UNIVERSITAT DE BARCELONA

# Chemical and ecotoxicological assessment of microplastics and emerging risks in the coastal environments

Gabriella Francesca Schirinzi

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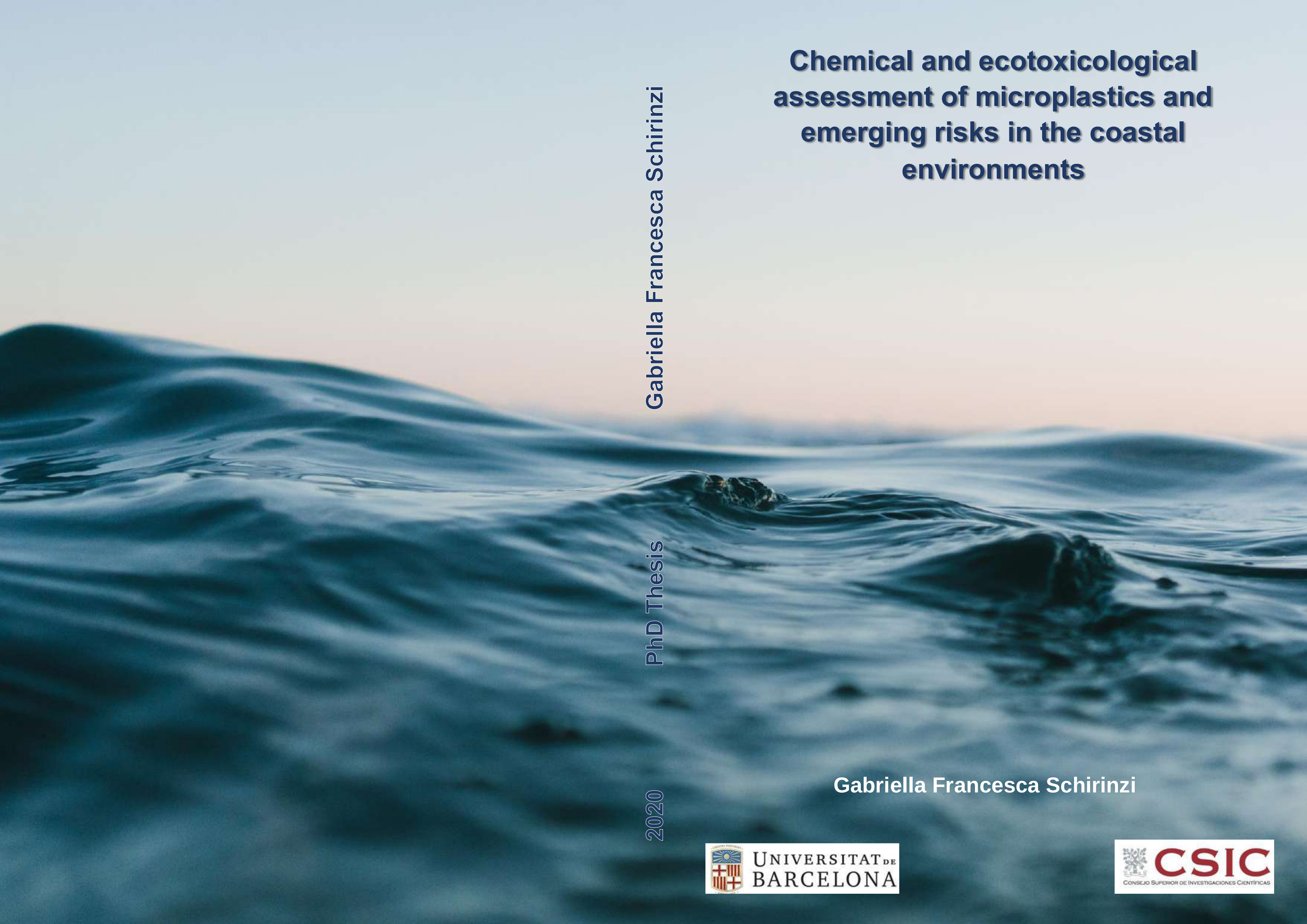

Gabriella Francesca Schirinzi

PhD Thesis

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CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

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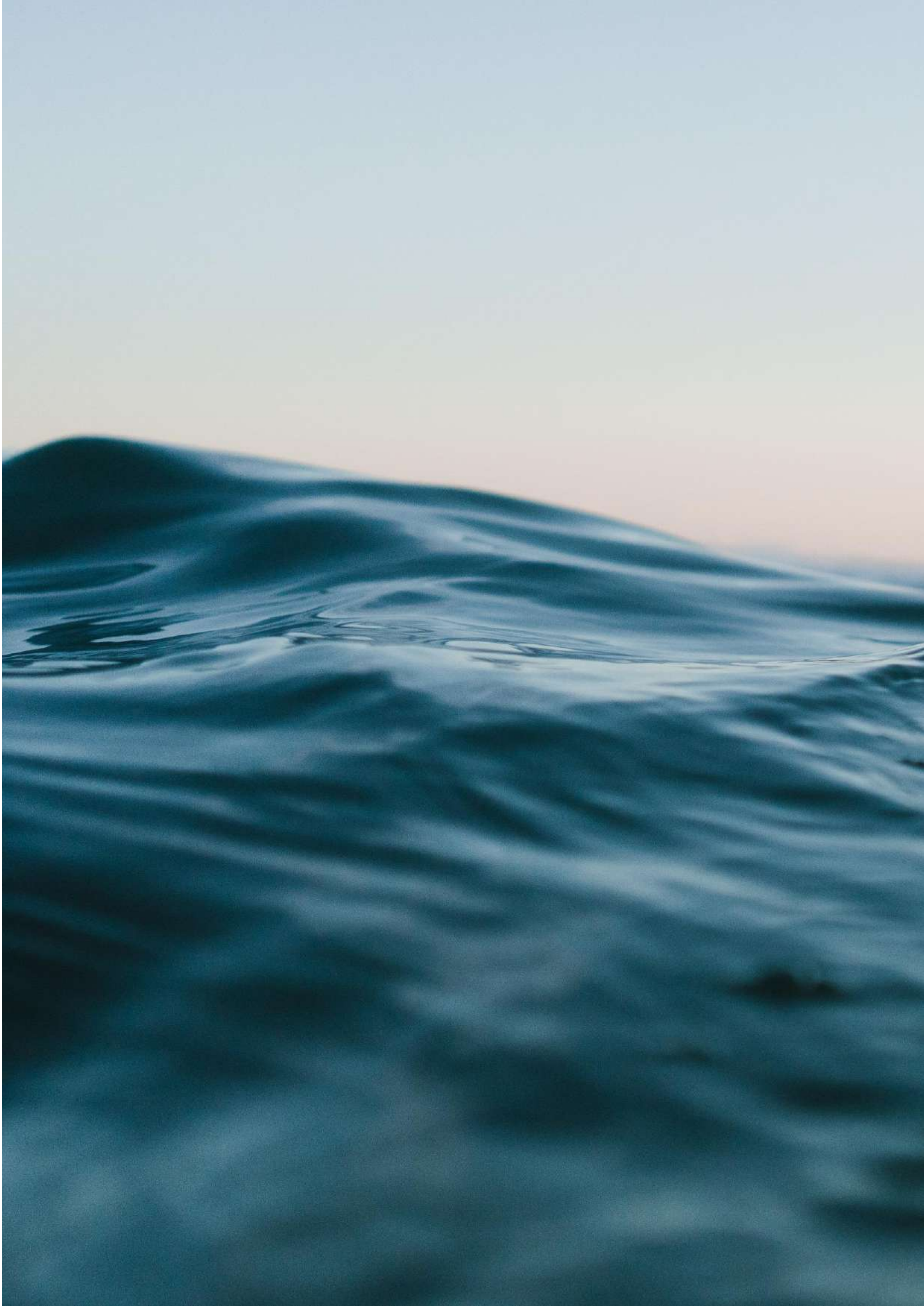

Programa de Doctorado
“Química Analítica i Medi Ambient”

# Chemical and ecotoxicological assessment of microplastics and emerging risks in the coastal environments

Memoria de Tesis presentada para optar al grado
Doctor por la Universidad de Barcelona a

**Gabriella Francesca Schirinzi**

| **Co-Directora** | **Co-Director** | **Tutor** |
|---|---|---|
| **Dra. Marinel·la Farré Urgell**<br>Científica titular<br>Dep. Química Ambiental<br>IDAEA-CSIC | **Dr. Damià Barceló Cullerès**<br>Profesor de investigación<br>Dep. Química Ambiental<br>IDAEA-CSIC | **Dr. Fco. J. Santos Vicente**<br>Profesor titular<br>Universidad de Barcelona |

Barcelona, May 2020

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La Dra. **Marinel·la Farré Urgell** y el Dr. **Damià Barceló Cullerès**, científica titular y Profesor de Investigación respectivamente del Instituto de Diagnóstico Ambiental y Estudios del Agua, IDAEA, perteneciente al Consejo Superior de Investigaciones Científicas, CSIC,

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Barcelona, 06 de mayo de 2020

Marinella Farré

Dra. Marinel·la Farré Urgell

Dr. Damià Barceló Cullerès

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*Whatever you do, do it well.*

*(W. Disney)*

*Without crisis there are no challenges,*
*without challenges life is a routine,*
*a slow agony.*

*(A. Einstein)*

*The real voyage of discovery consists*
*not in seeking new landscapes,*
*but in having new eyes.*

*(M. Proust)*

*Ai miei cari,*
*quelli sinceri, forse imperfetti,*
*ma comunque meravigliosi.*

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# List of abbreviations

| | |
|---|---|
| ABS | Acrylonitrile Butadiene Styrene |
| AFM | Atomic Force Microscopy |
| AFM-IR | Atomic Force Microscopy-based infrared spectroscopy |
| AMD | Anthropogenic Marine Debris |
| APC | Advanced Polymer Chromatography |
| APCI | Atmospheric Pressure Chemical Ionization |
| APPI | Atmospheric Pressure Photoionization |
| ATR | Attenuated Total Reflectance |
| BPA | Bisphenol-A |
| CPR | Continuous Plankton Recorder |
| DART | Direct Analysis in Real Time |
| DDD | 1,1-Dichloro-2,2-bis(4-chlorophenyl)ethane |
| DDE | 1,1-Dichloro-2,2-bis(4-chlorophenyl)ethylene |
| DDT | 1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane |
| DEHP | Diethylhexyl-phthalate |
| DESI | Desorption Electrospray Ionisation |
| DIDP | Diisodecyl Phthalate |
| DINP | Diisononyl Phthalate |
| DOM | Dissolved Organic Matter |
| DSC | Differential Scanning Calorimetry |
| DTA | Differential Thermal Analysis |
| EC-JRC | European Commission-Joint Research Centre |
| EDCs | Endocrine Disrupting Chemicals |
| EPS | Extended polystyrene |
| ESEM-EDS | Environmental Scanning Electron Microscope - Energy-dispersive X-ray spectroscopy |
| FADs | Fish Aggregating Devices |
| FPA | Focal Plane Array |
| FTIR | Fourier-Transform Infrared |
| GDP | Gross Domestic Product |
| GES | Good Environmental Status |
| GESAMP | Group of Experts on the Scientific Aspects of Marine Environmental Protection |
| GIT | Gastrointestinal tract |
| HD-PE | High-density polyethylene |
| HMW | High Molecular Weight |
| HOCs | Hydrophobic Organic Contaminants |
| ICC | International Coastal Clean-up |
| IR | Infrared |
| LC-HRMS | Liquid Chromatography coupled to High-Resolution Mass Spectrometry |
| LD-PE | Low-density polyethylene |
| LLD-PE | Linear low-density polyethylene |
| MALDI-TOF | Matrix-assisted Laser Desorption Ionisation coupled to time-of-flight |
| MD-PE | Medium-density polyethylene |
| MMPW | Mismanaged Plastic Waste |

## List of abbreviations

| | |
|---|---|
| MPLs | Microplastics |
| MPLs/NPLs | Micro(nano)plastics |
| MPSS | Munich Plastic Sediment Separator |
| MSFD | Marine Strategy Framework Directive |
| MSW | Mismanaged Solid Waste |
| NOAA | National Oceanic and Atmospheric Administration |
| NPLs | Nanoplastics |
| NR | Nile Red |
| PAHs | Polycyclic Aromatic Hydrocarbons |
| PBDEs | Polybrominated diphenylethers |
| PBT | Polybutylene terephthalate |
| PC | Polycarbonate |
| PCBs | Polychlorinated Biphenyls |
| PDI | Polydispersity Index |
| PES | Polyester |
| PET | Polyethylene terephthalate |
| PFASs | Polyfluoroalkyl substances |
| PFCAs | Perfluorocarboxylic acids |
| PFDA | Perfluorodecanoic acid |
| PFOA | Perfluorooctanoic acid |
| PFOS | Perfluorooctanesulfonate |
| PFPeA | Perfluoropentanoic acid |
| PMMA | Poly(methyl methacrylate) |
| POPs | Persistent Organic Pollutants |
| PP | Polypropylene |
| PS | Polystyrene |
| PTFE | Polytetrafluoroethylene |
| PUR | Polyurethane |
| PVC | Polyvinyl chloride |
| Pyr-GC-MS | Pyrolysis – gas chromatography-mass spectrometry |
| QA/QC | Quality Assurance/Quality Control |
| RIMMEL | Riverine and Marine floating macro-litter Monitoring and Modelling of Environmental Loading |
| ROS | Reactive Oxygen Species |
| RT | Room Temperature |
| SEM | Scanning Electron Microscopy |
| SEM-EDS | Scanning Electron Microscopy – Energy-dispersive X-ray spectroscopy |
| TA | Thermal Analysis |
| SPT | Sodium Polytungstate |
| TED-GC-MS | Thermal extraction-desorption – gas chromatography-mass spectrometry |
| TGA | Thermogravimetric analysis |
| TGA-GC-MS | Thermogravimetric analysis – gas chromatography-mass spectrometry |
| UNEP | United Nations Environment Programme |
| UNEP/IOC | United Nations Environment Programme/Intergovernmental Oceanographic Commission |

| | |
|---|---|
| UNEP/MAP | United Nations Environment Programme/Mediterranean Action Plan |
| UVA | Ultraviolet A |
| UVB | Ultraviolet B |
| WPO | Wet Peroxide Oxidation |
| WWTPs | Wastewater Treatment Plants |

# List of abbreviations

# Abstract

During the last decades, the global economy's evolution has been dominated by a linear model of production and consumption. In particular, new technologies have been based on the use of efficient and economical alternative materials, such as plastics. Over the years, demand and overconsumption of plastics have led to an increase in the amount of plastic waste. Even if many policy strategies on the prevention and recycling of waste have been applied, only a part of the total solid waste generated is collected to be correctly treated or recycled. The others are dispersed into nature causing significant impacts on the environment and ecosystems.

The presence of waste in the aquatic environment is a severe environmental problem, well known as *marine litter*, which is responsible for environmental, economic, health, and aesthetic problems at the ocean and regional sea level. In particular, the Mediterranean Sea is one of the most affected areas by marine litter worldwide. Most waste accumulated on the beaches, sea surface, and seafloor comes from land-based (up to 80%) rather than sea-based sources. In particular, the dominant litter category is the plastics, largely represented by single-use plastic products and abandoned or lost fishing gear. In this framework, several public policies have introduced different initiatives, such as Marine Strategy Framework Directive (MSFD, 2008/56/EC) and European Strategy for Plastics in a Circular Economy (COM/2018/028) at EU level, to prevent and limit plastic contamination.

Nevertheless, due to their chemical persistence, buoyancy and the increase of sources, plastic residues are already ubiquitous in the marine environment. In particular, plastic fragments with micro- and nano-size are heterogeneously distributed in the environment and they are generally known as '*microplastics'* (MPLs) and '*nanoplastics*' (NPLs), respectively. Their presence in the environment has been reported in diverse aquatic ecosystems and it is due to the direct introduction as micro(nano)spheres (primary MPLs/NPLs) or weathering breakdown of larger pieces (secondary MPLs/NPLs). Once in the marine environment, these particles can interact directly with biota, act as vectors of chemical contaminants and be introduced into the food chain.

Research on MPLs has long focused on their presence and distribution in the ocean. Given the increasing levels of plastic pollution in marine environments, MPLs/NPLs are currently considered emerging pollutants that may pose a serious threat to marine ecology and human health. This has resulted in changing research priorities to address the large gaps in knowledge about the fate, contamination and effects of MPLs on the Earth's natural cycles, ecosystems and organisms. However, despite constant scientific progress on MPLs/NPLs pollution, there is a lack of studies on their distribution, behaviour and fate.

In this framework, this thesis has aimed to expand knowledge about plastic pollution in freshwater, estuarine and coastal environments. The first part of the study was particularly focused on the river transport of plastics, and the fate and behaviour of MPLs/NPLs in estuarine and coastal waters. Focusing on the W Mediterranean, the contributions of floating waste from inland waters to the sea have been assessed. The results of visual observations of floating anthropogenic macro-litter (> 2.5 cm) from Llobregat and Besòs Rivers during an intensive one-year monitoring period have shown that large urban areas, such as the metropolitan area of Barcelona, substantially contribute to marine pollution. Especially, high quantities of plastics, mainly represented by food and beverage packaging, have been transported by these rivers ending up into the sea.

# Abstract

Since the significant contributions of plastic waste from rivers, valuable information can be achieved assessing the spatial distribution of surface MPLs/NPLs in estuarine environments. To carry out these studies, appropriate analytical methods have been developed to quantify and identify the behaviour of these compounds and their co-contaminates in these areas. Several analytical techniques (MALDI-TOF/MS, DART-MS, and LC-APPI-HRMS) have been compared for the quantitative analysis of trace MPLs/NPLs in water samples. Since the best results in term of sensitivity were obtained by dimensional exclusion-LC coupled with APPI-QExactive, a new method was optimized, validated and, lastly, applied to assess the presence of polystyrene in seawater and freshwater samples from Ebro Delta (Catalonia, Spain). This is an important Mediterranean estuarine coastal area which is under continuous environmental and anthropogenic pressures from tourism, agricultural activities, maritime traffic and fish farms. The results reported highest concentrations along the river near urban areas and in the bay where important fishing commercial activities are fixed.

In addition to plastic pollution, estuaries can be highly contaminated environments due to the possible indirect contributions of persistent organic pollutants from urban, industrial, hospital or agricultural wastewater discharges or runoff processes. Since environmental MPLs may also be responsible for the transport and distribution of pollutants, the absorption behaviour of MPLs in freshwater and seawater has been assessed in this thesis. The higher adsorption capacity was found for PS than for PE when exposed to real environmental concentrations of perfluoroalkylated substances (PFAs). Furthermore, for the same materials, a higher adsorption capacity was observed for aged MPLs.

As shown by these results, the plastic pollution of coastal ecosystems, from macro to nano, is a real problem that can lead to serious damage to both nature and society. In order to protect the coastal environment, its productivity and human health, it is necessary to assess the ecotoxicological impacts on marine organisms and human health. In this regard, the presence of MPLs in the gastrointestinal tracts (GITs) of the common dolphinfish of the Western Mediterranean Sea was evaluated for the first time by chemical digestion. The new approach applied as digestion method, based on a combination of basic-acid solutions, allowed to remove most of the organic and inorganic rest facilitating the identification of MPLs by FTIR. The high frequency of plastic presence and the types of polymer reported in this study have confirmed the negative impacts of local fishing gear on this species.

These results confirmed that the ingestion of MPLs by marine organisms is a widely recognised effect. However, as the degradation process of MPLs can progress to the formation of NPLs, exposure to these particles may be higher for both biota and humans. Evidence of the effects of NPLs on several marine organisms has been reported, while very limited data are available regarding the impact of these plastics on human health. Therefore, cytotoxic effects in terms of ROS (*Reactive Oxygen Species)* generation and cell viability were evaluated at the cellular level after 24 hours of exposure to a moderate concentration of nano-sized PS and PE particles. Significant results were reported only at high concentrations of PS-MPLs/NPLs.

In conclusion, the work presented in this thesis successfully filled some of the various gaps in knowledge about plastic pollution and its effects. In the future, further tools should be promoted to facilitate the overall analysis of the MPLs/NPLs pollution issue and the studies on the occurrence and impacts of NPLs should be more strongly encouraged.

# Resumen

Durante las últimas décadas, la evolución de la economía mundial ha estado dominada por un modelo lineal de producción y consumo. En particular, las nuevas tecnologías se han basado en el uso de materiales alternativos, eficientes y económicos, como los plásticos. A lo largo de los años, la demanda y el consumo excesivo de productos plásticos han provocado un aumento de la cantidad de estos residuos. Aunque se han implementado muchas estrategias políticas sobre la prevención y el reciclado de los residuos, sólo una parte del total de los residuos sólidos generados se recoge para realizar un correcto tratamiento. Los demás se dispersan en la naturaleza causando impactos significativos en el medio ambiente y los ecosistemas.

La presencia de residuos en el medio acuático es un grave problema ambiental, comúnmente conocido como *marine litter*, que es el responsable de problemas de tipo ecológicos, económicos, de salud y estéticos en el océano y a nivel del mar regional. En particular, el Mar Mediterráneo es una de las zonas más afectadas por esta problemática en todo el mundo. La mayoría de las basuras acumuladas en las playas, la superficie del mar y el fondo marino proceden de fuentes terrestres (hasta un 80%) y solo una pequeña porción proviene de fuentes marinas. En particular, la categoría dominante es la de los residuos plásticos, representada en gran medida por los productos de un solo uso y los utensilios de pesca que quedan abandonados o perdidos. En este aspecto, varias iniciativas políticas has sido introducidas, como, por ejemplo, la Directiva marco sobre la estrategia marina (MSFD, 2008/56/EC) y la Estrategia europea sobre los plásticos en una economía circular (COM/2018/028) a nivel de la UE, para prevenir y limitar la contaminación de los plásticos.

No obstante, debido a su persistencia química, su flotabilidad y el aumento de las fuentes, los residuos plásticos ya son omnipresentes en el medio marino. En particular, los fragmentos de plástico de tamaño micro y nano están distribuidos de manera heterogénea en el medio ambiente y se conocen generalmente como '*microplásticos*' (MPLs) y '*nanoplásticos*' (NPLs), respectivamente. La presencia de estos compuestos ya ha sido confirmada en diferentes ecosistemas acuáticos como resultado de la introducción directa como micro(nano)esferas (MPLs/NPLs primarios) o a la descomposición de piezas más grandes por factores ambiéntales (MPLs/NPLs secundarios). Una vez en el medio marino, los MPLs/NPLs pueden interactuar directamente con la biota, actuar como vectores de contaminantes químicos e introducirse en la cadena alimentaria.

Durante mucho tiempo la investigación sobre los MPLs se ha enfocado en sus presencia y distribución en el océano. Dados los crecientes niveles de contaminación plástica en los ambientes marinos, actualmente se considera que los MPLs/NPLs son contaminantes emergentes que pueden representar una seria amenaza potencial para la ecología marina y la salud humana. Esto ha dado lugar a un cambio en las prioridades de investigación para cubrir los vacíos de conocimientos sobre el destino, la contaminación y los efectos de los MPLs en los ciclos naturales, los ecosistemas, la flora y la fauna terrestre. Sin embargo, a pesar de los constantes avances científicos sobre la contaminación de los MPLs/NPLs, hay muy poco conocimiento sobre su distribución, comportamiento y destino.

En este sentido, la presente tesis doctoral ha tenido como objetivo ampliar los conocimientos sobre la contaminación plástica en los ambientes de agua dulce, estuarios y costeros. La primera parte del estudio se centró particularmente en el transporte fluvial de plásticos, y en el destino y comportamiento de los MPLs/NPLs en agua de estuario y agua costera.

# Resumen

Centrándose en el Mediterráneo occidental, se han evaluado las contribuciones de los residuos flotantes de las aguas interiores al mar. Los resultados de las observaciones visuales de la macro-basura antropogénica flotante (> 2,5 cm) en los ríos Llobregat y Besòs, durante un período de seguimiento intensivo de un año, han demostrado que las grandes zonas urbanas, como el área metropolitana de Barcelona, contribuyen sustancialmente en la contaminación marina. Especialmente, grandes cantidades de plásticos, representados principalmente por los envases de alimentos y bebidas, han sido transportados por estos ríos terminando en el mar.

Debido a las altas contribuciones de plásticos de los ríos, se puede obtener información importante evaluando la distribución espacial de los MPLs/NPLs superficiales en las zonas de los estuarios. Para llevar a cabo estos estudios, se han desarrollado métodos analíticos apropiados para cuantificar e identificar el comportamiento de estos compuestos y sus co-contaminantes en estas zonas. En particular, varias técnicas analíticas (MALDI-TOF/MS, DART-MS y LC-APPI-HRMS) se han comparado para el análisis cuantitativo de trazas de MPLs/NPLs en muestras de agua. Debido a los excelentes resultados en términos de sensibilidad, la técnica basada en exclusión dimensional-LC acoplada con APPI-QExactive se optimizó, validó y, por último, se aplicó, para evaluar la presencia de poliestireno en muestras de agua marina y de agua dulce en el Delta del Ebro (Cataluña, España). Esta zona es una importante reserva natural del Mediterráneo que está sometida a continuas presiones ambientales y antropogénicas por el turismo, las actividades agrícolas, el tráfico marítimo y las piscifactorías. Concentraciones moderadas de PS-MPLs/NPLs se han encontrado a lo largo del río, cerca de las zonas urbanas y en la bahía donde se encuentran importantes actividades comerciales de pesca.

Además de la contaminación plástica, los estuarios pueden ser ambientes altamente contaminados debido a las posibles contribuciones indirectas de contaminantes orgánicos persistentes procedentes de aguas residuales urbanas, industriales, hospitalarias y/o agrícolas o de procesos de escorrentía. Dado que los MPLs presentes en el medio ambiente también pueden ser responsables del transporte y la distribución de otros contaminantes, en esta tesis doctoral se ha evaluado el comportamiento de la absorción de los MPLs en el agua dulce y en el agua de mar. Se encontró que la capacidad de adsorción del PS es mayor que la del PE cuando se exponen a concentraciones ambientales de sustancias perfluoroalquiladas (PFAs). Además, para los mismos materiales, una mayor capacidad de adsorción se observó en el caso de los MPLs envejecidos.

Como demuestran estos resultados, la contaminación plástica de los ecosistemas costeros, desde la escala macro a la nano, es un problema real que puede provocar graves daños tanto a la naturaleza como a la sociedad. A fin de proteger el medio ambiente costero, su productividad y la salud humana, es necesario evaluar los efectos ecotoxicológicos en los organismos marinos y en los humanos. En este aspecto, se evaluó por primera vez, mediante digestión química, la presencia de MPLs en el tracto gastrointestinal de peces del Mar Mediterráneo occidental, *Coryphaena hippurus*. Un nuevo enfoque se ha aplicado como método de digestión. La combinación de una solución básica (KOH) y una ácida ($HNO_3$) a condiciones moderadas permitió eliminar la mayor parte de restos orgánicos e inorgánicos facilitando la identificación de los MPLs mediante FTIR. La alta frecuencia de la presencia de plásticos y los tipos de polímeros que se han reportado en este estudio han confirmado los impactos negativos de los útiles de pesca locales en esta especie.

Estos resultados confirmaron que la ingestión de MPLs por organismos marinos es efecto de la contaminación de plásticos en el mar. Sin embargo, como el proceso de degradación de los MPLs puede llevar a la formación de NPLs, la exposición a estas partículas puede ser mayor tanto para la biota como para los seres humanos. Los efectos de los NPLs se han reportado en varios organismos marinos, mientras hay muy pocos datos sobre el impacto de estos plásticos sobre la salud humana. Por lo tanto, en esta tesis doctoral se evaluaron los efectos citotóxicos a nivel celular, en términos de generación de especies reactivas al oxígeno, ROS (del inglés *Reactive Oxygen Species*) y viabilidad celular, después de 24 horas de exposición a una concentración moderada de partículas de PS y PE de tamaño micro y nano. Resultados significativos se han observado sólo a altas concentraciones de PS-MPLs/NPLs.

En conclusión, el trabajo presentado en esta tesis doctoral ha logrado hacer aportes significativos al conocimiento en relación a la contaminación de los plásticos y sus efectos. En el futuro, se deberían promover más instrumentos para facilitar el análisis general de la contaminación de los MPLs/NPLs, al igual que promover más estudios sobre la ocurrencia y los efectos de los NPLs en el medio ambiente, incluido los medios marinos, y en la salud humana.

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# 1 . Introduction

# 1.1 Plastics and polymers

## 1.1.1 Definition and origin

During the last century, technological developments triggered the promotion of new materials like plastic. The term "*plastic"* comes from Greek "πλαστικός" (*plastikos*), which means "capable of being shaped or moulded". In chemistry, plastic is a mix of polymers with additives, where polymers (*poly*-, "many"+ *-mer*, "parts") are made up of many identical or similar repeating units, known as monomers, linked by covalent bonds. Polymers are usually macromolecules with long chains and high average molecular weights. Natural polymers are abundant in nature, and the most common ones are cellulose, natural rubbers or proteins. Over the last century, man-made polymers with specific proprieties have started to be produced. Typically derived from fossil fuel or petroleum, synthetic polymers consist of long chains of monomers often much longer than those found in nature. The length of chains and the patterns in which they were arrayed confer to these materials many beneficial properties like strength, lightness, and flexibility. In other words, these features make them plastic.

Bakelite (phenol-formaldehyde resin), one of the first plastic made only with synthetic components, was developed by Leo Baekeland in New York in 1907. Due to its high heat-resistance and electrical non-conductivity, it was soon applied in electrical insulators, radio and telephone casings. Bakelite could be shaped or moulded into almost anything, making a variety of products such as jewellery, buttons or pipes (Figure 1.1).

THE SATURDAY EVENING POST

For Your Daughter's Jewelry and the Ponderous Dreadnaught

BAKELITE CORPORATION

Read this Fascinating Story—

The Material of a Thousand Uses

Divisions of BAKELITE CORPORATION

BAKELITE

Condensite

REDMANOL

BAKELITE

SHEET—ROD—TUBE

FOR WIRELESS APPARATUS

High mechanical and dielectric strength is combined with unsurpassed permanence—Bakelite is not affected by the cold of the Arctic regions nor by the heat and humidity of the tropics.

It is chemically inert; it does not bloom nor emit acid-sulphur compounds as does hard rubber, eliminating troubles from surface leakage.

It is impervious to moisture, oils and solvents, permanent in color, and is easily machined and engraved.

A Coil Impregnating Material Without a Melting Point

Bakelite is hardened by heat, not softened as are other impregnation materials. It has high dielectric strength; it is indifferent to steam, hot oil, solvents and most chemicals. It is chemically inert.

GENERAL BAKELITE COMPANY
2 RECTOR STREET, NEW YORK

**Figure 1.1** Advertisements published on 1924, showing one of the main applications of Bakelite, "*The material of a Thousand Uses*". *The Saturday Evening Post (January 27,1923)* and *(Shea 2016).*

## 1.1.2 Classification and properties

The major differences between smaller molecules and polymers mainly concern their physical, thermal, and mechanical properties. Most of them, such as density, tensile strength, glass transition temperature, and resistance to various chemical products, depend on polymer structure (Brinson et al. 2015). Depending on the basic structure, polymers can be classified as linear, branched, and cross-linked. Polymers with straight and unbranched chains are denser and harder. While covalent bonds between chains in cross-linked polymers make them stronger and more stable (Shrivastava 2018). According to their thermal properties, polymers can be classified as thermoplastics, thermosetting, and elastomers (Figure 1.2) (Peters 2015). Thermoplastics can be heated and reshaped many times and reused. Conversely, thermosets have rigid, cross-linked polymer structures that once set with heat cannot be reshaped. Thus, they can be softened or melted only once. Finally, elastomers can deform and then return to their original shape almost instantly.

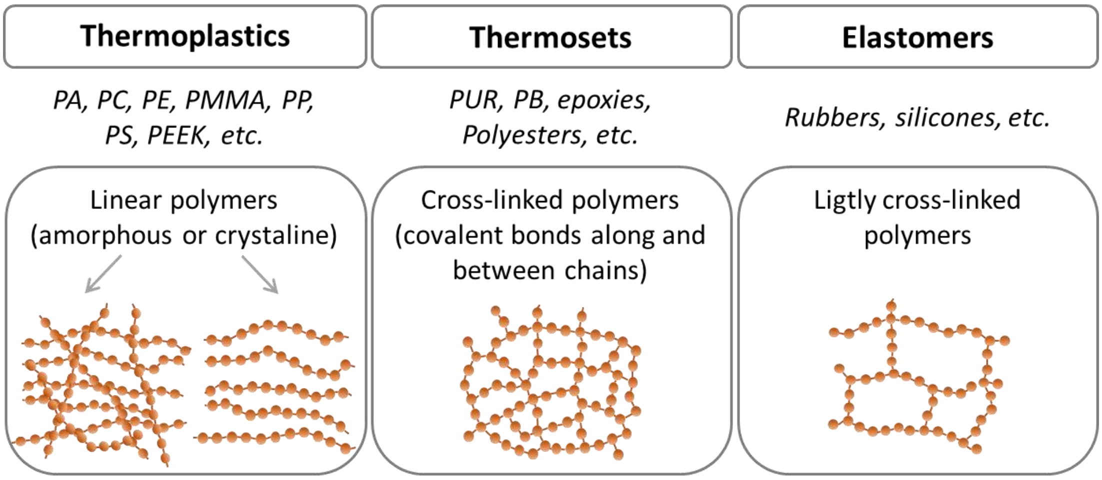


**Figure 1.2** Classification of polymers according to their thermal properties.

For instance, polyethylene (PE) is a versatile polymer that is widely used in consumer products. It can have different applications depending on its branching degree (Peacock 2000). Due to its short or long-chains branching, low-density polyethylene, LD-PE, is more flexible and it is used to make squeeze bottles or food storage containers. Less branching makes the denser form of this polymer, HD-PE, much more rigid to be applied for detergent jugs, trash cans, or children’s toys.

## 1.1.3 Plastic production and uses

### Synthesis

Most plastics are derived from primary materials such as oil, natural gas and coal (Bruckner et al. 2016). Monomers, resulting from refining processes of these raw materials, can chemically join together through polymerisation processes, creating large macromolecules with different shapes and sizes (Figure 1.3). The two main types of polymerisation are:

- *addition polymerisation.* Monomers use their multiple bonds during the coupling without the co-generation of other products. The addition polymerisation occurs mainly in a free radical mechanism, which includes three necessary steps: initiation, propagation, and termination. In general, a chemically active molecule, called initiator, is needed to initiate the chain reaction. The most common addition polymers are those formed by unsaturated monomers such as polyethylene, polypropylene, polystyrene, and acrylic.

- *condensation polymerisation.* A small molecule (e.g. water, ammonia or alcohol) is eliminated when the monomers combine. Condensation polymers can be formed when the monomers possess two or more kinds of functional groups such as an ester, amide or urethane. Nylons, some polyesters, and urethanes are examples of condensation polymers.

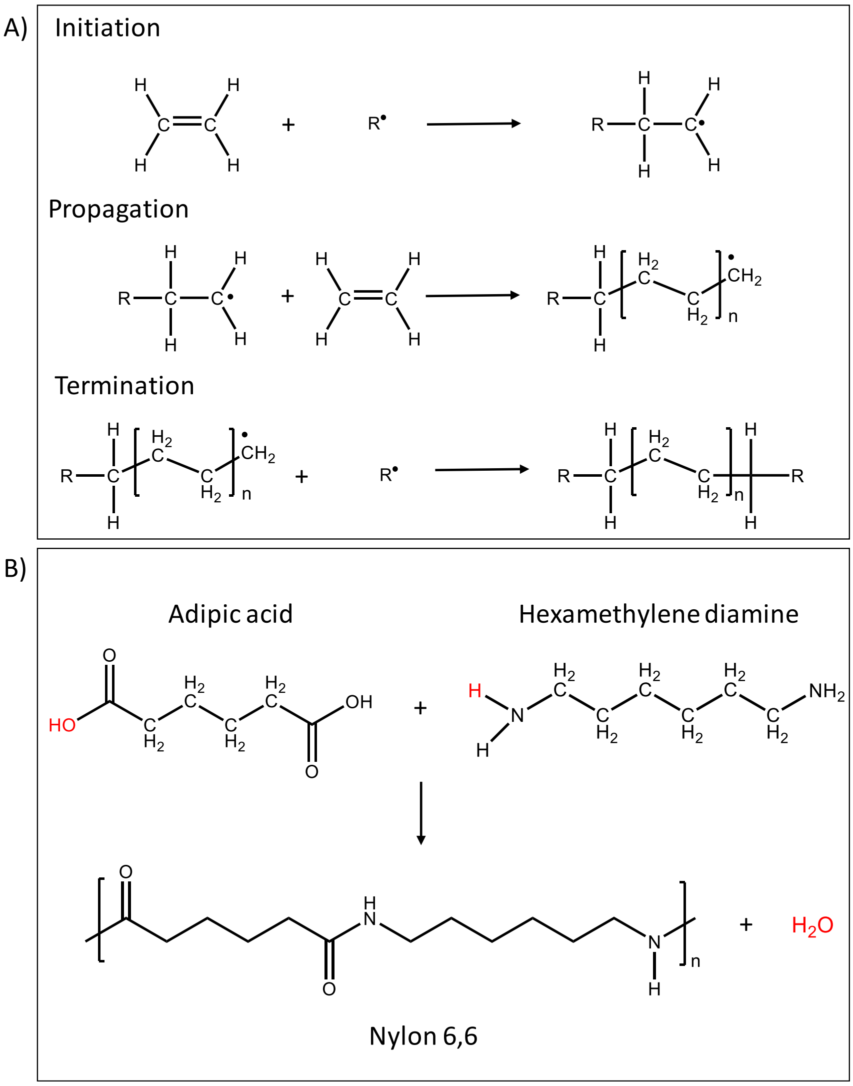


**Figure 1.3** Examples of (A) addition and (B) condensation polymer.

These reactions normally take place in large polymerization plants. The polymeric resins produced are collected and further processed.

## Plastic additives

To finalise the treatment process of plastic and prepare it for future use, additives can be incorporated to the synthesised polymer during their manufacturing or just added onto the surface to improve the performance, functionality, and ageing properties of the material (Coleman 2017). Additives mainly used in plastic materials are antioxidants, acid scavengers, UV and thermal stabilisers, flame retardants, lubricants, pigments, antistatic agents, and slip compounds (Hahladakis et al. 2018).

Plasticisers are the most common additives used in the plastics industry. Their main purpose is to increase the elasticity of the material and facilitate its processing. There are several general chemical families of plasticisers. Among these, the most used are phthalate esters (e.g. DEHP, DINP, and DIDP), colourless and odourless liquids, which are widely used as plasticisers for PVC. More than 85% of all plasticisers consumed in Europe are employed in flexible PVC applications, mainly for the construction, automotive and wire and cable sectors (Berard et al. 2005).

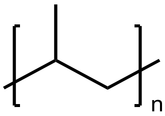
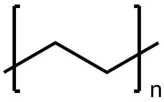
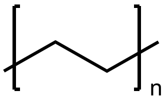
## Global production

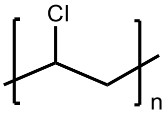
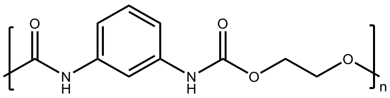
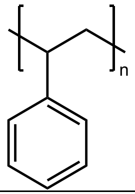
Since the 1940s, when the first industrial-scale production of plastics took place, plastic manufacturing has continued to grow steadily. During the Second World War, the need to preserve natural resources encouraged the use of these materials. For instance, nylon started to substitute silk in ropes and Plexiglas to replace the glass. Since then, due to excellent mechanical properties, durability, and low cost, the production of plastics increased very fast, replacing most traditional materials (Freinkel 2011). From 1950 to 2015, the annual production increased over 200 times, reaching 381 million tonnes. This value is still rising, and it is expected that in 2050 more than 1800 million tons will be produced globally (Geyer et al. 2017).

Europe is one of the largest producers of plastics after China, which alone accounts for over 29% of worldwide plastics (d'Ambrières 2019). The annual European converter demand for plastics is 51.2 million tonnes. Six countries, including Germany, Italy, France, Spain, the United Kingdom, and Poland, covered almost 70% of the European demand in 2017 (PlasticsEurope 2018).

Global demand for plastics is dominated by thermoplastics, polyurethane and other polymers used for adhesives, coatings and sealants. The most popular plastic materials, their main properties and applications are summarised in Table 1.1.

**Table 1.1** Summary of types and properties of common plastics in the order of European plastic demand.

| Polymers | Properties | Applications |
|---|---|---|
| Polypropylene<br>(PP)<br> | - translucent<br>- low moisture vapour transmission<br>- inertness toward | Food packaging, sweet and snack wrappers, hinged caps, microwave containers, pipes, automotive parts, bank notes, etc. |
| Low-density Polyethylene<br>(LD-PE)<br> | - relative transparency<br>- flexibility<br>- toughness<br>- excellent resistance to solvent | Reusable bags, trays and containers, agricultural film (LD-PE), food packaging film (LLD-PE), etc. |
| High density Polyethylene<br>(HD-PE)<br> | - relative transparency<br>- stronger<br>- more tensile strength<br>- excellent resistance to solvent | Toys, (HD-PE, MD-PE), milk bottles, shampoo bottles, pipes, houseware (HD-PE), etc. |

| Polymers | Properties | Applications |
|---|---|---|
| Polyvinyl chloride<br>(PVC)<br> | - brilliant clarity<br>- high impact strength<br>- excellent processing performance<br>- resistance to chemicals | Window frames, profiles, floor and wall covering, pipes, cable insulation, garden hoses, inflatable pools, etc. |
| Polyurethane<br>(PUR)<br> | - lightweight<br>- insulation<br>- strong bonding properties<br>- resistance to chemicals | Building insulation, pillows and mattresses, insulating foams for fridges, etc. |
| Polystyrene<br>(PS)<br> | - significant stiffness<br>- low thermal conductivity<br>- strong bonding properties<br>- resistance to chemicals | Eyeglasses frames, plastic cups, egg trays (PS); packaging, building insulation (EPS), etc. |
| Others:<br>- polyamide (PA)<br>- polycarbonate (PC)<br>- poly(methyl methacrylate) (PMMA)<br>- acrylonitrile butadiene styrene (ABS)<br>- polytetrafluoroethylene (PTFE)<br>- polybutylene terephthalate (PBT) | Depend on resin or combination of resins | Hub caps (ABS); optical fibres (PBT); eyeglasses lenses, roofing sheets (PC); touch screens (PMMA); cable coating in telecommunications (PTFE); and many others in aerospace, medical implants, surgical devices, membranes, valves & seals, protective coatings, etc. |

Thermoplastics may be melted to form different kinds of products with application in the main market activities. Due to the significant advantages for food preservation, lightweight (an important feature in the aviation and automotive industry to reduce fuel consumption) and because they are excellent thermal and electrical insulation materials, the primary uses are in the packaging, construction, furniture, textiles, and automotive sectors (Figure 1.4).

### 1.1.4 End-of-life of plastics

Depending on social-economical aspects, plastic lifetime may vary ranging from less than one year, for certain packaging applications, to decades (Geyer et al. 2017). Today, many plastic articles applied in the packaging and medical sector are designed to be used just once. In 2015, 47% of plastic waste generated globally was largely represented by plastic packaging with China responsible to produce almost 60 million tonnes. Whereas the main producers of packaging waste per capita were the United States, followed by Japan and Europe (Brooks et al. 2018).

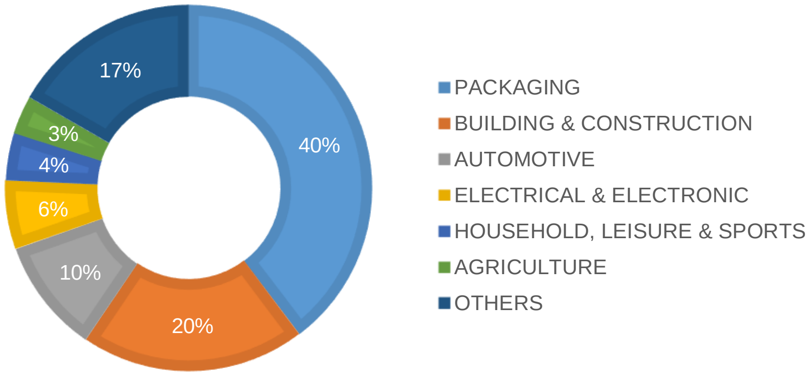


**Figure 1.4** Demand for plastics by market sectors.

After use, the plastic immediately enters the waste stream to be recycled, incinerated or, lastly, discarded in dumps or landfills. Depending on their base material and use, a percentage of plastic can be recycled after use by mechanical or chemical processes. In principle, all types of thermoplastics can be mechanically recycled. However, the replacement of virgin polymer by recovered plastic is often not possible for the same application. Intensive technology, selection, chemical depolymerisation or thermal degradation are necessary to recover most plastics making recycling more difficult and expensive.

Even if a considerable fraction is landfilled or cannot be recycled or incinerated for energy production, thanks to the EU strategies on prevention and recovery (EuropeanCommission 2018; PlasticsEurope 2017) the recycling of plastic waste has increased in recent years. In 2016, 27.1 million tonnes of plastic waste were collected to be treated. Among them, the percentages of plastic recycling and those used for energy recovery were 31.1% and 41.6%, respectively (PlasticsEurope 2018). However, due to collection, sorting and reprocessing costs and the low market value of recycled plastics, re-use and recycling of plastics at the end of their life remain lower than for paper, metal or glass (Woodford 2019; Boyle 2015). Part of plastic, which cannot be recycled or discarded in safety landfills, ends up in open dumps or the environment (Figure 1.5). Unfortunately, a non-negligible fraction of this waste is dispersed into the natural environment as litter, causing significant impacts on plants, animals, ecosystems, and human health (Geyer et al. 2017).

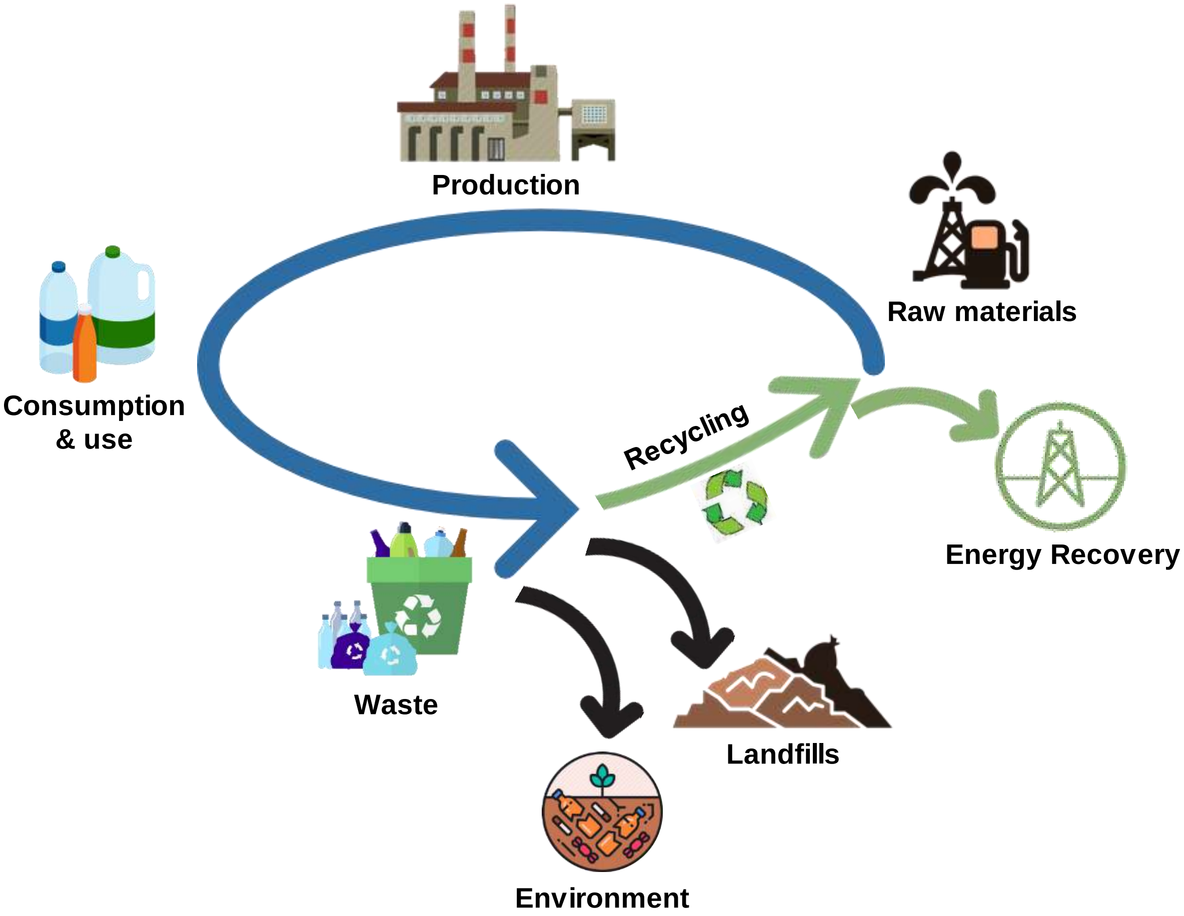


**Figure 1.5** The life cycle of a plastic products.

## 1.2 Plastics in the environment

The presence of waste in the aquatic environment is a global environmental concern, also well known as *marine litter* (or *marine debris*). The UNEP 2009 defined it as any persistent, manufactured, or man-made solid material which is discarded, disposed or abandoned in the marine and coastal environment (Commission 2009). Marine litter is responsible for environmental, economic, health, and aesthetic problems at the ocean and regional seas level. In general, the debris of different sizes comes from specific industrial sectors or human activities presented in the coastal area or has been transported to the sea by rivers (Rech et al. 2014; Thiel et al. 2013). The type and amount of debris found in open ocean areas or collected along beaches could also be influenced by other factors such as ocean current patterns, climate, run-off processes, wind, tides, proximity to urban centres, waste disposal sites, industrial and recreational areas, shipping lanes, and commercial fishing grounds (Ryan 2015a; Newman et al. 2015). This debris could affect beaches, coasts, oceans, seafloors, inland waterways, lands and also the inhabitants of these areas and their economies (Newman et al. 2015). Generally, the debris is classified according to the UNEP/IOC guidelines modified at region scale (Commission 2009). In the European Union, the Marine Strategy Framework Directive (MSFD_2008/56/EC) has created a common list of categories to compare marine debris from all European seas. The composition (e.g. plastic, glass, metal), overall shape (e.g. bottle, film, rope, net, bag), and the size are generally reported to know the possible sources of contamination.

Over the years, due to an increasing number of sources, plastic items have become ubiquitous in the marine environment (Ryan et al. 2009). Several marine litter monitoring surveys confirm that more than 60% of trash ending up in the ocean is plastic, followed by metal, glass, paper, cloth, rubber, and wood (Figure 1.6) (Wegener 2018). The latest report of International Coastal Clean-up (ICC) by Ocean Conservancy showed that more than 97 million items were collected around the world (in more than 100 countries) in total with an overwhelming percentage of cigarettes buds and plastics objects (OceanConservancy 2019).

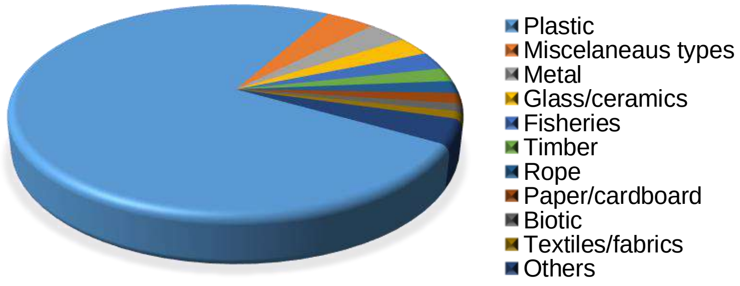


**Figure 1.6** Global composition of marine litter obtained matching 584 publications and 4171 locations (*LitterBase* source).

The type, distribution, and residence time of plastic debris in marine habitats can vary depending on numerous factors, including hydrodynamics, geomorphology, and human factors. Rivers or ocean currents are transport mechanisms, which allow the movement of plastics into and within the marine environment from various land- and sea-based sources. Hence, plastics debris can be transported over long distances before being deposited onto the shorelines or at the bottom of the oceans and seas. The presence of plastic debris is commonly reported on the beach (Moreira et al. 2016; Topçu et al. 2013) or floating on the sea surface

(Thiel et al. 2003; Eriksen et al. 2014). Buoyant plastics are the most abundant among the marine litter, and a large amount of them end up on sandy shores. Among these, plastic bags, fishing nets, and food or beverage packaging are the common items found on the beach. In the open sea, information about plastic abundance and distribution is still limited. High concentrations of floating plastic items have been reported in the North Atlantic (Law et al. 2010; Galgani et al. 2000) and Pacific Oceans (Goldstein et al. 2012; Wong et al. 1974). Also, ocean models report that subtropical ocean gyres are accumulation zones of plastic pollution, knows as “garbage-patches” (Barnes et al. 2009). Conversely, information on debris on the seabed is limited (Ioakeimidis et al. 2017; Gerigny et al. 2019). The type of waste accumulated on the seabed may vary depending on the presence of the nearest hotspots such as of urban centres, harbours or marine and hydrodynamic activities along the coast.

## 1.2.1 Sources

Plastic litter can be discharged directly into oceans or freshwater systems and transported by rivers from inland to the sea. On a global scale, diverse points and diffuse sources have been identified, showing that most plastics come from land (up to 80%) rather than the sea (Mehlhar et al. 2011) (Figure 1.7).

### Land-based sources

*Waste management.* The primary input of plastic debris from land into the ocean is related to mismanaged waste of waterfront areas (e.g. beaches, piers, harbours, riverbanks, etc.). In particular, coastal areas with a high and growing population density have been recognised as one of the main sources of marine debris. The improper trash disposal or inadequate waste collection facilities in these areas lead to an increase in the amount of waste in the marine environment (Jambeck et al. 2015). Plastic wastes from inland regions can also be carried by runoff processes or strong wind into waterways ending up into the ocean (Lebreton et al. 2017).

*Sewage discharges*. Plastic fragments, especially the smallest one, may enter the environment via untreated sewage, treated effluents from industrial and municipal wastewater treatment plants (WWTPs) (Conley et al. 2019), and stormwater drain (NOAA 2016; Axelsson et al. 2017). During heavy rainfall events, the volume of water may exceed the capacity of the sewage treatment plants, causing flood events. In this situation, untreated water is discharged directly into the water bodies (EuropeanCommission 2001).

*Agricultural activities.* Agricultural practices produce plastic waste that is not adequately managed. For instance, plastic sheets that are used to cover the soil to preserve moisture, improve fertility and reduce weed infestations are very often abandoned in fields or along watercourses, buried in the soil or disposed of in landfills (Vox et al. 2016).

*Tourism.* Coastal tourism is one of the main contributors to plastic marine debris. Waste can end up in the sea directly, deliberately or accidentally (de Araújo et al. 2006). For instance, in the Mediterranean Sea, over 200 million tourists have caused a 40% increase in marine litter during the summer period (WWF 2018b).

*Extreme natural events.* Hurricanes, tornadoes, tsunamis, floods and landslides are capable of carrying a huge amount of waste into the ocean (Kocasoy 2009).

### Sea-based sources

*Aquaculture and fishery.* The continued demand for seafood from markets leads to increased fishing activities and the development of aquaculture facilities in coastal locations and open waters. Fishing gear (e.g. nets, dredges, traps, hook and lines) are one of the most common plastic items lost or discarded at sea (Andrady 2011). Also, aquaculture activities or fish aggregating devices (FADs) use plastic-based lines, cages, or nets suspended from buoyant or submergible structures (partly plastic) and involve the application of biofouling and nanotechnological plastic-based paint. In due course, these plastics may degrade or fragment and represent an important contributor at the local level, particularly in coastal areas with intensive fishing activity (Good et al. 2010; Macfadyen et al. 2009).

*Stationary platforms.* Offshore oil and gas platforms are surrounded by water, and all plastic items lost from these structures (e.g. drill pipe thread protectors, hard hats, gloves, and storage drums) become marine debris.

*Shipping.* Items dropped or lost from commercial and recreational ships contribute significantly to plastic pollution (Čulin et al. 2016).

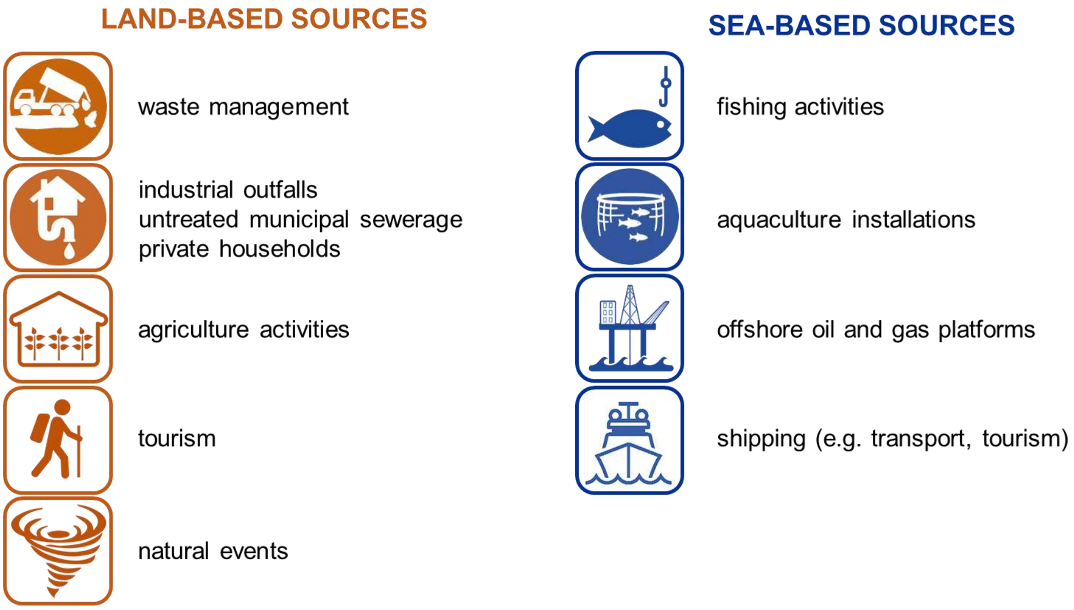


**Figure 1.7** Overview of land- and sea-based sources of marine plastic litter.

## 1.2.2 Plastic degradation

From largest to smallest, plastic results as persistent debris because of their durability and slow degradation. The life cycle of plastic items in the marine environment may vary from 55 years for a PE-plastic bag to 500 years for a disposable diaper or plastic toothbrush (WWF 2018a). The degradation of plastic in the environment is a multistage process influenced by a variety of biotic and abiotic factors (Strlič et al. 2005; Andrady 2015). Five different mechanisms can be distinguished: mechanical/physical degradation, hydrolysis, photo-degradation, thermo-oxidative degradation, and biodegradation (Andrady 2011).

The degradation rate is mainly influenced by the interaction with the natural environment (Fotopoulou et al. 2017). Waves are the leading causes of mechanical and physical deterioration in the aquatic ecosystem. Further degradation processes can be influenced by marine conditions like salinity, temperature, oxygen level, and light irradiation (Dash et al. 2013; De Tender et al. 2015). Although the hydrolysis of polymers contributes to the physical degradation of plastic marine debris (Zettler et al. 2013), the degradation rate in aquatic systems is mainly reduced due to low oxygen levels and seawater temperature. Indeed, photo-degradation processes in water are slower than those in the air or on land (Andrady et al. 1993; Andrady 1990) and also thermo-oxidative degradations are limited due to the moderate temperature (Hammer et al. 2012). Finally, microorganisms of the aquatic ecosystems (e.g. bacteria) may also play an important role in the fragmentation processes, breaking down plastic items. Furthermore, biofouling processes can affect the biodegradation rate and stability of polymers (Muthukumar et al. 2011).

In general, the effects of degradation are more visible on the beach and surface water than on settled plastics (Andrady 2011). Plastic items floating on the ocean surface are exposed to moderate temperatures, solar radiation (UVA and UVB), and oxidising conditions. Consequently, the most critical factors initiating abiotic degradation are oxygen and sunlight (Cooper et al. 2010). Generally, the first step starts on the polymer surface through UV-light that promotes the incorporation of oxygen atoms into the polymer. After a period of exposure, degradation is reflected in the properties of the material with colour changing, crazing, cracking, and erosion. Therefore, being more brittle, plastic is more exposed and available for chemical and enzymatic attacks, leading to breakage into smaller pieces. Finally, biodegradation processes are governed by different factors that include polymer characteristics (molecular weight, molecular structure, presence of additives, etc.) and the type of organism (Shah et al. 2008; Gu et al. 1994).

The entire process could be prolonged, and its effectiveness depends mainly on the type of plastic. For plastics with a carbon-carbon backbone (e.g. PE, PP, PS, and PVC), abiotic processes are required before biodegradation. Large polymers should first be depolymerised to pass through microbial cellular membranes. Also, abiotic degradation can produce functional groups, like carboxyl groups, which are more susceptible to attack by microorganisms (Vasile 2000). On the other hand, all degradation pathways (hydrolysis, photo-oxidation degradation, and biodegradation) are equally likely for plastics with heteroatoms in the main chain as in the case of PVC and PU (Szycher 1999; Pignatello 2013).

Regardless of the degradation pathways, large plastic items end up producing meso, micro and nano-sized particles. During this process, the molecular weight of polymer tends to decrease to produce monomers, which are successively mineralised into inorganic compounds over time (Eubeler et al. 2009; Andrady 1998) (Figure 1.8).

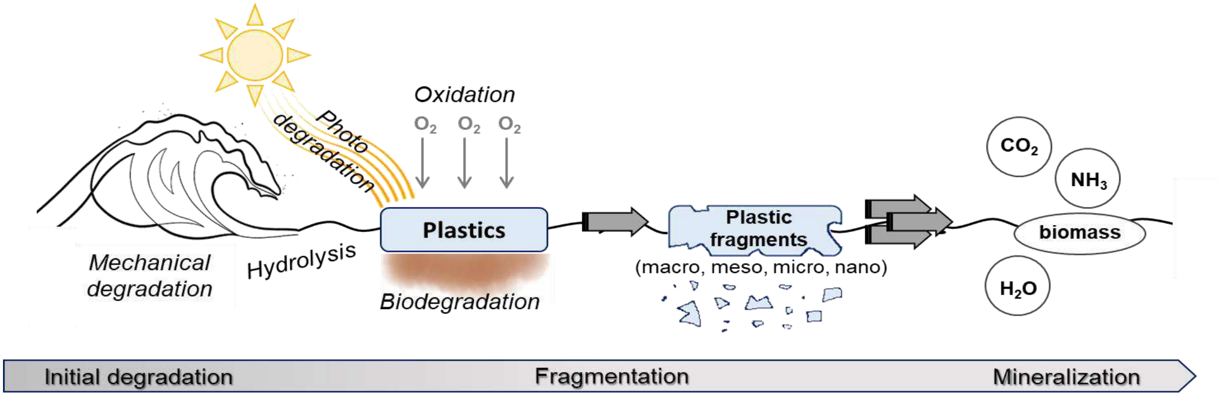


**Figure 1.8** Possible pathways for degradation of plastic polymers floating in the marine environment.

### 1.2.3 Plastic size-classification: from macro to nano

Plastic debris appears in a continuous size spectrum, ranging from nanoscopic particles to mega items, with different shapes. Both properties (size and shape) affect their fate in the environment, including further degradation, transport, and potential impacts. The definitions of the size categories of marine plastic litter evolve in parallel with sampling and analysis technology. A size-based classification proposed by the MSFD is shown in Figure 1.9 (MSFD_2008/56/EC).

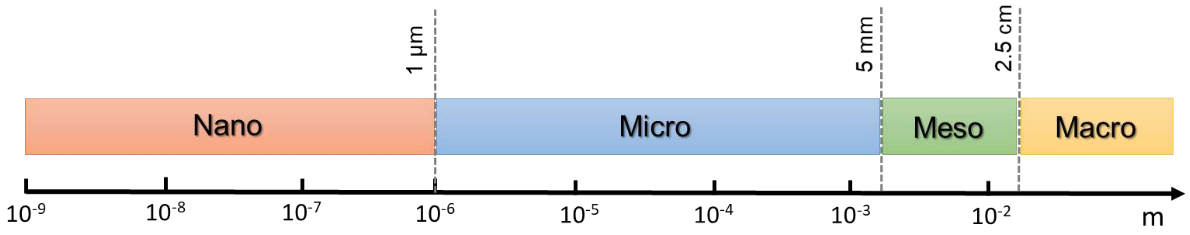


**Figure 1.9** Size-based classification of plastics debris observed in the marine environment based on the MSFD-GES.

The plastic pollution present on the sea surface is dominated by particles with a diameter of less than 1 cm with an increase in abundance decreasing in size (Suaria et al. 2016; Enders et al. 2015). The presence of these small pieces of plastics in the ocean was first reported in the scientific literature in the early 1970s (Carpenter et al. 1972a; Carpenter et al. 1972b). At the begging, the term “microplastic” included all plastic material that could be readily identified only by microscopy (Thompson et al. 2004). However, the need for an agreed terminology for sampling and measuring encouraged the scientific community to set an upper limit of 5 mm (Arthur et al. 2009). Since 2015, GESAMP has defined microplastics (MPLs) as “plastic particle with a size less than 5 mm in diameter, which includes particles in the nano-size range” (GESAMP 2015, 2016).

Nowadays, discrepancies in the range of small-size plastic have already been reported and the evolution of the terminology is still ongoing (da Costa et al. 2016a; Picó et al. 2019). Similarly to MPLs, the definition of NPLs has not yet been officially provided due to the lack of analytical methods. The European Commission has defined NPLs as particle < 100 nm in at least one of their sizes according to the nanomaterial criterion (Koelmans 2015; EuropeanCommission 2011). Other authors define NPLs as plastic particles < 20 µm (Wagner et al. 2014) or even < 1 µm (da Costa et al. 2016b; Ter Halle et al. 2017; GESAMP 2015).

### 1.2.4 Micro(nano)plastics

MPLs present in the environment are usually classified by their morphological characteristics (e.g. size, shape, and colour) to identify their potential sources. The main shape categories reported are fibres, sheets, fragments, foams or spheres, covering a broad spectrum of sizes (Figure 1.10) (Hidalgo-Ruz et al. 2012; Lusher et al. 2017a).

Concerning the origin, MPLs can be categorised as primary or secondary. The difference between them depends on whether the particles were originally manufactured to be of that size (primary), or they are the result of breaking larger plastics (secondary).

**Figure 1.10** Examples of MPLs shapes: 1) line/fibre 2) sheet, 3) film, 4) foam, 5) fragment, and 6) pellet/granule (Wu et al. 2018).

Primary sources may include engineered microscopic particles or industrial powders such as microbeads used in personal care and cosmetic products to improve the cleaning function (e.g. toothpaste, cleansing agents, skin exfoliators) (Napper et al. 2015; Fendall et al. 2009), resin beads used in the production of feedstock or plastic, abrasives to clean the surfaces of building or ship hulls (Browne et al. 2007), powders used in moulding, and polymeric microspheres applied in drug delivery systems (Patel et al. 2009). Conversely, secondary MPLs mainly include fibres or fragments originated from the progressive fragmentation and ageing of larger pieces by physical, chemical and biological processes in the environment or by mechanical abrasion.

Likewise, NPLs can be generated during fragmentation or ageing of MPLs or large plastic items as a result of mechanical abrasion, UV radiation, and microbiological activities (Corcoran et al. 2009; Koelmans et al. 2015). Additionally, a small portion of NPLs can be released directly into nature. Nano-sized particles are detected in commercial products (cosmetic products, paints, adhesives, medical applications, etc.) or can be originated during industrial processes (thermal treatments, 3-D printing, synthesis, etc.) (Hernandez et al. 2017; Stephens et al. 2013).

Once micro(nano)plastics (MPLs/NPLs) enter the ocean, the identification of the initial purpose and origin is challenging. Generally, MPLs/NPLs originated from fragmentation and degradation processes of larger items are the predominant form in the marine environment (Barnes et al. 2009). Nevertheless, small plastic particles can directly enter the marine environment through various activities on land and in the marine environment (Browne 2015).

Many sources of primary MPLs/NPLs have been identified in the literature. Among them, MPLs/NPLs used in facial cleansers, cosmetics or air blasting technology can widely enter waterways via domestic or industrial drainage systems. Like MPLs in cleaning products, those used in medicine are transferred to the sea through sewage but also through treating diseased animals in aquaculture and farming. In the same way, synthetic fibres can shake loose from the washing of synthetic clothing and end up in the oceans (Cesa et al. 2017). Sampling experiments of household washing machine wastewater have shown that more than 1900 fibres per wash can be discharged (Browne et al. 2011). In this framework, wastewater is considered as a pathway of MPLs/NPLs from treatment plants to the sea. Depending on their existence, design and effectiveness, wastewater treatment plants (WWTPs) can trap macro and mesoplastics. However, a large number of MPLs/NPLs still pass through the filtration systems.

Other routes for MPLs/NPLs are runoff processes and wind. For instance, city dust, including particles generated from tyres abrasion and road markings, can be washed in drains and waterways or transferred from the wind to the sea. Erosion of tyres and synthetic textiles contributes to more than half of MPLs releases to the ocean (Boucher et al. 2017). Finally, plastic pellets and marine coatings applied to vessels (i.e. polyurethane and epoxy coatings as well as vinyl and lacquers) can be released directly in the ocean.

Once these particles arrive in the ocean, they can be distributed in different compartments according to the size and physicochemical properties. A summary of different MPLs/NPLs sources and their main pathways is s shown in Figure 1.11.

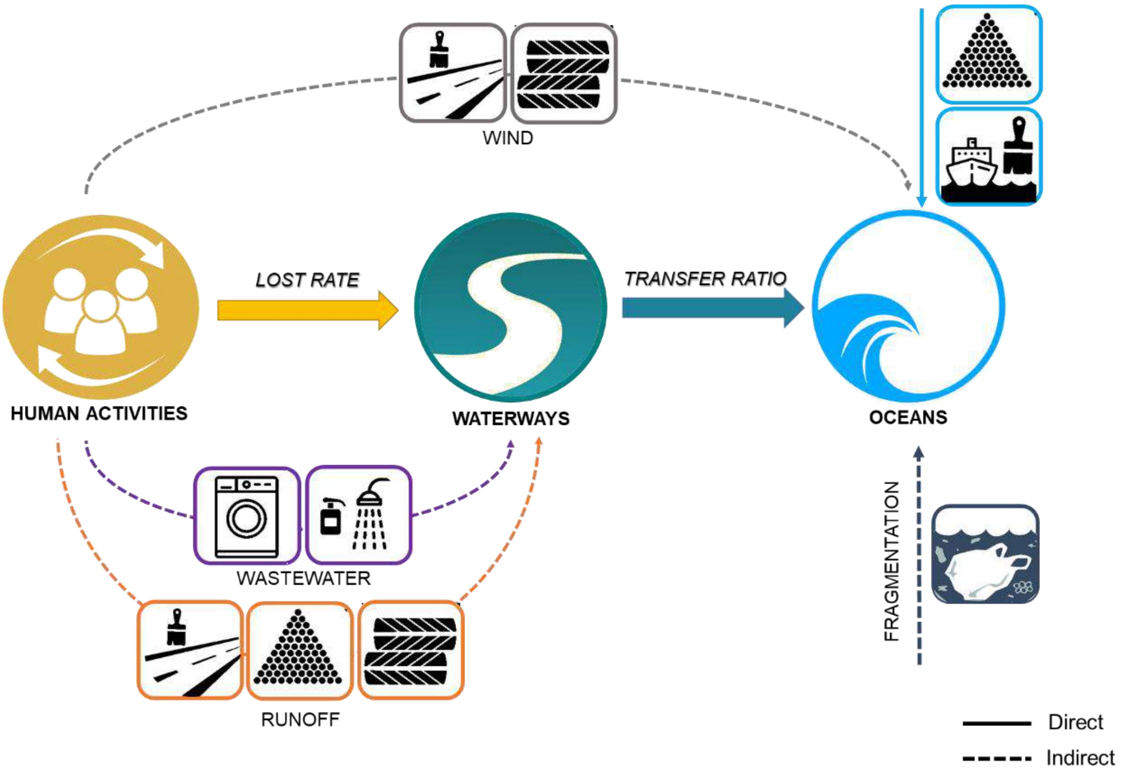


**Figure 1.11** Key sources (primary and secondary) and pathways of MPLs/NPLs found in the ocean. The *solid line* indicates direct input and *dotted lines* indicate the possible pathways (wind, runoff, wastewater, and fragmentation) of plastic particles (Boucher et al. 2017).

## 1.3 Analysis of microplastics

Plastics have been used since the 1950s. Because of their usage and resistance to degradation, their residues are ubiquitous in the environment. However, a concern about this type of contamination has only been raised in the last 10 years. Nowadays, plastic litter (macro, meso, MPLs, and NPLs) is considered emerging contaminants and a series of new regulations are being implemented to reduce the use of plastics and their potential impacts. The growing interest of the scientific community in the impacts caused by smaller plastic particles on the environment and, potentially, on human health has led to an increase in the number of studies focused on MPLs.

In the literature, most of the reported results are often difficult to compare due to the different methodological approaches applied for sampling, identification and quantification of different sizes and types of plastics. As a result, information on their spatial and temporal distribution in water, sediment, and biota remains limited mainly due to the lack of standardised methods.

### 1.3.1 Sampling

Different sampling methods are used to collect MPLs from the aquatic systems depending on the scope of the study and the cost. In this section the most common ones will be described.

#### Water

A variety of nets can be used for sea surface detection. Net tows are generally employed for sampling plankton, insects or another biota at sea. Currently, these devices have also been adapted for the sampling of plastic particles in different water layers. In particular, manta trawls sample the first 15-25 cm, while the neuston trawls sample up to 50 cm. Net tows are generally deployed from vessels, and they are usually towed for 15 - 30 min (rarely 60 min) at ~2 knots. The net can be between 1 and 8 m in length, and the mesh size is 50 - 500 µm, typically 300 or 333 µm being the most used mesh. When the frame is wholly immersed, using a flowmeter is possible to measure the filtered volume and estimate the sampled area.

Other more straightforward sampling methods for the assessment of plastic particles are the bulk water sampling and visual observation. Photographic and aerial surveys are generally used for the assessment of floating macroplastics. All methods at the sea surface are weather dependant.

Due to the vertical movements, the abundance of MPLs increases going down through the water column. Consequently, sampling methods for this compartment are generally focused on smaller microparticles. Bongo nets, or in some cases multi-level net tows, are typically used to collect particles from the bottom by replicating them. Also, Continuous Plankton Recorder (CPR) surveys are used for more extended monitoring programs. Generally, the CPR is towed to a depth of 5 - 10 metres (Thompson et al. 2004). Even if large volumes of water are collected quickly by net sampling, the nets are not fine-meshed enough to collect MPLs/NPLs or microfibres. Alternatively, other conventional methods reported are the bulk water pump and underway pump sampling. More than 100 L of water can be collected by bulk water using a container or pump; then, water is filtered through a small net mesh (20 - 80 µm). Pump sampling is not used for floating particles but is generally used by submerging the pump from a bridge or vessel at the preferred depth.

### Sediment

The sampling of MPLs in sediments requires different efforts and resources depending on the water depth. In general, shoreline sediments are more available than seabed ones (GESAMP 2015). For a sublittoral zone, grabs are normally used to sample surface and near-surface sediments from a bulk sample, whereas coring devices sample different depth strata providing information on MPLs distribution. The differences matrix type (sandy or muddy), sediment depth (0 - 32 cm), and accumulation zones (especially for beach sampling) play an important role in the quantification and comparison of studies (Hidalgo-Ruz et al. 2012). In the literature, reported abundances are often expressed in different units and conversion is possible if sufficient information on sampling depth and water content is available (Hanvey et al. 2017).

### Biota

To choose a species as a bioindicator of plastic contamination specific criteria are followed (Kershaw et al. 2019; Fossi et al. 2018). Generally, monitoring strategies focus on specific organisms based on their biology (size, age, development), behaviour (feeding strategies adopted) and their interaction with the environment and humans (Kershaw et al. 2019). For instance, sessile organisms (e.g. mussels and oysters) are chosen as global bio-indicator because they reflect local coastal plastic pollution and are species of human consumption (Li et al. 2019). On the other hand, organisms at high trophic levels and with higher mobility, such as fish, turtles, birds and mammals, may represent the availability of plastic litter on a larger spatial scale.

## 1.3.2 Sample pre-treatment

The analysis of MPLs in environmental samples is a multi-step process that requires some additional steps before the identification of polymer types. Once samples are obtained, plastic particles are separated from the matrix by filtering/ sieving or density separation. Further operations, involving chemical or biological agents, are aften necessary to reduce or eliminate the organic matter.

### Separation

Filtration is a simple and effective approach to separate MPLs from liquid samples. The most common filters used are glass fibre filters (0.2 - 0.7 µm), followed by cellulose nitrate or cellulose acetate filters (5 µm) or sieves (50 - 250 µm mesh size) (Lusher et al. 2017b). For sediment samples, physical separation via sieving is especially used to isolate the larger MPLs (> 1 mm) from the matrix. The mesh size of sieves may range between 0.035 and 4.75 mm and multi-tier sieving, with a decreasing mesh size, is used to reduce the volume of the sample before extraction. In contrast, the common strategy for extracting smaller MPLs (< 1 mm) from sediments is density separation combined with filtration. This method uses the buoyancy force of a saline solution with a density intermediate between that of plastic particles (0.8 - 1.70 g $cm^{-3}$) and that of organic/inorganic materials constituting sand or sediment which is typically 2.65 g $cm^{-3}$. The saline solution is mixed with the sample and shaken thoroughly (up to 2 hours). After sedimentation of the heavier materials in the liquid, the supernatant, where MPLs are assumed to be, is filtered. This operation can be repeated more than once to increase extraction efficiency. The saline solutions commonly employed for density separation are sodium chloride (NaCl, 1.2 g $cm^{-3}$), sodium polytungstate (SPT, 1.4 g $cm^{-3}$), sodium iodide

(NaI, 1.6 g cm$^{-3}$), and zinc chloride ($ZnCl_2$, 1.7 g cm$^{-3}$) (Hidalgo-Ruz et al. 2012; Claessens et al. 2013; Imhof et al. 2012). Imhof et al. (2012) significantly improved the classic density separation approach by launching the Munich Plastic Sediment Separator (MPSS).

#### Matrix removal by digestion

The presence of organic materials (e.g. plankton, biofilms, rest of organisms) in the samples may interfere with the analysis. In such cases, biological or chemical digestion is needed to purify the sample matrix. After digestion, the solutions can be directly filtered or extracted again by density separation. The National Oceanic and Atmospheric Administration (NOAA) recommends the use of wet peroxide oxidation (WPO) with hydrogen peroxide ($H_2O_2$) and Fe(II) as a catalyst to perform a more effective process to remove labile organic matter in sediment and water samples (Masura et al. 2015). Besides oxidising methods, digestion can also be acidic, alkaline or enzymatic (Table 1.2).

In marine organisms, depending on the species and its diet, different strategies could be followed to extract MPLs from biota samples. In any case, most of these chemical treatments damage synthetic polymers as the reagents involved are usually applied at high concentrations and temperatures. Common digestion methods for biota are discussed in the Chapter 4 and more details are provided.

### 1.3.3 Characterisation

MPLs in the environment have different physical, chemical and biological characteristics, depending on their sources and input pathways. Most of these properties play an essential role in assessing the effects of these particles on biota, ecosystems and human health. Specific analytical techniques can be used to determine the morphological/physical and chemical characteristics of MPLs. The combination of diverse analytical techniques is, in general, necessary for the analysis of these microparticles in environmental samples.

#### Physical characterisation

The physical characterisation allows classifying MPLs according to their morphology (fragments, fibres, sheets, foam, etc.), size and colour. This information is generally achieved by naked eyes or with the aid of optical microscopes. Visual identification by stereomicroscopes is widely recommended to distinguish MPLs below 1 mm from other particles. However, in complex environmental samples, the probability of error increases significantly when the particle size decreases if only optical microscopy is used (Song et al. 2015).

The characterisation of the surface morphology of smaller MPLs can also be performed by scanning electron microscopy (SEM) (Eriksen et al. 2013). In addition, scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDS) and environmental scanning microscopy-energy dispersive X-ray spectroscopy (ESEM-EDS) can be used to determine the elemental composition of polymers (Wang et al. 2017b). High-magnification images obtained with advanced microscopy techniques facilitate the discrimination of MPLs from organic particles; however, their application for routine analysis of a large number of samples is not feasible (Jiang 2018).

**Table 1.2** Digestion methods for the removal of organic matter in environmental samples.

| | Digestion | Exposure | Sample | Ref. |
|---|---|---|---|---|
| *Sedime* | $H_2O_2$ (30%) | RT (7 d) | Biogenic matter | (Nuelle et al. 2014) |
| | 0.05M Fe (II) + $H_2O_2$ (30%) | 75°C (45 min) | Seafloor sediment | (Masura et al. 2015) |
| *Biota* | $HNO_3$ (22.5M) | RT (12 h) + 100°C (2 h) | Blue mussels, oysters, lugworms | (Van Cauwenberghe et al. 2015) |
| | $HNO_3$ (100%) | RT (30 min) | Euphausids copepods | (Desforges et al. 2015) |
| | $HNO_3$ (≥69%) | 90°C (4 h) | Manilla clams | (Davidson et al. 2016) |
| | $HNO_3$ (65%) + $HClO_4$ (68%) (4:1) | RT (12 h) + 100°C (10 min) | Blue mussels, brown shrimp | (De Witte et al. 2014) |
| | KOH (10%) | 2 - 3 weeks | Fish | (Foekema et al. 2013) |
| | KOH (10%) | 60°C (24 h) | Seafood products | (Dehaut et al. 2016) |
| | KOH (10%) | 60°C (12 h) | Fish | (Rochman et al. 2015) |
| | NaOH (1M) | 60°C (1 h) | Blue mussels | (Catarino et al. 2017) |
| | NaOH (10M) | 60°C (24 h) | Zooplankton | (Cole et al. 2014) |
| | $H_2O_2$ (30%) | 65°C (24 h) + RT (< 48 h) | Bivalves | (Li et al. 2015) |
| | $H_2O_2$ (15%) | 55°C (3 d) | Fish | (Avio et al. 2015a) |
| | Proteinase-K | 50°C (2 h) | Zooplankton | (Cole et al. 2014) |
| | Proteinase-K + cellulase | 50°C (12 h) + 40°C (12 h) | Fish larvae | (Steer et al. 2017) |
| | Trypsin | 40°C (30 min) | Mussels | (Courtene-Jones et al. 2017) |

*RT: room temperature

## Chemical characterisation

The chemical characterisation is a final step that provides the chemical composition of the particles, allowing to distinguish MPLs from other natural materials in the sample. So far, spectroscopic methods (e.g. Fourier-Transform Infrared and Raman) are the most commonly used.

✓ *Fourier-Transform Infrared (FTIR) spectroscopy*

The change in the permanent dipole moment of a chemical bond after the absorption of infrared (IR) light is the physical principle on which the Fourier Transform Infrared (FTIR) spectroscopy is based. This technique is widely used for the characterisation of MPLs in environmental samples (Käppler et al. 2016). FTIR uses libraries of polymer spectra that allow the identification of most common polymers. In recent years several variants based on FTIR have been developed, improving the performance of the technique. For example, micro-FTIR (µ-FTIR) is commonly used for the detection and identification of smaller MPLs (up to 20 µm). In particular, the µ-FTIR in attenuated total reflectance (µ-ATR-FTIR) facilitates the identification of irregularly shaped particles (Harrison et al. 2012). Recently, the main limitations of ATR-FTIR, such as longer operating times and the minimum sample needed, have been partially overcome by µ-FTIR microscopes with focal plane array (FPA) imaging (FPA-FTIR). FPA-FTIR allows simultaneous chemical and physical characterisation of particles (Simon et al. 2018; Löder et al. 2015).

- ✓ *Raman spectroscopy*

Another vibrational spectroscopic technique commonly used to identify MPLs is Raman spectroscopy. In this case, when the laser beam falls on the particles, different frequencies of backscattered light result from them depending on the molecular structure and the atoms present. In this way, a unique spectrum for each polymer is provided, and non-polar functional groups are very well detected (Koenig 1999; Araujo et al. 2018). Analogously to FTIR, Raman is a non-destructive technique and allows further analysis. Furthermore, the identification of MPLs on the filter can be achieved after sample purification (Käppler et al. 2015). Raman spectroscopy can also be coupled to a microscope to identify particles in the µm range (µ-Raman). For example, µ-Raman associated with a confocal laser-scanning microscope is used to detect polymeric particles within biological tissues (Cole et al. 2013). However, compared to µ-FTIR, the manual analysis was slower and interference from organic material on MPLs can effectively influence the results (Käppler et al. 2015).

- ✓ *Thermal analysis*

Generally, thermal analyses (TA) measure changes in the physical and chemical properties of materials depending on their thermal stability and they are alternative methods to spectroscopy for MPLs identification. However, reference materials to identify polymer types are required. Among these, differential scanning calorimetry (DSC) or differential thermal analysis (DTA) have been applied for the identification of semi-crystalline plastic samples, such as PE, PET, and PP (Chialanza et al. 2018). Finally, thermogravimetry (TGA) combined with DSC can identify PE and PP, but it not suitable for other common polymers due to overlapping transition signals (Majewsky et al. 2016).

- ✓ *Novel approaches*

New approaches have recently been developed to try to speed up the process of MPLs identification by avoiding the time-consuming visual pre-sorting phase. In this context, the thermal decomposition gases from plastic particles can also be analysed by pyrolysis coupled to gas chromatography ((Pyr)-GC-MS). The chemical characterisation of several particles, including information on the type of plastic and the organic additives it contains, can be performed simultaneously in a single operation (Fries et al. 2013; Fischer et al. 2017).

For a high sample weight (up to 100 mg), another novel technique combines the advantages of TGA analysis with those of thermal extraction desorption coupled to gas chromatography-mass spectrometry (TED-GC-MS) for the identification of MPLs in complex matrices (Dümichen et al. 2015). As an alternative to TA, identification by Nile Red (NR) staining following by FTIR allows skipping pre-sorting, avoiding over- or underestimation of MPLs. Since NR is a selective hydrophobic dye, one of the main limitations of this method is the co-staining of natural organic materials. Therefore, any lipids or organic matter should be removed from the sample before NR staining (Shim et al. 2016).

Finally, atomic force microscopy (AFM) combined with IR or Raman spectroscopy is a surface analysis proposed for chemical characterisation at the nanoscale. AFM-IR can acquire IR absorption spectra and absorption images with spatial resolutions of 50 - 100 nm (Dazzi et al. 2012). The applicability of this system has not yet been demonstrated for environmental analysis.

### 1.3.4 Quality assurance and quality control

Due to the ubiquitous presence of MPLs in the environment, including in the air, the entire analytical process may be at risk of cross-contamination. In this framework, rigorous quality assurance and quality control (QA/QC) should be adopted to guaranty the quality of reported data. Some preventive measures include the use of glassware or metal objects, where possible, the careful rinsing of instruments and equipment (with acid and ultrapure water) and avoiding the use of synthetic fabrics during the sampling or sample handling and preferring the use of a 100% cotton laboratory coat. Airborne contamination could be limited by covering samples as much as possible and handling them in a fume hood. The presence of open Petri dishes or filter papers moistened is quite recommended, especially during visual observation, to minimize background plastic contamination (Wesch et al. 2017).

Sample contamination can also be controlled by procedural blanks from field to analysis. Laboratory blanks, replicate samples, spiked matrix and blank samples reduce the possibility of over- and underestimation of MPLs, enhancing data reliability (Lusher et al. 2017b; Silva et al. 2018; Hanvey et al. 2017).

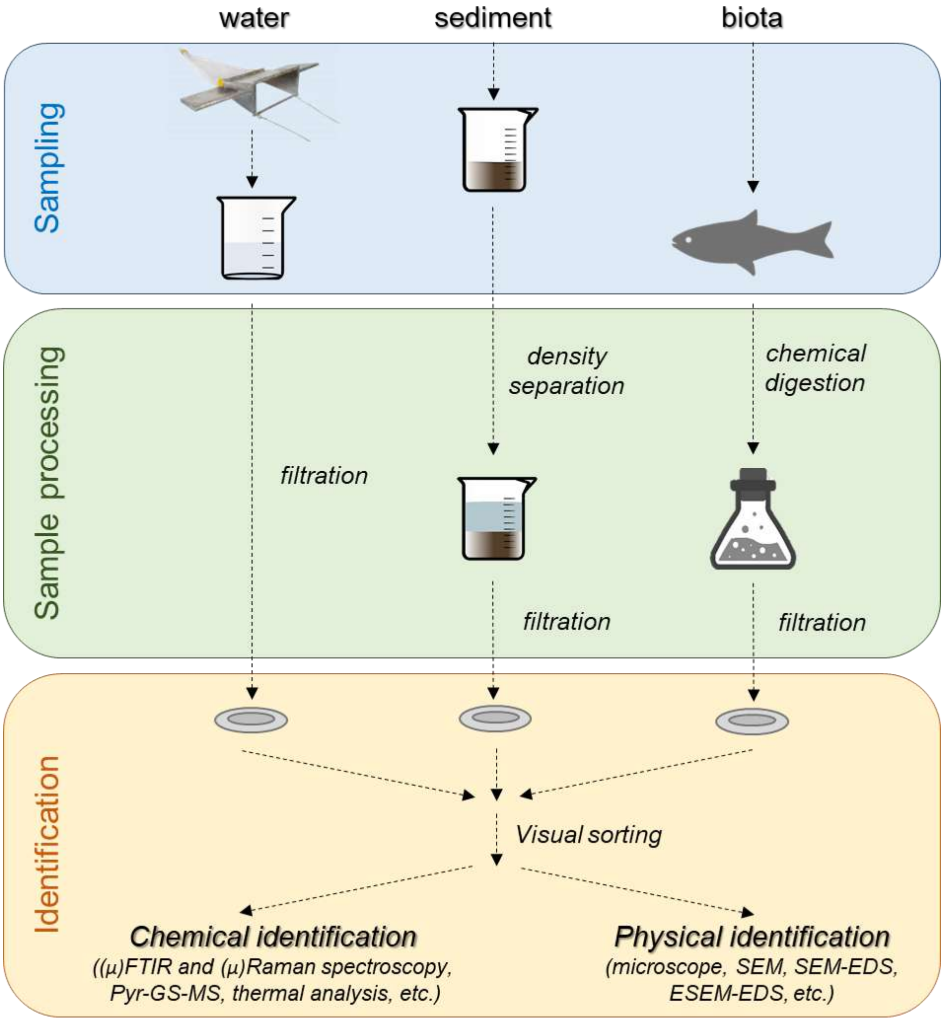


**Figure 1.12** Common strategies for the analysis of MPLs in water, sediment and biota samples including sampling, sample processing, and identification steps.

## 1.4 Environmental fate and impacts of microplastics

The potential impacts of MPLs on ecosystems are mainly influenced by their horizontal and vertical distribution. Vertical flow in aquatic systems is usually influenced by polymer density (Table 1.3). For this reason, low-density polymers, such as PP and PE, which tend to float, are commonly found on the surface of the sea. On the other hand, plastics made of polymers like PVC or PET tend to sink.

However, the density is not the only factor influencing their distribution. Adsorption of organic materials, biofouling and cold descending currents also affect the buoyancy of plastic particles. These facts explain why similar types of polymer are often found in the water column and sediments. Biofouling processes lead to an increase in particle density, especially those with a high surface area to volume ratios as sheets (Ryan 2015b). Also, phytoplankton aggregates can incorporate plastic particles that promote vertical movements (Long et al. 2015). In this way, the abundance of small particles (< 1 mm) decreases at the ocean surface (Kooi et al. 2017). Intermediate behaviours can be observed depending on other factors such as the physical properties of each MPLs, including size and shape (Thiel et al. 2003), and aqueous parameters (e.g. salinity and temperature) (Lusher et al. 2017a; Kowalski et al. 2016; Cole et al. 2016). In the ocean, the density of seawater gradually increases with depth following the profile of the thermocline. Therefore, plastic particles can be suspended if the polymer density is equal to that of seawater.

Finally, weather conditions also play an important role in the aquatic environment. Oceanic turbulence induced by wind and waves could change the vertical distribution and abundance in the seas (Kukulka et al. 2012; Reisser et al. 2015).

### 1.4.1 Occurrence in the aquatic environment

#### Surface and sub-surface water

The first evidence of MPLs in surface water dates back to more than 40 years ago (Carpenter et al. 1972a; Carpenter et al. 1972b). Nowadays, the presence of MPLs has been widely reported at (sub)surface water of the open oceans, coastal oceans, and semi-enclosed seas such as the Mediterranean Sea (Table 1.4). Due to vertical transport, biofouling processes, nano-fragmentation, and ingestion by biota, the amount of plastics particles below 1 mm gradually increases under the ocean surface (Cózar et al. 2014; Reisser et al. 2015). The widespread distribution of MPLs in sub-surface waters makes a wide range of aquatic organisms potentially susceptible to them and other compound used in their formulations.

MPLs were typically found in seawaters of the northern hemisphere. However, due to the dynamic nature of the North Atlantic and North Pacific oceans, their distribution in this area is not fully understood. So far, higher densities of plastics have been recorded in the open ocean than the coastal waters. This means that the currents act as a potent distribution chain (Reisser et al. 2015). Among them, oceanic currents, gyres, and recurring natural events are the main responsible for transporting smaller plastic particles from distant sources to polar latitudes or remote areas (Cózar et al. 2017). At contrast, high concentrations have been found in coastal waters of Asia near densely populated areas and estuarine zones. This suggests that models are needed to explain the distribution of MPLs including multifactorial parameters.

**Table 1.3** Densities and common applications of plastics found in the marine environment.

| Polymer | Acronym | Common uses | Density (g cm$^{-3}$) | Ref. |
|---|---|---|---|---|
| *Polystyrene expanded* | EPS | Insulation, packaging, furniture | < 0.05 | (Styrene ; Duis et al. 2016; Nuelle et al. 2014) |
| *Low Density Polyethylene* | LD-PE | Bags, storage containers, films, toys | 0.89 - 0.94 | (Nuelle et al. 2014; Andrady 2017; Driedger et al. 2015) |
| *High Density Polyethylene* | HD-PE | | 0.94 - 0.98 | (Hidalgo-Ruz et al. 2012; Morét-Ferguson et al. 2010; Duis et al. 2016) |
| *Polypropylene* | PP | Rope, bottles caps, netting, auto parts, food containers | 0.83 - 0.92 | (Andrady 2017; Driedger et al. 2015; Hidalgo-Ruz et al. 2012) |
| **Pure water** | | | **1.00** | |
| **Seawater** | | | **1.02 - 1.03*** | (Suaria et al. 2016; Alomar et al. 2016) |
| *Polystyrene* | PS | Utensils, food containers | 1.04 - 1.11 | (Driedger et al. 2015; Morét-Ferguson et al. 2010; Erni-Cassola et al. 2019) |
| *Polyamide or Nylon* | PA | Fishing nets, rope | 1.02 - 1.16 | (Andrady 2017; Duis et al. 2016; Erni-Cassola et al. 2019) |
| *Poly(methyl methacrylate)* | PMMA | Optical lenses, paint, shatterproof windows | 1.09 - 1.2 | (Driedger et al. 2015; Duis et al. 2016; Nuelle et al. 2014) |
| *Polyurethane* | PU | Furniture, automotive interiors, sponges, packaging | 1.17 - 1.28 | (Nuelle et al. 2014; Erni-Cassola et al. 2019) |
| *Polycarbonate* | PC | Optical disks, safety visors, rear lights of cars | 1.2 - 1.22 | (Driedger et al. 2015; Erni-Cassola et al. 2019) |
| *Polyvinyl chloride* | PVC | Films, pipes, containers | 1.16 - 1.58 | (Andrady 2017; Morét-Ferguson et al. 2010; Nuelle et al. 2014) |
| *Poly(ethylene terephthalate)* | PET | Beverage bottles, strapping, textiles | 1.29 - 1.45 | (Nuelle et al. 2014; Andrady 2017; Driedger et al. 2015) |
| *Polyester resin* | PES | Textiles, boats | 1.24 - 2.3 | (Hidalgo-Ruz et al. 2012; Duis et al. 2016) |
| *Polytetrafluoroethylene* | PTFE | Wires, cables, bearings, gears | 2.1 - 2.3 | (Driedger et al. 2015; Duis et al. 2016) |

*depending on temperature and salinity.

Abundant concentrations of secondary MPLs, especially PE, PP, and EPS, have been recorded in marine water in China, one of the biggest producers of plastic waste in the world (Kang et al. 2015; Zhao et al. 2014). Also, large amounts of microbeads and pellets (primary MPLs) have been detected in Japan and Hong Kong Seas (Cheung et al. 2016; Veerasingam et al. 2016).

In the case of closed basins, such as the Mediterranean Sea, the accumulation of plastic is getting a serious concern in recent years (Suaria et al. 2016). In general, there is a high variability on a small scale depending on seasonality, hydrographical conditions, population density, and industrial activities (Ruiz-Orejón et al. 2016; Gündoğdu 2017; Poulain et al. 2013a). The high concentrations found in the Mediterranean Sea can be compared with those reported in open oceans with a high accumulation of plastic (Cózar et al. 2015). In particular, the central area of this basin is affected by plastic pollution coming from North Africa and Western Asia (Gündoğdu 2017; Gündoğdu et al. 2017; Güven et al. 2017).

As most sources of plastics in the coastal environment are terrestrial (Andrady 2011), relatively new studies are focusing on inland waters. Rivers are considered as essential transport routes for plastic in coastal and lake environments. As a result, MPLs are ubiquitous in freshwater systems with concentration comparable to or higher than those observed in marine environments (Table 1.5). The abundance, distribution, and density of MPLs in waterways are especially affected by hydrodynamic conditions. MPLs presence has been widely reported in large European and Asiatic Rivers, especially in proximity to densely populated areas. In the case of inland waters in Asia, the high concentrations are also due to the lack of appropriate waste management measures in many urban and suburban regions (Free et al. 2014; Su et al. 2016).

The MPLs abundance reported in various studies is subject to the sampling methods, the size ranges studied, and the measurement units chosen to report (e.g. particles per $km^{-2}$, particles per $m^{-2}$, particles per $m^{-3}$, etc.). These disagreements in the literature make it difficult to compare surface water data, whether in a marine or riverine environment.

### Sediments

Plastic litter floating on the sea surface is just a small fraction of the waste that enters the world’s ocean every year (Eriksen et al. 2014). Sedimentation of the remaining fraction is considered the most likely process (Yao et al. 2019). Due to vertical and horizontal transfers, a significant amount of plastics may be accumulated on the shoreline or may sink from the water column to the seabed (Ivanov et al. 2004; Sanchez-Vidal et al. 2012; Talley 2002).

The first evidence of MPLs contamination in marine sediments was reported in the 2000s (Sweden 2007; Thompson et al. 2004; Ng et al. 2006). Today, the occurrence of small and large MPLs in beaches, subtidal, and offshore sediments worldwide is a known problem. In the same way that for surface waters, the differences in the MPLs distribution reported for sediments may depend on the sampled area and the methodologies and tools employed for extraction (Hidalgo-Ruz et al. 2012; Besley et al. 2017). Therefore, these studies are difficult to compare.

**Table 1.4** Examples of MPLs abundances reported for sea (sub)surface waters across the world since 2014.

| Location | Sample type | MPLs abundance | | Sampling methods | Ref. |
|---|---|---|---|---|---|
| ***Atlantic and Indian Ocean*** | | | | | |
| UK Channel, North, and Celtic Sea | Surface water | 0.14 | particles m$^{-3}$ | Manta trawl (333 µm) | (Maes et al. 2017) |
| North Atlantic accumulation zone | Surface and sub-surface water | 1.69 | particles m$^{-3}$ | Multi-level net tows (150 µm) | (Reisser et al. 2015) |
| European Coast - North Atlantic Subtropical Gyre (NASG) | Sub-surface water | 13 - 501 | particles m$^{-3}$ | Vessels (300 and 10 µm) | (Enders et al. 2015) |
| English Channel, Plymouth, UK | Sub-surface water | 0.27 | particles m$^{-3}$ | Net tows (500 and 200 µm) | (Cole et al. 2014) |
| NE Atlantic Ocean | Sub-surface water | 2.46 ± 2.43 | particles m$^{-3}$ | Vessels (250 µm) | (Lusher et al. 2014) |
| ***Pacific Ocean and Asiatic Seas*** | | | | | |
| Geoje Island | Surface water | 0.88 ± 0.81 | particles m$^{-3}$ | Manta trawl (330 µm) | (Song et al. 2014) |
| Bohai Sea | Surface water | 0.33 ± 0.34; 0.01 - 1.23 (mean; range) | particles m$^{-3}$ | Manta trawl (330 µm) | (Zhang et al. 2017) |
| East China Sea | Surface water | 0.17 ± 0.14; 0.03 - 0.45 (mean; range) | particles m$^{-3}$ | Neuston net (330 µm) | (Zhao et al. 2014) |
| Offshore NE Pacific Ocean | Sub-surface water | 8 - 9180 | particles m$^{-3}$ | Vessels (250, 125, and 62.5 µm) | (Desforges et al. 2014) |
| ***Mediterranean and European Seas*** | | | | | |
| Adriatic Sea | Surface water | 1.0 ± 1.8 | particles m$^{-3}$ | Neuston net (200 µm) | (Suaria et al. 2016) |
| Sardinia Sea | Surface water | 0.15 | particles m$^{-3}$ | Manta Trawl (500 µm) | (de Lucia et al. 2014) |
| Bay of Calvi, Corsica | Surface water | $62\cdot10^{3}$ | particles km$^{-2}$ | Neuston net (200 µm) | (Collignon et al. 2014) |
| Mediterranean cost of Turkey | Surface water | $1.6\cdot10^{4}$ - $5.2\cdot10^{4}$ | particles km$^{-2}$ | Manta trawl (330 µm) | (Güven et al. 2017) |
| Baltic Sea | Sub-surface water | ~$10^{2}$ - $10^{4}$ | particles m$^{-3}$ | Net tows (90 µm) | (Gorokhova 2015) |

**Table 1.4** (continued) Examples of MPLs abundances reported for sea (sub)surface waters across the world since 2014.

| Location | Sample type | MPLs abundance | | Sampling methods | Ref. |
|---|---|---|---|---|---|
| ***Arctic and Antarctic Ocean*** | | | | | |
| Beaufort and Chukchi Seas | Surface water | 38 - 234 | particles $m^{-3}$ | Ice core drilling | (Obbard et al. 2014) |
| Svalbard, Norway | Surface water | 0.34 ± 031; 0 - 1.31 (mean; range) | particles $m^{-3}$ | Manta trawl (333 µm) | (Lusher et al. 2015b) |
| | Sub-surface water | 2.68 ± 2.95; 0 - 11.5 (mean; range) | particles $m^{-3}$ | Vessels | |
| Southern Ocean | Surface water | 0.031 | particles $m^{-3}$ | Neuston net (350 µm) | (Isobe et al. 2017) |
| Ross Sea | Sub-surface water | 0.17 ± 0.34; 0.0032 - 1.18 (mean; range) | particles $m^{-3}$ | Vessels (60 µm) | (Cincinelli et al. 2017) |

**Table 1.5** Examples of MPLs abundances reported for surface freshwaters across the world since 2014.

| Location | Water body type | MPLs abundance | | Sampling methods | Ref. |
|---|---|---|---|---|---|
| ***America*** | | | | | |
| North Shore Channel, Chicago, USA | River | $7.3 \cdot 10^5$ - $6.7 \cdot 10^6$ | particles/km$^2$ | Neuston net (333 µm) | (McCormick et al. 2014) |
| Estuarine tributaries Chesapeake Bay, USA | Estuary | $5.5 \cdot 10^3$ - $3.0 \cdot 10^5$ | particles/km$^2$ | Manta trawl (330 µm) | (Yonkos et al. 2014) |
| Goiana Estuary, NE Brazil | Estuary | 0.26 | particles/m$^3$ | Plackton net (300 µm) | (Lima et al. 2014) |
| ***Asia*** | | | | | |
| Yangtze River Hanjiang River urban sections, China | River | 2516.7 ± 911.7<br>2933 ± 305.5 | particles/m$^3$ | 12 V DC Teflon pump and filtration (50 µm) | (Wang et al. 2017a) |
| Yangtze Estuary, East China | Estuary | 500-10200 | particles/m$^3$ | 12 V DC Teflon pump and filtration (32 µm) | (Zhao et al. 2014) |
| ***Europe*** | | | | | |
| Seine River, France | River | 0.35 | particles/m$^3$ | Manta trawl (300 µm) | (Dris et al. 2015) |
| River Seine,urban section (Paris) France | River | 0.54 (fragments)<br>45 (fibres) | particles/m$^3$ | Manta trawl (300 µm) | (Wagner et al. 2018) |
| Danube River, Austria | River | 0.32 ± 4.66 | particles/m$^3$ | Driftnets (500 µm) | (Lechner et al. 2014) |
| Rhine River, Germany | River | 892777 | particles/km$^2$ | Manta trawl (300 µm) | (Mani et al. 2015) |
| Tamar Estuary, UK | Estuary | 0.028 | particles/m$^3$ | Plankton net (330 µm) | (Sadri et al. 2014) |
| ***Africa*** | | | | | |
| Urban estuaries of KwaZulu-Natal, South Africa | Estuary | 7.03 ± 11.9; 2 - 48.7 (mean; range) | particles/m$^3$ | Zooplankton net (300 µm) | (Naidoo et al. 2015) |

Due to their easy accessibility, numerous studies focus mainly on sandy beaches. In Europe, a range of 1 - 2000 particles kg$^{-1}$ was found in beach sediments, dominated by PE and PP. As in water, wind, marine currents and tides play an important role as well in MPLs distribution in sediments and their accumulation on the shoreline. In the Mediterranean Sea, the eastern region is the most contaminated by MPLs, with values of about 387 ± 100 particles kg$^{-1}$ d.w., due to limited water circulation in this part of the basin. A large number of plastic microfibres (1.4-40 fibres per 50 mL) has been shown in deep-sea sediments from the Atlantic Ocean, the Mediterranean Sea and Indian Ocean (Woodall et al. 2014).

These data show that the occurrence of MPLs is more abundant in deeper habitats than surface water. However, there is still a large gap in the knowledge of the presence, fate and composition of plastic waste in sediments. For example, the abundance in sub-tidal sediments has not yet been fully studied, particularly in the areas far from the continental margins (Woodall et al. 2014; Peng et al. 2018). Recently, some studies have reported the first data for polar regions, confirming MPLs contamination in sediments in both the Arctic and Antarctic regions (Bergmann et al. 2017; Reed et al. 2018; Munari et al. 2017).

### Biota

Being ubiquitous in marine environments, MPLs interact with biota (Kühn et al. 2015). Because of their small size, marine organisms can ingest MPLs by confusing them with food (de Sá et al. 2015; Lusher et al. 2013). The presence of MPLs has been reported worldwide in a wide range of marine organisms from different trophic levels (Table 1.6), including zooplankton (Cole et al. 2013; Desforges et al. 2015). Predation of contaminated organisms contributes to their biomagnification through the marine food chain (Nelms et al. 2018; Farrell et al. 2013). Sun et al. (2017) confirmed that the rates between MPLs and organisms increase with the trophic level. On the other hand, filter-feeding organisms can also ingest MPLs/NPLs, by filtering or inspiration through ventilation mechanisms (Watts et al. 2016; Zhu et al. 2019; Koelmans et al. 2015).

At benthic level, primary organisms affected by MPLs contamination are invertebrates such as crustaceans or bivalves, especially those intended for human consumption (Murray et al. 2011; Van Cauwenberghe et al. 2014). For example, Andrade et al. (2017) reported the presence of plastic filaments between 3 and more than 20 mm long in the commercial crab (*Lithodes santolla*). In another study, Mathalon et al. (2014) found that farmed mussels have higher levels of microfibres than wild mussels in Nova Scotia.

In the pelagic area, several studies have shown high levels of plastic contamination in fish from various geographical locations and depths (Lusher et al. 2013; Wieczorek et al. 2018). High percentages of fragments, pellets, and films were found in the gastrointestinal tract (GIT) of mesopelagic fish from the Pacific Ocean (Davison et al. 2011; Choy et al. 2013). In particular, these fish are considered as an important source of MPLs for higher trophic-level organisms such as predator fish and marine mammals (Nelms et al. 2018; Burkhardt-Holm et al. 2019). Nelms et al. (2018) assessed the presence of MPLs (5.5 particles per animal) in the digestive tract of various raptorial feeders, including dolphins, porpoises, whales, and seals. The ingestion of MPLs has also been documented in seabirds and sea turtles (Avery-Gomm et al. 2013; Provencher et al. 2014; Caron et al. 2018). In some cases, the concentration of MPLs in seabirds is relatively low due to their ability to expel by regurgitation (Lindborg et al. 2012)

**Table 1.6** Examples of MPLs' occurrence in biota (recent studies since 2018).

| Habitat | Location | Organism | Tissue | MPLs type | MPLs | Ref. |
|---|---|---|---|---|---|---|
| *Water column* | East China Sea | Zooplanktons | - | Fibres, pellets, and fragments | 0.13 - 0.35 pieces/zooplankton | (Sun et al. 2018) |
| | Kenyan coastline | Zooplanktons | - | Fibres and fragments | 0.16 - 0.46 pieces/zooplankton | (Kosore et al. 2018) |
| *Benthic* | French Atlantic coasts | Bivalves (mussels and oysters) | soft tissue | PE and PP | 0.61 ± 0.56 particles/mussels and 2.10 ± 1.71 particles/oysters | (Phuong et al. 2018) |
| | California coast | Mole crabs | Gut | Fibres | 0.65 ± 1.64 particles/individual | (Horn et al. 2019) |
| | Persian Gulf | *Penaeus indicus* | Fish muscle | Fibres and fragments | 18.50 ± 4.55 items/ 10 g fish muscle | (Akhbarizadeh et al. 2018) |
| *Pelagic* | Spanish Mediterranean coast | *S. pilchardus* and *E. encrasicolus* | GIT | PET was dominant (30%) | 80% fibres | (Compa et al. 2018) |
| | Persian Gulf | *Sphyraena jello* | Fish muscle | Fibres and fragments | 5.66 ± 1.69 items/ 10 g fish muscle | (Akhbarizadeh et al. 2018) |
| | Cornwall, UK | Seal scats | GIT | PP, EP were dominant (both 27%) | 0-4 particles/scat | (Nelms et al. 2018) |
| | Scotia, UK | Marine mammals | Gut content | Fibres and fragments (PA, PET, PE, etc.) | 5.5 ± 2.7 particles/animal | (Nelms et al. 2019) |
| | Northern Cyprus | Loggerhead and green turtle | Gut content | Blue and black fibres and fragments | 10-15 particles/turtles | (Duncan et al. 2019) |
| | Parque Biológico de Gaia, Portugal | Seabirds | stomach content | Sheetlikes, fragments, threadlike, foam, etc. | MPLs > 65% | (Basto et al. 2019) |

### 1.4.2 Risk assessment

The impacts of MPLs and NPLs on ecosystems and their potential risks to human health continue to be uncertain. The risks assessment of MPLs/NPLs in the marine environment implies the evaluation of the adverse effects caused in an organism, population or ecological system as a result of interaction with these compounds, and other contaminants related to them, at the exposure levels. So far, due to the different methodological approaches applied for sampling, identification and quantification of different sizes and types of plastics, the information on MPLs/NPLs distribution of in the environment, including in living organisms, is still limited. Consequently, there are severe gaps in knowledge of hazard characterisation and exposure levels (Koelmans et al. 2017).

Recently, several laboratory experiments have been conducted to investigate the effects of marine organisms. In particular, ecotoxicological studies are focused on model species such as fish, crustaceans and molluscs (de Sá et al. 2018). A wide range of organisms may take up MPLs/NPLs from the natural environment by external or internal exposure based on the concentration and the size distribution of the particles (Lusher et al. 2013). Most organisms introduce plastic particles by ingestion than dermal uptake. Once in the organism, it has been demonstrated that MPLs/NPLs can induce physical and chemical toxicity depending on their size and shape (Lee et al. 2013). Smaller MPLs with variable surface roughness may attach more readily to internal and external surfaces of the body causing physical injuries, inducing inflammation and stress, or affecting feeding rate, growth, and reproduction (de Sá et al. 2018). At sub-micron size, plastic particles are potentially more hazardous due to the probability of penetrating cells causing biological effects such as oxidative stress, inflammatory responses, or cytotoxicity as demonstrated for nanomaterials (Lu et al. 2016; Fröhlich et al. 2010). In addition to physical effects, MPLs are carriers of hazardous chemicals that can cause adverse chemical effects. Plastic particles and their co-contaminants can pass through the food web while predators consume the prey, causing bioaccumulation or biomagnification (Koelmans 2015). However, higher trophic organisms (e.g. marine mammals) and the ecological and community-level effects of MPLs should be further explored (Browne 2015).

Finally, a major information gap is the fate and toxicity of MPLs/NPLs in humans (Wright et al. 2017). Also, although a wide range of foods are supposed to be contaminated with MPLs and NPLs, few studies have addressed the quantification of dietary exposure (Lusher et al. 2017a).

# 2 . Objectives and thesis structure

## 2.1 Objectives

Plastics have played a major role in technological and industrial development, improving the quality of human life. However, during the last decades, its overuse and poor waste management have led to accumulating plastics in the environment. Due to the high persistence, plastic debris is omnipresent and its presence in different environmental compartments has already been reported by several studies. In particular, the greatest concern is due to the smaller particles that come from the breakdown of this debris. Nowadays, micro(nano)plastics are considered hazardous materials that can cause serious damage to the environment and human health. In this context, several gaps need to be filled in terms of chemical and ecotoxicological assessment of micro(nano)plastics and emerging risks. For this reason, numerous research initiatives have been promoted to assess the presence, fate and impact of these contaminants, especially in marine environments.

Under this context, the overall objects of this doctoral thesis were:

1. To carry out an integrated study of the presence and fate of plastic pollution in the coastal environment. The study was particularly focused on the river transport of plastics, and the fate and behaviour of plastic particles in estuarine and coastal waters of the north western Mediterranean Sea. In this view, appropriate analytical methods have been developed to quantify and identify the behaviour of these compounds and their co-contaminants in the environment.
2. To assess the ecotoxicological implications of micro(nano)plastics on marine ecosystem and human health.

The specific objectives were:

- To contribute to enlarge our knowledge on anthropogenic riverine litter entering the Mediterranean Sea near the metropolitan area of Barcelona.
- To develop analytical methods for the analysis of micro(nano)plastics in water samples based on different mass spectrometric techniques. The different analytical techniques have been compared in terms of ionization and quantification. In this case, polystyrene (PS) was selected as representative polymer due to its wide use in consumer products, its environmental fate and behaviour, and its possible toxicological effects.
- To apply the analytical method based on liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) to study the occurrence of PS in natural water samples from the area of Ebro Delta (Catalonia, NE of Spain).
- To assess the adsorption behaviour of persistent organic pollutants, such as perfluoroalkyl substances (PFASs), on the surface of aged MPLs under real environmental conditions.
- To assess the impacts of MPLs on marine organisms filling gaps of knowledge concerning to medium scale bioindicators in the Mediterranean Sea. In particular, the presence of MPLs in the gastrointestinal tracts (GITs) of common dolphinfish caught under Fish Aggregating Devices (FADs) from the western Mediterranean was investigated.
- To study the cytotoxic effects of nano-size particles at cell levels. Human cells were exposed at different concentrations of PS-NPLs and PE-NPLs and other contaminants which could be present in the estuarine and coastal environments.

## 2.2 Thesis structure

This thesis is divided into five different chapters. The first two chapters contain a general introduction and the objectives of the thesis. In the introduction, various aspects such as the synthesis of plastics, global production and consumption, and environmental fate of plastic waste are discussed. Subsequently, the concept of micro(nano)plastics and their classification is presented, followed by a brief and general review of their occurrence in the environment and analysis.

Afterwards, the experimental work undertaken during this PhD thesis research is presented in Chapter 3 and 4. Each chapter is divided into a specific introduction, experimental results (presented through the corresponding publications) and discussion. In particular, Chapter 3 provides a more in-depth look at the problem of marine litter in the Mediterranean Sea and its possible sources of contamination. More information on the transport of plastics through river systems and the fate and behaviour of micro(nano)plastics in estuaries and coastal waters of the western Mediterranean Sea is reported.

On the other hand, Chapter 4 focuses more on the ecotoxicological assessment of micro (nano)plastics. Possible effects on Mediterranean marine organisms and human health are considered. Part of the results contained in this chapter corresponds to the experimental work carried out during the research activity at the Institute for Environmental Protection and Research (ISPRA) of Milazzo (Italy) under the framework of the Interreg Med project “Plastic Busters MPAs: Preserving biodiversity from plastics in Mediterranean Marine Protected Areas”.

Finally, Chapter 5 summarizes the general conclusions derived from the work carried out during this doctoral project and future trends. The cited literature can be found at the end of this work.

# 3. Plastics in estuarine environment, from macro to micro: transport, analysis, and behaviour

## 3.1 Introduction

### The Mediterranean plastic pollution

The Mediterranean Sea is one of the areas most affected by marine litter worldwide. The main reasons are densely populated coastlines, large rivers, highly developed tourism, intensive fishing and maritime activities, and a large amount of solid waste produced which is often not managed sufficiently. In addition, it is a semi-closed sea of 2.5 million km$^2$ characterised by a net inflow of surface water from the Atlantic Ocean, through the Strait of Gibraltar, with limited possibilities of deeper waters outflow. Because of these limited exchanges with the oceans, the water residence time in the basin can be very long up to a century (Candela 1991). Considering both the possible land and sea inputs in a model, Lebreton et al. (2012) identified the Mediterranean Sea as a potentially important accumulation zone.

A first assessment of the litter composition in the Mediterranean Sea has shown that the anthropogenic litter makes up the majority of waste accumulated on beaches, sea surface, and seafloor (Suaria et al. 2014; Ramirez-Llodra et al. 2013; Kordella et al. 2013). In particular, the main litter component is plastics (70-90%), largely represented by single-use plastic products and abandoned or lost fishing gear (UNEP/MAP 2015). Plastic debris is not evenly distributed in the basin and concentrations in the accumulation areas are generally comparable to those reported in subtropical oceanic gyres (Cózar et al. 2015).

Even though most of the waste load come from land-based sources (up to 69%), identifying the causes and amount of marine litter, including plastics, is a complex task. To address this critical issue, the United Nations Environment Programme/Mediterranean Action Plan (UNEP/MAP 2015) has developed a program to protect the Mediterranean marine environment and its coastal regions. Several implementations of the MAP have also been carried out in synergy with other relevant global and regional initiatives. Among them, the EU Marine Strategy Directive 2008 (MSFD_2008/56/EC 2008) set out a list of 11 qualitative descriptors of environmental status for which the Good Environmental Status (GES) of the EU marine waters will be achieved by 2020. In particular, the Descriptor 10 focuses mainly on protecting the coast and the marine environment against harm caused by marine litter. Therefore, an initial assessment of litter characteristics in the marine and coastal environment, potential sources and pathways, and accumulation dynamics has been requested from all EU Member States.

### Data collections

Data on quantities and types of marine litter in the Mediterranean is still incomplete. Most of the information comes from voluntary organisations with the aim of cleaning up the environment from waste. Consequently, studies assessing the composition, quantity and spatial/temporal distribution of macro- and MPLs in marine and river environments are scarce. Due to limited field data, the plastic mass present in the Mediterranean Sea is often estimated. For instance, Cózar et al. (2015) estimated that a much lower plastic load (756 to 2,969 tonnes) floating on the surface of the Mediterranean region compared to 23,150 tonnes calculated by Eriksen et al. (2014). The discrepancies in these studies could arise from different model setups.

Generally, different strategies based on quantity or flow measurement are adopted for data collection. Studies on floating litter in the Mediterranean are among the most reported in the literature and show an increase in plastic abundance from macro- to microparticles. The results of the first large-scale survey in the central and western Mediterranean in 2013 showed an average abundance of 24.9 floating items km$^{-2}$ of Anthropogenic Marine Debris (AMD), of which 95.6% was plastic (Suaria et al. 2014). Visual measurements allow for the detection of larger (> 2 cm) floating objects and are closely dependent on the observer. Also, weather conditions, sea currents and variability of debris input can influence the count (Aliani et al. 2003). On the other hand, trawl nets are typically used to quickly sample litter, especially small items (up to 200 µm), in larger areas. In these cases, the plastic density ranging from $1{\cdot}10^4$ to $1{\cdot}10^5$ of items per square kilometre (Ruiz-Orejón et al. 2016; Pham et al. 2014; Suaria et al. 2016).

## Possible plastic inputs

Nowadays, in agreement with MSFD 2008/56/EC, the interest of the scientific community also focuses on the sources and possible transport of macro- and MPLs into the environment.

The Mediterranean region is the 4th largest plastic producer, reaching almost 38 million tonnes of plastic goods in 2016 (76 Kg per person). After less than one year, most of the plastic produced becomes waste and most of it is highly related to tourism (UNEP/MAP 2017). Among these, 6.6 million tons of plastic waste are poorly managed, becoming the primary source of plastics in the Mediterranean Sea. Major contributors to the mismanagement of plastic waste are the Eastern Mediterranean countries such as Egypt and Turkey (WWF 2019).

Coastal activities contribute largely to the entry of plastics into the Mediterranean Sea. Nevertheless, anthropogenic and natural litter can be transported indirectly over long distances by rivers, streams, drains or sewage outflows from inland to the sea. In such cases, it is often difficult to assign a source with a robust level of accuracy due to the multiple factors involved. The amount and type of litter depends on length, size and (geo)morphological characteristics of the bed and the catchment area, as well as the population density along the river. Besides, Mediterranean rivers have a significantly higher level of seasonal variability (Cid et al. 2017), which can influence the waste load transported by them. Heavy rainfall and strong winds can play a crucial role in litter accumulation and distribution (Lebreton et al. 2017). Finally, the level of management of the catchment area, which depends on the presence of human infrastructure along the river (e.g. storage areas, dams, canals, canals, plants, etc.), can affect the river flow by retaining or releasing macro- and micro-litter.

Although a large proportion of plastics (from macro to micro) can reach the sea using rivers as waterways, few data on the presence of plastics in these systems have been reported, especially in estuarine environments. Rivers flowing into the Adriatic, the north-western basin and the Aegean Sea account for at least half of the actual annual average discharge in the Mediterranean Sea. Among these, the main contributions come from the Po, Ebro, and Rhone, which are also among the most studied rivers in terms of pollution. Focusing on MPLs/NPLs in the aquatic environment, one of the main contributions of these contaminates into the sea come from estuaries due to the discharge of WWTPs.

Identifying the pathways and fate of plastics could help the scientific community to quantify the likely coastal impacts of plastic pollution. In this context, numerical modelling of floating marine debris in the Mediterranean Sea has considered plastic inputs from large coastal cities, rivers and shipping lanes. The south-eastern part of the basin, especially Turkeys' Cilicia coastline, has been identified as one of the main accumulation zones in the Mediterranean (Liubartseva et al. 2018; Zambianchi et al. 2017). Other plastic pollution hotspots are placed in the coastal areas between Barcelona and Valencia, Algiers, Marseille's Bay, and Delta Po in the western Mediterranean (Liubartseva et al. 2018; Liubartseva et al. 2016). These accumulation areas may change mainly due to marine currents in the basin. Moreover, accumulation rates in these regions are influenced by various anthropogenic and natural factors, such as river discharges, sinking and degradation processes (Poulain et al. 2013b; Béranger et al. 2010; Mansui et al. 2015).

### Behaviour in the coastal environments

Human activities taking place in the coastal and marine areas of the Mediterranean Sea are also responsible for the chemical contamination. Nowadays, the interaction between plastic particles and these pollutants is a serious concern that is closely related to plastic contamination (Yu et al. 2019).

Once in the aquatic environment, due to their large surface-area-to-volume ratio, MPLs/NPLs can adsorb, and subsequently, transport and release to biota other organic contaminants present in the surrounding water (Haegerbaeumer et al. 2019; Teuten et al. 2009). Moreover, these plastic particles can also release monomers and leach other organic and inorganic compounds, known as additives, used in plastic manufacturing. Additives, including plasticisers, are chemical compounds incorporated into plastics to improve their properties. Since they are not chemically bound to the plastics, these compounds can easily migrate away from the polymer matrix ending up in the surrounding environment. Phthalates, bisphenol-A (BPA), nonylphenol, and brominated flame retardants are the most common additives recovered from the environment and organisms (León et al. 2019; Fossi et al. 2014).

As regards the transport of other contaminants present in the surrounding areas to the biota (*Trojan Horse effect*), the hydrophobic nature of MPLs plays a fundamental role. Several studies have reported the adsorption/desorption of persistent organic pollutants (POPs), such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and organochlorine pesticides and its degradants (e.g. DDT/DDE/DDD), on MPLs (Rodrigues et al. 2019). Other studies have also considered the absorption of endocrine disrupters (EDCs) or antibiotics (Tizaoui et al. 2017; Li et al. 2018).

The adsorption capacity of common MPLs (e.g. PE, PP, PS, and PVC) and their potential transport of POPs in the coastal environment have been widely studied (Bakir et al. 2014; Bakir et al. 2012; Frias et al. 2010; Mato et al. 2001). These researches show that the properties of polymer (e.g. chemical composition, shape, size, glass transition temperature ($T_g$), etc.) influence the adsorption dynamics (Rochman et al. 2013a; de Sá et al. 2018). For example, significant differences were observed in the adsorption capacity of PE-microbeads extracted from various commercial scrubs and exposed to $^{3}$H-phenanthrene and $^{14}$C-DDT in seawater, depending on their shape and surface morphology (Napper et al. 2015). Additionally, the changes on polymer surface due to a degradation or weathering processes increase the absorption capacity of MPLs. Fisner et al. (2017) reported a growing association of PAHs with plastic resins with a colour spectrum reflecting the actions of the environment. This behaviour was also confirmed by the fact that leaching plastic resin absorbed more trace metals than virgin pellets (Holmes et al. 2012). Apart from the type of plastic, parameters such as salinity, pH, temperature and presence of organic matter play an important role in absorption/desorption phenomena in aqueous environments.

## 3.2 Results

In this section, the transport, analysis and behaviour of MPLs in the coastal environment of the Western Mediterranean were discussed.

The experimental results are presented in the following publications:

- **Riverine anthropogenic litter load to the Mediterranean Sea near the metropolitan area of Barcelona.** Gabriella F. Schirinzi, Marianne Köck-Schulmeyer, María Cabrera, Daniel González-Fernández, Georg Hanke, Marinella Farré, Damià Barceló. *Science of The Total Environment 714 (2020): 136807.*

- **Trace analysis of polystyrene microplastics in natural waters.** Gabriella F. Schirinzi, Marta Llorca, Raquel Seró, Encarnación Moyano, Damià Barceló, Esteban Abad, Marinella Farré. *Chemosphere 236 (2019): 124321.*

- **Adsorption of perfluoroalkyl substances on microplastics under environmental conditions.** Marta Llorca, Gabriella F. Schirinzi, Mónica Martínez, Marinella Farré, Damià Barceló. *Environmental Pollution 235 (2018): 680-69.*

### 3.2.1 Publication I: Riverine anthropogenic litter load to the Mediterranean Sea near the metropolitan area of Barcelona

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# Riverine anthropogenic litter load to the Mediterranean Sea near the metropolitan area of Barcelona, Spain

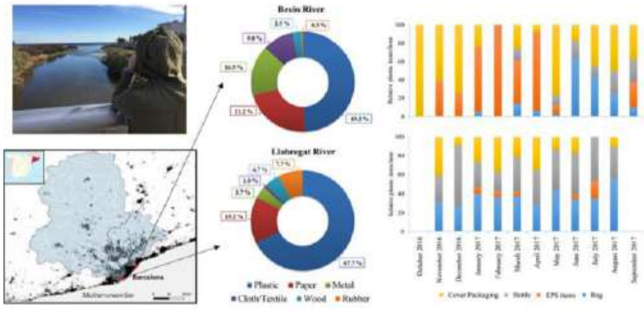
Gabriella F. Schirinzi [a], Marianne Köck-Schulmeyer [a], María Cabrera [c], Daniel González-Fernández [d,*], Georg Hanke [e], Marinella Farré [a], Damià Barceló [a,b]

[a] *Institute of Environmental Assessment and Water Research, IDAEA-CSIC, C/ Jordi Girona 18-26, 08034 Barcelona, Spain*
[b] *Catalan Institute for Water Research, ICRA, Emili Grahit 101, 17003, Girona, Spain*
[c] *Paisaje Limpio Association, C/ Santa Brígida 19, 28220 Majadahonda, Spain*
[d] *Department of Biology, International Campus of Excellence of the Sea (CEIMAR), University Marine Research Institute (INMAR), University of Cádiz, E-11510 Puerto Real, Spain*
[e] *European Commission - Joint Research Centre, Via E. Fermi 2749, 21027 Ispra, Italy*

## HIGHLIGHTS

- Large urban areas substantially contribute to marine pollution through rivers.
- 51% and 68% of the total floating litter in Llobregat and Besòs Rivers are plastic.
- Top plastic items were bags, bottles, EPS items and cover/packaging.
- ~0.4–0.6 tonnes/year of plastic were loaded into the Mediterranean Sea by Catalan Rivers.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Every year >4 million tonnes of plastic are estimated to enter the oceans and much of it comes from land-based sources through rivers and estuaries. To fill the lack of information related to plastic inputs from rivers, a harmonized approach based on visual observations for monitoring floating macro litter was followed in this work. We provide the results of one-year monitoring (October 2016–September 2017) in the Llobregat and El Besòs rivers, which are flowing through an industrialized and populated area nearby the city of Barcelona (Catalonia, NE Spain). Floating litter items categories were influenced by urban centres located along the rivers. Overall, similar litter composition was observed in both rivers with a prevalence of plastics, mainly related to the food and beverage sectors. Seasonal variability showed significant correlations with natural factors such as wind and rainfall. Approximately 0.4–0.6 tonnes of plastic per year were estimated to be loaded into the sea by these two Catalan rivers. This study contributes to enlarge our knowledge on anthropogenic riverine litter entering the NW Mediterranean Sea, providing a starting point for the development of further mitigation strategies.

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* Corresponding author.
*E-mail address:* daniel.gonzalez@uca.es (D. González-Fernández).

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## 1. Introduction

In the past decades, new technologies were based on the use of efficient and cheap alternative materials according to industrial interest. As a result, a lot of common commercial products are currently made of synthetic polymers such as plastic. A part of the plastic products is leaking from the production and use processes, e.g. by littering of single use plastics, or through waste mismanagement. The input of this litter into the environment by human activity contributes to increasing the amount of waste in coastal waters, estuaries and oceans (Vikas and Dwarakish, 2015). It represents not only an aesthetic problem but also potential damage to littoral areas, marine wildlife and the socio-economic activities linked to these regions (Rochman et al., 2016). In consequence, international organizations recognize marine litter as a severe environmental problem. In fact, several public policies have introduced different initiatives, e.g. at EU level: the Marine Strategy Framework Directive (MSFD, 2008/56/EC) (Commision, E., 2008) and the European Strategy for Plastics in a Circular Economy (COM/2018/028 final) (Commission, E, 2018a), in order to prevent and limit plastic contamination (Martin, 2013; Galgani et al., 2013; Commission, E., 2018b). However, these efforts are recent, while plastic residues are already abundant in the marine environment and have travelled long distances reaching remote areas (Cózar et al., 2017; Lavers and Bond, 2017) due to their chemical persistence and buoyancy properties. Further, plastic items in the environment are subject to fragmentation processes generating smaller pieces known as microplastics (<5 mm), which interact directly with marine organisms (Harrison et al., 2011) and may also act as vectors of emerging chemical contaminants (Bakir et al., 2014). Until recently, researchers have been mainly focused on litter already present in the marine enviroment (Ruiz-Orejón et al., 2016; Duhec et al., 2015; Martins and Sobral, 2011; Kako et al., 2018; Blettler et al., 2018), especially on the study of coastal waters adjacent to urban areas (Santos et al., 2009; Ramirez-Llodra et al., 2013). Every year between 4.8 and 12.7 million tonnes of plastic enter in the oceans (Jambeck et al., 2015). Some studies suggest that riverine litter can contribute up to 90% to all marine litter input (Öko-Institut, 2011) having an important impact on litter abundances in the marine environment (Schmidt et al., 2017; González et al., 2016) including coastal beaches (Lee et al., 2013; Veerasingam et al., 2016). Although a large share of litter entering the marine environment is assumed to come from land-based sources, these inputs have not been quantified (González-Fernández and Hanke, 2017). Rivers could transport plastic litter over long distances to downstream lakes and coastal environments, where they can interact with biota and the water ecosystem (Desforges et al., 2015; Wesch et al., 2016). The riverine transport is influenced by the morphologic and hydrologic characteristics of the catchment, which can affect the amount of waste emitted from rivers to the sea (González et al., 2016). The human population densities and urban development in the catchment area are among the factors determining the amount of mismanaged waste which could directly (or indirectly) end up in the rivers (Schmidt et al., 2017; Lebreton et al., 2017). Natural events connected to climatic and meteorological conditions (e.g. strong wind, rain or floods) play also an important role regarding the litter transport within the catchment area (Nizzetto et al., 2016; Browne et al., 2010; Yonkos et al., 2014). Therefore, the attribution of riverine litter input from mismanaged waste requires a complex previous study of the riverine area considering hotspots and possible sources, such as urban centres and wastewater treatment plants (WWTPs) (McCormick et al., 2014; Mintenig et al., 2017). In order to fill information gaps about plastic waste input from land to ocean (Jambeck et al., 2015; Schmidt et al., 2017; Lebreton et al., 2017), some initiatives have focused on riverine litter (Van der Wal et al., 2015; Surfrider Foundation Europe, 2014; Hohenblum et al., 2015). Based on needs identified within the MSFD context, the RIMMEL - Riverine and Marine floating macro litter Monitoring and Modelling of Environmental Loading - project (RIMMEL project, 2015) applied a harmonized approach for the monitoring of the litter transported from different European rivers into the marine environment. The objective of our work, within the RIMMEL project, was to estimate the riverine anthropogenic litter flux from Llobregat and Besòs River basins, which are flowing through an industrialized and populated area nearby the city of Barcelona (Catalonia, NE Spain) into the Mediterranean Sea.

## 2. Materials and methods

### 2.1. Study areas

Two rivers were studied, the Llobregat (basin size 4948 km$^2$) and Besòs (basin size 1024 km$^2$) located in Catalonia (NE of Spain) (Fig. 1). The lower courses of both rivers run through the densely populated Barcelona Metropolitan area, which encompasses a population of approximately 4.4 million inhabitants. These rivers provide water supply to urban, industrial and irrigation activities, and particularly, the Llobregat river is one of the most important water resources of Catalonia. Both delta areas, formerly devoted to irrigated agriculture, now support important industrial settlements (e.g. food, chemicals, motor vehicles, energy, and pharma industries). These activities are extensively located throughout these river basins and increase significantly downstream due to the influence of the Barcelona metropolitan area.

Furthermore, these waterways show a typical profile of Mediterranean rivers, subject to a large flow variation closely related to the rainfall regime (Fernández-Turiel et al., 2003). Due to some geographical factors (e.g. orography, proximity to the Mediterranean Sea and distance from the Atlantic coast), the annual rainfall regime in the Catalan region differs from the typical Mediterranean one (Martínez et al., 2007). In particular, the highest daily rainfalls were recorded in the north-eastern part of Catalonia and the Ebro Delta. Heavy rainfall occurs mainly in autumn, with occasional episodes in spring and summer. Significant flash floods have been also recorded in this zone (Llasat et al., 2010). For instance, Besòs river is dominated by a long dry spell punctuated by a short rainy season, resulting in a flow change from 1.5 to 1500 m per second during a flood (Bovet et al., n.d.; Ferrer, n.d.). For the Llobregat basin, which extends from the Pyrenees to the coast, there are several rainfall records depending on the altitude (Amengual et al., 2007). The river flux variation is also connected to urban and industrial wastewater discharges, which also increases the contamination, especially near the metropolitan area. WWTPs with tertiary treatment facilities, built along the basins, allow improving the quality of these watercourses. For example, in the Llobregat River, WWTP effluents contribute with >90% of the total flow; however, only 42 of 89 Catalan WWTPs operate with tertiary treatment (Pérez et al., 2010).

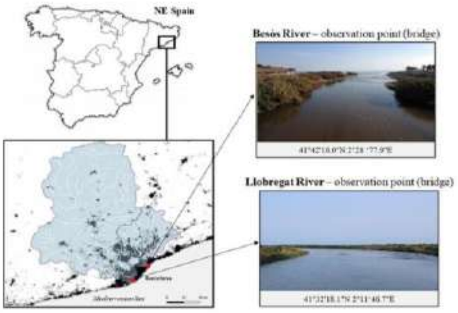

**Fig. 1.** Localization of Llobregat and Besòs rivers (Catalonia, NE Spain), including drainage basins and population density (white to black gradient, black color clipping for cells >2500 inhabitants/km$^2$), and the respective observation locations (red dots). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

### *2.2. Riverine litter monitoring*

The methodological approach was based on visual observations performed from an elevated position, corresponding to the first bridge upstream from the mouth of the river (Fig. 1). The three basic monitoring parameters: observation height (from river surface to observer's sight), observation track width (section where the observer scans for floating litter), and total river width at the monitoring site were 7.8 m, 36 m, and 36 m for Besòs River (BR), and 15 m, 26 m, and 150 m for Llobregat River (LR), respectively. The observations were done facing upstream from the middle of the bridge and targeted identification of floating macro litter items ($\geq$2.5 cm). For LR monitoring, the sighting was made by two observers, then using 12× binoculars for object identity confirmation. The monitoring sessions consisted of short observations ranging from 26 to 63 min. The specific time and frequency (weekly in most of the cases) of each monitoring session depended on the observers' availability and the weather conditions. A total of 36 and 51 sessions were carried out for BR and LR from October 2016 to September 2017, respectively.

Data were registered and reported using the JRC Floating Litter Monitoring App, which allowed the classification of the items based on material and size, following the harmonized approached proposed by RIMMEL. The App considers the floating items described in the MSFD "Master List of Categories of Litter Items" developed by the MSFD Technical Group on Marine Litter (Galgani et al., 2013). The use of a common list of items provides consistency in relation to the assessment of Descriptor 10 (Marine Litter) under the implementation of the MSFD and makes data from different monitoring schemes comparable. The monitoring approach has been further described in González-Fernández and Hanke (2017).

### *2.3. Data analysis*

In order to calculate floating macro litter flux from river to the sea, the following extrapolations have been performed: i) the monitoring results obtained in the observation track width were considered homogeneous over the cross-section for the total river width; ii) the litter counts were normalized over time and extrapolated to total river width to express litter flux as items per hour. In the LR, the percentage of observed track width compare to total river width was 15%, while in BR it was 100%.

Plastic items were classified into size ranges (2.5–5 cm, 5–10 cm, 10–20 cm, 20–30 cm, 30–50 cm, and $>$50 cm) based on their maximum length dimension, and their commercial purposes. Plastic mass per item was estimated according to this classification and the density of the main polymer used in the production of such items (Table 3). In particular, polyethylene (PE), polyethylene terephthalate (PET), and expanded polystyrene (EPS) were considered as the main polymers for bags, bottles, and EPS items, respectively. For the plastic mass input, only plastic items larger than 5 cm were considered. Smaller items were not taken into account as their mass contribution is expected to be low (Eriksen et al., 2014; van Emmerik et al., 2018). The computed dimensions per items included the minimum and maximum value of their size range (see above) as length (l). The thickness (t) was fixed as the minimum size used at industrial level to produce the items concerned. The chosen values were 0.015 cm, 0.02 cm, 1.3 cm and 0.05 cm for bags (Plastic, L, n.d.), bottles (Instruments, S, n.d.), EPS items (world, M. m, n.d.; WebstaurantStore, n.d.), and packaging material (molding, I. y. i, n.d.), respectively. The width (w) was fixed at 10 cm. These assumptions are considered conservative because the common items previously listed have usually substantially larger widths and thickness than those adopted in our estimation. Regarding cover/packaging, the average density of the most common polymers used for this purpose (e.g. PE, PET, polystyrene (PS), polypropylene (PP), and polyvinyl chloride (PVC)) was applied (Alavi et al., 2014). Finally, the total mass calculated for each category during the sampling year was divided by the total hours of monitoring for LR and BR (37.5 and 18.6 h, respectively), obtaining a range of the average plastic mass input per hour in each river, then extrapolated to the annual plastic mass load.

Additionally, an estimation of potential microplastics (MPLs) generation from macroplastics entering the sea via rivers was calculated considering ratios 1:16 from macro- ($>$2 cm) to mesoplastics (0.5–2 cm), and 1:625 from meso- to microplastics ($<$0.5 cm), adapted from a previous study reporting on plastics floating at sea (Eriksen et al., 2014). Fragmentation ratios were applied to the estimated annual number of macroplastic items delivered by LR and BR. For each river, the annual number of macroplastics was derived from the observed floating plastic items and the total hours of monitoring, similarly to the extrapolation of annual plastic mass load described above.

#### *2.3.1. Statistical tests*

Normality of the data was studied by Shapiro-Wilk test, showing a non-normal distribution in the litter flux datasets for each river. Furthermore, the litter statistics were determined by crossing the litter flux with rainfall events, and wind, and/or river flow patterns. In the case of natural factors, the average rainfall and wind values were calculated for a seven days' period prior to the monitoring date. Spearman's non-parametric correlation coefficient was used to identify a significant correlation between litter flux, natural factors and river flow. The level of statistical significance was set up at $p < 0.05$.

## **3. Results and discussion**

A total of 684 anthropogenic litter items were counted between October 2016 and September 2017. In particular, 297 and 387 floating items in LR and BR, respectively. Following the guidelines of the RIMMEL project, the registered items were attributed to six main categories of litter: plastic, paper, metal, rubber, textile, and wood, based on the MSFD Litter Category List (Galgani et al., 2013). Results showed similar waste composition in both rivers, mostly represented by plastic material: 67.7% in LR and 50.5% in BR. Regarding the size range, the majority of the observed plastic items were smaller than 20 cm in length, primarily bottles, bags, and cover/packaging (from food and beverage) (Fig. 2). Commonly sighted man-made items also included: cans, magazine and newspapers, clothing, boards, rubber and balls. The 10 most frequently occurring items for the two target rivers are presented in Table 1. These lists represent the 82.2% and 91.3% of the total litter items observed for LR and BR, respectively.

In general, the riverine litter composition observed in this study was comparable to that already reported from other rivers in different geographical areas (González-Fernández et al., 2018). The most frequently occurring 20 items in European rivers and streams monitored by the RIMMEL project reported average percentages of 73.7%, 79.3%, 55.8%, and 88.8% plastic items loaded as floating litter into the Mediterranean Sea, Black Sea, Baltic Sea, and North-East Atlantic regions, respectively (González-Fernández et al., 2018). In particular, the 17 rivers considered in the Mediterranean region showed 7 plastic litter categories (plastic pieces, bottles, bags, cover/packaging, polystyrene pieces, foam and others), 2 paper/cardboard (packaging and others) and 1 metal (cans) categories as the most frequent 10 items. Also, the monitoring conducted in Tiber and Rhone rivers, indicated a predominance of artificial polymers (82% and 77%, respectively) and paper/cardboard (8% and 14%, respectively) discharged as floating litter into the Western Mediterranean Sea (Crosti et al., 2018; Castro-Jiménez et al., 2019).

Following the RIMMEL approach, the identified floating litter was classified according to the MSFD Master List of Categories of Litter Items of the "Guidance on Monitoring of Marine Litter in European Seas". This list allowed to link the items to their use and, therefore, to their possible sources (Galgani et al., 2013). The observed floating litter suggested an input from urban centres and leisure activities along the river and, in a smaller proportion, from industry, taking into account

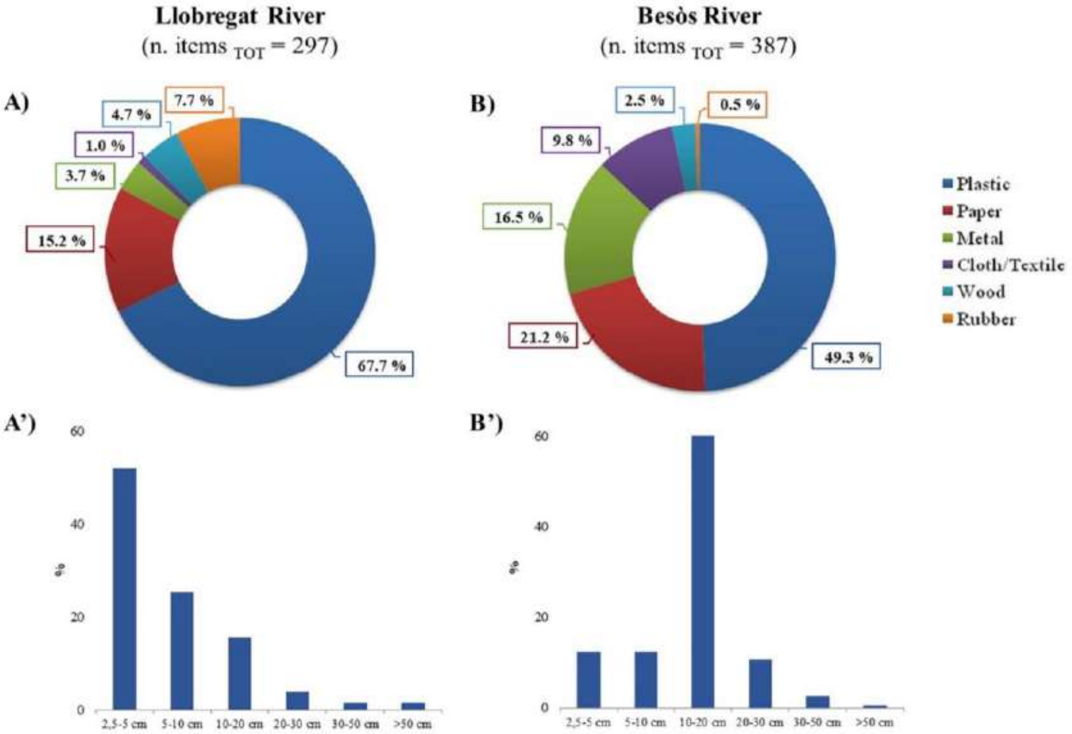

**Fig. 2.** Riverine litter composition by material (A, B) and contribution (%) of the macroplastics by size range (A', B') detected for Llobregat (A, A') and Besòs (B, B') rivers.

that rigorous waste management regulations are applied to reduce or eliminate the generation of mismanaged wastes by industries (Directive, E, 2008). Large urban areas contribute substantially to marine pollution through rivers; therefore, waste management in urban and sub-urban areas should be constantly investigated in order to identify the real causes of emissions of litter. As an example, the Catalan waste agency (ARC) showed that, in 2017, <40% of the total municipal solid waste generated by the population was collected to be correctly treated (Agencia de Residus de Catalunya, 2017) and the plastic fraction was <4%.

After data extrapolation, in LR, the floating litter flux ranged from 0 to 428.9 items/h (58.9 and 25.4 items/h as mean and median values, respectively). A growing trend from the autumn to the following spring was observed with the maximum occurrence in May 2017, mainly caused by rubber litter (Fig. 3a, Table S-1). The temporal litter flux profile of BR showed no apparent trend and the mean and median values were 20.6 and 21.7 items/h, respectively; varying from 0 items/h to a peak of 41.8 items/h registered in February 2017, related to an increase of textile items (Fig. 3b, Table S-2).

**Table 1**
Top 10 floating items for Llobregat and Besòs River basins.

| Top | Llobregat River | Besòs River |
|---|---|---|
| 1 | Cover/packaging | Plastic bottle |
| 2 | Other plastic/polystyrene items | Plastic bag |
| 3 | Plastic bag | Cans |
| 4 | Plastic pieces (2.5–50 cm) | Paper packaging |
| 5 | Polystyrene pieces (2.5–50 cm) | Cover/packaging |
| 6 | Rubber (balls and tyres) | Newspapers & magazines |
| 7 | Paper | Clothing |
| 8 | Plastic bottles | Sails/canvas |
| 9 | Paper packaging | Carpet & furnishing |
| 10 | Wood boards | Balls |

The contrast in catchment area size and the higher population density of 1685.6 inhabitants per km$^2$ in the LR basin area, compared to 1123.7 inhabitants per km$^2$ in BR's, supports the estimation of larger litter loads and more variable patterns as observed at the LR. However, these differences may not only be related to the potential quantity of mismanaged waste available for riverine transport in the basin, but also due to catchment characteristics (slope, land-cover, soil, geology, catchment area, flow regulation, and etc.) affecting hydrological response complexity to rainfall and runoff in rivers (Merheb et al., 2016; Schumm, 2007), and eventually litter transport. In fact, LR seems to be more affected by seasonal variations. Additionally, annual population variation caused by tourism, which is one of the most important economic activities in Catalonia (especially for Barcelona metropolitan area), should be also considered (Saladié, 2016). Conversely, BR showed a more homogenous temporal profile and equal distribution between plastic and non-plastic waste percentages. This area presents many social and leisure centres with recreation facilities, such as picnic sites, which can continuously generate waste, including single-use products made of plastic and paper materials.

Besides the importance of local sources, distribution of floating materials is influenced also by other factors, including natural ones, which can affect the transport of material far from the sources. Litter released by human activity on land can be washed by surface runoff or blown by the wind into rivers and other aquatic ecosystems. For example, wind may facilitate the transport of light plastic (e.g. cover/packaging) or paper. In addition, litter that remains stuck at the riverbank or entangled in vegetation near the shoreline may be remobilized by wind or water runoff.

Due to the short-term variability of the sources (e.g. discharge, weir opening, meteorological conditions, etc.), the quantities of waste

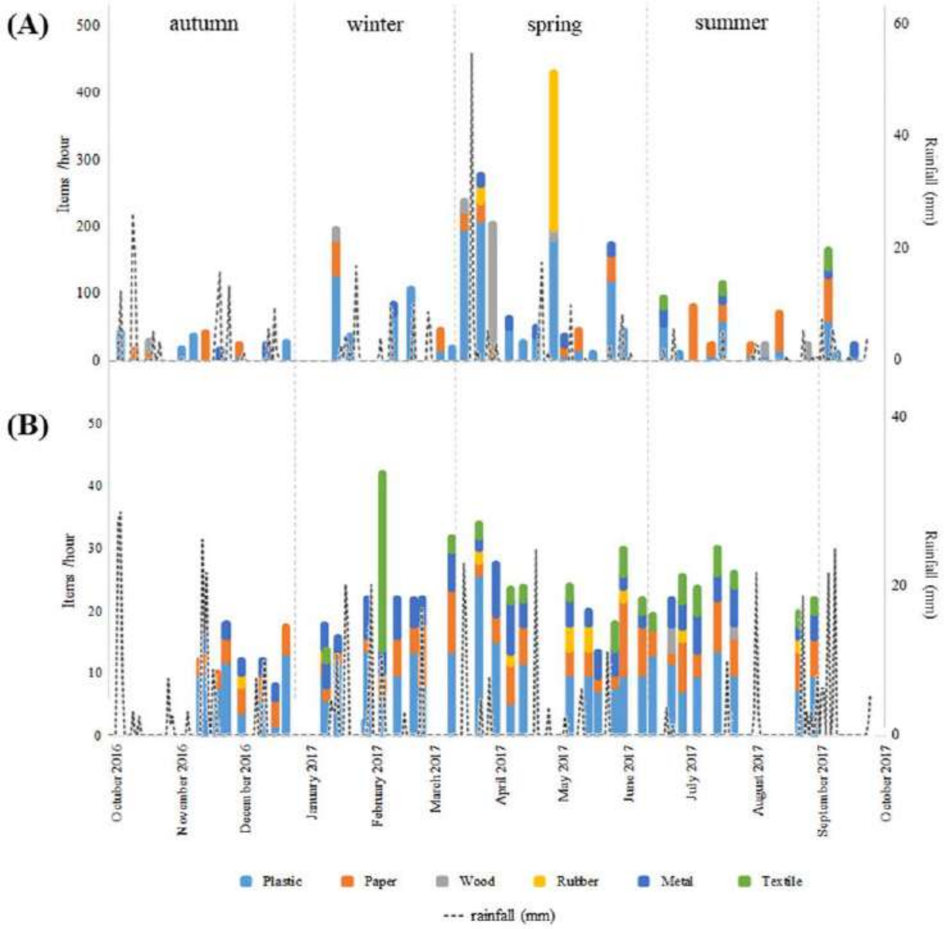

**Fig. 3.** Variation of litter flux (items/h) for A) Llobregat and B) Besòs river basin.

transported by rivers can be highly variable. Therefore, extreme weather events have not been considered in this study. Spearman's correlations between litter, wind and rainfall are summarized in Table 2. Significant positive correlation between plastic and rainfall were only observed in the case of LR during winter. Accordingly, higher plastic flux values (items/h) were observed after rainy periods due to probable inputs of anthropogenic litter from urban and riverbank run-off. Also, textile objects showed a positive correlation with the rainy events that occurred in LR during the summer. In addition to heavy rainfall events, other factors can influence the variability of litter flux. Human-controlled water releases, such as wastewater and dams discharge (Lahens et al., 2018; Zhang et al., 2015), can speed up the transport of litter away from their source. The presence of several dams and WWTPs in the LR catchment area could also act as a litter filter, reducing the amount of waste reaching the sea. Paper items (e.g. newspapers or magazines) were positively correlated with the wind in autumn and spring. On the contrary, the frequent rain events which characterized BR showed significant correlations with an increase in non-plastic items. Similarly, positive correlations have been observed, especially in the warmer seasons (April–June and July–September 2017), for rubber, metals and textiles items that are possibly related to leisure activities. Other significant correlations were found for non-plastic litter and wind. A positive correlation was found for metal objects, mainly cans, during the spring period, while a negative correlation was observed for wooden objects and textiles (i.e. carpet and furnishing) recorded during the summer period.

Finally, a year of monitoring with weekly sessions allowed obtaining information on seasonal changes and factors influencing the load of waste entering the sea through rivers. However, assessing short-time variability at daily and weekly scales would need more intense sampling effort (increasing sampling frequency) and implementation of further methodologies (e.g. nets or floating booms collecting integrated samples over longer time periods).

### 3.1. Plastic litter

Regarding plastic flux, the estimated mean and median values were 32.7 and 5.3 items/h for LR, and 9.9 and 9.8 items/h for BR, respectively. This flux was positively correlated to river flow ($R = 0.67$, $p < 0.005$; Spearman's correlation) for the LR, however not for the BR. Fig. 4 includes a comparison of monthly plastic flux, presenting bags, bottles, EPS items, and cover/packaging as the main observed items. On the one hand, LR showed that EPS items and cover/packaging items were mainly present until May 2017, whereas significantly higher numbers of plastic bags and bottles were recorded during the summer period (Fig. 4a). On the other hand, plastic items were homogenously distributed (except for EPS items), with no relevant monthly variations in BR (Fig. 4b). The amount of floating plastic transported by Catalan rivers

**Table 2**
Spearman's correlation of plastic items and non-plastic items with natural factors following season variability.

| | | Oct–Dec 2016 | Jan–Mar 2017 | Apr–Jun 2017 | Jul–Sep 2017 |
|---|---|---|---|---|---|
| Llobregat River basin | | | | | |
| Rainfall | Plastic | −0.34 | 0.66* | 0.42 | 0.53 |
| | Paper | 0.53 | 0.33 | 0.44 | −0.06 |
| | Wood | 0.48 | 0.46 | 0.27 | 0.28 |
| | Rubber | – | – | 0.4 | – |
| | Metal | −0.13 | 0.04 | −0.36 | 0.44 |
| | Textile | – | – | −0.08 | 0.58* |
| Wind | Plastic | 0.12 | 0.53 | 0.22 | 0.24 |
| | Paper | 0.67* | 0.05 | 0.57* | −0.02 |
| | Wood | 0.39 | – | 0.36 | 0.34 |
| | Rubber | – | 0.39 | 0.38 | – |
| | Metal | −0.38 | – | −0.04 | 0.48 |
| | Textile | – | – | −0.31 | 0.52 |
| Besòs River basin | | | | | |
| Rainfall | Plastic | 0.03 | 0.35 | −0.47 | −0.35 |
| | Paper | 0.57* | 0.75* | 0.26 | −0.79* |
| | Wood | – | – | −0.31 | −0.25 |
| | Rubber | 0.08 | −0.06 | 0.74* | −0.25 |
| | Metal | −0.2 | 0.48 | 0.27 | 0.56* |
| | Textile | – | 0.05 | 0.12 | 0.61* |
| Wind | Plastic | −0.19 | 0.15 | −0.38 | −0.10 |
| | Paper | −0.28 | 0.38 | 0.01 | −0.22 |
| | Wood | – | – | 0.1 | −0.71* |
| | Rubber | 0.58 | −0.47 | 0.47 | – |
| | Metal | −0.01 | −0.7* | 0.57* | 0.21 |
| | Textile | – | −0.35 | 0.35 | −0.86* |

* Statistically significant results ($p < 0.05$).

is comparable with that of other European rivers (mean, $2.5 \cdot 10^2$ items per hour) (van Calcar and van Emmerik, 2019). The variability of the plastic flow in several rivers could range up to two orders of magnitude, especially due to the strong seasonal variations. Instead, higher levels have been reported for Asian rivers, which on average can carry $7.1 \cdot 10^3$ items per hour (van Calcar and van Emmerik, 2019).

Floating plastic items observed in this study were composed of low- and high-density polymers, being mainly made of PE, PET, PP, and EPS. Plastic items may be buoyant or not, based on their physicochemical properties and environmental conditions (Andrady, 2017; Resmeriță et al., 2018). For this reason, low-density polymers (<1.2 g cm$^{-3}$), such as polyethylene and polypropylene, are usually the most common polymeric materials observed on the surface water (Gasperi et al., 2014). However, their presence on the surface may also be influenced by other factors in addition to density. Processes like biofouling may change the density of items (Barnes et al., 2009), leading them to sink in the water column. Also, denser polymers (e.g. PET ~1.380 g cm$^{-3}$) can be present in the river surface waters, as reported in this study and others (Gasperi et al., 2014; Sadri and Thompson, 2014), including items with air trapped compartments such as plastic bottles. Furthermore, the non-floating items could represent an important fraction of the plastic loads (Morritt et al., 2014), meaning waste entering the ocean could be much higher than the estimations made based on floating macro litter flux values.

Taking into account the item size and the density of the main polymeric material characterizing them, the lower and upper plastic loads (mass of plastic items per year) were estimated (Table 3). As a result, 0.0672–0.108 tonnes of bags, 0.150–0.237 tonnes of bottles, 0.0611–0.100 tonnes of EPS items, and 0.118–0.180 tonnes of cover/packaging were estimated to be loaded annually into the Mediterranean Sea from both rivers studied in this work. In particular, between 0.133 and 0.210 tonnes/year of plastic bottles and 0.0642 to 0.103 tonnes/year of bags were expected to be carried by BR, showing values one order of magnitude higher than those estimated for these items in LR (Table 3). Overall, 0.101–0.172 and 0.295–0.453 tonnes/year of plastic

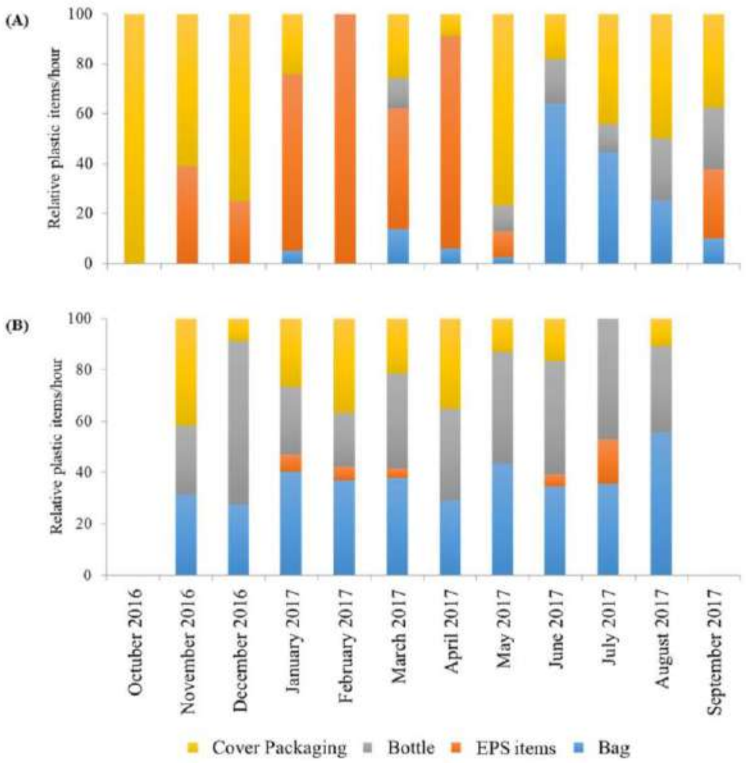

**Fig. 4.** Relative plastic items flux for A) Llobregat and B) Besòs river basins.

**Table 3**
Tonnes per year estimate of plastic litter (EPS items, bags, bottles and cover/packaging) load into the Mediterranean Sea from Llobregat (LR) and Besòs (BR) rivers.

| Common plastic item | Polymer | Density ($g/cm^3$) | Size l × w × t (cm × cm × cm) | Plastic mass (tonnes/year) | |
|---|---|---|---|---|---|
| | | | | LR | BR |
| Bag | PE | 0.94–0.97 | (≥5 × 10 × 0.015) | 0.00288–0.00577 | 0.0642–0.103 |
| Bottle | PET | 1.4 | (≥5 × 10 × 0.02) | 0.0167–0.0261 | 0.133–0.210 |
| EPS items | EPS | 0.05 | (≥5 × 10 × 1.3) | 0.0359–0.065 | 0.0252–0.0351 |
| Cover/packaging | PE, PS, PP, PET and PVC | 1.12[a] | (≥5 × 10 × 0.05) | 0.0459–0.0748 | 0.0724–0.105 |
| Total | | | | 0.101–0.172 | 0.295–0.453 |

[a] Average density of the most common polymers used for cover/packaging: LD-PE (0.91–0.93 $g/cm^3$), HD-PE (0.94–0.97 $g/cm^3$), PS (1.04–1.07 $g/cm^3$), PP (0.90–0.91 $g/cm^3$), PET (1.4 $g/cm^3$), and PVC (1.35–1.45 $g/cm^3$).

were loaded into the sea by LR and BR, respectively. Despite the catchment area differences, the estimated mass of plastic waste transported by LR was lower than BR. This difference could be justified by the higher amount of EPS items (lighter items) recorded in the LR. Also, the higher abundance of items <5 cm in the LR could indicate that macroplastics are subject to fragmentation processes in larger catchment areas, and therefore being excluded from our mass load calculation. Furthermore, the presence of artificial barriers (e.g. dams or weirs) along LR could be responsible for retaining >60% of waste (Lebreton et al., 2017). For LR, riverine plastic input models developed by Lebreton et al. (2017) and Schmidt et al. (2017) estimated 0.0244–0.249 tonnes/year of total plastics and 0.463 tonnes/year of macroplastics, respectively. These models provided orders of magnitude similar to the field-based results presented herein. Further, discordance between these two studies could be due to the different modelling setup, e.g. in the first model, only the catchment area downstream artificial barriers was included, while in the second case the whole catchment area was considered.

### 3.1.1. *Riverine microplastics*

In this work, an estimation of microplastics from an oceanographic model applied by Eriksen et al. was used (Eriksen et al., 2014). The application of an oceanographic rather than a riverine model was motivated by the idea of giving information about the amount of MPLs generated by floating macroplastics after reaching the ocean via rivers. Based on the estimated annual number of plastics items, a preliminary estimation of MPLs generation has been done using conversion rates per size range of each item (Table 4). As a result, it could be considered that 27 and 25 million MPLs would be generated over time by an annual input of $4.6 \cdot 10^4$ and $8.7 \cdot 10^4$ macroplastic items entering the sea respectively from LR and BR, considering the fragmentation processes as efficient as possible. Even if macroplastics fragmentation processes were not considered by models and smaller particles were underrepresented due to sampling limits, studies focused on MPLs occurrence in freshwater systems (Yonkos et al., 2014; Eerkes-Medrano et al., 2015; Wagner et al., 2014) confirmed that larger rivers could transport significant amounts of microplastics into the marine environment (Van der Wal et al., 2015; Lechner et al., 2014). The occurrence of these smaller particles in watercourses is largely due to various sources such as WWTP (Mani et al., 2015; Magnusson and Norén, 2014), car tyre and road wear (Siegfried et al., 2017), or fragmentation of litter that get stuck along the riverbanks (Rech et al., 2015).

**Table 4**
Potential generation of microplastics (MPLs/year) related to the estimated annual loads of macroplastics per size class (MaPLs/year) for LR and BR.

| Class size | MaPLs/year | | Fragmentation ratio | MPLs/year | |
|---|---|---|---|---|---|
| | LR | BR | | LR | BR |
| 2.5–5 cm | $2.4 \cdot 10^4$ | $1.1 \cdot 10^4$ | 1:625 | $1.5 \cdot 10^7$ | $3.7 \cdot 10^6$ |
| 5–10 cm | $1.2 \cdot 10^4$ | $1.1 \cdot 10^4$ | | $7.3 \cdot 10^6$ | $3.7 \cdot 10^6$ |
| 10–20 cm | $7.2 \cdot 10^3$ | $5.3 \cdot 10^4$ | | $4.5 \cdot 10^6$ | $1.8 \cdot 10^7$ |
| 20–30 cm | $1.9 \cdot 10^3$ | $9.4 \cdot 10^3$ | 1:10,000 | $3.0 \cdot 10^4$ | $8.2 \cdot 10^4$ |
| 30–50 cm | $7.0 \cdot 10^2$ | $2.3 \cdot 10^3$ | | $1.1 \cdot 10^4$ | $2.1 \cdot 10^4$ |
| >50 cm | $7.0 \cdot 10^2$ | $4.7 \cdot 10^3$ | | $1.1 \cdot 10^4$ | $4.1 \cdot 10^3$ |
| Total | $4.6 \cdot 10^4$ | $8.7 \cdot 10^4$ | | $2.7 \cdot 10^7$ | $2.5 \cdot 10^7$ |

## 4. Conclusions

This work provided a first monitoring study on floating macro litter items transported by rivers to the sea in two Catalan rivers, Llobregat and Besòs, influenced by the large metropolitan area of Barcelona. The analysis of most frequent anthropogenic items presented similar litter composition in both rivers. However, the difference in terms of litter loads highlighted that dimension of the catchment and the activities along the river rather influenced the amount of litter discharged. In addition, seasonal variations in the litter flux were observed especially for LR. Although many factors may affect the variation of the river flow changing the load of waste transported (e.g. presence of dams and WWTPs), higher litter loads were observed after heavy rainfall. On the other hand, a homogeneous profile and significant correlations between waste and natural factors were observed for BR. Generally, plastics have constituted the majority of the total litter in the two rivers. Above all, EPS items/fragments and cover/packaging were the main items observed for LR, and bags and bottles for BR. Estimations showed that 0.4–0.6 tonnes/year of floating macroplastics could be discharged into the Mediterranean Sea by these two rivers, where they could cause harm through entanglement and ingestion, while potentially generating a large number of microplastics, in addition to those already transported by rivers.

Mitigation strategies should be intensified in order to limit the inputs of plastic litter into aquatic environments before they reach marine ecosystems, especially near metropolitan areas. Following this line, more measures should be implemented to increase the percentage of treated waste reducing the amount of mismanaged waste associated to metropolitan areas. Also, clean-up activities should be supported at local scale.

To conclude, the information provided in this work can serve as a starting point for coordination and cooperation among environmental regulatory frameworks at national and European levels. Such strategies would expect interactions between the MSFD, the Water Framework Directive (WFD, 2000/60/EC), and the EU Plastic Strategy (COM/2018/028 final), as well with the Mediterranean Action Plan and the Regional Action Plan against marine litter, with regards to land-based sources of litter. In this context, further research should focus on the development of methodologies for river monitoring, with the aim of identifying litter flux patterns and source attributions. In addition to detailed descriptions of waste that could help identifying possible hotspots, high-resolution hydrological data could facilitate the process of understanding the transport and fate of floating litter in rivers. In this way, mitigation strategies, including macro and micro-litter, could be more targeted and thus efficient.

## Declaration of competing interest

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

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.136807.

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Supporting Information for:

# Riverine anthropogenic litter load to the Mediterranean Sea near the metropolitan area of Barcelona, Spain

Gabriella F. Schirinzi[1], Marianne Köck-Schulmeyer[1], María Cabrera[3], Daniel González-Fernández[4,*], Georg Hanke[5], Marinella Farré[1], Damià Barceló[1,2]

[1] *Institute of Environmental Assessment and Water Research, IDAEA-CSIC, C/ Jordi Girona 18-26, 08034 Barcelona, Spain*

[2] *Catalan Institute for Water research, ICRA, Emili Grahit 101, 17003, Girona, Spain*

[3] *Paisaje Limpio Association, C/ Santa Brigida 19, 28220, Majadahonda, Spain*

[4] *Department of Biology, International Campus of Excellence of the Sea (CEIMAR), University Marine Research Institute (INMAR), University of Cádiz, E-11510, Puerto Real, Spain*

[5] *European Commission - Joint Research Centre, Via E. Fermi 2749, 21027, Ispra, Italy*

*Corresponding author. Daniel González-Fernández *(daniel.gonzalez@uca.es)*

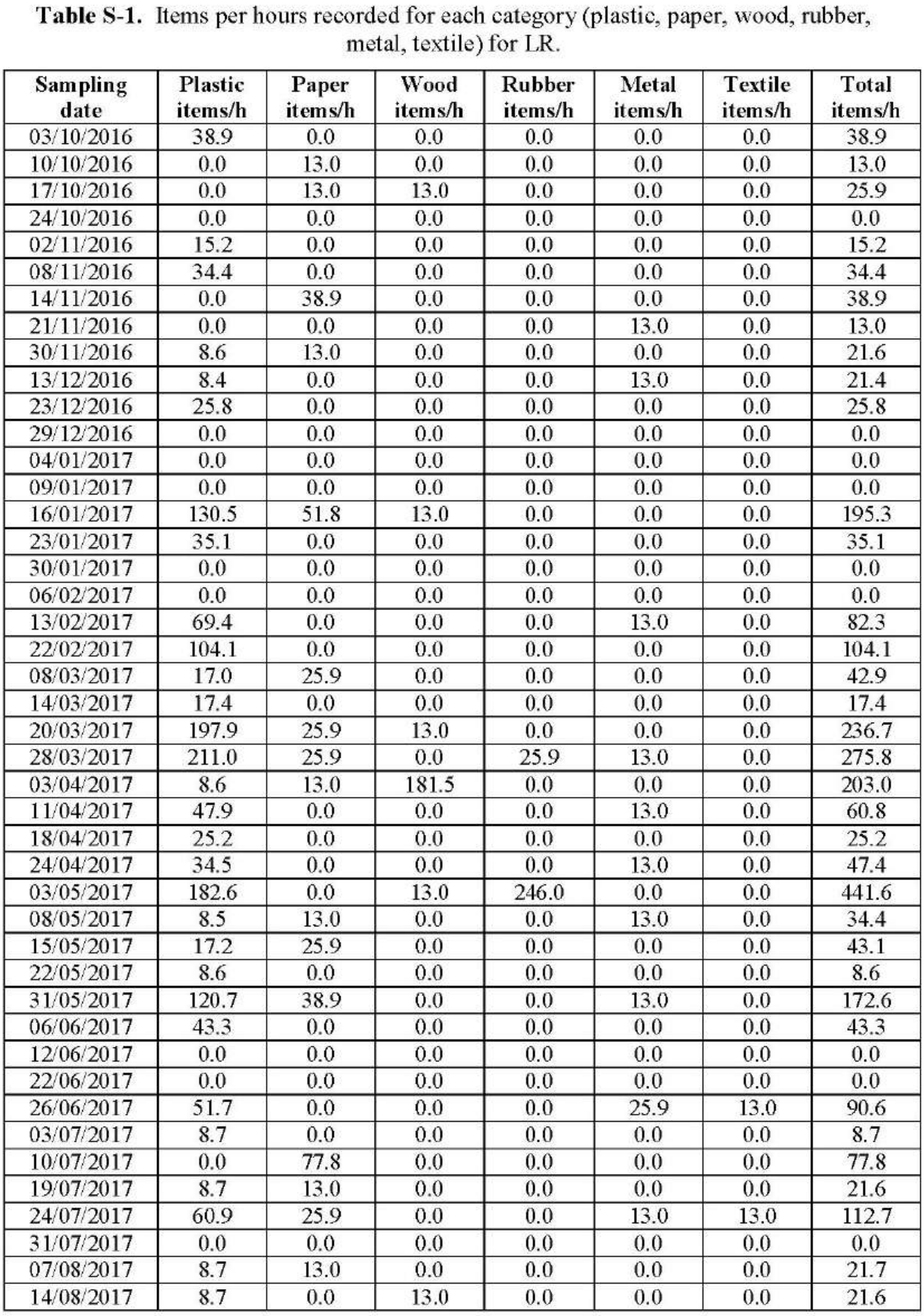
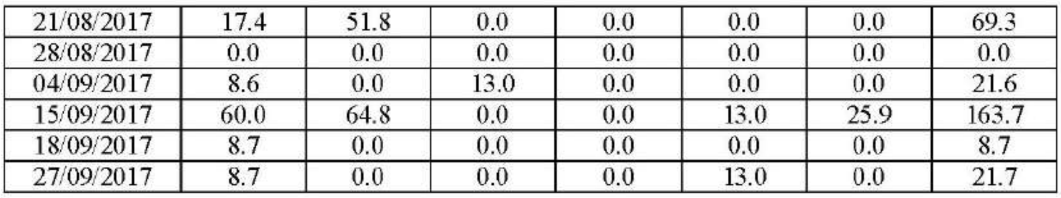


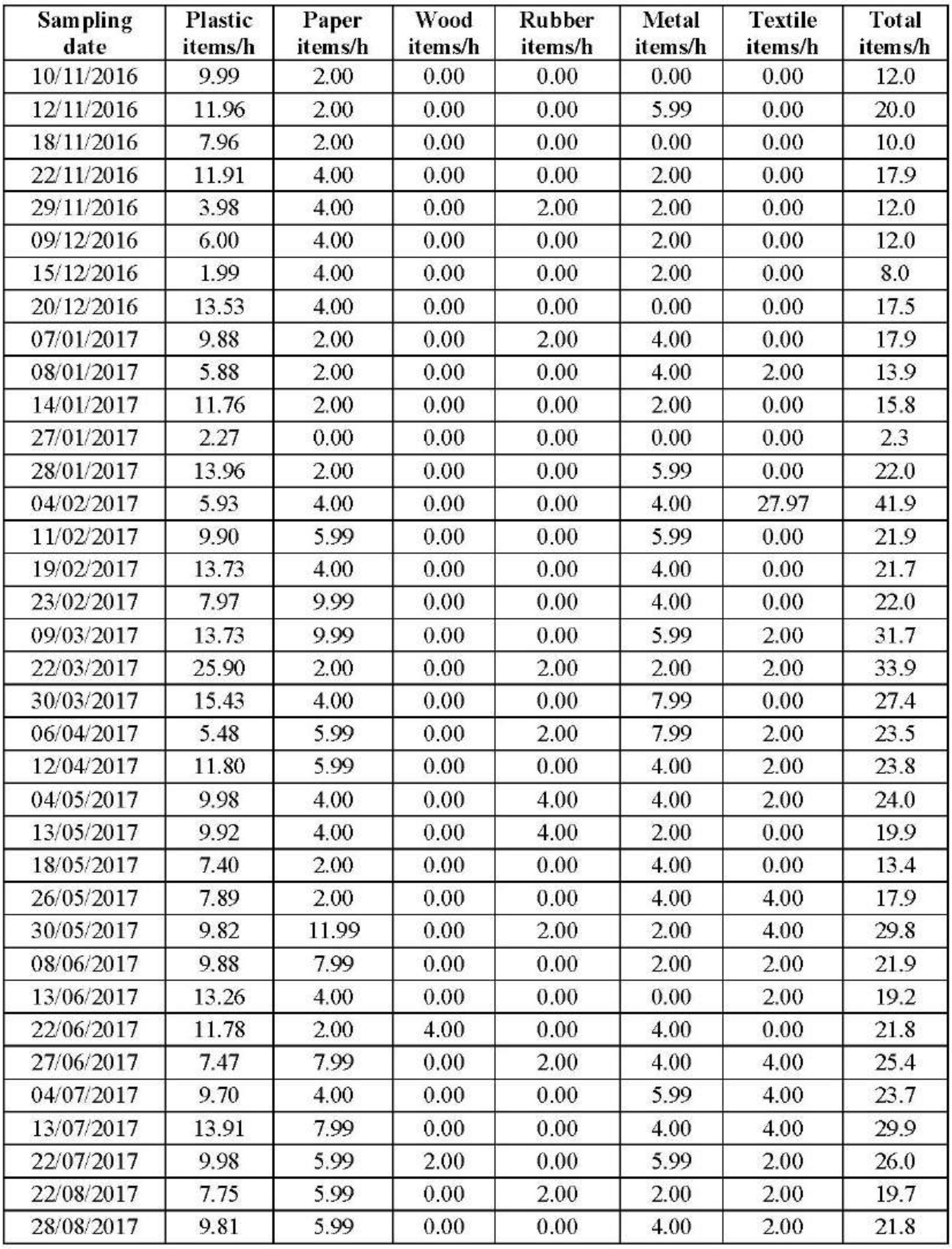
**Table S-1.** Items per hours recorded for each category (plastic, paper, wood, rubber, metal, textile) for LR.

| Sampling date | Plastic items/h | Paper items/h | Wood items/h | Rubber items/h | Metal items/h | Textile items/h | Total items/h |
|---|---|---|---|---|---|---|---|
| 03/10/2016 | 38.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 38.9 |
| 10/10/2016 | 0.0 | 13.0 | 0.0 | 0.0 | 0.0 | 0.0 | 13.0 |
| 17/10/2016 | 0.0 | 13.0 | 13.0 | 0.0 | 0.0 | 0.0 | 25.9 |
| 24/10/2016 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 02/11/2016 | 15.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 15.2 |
| 08/11/2016 | 34.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 34.4 |
| 14/11/2016 | 0.0 | 38.9 | 0.0 | 0.0 | 0.0 | 0.0 | 38.9 |
| 21/11/2016 | 0.0 | 0.0 | 0.0 | 0.0 | 13.0 | 0.0 | 13.0 |
| 30/11/2016 | 8.6 | 13.0 | 0.0 | 0.0 | 0.0 | 0.0 | 21.6 |
| 13/12/2016 | 8.4 | 0.0 | 0.0 | 0.0 | 13.0 | 0.0 | 21.4 |
| 23/12/2016 | 25.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 25.8 |
| 29/12/2016 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 04/01/2017 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 09/01/2017 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 16/01/2017 | 130.5 | 51.8 | 13.0 | 0.0 | 0.0 | 0.0 | 195.3 |
| 23/01/2017 | 35.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 35.1 |
| 30/01/2017 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 06/02/2017 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 13/02/2017 | 69.4 | 0.0 | 0.0 | 0.0 | 13.0 | 0.0 | 82.3 |
| 22/02/2017 | 104.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 104.1 |
| 08/03/2017 | 17.0 | 25.9 | 0.0 | 0.0 | 0.0 | 0.0 | 42.9 |
| 14/03/2017 | 17.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 17.4 |
| 20/03/2017 | 197.9 | 25.9 | 13.0 | 0.0 | 0.0 | 0.0 | 236.7 |
| 28/03/2017 | 211.0 | 25.9 | 0.0 | 25.9 | 13.0 | 0.0 | 275.8 |
| 03/04/2017 | 8.6 | 13.0 | 181.5 | 0.0 | 0.0 | 0.0 | 203.0 |
| 11/04/2017 | 47.9 | 0.0 | 0.0 | 0.0 | 13.0 | 0.0 | 60.8 |
| 18/04/2017 | 25.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 25.2 |
| 24/04/2017 | 34.5 | 0.0 | 0.0 | 0.0 | 13.0 | 0.0 | 47.4 |
| 03/05/2017 | 182.6 | 0.0 | 13.0 | 246.0 | 0.0 | 0.0 | 441.6 |
| 08/05/2017 | 8.5 | 13.0 | 0.0 | 0.0 | 13.0 | 0.0 | 34.4 |
| 15/05/2017 | 17.2 | 25.9 | 0.0 | 0.0 | 0.0 | 0.0 | 43.1 |
| 22/05/2017 | 8.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 8.6 |
| 31/05/2017 | 120.7 | 38.9 | 0.0 | 0.0 | 13.0 | 0.0 | 172.6 |
| 06/06/2017 | 43.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 43.3 |
| 12/06/2017 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 22/06/2017 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 26/06/2017 | 51.7 | 0.0 | 0.0 | 0.0 | 25.9 | 13.0 | 90.6 |
| 03/07/2017 | 8.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 8.7 |
| 10/07/2017 | 0.0 | 77.8 | 0.0 | 0.0 | 0.0 | 0.0 | 77.8 |
| 19/07/2017 | 8.7 | 13.0 | 0.0 | 0.0 | 0.0 | 0.0 | 21.6 |
| 24/07/2017 | 60.9 | 25.9 | 0.0 | 0.0 | 13.0 | 13.0 | 112.7 |
| 31/07/2017 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 07/08/2017 | 8.7 | 13.0 | 0.0 | 0.0 | 0.0 | 0.0 | 21.7 |
| 14/08/2017 | 8.7 | 0.0 | 13.0 | 0.0 | 0.0 | 0.0 | 21.6 |



| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 21/08/2017 | 17.4 | 51.8 | 0.0 | 0.0 | 0.0 | 0.0 | 69.3 |
| 28/08/2017 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 04/09/2017 | 8.6 | 0.0 | 13.0 | 0.0 | 0.0 | 0.0 | 21.6 |
| 15/09/2017 | 60.0 | 64.8 | 0.0 | 0.0 | 13.0 | 25.9 | 163.7 |
| 18/09/2017 | 8.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 8.7 |
| 27/09/2017 | 8.7 | 0.0 | 0.0 | 0.0 | 13.0 | 0.0 | 21.7 |

**Table S-2.** Items per hours recorded for each category (plastic, paper, wood, rubber, metal, textile) for BR.



| Sampling date | Plastic items/h | Paper items/h | Wood items/h | Rubber items/h | Metal items/h | Textile items/h | Total items/h |
|---|---|---|---|---|---|---|---|
| 10/11/2016 | 9.99 | 2.00 | 0.00 | 0.00 | 0.00 | 0.00 | 12.0 |
| 12/11/2016 | 11.96 | 2.00 | 0.00 | 0.00 | 5.99 | 0.00 | 20.0 |
| 18/11/2016 | 7.96 | 2.00 | 0.00 | 0.00 | 0.00 | 0.00 | 10.0 |
| 22/11/2016 | 11.91 | 4.00 | 0.00 | 0.00 | 2.00 | 0.00 | 17.9 |
| 29/11/2016 | 3.98 | 4.00 | 0.00 | 2.00 | 2.00 | 0.00 | 12.0 |
| 09/12/2016 | 6.00 | 4.00 | 0.00 | 0.00 | 2.00 | 0.00 | 12.0 |
| 15/12/2016 | 1.99 | 4.00 | 0.00 | 0.00 | 2.00 | 0.00 | 8.0 |
| 20/12/2016 | 13.53 | 4.00 | 0.00 | 0.00 | 0.00 | 0.00 | 17.5 |
| 07/01/2017 | 9.88 | 2.00 | 0.00 | 2.00 | 4.00 | 0.00 | 17.9 |
| 08/01/2017 | 5.88 | 2.00 | 0.00 | 0.00 | 4.00 | 2.00 | 13.9 |
| 14/01/2017 | 11.76 | 2.00 | 0.00 | 0.00 | 2.00 | 0.00 | 15.8 |
| 27/01/2017 | 2.27 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.3 |
| 28/01/2017 | 13.96 | 2.00 | 0.00 | 0.00 | 5.99 | 0.00 | 22.0 |
| 04/02/2017 | 5.93 | 4.00 | 0.00 | 0.00 | 4.00 | 27.97 | 41.9 |
| 11/02/2017 | 9.90 | 5.99 | 0.00 | 0.00 | 5.99 | 0.00 | 21.9 |
| 19/02/2017 | 13.73 | 4.00 | 0.00 | 0.00 | 4.00 | 0.00 | 21.7 |
| 23/02/2017 | 7.97 | 9.99 | 0.00 | 0.00 | 4.00 | 0.00 | 22.0 |
| 09/03/2017 | 13.73 | 9.99 | 0.00 | 0.00 | 5.99 | 2.00 | 31.7 |
| 22/03/2017 | 25.90 | 2.00 | 0.00 | 2.00 | 2.00 | 2.00 | 33.9 |
| 30/03/2017 | 15.43 | 4.00 | 0.00 | 0.00 | 7.99 | 0.00 | 27.4 |
| 06/04/2017 | 5.48 | 5.99 | 0.00 | 2.00 | 7.99 | 2.00 | 23.5 |
| 12/04/2017 | 11.80 | 5.99 | 0.00 | 0.00 | 4.00 | 2.00 | 23.8 |
| 04/05/2017 | 9.98 | 4.00 | 0.00 | 4.00 | 4.00 | 2.00 | 24.0 |
| 13/05/2017 | 9.92 | 4.00 | 0.00 | 4.00 | 2.00 | 0.00 | 19.9 |
| 18/05/2017 | 7.40 | 2.00 | 0.00 | 0.00 | 4.00 | 0.00 | 13.4 |
| 26/05/2017 | 7.89 | 2.00 | 0.00 | 0.00 | 4.00 | 4.00 | 17.9 |
| 30/05/2017 | 9.82 | 11.99 | 0.00 | 2.00 | 2.00 | 4.00 | 29.8 |
| 08/06/2017 | 9.88 | 7.99 | 0.00 | 0.00 | 2.00 | 2.00 | 21.9 |
| 13/06/2017 | 13.26 | 4.00 | 0.00 | 0.00 | 0.00 | 2.00 | 19.2 |
| 22/06/2017 | 11.78 | 2.00 | 4.00 | 0.00 | 4.00 | 0.00 | 21.8 |
| 27/06/2017 | 7.47 | 7.99 | 0.00 | 2.00 | 4.00 | 4.00 | 25.4 |
| 04/07/2017 | 9.70 | 4.00 | 0.00 | 0.00 | 5.99 | 4.00 | 23.7 |
| 13/07/2017 | 13.91 | 7.99 | 0.00 | 0.00 | 4.00 | 4.00 | 29.9 |
| 22/07/2017 | 9.98 | 5.99 | 2.00 | 0.00 | 5.99 | 2.00 | 26.0 |
| 22/08/2017 | 7.75 | 5.99 | 0.00 | 2.00 | 2.00 | 2.00 | 19.7 |
| 28/08/2017 | 9.81 | 5.99 | 0.00 | 0.00 | 4.00 | 2.00 | 21.8 |

### 3.2.2 Publication II: Trace analysis of polystyrene microplastics in natural waters

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# Trace analysis of polystyrene microplastics in natural waters

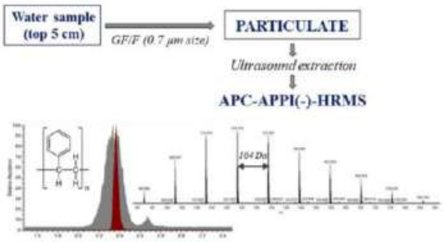
Gabriella F. Schirinzi [a], Marta Llorca [a], Raquel Seró [b], Encarnación Moyano [b], Damià Barceló [a, c], Esteban Abad [a], Marinella Farré [a, *]

[a] *Institute of Environmental Assessment and Water Research, IDAEA-CSIC, Barcelona, Catalonia, Spain*
[b] *University of Barcelona, Barcelona Catalonia, Spain*
[c] *Catalan Institute for Water Research, ICRA, Girona, Catalonia, Spain*

## HIGHLIGHTS

- LC-APPI(−)HRMS method for the quantitative analysis of polystyrene microplastics.
- LC separation was achieved using an advanced polymer chromatographic (APC) column.
- The instrumental limit of detection (ILOD) was 20 pg.

## GRAPHICAL ABSTRACT



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## ABSTRACT

The development of quantitative and qualitative analytical methods to assess micro-plastics (MPLs) and nano-plastics (NPLs) content in the environment is a central issue for realistic risk assessment studies. However, the quantitative analysis continues being a critical issue, in particular for MPLs from 100 µm down to the nano-sized range in complex environmental samples.

This paper evaluates the potential of mass spectrometry for the analysis of MPLs and NPLs. The performance of different techniques including matrix-assisted laser desorption ionisation (MALDI) coupled to time-of-flight mass spectrometry (TOF-MS), liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS), and the ambient ionisation approaches as desorption electrospray ionisation (DESI) and direct analysis real-time (DART), were assessed for the study of polystyrene (PS) MPLs and NPLs in natural waters.

A method based on LC-HRMS, equipped with an atmospheric pressure photoionisation source (APPI), operated in negative conditions for the quantitative analysis of PS MPLs and NPLs in natural waters, was developed. The chromatographic separation was achieved using an advanced polymer chromatographic (APC) column using toluene isocratic as the mobile phase. The optimal analytical method showed an instrumental limit of detection (ILOD) of 20 pg and methods limits of detection and quantification around 30 pg $L^{-1}$ and 100 pg $L^{-1}$, respectively. And, recoveries of 60 and 70% in samples from rivers and the marine coast, respectively. The performance of the new method was proved by the analysis of fortified samples and natural seawater samples.

## 1. Introduction

The occurrence of plastic litter in the environment is an

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[*] Corresponding author. Department of Environmental Chemistry of the Institute of Environmental Assessment and Water Research (IDAEA-CSIC), 08034, Barcelona, Spain.
*E-mail address:* mfuqam@cid.csic.es (M. Farré).

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emerging global threat (Browne et al., 2011; Barnes et al., 2009), that has been found distributed ubiquitously in both freshwater (Imhof et al., 2013; Rodrigues et al., 2018; Bordós et al., 2019) and marine systems (Browne et al., 2011; Cole et al., 2011; Lusher et al., 2015). Among plastic litter, microplastics (MPLs) are the particles of less than 5 mm that reach the environment by different processes: i) the degradation of larger plastic fragments, ii) by direct release of micro-particles used as abrasives, iii) by accidental industrial losses of raw materials, and iv) discharged in sewage sludge. Besides, under environmental conditions, MPLs can be degraded subsequently into nano-sized particles or nano-plastics (NPLs) (Lambert and Wagner, 2016). In the aquatic environment, due to their size, the floating MPLs and NPLs hamper the oxygen exchange and the transmission of light through the water column, which overall leads to eutrophication processes (Harrison et al., 2011), and since are similar in size to phytoplankton, are more prone to be ingested by the aquatic organisms (Browne et al., 2011). Furthermore, the high specific areas of MPLs can lead to the accumulation and transport to the biota of other contaminants (Bakir et al., 2014; Chua et al., 2014; Lu et al., 2018; Wang and Wang, 2018a) and pathogens (Kirstein et al., 2016). Besides, non-floating MPLs are deposited on the seafloor and river sediments (Wang et al., 2017a), affecting the biota communities living there. During recent years, different studies highlighted their effects in biota (Jeong and Choi, 2019; De Felice et al., 2019), such as accumulation (Huang et al., 2019), transport of co-contaminants (Wang and Wang, 2018a; D'Alessandro et al., 2018; Scopetani et al., 2018), functional diversity of enzymes, impairing living stages (Silva et al., 2018; Tallec et al., 2018) and behavioural changes. Various factors can influence their effects such as composition, size, ageing of the particles and shape among others. The ecological impact of NPLs and MPLs pollution is still unknown, and there is an urgent need to develop analytical methods to quantify and identify their composition in complex environmental samples as well as characterise the plastic particles.

Among the different analytical techniques currently in use for MPLs characterisation, scanning electron microscope (SEM) can provide high-resolution images of a sample with surface details and resolutions inferior to 0.5 nm. One of the main advantages of SEM is the characterisation of the surface morphology and the weathering processes of MPLs in the environment (Zhang et al., 2016). Moreover, the combined use of SEM and energy-dispersive X-ray spectroscopy (SEM-EDS) can provide detailed information about the elemental composition of MPLs and the inorganic additives composition differencing MPLs particles from other impurities (Crawford and Quinn, 2017). But, the main limitations of SEM-EDS are requirements of sample preparations, and qualitative data is only provided. Another technique that has been employed for MPLs characterisation is Fourier transform infrared (FTIR) spectroscopy. FTIR give an infrared spectrum for a specific type of chemical bond. This technique can accurately identify the polymer types and also can be used to characterise the weathering processes by analysing their oxidation degree (Corcoran et al., 2009). But, one of the main limitations of this approach is that FTIR is only capable of identifying the polymeric composition of MPLs with a size bigger than 10–20 μm (Huppertsberg and Knepper, 2018). Recently, different derivative technologies have been successfully used for MPLs such as FTIR micro-spectroscopy (micro-FTIR), that facilitates the detection of particles smaller than 10 μm, attenuated total reflectance-FTIR (ATR-FTIR) used for the analysis of particles bigger than 500 μm without a sample preparation (Löder and Gerdts, 2015), and focal plane array FTIR spectroscopy (FPA-FTIR) that can offer high throughput analysis of particles bigger than 20 μm (Tagg et al., 2015). Both FPA-FTIR and ATR-FTIR have been successfully applied for the analysis of complex samples. For example, ATR-FTIR has been used to identify aged and surface contaminated plastics within environmental samples without the use of clean-up processes combining the use of ATR-FTIR with a chemo-metric approach (Renner et al., 2017). In another example, FPA-FTIR has been used for the identification and quantification of MPLs in wastewater. In this case, a sample pre-treatment using 30% hydrogen peroxide was employed followed by FPA-FTIR. The method identified different types of MPLs of polyethylene, polypropylene, nylon-6, polyvinyl chloride, and polystyrene (Tagg et al., 2015).

Another prominent technique for the MPLs polymers composition identification in complex samples is Raman spectroscopy (Araujo et al., 2018). In this case, identification is undertaken by irradiating a monochromatic laser beam onto a sample resulting in a different frequency backscattered that identifies a specific molecular structure and atomic composition (Crawford and Quinn, 2017). The Raman shift can produce a unique spectrum for each polymer. Moreover, Raman spectroscopy is a non-destructive chemical characterisation technique (Shim et al., 2017). Some of the main advantages are the high reliability of this technique, the possibility for high throughput analysis, and low sample amount requirements (Wang and Wang, 2018b). A combination of Raman spectroscopy with microscopy (micro-Raman) makes it possible to identify MPLs down to 1 μm in size, which is an advantage. Raman spectroscopy has also being used combined with confocal laser scanning microscopy to locate MPLs in biological tissues (Imhof et al., 2013). However, the main limitation of Raman based techniques is that MPLs additives, pigments and other chemicals can interfere with the accuracy of the analysis (Huppertsberg and Knepper, 2018).

Pyrolysis–gas chromatography–mass (Pyr-GC-MS) spectrometry that has also been successfully employed in the chemical identification of MPLs in environmental samples (Fries et al., 2013). This is a destructive technique consisting in the analysis of MPLs by their thermal degradation products in comparison with reference pyrograms generated by known pure polymers. Pyr-GC-MS can provide detailed information about the chemical composition of the polymer and the organic additives of the MPLs (Käppler et al., 2018). However, this technique does not give information on shape and size (Dekiff et al., 2014), and only particles that are large enough (>100 μm) to be manually inserted into the pyrolysis tube are suitable to be analysed (Dekiff et al., 2014). In this regard, different variants have been developed to overcome these limitations, such as thermal extraction and desorption–gas chromatography-mass spectrometry (TED-GC-MS) (Duemichen et al., 2014), that combines thermal extraction with thermogravimetric analysis (TGA) and thermal desorption gas chromatography-mass spectrometry (TD-GC-MS). Very recently, TGA-MS approach was also applied for the study of poly(ethylene terephthalate) (PET) in soil (David et al., 2018). Also, liquid chromatography coupled with mass spectrometry (LC-MS) was applied to quantifying polymer MPLs by depolymerization (Wang et al., 2017b).

In spite of the efforts to develop practical analytical procedures for the detection of NPLs in the environment is not yet possible (Oliveira and Almeida, 2019), at least using a single technique. There is still lacking standardized analytical procedures, there are very few test materials (Kühn et al., 2018), and there is a need of analytical approaches able to identify and quantify MPLs and NPLs with enough sensitivity to assess it in the environment. The main difficulty starts with sampling approaches. For example, to evaluate the presence of MPLs and plastics at the surface layer, neuston nets are frequently used. After collection, the identification of the polymer composition of MPLs can be achieved by FTIR, Raman, and

TED-GC-MS techniques. However, these approaches are not suitable for quantitative results, only qualitative or in some cases, semi-quantitative measures of enough large particles can be achieved, and in any case, these are methods not suitable for the investigation of NPLs. Other separation techniques based on filtration, nano-filtration or field-flow fractionation (FFF) combined with microscopy (TEM, SEM, AFM, and SRS) can be used for semi-quantitative assessment of NPLs, but then the polymer composition later is not identified. It should be highlighted that the analysis of NPLs is of high interest because, while MPLs cannot be digested, in the case of NPLs, it cannot be discarded due to their size translocation to different tissues. Therefore, combined analytical approaches should be considered to obtain the maximum of information including the study of NPLs.

In this regard, the first objective of this work was to develop a quantitative analytical method able to determine the chemical composition of the polymers of NPLs and MPLs in natural waters. As a proof of concept, the method was developed explicitly for polystyrene (PS) determination, one of the most commonly used materials, largely found in costal environment as debris (Poeta et al., 2014). Additionally, the negative effects of polystyrene microplastics on marine organisms (Wang et al., 2019; Cole et al., 2015) and its capacity to adsorb and transport other contaminants (Llorca et al., 2018; Rochman et al., 2013) have been already reported in several studies. The analytical method is based on liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS), equipped with an atmospheric pressure photoionisation source (APPI), operated in negative conditions. The performance of the method was evaluated by spiking experiments and finally by assessing the occurrence of PS MPLs in 17 natural water samples. The potential of other mass spectrometric techniques for the rapid screening was as well explored such as desorption electrospray ionisation (DESI) and direct analysis real-time (DART) as well as the performance of more classical approaches such as matrix-assisted laser desorption ionisation (MALDI) coupled to time-of-flight mass spectrometry (TOF-MS).

The results of this study demonstrated that MS techniques can be an excellent complementary analytical approach for target quantification of MPLs and NPLs in environmental samples.

## 2. Experimental section

### 2.1. Chemicals and reagents

Polystyrene analytical standard for GPC/SEC (MW~1200Da) was supplied by Polymer Standard Service GmbH (PSS, Mainz, Germany). Industrial-grade polystyrene (MW~180 kDa) was purchased from Repsol© (Madrid, Spain) and expanded polystyrene (EPS) with HMW derived from common packaging. Toluene (≥99.7% purity), and HPLC-grade water were purchased from J.T.Baker®-VWR (Radnor, PA, USA). Nitrogen used as drying gas with 99.995% purity was acquired from Air Liquide (Spain). For the MALDI analysis, tetrahydrofuran (THF), matrices (trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene] malononitrile (DCTB), dithranol (DIT) and salicylic acid (SA)) and silver trifluoroacetate salt (≥99.99%) were purchased from Sigma Aldrich (Steinheim, Germany).

### 2.2. Standard solutions

To optimise LC-HRMS, DESI, and DART analytical approaches, a stock standard solution of polystyrene was prepared by dissolving 100 mg in 10 mL of toluene to obtain a stock solution of 10 g $L^{-1}$ and stored at −20 °C. Standard working mixtures, required for calibration curve and spiking tests, were prepared daily, at concentrations from 100 ng $L^{-1}$ to 10 mg $L^{-1}$ in toluene. In the case of MALDI-TOF-MS, a standard solution (100 mg $L^{-1}$) of PS 1200 Da was prepared in THF.

### 2.3. Sampling and sample preparation

To assess the good performance of the optimised analytical method, 17 samples from the Ebro Delta (Catalonia, North-East of Spain) were taken during winter 2017 in order to characterise MPLs. Ebro Delta is the 3rd largest delta in the Mediterranean Sea, and it is an area under the pressure of industry, agriculture and tourism. Because of this, the investigation of the presence of MPLs in this area is of high interest.

In the specific case of MPLs, water samples were collected from the upper water column (top 5 cm) where the presence of low-density PS is mainly expected. 10 L samples were taken in amber glass bottles (previously cleaned in the laboratory with toluene, methanol and water successively) cleaned with sample water 3 times before taking the final sample. Afterwards, the samples were transported to the laboratory at 4 °C. Then, 2 L were filtered using a dried and weighted glass fiber filters 0.70 μm (GF/F, Whatman™, Maidstone, United Kingdom). The filters were, successively, dried overnight at 60 °C until constant weight, and stored at −20 °C until their extraction. All the analyses were carried out working in triplicates. The extraction of PS from filters was achieved by ultrasonic assisted extraction (UASE) with toluene (3 × 10 mL) during 10 min each cycle. Finally, the extracts were dried under a gentle stream of $N_2$ near to dryness and reconstituted up to 1.5 mL of toluene in LC-vial for their analysis.

### 2.4. Instrumental analyses

#### 2.4.1. LC-HRMS

Chromatographic separation was achieved using an Acquity LC (Waters, Milford, MA, USA) chromatograph system equipped with an advanced polymer chromatography (APC) column (Acquity APC™ XT45 1.7 μm). The mobile phase consisted of toluene 100% working with isocratic conditions. The flow rate was kept at 0.5 mL $min^{-1}$ throughout the run, and the sample volume injected was 20 μL. The total chromatographic run had a duration of 5 min. The LC system was coupled to a Q-Exactive (Thermo Fisher Scientific, San Jose, CA) hybrid quadrupole–Orbitrap mass spectrometer, equipped with an atmospheric pressure photoionisation source operating in negative ionisation mode ((−) APPI). The acquisition was performed in full scan mode ($m/z$ 500–3000) with a resolution of 17,500 full widths at half maximum (FWHM). The optimal APPI source conditions were as follows: sheath gas at 60 a.u., auxiliary gas at 35 a.u., S-lens RF at 100 and the capillary and probe temperatures were maintained at 400 °C.

One of the main issues in MS of polymers remains in the data mining since the mass spectra are often complex because of the composition of technical blended polymers, isotope distribution, adducts, multiply charged and fragment ion series (Fouquet and Sato, 2017). For these reasons, for identification purposes and data mining of mass spectra, the Kendrick Mass Defect (KMD) analysis was applied as proposed by Sato et al. (2014). The $m/z$ obtained by LC-HRMS were converted to Kendrick Mass (KM) according to eq. (1):

$$\mathrm{KM\ (ion)} = \frac{m}{z}\mathrm{(ion)}\cdot\frac{\mathrm{round(R/X)}}{\mathrm{R/X}} \tag{1}$$

where Nominal Kendrick Mass (NKM) and Correct Nominal Kendrick Mass (CNKM) are represented in eq. (2) to eq. (4):

$$\text{NKM (ion)} = \text{round (KM(ion))} \tag{2}$$

$$\Delta\text{KMD (ion)} = \text{NKM(ion)} - \text{KM(ion)} \tag{3}$$

Correct Nominal Kendrick mass.

$$\text{CNKM(ion)} = \text{NKM(ion)} - \text{ceiling(NKM(ion)} - m/z\text{ (ion))} \tag{4}$$

where R is the mass of a repeat unit and X is a positive integer used as a base unit for the calculation of KM(ion). Normally, X = 1 for a standard KMD analysis. However, the concept of fractional base unit was recently introduced by Fouquet et al. (2017) to increase the resolution of KMD plots. The KMD plot present in the paper is the result of the application of this concept in order to obtain a distinct separation of $^{12}$C and $^{13}C_X$ isotopes of polystyrene for each adduct generated by LC-HRMS (Figure S-1). Finally, the best option was when (X ~ round (R)) or X = 102 which offer a clear visualisation of the isotope distribution ($^{12}$C and $^{13}C_X$ isotopes) of polystyrene.

In the specific case of polymers analysed by LC-HRMS, just the high-resolution mass spectra followed by KMD analysis can be used to achieve the minimum number of identification points, since the retention time (Rt) by APC column depends on molecular weight (MW) and not on polymer type. In this context, a conversion of concentration was required due to the possible difference in terms of MW between the polymer used as standard and the polymer present in samples. As an example, the area of the highest peak of the mass spectra profile in samples was used to quantify a polymer with MW different from 1200 Da. The concentration obtained by calibration curve $[PS]_{CC}$ was converted to equivalent concentration $[PS]_{eq}$ according to eq. (5). The equivalent factor ($F_{eq}$) depends on the number of monomers present in the standard $(n.mon.)_{std}$ and in the sample $(n.mon.)_{sample}$ (eq. (6)). The *n.mon* are the rounded values obtained by dividing the mass of the highest peak of the polymer's profile by 104, the mass of PS-monomer (eq. (7)).

$$[PS]_{eq} = \frac{[PS]_{CC}}{F_{eq}} \tag{5}$$

$$F_{eq} = \frac{(n.mon)_{sample}}{(n.mon)_{std}} \tag{6}$$

$$(n.mon) = \frac{(mass)_{highest\ peak}}{104} \tag{7}$$

### 2.4.2. Ambient ionisation techniques-MS

The applicability of ambient ionisation techniques to obtain qualitative information of the polymer composition of MPLs in environmental samples was explored. The advantage of these techniques is the possibility to obtain a mass spectrum without sample pre-treatment or with minimal treatment. Two different techniques were employed: direct analysis in real time (DART) and desorption electrospray ionisation (DESI).

DART-SVP ion source (Ion Sense, Saugus, MA, USA) interfaced to an LTQ-Velos Orbitrap dual-cell linear ion trap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) with a Vapur® API interface (IonSense, Saugus, MA, U.S.A.) was used. The optimised DART settings were as follows: DART ion source was positioned 47 mm away from the sample cards and 1.5 cm away from the mass spectrometer inlet; gas temperature of 450 °C; and grid electrode voltage of +100 V; helium gas at a flow rate of 3.0 L min $^{1}$; and a needle potential of 3 kV 3 μL of the standard solutions of PS1200, industrial PS and EPS in toluene were deposited on a transmission module stainless steel wire mesh and left to dry. A constant speed of 0.2 mm s $^{1}$ was used for the transmission module rail system. Polymer mass spectra were acquired in full scan mode for the mass range of 200–3000, and the summed mass spectra were stored at a rate of 5 spectrum s $^{1}$. Xcalibur 3.1 (Thermo Fisher Scientific) software was used for instrument control and data processing.

DESI ion source (Omnispray Ion Source, Prosolia Inc., Indianapolis, IN) with a 1D moving stage and interfaced to a quadrupole-Orbitrap mass spectrometer (Q-Exactive, Thermo Fisher Scientific, San José, CA, USA) was used. A red permanent marker that contains rhodamine 6G (ion at *m/z* 443.2329, positive ion mode), purchased from Fine Sharpie (Stanford Corp., Oak Brook, IL), was used to establish the DESI geometric source conditions. The optimised DESI settings were as follows: acetonitrile:water (80:20, v:v) was used as DESI solvent at a flow rate of 3.5 μL min $^{1}$; sprayed solvent was directed onto the sample surface at a nebulization capillary angle of 55°; spray tip-to-mass spectrometry inlet distance was 9.2 mm; nitrogen (99.95% pure, Air Liquide, Madrid, Spain) at a pressure of 8 bars was used as nebulizer gas; electrospray voltage was set at 4.5 kV in positive ion mode and 5 kV in negative ion mode; transfer capillary temperature was 250 °C. Q-Exactive mass spectrometer operated in positive and negative modes in full-scan at a mass resolution of 70,000 FWHM (full width at half maximum) at *m/z* 200, with a maximum injection time of 300 ms and a scan range from *m/z* 200 to 3000. Automatic gain control (AGC) values were set at $1 \times 10^6$. Omni Spray ion source software v2.0 (Omnispray Ion Source, Prosolia Inc., Indianapolis, IN) was used to control the DESI source, while Xcalibur software v2.2 (Thermo Fisher Scientific, San José, CA, USA) was used for data acquisition and data processing. For sample analysis, 3 μL of a liquid sample was deposited onto microscope glass slides of 7.1 mm polytetrafluoroethylene (PTFE) (Teflon; McMaster-Carr, Santa Fe, CA, USA) printed spots.

### 2.4.3. MALDI-TOF-MS

This analysis was performed using an AutoFlex III MALDI-TOF/MS instrument (Bruker Daltonik GmbH, Bremen, Germany) equipped with a 200 Hz Smart beam laser and were controlled using Flex Analysis 3.4 and PolyTools 1.0 (Bruker Daltonik GmbH, Bremen, Germany).

The acquisition was conducted in positive reflectron and linear ion modes for low and high-molecular-weight polymers, respectively. The voltage of the ion source 1 was set at 19.2 kV, and for the ion source 2 at 16.2 kV, the lens voltage was set at 8.5 kV, and mass spectra were collected in a *m/z* range between 500 and 3000. The mass spectra were acquired by averaging 500 laser shots. Laser irradiance was maintained slightly above threshold.

As sample matrix a solution of Trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene] malononitrile (DCTB) in tetrahydrofuran (THF) at 100 mg mL $^{1}$ was used. While silver trifluoroacetate (Ag-TFA) in THF was used as ion pairing agent at 10 mg mL $^{1}$. Finally, 100 mg L $^{1}$ of PS 1200 Da in THF was used for tests. Volumes ratio 1:10:1 V/V of the polymer solution, matrix and salt solution were used. The same mix was used for the study of the behaviour using industrial origin polymers and EPS. Then, 1 μL of the mixture was deposited onto the stainless-steel target plate, which was then dried in air before insertion into the ion source chamber.

## 2.5. Precautions and safety considerations

In order to minimise the potential losses by adsorption, complete dryness was prevented during the evaporation of the extract. The instrumental analysis was always carried out within the 12 h after extraction. All manipulation of standard solutions and extracts were performed under a fume hood. Since toluene was used as mobile phase, the LC system was vented into a fume hood.

## 3. Results and discussion

### 3.1. Sample preparation

PS is soluble in toluene, tetrahydrofuran (THF) and acetone, and partially in methanol. Therefore, according to their solubility, toluene, THF, and acetone, can be selected as PS solvents. However, THF and acetone were discarded since they were not compatible with the instrument pumps, and toluene was chosen as the optimal one.

### 3.2. Liquid chromatography-high resolution mass spectrometry analysis

The following parameters were optimised: i) selection of the filter type; ii) UASE extraction time; iii) UASE extraction volume, and iv) number of cycles. Regarding filter type, cellulose nitrate 0.45 μm (NC45, Whatman™, Maidstone, United Kingdom) and glass fibre 0.70 μm (GF/F, Whatman™, Maidstone, United Kingdom) were tested because their chemical composition would not produce cross-contamination effects. In the case of extraction time, extraction volume, and the number of cycles, 10, 20 and 30 min and 10 and 20 mL were tested for each cycle, and up to 3 cycles were tested. The results are summarised in Fig. 1. As can be seen, the best recoveries were obtained with 0.70 μm glass fibre filter (GF/F), which was selected.

Regarding extraction time and volume, no significant differences were observed between 10 and 30 min, neither comparing extraction volumes of 10 mL vs 20 mL. The optimum method includes 10 mL of toluene for extraction, with recoveries higher than 80% (Fig. 1), with extraction of 10 min three times. Three cycles of extraction were finally chosen in fact no significant differences were observed when the number of cycles was increased, but more than two cycles of extraction are normally suggested for the extraction of complex sample (Navarro et al., 2009; Picó, 2013; Pavlović et al., 2007). In spite of slightly higher recoveries are obtained with 20 mL and 10 mL were selected since it is more environmentally friendly.

#### 3.2.1. LC-HRMS optimisation

To optimise the mass spectrometry parameters flow injection analysis with PS standard solution (200 $\mu L\ min^{-1}$) was carried out. The performance of the following ionisation sources: electrospray ionisation (ESI), atmospheric pressure ionisation (API), atmospheric pressure chemical ionisation (APCI) and atmospheric pressure photoionisation (APPI), were tested. ESI was not efficient enough due to the low polarity of PS, as expected. Finally, APPI was selected because the ionisation was milder than APCI and, consequently, the matrix effect could be reduced (Figure S-2).

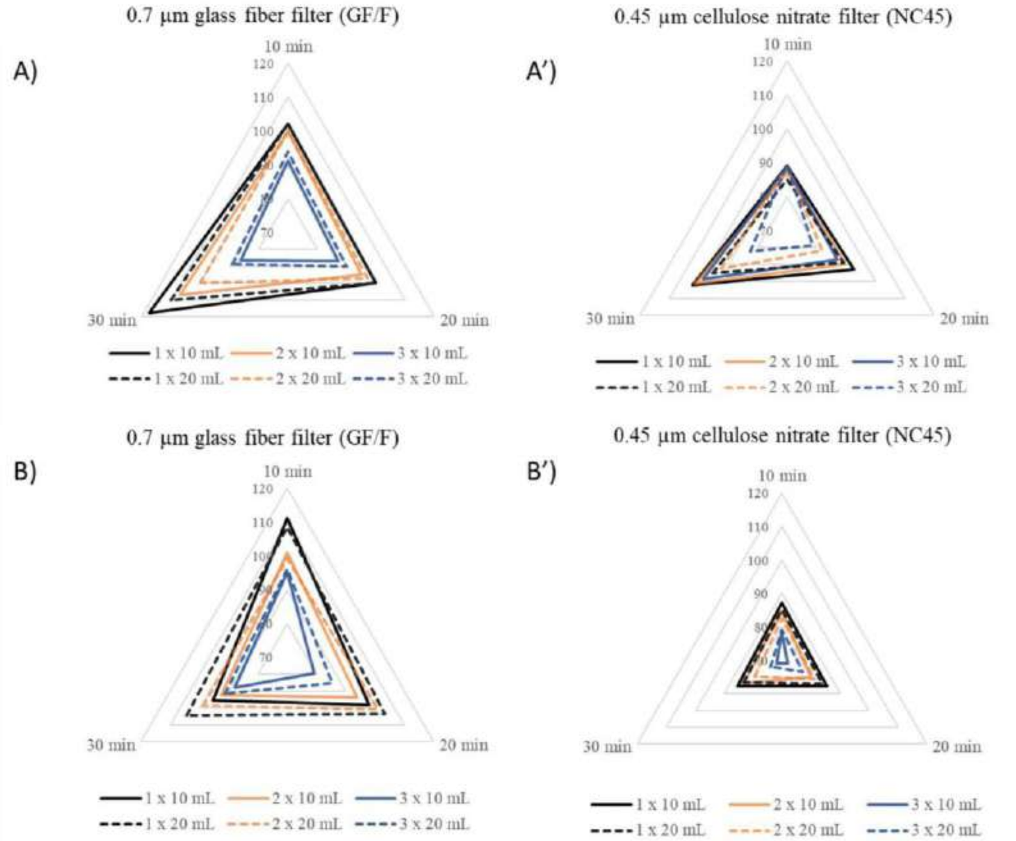

**Fig. 1.** Radial graphs of the recoveries of polystyrene with different filters, extraction volumes and times for freshwater (**A** and **A'**) and seawater (**B** and **B'**).

As can be seen in Figure S-3, working in positive conditions the formation of adducts decreases the signal of the molecular ion significantly, for this reason, negative ionisation conditions were selected. The sheath and auxiliary gas were ranging between 5 and 70 a.u., the tube S-lens RF between 50 and 100 and the capillary and probe temperature were varied from 150 to 500 °C (data not shown). Finally, the optimum APPI conditions were as follows: sheath gas pressure 60 a.u, auxiliary gas 35 a.u, capillary and probe temperatures at 400 °C.

The chromatographic separation was optimised using two size exclusion chromatography (SEC) columns: a conventional gel permeation chromatography (GPC) column (Phenogel™ Phenomenex, 5 µm, 50 A, 300 × 7.8 mm) and an Advanced Polymer Chromatography (APC) column (Acquity APC™ XT45, 1.7 µm, 150 × 4.6 mm). In general, SEC is used to characterise the complete molecular weight distribution of a polymer. The use of this type of columns coupled to HRMS offers the possibility not only to determine the polymer type by their unequivocal *m/z* but also to determine the MW of the polymer since plastics are decomposed in the environment in different MPLs weight' depending on the polymer monomers' and weathering conditions. The columns were compared regarding the retention time, peak resolution and separation efficiency among different MWs. It is important to mention that no gradient ramps with, for example, methanol were tested since selected columns do not afford the use of other solvents than toluene and THF (this last one is not recommended for the pumps of the instrument). In this sense, isocratic conditions with toluene were used in both cases, and this solvent was also used as a dopant for APPI. Different flow rates were tested ranging from 0.3 to 0.7 mL min-1. Finally, the best chromatographic separation in terms of peak resolution, retention time and separation efficiency was achieved with the APC column (Figure S-4).

In Fig. 2A, a total ion current (TIC) chromatogram and an extracted ion chromatogram (XIC) of PS is shown. The *m/z* (1339.8131) is one of the highest signals in the isotopic distribution of the PS-polymer. In Fig. 2B the relative mass spectra of PS is shown. The isotopic profile is characterised by the highest peaks referred to oxygenated analyte product ions such as [M-O2]·- or [M-H + O]- due to the presence of high quantities of oxygen in APPI ionisation source operating under ambient conditions. The ionisation source produces superoxide ion (O2·-). This ion can react with the analytes generating a negative radical adduct or can do a substitute reaction depending on ionisation conditions (Kauppila and Kostiainen, 2017; Kostiainen and Kauppila, 2009). Finally, the molecular ion distribution of PS (N = 12) is shown in Fig. 2B, where the monoisotopic mass (1338.8178) is the most abundant, followed by the nominal mass (1337.8116) and the average mass (1339.8131). This non-symmetrical profile of PS agrees with the most probable profile when the mass range of polymer is within 1000–3000 *m/z* (Yergey et al., 1983).

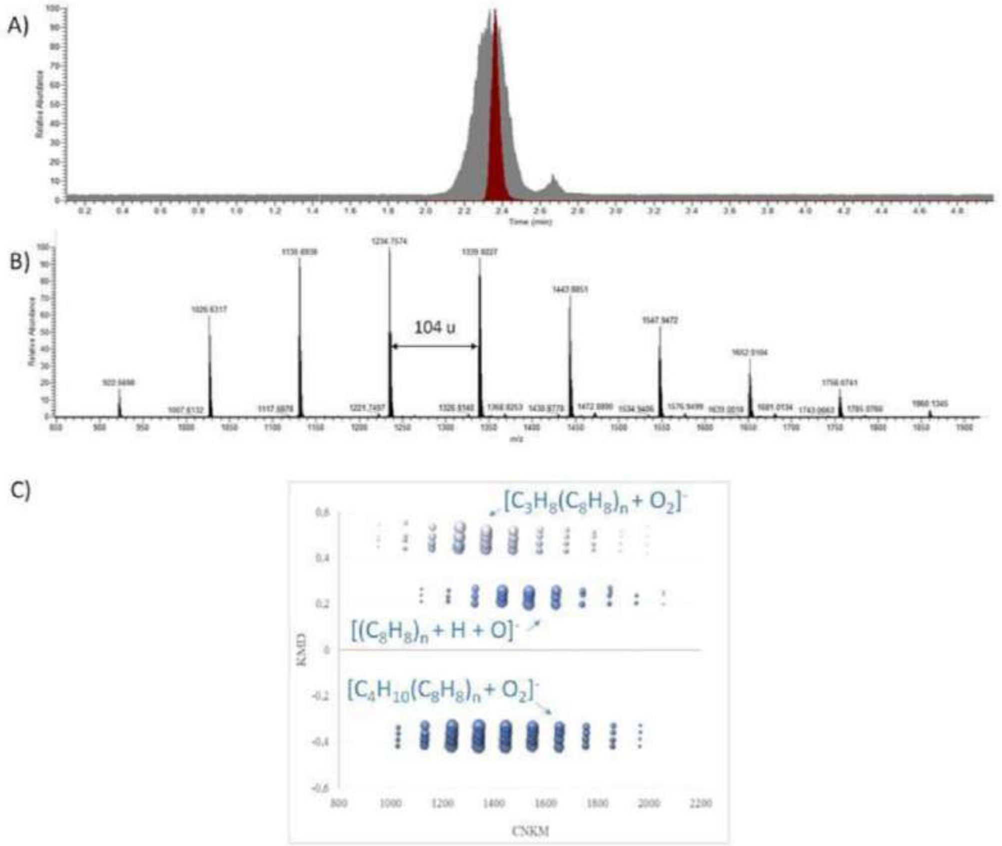

**Fig. 2.** A) Separation by APC-APPI-HRMS of polystyrene. The total ion current (grey) and extracted ion chromatogram (red) of one fraction. B) APPI-HRMS mass spectrum of polystyrene 1200. Negative ion mode, solvent/dopant = toluene, T = 400 °C, flow rate = 500 µL min-1. C) KMD plots using PS/102 as the base unit. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

**Table 1**
Instrumental and method quality parameters for the analysis of polystyrene by LC-APPI(−)-HRMS in freshwater and seawater.

| | | Parameters | | PS (MW = 1.2 kDa) |
|---|---|---|---|---|
| Instrumental parameters | | ILOD (pg) | | 20 |
| | | Linearity (µg L$^{-1}$), $R^2$ | | 0.5–50, 0.998 |
| | | Linearity (µg L$^{-1}$), $R^2$ | | 50–1000, 0.996 |
| | | Accuracy (%) | | <20 |
| | | Inter-day (n = 5) repeatability (%) | 5 µg L$^{-1}$ | 15.5 |
| | | | 50 µg L$^{-1}$ | 20.0 |
| | | | 500 µg L$^{-1}$ | 25.6 |
| | | Intra-day (n = 5) repeatability (%) | 5 µg L$^{-1}$ | 2.4 |
| | | | 50 µg L$^{-1}$ | 1.9 |
| | | | 500 µg L$^{-1}$ | 1.8 |
| Method parameters | Fresh water | $MLOD_{FW}$ (pg L$^{-1}$) | | 32.5 |
| | | $MLOQ_{FW}$ (pg L$^{-1}$) | | 110 |
| | | Recovery $_{FW}$ (%)[b] | 5 µg L$^{-1}$ | 63.4 |
| | | | 50 µg L$^{-1}$ | 57.7 |
| | | | 500 µg L$^{-1}$ | 57.0 |
| | | Inter-day $_{FW}$ (%)[a] | | <10 |
| | | Matrix effect $_{FW}$ (%)[c] | | −44.8 |
| | Sea water | $MLOD_{SW}$ (pg L$^{-1}$) | | 30.0 |
| | | $MLOQ_{SW}$ (pg L$^{-1}$) | | 102.5 |
| | | Recovery $_{SW}$ (%)[b] | 5 µg L$^{-1}$ | 60.4 |
| | | | 50 µg L$^{-1}$ | 72.7 |
| | | | 500 µg L$^{-1}$ | 67.1 |
| | | Inter-day $_{SW}$ (%)[a] | | <10 |
| | | Matrix effect $_{SW}$ (%)[c] | | −71.4 |

[a] Method repeatability was assessed in triplicate.
[b] RSD value for the recovery is lower than 20% or 25% for freshwater and seawater, respectively.
[c] ME (%) = (slope matrix/slope solvent −1)*100.

The profile of the PS and its isotopic pattern was confirmed by the KMD analysis, with two PS-polymer chains with different end-group combinations, one end-group negatively charged [M-O2]·- and the other one with an adduct of oxygen formed by the substitution reaction previously described [M-H + O]-. In Fig. 2C, these results are summarised.

The good performance of the analytical method was evaluated in terms of linearity, accuracy, precision, and instrumental limit of detection (Table 1). The instrumental quality parameters were calculated using ten points calibration curve in toluene working with two ranges: from 0.5 to 50 µg L$^{-1}$ and from 50 to 1000 µg L$^{-1}$. Instrumental linearity and ranges were estimated using linear regressions with good results in both ranges ($R^2 > 0.995$). Instrumental precision (n = 5), expressed as the relative standard deviation (RSD%) of intra-day and inter-day repeatability of three different concentration levels (5, 50, and 500 µg L$^{-1}$) was evaluated. In all the cases the precision was lower than 5% and 25% for intra- and interday evaluations, respectively. The ILOD was estimated from the injection of the lowest calibration curve point (20 pg).

### 3.2.2. *Method validation*

The method limits of detection and quantification (MLOD and MLOQ, respectively), recoveries and matrix effects for freshwater and seawater are summarised in Table 1. Regarding extraction method parameters, recoveries of quintuplicates at three spiking concentrations (5, 50, and 500 µg L$^{-1}$) were calculated. The MLOD and MLOQ achieved were of 32.5 and 110 pg L$^{-1}$ for freshwater and 30 and 102.5 pg L$^{-1}$ for seawater, respectively. The method was shown to be sensitive enough for the analysis of polymers in environmental samples. In this work, the matrix effect was estimated by comparing the slopes of standard curves dissolved in extraction solvent and matrix-matched calibration curves. The results confirm up to 40% of ion suppression for freshwater while in seawater the suppression was much higher with values up to 70%. These results highlight the need to use matrix match standard calibration curves for quantitative analyses.

Therefore, the performance of the method was tested as well with an industrial polystyrene (MW > 150 kDa) (HMW), which have impurities compared with standards. The mass spectra are shown in Figure S-5. As can be seen, the profile of PS was clearly identified and the number of chains with a reduced molecular weight form is constant and it is enough for the identification of the polymer.

### 3.2.3. *Analysis of real samples*

As a proof of concept, the method was applied to the analysis of 17 samples (8 freshwater and 9 seawater samples) from the Ebro Delta from Catalonia (North-East of Spain). In Fig. 3 and Tables S−1 of Supporting Information, the main results were summarised. After the analysis, five samples were positive in PS showing a concentration between 1 and 140 ng L$^{-1}$ (RSD < 5%). The highest concentration of PS (136.74 ng L$^{-1}$) with a MW range of 1500–2000 Da corresponded to Port d'Illa de Mar, the main industrial harbour of seafood in Fangar Bay. To the authors knowledge, this is the first time that PS ranging from 800 to 2000 Da has been detected in Ebro Delta area.

### 3.3. *Dart- orbitrap-ms analysis*

The DART transmission was employed, where the heated ionizing gas flows through a metal screen onto which samples have been deposited, increasing the surface area available for sample deposition, and thus increasing sensitivity and uniform thermal desorption. Helium pressure and heater temperature, distance from the source to the MS, and sample speed through the DART source were optimised using a solution of PS (6 µL). The ionisation conditions were set in positive. The temperature of the helium gas in the DART was optimised in steps of 50 °C (Figure S−6A). Below 200 °C, no signal was observed while at higher temperatures than 450 °C intensity decreased. In regards to the exit grid voltage, the intensity of the signal decreased by the increase of the voltage (Figure S−6B). The optimum results were obtained when the exit grid voltage was set at +100 V and the temperature of gas ionisation at 450 °C. Finally, the entrance hole was set at 4.6 cm of the

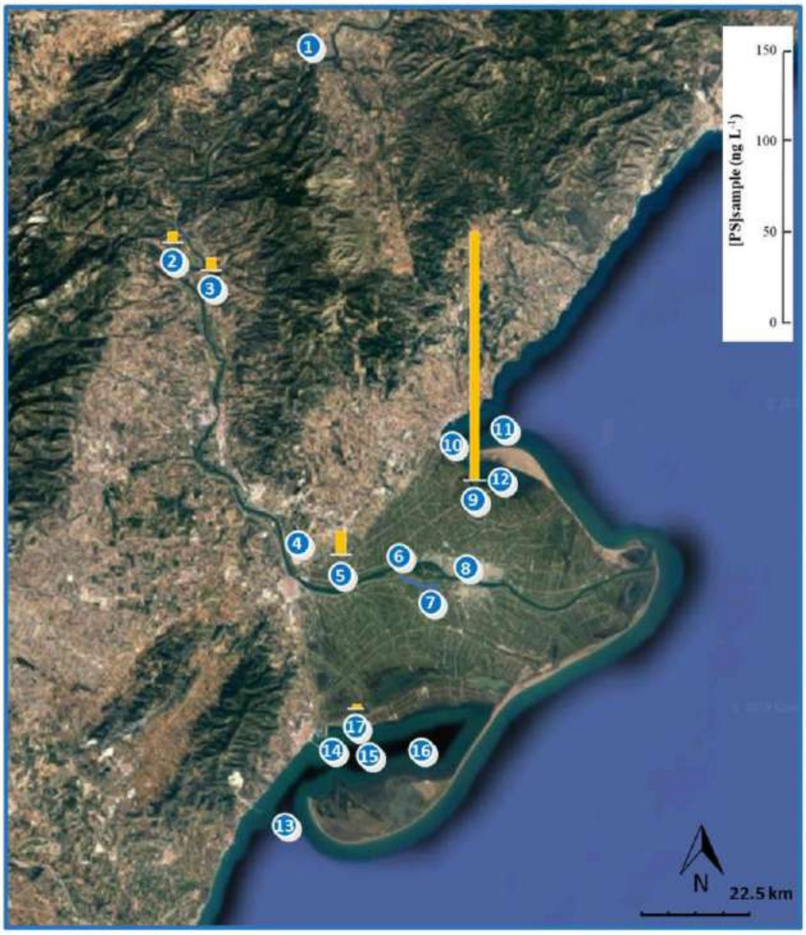

**Fig. 3.** Main results from the sampling sites located in the Ebro Delta area (NE Spain).

DART SVP railing guide.

Once the optimal parameters were set, the analytical performance was explored with a PS standard in toluene ranging from 0.1 to 10 mg L$^{-1}$ (Fig. 4) As can be seen in Fig. 4B, the least squares linear regression indicates good linearity ($R^2 = 0.9947$). In Fig. 4C, the DART mass spectra are presented. As can be seen, the molecular ion $[M]^{+\cdot}$ was less intense than the ammonia adduct and the oxidation products such as $[M-O_2]^{+\cdot}$. In contrast, adducts with ammonium give up to 5-fold intensity compared to molecular ion.

The use of DART is a promising tool for the rapid identification and quantification of MPLs, and further work will be done in this field. However, it is noteworthy to mention that ambient ionisation techniques are not suitable for the ionisation of molecules with MW higher than 1500–2000 Da.

Although DESI has been previously used to detect and characterise polar low molecular weight polymers such as polyethylene glycol in a complex sample (Sero et al., 2019) this ambient ionisation technique was not suitable for the analysis of no polar polymers such as PS, and, consequently, any results were obtained.

### 3.4. Maldi-tof-ms

The parameters of MALDI were optimised, and they are presented in Tables S–2 of the Supporting Information. Three different matrices (DCTB, DIT and SA) were prepared in THF at different concentrations. Matrix, polymer and cationization agent were mixed at three different ratios (20:30:1, 100:10:1 and 10:1:1). The results of the MALDI analyses were obtained in positive ionisation mode. The AgTFA salt was used because its efficiency to ionise long and short polymer chains (Pruns et al., 2002; Cvačka and Svatoš, 2003; Dutta and Harayama, 2001). Especially, in the case of polystyrene, the presence of phenyl rings facilitates π-complexation of metal cations such as $Ag^+$ or $Cu^+$. One of the central parameters to a successful characterisation of a polymer is the proportion between the matrix and polymer. Initial experiments were performed using PS with a molecular weight of 1200 Da and low polydispersity (PDI < 1.1). Matrix, polymer and salt were mixed at a ratio of 20:30:1, 1 μL of the mix was put on the target plate and finally air-dried at room temperature. In this analysis, DCTB and DIT matrices were used because they have been previously reported for the analysis of polystyrene by MALDI (Aksenov and Bier, 2008; Wetzel et al., 2006; Gabriel et al., 2015; Schnöll-Bitai et al., 2007). An example of mass spectra using a reflectron TOF mass spectrometer can be seen in Figure S-7. As it can be observed, the difference between two adjacent mass peaks is of 104 Da (repeating unit of PS -($C_8H_8$)-) for series of single peaks

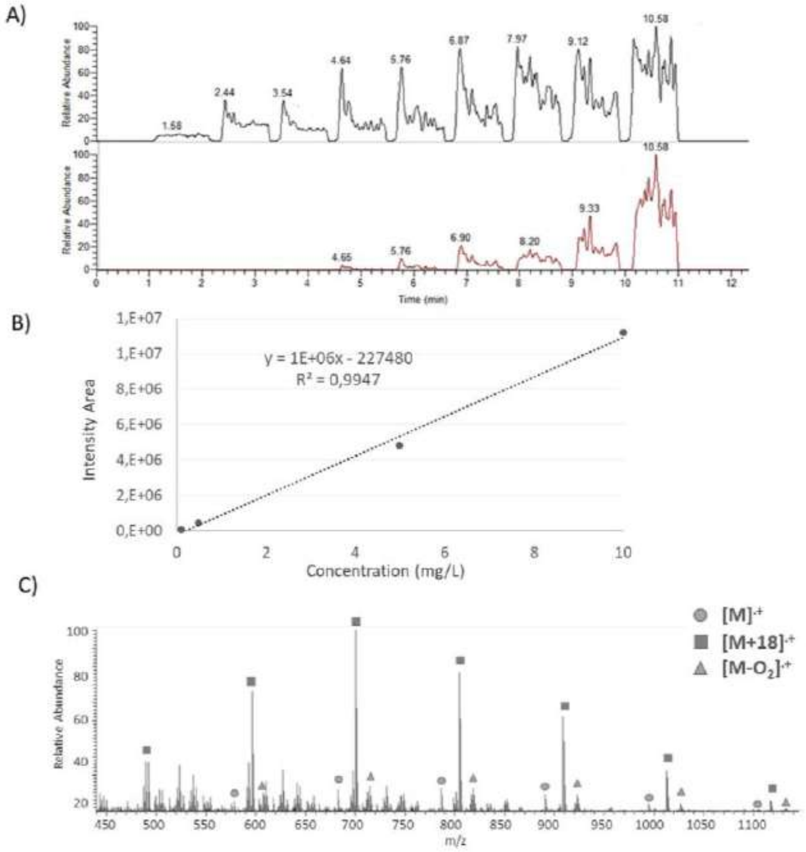

Fig. 4. (+)DART-Orbitrap of polystyrene; heater temperature 450 °C and exit grid voltage +100 V: **A)** analytical response (calibration) of PS in the range of 0.1–10 mg $L^{-1}$ with $R^2 = 0.9947$; **B)** TIC and mass chromatogram of PS at *m/z* 699.99–700.99 whereby the mass signal was dependent on positioning; **C)** Major ions observed in (+)DART mass spectra of polystyrene.

ranging from 800 ± 2000 *m/z*, and with values of *n* between 7 and 18. Comparing the results obtained with different matrices (Figure S-8), the PS distribution showed higher intensities when working with DTCB.

Finally, MALDI-TOF/MS method was applied to HMW polymers (industrial PS and EPS) and samples from Ebro Delta. In this case, the proportion of matrix/polymer/salt was optimised again in order to have the highest response and settled at 10:1:1. It should be noticed the necessity to increase the molar amount of matrix for higher MWs due to the decrease in sensitivity. According to the results shown in Figure S-9, the methodology gives different response depending on the nature of the polymer (i.e., a peak intensity of HMW is lower compared to a standard sample). In the case of Ebro Delta samples, the sensitivity given by this methodology is lower than LC-HRMS, and none of the analysed samples gave positive in PS.

## 4. Conclusions

This work showed the suitability of LC-APPI-HRMS for quantitative analysis of small size-range PS-MPLs and NPLs with MW below 3000 Da in the suspended fraction of natural environmental waters. The method presented high sensitivity and robustness, allowing the analysis in real environmental samples. As a proof of concept, the method was applied to the analysis of 17 samples of freshwater and seawater, and the concentration founded were at the ppt level. For the analysis of environmental water samples, it should be necessary to optimise the sampling, also to have more information about the particles size collected. Moreover, follow-up studies will need to show whether a similar analytical setup could be extended to the analysis of biota samples. Among the different analytical methods assessed in this study (MALDI-TOF/MS, DART-MS, and LC-APPI-HRMS) for the analysis of PS-MPLs/NPLs, LC-APPI-HRMS provided the best sensitivity. The method developed here can be an excellent complement to the existing procedures, to carry out quantitative measures.

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## Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2019.07.052.

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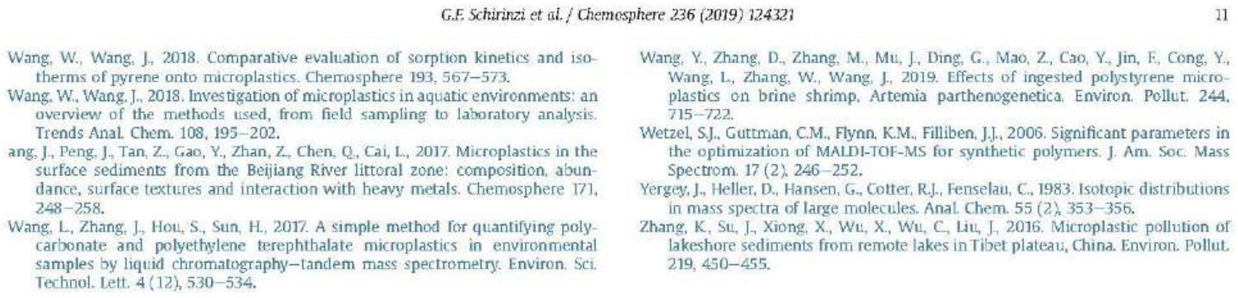

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Supporting Information for:

# TRACE ANALYSIS OF POLYSTYRENE MICROPLASTICS IN NATURAL WATERS

Gabriella F. Schirinzi[a], Marta Llorca[a], Raquel Seró[b], Encarnación Moyano[b], Damià Barceló[a,c], Esteban Abad[a], Marinella Farré[a,*]

[a] Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona (Catalonia, Spain)
[b] University of Barcelona, Barcelona (Catalonia, Spain)
[c] Catalan Institute for Water research (ICRA), Girona (Catalonia, Spain)

## Content

**Table S-1.** Samples information with physicochemical parameters and results concentration of PS (ng $L^{-1}$).

| Sample information | | | | | Physicochemical parameters | | | | Concentration | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Area** | **GPS coordinates** | **Water type** | **Origin** | **Temperature (°C)** | **pH** | **Conductivity (uS/cm)** | **Salinity (ppt)** | **$[PS]_{sample}$ (ng/L)** | **RSD (%)** | **MW Range (Da)** |
| 1 | *Before Xerta* | 41.032962,<br>0.608565 | FW | river | 24.9 | 8.1 | 759 | 0.30 | - | - | - |
| 2 | *Xerta* | 40.976154,<br>0.518484 | FW | river | 25.0 | 8.2 | 767 | 0.30 | 5.84 | 3.20 | 500-1000 |
| 3 | *After Xerta* | 40.974850,<br>0.516012 | FW | river | 25.0 | 8.0 | 765 | 0.29 | 6.77 | 4.43 | 500-1000 |
| 4 | *Amposta* | 40.715022,<br>0.581698 | FW | river | 25.2 | 7.9 | 774 | 0.31 | - | - | - |
| 5 | *After Amposta* | 40.713142,<br>0.689317 | FW | river | 25.1 | 7.5 | 781 | 0.29 | 13.0 | 2.83 | 500-1000 |
| 6 | *Before Deltebre* | 40.712942,<br>0.717407 | FW | river | 28.0 | 8.1 | 1294 | 0.60 | - | - | - |
| 7 | *Deltebre* | 40.713967,<br>0.716723 | FW | river | 27.2 | 7.7 | 1026 | 0.48 | - | - | - |
| 8 | *After Deltebre* | 40.714857,<br>0.734523 | FW | river | 27.9 | 7.6 | 1011 | 0.54 | - | - | - |
| 9 | *Fangar Bay beach (Port d'Illa de Mar)* | 40.766660,<br>0.737865 | SW | beach | 32.8 | 8.2 | 46286 | 25.5 | 136.7 | 1.46 | 1000-1500 |
| 10 | *Fangar Bay harbor* | 40.803269,<br>0.710630 | SW | harbor | 26.7 | 7.8 | 41752 | 26.1 | - | - | - |
| 11 | *Fangar Bay open sea 1* | 40.795664,<br>0.728952 | SW | open sea | 26.7 | 7.6 | 45335 | 26.7 | - | - | - |
| 12 | *Fangar Bay open sea 2* | 40.814594,<br>0.743862 | SW | open sea | 25.9 | 7.4 | 50119 | 27.2 | - | - | - |
| 13 | *Alfacs Bay beach* | 40.601594,<br>0.600383 | SW | beach | 31.9 | 8.1 | 52300 | 32.1 | - | - | - |
| 14 | *Alfacs Bay harbor* | 40.614640,<br>0.605632 | SW | harbor | 29.1 | 7.8 | 51104 | 30.8 | - | - | - |
| 15 | *Alfacs Bay open sea 1* | 40.622999,<br>0.661707 | SW | open sea | 29.3 | 7.9 | 50946 | 30.8 | - | - | - |
| 16 | *Alfacs Bay open sea 2* | 40.600416,<br>0.652181 | SW | open sea | 28.1 | 7.4 | 51207 | 31.4 | - | - | - |
| 17 | *Emissary Sant Carles de la Rapita* | 40.622579,<br>0.622754 | SW | emissary | 20.9 | 7.4 | 20046 | 13.05 | 1.08 | 3.58 | 1500-2000 |

**Table S-2.** Summary of parameters to optimize MALDI-TOF/MS method for PS-polymers.

| | | | | |
|---|---|---|---|---|
| **Matrix** | Name | DTCB | DIT | SA |
| | Conc. | 10 mg $mL^{-1}$ or 100 mg $mL^{-1}$ | | |
| **Polymers** | Name | PS standard (MW~1.2 kDa) | PS industrial (MW~180kDa) | EPS (HMW) |
| | Conc. | 10 mg $mL^{-1}$ or 100 mg $mL^{-1}$ | | |
| **Cationization Agent (AgTFA)** | Conc. | 10 mg $mL^{-1}$ | | |
| **Proportion Matrix : Polymer : Cat. Agent** | | 20:30:1 | 100:10:1 | 10:1:1 |

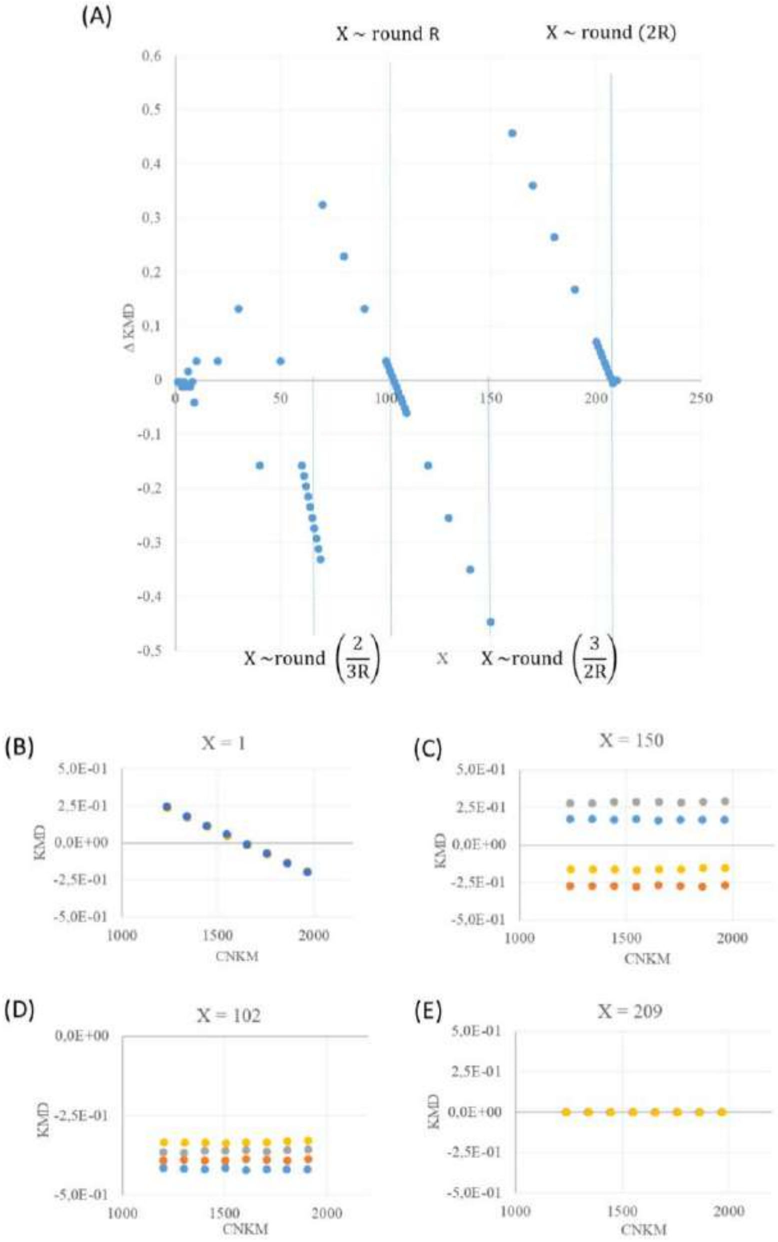

**Figure S-1. A)** ΔKMD plots using PS (=104) as base unit and X = 1 – 209. The recommended values for X are between (round (2/3R)) and (round (2R)). For PS 1200Da the X values tested are **B)** PS/1, **C)** PS/150, **D)** PS/102 and, **E)** PS/209 as the base units.

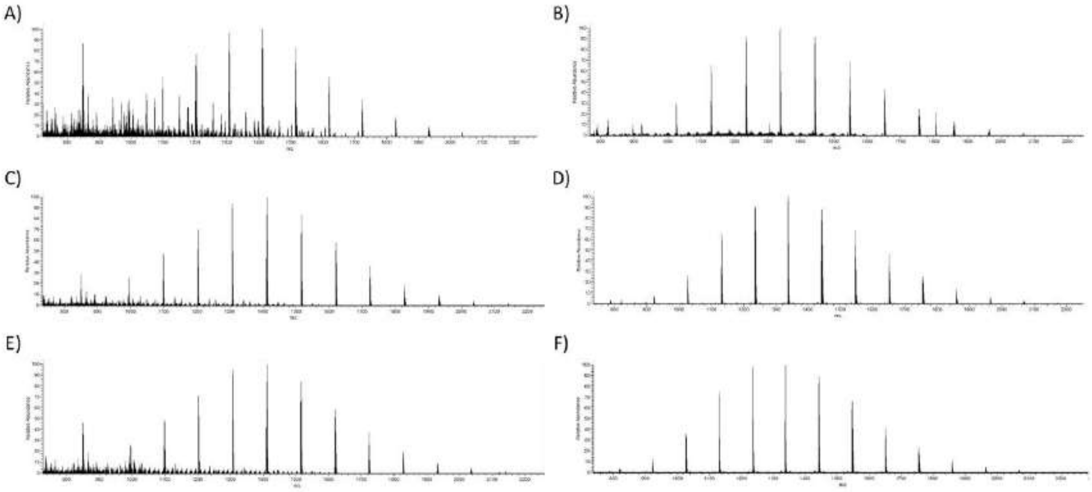

**Figure S-2.** Influence of ion suorce. Comparison of PS 1200 mass spectra with: **A)** positive-ion, and **B)** negative-ion APCI; **C)** positive-ion, and **D)** negative-ion APPI; **E)** positive-ion, and **F)** negative-ion APPI/APCI by flow injection analysis (flow rate = 200 µL min$^{-1}$, sheat gas = 15, aux gas = 10, S-Lens RF level = 100).

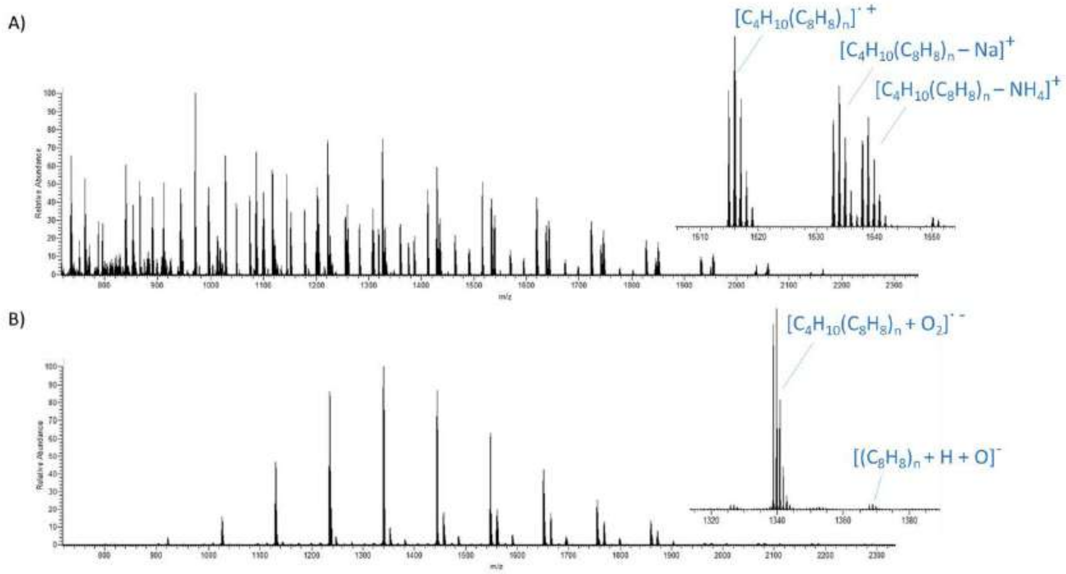

**Figure S-3.** Comparison of APPI mass spectra of PS 1200: full scan **A)** positive-ion, and **B)** negative-ion APPI spectrum.

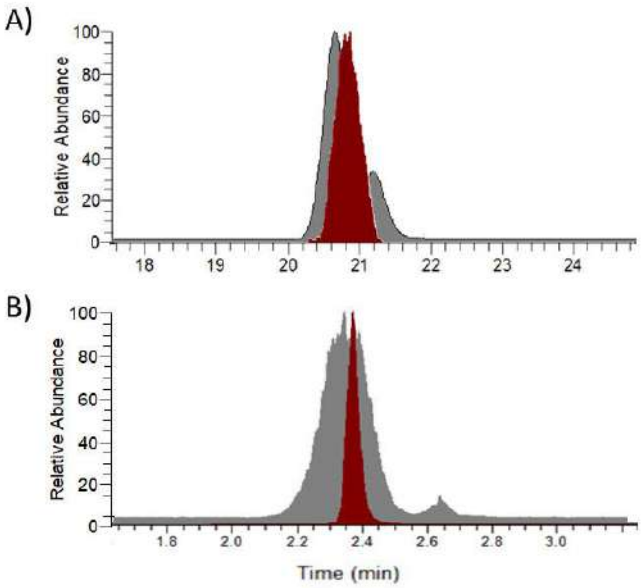

**Figure S-4.** The chromatographic separation by **A)** conventional gel permeation chromatography (GPC) column (Phenogel™ Phenomenex, 5µm, 50 A, 300 x 7.8 mm,) and **B)** Advanced Polymer Chromatography (APC) column (Acquity APC™ XT45, 1.7 µm, 150 x 4.6 mm). The total ion current (grey) and extracted ion chromatogram (red) of one fraction.

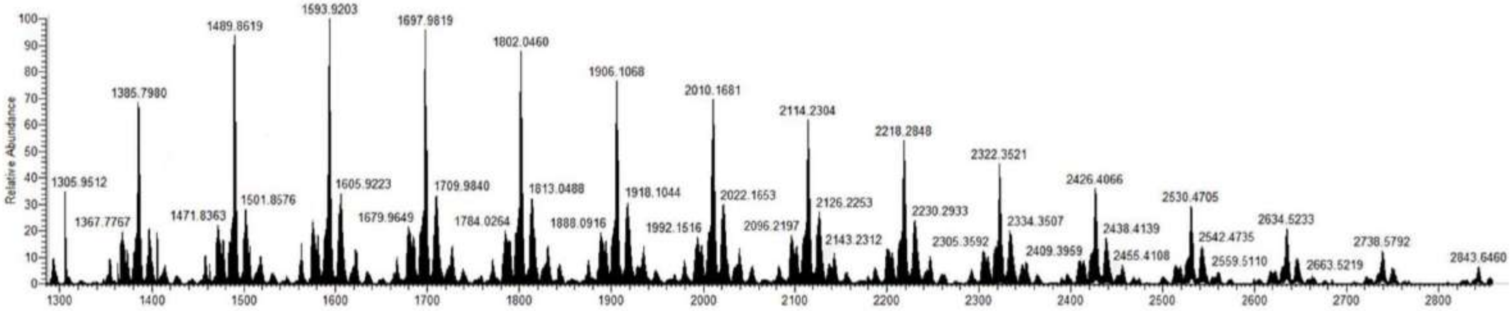

**Figure S-5.** LC-HRMS mass spectra of industrial PS (MW = 180kDa) using a APPI-negative ion mode, solvent/dopant = toluene, T = 400°C, flow rate = 500 μL min$^{-1}$.

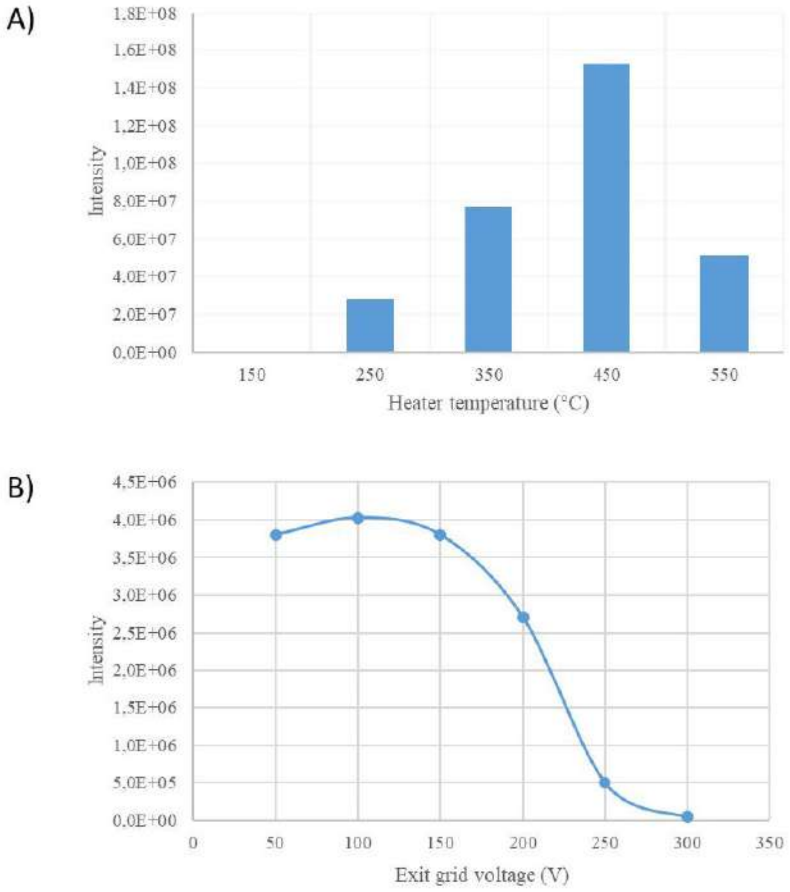

**Figure S-6.** Effect of helium heater temperature **A)** and exit grid voltage **B)** in the signals intensity of polystyrene (XIC = 700.4852).

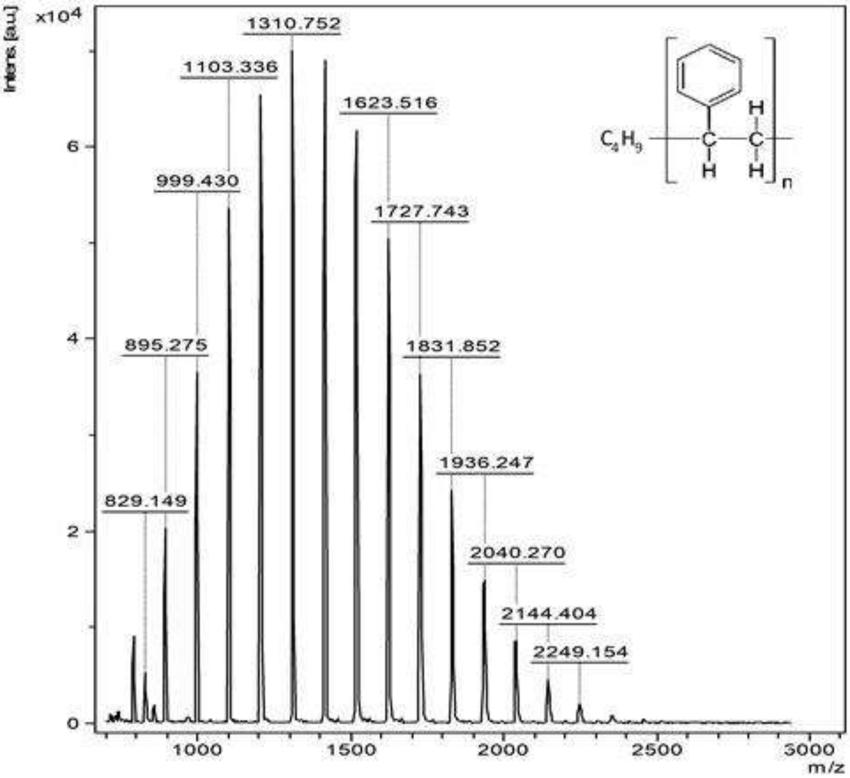

**Figure S-7.** MALDI-TOF spectra of polystyrene 1200 (10 mg $L^{-1}$) using DCTB matrix (100 mg $L^{-1}$); end group = butyl; Mn = 1430 Da; Mw = 1449 Da; PDI= 1.01

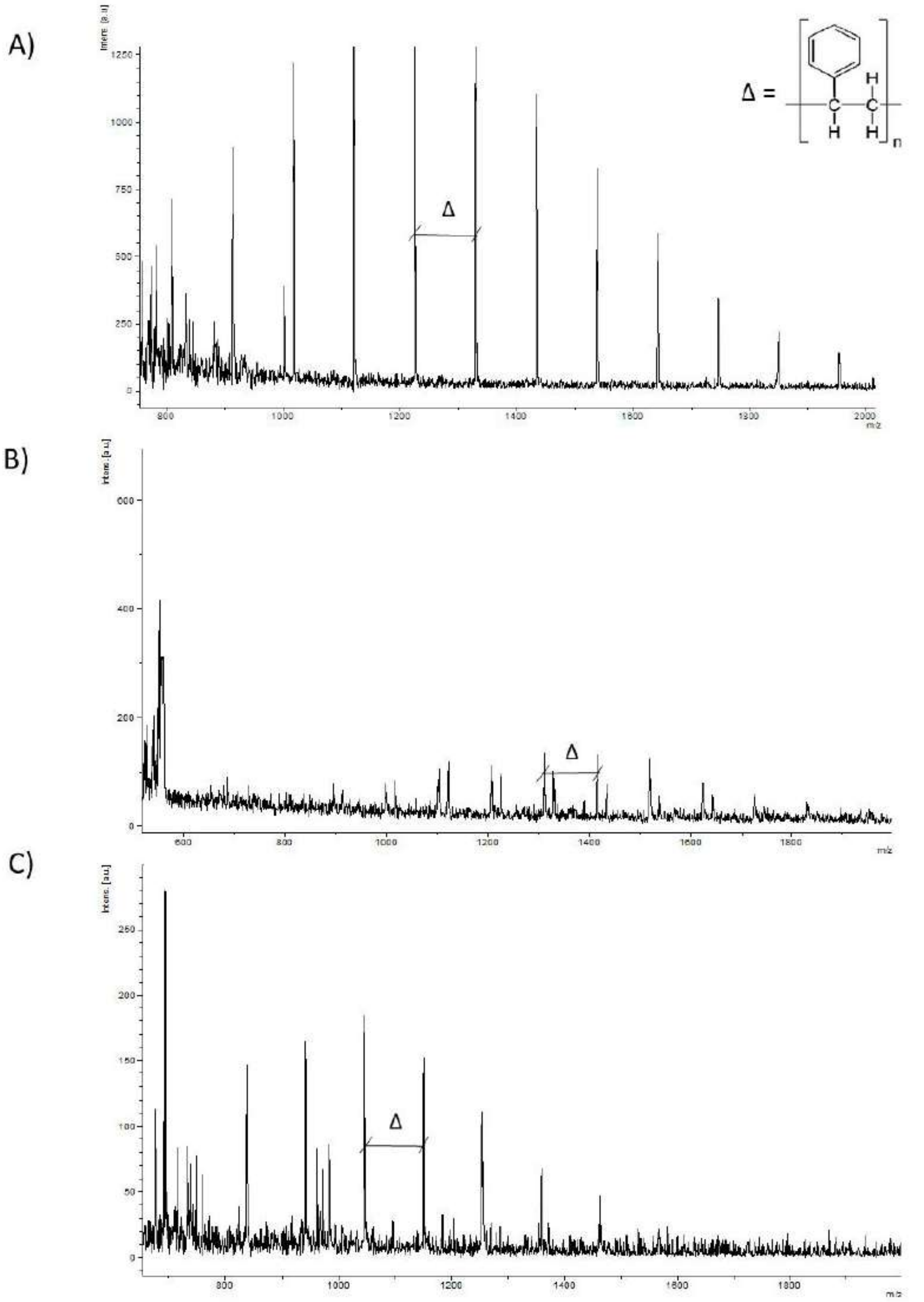

**Figure S-8.** MALDI mass spectra of PS1200 using **A)** DTCB, **B)** DIT, and **C)** SA matrix obtained by using a reflectron TOF mass spectrometer.

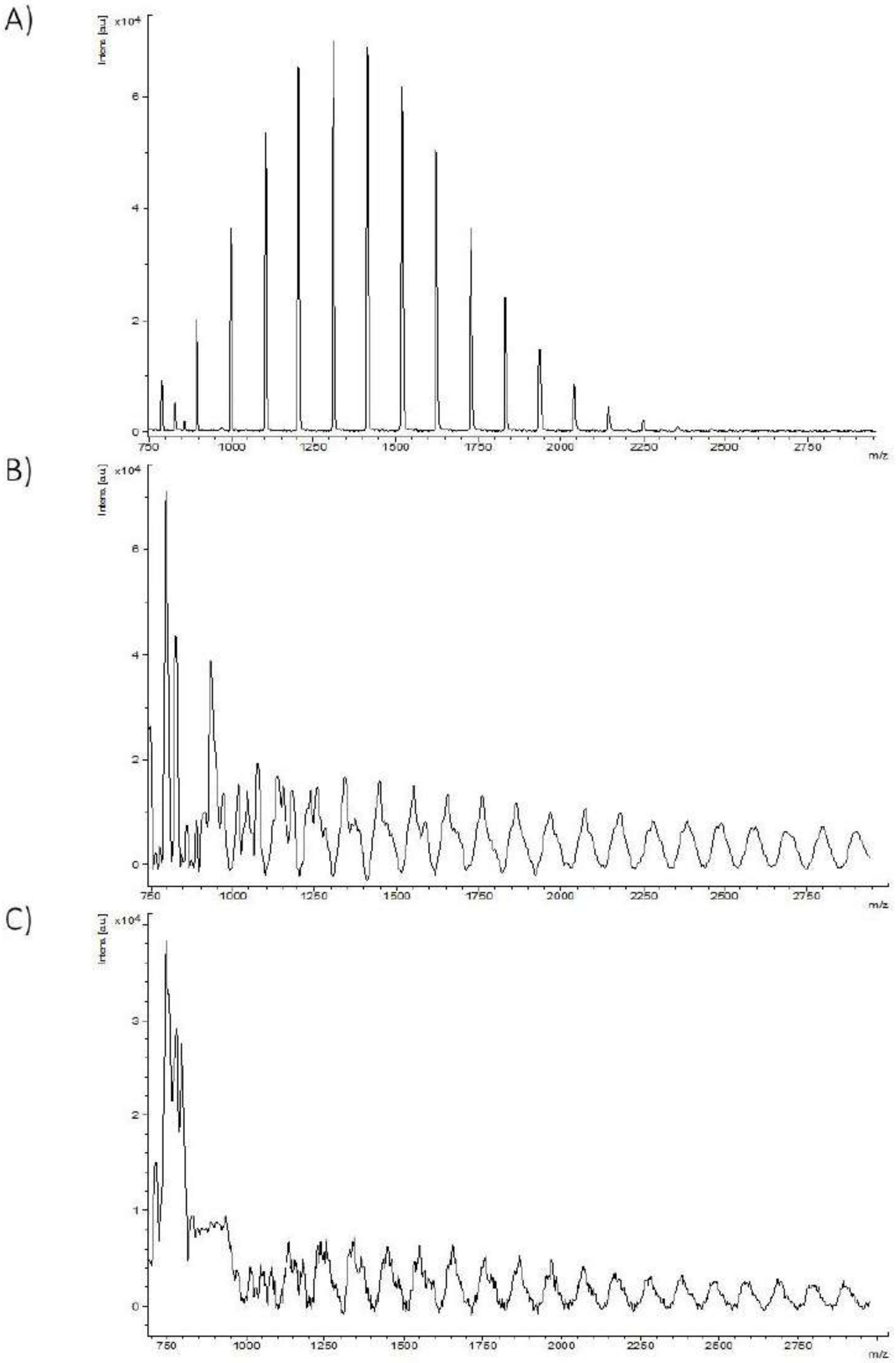

**Figure S-9.** MALDI mass spectra of **A)** PS1200, **B)** industrial PS (MW > 150kDa), and **C)** EPS obtained using a reflectron and linear TOF mass spectrometer.

### 3.2.3 Publication III: Adsorption of perfluoroalkyl substances on microplastics under environmental condition

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# Adsorption of perfluoroalkyl substances on microplastics under environmental conditions☆

Marta Llorca [a], Gabriella Schirinzi [a], Mònica Martínez [a, b], Damià Barceló [a, c], Marinella Farré [a, *]

[a] Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain
[b] Department of Materials Science and Physical Chemistry, Faculty of Chemistry, Martí i Franquès 1, 08028 Barcelona, Spain
[c] Catalan Institute for Water Research (ICRA), H2O Building, Scientific and Technological Park of the University of Girona, Emili Grahit 101, 17003 Girona, Spain

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ABSTRACT

Plastic debris has become an environmental problem during recent years. Among the plastic debris, microplastics (<5 mm; MPLs) imply an extra problem due to their capacity to enter into the fauna through ingestion. In this work, we study the capacity of three MPLs, that include high-density polyethylene (HDPE), polystyrene (PS) and polystyrene carboxylate (PS-COOH), to sorb 18 perfluoroalkyl substances (PFASs; including carboxylic acids, sulphonates and one sulphonamide) from the surrounding waters (freshwater and seawater).

Conclusions drawn from the results are that perfluoro sulphonates and sulphonamides have more tendency to be sorbed onto MPLs. In addition, PS and PS-COOH have more affinity for PFASs than HDPE. Finally, the increment of conductivity and pH of the water decreases the exposure time that is necessary to reach equilibrium. However, the presence of salts decreases the tendency of PFASs to be sorbed onto plastic surfaces. These results highlight the problem associated with the presence of MPLs in inland and marine waters since toxic compounds can be sorbed onto surrounding plastics that could be ingested by aquatic fauna.

## 1. Introduction

Nowadays, the unsustainable disposal of plastic residues is an emerging environmental problem for surface water (Mani et al., 2015; Van Sebille et al., 2015) and the ocean is presented as final sink (Avio et al., 2016; Llorca et al., 2014; PlasticsEurope, 2011). Plastic debris represents an aesthetic problem but also implies harmful damages for coastal areas and socio-economic activities that are linked to contaminated regions (Cole et al., 2011; Harrison et al., 2011; US_EPA, 2002). The low degradability of these materials and the presence of low-density polymers in upper layers of aquatic systems hamper oxygen exchange and obstruct light, which leads to eutrophication processes (Harrison et al., 2011). Recent studies have demonstrated that zooplankton (Setälä et al., 2014), fish (Romeo et al., 2015) and birds (Holland et al., 2016) can ingest microplastics (MPLs <5 mm). The ingestion of MPLs can alter different functions on aquatic biota, for example, polystyrene can significantly change the feeding capacity of zooplankton, and prolonged exposure significantly decreases their reproductive output (Cole et al., 2015). In addition, ingestion by zooplankton may assist in the marine food web transfer of MPLs. However, their impact has not yet been fully evaluated (Bouwmeester et al., 2015; Cole et al., 2011; Lusher, 2015). On the other hand, MPLs can accumulate persistent organic contaminants and pathogens, that are also an additional transport vector for these contaminants (Bakir et al., 2014a; Lusher, 2015; Rios et al., 2007). Therefore, the effects that MPLs may cause to the marine food web are not only associated with the consequences of their direct ingestion, but they are also associated with the release of compounds adsorbed onto surfaces of MPLs (Deudero and Alomar, 2015; Engler, 2012).

Perfluoroalkyl substances (PFASs) are a large group of chemicals that have been synthesised for more than 60 years. These are used in industrial and commercial applications due to their inertia and resistance to physical, chemical and biological degradation (Llorca

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* Corresponding author.
*E-mail address:* mfuqam@cid.csic.es (M. Farré).

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0269-7491/

et al., 2012). In addition, these compounds have been related to different toxicological effects (e.g., disruption of the thyroid hormones and the metabolism of high-density lipoproteins, cholesterol and triglycerides (Lau et al., 2007; Peden-Adams et al., 2008)). The high stability of PFASs and their wide distribution around the environment make them considered as being emerging persistent organic pollutants (POPs). Their presence in surface waters varies from few to hundred ng/L, even μg/L. For example, recently, average concentrations of 162 ng/l have been reported in the Qiantang River in China (Lu et al., 2017), while in other areas only few ng-pg/L levels have been reported (Sammut et al., 2017). But their occurrence is in general confirmed even in pristine areas (Casal et al., 2017) and open oceans waters (González-Gaya et al., 2014). Their environmental transport has been related to geophysical phenomena (Lohmann et al., 2007) as well as other vectors, such as sorption, onto marine plastic debris (Bakir et al., 2014b; Llorca et al., 2014). Sorption is also an additional vector for the entrance of PFASs in marine biota. That phenomenon has been previously identified for other POPs such as polychlorinated biphenyls (PCBs) (Colabuono et al., 2009; Rochman et al., 2013) and personal care products (Wardrop et al., 2016). Recently, different authors highlighted the limited evidence or the low role of MPLs as a vector to the biota of persistent organic pollutant (POPs) (Koelmans et al., 2016; Lohmann, 2017) while the importance of the study of some additives used in plastic manufacture is pointed out (Kwon et al., 2017). However, some controversy arises at the light of some other studies. Hartmann et al. indicated the need to improve the estimations of the role of MPs as vectors for hydrophobic organic compounds transfer into biota under environmental conditions (Hartmann et al., 2017). Bakir et al. (2014b) studied the desorption of organic contaminants such as phenanthrene, dichlorodiphenyltrichloroethane (DDT), diethylhexyl phthalate (DEHP) and perfluorooctanoic acid (PFOA) from polyethylene (PE) and polyvinyl chloride (PVC) under simulated physiological conditions. The results of that study proved that desorption was enhanced in the physiological medium and induced a hypothesis that the presence of selected pollutants adsorbed onto MPLs could enhance their bioavailability via marine organisms (Bakir et al., 2014b). But, in the case of PFOA, the environmental transport was shown to be negligible.

In addition, while the adsorption of highly hydrophobic compounds has been widely studied other groups of compounds with more hydrophilic properties have been much less considered as well as those with surfactant properties as PFASs. PFASs are a large group of industrial compounds with a plethora of uses. Moreover, currently, legacy PFASs, such as PFOA and perfluorooctanesulfonate (PFOS) have been substituted by shorter-chain compounds.

The main objectives of this work were to characterise the sorption capacity of 18 PFASs by three different types of microplastics (size between 3 and 12 μm) and to carry out the evaluation of a mixture of PFASs, as in real emissions. The selected MPLs were high-density polyethylene (HDPE), polystyrene (PS) and polystyrene carboxylic acid (PS-COOH) due to their high production and uses, low biodegradability in the environment and their increase as a litter in different water bodies (Kwon et al., 2014; PlasticsEurope, 2011). To assess the capabilities of these MPLs in the aquatic environment, the experimental setup was emulating realistic environmental conditions of freshwater and seawater ecosystems.

## 2. Materials and methods

### 2.1. Materials

The studied MPLs consisted of HDPE microspheres, ranging from 3 to 16 μm, which were supplied by Cospheric (Santa Barbara, California, USA); and 10 μm PS and PS-COOH, which were supplied by Phosphorex (Hopkinton, Massachusetts, USA).

A mixture of PFASs [MXB; > 98%] in methanol containing: i) perfluorobutanoic (PFBA), perfluoropentanoic (PFPeA), perfluorohexanoic (PFHxA), perfluoroheptanoic (PFHpA), perfluorooctanoic (PFOA), perfluorononanoic (PFNA), perfluorodecanoic (PFDA), perfluoroundecanoic (PFUdA), perfluorododecanoic (PFDoA), perfluorotridecanoic (PFTrA), perfluorotetradecanoic (PFTeA), perfluorohexadecanoic (PFHxDA) and perfluorooctadecanoic (PFODA) acids; and ii) perfluorobutanesulfonate (PFBS), perfluorohexasulfonate (PFHxS), perfluorooctanesulfonate (PFOS) and perfluorodecanesulfonate (PFDS), was mixed with the sulphonamide perfluorooctanesulphonamide (PFOSA), >99%. A mixture of labelled PFASs [MXA; > 98%] in methanol containing: [$^{13}C_4$]-perfluorobutanoic acid (MPFBA ($^{13}C_4$)), ion [$^{18}O_2$]-perfluorohexanesulfonate (MPFHxS ($^{18}O_2$)), [$^{13}C_2$]-Perfluorohexanoic acid (MPFHxA ($^{13}C_2$)), ion [$^{13}C_4$]-perfluorooctanesulfonate (MPFOS ($^{13}C_4$)), [$^{13}C_4$]-perfluorooctanoic acid (MPFOA ($^{13}C_4$)), [$^{13}C_5$]-perfluorononanoic acid (MPFNA ($^{13}C_5$)), [$^{13}C_2$]-perfluorododecanoic acid (MPFDoA ($^{13}C_2$)), [$^{13}C_2$]-perfluorodecanoic acid (MPFDA ($^{13}C_2$)), [$^{13}C_2$]-perfluoroundecanoic acid (MPFUdA ($^{13}C_2$)), was mixed with [$^{13}C_8$]-perfluorooctane sulphonamide (M8FOSA ($^{13}C_8$)), that was added before analysis, and they were used as internal standards in order to normalise the instrumental analysis results. All of the standards were supplied by *Wellington Laboratories Inc., Canada.* In Table S1 of the Supporting Information, the list of compounds and the carbon chain length of each one is presented.

Water and Methanol CHROMASOLV®Plus, for HPLC grade, ammonium acetate salt (AcNH$_4$: 77.08 g/mol; 98%) were provided by Sigma-Aldrich, Steinheim, Germany.

### 2.2. Freshwater and seawater sampling

The experiments were carried out using fortified real environmental waters at 10 μg/l with the 18 PFASs. For this purpose, in February 2016, 2.5 l of water from the Ebro River (40.976154,0.518484; NE of Spain) and 2.5 l of seawater from the Alfacs Bay (40.584421,0.579456; Ebro Delta, NE of Spain) were collected. The main physicochemical parameters including pH, dissolved oxygen, conductivity and total organic carbon (TOC) are shown in section S1 from supplementary material. Natural waters were transported to the laboratory under cool conditions. To assess the initial concentration of PFASs, 1 ml of each type of sample was directly analysed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS; according to the methodology described elsewhere (Llorca et al., 2014)). The samples were analysed in triplicates. The results showed that no PFASs were present at concentrations higher than limits of 10 ng/l.

### 2.3. Adsorption experiments

Adsorption experiments were conducted according to a batch method at room temperature (20 °C) using natural waters fortified with a mixture of PFASs. The experiments were designed as follows: 48 glass vials were filled with 10 ml of freshwater and a further set of 48 glass vials were filled with 10 ml of seawater. All of them were fortified with a mix of PFASs for a final concentration of 10 μg/l of each individual compound (180 μg/l ΣPFAS). This concentration was selected in consideration of background concentration of PFASs in the environment (Llorca et al., 2012). Then, 12 vials of each type of water were spiked with HDPE at 5 mg/l, PS at 2 mg/l and PS-COOH at the same concentration. Finally, the last 12 vials containing only PFASs were used as blanks in order to monitor the adsorption of PFASs onto the inner glass walls.

Then, the vials were settled in an orbital digester working at 120 rpm and 20 °C along the experiment period. In addition, the pH was continuously monitored, and the exposure to light/dark was carried out by simulating day and night hours.

The adsorption was assessed at 0, 4, 7 and 50 days. After each period, 3 blanks and 3 vials of each type were stopped. The vials were homogenised in a shaker, and a 0.1 ml aliquot was added to LC vials with inserts of 200 μl. Then, the aliquots were spiked with the mix of internal labelled standards at a final concentration of 10 μg/l of each compound. Finally, the samples were directly analysed by LC-MS/MS, as described elsewhere (Llorca et al., 2014).

The percentage of adsorption was calculated according to Equation (1):

$$\% Ads = 100 - \frac{[A]_t}{[A]_0} \times 100 \tag{1}$$

Where, % Ads the percentage of adsorption, $[A]_t$ is the concentration of compound A remaining in solution after time *t*, and $[A]_0$ is the concentration of compound A at time 0.

The partition/distribution coefficients ($K_d$) were calculated in agreement with Equation (2), based on OECD guideline 106 (OECD_106):

$$K_d = \frac{\%Ads_{eq.}}{100 - \%Ads_{eq.}} \cdot \frac{V_0}{m_{sorbent}} (ml/g) \tag{2}$$

Where $\%Ads_{eq}$ is the percentage of adsorption at equilibrium, $V_0$ is initial volume of the aqueous phase, and the $m_{sorbent}$ is the mass of the MPL. According to Equation (2), $K_d$ of each compound is directly proportional to their tendency to be adsorbed onto a type of MPL in a particular medium. In addition, Equation (2) assumes negligible loss of compounds.

### 2.4. Adsorption isotherm experiments

To assess the adsorption isotherms of PFASs onto HDPE, PS and PS-COOH, the range of concentrations of PFASs that were assayed was between 1 and 20 μg/l. The experimental design was the same as described above for adsorption experiments. In this case, the experiments were run for just 7 days, according to the results obtained during adsorption experiments and the apparent distribution was used for isotherms.

The experiments were carried out under the same conditions as described above.

### 2.5. Analysis by LC-MS/MS

The analysis of water samples was according to the method previously described by Llorca et al. (2014). Briefly, LC separation was achieved using a Thermo Scientific Aria TLX-1 system (Thermo Fisher Scientific, Franklin, MA, USA) equipped with a Hypersil GOLD™ PFP LC analytical column (50 × 3) (Thermo Scientific). Mobile phases were (A) aqueous ammonium acetate 20 mM and (B) methanol ammonium acetate 20 mM, used under gradient conditions. An extra column, BDS Hypersil C8 (50 × 3) (Thermo Scientific) was used after the LC pumps, and before the injection system, in order to delay the contamination from the system pumps. The total run time for each injection was 9 min with a flow rate of 0.4 ml/min. The injection volume was 20 μl.

The LC system was coupled to a Thermo Scientific Quantiva triple quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA), equipped with a Heated IonSpray source. All analyses were performed operating in the negative electrospray ionisation (ESI (-)) mode. The acquisition was performed in selected reaction monitoring mode (SRM) to obtain enough identification points (two transitions for each compound) according to current legislations (Commission_Decision, 2002/657/EC). Selected m/z for each compound can be seen elsewhere (Llorca et al., 2012).

## 3. Results

In Fig. 1, the main results of adsorption of PFASs onto surfaces of MPLs, expressed in percentage, are summarised, and Table S2, experimental results are quantitatively presented. In Table 1, the results of $K_d$ are summarised. The calculation of $K_d$ has been based on 7 days concentrations and according to the compounds that an adsorption percentage lower than 15% between 7 and 50 days.

### 3.1. Adsorption behaviour of PFAS onto HDPE

In general, low adsorption percentages onto HDPE, where maximum values were, in general, inferior to 50% with the exception of PFDoA, PFTrA, PFTeA and PFHxDA in fresh water. Notwithstanding, slow adsorption behaviour was exhibited, initiating after 4 days and reaching maximum values after 7 days. The main hypothesis is that adsorption kinetics between PFASs mixture and HDPE was affected by the granular shape of the surface of HDPE micro-particles (diameter size 3–16 μm), assuming that the sorption rate is limited by the rate of sorbate diffusion in the pores of sorbent (intraparticle diffusion model) (Plazinski et al., 2013) which, then, influences the adsorption rate constant ($k_2$; *Kg/(g min)*) obtained by the pseudo-second-order model, that drives the trend of their equilibrium time according to the following expression (Equation (3)).

$$\frac{dq_t}{dt} = k_2 \left(q_{eq} - q_t\right)^2 \tag{3}$$

This is in agreement with previous studies on the adsorption behaviour of individual PFASs as PFOS on different types of sorbents, being rapid in powdered sorbents with high surface area and slow in granular sorbents, such as in a cross-linked chitosan bead (Du et al., 2014).

The adsorption on HDPE can be explained as being a result of the combined effects that include hydrophobic and van der Waals forces, PFASs self-aggregations as micelles or hemimicelles, and competition between adsorption rates in natural organic material and on HDPE (Du et al., 2014). In addition, it must be considered that the influence of van del Waals forces is negligible because of the low polarisability and the molecular sizes of PFASs. As a result, low adsorption rates onto HDPE were obtained.

In freshwater, after 50 days, only longer-chain compounds PFTrA (C13), PFTeA (C14) and PFHxDA (C16) were adsorbed with rates superior to 50% (Fig. 1). This is in agreement with the increment of hydrophobic interactions for longer-chain compounds, which, have been already reported elsewhere (Milinovic et al., 2015, 2016). In PFAS adsorption there is an important influence of hydrophobic forces, particularly in the case of hydrophobic adsorbents such as HDPE (Du et al., 2014). As can be seen in Table 1, $K_d$ was in general directly proportional to the carbon chain length (Fig. S1). This is an indication that the aliphatic part of the molecules is a predominant factor in their partitioning, as has been informed on before for other groups of organic compounds, as well as such compounds as hydrocarbons (Sacks and Lohmann, 2011). In this case, the exception was PFODA (C18), for which maximum adsorption percentage was 37.4% after 50 days. But this compound, with the longest aliphatic chain of those studied here, presented an increasing adsorption after 4 days, a tendency that was maintained during the rest of the experiment. Since the critical micelle concentration (CMC)

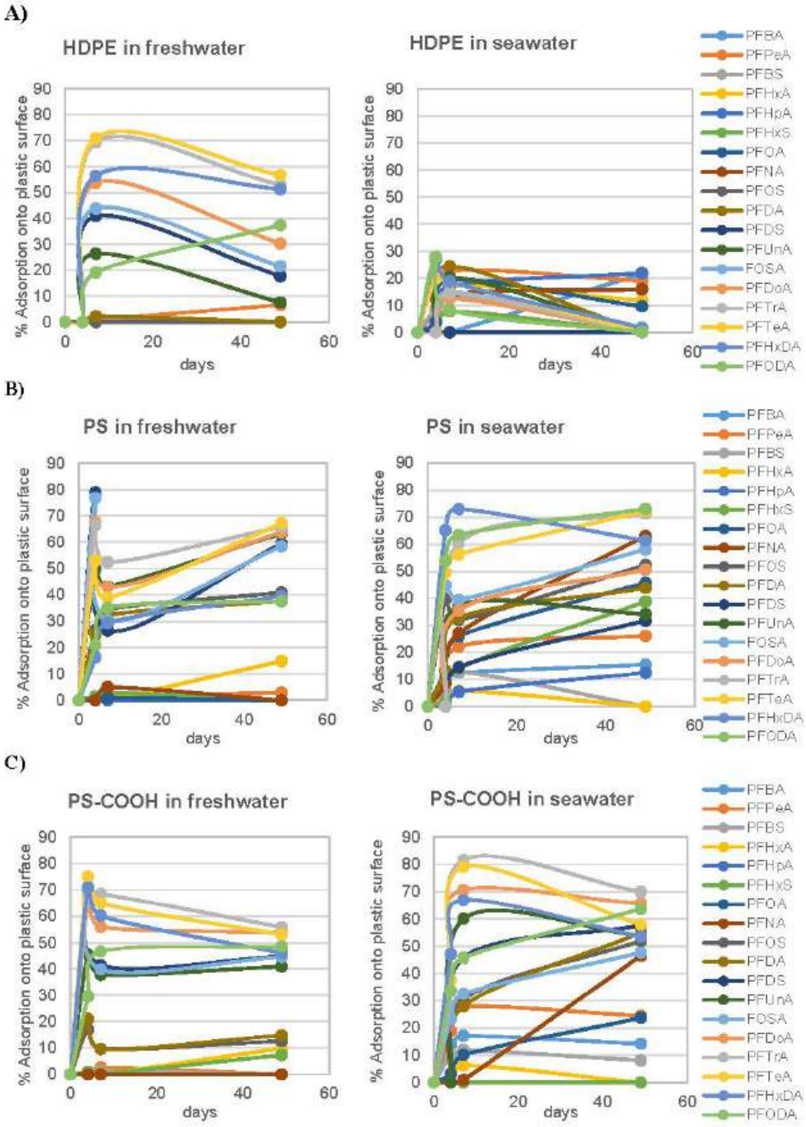

Fig. 1. Adsorption percentages of PFASs onto A) HDPE, B) PS and C) PS-COOH surfaces in freshwater (left column) and seawater (right column).

decreases with increasing chain length of the fluorosurfactant and is lower for the molecules with carboxylate groups than for molecules with sulphonates groups (Kissa, 2001), PFODA has a much lower CMC than the other PFASs in this study. We hypothesise that over the CMC, the apparent solubility of PFDA in water is initially increased, but also through micelle formation does the adsorption slowly continue increasing over time.

Most of the PFASs presented their maximum adsorption percentage after 7 days; and then, they started to be slowly desorbed. Carboxylated compounds with carbon chains between 4 and 10, presented low adsorption percentages, except PFPeA (C5). PFPeA, after 4 days until the end of the experiment, presented an increasing tendency to be adsorbed onto HDPE surface.

In seawater, salinity, natural organic material and pH were higher than in freshwater. When only considering the effect of salinity, in seawater it is expected that the solubility of PFASs will sharply decrease (Yu et al., 2008); thus, increasing their adsorption onto MPLs, as also happens with other hydrophobic contaminants (Lohmann, 2011; Sacks and Lohmann, 2011). However, on the contrary, in this study the adsorption rates of PFASs on HDPE in seawater were lower (Fig. 1 and Fig. S1). On the other hand, it has been proven that pH significantly influences the adsorption behaviour of PFAS, decreasing when the pH increases (Deng et al., 2010; Qu et al., 2009; Yu et al., 2009), as has been reported for other anionic contaminants (Du et al., 2014). In addition, natural organic matters can also play a major role on PFASs adsorption on HDPE. In fact, organic matter can be the main competitor of MPLs for PFASs adsorption (Du et al., 2014; Yang et al., 2013). Moreover, several studies (Deng et al., 2014; Hansen et al., 2010; Xiao et al., 2011) reported the competition between PFASs and other organic

**Table 1**
$K_d$ for PFASs after 7 days of exposure with the presence of HDPE, PS and PS-COOH in freshwater and seawater.

| MPLs | Functional group | Number carbon atoms | Acronyms | $K_d$ (ml/g) | |
|---|---|---|---|---|---|
| | | | | Freshwater | Seawater |
| HDPE | Carboxylic acids | 4 | PFBA | n.a | n.a |
| | | 5 | PFPeA | n.eq | 76.93 |
| | | 6 | PFHxA | n.a | 53.31 |
| | | 7 | PFHpA | n.a | 54.29 |
| | | 8 | PFOA | n.a | 64.05 |
| | | 9 | PFNA | n.a | 41.74 |
| | | 10 | PFDA | n.eq | n.eq |
| | | 11 | PFUnA | n.eq | n.eq |
| | | 12 | PFDoA | n.eq | n.eq |
| | | 13 | PFTrA | 595.91 | n.eq |
| | | 14 | PFTeA | 642.30 | n.eq |
| | | 16 | PFHxDA | 341.33 | n.eq |
| | | 18 | PFODA | n.eq | n.eq |
| | Sulphonates | 4 | PFBS | n.eq | n.eq |
| | | 6 | PFHxS | n.a | n.eq |
| | | 8 | PFOS | n.a | n.a |
| | | 10 | PFDS | n.eq | n.a |
| | Sulphonamide | 8 | FOSA | n.eq | n.eq |
| PS | Carboxylic acids | 4 | PFBA | 4.40 | 136.02 |
| | | 5 | PFPeA | 11.96 | 270.49 |
| | | 6 | PFHxA | n.eq | 57.60 |
| | | 7 | PFHpA | n.a | 53.87 |
| | | 8 | PFOA | 8.37 | n.eq |
| | | 9 | PFNA | n.eq | n.eq |
| | | 10 | PFDA | 455.14 | 466.41 |
| | | 11 | PFUnA | n.eq | 565.01 |
| | | 12 | PFDoA | n.eq | 524.84 |
| | | 13 | PFTrA | 1052.10 | 1451.90 |
| | | 14 | PFTeA | n.eq | 1223.48 |
| | | 16 | PFHxDA | 396.33 | n.eq |
| | | 18 | PFODA | 515.08 | 1657.85 |
| | Sulphonates | 4 | PFBS | 27.67 | 140.44 |
| | | 6 | PFHxS | 27.09 | n.eq |
| | | 8 | PFOS | 501.41 | n.eq |
| | | 10 | PFDS | n.eq | n.eq |
| | Sulphonamide | 8 | FOSA | n.eq | 619.29 |
| PS-COOH | Carboxylic acids | 4 | PFBA | 9.33 | 197.05 |
| | | 5 | PFPeA | 25.77 | 366.89 |
| | | 6 | PFHxA | n.a | 63.97 |
| | | 7 | PFHpA | n.a | n.a |
| | | 8 | PFOA | n.a | n.eq |
| | | 9 | PFNA | n.a | n.eq |
| | | 10 | PFDA | 100.20 | 370.89 |
| | | 11 | PFUnA | 578.00 | 1432.11 |
| | | 12 | PFDoA | 1208.95 | 2293.60 |
| | | 13 | PFTrA | 2071.60 | 4240.63 |
| | | 14 | PFTeA | 1773.70 | n.eq |
| | | 16 | PFHxDA | n.eq | 1931.16 |
| | | 18 | PFODA | 832.82 | 802.53 |
| | Sulphonates | 4 | PFBS | 12.50 | 128.33 |
| | | 6 | PFHxS | 2.21 | n.a |
| | | 8 | PFOS | 100.51 | n.eq |
| | | 10 | PFDS | 673.40 | 801.30 |
| | Sulphonamide | 8 | FOSA | 630.35 | 459.55 |

n.a: not applicable / n.eq.: the equilibrium was not reached between 7 and 50 days.

matter by the active sites of sorbent materials. Therefore, the combined effects of solution chemistry in seawater prominently reduce the adsorption of PFASs onto HDPE, as can be seen in Fig. 1, with percentages of adsorption below 30%. In general, maximum levels were achieved between 4 and 7 days, except for PFOS and PFDS that did not present any adsorption along the experiment, and PFNA and PFHpA, which were partitioning onto HDPE, continued increasing along the 50 days, until maximums values of around 23%. The compounds with carboxylic groups such as PFHxA (C6), PFHpA (C7), PFOA (C8) and PFNA (C9), after 7 days started to be progressively desorbed, but not totally. On the contrary, the rest of the compounds, corresponding to longer-chain and sulphonates presented a strong desorption behaviour after reaching the maximum and were totally desorbed at the end of the experiment. These facts are in agreement with the amphoteric behaviour of longer-chain compounds in a more conductive medium such as seawater (Yu et al., 2008).

For most of the compounds, the adsorption was lower than 25% and at the end of the experiment only the carboxylic acids PFBA (C4), PFPeA (C5), PFHxA (C6), PFHpA (C7), PFOA (C8) and PFNA (C9), presented adsorption rates between 10 and 21%. The carboxylic acids PFDA (C10), PFUnA (C11), PFDoA (C12), PFTrA (C13), PFTeA (C14), PFHxDA (C16) and PFODA (C18) and the sulphates PFBS (C4), PFHxS (C6) and FOSA (C8), after reaching the maximum adsorption at 7 days were totally desorbed at the end of the experiment (50 days).

Comparing the different functional groups, carboxylic acids presented a higher tendency to be sorbed than sulphonates (PFDA vs. PFDS and PFOA vs. PFOS) (Fig. 2) and sulphonamides (PFOA vs. FOSA). The different behaviour among functional groups of the same carbon chain in freshwaters and seawaters could be attributed not only to the different ionic strength of water but also to the pH. For example, in freshwaters, the equilibrium was reached after longer experiment times (if the amount adsorbed does not change after 50 days), but the $K_d$ at the assumed equilibrium for most of the compounds (7 days) were higher than in seawater for compounds with more than 10 carbons. On the other hand, compounds with less than 10 carbons showed more affinity for HDPE in seawater than in fresh water. In this latter case, the high ionic strength of water increased the hydrophobicity of the molecules from C4 to C10 (Fig. 3).

Comparing our results for PFASs with those from the body of literature, just one study reporting the adsorption for PFOA onto HDPE was found (Bakir et al., 2014b). The authors used similar concentrations of MPLs, but just PFOA was studied individually and at much lower concentration. This could explain why we observed one order of magnitude lower for PFOA $K_d$ in seawater than in the study by Bakir et al. (2014b).

### 3.2. Adsorption behaviour of PFAS onto PS

As can be seen in Fig. 1B, the experiments onto PS in freshwater showed, in general, slightly higher rates of adsorption than in HDPE, especially at 50 days. In addition, for most of the compounds the adsorption onto PS was faster than on HDPE. Again, adsorption kinetics is affected by the adsorbent particle size, which is smaller in the case of PS than in HDPE. Therefore, a higher speed of adsorption was shown. The longer carbon-chain acidic compounds PFUdA (C11), PFDoA (C12), PFTrA (C13) and PFTeA (C14) presented the higher adsorption percentages after 50 days. The rates of adsorption of these compounds were relatively increasing, with the carbon chain of PFTeA in the most adsorbed with 67% of adsorption on PS. However, for the longest compounds PFHxDA (C16) and PFODA (C18) this tendency was not maintained again because of the equilibrium between micelles formation and adsorption, presenting adsorption percentages of around 38%. The PFASs with carboxylic groups and carbon chains between C4 and C9 were almost not adsorbed onto PS showing a similar pattern as onto HDPE (Fig. 1). Only perfluorohexanoic acid (C6) presented a certain adsorption after 50 days, reaching about 15% of adsorption. These results agree with $K_d$ values for C16 and C18 which are lower than those for C13. In general, compounds with longer carbon chain exhibit higher $K_d$ values (Table 1) and, hence, major adsorption onto PS surface (Fig. S2). Finally, comparing same carbon chains with different functional groups, carboxylic acids have a lower tendency to be sorbed onto PS than sulphonates (PFDA vs. PFDS and PFOA vs. PFOS (Table 1 and Fig. 2); while the PFASs with the sulphonamide group are more favoured for adsorption onto PS than those with carboxylate or sulphonate groups (PFOA and PFOS vs. FOSA (Table 1)).

In seawater, longer chain compounds C13-C18 were those exhibiting higher adsorption after 50 days. In this case, salting-out increased the hydrophobicity of longer-chain compounds, and the aromatic rings of PS monomers helped to stabilise the adsorption between PS and PFASs in the face of competition with auto-aggregation by micelles formation and the organic matter.

Under % of adsorption, sulphonates behave quite similarly in freshwater. Sulphonamide showed more affinity for PS in freshwater than in seawater after 4 days of experiments (Fig. 1). Finally, the adsorption capacity of C8 and C10 with different functional groups showed that carboxylic acids were more readable for adsorption than sulphonates (Fig. 2).

### 3.3. Adsorption behaviour of PFAS onto PS-COOH

In freshwater, the adsorption behaviour of PFASs onto PS-COOH was similar to that onto PS, but slightly lower. Long-chain acidic PFASs between C11 and C18 were the most adsorbed compounds. Once again, the higher chain compounds PFODA and PFHxDA were slightly affected by micelles formation. All of these compounds, after 50 days, presented adsorption percentages of between 48 and 68%. From C11 to C14, as can be seen in Fig. S3 of the Supporting Information, $K_d$ increases with the long-chain (Table 1) and the percentage of non-polar atoms in the molecules. However, C16 and C18 did not follow this pattern. In addition, the percentage of moles sorbed onto PS-COOH did not change over time. FOSA and PFDS presented adsorption rates of around 45% with similar $K_d$ (Table 1). While the rest of compounds (PFDA, PFOS, PFHxS, PFHxA, PFNA, PFOA, PFHpA, PFBS, PFPeA and PFBA) were adsorbed in percentages that were inferior to 20%. In the case of sulphonates, adsorption capacity onto plastic surface was increased with the increase of aliphatic chain from C6 to C10 (Table 1). Comparing the same carbon chain with different functional groups, carboxylic acids were less sorbed onto the plastic surface (PFDA vs. PFDS and PFOA vs. PFOS) (Fig. 2), while sulphonamide functional group is more readable as being sorbed than sulphonates (FOSA vs. PFOS) (Table 1). This pattern was detected for all tested plastics during this work.

In seawater, the pH and the presence of carboxylic groups in the adsorbent increase the adsorption rates of carboxylic acids. PFASs carboxylates with carbon chains between C9 and C18 presented adsorption rates between 45 and 70% after 50 days. In general, $K_d$ increments were directly proportional to carbon chain except for C6, C16 and C18 (Table 1). PFPeA behaves differently because this compound had higher $K_d$ than other shorter-carbon chain compounds. However, again the high ionic strength increments the hydrophobic character of shorter molecules, increasing their affinity for MPLs. Finally, comparing the different functional groups, carboxylic acids have less tendency for the adsorption onto plastic surface (PFDA vs. PFDS and PFOA vs. PFOS) (Fig. 2), and sulphonates and sulphonamides have similar capacities for adsorption (FOSA vs. PFOS).

Comparing the results in freshwater and seawater, the main difference is the higher adsorption of shorter-carbon chain carboxylates (Fig. 3) in seawater due to the increase in ionic strength, as observed by Sacks et al. (Sacks and Lohmann, 2011). Finally, a similar pattern was observed for sulphonates while FOSA behaved comparably in both matrix types.

### 3.4. Adsorption isotherms

Adsorption isotherms of common PFASs, such as PFOS, PFOA, PFBA, PFBS, PFHxA and PFHxS, in different types of materials such as granular activated carbons, anionic-exchange and non-ionic resins, minerals and biomaterials have been studied before (Andersen et al., 2014; Cao et al., 2016; Chen et al., 2009, 2011, 2012, 2016; Conte et al., 2015; Du et al., 2014; Li et al., 2011; Zhang et al., 2016). In these works, the individual behaviour of the compounds was considered, and the adsorption isotherms have usually been described by linear, Langmuir, and Freundlich models (see section S3).

The isotherms with concentrations of PFASs onto plastic surface vs. concentrations of PFASs in the aqueous phase are graphically represented in Fig. S4. Also, Fig. 4 shows the adsorption isotherms for the experimental and theoretical results of the total sum of PFASs, the sum of acids, and the sum of sulphonates. The results

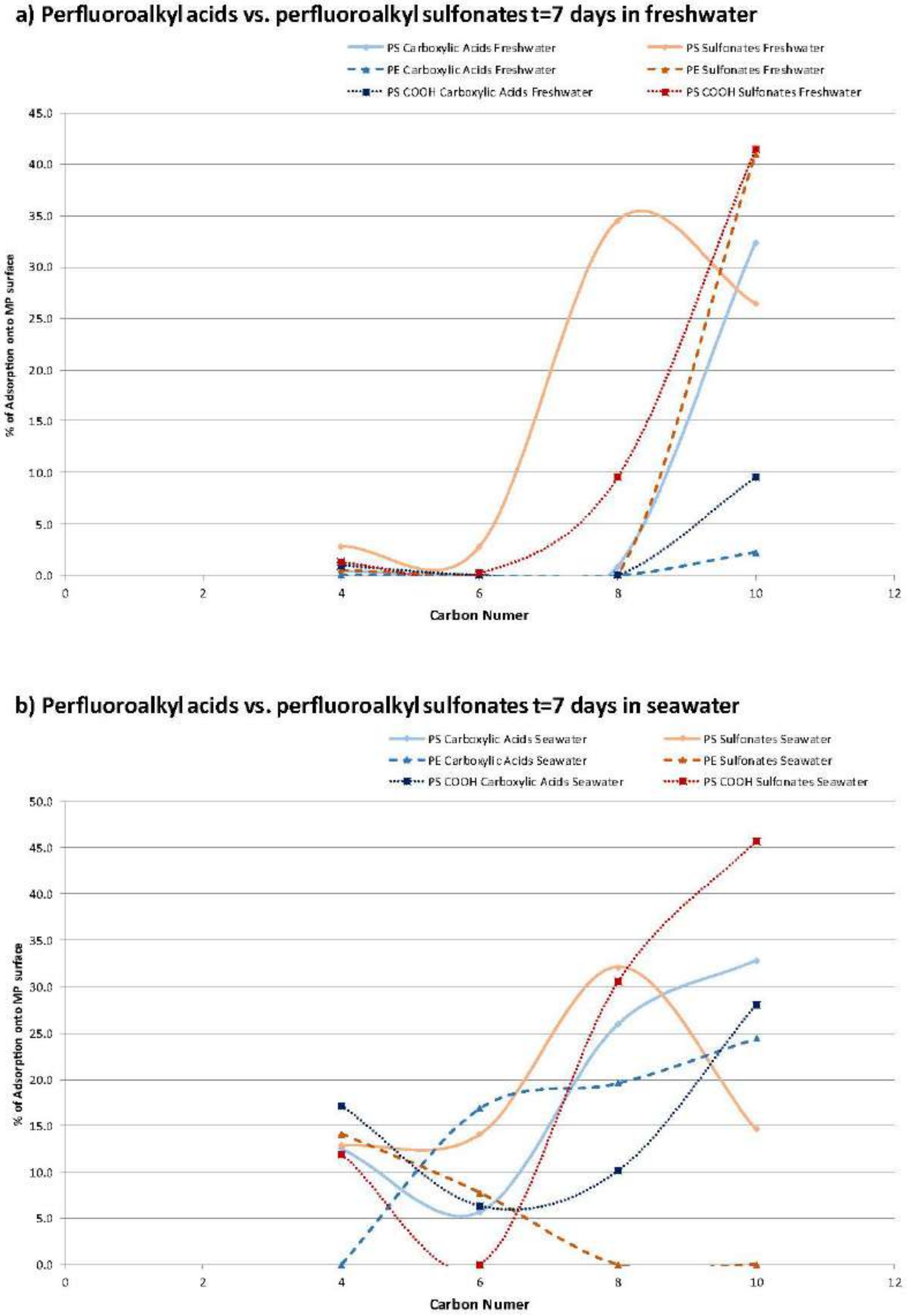

**Fig. 2.** Sorption percentage of perfluoroalkyl acids [PFBA (C4), PFHxA (C6), PFOA (C8) and PFDA (C10)] and perlfuoroalkyl sulphonates [PFBS (C4), PFHxS (C6), PFOS (C8) and PFDS (C10)] onto MPLs surface after 7 days in a) freshwater and b) seawater.

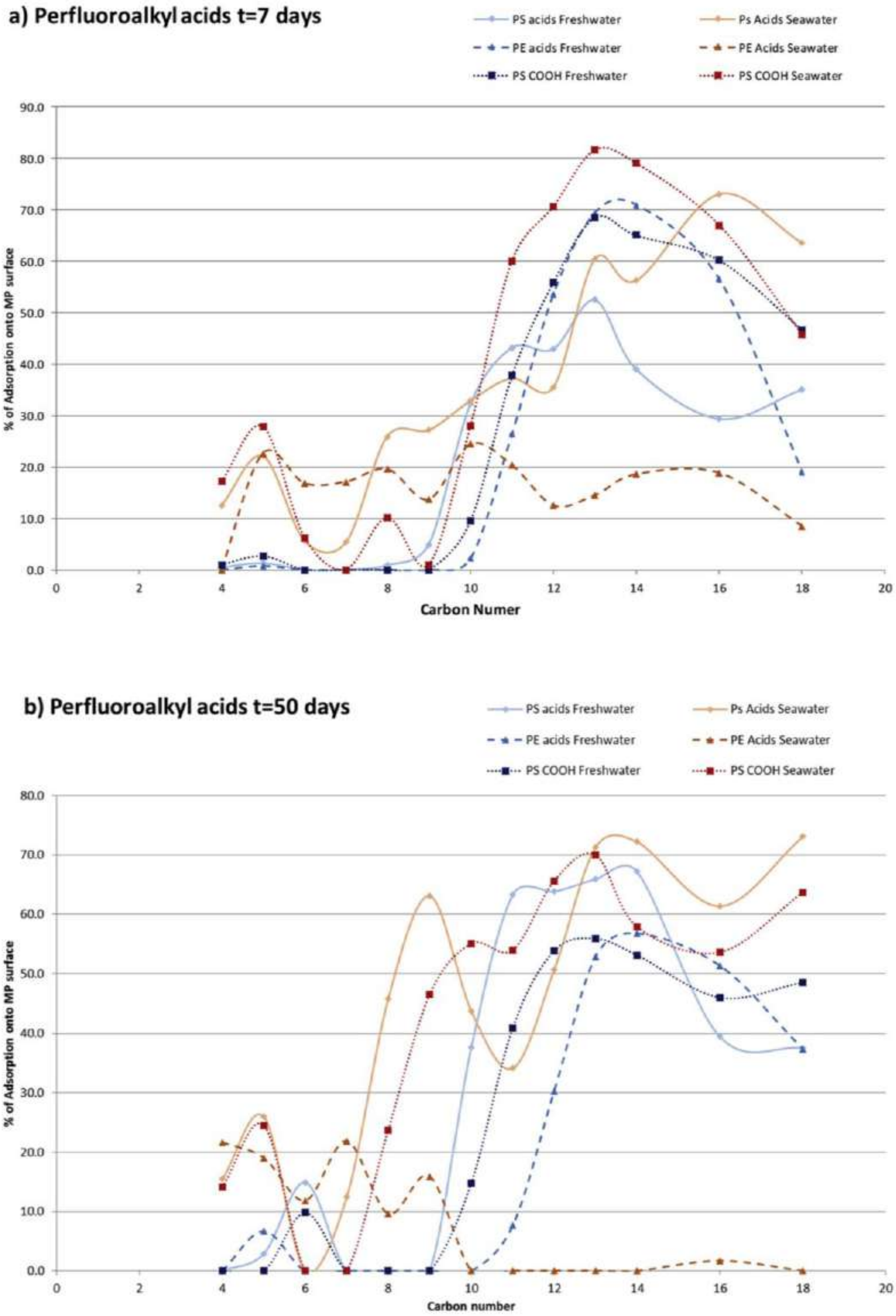

**Fig. 3.** Adsorption percentage of perfluoroalkyl acids onto MPLs surface after a) 7 days and b) 50 days of contact in seawater and freshwater.

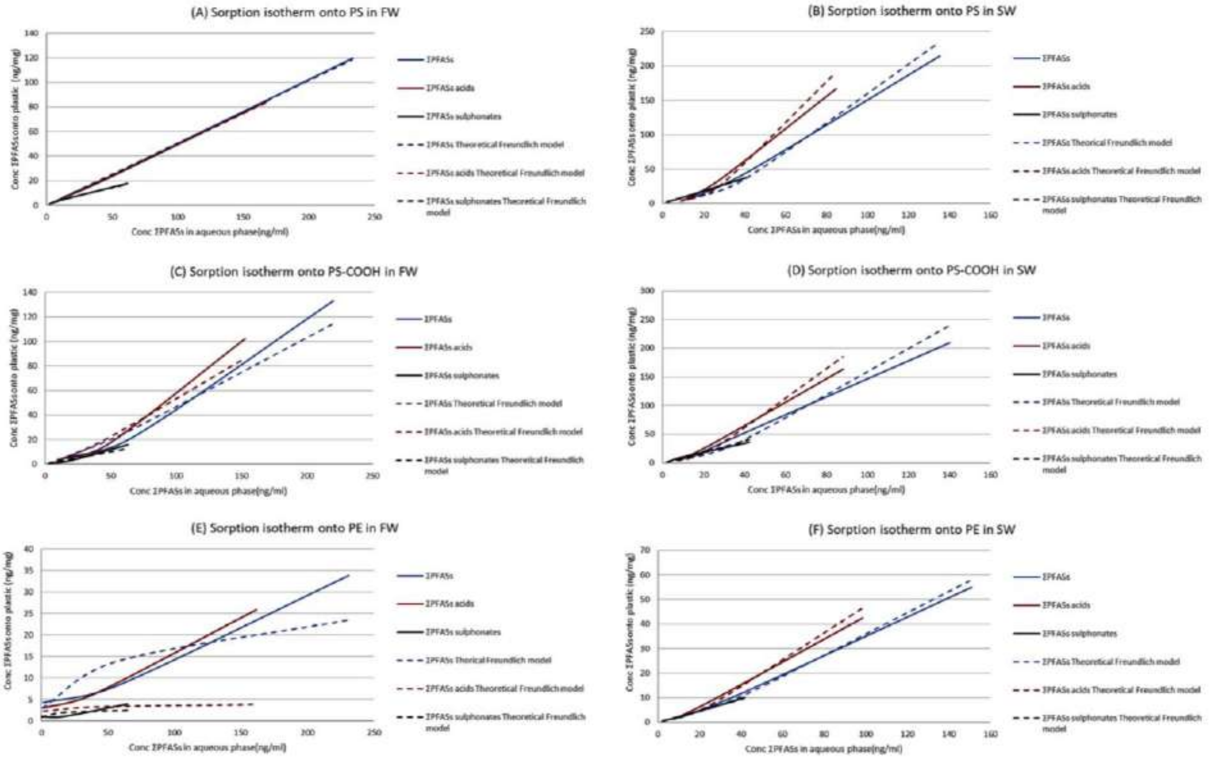

**Fig. 4.** Adsorption isotherms for experimental results of ΣPFASs, ΣPFASs acids and ΣPFASs sulphonates (solid lines); Theoretical Freundlich model fitting for ΣPFASs, ΣPFASs acids and ΣPFASs sulphonates (pecked lines). Experiments with (A) PS in freshwater; (B) PS in seawater; (C) PS-COOH in freshwater; (D) PS-COOH in seawater; (E) PE in freshwater; and (F) PE in seawater.

show that most of the compounds followed the Freundlich equation. At this point, Freundlich equation (see section S3) was linearized in its logarithmic form (Equation (4)) to obtain the Freundlich constants and for the convenience of data fitting (Zhaoyi et al., 1997).

$$\log q = \log K_F + \frac{1}{n}\log C \tag{4}$$

Where "$K_F$" is an indicator of adsorption capacity (Zhaoyi et al., 1997). The higher the value of "$K_F$", the higher is the adsorption capacity (Zhaoyi et al., 1997). The $n$ value can range from 0 to 10, between 2 and 10 indicates good, between 1 and 2 indicates moderate, and less than 1 indicates poor adsorption capacity (Subramanyam and Das, 2009; Treybal, 1981). In Table 2, the compounds fitting into the Freundlich model and the corresponding parameters are summarised. The isotherms have been built assuming that all the compounds reached the equilibrium after 7 days of experimental time.

As can be seen in Table 2, according to the results obtained for $K_F$, the adsorption tendency of PFASs onto MPLs is mainly favoured by major salinity because of hydrophobic forces and salting-out phenomenon (Lohmann, 2011; Sacks and Lohmann, 2011). One exception was FOSA, in agreement with previous results reported by Wang (2013). The authors observed that the adsorption of PFOS onto PE, PS and PVC was higher with the increase of salinity, while not in the case of FOSA, as in the present study (Table 2). In addition, a highly linear adsorption of both compounds onto HDPE and PS surfaces (wang, 2013) was informed upon. In the present study, PFOS presented a moderate adsorption ($1 < n < 2$) onto the three types of MPLs tested in seawater. In freshwater, poor adsorption was obtained onto PS and PS-COOH; and, the PFOS adsorption onto HDPE did not fit with the Freundlich model. In general, in the present study, PFASs followed the Freundlich model. In the case of freshwater, the sorption capacity, for the compounds with carbon chains between 4 and 9 units is poorly favoured ($n_F < 1$) for all MPLs studied. In addition, for the particular case of PS-COOH, $n_F$ was also <1 for the compounds between carbon chains 12–14. In general, higher adsorption capacity was obtained in the range of carbon chains between 10 and 14, because the aliphatic moiety favoured the hydrophobicity and the adsorption. However, for the longer-chain compounds the surfactant character of the molecule is the dominant trend, and then the adsorption capacity is also reduced. In addition, these experiments revealed that the mixture of surfactants in the real world are probably less adsorbed, as can be expected from their individual adsorption capacities.

The Freundlich isotherms of the total PFASs mixture, acidic compounds and sulphonates are shown in Fig. 4 and the calculated parameters are summarised in Table 3. In general, good fitting was obtained for the different combinations with the Freundlich model. However, as aforementioned, poor affinity was observed. The only deviation to this model was observed in the case of HDPE in freshwater (Fig. 4E).

### 3.5. *Implications for predicting environmental behaviour*

The results of this study show that mixtures of PFASs can be sorbed onto HDPE, PS and PS-COOH surface. Adsorption kinetics

**Table 2**
Freundlich model parameters calculated for PFASs fitting linearized equation with $R^2 > 0.75$. (n.a: not applicable).

| | $R^2$ | log $K_F$ | $K_F$ (ng/mg) | $n_F$ | $R^2$ | log $K_F$ | $K_F$ (ng/mg) | $n_F$ |
|---|---|---|---|---|---|---|---|---|
| | PS freshwater | | | | PS seawater | | | |
| **PFBA** | 0.9994 | −2.2516 | 0.01 | 0.393 | 0.9609 | −0.4667 | 0.34 | 0.512 |
| **PFPeA** | 1 | −1.0377 | 0.09 | 0.669 | 0.9962 | −0.9378 | 0.12 | 0.566 |
| **PFBS** | n.a | | | | 0.8091 | −0.6939 | 0.20 | 1.17 |
| **PFHxA** | 0.9952 | −0.6855 | 0.21 | 0.809 | 0.9981 | −0.8092 | 0.16 | 0.691 |
| **PFHpA** | 0.9987 | −1.1825 | 0.07 | 0.599 | 0.9898 | −1.01 | 0.10 | 0.693 |
| **PFHxS** | 0.9979 | −0.7383 | 0.18 | 0.720 | 0.9990 | −0.2913 | 0.51 | 0.965 |
| **PFOA** | 0.9277 | −0.5031 | 0.31 | 0.00008 | 0.9982 | −0.5512 | 0.28 | 0.854 |
| **PFNA** | 0.9913 | −0.6574 | 0.22 | 0.765 | 0.9991 | −0.0494 | 0.89 | 0.999 |
| **PFOS** | 0.9992 | −0.208 | 0.62 | 1.56 | 0.9987 | 0.3904 | 2.5 | 1.74 |
| **PFDA** | 0.9721 | −0.1717 | 0.67 | 1.53 | 0.9703 | 0.3038 | 2.0 | 0.889 |
| **PFDS** | 0.9894 | 0.1453 | 1.4 | 1.61 | 0.9698 | 0.4957 | 3.1 | 0.349 |
| **PFUnA** | 0.9939 | 0.4837 | 3.1 | 1.36 | 0.8121 | 0.7216 | 5.3 | 0.335 |
| **FOSA** | 0.9813 | 0.3746 | 2.4 | 0.431 | 0.9999 | −0.3521 | 0.44 | 0.740 |
| **PFDoA** | 0.9997 | 0.2229 | 1.7 | 1.11 | 0.9133 | 1.0339 | 11 | 0.0824 |
| **PFTrA** | 0.9979 | 0.5792 | 3.8 | 1.02 | 0.9974 | 5.3359 | 216721 | 0.0233 |
| **PFTeA** | 0.9913 | 0.2128 | 1.6 | 1.48 | 0.4617 | | | |
| **PFHxDA** | n.a | | | | 0.4676 | | | |
| **PFODA** | n.a | | | | 0.1176 | | | |
| | **PSCOOH freshwater** | | | | PSCOOH seawater | | | |
| **PFBA** | 0.3443 | | | | 0.9946 | −0.1543 | 0.70 | 0.622 |
| **PFPeA** | 0.9904 | −1.1744 | 0.07 | 0.674 | 0.9995 | −0.7518 | 0.18 | 0.644 |
| **PFBS** | 0.7906 | −1.312 | 0.05 | 0.830 | 0.9529 | −0.4795 | 0.33 | 1.16 |
| **PFHxA** | 0.9911 | −0.6324 | 0.23 | 0.893 | 0.9909 | −0.8081 | 0.16 | 0.721 |
| **PFHpA** | 0.9284 | −0.7042 | 0.20 | 0.984 | 0.9504 | −0.7441 | 0.18 | 0.939 |
| **PFHxS** | 1 | −0.802 | 0.16 | 0.705 | 0.9777 | −0.5471 | 0.28 | 0.757 |
| **PFOA** | 0.9954 | −0.7505 | 0.18 | 0.711 | 0.9597 | −0.415 | 0.38 | 0.948 |
| **PFNA** | 0.982 | −1.0172 | 0.10 | 0.601 | 0.9305 | −0.0742 | 0.84 | 0.996 |
| **PFOS** | 0.8608 | −0.7826 | 0.16 | 1.10 | 0.9877 | 0.2065 | 1.6 | 1.37 |
| **PFDA** | 0.9911 | −0.456 | 0.35 | 1.37 | 0.9314 | 0.2589 | 1.8 | 0.874 |
| **PFDS** | 0.8556 | −0.2141 | 0.61 | 1.28 | 0.9985 | 0.4384 | 2.7 | 0.297 |
| **PFUnA** | 0.9445 | 0.2331 | 1.7 | 1.18 | 0.9217 | 0.6874 | 4.9 | 0.164 |
| **FOSA** | 1 | 0.2521 | 1.8 | 0.664 | 0.9074 | −0.6056 | 0.25 | 0.572 |
| **PFDoA** | 0.9494 | −0.1533 | 0.70 | 0.791 | 0.8981 | 1.1566 | 14 | 0.201 |
| **PFTrA** | 0.8483 | 0.6136 | 4.1 | 0.565 | 0.9915 | 2.0505 | 112 | 0.120 |
| **PFTeA** | 0.9119 | 0.4637 | 2.9 | 0.442 | 0.7484 | | | |
| **PFHxDA** | 0.6560 | | | | 0.9654 | −0.8693 | 0.14 | 0.263 |
| **PFODA** | 0.9727 | −0.6391 | 0.23 | 0.903 | 0.2478 | | | |
| | **HDPE freshwater** | | | | HDPE seawater | | | |
| **PFBA** | 0.1767 | | | | 0.9881 | −0.5926 | 0.26 | 0.701 |
| **PFPeA** | 0.434 | | | | 0.9801 | −1.3355 | 0.05 | 0.587 |
| **PFBS** | 0.0467 | | | | 0.9846 | −1.3076 | 0.05 | 1.05 |
| **PFHxA** | 0.4113 | | | | 0.9971 | −1.3428 | 0.05 | 0.740 |
| **PFHpA** | 0.2883 | | | | 0.9882 | −1.2984 | 0.05 | 0.916 |
| **PFHxS** | 0.4262 | | | | 1 | −1.0433 | 0.09 | 0.840 |
| **PFOA** | 0.5885 | | | | 0.9988 | −0.956 | 0.11 | 0.929 |
| **PFNA** | 0.4924 | | | | 0.9946 | −0.5589 | 0.28 | 1.14 |
| **PFOS** | 0.1153 | | | | 0.9886 | −0.2844 | 0.52 | 1.55 |
| **PFDA** | 0.4806 | | | | 1 | −0.2443 | 0.57 | 1.05 |
| **PFDS** | 0.9737 | −0.3448 | 0.45 | 2.64 | 0.9971 | 0.1342 | 1.4 | 0.474 |
| **PFUnA** | 0.9920 | −0.0661 | 0.86 | 1.89 | 0.9282 | 0.2704 | 1.9 | 0.424 |
| **FOSA** | 0.8543 | −0.3697 | 0.43 | 0.654 | 0.9812 | −1.034 | 0.09 | 0.757 |
| **PFDoA** | 0.9999 | −0.1825 | 0.66 | 1.23 | 0.8774 | 0.4841 | 3.1 | 0.231 |
| **PFTrA** | 0.4075 | | | | 0.3987 | | | |
| **PFTeA** | 0.9507 | 0.4259 | 2.7 | 1.67 | 0.9621 | −0.8824 | 0.13 | 0.206 |
| **PFHxDA** | 0.0072 | | | | 0.9433 | −0.9339 | 0.12 | 0.668 |
| **PFODA** | 0.7380 | | | | 0.8156 | −0.096 | 0.80 | 0.888 |

was mainly influenced by particle size of MPLs being higher for smaller diameters. This fact indicates that aged MPLs facilitate adsorption of PFASs, and at the same time, the small size facilitates their ingestion by the aquatic biota, facilitating the transfer of PFASs to the aquatic food chain. Nonetheless, it is important to study the fugacity gradient of MPLs with sorbed PFASs between water and aquatic food chain since this is the main driving force of transfer. In addition, adsorption kinetics was favoured in seawater in contrast to freshwater. However, in general, the adsorption amount was higher in freshwater than in seawater. The adsorption capacity of PFASs onto the studied MPLs is also influenced by pH, which in general is decreasing when the pH increases, as happens in seawater. However, this effect can be buffered by the presence of divalent cations such as $Ca^{2+}$ and $Mg^{2+}$ through a divalent cation bridging effect.

The presence of organic material is in accordance with the competence of MPLs to adsorb PFASs. However, while bigger particulate organic material tends to be deposited at the bottom of aquatic environments, low-density MPLs are more likely to be in the water column facilitating their ingestion by biota as well as

**Table 3**
Freundlich model parameters calculated for ΣPFASs, ΣPFASs acids and ΣPFASs sulphonates fitting linearized equation with $R^2 > 0.75$.

| | $R^2$ | log $K_F$ | $K_F$ (ng/mg) | $n_F$ | $R^2$ | log $K_F$ | $K_F$ (ng/mg) | $n_F$ |
|---|---|---|---|---|---|---|---|---|
| | PS freshwater | | | | PS seawater | | | |
| **ΣPFASs** | 0.9998 | −0.3095 | 0.49 | 0.994 | 0.9924 | −0.9305 | 0.12 | 0.645 |
| **ΣPFASs acids** | 0.9998 | −0.3749 | 0.42 | 0.969 | 0.9843 | −1.2109 | 0.06 | 0.552 |
| **ΣPFASs sulphonates** | 0.9975 | −0.1570 | 0.70 | 1.29 | 0.9998 | −0.0770 | 0.84 | 0.980 |
| | **PSCOOH freshwater** | | | | **PSCOOH seawater** | | | |
| **ΣPFASs** | 0.9813 | −0.6786 | 0.21 | 0.856 | 0.9807 | −0.7177 | 0.19 | 0.693 |
| **ΣPFASs acids** | 0.9791 | −0.6964 | 0.20 | 0.829 | 0.9841 | −0.7594 | 0.17 | 0.642 |
| **ΣPFASs sulphonates** | 0.9488 | −0.6689 | 0.21 | 1.01 | 0.9818 | −0.2216 | 0.60 | 0.888 |
| | **HDPE freshwater** | | | | **HDPE seawater** | | | |
| **ΣPFASs** | 0.8123 | 0.4797 | 3.02 | 2.66 | 0.9973 | −1.033 | 0.09 | 0.779 |
| **ΣPFASs acids** | 0.8457 | 0.3396 | 2.19 | 2.39 | 0.9920 | −1.0736 | 0.08 | 0.727 |
| **ΣPFASs sulphonates** | 0.4534 | | | | 0.9996 | −0.6717 | 0.21 | 0.974 |

smaller organic matter or dissolved organic matter that also play an important role in bioavailability of hydrophobic organic chemicals. Hydrophobic interaction seems to be an important mechanism in adsorption of PFASs, increasing the tendency to be adsorbed by longer-chain compounds. However, for the longer-chain compounds, if the CMC is overcome then this changes this behaviour drastically.

The adsorption capacity of MPLs for sulphonates and sulphonamide compounds was, in general, shown to be higher than for compounds with carboxylic groups. In general, plastic debris made of, or containing PS and PS-COOH, presented higher adsorption capacity and also presented an affinity for a large variety of PFASs than in the case of HDPE. The adsorption affinity of MPLs for the PFASs of this study was shown to be lower than for other non-polar organic compounds (Hüffer and Hofmann, 2016; Lohmann, 2011; Rochman et al., 2013; Sacks and Lohmann, 2011). In particular, this fact is due to the formation of micelles and the ionic character of PFASs. However, in most of the experiments after 50 days, for most of the compounds, the percentages of adsorption were superior to 40%.

## 4. Conclusions

It can be concluded that MPLs of HDPE, PS and PS-COOH can adsorb and stabilise residues of PFASs that are present in the surrounding waters. For the same materials, aged MPLs or/and smaller particles present higher adsorption capacities. In general, the adsorption capacity of MPLs studied here were PS > PS-COOH > HDPE if we consider individual PFASs. Considering adsorption mechanisms, hydrophobic interactions increase the adsorption affinity for longer-chain compounds while the CMC is not overcome. Electrostatic interactions are affected by the solution pH, which can change the surface charge of some MPLs. However, in general the effect of hydrophobic interaction and salting out is a major influence.

In general, the adsorption isotherm followed by PFASs is based on Freundlich equation in most of the cases. However, it is noteworthy that the behaviour of the compounds that did not follow the Freundlich isotherm could not be adjusted to a linear or a Langmuir isotherm.

Finally, due to the sorption capacity of MPLs for PFASs, these should be considered as an extra transport vector for these contaminants in the environment once are sorbed onto MPL surface'. However, more in deep studies are necessary to establish the fugacity factor for MPLs with sorbed PFASs since this is the main parameter that regulates their transferability among environmental compartments.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2017.12.075.

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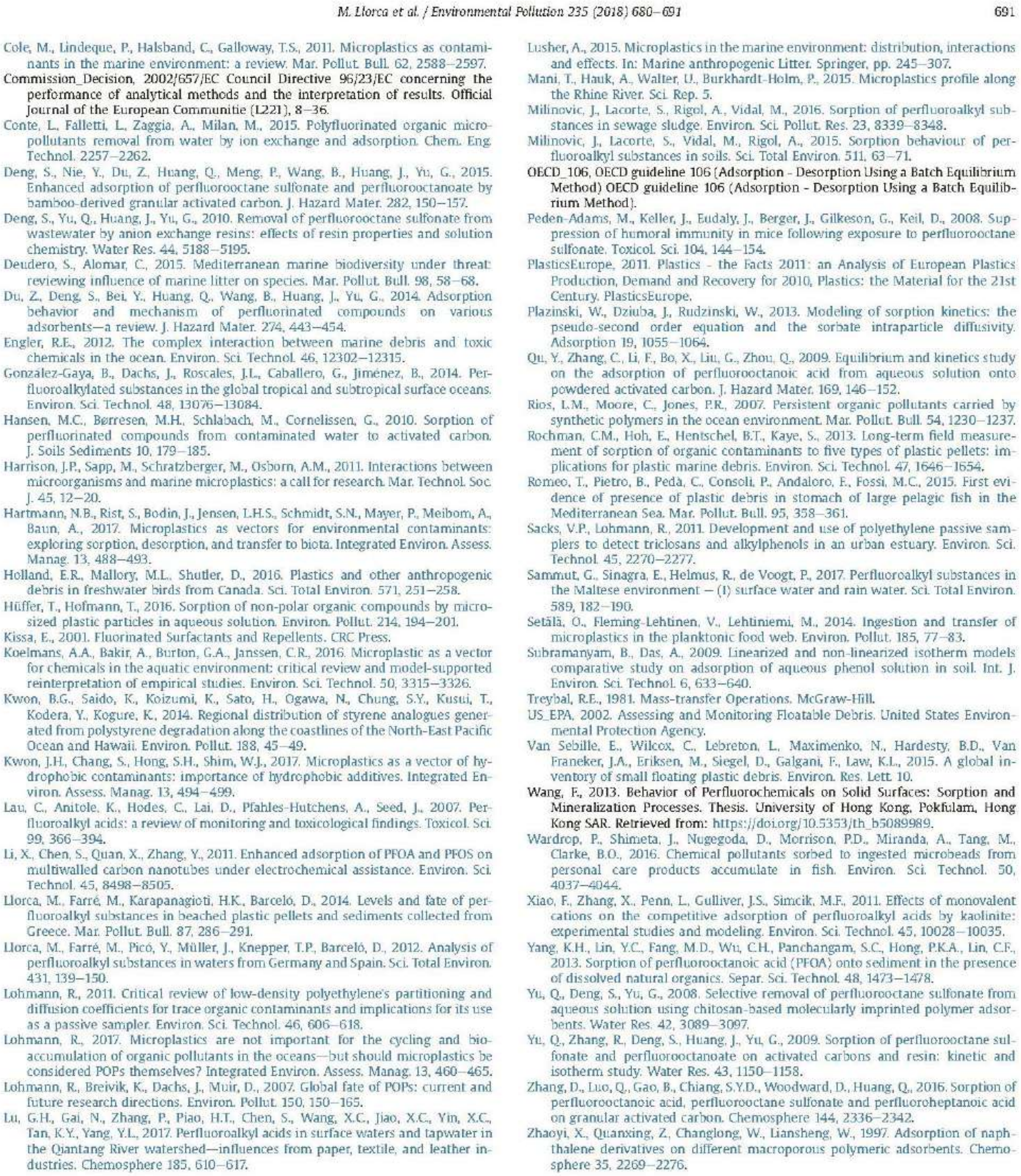

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# Supporting Information

## SORPTION OF PERFLUOROALKYL SUBSTANCES ON MICROPLASTICS UNDER ENVIRONMENTAL CONDITIONS

Marta Llorca[1,*], Gabriella Schirinzi[1], Mònica Martínez[1,2], Damià Barceló[1,3], Marinella Farré[1]

[1] Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain

[2] Department of Materials Science and Physical Chemistry. Faculty of Chemistry. Martí i Franquès 1, 08028 Barcelona, Spain

[3] Catalan Institute for Water Research (ICRA), H2O Building, Scientific and Technological Park of the University of Girona, Emili Grahit 101, 17003 Girona, Spain

*Corresponding author

**S1** Physicochemical parameters of freshwater and seawater.

| | pH | $O_2$ (mg/L) | Conductivity (µS/cm) | TOC |
|---|---|---|---|---|
| Ebro river (freshwater) | 7.78 | 8.99 | 1362 | 4.386 |
| Alfacs Bay (seawater) | 7.70 | 7 | 45332 | 7.735 |

$O_2$: dissolved oxigen
TOC: total organic carbon

**S2** Calculation of the concentration of PFASs adsorbed onto plastic surfaces expressed in pmoles according to Equation S1:

$$\text{Conc PFAS}_i\ [\text{pmoles}] = \text{weight of MP [mg]} \times \text{Conc PFAS}_i\ [\text{ng/mg}] \times \frac{1000\ \text{pmole}/1\ \text{nmole}}{\text{MW [ng/nmole]}}$$

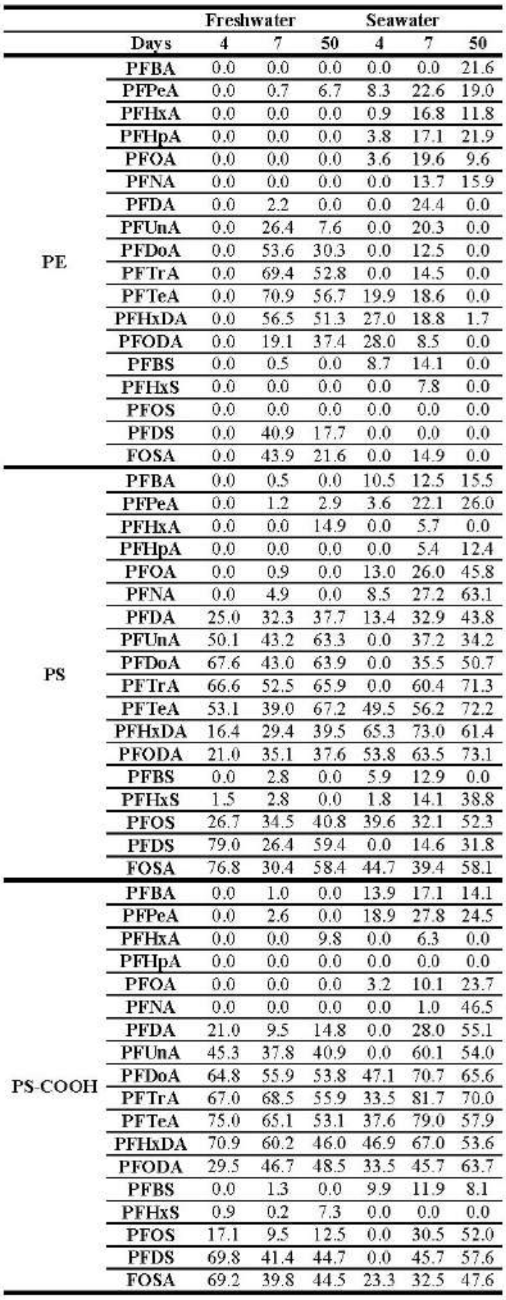
(**Equation S1**)

Where Conc $PFAS_i$ indicates the concentration of compound "i", and MW indicates the molecular weight of compound "i" expressed in ng/nmole. The units of each parameter are indicated in brackets in Equation S1.

**Table S1**: molecular weight (MW), number of C, F, O, S, H and N atoms as well as total number of atoms in molecules, and percentages of polar and non-polar molecules.

|  | MW (g/mole = ng/nmole) | Number of atoms in molecule |  |  |  |  |  | Total number of atoms in molecule | % polar molecule (O, N, H) | % non-polar molecule |
|---|---|---|---|---|---|---|---|---|---|---|
|  |  | C | F | O | S | H | N |  |  |  |
| **PFBA** | 214 | 4 | 7 | 2 | 0 | 1 | 0 | 14 | 21.43 | 78.57 |
| **PFPeA** | 264 | 5 | 9 | 2 | 0 | 1 | 0 | 17 | 17.65 | 82.35 |
| **PFHxA** | 314 | 6 | 11 | 2 | 0 | 1 | 0 | 20 | 15.00 | 85.00 |
| **PFHpA** | 364 | 7 | 13 | 2 | 0 | 1 | 0 | 23 | 13.04 | 86.96 |
| **PFOA** | 414 | 8 | 15 | 2 | 0 | 1 | 0 | 26 | 11.54 | 88.46 |
| **PFNA** | 464 | 9 | 17 | 2 | 0 | 1 | 0 | 29 | 10.34 | 89.66 |
| **PFDA** | 514 | 10 | 19 | 2 | 0 | 1 | 0 | 32 | 9.38 | 90.63 |
| **PFUnA** | 564 | 11 | 21 | 2 | 0 | 1 | 0 | 35 | 8.57 | 91.43 |
| **PFDoA** | 614 | 12 | 23 | 2 | 0 | 1 | 0 | 38 | 7.89 | 92.11 |
| **PFTrA** | 664 | 13 | 25 | 2 | 0 | 1 | 0 | 41 | 7.32 | 92.68 |
| **PFTeA** | 714 | 14 | 27 | 2 | 0 | 1 | 0 | 44 | 6.82 | 93.18 |
| **PFHxDA** | 814 | 16 | 31 | 2 | 0 | 1 | 0 | 50 | 6.00 | 94.00 |
| **PFODA** | 914 | 18 | 35 | 2 | 0 | 1 | 0 | 56 | 5.36 | 94.64 |
| **PFBS** | 300 | 4 | 9 | 3 | 1 | 1 | 0 | 18 | 22.22 | 77.78 |
| **PFHxS** | 400 | 6 | 13 | 3 | 1 | 1 | 0 | 24 | 16.67 | 83.33 |
| **PFOS** | 500 | 8 | 17 | 3 | 1 | 1 | 0 | 30 | 13.33 | 86.67 |
| **PFDS** | 562 | 10 | 19 | 3 | 1 | 1 | 0 | 34 | 11.76 | 88.24 |
| **FOSA** | 499 | 8 | 17 | 2 | 1 | 2 | 1 | 31 | 16.13 | 83.87 |

**Table S2:** % of Adsorption onto MPL surface



| | | Freshwater | | | Seawater | | |
|---|---|---|---|---|---|---|---|
| | **Days** | **4** | **7** | **50** | **4** | **7** | **50** |
| **PE** | **PFBA** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 21.6 |
| | **PFPeA** | 0.0 | 0.7 | 6.7 | 8.3 | 22.6 | 19.0 |
| | **PFHxA** | 0.0 | 0.0 | 0.0 | 0.9 | 16.8 | 11.8 |
| | **PFHpA** | 0.0 | 0.0 | 0.0 | 3.8 | 17.1 | 21.9 |
| | **PFOA** | 0.0 | 0.0 | 0.0 | 3.6 | 19.6 | 9.6 |
| | **PFNA** | 0.0 | 0.0 | 0.0 | 0.0 | 13.7 | 15.9 |
| | **PFDA** | 0.0 | 2.2 | 0.0 | 0.0 | 24.4 | 0.0 |
| | **PFUnA** | 0.0 | 26.4 | 7.6 | 0.0 | 20.3 | 0.0 |
| | **PFDoA** | 0.0 | 53.6 | 30.3 | 0.0 | 12.5 | 0.0 |
| | **PFTrA** | 0.0 | 69.4 | 52.8 | 0.0 | 14.5 | 0.0 |
| | **PFTeA** | 0.0 | 70.9 | 56.7 | 19.9 | 18.6 | 0.0 |
| | **PFHxDA** | 0.0 | 56.5 | 51.3 | 27.0 | 18.8 | 1.7 |
| | **PFODA** | 0.0 | 19.1 | 37.4 | 28.0 | 8.5 | 0.0 |
| | **PFBS** | 0.0 | 0.5 | 0.0 | 8.7 | 14.1 | 0.0 |
| | **PFHxS** | 0.0 | 0.0 | 0.0 | 0.0 | 7.8 | 0.0 |
| | **PFOS** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | **PFDS** | 0.0 | 40.9 | 17.7 | 0.0 | 0.0 | 0.0 |
| | **FOSA** | 0.0 | 43.9 | 21.6 | 0.0 | 14.9 | 0.0 |
| **PS** | **PFBA** | 0.0 | 0.5 | 0.0 | 10.5 | 12.5 | 15.5 |
| | **PFPeA** | 0.0 | 1.2 | 2.9 | 3.6 | 22.1 | 26.0 |
| | **PFHxA** | 0.0 | 0.0 | 14.9 | 0.0 | 5.7 | 0.0 |
| | **PFHpA** | 0.0 | 0.0 | 0.0 | 0.0 | 5.4 | 12.4 |
| | **PFOA** | 0.0 | 0.9 | 0.0 | 13.0 | 26.0 | 45.8 |
| | **PFNA** | 0.0 | 4.9 | 0.0 | 8.5 | 27.2 | 63.1 |
| | **PFDA** | 25.0 | 32.3 | 37.7 | 13.4 | 32.9 | 43.8 |
| | **PFUnA** | 50.1 | 43.2 | 63.3 | 0.0 | 37.2 | 34.2 |
| | **PFDoA** | 67.6 | 43.0 | 63.9 | 0.0 | 35.5 | 50.7 |
| | **PFTrA** | 66.6 | 52.5 | 65.9 | 0.0 | 60.4 | 71.3 |
| | **PFTeA** | 53.1 | 39.0 | 67.2 | 49.5 | 56.2 | 72.2 |
| | **PFHxDA** | 16.4 | 29.4 | 39.5 | 65.3 | 73.0 | 61.4 |
| | **PFODA** | 21.0 | 35.1 | 37.6 | 53.8 | 63.5 | 73.1 |
| | **PFBS** | 0.0 | 2.8 | 0.0 | 5.9 | 12.9 | 0.0 |
| | **PFHxS** | 1.5 | 2.8 | 0.0 | 1.8 | 14.1 | 38.8 |
| | **PFOS** | 26.7 | 34.5 | 40.8 | 39.6 | 32.1 | 52.3 |
| | **PFDS** | 79.0 | 26.4 | 59.4 | 0.0 | 14.6 | 31.8 |
| | **FOSA** | 76.8 | 30.4 | 58.4 | 44.7 | 39.4 | 58.1 |
| **PS-COOH** | **PFBA** | 0.0 | 1.0 | 0.0 | 13.9 | 17.1 | 14.1 |
| | **PFPeA** | 0.0 | 2.6 | 0.0 | 18.9 | 27.8 | 24.5 |
| | **PFHxA** | 0.0 | 0.0 | 9.8 | 0.0 | 6.3 | 0.0 |
| | **PFHpA** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | **PFOA** | 0.0 | 0.0 | 0.0 | 3.2 | 10.1 | 23.7 |
| | **PFNA** | 0.0 | 0.0 | 0.0 | 0.0 | 1.0 | 46.5 |
| | **PFDA** | 21.0 | 9.5 | 14.8 | 0.0 | 28.0 | 55.1 |
| | **PFUnA** | 45.3 | 37.8 | 40.9 | 0.0 | 60.1 | 54.0 |
| | **PFDoA** | 64.8 | 55.9 | 53.8 | 47.1 | 70.7 | 65.6 |
| | **PFTrA** | 67.0 | 68.5 | 55.9 | 33.5 | 81.7 | 70.0 |
| | **PFTeA** | 75.0 | 65.1 | 53.1 | 37.6 | 79.0 | 57.9 |
| | **PFHxDA** | 70.9 | 60.2 | 46.0 | 46.9 | 67.0 | 53.6 |
| | **PFODA** | 29.5 | 46.7 | 48.5 | 33.5 | 45.7 | 63.7 |
| | **PFBS** | 0.0 | 1.3 | 0.0 | 9.9 | 11.9 | 8.1 |
| | **PFHxS** | 0.9 | 0.2 | 7.3 | 0.0 | 0.0 | 0.0 |
| | **PFOS** | 17.1 | 9.5 | 12.5 | 0.0 | 30.5 | 52.0 |
| | **PFDS** | 69.8 | 41.4 | 44.7 | 0.0 | 45.7 | 57.6 |
| | **FOSA** | 69.2 | 39.8 | 44.5 | 23.3 | 32.5 | 47.6 |

**S3.** adsorption isotherms most commonly followed by PFASs according to the literature:

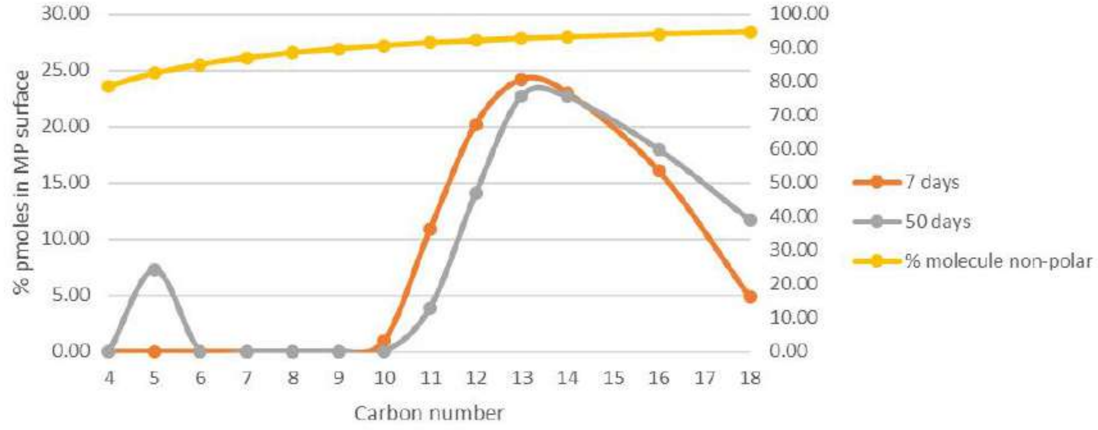
$q = K_{lin}C$ (Linear);

$q = q_{max}\frac{K_L C}{1 + K_L C}$ (Langmuir);

$q = K_F C^n$ (Freundlich)

Where "$q$" is the concentration of PFASs onto the plastic surface, "C" is the concentration of PFASs in the aqueous phase, and "$K$" and "$n$" are characteristic constants.

A) % pmoles PFASs acids on HDPE in Freshwater



B) % pmoles PFASs acids on HDPE in Sea water

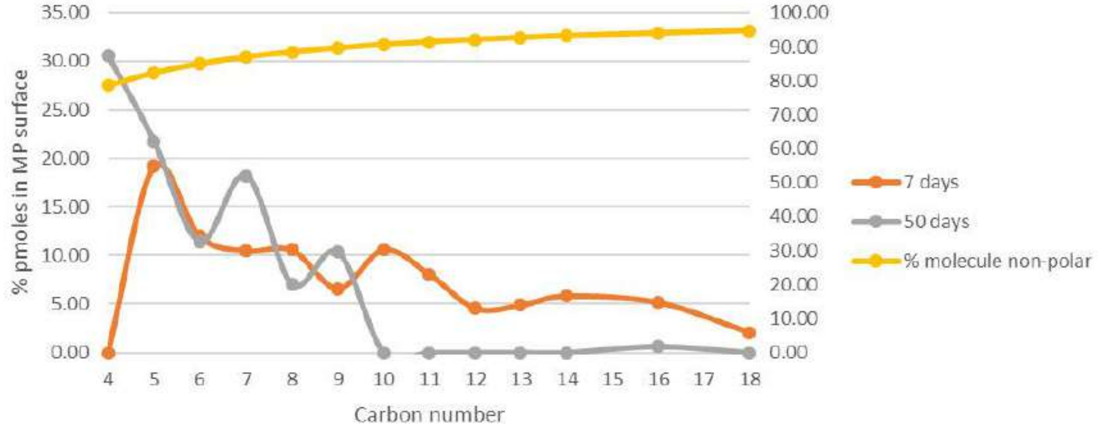
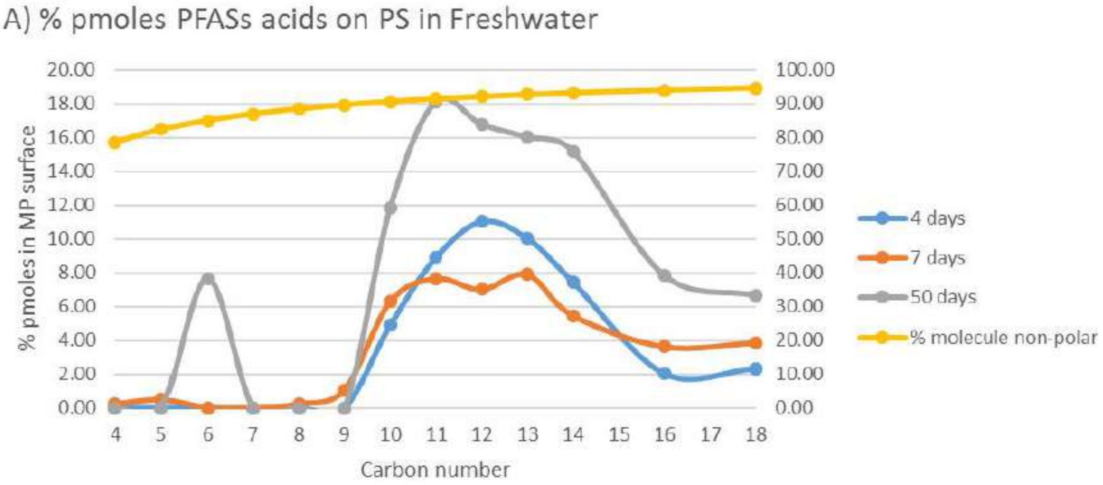


**Fig. S1**. Percentage of pmoles of carboxylic acids adsorbed onto HDPE surface and percentage of non-polar molecule (yellow line corresponds to the right axis calculated from Table S1) for each carbon number for A) freshwater and B) seawater.



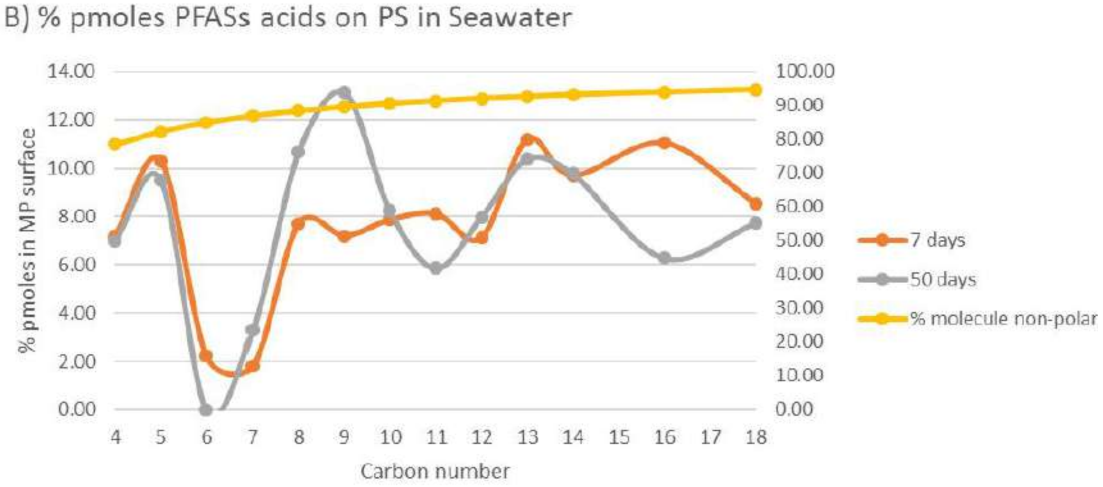
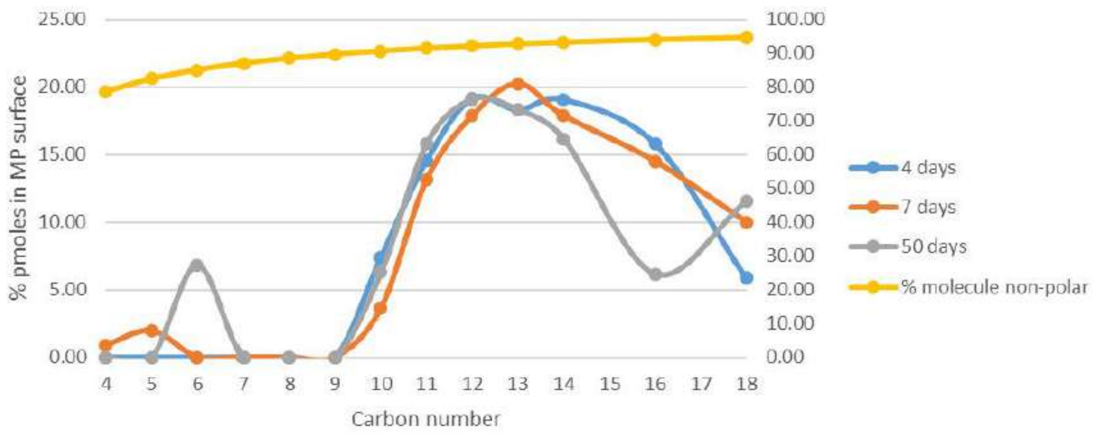


**Fig. S2**. Percentage of pmoles of carboxylic acids adsorbed onto PS surface and percentage of non-polar molecule (yellow line corresponds to the right axis calculated from Table S1) for each carbon number for A) freshwater and B) seawater.

A) % pmoles PFASs acids on PS-COOH in Freshwater



B) % pmoles PFASs acids on PS-COOH in Seawater

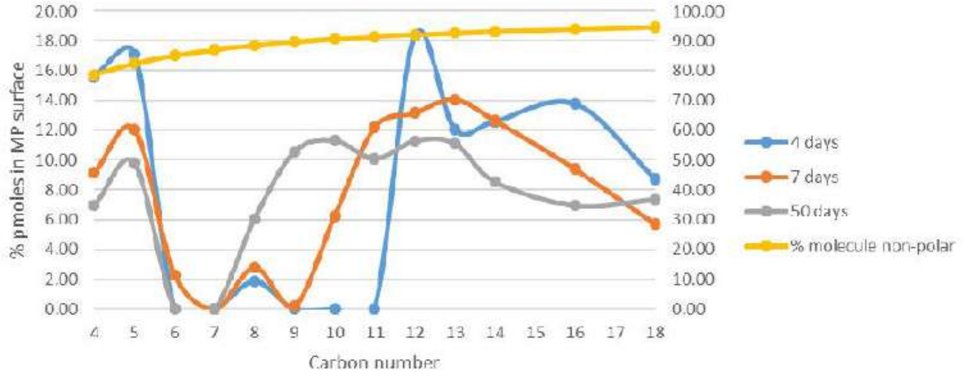


**Fig. S3**. Percentage of pmoles of carboxylic acids adsorbed onto PS-COOH surface and percentage of non-polar molecule (yellow line corresponds to the right axis calculated from Table S1) for each carbon number for A) freshwater and B) seawater.

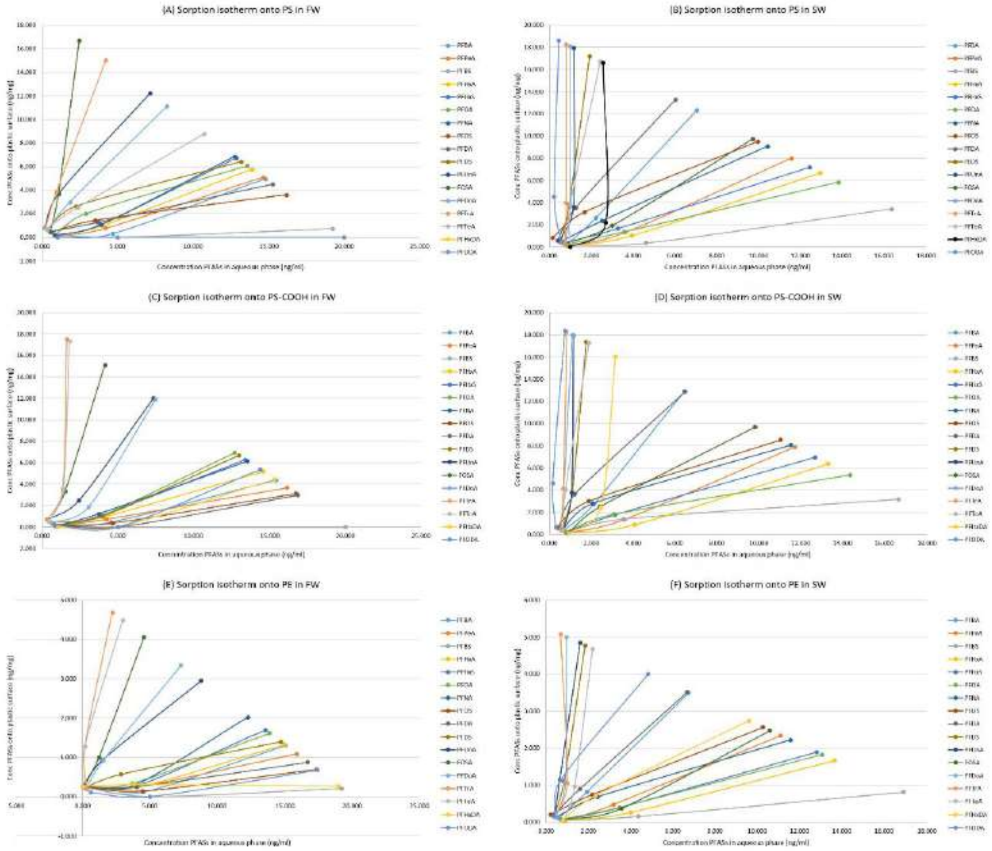


**Fig. S4.** Sorption isotherms for individual PFASs in (A) PS in freshwater; (B) PS in seawater; (C) PS-COOH in freshwater; (D) PS-COOH in seawater; (E) HDPE in freshwater; and (F) HDPE in seawater.

## 3.3 Discussion

### 3.3.1 Riverine plastic loads in the Western Mediterranean Sea

The Mediterranean Sea is a semi-enclosed sea with a high number of hotspots of contamination. In regions with higher population density on the coast, such as the Western Mediterranean Sea, high concentrations of floating plastic have been found. However, the sources and transport of MPLs and NPLs at sea have not yet been understood and a study of the influence of major rivers and estuarine areas is necessary to fill the information gap. In this context, the occurrence of floating macro-litter, especially plastic items, loaded by the Llobregat and Besòs Rivers into the Catalan Sea (Western Mediterranean Sea) has been provided (Publication I). The observations were performed within the framework of the RIMMEL project (*Riverine and Marine floating macro-litter Monitoring and Modelling of Environmental Loading*) from the EC-JRC (González-Fernández et al. 2017). Under the same project, the Rhone River (Switzerland/France) (Castro-Jiménez et al. 2019) and the Tiber River (Italy) (Crosti et al. 2018), two main European rivers flowing into the western part of the basin, were monitored during 2016-2017. The results of these recent works have been compared with those obtained for Catalan Rivers to assess the amount, the composition and the spatial-temporal variation of macro floating debris loaded by these watercourses in the Western Mediterranean, including the Tyrrhenian Sea.

As confirmed by Top Items List reported by González-Fernández et al. (2018), the 17 rivers and streams monitored for the Mediterranean region have presented the same composition showing the plastic as the main debris, followed by paper and metal or rubber. Within each category, the composition of floating debris varies among countries. Nevertheless, items mainly related to the food and beverage sectors and packaging are the dominant plastic categories reported. Despite a similar waste composition was observed, the number of items discharged into the sea varies from one river to another depending on the characteristics of the basin (size, river flow, and population) and on basic monitoring parameters (observation height and observation track width). Table 3.1 shows the characteristics of the river basins and the methodological approaches used in these studies.

**Table 3.1** Characteristics of the river basins loaded in North-Western of the Mediterranean Sea and the methodological approaches used during the floating macro-litter monitoring.

| | *River basin properties* | | | | *River monitoring parameters* | | |
|---|---|---|---|---|---|---|---|
| **River** | **Catchment area [km$^2$]** | **Discharge [m$^3$ s$^{-1}$]** | **Length [km]** | **Width [m]** | **Distance from the sea [km]** | **Observation height [m]** | **Observation track width [m]** |
| *Rhone* | 95000 | 1710 | 816 | 150 | 40 | 13 | 65 |
| *Tiber* | 17375 | 240 | 405 | 60 | 0.8 | < 5 | 26 |
| *Llobregat* | 4948 | 19.8 | 175 | 150 | 3.5 | 15 | 15 |
| *Besòs* | 1024 | 4.33 | 18 | 36 | 0.5 | 7.8 | 36 |

Although the Rhone covers a large catchment area, the highest number of items has been observed for the Tiber River (Figure 3.1). The high population density combined with poor waste management in this area (i.e. illegal dumping along the Tiber River), contributes to an increase in the amount of mismanaged waste that ends up in the sea. In fact, even if with differences at the regional level, the daily quantity of waste produced in Italy (2.23 Kg/inhabitant/day) is higher than that produced in Spain and France (2.13 and 1.92 Kg/ inhabitant /day, respectively).

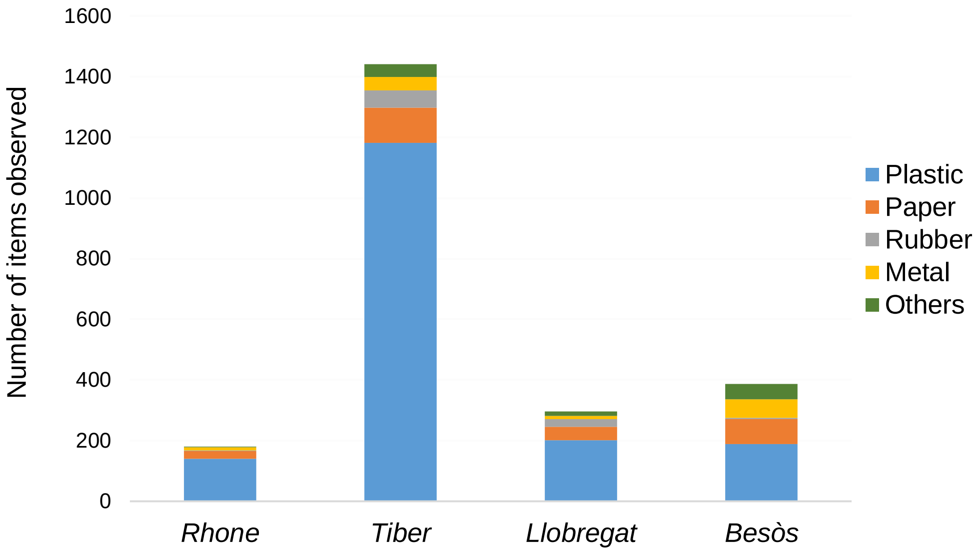


**Figure 3.1** Riverine litter composition by material detected for Rhone, Tiber, Llobregat, and Besòs rivers.

Furthermore, the final result could depend on the characteristics of the observation sites. Since small objects are more abundant, a greater number of items were recorded in the Tiber and Besòs Rivers where the observation point was closer to the surface of the river. Alternatively, the use of binoculars during the monitoring, like for the Llobregat River, facilitated the visual observation from upper bridges. In addition, the proximity of the observation point to the mouth of the river is relevant because of the waste accumulation at the end of the river.

In these studies, the litter flux (measured as the number of elements per hour) was extrapolated on the assumption that the floating waste is evenly distributed over the cross-section of the river. A growing trend from the autumn to the following spring was observed for the Llobregat and Rhone Rivers with a maximum load in May 2017. On the contrary, the Tiber showed its highest values in autumn 2016, followed by a decrease in load during spring and summer. Generally, the litter peaks were registered in no precipitation days after important rainfall events. The dependence of the waste flow on the river discharge observed is mainly due to the high seasonal variability of Mediterranean rivers (Cid et al. 2017). The rainfall regime in Mediterranean climate regions changes monthly depending on the orography of the area. The north-western area, which includes Catalonia, Southern France and North Italy, is also characterised by major flash flood events (Tarolli et al. 2012). The steep orography surrounding this part of the basin favours the occurrence of intense precipitation events from autumn to summer. Overall, runoff processes or strong winds play an important role in the final fate of waste. In particular, the plastic flow is mainly affected by extreme events as this debris is largely represented by light objects such as bottles, bags and covers/packaging.

Concerning to the plastic loads (mass of plastic items per year) entering the W Mediterranean, the values estimated were 0.71 tonnes per years for Rhone River, assuming an item-to-mass conversion factor of 3.2 g per item; while those calculated for the Llobregat and Besòs Rivers ranged between 0.10 - 0.17 and 0.29 - 0.45 tonnes per years, respectively. The total loading in the North-western Mediterranean Sea amounts to 1.1 - 1.3 tonnes per year of macroplastics. This is an underestimation considering that only floating products have been considered and that there is no information on the load carried by other rivers in the same area.

Due to a lack of field data, the contribution of rivers to marine litter has been estimated by several studies, especially for plastic debris. Schmidt et al. (2017) developed a regression model including properties of the catchment area, population data and the generation of mismanaged plastic waste (MMPW). This model has shown that the estimated mass loads of macroplastics discharged into the western part of the basin by the Rhone, Ebro, Llobregat, Besòs, and Ter Rivers are 5.2, 0.86, 0.46, 0.32, and 0.046 tonnes per year, respectively. On the other hand, the plastic loads reported by Lebreton et al. (2017) ranged between 0.01 - 0.014, 0.024 - 0.25 and 0.007 - 0.092 for the Rhone, Llobregat and Besòs Rivers. Lastly, the Lagrangian 2D model proposed by Liusbartseva et al. (2018) indicated the Rhone as one of the largest river inputs in the Mediterranean Sea and Barcelona as the biggest terrestrial inputs of the Western Mediterranean. The estimated plastic loads for the Rhone River and Barcelona city were 1454 and 1787 tonnes per year, respectively. The disagreement between these models and the loads estimated from field data increases especially for larger rivers depending on the natural (rain, wind, etc.) or anthropogenic (presence of artificial barriers or WWTPs) factors considered.

### 3.3.2 MPLs in W Mediterranean coastal area

As seen above, rivers can carry huge amounts of large plastic items which, once at sea, can break into small pieces by erosion (secondary MPLs). Additionally, significant amounts of primary MPLs could be transported into the marine environment. For instance, textile fibres are not retained or degraded in WWTPs reaching the aquatic systems. Among the main rivers discharging in the W Mediterranean, the river systems of the Gulf of Lion (Rhône and Têt Rivers) have been monitored for one complete hydrological year. The mean concentration of MPLs in the Rhône and Têt rivers were 11.6 ± 17.7 and 42.3 ± 107.3 particles $m^{-3}$, respectively. Despite a large predominance of fibres (> 92%), the main plastic items were fragments and films mainly made of polyolefin polymers (PE and PP) (Constant et al. 2020). After the Rhone, the Ebro River is another largest Mediterranean river and it is also an important water resource in the NE of Spain. Early information reported on plastic pollution in the Ebro Delta area showed an abundance of MPL at the water surface of 3.5 ± 1.4 particles $m^{-3}$ (Simon-Sánchez et al. 2019). However, the limited number of sampling points in this study does not allow a proper evaluation of the distribution of MPLs in this area.

Despite the significant contribution of plastic waste from rivers, most studies focus on MPL abundance on sea surface waters. Valuable information can be achieved assessing the spatial distribution of surface MPLs near the coast, especially near the mouth of the main rivers. Focusing on the W Mediterranean, field data has been reported only for the northern areas of this sub-basin (Table 3.2).

The reported abundances are relatively close to each other and with orders of a magnitude similar to those found in the North Pacific and North Atlantic oceans (Law et al. 2010; Law et al. 2014). Going even more in detail, higher abundances were found offshore the North Catalan coast due to the discharge of several Mediterranean rivers, including those near the metropolitan area of Barcelona.

**Table 3.2** Abundance of floating MPLs in NW Mediterranean Sea.

| Area | MPLs abundance (particles m$^{-2}$) | Polymers | Ref. |
|---|---|---|---|
| Barcelona nearshore | 0.112 ± 0.131 | PE (64%), PP (16%) and PS (1%) | (Camins et al. 2020) |
| Barcelona offshore | 0.227 ± 0.174 | | |
| North Catalan coast | 0.183 ± 0.158 | PE (65%), PP (14%) and PS (10%) | (de Haan et al. 2019) |
| Rhone mouth | 0.033 ± 0.019 | Fragments: PE (54%) and PP (17%); foams: PS (67%) | (Constant et al. 2018) |
| Têt mouth | 0.062 ± 0.081 | | |
| Gulf of Lion (Marseille Bay) | 0.096 | - | (Schmidt et al. 2018) |
| Gulf of Lion (Corsica) | 0.062 | - | (Collignon et al. 2012) |
| Gulf of Lion, Balearic Islands, Sardinia and Corsica | 0.130 | - | (Faure et al. 2015) |
| Ligurian Sea | 0.125 ± 0.132 | PE (62-76%) and PP (7-14%) | (Pedrotti et al. 2016) |
| Pelagos Sanctuary (Tuscany) | 0.082 ± 0.079 | PE (76%), PP (20%) and PS (2%) | (Fossi et al. 2017) |
| Tuscany | 0.069 ± 0.083 | PE (>66%), PP (28%) and PS (5%) | (Baini et al. 2018) |

Besides, the Northern current, that cyclically flows from the continental side from the Gulf of Genoa to the Gulf of Valencia, can displace MPLs in this area. Even if the abundance and chemical composition of MPLs at sea is surely subject to continuous temporal changes, similar distribution was reported by these studies. The composition of the western Mediterranean was dominated by low-density polymers such as PE, PP, and PS. Fibres and fragments resulted as principal shape followed by foams and sheets.

These works, as most of those on MPLs analysis in surface waters, are based on sampling by manta or Neuston trawl (~330 µm). Afterwards, MPLs are visual identified (naked eye or by microscope), counted and, eventually, characterised for the confirmation. Although this is a well-established scheme for the analysis of MPLs in water samples, advantages and disadvantages for each step of the analysis, especially for the identification/confirmation, should be discussed (Table 3.3.).

Firstly, the mesh size of sampling nets is a critical point that determines the minimum size of MPLs detected and, consequently, the concentrations reported (expressed as the number of particles per volume). In addition, even if in most of the case samples are treated with chemical agents, the plastic particles may be easily confused with biological material during counting. Finally, the identification by visual sorting is time-consuming and prone to a human error leading to overestimate or underestimate MPLs in the sample. Other important limitations may be found if analytical techniques for MPLs confirmation are applied. (µ)FTIR and (µ)Raman spectroscopic techniques are commonly used for this purpose. These can distinguish among different types of polymers, but they are quite limited in terms of sensibility or particle size. Raman spectroscopy combined with imaging techniques offers the detection of MPLs up to 1 µm size. Other methods are based on selected scanning microscopy (SEM), but sample preparation is required, and qualitative data is only provided in this case. Generally, one of the main limitations of MPLs analysis in the aquatic environment is the lack of a standardized method. With this aim, a qualitative analytical method based on validated parameters, such as recovery, linearity, accuracy or sensitivity, is required. Not many analytical techniques combining the quantification and characterisation of MPLs are available. Recently, (Pyr)-GC-MS and TED-GC-MS are recently applied in MPLs analysis. However, their main limitation is the large amount of matrix required to obtain between 5 and 200 µg of polymer/copolymer solid sample.

**Table 3.3** Advantages and disadvantages for the methods used for MPLs identification/confirmation.

| Method | Advantages | Disadvantages |
|---|---|---|
| Visual identification:<br>*necked eyes, microscope, SEM, TEM, etc.* | ○ Low-cost analysis<br>○ Physical characterisation | ○ Time consuming<br>○ High possibility of false positives<br>○ No chemical<br>○ No quantitative information<br>○ Size limitation |
| Spectroscopic techniques:<br>*FTIR, (µ)FTIR, Raman, (µ)Raman, ATR-FTIR, etc*. | ○ Common techniques<br>○ Non-destructive<br>○ Chemical characterisation<br>○ Assessing polymer ageing | ○ Time consuming<br>○ Sample pre-treatment<br>○ Low sensitivity<br>○ Size limitation<br>○ No quantitative information |
| Thermal analysis:<br>*DSC-TGA, TED-GC-MS, (Pyr)-GC-MS etc.* | ○ No sample pre-treatment<br>○ Simultaneous analysis for polymer type and additive chemicals<br>○ Quantitative information | ○ Big amount of sample<br>○ No physical characterisation<br>○ Low sensitivity<br>○ Complex data<br>○ Destructive analysis |

All these detection methods are focused on MPLs analysis and there are no recent developments aiming for NPLs detection in natural aquatic systems. This big limitation moved our research to develop a quantitative analytical method based on mass spectrometry (MS) capable of determining MPLs from 100 µm down to the nano-sized range in environmental samples (Publication II). In this work, the performance of several MS-techniques including matrix-assisted laser desorption ionisation (MALDI) coupled to time-of-flight mass spectrometry (TOF-MS), liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS), and the ambient ionization approaches as desorption electrospray ionisation (DESI) and direct analysis real-time (DART), were assessed for the study of PS-MPLs/NPLs. These ionization methods are not usually applied for MPLs analysis in environmental samples. In this case, more attention should be paid to the sensitivity of the methods and the target polymer. As a proof of concept, PS polymer was selected in our study because of its high occurrence in the western Mediterranean Sea as surface MPLs. Being PS nanoparticles easy to synthesise, they are widely used in research and other applications and can therefore easily end up in the environment. Additionally, the fragmentation of EPS beads to micro and nano-size particles by mechanical abrasion has been already reported by Shim et al. (2014).

Plastic debris present in the environment are constituted by industrial polymers with high molecular weight (HMW). These polymers are a mixture of polymer chains of various lengths which are linked by weak interactions. Possible breakages due to external forces can lead to the formation of MPLs and NPLs which could be represented by short-chain polymers. Due to the limitation of analysable mass range in mass spectrometry, all methods tested in our study were first applied to a standard of PS (MW ~1200 Da) and then to industrial PS (HMW > 150kDa). As reported in polymer analysis, the performance of the analytical methods could be influenced by the distribution of molecular mass of polymer, which is represented by the polydispersity index (PDI). The PDI is defined by *Mw/Mn*, where *Mw* is the weight average molecular weight and *Mn* is the number average molecular weight of the polymer. A standard polymer (often referred to as a monodisperse polymer) is composed of molecules of the same mass; whereas an industrial polymer is generally polydisperse, or non-uniform, and its chain lengths vary over a wide range of molecular masses (Figure 3.2).

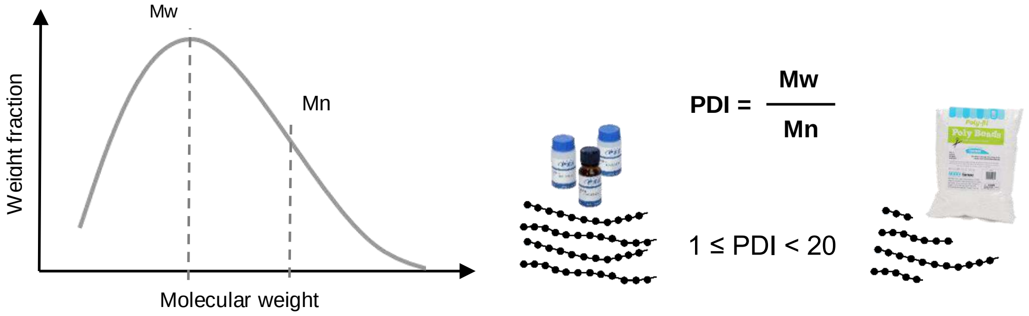


**Figure 3.2** Molecular weight distribution of polymer; the polydispersity index (PDI) is used as a measure of the broadness of a molecular weight distribution of a polymer. It is defined by Mw/Mn and normally range between 1-20.

Different behaviours for standard and industrial polymers have been observed in our work, showing the MALDI-TOF and the ambient-air techniques as unsuitable methods for the analysis of HMW polymers. The difficulties to analyse polydisperse polymers by MALDI-TOF were also previously reported by Schriemer et al. (1997) . On the other hand, the performance of DESI-HRMS and MALDI-TOF-MS for the analysis of various industrial synthetic polymers such as poly(ethylene glycol) (PEG), poly(propylene glycol) (PPG), poly(methylmethacrylate) (PMMA), poly(dimethylsiloxane) (PMDS) and copolymers, with masses ranging from 500 g mol$^{-1}$ up to more than 20,000 g mol$^{-1}$, was compared. The results shown that DESI-Orbitrap MS is fully suitable for the detection of low molecular weight synthetic polymers (Mn < 5000 g mol$^{-1}$) in complex matrices, such as cosmetics. However, the low sensitivity of these methods did not allow their application for environmental analyses (Friia et al. 2012). Finally, a recent publication by Okoffo et al. (2020) reported the extraction of pressurized liquids (PLE) coupled with (Pyr)-GC-MS as a suitable method for the rapid and effective identification and quantification of different polymers in a complex matrix, such as biosolid. However, even in this case, the detection limit is in the order of mg g$^{-1}$.

Hence, compared to previous works and the methods applied in this study, the APC/APPI-LTQ-Orbitrap-MS is more sensitive and robust, allowing to determine PS at trace and ultra-trace level in environmental water samples. The high sensitivity achieved in this case allows detecting the shortest polymer chains obtaining semi-quantitative results also for HMW polymers. In particular, the high-resolution capability of LTQ-Orbitrap mass spectrometer and the selectivity of APC column were successfully applied to identify and quantify PS in particulate matter derives from freshwater and seawater samples of the Ebro Delta (NE of Spain).

### 3.3.3 MPLs/NPLs in the estuarine environment

Despite the information reported for NW Mediterranean coast, there is a lack of knowledge about plastic contamination on the southern coast of Catalonia. Ebro Delta is a wetland area of 320 km$^2$ with a typical Mediterranean climate (heavy rainfall events in autumn and spring (200 - 300 mm) and intense summer drought (< 50 mm)). The international importance of this area is especially due to the fauna and flora preservation. Almost 13% of its total surface is composed of natural lagoons, bays, and marshes. It also devoted to intensive rice production and aquaculture, with high touristic impact. Consequently, the river, irrigation channels, and coastal areas are under continuous stress from a huge number of anthropogenic factors. Because of the possible impacts that contamination could have on all these activities and on the environment, it is important to investigate the presence of emerging contaminants, including MPLs, in this area.

The performance of the LC-HRMS methods developed in this thesis was evaluated by assessing the occurrence of PS-MPLs/NPLs in 17 natural water samples of Ebro Delta River. PS-MPLs/NPLs have been detected in 5 samples of natural waters with a concentration range between 1 and 140 ng L$^{-1}$. Significant concentrations were found in Port d'Illa de Mar (Fangar Bay), where there is an intense fishing activity, and near populated areas along the river, especially after Amposta, one of the main towns towards the mouth. Positive concentrations of MPLs/NPLs in the estuarine environment could be also due to microgel formation caused by an increase of hydrophobic interactions between NPLs and dissolved organic matter (DOM). The aggregate formation and abundance increase at specific salinities (Shiu et al. 2020; Chen et al. 2018).

Contemporary study on MPLs in Ebro River reported the dominance of fibres and fragments sizing less than 1000 µm. Possible sources of primary MPLs, such as fibres, could be WWTPs or atmospheric contamination. Whereas run-off processes or agricultural activities could be responsible for the presence of secondary MPLs (i.e. fragments, sheets, foams). Any information regarding the polymer identification was reported for these samples.

Due to their ability to act as transport vectors, the presence of MPLs/NPLs in the environment is often linked to other persistent organic contaminants. The adsorption of highly hydrophobic compounds on plastic particles has been widely explored, whereas limited information is available regarding slightly polar molecules, such as surfactants. Among these, polyfluoroalkyl substances (PFASs) are chemicals found in a wide range of consumer products. Due to their high stability in the environment, PFASs are considered as persistent organic pollutants (POPs). Additionally, being PFASs hydrophobic and hydrophilic compounds, the probability to be sorbed onto marine debris surface increases being a risk for aquatic organisms.

Llorca et al. (2014) reported for the first time the occurrence of different PFASs in different plastic pellets widely distributed around the coastal areas of Greece. The PFASs concentrations reported ranging from 10 to 180 ng/Kg and they were higher than those found in the sediments of the same area. PFPeA was the predominant compound followed by PFDA. The sorption of PFASs with shorter-chain (C3–C5) on plastics was attributed to more steric effects and not to van der Waals forces or hydrophobic interaction as in the case of longer-chain fluorinated compounds. These preliminary results lead to characterise the adsorption capacity of 18 PFASs on the surface of three different types of MPLs (HDPE, PS and PS-COOH) (Publication III). Since the distribution of PFASs in the Ebro Delta was previously assessed (Pignotti et al. 2017), the experimental setup was performed by emulating the realistic environmental conditions of this estuarine area. In the previous study, the concentrations reported in water samples ranged from 0 to 15 ng $L^{-1}$ up to 2500 ng $L^{-1}$ in wastewater influents. Also, perfluorocarboxylic acids (PFCAs) are the most abundant compounds in water, especially shorten chains compounds, such as perfluoropentanoic (PFPeA) and perfluorooctanoic (PFOA). Low concentrations of perfluorooctanesulfonate (PFOS), which is the most common among perfluorinated sulfonates, were also reported. According to these results, the concentrations of PFASs in freshwater and seawater set in the experiments was 10 µg $L^{-1}$.

The results of the experiments revealed the order of PS > PS-COOH > HDPE for the adsorption of PFAS. Among them, perfluoro sulfonate and sulfonamide tended to be sorbed more on the surface of MPLs than carboxylic acids. However, differences in polarity between functional groups can influence the sorption affinity towards MPLs with different composition. Overall, according to their hydrophobicity, longer chain PFASs (C11 - C18) were highly sorbed on the surface of MPLs. Moreover, salinity, pH and the presence of organic matter can significantly influence the adsorption process in the aquatic environment (Figure 3.3 and 3.4).

Largely, these results are in agreement with those obtained from the study of the presence of PFAS in the aqueous environment of the Ebro Delta. In fact, higher concentrations of PFCAs, particularly those with the shortest chains (i.e. PFPeA and PFOA), were found in the coastal lagoons. Despite the high salinity of these waterbodies, the uptake of PFASs onto MPLs surface is not favoured because of the prevalence of the organic matter. Finally, the detection of PFOS also in water samples may be due to either the presence of organic matter or plastic debris, as demonstrated by these results.

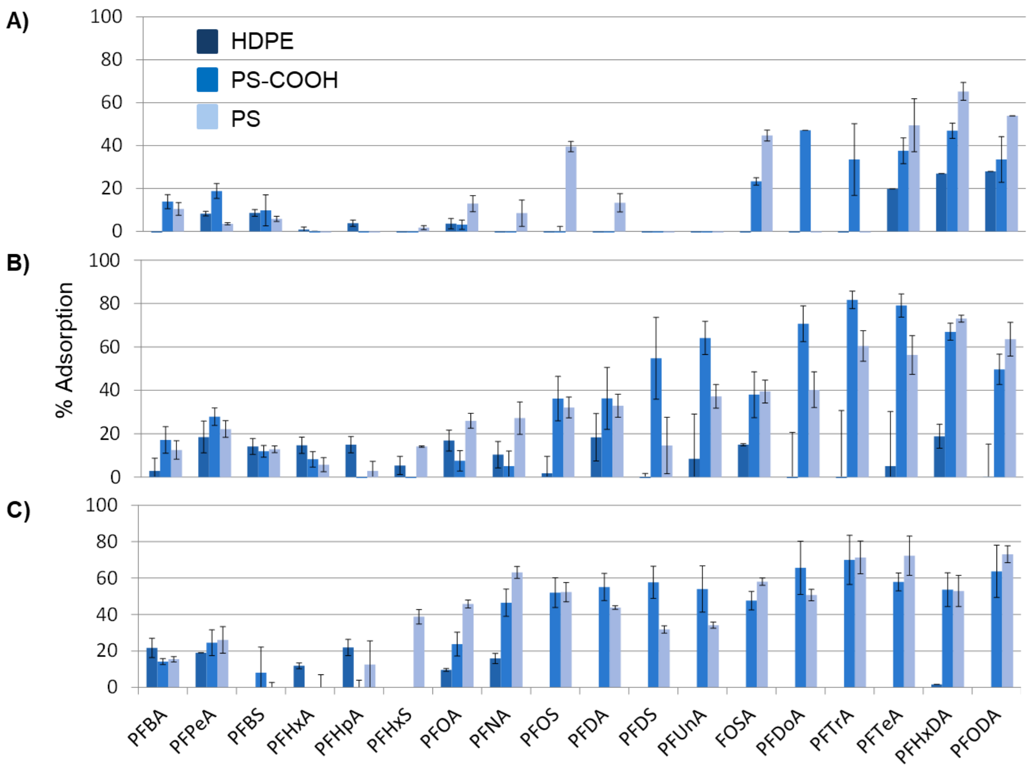


**Figure 3.3** Adsorption percentages of PFAs onto HPDE, PS and PS-COOH microspheres surface in freshwater after (A) 4, (B) 7, and (C) 49 days.

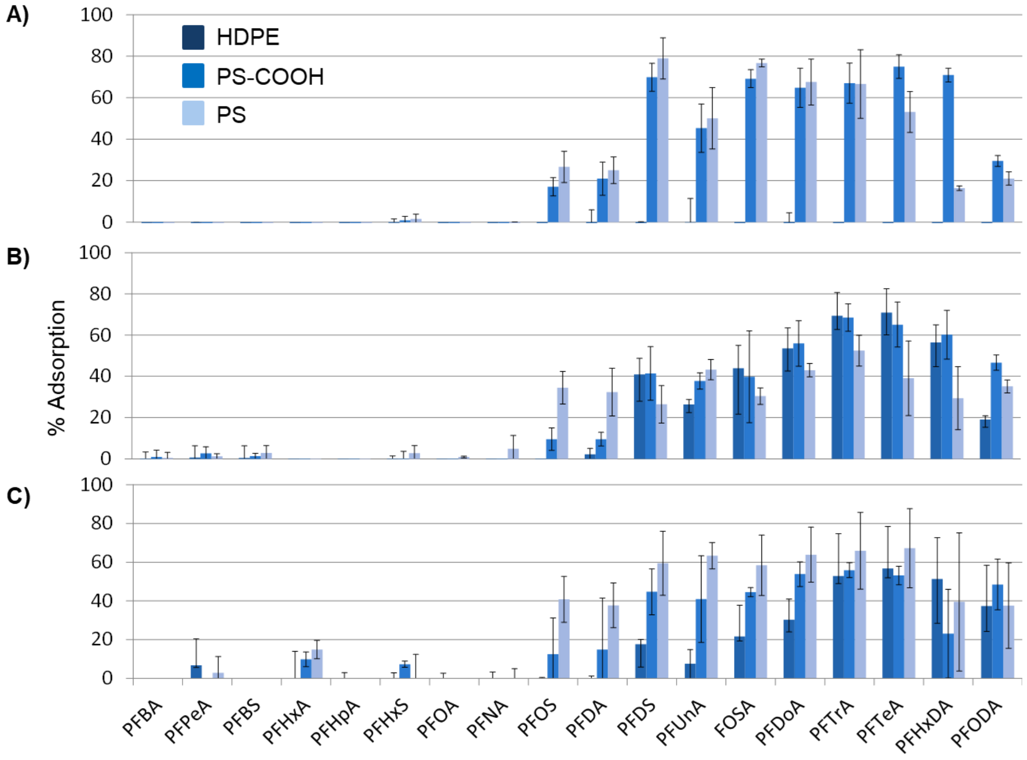


**Figure 3.4** Adsorption percentages of PFAs onto HPDE, PS and PS-COOH microspheres surface in seawater after (A) 4, (B) 7, and (C) 49 days.

# 4 . Effects of micro(nano)plastics on the environment and human health

## 4.1 Introduction

The presence of plastic waste at sea causes aesthetic problems that have a significant impact on society and the economies of coastal activities, such as tourism, fisheries and the maritime industry. However, the main concern that comes from plastic pollution is the damage it can cause to nature and human health.

### Effects on the marine environment

Plastic litter can affect marine systems at different levels, ranging from impacts at the individual to population and ecosystem level. The consequences for ocean ecosystems are still almost unknown, while impacts on marine biota are among the most explored.

The threats to marine life are mainly physical or mechanical due to ingestion of plastic debris and entanglement in larger plastics objects (e.g. packaging bands, synthetic ropes and lines, nets, etc.). Recent studies focus on the effects of ingestion of smaller plastic particles by organisms. Concerning MPLs, their detrimental effects can be classified as those caused by polymer itself (*primary effects*), by the contaminants leaching from the polymeric matrix, and by contaminants being transported and released by them (*secondary* or *chemical effects*). In addition, the risks associated with the ingestion of MPLs are difficult to estimate due to the variety in the chemical composition, shape and size of plastic particles. In general, impacts have been studied more on the health of marine species than freshwater or terrestrial species (de Sá et al. 2018; Haegerbaeumer et al. 2019).

At the lower trophic level, planktons are the primary consumers of aquatic ecosystems. In the coastal environment, MPLs can interact with these organisms affecting their biological functions. In particular, smaller MPLs may have negative effects on the growth of microalgae (Sjollema et al. 2016; Prata et al. 2019). On the other hand, zooplanktons can capture and ingest smaller and larger MPLs having the same size as their natural prey. The effects on feeding, reproduction, growth and development have been reported mainly for various species of copepods (Botterell et al. 2019). Likewise, corals prefer eating plastic microbeads instead of natural food, inhibiting the nutritional intake and reducing energy reserves (Rotjan et al. 2019; Reichert et al. 2018). Suspended bivalves are doubly exposed to plastic contamination because they are filter feeders. Studies on mussels have reported structural changes in the digestive system and the gill (Kolandhasamy et al. 2018; Paul-Pont et al. 2016). Furthermore, small plastic particles could pass from the digestive tract to the tissues and the circulatory system, increasing the likelihood of being transferred to higher trophic levels (Browne et al. 2008; Von Moos et al. 2012b).

Moreover, since MPLs are similar in size to phytoplankton, they can be mistakenly ingested by higher trophic species. No enzymatic pathways can break down or digest MPLs/NPLs in any of these aquatic organisms, and therefore, they can be bioaccumulated. At benthic level, other effects related to feeding and reproduction have been identified. Sussarellu et al. (2016) reported that oyster's reproduction was affected by exposure to PS microspheres (2 and 6 μm in diameter; 23 μg $L^{-1}$). Other studies reported a reduction in feeding activity and lysosomal membrane stability and an increase in energy consumption in marine deposit-feeding, such as polychaete *Arenicola marina* exposed at high concentrations of PS-MPLs (Besseling et al. 2012; Della Torre et al. 2014). In the case of fish, in-vitro and in-vivo exposure studies have shown effects like neurotoxicity, histopathological alterations in the intestine, oxidative stress in the immune system and changes in the metabolism (Espinosa et al. 2018; Lu et al. 2016;

Pedà et al. 2016; Rochman et al. 2013b; Oliveira et al. 2013). The results of MPLs ingestion have been largely studied for pelagic organisms. At higher trophic levels, ingested plastics could cause physical damages to the digestive system (e.g. mechanical injuries and ulceration) or a false sense of satiety leading to a reduction in predatory performance and starvation (de Sá et al. 2015; Mazurais et al. 2015; Critchell et al. 2018; Lusher et al. 2015a; Rodrigue 2017).

Even if MPLs are considered biochemically inert, chemical contaminants transported by them may be toxicologically harmful (Vanden Bilcke 2003). Once MPLs or NPLs are ingested by biota, additives and monomers can be leached within their body. Possible toxic effects depend on the species, the type of plastic and plasticisers used during its production (Hermabessiere et al. 2017). Phthalates, mainly used in PVC production, have been found in a wide range of organisms at different trophic levels (Mackintosh et al. 2004). Exposure to DEHP and MEHP causes reproductive dysfunction and endocrine disruption (Ye et al. 2014). Also, nonylphenol and BPA are recognised as endocrine disrupters (Oehlmann et al. 2009). Reproductive defects and reduced fertility have been reported for aquatic vertebrates exposed to phthalates and BPA (Mathieu-Denoncourt et al. 2015). While flame retardants, such as PBDEs, induced hepatic stress (Rochman et al. 2013b).

Apart from the results of leaching, another part of the research focuses on the effects of organic contaminants adsorbed and, then, desorbed by MPLs. In general, filter feeders can passively uptake these contaminants from water and surrounding sediments (bioconcentration/bioaccumulation) (Teuten et al. 2009; Reichenberg et al. 2006) or can accumulate them after MPLs ingestion (Rodrigues et al. 2019; Thompson et al. 2004). The chemical transfer of additives or associated contaminants from plastics to organism tissues, especially lipids, occurs only if MPLs are subjected to the gastric fluids and/or if residence times in the gastrointestinal tract (GIT) are sufficiently long (Koelmans 2015). For instance, worms use surfactants in their digestive tract to mobilise hydrophobic organic contaminants from sediments or MPLs (Voparil et al. 2000). Significant concentrations of hydrophobic organic contaminants (HOCs) have also been reported for the filtered feed species (Pittura et al. 2018; Ogata et al. 2009). In these cases, the long residence times of the plastic particles favour the desorption processes, contributing to the bioaccumulation of the carried chemicals (Avio et al. 2015b).

After the plastic particles have been ingested by benthic fauna, they and some of the co-contaminants can be biomagnified through the marine food-web. Marine mammals are considered as sentinel species to understand the transfer of toxic compounds at the top of the food chain and the quality of the marine environment (Fossi et al. 2012; Ross 2000). Higher concentrations of PCBs have been reported for many seals, orcas, and dolphins (Letcher et al. 2010; Stockin et al. 2007).

Generally, biomagnification does not affect all compounds (Mackintosh et al. 2004). They are less likely for degradable compounds such as PAHs and PBDEs, which could be degraded by water or metabolised by organisms (Rochman et al. 2013b; Di Paolo et al. 2010). Higher organisms, as seabirds, are generally exposed to POPs only through diet. Significant concentrations of persistent, bioaccumulative, and toxic substances have been found in their tissues due to the long residence times of plastic in their organisms (Teuten et al. 2009; Takada et al. 2005; Colabuono et al. 2010). Once in the body, due to their lipophilic nature, HOCs tend to accumulate in the fatty tissues leading to substantial effects on several biochemical mechanisms (O'Donovan et al. 2018). Biomarkers assessment at a cellular and sub-cellular level shows alterations in oxidative stress, immune and neurological responses, as well as in gene expression profiles (Browne et al. 2013; Avio et al. 2015b; Paul-Pont et al. 2016; von Moos et al. 2012a).

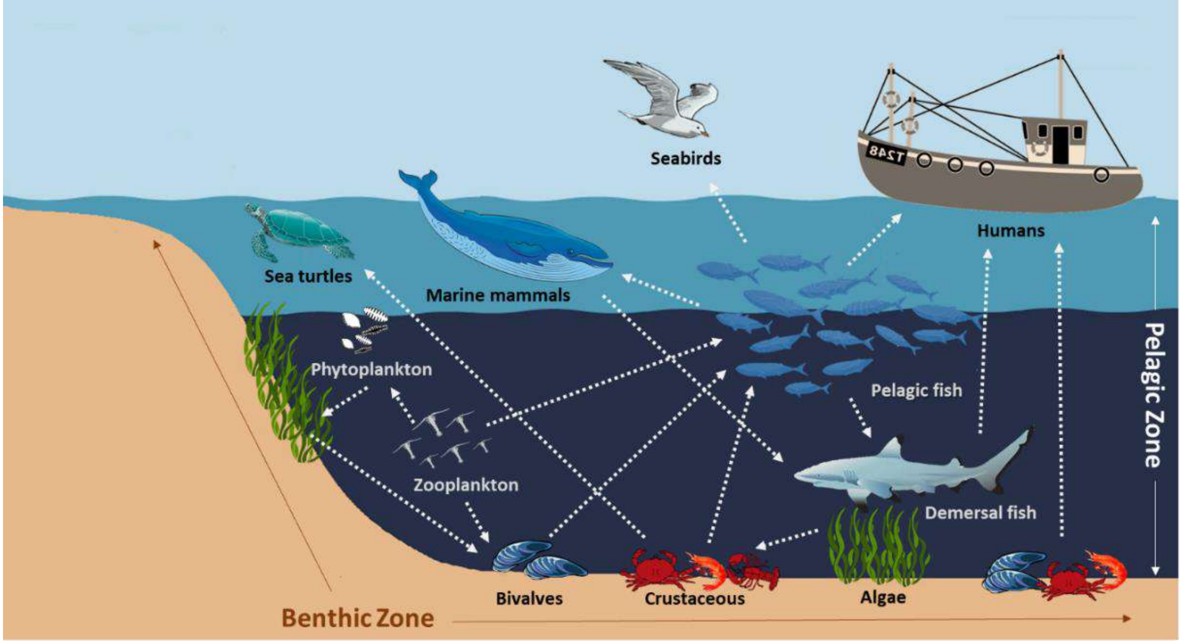


**Figure 4.1** Potential biological interactions in the marine environment**.**

## Effects of humans

✓ *Exposure pathways*

As plastic is used in various everyday products and is ubiquitous in the environment, human exposure to MPLs/NPLs is unavoidable and the most common routes of exposure are ingestion (food and drinking water) and inhalation (Cox et al. 2019; Dris et al. 2015).

The main human exposure may occur by ingestion. The evidence of MPLs has already been reported for several types of food and beverages such as table salt (Kosuth et al. 2018; Yang et al. 2015; Iñiguez et al. 2017; Gündoğdu 2018; Karami et al. 2017), sugar (Liebezeit et al. 2013), honey (Liebezeit et al. 2013), beer (Liebezeit et al. 2014; Kosuth et al. 2018), drinking water (Mintenig et al. 2019; Eerkes-Medrano et al. 2015; Schymanski et al. 2018), and others. Liebezeit et al. (2013) assumed that the presence of non-pollen particulates in honey, especially fibres (166 ± 147 kg$^{-1}$), could be due to airborne contamination. The occurrence of MPLs in drinking water could be due to the contamination of its freshwater sources (rivers, lakes, groundwater, and wastewater) or directly to packaging materials. This is a risk to human health that should be well assessed by improving the quality assurance of water sampling and analysis (Koelmans et al. 2019).

Due to the long-time stability of MPLs/NPLs in the marine environment, one of the main routes of human exposure is via seafood (Hantoro et al. 2019; Smith et al. 2018). As previously reported in this chapter, the capacity for marine organisms to ingest MPLs has been demonstrated in many laboratory studies and in nature. Important sources of dietary exposure to MPLs are filter-feeding organisms (e.g. molluscs, bivalves etc.), which can retain these particles in the gills (De Witte et al. 2014; Van Cauwenberghe et al. 2015). Even after a purification period, high levels of plastics could be accumulated in farm mussels. Fibres, fragments, and pellets MPLs (2.1 - 10.5 items $g^{-1}$) were found in nine commercial bivalves species from China, where aquaculture activities are intense (Li et al. 2015). Generally, the presence of MPLs in GITs of commercial fish is not considered as direct evidence of human exposure, as this organ is usually not consumed. In this case, exposure is only possible if there is the absorption of these particles into the gut of the fish or from the respiratory epithelium of the gill. In contrast, the contaminants, pathogens or additives associated with such particles can be transferred from the litter to the fish tissue becoming a risk to human health (Vanden Bilcke 2003).

Recently, the evidence of MPLs in atmospheric fallout has been reported (Dris et al. 2016). Among sources of airborne MPLs, synthetic textiles (Zubris et al. 2005; Dris et al. 2017), the synthetic rubber tires erosion (Kole et al. 2017; Magnusson et al. 2016) and city dust (Dehghani et al. 2017; Cai et al. 2017) are the most important. The assessment of air in several urban and rural areas of Europe, USA and Japan confirms that tyre and road wear particles represent an average $PM_{10}$ contribution of 0.84% (Panko et al. 2013). In the urban area of Paris, a great abundance of fibres has been reported and almost 30% of them are of synthetic origin (Dris et al. 2015; Dris et al. 2016). Further studies have shown a higher concentration of MPLs indoors than outdoors increasing potential human exposure to MPLs by inhalation (Dris et al. 2017; Gasperi et al. 2015; Prata 2018). In particular, workers in the textile, wool, and PVC industries are the most affected by airborne contamination (Prata 2018).

- ✓ *Potential human health risks*

Although there are various routes of human exposure to MPLs, there is currently no evidence of translocation or absorption of these particles after ingestion of contaminated food or inhalation (SAPEA 2019). The potential hazards to human health from exposure to plastic depend mainly on the properties of these particles (size, shape, surface-charge, etc.) and generally concern NPLs. The three main routes for such particles into the human body are the respiratory system, the GIT and the skin. Among these, absorption into the lungs is limited as it depends on the size. The lung tissue barrier is very thin (< 1 µm) only allows nanoparticles to pass into the blood system. Similarly, NPLs from microspheres used in cosmetics is difficult to penetrate the skin. In this case, the introduction of these plastic particles may be possible through hair follicles, sweat glands and injured skin areas (Alvarez-Román et al. 2004; Campbell et al. 2012).

Consequently, GIT remains the primary exposure site for MPLs/NPLs uptake. Once they are introduced, the gut mucosa blocks potentially toxic substances and organisms as well as the larger plastic particles. Whilst inert and nano/micron-size particles continue their course along the GIT. In vitro studies using intestinal cells have provided information about the internalization and translocation of NPLs in this tract. Generally, there are several pathways by which NPLs can be uptake by cells. Among these, endocytosis, including phagocytosis and pinocytosis, is the most likely to proceed (Galloway 2015). In this step, the cellular uptake is strongly influenced by the size and chemical composition of the surface of the particle. These smaller particles can adsorb other biological macromolecules (e.g. proteins, phospholipids or carbohydrates) from the surrounding body fluid on their surface to form a ‘corona’. Biomolecules can easily bind to NPLs in a variety of ways, including through Van der Waals forces, hydrophobic interactions, dipole-dipole bonding or through interactions between amino acids structure and the benzene ring of polymers. In this way, the density and surface charge of particles increase influencing their bioavailability and toxicity (Galloway 2015). In nanomedicine, this process has been studied for PS-nanoparticles which are used to be functionalized for therapeutic uses. The induced surface charge facilitates the uptake but also produce toxic effects (Kim et al. 2016). Consequent biological impacts reported were uptake of nanoparticles, thrombocyte activation, effects on haemolysis, death of endothelial cells at an early exposure time (Tenzer et al. 2013).

Other biological responses are connected to MPLs assimilation in tissues, such as inflammation and immune responses. Wear particles from plastic prosthetic implants are generally found in the joint capsule, cavity and surrounding tissues. Depending on their size, these particles (in general PE or PET residues) may remain extracellular in the tissue or may pass into lymphatic systems moving throughout the body. The main effects reported were tissue necrosis or granulomatous inflammation (Willert et al. 1996; Urban et al. 2000).

As with marine organisms, the introduction of MPLs/NPLs into the human body leads not only physical effects but also to potentially toxic effects as a result of the transfer of chemicals or pathogenic pollutants. The desorption mechanisms of HOCs from plastic particles in the human digestive systems have not been properly studied to confirm a high risk of exposure to these contaminants. On the other hand, chemical additives incorporated in MPLs and monomers can be released from plastic to tissues and fluids. Among these, brominated flame retardants, phthalate plasticisers (e.g. DEHP), and BPA are the most harmful for human health (Rist et al. 2018) leading to reproductive toxicity, carcinogenicity, and mutagenicity (Mariana et al. 2016; Tickner et al. 2001; Shaw et al. 2014). Besides, during GIT transit or in the lung, the plastic may also be colonised by harmful human pathogens following the ‘corona’ mechanism explained before. Since microbiomes can metabolise a wide range of toxic substances, the effects reported include changes in the immune system that stimulate inflammation and oxidative stress (Kish et al. 2013; Adar et al. 2016; Vanhaecke et al. 2006).

## 4.2 Results

In this section, the ecotoxicological effects of micro(nano)plastics on the marine ecosystem and human health were assessed.

The experimental results are presented in the following publications:

- **A new digestion approach for the extraction of microplastics from gastrointestinal tracts (GITs) of the common dolphinfish (*Coryphaena hippurus*) from the western Mediterranean Sea.** Gabriella F. Schirinzi, Cristina Pedá, Pietro Battaglia, Federica Laface, Matteo Galli, Matteo Baini, Pierpaolo Consoli, Gianfranco Scotti, Valentina Esposito, Caterina Faggio, Marinella Farré, Damiá Barcelò, M. Cristina Fossi, Franco Andaloro, Teresa Romeo*. Journal of Hazardous Materials 397 (2020): 122794.*

- **Cytotoxicity effects of commonly used nanomaterials and microplastics.** Gabriella F. Schirinzi, Ignacio Pérez-Pomeda, Josep Sanchís, Cesare Rossini, Marinella Farré, Damià Barceló. *Environmental Research 159 (2017): 579-587.*

### 4.2.1 Publication IV: A new digestion approach for the extraction of microplastics from gastrointestinal tracts (GITs) of the common dolphinfish (*Coryphaena hippurus*) from the western Mediterranean Sea

*Journal of Hazardous Materials 397 (2020): 122794*

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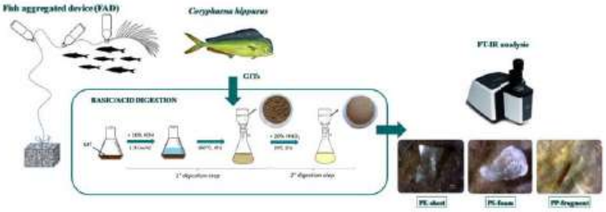
# A new digestion approach for the extraction of microplastics from gastrointestinal tracts (GITs) of the common dolphinfish (*Coryphaena hippurus*) from the western Mediterranean Sea

Gabriella F. Schirinzi[a], Cristina Pedà[b,*], Pietro Battaglia[c], Federica Laface[c], Matteo Galli[d], Matteo Baini[d], Pierpaolo Consoli[c], Gianfranco Scotti[b], Valentina Esposito[f], Caterina Faggio[g], Marinella Farré[a], Damià Barceló[a,e], M. Cristina Fossi[d], Franco Andaloro[c], Teresa Romeo[b,c]

[a] *Institute of Environmental Assessment and Water Research, IDAEA-CSIC, Barcelona, Spain*
[b] *Institute for Environmental Protection and Research, ISPRA, Milazzo, Italy*
[c] *Stazione Zoologica Anton Dohrn, Department of Integrative Marine Ecology, SZN, Messina, Italy*
[d] *University of Siena, Department of Physical, Earth and Environmental Sciences, Siena, Italy*
[e] *Catalan Institute for Water Research, ICRA, Girona, Spain*
[f] *Istituto Nazionale di Oceanografia e di Geofisica Sperimentale, OGS, Trieste, Italy*
[g] *University of Messina, Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, Messina, Italy*

## GRAPHICAL ABSTRACT



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## ABSTRACT

Plastic ingestion is one of the main impacts of marine litter on organisms. The occurrence of microplastics (MPs < 5 mm) in the stomachs of Mediterranean species was already reported in several studies. In this context, the present study aims to develop a new approach of digestion for the identification of MPs in the gastrointestinal tracts (GITs) of marine organisms. The new approach combines two digestion protocols, including potassium hydroxide (KOH) and nitric acid ($HNO_3$), to remove most organic and inorganic materials. This digestion allows recording small MPs that are difficult to find via routinely stomach content analysis and also to minimize the overestimation of the phenomenon trough the control of airborne contamination. The new approach was tested on a voracious pelagic opportunistic predator, the common dolphinfish, a fishery resource exploited in several Mediterranean areas. The results showed that a large amount of ingested meso- and microplastics, such as fragments or sheets, was recorded in GITs (F = 65.5 %). The FTIR analysis on litter samples allowed to identify polyethylene, polypropylene and polystyrene as dominant constituent polymers of microplastics. These results confirmed that our novel combined digestion protocol represents a reliable approach to detect MPs in opportunistic pelagic predators.

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[*] Corresponding author.
*E-mail address:* cristina.peda@isprambiente.it (C. Pedà).

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## 1. Introduction

Marine anthropogenic litter represents a serious problem of the present age which affects the world's oceans (Barnes et al., 2009), including the Mediterranean Sea (Zambianchi et al., 2014; Cózar et al., 2015; UNEP/MAP, 2015). As a result of the growing plastic consumption (Comăniță et al., 2016), plastic items are the most common waste found on the beaches (Topçu et al., 2013; Turner and Holmes, 2011), floating on the sea surface (de Haan et al., 2019d; Suaria et al., 2016), throughout the water column (Choy et al., 2019) and on the seafloor (Derraik, 2002; Watters et al., 2010). In particular, the attention of the scientific community is focused on plastic particles smaller than 5 mm, which are commonly known as microplastics (MPs) (Resmeriță et al., 2018; Arthur et al., 2009). In the marine environment, MPs can derive from direct introduction such as microbeads, which are largely used in consumer and personal care products (primary MPs), or from the fragmentation of meso- and macroplastics exposed to abiotic and biotic factors (secondary MPs) (Andrady, 2011, 2017). Due to the increase in sources and properties of these particles, such as size range, shape and physicochemical properties of polymers (e.g. density), MPs are considered persistent and ubiquitous pollutants in all compartments of the aquatic environment (Sul et al., 2014; Valavanidis and Vlachogianni, 2014; Strungaru et al., 2019). High concentrations of plastics were recorded in the Mediterranean Sea from the sea surface (Panti et al., 2015; Fossi et al., 2017) to the sea bottom (Guerranti et al., 2017; Consoli et al., 2018). The high availability of MPs facilitates the uptake processes by marine biota at different trophic levels (de Sá et al., 2018). Small plastic particles could be accidentally ingested by organisms via filter-feeding or during foraging activities. Due to their shape, size and colour, predators may mistake MPs for potential preys. Moreover, plastics could be found in the stomach contents of marine organisms as a result of secondary ingestion of organisms which have already ingested plastics (Ryan, 2019). The potential impacts of MPs ingestion on marine biodiversity has only recently become an important subject of research (Egbeocha et al., 2018; Rezania et al., 2018; Anbumani and Kakkar, 2018; Guzzetti et al., 2018). Reported adverse effects include mechanical/physical sub-lethal damages (Stamper et al., 2009, 2006; Wright et al., 2013; Jovanović, 2017; Critchell and Hoogenboom., 2018), such as blockage of the intestinal tracts and/or feeding appendages or pseudo-satiation, and chemical harms due to direct (i.e. additives) and/or indirect (i.e. POPs, heavy metals) transfer and release of toxic substances from plastic particles (Betts, 2008; Rochman et al., 2013; Gouin et al., 2011).

The ingestion of microplastics has been already reported in a wide range of Mediterranean marine vertebrate species (e.g. fish, mammalians, etc.) (Bellas et al., 2016; Battaglia et al., 2016; Romeo et al., 2015; Duncan et al., 2019; Romeo et al., 2016; Fossi et al., 2012, 2014; Savoca et al., 2019) as well as invertebrates (molluscs, arthropods, etc.) (Digka et al., 2018; Remy et al., 2015). At a regional level, the European Union has adopted the Marine Strategy Framework Directive (MSFD, 2008/56/EC) which identifies 11 Descriptors to achieve and maintain a Good Environmental Status (GES) of the marine environment. In particular, the MSFD Descriptor 10 "Marine litter" and the respective criteria D10C3 "*The amount of litter and micro-litter ingested by marine organisms is at a level that does not adversely affect the health of the species concerned*" indicate the importance of countering the marine litter impacts and providing new tools to monitor and preserve the marine environment and its organisms from the plastics litter (MSFD, 2013).

Despite several methods have been reported in the literature (Lusher et al., 2017), unfortunately, a standard protocol for the extraction of microplastics from biological tissues has not been established yet. As a matter of fact, Proteinase K currently represents the most efficient digestion method, but the high costs do not make it suitable for large scale samplings (Cole et al., 2014; Löder et al., 2017). Consequently, digestion methods based on acid, alkaline, and oxidative reagents are mostly used for MPs extraction from GITs of fish (Dehaut et al., 2016; Catarino et al., 2017; Courtene-Jones et al., 2017; Cole et al., 2014; Karami et al., 2017; Kühn et al., 2017). However, depending on the reagent and conditions, several limitations were reported. For instance, acids can damage or even destroy some polymeric matrices because of high concentrations and temperatures (Nuelle et al., 2014; Cole et al., 2014), while long exposure time is often required for alkaline and oxidative digestions (Lusher et al., 2017).

In this work, another digestion protocol was tested on a highly migratory species and an opportunistic and voracious medium pelagic predator, the common dolphinfish, *Coryphaena hippurus* Linnaeus, 1758, which is an important fishery resource and represents a target species of medium-scale fishing in several areas of the Mediterranean Sea (Lleonart et al., 1999; Sinopoli et al., 2020). Although some information on the plastic ingestion by common dolphinfish have already been provided through previous dietary or litter studies (Massutí et al., 1998; Deudero and Alomar, 2015; Menezes et al., 2019; Phillips and Bonner, 2015), no chemical digestion has been applied to investigate the presence of plastic in the stomach contents of this species.

In this framework, the aim of this study was to develop a new approach to digestion, based on a combination of basic-acid solutions, to identify MPs in the gastrointestinal tracts (GITs) of those marine organisms with a complex diet, such as *C. hippurus*. Based on the two most common digestion protocols, i.e., the potassium hydroxide (KOH) (Rochman et al., 2015) and the hydrogen peroxide ($H_2O_2$) (Digka et al., 2018), a further digestion step with nitric acid ($HNO_3$) at moderate condition was added to improve the digestion efficiency. In this way, a reliable assessment of the presence of MPs can be achieved and, with a control airborne contamination procedure, overestimation problems can also be avoided. The most efficient method has been validated and applied for the identification of plastic litter in GITs of the common dolphinfish from the western Mediterranean Sea.

## 2. Materials and method

### 2.1. *Samples collection*

Overall, 29 samples of common dolphinfish were collected during 2015.The sampling was carried out by purse-seines and trolling lines under Fish Aggregates Devices (FADs) in the western Mediterranean Sea (southern Tyrrhenian Sea) (Fig. 1). The fork length, (FL, mm) and the total weight (W, g) of each specimen were recorded. Finally, gastrointestinal tracts (GITs) were removed and stored at −20 °C until analysis.

### 2.2. *GITs digestion method*

In this work, a combination of two approaches was applied to digest *C. hippurus*' GITs. In particular, at first, the efficacy of a digestion based on two exiting and independent protocols previously reported for the extraction of MPs from biota (Rochman et al. (2015) and Digka et al. (2018) with minor modifications) was tested. Then, a second digestion step with nitric acid was performed. Overall, the first protocol combined potassium hydroxide and nitric acid (protocol 1: KOH/$HNO_3$), while the second one involved hydrogen peroxide and nitric acid (protocol 2: $H_2O_2$/$HNO_3$).

GITs were firstly weighed, chopped and placed into glass beakers in 1:3 (w/v) with 10 % KOH (Labochimica Srl, Padova, Italy) and 1:20 (w/v) with 30 % $H_2O_2$ (Carlo Erba reagents Srl, Milano, Italy), respectively. The solutions were incubated in a stove at 60 ± 5 °C for 6 h and, then, left at room temperature overnight. Afterwards, the samples were filtered through fibreglass filters (pore size 1.6 μm, GF/A Whatman, GE Healthcare, United Kingdom) using a vacuum pump (1st digestion step). Immediately after the filtration, 40 mL of 20 % $HNO_3$ (≥ 65 % Honeywell Fluka™, Rodano, Italy) were added to the clogged filters generated from the first digestions (2nd digestion step). The acid solution remains in contact with the filter for 60 min at room temperature

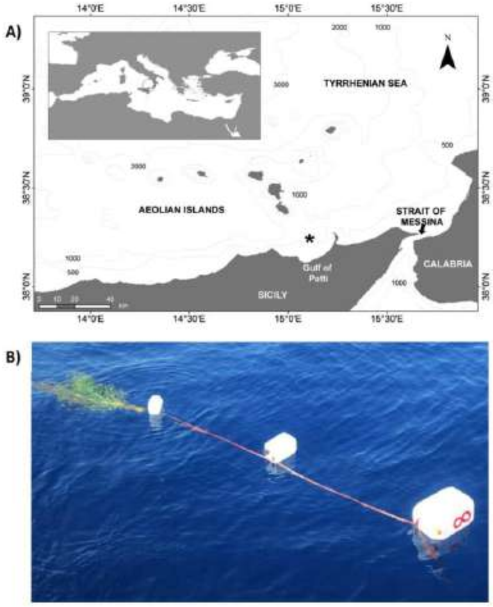

**Fig. 1.** **A)** Map of the study area indicating the sampling site (*) in the western Mediterranean Sea and **B)** Example of Sicilian Fish Aggregate Devices (FADs).

before being removed by filtration. Then, the filters were cleaned with few millilitres of filtered water (2–3 times) and were placed in Petri dishes. Before and after each digestion step, the filters were dried overnight at 40 °C and were weighted to assess the sample weight loss on the filter. The two protocols were compared in terms of digestion efficiency (%*DE*) with a total of n = 5 replicates. The digestion efficiency was estimated as follows: $\%DE = ((W_i - (W_a - W_b))/\, W_i) * 100$, where $W_a$ and $W_b$ represent the dry weights of the filter after and before filtration, respectively; while $W_i$ corresponds to the weight of the digested tissue. Treatment resulting in the digestion efficacy ≥ 95 % was validated (Thiele et al., 2019; Karami et al., 2017; Dehaut et al., 2016).

### 2.3. Recovery and effects of digesting solutions on polymer types

Based on the previous results, the most suitable protocol has been tested for the recovery of microplastics. Plastic particles were created from common items, which are identified by the recycling code appearing on commercial products. The effects of digestion method were assessed on polypropylene (PP, food packaging), polystyrene (PS, coffee cup), expanded polystyrene (EPS, packaging material), polyethylene terephthalate (PET, food packaging), and polyamide or nylon (PA, fishing line). They were cut as rectangular or triangular fragments (except for the nylon which had a cylindrical shape) with sizes ranging between 1 and 5 mm. Each particle was weighed, photographed, and measured using stereomicroscope Zeiss Discovery V.8 coupled with AxioVision V.4.8.2.0 digital image processing software (CTRL). The plastic particles were added to *C. hippurus'* GITs tissue and extracted according to protocol 1 described above (OM). After each digestion step, the particles were recovered, dried overnight at 40 °C, photographed, weighed, and measured again; changes in colour and shape were also recorded. The same procedure was applied without GITs tissue (B) to assess the effects of digestion strictly related to the reagents and temperature. The validation tests were performed in triplicate adding three plastic particles for each polymer with and without organic matter. Finally, to assess the effect of basic-acid agents on polymers identification and possible changes in their chemo-physical properties, plastic particles were analysed by Fourier transform infrared (FTIR) spectroscopy technique (see FTIR analysis section) before and after both digestion steps.

### 2.4. Microplastics identification

The filters obtained from the digestion of the GITs samples were examined under a stereomicroscope Zeiss Discovery V.8 coupled with AxioVision digital image processing software. All particles recovered on the filter were photographed, counted, and measured (length, width, and thickness). Then, they were classified based on their shape (fibres, foams, fragments, sheets and spheres), size (small micro: 0.1 – 1 mm; large micro: 1 – 5 mm; meso: 5 – 25 mm; macro: > 25 mm) and colour following the protocol of the Marine Strategy Framework Directive (2013) (Galgani et al., 2013).

### 2.5. FTIR analysis

Fourier transform infrared (FTIR) spectroscopy technique was used to identify polymers nature. Due to the instrumental limit of detection, only particles with a size > 100 μm were examined, according to Lenz et al. (2015) and Giani et al. (2019). The FTIR analysis was carried out on an Agilent Cary 630 spectrophotometer using specific libraries to identify the polymer composition according to the procedure reported by Fossi et al. (2017) and Bernardini et al. (2018). During the validation phase, considerable changes in chemical composition were considered only if below 70 % of the quality percentage. During the identification of microplastics, polymers matching reference spectra for more than 70 % were accepted as plastics.

### 2.6. Contamination precautions and quality control

To mitigate laboratory contamination, glassware was recommended to be used and all instruments and equipment (including tweezers and scissors) were rinsed thoroughly with filtered water. To evaluate possible cross-contamination during the application of the method, procedural blanks without tissue were also run concurrently with samples. One blank for each batch of samples (4 or 5). Blank samples include 20 mL of 10 % KOH and, successively, 20 mL of 20 % $HNO_3$.

All experiments were conducted under the fume hood and the beakers were covered with paper or aluminium foil between one step and another to limit airborne contamination. Additionally, cotton coats were worn by operators. To avoid fibres overestimation, moist filters in Petri dishes were exposed to the laboratory air and put under the fume hood and near the stereomicroscope. Fibres and particles fixed on these filters were recorded and photographed. Finally, all particles found in the samples presenting the same shape and colour of those in blank samples were excluded from results, as they were considered airborne contamination.

### 2.7. Statistical analysis

Data were tested using non-parametric tests. Differences in %*DE* of each of the extraction protocol tested in this study (protocol 1: KOH/$HNO_3$; protocol 2: $H_2O_2$/$HNO_3$) were analyzed using a one-way non-parametric multivariate analysis (PERMANOVA). Then, the same statistical test was also used to examine differences in weight and size (length and width) between the virgin polymers (CTRL) and those resulting from each of the digestion steps in protocol 1, in the presence (OM) and absence (B) of biological tissue. A two-way crossed (or nested for PA-polymer) PERMANOVA was applied using "digestion step" and "OM/B" as factors. All these analyses were based on Euclidean distances and were carried out using 4999 random permutations. Prior to the analyses, data were square-root transformed. Pair-wise comparison was computed when significant differences ($p < 0.05$) among factors levels were detected. Statistical analyses were performed using the software PRIMER v.6 with PERMANOVA+ (Gorley and Clarke, 2006; Anderson, 2001). All data are expressed as mean percentage ± SEM (standard error of the mean).

## 3. Results

### 3.1. *GITs digestion protocol efficiencies*

The two protocols described above were compared in term of digestion efficiency (*%DE*). As reported in Fig. 2, the extraction yields obtained for the first digestion step were 98.0 % ± 0.16 and 92.9 % ± 1.31 (mean% ± SEM) for KOH and $H_2O_2$, respectively. Even if the *%DE* was high in both cases, a carryover of organic and inorganic matrix was observed on the filters' surface (Fig. 3A and B). After the second digestion step, the efficiency increased to 99.6 % ± 0.11 and 94.8 % ± 1.14 for protocol 1 and 2, respectively. Significant differences between the two treatments ($F_{3,12}$ = 12.903; $p < 0.01$) and within the protocol 1 (KOH *vs* KOH/$HNO_3$, $p < 0.05$) were observed. Conversely, no difference was recorded within the protocol 2 ($H_2O_2$ *vs.* $H_2O_2$/$HNO_3$). Filters surface obtained by protocol 1 appeared clear and homogenous with minor traces of tissues after nitric acid action (Fig. 3C). On the contrary, clumps of undigested tissues were found on the filters obtained by protocol 2 (Fig. 3D). Differences among protocols and their steps were confirmed by statistical analysis (PERMANOVA). As a result, due to its high efficiency, protocol 1 was validated and applied to extract microplastics from GITs of *C. hippurus*.

### 3.2. *Effects of protocol 1 (KOH/$HNO_3$) on five polymer classes and recovery*

Fig. 4 summarizes the effects on five polymer classes after each step of protocol 1. Polymers tested appeared sufficiently resistant to basic and acid solutions used in this experiment. Fig. S-1 and S-2 show the visual effects observed for each polymer, while Fig. S-3 and Fig. S-4 report the observed changes in size (length and width) and weight, respectively. Overall, PP, PS, and EPS appeared widely resistant to the digestion agents used. The results of PERMANOVA evidenced significant differences in EPS and PS length between OM and B experiments ($p < 0.05$). An increase in weight was observed for EPS, but this was not statistically significant. Differently, weight losses were recorded for PET fragments whose sides appeared deformed into a curve with a switch of colour from transparent to opaque. Overall, apart from the PA, no significant weight differences were found within protocol 1 and similar results were recorded with (OM) and without (B) tissue ($F_{1,26}$ = 11.221; $p < 0.01$). After the application of basic conditions, size changes were visible for PA in both B and OM experiments, with highly significant differences in width ($p < 0.005$). After the addition of the nitric acid, PA was recovered only in the presence of organic material (OM). In this case, the pair-wise comparison showed highly significant differences ($p < 0.01$) after each digestion step of protocol 1 with changes in shape (with an increase in width) and colour (Fig. S-2 of Supporting Information). In summary, except for PA, all plastic particles were recovered with 100 % efficiency in both B and OM experiments, and changes in the chemical composition of polymers were not observed after FTIR analysis (Fig. S-5).

### 3.3. *Identification of microplastics in GITs*

A total of 29 *C. hippurus* (FL: 266 ± 30.7 mm, W: 201.9 ± 53.9 g) were analysed. Overall, 65.5 % of the examined GITs were identified to have ingested anthropogenic litter (Table 1). A total of 106 potential plastic particles were recorded of which only 70.7 % (n = 75) were identified as anthropogenic litter. The items that have been positively identified as plastics include 7 mesoplastics and 68 microplastics. In particular, small MPs were the majority (61.7 %) with a size ranging from 0.21 to 0.95 mm, while large MPs (38.3 %) ranged from 1.17 to 5.02 mm. Overall, the average number of plastics per individual in all examined samples was 2.6 items/specimen; while in those individuals containing plastics was 3.9 items/specimen. Fibres (42.6 %) and fragments (34.7 %) result as predominant shape categories, followed by sheets (20 %) and foams (2.7 %) (Fig. 5A). Whereas, the most common colours observed were blue, transparent, and white (Fig. 5B). Finally, the main polymer types identified by FTIR were polyethylene (PE, 36 %), polystyrene (PS, 14 %), polypropylene (PP, 11 %), and polyethylene terephthalate (PET, 5%) followed by ethylene propylene diene monomer (EPDM), polyvinyl chloride (PVC), neoprene, styrene butadiene rubber (SBR), and polyacrylate polyester (Fig. 5C). In the case of fibres, the identification of polymers was not achieved by FTIR due to their thickness. Fig. 6 reports some examples of meso- and microplastics found in fish GITs.

## 4. Discussion

The present study aimed to develop a new digestion method for the identification of microplastics in GITs of marine organisms. To increase the efficiency of the method and to minimize processing time, two digestion methods were combined. As a starting point, alkaline (10 % KOH) and oxidizing (30 % $H_2O_2$) digestion were tested due to their broad application in MPs extraction from biological tissue. However, the long processing time, ranging from 12 h to one week, makes inconvenient their application in large scale. Additionally, depending on the species, the presence of tissue carryover on the filter makes the MPs identification difficult. As a matter of fact, opportunistic species as *C. hippurus* are active predators and, therefore, may eat a wide variety of species including fish, squids and crustaceans. This results in the presence of skeletons remains in the filter (e.g. vertebrae, otoliths, beak of cephalopods and crustaceous carapaces, etc.) after the implementation of common digestion methods. Here, the introduction of an additional step with $HNO_3$ was proposed allowing an important reduction in the amount of non-digested tissues on the filter, especially the inorganic matrix. In this way, potential underestimation or overestimation of MPs is prevented. The procedure resulting in the smallest tissue fraction remaining on the filters was then further validated. Higher efficiency was observed when basic and acid agents were combined as in protocol 1 (KOH/$HNO_3$). The higher temperatures (T = 60 °C) applied during the first digestion step also allowed to reduce the incubation time to 6 h.

To assess the effects of the selected protocol on polymers, five categories of plastics (PS, EPS, PP, PET and PA) were analysed before and after each digestion step. These polymers were chosen matching the information reported from previous studies (Lusher et al., 2017; Avio et al., 2015; Karami et al., 2017). Negative effects on pH-sensitive polymers by basic and acid treatments have been previously reported.

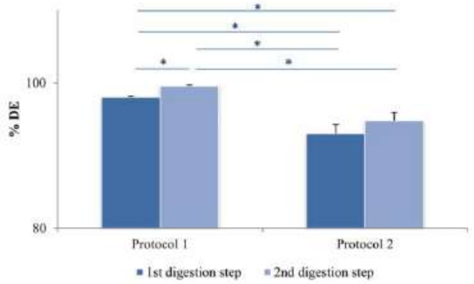

**Fig. 2.** Digestion efficiencies (*%DE*) on *C. hippurus*' GITs for protocol 1 (10 % KOH at 60 °C, 6 h + 20 % $HNO_3$ at room temperature, 1 h) and protocol 2 (30 % $H_2O_2$ at 60 °C, 6 h + 20 % $HNO_3$ at room temperature, 1 h). Statistically significant differences ($p < 0.05$) were indicated in the graph (*). Data are expressed as mean percentage ± SEM (N = 5).

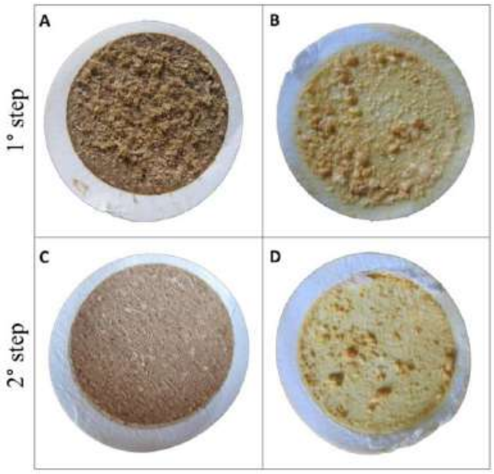

**Fig. 3.** Filters obtained from digestion of *C. hippurus*' GITs with protocol 1 (10 % KOH at 60 °C, 6 h + 20 % $HNO_3$ at room temperature, 1 h) (A and C) and protocol 2 (30 % $H_2O_2$ at 60 °C, 6 h + 20 % $HNO_3$ at room temperature, 1 h) (B and D).

Tabulated data confirmed that PA, PS, and PP resist to the alkaline reagents, while PC and PET are partially degraded by 10 % KOH (Lusher et al., 2017; Karami et al., 2017). In particular, Karami et al. (2017) reported a decrease in recovery rate of no more than 18 % for PET particles (< 300 µm), treated with KOH for 12 h, by increasing the temperature to 60 °C.

Additionally, previous studies reported that the majority of polymers are not resistant to high concentrations of nitric acid (≥ 69 % $HNO_3$) when high temperatures were applied (T ≥ 50 °C) (Lusher et al., 2017; Claessens et al., 2013). However, reducing acid concentration may lead to an increase of the resistance of several polymers (e.g. PA, PC, PE, PET, PP, PS and PVC) at room temperature. Within this framework, the degradation effects on selected plastics have been limited by developing a method with a strong base and acid under soft conditions. Similar strategies were previously proposed to enhance digestion efficiency (Roch and Brinker, 2017; Collard et al., 2015), but higher temperature and concentrations were applied. The results of the validation test confirmed that particles were efficiently recovered from spiked GITs samples with minimal modifications. Among others, more important damages were recorded for PA confirming its sensitivity in acid medium.

The polymer type identification by FTIR showed that PE was the most abundant plastic compound, followed by PS and PP. Generally, they represent the most common polymers widespread in the marine environment (Andrady, 2011). Actually, more than 50 % of floating litter in the Western Mediterranean Sea is represented by high- and low-density polyethylene (HD-PE and LD-PE) (Suaria et al., 2016), which is mainly used for packaging as well as fishing gear manufactures (along with polypropylene and nylons) (Andrady, 2011). Also, PP is largely used for bottles or jars, as rigid packaging, while PS (solid and foam) is widely used to protect consumer products or in food packaging. These polymer categories are largely found as plastic debris. Finally, the other types of polymers identified are less frequent in the Mediterranean Sea (Suaria et al., 2016).

In general, the occurrence of plastic particles in *C. hippurus*' GITs may be due to its generalist feeding behaviour and predation on schooling prey (as observed for other pelagic predators) (Romeo et al., 2015; Battaglia et al., 2016). Most of the information about plastic ingestion by common dolphinfish from different areas come from diet and feeding habits studies (Massutí et al., 1998; Deudero and Alomar, 2015; Menezes et al., 2019). Few details on ingested litter are reported, but the meso- and macroplastic fragments found in their stomachs generally come from fishing gear manufactures and other common trash (e.g. bottle caps, balloon fragments, and bags) (Menezes et al., 2019). The common dolphinfish normally lives in aggregate shoals under large floating objects, mainly debris, for its protection and/or predation (Taquet et al., 2007; Massutí et al., 1998; Gatt et al., 2015; Castriota et al., 2007). In Mediterranean areas, during the period from the end of summer to the winter, fishermen use FADs to catch this species by surrounding nets (Potoschi et al., 1999). These artificial structures may enhance food availability because of an increase in plankton concentrations around the floating objects and provide shelter from predators. Nevertheless, FADs could also represent potential sources of debris in the marine environment as well as accumulation areas of litter and other contaminants (Sinopoli et al., 2019). Therefore, plastic ingestion can be strongly promoted by the presence of FADs. More than 65,000 FADs per year are employed in the Mediterranean (Morales-Nin et al., 1999). Anchored FADs are largely used in the southern Mediterranean, especially in the coastal areas of Sicily, Tunisia, Malta, and Majorca (Spain) (Consoli et al., 2020). These devices are normally equipped with palm leaves connected to a float (usually empty plastic bottles or PS-slabs tied together) using synthetic rope or PE/nylon cables for anchoring to large stones or blocks (Andaloro et al., 2007; Sinopoli et al., 2020). In winter, at the end of the fishing season, most FADs are lost (76 %), especially those far from the coast, or destroyed

| **Digestion step** | | **PP** | **PS** | **EPS** | **PET** | **PA** |
|---|---|---|---|---|---|---|
| **1.** 10% KOH (60°C, 6h) | Weighing | ○ | ○ | ○ | ○ | ○ |
| | Visual | ○ | ○ | ○ | ◐ | ○ |
| | Size | ○ | ○ | ○ | ○ | ◐ |
| | FTIR | ○ | ○ | ○ | ○ | ○ |
| **2.** 10% KOH (60°C, 6h) + 20% $HNO_3$ (RT, 1h) | Weighing | ○ | ○ | ○ | ○ | ○ |
| | Visual | ○ | ○ | ○ | ◐ | ● |
| | Size | ○ | ○ | ○ | ○ | ◐ |
| | FTIR | ○ | ○ | ○ | ○ | ○ |

○ = no significant changes
◐ = partial changes
● = significant changes

**Fig. 4.** Summary of the changes on polymers for each step of protocol 1 (KOH/$HNO_3$).

**Table 1**
Frequency of occurrence (%) of ingested plastic particles, average numbers of plastic items per GITs of common dolphinfish sampled in the western Mediterranean Sea.

| Species | Number of GITs examined | Number of GITs with plastics | Frequency of occurrence (%) | Average number of items in all individuals examined | Average number of items in individuals containing plastics |
|---|---|---|---|---|---|
| *Coryphaena hippurus* | 29 | 19 | 65.5 | 2.6 | 3.9 |

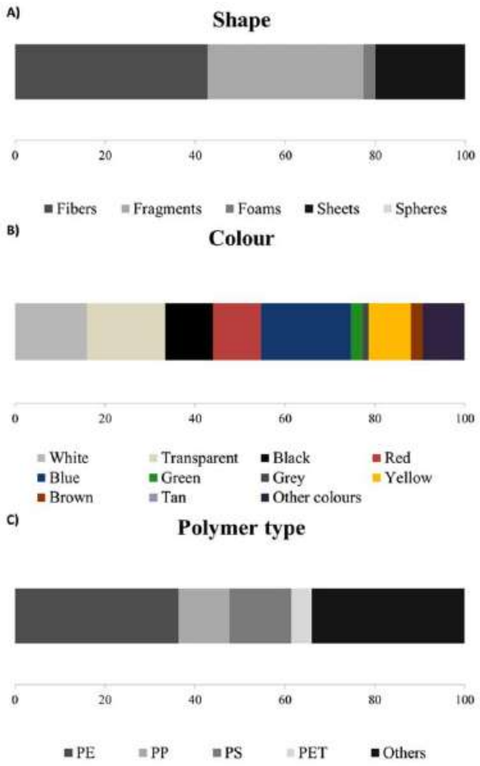

**Fig. 5.** Shape (A), colour (B) and polymer type (C) of plastics particles detected in 29 GITs of *C. hippurus*.

**Fig. 6.** Examples of meso- and microplastics found in fish GITs of *C. hippurus*. A) PE-sheet, B) PP-fragment, C) PS-foam, and D) SBR- fragment.

by adverse weather conditions. It has been estimated that more than 1.5 million of FADs were abandoned in the Mediterranean basin from 1961 to 2017. A high number of FAD components were reported as litter in the Sicilian area, including PS slabs and PE cables/ropes (Sinopoli et al., 2020). As a result, FADs are identified as potential sources of marine debris and the degradation of their plastic components may increase the chance for the introduction of synthetic materials into the marine food web (Rochman et al., 2015).

## 5. Conclusions

The obtained results confirmed that the combination of basic-acid digestion (KOH/$HNO_3$) represents a reliable approach to detect MPs in the GITs of a pelagic fish predator, avoiding overestimation problems through an airborne contamination control procedure. Although this approach has been tested on a medium pelagic species with a large spectrum of prey ranging from zooplankton to nekton, the digestion protocol can also be adapted to other species. The common dolphinfish has been already considered among pelagic sentinel species for marine litter ingestion at medium-spatial scale (Mediterranean UN Environment/MPA sub-regions), according to biological and ecological criteria (habitat, distribution, home range, etc.) by Fossi et al. (2018). In view of this, the present study suggests that common dolphinfish provides good information on the ingestion of MPs and future monitoring studies on this species should be encouraged.

## CRediT authorship contribution statement

**Gabriella F. Schirinzi:** Methodology, Investigation, Validation, Writing - original draft. **Cristina Pedà:** Methodology, Investigation, Validation, Visualization, Data curation, Writing - review & editing. **Pietro Battaglia:** Writing - review & editing. **Federica Laface:** Validation. **Matteo Galli:** Methodology. **Matteo Baini:** Methodology. **Pierpaolo Consoli:** Resources, Formal analysis. **Gianfranco Scotti:** Resources. **Valentina Esposito:** Resources, Formal analysis. **Caterina Faggio:** Supervision. **Marinella Farré:** Supervision. **Damià Barceló:** Supervision. **M. Cristina Fossi:** Supervision. **Franco Andaloro:** Supervision. **Teresa Romeo:** Conceptualization, Supervision, Project administration, Funding acquisition.

## Declaration of Competing Interest

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

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2020.122794.

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*Supporting Information for:*

# A new digestion-approach for the extraction of microplastics from gastrointestinal tracts (GITs) of the common dolphinfish (*Coryphaena hippurus*) from the western Mediterranean Sea

Gabriella F. Schirinzi[1], Cristina Pedà[2,*], Pietro Battaglia[3], Federica Laface[3], Matteo Galli[4],Matteo Baini[4],Pierpaolo Consoli[3], Gianfranco Scotti[2], Valentina Esposito[6],Caterina Faggio[7], Marinella Farré[1], Damiá Barcelò[1,5], M. Cristina Fossi[4], Franco Andaloro[3],Teresa Romeo[2,3]

[1]Institute of Environmental Assessment and Water Research, IDAEA-CSIC (Barcelona, Spain)
[2]Institute for Environmental Protection and Research, ISPRA (Milazzo, Italy)
[3]Stazione Zoologica Anton Dohrn, Department of Integrative Marine Ecology, SZN, (Messina, Italy)
[4]University of Siena, Department of Physical, Earth and Environmental Sciences (Siena, Italy)
[5]Catalan Institute for Water research, ICRA (Girona, Spain)
[6]Istituto Nazionale di Oceanografia e di Geofisica Sperimentale, OGS (Trieste, Italy)
[7]University of Messina, Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, (Messina, Italy)

*Corresponding author, e-mail: Cristina Pedà, cristina.peda@isprambiente.it

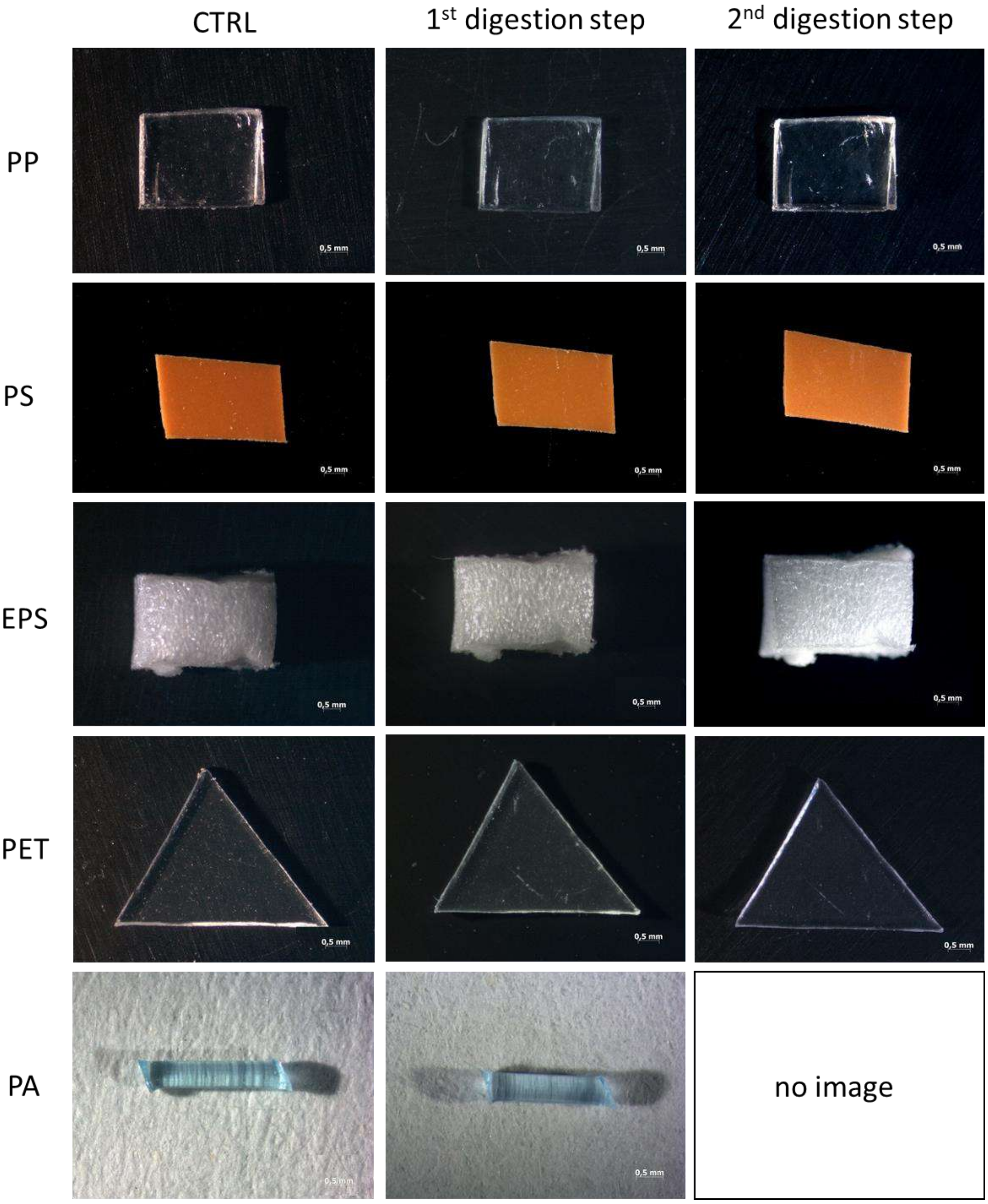


**Figure S-1.** Visual effects after the application of protocol 1 on five polymers (Blk test; N = 3); polypropylene (PP), polystyrene (PS), expanded polystyrene (EPS), polyethylene terephthalate (PET), and polyamide (PA) before digestion (CTRL), after the first digestion step (10% KOH at 60°C, 6h), and the second digestion step (10% KOH at 60°C, 6h + 20% HNO3 at room temperature, 1h).

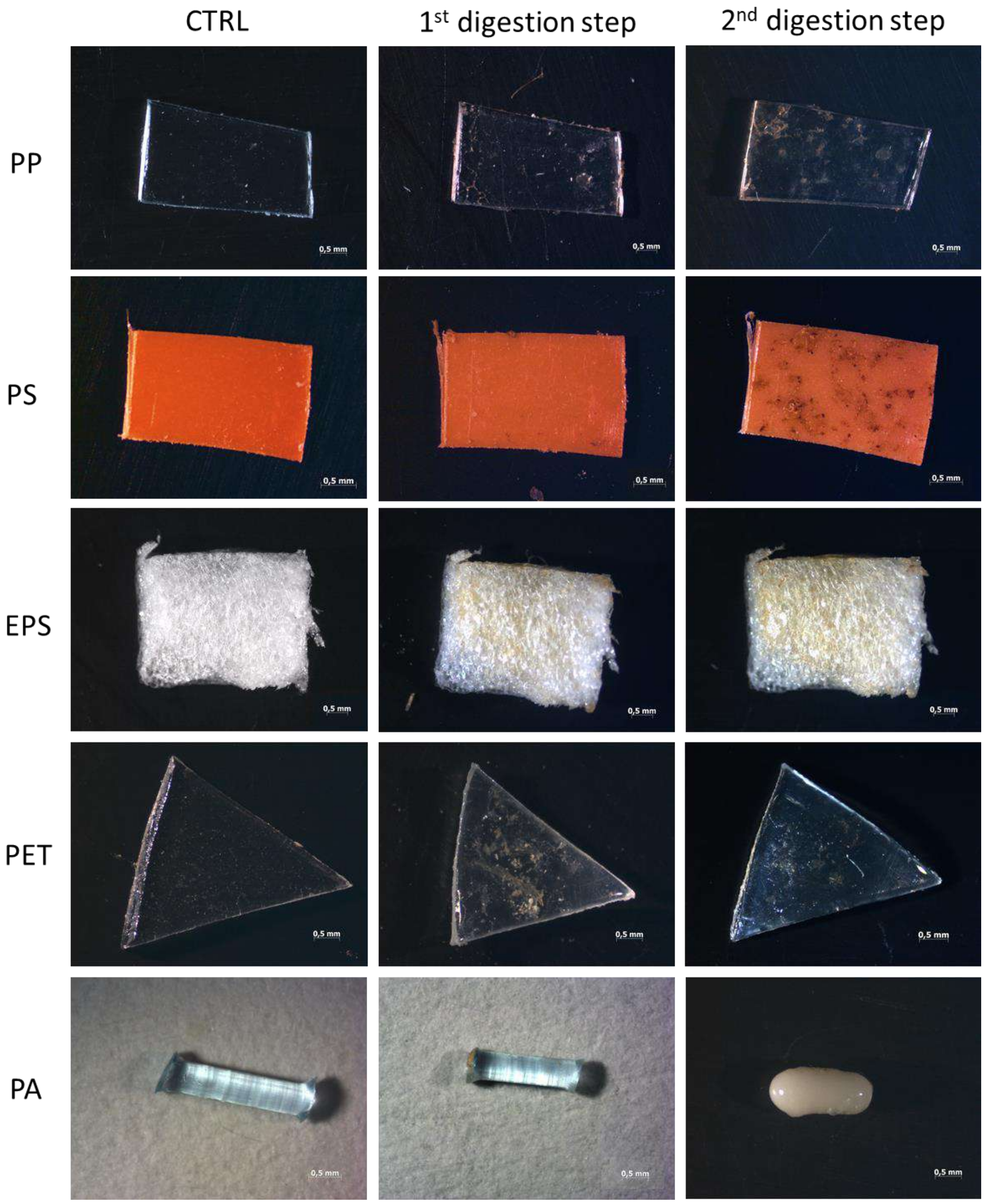


**Figure S-2.** Visual effects after the application of protocol 1 on five polymers with the organic matter (N = 3); polypropylene (PP), polystyrene (PS), expanded polystyrene (EPS), polyethylene terephthalate (PET), and polyamide (PA) before digestion (CTRL), after the first digestion step (10% KOH at 60°C, 6h) and the second digestion step (10% KOH at 60°C, 6h + 20% HNO3 at room temperature, 1h).

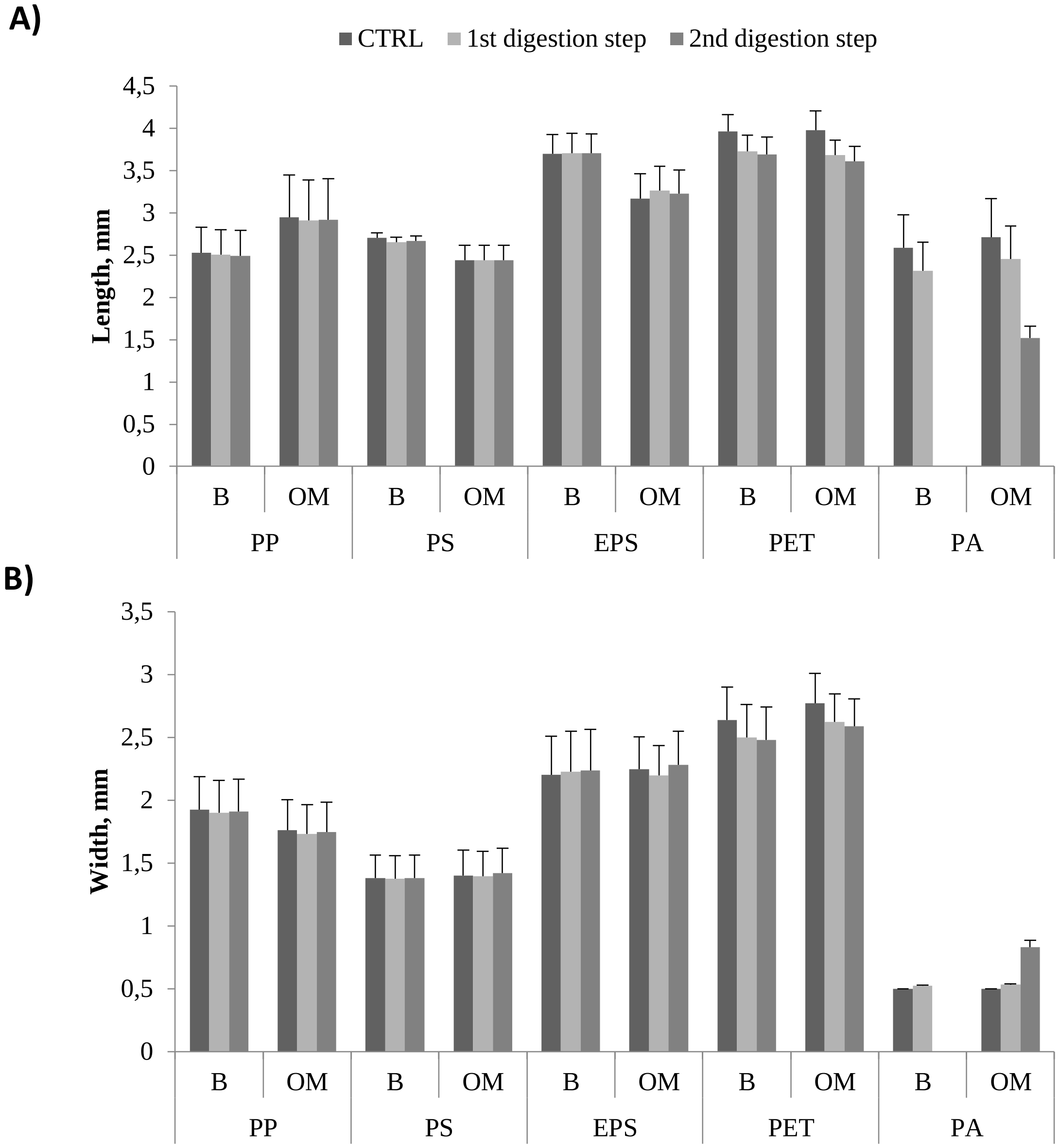


**Figure S-3.** Bar chart describing the changes in length (A) and width (B) of polypropylene (PP), polystyrene (PS), expanded polystyrene (EPS), polyethylene terephthalate (PET) and nylon or polyamide (PA) polymers before (CTRL) and after each digestion step in presence (OM) and absence (B) of the organic matrix (N = 3). Data are expressed as mean ± SEM (N = 9)

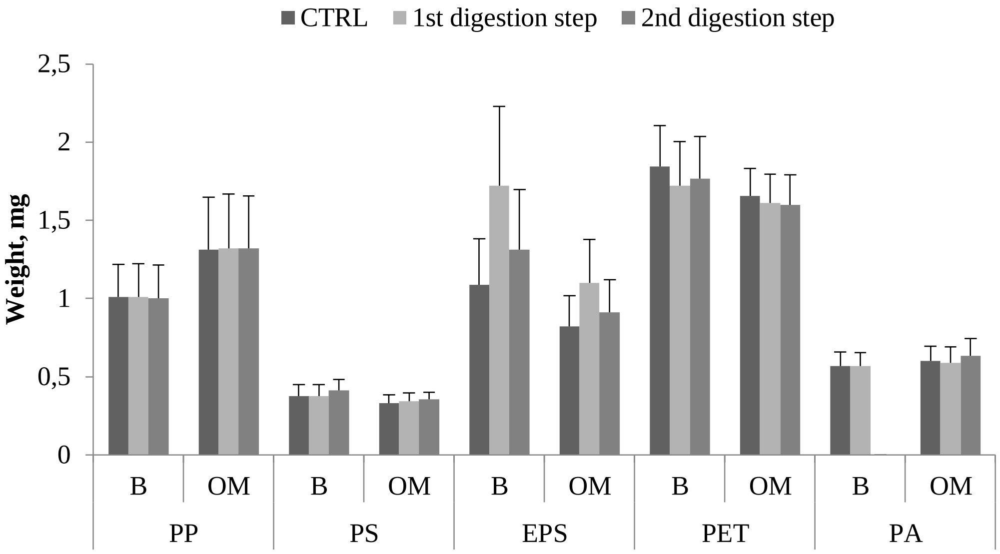
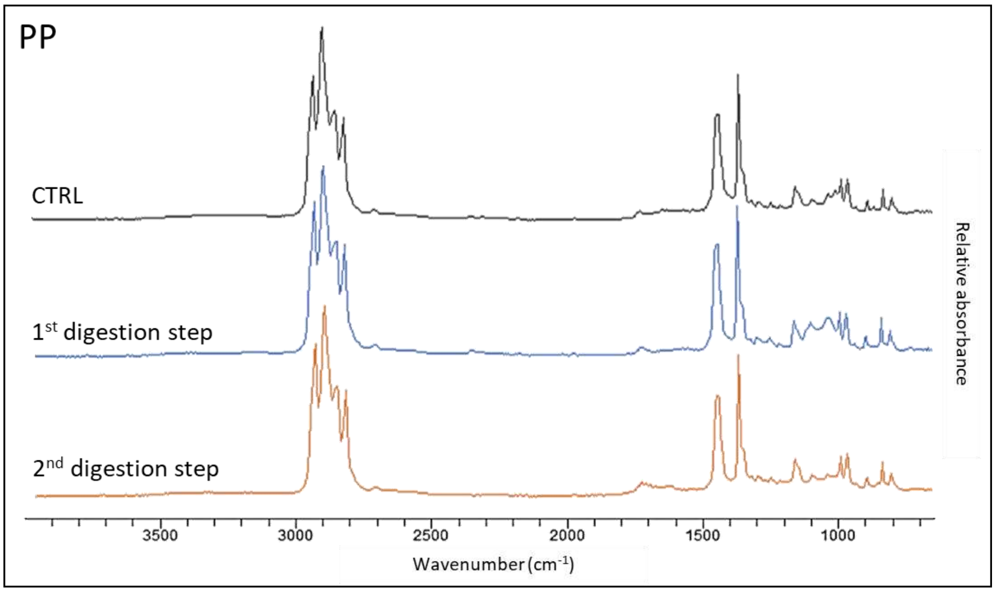


**Figure S-4.** Bar chart describing the differences in weight of polypropylene (PP), polystyrene (PS), expanded polystyrene (EPS), polyethylene terephthalate (PET) and nylon or polyamide (PA) polymers before (CTRL) and after each digestion step in presence (OM) and absence (B) of the organic matrix (N = 3). Data are expressed as mean ± SEM (N = 9).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CTRL
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2nd digestion step
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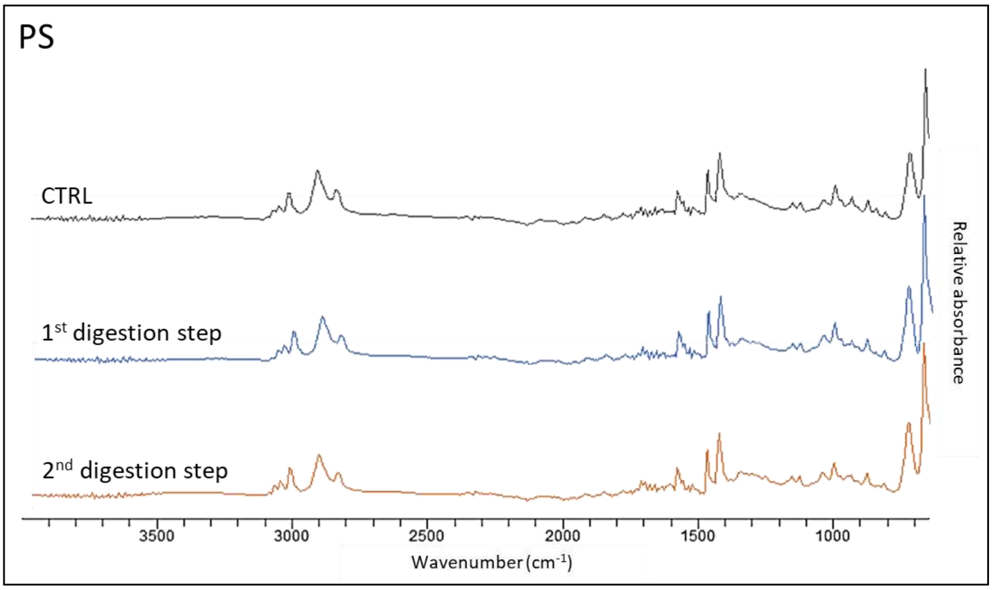
3000
2500
2000
1500
1000
Wavenumber (cm-1)
Relative absorbance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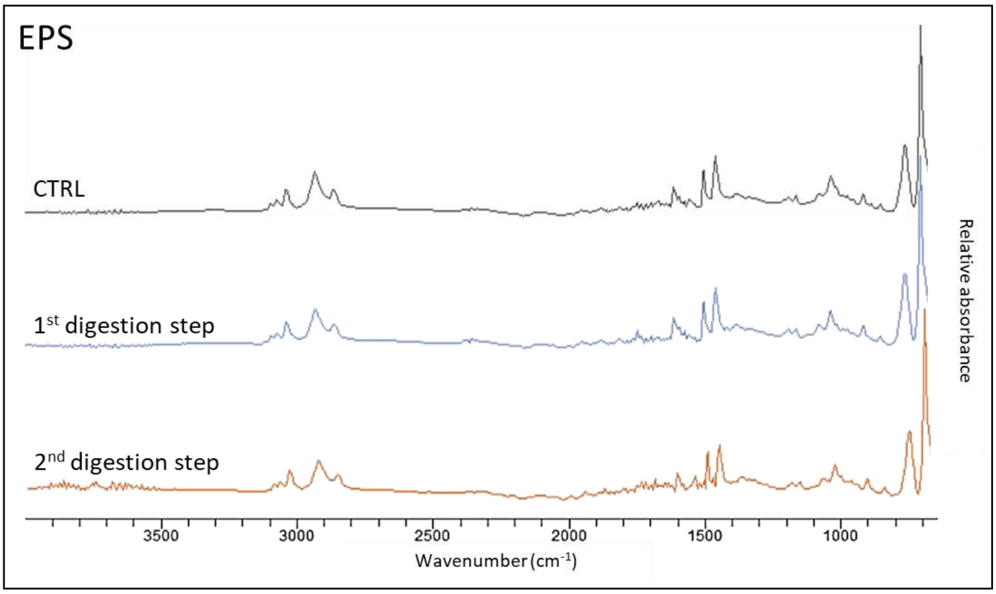
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3500
3000
2500
2000
1500
1000
Wavenumber (cm-1)
Relative absorbance

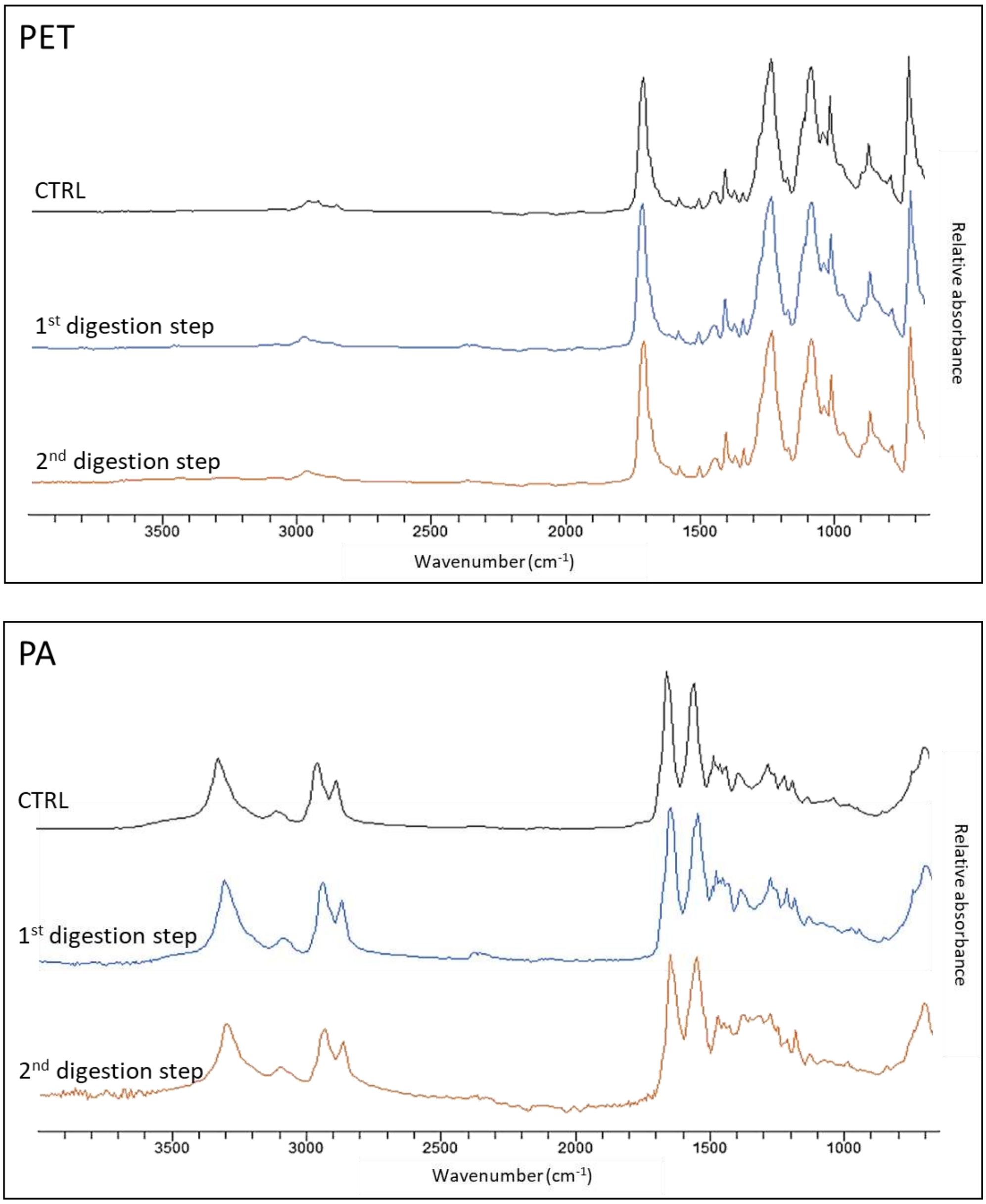

**Figure S-5.** FTIR spectra of polypropylene (PP), polystyrene (PS), expanded polystyrene (EPS), polyethylene terephthalate (PET) and nylon or polyamide (PA) polymers before (CTRL) and after each digestion step with (OM) organic matter (N = 3).

### 4.2.2 Publication V: Cytotoxic effects of commonly used nanomaterials and microplastics on cerebral and epithelial human cells

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# Cytotoxic effects of commonly used nanomaterials and microplastics on cerebral and epithelial human cells

Gabriella F. Schirinzi[a,d], Ignacio Pérez-Pomeda[b,d], Josep Sanchís[a,d], Cesare Rossini[c,d], Marinella Farré[a,d,*], Damià Barceló[a,b,d]

[a] *Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Catalonia, Spain*
[b] *Institute for Advanced Chemistry of Catalonia (IQAC), Barcelona, Catalonia, Spain*
[c] *Thermo Fisher Scientific, StradaRivoltana, 20090 Rodano, Milano, Italy*
[d] *Catalan Institute for Water Research (ICRA), Girona, Catalonia, Spain*

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ABSTRACT

Plastic wastes are among the major inputs of detritus into aquatic ecosystems. Also, during recent years the increasing use of new materials such as nanomaterials (NMs) in industrial and household applications has contributed to the complexity of waste mixtures in aquatic systems. The current effects and the synergism and antagonisms of mixtures of microplastics (MPLs), NMs and organic compounds on the environment and in human health have, to date, not been well understood but instead they are a cause for general concern.

The aim of this work is to contribute to a better understanding of the cytotoxicity of NMs and microplastics/nanoplastics (MPLs/NPLs), at cell level in terms of oxidative stress (evaluating Reactive Oxygen Species effect) and cell viability. Firstly, the individual cytotoxicity of metal nanoparticles (NPs) (AgNPs and AuNPs), of metal oxide NPs ($ZrO_2$NPs, $CeO_2$NPs, $TiO_2$NPs, and $Al_2O_3$NPs), carbon nanomaterials ($C_{60}$fullerene, graphene), and MPLs of polyethylene (PE) and polystyrene (PS) has been evaluated *in vitro*. Two different cellular lines T98G and HeLa, cerebral and epithelial human cells, respectively, were employed. The cells were exposed during 24–48 h to different levels of contaminants, from 10 ng/mL to 10 µg/mL, under the same conditions. Secondly, the synergistic and antagonistic relationships between fullerenes and other organic contaminants, including an organophosphate insecticide (malathion), a surfactant (sodium dodecylbenzenesulfonate) and a plasticiser (diethyl phthalate) were assessed. The obtained results confirm that oxidative stress is one of the mechanisms of cytotoxicity at cell level, as has been observed for both cell lines and contributes to the current knowledge of the effects of NMs and MPLs-NPLs.

## 1. Introduction

During the last century, technological developments triggered the promotion of new materials such as plastics which, due to their excellent mechanical properties, versatility and low cost impelled their consumption. Therefore, since the 1940s, when the first production of plastics at the industrial scale took place, their manufacture has steadily grown (Al-Salem et al., 2010). In addition, from 2000 to 2010 a massive growth of the world's yearly consumption was produced in relation to the growing demand in developed economies such as the EU, Canada and the USA, together with the new demand and enhanced consumerism in emerging economies such as China. However, this expansion comes with the generation of tonnes of waste, some of which are non-biodegradable and toxic by-products, resulting in new undesirable impacts on the environment (Tongesayi and Tongesayi, 2016). Moreover, to date, the environmental fate and time of total degradation have not been well understood. In particular, microplastics (MPLs), defined as small particles at the millimetre to sub-millimetre size range with high densities, are a new environmental risk (da Costa et al., 2017; Horton et al., 2016). The origin of MPLs can be from the manufacturing of micro-beads that are used in cosmetic facial cleansers (Fendall and Sewell, 2009). Alternatively, secondary MPLs can result from the fragmentation and erosion of plastic items (Aueviriyavit et al., 2014). During recent years, the presence of plastics and MPLs in marine and coastal areas has been a cause for concern (Cole et al., 2011), attracting the attention of both researchers and the general public. Low density MPLs contaminate the sea surface and due to their small size, which is similar to plankton, they could be introduced into the food chain (Wright et al., 2013). Another important uncertainty is whether these compounds can act as vector for transferring pollutants to biota,

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* Corresponding author at: Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Catalonia, Spain.
*E-mail address:* mfuqam@cid.csic.es (M. Farré).

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thus leading to their bioaccumulation (Llorca et al., 2014; Cedervall et al., 2012).

On the other hand, during the last decade, developing nanotechnology has promoted a new series of materials and opportunities for different industrial and consumer products. Nanomaterials (NMs) are characterized by their dimensions in the range of 1–100 nm. Key factors driving the fact that NMs differ significantly from pollutants at the macro-scale are their high surface area and quantum effects (Farré and Barceló, 2012). These factors can modify some properties such as electrical or optical reactivity and strength, thereby altering the fundamental physical and chemical properties of conventional materials. Current applications in nanotechnology span from electronics (Sanchez et al., 2011), household products, food additives and food packaging (Eleftheriadou et al., 2017; Ranjan et al., 2014), personal care products, nanomedicine (Marchesan and Prato, 2013), sports equipment, textiles (Contado, 2015), among many others. With the ongoing commercialization of NMs, human exposure to nanoparticles will dramatically increase, and evaluation of their potential toxicity is essential (Holden et al., 2016).

During recent years, a high number of studies have evaluated the ecotoxicological effects on NMs (Yan et al., 2011; Fu et al., 2014) as well as the environmental fate and behaviour of NPs (Nickel et al., 2014). In particular, NMs and NPs, which currently have an extensive range of applications such as metal and metal oxide NPs (among which there are silver nanoparticles (AgNPs), gold nanoparticles (AuNPs), and titanium dioxide nanoparticles ($TiO_2$), and more) have been studied more by using *in vivo* and *in vitro* approaches (Johnston et al., 2010; Wijnhoven et al., 2009). For instance, due to the antibacterial properties of AgNPs, they are exploited in an increasing number of consumer and medical products such as packaging materials (Aueviriyavit et al., 2014), clothing, wound dressings, deodorants and spray rooms (Vigneshwaran et al., 2007; Lee et al., 2007). Regarding Au-NPs, they are used in current biomedical applications including diagnostics, photo-thermal and photodynamic therapies and delivery of target molecules such as drugs or peptides (Austin et al., 2015; Dykman and Khlebtsov, 2012). On the other hand, recent research has investigated the uptake in plants of some metal oxide NPs such as $nCeO_2$ (nano-scaled cerium oxide) and the introduction of these into the food chain (Rico et al., 2011). On the other hand, other NMs such as fullerenes and carbon nanotubes (CNTs) have recently been considered in different toxicological and bioaccumulation studies (Maes et al., 2014) because, firstly, in addition to nanotechnology they can be emitted by incidental and natural sources into the environment. Secondly, because of their adsorption/desorption capabilities, they could influence the toxicity of other co-contaminants (Sanchís et al., 2016).

The lack of information on the toxicology of NMs under certain scenarios of exposure has led to the restricted use of some of them for certain applications that are directly in contact with humans, such as inclusion in cosmetics, detergents and food, in the prevention of their potential toxicity or long-term secondary adverse effects (Yoo-Iam et al., 2014; Khanna et al., 2015). But, due to the impact of anthropogenic activities, the concentrations of some NPs in the aquatic environment increase and organisms can uptake them by ingestion or through the skin. Therefore, the increasing bioconcentration of these substances in the tissue of organisms leads to successively higher levels that move along the food chain. However, this field continues being in an initial phase of development and most of the current data are regarding high concentrations of exposure and the effects of NPs and NMs at low concentrations of exposure in complex mixtures that, to date, have not been well understood (Holden et al., 2016).

The aim of this study is to contribute to the better understanding of toxicity of NMs including AgNPs, AuNPs, $ZrO_2$NPs, $CeO_2$NPs, $TiO_2$NPs, $Al_2O_3$NPs, $C_{60}$ fullerene, graphene, and MPLs (polyethylene and polystyrene) at cell level in terms of oxidative stress and cell viability. The cytotoxic responses of two different cell lines T98G and HeLa, cerebral and epithelial human cells, respectively, that were exposed to the same conditions, were evaluated. Moreover, the synergistic and antagonistic relationships between fullerenes and other organic contaminants, an organophosphate insecticide (malathion), a surfactant (sodium dodecylbenzenesulfonate) and a plasticizer (diethyl phthalate) were assessed. The selection of these three organic compounds was based on their use, their presence and their toxicity in the aquatic environment, as well as their physicochemical properties. Malathion is a commonly used insecticide and in different studies has been related with oxidative stress induction. Sodium dodecylbenzenesulfonate is a commonly detected surfactant in rivers and coastal waters. Furthermore, diethyl phthalate is a widely used plasticizer.

Most of the reported works have explored the cytotoxicity at much higher concentrations in relation to, for example, their applications as drug carriers or nanomedicine. Here, we consider much lower levels, such as those that can be considered by incidental exposure. Notwithstanding, in the case of MPLs almost no data have been reported to date.

## 2. Materials and methods

### 2.1. Chemicals

To the best of our knowledge, the standards and mediums used in this study were of the highest purity available. Dulbecco Modified Eagle's Media (DMEM) and trypsin-EDTA were supplied by Sigma-Aldrich. Fetal Bovine Serum (FBS) and Penicillin/Streptomycin Solution were obtained from Invitrogen™ (Thermo-Fisher Scientific). Dulbecco's phosphate-buffered saline (PBS), dimethyl sulfoxide (DMSO), Hoechst 33258 (bisBenzimide H 33258 ≥ 98%) and dihydroethidium (DHE) (≥ 95%) were purchased from Merck Millipore (Darmstadt, Germany). Salts and analytical standards silver nitrate (ACS reagent, ≥ 99%), trisodium citrate (≥ 98%), gold nanoparticles (10 nm particles size, stabilised suspension in 0.1 mM PBS), zirconium (IV) oxide (suspension 10% wt in $H_2O$, < 100 nm particles size), cerium (IV) oxide (suspension 10% wt in $H_2O$, < 25 nm particles size), titanium (IV) oxide (~21 nm particles size, ≥ 99.5% trace metals basis), fullerene-$C_{60}$, fullerol and fullerene soot (76% of $C_{60}$ fullerene, 22% of $C_{70}$ fullerene and 2% of higher-order fullerenes) produced by the Krätschmer – Huffman method (Kratschmer et al., 1990) was purchased from Sigma-Aldrich (Steinheim, Germany). Aluminium oxide 90 active neutral (0.063–0.200 mm particle size) was purchased from Merck Millipore (Darmstadt, Germany). Graphene nano-powder consisting of 1.5–10 µm particles (4.5 µm mean size) composed of 12 nm graphene flakes (equivalent to 30–50 graphene layers) was supplied by Graphene Supermarket (Reading, MA, USA). The organic compounds used, such as malathion (≥ 99%), diethyl phthalate PESTANAL® (DEHP) (≥ 99.5%) and sodium dodecylbenzenesulfonate ($C_{12}$-LAS) (~80%), were purchased from Sigma-Aldrich.

Silver nanoparticles (AgNPs) were synthetized using trisodium citrate as a reducing agent (Rashid et al., 2013). In brief, all solutions of reacting materials were prepared in HPLC water. Fifty (50) mL of 0.001 M $AgNO_3$ was heated to its boiling point and then 5 mL of 1% trisodium citrate was added, drop by drop. The solutions were heated and stirred vigorously until there was a change of color to pale yellow. Then, the obtained suspension was allowed to reach room temperature. The silver colloid suspension was characterized by nanoparticle tracking analysis (NTA) using a NanoSight LM10 (NanoSight Ltd., Salisbury, UK). This technique allows visualizing particles in liquids, relating Brownian motion and particle size. For this characterization, measurements were carried out in static mode. To adjust camera level and threshold, a pre-scan was carried out.

Polyethylene (PE) microspheres (3–16 µm) accompanied by NPs with sizes between 100 and 600 nm were purchased from Cospheric LLC (Santa Barbara, CA, USA) and polystyrene (PS) (10 µm, 1% solids) accompanied by NP with sizes between 40 and 250 nm were obtained from PolySpherex™ Polystyrene microspheres which are purchased from Phosphorex (Hopkinton, MA, USA). The presence of NPs in each case was validated by NTA (see Fig. S1 of Supporting Information section). The cell lines employed in these experiments were human glioblastomamultiforme T98G cells and human cervical carcinoma HeLa cells that were kindly provided by Dr. Gemma Fabriàs from the Institute for Advanced Chemistry of Catalonia (IQAC-CSIC).

### 2.2. *Standard suspension and solutions*

Dry powder of metal oxide NPs ($ZrO_2$, $TiO_2$, $CeO_2$, $Al_2O_3$), metal NP (Au and Ag), fullerol, and MPLs of PE and PS microspheres were weighed and diluted in PBS in order to obtain a mother solution of 100 mg/L and then with the cellular medium DMEM. While AuNPs and AgNPs were diluted from stable suspensions. Mother solutions were subsequently diluted in the assay range of 50 µg/L to 1–10 mg/L.

### 2.3. *Carbon NMs dispersion procedure*

Fullerenes ($C_{60}$ 76%, $C_{70}$ 22%, with the remainder being higher-order fullerenes) and graphene standard had been aged during 40 d in mesocosms, simulating the aging of materials under real estuary conditions with controlled salinity, pH and organic matter content (Sanchís et al., 2015). The use of artificial dispersing agents as solvents was avoided in order to reproduce relevant environmental conditions (Fig. S2). Mother solutions were subsequently diluted in the assay range of 50 µg/L to 10 mg/L.

### 2.4. *Binary mixture*

Binary mixtures containing fullerene soot and organic contaminant were prepared at different concentration ratios. In each experiment, increasing concentrations of organic contaminants (0 − 60 mg/L) were tested in triplicate. Each series was prepared with a constant concentration of fullerene soot suspension 0, 1.0 and 5.0 mg/L.

### 2.5. *Cell culture*

The cell lines employed in this experiment were T98G and HeLa cells, which are both human cells that are commonly used in research. Both cell lines were grown in cell culture flasks containing DMEM supplemented with 10% FBS and 1% antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin). The cells were incubated at 37 °C in a humidified incubator set at 5% $CO_2$. Cells were subcultured according to standard cell culture protocols prior to cytotoxicity assays.

### 2.6. *In vitro cytotoxicity assay*

T98G and HeLa cells were seeded in 96-well plates at 25,000 cells/well. After 24 h, the medium was removed and cells were exposed for 24–48 h to the different NMs, polymers and mixtures at concentrations from 10 ng/mL to 10 µg/mL. At the end of exposure, the tested solutions were removed and the cells were stained with two fluorescent biomarkers for 30 min at 37 °C in a 5% $CO_2$ atmosphere. Fluorescent biomarkers were (1) 2-[2-(4-hydroxyphenyl) − 6-benzimidazolel-6-(1-methyl-4-piperazyl)-benzimidazole trihydrochloride (Hoechst 33258), which is a fluorescent compound that interacts with DNA (Excitation/Emission maxima = 352/461 nm). A 10 µM Hoechst 33258 solution was used as an indicator of cell viability, and (2) DHE solution 25 µM was used as a superoxide ($O_2^{\cdot-}$) generation indicator.

Then, the labeling solutions were aspirated and cells were rinsed with PBS. Fluorescence measures were determined at 485 nm (Hoechst) and 549 nm (DHE) emission wavelengths using CellInsight™ NXT High Content Screening (HCS) Platform (Thermo Scientific).

However, DHE is a colourless compound but it is cell-permeable. If there is the presence of superoxide anion ($O_2^{\cdot-}$) in the cytoplasm, DHE is oxidated to ethidium which intercalates into the DNA in the nucleus and fluoresces red color (Excitation/Emission maxima = 510/595 nm).

### 2.7. *High-content analysis*

The cell viability and the oxidative stress were measured via High-Content Analysis (HCA). This technique combines automated microscopy and cell fluorescence-tagging with Hoechst 33258 and DHE. The acquisition was performed with a 10 × lens using two channels with two different wavelengths ($\lambda_1$ = 485 nm and $\lambda_2$ = 549 nm) that are close to the Hoechst and DHE emission maximum, respectively. Optics focus and acquisition brightness were optimized. The obtained micrograph is a result of a magnified image of the well, which permits to recognise automatically the cells stained with fluorescent compound. Primary objects were validated or rejected according to the following criteria: emplacement in the micrograph, shape, area and light intensity; see Fig. S3 of Supporting information section on the micrograph of T98G cells.

### 2.8. *Statistics*

The data were expressed as mean ± SD of three independent experiments. Wherever appropriate, the data were subjected to statistical analysis using a one-way analysis of variance (ANOVA) test, followed by Dunnett's test to compare all data with the control values. A value of $P < 0.05$ was considered to be statistically significant only for a high concentration of contaminant. GraphPad 4.0 Software was used for the statistical analysis.

## 3. Results and discussion

### 3.1. *Cell viability*

The effects of NMs and MPLs on T98G and HeLa cells survival were evaluated using Hoechst 33258 as staining solution. Different concentrations between 50 µg/L and 10 mg/L of the selected NMs and MPLs in this work were studied individually. To obtain information regarding cell viability, the channel with 485 nm was selected in the HCA. However, no well-differentiated images were obtained for cells stained with Hoechst 33258 compared to those stained with DHE, as can be seen in Fig. S3 in Supporting Information. The main results of the cell viability study showed that none of the NMs or MPLs studied here did lead to a significant reduction of cell viability, as shown in Fig. 1. Therefore, cytolysis was not produced.

### 3.2. *Oxidative stress of nanomaterials*

A cytotoxic assay using DHE as staining solution was used to obtain information about the oxidative stress of selected NMs and MPLs on T98G and HeLa cell lines after 24 h of exposure.

As can be seen in Fig. 2A, in general, dose-dependent effects were obtained, with the exception of fullerol and $ZrO_2$-NPs in T98G cultured cells. In the case of fullerol, formation of aggregates drove the limitation of ROS production. On the other hand, in the case of $ZrO_2$-NPs, these were the biggest metal dioxide NPs studied here, and when the concentrations were over 0.5 mg/L, they tended to aggregate, and the ROS effects were modulated. Meanwhile, the greatest effects for T98G cells were produced by fullerene soot followed by $TiO_2$-NPs. It is noteworthy that cytotoxicity is highly influenced by the size and shape of NMs. For cytotoxicity comparison purposes, the particular characteristics of assayed NMs should be considered. The cells uptake biomolecules through endocytosis or can involve and entrap them by clathrin or caveolin pits or some proteins, and cell uptake and sub cellular distribution depend on their size (Shang et al., 2014). In addition, the medium can influence the size of NPs because of a change of ionic strength (Wyrwoll et al., 2016). In this study, the maximum of ROS production was shown to be limited by the aggregates generation in the

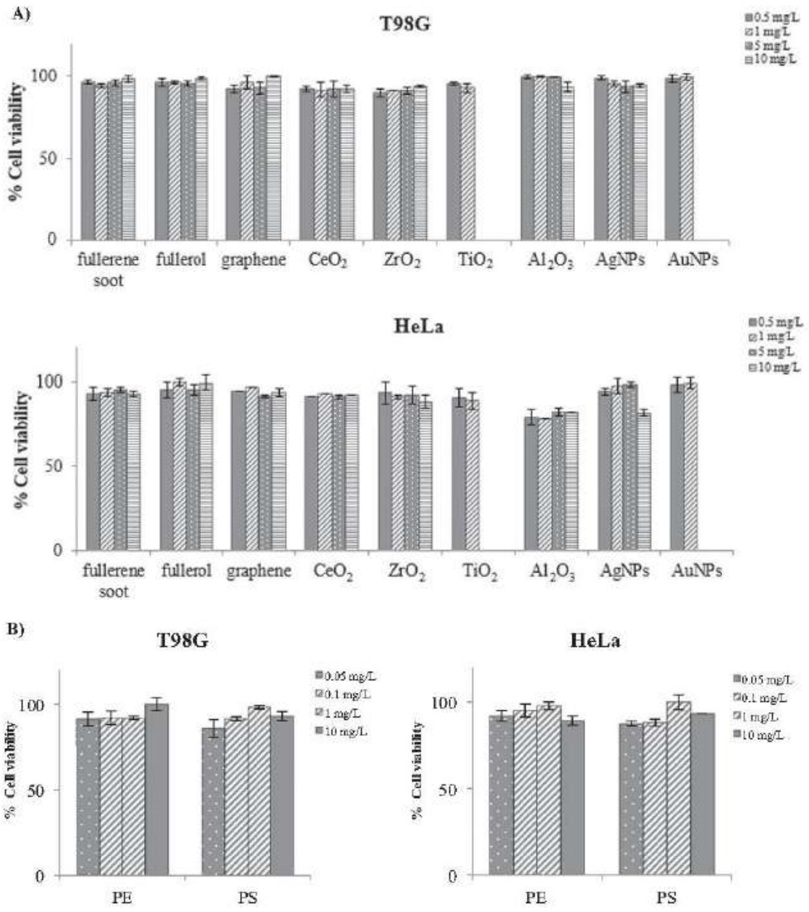

Fig. 1. Data obtained from HCA assay for metal oxide NPs, metal NPs, carbon NMs (A) and microplastics MPLs (B) on cerebral (T98G) and epithelial (HeLa) human cells. Data represented as live cells content. The y-axis represents the percentage of cell viability compared to the control. The x-axis represents the compounds used in the assay after a time period of 24 h of incubation. The different colors of the bars identify the concentration of the compounds. The values represent the mean ± standard deviation (SD) of three experiments.

cases of Fullerol and $ZrO_2$-NPs. However, the aggregation was previously characterized by NTA and in was shown stable during exposure, as in previous works. For example, Guadagnini et al., showed that the size of NPs in the cellular medium normally do not change at least 1 day, indicating the feasibility of in vitro testing without influence from potential agglomeration (Guadagnini et al., 2015).

Graphene is a two-dimensional layer of $sp^2$-hybridised carbon atoms. In previous studies, pristine graphene has been found to increase ROS and apoptosis. For example, in murine RAW 264.7 macrophages, the depletion of mitochondrial membrane potential (MMP) and ROS-triggered apoptosis was produced by the activation of the mitochondrial pathway (Li et al., 2012; Sasidharan et al., 2012). In another work, the potential influence of graphene on cell morphology, mortality, membrane integrity and cell viability was studied using the human glioblasoma U87 and U118 cells. In this study, it was found that graphene sheets had a strong tendency to localize close to the cells, but not enter inside the cells (Jaworski et al., 2013). However, in our findings, the exposure of cultured T98G and HeLa cells to fullerene soot led to an increase of intracellular reactive oxygen species only in HeLa cells and this effect was not very pronounced. It is noteworthy that previous studies showing a stronger ROS effects used, in general, graphene flakes composed of around 10 layers, and here we have used flakes composed of 30–50 layers. Both types of graphene are currently on the market, however, their effects are highly influenced by their particular characteristics. In addition, as already noted the most obvious difference between T98G cells and HeLa, which can induce DNA synthesis in senescent nuclei, is that T98G cells exhibit normal G1 growth regulation, whereas HeLa cells do not. We assume that this factor can influence on the major ROS response in HeLa cells.

While the graphene assayed here produced only effects at concentrations higher of 5 mg/L in HeLa cells, the results were contrary in the case of fullerol and fullerene soot, producing effects only on T98G cells. Fullerene soot was the NM producing the greatest effect on T98G cells in this study, while in HeLa cells no effects were observed.

In HeLa culture cells, major effects were produced by$TiO_2$-NPs followed by $ZrO_2$-NPs. In general, cytotoxicity of the metal oxide NPs depended on the particle composition and their uptake. Consequently, their incorporation into the cell could alter the membrane properties due to the generation of the ROS. The subcellular location in endosomal and caveolae compartments, or the lysosomes accumulation, could generate several effects such as organelle clumping, oxidative cell injury, mitochondrial depolarisation, cytokine release and cytotoxicity (Zbyszewski et al., 2014). Consequently, membrane fluidity decreases with relative modification of membrane-bound proteins and other radical species could also be generated (Cabiscol et al., 2010). Serum proteins and lipids present in the cellular medium improve NP dispersion or solution (Sager et al., 2007). In fact, the change from aqueous medium (PBS) to cellular medium (DMEM) during the standard preparation may influence NP size and consequently intracellular diffusion.

We have observed that $TiO_2$-NPs have induced ROS generation in both cell lines. Moreover, it was the NMs producing higher effects in HeLa cells. Previous studies on $TiO_2$-NPs toxicity provided conflicting results. The toxic effects have been reported by several studies, for

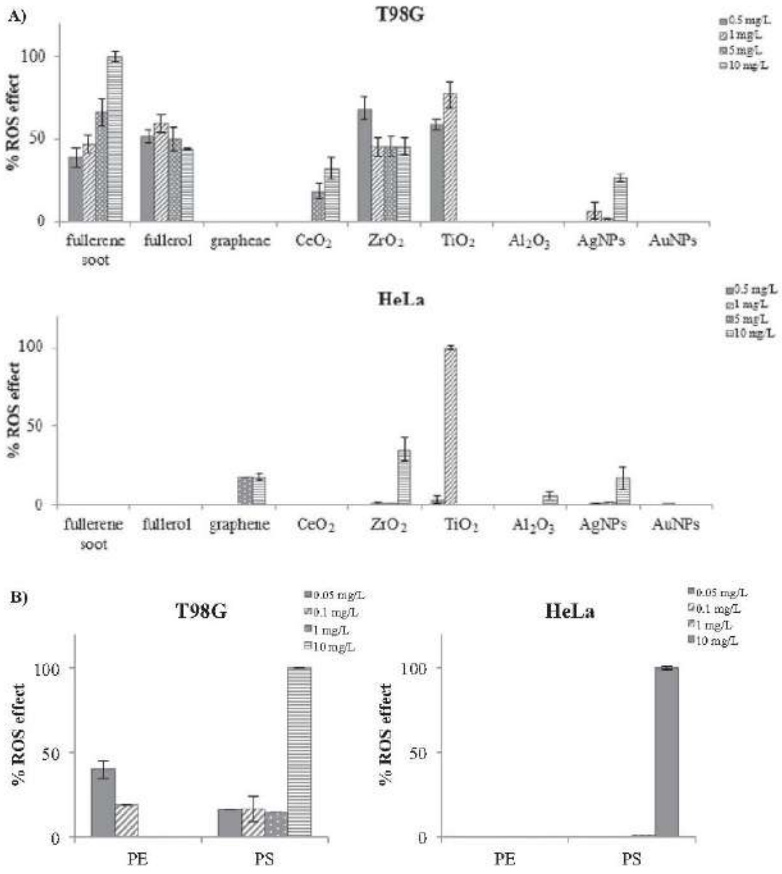

**Fig. 2.** Data obtained from HCA assay for metal oxide NPs, metal NPs, carbon NMs (A) and microplastics MPLs (B) on cerebral (T98G) and epithelial (HeLa) human cells. Data represented as Reactive Oxygen Species (ROS) effect. The *y* axis represents the percent ROS effect compared to the maximum effect observed after a time period of 24 h of exposure. The *x*-axis represents the compounds used in the HCA assay. The different colors of the bars identify the concentration of the compounds. The values represent the mean ± standard deviation of three experiments.

example, anatase $TiO_2$ nanoparticles were found to induced oxidative DNA damage, lipid peroxidation, micronuclei formation in a human bronchial epithelial cell line, in the absence of photo-activation (Gurr et al., 2005). Conversely, $TiO_2$-NPs in dark conditions were found not cytotoxic and not causing ROS generation in different studies (Hou et al., 2015; Tong et al., 2017; Zhang et al., 2014). For both type of results, it should be mentioned that the concentrations were much higher than those employed in the present study. In summary, cytotoxicity and oxidative stress of $TiO_2$ nanoparticles have been shown dependent on physicochemical properties as their structure or photocatalytic potency (Park et al., 2011). However, the concentration and aggregation could also be a modulation factor. In the present work, the maximum of ROS generation was obtained at 1 mg/L, and at higher concentrations, the ROS generation was not observed. This behaviour, observed in both cell lines, could be explained by the possibility of aggregates formation and the consequent depletion of cellular uptake at higher concentrations in dark conditions. The increase of ROS generation has been observed in several studies, under UV irradiation because of the photo-catalytic potential of $TiO_2$-NP, but also, probably, because the number of individual excited NPs able to be up-taken is increased by UV radiation. For example, Park et al. (2011) compared the cellular effects of $TiO_2$-NP with different photo-catalytic potential in human keratinocyte, HaCaT cells (Park et al., 2011) at concentrations from 50 to 150 mg/L, and much higher values of ROS effect was encountered after UV-irradiation. Consistent with these results, are the reported effects by irradiated $TiO_2$-NPs in HeLa cells by Zhang et al., but also with more recent studies on toxicity and ROS generation by irradiated $TiO_2$-NPs (Lu et al., 2017; Tong et al., 2017; Wang et al., 2014; Yamada et al., 2016). Therefore, the present finding can complement previous the information and underpinned the need to assess the effects at environmental concentrations since the behaviour of NPs is very much influenced by their aggregation and in general is not directly proportional to concentration.

Regarding metal NPs, at the highest concentration (1 mg/L) of AgNPs, ROS generation was increased in both cell lines. The toxicity of AgNPs is related to their surface charge (El Badawy et al., 2011), which varies according to the type and medium used during their synthesis. In this study, AgNPs were coated by the citrate functional group, which gives a negative surface charge. Therefore, citrate-AgNPs were repelled by the negative charge of cellular membranes. However, once the electrostatic barrier is overcome, at high concentrations of NPs, NPs can penetrate cellular membranes and interact physically or chemically with the mitochondria, resulting in oxidative stress. Cytotoxicity of NPs can be an effect of alteration of mitochondrial function, expressly with an uncoupling oxidation of the phosphorylation membrane system.

However, there was no significant variation in T98G neither in HeLa cells exposed to AuNPs, which is in agreement with previous studies with different cell lines (Austin et al., 2015; Ristig et al., 2015; Connor et al., 2005). With respect to general trends, the ROS generation in both cell lines followed a different trend.

It is noteworthy that at the low concentrations assayed (low in comparison to those that are, in general, studied for the use of NPs in nanomedicine), a different picture was obtained. For example, in the case of AgNPs, strong effects are, in general, reported as being obtained at higher concentrations, while in the case of carbon NMs the association of aggregates inhibits their bioavailability.

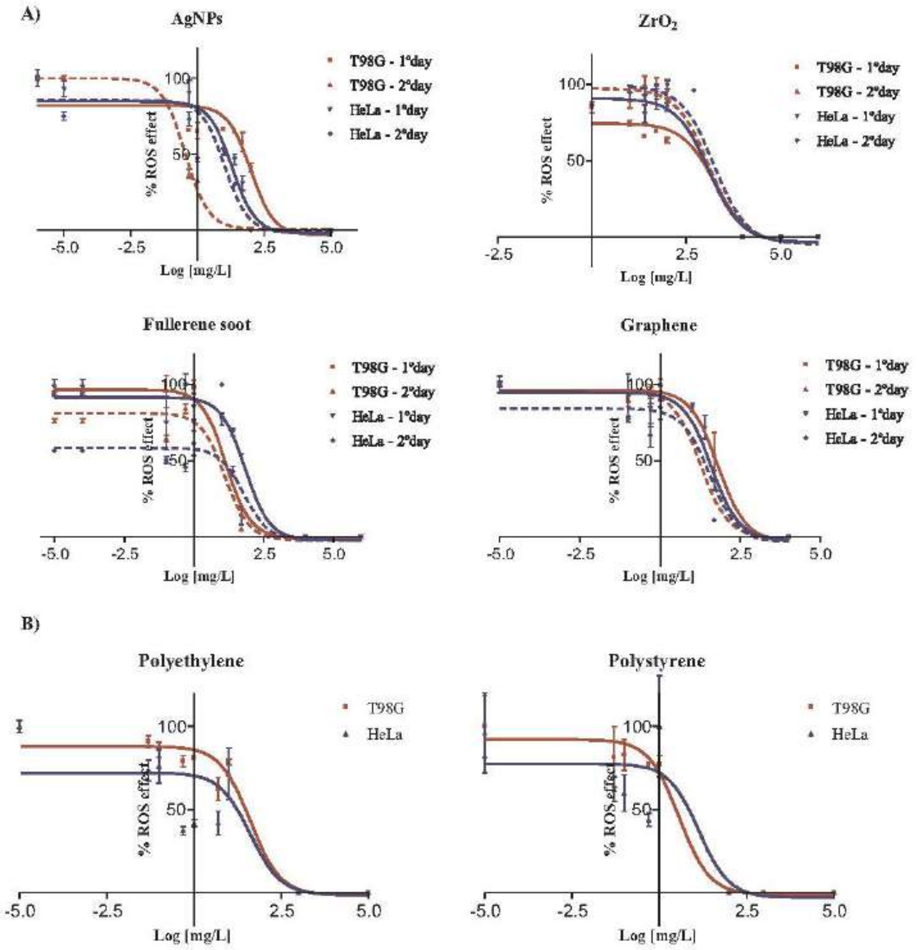

**Fig. 3.** Dose response curve showing the ROS effect (%) of T98G and HeLa cell lines exposed for 48 h to increasing doses of metal oxide NPs, metal NPs, carbon NMs (A). Dose response curve showing the ROS effect (%) of T98G and HeLa cell lines exposed for a time period of 24 h to increasing doses of microplastics (B). The data presented are the mean ± SD of three independent experiments.

**Table 1**
$EC_{50}$ values for the exposure of AgNPs, $ZrO_2$, graphene and fullerene soot to cerebral (T98G) and epithelial (HeLa) human cells for 72 h, as determined by HCA assay.

| **Compounds** | **$EC_{50}$ (mg/L)** (95% confidence intervals) **T98G** *1° day* | *2° day* | **HeLa** *1° day* | *2° day* |
|---|---|---|---|---|
| **AgNPs** | 101.5<br>(54.9–187.6) | 0.4<br>(0.3–0.5) | 20.4<br>(9.9–41) | 10.8<br>(4.1–29) |
| **$ZrO_2$** | 1831<br>(938.7 – 3572) | 1265<br>(398.4 – 3824) | 1257<br>(711.9 – 2221) | 1730<br>(791.9 – 3781) |
| **Graphene** | 64.56<br>(35.7–116.7) | 18.13<br>(17 – 193.2) | 39.92<br>(20.4–78) | 36.36<br>(15.7–84.1) |
| **Fullerene soot** | 14.16<br>(6.7–29.8) | 13.13<br>(2.5–40) | 68.76<br>(33.9–139.4) | 61.60<br>(20.5–185.3) |

As second step, both cells lines were exposed for 48 h to the contaminants that showed a more notorious cytotoxic effect, such as AgNPs, $ZrO_2$, graphene, and fullerene soot. Moreover, the maximum concentration assayed was at least 10 times greater. The dose response curves were fitted (Fig. 3) and the half maximal effective concentrations ($EC_{50}$) were calculated (Table 1). As can be shown in T98G cells, in the two periods of exposure, fullerene soot presented the lower $EC_{50}$ but the maximum effect was at the 60%. This indicates that over a concentration the hydrophobicity of fullerenes drives to auto-aggregation limiting their ROS generation. While, in the case of AgNPs ROS generation is slowly produced.

It should be highlighted that we have studied the potential effects at concentrations that can be the result of bioaccumulation from the environment. Therefore, higher than environmental reported or estimated concentrations and much lower than concentrations previously explored when the potential toxicity of NMs for it use as drug carriers was studied, in occupational exposure studies, or when what is explore it's the elucidation of the general mode of action.

**Table 2**
$EC_{50}$ values for the exposure of polyethylene and polystyrene to cerebral (T98G) and epithelial (HeLa) human cells for 24 h, as determined by HCA assay.

| | $EC_{50}$ (mg/L) (95% confidence intervals) | |
|---|---|---|
| Compounds | T98G | HeLa |
| Polyethylene | 41.22<br>(12.8–133) | 40.96<br>(17.8–178.8) |
| Polystyrene | 9.617<br>(3.9–23.8) | 13.56<br>(2–96) |

### 3.3. Oxidative stress of microplastics

In the case of PE, ROS generation was only significant on T98G. However in both cell cultures PS presented a higher ROS generation. Probably, this effect can be related to the smaller size of PS particles (Fig. 2 B, Table 2). After control substraction, no significant ROS generation was observed in some cases when cells were exposed to PE.

#### 3.3.1. Trojan Horse effect

The synergistic and antagonistic relationships between fullerenes and three selected organic contaminants (malathion, C12-LAS and DEHP) were assessed. The ROS generation of a range of different nominal concentrations of these contaminants (malathion between 5 and 30 mg/L; C12-LAS between 5 and 50 mg/L and DEHP between 0.5 and 50 mg/L) was assessed with and without fullerenes. In the mixtures with fullerene soot, two concentrations (1 and 5 mg/L) were tested. The experimental results were compared with the theoretical values according to a simple additive model.

As can be seen in Fig. 4, in the case of malathion, this compound has been related to ROS generation at concentrations around 40 mg/L in PC12 cells. In our study using T98G cell, also almost no ROS generation (around 5%) was observed for concentrations of exposure inferior to 30 mg/L. Therefore, according to a simple additive model, the ROS generation in this mixture should be attributed mainly to fullerenes.

Due to the hydrophobicity of malathion (log Kow = 2.7 at 25 °C), in a first step fullerene soot will adsorb malathion and tend to be deposited onto the membrane cells. When fullerene soot concentrations were high enough, malathion was efficiently retained and probably assisted in the formation of bigger fullerene aggregates. This can contribute to decreasing the bioavailability in agreement with the observed antagonistic effect. Whereas, in those binary mixtures where fullerene aggregates surface was saturated, loosely attached malathion molecules were released directly on membranes surface and the synergistic effect was then produced. The same behaviour was observed in the case of *D. magna* exposed to mixtures of malathion and fullerene soot (Sanchís et al., 2015).

A similar behaviour was observed in the case of C12-LAS. This surfactant alone did not present ROS generation on T98g cells. Again, ideally, the ROS generation of the mixture should be only the contribution of fullerene soot following a simple additive model. However, for the lower concentrations of C12-LAS between 5 and 25 mg/L we have observed antagonism. And, this effect was clearly more pronounced when the ratio between fullerene soot and C2-LAS was higher. In a first step, fullerenes are sequestering C12-LAS from solution and when fullerene soot concentrations were high enough, C12-LAS was efficiently retained. The surfactant character of C12-LAS contributes again to the formation of hetero-aggregates contributing to the bioavailability decrease. However, in one case, when the concentration of fullerenes was 50 mg/L and the concentration of fullerene soot was 1 mg/L, synergism was produced. In this case, fullerenes surface was saturated, free C12-LAS surfactants did not reach their critical micelle concentration (100 mg/L) for auto-aggregation and, therefore, tended to be associated to membrane cells and membrane solubilisation. Notwithstanding, for the same concentration of C12-LAS, but with 10 times more fullerene soot, then C12- is attached to fullerene surface, hetero-aggregates are favored, bioavailability is decreased and an antagonistic effect was observed.

Finally, DEHP presented ROS generation that was directly proportional with concentration. Once again, DEHP molecules tended to be adsorbed on the fullerene aggregate surface and hetero-aggregates and synergistic effects were shown. Only when concentrations of DEHP were high enough was 100% of effect reached, as expected.

These results are a pioneer observation of the Trojan-Horse mechanism at cell level and they underpin the necessity of assessing the presence and effect of carbon nanomaterials in the marine environment, since they are active agents, which are changing the bioavailability of xenobiotics.

## 4. Conclusions

HCA has proven to be a powerful, robust, reliable technique for the simultaneous observation and toxicity assessment of cells exposed to nanoparticles and common organic contaminants, and it has allowed assessment of the toxic effects of nanomaterials and two types of microplastics of two different types of cell. The data provided in this work complement previous studies conducted and much higher concentrations, which is important since toxicity of NPs is not always dose-dependent. Also, these results are focussed on potential toxicity produced by bioaccumulation in the environment offering data relevant for posterior risk-assessment studies evaluating the risks for human health. For example, considering that it has been widely reported that ROS generation had a critical role in the growth and proliferation of cancer cells (Tang et al., 2011; Manda et al., 2015; Poillet-Perez et al., 2015). During the recent years, the use of in vitro approaches has to gain interest for assessing the risks of chemicals and drugs in a more mechanistic and high throughput manner than in vivo tests. Different models have been developed in recent years for the quantitative in-vitro to in-vivo toxicity assays, in particular, in the case of cell-lines. Physiologically based pharmacokinetic (PBPK) modelling provides a practical framework for conducting quantitative in vitro to in vivo extrapolation (Yoon et al., 2012; Wetmore, 2015).

The obtained results contribute to the current knowledge of the cytotoxcity of both nanomaterials and microplastics, whereby oxidative stress is one mechanism that explains the toxicity of these emerging contaminants at cell level, as has been observed for both T98G and HeLa lines.

In addition, carbon nanomaterials have the potential to interact in a complex way with the co-occurring contaminants, changing the bioavailability and, therefore, their resulting toxicity in either a synergistic or an antagonistic way. The result of this study showed that fullerene soot presented a predominantly antagonistic effect in binary mixtures with other organic contaminants. To the best of knowledge, this fact agrees with previous results obtained in ecotoxicological studies.

In general, these phenomena have been overlooked during risk assessments of these substances and more attention should be paid to them in the future. Other nano-structured materials, such as nano-sized plastics or metal-oxide nanoparticles, may modulate the toxicity of co-contaminants (Sanchís et al., 2015). In the same manner, recent studies (Vidali et al., 2016) provide evidence that the toxic behaviour of NMs such as carbon nanotubes could be altered with unknown effects on humans, and the environment by environmental factors and these topics must be further studied.

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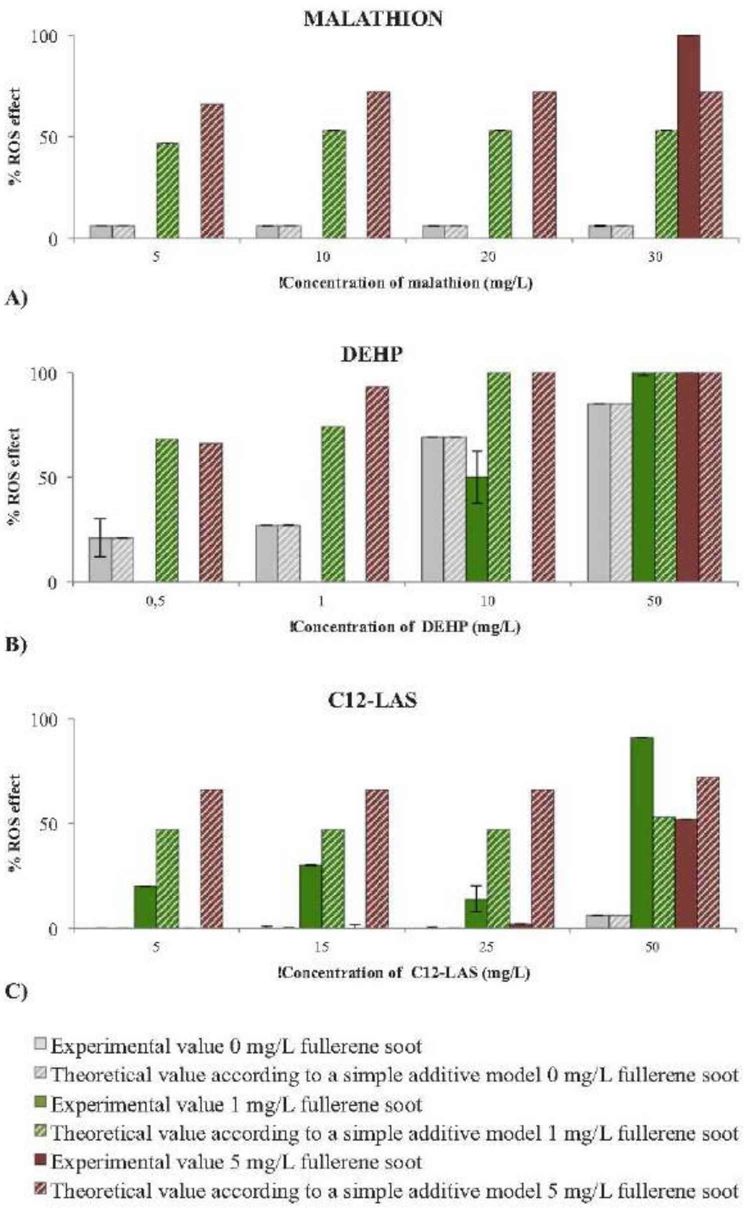

**Fig. 4.** Data obtained from HCA assay for binary mixture of fullerenes soot and organic compounds such as malathion (A), DEHP (B) and C12-LAS (C) on the cerebral human cells (T98G). Data represented as Reactive Oxygen Species (ROS) effect. The *y*-axis represents the percent ROS effect compared to the maxim effect observed after 24 h of time of exposure. The *x*-axis represents the concentration of fullerenes soot in the binary mixture. The different colors of the bars identify the concentration of the compounds. The values represent the mean ± standard deviation of three experiments.

to Thermo Scientific CellInsight™ NXT High Content Screening (HCS) Platform.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2017.08.043.

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## Supporting Information

# CYTOTOXIC EFFECTS OF COMMONLY USED NANOMATERIALS AND MICROPLASTICS ON CEREBRAL AND EPITHELIAL HUMAN CELLS

Gabriella F. Schirinzi[1], Ignacio Pérez-Pomeda[2], Josep Sanchís[1], Cesare Rossini[3], Marinella Farré[1,*], Damià Barceló[1,2]

[1]Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona (Catalonia, Spain)

[2]Institute for Advanced Chemistry of Catalonia (IQAC), Barcelona (Catalonia, Spain)

[3]Thermo Fisher Scientific, StradaRivoltana, 20090, Rodano (Milano, Italy)

[4]Catalan Institute for Water research (ICRA), Girona (Catalonia, Spain)

- Corresponding Author: Marinella Farré E-mail: mfuqam@cid.csic.es

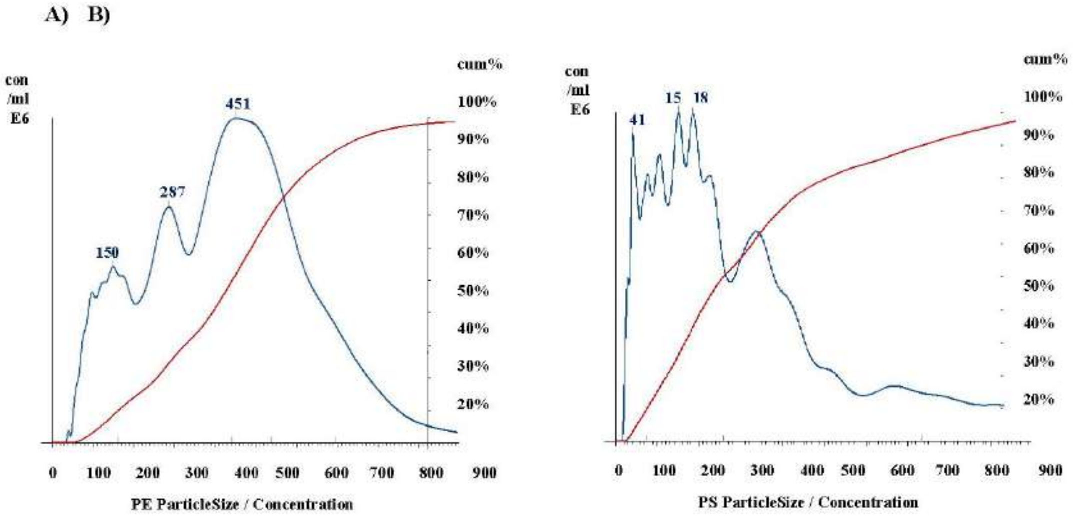

**Figure S1** Characterization of polyethylene (A) and polystyrene (B) NPLs by Nanoparticle Tracking Analysis (NTA)

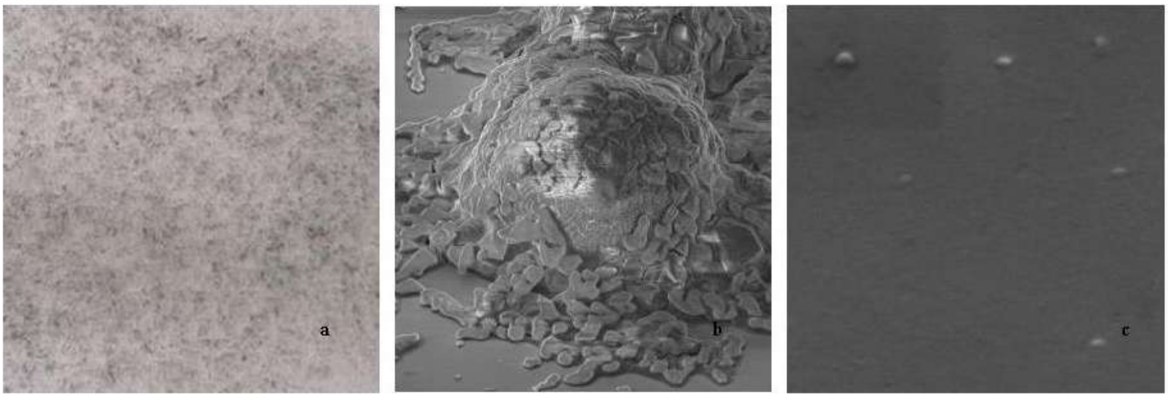

**Figure S2:** Real image (a) and low (b) - high (c) magnification SEM images of fullerenes soot used to reproduce ecologically relevant conditions

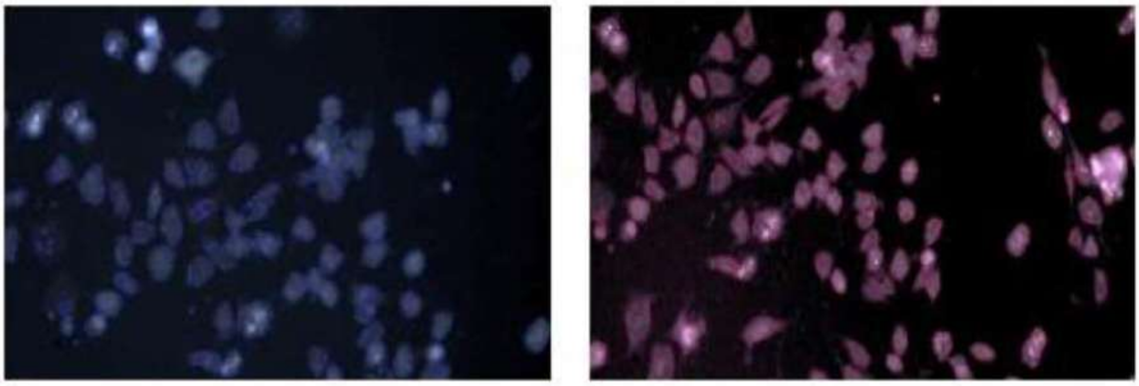

**Figure S3** Micrograph of T98g cells in Channel 1 with $\lambda_1$=485 nm (A) and Channel 2 with $\lambda_2$=549 nm (B). Blue objects identify live cells that were identified by objects of blue colour, while the presence of Reactive Oxygen Species (ROS) was indicated through objects of red colour

## 4.3 Discussion

### 4.3.1 MPLs occurrence in Mediterranean species

The Mediterranean Sea is currently one of the areas most affected by marine litter due to critical levels of contamination and its consequent damage to biodiversity. Although this is a most important threat to Mediterranean marine organisms, the scientific community has focused on interactions with biota and the trophic web only in recent years. Almost 134 species were affected by marine litter at basin scale (Deudero et al. 2014). Most of the studies have been reported for the Western Mediterranean Sea documenting litter ingestion for different taxonomic groups including invertebrates, cetacean, fish, sea turtles, seabirds, and marine mammals. Among these, cetacean and fish are the most studied. Particularly, taking into account the habitat, the demersal and pelagic species seem to be most affected by plastic ingestion than benthic species (Fossi et al. 2018).

Sentinel species have been selected to monitor the impacts of marine litter on Mediterranean fauna. The selection criteria have been based on their marine habitats and other specific ecological and biological criteria, such as trophic information, feeding behaviour, spatial distribution and commercial importance. Based on data available in the literature, different bioindicators from different ecological compartments and geographical scales have been proposed (Fossi et al. 2018). The first approach consists in the analysis of the gastrointestinal content of marine organisms in order to define the threshold levels of the negative effects of plastic ingestion on biota. Most studies on the ingestion of MPLs by Mediterranean species are generally based on direct microscopic examination of the stomach and/or intestinal contents. However, this strategy may lead to misidentifying natural materials as polymers (false positives) or to underestimating the number of MPLs as they could be camouflaged with natural materials. In this way, further steps (i.e. separation by density and chemical and biological digestion) are needed to purify the sample and aiding the subsequent chemical characterisation.

The extraction procedures most recently developed for the extraction of MPLs from biota include different digestion protocols (oxidative, acid, alkaline, and enzymatic). These protocols can be applied independently or in combination with others. Concerning the studies on Mediterranean species, a limited number the works have applied a digestion step before the chemical characterisation (Table 4.1).

The presence of MPLs in the juvenile *Coryphaena hippurus* from the Mediterranean Sea has been investigated to fill this gap of information concerning sentinel species (Publication IV). This species is a valuable fishing resource, which is widely distributed in several sub-regions of the basin. It has been classified as a medium-scale bioindicator of MPLs in open waters together with *Thunnus alalunga*, another large pelagic predator (Fossi et al. 2018). So far, major reports on this species from different areas were mainly focused on its diet showing the occurrence of meso- and macroplastic fragments, deriving from fishing gear manufactures and other common trash (e.g., bottle caps, balloon fragment and bags).

**Table 4.1** Studies applying digestion methods for the extraction of MPLs from GITs of Mediterranean fish.

| Mediterranean basin areas | Num. samples analysed | Species | Habitat | MPLs Analysis | | % *F* $^{a}$ | Polymers | Ref. |
|---|---|---|---|---|---|---|---|---|
| | | | | Extraction method | Instrument | | | |
| Western (Spanish coast) | 72 | *Scyliorhinus canicula* | demersal | NaOH 1 M (~21 days) | - | 15 | - | (Bellas et al. 2016) |
| | 12 | *Merluccius merluccius* | benthopelagic | | | 17 | | |
| | 128 | *Mullus barbatus* | demersal | | | 19 | | |
| Aegean-Levantine Sea (Israeli coast) | 88 | *Siganus rivulatus*<br>*Siganus luridus* | demersal | 10% KOH (RT, 2 weeks) | - | 92 | - | (van der Hal et al. 2018) |
| Adriatic Sea and NE Ionian Sea (Croatian Sea, Corfu island, and Slovenian Sea) | 55 | *Mullus surmuletus* | demersal | 1:20 (w/v) 30% $H_2O_2$ (~60°C, until evaporation) | - | 53 | - | (Anastasopoulou et al. 2018) |
| | 49 | *Pagellus erythrinus* | demersal | | | 47 | | |
| | 66 | *Sardina pilchardus* | pelagic | | | 42 | | |
| | 20 | *Liza aurata* | benthopelagic | | | 95 | | |
| | 20 | *Sparus aurata* | demersal | | | 100 | | |
| | 20 | *Solea solea* | benthic | | | 65 | | |
| Western, Central and Adriatic Sea | 132 | *Mullus barbatus* | demersal | 1:3 (w/v) 10% KOH (~60°C, overnight) | - | 20 | - | (Giani et al. 2019) |
| | 97 | *Merluccius merluccius* | benthopelagic | | | 27 | | |
| North-Western (Gulf of Lions) | 20 | *Engraulis encrasicolus* | pelagic | 1:3 (v/v) 9% NaClO (overnight) + $HNO_3$/NaClO | Raman | 40 | PE (37%), PP (26%), and PET (16%) | (Collard et al. 2017) |
| Adriatic Sea | 99 | *Sardina pilchardus,* | pelagic | NaCl + 15% $H_2O_2$ (until evaporation) | FTIR | 19 | PE (65%), PET (19%) and PP/PS (4%) | (Avio et al. 2015a) |
| | 9 | *Squalus acanthias* | demersal | | | 44 | | |
| | 3 | *Merluccius merluccius* | benthopelagic | | | 100 | | |
| | 11 | *Mullus barbatus* | demersal | | | 65 | | |
| | 3 | *Chelidonichthys lucernus* | benthic | | | 67 | | |

**Table 4.1** (continued) Studies applying digestion methods for the extraction of MPLs from GITs of Mediterranean fish.

| Mediterranean basin areas | Num. samples analysed | Species | Species | MPLs Analysis | | % *F* [a] | Polymers | Ref. |
|---|---|---|---|---|---|---|---|---|
| | | | | Extraction method | Instrument | | | |
| Central (Northern Ionian Sea) | 36 | *Sardina pilchardus* | pelagic | 1:20 (w/v) 30% $H_2O_2$ (~60°C, until evaporation) | FTIR | 47 | PE (62%), PP (23%) and PS/PET (4%) | (Digka et al. 2018) |
| | 19 | *Pagellus erythrinus* | demersal | | | 42 | | |
| | 25 | *Mullus barbatus* | demersal | | | 32 | | |
| Adriatic Sea | 533 | *Solea solea* | benthic | 1:5 (w/v) 10% KOH (55°C, 36h) + NaI | µFTIR | 95 | PE/PP/PA (21%), PES (20%), and PVC (17%) | (Pellini et al. 2018) |
| Aegean-Levantine Sea (Turkish coast) | 1337 | 28 species | benthopelagic, demersal, pelagic | 35% $H_2O_2$ until complete digestion | FTIR | 58 | PE, PP and their copolymers | (Güven et al. 2017) |

[a] Frequency % = number of species with MPLs / number of species analysed * 100

In this context, the main purpose of this work was to obtain for the first-time information on MPLs ingested by common dolphins (*C. hippurus*) by applying chemical digestion. Despite the fact that several methods have been developed on different species, a standard approach for the detection of MPLs in biota has not yet been provided. Therefore, to choose the best digestion method, the advantages and disadvantages of each approach have been evaluated (Table 4.2).

**Table 4.2** Digestion methods generally used for MPLs extraction from GITs of marine organisms.

| Digestion | Most applied method | Advantages | Disadvantages |
|---|---|---|---|
| **Oxidative**<br>*($H_2O_2$ and Fenton's reagent + $H_2O_2$)* | ~30% $H_2O_2$ (60°C, until evaporation) | Inexpensive | ○ Time-consuming<br>○ Several applications could be needed |
| **Alkaline**<br>*(KOH and NaOH)* | 10% KOH (60°C, overnight) | ○ Effective<br>○ Minimal damage to polymers | ○ Attacks cellulose acetate |
| **Acid**<br>*($HNO_3$, $H_2SO_4$ and HCl)* | ≥ 65% $HNO_3$ (overnight to 2 h) | Rapid | ○ Damage to polymers |
| **Enzymatic**<br>*(proteinase, trypsin, cellulose, etc.)* | Proteinase-K (50°C, 2 h) | ○ Effective<br>○ Minimal damage to polymers | ○ Expensive<br>○ Several preparation steps |

Afterwards, the first approach was to test the alkaline (10% KOH) and oxidative (30% $H_2O_2$) digestion due to their broad application in MPLs extraction from biological tissue. The best results in terms of time and digestion efficiency were obtained with 10% KOH solution. Despite that, the method was combined with another one because of the presence of skeletons remains on the filter. The feeding behaviour of the species, in this case of *C. hippurus*, has a significant influence on the digestion result. Subsequent application of nitric acid under controlled conditions has led to an excellent result in terms of recovery and identification. This new approach has been validated using 5 types of polymers (PP, PET, PS, EPS and PA). These polymers have been selected based on the information reported in the latest publications about the chemical resistance data of polymer on digestion agents (Lusher et al. 2017b). However, it must be considered that plastics exposed to the environment do not have the same resistance to chemicals as virgin plastics. Weathering and ongoing degradation processes can affect their integrity by reducing the resistance of plastics to chemical agents.

Finally, the digestion method was applied to 29 samples of common dolphinfish from the western Mediterranean, confirming the MPLs ingestion. In general, the occurrence of plastics in *C. hippurus* may be due to its generalist feeding behaviour and predation on schooling prey, as observed for other pelagic predators. Besides, the type of polymers found in the biota can also be related to marine debris from the loss or degradation of fishing gear. The common dolphin fishery in the Southern Mediterranean (i.e. Sicily, Malta, Tunisia) is based on the use of Fish Aggregated Devices (FADs). The structure of a typical anchored FAD employed in these areas is made of palm leaves or plastic sheets for the provision of shade, plastic bottles or PS-slabs as floaters, and PE-ropes anchored on a stone. The highest number of these items were found as abandoned submerged objects from 1961 to 2017 in the Sicilian area (Sinopoli et al. 2020). The negative impacts of local fishing gear (e.g. FADs) on benthic habitat and species has been recently reported (Consoli et al. 2020).

Significant percentages of PE (36%), the most frequent polymers found in Mediterranean fish, were recorded followed by PS (14%) and PP (11%). These results were compared with those reported for other Mediterranean fish species of commercial interest. In this case, only those works where a digestion method was applied, and the polymers were subsequently identified have been considered (Table 4.1). Although the number of these studies is quite limited, the types of plastics identified, and their composition are in the same range of those reported in the respective sub-basin. For example, in agreement with the polymeric composition of MPLs floating in the Adriatic Sea and the Northern Ionian Sea, high percentages of PE (> 60%) were found in species of the same region (Zeri et al. 2018; Vianello et al. 2018). However, in the case of Western Mediterranean fish, the variety of polymers is even wider, and it depends on the sampling area as a consequence of the multiple hotspots present and the circulation pattern in this part of the basin.

### 4.3.2 Nanotoxicity

The ingestion of MPLs by marine organisms is a wide recognised concern. Additionally, since the degradation process of MPLs can progress to the formation of NPLs, the exposure to such particles may be higher. Despite their ubiquity in the aquatic environment, their presence in biota has not yet been confirmed due to the lack of analytical methods. However, the effects of NPLs on marine organisms has been reported showing different behavioural, morphological and reproductive consequences (da Costa et al. 2016b).

At the contrary, very limited data is available regarding the impact of these plastics on human health. Since NPLs can overcome tissue barriers in the human body, it is important also to evaluate the possible interactions with individual cells and the effects. Being considered as nanomaterials (NMs), cytotoxicity, (pro-)inflammation, or production of reactive oxygen species (ROS) are among the most likely consequences of the uptake at the cellular level. Although plastic particles can be of numerous types, shapes and sizes, most of the in vitro experiments described in the literature have been mainly based on PS-NPLs (Lehner et al. 2018). The introduction of these particles into the biological system depends on the binding mechanism of protein with NPLs. The biomolecules can easily bind on the NPLs through several ways including via Van der Waals forces, hydrophobic interactions, dipole-dipole bonding or for the interactions between amino acids structure and the benzene ring of PS-NPLs.

The effects studied show adverse effects depending on the size and surface modification of the particles and, lastly, the cell-type. Pro-inflammatory responses in the human gastric and lung adenocarcinoma cells have been reported for PS-particles with a size less than 100 nm, independently from their charge-surface (Forte et al. 2016; Brown et al. 2001). Changes in cell structure have also been observed after PS-NPLs internalization, which induced to binucleated cells formation (Xia et al. 2016; Inkielewicz-Stepniak et al. 2018). The acute exposure to PS-carboxylated and amino-functionalized particles can affect the physiological processes, which include activation of ion channels or disruption of iron transport (McCarthy et al. 2011; Mahler et al. 2012). Lastly, Liu et al. (2018) have reported that PS (> 50 µg mL$^{-1}$) can rapidly stimulate the generation of $O_2^{\cdot -}$. Also, the cell viability decreases more with cationic ($NH_2$-PS) than anionic (PS-COOH) or unmodified particles due to their strong electrostatic interaction with the cell membrane. In this case, the cell death was dependent on both the concentration and time of exposure (Chiu et al. 2015). So far, aside from PS, there is little information on the adverse effects of other NPLs in the human body. Information reported for PE nanotoxicity includes immune system responses and osteolysis for the presence of PE particles from joint replacements (Nich et al. 2014).

Monodisperse nanospheres with few defects and homogeneous chemical composition were used in all these studies. However, since the NPLs dispersed in the environment usually have an irregular shape, a complex surface area and a wide size-range, the toxicological consequences may be different. In this context, Magrí et al. (2018) evaluated the cytotoxic effects of PET nanoparticles that were obtained through a top-down laser ablation technique to simulate the behaviour of NPLs in the environment. Despite 96h exposure, in vitro test results showed that PET-NPLs did not produce short-term toxicity on colon carcinoma cell lines.

Follow this line, our study (Publication V) contributed to a better understanding of the toxicity behaviour of PE and PS-MPLs/NPLs in terms of oxidative stress and cell viability to two cell lines (T98G and HeLa). In this work, dispersions of MPLs were used, which included nanostructures with size ranges of 100 - 600 nm and 40 - 250 nm for PE and PS, respectively. In addition, exposure concentrations lower than those generally applied in vitro nanotoxicological studies have been applied to reproduce a scenario close to environmental conditions. Results showed an increase in ROS generation for both cell lines after 24h exposure to PS-NPLs, especially at higher concentrations (10 µg mL$^{-1}$). While, in the case of PE-NPLs, ROS generation was significant only for T98G at lower levels (0.05 - 0.1 µg mL$^{-1}$). The $EC_{50}$ reported for the exposure of PS-MPLs/NPLs to cerebral (T98G) and epithelial (HeLa) human cells were 9.61 and 13.5 µg mL$^{-1}$, respectively; while values > 40 µg mL$^{-1}$ were reported after PE-MPLs/NPLs exposure for both cell lines. On the contrary, cell viability has not been affected, indicating the low cytotoxicity of PS-MPLs/NPLs.

Similar results were also reported by Wu et al. (2019) demonstrating that PS-MPLs did not cause a significant reduction in cell viability in intestinal cells. While, significant increases in intracellular ROS levels were induced after the exposure to a higher concentration of PS (200 µg mL$^{-1}$). Lastly, mitochondrial depolarization and inhibition of efflux pumps activity have been observed depending on particle size (0.1 and 0.5 µm). The similarity between MPLs/NPLs and NMs in terms of cellular toxicity was also confirmed in this study. The impacts, the interaction and the translocation of PS-NPLs (50 nm) and PS-MPLs (0.5 µm) with carboxy-modified surface on placental and intestinal cells have also been investigated in different biological models in vitro. Even if a cellular uptake and intracellular accumulation of PS MPLs/NPLs were determined, no acutely toxic effects were observed. In summary, the results show different responses in the cells depending on the robustness of the tissue types and the mucus layer (Hesler et al. 2019).

Although the polymers of MPLs/NPLs may be bio-inert, additives not chemically bound to polymer chains may migrate and filter out of the material over time contributing to the risk of human exposure. Plasticisers represent a large fraction of polymer additives. Among these, DEHP is a plasticiser widely used in PVC products such as household products, dust, food packaging and medical devices. Due to its highly hydrophobic behaviour, leaching of DEHP from plastics to the environment, food, blood or lipophilic fluids can easily occur (Erythropel et al. 2014).

For instance, high concentrations of DEHP were detected in blood storage bags (150 - 300 μg $mL^{-1}$) and the patient’s blood and urine samples (20 - 30 μg $mL^{-1}$). Thus, it was already classified as CMR 1B (carcinogenic, mutagenic or toxic to reproduction) according to the Regulation on Classification, Labelling and Packaging (CLP) in the EU (EuropeanRegulation_(EC)_1272/2008)). An overview of the endocrine, testicular, ovarian, neural, hepatotoxic, and cardiotoxic effects of DEHP on animal models and humans in vitro and in vivo was recently published by Rowdhwal et al. (2018). Even though the use of DEHP in PVC medical devices has been limited, its presence in several food products continues to be a valuable exposure route for the human population.

Evidence of detrimental effects on human health associated with exposure to DEHP is relatively limited, and risk assessments have been based primarily on the results of animal studies. The cytotoxic potential of DEHP at relatively low concentrations (≥ 10 μM) in Vero cells, a monkey kidney-derived cell line, was previously analysed by Peropadre et al. (2013) reporting lysosomal destabilization and late plasma membrane damage. However, these sublethal cytotoxic effects should also be verified in human cell lines.

In this context, the cytotoxic behaviour of some organic contaminants, including DEHP, fullerenes soot and, lastly, synergistic and antagonistic relationships of the binary mixture were also assessed in our work. Cytotoxic effects with a generation of ROS directly proportional to the concentration (0.5 - 50 μg $mL^{-1}$) were reported for the cerebral human cells (T98G) after 24h-exposure of DEHP. Synergic effects were observed when the compound was mixed with fullerene aggregate. In addition, delays in cell cycle progression and reduction in mitotic activity were observed by Peropadre et al. (2015) in human mammalians cells (HaCaT) exposed to the same DEHP concentration range. These results demonstrated that the cytotoxic effects of DEHP in human cells are not cell type specific.

# 5 . Future trends and conclusions

## 5.1 Future trends

Despite mitigation strategies adopted during the last years, plastic waste is still a major global concern. Therefore, more political and public efforts are needed. First, recycling and re-use measures should be implemented and simplified, and public information and awareness-raising activities should be supported. Besides this, circular economy actions and industrial change, including a ban on the use of plastic microspheres in commercial products, should be further encouraged to reduce plastic pollution.

In addition, further national or regional monitoring programmes are needed to increase knowledge of marine litter. In this context, citizens are very important resources in marine waste research. Citizen science should continue to be promoted as the first rapid assessment survey for the identification of macro-litters, including macroplastics, especially in beaches and coastal waters. In this way, more quantitative data on litter density and its impact on the environment could be achieved. Also, details on the litter found should be reported to know the specific contamination sources.

Despite public involvement in research on macro-sized marine litter, more attention should be paid to the issue of MPLs, including NPLs. These contaminants continue to be the focus of research, public engagement and government action. Since some MPLs are identifiable to the naked eye, initial data on the extent and impact of these pollutants on the surrounding environment could be obtained by specialised volunteers. This data combined with the work supported by agencies, NGOs and private sector partners can create a new global platform.

In terms of assessing the occurrence of MPLs and NPLs in the environment, sampling procedures in water, sediment and soil need to be improved. The lack of a single definition and, thus, comparability of the results obtained in current studies limits the possibility of carrying out risk assessment studies. Therefore, harmonised approaches are necessary, starting from sampling protocols, to facilitate the global analysis of the MPLs/NPLs pollution problem. In this line, methodologies and measurements should be as well consolidated. For some analytical techniques, the first interlaboratory studies have already been organised to ensure the quality of the results. However, interlaboratory activities should be extended to other techniques and on different matrix samples. Other difficulties that should be overcome are the lack of reference materials and MPLs/NPLs standards with varying sizes and chemical composition. Furthermore, these are particularly necessary to develop quantitative analytical methods for smaller plastic particles, especially those with high cross-linking percentages that cannot be solubilised.

On the other hand, the main environmental paths followed by plastic particles, their fate and behaviour should be better addressed. Therefore, the properties of weathering MPLs should be well described. Because of the daily human interaction with the environment, it will be necessary to define and characterise human exposure to plastic pollution. Further investigations into the potential adverse effects of plastic nanoparticles on human health should be investigated through toxicological studies, assessing long-term effects and the potential accumulation and translocation in the human body. Therefore, expanding studies evaluating toxicological response on different human cell lines and research on biomarkers of exposure are in high demand.

The results of such monitoring programmes could serve as a model to guide large-scale government action and future environmental research projects targeting MPLs/NPLs.

## 5.2 Conclusions

According to the objectives of this thesis, the research carried out, and the results described in the previous chapters, the following conclusions can be drawn.

- Large urban areas substantially contribute to marine pollution through rivers. Riverine litter inputs of two Catalan rivers into the Western Mediterranean have been quantified. In particular, the results of visual observations of floating anthropogenic macro-litter (> 2.5 cm) have been reported from Llobregat and Besòs Rivers during an intensive one-year monitoring period. The lower courses of both rivers run through an industrialized and populated area nearby the city of Barcelona (Catalonia, NE Spain), entering the sea. The results show a similarity in litter categories which are mainly influenced by urban centres located along rivers. Plastics is the dominant waste category and bottles, bags and cover/packaging (from food and beverages) are among the most observed items. Seasonal variability was reported for Llobregat as well as other Mediterranean rivers of the closer area. The runoff processes or strong winds play an important role in the final fate of waste. Finally, a new approach to estimate the annual loading (mass) of floating macroplastics shown that 0.4 - 0.6 tonnes per year of plastic were loaded into the Mediterranean Sea by Catalan Rivers. These results could be interesting to further model riverine plastic inputs at a larger scale.

- Several analytical techniques (MALDI-TOF/MS, DESI-MS, DART-MS, and LC-APPI-HRMS) were compared for the quantitative and qualitative analysis of MPLs/NPLs. The comparison of these approaches showed the technique based on dimensional exclusion-LC coupled with HRMS to be slightly more sensitive (ILOD = 2 pg). This technique resulted suitable for the analysis of low molecular weight polymers (< 3000 Da). The analytical method based on HPLC-HRMS equipped with atmospheric pressure photo-ionisation (APPI) source operating in negative mode was developed and validated for the analysis of PS-MPLs/NPLs in the suspended fraction of natural environmental waters. Due to the nature of these plastic particles, the chromatographic separation was achieved with advanced polymer chromatography (APC) column using toluene 100% as the mobile phase. The results of methods validation showed high recoveries (> 60%), robustness, and high sensitivity (MLOD = 30 pg $L^{-1}$ and MLOQ = 100 pg $L^{-1}$). Finally, the good applicability of the method was proved to analyse 17 natural water samples (8 freshwater and 9 seawater samples) from Ebro Delta (Catalonia, NE Spain), an important Mediterranean estuarine coastal area. The presence of PS-MPLs/NPLs was confirmed in 5 water samples with a concentration ranging from 1 to 150 ng $L^{-1}$ (RSD < 5%).

- The adsorption behaviour of HDPE, PS and PS-COOH MPLs was studied conforming their capacity to adsorb PFAs compounds from the surrounding water. The adsorption capacity observed was PS > PS-COOH > HDPE and Freundlich equation was the adsorption isotherms reported in most of the cases. Generally, an increase in adsorption rate for longer-chain compounds was observed. For example, till 35% of short-chain PFAS were adsorbed in PS and PS-COOH in the seawater experiments. The 8-carbon chain compounds, such as PFOS and PFOA, were adsorbed in the same type of MPLs reaching higher percentages (> 50%) after 46 days of exposure under ambient conditions. Long-chain compounds were shown to be stabilized in all types of MPLs reaching up to 80%. It should be highlighted that different behaviours were observed depending on water type.

Overall, the stabilization of organic contaminates onto MPLs surface in seawater was favoured.

- The MPLs ingestion by common dolphinfish (*C. hippurus*) from the western Mediterranean was assessed. In this context, a new digestion approach, combing basic (KOH) and acid ($HNO_3$) reagents, was considered. The method was validated and applied to 29 samples of GITs. The results showed a high frequency of plastic occurrence (65.5%). Most of ingested MPLs were fragments with varying colours and size. FTIR analysis indicated PE as the most common polymer type followed by PS and PP. In general, the presence of plastic particles in the GIT of this species may be due to its general feeding behaviour and/or predation. However, considering the type of plastics identified, a most likely microplastic source could be local fishing gears which were abandoned or lost in this part of the basin, such as FADs. As a result, *C. hippurus* can be considered as a good bio-indicator for the impact of litter ingestion (micro and macro litter) in open waters at medium-spatial scale (Mediterranean UN Environment/MPA sub-regions). Finally, the newly developed digestion method ($KOH/HNO_3$) applied for this study was considered as a reliable approach to detect MPLs in the GIT of opportunistic pelagic predators, feeding on a wide variety of prey items.
- The acute toxicity of nanomaterials, common organic contaminants and two types of micro(nano)plastics was studied against two human cell lines in terms of cell viability and oxidative stress. The High-Content Analysis (HCA) was a suitable technique for the simultaneous observation and toxicity assessment of cells exposed to these contaminants. In particular, cells exposed to PE-MPLs/NPLs showed no significant variation in ROS levels. On the contrary, the cytotoxic effect of PS-MPLs/NPLs increased in a dose-dependent manner. Even if both cell lines behaved similarly, ROS effect is more evident in the HeLa cell line compared to T98G cells. Finally, the cytotoxic assessment of one of the most common plasticisers, DEHP, showed a generation of ROS in a concentration-dependent manner in T98G cells and synergic effects with the presence of fullerene aggregates.

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