfish samples with higher proportions of PAN and PVC, however the polymer risk index was between III and IV. The polymer risk index calculated provide a preliminary discussion for risk assessment by mollusc's consumption, and it can be concluded that the majority of samples contain chemical compounds that pose a threat to the human health.

PVC, as denser polymer (density 1.16-1.58 ~1.38 g/cm3), commonly accumulated in the deeper layers of the water or the benthic zone (Ding et al., 2021; Ding et al., 2022). PVC had more opportunities to encounter the infaunal bivalves, such as clams (sediments), therefore PVC MPs ingested by bivalves might pose a hazardous threat to the marine ecosystem and human health (Cox et al., 2019). From the perspective of human health, bivalves can act as the transporter to carry MPs into humans, potentially hazardous contaminants, such as MPs chemical additives are entering and producing potential hazards to human health, thus according Proshad et al., (2017), PVC could disrupt the function of the endocrine system, and PS could induce neurotoxic and genotoxic effects (Oliveira and Almeida, 2019).

Phthalate esters are used as plasticizers in the manufacturing of PVC polymers and plastisol to achieve enhanced flexibility and durability (Kim et al., 2020). Human exposure to phthalate esters is potentially harmful and may cause abnormal sexual development and birth defects (Cheng et al., 2013). Additionally, butyl benzyl phthalate (BBP) has been named as a probable carcinogen, and di-2-ethylhexyl phthalate (DEHP) has been cited as a possible carcinogen by U.S. EPA (Karbalaei et al., 2018).

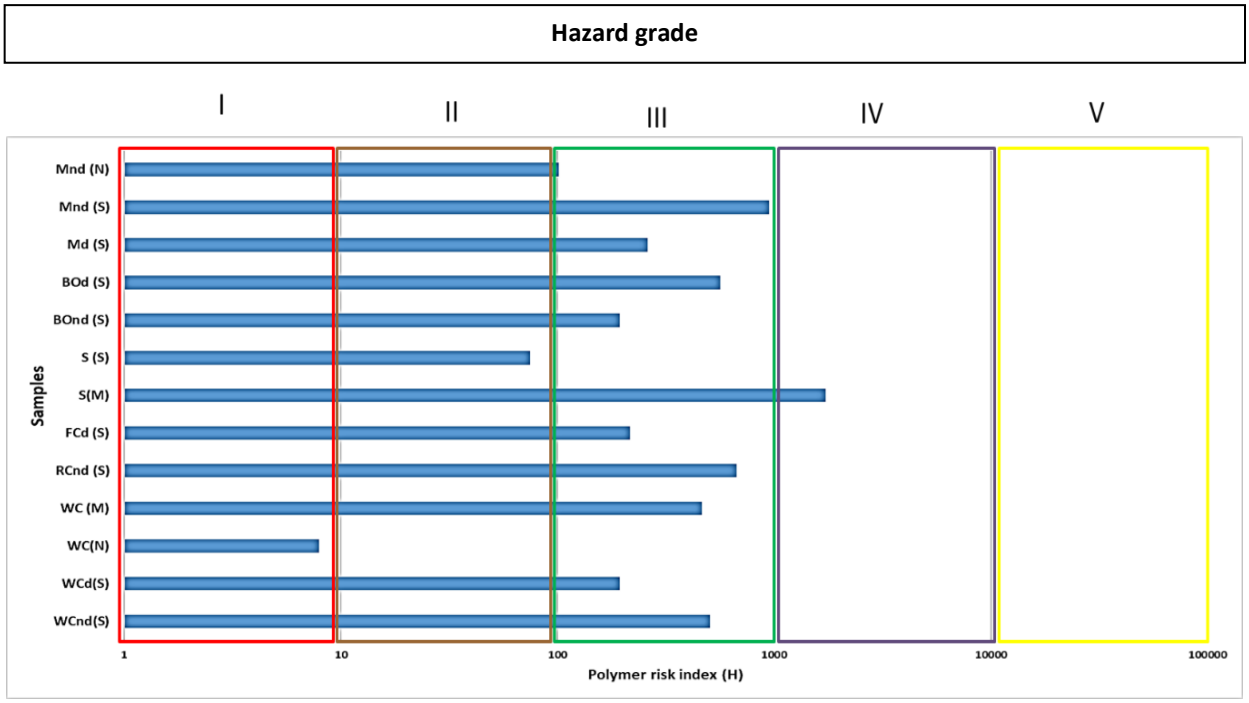


Figure 8. Polymer risk index (H) and hazard grade of microplastics in each specie, catchment zone and depuration condition.

3.2.3 Persistent organic pollutants adhered and additives in microplastics

According Fu et al., (2021), hydrophobic interaction (octanol-water partition coeffcient (Kow)), is the most common mechanism by which MPs adsorb organic pollutants, Additionally, electrostatic interaction and other non-covalent forces, such as hydrogen bonds, halogen bonds, and π–π interactions, are also mechanisms of organic pollutant adsorption on MPs. Thus, its reversible nature preserves the likelihood of chemical desorption from the matrix (Endo and Koelmans, 2016).

Another concerning effect of MPs is the adsorption of toxic pollutants from the marine environment, specifically persistent organic pollutants (POPs) of concern to human health (with possible endocrine disruption and/or carcinogenicity) that can also be transferred by ingestion such as polychlorinated biphenyls; PCBs, 2,2´-bis(p-chlorophenyl)1,1-trichloroethane; DDTs (or

it metabolites DDD and DDE), hexachlorocyclohexans; HCHs and polycyclic aromatic hydrocarbons; PAHs, as well as adsorb metals, including cadmium and lead (EFSA, 2016; Mato et al., 2001; Rochman et al., 2015; Teuten et al., 2009) determined the concentrations of PCBs and DDE on plastic resin pellets collected at the coast of Japan and found the levels to be 105 – 106 times higher than in the surrounding seawater.

Absorption mainly occurs onto rubbery polymers (i.e., PE and PP), where external molecules pass through and associate within their matrix (Teuten et al., 2009; Wang et al., 2018a). These polymers are generally recognized as the ones concentrating the highest amounts of HOCs (Hydrophobic Organic Chemicals) and are then possibly more dangerous for marine life (Fisner et al., 2017; Wang and Wang, 2018; Wang et al., 2018b) and possibly to human health

Some polymers present several functional groups on their surface, conferring a certain degree of polarity. These are mostly PS and PVC, whose glassy nature yields the formation of nanovoids and pores on the surface, which are the sites of sorption. In this case, the process is mainly led by adsorption, a mechanism through which the chemicals more effciently bind to the plastic polymer through ionic strength (Gamarro and Constanzo, 2022). Polymers as PS and PET showed high adsorption capacity for stronger hydrophobic and aromatic character compounds (Munoz et al., 2021)

According to Fu et al., (2021), the particle size, specific surface area (surface area to volume ratio of a plastic particle, which increases as the size decreases), aging degree, crystallinity, and polarity of MPs, and organic pollutant properties (hydrophobicity and dissociated forms) are key factors affecting adsorption capacity (Ma et al., 2016; Teuten et al., 2009; Zhang et al, 2018). Adsorption capacity, which was found to increase with decreasing the particle size, thus sizes between 20 to 500 µm of PS particles showed equilibrium adsorption capacity between >70 to 120 µg/g of hydrophobic compound as diclofenac ($C_{14}H_{11}Cl_2NO_2$) (Munoz et al., 2021). It is important to highlight, environmental parameters as temperature, pH (which modulates the ionization of ionic organic pollutant), and salinity are among the determinants of the sorption/desorption processes (Gamarro and Constanzo, 2022).

Plastic surface exposed to weathering, can be subjected to embrittlement and fragmentation, steps that increase the surface/volume and provide more space, a larger contact area, and new sorption sites for external molecules (Napper et al., 2015; Wang et al., 2018b).
However, UV rays-induced weathering, or photo-oxidation, can also lead to chemical alterations and loss of hydrophobicity through the creation of new oxygen-rich functional groups (i.e., carbonyl fractions). Oxidized MPs exhibited acidic surface properties, which led to a strong

decrease on the adsorption of the hydrophobic micropollutant but to an increase with the hydrophilic one (Gamarro and Constanzo, 2022; Munoz et al., 2021), such as metals.

Regard plastics additives, (plasticizers, PBDEs flame retardants, antioxidants, and stabilizers) residual monomers, catalysing agents, many of them, are not chemically bound to the polymer and can thus more easily migrate from the material. Only some reactive organic additives, such as some flame-retardants, are polymerized with the plastic molecules becoming part of the polymer chain (Gamarro and Constanzo, 2022). Additionally, plastics additives (di(2-ethylhexyl) phthalate (DEHP), (3) 3,3′,4,4′,5-) in MPs may be as harmful as listed POPs in terms of behaviour and consequences since they may have an activity level, widespread distribution, toxic risk and bioaccumulation comparable to that of POPs (SCP/RAC, 2020).

There is a synergistic effect between MPs (PVC) and organic compounds (benzo(a)pyrene), indicating the significant role of MPs as a vector for organic pollutants (Caruso, 2019; Gomiero et al., 2018). In the gastrointestinal system, some digestive solutions as bile salts (as detergents) are present and, through their surfactant properties, could induce the release of toxic substances from the ingested MPs (Ahrens et al., 2001; Heinrich and Braunbeck, 2019). This could suggest that bioavailability of liphophilic contaminants can be increase due to the action of gastrointestinal bile acids.

EFSA, (2016) reported a worst-case exposure scenario based on a portion of 225 g of mussels eaten by French volunteers in a day. Lusher et al., (2017) estimated a ratio intake by microplastic/total dietary intake by ingestion (percentage) of hazardous compounds in 7 µg of plastics. The estimations were based in highest portion of mussels consumed, MPs concentration reported by Li et al., (2015) with median value 4 MPs/g wet weight, spherical particles with an average size diameter of 25 µm (van Cauwenberghe and Janssen, 2014), density of 0.92 g/cm^3 (density of low-density polyethylene –LDPE), and an average human individual of 70 kg. In the following, the highest reported contents of contaminants and additives in MPs were used and total intake from the diet (pg/kg bw*day) of each compound (Table 10) to calculate exposure to contaminants and additives via microplastic ingestion.

Table 10. Highest concentration in microplastics and total intake from the diet of organic persistant pollutants and additives obtained by EFSA.

Compound	Highest concentration in microplastics (ng/g)	Total intake from the diet (pg/kg bw*day)	Observations
Non-dioxin like PCBs	**2970**	**4300**	**Lowest intake of 6 indicators of non-dioxin like PCBs, representing about 50 percent of all non-dioxin like PCBs (EFSA, 2008)**
PAHs	**44800**	**28800**	**Median intake (EFSA, 2008)**
DDT	**2100**	**5000**	**Lowest intake, DDT and related compounds (EFSA, 2006)**
Bisphenol A	**200**	**130000**	**Average intake adults (EFSA, 2015)**
PBDEs	**50**	**700**	**Lowest intake, sum of BDE-47, -209, -153, -154 (EFSA, 2011)**

Based on new assumptions and data obtained in this study, the contribution of MPs from mussels to the total dietary exposure to contaminants and additives was estimated assuming that all adhered contaminants and additives are totally released from the particle. Average and maximum MPs concentration of mussels from north and south with or without depurated condition were considered (g wet weight), only morphology fragments and sizes range accumulated from 75 to 85% (average of range) were also considered. Similarly, to Lusher et al., (2017), spherical fragments, a density of 0.92 g/cm^3 and an average human individual of 70 kg were assumed (Table 11).

Table 11. Average size, concentration and total plastic weight in mussels samples from Catalonia Coast.

Sample	% Sizes 0.02-1 mm	Average size (mm)	Fragments (%)	Average concentration MPs/g	Highest concentration MPs/g	Plastic weight (g)
Mussel not depurated North	84	0.21	64	8.17		0.00553
			80	-	34.4	0.02912
Mussel not depurated South	78	0.467	3	3.5		0.00016
			4	-	6.62	0.00219
Mussel depurated South	75	0.428	5	4.78		0.00203
			3	-	9.65	0.00267

The contribution of mussels' MPs to the total dietary exposure to contaminants and additives is shown in Table 12, not depurated mussels from north presented the highest contribution for organic persistent pollutants and additives. The highest contributions to the total dietary intake of contaminants were: PCBs, 29%, PAHs 65%, DDT 17%, while additives were smaller bisphenol A 0.06% and PBDEs 3%. The pollutants contributions compared with obtained by Lusher et al., (2017), were up to 4400 times higher and it can be concluded that the effect of consumption of MPs on the intake of contaminants and additives is not negligible for organic contaminants. For additives, according to Lusher et al., (2017), the contribution of seafood may even be overestimated considering that the diet is not the only route of exposure, for PBDE's indoor dust (polymeric material originating from clothing and textiles) and for BPA dermal uptake are relevant. However, despite low contribution of additives, the contribution obtained here compared with estimated by Lusher et al., (2017) were 4200 times higher and important implications for human health should be considered even more if not depurated organisms from high MPs polluted zones are consumed.

These fragments and associated toxic compounds will probably not pass through the intestinal barrier because they are a bit larger than 150 µm (EFSA, 2016). However, the majority bivalves, are most often heat-treated prior to consumption together to the effect of chewing and digestion

causes fragmentation and disaggregation to even more smaller fractions, when are consumed, with the production of smaller MPs <150 µm and even nanoplastics increasing the risk of passage of the membrane carrying toxic compounds.

Table 12 Ratio of the calculated intake of contaminants and additives (worst-case scenario) directly from microplastics in mussels of Catalonia Coast and the total dietary intake of these compounds. Comparison with ratio calculated by Lusher et al., (2017).

	Mussel not depurated North		Mussel not depurated South		Mussel depurated South		
Compound	Average ratio intake microplastic /total dietary intake (%)	Maximun ratio intake microplastic /total dietary intake (%)	Average ratio intake microplastic/ total dietary intake (%)	Maximun ratio intake microplastic /total dietary intake (%)	Average ratio intake microplastic/total dietary intake (%)	Maximun ratio intake microplastic/total dietary intake (%)	FAO Ratio intake microplastic/total dietary intake (%)
Non-dioxin like PCBs	5.46	28.74	0.16	2.16	2.00	2.64	0.007
PAHs	12.29	64.72	0.36	4.87	4.51	5.94	0.02
DDT	3.32	17.47	0.10	1.32	1.22	1.60	0.004
Bisphenol A	0.01	0.06	0.0004	0.005	0.004	0.01	0.00002
PBDEs	0.56	2.97	0.02	0.22	0.21	0.27	0.0007

Preliminary assessments suggest that the contributions of these chemicals from MPs in bivalves among top consumers in Europe are very small compared to other sources. However, the MPs concentrations in a great edible molluscs variety, composition, morphology and sizes found here make the contribution considerable, especially by not depurated mussels with possible synergistic toxicity by MPs and chemical compound associated with. To get to further conclusions, an exposure assessment to chemical compound associated with from the whole diet (other food items) should be estimated and the toxicity of the most common polymers and possible associated chemical compounds (e.g. HCH's), as well as their mixtures, needs to be evaluated.

According to Gamarro and Costanzo (2022), consequences of the dietary intake of different concentrations of plastic additives, monomers, and contaminants should be clarified, as many of these chemicals might trigger response mechanisms even at low doses

3.2.4 Human health implications

The ingestion of bivalves is a main a route of human exposure to MPs because they filter a large volume of seawater polluted with MPs, with filtration rate up to 100-120 L/day (FAO, 2022; Simal-Lozano and Ameijeiras, 1994). The fact that bivalves are eaten as a whole, without gut removal are considered a main pathway of exposure to MPs by humans (Lusher et al., 2017). The MPs pollution is widespread in bivalves sold in markets, suggesting their potential as a route of microplastic exposure for humans and potential human risks because the highest intakes especially in elderly and pregnant ages group observed only by depurated molluscs as clams, mussels and oyster. In case, consumption molluscs no depurated there will be an additional risk for mussels from northern because higher MPs concentration especially of smaller size.

Transference of MPs from molluscs to humans in Mediterranean Sea by direct or indirect consumption through food chains is feasible; although it was demonstrated MPs shows trophic dilution rather than magnification in edible parts of seafood. The transferring will depend of organism capacity of ingestion and egestion (bioaccumulation) physical and chemical properties of the consumed microplastic and trophic level (Akhbarizadeh et al., 2018; Gündogdu et al., 2022). In case of bigger and medium pelagic or benthonic fish consumption and crustaceans with gut removals, the MPs transference could be through the muscles (Abassi et al., 2018; Ferrante et al., 2022; Karami et al., 2017, 2018) and would be to a lesser extent.

In figure 17, it is shown the possible routes for human and organism MPs exposure from molluscs consumption in Mediterranean Sea consumption interrelations between edible fishes and shellfish with humans were established. The first level (1st consumers), is constituted by bivalves *Donax, Ensis, Tapes, Mytilus and Crassosstrea* depending on feeding habits, MPs consumptions comes from water column or interface sediment-water. Snail *Bolinus*, fishes and crustaceans constitute the second level. *Bolinus* is a depredator of bivalves as well as the crab *Callinectes* (invasive species), the lobster *Homarus* (bogavante) and fishes as: *Sparus aurata* (dorada), *Mullus* (salmonete), *Pagellus acarne* (aligoté), *Pagellus bogaraveo* (besugo), *Brama brama* (Japuta) and *Diplodus vulgaris* (mojarra). In turn, lobster *Palinurus* and fish *Pagrus pagrus* (pargo), constitute the third level, they are depredators of crabs. Voracious predators as *Dicentrarchus labrax* (lubina) and *Epinephelus marginatus* (mero) eating on other fishes and crustaceans and constitute the last level, the fourth. Dotted lines mean MPs consumption by presence in muscles or body parts different from digestive system, continuous line mean MPs consumption by whole organism (without digestive system removals) or including digestive system consumption.

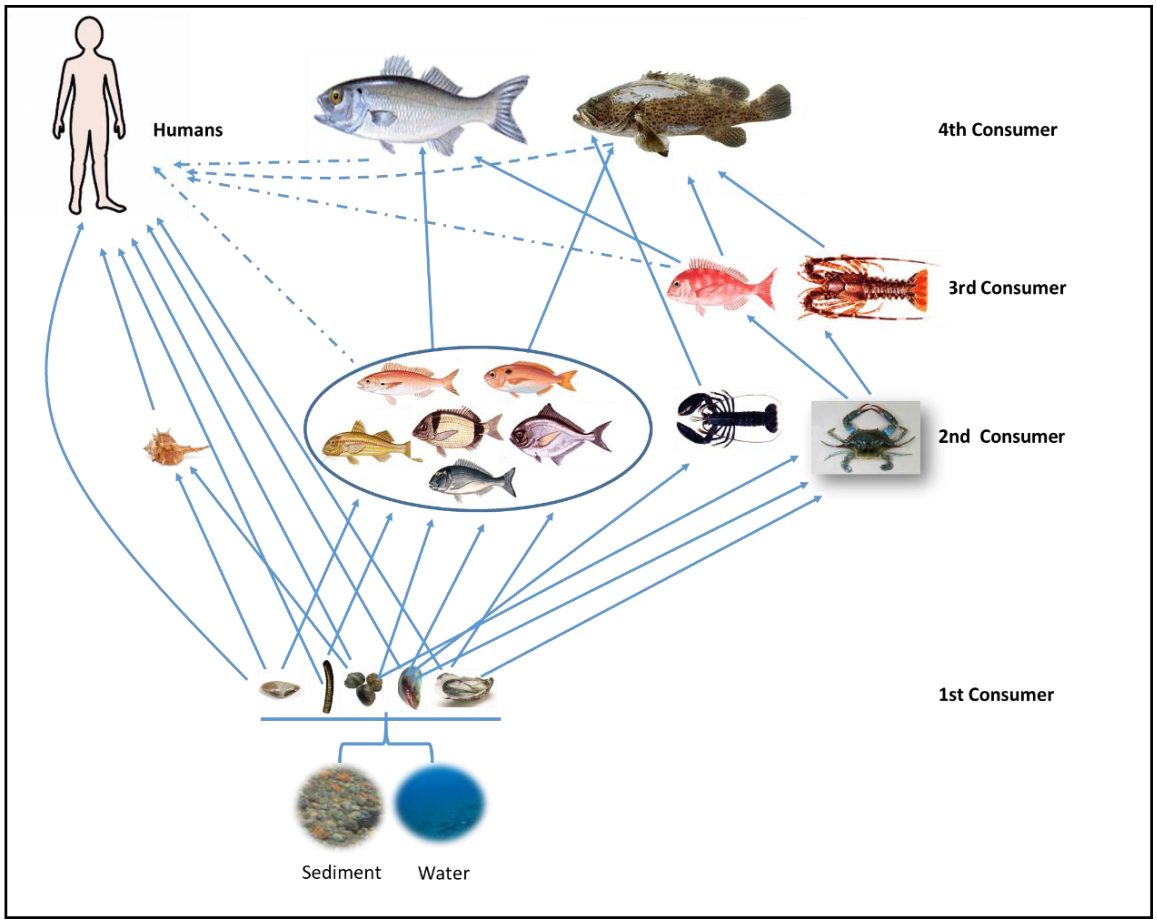


Figure 17. Proposed pathways for human and benthic-pelagic edible marine organisms microplastics exposure by molluscs consumption in Mediterranean Sea. (Gencat.Agricultura y Ganaderia. Pesca, 2022; PecesMediterraneo, 2022; Gencat.Agricultura, 2022-50 species pesquers; Gencat.Agricultura,2022, estadistiques; Gencat.Agricultura,2022, pesca recreativa a la costa catalana; Gencat.Agricultura,2022, mol-luscs; Gencat.Agricultura,2022, crustacis; Ictioterm, 2022; SeaLifeBase, 2022; Jomenjopeix, 2022; Cabidigitallibrary, 2022)

The assessment of human intake of MPs highlights the possible risks posed by seafood consumption to the human population depending largely on a seafood diet, MPs composition, associated contaminants and frequency of consumption. In addition, toxicity and the relative ease with which MPs cross biological barriers are expected to increase with decreasing size with concerns about very smaller MPs, and in particular, nanoplastics.

There is no risk assessment framework that takes into account the multidimensionality of microplastic particles that encompass an infinite combination of sizes, shapes, densities and chemical composition (Koelmans et al., 2022). Based on Steps for Conducting a Human Health Risk Assessment framework provided by US-EPA (2022), a preview of possible risk for Spanish human health by MPs consumption from edible molluscs is shown in figure 18.

Hazard identification (step 1): MPs (heterogeneous particle mixture), have the coexistence of three classes of hazards, physical, chemical and microbiological (Koelmans et al., 2022). According EFSA, (2016), only MPs smaller than 150 μm may translocate across the gut epithelium causing systemic exposure. The absorption of these MPs is expected to be limited (≤0.3%). Only the smallest fraction (size<1.5 μm) may penetrate deeply into organs (EFSA, 2016). Previous studies have revealed that microsized particles between 1 and 20 μm can be absorbed by the body through oral ingestion (Jani et al., 1992; Stock et al., 2019; van Raamsdonk et al., 2020; Wright and Kelly, 2017). Fragments, fibres and films are the most frequently reported microplastic shape categories. Persistence and longevity of environmental plastic are very high (average 10^5 days) and the possibility to be ingested and transferred throught food webs is high (Koelmans et al., 2022).

Energy and metabolism disruption, traslocation, chronic inflammation, unwanted immune response (by PP MPs 20-200 μm), disruption of immune function, neurotoxicity, cytotoxic, hemolysis, induced ROS generation, loss of cell viability (very high dosage), disruption of mitochondrial membrane potential, increase level pro-inflammatory cytokines and histamines in human peripheral blood mononuclear cells (PBMCs) and HMC-1 cells, human microbial colonic community composition changes (by irregular PET particles 160 μm ± 110 μm), among many others have been described as potential health effects associated with MP exposure (Hwang et al., 2019; Prata et al., 2020; Schirinzi et al., 2017; Stock et al., 2019; Tamargo et al., 2022; Wu et al., 2019). Toxicity due to chemical leachates from plastics as PU and PVC "*in vitro*" assays, comprise baseline toxicity, oxidative stress, cytotoxicity, estrogenicity, and antiandrogenicity (Zimmermann et al., 2019).

Persistent organic pollutants (POPs) could affect and alter endocrine functions in the organism (i.e., PCBs, PBDEs) but also promote carcinogenicity (PAH). These outcomes mainly arise from the interaction with intracellular receptors and gene expression induction (JECFA, 2016; Pocar et al., 2005). According to the strength and type of interaction they have with the polymer's matrix, they could be released at different degree (Gamarro and Constanzo, 2022).

Nanoplastics derived from MPs through ingestion process can cause in addition lysosomal dysfunction and genotoxicity (Ferraro et al., 2016; Meindl et al., 2015; Poma et al., 2019; Zhang et al., 2011) as well as synergistics, effects, thus Liang et al., (2021), found that the combined toxicity of PS micro- and nanoplastics on intestinal barrier dysfunction was caused primarily by reactive oxygen species (ROS)-mediated epithelial cell apoptosis in the mice. In addition, the combined toxicity of PS micro- and nanoplastics was also found in the mice after a 28-day

repeated dose exposure. Cooking molluscs also increase the possibility size reduction of the MPs with nanoplastics potential generation (Renzi et al., 2018).
According Mohamed Nor et al., (2021) there is uncertainty in translations to "*in vivo*" scenarios and in addition, particles used in tests are not representative of MPs found in the environment

Dose response assessment (step 2), summarize de effects in vivo assays and concentrations used, until now a Reference Dose (RfD), acute reference dose (ARfD); estimation of the margin of exposure (MOE) or tolerable daily intake (TDI) for particles or mass has not been stablished due possibly the MPs features of heterogeneous particle mixture implying that there are multiple relevant dose metrics (Koelmans et al., 2022). Moreover, the toxic effects strongly depend on the dose, dose rate, and duration of exposure used in the experiments (Pironti et al., 2021).

The toxicity of MPs "in vivo" experiments has been showed in some studies as:

- Stock et al., (2019) conducted a later investigation on rodents by oral gavage. It was indicated that the oral-systemic availability of MPs was low, and most particles were excreted by fecal excretion following 28-day oral exposure. This experiment also indicated that a very small number of MPs was found in the intestines but not in other remote organs such as the kidneys, spleen, or liver. They used a mixture of spherical fluorescent PS particles of 1 µm ($4.55x10^7$ particles), 4 µm ($4.55x10^7$ particles), 10 µm ($1.49x10^6$ particles) at a volume of 10 mL/kg/bw.

- Lu et al., (2018) observed a decrease of mucin secretion in the gut, induction of gut microbiota dysbiosis, and hepatic lipid metabolism disorder in mice following oral exposure to polystyrene MPs (0.5 and 50 µm) at concentrations of 100 µg/L (1.456×10^{10} items/L for 0.5 µm and 1.456×10^4 items/L for 50 µm) and 1,000 µg/L in drinking water for 35 days. Others effects were reduced body and liver weight

- Jin et al., (2019), found an increase of bile acid secretion in the liver and a decrease in mucus secretion in the colon were observed in mice after exposure to polystyrene MPs (5 µm) at 100 µg/L (1.456×10^6 items/L) and 1,000 µg/L (1.456×10^7 items/L), in drinking water for six weeks.

- Zheng et al., (2021), reported that polystyrene (5 µm) caused an increase of intestinal permeability, differentiation of adipocytes and lipid disorders in the liver of mice with acute colitis at a concentration of 500 µg/L in drinking water for 28-day exposure.

- Luo et al., (2019), maternal exposure of polystyrene MPs (0.5 and 5 μm) at concentrations of 100-1,000 μg/L for six weeks in drinking water during the gestation and lactation periods increases the risk of metabolic disorders, gut microbiota dysbiosis, and barrier dysfunction in the offspring of mice.

- Li et al., (2020), polyethylene MPs (10-150 μm) at concentrations 6, 60, and 600 μg/day for 5-week oral exposure in mice, showed minor intestinal inflammation.

- Exposure to PAEs mixtures (phthalate esters, DEHP, DBP, DEP, and DMP) accumulated on PE-MPs with maximum absorption of 176.07 ± 20.54 μg/g of particles (0.4 to 5 μm) were given to male mice by oral gavage in a dose of 100 mg/kg bw per day for a period of 30 days. The concentrations of PAE-contamined MPs were 0.2 g/L MPs+5 μg/L PAE mixture and 0.2 g/L MPs +50 ug/L. Significantly increased accumulation of PAEs in the liver and gut of (469.5±37.3 ng/g dry weight) was observed. In addition, induced enhanced reproductive toxicities manifested by greater alterations in sperm physiology and spermatogenesis was due synergistic effect of both MPs (oxidative stress) and PAEs. (Deng et al., 2021).

- Fluorescent and pristine polystyrene MPs (PS-MPs) particles with two diameters (5 μm and 20 μm), revealed significant alteration in several biomarkers related to energy metabolism, lipid metabolism, oxidative stress and neurotoxicity in mice indicating potential toxicity from MPs exposure, with doses from 0.01 mg/day (1×10^5 items for 5 μm and 2×10^3 items for 20 μm PS-MPs) to 0.5 mg/day (5×10^6 items for 5 μm and 1×10^5 items for 20 μm) by oral gavage and 4 weeks of exposure (Deng et al., 2017)

All studies indicated that prolonged exposure of MPs could be hazardous for mammal consumers

Furthermore, toxicity of NPs "*in vivo*" experiments has been also showed in some studies as:

- Gündogdu et al., (2022), indicated that there was an inverse relationship between particle size and systemic bioavailability among rats after oral exposure, particles smaller than 100 nm were distributed systemically and found in the liver, spleen, blood, and bone marrow.

- Amereh et al., (2019), found signs of nephrotoxicity, with significantly higher serum levels of creatinine reported in rats after oral ingestion of polystyrene MP (25 and 50 nm) at doses of 1, 3, 6, and 10 mg kg/day for 35 days.

- Deng et al., (2018), studied co-exposure of PE-MPs (0.5-1.0 μm) and organophosphorus flame retardants (OPFRs) and found these particles induced greater oxidative stress, neurotoxicity and enhanced disruption of amino acids as well as energy metabolism in mice after 90 days exposure of compounds (MP: 2 mg/L, OPFRs + PS: 10 μg/L and 100 μg/L).

- Amereh et al., (2020), suggested that polystyrene MPs (average size: 39 nm) caused signs of reproductive toxicity and a significant effect on gene expressions, as well as endocrine disturbances and histological lesions in rats after oral administration at doses of 1, 3, 6, and 10 mg/kg for 35 days.

In addition, the translocation of NPs to the intestinal serous was significantly increased in the ulcerative intestinal mucosa of human patients. This may cause transfer into the systemic circulation and organs to become even a higher risk (Gündogdu et al., 2022).

Exposure assessment (step 3) data of people exposed, according to data from this thesis is described as follow:

- Annual dietary only by depurated molluscs ranging from 3530 to 8103 MPs in adults (average rate consumption) and 8431 to 22338 in elderly groups (95^{th} percentile rate consumption).
- Exceptional range (measured with highest mussels concentration found of 34.43 MPs/g.w. w) varied from 19243 to 40426 MPs (average rate consumption) and from 54430-138054 MPs calculated in ederly groups (95^{th} percentile rate consumption).
- It is possible the annual MPs intakes value increase to beyond hundred thousand supposing ingestion of canned foods and intake through pelagic fish (through muscles).
- About a 56-77 % of microplastic ingested by principal food item coming from edible molluscs when the drinking water source is only tap water.
- In worst-case exposure, scenario (consumption of 225 g of mussels per day) average intake ratio MPs/dietary intake (percentage) of additives ranged from 0.004 to 0.27% in mussels depurated and 0.0004 and 2.97% in mussels not depurated. Regard, organic

persistent associated compound, the average intake ratio ranged from 1.22 to 5.94 % for depurated mussels and 3.32 to 65 % for not depurated mussels.

- Hazard polymeric risk (H) of molluscs samples reach the hazard levels III and IV.
- In edible molluscs, 74 % of the MPs sizes were from 0.02 to 1 mm. In not depurated mussels no depurated (from the north) the 50 % of sizes ranged from 0.02 to 0.125 mm.
- High concentration of polymers as PE and PET were found in edible molluscs. It is recognized as the ones concentrating the highest amounts of HOCs and high adsorption capacity for stronger hydrophobic and aromatic character compounds respectively.
- Gender differences was not considered

Risk characterization (step 4): The potentially hazardous effects of different types of micro- and nanoplastics to human health remain largely unknown (Leslie and Depledge, 2020). Due to lack of any reference dose for risk characterization, and the observed high MPs concentrations in edible Catalan molluscs and high intake by person/year, it is proposed that high and frequent consumption of mussels specifically not depurated and coming from high MPs polluted zones, as well as fine clams should be controlled for vulnerable groups such as pregnant and elderly groups.

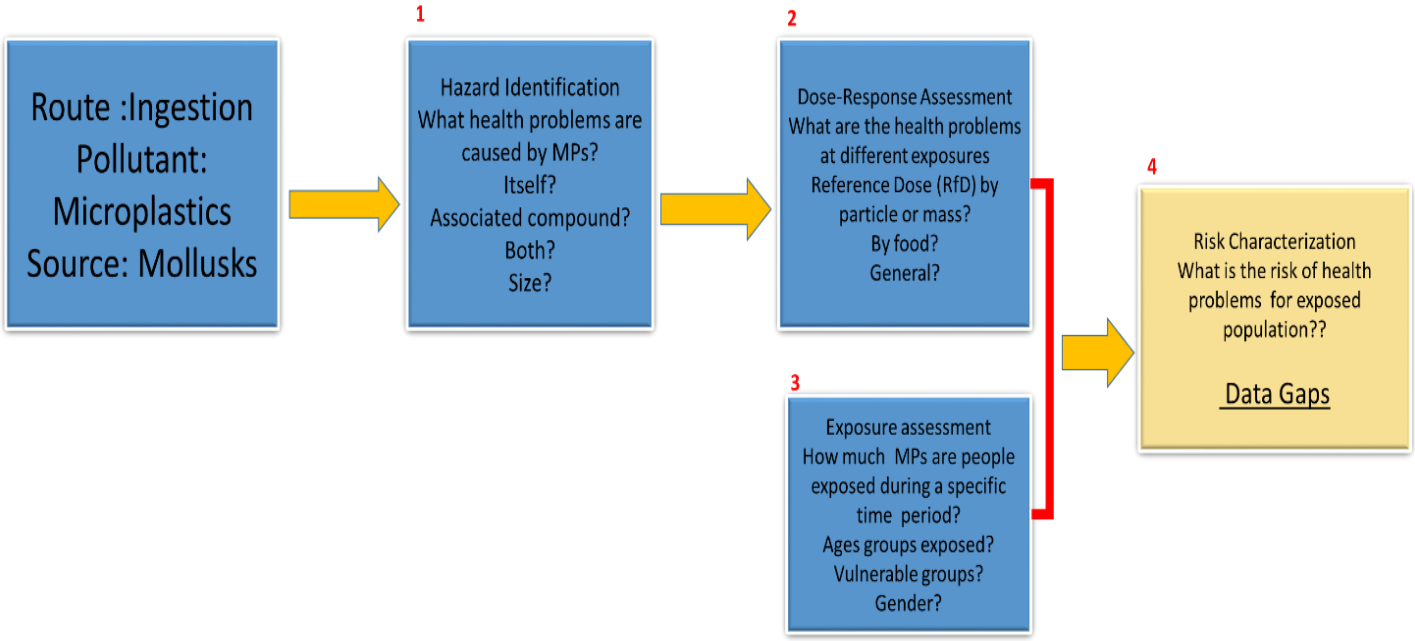


Figure 18 The 4 steps to conducting a Human Health Risk Assessment by microplastics in molluscs. Adapted from US EPA (US EPA, 2022).

For risk assessment development, many gaps should be solved:

- Standard tissue digestion of seafood for MPs and NPs identification and quantification should be determined. The concentration units should be expressed in both way as weight and particles number.

- More research is needed regarding additive leaching and contaminant associated desorption processes under gut conditions to understand the exposure to chemicals compound through microplastic ingestion (bioavailability/absortion rates).

- Chronic exposure to MPs and nanoplastics in single and mixture in humans via ingestion. MPs seems almost constant and inevitable at the current time; therefore, full chronic toxicity assessment should be performed (long lasting exposure toxicity studies).

- Environmentally relevant concentrations of plastics, their additives, and sorbed pollutants should be used "in vivo" dose-response assays for a better understanding of their toxicity.

- The exposure to microorganisms presents in biofilm (pathogens might also be found on them) on plastic debris should be evaluated as well as effects on autochthonous human gut microbiota communities.

- Changes in the structure and chemical composition of MPs including nanoplastic formation, during foods processing and/or cooking as well as the interactions between food matrix and plastic surfaces.

- There are conflicting claims about the toxic effects of MPs on the human body, but according Danopoulus et al., (2020), and Leslie and Depledge, (2020), no evidence exists that microplastic exposures to humans are safe. In consequence, more efforts need to be focused on the microplastic pollution in bivalve.

- For reducing microplastic pollution in bivalves may go away in cutting back the microplastic exposure to humans and health risks, measures as (i) less usage of plastics materials in maricultural zones or replace by bio-based or biodegradable materials, plastic waste from farming materials should be recovered and disposed do not throw them into the sea, (ii) guidelines for handling, storing preparing and sale of alive bivalves packages products (for farming) without plastics contaminations (iii) sequence proposals for

improvement depuration processes for MPs removals from bivalves and others edible molluscs. The relevant organizations need to take steps for, storing, and preparing seafood products to minimize plastic usage

3.2.6 Discussion Summary

Spain annual intakes by MPs concentrations average in total and depurated molluscs were higher than obtained for other worldwide zones due mainly to higher MPs concentration in different molluscs groups of Catalonia coast and relatively higher consumption for Spain population. Molluscs contamination with MPs pose a potential threat to Spanish consumers.

Polymer Risk Index between III to IV with possible human health implications were observed in mussels, snail from middle zone, big oysters, fine clams, razor clams, and wedge clams from middle and southern zone. By contrast low grade of I and II were observed only in wedge clams from north WC(N) and marine snail from south S(S).

MPs sizes smaller than 150 µm observed in mussels no depurated in Catalonia coast, mostly enhance negative consequences on the organism because both of their ability to cross biological barriers and their higher sorption capacity. Small MPs (high surface/volume) combined with their composition (high hydrophobicity of polymers) could concentrate organic contaminants by several folds compared to the water column. The worst-case estimate of the exposure to contaminants and additives through mussels from Catalonia coast indicates the contribution of toxics compounds and plastics additives are not so negligible compared to other sources, especially in case of mussels no depurated consumption. Thus, Bivalve consumption is a non-negligible pathway for human exposure to toxic compounds considering high consumption of bivalves by Spanish people

Efforts in the construction of human health risk frameworks should be conducted as soon as possible to understand the actual microplastic risks to humans. MPs dietary risk assessment reports should involve total diet studies.

References

Abbasi, S., Soltani, N., Keshavarzi, B., Moore, F., Turner, A., Hassanaghaei, M., 2018. Microplastics in different tissues of fish and prawn from the Musa Estuary, Persian Gulf, Chemosphere 205, 80-87.

Abbasi, S., Soltani, N., Keshavarzi, B., Moore, F., Turner, A., Hassanaghaei, M., 2018. Micropl astics in different tissues of fish and prawn from the Musa estuary, Persian Gulf. Chemosphere 205, 80-87.

Abidli, S., Lahbib, Y., Trigui El Menif, N., 2019. Microplastics in commercial molluscs from the lagoon of Bizerte (Northern Tunisia). Mar. Pollut. Bull. 142, 243–252.

AESAN, Spanish Agency for Food Safety and Nutrition, 2016a. Encuesta ENALIA 2. Encuesta Nacional de Alimentación en población adulta, mayores y embarazadas. Last accesed: February 2022. [Online] https://www.aesan.gob.es/AECOSAN/web/seguridad_alimentaria/subdetalle/enalia_2.htm

AESAN, Spanish Agency for Food Safety and Nutrition, 2016b. Encuesta ENALIA. Encuesta Nacional de Alimentación en la población Infantil y Adolescente. Last accesed: February 2022. [Online] (https://www.aesan.gob.es/AECOSAN/web/seguridad_alimentaria/subdetalle/enalia.htm

Ahrens, M.J., Hertz, J., Lamoureux, E.M., Lopez, G.R., McElroy, A.E., Brownawell, B.J. 2001. The role of digestive surfactants in determining bioavailability of sediment-bound hydrophobic organic contaminants to 2 deposit-feeding polychaetes. Mar. Ecol. Prog. Ser. 212,145-157.

Akhbarizadeh, R., Moore, F., Keshavarzi, B., 2018. Investigating a probable relationship between microplastics and potentially toxic elements in fish muscles from northeast of Persian Gulf. Environ Pollut. 232, 154-163.

Aldenberg, T., Jaworska, J.S., 2000. Uncertainty of the hazardous concentration and fraction affected for normal species sensitivity distributions. Ecotoxicol. Environ. Saf. 46(1), 1-18.

Amereh, F., Babaei, M., Eslami, A., Fazelipour, S., Rafiee, M., 2020. The emerging risk of exposure to nano(micro)plastics on endocrine disturbance and reproductive toxicity: From a hypothetical scenario to a global public health challenge. Environ. Pollut. 261, 114158.

Amereh, F., Eslami, A., Fazelipour, S., Rafiee, M., Zibaii, M.I., Babaei, M., 2019. Thyroid endocrine status and biochemical stress responses in adult male Wistar rats chronically exposed to pristine polystyrene nanoplastics. Toxicol. Res. 8(6), 953-963.

Bakir, A., O'Connor, I.A., Rowland, S.J., Hendriks, A.J., Thompson, R.C., 2017. Relative importance of microplastics as a pathway for the transfer of hydrophobic organic chemicals to marine life. Environ. Pollut. 219, 56-65.

Besseling, E., Redondo-Hasselerharm, P., Foekema, E.M., Koelmans, A.A., 2019. Quantifying ecological risks of aquatic micro- and nanoplastic. Crit. Rev. Environ. Sci. Technol. 49(1), 32-80

Besseling, E., Wegner, A., Foekema, E.M., van den Heuvel-Greve, M., Koelmans, A.A., 2013. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.). Environ. Sci. Technol. 47(1), 593-600.

Besseling. E., Foekema, E.M., van den Heuvel-Greve, M.J., Koelmans, A.A., 2017. The effect of microplastic on the uptake of chemicals by the lugworm *Arenicola marina* (L.) under environmentally relevant exposure conditions. Environ. Sci. Technol. 51(15), 8795-8804.

Bhatt, P., Pathak, V.M., Bagheri, A.R., Bilal, M., 2021. Microplastic contaminants in the aqueous environment, fate, toxicity consequences, and remediation strategies. Environ. Res. 200, 111762.

Bhatt, P., Pathak, V.M., Bagheri, A.R., Bilal, M., 2021. Microplastic contaminants in the aqueous environment, fate, toxicity consequences, and remediation strategies. Environ. Res. 200, 111762.

Bonello, G., Varrella, P., Pane, L., 2018. First evaluation of micro-plastic content in benthicfilter-feeders of the gulf of La Spezia (Ligurian Sea). J. Aquat. Food Prod. Technol. 27(3), 284-291.

Brede, C., Fjeldal, P., Skjevrak, I., Herikstad, H., 2003. Increased migration levels of bisphenol A from polycarbonate baby bottles after dishwashing, boiling and brushing. Food Addit. Contam. 20(7), 684-689.

Browne, M.A., Niven, S.J., Galloway, T.S., Rowland, S.J., Thompson, R.C., 2013. Microplastic moves pollutants and additives to worms, reducing functions linked to health and biodiversity. Curr. Biol., 23(23), 2388-2392

Buhl, K.J., Hamilton, S.J., Schmulbach, J.C., 1993. Chronic toxicity of the bromoxynil formulation Buctril to Daphnia magna exposed continuously and intermittently. Arch. Environ. Contam. Toxicol. 25, 152-159.

Burns, E.E., Boxall, A.B.A., 2018. Microplastics in the aquatic environment: Evidence for or against adverse impacts and major. Knowledge Gaps 37(11), 2776-2796.

Cabidigitallibrary, 2022 Last accessed: July 2022. [Online] https://www.cabidigitallibrary.org/

Caruso, G., 2019. Microplastics as vectors of contaminants. Mar. Pollut. Bull., 146, 921-924.

Catarino, A.I., Macchia, V., Sanderson, W.G., Thompson, R.C., Henry, T.B., 2018. Low levels of microplastics (MP) in wild mussels indicate that MP ingestion by humans is minimal

compared to exposure via household fibres fallout during a meal. Environ. Pollut. 237, 675–684.

Chemsafetypro. Last accesed December, 2022 https://chemsafetypro.com/Topics/CRA/How_to_Calculate_Predicted_No-Effect_Concentration_(PNEC).html

Cheng, Z., Nie, X.-P., Wang, H.-S., Wong, M.-H., 2013. Risk assessments of human exposure to bioaccessible phthalate esters through market fish consumption. Environ. Int. 57-58, 75-80.

Cho, Y., Shim, W.J., Jang, M., Han, G.M., Hong, S.H., 2019. Abundance and characteristics of microplastics in market bivalves from South Korea. Environ. Pollut. 245, 1107–1116.

Cincinelli, A., Scopetani, C., Chelazzi, D., Lombardini, E., Martellini, T., Katsoyiannis, A., Fossi, M.C., Corsolini, S., 2017. Microplastic in the surface waters of the Ross Sea (Antarctica): Occurrence, distribution and characterization by FTIR. Chemosphere 175, 391-400.

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Galloway, T.S., 2015. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod Calanus helgolandicus. Environ. Sci. Technol. 49(2), 1130-1137.

Cox, K.D., Covernton, G.A., Davies, H.L., Dower, J.F., Juanes, F., Dudas, S.E., 2019. Human consumption of microplastics. Environ. Sci. Technol. 53(12), 7068-7074.

Danopoulos, E., Jenner, L.C., Twiddy, M., Rotchell, J.M., 2020. Microplastic contamination of seafood intended for human consumption: a systematic review and metaanalysis. Environ. Health. Perspect. 128, 126002.

de Lucia, G.A., Caliani, I., Marra, S., Camedda, A., Coppa, S., Alcaro, L., Campani, T., Giannetti, M., Coppola, D., Cicero, A.M., Panti, C., Baini, M., Guerranti, C., Marsili, L., Massaro, G., Fossi, M.C., Matiddi, M., 2014. Amount and distribution of neustonic micro-plastic off the Western Sardinian coast (Central-Western Mediterranean Sea). Mar. Environ. Res. 100, 10-16.

De Witte, B., Devriese, L., Bekaert, K., Hoffman, S., Vandermeersch, G., Cooreman, K., Robbens, J., 2014. Quality assessment of the blue mussel (Mytilus edulis): comparison between commercial and wild types. Mar. Pollut. Bull. 85(1), 146-155.

Deng, Y., Yan, Z., Shen, R., Huang, Y., Ren, H., Zhang, Y., 2021. Enhanced reproductive toxicities induced by phthalates contaminated microplastics in male mice (Mus musculus). J. Hazard Mater. 406, 124644.

Deng, Y., Zhang, Y., Lemos, B., Ren, H., 2017. Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. Sci. Rep. 7, 46687.

Deng, Y., Zhang, Y., Qiao, R., Bonilla, M.M., Yang, X., Ren, H., Lemos, B., 2018. Evidence that microplastics aggravate the toxicity of organophosphorus flame retardants in mice (Mus musculus). J. Hazard. Mater. 357,348-354

Ding, J., Li, J., Sun, C., Jiang, F., He, C., Zhang, M., Ju, P., Xiangyu, N., 2020. An examination of the occurrence and potential risks of microplastics across various shellfish. Sci. Total Environ. 739, 139887.

Ding, J., Sun, C., He, C., Li, J., Ju, P., Li, F., 2021. Microplastics in four bivalve species and basis for using bivalves as bioindicators of microplastic pollution. Sci. Total Environ. 782, 146830.

Ding, J., Sun, Y., He, C., Li, J., Li, F., 2022. Towards risk assessments of microplastics in bivalve mollusks globally. J. Mar. Sci. Eng. 10(2), 288.

Dyer, S.D., Versteeg, D.J., Belanger, S.E., Chaney, J.G., Mayer, F.L., 2006. Interspecies correlation estimates predict protective environmental concentrations. Environ. Sci. Technol. 40(9), 3102-3111.

Dyer, S.D., Versteeg, D.J., Belanger, S.E., Chaney, J.G., Raimondo, S., Barron, M.G., 2008. Comparison of species sensitivity distributions derived from interspecies correlation models to distributions used to derive water quality criteria. Environ. Sci. Technol. 42(8), 3076-3083.

EC, European Commission, 2001. Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Last accessed: September 2022. [Online] https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2001L0018:20080321:EN:PDF

ECHA, European Chemical Agency, 2008. Chapter R10: Characterisation of dose [concentration]-response for environment. In: Guidance on information requirements and chemical safety assessment. Last accessed: September 2022. [Online] https://echa.europa.eu/documents/10162/13632/information_requirements_r10_en.pdf/bb902be7-a503-4ab7-9036-d866b8ddce69

ECHA, European Chemical Agency, 2012. Chapter R19: Uncertainty Analysis. In: Guidance on Information Requirements and Chemical Safety Assessment. Last accessed: September 2022. [Online] https://echa.europa.eu/documents/10162/13632/information_requirements_r19_en.pdf/d5bd6c3f-3383-49df-894e-dea410ba4335

ECHA, European Chemical Agency, 2018a. Mapping exercise – Plastic additives initiative. Last accessed: September 2022. [Online] https://echa.europa.eu/es/mapping-exercise-plastic-additives-initiative

ECHA, European Chemical Agency, 2018b. Plastic additives initiative. Last accessed: September 2022. [Online] https://echa.europa.eu/es/plastic-additives-initiative

ECHA, European Chemical Agency, 2019. Plastic additives initiative Supplementary Information on Scope and Methods 15.02.2019. Last accessed: September 2022. [Online] https://echa.europa.eu/documents/10162/17228/plastic_additives_supplementary_en.pdf/79bea2d6-8e45-f38c-a318-7d7e812890a1

ECHA, European Chemical Agency, 2022. Substance evaluation CoRAP. Last accessed: September 2022. [Online] https://echa.europa.eu/information-on-chemicals/evaluation/community-rolling-action-plan/corap-table

- ECHA, European Chemical Agency, 2023. Substance information. Last accessed: January 2023 [Online] https://www.echa.europa.eu/substance-information/-/substanceinfo/

EFSA, European Food and Safe Authority, 2006. Scientific Opinion on contaminants in the food chain [CONTAM] related to DDT as an undesirable substance in animal feed, EFSA Journal 4(12), 433, 69 pp.

EFSA, European Food and Safe Authority, 2008. Scientific Opinion on polycyclic aromatic hydrocarbons in food. The EFSA Journal 724, 1-114.

EFSA, European Food and Safe Authority, 2010. Scientific opinion on the assessment of potential impacts of genetically modified plants on non-target organisms. The EFSA Journal 8(11), 1877.

EFSA, European Food and Safe Authority, 2011. Scientific Opinion on polybrominated diphenyl ethers (PBDEs) in Food. EFSA Journal 9(5), 2156, 274 pp.

EFSA, European Food and Safe Authority, 2015. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs: Executive summary. EFSA Journal 13(1), 3978, 23 pp.

EFSA, European Food and Safe Authority, 2016. Statement on the presence of microplastics and nanoplastics in food, with particular focus on seafood. EFSA Journal 14(6), 4501, 30 pp.

EFSA, European Food and Safe Authority, 2021. Scientific Colloquium 25: A coordinated approach to assess the human health risks of micro- and nanoplastics in food. Last accessed: July 2022. [Online] https://www.efsa.europa.eu/en/events/event/update-scientific-colloquium-25-coordinated-approach-assess-human-health

Elgarahy, A.M., Akhdhar, A., Elwakeel, K.Z, 2021. Microplastics prevalence, interactions, and remediation in the aquatic environment: A critical review. J. Environ. Chem. Eng. 9(5), 106224.

Endo, S., Koelmans, A.A., 2016. Sorption of hydrophobic organic compounds to plastics in the marine environment: Equilibrium (185-204 pp.). In: Takada, H., Karapanagioti, H. (eds) Hazardous chemicals associated with plastics in the marine environment. The handbook of environmental chemistry. Vol 78. Springer, Cham.

Engler, R.E., 2012. The complex interaction between marine debris and toxic chemicals in the ocean. Environ. Sci. Technol. 46(22), 12302-12315.

EP, European Parliament, 2006. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation

and Restriction of Chemicals (REACH). Last accessed: December 2022. [Online] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02006R1907-20140410

EP, European Parliament, 2016. European Parliament targets DEHP plasticizer in recycled PVC. Health & Safety Environment, Health and Safety Issues. Addit. Polym. 2016(2), 11.

EPA, US Environmental Protection Agency, 2022. Ecological Risk Assessment. Last accessed: March 2022. [Online] https://www.epa.gov/risk/ecological-risk-assessment

Eriksen, M., Lebreton, L.C., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G., Reisser, J., 2014. Plastic pollution in the world's oceans: More than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLoS One 9(12), e111913.

Everaert, G., De Rijcke, M., Lonneville, B., Janssen, C.R., Backhaus, T., Mees, J., van Sebille, E., Koelmans, A.A., Catarino, A.I., Vandegehuchte, M.B., 2020. Risks of floating microplastic in the global ocean. Environ. Pollut. 267, 115499.

Everaert, G., Van Cauwenberghe, L., De Rijcke, M., Koelmans, A.A., Mees, J., Vandegehuchte, M., Janssen, C.R., 2018. Risk assessment of microplastics in the ocean: Modelling approach and first conclusions. Environ. Pollut. 242(B),1930-1938.

Fang, C., Zheng, R., Chen, H., Hong, F., Lin, L., Lin, H., Guo, H., Bailey, C., Segner, H., Mu, J., Bo, J., 2019. Comparison of microplastic contamination in fish and bivalves from two major cities in Fujian province, China and the implications for human health. Aquaculture 512, 734322.

Fantke, P., Weber, R., Scheringer, M., 2015. From incremental to fundamental substitution in chemical alternatives assessment. Sustain. Chem. Pharm. 1, pp. 1-8.

FAO, Food and Agriculture Organization of the United Nations, 2021. Food Balances (2010-). Last accessed: July 2022. [Online] http://www.fao.org/faostat/en/#data/FBS

FAO, Food and Agriculture Organization of the United Nations, 2022. Cultured Aquatic Species Information Programme (CASIP). Fisheries and Aquaculture Division. Rome. Last accessed: March 2022. [Online] https://www.fao.org/fishery/en/collection/culturedspecies

FAO/WHO, 2016. Evaluation of certain food additives and contaminants. Eightieth report of the Joint FAO/WHO Expert Committee on Food Additives, Geneva. WHO technical report series – 995 (94 p.). World Heal. Organ. Rep. Ser.

Ferrante, M., Pietro, Z., Allegui, C., Maria, F., Antonio, C., Pulvirenti, E., Favara, C., Chiara, C., Grasso, A., Omayma, M., Gea, O.C., Banni, M., 2022. Microplastics in fillets of Mediterranean seafood. A risk assessment study. Environ Res. 204(Pt C),112247.

Ferraro, D., Anselmi-Tamburini, U., Tredici, I.G., Ricci, V., Sommi, P., 2016. Overestimation of nanoparticles-induced DNA damage determined by the Comet assay. Nanotoxicology 10(7), 861-870.

Fisner, M., Majer, A., Taniguchi, S., Bícego, M., Turra, A., Gorman, D., 2017. Colour spectrum and resin-type determine the concentration and composition of Polycyclic Aromatic Hydrocarbons (PAHs) in plastic pellets. Mar. Pollut. Bull. 122(1-2), 323-330.

Fu, L., Li, J., Wang, G., Luan, Y., Dai, W., 2021. Adsorption behavior of organic pollutants on microplastics. Ecotoxicol. Environ. Saf. 217, 112207.

Galloway, T.S., Lewis, C.N., 2016. Marine microplastics spell big problems for future generations. Proc. Natl. Acad. Sci. 113(9), 2331-2333.

Garrido Gamarro, E., Costanzo, V., 2022. Dietary Exposure to Additives and Sorbed Contaminants from Ingested Microplastic Particles Through the Consumption of Fisheries and Aquaculture Products (261-310 pp.). In: Bank, M.S. (Eds). Microplastic in the environment: Pattern and process. Environmental contamination, remediation and management. Springer, Cham.

GENCAT, Generalitat de Catalunya, 2022. Agricultura. 50 principals espècies pesquers comercials de Catalunya. Last accessed: July 2022. [Online] http://;agricultura.gencat.cat/web/.content/pe_pesca_aquicultura/pe03_comercialitzacio_peix/documents/fitxers_estatics/poster_50-especies.pdf

GENCAT, Generalitat de Catalunya, 2022. Agricultura. Pesca i aqüicultura. Estadístiques de pesca. Dades per espècies. Last accessed: July 2022. [Online] https://agricultura.gencat.cat/ca/ambits/pesca/dar_estadistiques_pesca_subhastada/dar_captures_especies

GENCAT, Generalitat de Catalunya, 2022. Agricultura. Pesca i aqüicultura. Gestió pesquera. Espècies d'interès pesquer. Mol-luscs. Last accessed: July 2022. [Online] https://agricultura.gencat.cat/ca/ambits/pesca/gestio-pesquera/especies-interes-pesquer/molluscs

GENCAT, Generalitat de Catalunya, 2022. Agricultura. Pesca i aqüicultura. Gestió pesquera. Espècies d'interès pesquer. Crustacis. Last accessed: July 2022. [Online] https://agricultura.gencat.cat/ca/ambits/pesca/gestio-pesquera/especies-interes-pesquer/crustacis

GENCAT, Generalitat de Catalunya, 2022. Agricultura. Pesca recreativa a la costa catalana: Guía de especies. Last accessed: July 2022. [Online] https://agricultura.gencat.cat/web/.content/02-tramits/enllacos-documents/X0011/guia_especies_peix.pdf

GENCAT, Generalitat de Catalunya, 2022. Agriculture and livestock. Fisheries. Last accessed: July 2022. [Online] https://web.gencat.cat/en/temes/agricultura/index.html

Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 3(7), e1700782.

Gola, D., Tyagi, P.K., Arya, A., Chauhan, N., Agarwal, M., Singh, S.K. Gola, S., 2021. The impact of microplastics on marine environment: A review. Environ. Nanotechnol. Monit. Manag. 16, 100552.

Golsteijn, L., Hendriks, H.W.M., van Zelm, R., Ragas, A.M.J., Huijbregts, M.A.J., 2012. Do interspecies correlation estimations increase the reliability of toxicity estimates for wildlife? Ecotoxicol. Environ. Saf. 80, 238-243.

Gomiero, A., Strafella, P., Pellini, G., Salvalaggio, V., Fabi, G., 2018. Comparative effects of ingested PVC micro particles with and without adsorbed benzo(a)pyrene vs. spiked sediments on the cellular and sub cellular processes of the benthic organism Hediste diversicolor. Front. Mar. Sci. 5, 99.

Granek, E.F., Brander, S.M., Holland, E.B., 2020. Microplastics in aquatic organisms: Improving understanding and identifying research directions for the next decade. Limnol. Oceanogr. Lett. 5(1), 1-4.

Gray, A.D., Weinstein, J.E., 2017. Size- and shape-dependent effects of microplastic particles on adult daggerblade grass shrimp (Palaemonetes pugio). Environ. Toxicol. Chem. 36(11), 3074-3080.

Green, D.S., 2016. Effects of microplastics on European flat oysters, Ostrea edulis and in the environment. Environ. Pollut. 216, 95-103.

Green, D.S., Boots, B., Sigwart, J., Jiang, S., Rocha, C., 2016. Effects of conventional and biodegradable microplastics on a marine ecosystem engineer (*Arenicola marina*) and sediment nutrient cycling. Environ Pollut. 208(Part B), 426-434.

Gündogdu, S., Rathod, N., Hassoun, A., Jamroz, E., Kulawik, P., Gokbulut, C., Aït-Kaddour, A., Özogul, F., 2022. The impact of nano/micro-plastics toxicity on seafood quality and human health: facts and gaps. Crit. Rev. Food Sci. Nutr., 1-19.

Hahladakis, J.N., Velisa, C.A., Weberb, R., Iacovidoua, E., Purnella, P., 2018. An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. J. Hazard. Mater. 344, 179-199.

Hakanson, L.J., 1980. An ecological risk index for aquatic pollution control: a sedimentological approach. Water Res. 14(8), 975-1001.

Harris, L.S.T., Carrington, E., 2020. Impacts of microplastic vs. natural abiotic particles on the clearance rate of a marine mussel. Limnol. Oceanogr. Lett, 5(1), 66-73.

Hartmann, N.B., Rist, S., Bodin, J., Jensen, L.Hs., Schmidt, S.N., Mayer, P., Meibom, A., Baun., A., 2017. Microplastics as vectors for environmental contaminants: exploring sorption, desorption, and transfer to biota. Integr. Environ. Assess. Manag. 13(3), 488-493.

Hauser, R., Calafat, A.M., 2005. Phthalates and human health. Occup. Environ. Med. 62(11): 806-818.

Hauser, R., Calafat, A.M., 2005. Phthalates and human health. Occup. Environ. Med. 62(11), 806-818.

Heinrich, P., Braunbeck, T., 2019. Bioavailability of microplastic-bound pollutants in vitro: the

role of adsorbate lipophilicity and surfactants. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 221, 59-67.

Hennebert, P., 2021. The substitution of regulated brominated flame retardants in plastic products and waste and the declared properties of the substitutes in reach. Detritus 16, 16-25.

Hermabessiere, L., Dehaut, A., Paul-Pont, I., Lacroix, C., Jezequel, R., Soudant, P., Duflos, G., 2017. Occurrence and effects of plastic additives on marine environments and organisms: A review. Chemosphere 182, 781-793.

Hwang, J., Choi, D., Han, S., Choi, J., Hong, J., 2019. An assessment of the toxicity of polypropylene microplastics in human derived cells. Sci. Total Environ. 684, 657-669.

Ictioterm, 2022. Base de datos terminológica y de identificación de especies pesqueras de las costas de Andalucía. Nombre científico. *Bolinus brandaris* (Linnaeus, 1758). Last accessed: July 2022. [Online] http://www.ictioterm.es/nombre_cientifico.php?nc=207

Isobe, A., Kubo, K., Tamura, Y., Kako, S., Nakashima, E., Fujii, N., 2014. Selective transport of microplastics and mesoplastics by drifting in coastal waters. Mar. Pollut. Bull. 89(1-2), 324-330.

Issac, M.N., Kandasubramanian, B., 2021. Effect of microplastics in water and aquatic systems. Environ. Sci. Pollut. Res. Int. 28(16), 19544-19562.

Jani, P.U., McCarthy, D.E., Florence, A.T., 1992. Nanosphere and microsphere uptake via Peyer's patches: Observation of the rate of uptake in the rat after a single oral dose. Int. J. Pharm. 86(2-3), 239-246.

Jeong, C.-B., Won, E.-J., Kang, H.-M., Lee, M.-C., Hwang, D.-S., Hwang, U.-K., Zhou, B., Souissi, S., Lee, S.-J., Lee, J.-S., 2016. Microplastic size-dependent toxicity, oxidative stress induction, and p-JNK and p-p38 activation in the Monogonont Rotifer (Brachionus koreanus). Environ. Sci. Technol. 50(16), 8849-8857.

Jin, Y., Lu, L., Tu, W., Luo, T., Fu, Z., 2019. Impacts of polystyrene microplastic on the gut barrier, microbiota and metabolism of mice. Sci. Total Environ. 649, 308-317.

Jomenjopeix, 2022. Conoce el pez y el marisco. Last accessed: July 2022. [Online] https://jomenjopeix.com/es/pez-marisco

Jung, J.-W., Park, J.-W., Eo, S., Choi, J., Song, J.K., Cho, Y., Hong, S.H., Shim, W.J., 2021. Ecological risk assessment of microplastics in coastal, shelf, and deep-sea waters with a consideration of environmentally relevant size and shape. Environ. Pollut. 270, 116217.

Karami, A., Golieskardi, A., Choo, C.K., Larat, V., Karbalaei, S., Salamatinia, B., 2018. Microplastic and mesoplastic contamination in canned sardines and sprats. Sci Total Environ. 612, 1380-1386.

Karami, A., Golieskardi, A., Choo, C.K., Romano, N., Ho, Y.B., Salamatinia, B. 2017. A high-performance protocol for extraction of microplastics in fish. Sci. Total Environ. 578, 485-494.

Karbalaei, S., Hanachi, P., Walker, T.R., Cole, M., 2018. Occurrence, sources, human health impacts and mitigation of microplastic pollution. Environ. Sci. Pollut. Res. 25(36), 36046–36063.

Kim, D.Y., Chun, S.-H., Jung, Y., Mohamed, D.F.M.S, Kim, H.-S., Kang, D.Y, An, J.-W., Park, S.-Y., Kwon, H.-W., Kwon, J.-H., 2020. Phthalate plasticizers in children's products and estimation of exposure: Importance of migration rate. Int. J. Environ. Res. Public Health. 17(22), 8582.

Kim, Y.-J., Osako, M., Osako, S.-i., 2006. Leaching characteristics of polybrominated-diphenyl ethers (PBDEs) from flame-retardant plastics. Chemosphere 65(3), 506-513.

Koelmans, A.A., Bakir, A., Burton, G.A., Janssen, C.R., 2016. Although the relative role of MPs as vectors for HOCs to organisms is generally considered minor in comparison to that of natural exposure pathways (such as water, food, and natural particulate matter). Environ Sci Technol. 50(7), 3315-3326.

Koelmans, A.A., Redondo-Hasselerharm, P.E., Nor, N.H.M., de Ruijter, V.N. Mintenig, S.M., Kooi, M., 2022. Risk assessment of microplastic particles. Nat. Rev. Mater. 7, 138-152.

Korez, Š., Gutow L., Saborowski, R., 2019. Microplastics at the strandlines of Slovenian beaches. Mar. Pollut. Bull. 145, 334-342.

Lacerda, A.L.F., Rodrigues, L.S., van Sebille, E., Rodrigues, F.L., Ribeiro, L., Secchi, E.R.,

Kessler, F., Proietti, M.C., 2019. Plastics in sea surface waters around the Antarctic Peninsula. Sci. Rep. 9, 3977.

Leslie, H.; Depledge, M., 2020. Where is the evidence that human exposure to microplastics is safe? Environ. Int. 142, 105807.

Li, J., Yang, D., Li, L., Jabeen, K., Shi, H., 2015. Microplastics in commercial bivalves from China. Environ. Pollut. 207, 190–195.

Li, Z., Zhu, S., Liu, Q., Wei, J., Jin, Y., Wang, X., Zhang, L., 2020. Polystyrene microplastics cause cardiac fibrosis by activating Wnt/β-catenin signaling pathway and promoting cardiomyocyte apoptosis in rats. Environ. Pollut. 265, 115025.

Liang, B., Zhong, Y., Huang, Y., Lin, X., Liu, J., Lin, L., Hu, M., Jiang, J., Dai, M., Wang, B., Zhang, B., Meng, H., Lelaka, J.J.J., Sui, H., Yang, X., Huang, Z., 2021. Underestimated health risks: polystyrene micro- and nanoplastics jointly induce intestinal barrier dysfunction by ROS-mediated epithelial cell apoptosis. Part. Fibre Toxicol. 18, 20.

Lithner, D., Larsson, Å., Dave, G., 2011. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Sci. Total Environ. 409(18), 3309-3324.

Liu, Z., Huang, Q., Wang, H., Zhang, S., 2022. An enhanced risk assessment framework for microplastics occurring in the Westerscheldt estuary. Sci. Total Environ. 817, 153006.

Lo, H.K.A., Chan, K.Y.K., 2018. Negative effects of microplastic exposure on growth and development of *Crepidula onyx*. Environ. Pollut. 233, 588-595.

Lohmann, R., 2017. Microplastics are not important for the cycling and bioaccumulation of organic pollutants in the oceans—but should microplastics be considered POPs themselves? Integr. Environ. Assess. Manag. 13(3), 460-465.

Lu, L., Wan, Z., Luo, T., Fu, Z., Jin, Y., 2018. Polystyrene microplastics induce gut microbiota dysbiosis and hepatic lipid metabolism disorder in mice. Sci. Total Environ. 631-632, 449-458.

Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., Ding, L., Ren, H., 2016. Uptake and accumulation of polystyrene microplastics in zebrafish (*Danio rerio*) and toxic effects in liver. Environ. Sci. Technol. 50(7), 4054-4060.

Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., Ding, L., Ren, H., 2016. Uptake and accumulation of polystyrene microplastics in Zebrafish (Danio rerio) and toxic effects in liver. Environ. Sci. Technol. 50(7), 4054-4060.

Luo, T., Wang, C., Pan, Z., Jin, C., Fu, Z., Jin, Y., 2019. Maternal polystyrene microplastic exposure during gestation and lactation altered metabolic homeostasis in the dams and their F1 and F2 offspring. Environ. Sci. Technol. 53(18), 10978-10992.

Lusher, A.L., Hollman, P.C.H., Mendoza-Hill, J.J., 2017. Microplastics in fisheries and aquaculture: status of knowledge on their occurrence and implications for aquatic organisms and food safety (140 p.). FAO Fisheries and Aquaculture Technical Paper No. 615. Rome, Italy.

Ma, H., Pu, S., Liu, S., Bai, Y., Mandal, S., Xing, B., 2020. Microplastics in aquatic environments: toxicity to trigger ecological consequences. Environ. Pollut. 261, 114089.

Ma, Y., Huang, A., Cao, S., Sun, F., Wang, L., Guo, H, Ji, R., 2016. Effects of nanoplastics and microplastics on toxicity, bioaccumulation, and environmental fate of phenanthrene in fresh water. Environ. Pollut. 219, 166-173.

Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C., Kaminuma, T., 2001. Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. Environ. Sci. Technol. 35(2), 318-324.

Meeker, J.D, Sathyanarayana, S., Swan, S.H., 2009. Phthalates and other additives in plastics: human exposure and associated health outcomes. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 364(1526), 2097-2113.

Mehinto, A.C., Coffin, S., Koelmans, A.A., Brander, S.M., Wagner, M., Hampton, L.M.T., Burton Jr, A.G., Miller, E., Gouin, T., Weisberg, S.B., Rochman, C.M., 2022. Risk-based management framework for microplastics in aquatic ecosystems. Microplastics and Nanoplastics 2, 17.

Meindl, C., Kueznik, T., Bösch, M., Roblegg, E., Fröhlich, E., 2015. Intracellular calcium levels

as screening tool for nanoparticle toxicity. J. Appl. Toxicol. 35(10), 1150-1159.
Messinetti, S., Mercurio, S., Scarì, G., Pennati, A., Pennati, R., 2019. Ingested microscopic plastics translocate from the gut cavity of juveniles of the ascidian *Ciona intestinalis*. Eur. Zool. J. 86(1), 189-195.
Ministry of Environment, Lands and Parks of Canada, 2000. Environmental risk assessment (ERA): An approach for assessing and reporting environmental conditions. Canada. 84 pp.
Mistri, M., Scoponi, M., Granata, T., Moruzzi, L., Massara, F., Munari, C., 2020. Types, occurrence and distribution of microplastics in sediments from the northern Tyrrhenian Sea, Mar. Pollut. Bull. 153, 111016.
Munoz, M., Ortiz, D., Nieto-Sandoval, J., de Pedro Z.M., Casas, J.A., 2021. Adsorption of micropollutants onto realistic microplastics: Role of microplastic nature, size, age, and NOM fouling. Chemosphere 283, 131085.
Murphy, C.L., 2018. A comparison of microplastics in farmed and wild shellfish near Vancouver Island and potential implications for contaminant transfer to humans. Available from ProQuest Dissertations Publishing. Retrieved from: https://search. proquest.com/docview/2027207899?accountid¼11528.Last accessed March 2022
Napper, I.E., Bakir, A., Rowland, S.J., Thompson, R.C., 2015. Characterisation, quantity and sorptive properties of microplastics extracted from cosmetics. Mar. Pollut. Bull. 99(1-2), 178-185.
Nor, N.H.M., Kooi, M., Diepens, N.J., Koelmans, A.A., 2021. Lifetime accumulation of microplastic in children and adults. Environ Sci Technol. 55(8), 5084-5096.
OECD, 1995. Guidance document for aquatic effects assessment. Organisation for economic cooperation and development, Paris, France, p. 116.
Oliveira, M., Almeida, M., 2019. The how and why of micro(nano) plastic research. TrAC Trends Anal. Chem. 114, 196-201.
Palomares, M.L.D., Pauly, D. (Eds.), 2022. Sea Life Base. Last accessed: July 2022. [Online] https://www.sealifebase.se/
Peces Mediterraneo, 2022. Clasificación de los peces. Last accessed: July 2022. [Online] https://pecesmediterraneo.com/peces-del-mar-mediterraneo
Peng, G., Xu, P., Zhu, B., Bai, M., Li., D., 2018. Microplastics in freshwater river sediments in Shanghai, China: a case study of risk assessment in mega-cities. Environ. Pollut. 234, 448-456.
Peretz, J., Vrooman, L., Ricke, W.A., Hunt, P.A., Ehrlich, S., Hauser, R., Padmanabhan, V., Taylor, H.S., Swan, S.H., VandeVoort, C.A., Flaws, J.A., 2014. Bisphenol a and reproductive health: update of experimental and human evidence, 2007-2013. Environ. Health Perspect. 122(8), 775-86.
Phuong, N.N., Poirier, L., Pham, Q.T., Lagarde, F., Zalouk-Vergnoux, A., 2018. Factors influencing the microplastic contamination of bivalves from the French Atlantic coast: location, season and/or mode of life?. Mar. Pollut. Bull. 129, 664–674.
Pironti, C., Ricciardi, M., Motta, O., Miele, Y., Proto, A., Montano, L., 2021. Microplastics in the environment: Intake through the food web, human exposure and toxicological effects. Toxics 9(9), 224.
Pocar, P., Fischer, B., Klonisch, T., Hombach-Klonisch, S., 2005. Molecular interactions of the aryl hydrocarbon receptor and its biological and toxicological relevance for reproduction. Reproduction 129(4), 379-389.
Poma, A., Vecchiotti, G., Colafarina, S., Zarivi, O., Aloisi, M., Arrizza, L., Chichiriccò, G., di Carlo, P., 2019. In vitro genotoxicity of polystyrene nanoparticles on the human fibroblast Hs27 cell line. Nanomaterials 9(9), 1299.
Prata, J.C., da Costa, J.P., Lopes, I., Duarte, A.C., Rocha-Santos, T., 2020. Environmental exposure to microplastics: An overview on possible human health effects. Sci. Total Environ. 702, 134455.
Proshad, R., Kormoker, T., Islam, M.S., Haque, M.A., Rahman, M.M., Mithu, M.M.R., 2017. Toxic effects of plastic on human health and environment: A consequences of health risk assessment in Bangladesh. Int. J. Health 6(1), 1-5.
Qiao, R., Deng, Y., Zhang, S., Wolosker, M.B., Zhu, Q., Ren, H., Zhang, Y., 2019. Accumulation

of different shapes of microplastics initiates intestinal injury and gut microbiota dysbiosis in the gut of zebrafish. Chemosphere 236, 124334.

Renzi, M., Guerranti, C., Blašković, A., 2018. Microplastic contents from maricultured and natural mussels. Mar. Pollut. Bull. 131, 248–251

Rijk, R., Ehlert, K., 2001. Final Report. Migration of Phthalate Plasticisers from soft PVC toys and child-care articles. TNO Report V3932. TNO Nutrition and Food Research, Zeist.

Rochman, C.M., Tahir, A., Williams, S.L., Baxa, D.V., Lam, R., Miller, J.T., Teh, F.-C., Werorilangi, S., Teh, S.J. 2015. Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. Sci. Rep. 5, 14340.

Rochman, C.M., Tahir, A., Williams, S.L., Baxa, D.V., Lam, R., Miller, J.T., The, F.-C., Werorilangi, S., Teh, S.J., 2015. Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. Sci. Rep. 5(1), 14340.

Rockstrom, J., Steffen, W., Noone, K., Persson, Å., Chapin, F.S., Lambin, E.F.,Lenton, T.M., Scheffer, M., Folke, C., Schellnhuber, H.J., Nykvist, B., de Wit, C.A.,Hughes, T., van der Leeuw, S., Rodhe, H., Sorlin, S., Snyder, P.K., Costanza, R.,Svedin, U., Falkenmark, M., Karlberg, L., Corell, R.W., Fabry, V.J., Hansen, J.,Walker, B., Liverman, D., Richardson, K., Crutzen, P., Foley, J.A., 2009. A safe operating space for humanity. Nature 461, 472-475

Sajiki, J., Yonekubo, J., 2003. Leaching of bisphenol A (BPA) to seawater from polycarbonate plastic and its degradation by reactive oxygen species. Chemosphere 51(1), 55-62.

Schirinzi, G.F., Pérez-Pomeda, I., Sanchís, J., Rossini, C., Farré, M., Barceló, D., 2017. Cytotoxic effects of commonly used nanomaterials and microplastics on cerebral and epithelial human cells. Environ. Res., 159, 579-587.

SCP/RAC, Regional Activity Center for Sustainable Consumption and Production, 2020. SCP/RAC joined global efforts to tackle the plastic waste crisis. Last accessed: March 2022. [Online] http://www.cprac.org/en/news-archive/general/scp/rac-joined-global-efforts-to-tackle-the-plastic-waste-crisis

Setälä, O., Fleming-Lehtinen, V., Lehtiniemi, M., 2014. Ingestion and transfer of microplastics in the planktonic food web. Environ. Pollut. 185, 77-83.

Shang, X., Lu, J., Feng, C., Ying, Y., He, Y., Fang, S., Lin, Y., Dahlgren, R., Ju, J., 2020. Microplastic (1 and 5 μm) exposure disturbs lifespan and intestine function in the nematode *Caenorhabditis elegans*. Sci. Total Environ. 705, 135837.

Simal-Lozano, J., Ameijeiras, A.H., 1994. El mejillón: Desde su desarrollo en una batea hasta su depuración. Revista de tecnología e higiene de los alimentos, 255, 35-36.

Solomon, K., Giesy, J., Jones, P., 2000. Probabilistic risk assessment of agrochemicals. Crop Prot. 19(8-10), 649-655.

Song, Y.K., Hong, S.H., Jang, M., Kang, J.H., Kwon, O.Y., Han, G.M., Shim, W.J., 2014. Large accumulation of micro-sized synthetic polymer particles in the sea surface microlayer. Environ. Sci. Technol. 48(16), 9014-9021.

Stienbarger, C.D., Joseph, J., Athey, S.N., Monteleone, B., Andrady, A.L., Watanabe, W.O., Seaton, P., Taylor, A.R., Brander, S.M., 2021. Direct ingestion, trophic transfer, and physiological effects of microplastics in the early life stages of Centropristis striata, a commercially and recreationally valuable fishery species. Environ. Pollut. 285, 117653.

Stock, V., Böhmert, L., Lisicki, E., Block, R., Cara-Carmona, J., Pack, L.K., Selb, R., Lichtenstein, D., Voss, L., Henderson, C.J., Zabinsky, E., Sieg, H., Braeuning, A., Lampen, A., 2019. Uptake and effects of orally ingested polystyrene microplastic particles in vitro and in vivo. Arch. Toxicol. 93(7), 1817-1833.

Stockholm Convention, 2015. Risk management evaluation on decabromodiphenyl ether (commercial mixture, c-decaBDE). Persistent Organic Pollutants Review Committee Eleventh meeting. In: InforMEA, United Nations Information Portal on Multilateral Environmental Agreements. Last accessed: September 2022. [Online] https://www.informea.org/en/draft-risk-management-evaluation-decabromodiphenyl-ether-commercial-mixture-c-decabde

Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Le Goïc, N., Quillien, V., Mingant, C., Epelboin, Y., Corporeau, C., Guyomarch, J., Robbens, J., Paul-Pont, I., Soudant, P., Huvet, A., 2016. Oyster reproduction is affected by exposure to

polystyrene microplastics. Proc. Natl. Acad. Sci. 113(9), 2430-2435.
Talsness, C.E., Andrade, A.J.M., Kuriyama, S.N., Taylor, J.A., vom Saal, F.S., 2009. Components of plastic: Experimental studies in animals and relevance human health. Phil. Trans. R. Soc. B. 364(1526): 2079-2096.
Tamargo, A., Molinero, N., Reinosa, J.J., Alcolea-Rodriguez, V., Portela, R., Bañares, M.A., Fernández, J.F., Moreno-Arribas, M.V., 2022. PET microplastics affect human gut microbiota communities during simulated gastrointestinal digestion, first evidence of plausible polymer biodegradation during human digestion. Sci. Rep. 12, 528.
Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Phil. Trans. R. Soc. Lond. B. Biol. Sci. 364(1526), 2027-2045.
Tomlinson, D.L., Wilson, J.G., Harris, C.R., Jeffrey, D.W., 1980. Problems in the assessment of heavy-metal levels in estuaries and the formation of a pollution index. Helgol. Meeresunters 33, 566-575,
U.S. EPA, United States Environmental Protection Agency, 1992. Framework for ecological risk assessment. Risk Assessment Forum EPA/630/R-92/001. U.S. Environmental Protection Agency.
U.S. EPA, United States Environmental Protection Agency, 1998. Guidelines for ecological risk assessment. EPA/630/R-95/002F. U.S. Environmental Protection Agency.
U.S.EPA, United States Environmental Protection Agency, 1985. Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses (PB 85-227049). Office of Research and Development, Environmental Research Laboratories, US Environmental Protection Agency.
Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M.B., Janssen, C.R., 2015. Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats. Environ Pollut. 199, 10-17.
Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human consumption. Environ. Pollut. 193, 65–70.
van Raamsdonk, L.W.D., van der Zande, M., Koelmans, A.A., Hoogenboom, R.L.A.P., Peters, R.J.B., Groot, M.J., Peijnenburg, A.A.C.M., Weesepoel, Y.J.A., 2020. Current insights into monitoring, bioaccumulation, and potential health effects of microplastics present in the food chain. Foods 9(1), 72.
Wang, F., Wang, F., Zeng, E.Y., 2018a. Sorption of toxic chemicals on microplastics. In: Microplastic contamination in aquatic environments. In: Zeng, E.Y. (Ed.), Microplastic contamination in aquatic environments: An emerging matter of environmental urgency. Elsevier, pp 225-247.
Wang, W., Wang, J., 2018. Comparative evaluation of sorption kinetics and isotherms of pyrene onto microplastics. Chemosphere 193, 567-573.
Wang, Z., Chen, M., Zhang, L., Wang, K., Yu, X., Zheng, Z., Zheng, R., 2018b. Sorption behaviors of phenanthrene on the microplastics identified in a mariculture farm in Xiangshan Bay, southeastern China. Sci. Total Environ. 628-629, 1617-1626.
Weber, R., Fantke, P., Hamouda, A.B., Mahjoub, B., 2018. 20 Case studies on how to prevent the use of toxic chemicals frequently found in the Mediterranean Region. Regional Activity Centre for Sustainable Consumption and Production (SCP/RAC).
Wright, S.L., Rowe, D., Thompson, R.C., Galloway, T.S., 2013. Microplastic ingestion decreases energy reserves in marine worms. Curr. Biol. 23(23), 1031-1033.
Wright, S.L., Kelly, F.J., 2017. Plastic and human health: A micro issue? Environ. Sci. Technol. 51(12), 6634-6647.
Wu, J., Zhao, X., Gao, L., Li,Y., Wang, D., 2022. Use of interspecies correlation estimation (ICE) models to derive water quality criteria of microplastics for protecting aquatic organisms. Int. J. Environ. Res. Public Health 19(16), 10307.
Wu, P., Huang, J., Zheng, Y., Yang, Y., Zhang, Y., He, F., Chen, H., Quan, G., Yan, J., Li, T.,

Gao, B., 2019. Environmental occurrences, fate, and impacts of microplastics. Ecotoxicol. Environ. Saf. 184, 109612

Xu, P., Peng, G., Su, L., Gao, Y., Gao, L., Li., D., 2018. Microplastic risk assessment in surface waters: a case study in the Changjiang Estuary, China. Mar. Pollut. Bull. 133, 647-654.

Yin, K., Wang, D., Zhao, H., Wang, Y., Guo, M., Liu, Y., Li, B., Xing, M., 2021. Microplastics pollution and risk assessment in water bodies of two nature reserves in Jilin Province: Correlation analysis with the degree of human activity. Sci Total Environ. 799, 149390.

Yu, Y., Chen, H., Hua, X., Dang, Y., Han, Y., Yu, Z., Chen, X., Ding, P., Li, H., 2020. Polystyrene microplastics (PS-MPs) toxicity induced oxidative stress and intestinal injury in nematode *Caenorhabditis elegans*. Sci. Total Environ. 726, 138679.

Zhang, M., Li, J., Xing, G., He, R., Li, W., Song, Y., Guo, H., 2011. Variation in the internalization of differently sized nanoparticles induces different DNA-damaging effects on a macrophage cell line. Arch. Toxicol. 85(12), 1575-1588.

Zhang, X., Zheng, M., Wang, L., Lou, Y., Shi, L., Jiang, S., 2018. Sorption of three synthetic musks by microplastics. Mar. Pollut. Bull. 126, 606-609.

Zhang, Z., Wu, H., Peng, G., Xu, P., Li, D., 2020. Coastal ocean dynamics reduce the export of microplastics to the open ocean. Sci. Total Environ. 713, 136634.

Zheng, H., Wang, J., Wei, X., Chang, L., Liu, S., 2021. Proinflammatory properties and lipid disturbance of polystyrene microplastics in the livers of mice with acute colitis. The Sci. Total Environ. 750, 143085.

Ziajahromi, S., Neale, P.A., Rintoul, L., Leusch, F.D.L., 2017. Wastewater treatment plants as a pathway for microplastics: Development of a new approach to sample wastewater-based microplastics. Water Res. 112, 93-99

Ziccardi, L.M., Edgington, A., Hentz, K., Kulacki, K.J., Driscoll, S.K., 2016. Microplastics as vectors for bioaccumulation of hydrophobic organic chemicals in the marine environment: A state-of-the-science review. Environ. Toxicol. Chem. 35(7), 1667-1676.

Zimmermann, L., Dierkes, G., Ternes, T.A., Völker, C., Wagner, M., 2019. Benchmarking the in vitro toxicity and chemical composition of plastic consumer products. Environ. Sci. Technol. 53(19), 11467-11477.

4.-General Conclusions

-In the present study, methods of analysis of MPs, with different sequential steps, including visual sorting, density separation, alkaline hydrolysis, oxidation (Fenton/hydrogen peroxide), surfactant use, enzymatic hydrolysis, fluorescence with Nile Red staining and spectroscopic techniques (FTIR/RAMAN), have been proposed. It has shown to be effective for the extraction, quantification, classification and polymer characterization of MPs in seawater, subtidal marine sediments, sandy beach sediments and molluscs with recovery rates spanning from 60 to 100 % in spheres from 53 to 500 µm diameter.

-MPs abundance, size, morphology and composition were determined in superficial seawater, subtidal sediments and sandy beaches in Catalonia coast. Microplastics mean abundance in surface seawater was 1.3 MPs/m^3 with sizes from 80 µm to 2 mm, similar to other areas of Mediterranean Sea with high MPs accumulation, corroborating the Western Mediterranean Sea basin as a region of MPs accumulation.

-Nine (9) different plastic polymers were found in superficial seawater (PE, PP, PS, PU, PET, PC, ABS, PMMA, PVC). The highly demanded polymers (PE and PP) were the most abundant for fragments, pellets, and films, for fibres, polyester (polyethylene terephthalate) was the most abundant.

-MPs distribution on marine sediment on Tarragona coast seems is by patching. Concentration of MPs ≥0.5 mm tends to be low with values from 2.4 to 88.6 MPs/kg d.w., and for <0.5 mm tends to be high specially in front of Pineda beach, **Pin N** and Cambrils **Cam**, Cavet beach with values from 319 to 1266 MPs/kg d.w. (FTIR) or 108 to 1008 MPs/kg d.w (Fluorescence).

-Eight (8) different plastic polymers were found in subtidal sediments (PE, PP, PS, PVC, PA, PET, ABS, PP-PE copolymer), resins and additives, being PE was common polymer in all zones while high density polymers proportion tend to be high specially for PVC, PA and PET.

-Fibres were the dominant MPs morphology in bottom sediments and seawater, probably comes from effluents from WWTP, river dischargues and urban runoff. Fibres balls associated with bottom sediments, organic debris and plankton were abundant, masking the real amounts of fibres in each reservoir.

-There was no relationship between MPs high concentration and sand grain size or physicochemical parameters in subtidal sediments. The values of MPs concentration obtained with fluorescent methodology were smaller than with µFTIR in almost all sites but following the

same trend. Fluorescence methodology enhances fibres identification in marine submerged sediment samples.

-MPs distribution in Catalonia Coast is heterogeneous and vary spatially. MPs were present in almost all beaches along Catalonia coast with a high variability between them and within beaches sampling sites. The highest MPs pollution is registred in southern part of Catalonia (South Barcelona, Tarragona and South Catalonia zones) with MPs abundance from <0.55 to 56.8 MPs/kg for MPs≥0.5 mm and from 261 to 1473 MPs/kg (FTIR)/73 to 2013 MPs/kg (fluorescence) for MPs<0.5 mm. Fibres predominated in fractions MPs<0.5 mm and fragments in MPs≥0.5 mm. Pellets, although in low proportion, were abundant in beaches near to industrialized areas in Tarragona zone (Pineda beach, 14 MPs/kg) and near river discharges.

- Regarding MPs composition in sandy beaches, particles (fragments, films, pellets and foam) composition, for both fractions (<0.5 mm and ≥ 0.5 mm) majority polymers were PE, PP, PET and PS. PVC appeared abundant only in particles MPs<0.5 mm fraction. For fibres, the majority composition was polyester (PES), PA, PP and PAN.

-Beaches with fine sand showed higher concentration (575±460 MPs/kg) than coarse sand (251±209 MPs/kg) and in turn with very coarse sand (118±150 MPs/kg).

-The size range 4-5 mm was not observed in superficial seawater samples. For seawater the most abundant size range was 2-1mm and 0.5-0.08 mm, for MPs ≥0.5 mm in subtidal sediments was 1-0.5 mm and 2-5 mm whereas for MPs ≥0.5 mm in sandy beach sediments was 2-1 mm.

-For MPs <0.5 mm in subtidal sediments, the average fibres size with µFTIR methodology was 202±190 µm while with fluorescence was 508±466 µm. For particles (fragments and films) the average size was 114±98 µm with µFTIR and 92±38 µm with fluorescence. For sandy beach sediments, MPs <0.5 mm, the average fibres sizes varied from 93±12 to 1600±1457 µm and particles (fragments and films) from 80±8 to 180±215 µm.

-The high proportion of 1-2 mm observed in seawater and sandy beach suggests seawater is the media that receive and facilitates MPs dispersion, the shoreline acts as an intermediate reservoir with constant weathering and active exchange with seawater surface and the sediments acts as a significant sink for MPs 0.5-1 mm from seawater

-The composition, morphology and abundance of the MPs observed in coastline, suggests that the main contribution comes from numerous land base-sources: through streams, direct or through river WWTP discharges, wide distribution of plume, industrial and commercial port activities, urban and agriculture runoff, touristic activities. The MPs distribution and accumulation are

favoured by fine size grain sand presence, artificial and natural barriers, wind direction opposite to beach orientation or facing to as well as local surface currents coming at the same direction to beach orientation Fisheries and marine transport were less important sources of MPs on Catalonia coast.

-Currently there is no threat or risk by MPs pollution >80µm sizes in seawater surface (upper pelagic compartiment) on Tarragona coast according RCR index and RI index. Zones presented high levels of MPs pollution (danger) according PLI index as Tarragona harbour.

-All seawater surface sites showed high hazard polymer index risk (H), composited by polymers with high hazard score (PVC, PU, ABS). The exposure concentration in the upper pelagic compartiment remains below the safe concentration.

-MPs ≥0.5 mm in subtidal marine sediments on Tarragona coast not showed threat or risk by MPs pollution according RCR and PLI index, but in Tarragona harbour (industrial zone) sediments high pollution levels were observed. MPs <0.5 mm cause pollution risks according RDR, PLI and RI indexes in subtidal marine sediments of: Miracle beach north, Pineda beach north, Pineda beach south, Cambrils-Cavet beach and Hospitalet beach of Tarragona centre-south zone. Almost all sites showed high hazard polymer index risk (H), composited by polymers with high hazard score as PVC and ABS.

-According PLI index estimated with MPs <0.5 mm abundance, more polluted beaches from Catalonia coast are ubicate in South Barcelona, Tarragona and South Catalonia zones. Zone less polluted resulted North Catalonia and North Barcelona. For South Barcelona zone beaches as Cala de Vallcarca- and Platja Far de Sant Cristòfol (Vilanova) were the most polluted, for Tarragona zone beaches as l'Arrabasada, Miracle, La Pineda and Salou, finally for South Catalonia zone beaches as Bahía del Fangar, La Marquesa, Riumar, Los Eucaliptos, Trabucador, Alfacs Bay (Playa del Delta). In majority Tarragona city coast beaches and subtidal sediments sites showed the same PLI and hazard level.

-All commercial molluscs from the Catalan coast, contained MPs. Wedge clams and snails showed the lowest concentration of MPs regarding individual and wet weight: 0.49±0.23 MP/individual and 0.94±0.62 MP/$g_{w.w}$, respectively. Mussels and fine clams showed the highest level by wet weight with values of 6.47±7.95 and 4.97±4.78 MPs/$g_{w.w.}$, respectively. Big oysters and mussels showed the highest concentration per individual: 22.8±14.4 and 18.6±23.0 MPs/individual, respectively. Mussels showed the highest level by wet weight with values ranging from 0.68 to 34.4 MPs/g $_{w.w.}$ Fibres were the predominant morphology

-Of the total MPs measured in molluscs 74% showed sizes smaller than 1 mm, whereas 20% were between 0.02 and 0.150 mm, a critical range for negative consequences on the organism because both of their ability to cross biological barriers and their higher sorption capacity. Small MPs observed in bivalves (high surface/volume) combined with their composition (high hydrophobicity of polymers) could concentrate organic contaminants by several folds compared to the water column. Bivalve consumption is a non-negligible pathway for human exposure to toxic compounds considering high consumption of bivalves by Spanish people

-Twenty-one (21) different plastic polymers were found in molluscs tissuues (PES, PE, PVDF, PP, PA, PAN, PC, PU, PS, EPS, EVOH/EVA, PET, PVC, PEVA, PVA, PMMA, PU, PP-PE diene, polystyrene acrylate ester, PE-vinylchloride, polyacrylate). PES, PVDF and PA, were abundant in depurated organisms and PE in non-depurated organisms. A similar number of polymers for both conditions were observed. PE was the predominant polymer in the organisms from the Northern zone, while PES, PP, PAN and PA were mainly found in the organisms from the Central zone. PVDF, PC, PMMA, PVC, PET, PVA, PEVA, EVOH/EVA, PS and EPS were predominant in the organisms from the Southern coast of Catalonia.

-Polymer Risk Index (H) between III to IV with possible human health implications were observed in mussels, snail from middle zone, big oysters, fine clams, razor clams, and wedge clams from middle and southern zone. By contrast low grade of I and II were observed only in wedge clams from north WC(N) and marine snail from south S(S).

-The bivalve depuration process did not remove MPs from the organisms. Only changes in their morphology, size and composition were observed. The time of depuration could be a key parameter.

-Consumption of molluscs is as an important route of exposure to MPs for the population of Catalonia coast. The daily intake of MPs through molluscs consumption was estimated to be 22.2, 20.4 and 9.67 MPs, for adults, elderly and pregnant women, respectively. The mean annual MPs ($\geq 20\mu m$) consumption for the adult population was estimated in 8,103 MPs, with 95^{th} percentile of 19,418 MPs/year. Spain annual intakes by MPs concentrations were higher than obtained for other worldwide zones due mainly to higher MPs concentration in different molluscs groups of Catalonia coast and relatively higher consumption for Spain population.

ANNEXES

CHAPTER 1-MICROSPLASTICS IN COASTAL AREAS

1.-Sub-chapter 1.1. Microplastics in marine environmental compartments

Microplastics levels, size, morphology and composition in marine water, sediments and sand beaches. Case study of Tarragona coast (western Mediterranean).

Nora Expósito[1], Joaquim Rovira*[1,2], Jordi Sierra[1,3], Jaume Folch[4], Marta Schuhmacher[1].

Table S1. Location and characteristics of superficial seawater, sediments and sand beach sampling points.

	Surface waters			
	Coordinates of the transect	**Distance from shoreline (m)**	**Place**	
T1	From: N 41° 07’ 13’’; E 1° 20’ 19’’ To: N 41° 07’ 10’’; E 1° 19’ 49’’	924	La Mora-Llarga beach	
T2	From: N 41° 07’ 25’’; E 1° 18’ 23’’ To: N 41° 07’ 9’’; E 1° 17’ 50’’	765	Llarga-Arrabassada	
T3	From: N 41° 06’ 39’’; E 1° 16’ 19’’ To: N 41° 06’ 23’’; E 1° 15’ 43’’	702	Arrabassada -Miracle	
T4	From: N 41° 05’ 21’’; E 1° 13’ 47’’ To: N 41° 05’ 40’’; E 1° 14’ 22’’	500	Tarragona Harbour	
	Marine sediments			
	Coordinates	**Depth (m)**	**Place**	
S1	N41° 07´ 14´´; E1° 20´ 27´´	13	La Mora	
S2	N41° 07´ 15´´; E1° 20´ 13´´	14.4	Cala de Roca Plana	
S3	N41° 07´ 27´´; E1° 18´ 26´´	15.3	Llarga	
S4	N41° 06´ 40´´; E1° 16´ 19´´	14	Miracle-Arrabassada	
S4	N41° 06´ 34´´; E1° 16´ 02´´	15	Miracle	
S5	N41° 06´.03´´; E1° 14´ 53´´	14	Tarragona Harbour (Marina)	
S6	N41° 05´ 22´´; E1° 13´ 47´´	17	Tarragona Harbour (Industrial)	
	Beach sand			
	Coordinates	**Beach length (m)**	**Beach**	**Sub-samples**
B1	From: N 41° 07’ 40’’; E 1° 20’ 56’’ To: N 41° 07’ 36’’; E 1° 20’ 38’’	447	La Mora	1
B2	From: N 41° 07’ 23’’; E 1° 17’ 16’’ To: N 41° 07’ 20’’; E 1° 17’ 02’’	348	Savinosa	1
B3	From: N 41° 07’ 45’’; E 1° 19’ 22’’ To: N 41° 07’ 34’’; E 1° 17’ 34’’	2619	Llarga	4
B4	From: N 41° 07’ 13’’; E 1° 16’ 45’’ To: N 41° 07’ 03’’; E 1° 16’ 26’’	623	Arrabasada	2

B5.S	From: N 41° 06’ 52’’; E 1° 15’ 49’’	1074	Miracle (September)	3
B5.J			Miracle (June)	3
	To: N 41° 06’ 36’’; E 1° 15’ 11’’			
B6	From: N 41° 05’ 06’’; E 1° 11’ 27’’	2264	Pineda	3
	To: N 41° 04’ 04’’; E 1° 10’ 46’’			

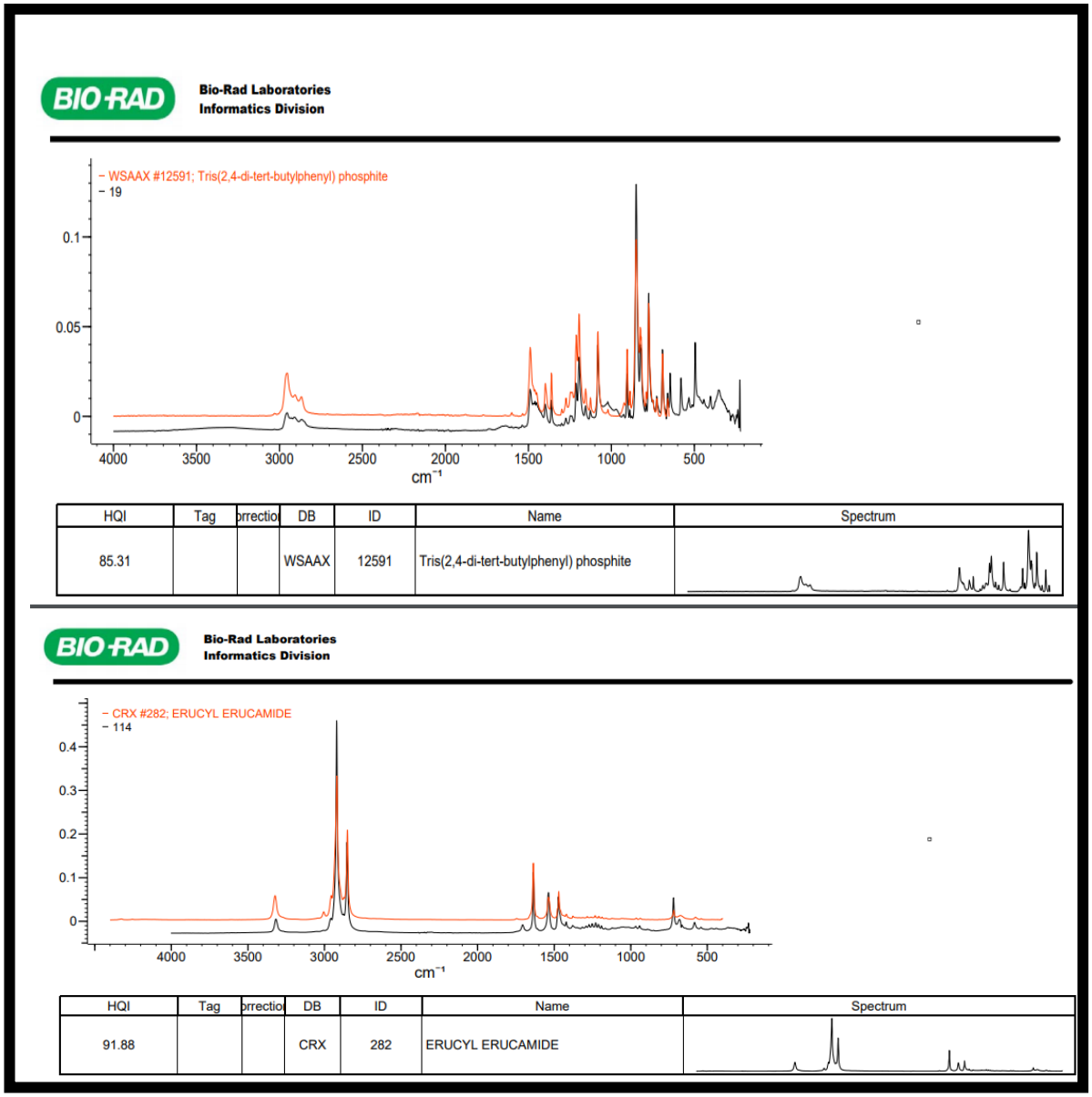


Figure S1. Spectra Comparison with BIO-RAD database

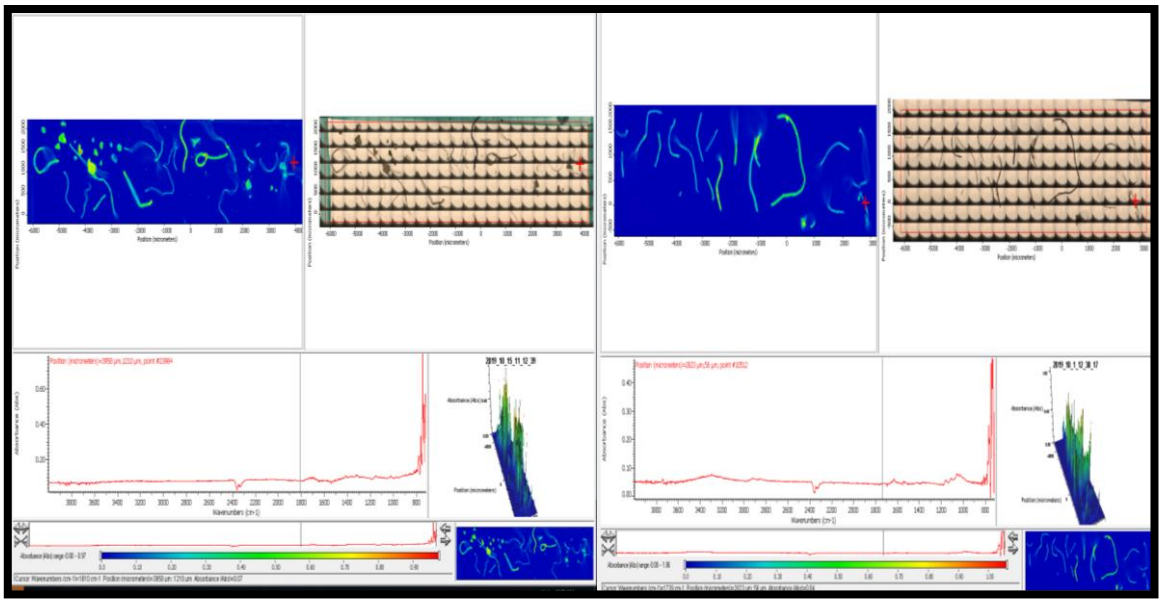


Figure S2. Fibres and small fragments identification with µFTIR technique.

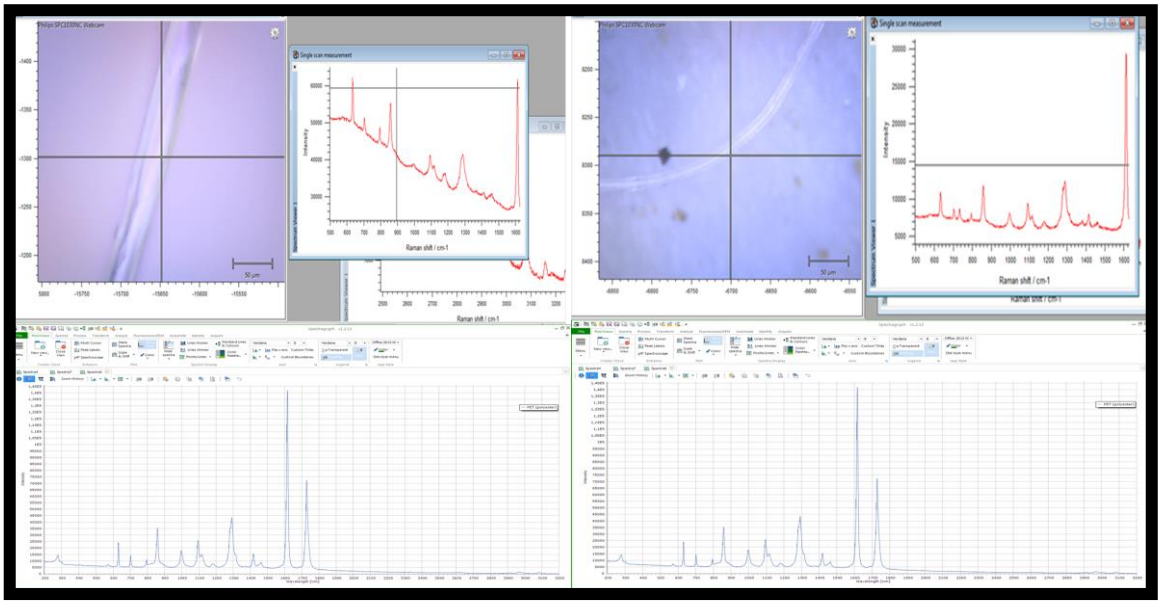


Figure S3. Raman spectra of fibres from seawater samples and comparison with spectra from libraries made with plastics found in the market.

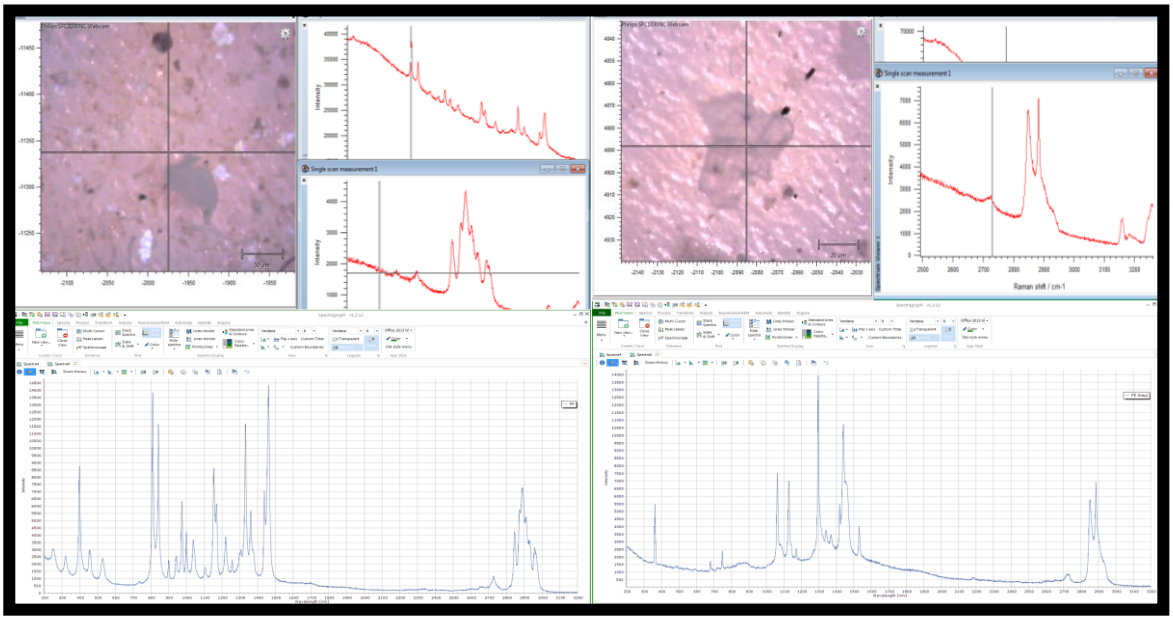


Figure S4. Raman spectra of small fragments from seawater samples and comparison with spectra from libraries made with plastics found in the market.

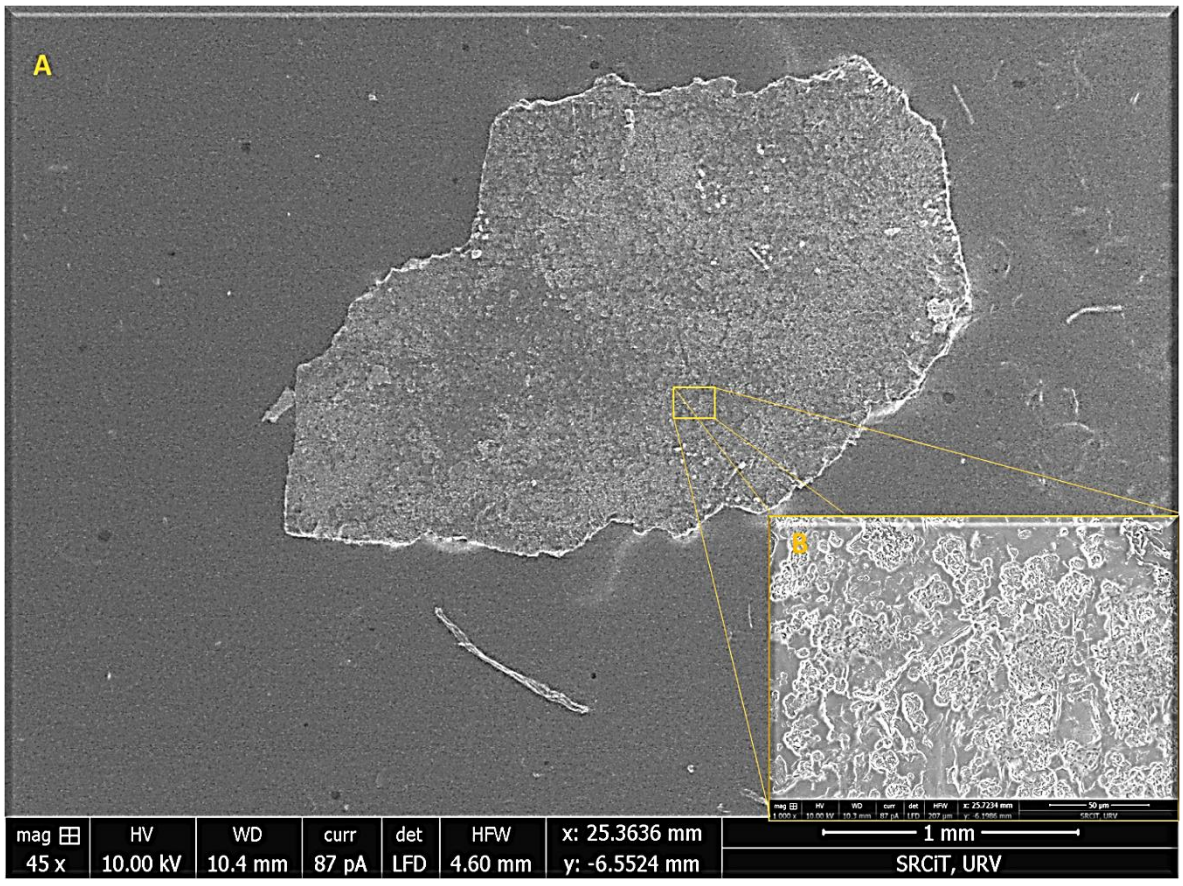


Figure S5a. Scanning electron microscope (SEM) of PE film surface found in seawater samples (Note irregular surface). Magnification: A.45x, B.1000x (scale 50µm)

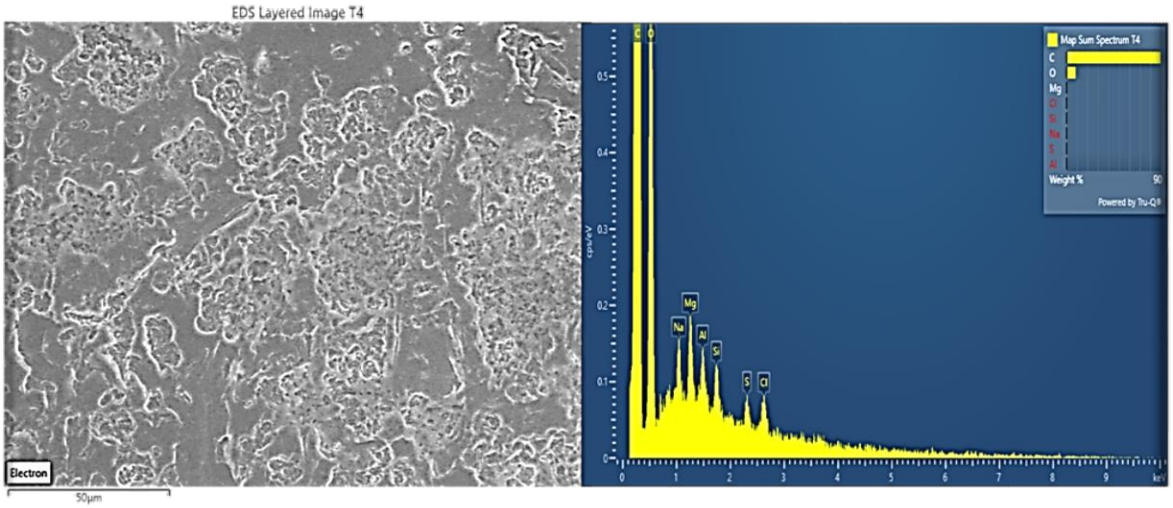


Figure S5b. Scanning electron microscope (SEM) image and X-rays analysis of PE film surface found in seawater samples (Note irregular surface)

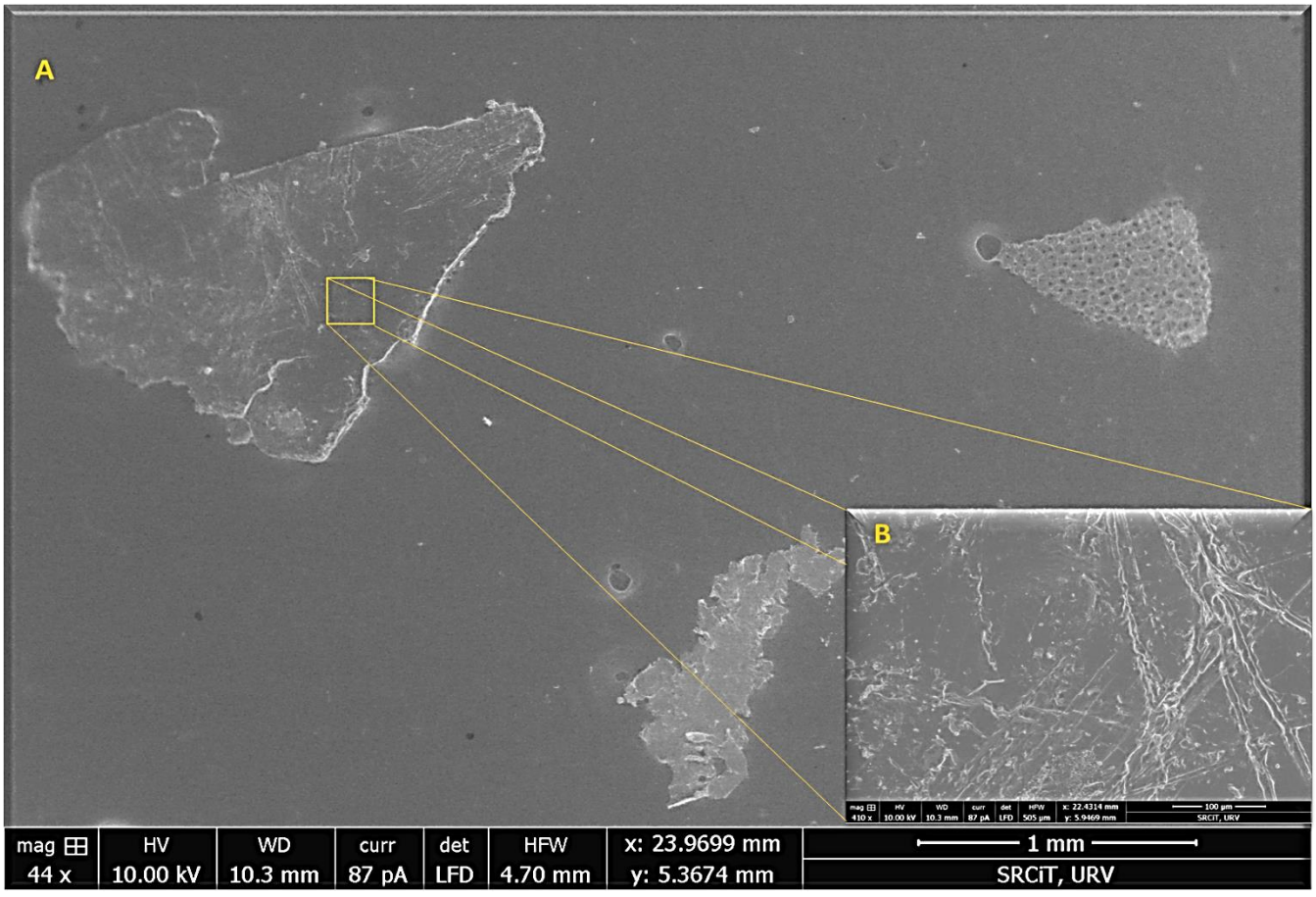


Figure S5c. Scanning electron microscope (SEM) of PE film surface found in seawater samples (Note irregular surface). Magnification: A.44x, B.410x (scale 100 µm)

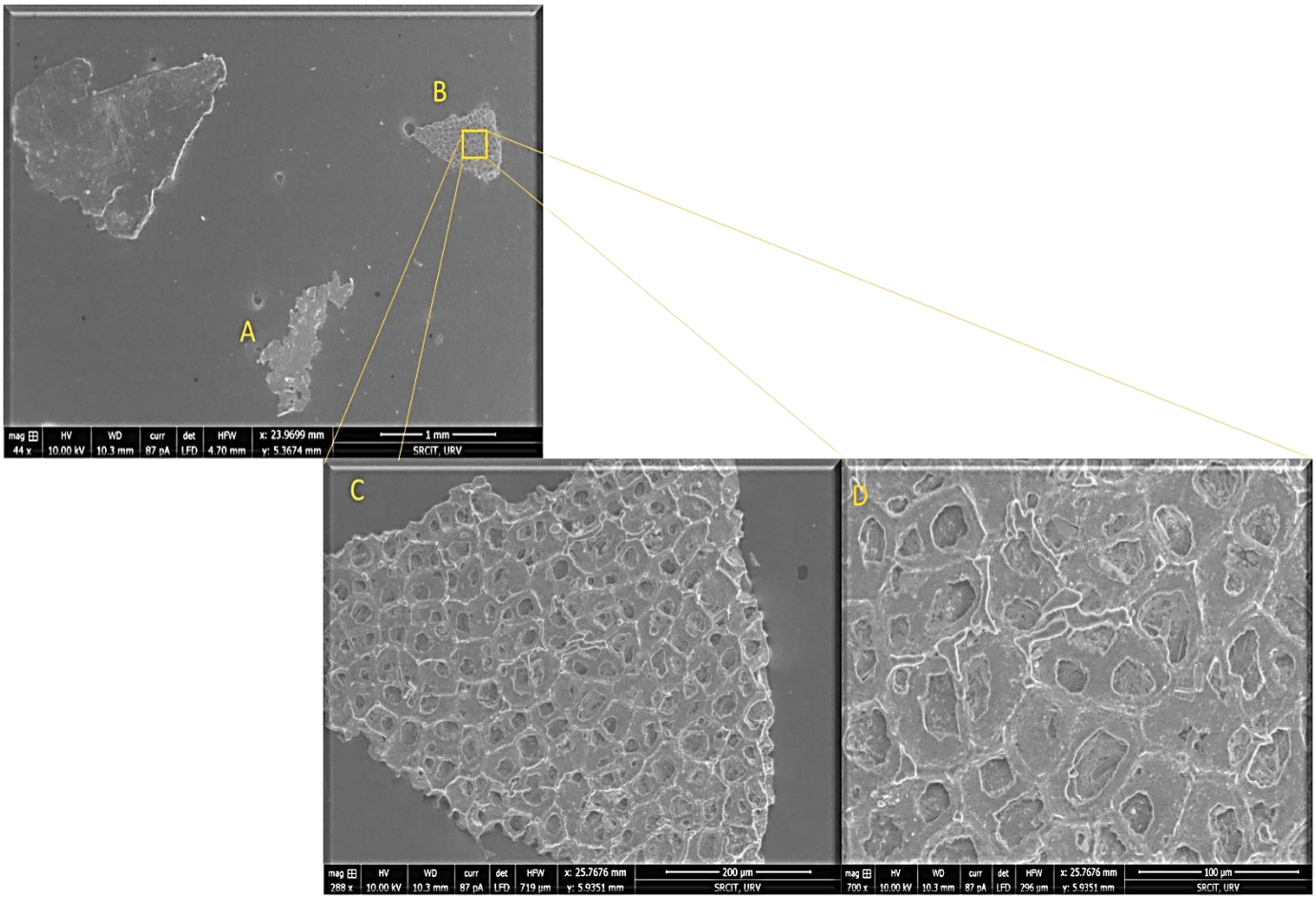


Figure S6a. Scanning electron microscope (SEM) image of PE films surface found in subtidal sediments samples. Magnification: A- 44x, B. 44x (scale 1 mm), C. 288x (scale 200µm), D. 700X (scale 100µm).

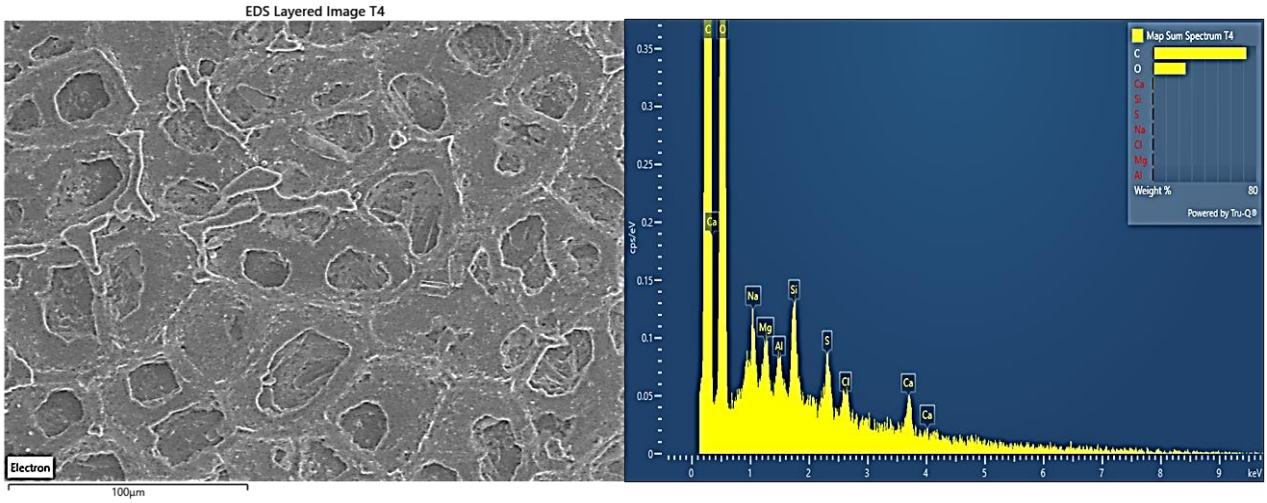


Figure S6b. Scanning electron microscope (SEM) image and X-rays analysis of PE film surface found in subtidal sediments samples (Note irregular holes).(Scale 100 µm)

1.-Sub-chapter 1.2. Microplastics in subtidal marine sediments

Pin N, Pin S: the principal emissary is located at 2400 m away from shoreline and 32 m depth with a flow of 15833 m3/day and an emergency emissary 950 m away from shoreline 20 m depth. River discharges with WWTP effluents (Francolí River), pluvial emissaries, high intensity touristic activity, commercial port activities and urban-industrial run-offs (spillages) mainly (GENCAT, 2022)

In case **Cam**, this zone is influenced by touristic activities, WWTP discharges effluents influence from northern (Cap of Salou) with total flow 47000 m^3/day (two pipeline 1.1 km and 1.3 km in 35 and 30 m depth), direct WWTP discharges of 25000 m^3/day (principal pipeline 2 km away from the coast, 29 m depth, four emergency emissaries 860-1420 m away from the coast and 12-18 m depth), urban runoff, directly little stream discharges (ACA, 2022; GENCAT 2022).

For **Hos** site, there is an influence of WWTP discharges from northern with flow 8800 m^3/day (pipeline 2.4 km on local stream ending to Mediterranean Sea) and direct discharges through two pipelines discharging a total of 3750 m^3/day away to shoreline 1.76 km into Mediterranean Sea at 25 m plus urban runoff. (ACA, 2022; GENCAT, 2022).

For **Tg N, S** site, there is an influence of WWTP discharges from northern with flow 8292 m^3/day pipeline 1.6 km ending to Mediterranean Sea at 32 m (Altafulla). Direct discharges through emergency pipelines, discharging a total of 35000 m^3/day away to shoreline 930 m into Mediterranean Sea at 24 m, pluvial emissaries, urban runoff and industrial port activities (ACA, 2022; GENCAT, 2022).

1.-Sub-chapter 1.3. Microplastics in intertidal sandy beach sediments

Microplastics levels along Catalonia coast (western Mediterranean): Approach for development of a fast and low-cost methodology for coastal hot spots establishment and mitigation measures application.

Nora Expósito[1], Jordi Sierra[1,3], Joaquim Rovira*[1,2], Esther Marti[3], Jaume Folch[4], Marta Schuhmacher[1].

Samples, meteorological parameters, and sand characteristics

The Catalan coastline is a densely populated coast, with 10,000 inhabitants per kilometer of coastline, and highly urbanized in a 59% of the coastal strip (Generalitat de Catalunya -GENCAT, 2019). The study took place along the beaches of Catalonia, a coastal segment approximately of 580 km long between Cape Creus and Ebro Delta. The types of coasts and their extensions are as follows: cliffs, 208 km; low coast, 52 km; beaches, 280 km; ports and maritime structures, 40 km.

The Catalan coast presents a microtidal coastal regime, with tidal amplitude of less than 0.2 m (Bowman et al., 2009) and waves being the main mechanism for controlling coastal evolution (Ojeda and Guillén, 2008). According to the dynamics of Catalan coasts in spring/summer season the beaches tend to recover the sands which was mobilized towards submerged areas during winter storms, it means the sampling campaign was carried out in the late summer (after the peak tourist season), therefore, the sampling was carried out during the period of sediment deposition on the shore.

The waves vary along the Catalan coast, decreasing gradually in energy from north to south (Bowman et al., 2009), following a marked seasonal pattern, with periods of low energy during the summer, while during the fall and winter high-energy conditions dominate (Bowman et al., 2009). During the sampling period, the wind velocity was between 5 to 20 km/h (3.1-12.43 mph) with a Beaufort Index of 1-3 between **Light Air** and formed ripples with the appearance of scales but without foam crests and **Gentle Breeze** and large wavelets crests begin to break, foam of glassy appearance, perhaps scattered white horses (National Weather Service-NOAA, 2022) https://www.weather.gov/mfl/beaufort

Taking in account the gusts, the Beaufort index increase in at specific moments from 3 to 6 with characteristics of **Strong Breeze,** large waves begin to form; the white foam crests are more extensive everywhere, large branches in motion; umbrellas used with difficulty. Regarding, the slope in beaches, the slope of entrance to the seawater in Catalonia coast beaches is considered between very strong and smooth according GENCAT-ACA 2022 https://acanet.gencat.cat/geco/platges/perfilszonabany.asp.

Catalonia coast was divided in five zones which include the following beaches:

North Catalonia: Platja de la Vall -Cap de Creus (PDV), El Port de la Selva (PPS), Roses (RO1), Empuriabrava, Roses (RO2), Platja Can Sopa, Roses (RO3), L' Escala (RO4), Els Griells- L'estartit (ELG), Platja del Racó (Pals) (PPA), Palamós (PAL), Platja d'Aro (PAR).

North Barcelona: Blanes (BLA), Les Creus –Calella (CAL), Platja Del Balís (Caldetas) (CTA), Premià del mar (PMA), Montgat -Platja Barca Maria (MONT), Badalona (BADAL), Sant Adrià del Besòs (STA), Fòrum, Barcelona (FORUM), Poblenou (POBLE), Bogatell (BOGA), Barcelona (BCN).

South Barcelona: Airport of Barcelona (EPLL), Gavà (GAVA), Castelldefels (CSDFLS), Cala Morisca (MORI), Cala de Vallcarca CAV), Platja Far de Sant Cristòfol, Vilanova (VIL01), Vilanova-Platja Ribes Roges (VIL02), Vilanova-Platja I'broig (VIL03), Platja Segur de Calafell (SER), Platja Comarruga (COM).

Tarragona Torredembarra (TOR), La Mora (MOR), Playa Larga North (PLA 1), Playa Larga Centre (PLA2), Playa Larga Centre (PLA3), Playa Larga South (PLA4), Playa de la Savinosa (SAV), l'Arrabasada North (ARRA 1), l'Arrabasada South (ARRA 2), Playa Miracle North (MIR 1), Playa Miracle Centre (MIR 2), Playa Miracle South (MIR 3), La Pineda North (PIN 1), La Pineda Centre (PIN 2), La Pineda South (PIN 3), Salou North (SAL 1), Salou South (SAL 2), Cambrils North (CAM 1), Cambrils Centre (CAM 2), Cambrils South (CAM 3).

South Catalonia: Playa Miami (MIA N), Playa Miami (MIA S), L'Hospitalet de l'Infant (HOS), L'Almadrava (ALM), L'Ametlla de Mar (LAM), Gola de la Bassa Olles (LOL), Bahía del Fangar North (BF N), Bahía del Fangar Faro (BF FAR), Bahía del Fangar South (BF S1), Bahía del Fangar South (BF S2), Playa la Marquesa (PLM), Riumar Playa (RIU), Playa los Eucaliptos (PEC), Playa Trabucador East (TRA E), Playa Trabucador West (TRA W), Playa del Delta East (PDE E), Playa del Delta West (PDE W), Sant Carles de la Rápita (SCR), Vinaròs (VIN).

In summary, to see the influence of local winds and surface currents on MPs accumulation in beaches, in table S1 are shown the coordinates of each sampling point, the facing or sampling site on beach orientation, the prevailing surface local currents and winds (velocity and direction) from the day before the sampling activities in each beach.

Data of wind velocity and direction was acquired from the following meteorological stations from the Meteorological Service of Catalonia (www.meteocat.cat): Illa de Buda, El Perelló, Ametlla de mar, Montroig del Camp, Tarragona-complex educatiu, Torredembarra, Cunit, PN Garraf, Barcelona-el Raval, Badalona-museu, Cabrils, Canet de mar, Platja d'Aro, Palafrugell, Sant Pere Pescador, Roses and Portbou and data of currents from the Balearic Islands Coastal Observing and Forecasting System (www.socib.es) . Current velocity during sampling campaign was between 0.5 and 1 m/s.

Table S1. Name of the beaches selected, reference, position (coordinates), beach facing and prevailing currents and winds the day before the sampling date.

Sand Beach	Name of the Beach Location	Coordinates	Beach facing	Day before Seawater current direction comes from	Day before Wind velocity range (km/h) (gust) and mainly directions
	Northern Catalonia				
PDV	Platja de la Vall (Cap de Creus)	42°20'35.51''N 3°11'12.13''E	**NE**	**N**	**10-20 (40) N/S**
PPS	El Port de la Selva	42°20'07.22''N 3°12'02.95''E	**N**	**N**	**10-20 (40) N/S**
RO1	Roses	42°15'48.50''N 3°10'24.06''E	**S**	**SE**	**10-20 (42) N/NE**
RO2	Empuriabrava, Roses	42°14'31.77''N 3°07'50.95''E	**SE**	**SE**	**10-20 (42) N/NE**
RO3	Platja Can Sopa, Roses	42°10'56.34''N 3°06'56.34''E	**E**	**SE**	**10-20 (42) N/NE**
RO4	L' Escala	42°06'56.10''N 3°08'18.07''E	**N**	**SE**	**5-20 (31) E/W**
ELG	Els Griells- L'estartit	42°02'36.71''N 3°11'48.27''E	**E**	**SE**	**5-20 (31) E/W**
PPA	Platja del Racó (Pals)	41°58'50.60''N 3°12'28.95''E	**E**	**SE**	**5-20 (31) E/W**
PAL	Palamós	41°50'54.78''N 3°07'04.75''E	**S**	**SW**	**5-20 (33) E/W**
PAR	Platja d'Aro	41°48'40.32''N 3°04'01.61''E	**E**	**SW**	**5-20 (33) E/W**
	North of Barcelona				
BLA	Blanes	41°39'46.68''N 2°47'03.79''E	**SE**	**SW**	**10-20 (33) E/N**
CAL*	Les Creus –Calella	41°36'38.17''N 2°39'22.11''E	**SE**	**SW**	**10-20 (33) E/N**
CTA	Platja Del Balís (Caldetas)	41°33'39.12''N 2°30'43.05''E	**SW**	**S/SW**	**10-20 (33) E/N**
PMA	Premià del mar	41°29'25.54''N 2°22'14.71''E	**SE**	**NW**	**5-10(20) S/NW**
MONT	Montgat, Platja Barca Maria	41°27'30.50''N 2°15'57.24''E	**SE**	**NW**	**5-10(26) SE/W**
BADAL	Badalona	41°26'18.27''N 2°14'41.47''E	**E**	**NW**	**5-10(26) SE/W**
STA	Sant Adrià del Besòs	41°25'18.82''N 2°14'03.66''E	**E**	**NW**	**5-10(19) S/W**
FORUM	Fòrum, Barcelona	41°25'02.41''N 2°13'56.61''E	**E**	**NW**	**5-10(19) S/W**
POBLE	Poblenou	41°24'13.36''N 2°12'58.57''E	**E**	**NW**	**5-10(19) S/W**
BOGA	Bogatell	41°23'36.09''N 2°12'23.27''E	**E**	**NW**	**5-10(19)S/W**
BCN	Barcelona	41°22'43.76''N 2°11'33.36''E	**E**	**NW**	**5-10(19)S/W**
	South of Barcelona				
EPLL	Airport of Barcelona	41°17'10.98''N 2°06'16.79''E	**S**	**E**	**5-10(17) E/N**
GAVA	Gavà	41°15'57.54''N 2°01'14.11''E	**S**	**E**	**5-10(17) E/N**
CSDFLS	Castelldefels	41°15'46.61''N 1°56'19.27''E	**S**	**E**	**5-10(17) E/N**
MORI	Cala Morisca	41°14'33.38''N 1°52'08.33''E	**SE**	**SE**	**5-10(18) E/S**
CAV	Cala de Vallcarca	41°14'22.27''N 1°51'58.95''E	**SE**	**SE**	**5-10(18) E/S**
VIL 01	Platja Far de Sant Cristòfol, Vilanova	41°12'53.53''N 1°44'15.67''E	**SE**	**S/SE**	**5-10(19) S/E**
VIL 02	Vilanova-Platja Ribes Roges	41°12'40.45''N 1°43'17.58''E	**S**	**S/SE**	**5-10(19) S/E**
VIL 03	Vilanova-Platja l'broig	41°12'12.85''N 1°41'08.34''E	**SE**	**S/SE**	**5-10(19) S/E**
SER	Platja Segur de Calafell	41°11'20.97''N 1°36'20.97''E	**S**	**S**	**5-10(19) S/E**
COM	Platja Comarruga	41°10'44.43''N 1°31'38.37''E	**S**	**S**	**5-10(19) S/E**
	Tarragona				
TOR	Torredembarra	41°08'19.94''N 1°24'18.19''E	**SE**	**S**	**10-20(21) E**
MOR	La Mora	41°07'40.52''N 1°20'45.75''E	**SE**	**SW**	**10-20(21) E**
PLA 1	Playa Larga North	41°07'45.95''N 1°19'16.11''E	**S**	**SW**	**5-15(21) W/S**
PLA 2	Playa Larga Centre	41°07'46.69''N 1°18'46.70''E	**S**	**SW**	**5-15(21) W/S**
PLA 3	Playa Larga Centre	41°07'43.71''N 1°18'10.95''E	**S**	**SW**	**5-15(21) W/S**
PLA 4	Playa Larga South	41°07'34.53''N 1°17'35.61''E	**S**	**SW**	**5-15(21) W/S**
SAV	Playa de la Savinosa	41°07'22.69''N 1°17'06.07''E	**S**	**SW/SE**	**5-15(26) E**

ARRA 1	l'Arrabasada North	41°07'11.94''N	1°16'41.97''E	**S**	**SE**	**5-15(26) E**
ARRA 2	l'Arrabasada South	41°07'05.35''N	1°16'05.35''E	**SE**	**SE**	**5-15(26) E**
MIR 1	Playa Miracle North	41°06'51.12''N	1°15'49.74''E	**S**	**SW**	**5-15 (21) W/S**
MIR 2	Playa Miracle Centre	41°06'46.74''N	1°15'29.02''E	**SE**	**SW**	**5-15 (21) W/S**
MIR 3	Playa Miracle South	41°06'36.44''N	1°15'13.52''E	**E**	**SW**	**5-15 (21) W/S**
PIN 1	La Pineda North	41°05'02.23''N	1°11'20.55''E	**SE**	**SE**	**5-10(19) N/S**
PIN 2	La Pineda Centre	41°04'35.24''N	1°10'58.95''E	**SE**	**SE**	**5-10(19) N/S**
PIN 3	La Pineda South	41°04'04.63''N	1°10'44.55''E	**E**	**SE**	**5-10(19) N/S**
SAL 1	Salou North	41°04'29.04''N	1°07'29.02''E	**S**	**SE**	**5-10(19) N/S**
SAL 2	Salou South	41°04'03.77''N	1°05'41.76''E	**S**	**SE**	**5-10(19) N/S**
CAM 1	Cambrils North	41°03'49.49''N	1°02'57.55''E	**S**	**SE**	**5-10(21)NW/SE**
CAM 2	Cambrils Centre	41°03'31.76''N	1°02'15.11''E	**S**	**SE**	**5-10(21) NW/SE**
CAM 3	Cambrils South	41°03'16.18''N	1°01'38.60''E	**S**	**SE**	**5-10(21) NW/SE**
	Southern Catalonia					
MIA N	Playa Miami	41°02'47.93''N	1°00'30.11''E	**SE**	**NW**	**10-20(34) W/S**
MIA S	Playa Miami	41°01'20.40''N	0°57'31.12''E	**SE**	**NW**	**10-20(34) W/S**
HOS	L'Hospitalet de l'Infant	41°01'20.40''N	0°57'31.12''E	**SE**	**NW**	**10-20(34) W/S**
ALM	L'Almadrava	40°56'16.24''N	0°51'26.72''E	**E**	**NW**	**5-10(22) NW/S**
LAM	L'Ametlla de Mar	40°53'36.60''N	0°48'58.62''E	**SE**	**NE**	**5-10(22) NW/S**
LOL	Gola de la Bassa Olles	40°47'07.94''N	0°42'36.77''E	**NE**	**NE**	**5-10(22) NW/S**
BF N	Bahía del Fangar North	40°47'35.58''N	0°44'35.56''E	**NE**	**NE**	**10-20 (29) S/NW**
BF FAR	Bahía del Fangar Faro	40°47'27.08''N	0°46'09.83''E	**NE**	**NE**	**10-20 (29) S/NW**
BF S 1	Bahía del Fangar South	40°46'58.81''N	0°45'10.65''E	**W**	**NE**	**10-20 (29) S/NW**
BF S 2	Bahía del Fangar South	40°46'39.32''N	0°46'11.05''E	**W**	**NE**	**10-20 (29) S/NW**
PLM	Playa la Marquesa	40°45'42.16''N	0°47'53.30''E	**NE**	**NE**	**10-15 (22) E**
RIU	Riumar Playa	40°43'51.07''N	0°50'14.41''E	**N**	**E**	**10-15 (22) E**
PEC	Playa los Eucaliptos	40°39'19.98''N	0°47'30.45''E	**E**	**E**	**10-15 (22) E**
TRA E	Playa Trabucador East	40°37'37.63''N	0°44'31.69''E	**E**	**S**	**10-15 (22) E**
TRA W	Playa Trabucador West	40°37'42.12''N	0°44'24.25''E	**W**	**S**	**10-15 (22) E**
PDE E	Playa del Delta East	40°35'33.17''N	0°42'41.99''E	**E**	**S**	**10-15 (22) E**
PDE W	Playa del Delta West	40°35'37.68''N	0°42'38.16''E	**W**	**S**	**10-15 (22) E**
SCR	Sant Carles de la Rápita	40°36'48.15''N	0°35'29.66''E	**S**	**S**	**5-10 (18)E**
VIN	Vinaròs	40°29'05.22''N	0°29'17.94''E	**E**	**S**	**5-10 (17)E**

Catalonia coast is a sedimentary coast with heterogeneous sand size grain distribution. The coast is mainly composed by medium and fine sandy beaches together with some coarse, mixture very coarse-coarse sand, coarse-fine sand and gravel ones. Thus 39% of beaches analysed were fine sand type, 27% medium sand, 20 % coarse sand, 10 % very coarse sand, 1 % very fine gravel and 7 % of sand types mixture.

The grain size distribution of the beaches of Northern Catalonia is composed from medium to very coarse sands, with the exception of beach of the Platja d'Aro (PAR) that contain high percentages of very fine gravels (Table S2). In Barcelona northern predominate coarse sands with only three beaches with very coarse sand as Blanes (BLA), Les Creus –Calella (CAL) and Barcelona (FORUM), while in Barcelona southern, coarse and medium sands predominate, although there are beaches as Vilanova beaches (VIL 03) that contain also very coarse sands and Platja Comarruga (COM) with fine sand.

By contrast, In the area of Tarragona, fine sands predominate with four beaches with medium sand as: Playa Larga Centre (PLA 3), Playa Larga South (PLA 4), Salou South (SAL 2), Cambrils

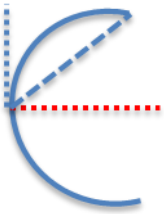
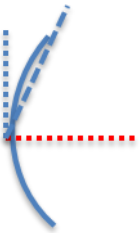
South (CAM 3). Similarly, in the south of Catalonia fine sands predominate, with the exception of the beach of Vinaròs (VIN), which has coarse sands and gravels.

Table S2. Sand grain size distribution and length of selected beaches.

Sand Beach	Length (m)	% Size category (mm)						
		Fine gravel	Very fine gravel	Very coarse sand	Coarse sand	Medium sand	Fine sand	Very fine sand
Code		**4-8**	**2-4**	**1-2**	**0.5-1**	**0.25-0.5**	**0.1-0.25**	**0.05-0.1**
PDV	191	9.8	21.0	32.5	15.6	12.6	8.1	0.2
PPS	546	13.4	4.2	0.2	19.4	38.7	22.5	1.6
RO1	808	0.0	0.0	0.2	7.0	73.1	19.7	0.0
RO2	1180	0.0	0.0	0.4	57.4	39.2	3.0	0.0
RO3	2490	0.0	0.0	0.2	2.8	79.1	17.8	0.0
RO4	649	0.0	0.0	0.6	74.7	17.0	7.6	0.2
ELG	2300	0.0	0.0	1.1	8.3	72.3	18.1	0.2
PPA	754	1.7	21.6	75.3	1.4	0.0	0.0	0.0
PAL	997	1.2	17.4	54.3	24.1	2.9	0.1	0.0
PAR	1950	6.85	60.43	25.9	6.82	0.0	0.0	0.0
BLA	2330	0.0	17.0	53.9	28.1	1.0	0.0	0.0
CAL	1300	0.0	7.8	79.0	12.5	0.5	0.3	0.0
CTA	1020	0.0	0.5	13.8	82.0	3.5	0.2	0.0
PMA	1830	0.0	0.9	18.2	75.1	5.8	0.0	0.0
MONT	1400	0.0	0.0	7.6	91.3	0.8	0.3	0.0
BADAL	993	0.0	3.5	37.0	58.3	0.8	0.4	0.0
STA	1380	0.0	0.0	0.0	85.34	14.66	0.0	0.0
FORUM	429	0.0	19.3	49.2	23.6	6.7	1.2	0.0
POBLE	377	0.0	0.7	2.9	86.6	9.4	0.4	0.0
BOGA	604	0.0	1.7	11.4	77.9	9.1	0.0	0.0
BCN	459	0.0	0.0	1.1	87.6	11.3	0.0	0.0
EPLL	1820	0.0	0.0	0.6	23.9	58.4	17.1	0.0
GAVA	2970	0.0	0.1	0.3	2.5	76.7	20.3	0.0
CSDFLS	5740	0.0	0.0	0.5	10.5	74.2	14.7	0.0
MORI	141	0.0	0.0	23.3	33.5	25.2	18.0	0.0
CAV	124	0.0	0.0	23.3	33.5	25.2	18.0	0.0
VIL 01	622	0.0	0.0	31.7	32.1	27.0	9.2	0.0
VIL 02	893	0.0	0.0	0.0	32.0	7.8	60.3	0.0
VIL 03	150	0.0	0.0	33.0	32.9	21.1	12.9	0.0
SER	1040	0.0	0.1	5.6	2.5	57.5	34.3	0.0
COM	611	0.0	0.0	0.0	50.0	0.0	0.0	50.0
TOR	2480	0.0	0.0	0.0	0.0	15.6	84.4	0.0
MOR	472	0.0	0.0	0.0	0.6	23.4	75.6	0.3
PLA 1	2540	0.0	0.1	0.0	0.3	23.3	76.2	0.1
PLA 2		0.0	0.1	0.0	0.1	30.7	68.9	0.1
PLA 3		0.0	0.2	0.3	0.6	56.0	42.9	0.0
PLA 4		0.0	0.2	0.0	0.3	80.0	19.4	0.0
SAV	350	0.0	0.3	0.3	3.2	18.7	77.5	0.0
ARRA 1	578	0.0	0.6	0.5	1.6	20.5	76.7	0.0
ARRA 2		0.0	0.9	0.4	1.7	42.6	54.4	0.0
MIR 1	1140	0.0	0.1	0.0	0.1	34.5	65.3	0.0
MIR 2		0.0	0.2	0.1	0.2	22.8	76.7	0.0
MIR 3		0.0	0.1	0.1	0.1	35.5	64.1	0.0
PIN 1	2520	0.0	0.9	1.1	1.8	10.6	85.6	0.0
PIN 2		0.0	0.2	0.1	0.5	4.3	94.9	0.1
PIN 3		0.0	1.0	0.3	0.5	3.9	94.3	0.1
SAL 1	2010	0.0	0.0	0.0	0.9	25.1	74.0	0.1
SAL 2	1750	0.0	5.9	13.3	5.5	41.9	33.4	0.0
CAM 1	224	0.0	0.2	1.5	19.3	17.9	57.1	3.9
CAM 2	469	0.0	0.0	0.1	0.8	11.5	87.2	0.4

CAM 3	432	0.0	0.3	1.7	25.8	47.5	24.5	0.2
MIA N	1930	0.0	0.0	0.6	2.2	26.0	70.9	0.3
MIA S	1320	0.0	0.1	5.4	14.6	51.8	27.8	0.2
HOS	2230	0.0	0.7	1.2	37.7	59.2	1.3	0.0
ALM	706	0.0	0.0	0.0	0.7	73.2	26.1	0.0
LAM	55	0.0	2.7	3.1	4.6	49.4	40.0	0.1
LOL	1490	0.3	0.5	0.1	0.8	6.3	91.7	0.2
BF N	2890	0.0	0.0	0.5	1.8	28.3	69.4	0.0
BF FAR	4390	0.0	0.0	0.0	0.6	21.6	77.6	0.1
BF S 1	2360	0.0	0.4	0.2	1.1	33.8	63.6	0.8
BF S 2		0.0	0.4	0.1	1.4	51.8	46.3	0.0
PLM	1530	0.0	0.0	0.0	0.6	53.1	46.3	0.0
RIU	2200	0.0	0.6	0.0	0.5	26.9	71.3	0.6
PEC	4550	0.0	0.0	0.0	1.0	31.1	67.9	0.1
TRA E	4370	0.0	0.0	0.0	0.0	14.0	86.0	0.0
TRA W		0.0	0.0	0.9	0.4	47.4	51.0	0.3
PDE E	1210	0.0	0.0	0.0	0.2	60.9	38.9	0.0
PDE W		0.0	0.0	0.2	1.1	40.9	57.8	0.1
SCR	500	0.0	0.0	0.3	0.8	12.9	85.8	0.1
VIN	189	10.1	7.0	20.9	33.5	23.7	4.7	0.0

Table S3. Catalonia coast beaches morphology criteria

Code Beach morphology		Description	Angle between the sides of the beach and imaginary central axis of the sampling point
Enclosed: Located in sheltered areas and protected by natural elements (capes) or artificial ones such as ports, breakwaters or dikes		  	**One or two sides 0°>X ≤ 45°**
Semi-Enclosed Supported beaches, their ends with a support element such as a breakwater, cape, industrial or marina sports harbour but not protected at all			**One or two sides 45°< X <90°**

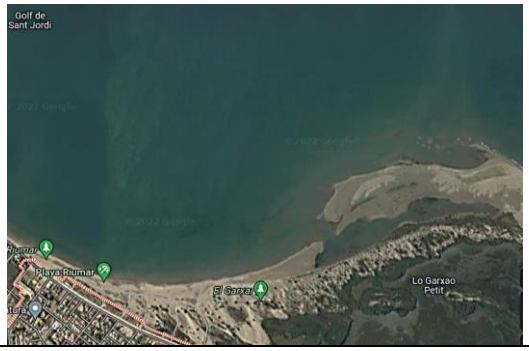
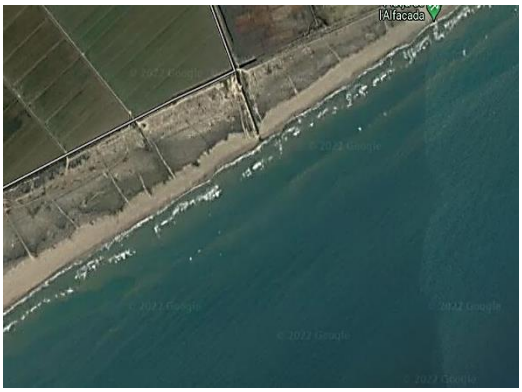
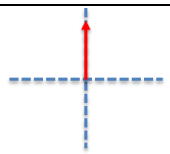
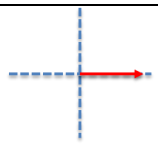
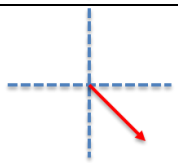
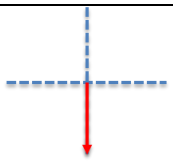
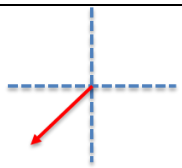
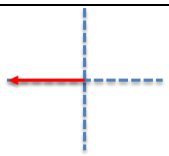
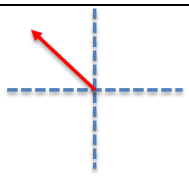
			**One or two sides** **45°< X <90°**
Open Totally unprotected beaches without any cape or breakwater at their ends			**Both sides** **X = 90°**

Table S4. Currents and wind directions nomenclature

Direction	
N (0°)	
NE (45°)	
E (90°)	
SE (135°)	
S (180°)	
SW (225°)	
W (270°)	
NW (315°)	

From table S5 to S9 were elaborated with anthropogenic pressures, slope and shoreline morphology for beaches that showed low and high MPs concentrations for MPs<0.5 mm with values >150 MPs/kg, by each region.

Table S5. Beaches with lowest concentration in entire Catalonia Coast zone, morphology, anthropogenic sources and profile

Beach Code	Beach profile (slope of entrance to the seawater)	Morphology type criteria	Close anthropogenic sources influence within 7-12 km radius (Sources Nº)
PPA	Strong	Open	Semi-urbane (0 sources)
PAR	Very strong	Open	Urbane, WWTP discharges principal emissary away from shoreline 350 m and 24 m depth and 35000 m^3/day, Ridaura river discharges. (2 sources)
CAL	Strong	Open	Urbane, WWTP discharges emergency emissary away from shoreline 780 m and 8 m depth 40.000 m^3/day, little streams, pluvial emissaries (13 sources)
MOR	Strong	Enclosed	Semi-urbane, little streams, pluvial emissaries, WWTP discharges principal emissary 1600 m away from shoreline and 32 m depth and emergency emissary 1200 m and 27 m depth 8292 m^3/day. (4 sources)

Table S6. Beaches with high concentration in Catalonia Northern zone, morphology, anthropogenic sources and profile

Beach Code	Beach profile (slope of entrance to the seawater)	Morphology type criteria	Close anthropogenic sources influence within 7-12 km radius (Sources Nº)
PPS	Smooth	Enclosed	Urban, little streams, pluvial emissary, Port activities, WWTP discharges principal emissary away from shoreline 1275m and 42 m depth and 2625 m3/day. (5 sources)
R01	Smooth	Semi-enclosed	Urban, little streams, pluvial emissary, WWTP discharges principal emissary away from shoreline 1532m and 18 m depth and 25000 m^3/day. (5 sources)
R04	Very strong	Enclosed	Urban, port activities, pluvial emissaries. (7 sources)

ELG	Strong	Semi-enclosed	Semi-urbane, port activities, Ter River discharges, little stream, pluvial emissary. (4 sources)
PAL	Very strong	Semi-enclosed	Urban, little stream, port activities. (2 sources)

Table S7. Beaches with high concentration in Barcelona Northern and Southern zone, morphology, anthropogenic sources and profile

Beach Code	Beach profile (slope of entrance to the seawater)	Morphology type criteria	Close anthropogenic sources influence within 7-12 km radius (Sources Nº)
STA	Very strong	Open	Urban, Besos river discharges, WWTP discharges principal emissary 2830 m away from shoreline and 48 m depth, emergency emissary 580 m away from shoreline and 14 m depth, 525000 m^3/day. (2 sources)
FORUM	Strong	Semi-enclosed	Urban, port activities, Besos river discharges, WWTP discharges principal emissary 2830 m away from shoreline and 48 m depth, emergency emissary 580 m away from shoreline and 14 m depth, 525000 m^3/day. (3 sources)
BOGA	Very strong	Semi-enclosed	Urban, pluvial emissaries. (2 sources)
CSTDFLS	Strong	Semi-enclosed	Semi-Urban, pluvial emissaries, port activities. (9 sources)
MORI	Very strong	Enclosed	Semi urban, industrial port activities, WWTP discharges principal emissary 0 m away from shoreline and 0 m depth, 160 m^3/day. (2 sources)
CAV	Very strong	Enclosed	
Vil 01	Moderate	Enclosed	Urban, port activities, little stream, WWTP discharges principal emissary 1950 m away from shoreline and 15 m depth, 25000 m3/day. (3 sources)
Vil 02	Moderate	Enclosed	

Table S8. Beaches with high concentration in Tarragona zone, morphology, anthropogenic sources and profile

Beach Code	Beach profile (slope of entrance to the seawater)	Morphology type criteria	Close anthropogenic sources influence within 7-12 km radius (Sources Nº)
PLA 3	Moderate	Open	Semi-urban, WWTP discharges emergency emissary 780 m away from shoreline and 21 m depth, 8.292 m^3 /day. (1 source)
ARRA 1	Moderate	Enclosed	Semi-urban, WWTP discharges emergency emissary 710 m away from shoreline and 22 m depth, 8.292 m^3 /day, little streams. (2 sources)
ARRA 2			
MIR 1	Very strong	Enclosed	Urban, WWTP discharges emergency emissary 930 m away from shoreline and 24 m depth, /day, 35.000 m^3 day, pluvial emissaries. (2 sources)
MIR 2		Open	
MIR 3		Enclosed	Urban, WWTP discharges emergency emissary 930 m away from shoreline and 24 m depth, 35.000 m3/ day, pluvial emissary, industrial port activities. (3 sources)
PIN 1	Strong	Enclosed	Urban, industrial port activities, Francolí river discharges, pluvial emissary, WWTP discharges principal emissary 2400 m away from shoreline and 32 m depth, 15833 m^3/day, emergency emissary 950 m away from shoreline 20 m depth. (5 sources)
PIN 2		Open	Urban, industrial port activities, Francolí river discharges, pluvial emissaries, WWTP discharges principal emissary 2400 m away from shoreline and 32 m depth, 15833 m3/day, emergency emissary 950 m away from shoreline 20 m depth. (6 sources)
PIN 3		Enclosed	Urban, industrial port activities, Francolí river discharges, WWTP discharges principal emissary 2400 m away from shoreline and 32 m depth, 15833 m3/day, emergency emissary 950 m away from shoreline 20 m depth. (4 sources)
SAL 1	Moderate	semi-enclosed	Semi-urban, port activities, pluvial emissaries, WWTP discharges 47500 m^3/day: principal emissary 1300 m away from shoreline and 30 m depth, emergency emissary 500 m away from shoreline 7 m depth, emergency emissary 460 m away from shoreline 7.5 m depth, emergency emissary 350 m away from shoreline 11 m depth, emergency emissary 1350 m away from shoreline 18 m depth principal emissary 1550 m away from shoreline and 18 m depth. (18 sources)

SAL 2	Strong	Open	Urban, pluvial emissaries, WWTP discharges 47500 m^3/day: principal emissary 1550 m away from shoreline and 18 m depth; WWTP discharges 25000 m^3/day: emergency emissary 860 m away from shoreline 12 m depth, emergency emissary 850 m away from shoreline 12 m depth, principal emissary 2000 m away from shoreline 15 m depth. (10 sources)

Table S9. Beaches with high concentration in Catalonia Southern zone, morphology, anthropogenic sources and profile

Beach Code	Beach profile (slope of entrance to the seawater)	Morphology type Criteria	Close anthropogenic sources influence within 7-12 km radius (Sources Nº)
LOL	Moderate	Open	Semi-urban, agricultural runoff, little stream, WWTP discharges principal emissary 1300 m away from shoreline, 3200 m^3/day, 6 m depth. (3 sources)
BF N	Moderate	Open	Natural, WWTP discharges principal emissary 1300 m away from shoreline, 3200 m3/day, 6 m depth. (1 source)
BF F			Natural, WWTP discharges principal emissary 1300 m away from shoreline, 3200 m3/day, 6 m depth. Ebro River plumes discharges influence. (2 sources)
PLM	Moderate	Open	Natural, Ebro River mouth (WWTP discharges on Ebro River 5000 m^3/day), agriculture runoff, little stream. (3 sources)
RIU		Semi-enclosed	Semi-urban, Ebro River mouth (WWTP discharges on Ebro River 5000 m^3/day), agriculture runoff, little stream. (3 sources)
PEC		Open	Natural, Ebro River mouth (WWTP discharges on Ebro River 5000 m^3/day), agriculture runoff, little stream. (4 sources)
TRA E		Open	Natural, Ebro River mouth (WWTP discharges on Ebro River 5000 m^3/day). (1 source)
PDE E		Open	Natural, Ebro River mouth (WWTP discharges on Ebro River 5000 m^3/day), agriculture runoff. (2 sources)

CHAPTER 2-MICROSPLASTICS IN BIOTA

Levels of microplastics and their characteristics in molluscs from North-West Mediterranean Sea: Human intake

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Numerous sub-samples of 50-70 g of only soft tissues for samples were prepared and considered for analysis.

Table A1. Weight and individuals analysed by sample type

Code	Scientific name	g wet weight/ind.	Nº of organisms analyzed	Total weight analysed (g)
WCnd(S)	*Donax trunculus*	0,263	383	**100,7**
WCd(S)	*Donax trunculus*	0,263	387	**101,8**
S (S)	*Bolinus brandaris*	3,53	68	**241,8**
RCnd (S)	*Ensis silicua*	5,11	59	**300,4**
Md (S)	*Mytilus galloprovincialis*	2,37	88	**209,4**
Mnd (S)	*Mytilus galloprovincialis*	3,37	62	**210,5**
FCd (S)	*Tapes decussatus*	2,09	74	**154,6**
BOnd (S)	*Crassostrea gigas*	12,32	19	**231,4**
Bod (S)	*Crassostrea gigas*	9,78	28	**270,0**
Mnd (N)	*Mytilus galloprovincialis*	2,9	223	**645,7**
S(M)	*Bolinus brandaris*	5,42	55	**296,6**
WC(N)	*Donax trunculus*	0,3065	373	**114,3**
WC (M)	*Donax trunculus*	0,2195	489	**107,4**
Total			***2310***	***2985***

Table A2. Molluscs production subzones coordinates in Catalan Coast and anthropogenic activities associated with.

Molluscs production sub-zones in Catalan Coast (FAO 37-1)	Coordinates (From)	Coordinates (to)	Samples	Anthropogenic activities for plastic pollution and MPs concentration (a,b)
CAT-1-01	40° 31’ 50´´ N 0° 31´ 00´´ E	40° 36’ 53.5´´ N 0° 35´ 50´´ E	Md (S) Mnd(S) FCd (S) RCnd (S) BOnd (S) BOd (S)	1.-Aquaculture, 2.-Agricultural runoffs 3.-WWTP effluents discharges (6,300 m^3/d flow), submarine outflow up to 500 m. 4.-Touristic 5.- Urban runoffl
	40° 31’ 50´´ N 0° 31´ 00´´ E	40° 34’ 45´´ N 0° 35´ 47.53´´ E		
CAT-1-02	40° 36’ 53.5´´N 0° 35´ 50´´ E	40° 34’ 45´´N 0° 35´ 47.53´E		
CAT-1-03/01	40° 36’ 53.5´´N 0° 35´ 50´´E	40° 34’ 45´´N 0° 35´ 47.53´´E	WCd (S)	1.-Ebro River discharges 5.2 *10^7 m^3/d (receiving WWTP effluents, urban and agricultural runoff). Ebro surface water represents an input of 2.14 × 10^9 MPs/year to the Mediterranean Sea, The Ebro River discharges affect surrounding environment as estuarine sediments, sandy beaches (northern edge) and seawater surface with MPs concentrations higher concentration in three environment matrices:422 ± 119 MPs/kg d.w. in sediments, 2052 ± 746 MPs·/kg d.w. in sands and 3.5 ± 1.4 MPs/m3 in water), being the fibres the predominant morphology. (Simón-Sánchez et al., 2019) 2.-Agricultural runoffs 3.-WWTP effluents discharges (3,000 m^3/d flow), submarine outflow up to 500 m.
	40° 36’ 53.5´´N 0° 35´ 50´´E	40° 39’ 12´´N 0° 46´ 54´´E		
CAT 04-02	40° 44’ 36´´N 0° 49´ 12´´E	40° 49’ 28´´N 0° 44´ 33´´E	WCnd (S)	
	40° 47’ 31´´N 0° 44´ 07´´E	40° 49’ 28´´N 0° 44´ 07´´E		
CAT 1-09	40° 59’ 22´´N 1° 23´ 45.5´´E	40° 59’ 22´´N 1° 43´ 51´´E	WC (C) S (C)	1.-Three WWTP effluents discharges (25,500; 18,000; 16,200 m^3/d flow), submarine outflows up to 2-3km. 2.-Touristic 3.-Urban runoffs 4.-Microplastics concentration in surrounding areas was <0.4 MPs/m^2 at up to 237 m distance from the coast. (de Hann et al., 2022)
CAT 1-14	41° 23’ 18.7´´N 2° 16´ 51.8´´E	41° 23’ 18.7´´N 2° 16´ 51.8´´	Mnd (N)	1.-Industrial 2.- Urban runoff 3.- WWTP effluents discharges 525,000 m^3/d submarine, outflow up to 3.6 km, two outfalls 4.-Microplastics concentration in Barcelona city coast and marginal zones ranged from 4.07 to 5.62 MPs/m^2 at up to 205 m distance from the coast (de Hann et al., 2022). At 4 km from shore microplastics concentration ranged from 0.44 to 1.99 MPs/m^3 (de Hann et al., 2019)
CAT 1-15	41° 23’ 18.7´´N 2° 16´ 51.8´´E	41° 23’ 18.7´´N 2° 26´ 44.4´´E		
CAT 1-21	41° 58’ 12.4´´N 3° 13´ 56.6´´E	42° 3’ 18´´N 3° 13´ 56.6´´	WC (N)	1.- Urban runoff
CAT 1-22	42° 3’ 18´´N	42° 17’ 7.5´´N		

	3° 13´ 56.6´´E	3° 13´ 56.6´´E		2.- Three WWTP effluents discharges 25,000; 21,000 17,000 m^3/d submarine, outflows up to 1.5 km. 3.- Touristic 4.-Two rivers' discharges (Muga and Fluviá with average flow of 3.34 and 10.67 m^3/s respectively) in Roses Gulf (de Hann et al., 2019). 4.- Microplastics concentration in Roses Gulf coast and marginal zones ranged from 0.15 to 0.009 MPs/m^2 and 0.59 to 0.04 MPs/m^3 at up to 4km distance from the coast (de Hann et al., 2019).

(a) Agencia Catalana del Agua 2022. Available from: https://aca.gencat.cat/es/inici/index.html. Last accessed March 2022

(b) GENCAT 2022. Available from: https://territori.gencat.cat/ca/06_territori_i_urbanisme/. Last accessed March 2022

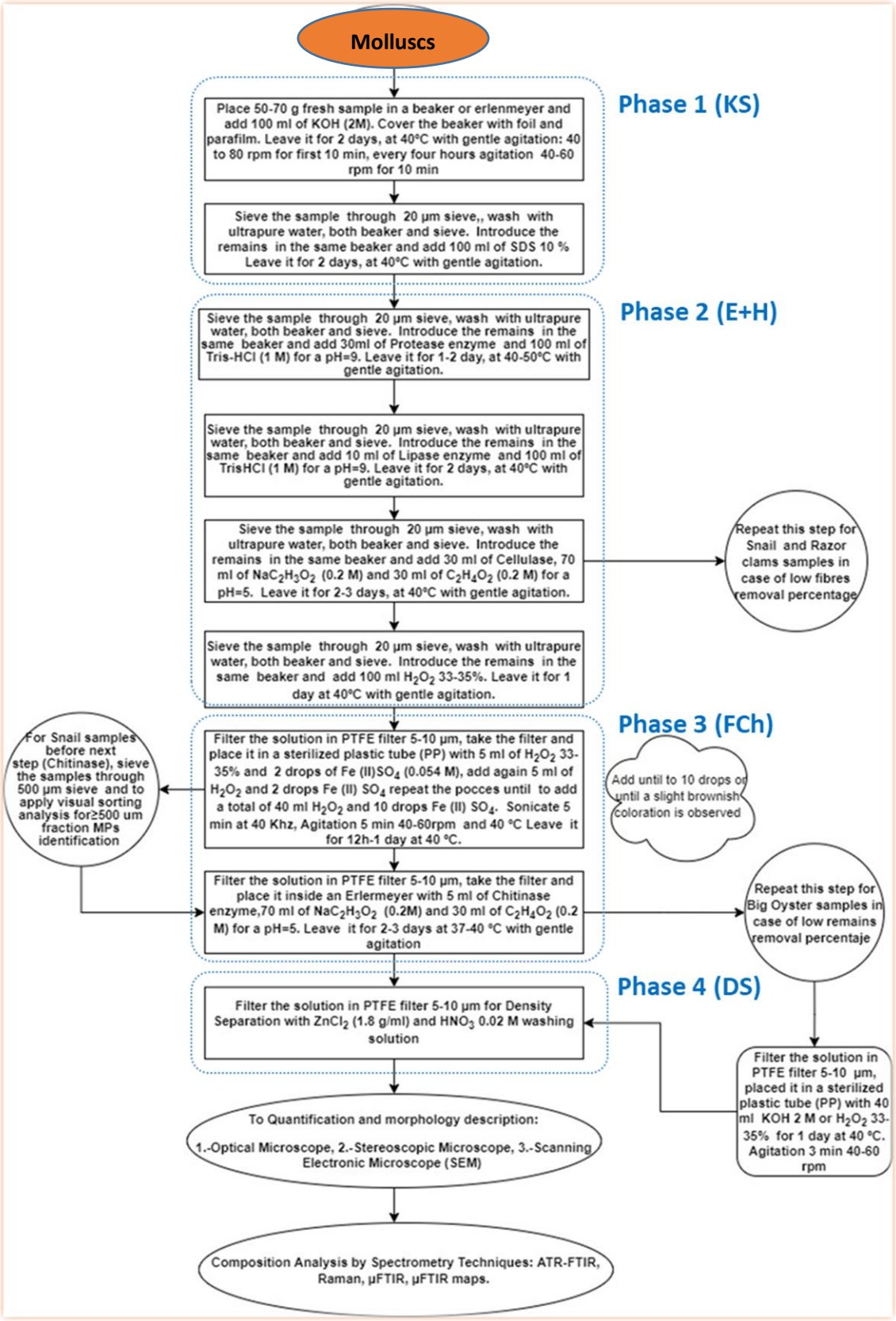

Figure A1. General methodology for microplastics (MPs) extractions and analysis from molluscs samples

Additional information for each phase:

a. After samples washing, the remains were introduced (as dray as possible) to beaker or Erlenmeyer
b. The density separation steps are detailed in figure A2:

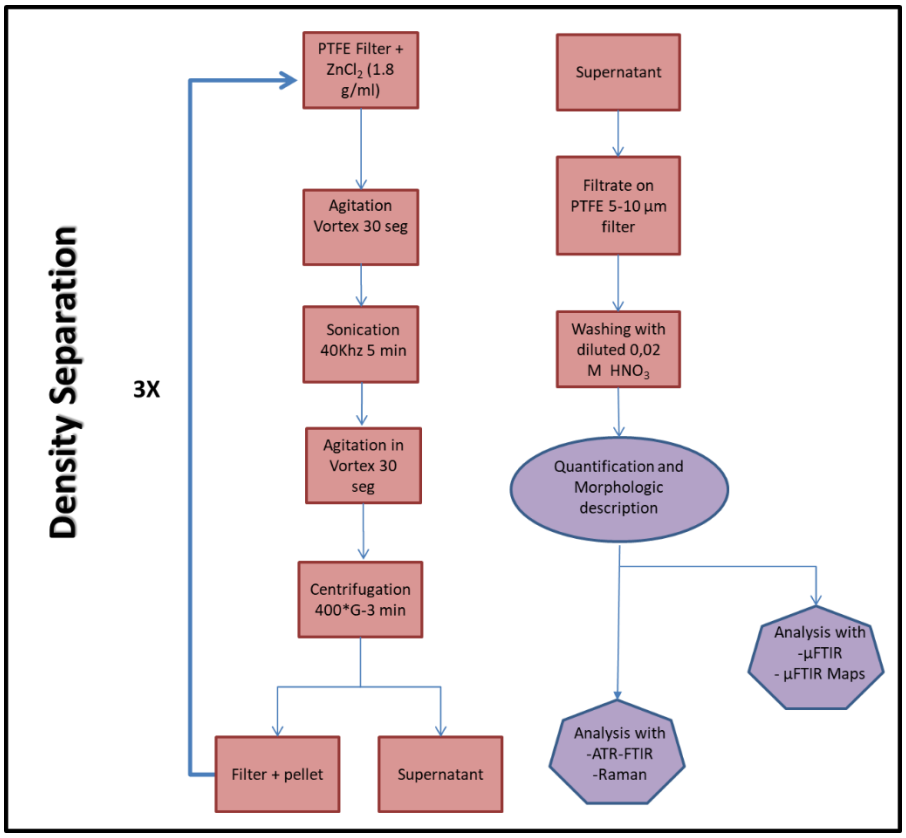

Figure A2. Steps for microplastics (MPs) extractions by Density Separation from molluscs samples (1 day duration)

c. During density separation procedure, all remains from the filters were removed as much as possible for close contact with $ZnCl_2$. At final centrifugation step, the pellet and filter are checked for microplastics presence specially fibres.
d. The procedure for washing samples was as follow: the 20 µm (20 cm diameter) sieve was washed three time, at the beginning, between samples and final of the processes with tap water high pressure for 5 min, after that with distilled water (MilliQ® water) high pressure for 3 min and finally with ultrapure water high pressure for 3 min. The clean sieves were wrapped in foil paper for storage in clean fume hood.
e. Before washing the samples on 20 µm sieve, the control was washed at the beginning to check the sieve washing efficiency.
f. In case snail and oyster samples, the density separation could be with NaCl 1.2 g/ml instead $ZnCl_2$in case very fine calcium carbonate remains.
g. The buffer concentrations were based on www.sigmaaldrich.com/life-science/core-bioreagents/biological-buffers/learning-center/buffer-reference-center.html
h. For buffer pH adjustment, NaOH or KOH concentrate solutions were used

Phase completed by Samples:

The use of substrate specific enzymes for digesting high fresh quantity bivalves tissues showed advantages in reducing organic matter. In agreement with many authors as Catarino et al., (2017), Courtene-Jones et al., (2017); Karlsson et al., (2017) and Sinja et al., (2018), the enzymes use in digestion protocols for microplastics extraction from mollusks is highly recommendable due they are relatively inexpensive compounds, mostly supplied in liquid form and therefore easy to handle with a low hazard for technician and not visual alterations to microplastics particles. The use of the enzyme Chitinase was useful for the degradation of fresh tissue adhered to shell remains, especially in subsamples of snails and oysters.

For wedge clams, in the majority samples the digestion processes only reached up to the phase 2, E-H, only sample of wedge clams depurated from south WCd (S), reached up phase 3, F (Fenton processes). For snails, the digestion processes reached up to the phase 3, F-Ch and additional step density separation DS. For razor clam not depurated from south, RCnd (S), the digestion processes reached up to phase 2, E-H and phase 3, F (only Fenton processes). For fine clams depurated from south, FCd (S), reached up all phases and additional step: phase 2 E-H, phase 3 F-Ch, phase 3 F (only Fenton processes), DS.

For big oyster not depurated from southern, BOnd (S), the digestion processes reached up to phase 3, F-Ch, phase 3, F, Chitinase enzyme followed by KOH 2M steps, Ch-K and DS. In big oyster depurated from southern, BOd (S), reached up to phase 2, E-H, phase 3, Ch-K and DS. By in contrast for mussels group, mussels from south depurated, Md (S) reached up to phase 2, E-H, phase 2 with only Lipase enzyme E (Lipase) and phase 1, K-S. Mussel no depurated in same zone Mnd (S) reached phase 2 with Cellulase enzyme, E (Cellulase) and phase 3, F-Ch. Ultimately, mussels from northern and no depurated, Mnd (N), reached up to phase 2, E (Lipase), phase 2 E-H, phase 3, F-Ch.

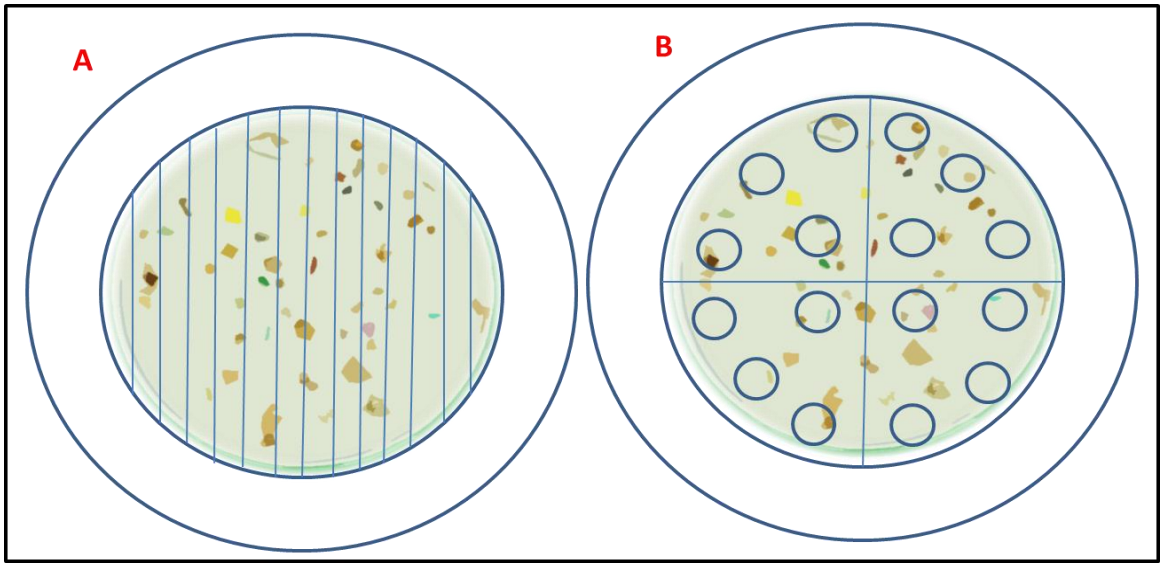

Figure A3a. Filters division for Microplastics identification and measures: A. by columns, B. by quadrants and random fields. Modified from Paul-Pont et al., (2018).

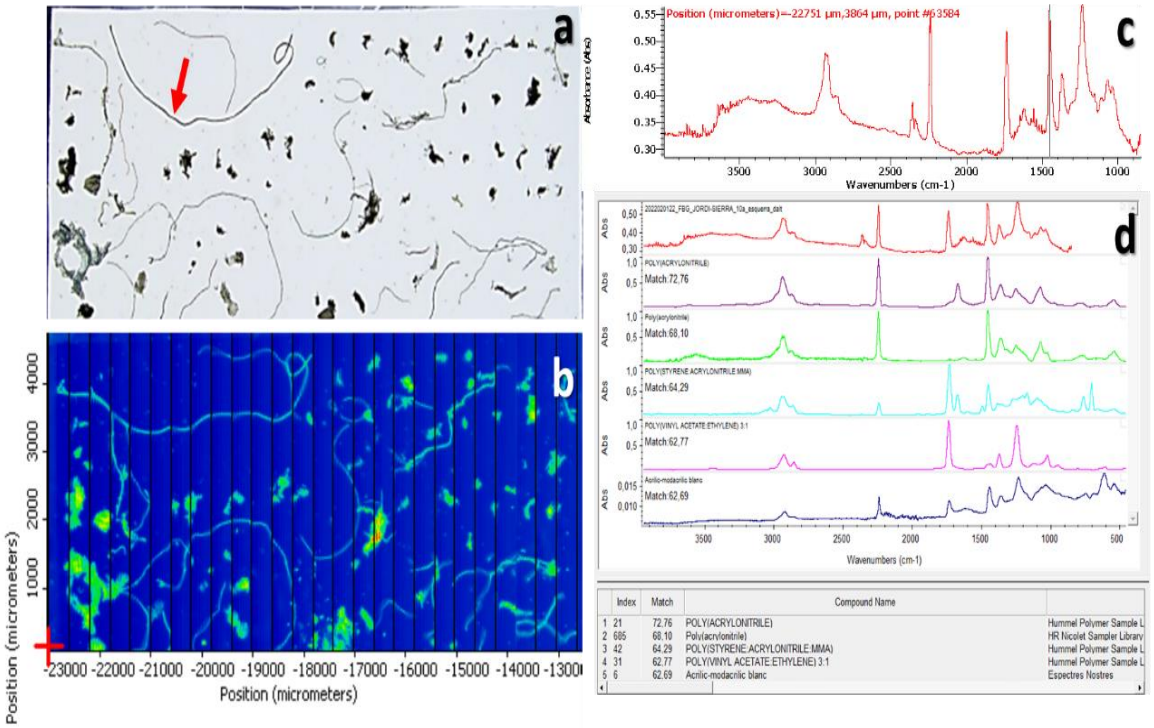

Figure A3b. Fibres and fragments isolated from a mussels sample (a); IR map (b); IR spectrum of an acrylic fibre (c); and the comparison with different spectrum libraries (d).

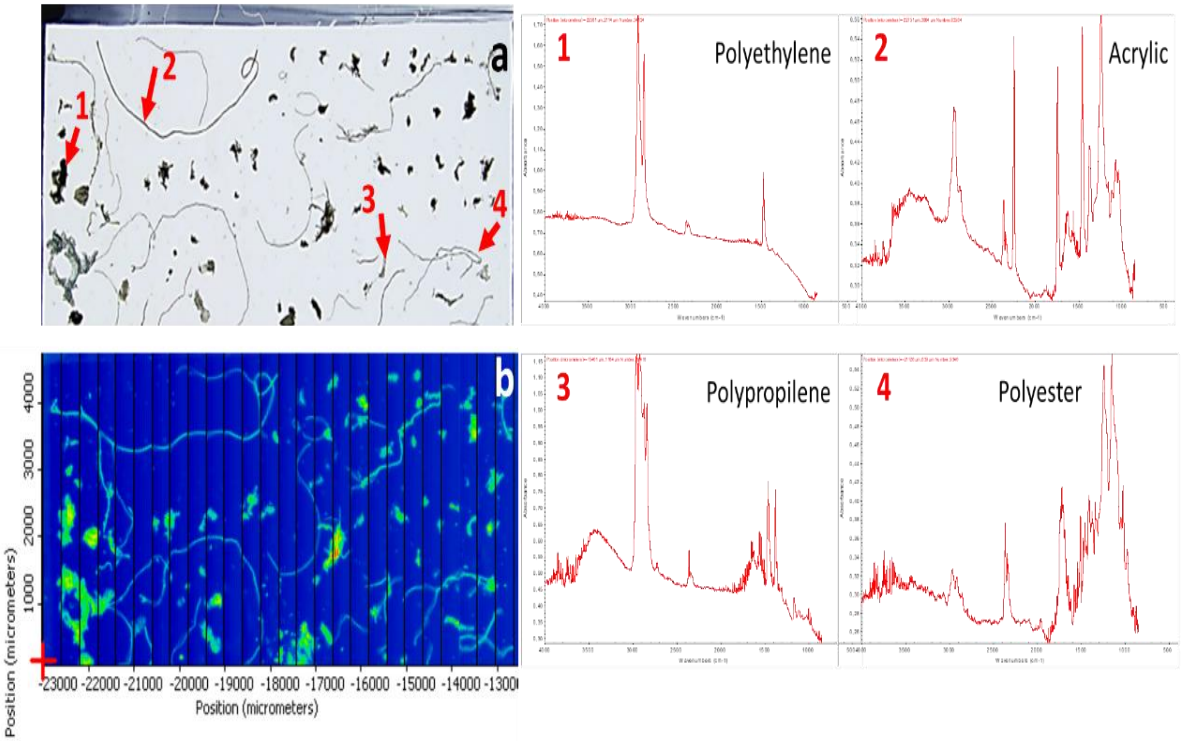

Figure A3c. Fibres and fragments isolated from a mussel sample (a); IR map (b), IR spectrum of polyethylene fragment (1), an acrylic fibre (2), a polypropylene fragment (3) and a polyester fibre (4).

Recovery rates:

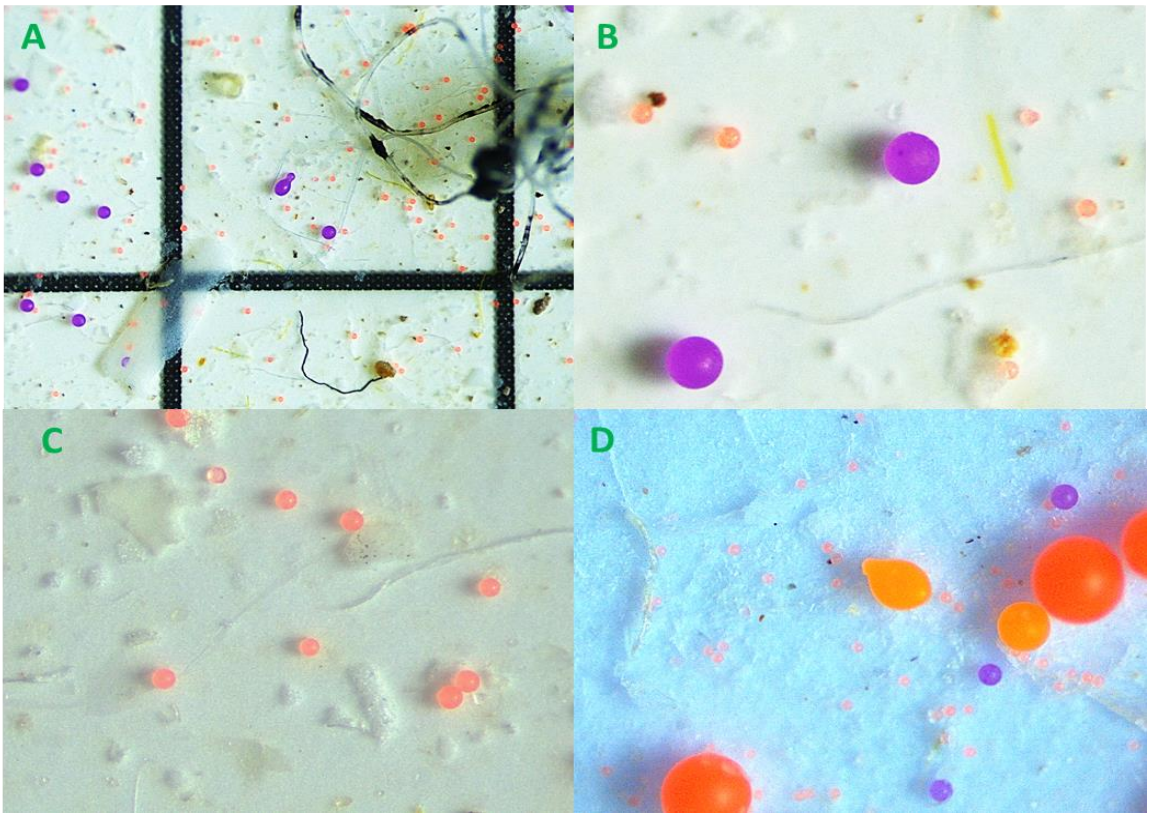

Figure A4. Polyethylene (PE) spheres after recovery rates analysis up to phase 3 in molluscs soft tissues: A. 53 and 125 um spheres 20 X. B. 53 and 125 µm spheres 40 X. C. 53 µm spheres 80 X. D. 53,125,250, 425 µm mixed 12.5 X.

Spectra FTIR identification criteria

For temporary unidentified spectra with libraries, other criteria for spectral analysis was to applied:

a.- The identification of the IR spectra was supported by comparing spectra with a library of own elaboration taking into account environmental weathered particles analyzed, more than 80 IR spectra have been made. Apart from conventional plastics, elastomers, resins and chemical additives were included in the library. Only the spectra whose peaks were 60-70% or more coincident with the peaks of the reference spectra were identified.

b.- The rejected items were analysed according their characteristics absorption band or vibration modes of each polymer chemical grouping bonds for frequencies ranging mostly from 3650-500 cm-1(Campbell et al., 2000; Coates, 2000; Primpke et al., 2018): 1480-1430 cm-1 for C-C aromatic ring stretching, 1790-1700 cm-1 for double binding C-O stretching, 2980-2780 cm-1 for C-H stretching of aliphatic and 3150-3030 cm-1 of aromatics. For aliphatic organohalogen, natural and synthetic cellulose identification, frequencies from 1150 to 550 cm-1 and from 3600 to 3000 cm-1 were also evaluated. The rejected items were counted as the definitive unidentified category.

Microplastics maps on CaF_2 slides:

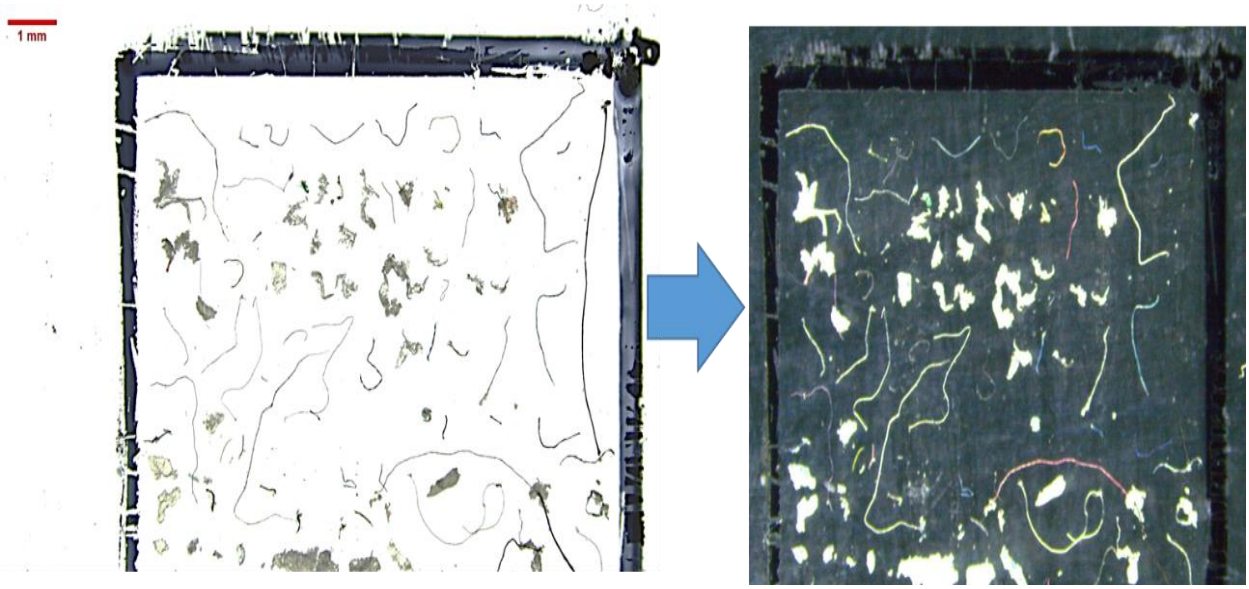

Figure A5. FTIR maps of MPs found in an oyster sample

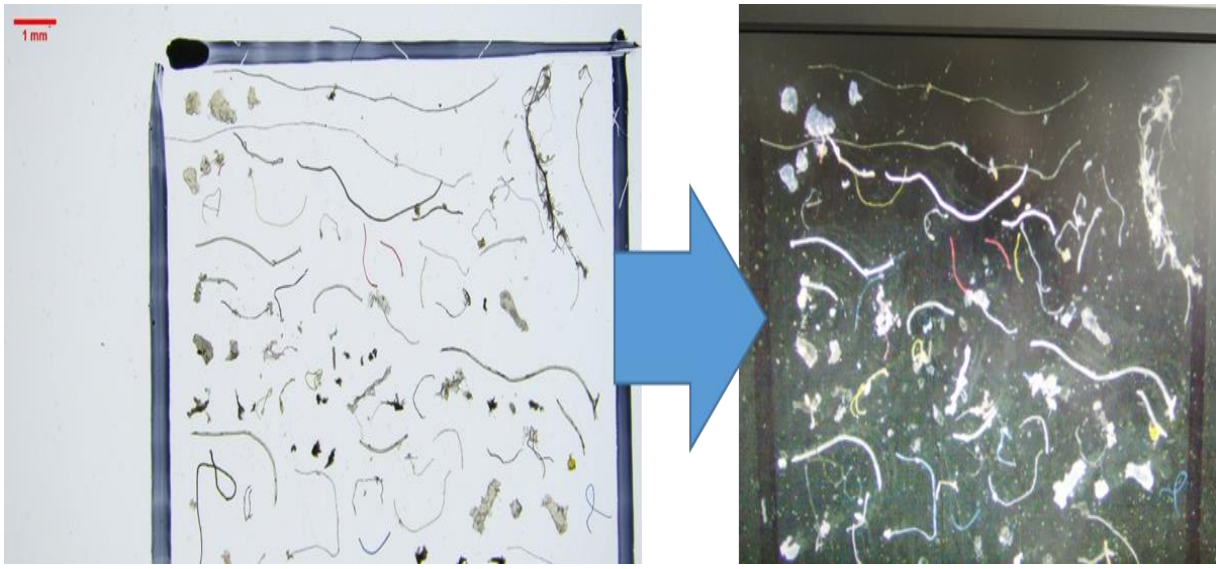

Figure A6. FTIR map of MPs found in a mussel sample

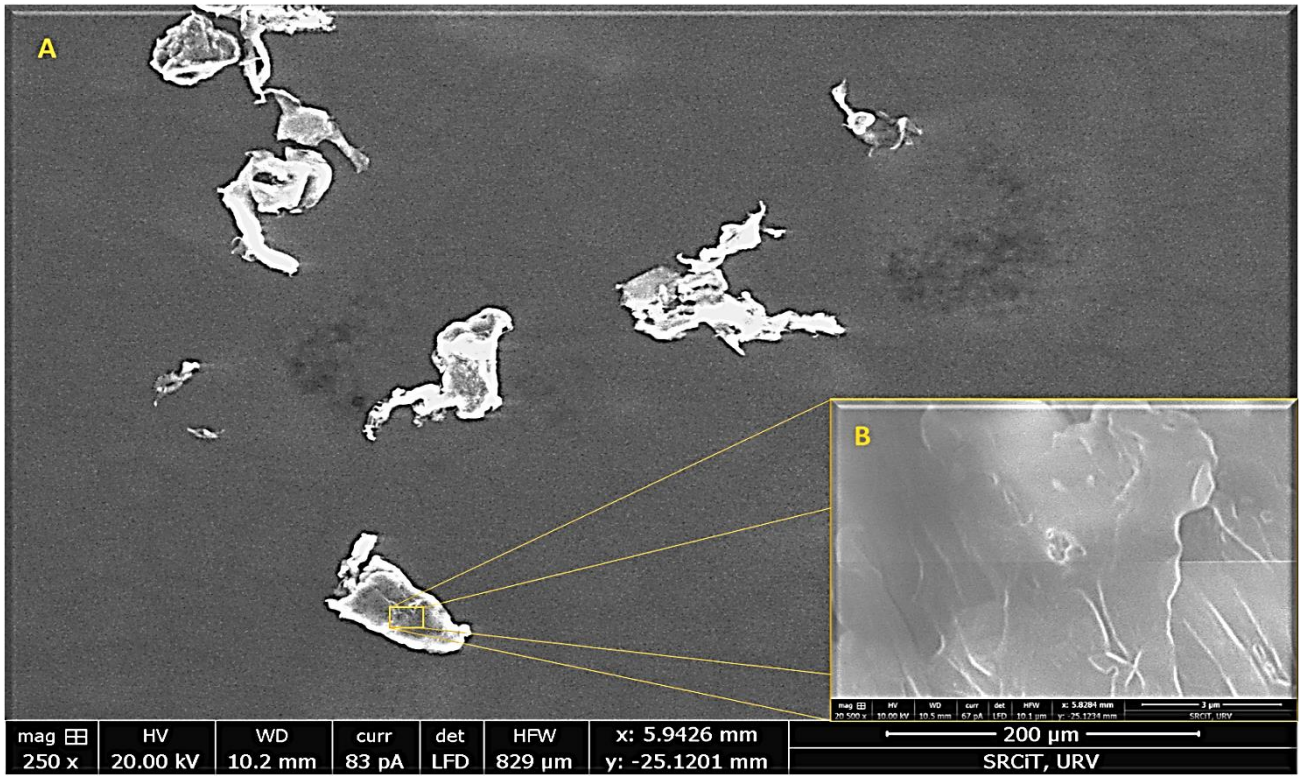

Figure A7a. Scanning electron microscope (SEM) image of PE fragment surface found in mussels samples (Note the cracks and cavities). Magnification: A. 250x, B. 20500x (Scale 3 µm)

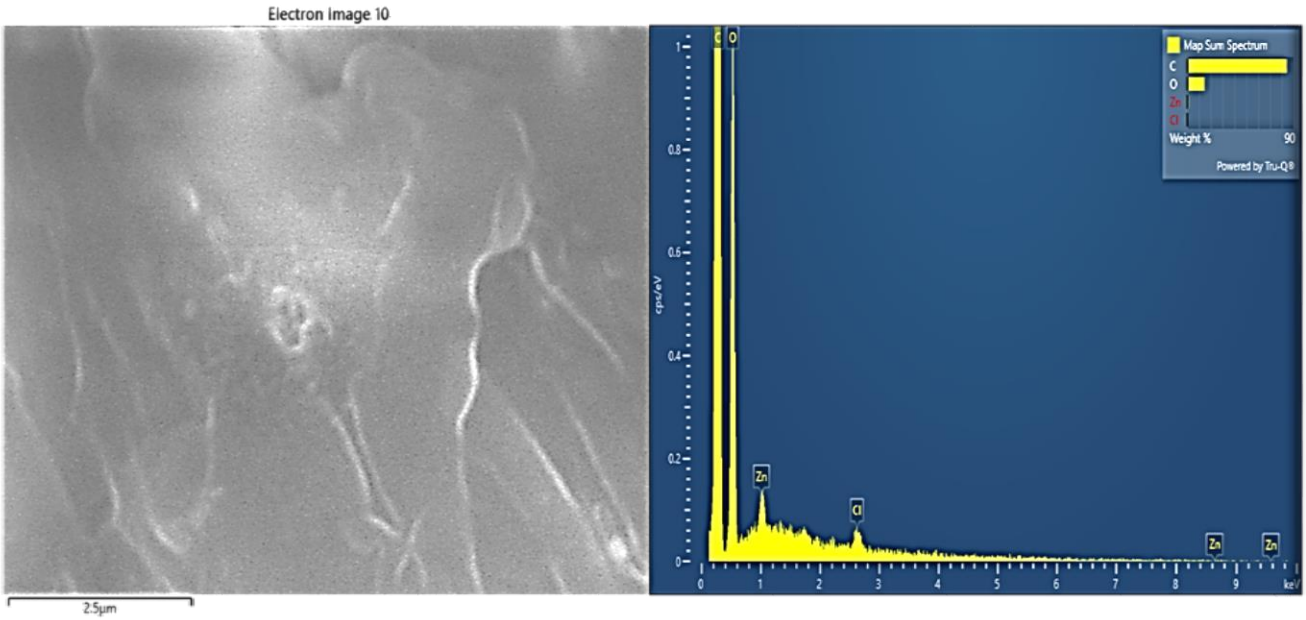

Figure A7b. Scanning electron microscope (SEM) image and X-rays analysis of PE fragment surface found in mussels samples (Note the cracks and cavities).

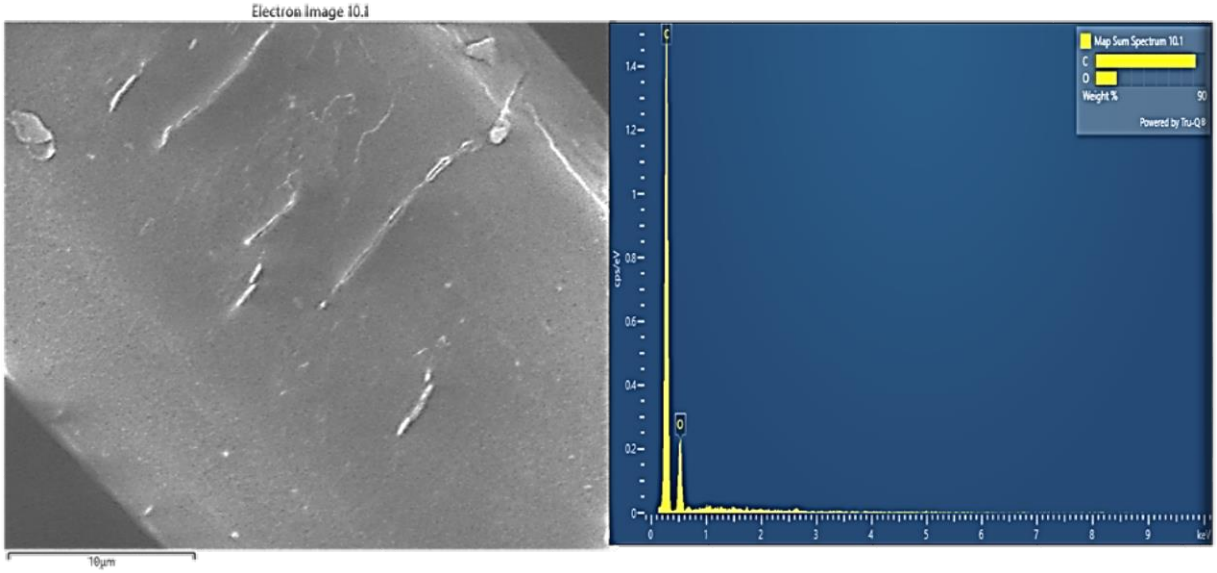

Figure A7c. Scanning electron microscope (SEM) image and X-rays analysis of fibre surface found in mussels samples (Note the cracks).

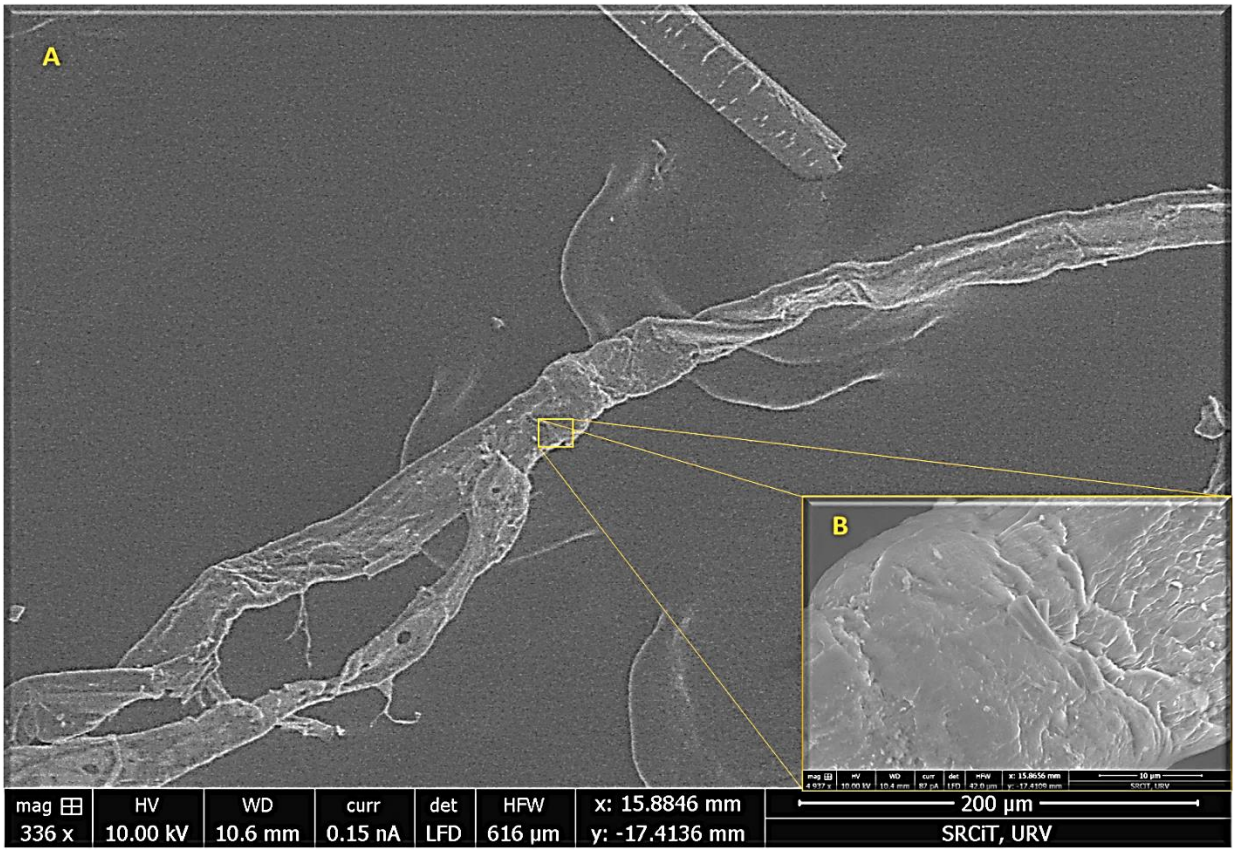

Figure A7d. Scanning electron microscope (SEM) of fibre surface found in mussels samples (Note the loss of material). Magnification: A. 336x, B. 4937x (Scale 10µm)

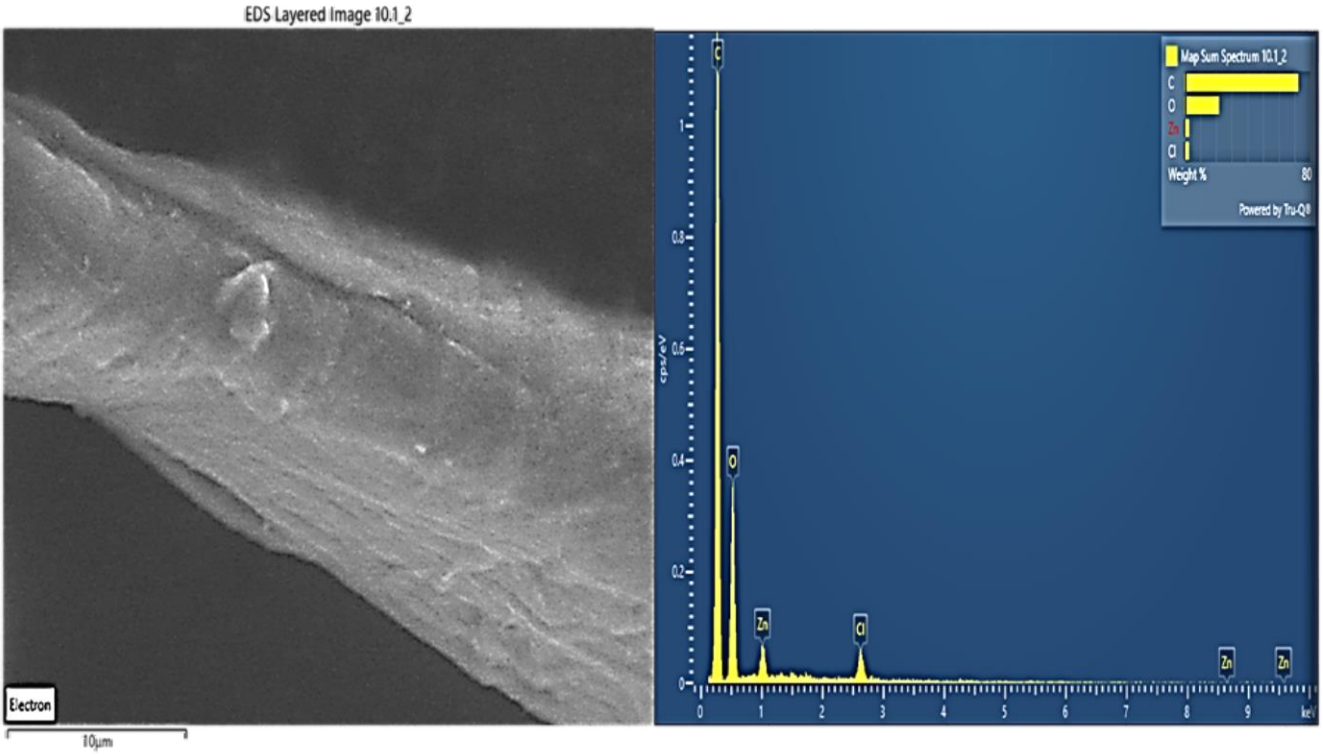

Figure A7e. Scanning electron microscope (SEM) image and X-rays analysis of fibre surface found in mussels samples.

Table A3. Molluscs groups and particles number analysed

Mollusc group	Nº organisms analysed	Organism analysed (g)	Microplastic items analysed for size and composition
Wedge Clams (WC)	1632	424.2	194
Fine clams (FC)	74	154.6	51
Razor clams (RC)	59	300.4	88
Mussels (M)	373	1065.6	755
Big Oysters (BO)	47	501.4	245
Snails (S)	123	537.6	127
Total	**2310**	**2984.8**	**1460**

Table A4. Plastics sources and MPs concentration in worldwide regions

Region	Plastics Sources	MPs environmental concentrations	References
Spain	High urban and industrial pressure on the surroundings. Human population, tourism, and fishing port activities. Proximity to a sewage treatment plant.	-	Reguera et al., (2019)
China (North China-Quingdao) (South China-Xiamen)	Municipal waste, sewage discharges, industrial sources, river discharges (Pearl River along Guangzhou City-South China).	High microplastics concentration in sediments,1669 MPs/kg d.w. with high proportion of Polypropylene.	Li et al., (2016), Fang et al., (2019) Ding et al., (2020)
South Korea	Use of high number of plastics materials (EPS bouys) in aquaculture industry.	-	Cho et al., (2019)
China (Jiaozhou Bay)	High coastal population density along the bank, more than ten (10) rivers discharges into Jiaozhou Bay.	The concentration of microplastics in the bay seawater ranged between 20 MPs/m³ and 120 MPs/m³. In sediment samples the concentration were 7 MPs/kg d.w. and 25 MPs/kg d.w.	Zhang et al., (2022) Zheng et al., (2019)
China (Along the coastal waters)	China has the greatest plastic waste originating from the land and deposited into the ocean. High levels of microplastics have also been observed in surface waters of the Yantgze Estuary and adjacent waters.	Severe microplastic pollution has been reported in the seawater and sediments of China. Up to 8720 MPs/kg d.w. were found in sediments, while 4137 ± 2462 MPs/m^3 and 0.167±0.138 MPs/m^3, were found in the estuarine and the sea surface samples respectively.	Li et al., (2015) Qiu, et al., (2015) Zhao et al., (2014)
UK (Along the coastal waters)	-	Concentration MPs in sea water ranged from 1.5 to 6.7 MPs/L	Li et al., (2018)
France, Belgium, Netherlands	Close to coastal harbours where shipping and industrial activity is high	Concentration in seawater ranged from 0 to 0.8 MPs/L, while in sediments ranged from 1.5 to 23.4 MPs/kg	Van Cauwenberghe et al., (2015)
Norway (Along coastal areas)	Pristine and highly urbanized sites, atmospheric deposition of airborne MPs, low tidal flow and amplitude, as well as limited circulation.	Concentration in seawater surface from 3.4 *10^{-4} -3.2 *10^{-3} MPs/L and in marine sediment from 6.3 to 300 MPs/kg d.w.	Booth et al., (2017)
Tunisia (Lagoon of Bizerte- 8 m depth)	WWTP effluents discharges by surrounding the Lagoon of Bizerte, textile industries, fragmentation of fishing gear as ropes and nets and mainly shellfish farming material	On the Tunisia coast, MPs concentrations in sediments varied from 141 ± 26 to 461 ± 30 MPs/ kg d.w. The number of MPs in lagoon Bizerte sediment was at the range of 3000–18,000 MPs/kg d. w.	Abidli et al., (2017), Abidli et al., (2018),
Greece (North Ionian Sea)	River outflows into the North Ionian Sea. Shoreline tourism and recreational, fisheries, aquaculture, and shipping activities including poor waste management practices,	In western Ionian Sea, the MPs mean concentration was 0.134 ± 0.084 MPs/m^2.	Galli et al., (2022)