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Micro and nanoplastics in the environment: development of mass-quantitative analytical methodologies for the global impact assessment

Albert Vega Herrera

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Albert Vega Herrera PhD Thesis

2024

Micro and nanoplastics in the environment: development of mass-quantitative analytical methodologies for the global impact assessment

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CSIC
Consejo Superior de Investigaciones Científicas

Universitat de Barcelona

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

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Programa de doctorado “Química Analítica y Medio Ambiente”

MICRO AND NANOPLASTICS IN THE ENVIRONMENT: DEVELOPMENT OF MASS-QUANTITATIVE ANALYTICAL METHODOLOGIES FOR THE GLOBAL IMPACT ASSESSMENT

Memoria presentada en compendio de artículos científicos para optar
al título académico de Doctor por la Universidad de Barcelona

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Sr. Albert Vega Herrera
En Barcelona, a 10 de junio de 2024

La Dra. Marinella Farré Urgell, Investigadora Científica en el Instituto de Diagnóstico Ambiental y Estudios del Agua – Consejo Superior de Investigaciones Científicas (IDAEA-CSIC), y el Dr. Esteban Abad Holgado, Investigador Científico del IDAEA-CSIC, certifican:

Que la presente memoria presentada para optar al título académico de Doctor ("Doctor of Philosophy") cuyo nombre es “Micro and nanoplastics in the environment: development of mass-quantitative methodologies for the global impact assessment”, se ha realizado principalmente bajo nuestra supervisión por el Sr. Albert Vega Herrera en el IDAEA-CSIC, y que todos los resultados presentados son fruto de trabajos de investigación llevados a cabo por el mencionado candidato a doctor.

En Barcelona, a 10 de junio de 2024

Dra. Marinella Farré Urgell **Dr. Esteban Abad Holgado**

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“**Si quieres hacer algo en la vida, no creas en la palabra imposible. Nada hay imposible para una voluntad enérgica**”

Pío Baroja

“**Es arriesgado, dijo la experiencia. No tiene sentido, dijo la razón. Inténtalo, dijo el corazón**”

Mario Alonso Puig

La investigación es un proyecto personal, un aprendizaje constante sobre ti mismo.

Por los que ya no están, y por los que siempre han estado.

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¡GRACIAS!

¡GRÀCIES!

¡MERCI!

THANK YOU!

TABLE OF CONTENTS

A

ABS	Acrylonitrile Butadiene Styrene
AI	Artificial Intelligence
AOPs	Advanced Oxidation Processes
ADI	Acceptable Daily Intake
ATR-FTIR	Attenuated Total Reflectance - FTIR
AFM	Atomic Force Microscopy
AF4	Asymmetric Flow Field – Flow Fractionation
APCI	Atmospheric Pressure Chemical Ionisation
APPI	Atomospheric Pressure PhotoIonisation

B

BMD_{10}	Benchmark Dose at 10%
BW	Body Weight
BPA	Bisphenol A
BBP	Butyl Benzyl Phthalate

C

CECs	Contaminants of Emerging Concern
CO	Carbon Monoxide
CO_2	Carbon Dioxide
Ca	Calcium
CRC	Concentration-Response Curves
C_e	Average Compound Concentration
CTD	Conductivity-Temperature-Depth
CCC	Critical Coagulation Concentration
CE	Capillary Electrophoresis
CI	Chemical Ionisation

D

DME	Dimethyl Ether
DBD	Dielectric Barrier Discharge
DWTPs	Drinking Water Treatment Plants
DI	Daily Intake
DLS	Dynamic Light Scattering
DART-MS	Direct Analysis in Real Time coupled with MS
DDA	Data Dependent Acquisition
DIA	Data Independent Acquisition
DDT	Dichlorodiphenyltrichloroethane
DEHP	Di(2-Ethylhexyl) Phthalate
DBP	Dibutyl Phthalate
DIBP	Diisobutyl Phthalate

E

EU	European Union
ECHA	European Chemicals Agency
EPA	United States Environmental Protection Agency
ENMs	Engineered Nanomaterials
EFSA	European Food Safety Authority
EDI	Estimated Daily Intake
EIS	Electrochemical Impedance Spectroscopy
ESI	Electrospray Ionisation
EI	Electron Ionisation
ETM	Estuarine Turbidity Maximum

F

Fe^{+2}	Iron ion (II)
FLEXPART	Flexible Particle Dispersion
μ-FTIR	Micro-Fourier Transform Infrared Spectroscopy

G

GIT	Gastrointestinal Tract

H

HD-PE	High-Density Polyethylene
H_2	Hydrogen
H_2O_2	Hydrogen Peroxide
HQs	Hazard Quotients
HI	Hazard Index
HNO_3	Nitric Acid
HILIC	Hidrophilic Interaction Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
HYSPLIT	Hybrid Single-Particle Lagrangian Integrated Trajectory
HCH	Hexachlorocyclohexene

I

ISO	International Organisation for Standardisation
INC	Intergovernmental Negotiating Committee
IC_x	Concentration required to cause x% of inhibition
ICP-MS	Inductively Coupled Plasma – Mass Spectrometry
IC	Ion Chromatography

K

$K_2S_2O_8$	Disodium Persulfate
KOH	Potassium Hydroxide

L

LD-PE	Low-Density Polyethylene
LCA	Life Cycle Assessment
LRAT	Long-Range Atmospheric Transport
LD_{50}	Dose that is lethal to 50% in an organism
LOAEL	Lowest Observed Adverse Effect Level
LC_x	Concentration required to cause x% of lethal response
LDIR	Laser Direct Infrared Spectroscopy
LC	Liquid Chromatography
LC x LC	Comprehensive Two-Dimensional LC
LRMS	Low Resolution Mass Spectrometry
LAGRANTO	Lagrangian Analysis Tool
Log $k_{o/w}$	Logarithm Octanol-Water Partition Coefficient

M

MNPLs	Micro and Nanoplastics or Micro(nano)plastics
Mt	Metric Tonnes
MeOH	Methanol
MPLs	Microplastics
MALDI-MS	Matrix-Assisted Laser Desorption/Ionisation coupled with MS
m/z	Mass-To-Charge Ratio
MRM	Multiple Reaction Monitoring
Mg	Magnesium

N

N_2	Nitrogen
NPLs	Nanoplastics

NOM	Natural Organic Matter
NOAEL	No Observed Adverse Effect Level
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NTA	Nanoparticle Tracking Analysis
NTS	Non-Target Screening
NORMAN	Network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances

O

OCDE	Organisation for Economic Cooperation and Development
O_2	Oxygen
·OH	Hydroxide Radical

P

PP	Polypropylene
PS	Polystyrene
PBD	Polybutadiene
PI	Polyisoprene
PET	Polyethylene Terephthalate
PVC	Polyvinyl Chloride
PUR	Polyurethane
PC	Polycarbonate
PDMS	Polydimethylsiloxanes
PMMA	Polymethylmethacrylate
PTFE	Polytetrafluoroethylene
PBT	Polybutylene Terephthalate
PLA	Polylactic Acid
PGA	Polyglycolic Acid
PHB	Polyhydroxybutyrate
PHAs	Polyhydroxyalkanoates
PCL	Polycaprolactone
PEA	Polyesteramides
PBAT	Polybutylene Adipate Terephthalate
PBS	Polybutylene Succinate
PTT	Polytrimethylene Terephthalate
PA	Plastic Additive(s)
POPs	Persistent Organic Pollutants
PCPs	Personal Care Products
$PM_{2.5}$	Particulate Matter less than 2.5 µm
Py-GC-MS	Pyrolysis coupled to Gas Chromatography and Mass Spectrometry
PLEX	Plastic Explore
PUF	Polyurethane Foam
PCBs	Polychlorinated Biphenyls
PFASs	Perfluoroalkyl Substances
PAHs	Polycyclic Aromatic Hydrocarbons
PFOA	Perfluorooctanoic
PFBS	Perfluorobutanesulfonate
PFHxS	Perfluorohexasulfonate
PFOS	Perfluorooctanesulfonate
PBDEs	Polybrominated Diphenyl Ethers

Q

qQq	Triple Quadrupole
q-IT	Quadrupole-Ion trap
q-Orbitrap	Quadrupole-Orbitrap
q-TOF	Quadrupole-Time Of Flight

QSAR	Quantitative Structure-Activity Relationship
QA/QC	Quality Assurance/Quality Control

R

REACH	Registration, Evaluation, Authorisation, and restriction of Chemicals
ROS	Reactive Oxygen Species
RNS	Reactive Nitrogen Species
RNA	Ribonucleic Acid
RC_{50}	Concentration required to cause 50% of the response
μ-Raman	Micro-Raman Spectroscopy
RP	Reversed Phase

S

SDGs	Sustainable Development Goals
SERS	Surface Enhanced Raman Scattering
SFC	Supercritical Fluid Chromatography
SML	Surface Microlayer
SIM	Selected Ion Monitoring

T

TGA	Thermogravimetry
TED-GC-MS	Thermal Extraction Desorption coupled with GC and MS
TOF	Time Of Flight
$T_{1/2}$	Half-Life Time
T_D	Decreasing Time

U

UN	United Nations
UNEA	United Nations Environmental Assembly
UV	Ultraviolet
UAE	Ultrasonic-Assisted Extraction
ULW	Underlying Seawater at 5 m Depth

V

VOCs	Volatile Organic Compounds
v/v	Volume per Volume

W

WWTPs	Wastewater Treatment Plants
WHO	World Health Organisation
ww	Wet Weight

Z

$ZnCl_2$	Zinc (II) Chloride

Micro and nanoplastics (MNPLs) are considered contaminants of emerging concern (CECs) due to their detrimental environmental and human health effects already reported in recent literature and because current legislation applied in developed countries is not able to regulate these effectively enough or simply does not exist in most developing countries. Plastic pollution concerns the scientific community because of its continuous introduction to the environment and the poor consensus regarding the impact at global scale and its potential toxic effects associated with living organisms that are still poorly understood. Moreover, there is a lack of harmonised quantitative analytical methodologies able to estimate plastic pollution's impact in terms of mass concentration. The main current scientifc advances published for these purposes are related to microscopy, spectroscopy or nanometric techniques that are more suitable for successful particle characterisation (nature, size and shape) and qualitative extrapolations based on plastic particle-counting approaches which are unsuitable to provide real quantitative measurements.

In the present context of climate change, circular economy processes acquire increased importance and there is a need to understand what micro(nano) plastics are, as well as their different origin sources, their occurrence, fate, behaviour in the environment, and the different human exposure routes. In this sense, in this doctoral thesis, mass-quantitative analytical methodologies based on liquid and pyrolysis-gas chromatography coupled with mass spectrometry have been developed and applied to samples collected from different environmental compartments and marine biota in order to assess the plastic pollution by MNPLs abundance and to give a comprehensive global view. Target and suspect screening approaches have been applied to obtain the polymeric and plastic additives composition. Moreover, various MNPLs human exposure routes have been also evaluated through contaminated food (mussels previously exposed), drinking water taken from household taps located in the Barcelona Metropolitan Area, and bottled water in single-use plastic bottles from the 20 most popular commercial brands in Spain.

Thus, this thesis contributes to enhance the understanding and knowledge about MNPLs pollution, putting special emphasis on the occurrence, fate, and behaviour of these contaminants in atmospheric and aquatic ecosystems from areas close to populated zones to remote regions, the potential exposure to marine biota, and ultimately, to humans. In addition, the analytical methodologies presented here will help to address the scientific community regarding the current lack of mass-quantitative methods available in order to tackle plastic pollution through real and more accurate MNPLs-polymers abundances at ultra-trace levels, and finally, to obtain a more comprehensive view of the real impact of these contaminants on the environment and the subsequent potential threats associated with living organisms through the main exposure pathways.

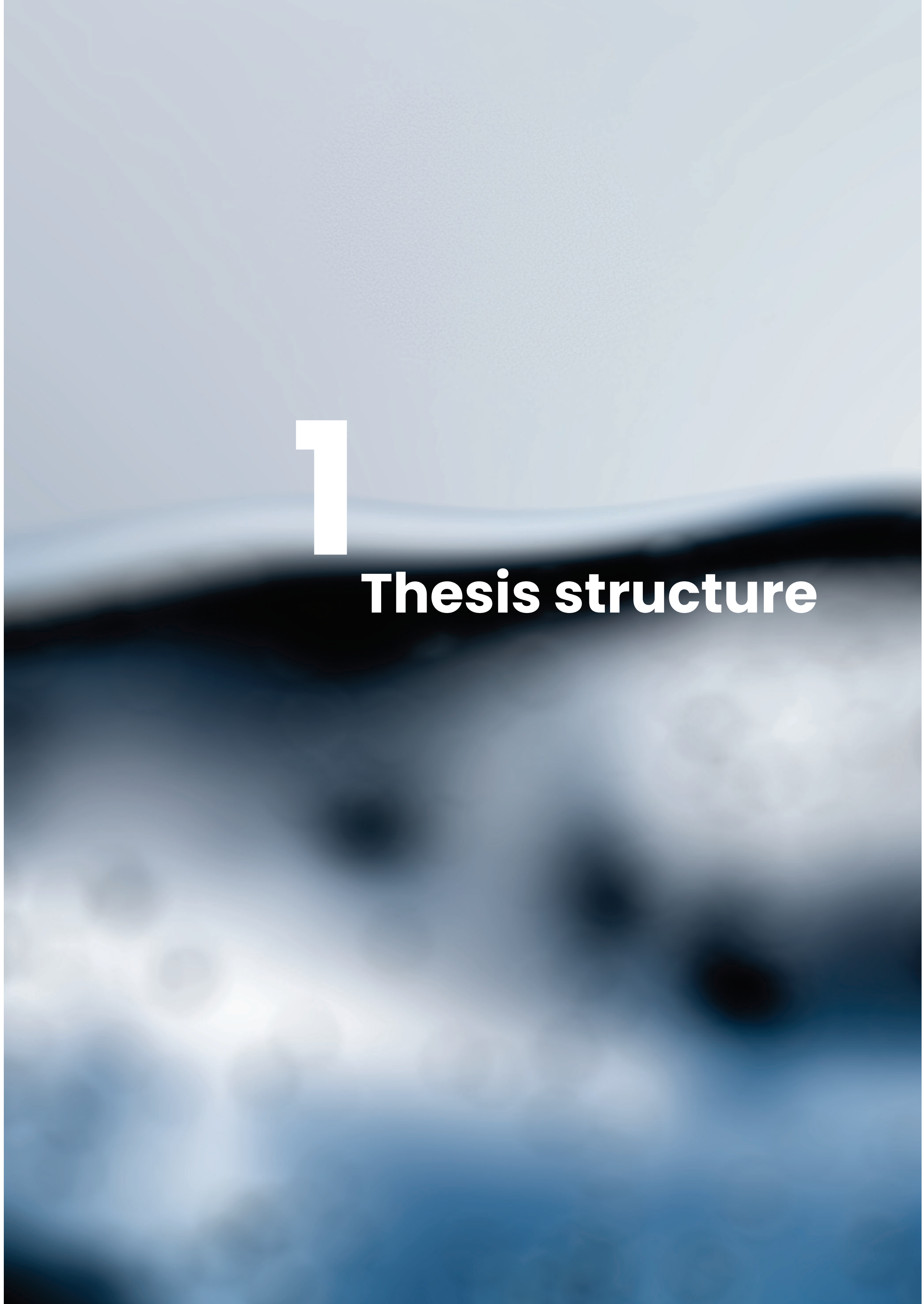
Los micro y nanoplásticos (MNPLs) son considerados contaminantes de preocupación emergente (CECs) debido a que algunos de sus efectos perjudiciales para el medio ambiente y la salud humana ya han sido reportados en la literatura científica. Además, en países desarrollados no han sido legislados de manera eficaz, mientras que en los países en vías de desarrollo, dicha legislación, simplemente no existe. La contaminación plástica preocupa a la comunidad científica por su continua introducción en el medio ambiente, el escaso consenso sobre su impacto a escala global, y sus potenciales efectos tóxicos asociados a organismos vivos aún poco estudiados. Es necesario destacar la dificultad del análisis de MNPLs, ya que actualmente hay una carencia importante de armonización de métodos analíticos en uso y un número limitado de métodos analíticos cuantitativos. Los principales hallazgos científicos publicados con este fin están relacionados con la microscopía, la espectroscopía o técnicas nanométricas más adecuadas para la caracterización exitosa de partículas (naturaleza, tamaño y forma) y extrapolaciones cualitativas basadas en enfoques de recuento de partículas de plástico, las cuales son inadecuadas para proporcionar mediciones cuantitativas reales.

En el contexto actual de cambio climático, los procesos de economía circular adquieren una importancia relevante y surge la necesidad de comprender qué son los micro(nano)plásticos, así como sus diferentes fuentes de origen, su presencia, destino y comportamiento en el medio ambiente, y las diferentes rutas de exposición. En este sentido, en esta tesis, se han desarrollado y validado diferentes metodologías analíticas cuantitativas de masa basadas en cromatografía de líquidos y pirólisis-gases acoplada a la espectrometría de masas para su posterior aplicación a muestras recogidas en diferentes compartimentos ambientales y biota marina para evaluar la contaminación plástica a través de las abundancias de MNPLs e intentando proporcionar una visión global integral. Métodos de detección “target” y de cribado de sospechosos han sido aplicados con la finalidad de obtener la composición polimérica y de aditivos plásticos. Además, varias rutas de exposición humana a los MNPLs también han sido evaluadas a partir de alimentos contaminados (mejillones previamente expuestos), agua apta para el consumo humano a partir de grifos domésticos del Área Metropolitana de Barcelona, y agua potable embotellada en botellas de plástico de un solo uso a través de las 20 marcas comerciales más populares en España.

Así, esta tesis doctoral contribuye a mejorar la comprensión y el conocimiento actual sobre la contaminación plástica por MNPLs, poniendo especial énfasis en los efectos perjudiciales que pueden ocasionar en ecosistemas marinos y atmosféricos desde zonas cercanas a áreas pobladas hasta regiones remotas, la potencial exposición en organismos vivos marinos, y en último lugar, en humanos. Además, las metodologías aquí presentadas ayudarán a abordar a la comunidad científica la falta de métodos cuantitativos de masa disponibles para abordar la contaminación por plásticos a partir de abundancias reales y más precisas de polímeros de MNPLs a nivel de ultratraza, y finalmente, a obtener una visión más completa y abarcadora sobre el impacto real de estos contaminantes en el medio ambiente y sus posteriores amenazas potenciales asociadas a organismos vivos a través de las principales rutas de exposición.

1

Thesis structure



1.1 Thesis structure

This doctoral thesis is presented as a compendium of 8 scientific articles and it is divided into 7 chapters and the appendix. **Chapter 1** includes the structure followed throughout this doctoral thesis.

In **chapter 2**, a state of art of plastics is provided including crucial definitions and keywords, the different polymers most produced for the human activities, and the specifics on which this thesis is focused. In this context, the different plastic additives formulations used for each type of polymer and the most respresentatives are also described. In addition, the previous, current, and upcoming legislation and regulation regarding plastics at global-, European-, and Spain-scale are summarised. Fundamentals on the occurrence, fate and behaviour of MNPLs in various environmental compartments such as aquatic and atmospheric ecosystems, as well as the impact on biota, paying special attention in marine living organisms are also shown. Then, the main human exposure routes and the potential adverse effects associated to MNPLs are described. Finally, a review of the analytical methodologies available, as well as the application of target, suspect screening, and non-target screening (NTS) approaches for the MNPLs analysis and characterisation are detailed.

Chapter 3 explains the primary and the specifics derived goals of this doctoral thesis.

Chapters 4, **5**, and **6** include the scientifics articles already published or (to be) submitted to scientific journals related with the field, where the experimental work, instrumental analysis, results achieved, and their discussions are shown. All these chapters are divided by topic, following a circular structure since the assessment of the MNPLs occurrence, fate and behaviour in various aquatic and atmospheric ecosystems from areas nearby to populated zones to remote regions (**chapter 4**), the study of the possible interactions between MNPLs and other contaminants present in surroundings environments by the characterisation of their adsorption-desorption mechanisms (**chapter 5**), as well as an exhaustive evaluation of the common MNPLs human oral exposure routes by the food and drinking water daily intake (**chapter 6**). All these chapters have the same structure, including a brief introduction of the subject mentioning the analytical methodologies employed, followed by the scientific articles included in the chapter, a transversal discussion, and the corresponding references taken from the literature.

Chapter 4 provides novel insights about the MNPLs occurrence in air and surface seawater samples collected from estuarine areas, the North and South Atlantic ocean, and the Southern ocean covering the Bellingshausen sea (west Antarctica peninsula). It includes 4 scientific publications reporting the MNPLs-polymers abundances and seasonal variations in 2 protected Mediterranean areas with particular ecosystems of relevant ecological interest, as well as the MNPLs-polymers spatial distribution in the marine low-atmosphere and surface water compartments from oceanic regions. Besides, the origin sources of these airborne MNPLs found in the marine atmosphere and their air-water mass exchanges have been also studied:

- **Publication nº1:** Llorca, M., Vega-Herrera, A., Schirinzi, G., Savva, K., Abad, E., Farré, M., Screening of suspected micro(nano)plastics in the Ebro Delta (Mediterranean Sea), 2021, Journal of Hazardous Materials, https://doi.org/10.1016/j.jhazmat.2020.124022

- **Publication nº2:** Vega-Herrera, A., Llorca, M., Savva, K., M. León, V., Abad, E., Farré, M., Screening and quantification of micro(nano)plastics and plastic additives in the seawater of Mar Menor lagoon, 2021, Frontiers in Marine Science, https://doi.org/10.3389/fmars.2021.697424

- **Publication nº3**: Caracci, E., Vega-Herrera, A., Dachs, J., Berrojalbiz, N., Buonanno, G., Abad, E., Llorca, M., Moreno, T., Farré, M., Micro(nano)plastics in the atmosphere of the Atlantic ocean, 2023, Journal of Hazardous Materials, https://doi.org/10.1016/j.jhazmat.2023.131036

- **Publication nº4**: Vega-Herrera, A., Le Bagousse, M., Berrojalbiz, N., Abad, E., Llorca, M., Dachs, J., Gigault, J., Farré, M., Micro and nanoplastics in marine waters from the Atlantic and Southern oceans, To be submitted in Nature Communications.

In **chapter 5**, the characterisation of the different adsorption and desorption mechanisms between different polymers of microplastics and persistent organic contaminants carried out in simulated estuarine environments by seawater-sediment systems at laboratory scale is included:

- **Publication nº5**: Llorca, M., Ábalos, M., Vega-Herrera, A., Adrados, M. A., Abad, E., Farré, M., Adsorption and desorption behaviour of polychlorinated biphenyls onto microplastics' surfaces in water/sediment systems, 2020, Toxics, https://doi.org/10.3390/toxics8030059

In **chapter 6**, three studies assessing the MNPLs human oral exposure by the daily consumption of food and drinking water are reported. The drinking water analysed has been collected through household taps and single-use plastic bottles commercialised in the Barcelona Metropolitan Area. Regarding the human oral exposure to MNPLs through the bottled water in single-use plastic bottles, a prioritisation study based on a multi-QSAR approach is also provided to elucidate the migrated plastic additives composition from the plastic bottle to the water. Furthermore, the human dietary bioaccessibility of various perfluoroalkyl substances individually and co-exposed with MPLs through Mediterranean mussels is also evaluated by *in vitro* assays.

- **Publication nº6**: Vega-Herrera, A., Llorca, M., Borrell-Diaz, X., Redondo-Hasselerharm, P. E., Abad, E., Villanueva, C. M., Farré, M., Polymers of micro(nano)plastic in household tap water of the Barcelona metropolitan area, 2022, Water Research, https://doi.org/10.1016/j.watres.2022.118645

- **Publication nº7**: Vega-Herrera, A., Garcia-Torné, M., Borrell-Diaz, X., Abad, E., Llorca, M., Villanueva, C. M., Farré, M., Exposure to micro(nano)plastics polymers in water stored in single-use plastic bottles, 2023, Chemosphere, https://doi.org/10.1016/j.chemosphere.2023.140106

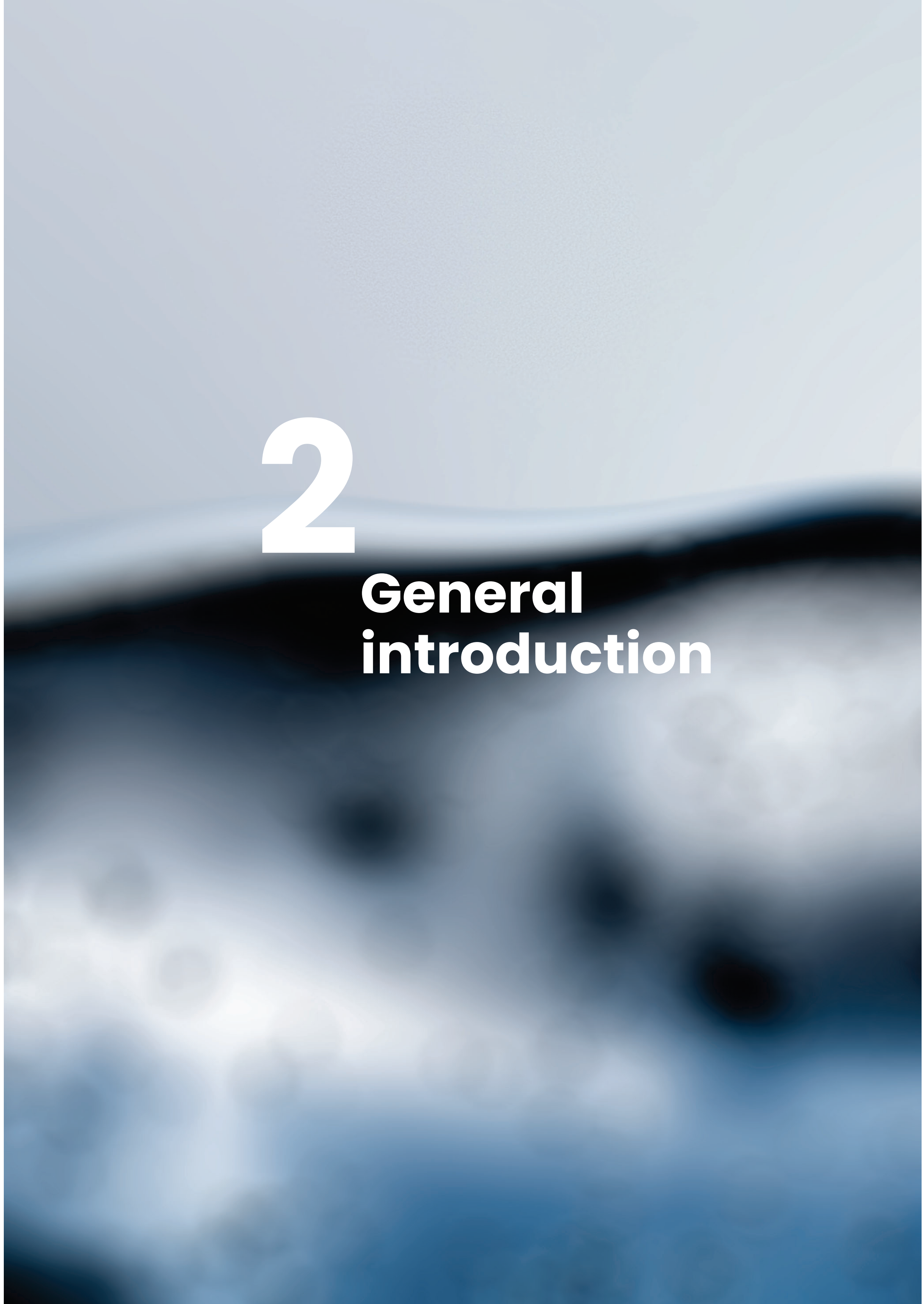
- **Publication nº8**: Vega-Herrera, A., Savva, K., Lacoma, P., Santos, Lúcia H.M.L.M., Hernández, A., Marmelo, I., Marques, A., Llorca, M., Farré, M., Bioaccumulation and dietary bioaccessibility of microplastics composition and cocontaminants in Mediterranean mussels, Submitted to Chemosphere.

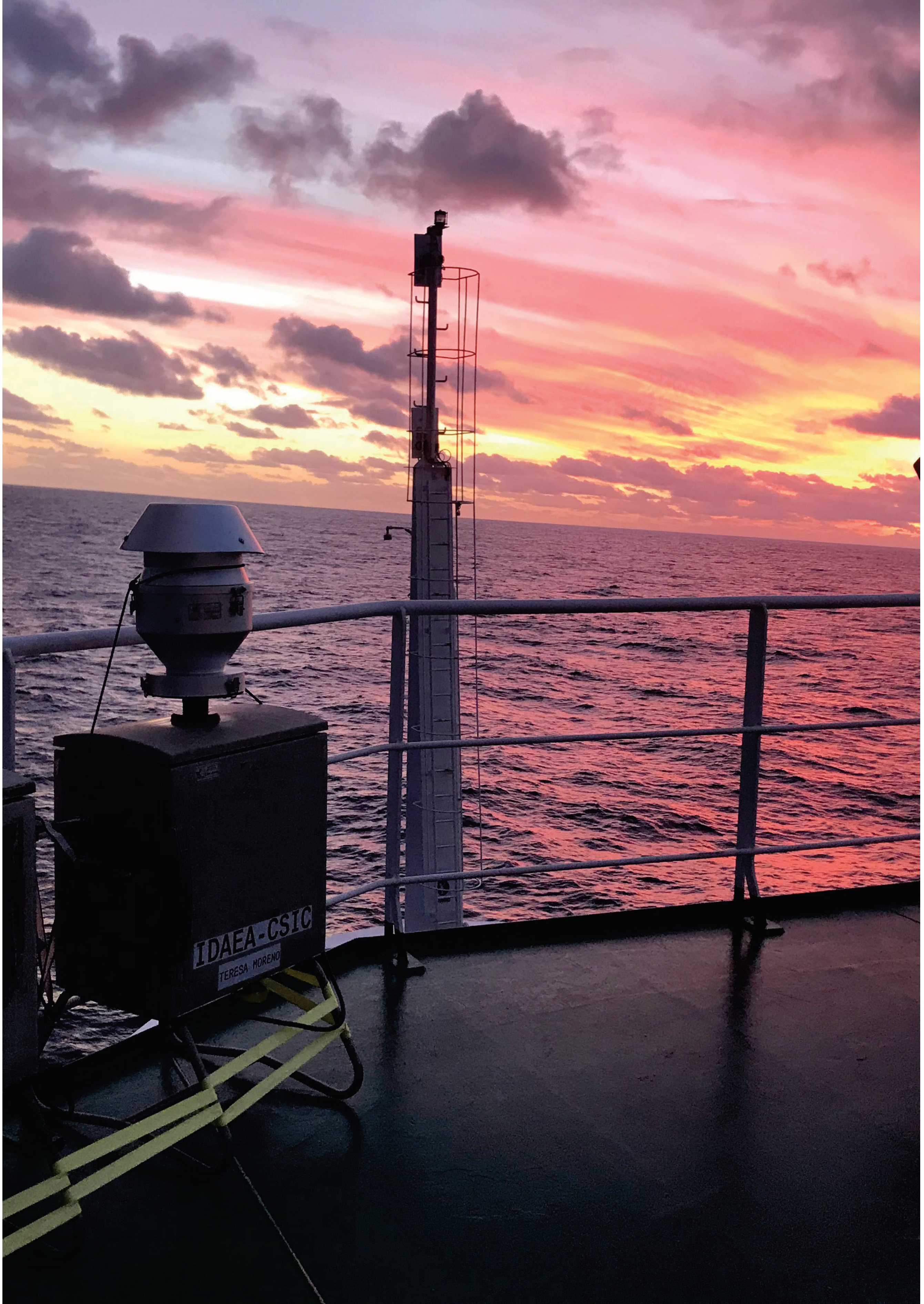
The conclusions of this doctoral thesis and the future trends are provided in **chapter 7**.

Finally, the appendix summarising the figures and tables that appear in this thesis, as well as the financial support, are included at the end.

2

General introduction





2.1 Plastics as contaminants of emerging concern

Contaminants of emerging concern (CECs) are substances that have been identified in the environment, and have the potential to provoke adverse human health or ecological impacts, but are not regulated. The group of compounds that collectively fall within this description has historically been a 'moving target', which began with global lead pollution that was incurred by the Romans two millennia ago, then included chlorofluorocarbons or dichlorodiphenyltrichloroethane at the beginning of the 1960s with the consequent environmental protests, and more recently, includes a wide range of pharmaceuticals, personal care products, engineered nanomaterials, and plastics (Mitrano et al., 2021).

Nowadays, we live in what is termed the 'plastic era'. Plastic materials are used in a wide variety of industrial and consumer products, from personal care products or the clothes we wear daily to single-use and permanent items that are applied in healthcare, as well as in the food, construction, or automotive industries. Plastics are everywhere, being hardly avoidable, and the reason for this lies in four of the main characteristics of plastics: versatile, lightweight, cheap, and durable.

Humanity started the industrial production of plastic materials in the mid-20th century, and since then, 8.7 billion metric tonnes (Mt) have been produced (**Figure 2.1**). Annually, production amounts to 391 million Mt and the Organisation for Economic Cooperation and Development (OECD) estimates that if this trend continues then, by 2060, humanity will reach production of 1 billion Mt annually, thus tripling the current amount (OECD, 2023).

The onset of the plastic era represented progress and was one of the great revolutions in history, but it also initiated a significant threat to the environment. As well as two major advantages of plastics, being their versatility and durability, the poor resistance of plastics to degradation, once they are exposed to external factors, is a disadvantage and also one of their major environmental problems. The current extensive use of plastics, together with an insufficiency regarding their proper management, have led to the attainment of considerably high levels of terrestrial, atmospheric, and aquatic pollution, and these levels will continue to increase sharply over the next decades. In fact, around 20 Mt of plastic are released into lakes, rivers, seas, and oceans each year, while the widespread use of plastic mulch in agriculture is one of the most important causes of soil degradation and groundwater contamination (Hofmann et al., 2023).

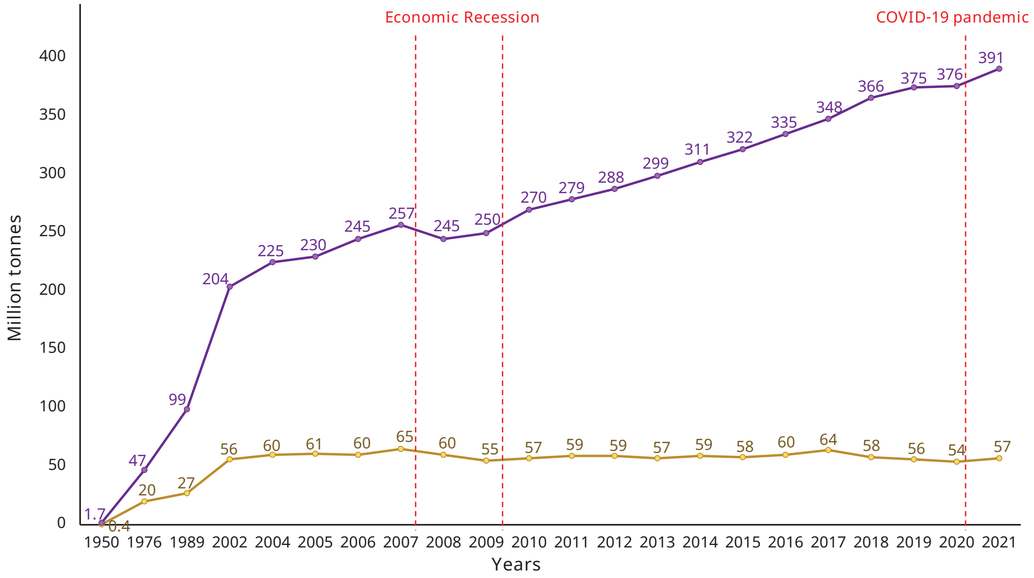


Figure 2.1 World (purple) and European Union (EU; yellow) plastic production since 1950, expressed in million metric tonnes.

Furthermore, recently (in 2020), the COVID-19 pandemic shook the world population and turned plastics into a public health necessity, and they were prioritised at that time over environmental issues. The use of plastics multiplied: in addition to the regular utensils, masks, gloves, hydroalcoholic gel containers, more products were added. Now that the COVID-19 crisis has (to the best of our knowledge) been managed, there exists in our society what is described as a rebound effect, in that, 'the more widespread recycling practices become, the more the population does tend to consume', while relying on the belief that the material will be fully recycled (PlasticsEurope, 2022), which is certainly not the case. In **Figure 2.2**, the evolution of world plastic production from 2017 to 2021, as well as plastic origin during 2021, are shown.

As can be seen in **Figures 2.1** and **2.2**, in general, in the EU, North America, and Japan, plastic production has been either reduced or maintained due to the implementation of the first directives that are aimed at mitigating plastic pollution, and also due to the externalisation of the most contaminating industrial activities. In China and other countries that are currently undergoing a rapid socioeconomic expansion, plastic production continues to rise, and it is projected to be an exponential increase. The cases of China and India are noteworthy, as they are home to two-fifths of the world's population with a strong industrial weight which includes the plastics sector, which is the main industry. In addition, particularly for China, the country's notable increase in production between 2017 and 2021 can be attributed primarily to its response to the COVID-19 pandemic, by the manufacturing of protective equipment against the SARS-CoV-2 virus for the Chinese population, as well as for other countries in the world, notwithstanding carrying on with the Chinese production of cheaper and low-quality plastics for traditional consumer products internationally. Without doubt, the historical high percentage of people living in China, compared to the world's population, together with a poor awareness regarding the environment and good labour practices, have currently allowed China to become the most profitable industrial cluster worldwide.

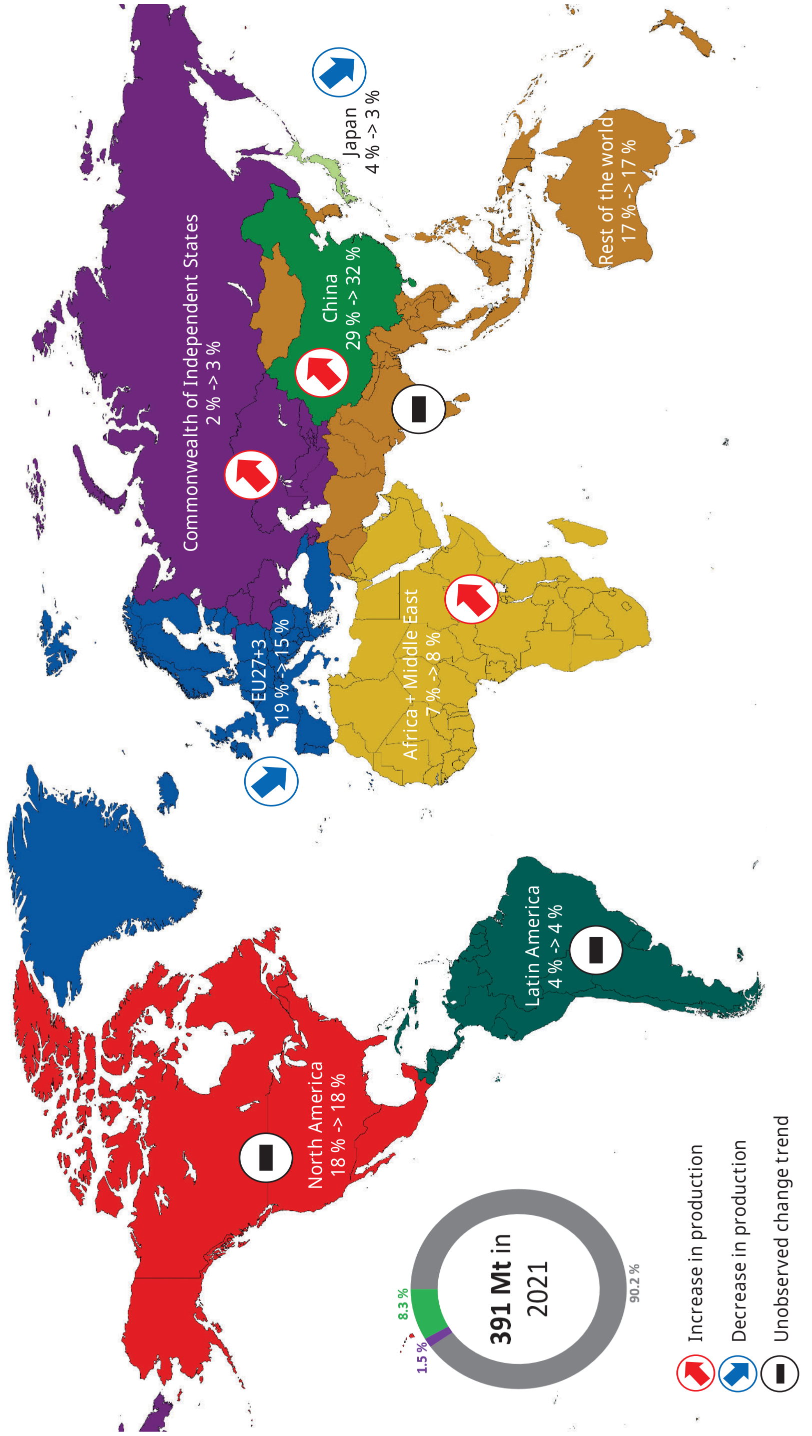


Figure 2.2 Evolution of world plastic production from 2017 to 2021 and plastic origin for production purposes in 2021: fossil- (grey), bio-based (pink), and recycled (green). Adapted from PlasticsEurope (2022).

Thus, the rise in global plastics production has shown a non-linear trend in recent years, indicating a concurrent increase in per-capita plastic consumption (**Figure 2.3**). A significant exponential increment in plastic consumption has been specifically shown during these last 20 years, unlike the second half of the 20th century, due to the rapid growth of the world's population, among other reasons. Hence, the ultimate objective must be to change the established linear economy model, so that these materials can be reintegrated into the human production chain, but this time following the concept of a circular economy (CE) (Andrady, 2017).

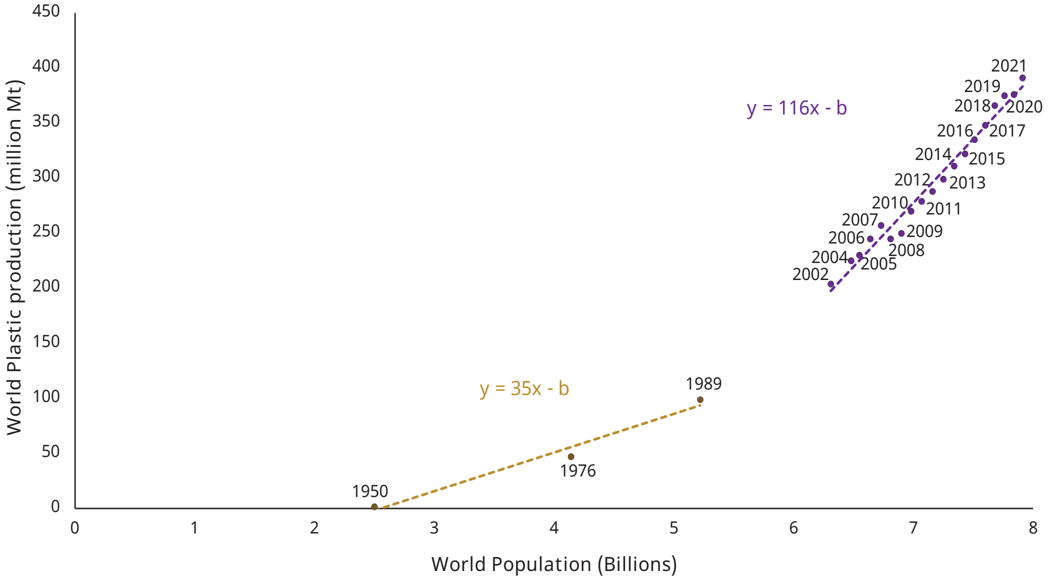


Figure 2.3 Change in global plastic production and the world population since 1950.

In this context, the sustainable use of plastics must involve all the stakeholders: consumers, producers, policymakers, and the scientific community. Moreover, a successful transition requires not only individual and consensual technical solutions, but also education and the full acceptance by all the social and business parties.

2.1.1 Plastics

Plastics are defined by the International Organisation for Standardisation (ISO, 2020), as macromolecules that are made of repetitive units (monomers), which are mainly composed of carbon and hydrogen atoms. The properties of these basic polymeric matrices are tuned using a wide variety of plastic additives, and sometimes by cross-linking with other polymers in order to modulate and enhance their own mechanical, physical, and chemical properties that are required for fit-for-purpose applications. Therefore, plastics are complex mixtures which encompass a wide range of materials with different properties, environmental behaviour, and potential adverse effects.

Following the present drastic rise in plastic use at a global scale in the last twenty years, the increased concern of the scientific community regarding the environmental risks and subsequent potential threats to living organisms that are associated with plastic pollution have promoted the research of plastic litter since 2007 (**Figure 2.4 A**). Further, special emphasis has been placed on the smaller-sized fractions from 2015 and 2019, in the case of micro- and nanoplastics, respectively, as shown in **Figure 2.4 B**.

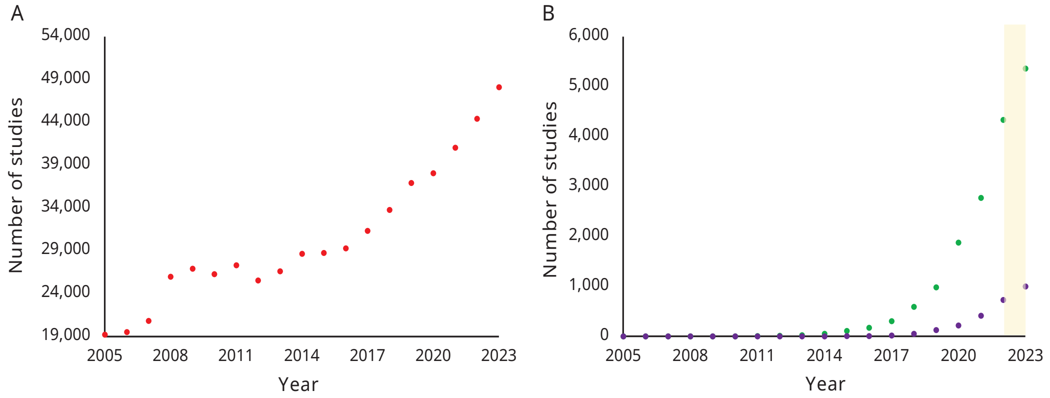


Figure 2.4 Number of studies available in Scopus database using the terms A) 'plastics', and B) 'microplastics' (green) AND 'nanoplastics' (purple) along the last two decades.

Moreover, the vertical yellow bar in **Figure 2.4 B** highlights the coincidence between the decrease in the number of publications and the exponential increase that occurs when the search terms are changed from 'microplastics' to 'nanoplastics' during this last year (2023), retrieving a total of 5,366 and 1,003 scientific articles, respectively, and emphasising a change of trend in plastic pollution research for the coming years, focusing more on studying the understanding and potential threats of nanoplastics instead of microplastics while, obviously, the general term 'plastics' is still growing.

Polymers

The main physical, mechanical, or thermal properties of polymers are governed by the disposition of their chains in the structure. They can be classified as linear, branched, and cross-linked. Thermoplastics with linear and unbranched polymeric chains are denser and harder and could be both amorphous or crystalline or heated and reshaped several times and reused. In the case of thermosets, cross-linked polymerics are rigidly linked by covalent bonds among chains that make them more stable and stronger, but once that are set with heat, then they cannot be reshaped. However, while elastomers may be deformed, they can still return to their original shape and size almost instantly (Peters, 2015).

Polymers can also be classified by their origin. In this sense, we distinguish between fossil- and bio-based materials. In 2021, 90.2% of plastics produced globally was largely based on petrochemical virgin polymers, due to their low costs associated with industrial production and the large number of applications; while only 8.3 and 1.5% of plastics were fabricated by recycled and biological materials, respectively (**Figure 2.2**).

In **Table 2.1**, the principal fossil-based polymers manufactured nowadays around the world are summarised according to their main properties and industrial applications (Li et al., 2016; Luzi et al., 2019).

Table 2.1 Current fossil-based polymers widely produced by humans at the global scale according to their density, main properties, and commercial applications.

Polymers	Density	Properties	Applications
Polypropylene (PP) $-[C_3H_6]_n-$	0.83-0.85	Translucent, low moisture vapour, transmission, inertness.	Plastic cups, pipes, food packaging, electro-domestics, automotive components, garden furniture, etc.
Low-density polyethylene (LDPE) $-[C_2H_4]_n-$	0.91-0.93	Relative transparency, flexibility, toughness, excellent resistance to solvents.	Plastic cups and bags, plastic films for agriculture, trays, containers, etc.
High-density polyethylene (HDPE) $-[C_2H_4]_n-$	0.94-0.96	Relative transparency, stronger, more tensile strength, excellent resistance to solvents.	Abrasive products, microspheres for cosmetic products, toys, etc.
Polystyrene (PS) $-[C_8H_8]_n-$	1.04	Significant stiffness, low thermal conductivity, resistance to chemicals.	Building insulation, packaging, egg trays, spectacle frames, etc.
Polybutadiene (PBD) $-[C_4H_6]_n-$	0.90-0.94	High elasticity and flexibility, good resistance to chemicals, water and abrasion, good electrical insulation.	Engineering, automotive industry, consumer products, etc.
Polyisoprene (PI) $-[C_5H_8]_n-$	0.92-0.94	Tear, chemical, ozone and weathering resistance, low gas permeability.	Adhesive formulations, tyre manufacturing, production of shoe soles and conveyor belts, O-rings, etc.
Polyethylene terephthalate (PET) $-[C_{10}H_8O_4]_n-$	1.37	High transparency, strength, and durability; lightweight material; good barrier against gases and moisture; recyclability, versatility and biocompatibility.	Food packaging, textile fibres, X-ray film, medical packaging, electronic applications, 3D printing, laminates and coatings, construction materials.
Polyvinyl chloride (PVC) $-[C_2H_3Cl]_n-$	1.37-1.39	Resistance to chemicals, brilliant clarity, high impact strength, excellent processing performance.	Pipes and accessories, windows, cable insulation, valves, floor and wall covering, etc.
Polyurethanes (PUR) $-[C_xH_yO_2N_2]_n-$	1.02-1.12	Abrasion resistance, resistance to oils and greases, rebound resilience, load-bearing capacity, damping properties.	Wearing parts, wheels and solid tyres, rollers, damping components, drive elements, cable sheathing, coated fabric, films, membranes, etc.
Polycarbonate (PC) Polydimethylsiloxanes (PDMS) Polymethylmethacrylate (PMMA) Acrylonitrile Butadiene Styrene (ABS) Polytetrafluoroethylene (PTFE) Polybutylene Terephthalate (PBT)	1.20-1.22 (PC), 1.07 (ABS)	Dependent on resin or mix of them.	Optical fibres (PBT), hub cups (ABS), roofing sheets (PC), touchscreens (PMMA), telecommunications (PTFE), aerospace, medical implants, surgical devices, membranes, seals, paintings, coatings, etc.

Otherwise, bio-based and petrochemical biodegradable polymers have emerged as very important alternatives to fossil raw materials. After the addition of plastic additives to the polymeric matrix, these materials can exhibit similar properties to fossil-based materials, but with the advantage of having a reduced carbon footprint. The term 'bioplastic' encompasses a wide range of polymers, each of which has different attributes in terms of its environmental impact, but there is no unique classification system. In **Table 2.2**, the most produced bio- and petrochemical biodegradable polymers are summarised (Asgher et al., 2020; Rosenboom et al., 2022).

In this context, bio-based and petrochemical-based biodegradable polymers are of special interest for products that are designed to intentionally end up in the environment or have a high-risk of doing so (examples are mulching films, plant nurseries, or materials used in fishing, and cigarette tips or filters), as well as for plastic materials that are able to generate contamination during their use (such as car tyres, wheels, textiles, paints, or aquaculture nets).

Table 2.2 Current bio- and petrochemical biodegradable polymers widely manufactured at the global scale, classified according to origin and main applications.

Polymers	Origin	Applications
Polylactic acid (PLA) $-[C_3H_4O_2]_n-$	Bio-based and partially biodegradable from polymerisation of monomers of biomass	Waste carrier bags, injection moulding, paper coatings, food packaging, 3D-printing, electronics, infant care.
Polyglycolic acid (PGA) $-[C_2H_2O_2]_n-$	Bio-based and partially biodegradable from monomers of biomass	Paints and coatings, adhesives, sealants, artificial suede, synthetic leather.
Polyhydroxy butyrate (PHB) $-[C_4H_6O_2]_n-$	Bio-based and partially biodegradable from microorganisms	Agricultural mulch films, packaging, utensils, plates, and cups; medical implants; wastewater treatments, 3D-printing, fishing nets, etc.
Other polyhydroxy alkenoates (PHAs)	Bio-based and partially biodegradable from microorganisms	Production of adhesives and sealants; tissue engineering; disposable diapers; sutures, wound dressings, and drug delivery systems, etc.
Cellulose acetate	Bio-based and partially biodegradable from polysaccharides	Personal care products, packaging, films, and photography; eyewear frames; cigarette filters; membranes; coating and lacquers; manufacture of binders, folders, clear sheet protectors; etc.
Other biopolymers	Bio-based and partially biodegradable from biomass by proteins & lipids	Single-use packaging sector, cosmetics and personal care products, textiles, etc.
Polycaprolactone (PCL) - $[C_6H_{10}O_2]_n-$	Petrochemical biodegradable polymer	Textile finishing; toys and craft materials; coatings; orthopaedic implants; packaging; tissue engineering, etc.
Polyester amides (PEA)	Petrochemical biodegradable polymer	Sporting and consumer goods; lubricants; electrical insulation; films and packaging; engineered plastics; textiles; automotive parts, etc.
Copolyesters (PBAT, PBS, etc.)	Petrochemical biodegradable polymer	Plastic mulch, medical devices, disposable tableware, polyurethane, elastomers, gas barrier packaging.

Nevertheless, bio-based polymers are not commercialised yet in the same amounts as fossil-based polymers, due to the associated current higher production costs and poorly improvable material properties they provide. Moreover, most of these biopolymers are based on the fermentation of vegetables such as corn, cassava (Brazilian arrowroot), and sugar cane, among others, that compete with food production and promote intensive agriculture, which also entails a strong environmental soil and the use of pesticides. Therefore, future research efforts must be focused on the identification of low- or zero-cost raw materials (such as agricultural residues after food production), more efficient bioprocessing strategies, and the enhancement of their recycling, which is currently limited.

On the other hand, to improve the circularity of bioplastics, it would be necessary to develop new anaerobic treatments that provide effective conver-

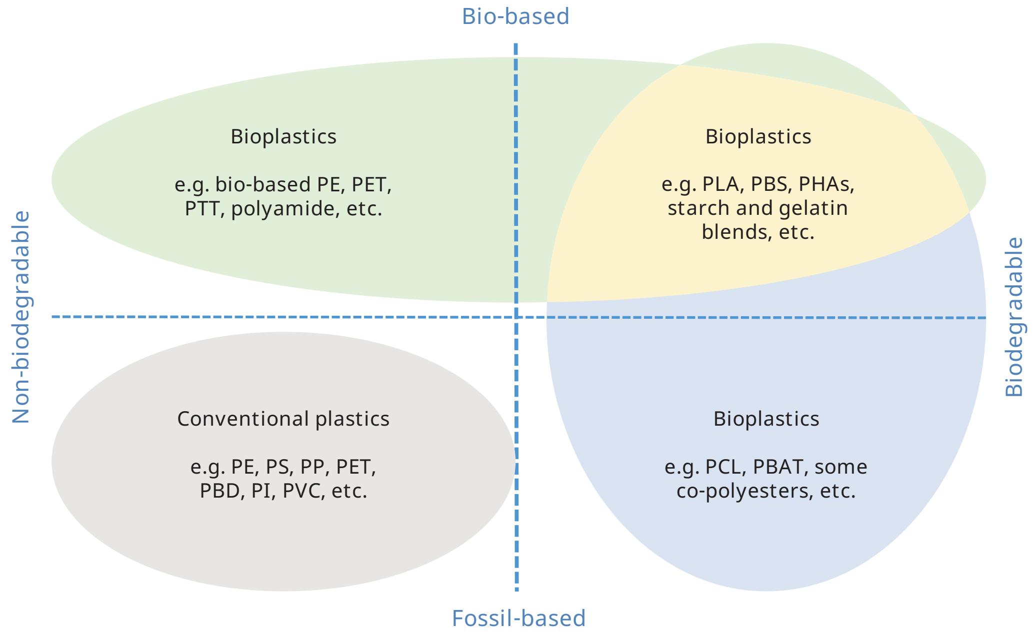


Figure 2.5 Different types of plastics that are available, from perspectives of origin and degradability.

sions into biogas during their degradation, thus aligning with the principles of 'green chemistry'. Finally, more research is also needed to assess the occurrence, fate, and behaviour in the environment of these recent anthropogenic materials and their toxicity regarding human health, as well as to elucidate their subsequent transformation products, in order to guarantee the safety of bioplastics, if we wish to consider them as a future possible global solution to mitigate the plastic pollution threat (Thomas et al., 2023).

Plastic additives

Plastics are composed of polymers and plastic additives which are added to polymeric chains in order to modify and enhance the final characteristics. The value of the market of plastic additives (PAs) reached 48 billion US$ in 2020, and it is projected to grow up to 75 billion by 2028, with an average annual growth rate of 5.7% (da Costa et al., 2022), while the global production was 20 million Mt in 2019 and is expected to rise by 100% in the next 30 years (Geyer et al., 2017).

According to Wiesinger et al. (2021), in 2021 there were around 10,000 additives that were used and only across 25 different applications, where the inclusion of a single PA in the final material depends on the intended use (Do et al., 2022). In this sense, plastic additives can be classified as:

- Functional: plasticisers, flame retardants, stabilisers, antioxidants, lubricants, biocides, antistatic and nucleating agents, etc.,
- Colourants: organic and inorganic pigments,
- Fillers: mica, talc, kaolin, clay, calcium carbonate, barium sulphate, etc.,
- Reinforcements: glass and carbon fibres, for example,

where the industrial usage of functional plastic additives is as follows: plasticisers (~70%) > flame retardants (~25 %) > antioxidants and stabilisers (~ 0.10–3%) > other PA (< 0.1%) (Hahladakis et al. 2018).

Plasticisers are the most widely used materials with which to enhance the durability, stretchability, and flexibility of the polymers from plastic materials, while simultaneously avoiding melt flow. Meanwhile, in the case of antioxidants, plasticisers are embedded in some polymeric resins to delay the overall oxidative degradation if or when plastics are exposed to ultraviolet light, particularly in food packaging where oxidative processes increase when materials are exposed to infrared and microwave heating. In addition, stabilisers are responsible for preventing thermal, light, or other kinds of polymeric degradations that might occur; whereas flame retardants are used for avoiding or decreasing fire propagation, this providing an extra time period for escape purposes (Maddela et al., 2023).

In this context, shown in **Table 2.3** are some plastic additives classified according to their main functions, and the typical concentration expressed as wet weight (ww) % in the associated polymers (Andrade et al., 2021; ECHA, 2023; Hansen et al., 2013).

Furthermore, a small part of the current body of literature on plastic additives is focused on the potential threat of these chemicals that is commonly governed by their ability to be leached from the original plastic bulk to the environment. This includes being able to be transported by air, water, and terrestrial compartments showing a high global ubiquity, as well as being finally uptaken and bioaccessible in biota and human organisms that might produce a certain toxicity at the cellular and molecular scales, which are more prone when ageing factors are present, such as low pH conditions, the presence of ultraviolet radiation, or under the occurrence of some microbial communities, as will described in more detail in section 2.3. In this sense, a few examples of scientific evidence have already been brought to light regarding human exposure to plastic additive substances, and assessing their possible toxicity via *in vitro* models (such as those described by Savva et al., 2023), thus revealing the shortcomings in the existing legal guidelines that are aimed at managing pollution through plastic additives, while highlighting the lack of toxicological information for the main types of these substances that are currently used, and the transparency in the clear labelling of additives on the final products.

Indeed, some PAs are currently banned or under review by some environmental health agencies (e.g. European Chemicals Agency (ECHA), Environment Canada, or the United States Environmental Protection Agency (EPA)) as in the case of many orthophthalates like DEHP or DBP, which have been proved to be toxic regarding reproduction and in consequence they are restricted in a wide range of products since July 2020 by ECHA (2024). Therefore, accurate estimations of the levels and types of additives that are em-

Table 2.3 Examples of functional plastic additives used in plastic materials.

Function	Plastic Additive	CAS number	Polymer associated	Concentration (% ww)
Plasticisers	Decanedioic acid	122–62–3	PUR, PVC	10-35
	Di-allyl phthalate	131–17–9	PUR, PVC	10-35
	Bis(tridecyl) adipate	16958–92–2	PUR, PVC	10-35
	Isobutyric acid	25265–77–4	PVC	-
	Diisononyl adipate	33703–08–1	PUR, PVC	10-35
	1,2-Benzenedicarboxylic acid	90193–76–3	PUR, PVC	10-35
	Triethyl phosphate	78–40–0	PUR	10
	Melamine	108–78–1	PUR	25
	Dibutyl phthalate	84–74–2	PUR, PVC	10-35
	Bis(2-ethylhexyl) adipate	103–23–1	PUR, PVC	10-35
	Dibutyl adipate	105–99–7	PUR, PVC	10-35
	Di-2-ethylhexyl phthalate	117–81–7	PUR, PVC, ABS, PS	2-35
	Hexaboron dizinc undecaoxide	12767–90–7	PE, PP	0.3-0.4
Light Stabilisers	Octabenzone	1843–05–6	PE, PP, PUR, ABS, PVC, PS, PMMA	0.2-5
	2-(2 H-benzotriazol-2-yl)-p-cresol	2440–22–4	PE, PP, ABS, PVC, PS, PMMA	0.002-0.5
	Bumetrizole	05-11-3896	PE, PP, PUR, ABS, PVC, PS, PC	0.3-1
	N-(2-ethoxyphenyl)-N′-(2-ethylphenyl) oxamide	23949–66–8	PVC, ABS, PC, PS	0.7
	Dibutyltin dilaurate	77–58–7	PE, PP, PVC	3
Heat Stabilizers	2-ethylhexyl diphenyl phosphite	15647–08–2	PVC	2
	Triisodecyl phosphite	25448–25–3	PVC	2
	Diisodecyl phenyl phosphite	25550–98–5	PVC	3
	Isodecyl diphenyl phosphite	26544–23–0	PVC	3
	N,N′-ethylenedi(stearamide)	110–30–5	PE, PP, ABS	0.3-0.5
Other Stabilisers	1,3-diphenylpropane-1,3-dione	120–46–7	PVC, PET	-
	Magnesium carbonate	546–93–0	PE, PP	1
	6,6′-di-tert-butyl-4,4′-butylidenedi-m-cresol	85–60–9	PVC	0.5
Antioxidants	6,6′-di-tert-butyl-4,4′-thiodi-m-cresol	96–69–5	PE, PP	0.001
	6,6′-di-tert-butyl-2,2′-methylenedi-pcresol	119–47–1	PE, PP, ABS	0.5
	Dioctadecyl 3,3′-thiodipropionate	693–36–7	PE, PP, ABS, PET	0.25-3
	Sodium benzoate	532–32–1	PE, PP	0.2
	2,2′-methylene bis-(4,6-di-tert. butylphenyl) sodium phosphate	85209–91–2	PE, PP	0.2
Nucleating Agents	Copper	27614–71–7	PE, PP	0.05
	Tributyl-O-Acetyl citrate	77–90–7	PUR, PVC	10-35
	Triethyl citrate	77–93–0	PUR, PVC	10-35
Antistatic	Oxydiethylene dibenzoate	120–55–8	PVC	-
Flame Retardants	Decabromodiphenylethane	84852-53-9	PS	3-25
	Tetrabromobisphenol A	79-94-7	PC, PBT, PET, PU	3-25
	Tris(2-chloroethyl)phosphate	115-96-8	PVC, PS, PE, PP	3-25
	Tris(2-chlorisopropyl)phosphate	6145-73-9	PVC, PE, PP, PET, PS, ABS	3.25
	Hexabromocyclohexane	1837-91-8	PS	0.7-3

ployed industrially in different daily-used plastic materials is challenging, due to the limited disclosure or lack of transparency by manufacturers and the constant development of new similar modified types of additives that are not regulated by the existing regulations, thus making it extremely difficult, for identification and monitoring purposes, for the final assessment which is in search of potential environmental and human threats. Notwithstanding, although PAs can be present in small traces (< 0.01% ww), as well as with significant levels (> 50% ww) in the final products, data are not available in most cases (Bilitewski et al., 2012). Filling these gaps might significantly impact the industries in minimising the indiscriminate use of toxic plastic additives, and the global health agencies in framework of new, or the reviewing of, more effective and accurate regulations.

2.1.2 The plastics life cycle

In **Figure 2.6**, the life cycle of plastics with their different steps is shown. The cycle begins with the extraction of raw materials that are used for polymerisation (1), and ends with the production of plastics such as crude oil and natural gas or starch and sugarcane for fossil- and bio-based plastics, respectively, as well as plastic additives. The newly formed plastics or those that are recycled and then employed in manufacturing processes (2) in order to create a wide range of products that is ready to be used for consumption, including, among others, packaging materials, consumer goods, construction materials, automotive parts, electronics, healthcare, and so on (3). Once used, plastic waste may end up in landfills (4) that can contribute directly to environmental pollution, such as fossil-based plastics or that can be broken down into smaller particles more easily, as in the case of biodegradable plastics. Nevertheless, the conditions that are required for effective biodegradation might not be present in typical landfill environments. Finally, recycling and upcycling (or creative reuse) technologies (5) can be employed to minimise their impact and promote resource recovery, or reduce the demand for new raw materials. These technologies are described in detail in the next subsection.

It is noteworthy that the plastics life cycle might vary depending on the type of plastic, its application, as well as regional production and waste management practises.

2.1.3 Strategies to tackle plastic pollution

The goal of new strategies in plastic waste management is to achieve a more circular economy (i.e. Reduce, Reuse, Recycle). The circular economy (CE) is an economic system in which materials are designed to be used, not used up. From the outset, products, and the systems within which they sit should be designed to ensure no materials are lost, no toxins are leaked, and the maximum use is achieved from every process, material, and component. If applied properly, the circular economy (CE) benefits the society, the environment, and the economy.

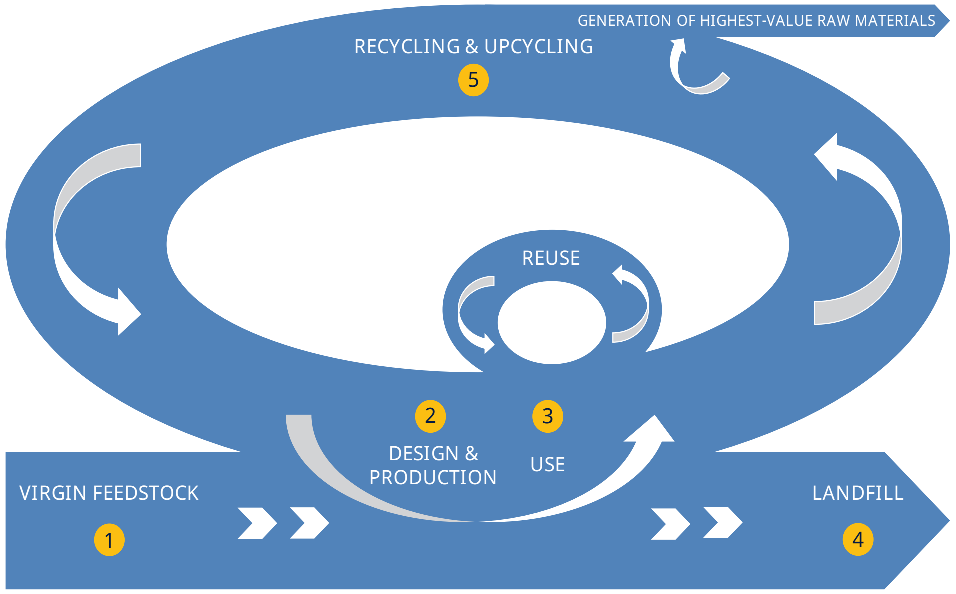


Figure 2.6 The life cycle of plastics.

Recycling and upcycling technologies

Recycling involves the gathering of waste materials and transforming them into useful consumer goods, using less energy and aiming to reduce global CO_2 emissions from the atmosphere. However, with respect to plastic waste, these processes normally generate greenhouse gases (GHGs), sulphur and carbon subproducts, which contribute towards acid rain and global warming (Szostak et al., 2021).

The conventional waste management practices are incineration, landfills, or bioremediation, while recent upcycling technologies such as mechanical (sorting and reprocessing) and pyrolysis, gasification, hydrocracking, or biodegradation have emerged and been adopted because they are more cost-effective and can be employed more easily at a large scale thus producing low emissions in comparison to older waste management practices (Anjum et al., 2016). The chemical upcycling technologies degrade the hydrocarbon content of plastics into energy, fuels, or other value-added chemicals.

Mechanical recycling is the most employed method in Europe. In general, it involves 5 steps: collection, sorting, washing, milling, and crushing and melting. Despite its success, it requires improvements in capacity and efficiency. It can be applied to fabrics and packaging (PET or PP), construction materials (PP), and almost any product made with PE, one of the most used and versatile plastics. PE is used, for example, in shopping bags or folding chairs.

Incineration is the energy generation by temperature (870–1,200 °C) burning of plastic waste where HCl, ash, and other volatile organic compounds (VOCs) are formed during the process. The benefits of incineration processes are confined to lowering waste volume; nevertheless, the costly set-up and the release of harmful subproducts into the atmosphere has adverse effects on the environment and on human health (Netzer et al., 2021). Integration of pyrolysis plants with existing chemical cracking units should be preferred, in order to reduce the drawbacks of incineration, and in some cases when a separate product separation section is necessary (Westerhout et al., 1998).

In the case of landfills, the disposal of solid wastes and allowing them to decay is an easy to achieve, but dangerous practice due to different biodegradation processes in which CO_2 and CH_4 are continuously generated as main subproducts. Notwithstanding, landfill arrangements should be designed to offer a safer area for the disposal of plastic waste, besides protecting marine life, airspace, or landfill sites. Specifically, the dumping of plastic garbage in a landfill due to the presence of toxic chemicals such as hydrocarbons, dioxins, furans, and metal or ion traces has a huge potential for contamination (Liang et al., 2021).

Meanwhile, for bioremediation practices, the microbial activities of microorganisms are used to decompose plastic waste. In order to work efficiently, bioremediation requires optimal conditions (pressure and temperature), nutrients, and enzymes that must be adjusted to ideal conditions for efficient growth of microorganisms. Nevertheless, the efficiency of this method decreases when one of the previously stated elements is missing, or there are inhibitors present in the medium. Common subproducts are obtained by bioremediation processes e.g. chlorofluorocarbons, PVC, and vinyl monomers during the natural breakdown of plastics (Haider et al., 2019). Moreover, bringing this practice to an industrial scale is very expensive due to the cost of the enzymes that are needed for the breakdown of synthetic polymers, and also because of some hindrances, such as the time for incubation, as well as the optimised level of the degradation process (Ragaert et al., 2017). Different degradation or depolymerisation technologies for plastic waste management have been implemented during the last 20 years, such as degradation with invertebrates, composting treatments, and oxo-biodegradation processes (Tiwari et al., 2023).

Current trends show that thermal conversion pathways such as pyrolysis and gasification provide promising avenues for the conversion of plastic waste into valuable products, and this might be integrated into other subsystems in order to improve the economic and environmental performance of the overall system.

Pyrolysis is used for depolymerisation of plastic at temperatures from 300 –1,300 °C and 1-2 atm of pressure by radical chain mechanisms without oxygen, depending on the different pyrolysis technologies: slow, fast, or flash. These processes are also mainly influenced by the structure or the presence of impurities and additives in the plastic waste. One of the advantages of this technique is that it can handle extremely heterogeneous combinations of plastics like PE, PS, PP, PTFE, or PMMA, while some of the main challenges are the absence of compact and comprehensive dynamic prototypes that have been confirmed with reliable empirical data, composition, and quality of plastic waste (Angyal et al., 2007). Common products obtained by pyrolysis processes are bio-oil, biochar, and gases (Jenkins et al., 2016).

In the case of gasification, plastic waste is reacted with gasifying agents at high temperatures (600–1,000 °C) to transform them into primarily gaseous output. This practice involves several steps that can be summarised as: drying, pyrolysis, cracking, and reforming reactions in gas phase and heterogeneous char gasification (Lopez et al., 2018). Mainly, gasification products are solid, liquid, and gaseous, and where these might account for 30-50% of the input weight, liquid is mixed with tar and oil of around 10–20%, while gas yields range between 30 to 60%. In some cases, gaseous or syngas are desirable products that contain H_2, CO, CO_2, N_2, small hydrocarbons, and the key product syngas that can be further used for producing downstream chemicals (Sanlisoy and Carpinlioglu, 2017). Overall, these products are very influenced by the temperature and the kind of gasifying agent and plastics. Regarding the gasifying agents, most developed technologies include air, steam, and other more innovative products, such as supercritical and plasma gasification (Qian and Ren, 2024).

In **Figure 2.7**, an outline of the main plastics chemical upcycling technologies is shown.

The first-stage products are mainly generated by the upcycling processes that are presented in the above, which are predominantly syngas (H_2, CO, CO_2, CH_4, etc.), raw oil (ethylene, propylene, butene, pentene, etc.), and char. Meanwhile, to produce other final valuable products (MeOH, LNG, Power, Gasoline, Diesel, etc.), other conversion subsystems need to be linked to these primary practices, such as pyrolysis or gasification processes. Some of them are steam methane reforming or hydrogenation for hydrogen production and power generation, chemical looping techniques to finally produce methanol, combined heat, a power cycle for generating energy, or oil processing to obtain refined gasoline or diesel from plastic waste. In fact, for each type of process that is implanted at an industrial scale, a deep optimisation and evaluation in regards to techno-economic viability and environmental simulation analyses, and safety and life cycle assessment must be undertaken along the entire life cycle (Chari et al., 2023).

Life cycle assessment & supply chain management

The implementation of the life cycle assessment (LCA), as well as the integration of renewable energies and the expansion of value chains, is a crucial step to evaluate and minimise risks associated with energy consumption and the generation of wastes and emissions.

Four steps have been defined to perform a LCA (Hellweg and Milà i Canals, 2014):

1) **Goal and scope definition:** describe the goal and scope, including defining objectives and setting the system boundaries.

2) **Inventory analysis:** compiling inputs and outputs for every process in the life cycle and accumulating these across the whole system.

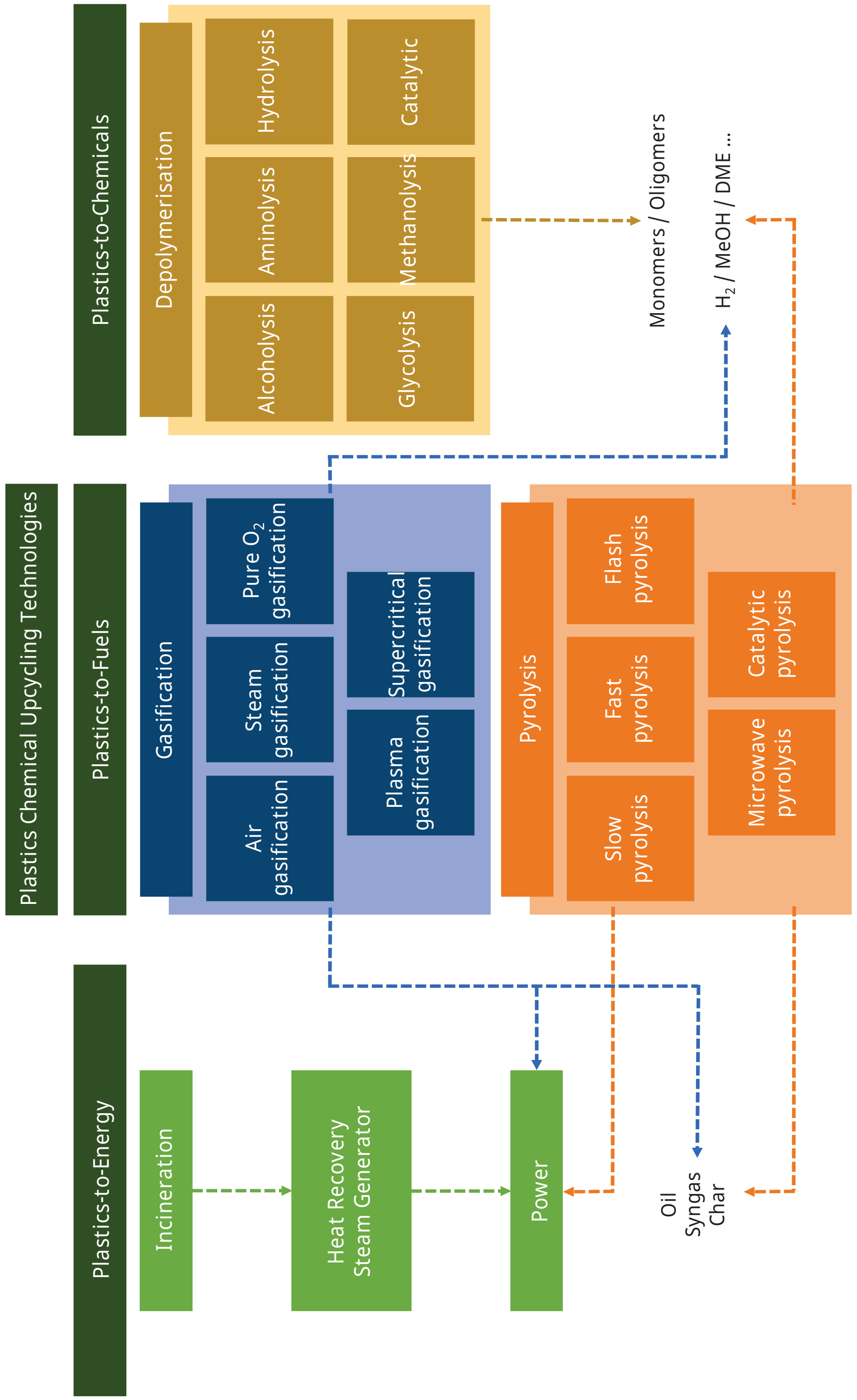


Figure 2.7 Main chemical upcycling technologies for the conversion of plastic waste into valuable products.

3) **Life-cycle impact assessment:** where emissions and resources are gathered with respect to their impact units, in order to make them comparable (e.g. climate change or photochemical ozone creation impact on human health, eutrophication and acidification in the environment, or land and water stress regarding natural resources).

4) **Interpretation:** understanding of the inventory and impact assessment results.

The high number of LCA techniques and the requirement for researchers to establish project-specific parameters, result in the consequence that LCA results are difficult to compare among themselves. However, the effort of comparing different LCA studies can provide a deeper understanding of how these parameters influence the impact on the environment and human health (Alhazmi et al., 2021).

A supply chain refers to a set of facilities, suppliers, customers, products, and methods with which to control inventory, purchasing, and distribution, where the chain links suppliers with raw materials production and customers with product consumption. The economic and environmental impacts of implementing a circular economy (CE) in plastics waste supply chains also need comprehensive investigation (Vidal and Goetschalckx, 1997).

Recently, green chains have been attractive, and the closed-loop supply chain has gained attention in sustainability matters, while artificial intelligence (AI) algorithms have been successfully used in some aspects, as in the acceleration of the optimisation procedures, in the eco-design of plastic materials, in plastic waste recycling network management, or to solve complex problems about engineering (Goodarzian et al., 2020). To sum up, governments and regulatory bodies play an important role in promoting the adoption of innovative recycling and upcycling techniques. Policies, incentives, and regulations that encourage the revolution of the current plastic waste recycling industry can create a conducive environment for investment and market growth.

Advanced oxidation processes for plastics remediation

Based on natural processes, current research efforts in the removal of plastics from the environment are focused on the optimisation of advanced oxidation processes (AOPs) and photo-oxidation processes at the laboratory scale.

AOPs are widely used to accelerate plastic ageing due to environmental friendliness and high efficiency. This is the case of the Fenton reaction that may occur in realistic environments, where Fe^{2+} and H_2O_2 are employed to catalyse ·OH formation as reactive oxygen species (ROS) (Zhang and Chen, 2020). In the case of ozone, this is also an ubiquitous advanced oxidant that can produce amounts of free radicals in a short period of time with the presence of promoters, increasing its oxidation capacity as highly reactive radicals are generated. Another kind of advanced oxidation is the heat-activation of disodium persulfate ($K_2S_2O_8$), where $K_2S_2O_8$ is ionised in water, breaks under thermal radiation, and is then decomposed into $\cdot SO_4^-$, as the reactive species. Among these 3 AOPs, heat-activated $K_2S_2O_8$ has presented the strongest oxidative capacity; nevertheless, it is susceptible to environmental factors due to the fact that the temperature required for laboratory assays is higher and different from conditions in the environment (Luo et al., 2021).

Discharge plasma oxidation approaches have also received attention regarding the anthropogenic matter remediation in the environment, where dielectric barrier discharge (DBD) is the most widely used technique. In DBD, high-energy electrons bombard aqueous solution in the low-temperature plasma. Water molecules are ionised and excited to produce ROS and reactive nitrogen species (RNS), and these compounds are regenerated into active free radicals that may oxidase plastics. DBS exhibits traits such as high-energy electron bombardment, ozone oxidation, ultraviolet photolysis, and their combined effects may render a treatment outcome that is more akin to real environmental conditions, surpassing the simulation capacities of Fenton, persulfate, or other OAPs processes (Zhou et al., 2020). Nonetheless, further research should be undertaken in order to understand the knowledge gaps between natural ageing processes and discharge plasma oxidation.

In **Figure 2.8**, the more promising advanced oxidation processes at the laboratory scale to accelerate plastic ageing for facilitating their environmental remediation are shown.

On the other hand, photo-oxidation processes are the most widespread abiotic degradation pathway in the environment, where light irradiation can also accelerate the plastic ageing, firstly decomposing the chemical bonds from the polymer backbone and then, triggering the initiation of polymer degradation for its final remediation. In general, these kinds of processes can be divided into three main steps: initiation, propagation, and termination (Yu et al., 2021a):

Initiation:
(1) Initiator —> $R_i\cdot$
(2) $R_i\cdot + O_2$ —> $R_iOO\cdot$
(3) $R_iOO\cdot$ + RH —> R_iOOH + R·

Propagation:
(1) $R\cdot + O_2$ —> ROO·
(2) ROO· + RH —> ROOH + R·

Termination:
(1) R· + R· —> R-R
(2) R· + ROO· —> ROOR

Notwithstanding, several initiation mechanisms can occur in plastic degradation via photo-oxidation processes. Ultraviolet (UV) light usually initiates photolysis reactions of C-C and C-H bonds. Indeed, for polymers that contain chromophores groups on the plastic surface, hydrogen atom abstraction and other types of reactions are prone to forming free radicals and causing main-chain scissions under light radiation (Ammala et al., 2011). In the case of metal oxidants, as photoactive additives added during plastic production, can be excited to generate ROS, thus also triggering initiation reactions by light irradiation (Yu et al., 2021b). In addition, studies in the recent body of literature have demonstrated that mechanisms involved during photo-oxidation of plastics may be strongly ascribed to ROS-mediated chemical bonds that are broken, and oxygen-containing functional groups that are formed, where OH radicals tend to be added onto benzene rings with higher electron density to produce easily formed intermediates, such as phenols or monohydroxy functional groups on the hydrocarbon plastic surface, without the presence of other heteroatoms like nitrogen or sulphur (Zhu et al., 2019).

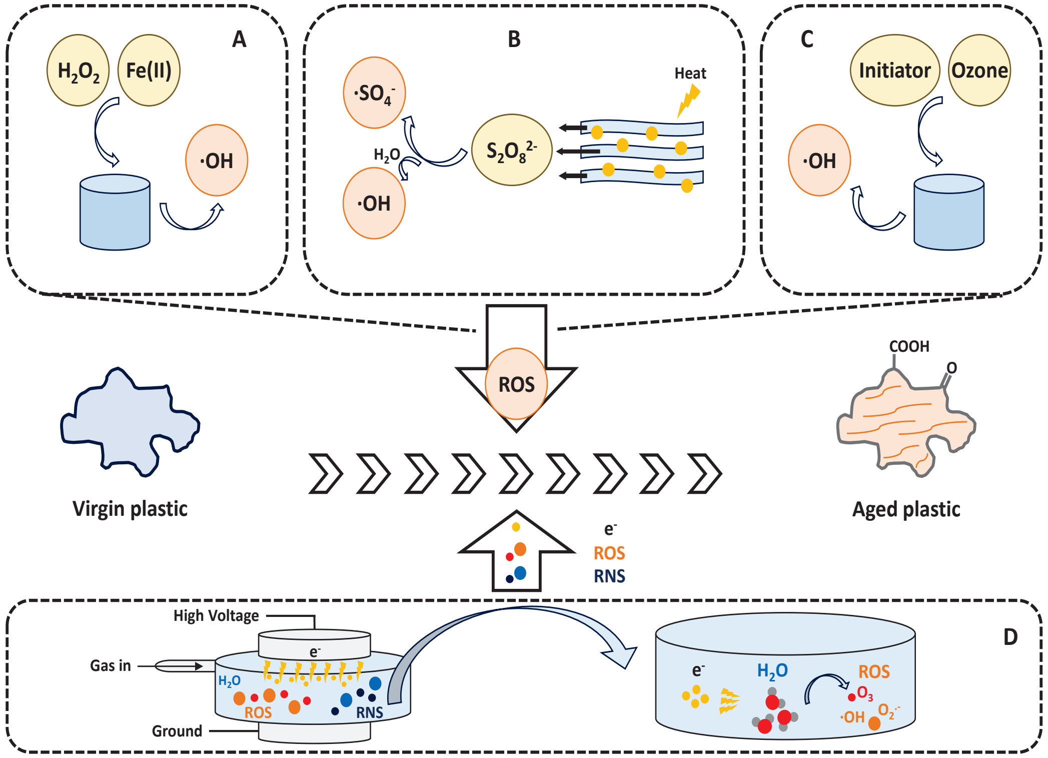


Figure 2.8 Scheme of the more common AOPs optimised at laboratory scale for plastic degradation purposes in the environment. (A) Fenton's reaction, (B) heat-activated $K_2S_2O_8$, (C) ozone, and (D) discharge plasma oxidation (adapted from He et al., (2023)).

2.2 Plastics regulation & legislation

Several international agreements, as well as different regulations and directives, have been implemented simultaneously in a consensual manner by different countries, or individually. In this section, a summary of the main agreements, regulations and directives at global-, European-, and Spain-scale is detailed, as well as the current lack of harmonisation and effective measures to tackle plastic pollution are described.

2.2.1 Global efforts for plastic legislation

The 2030 Agenda for Sustainable Development, adopted by all United Nations Member States in 2015, provides a shared blueprint for prosperity for people and the planet, both now and into the future. To achieve it, **17 Sustainable Development Goals (SDGs)** are an urgent call for action by all countries in a global partnership. The SDGs should be completed by 2030, and they recognise that people's well-being should be encompassed by tackling climate change and working towards preserving our oceans and forests.

In February and March of 2022, the United Nations Environmental Assembly (UNEA) met in Nairobi, Kenya, to discuss what could be the most influential environmental policy since the Paris Agreement (2015; effective 2016), which is a legally binding international treaty on climate change. The **Nairobi meeting** aimed to find the tools to **end plastic pollution and forge an international legally binding agreement by 2024, the Global Plastic Treaty**, by creating an Intergovernmental Negotiating Committee (INC), that is expected to be ready by 2024. This agreement will set the legal framework to:

- Reduce the use and production of plastics.
- Enhance waste treatment capabilities, including recycling and upcycling capacity.
- Search for sustainable alternative plastics as substitutes for fossil-based plastics.

In addition, there are three more relevant international agreements that include initiatives to tackle plastic pollution that must be specially mentioned:

- **The Stockholm Convention** (2001) on persistent organic pollutants (POPs), a global treaty aimed at protecting human health and the environment from persistent chemicals.
- **The Rotterdam Convention** (1998; effective 2004), a commitment by the European Union (EU) and other countries to exercise adequate control over the trade and use of hazardous chemicals worldwide.
- **The Basel Convention** (1989; effective 1992), to minimise the generation of hazardous wastes and their cross-border movement while ensuring environmentally sound management.

However, other previous international binding agreements/conventions, protocols, initiatives, and cooperation processes have already been launched. In **Figure 2.9**, some of these previous measures since 1960 are summarised.

Furthermore, over the last thirty years, a wide range of regulations and legislations worldwide have aimed to manage the risks and impacts of plastic pollution. Some strategies, such as levies and bans, have been commonly applied.

Bangladesh and Kenya have enforced prohibitions on LDPE bags, prompting a ripple effect with many other nations adopting similar measures. Wales has implemented charges for single-use plastics bags, which is also true of various European countries such as Switzerland, Germany, Portugal, and Spain that have urged fees for plastic bags in supermarkets, resulting in notable declines in their consumption. Ireland's imposition of a levy resulted in a remarkable reduction of over 90% in plastic bag usage. Additionally, China has launched the Economic Structure Adjustment Guidance Catalogue, which forbids the use of lightweight plastic bags (thickness less than 0.025 mm), disposable foamed plastic tableware or plastic swabs, and daily chemical products, among other items. In regards to MPLs pollution, several European countries and states from the United States have adopted bans on microspheres in Personal Care Products (PCPs). Amendments to the Canadian Environmental Protection Act have also introduced restrictions on the use of PCPs. Concerns raised by European countries have led to the formulation of the European Recommendation on Solid Plastic Particles in Cosmetics, thus influencing global legislation. Furthermore, countries including the United Kingdom, France, South Korea, Italy, New Zealand, India, and South Africa have actively joined the 'no microbeads' movement (Zeb et al., 2024).

These actions demonstrate global efforts. However, they are not enough to impact on reducing plastic pollution. The implementation of essential measures, including enacting effective legislation and establishing international agreements to regulate plastic manufacturing, usage, and management of the residues at all the scales is critical.

In this sense, the Plastic Pollution Coalition, with key partners Break Free From Plastic Europe, Environmental Law Alliance Worldwide, and Surfrider Foundation, have created a collaborative project called The Global Plastic

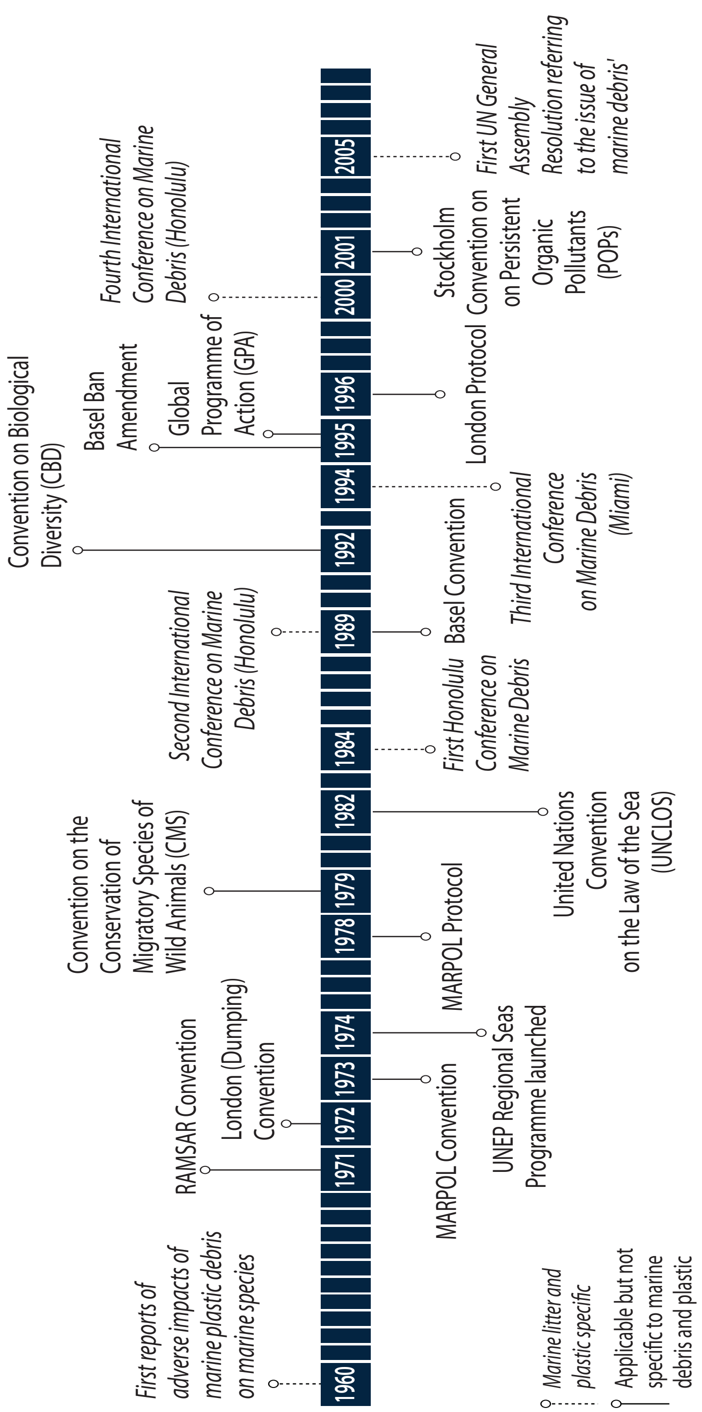


Figure 2.9 Timeline for selected international marine litter and plastic pollution initiatives, laws, and policies between 1960-2009 (taken from GRID-Arendal (2021)).

Laws Database, a portal and resource library agreed after the UN Environmental Assembly in March 2022 under the frame of The Plastic Treaty, to bring together all the laws worldwide for policymakers, advocates, organisations, researchers, educators, journalists, etc., with a focus on addressing a policy based on the full life cycle of plastics (Coalition et al., 2023).

Nevertheless, The Plastic Treaty is under negotiation, and is expected to be approved, but not before the end of 2024. In fact, during the last negotiation meeting held in Nairobi at the UN headquarters during 13-19th November 2023, no clear and significant advances were reached. The next meetings will be held in April and October-November 2024 in Canada and South Korea, respectively. Following the agreement and adoption of the UN Plastic Treaty, the database will be useful to support its implementation and then necessary to track and monitor this implementation.

2.2.2 European Union policies on plastics

At the European scale, a series of regulations and directives with direct applicability to EU member countries has already been established.

Regulations:

- Regulation (EU) 1907/2006, REACH, concerning the registration, evaluation, authorisation, and restriction of chemicals.
- Regulation (EU) 2020/2017, December 22, introducing a ban on the export of plastic waste to non-OECD countries.
- Regulation (EU) 2019/2021, on persistent organic pollutants.
- Regulation (EU) 2023/2055, establishing the ban on synthetic polymer microparticles on their own or in mixtures in a concentration > 0.01% by weight.

Directives:

- Directive (EU) 2018/851, amending Directive 2008/98/EC on waste.
- Directive (EU) 2019/904, regarding single-use plastics.
- Directive (EU) 2018/852, amending Directive 94/62/EC on packaging and packaging waste (the corresponding regulation is already in force).

Furthermore, a particular concern is paid to the marine environment because it is a major sink for most plastic pollution. EU member states must monitor MNPLs and promote research initiatives from the Horizon 2021-2027 Programme to reduce their environmental levels. In this context, the EU Plastics Strategy should be highlighted, as it aims to transform the way plastic products are designed, produced, used, and recycled in the EU, and the connected strategies such as the European Green Deal, the Circular Economy Action Plan, the European Industrial Strategy, the Chemicals Strategy for Sustainability, the Zero Pollution Action Plan, and the Biodiversity Strategy for 2030. Moreover, other recommendations were previously implemented, such as the Mediterranean Action Plan, followed by the Integrated Coastal Zone Management along the Mediterranean Sea coasts during the Barcelona Convention in 1995, and the Marine Strategy Framework Directive (Descriptor 10 – Marine Litter) which helped to provide a preliminary perspective in order to provide protection for all European coastal and marine waters (Llorca et al., 2020).

The EU plastic-related policies introduced from 2023 onwards are:

- The proposed revision of the EU legislation on Packaging and Packaging Waste that has come into play in 2023. This legislation aims to put the packaging sector on track to climate change neutrality by 2050.
- The duty of caterers, delivery services, and restaurants to make their contribution to plastic waste prevention. From 2023, they must offer reusable containers as an alternative to single-use plastics for takeaway food.
- From 2025, disposable beverage bottles must also be made from at least 25% recycled plastic.

2.2.3 Spain policies on plastics

National action plans are strategies or programs that a government or a national entity implements to address specific issues or problems. These plans can cover a wide range of topics, from the economy and health to the environment and education. In Spain, European regulations or directives are adapted through laws and royal decrees, and they extend their scope of application to producers by 'extended producer responsibility', in order to address the specific challenges it faces regarding plastic pollution and the sustainable management of these materials. These plans often rely on specific objectives and goals, as well as collaboration with various stakeholders, such as the scientific community, industry, and the civil society, to achieve effective results.

Currently, in the context of plastic pollution, the following regulations are in force in Spain:

- Law 7/2022, April 8, on waste and contaminated soils for a circular economy (CE).
- Royal Decree 1055/2022, April 27, on packaging and waste packaging.
- Royal Decree 1021/2022, December 13, regulating certain hygiene requirements for the production and marketing of food products in retail establishments.
- Royal Decree 646/2020, July 7, regulating waste disposal by dumping.
- Royal Decree 293/2018, May 18, regarding the reduction in the consumption of plastic bags and the creation of the Registry of Producers.

Other local regulations in Spain have already been implemented, and they are:

- Law 3/2023, March 30, on the Circular Economy of Andalusia.
- Law 3/2020, March 11, on Food Loss and Wastage Prevention (Catalonia).
- Regional Law 14/2018, October 20, on Waste and its Taxation (Navarra).
- Law 5/2022, November 29, on waste and polluted soils for the circular economy promotion in the Community of Valencia.
- Law 6/2021, February 17, on waste and polluted soils of Galicia.
- Law 8/2019, February 19, on waste and contaminated soils on the Balearic Islands.
- Decree-Law 3/2022, February 11, on urgent measures for the sustainability and circularity of tourism on the Balearic Islands.

Overall, the government of the state of Spain does promote the fight against climate change and the energy transition since 2021 (Law 7/2021, May 2020, aligned with European Climate Law that was also launched during 2021) by legislation and through other economic instruments by financial agencies of the state, such as green bonds, which are used to fund projects and activities that have positive environmental benefits and contribute to sustainability and climate change mitigation. Projects funded normally include initiatives related to renewable energy, energy efficiency, sustainable packaging, transportation, or natural resource management, among others. These green bonds have been made to adhere to specific principles and standards in order to ensure transparency and integrity in green financing.

2.3 Occurrence, fate, and behaviour of MNPLs in the environment & their impact on biota

Despite the many advantages of using plastics, in terms of malleable, tuneable properties, and lightweight materials with hygiene-enhancing applications in healthcare, in the food industry, for transportation and storage of foodstuffs, or corrosion-resistant applications in construction, it is necessary to make efforts to reduce the anthropogenic footprint that is associated with these kinds of materials. As mentioned in the previous sections, the overuse of plastics, and the lack of recycling, upcycling, and reuse initiatives have posed one of the most significant environmental crises on the planet. Hence, it is also necessary to understand the problem from the sources of plastics and all the way to their environmental fate and behaviour, as well as their exposure to living organisms and the adverse effects that are generated in the environment.

2.3.1 Micro(nano)plastics

Once released into the environment, plastics can undergo diverse ageing processes under different conditions, such as physical abrasion, chemical oxidation, and biodegradation. Thereby, plastic litter can suffer changes according to its different physicochemical properties, which can increase its ecological risk via mechanisms that break down original plastic bulk, thus transforming the plastic litter into smaller particles.

In this context, plastic wastes can be classified according to their size, in macroplastics (> 25 mm), mesoplastics (5–25 mm), microplastics (MPLs, 1 µm – 5 mm), and nanoplastics (NPLs, < 1 µm); and depending on their origin, whereby they can reach the environment as primary MNPLs (e.g. fabric fibres, pellets, or virgin plastics included in PCPs formulations that are released directly), as well as secondary plastics, which are those plastics which are produced incidentally, e.g. MNPLs, by different environmental ageing processes over time.

Furthermore, NPLs are anthropogenic materials that, apparently, might be considered similar to the engineered nanomaterials (ENMs). However, nanoplastic particles are completely different materials to ENMs, due to the fact that they can present multiple random shapes and sizes in the media, and a wide range of surface properties that are governed mainly by their higher heterogeneity, which is presented through hundreds of thousands of substances and microorganisms that are adsorbed onto the plastic surfaces. Plus, that is apart from the nature of the polymeric matrix including, for example, ions or dissolved metals, polysaccharides, natural organic matter (NOM) as fulvic and humic aggregates, Fe-Mn hydroxides, dust, volcanic ash, viruses, pathogens, plankton, etc. While the ENMs, according to the International Organisation for Standardisation (ISO), are defined as manufactured materials (between 1-100 nm diameter) with at least one dimension at the nano-range designed and produced intentionally for desirable specifications, typically with a uniform composition that may include polymer formulations, and even favour the release of more NPLs incidentally into the environment (Gigault et al., 2021; Ter Halle et al., 2017).

In this sense, as MNPLs are unintentionally generated in the environment, the formation of heteroaggregates from multiple sources of bio-, geo-, or anthropogenic matter that is present in the same compartments, it is highly probable that instead there would be homo-aggregation, as with ENMs. Consequently, MNPLs surfaces and structures are uncontrolled and very conditioned through the physical and chemical parameters that govern the environment (salinity-ionic strength, pH, etc.), thus obtaining a wide range of sizes, shapes, and overall compositions, even when they have been originated from a common source. The resulting physicochemical heterogeneity of MNPLs may change the crystallinity or polarity of these particles, thus increasing their affinity to adsorb other substances, which is known as the Trojan Horse effect mechanism (Hotze et al., 2010; Liu et al., 2020a).

2.3.2 Plastic waste sources

Ocean plastic litter is mainly produced by inland activities, whereby 80% of the litter that is found comes from the densely populated and industrialised areas (Sherrington et al., 2016). It is noteworthy that approximately half the world's population resides within 100 kilometres (62 miles) of a coastline.

Plastic litter is commonly transported to the sea by wind and run-off processes through beaches, rivers, or effluents from wastewater treatment plants (WWTPs). However, rubbish dumped from ships, vessels, oceanic platforms, and loss of packaging in addition to accidental releases, is another important source of plastic pollution in the sea, representing about 20% of the marine litter that is found. Fishing activities also have an important contribution, and it has been estimated that 640,000 tonnes of discarded fishing gear is thrown into the seas and oceans annually. In addition, extreme weather events such as storms, hurricanes, or floods, can increase this inland debris transfer from terrestrial to marine ecosystems at greater distances from estuarine zones (Li et al., 2016).

The long-range atmospheric transport (LRAT) of persistent contaminants, including MNPLs, has been reported (Caracci et al., 2023; Chen et al., 2023), and is a non-negligible pathway for the transport of primary and secondary MNPLs, as well as trace and ultrafine particulate material. Notwithstanding, MNPLs can be transported throughout the whole ocean until reach the remote regions of the North Pole and the South Pole, for which both areas have typically been used by the scientific community as a sentinel for the identification of what is considered to be the ubiquitous worldwide anthropogenic marine debris.

In the case of MPLs, marine and air processes that govern their transport from the coast to the open sea or ocean are known (Van Sebille et al., 2020). MPLs can be used as tracers to learn more about the physics and dynamics of our oceans across several scales. Some studies showed the existence of huge global accumulation zones of plastic, which are mainly in the convergence areas from each of the 5 large subtropical gyres that are caused, in general, by global wind patterns and their effect on ocean surface currents. The north-pacific subtropical gyre accumulates the largest amount of plastics, which is around one-third of the total worldwide amount or higher (Barboza et al., 2019). MNPLs water-air exchanges and plastic behaviour in coastal and polar regions will be detailed in chapter 4. In **Figure 2.10**, a scheme of the major physical processes involved in plastic litter transport from river-coastal areas to open sea-ocean is presented.

For terrestrial environments, to the best of our knowledge, the transport of MNPLs has not been studied extensively as in marine ecosystems, but some

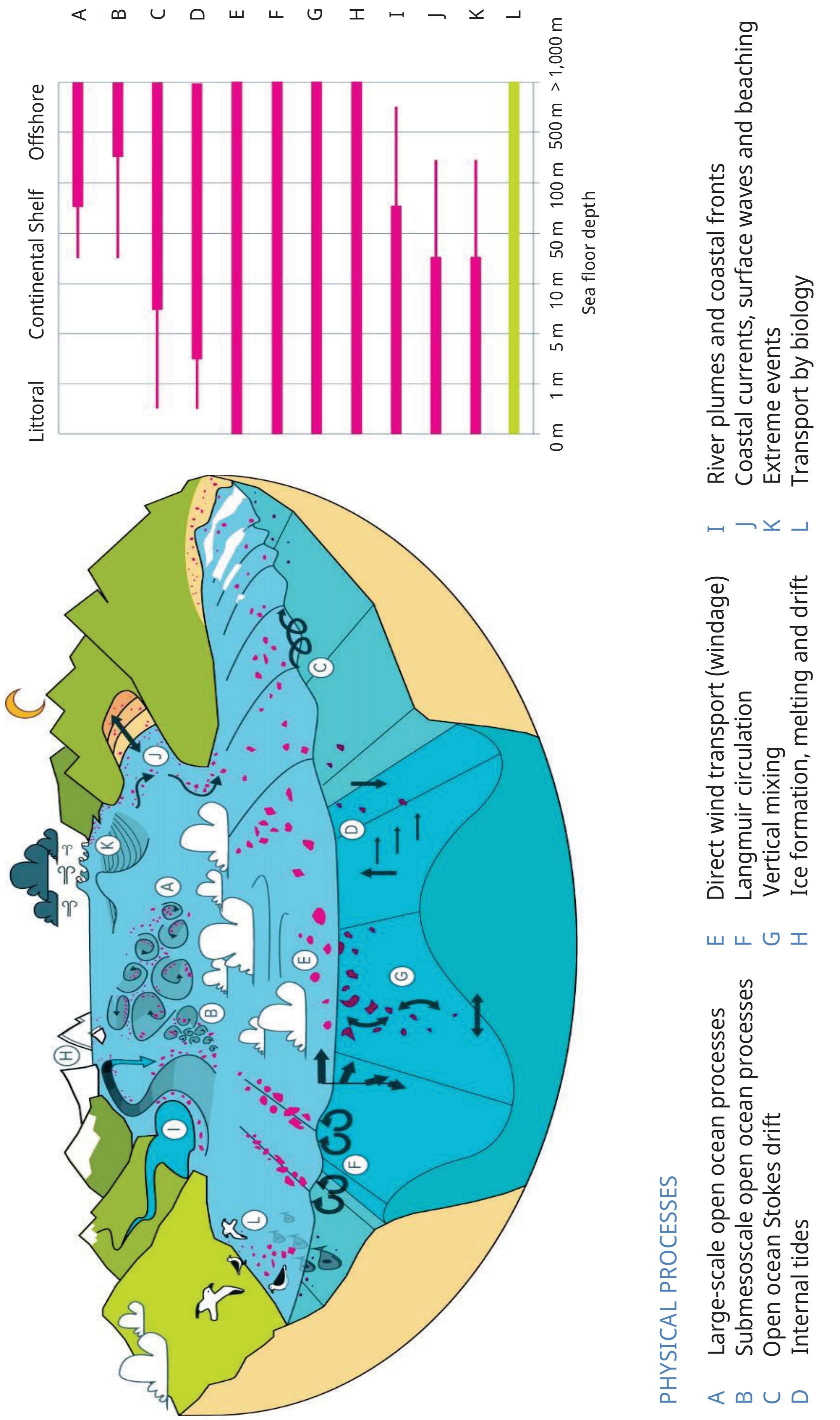


Figure 2.10 Physical processes involved in microplastic (pink items) transport. On the right side of the figure, it is shown at which depth different processes are the most relevant, while the pink lines mean that the process may be present as a secondary process. Plastic transport by living organisms is not a physical process, hence it is represented by a green line instead of a pink line (adapted from Van Sebille et al. (2020)).

evidence affecting the physical, chemical, and biological properties of the soil have also been reported. This includes soil's low removal efficiency from some critical water cleaning processes that are carried out by wastewater treatment plants (WWTPs) and drinking water treatment plants (DWTPs), or even plastic pollution from so many human activities such as agriculture, despite their multiple applications and benefits (Hofmann et al., 2023). Plastic and soil (hydraulic and physicochemical conditions or biogenic activity) properties, and the particle interactions of soil-MNPLs, determine the transport of plastics through soils and groundwater. Nonetheless, recent studies have shown that plastics are not the principal vector for the co-transport of pollutants in deeper layers. Further, soil erosion is an important diffusive pathway for plastics to travel into water bodies, and plastic fragment size is a sensitive factor in determining erosion (Rehm et al., 2021). In addition, as MNPLs act as stressors in terrestrial ecosystems, the residues of conventional mulch films in the soil can hinder water infiltration, decrease water holding capacity, impact on microbial communities and macrofauna, and decrease soil fertility (Qi et al., 2020). Regards soil-air MNPLs exchanges, the atmospheric transport involves (re)suspension-deposition cycles, and owing to the lower density of plastics in relation to soil particles, concentrations of plastics may be higher in wind-eroded soil materials than in the original agricultural soils in crop production (Aeschlimann et al., 2022). However, knowledge of MNPLs occurrence and behaviour as well as potential origin sources, removal capabilities, or ecological impacts, is still in its infancy because these factors have, to date, been poorly evaluated by the scientific community during controlled wetland ecosystems experiments (Zhong et al., 2023).

Moreover, either in terrestrial, atmospheric, or aquatic compartments, the release of plastic additives from polymeric matrices must be taken into consideration. Some studies have shown that the leaching rates of additives might differ according to the size of the MNPLs particles, being in general 2-3 orders of magnitude higher in NPLs than in MPLs, due to the shorter distances for diffusive transport having thus, a major impact on the environment. Indeed, the nature of either polymer and additives as well as environmental factors that are present will lead to the final release rate (Mercea et al., 2018).

2.3.3 Plastic waste sinks and degradation mechanisms

Sink mechanisms

Initially, depending on the composition, density, and shape of plastics, they can be buoyant or neutral, or they can sink, and thus, will be distributed vertically and horizontally along the atmosphere and the marine water column, including the seafloor (Cole et al., 2011). Polyethylene and many polystyrene foams are examples of materials that are less dense than seawater, while vinyl chloride sinks. In addition, the increase in particle weight due to particle fouling should also be considered. Some authors estimate that most of the plastic waste in the marine environment is deposited on the seafloor, and only a small percentage is on the ocean surface (Sherrington et al., 2016).

On the other hand, the distribution also depends on other factors such as winds, currents, and coastline geography, among others (Li et al., 2016). Furthermore, due to their low degradation rates, plastics remain in the ocean for decades until they become MNPLs. However, marine biota including microbiome may degrade these plastics faster once they have been ingested and excreted into the marine environment again. This process and biomagnification routes along the food chain will be detailed in chapter 4.

Overall, MNPLs are ubiquitous in multiple environmental compartments such as surface and deep compartments from the water column, sediments, sea ice, snow, biota, or the atmosphere (Van Sebille et al., 2020). Nonetheless, all of these studies have been based on merely qualitative particle-counting estimations and the characterisation of a few MPLs particles, thus providing concentrations based on items found per volume or weight unit.

Physical abrasion

Plastics are susceptible to undergoing physical abrasion, which can be related to environmental loadings such as sand, other particles in the same compartments, tides, or waves. Several simulations at the laboratory scale have been carried out, whereby the principal results have shown that plastic particles are prone to be cracked, ripped, or scratched from silt/sand by observing depressions, racks, and yellowish discolouration causing damage on plastic surfaces and altering their morphology, mechanical properties and even their global charge (Ravishankar et al., 2018). To sum up, physical ageing processes of plastics occur when plastic particles experience the Brownian motion of chain segments that upset condensed plastic structures from a non-equilibrium state to an equilibrium state (Luo et al., 2022).

Chemical oxidation

Regarding chemical oxidation processes, which are governed by redox mechanisms, the best known are those where plastic particles can interact with a wide range of reactive oxygen species (ROS), that are produced through some natural processes from environmental stressors in the presence of oxygen (Sun et al., 2020).

Sunlight irradiation is the most important process for the ageing of MNPLs in the environment, where the high-energy ultraviolet (UV) fraction of light irradiation plays a crucial role in the weathering of MNPLs. Initially, the yellowing of the polymers, which is a typical sign of the generation of chromophore functional groups on the surfaces by ROS radicals (e.g. alkyl, peroxyl, or alkoxyl groups), is achieved. Then, with further oxidation over time, the molecular weight of the polymers is reduced; thus, also modifying the original physical properties and making plastics more fragile and prone to breaking down into smaller pieces and favouring the leaching of dissolved organic carbon and plastic additives, as well as further fragmentation until the MNPLs ranges are achieved, and also their subsequent final mineralisation (Xu et al., 2024). Moreover, aromatic MNPLs, such as PS and PET, are able to produce an excited triplet state upon exposure to UV radiation, which can transfer energy to dissolved O_2 and water molecules, in order to generate other ROS-like $^1O_2$ and O_2^{*-} and catalyse the photo-oxidation of these aromatic MNPLs (Yousif and Haddad, 2013).

Nonetheless, in real environments, MNPLs do not undergo chemical oxidation processes in isolation. Various other environmental substances are present, such as inorganic ions, dissolved organic matter, or natural colloids. Under UV sunlight irradiation, these co-substances may also suffer photo-chemical processes, absorbing photons and either consuming or generating reactive species, thus promoting the inhibition or catalysation of the photo-chemical oxidation of MNPLs in the environment, respectively. As an example, in a study by Berg et al. (2023), the chromophores in dissolved organic matter could absorb UV energy; thus, promoting excited states with higher energy, which initiate reactions with dissolved oxygen and water molecules, resulting in the generation of ROS by energy transfer.

Inorganic ions in aquatic ecosystems might influence UV penetration depth and the free radical reactions, inducing different degrees of photo-oxidation of MNPLs. Several examples of scientific evidence have found that PE, PS, and PP-MPLs have been aged and degraded more efficiently in ultrapure water, rather than in seawater, owing to the high salt concentrations which promoted a higher refractive index of water, and salt crystals aggregation onto MPLs surfaces (Cai et al., 2018). In addition, other studies suggest that Cl^- may capture ·OH radicals and inhibit thus the formation of O_2^{*-}, decreasing the generation of ROS and their role in relation to the photo-oxidation of MNPLs (Wu et al., 2021b). However, the real role of inorganic matter regarding chemical oxidation processes is still in its infancy. In fact, halide ions have exhibited ROS-scavenging effects; thus, inhibiting the chemical ageing of aliphatic MNPLs (Wu et al., 2021b), whereas for aromatic MNPLs these ions could interact with the excited state to generate highly reactive halide radicals that are able to promote photo-oxidation and increase the abundance of ·OH (Zhu et al., 2022).

As the main colloidal in aquatic compartments, natural minerals such as kaolinite, goethite, hematite, or pyrite, might take part in, and promote, the chemical oxidation processes of MNPLs. For instance, under UV irradiation, the surface Fe(II) phases of goethite and hematite could catalyse the production of H_2O_2 and Fe^{2+}, thus favouring the initiation of the Fenton reaction by the generation of a large amount of ·OH, and that would accelerate the final ageing of PE, and PP-MPLs (Ding et al., 2022).

In the case of dissolved organic matter, the influence of chemical oxidation is not clear. Humic and fulvic acids have been reported to inhibit the photo-oxidation of PP-MPLs (Wu et al., 2021a), and to accelerate the degradation of PS-MPLs (Zhang et al., 2022c). Nonetheless, in their study, Cao et al. (2022) suggest that humic and fulvic acids catalysed the photo-oxidation of aliphatic PP-MPLs owing to the production of ·OH by photosensitisation, whereas it had only a minor effect on the degradation of aromatic PS, and PC-MNPLs. The reason argued by those authors was that PS-NPs can adsorb enough dissolved organic matter via π-π interactions, thus delaying photoageing by competing for photon absorption sites, whereas the released phenolic groups from aromatic MPLs weaken the ageing process via the quenching effect of ·OH.

Biodegradation

Most microorganisms, such as bacteria, algae, fungi, etc., that are ubiquitous in nature, can adapt to changing environments and use some substances from those compartments to facilitate plastic degradation into MNPLs. Mainly, biodegradation processes occur as follows: (1) microorganisms colonise the plastic surfaces forming the biofilm, which is governed by particle size, the surface structure, or its hydrophilicity, and by interferences from the external environment; (2) plastic particles may break down into smaller particles due to possible hydrolysis or ionisation processes led by the microbial growth; (3) then, the plastic molecular structure is depolymerised by enzymes secreted by the microorganisms; (4) assimilation is carried out, thus generating primary and secondary metabolites; (5) finally, those metabolites are further mineralised into biomass, energy, CO_2, and H_2O as end products (Arpia et al., 2021). In **Figure 2.11**, a scheme of MNPLs biodegradation by microorganisms as well as their different steps in time are shown.

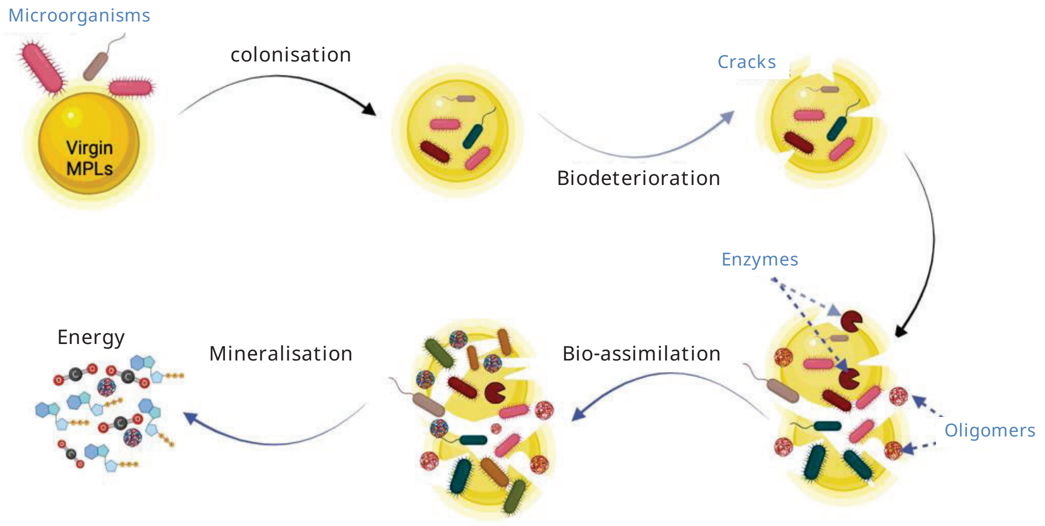


Figure 2.11 Ageing and degradation steps for MNPLs by microorganisms (adapted from Duan et al., (2021)).

It is noteworthy that only compounds with low molecular weights may be assimilated and degraded by enzymes from some microorganisms. Ng et al. (2018) reported that biodegradation does not occur for plastic particles with molecular weights higher than 1,000 $g{\cdot}mol^{-1}$. Furthermore, the time required to degrade fuel-based plastics and their efficiency are low, requiring a long period of time. Biodegradable plastics present higher rates of biodegradation but under conditions, such as aerobic composting (Napper and Thompson, 2019), which are not the natural conditions in the sea. Therefore, biodegradable plastics have a similar impact under natural conditions.

According to Liu et al. (2022), regarding weathering-modified MPLs compared to pristine MPLs, those MPLs that are collected in the marine environment, tend to present rough surfaces with higher degrees of crystallinity and negative charges, and they contain more functional oxygen groups. Consequently, higher hydrophobicity improves the adsorption capabilities of MPLs surfaces, compared to other compounds in surrounding continental environments.

In nature, several ageing mechanisms might act simultaneously with synergistic effects. For example, some chemical oxidation reactions between MNPLs and ROS species, that are produced from pigments and natural chromophore radiation, can be activated by sunlight. Particularly, in the case of ageing and plastic degradation processes via microorganisms, these may be affected by different factors such as the salinity of seawater, temperature, the presence of organic and inorganic matter, pH, exposure time, etc. In addition, biodegradation further depends on the biomass that is deposited on MNPLs particles. Physical mechanisms cannot modify the chemical properties of plastic particles, but they may damage the surfaces of plastic particles by producing racks or holes that improve the penetration of oxygen and light energy to the deeper layers of MNPLs, further enhancing the ageing and degradation rate of plastics.

2.3.4 Environmental fate & behaviour of MNPLs in terrestrial, atmospheric, and aquatic ecosystems

When MPLs break down into NPLs, the monomer mass remains constant, while the total particle number and active surface area (or surface-to-volume ratio) increase promoting and enhancing; thus, there is the aforementioned

Trojan Horse effect of plastic with respect to other co-substances or organisms that are present in the same compartments. Indeed, in other words, there is the same kind of substance due to nature of the particles that does not change, but once the particle sizes decrease around and lower than 200 nm, the high surface-to-volume ratio begins to become a critical parameter, conferring unique features that may influence the physical chemistry and mobility of these particles in the environment, which does not occur in the case of their bulk counterparts (Mitrano et al., 2021). However, under no circumstances is the appearance of size-emergent properties (e.g. quantum-physical properties) in the smallest nanoplastics expected, as in the case of fluorescence nanocrystals or quantum dots, a novel class of materials that can be tuned by the particle size determination; as in, the quantum mechanical states of the material's charge carriers (NobelPrize.org, 2023).

In accordance with the studies by Gigault et al. (2021) and Gigault et al. (2018), the primary characteristics ruling a different fate and behaviour between MPLs and NPLs are summarised in **Table 2.4**:

Table 2.4 Main features that distinguish between nanoplastics (NPLs) and microplastics (MPLs) in the environment.

	Nanoplastics vs. Microplastics
1	The prevalence of Brownian motion, led by the unpredictable movements of particles in a suspension medium (i.e. NPLs), outweighs sedimentation and buoyancy dominance (i.e. MPLs).
2	Deviation from the geometric-ray approximation that occurs during light-matter interactions. While modelling light as a straight ray adequately approximates the interaction with MPLs, the wave-like nature of light becomes significant and more successful for NPLs, as colloids.
3	For NPLs, a significant concentration of molecules on the surface leads to an increased relative significance of surface interactions when compared to physical interactions.
4	For MPLs, size can be comparable to the dimensions of environmental macromolecules, also favouring homo- or hetero-aggregation processes, but it remains small in comparison to the size of microorganisms. In the case of NPLs, sizes are optimal with bio-uptake, translocation mechanisms, and the transport across biological membranes.
5	Smaller particle sizes may accelerate the diffusive leaching of non-intentionally plastic additives, and other substances added to the initial plastic bulk.

Due to the colloidal nature over sedimentation and buoyancy processes, NPLs show the greatest vertical transport compared to MPLs when both have the same polymeric composition, in that nanoplastics are more likely to stay homogeneously dispersed in aquatic and atmospheric compartments, as well forming so many small heteroaggregates through natural and anthropogenic material that may also be suspended in the same aquatic and air compartments. Nevertheless, aggregation processes that involve NPLs and the smallest range of MPLs (1-25 μm) with other dissolved compounds or aerosols and ultrafine particles, respectively, tend to increase effective settling rates, in either water or air (Wagner and Lambert, 2018).

Particularly in aquatic ecosystems, the success of hetero-aggregation and the conformation of charged macromolecules are likely to be influenced by environmental factors, such as ionic strength and pH. The attachment is more efficient for NPLs than MPLs, due to the dominant role of surfaces at the nanoscale, and because of the dominance of Brownian diffusion rather than through settling, buoyancy, or fluid motion, as in the case of MPLs. However, for the interactions of plastic microorganism, the size implies differences in biofilm formation and microbial-bacterial interaction. In the case of MPLs, they may host a community of microorganisms to end up forming a complex and significant biofilm that might alter global density of microplastics, but the overall particle would still be, primarily, the plastic matrix. Otherwise, NPLs will tend to have a low mass of hetero-aggregates adsorbed onto their surfaces as a consequence of their small polymeric matrix sizes, but the properties of these hetero-aggregate NPLs will not be mainly governed by the polymer properties, due to the fact that the volume ratio between hetero-aggregates and the polymer matrix for a given particle would likely be high. Indeed, the global fate and behaviour of NPLs in aquatic systems (transport, accumulation, and bioaccumulation in living organisms, and so on) will be strongly led by the contribution of NOM aggregated to these NPLs particles (Amaral-Zettler et al., 2020).

2.3.5 Impact of MNPLs on biota

Exposure and trophic transfer of MNPLs to biota

Once the ubiquity of MNPLs has been proved from several pollution sources and transport pathways that are discussed here, as well as from the different environmental stressor factors presented that govern the main occurrence, fate, and behaviour of MNPLs in the environment, such as the physical abrasion and photo-chemical mechanisms, the interaction with NOM and other nanoparticles to form hetero-aggregates, and the marine microbiome which may also produce biodegradation effects in those plastic particles, apart from aggregation, the potential exposure and trophic transfer of these kinds of contaminants in biota is shown by the currently scant body of available literature.

Some *in vivo* studies at the laboratory scale have already highlighted the trophic transfer of MNPLs along the food chain in freshwater ecosystems. In a study conducted by Mateos-Cárdenas et al. (2019) and Mateos-Cárdenas et al. (2022), the amphipod Gammarus duebeni (invertebrate) that was fed duckweed Lemna minor (plant) that was previously exposed for 72 h to MPLs of polyethylene (PE) and polystyrene (PS) (1-45 µm). The outcomes showed that G. duebeni did not present any food-avoiding behaviour, as well as no apparent acute impacts on its survival, mobility, and weight after 24-48 h of feeding. Nonetheless, small plastic particles including NPLs (86-695 nm) were found in the digestive tracts of G. duebeni, but according to Waring et al. (2018), in the case of the main aquatic invertebrates such as fish, the uptake of MNPLs is prone to be bioaccumulated in their gills, liver, and gut, which might not be relevant to human consumption since these tissues are not usually consumed.

Previous studies also reported that MPLs were able to be broken down into NPLs by the digestive tract activity of Euphausia superba (Antarctic krill; Dawson et al., 2018) and Eisenia andrei (earthworm; Kwak and An, 2021). Particularly, Euphausia superba is considered to be a key organism in the Antarctic ecosystem, due to its ability to travel long distances along the marine water column in order to feed on planktonic species, and for being simultaneously the common prey for multiple living organisms of higher trophic levels from the epipelagic to the bathypelagic.

In addition, mussels and other bivalve organisms, owing to their filter-feeding behaviour, are of especial interest for assessing and enhancing the understanding of dermal absorption dynamics during exposure to MNPLs, and because they are also a common prey for a wide range of other marine living organisms. In fact, many studies have already been carried out during recent years, that demonstrate that mussels and other bivalve organisms can bioaccumulate contaminants from their surroundings, as they have lower biotransformation rates than fish and other vertebrates, and because they are a very suitable species for pollution rate evaluation in coastal, estuarine, or freshwater areas, as well as in the deep ocean (Álvarez-Ruiz et al., 2021). For instance, a simple food chain was assessed by Crooks et al. (2019), where velvet swimming crabs Necora puber fed for up to 21 days on mussels M. edulis that were previously exposed to PS-NPLs during 90 min, thus showing a final accumulation of these PS-NPLs in crabs tissues (gill, stomach, and brain). The number of particles of NPLs decreased in the stomach and gills, but stayed unchanged in the brain throughout the course of the experiment. Whereas in a study by Santana et al. (2017), through a complex food chain study involving 3 species, PVC-NPLs (100-1,000 nm) transfered from prey mussel Perna perna (3 h incubation) to predator crab Callinectes ornatus and puffer fish Spheoeroides greeleyi after feeding for 10 days, and 10 days of depuration, results showed that there was no persistence of NPLs in tissues, hence there was a lower risk of their direct effects on higher trophic levels.

In **Figure 2.12**, the different ranges of plastics according to their sizes are shown, with special emphasis on the micro- and nano-fractions, due to their optimum size for bioaccumulation and biomagnification processes in biota along trophic levels from the food chain.

Ecotoxicological tools and potential adverse effects on biota

At the end of 2019, the World Health Organisation (WHO) pointed out the ubiquity of plastics in the environment, and aroused great concern regarding exposure and subsequent effects of MNPLs in biota, and consequently on human health (Campanale et al., 2020). The bioavailable MNPLs fraction in living organisms is expected to be low; however, recent outcomes suggest that, as a consequence of the lack of mass-quantitative analytical methods, the concentrations that were previously reported might be underestimated.

Despite this current lack of consensus concerning mass-quantitative approaches, that are able to measure concentrations of MNPLs at ultra-trace level in the environment and in different tissues or fluids from living organisms, several studies have suggested that MNPLs, once accumulated and subsequently translocated in the liver, muscle, or in other vital organs, might

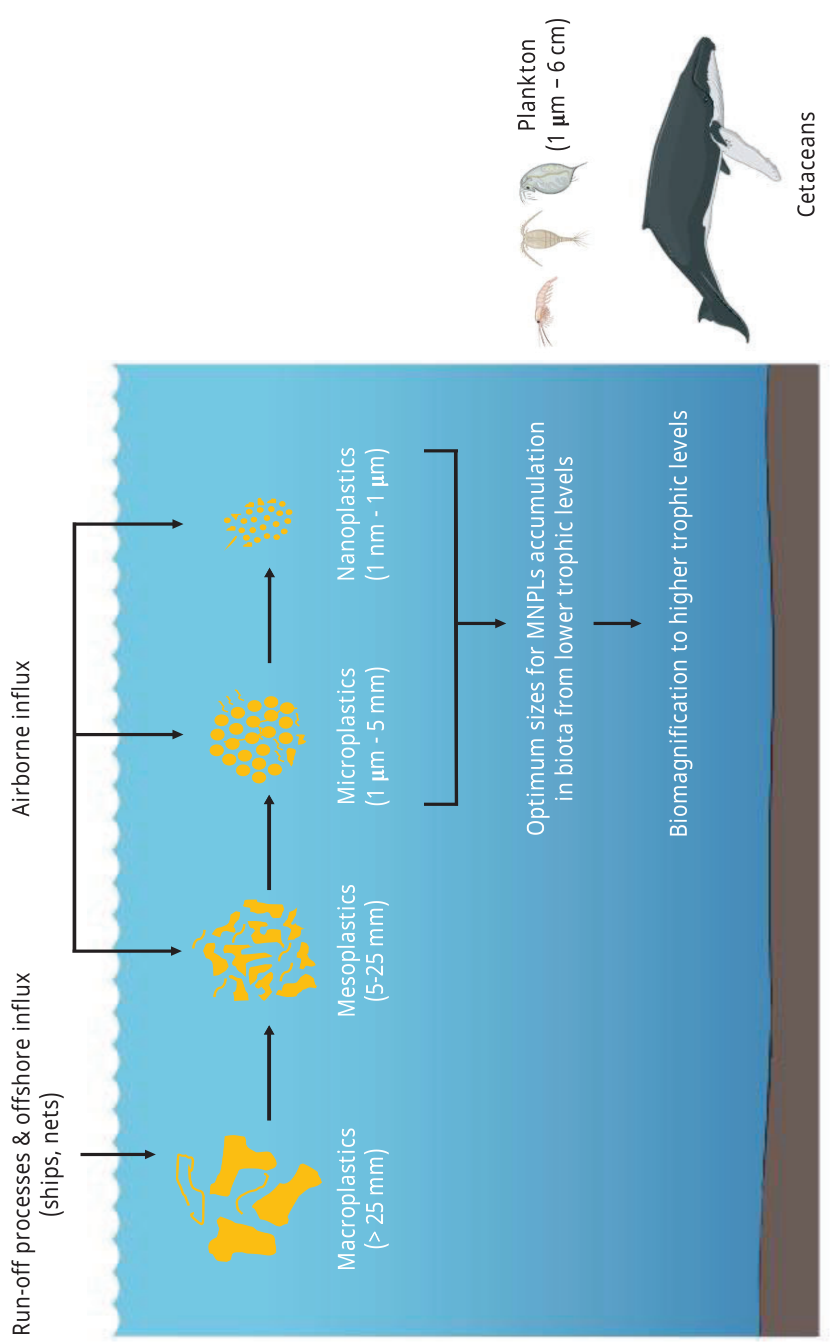


Figure 2.12 Plastic waste sources and ranges according to their size, with special emphasis on MNPLs particles, owing to their optimum size for their bioaccumulation and biomagnification in marine biota along the food chain.

induce cellular toxicity. This toxicity results in respiratory distress, cytotoxicity, inflammatory effects, as well as autoimmune diseases, among others, owing to the interaction between surface functional groups from primary and secondary persistent small plastic particles uptake and the tissues of different living organisms (Wright and Kelly, 2017). In fact, as a rule, the potential damage in a single organism will basically depend on the dose it is exposed to over time, initiating the toxicity at the biomolecule and cellular levels, respectively.

A response at the molecular and cellular levels does not necessarily translate into higher-order effects, but rather into chemical-mediated toxicity, and higher-order effects cannot occur without a preliminary molecular- or cellular-level effect.

Currently, several bioanalytical tools have already been widely employed for the toxicological assessment of MNPLs in biota, such as *in vitro* assays at the cellular scale that employ engineered MNPLs and primary cells or cell lines to simulate and understand the fate and behaviour of these smallest of plastic particles during cellular internalisation processes. Such tools are also used for assessing their possible adverse responses (biomarkers) of plastics in those processes as simply a biological effect or a triggering in cytotoxicity, being apoptosis, oxidative stress by ROS, fertility problems, or growth inhibition effects, among others, as the most commonly elucidated responses, to date. In general, when the initiating event triggers the cellular response, the reporter gene is transcribed to messenger ribonucleic acid (RNA), which is then translated as a fluorescent protein or enzyme that may be measured by fluorescence or enzymatic assays. In addition, robustness and specificity must be characterised and optimised before their implementation as monitoring tool (Escher et al., 2021).

Biota exposure-controlled experiments on anthropogenic and biogenic substances that are individually or co-exposed at relevant concentrations, that have previously been reported in the environment at the laboratory scale in meso and microcosms simulating real environmental ecosystems, are also widely used as a first *in vivo* estimation of the bioaccumulation rate in reference living organisms, as well as to determine the probable distribution of these substances, such as MNPLs, across the whole body of an organism. Some biota are impacted by human activities, and are connected between different tropic levels from the food chain; for example, filter feeders or vertebrate organisms such as mussels and zebrafish are mainly used as model organisms, due to their easy management during the execution of experiments and the absence of otherwise necessary ethical requirements. Once bioaccumulation and translocation rates have been evaluated, *in vitro* assays followed by a dose-response assessment (Concentration-Response Curves, CRCs) are commonly carried out, using available specific cell lines from the organs tissues where the bioaccumulation and translocation of MNPLs are likely to provide a first comprehensive view of the potential toxicity of these MNPLs in those individuals.

Table 2.5 shows some scientific evidences from previous *in vitro* or *in vivo* studies followed by *in vitro* assays performed with MNPLs reference materials simulating a plastic exposure to biota and describing their toxicological effects.

Although these bioanalytical tools are useful for the understanding and elucidation of several specific possible toxicological adverse effects from a wide range of tissues from different living organisms, the use of engineered or standard MNPLs materials with specific sizes, shapes, surface properties, and under controlled factors, simplifies the real effect produced in comparison to those MNPLs that are generated in the environment. These environmental-based MNPLs can suffer continuous ageing, homo-aggregation, and hetero-aggregation processes from NOM, microorganisms, and other co-contaminants that are present in the same environmental compartment, which may favour or inhibit the toxicological adverse effects of MNPLs in living organisms due to the constant changing in the surface properties after photo-oxidation processes from random sizes and shapes of aggregates of plastic particles. This is in contrast to uniform plastic particles that are employed in *in vivo* and *in vitro* assays. Notwithstanding, the potential entrance for the MNPLs in the food chain from organisms by lower trophic levels, as well as their subsequent bioaccumulation and biomagnification along living organisms from higher trophic levels should also be taken into account (Mancia et al., 2020).

In addition, according to Schirinzi et al. (2017), pollutants adsorbed to plastic surfaces such as carbon-based nanoparticles or metal-oxide nanoparticles may also have the potential to interact with plastics, thus changing the final

Table 2.5 Examples of *in vitro* and *in vivo* + *in vitro* assays from the body of literature, describing possible toxicological adverse responses in biota by the exposure of MNPLs.

Organism	Adverse responses	MNPLs (size; concentration)	Reference
Plant – *Oryza sativa*	Loss in height and biomass; Oxidative stress; Decrease in photosynthetic rate	PBAT and PE (50 μm; 10 g·Kg^{-1})	(Yang and Gao, 2022)
Plant – *Skeletonema costatum*	Decreased algae density; Decrease in photosynthetic rate	PVC (1 μm; 20, 100, 200 mg·L^{-1})	(Zhang et al., 2017)
Plant – *Cucumis sativus*	Increased root activity; Oxidative stress; Increased soluble protein in cucumber fruits; Decreased Mg, Ca, Fe accumulation	PS (100, 300, 500, 700 nm; 50 mg·L^{-1})	(Li et al., 2021)
Chicken - *Gallus domesticus*	Myocardial pyroptosis; Inflammatory cell infiltration; Mitochondrial lesions; Energy metabolism disorders	PS (5 μm; 1, 10, 100 mg·L^{-1})	(Zhang et al., 2022b)
Fly – *Hermetia ilucens*	Gut inflammation; Oxidative stress; Faecal hardening	PVC (200 nm; 20 μg·Kg^{-1})	(Xu et al., 2023)
Frog – *Physalaemus cuvieri*	Mutagenic effects; Increase of abnormalities and melanophores in the skin and pigmentation rate	PE (< 150 μm; 60 mg·L^{-1})	(da Costa Araújo et al., 2020)
Goldfish – *Carassius auratus*	Genotoxicity effects by an increase in erythrocyte nuclear abnormalities	PS (44 nm; 15, 100 ng·g^{-1})	(Brandts et al., 2022)
Zebrafish – *Danio rerio*	Spontaneous tail coiling, increase of the heart rate, MNPLs accumulation on the chorion surface, and potential morphological changes in interocular distance	PET (1,305-2,032 μm and 68-955 nm; 0.5, 1, 5, 10, and 20 mg·L^{-1})	(de Souza Teodoro et al., 2024)
Mussel – *Myt. galloprovincialis*	Reduction of AChE activity, nuclear distortions, and DNA damage	PE (1-100 μm; 1,000 mg·L^{-1})	(Avio et al., 2015)
Mussel – *Mytilus galloprovincialis*	Genotoxicity, lipid peroxidation inducing oxidative damage, effects on neurotransmission	PS (96-116 nm; 0.005-50 mg·L^{-1})	(Brandts et al., 2018)

bioavailability of those contaminants and, therefore, resulting in their toxicity in either a synergistic or an antagonistic mode. Specifically, in the above study, fullerene soot showed a predominantly antagonistic effect in binary mixtures when PE-NPLs and PS-NPLs were presented. In general, to our knowledge, these phenomena have been overlooked in the main MNPLs risk assessment studies that have been published to date, and in future research works more attention should be paid to obtaining a more realistic view regarding the real potential threat of MNPLs in the environment and in biota.

Regarding dose-response assessment, employing a well-designed battery of *in vitro* bioassays therefore allows a sensitive assessment of toxic potential. On the left of the log dose-response curve (CRC for cell-based bioassays) at very low doses the relationship between dose and response is linear. In fact, several parameters can be measured as the dose that is lethal to 50% in an organism (LD_{50}), the highest dose tested which is not statistically different from the negative control referred to as 'no observed adverse effect level' (NOAEL), or the 'lowest observed adverse effect level' (LOAEL) as the lowest dose tested that is statistically different from the negative control. However, LOAEL and NOAEL as expressions of toxicity are strongly dependent on the experimental design and variability of the data or system, and several replicates for each dose are needed. For these reasons, benchmark values are more suitable, and are used in terms of toxicity assessment since they do not depend on the experimental doses, and the shape of the full dose-response curve is considered. For example, the benchmark dose (BMD) at 10% (BMD_{10}) is the dose estimated from the dose-response curve that is required to generate 10% of the maximum response (Escher et al., 2021).

The difference between the 'dose' and 'concentration' terms is also noteworthy. The dose in an *in vitro* assay would be related to the amount of substance added to the well of a microtiter plate. How much of that dose reaches the cells depends on the number of cells, and especially the volume of the medium. For the same number of cells and the same dose added, there will be a threefold increase in concentration if the volume of the medium is reduced by a factor of 3. Therefore, 'concentration' is a more accurate term instead of 'dose'; for example, in aquatic ecotoxicology and in cell-based bioassays. Moreover, parameters used for CRC linear regressions are the same parameters for dose-response curves, exchanging the term 'dose' for 'concentration'. The most used value is the RC_{50}, the concentration required to cause 50% of the response. In assays for cytotoxicity assessment, the response is usually referred to as lethal, and the concentration is referred to as lethal concentration (LC_x). Likewise, inhibitory concentrations (IC_x) are used when the assay presents an inhibitory response (Escher et al., 2021).

2.4 Human exposure to MNPLs & potential adverse effects

MNPLs are present in the air we breathe, the water we drink, and the food we eat. It is genuinely challenging to assess in detail the damage that MNPLs might cause to human health at those scales. It certainly is a challenge that is embedded within another challenge, in that reliable and comparable data must be generated using various effective and harmonised tracking methodologies from complex biomatrices to thoroughly study the effects, and then take an appropriate action. In this sense, in this section, a discussion about the different exposure routes of micro(nano)plastics with respect to humans, as well as the current insights regarding risk and toxicological assessment is provided.

2.4.1 Main routes of human exposure

In brief, it is known that humans can be exposed to MNPLs by 3 principal routes: ingestion, inhalation, and dermal absorption. Nevertheless, the total contribution of MNPLs to exogenous particles exposure is still unknown, and due to their unique composition and properties, the human biological responses will differ from those invoked by other exogenous materials. For example, as MNPLs tend to form hetero-aggregates in the environment, once they have been internalised in the human body, the potential damages caused might arise through the release of these aggregates, as well as the plastic additives that are initially adsorbed, apart from the expected polymeric particles. Furthermore, as many of the biggest types of cells from living organisms have dimensions in the range of 20-150 μm, here we focus on particles of MNPLs from 1 nm to the smallest size fraction of MPLs (1-25 μm) that are likely to be more easily prone to uptake.

Ingestion

Ingestion is considered as the major route of human exposure to MNPLs, being the tissues of the human gastrointestinal tract (GIT) that are among the most exposed parts. Ingestion encompasses consecutive steps that might favour possible changes in the original surface properties of MNPLs and, therefore, their interactions with the different tissues that form part of the digestive system along the process; thus, having the capability of being able to change the bioaccessibility of those MNPLs following their initial uptake.

Firstly, once MNPLs are in the mouth and in contact with salivary fluids, size-dependent differences have been reported in *in vitro* assays where charged MNPLs particles were used, in that MPLs are prone to homo-aggregation whereas in the case of NPLs they tend to be more diffused. In the case of neutral MNPLs, no significant differences were observed (Teubl et al., 2018). In addition, MNPLs might be translocated in the tongue, as well as in the esophagus before they reach the stomach by way of the epithelial tissues. Once in the stomach, MNPLs are in contact with acidic gastric juice (pH ~ 2) for approximately 2 h, where no significant chemical degradation processes are expected, due to the common high hydrophobicity behaviour of these contaminants and the lack of heteroatoms in their surface structures, in relation to other organic and inorganic substances. Notwithstanding, the lack of oxygenase enzymes present in human stomachs, which are present in some particular microbes that are able to add oxygen atoms to carbon-based substances ingested to favour their chemical biodegradation, avoids the breakdown processes of MNPLs into smaller particles (Ghosh et al., 2013). After that, MNPLs reach the epithelium of the intestine, the main region of nutrient absorption. The small size and high levels of hydrophobicity in MNPLs can facilitate MNPL transport through the mucus layer, where these characteristics also influence the adsorption of proteins and biomolecules to the plastic particle surface, known as corona formation (Lunov et al., 2011). Specifically, the Peyer's patches of the ileum in the small intestine have potentially been defined as one of the main sites of uptake and translocation of anthropogenic particles. The Peyer's patches are part of the gut associated to lymphoid tissues (Powell et al., 2010), where the M cells of these patches can transport particles (NPLs – 10 µm) from the intestine to the mucosal lymphoid tissues, which are responsible for initiating immune responses to specific antigens that are encountered along all mucosal surfaces. The subepithelial area of the Peyer's patches can also store non-degradable particles and, consequently, they may disrupt the immunity processes (Campanale et al., 2020). For bigger particles up to 130 µm diameter, persorption performed by GIT epithelial cells is another route of MNPLs uptake, but not of adsorption, and this is the cellular passage of large particles through the epithelial layer of the GIT.

In this sense, the European Food Safety Authority (EFSA) considered that MPLs have a really limited bioavailability that is less than 0.3%, whereby plastic particles smaller than 150 µm might be able to interact with the intestinal epithelium tissues, and while only plastic particles up to 1.5 µm including NPLs might be internalised to the bloodstream and be successfully translocated systematically in tissues and other vital organs by cellular uptake processes (Walczak et al., 2015). The main uptake of MNPLs would thus be excreted from the body via urine and faeces.

Food and drinking water are key factors in the assessment of human MNPLs exposure through ingestion, but most of the studies published to date have been focused on seafoods such as mussels, rather than on other foodstuffs. In a review study by Llorca and Farré (2021), a review of recent studies assessing MNPLs in a wide number of foodstuffs is exhaustively detailed.

Inhalation

MNPLs through airborne fine or ultrafine particulate matter have a direct penetrating capacity into the respiratory system, reaching the respiratory epithelium via tracheal and bronchial secretions, and initially avoiding the vibrissae accumulated in nasal passages, which is able to inhibit the inhalation of exogenous substances, including MPLs particles, above 10 µm (Prata, 2018). It is expected that MNPLs may be translocated via diffusion, direct cellular penetration, or active cellular uptake through endocytic and phagocytic processes. In the lung alveoli, phagocytosis is the main pathway for particles with sizes in the range of 1-3 µm, whereas NPLs could be passively transported via diffusion across membrane pores, thus finally reaching the bloodstream (Wright and Kelly, 2017).

However, to our knowledge, the number of studies in the body of literature that assesses MNPLs in indoor and outdoor environments continues to be scant, and most of those studies are based on the use of µ-FTIR, which limits the assessment of NPLs. Particularly, in the case of human exposure from indoor environments, few studies have demonstrated that the presence of MPLs is greater than in outdoor environments, where synthetic textile is the more frequent source from fibres based on polyester, acrylic, or polyamide that might be photodegraded after 10 months under UV exposure. Other non-negligible exposure routes may come from airflow turbulence generated by air conditioners, which might increase the migrations of MNPLs in indoor areas (Sait et al., 2021). Moreover, workers from specific industries can be more prone to inhalation of airborne particles, including MNPLs, through fine or ultrafine particles (Prata, 2018).

In **Figure 2.13**, human oral exposure to MNPLs by the two common pathways is shown.

Dermal

On the other hand, the possible direct contact of MNPLs with the human epidermis may occur by skin perfusions from the daily use of personal care products (PCPs), which can contain NPLs and microbeads in their formulations such as skincare or cleaning products. In a study led by Jatana et al. (2016), some evidence has revealed that skincare lotion ingredients may improve the accessibility of MNPLs although their final penetration is highly reliant on particle size and surface chemistry. Nonetheless, there is some uncertainty as to how MNPLs might be internalised through skin, because the stratum corneum serves as a substantial barricade where NPLs in the range of 20-200 nm were merely able to penetrate the stratum to a depth of 2-3 cm in a study led by Campbell et al. (2012).

Moreover, MNPLs can interact with the epidermis indirectly by continuous short- and long-term dermal exposures of MNPLs from polluted water/air systems. Particularly, for aquatic ecosystems, despite the common high non-polar behaviour of MNPLs, in the first instance, the dermal exposure to MNPLs might be favoured when skin is in contact with water (Lehner et al., 2019). Thereby, generalisation is not feasible and MNPLs impacts, making especial emphasis in NPLs, need to be studied more to understand the real mechanisms involved during skin absorption, as well as their potential associated effects.

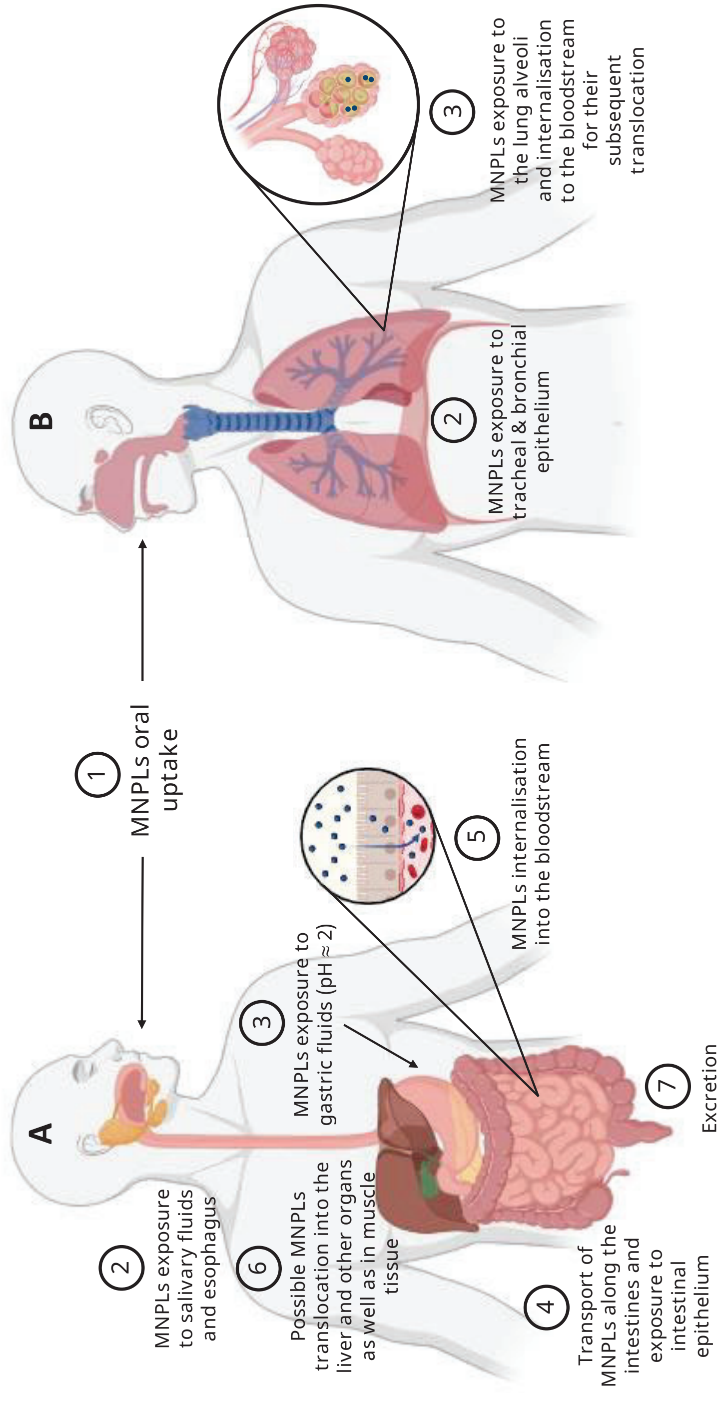


Figure 2.13 Human oral exposure to MNPLs (blue particles) by: A) ingestion and B) inhalation routes.

2.4.2 Pathways of MNPLs in human toxicity

Polymers

The human toxicity of MNPLs must consider the physical particle damages that can cause inflammatory lesions originating from the potential of their surface to interact with the tissues, as well as the long-term toxicity produced by the plastic additives, which are not covalently bound to the polymer, and once internalised might be easily released.

Nowadays, most of the information about the toxicity of MNPLs-polymers impacting on human health is, to our knowledge, from a limited number of *in vitro* studies which thus highlight the current relevant information gaps. In this sense, according to the information gathered to date, the Science Advice for Policy by European Academies project considered in 2019 the lack of evidence on direct adverse effects of MPLs on human health (Sapea, 2019). Furthermore, considering the number of current uncertainties, the European Commission Scientific Advice Mechanism also considered the urgent need to research this field to obtain a global comprehensive perspective. In **Table 2.6**, some adverse effects against human health that have been demonstrated by *in vitro* assays during recent years are summarised, but the reader should exercise caution when comparing these.

Since ingestion is one of the first modes of human exposure to MNPLs, the GIT is one of the first exposure tissues. For this reason, several toxicological studies have been carried out to determine the potential responses in the human colon adenocarcinoma Caco-2 cells, as a model. According to the study by Wu et al. (2019), PS particles of 0.1 and 5 µm exhibited low toxicity on cell viability, oxidative stress, and membrane integrity. Nevertheless, the mitochondrial membrane potential was disrupted by both, and on the contrary, as expected, the 5 µm particles induced higher effects than 100 nm particles. In this case, those authors explained that this might be due to the different mechanisms of induced mitochondrial depolarisation. It was also found that 100 nm PS-NPLs accumulated in lysosomes, whereas 5 µm PS-MPLs analyses did not reveal accumulation. Thus, large particles at a micrometre scale may escape from the lysosomes after endocytosis, and being localised in the intracytoplasmic vacuoles or randomly in the cell cytoplasm, which might further damage lysosomes, inducing mitochondrial depolarisation, and finally cell apoptosis.

Table 2.6 Selection of toxicological *in vitro* studies already performed using human cell-lines and MNPLs-polymers.

Cells (cell-tissues)	Effect assessed	MNPLs characteristics	Reference
T98G and HeLa (brain and epithelial)	Cytotoxicity (cell viability and oxidative stress by ROS)	PE, PS (100 – 600 nm; 0.05 – 10 mg·L^{-1})	(Schirinzi et al., 2017)
HEK 293 and HepG2 (kidney and liver)	Reduction in cellular proliferation; Morphological changes; Oxidative stress	PS (1 µm; 0.05 – 100 mg·L^{-1})	(Goodman et al., 2022)
L02 and BEAS-2B (liver and lung)	Mitochondrial damage; Oxidative stress; Alterations in mitochondrial membrane potential; Suppression of mitochondrial respiration	PS (80 nm; 6, 13, 32, 63, 125, 250 g·L^{-1})	(Lin et al., 2022)
CCD841CoN and HIEC-6 (colon and intestinal epithelial)	Oxidative stress; Decrease of mitochondrial membrane potential; Membrane damage	PS (0.1 – 5 µm; 100 mg·L^{-1})	(Zhang et al., 2022a)
DMBM-2 and THP-1 (peripheral blood monocytic cells)	Oxidative burst in monocytes, granulocytes; Cytotoxicity	PS (0.02 – 1 µm; 200 mg·L^{-1})	(Prietl et al., 2014)
A549 (lung alveolar epithelial)	Internalisation; Cell viability; Cell cycle; Apoptosis; And associated gene transcription and protein expression	PS (25 and 70 nm; 1.14 µg·mL^{-1})	(Xu et al., 2019)
A549 (lung alveolar epithelial)	Cell proliferation; Cytotoxicity; Metabolic activity	PS (1-10 µm; 100 µg·mL^{-1})	(Goodman et al., 2021)
Caco-2, HepG2, and HepaRG (colon and liver)	Uptake; Transport; Cytotoxicity	PE, PP, PET, and PVC (1-137 µm; 1-100 mg·mL^{-1})	(Stock et al., 2021)
BEAS-2B (lung)	Cytotoxicity; Inflammatory effects by ROS	PS (1-3 µm; 10 and 1,000 µg·mL^{-1})	(Dong et al., 2020)
Caco-2 (colon)	Cytotoxicity; Cell viability; Oxidative stress; Membrane integrity	PS (0.1 and 5 µm; 1-200 µg·mL^{-1})	(Wu et al., 2019)

In another interesting study by Liu et al. (2020b), where PS-MNPLs of the same size and Caco-2 cells were also used as in the previous work noted above, the toxicity was also assessed, taking into account the original and transformed surface compositions that these plastic particles might suffer during the *in vitro* digestive process by the gastric fluids treatment. Results showed that the digestive process did not alter the chemical surface composition, but favoured the corona formation onto MNPLs surfaces, where the 100 nm PS-NPLs provided higher intestinal toxicity than 5 μm PS-MPLs. Digestive treatment relieved cytotoxicity and transport function disorder of the Caco-2 monolayer that was induced by non-treated PS-MPLs. However, the *in vitro* digestive process increased the proinflammatory effects of PS-MPLs. Thereby, the formation of a corona on the surface leads to a change in size, zeta potential, and adsorbed compounds, as well as increases in hepatic cytotoxicity after the transformation of MNPLs by simulated gastric fluid.

The GIT uptake and effects of different MPLs of most common polymers (PE, PP, PET, and PVC) were investigated by Stock et al. (2021) using the Caco-2 cell-line for the *in vitro* model and evaluating their cytotoxicity in Caco-2, as well as in HepG2, and HepaRG cell-lines in order to detect possible responses in the organs which are first to come into contact with ingested particles: the intestine and the liver. The results of the study demonstrate that specially 1–4 μm PE-MPLs were transported through the intestinal epithelium, and that intestinal exposure to MPLs is material-dependent and size-dependent. However, it is noteworthy that only a high concentration far beyond realistic dietary exposure of consumers induced cytotoxic effects.

Overall, the main current toxicological effects at the cellular level that can be attributed to MNPLs-polymers particles are oxidative stress by the generation of reactive oxygen species (ROS), metabolic changes, as well as cytotoxicity and genotoxicity responses that are governed, basically, by the size, shape, surface composition, and the dose.

Plastic additives

As aforementioned, when particle size decreases, the surface-active area in contact with the external medium increase, thus facilitating the leach. Thereby, if MNPLs are accumulated in human organisms, they will provide an extra source of chemical plastic additives that will be in contact with the human tissues and biofluids, apart from the polymeric composition that is already expected.

Some of these MNPLs-plastic additives have recently been related to carcinogenicity, neurotoxicity (Mustieles and Fernández, 2020), obesity (Andújar et al., 2019), and endocrine disruption (Ma et al., 2019). Notwithstanding, previously published human studies show that the concentrations of these additives in young people, a susceptible population segment, are typically higher, indicating the need to decrease exposure to MNPLs (Ouyang et al., 2020). Thereby, a wide variety of substitution compounds have arisen in new plastic formulations, including emerging plasticisers, brominated flame retardants, and organophosphate esters (OPEs) in substituting legacies of polybrominated diphenyl ethers (PBDEs), or those compounds that are used in new plastic materials such as bioplastics. Bioplastics derived from renewable biomass sources such as polylactic acid (PLA), polyhydroxy alkenoates (PHA), bio-derived polyethylene, among many others, offer a greener solution for plastic production. Nonetheless, to our knowledge, very little information is currently available on their impact on living organisms, including humans. In this context, Zimmermann et al. (2019) demonstrated that consumer products made of PLA induced strong baseline toxicity similar to the toxicity of PVC items. This demonstrates that this bio-based and biodegradable material, despite being marketed as a better alternative, is not necessarily safer than conventional plastics. Despite the amount of research that has been devoted to assessing the potential impact of certain groups of plastic additives, such as some plasticisers and flame retardants, to the best of our knowledge, the information about the other groups continues to be scant.

Moreover, in general, the potential migration of additives from the plastics in use is the considered factor for the evaluation of their potential impact. In the case of MNPLs contamination from different sources, the potential migration from MNPLs particles during long-term exposure should be assessed. Lixiviation should be evaluated from particles of the smallest range of sizes, with modified surface properties, considering the potential accumulation in tissues, and sometimes coming from plastic formulations that are no longer in use, with residues that are still in the environment.

In **Figure 2.14**, the common potential adverse effects on human health derived from MNPLs by the different exposure routes are shown.

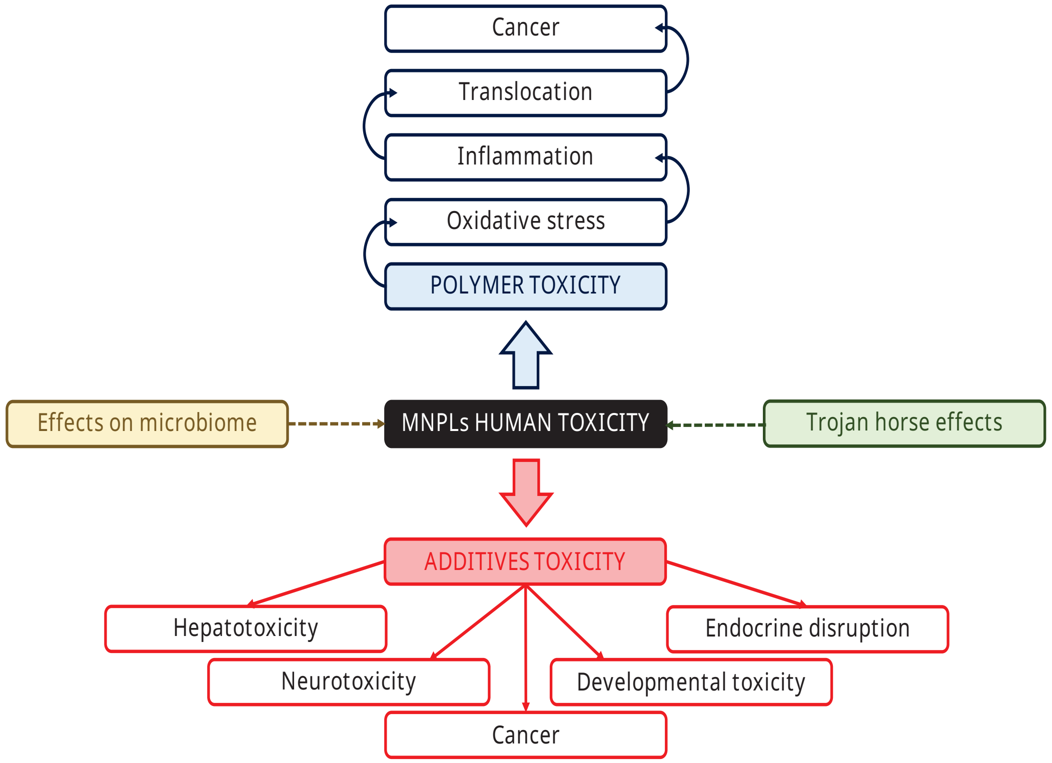


Figure 2.14 Summary of the potential adverse effects on human health generated by MNPLs via the ingestion, inhalation, and dermal exposure routes.

2.4.3 Adsorption and desorption and Trojan Horse effects

If the increased surface area of MNPLs favours the lixiviation of plastic additives, due to the increase in active surface area, the accumulation and subsequent adsorption of co-contaminants and pathogens from surrounding environments onto plastic surfaces are also prone. This environmental characteristic of MNPLs is well-known as the aforementioned Trojan Horse effect, and this shall be discussed more deeply in chapter 5.

This pathway of human exposure also needs to be addressed. In general, the toxicological interaction between MNPLs and other contaminants has already been assessed using *in vivo* studies, the majority of which were focused on the effect on model organisms exposed to a mixture of chemicals, or an individual chemical, but in both cases at a single environmental relevant concentration. Therefore, to our knowledge, dose-response curves have not been tackled from a comprehensive viewpoint to date, and a complete description of these interactions has not assessed. In this sense, several factors that rule the modulation of the toxicity of MNPLs and other co-contaminants toxicity such as physicochemical properties of MNPLs and contaminant mixtures, and therefore, their adsorption/desorption behaviour, and that of the selected model organisms, must first be considered. Other essential factors are the size and shape of MNPLs, and even colour is important for some test organisms (Bhagat et al., 2021).

On the other hand, MNPLs surfaces might also be colonised by microbes. In the natural environment, for example, biofilms can be established on plastic particle surfaces within a period of 7 days. Thereby, the potential transfer of pathogens via microplastics that are inhaled or ingested should also be investigated.

Finally, another potential effect that should be closely studied is the potential of MNPLs to disrupt the human microbiome. The chronic ingestion of MNPLs could impact on the natural community and abundance pattern of the gut microbiota. This so-called dysbiosis may be associated to mechanical disruption within the GIT, the ingestion of foreign and potentially pathogenic bacteria, as well as chemicals, which constitute or adhere to MNPLs. The impact on the microbiome can result in the immune system response and trigger chronic diseases, promote infections, and alter the gut microbiota. For example, lipopolysaccharides are able to adjust the hydrophobicity in their cell walls by changing their composition in the outer membrane, to better interact with other hydrophobic substances (Rogers et al., 2020). In this regard, Fackelmann and Sommer (2019) have summarised microplastics-induced gut dysbiosis effects on host health, and a few pioneering studies have been carried out during recent years, that studied responses on mice (Lu et al., 2018) and aquatic organisms (Evariste et al., 2019), but this is another open question regarding human health implications.

2.4.4 Human risk assessment

Various criteria and frameworks for evaluating risk levels of micropollutants have been proposed in the past decades, such as risk-based prioritisation methods (the fish plasma model and effect-directed analysis for example) in order to address which pollutants deserve more attention globally and which pollutants should deserve higher priority for their removal. Nonetheless, other kinds of prioritisation approaches, known as the hazard quotient (HQ) methodologies, have been found to be a feasible and effective approach, with which to prioritise contaminants on a large scale due to the relatively simple assessment procedure and extensive use of the HQ (Yang et al., 2021). In this context, HQ prioritisation methods are currently booming for the human risk assessment estimation of individual chemicals in relation to the maximum daily intake rate:

$$HQ = \frac{EDI}{ADI} \qquad (\textbf{Equation 1})$$

where ADI is the acceptable daily intake obtained from the appropriate international or national agency or authority. Particularly, for the different types of polymers, consensual ADI parameters are likely to be difficult to find due to the lack of regulations and legislations at the global scale. However, when the ADI data are insufficient or they seem to lack clarity, it might be estimated as stated by the World Health Organisation (WHO) in the case of pharmaceuticals in drinking water, by dividing the no observed adverse effect level (NOAEL) by a factor of 1,000 (WHO, 2012). EDI is the estimated daily intake calculated as follows:

$$EDI = \frac{DI \cdot Ce}{BW} \qquad (\textbf{Equation 2})$$

where C_e is the average substance concentration, DI the daily dietary intake of a particular foodstuff by an individual, and BW is the reference average body weight of a population from a studied area. Once the HQ is calculated for a substance, the higher the value obtained, the more risk there is to human health. Indeed, HQ values close to, or higher than, 1 can be considered as indicating that a substance may be potentially harmful to humans.

Finally, using a linear approach, the hazard index (HI) is given, considering the cumulative risk posed by a group of substances of interest ‘i’ evaluated in the same type of matrix and for a specific population group:

$$HI = \sum_i HQs, i \qquad (\textbf{Equation 3})$$

2.5 Analytical methodologies for the analysis of MNPLs

Several analytical strategies have been developed by the scientific community to assess and understand plastic pollution on Earth, and their consequences for the health of living organisms and for the environment. Nonetheless, as discussed in section 1.2 regarding the different current regulations and legislations that have been launched by many governments or international agencies in order to tackle plastic pollution; although there are many available methodologies for the characterisation of micro- and nanoplastic polymers, there is evidence of the lack of effective quantitative analytical methodologies that are able to provide accurate mass-based concentrations of MPLs and NPLs that are simultaneously more suitable and robust for monitoring and toxicological-risk assessment purposes. Thus, in this section, the current main approaches in relation to the sampling, sample pre-treatment, and the different instrumental techniques that are available for the characterisation of polymers from MPLs and NPLs are summarised. In addition, recent outcomes of pyrolysis coupled to gas chromatography and mass spectrometry (Py-GC-MS), as well as its advantages and disadvantages are shown as a potential candidate to provide quantitative MNPLs measurements, notwithstanding the fact that it is still in its infancy.

2.5.1 Polymers of micro(nano)plastics

The fate and behaviour of plastics in the environment and living organisms are prone to be governed by their chemical nature in the case of MPLs, whereas for the smallest NPLs by their size, thus different analytical strategies regarding the sampling, sample pretreatment and instrumental analysis must be employed for the optimal characterisation of MNPLs in natural and biological matrices.

Sampling

Sampling and sample pre-treatment are transcendental steps for the subsequent successful identification and characterisation of micro- and nanoplastics, but there are so many challenges because of the diversity of MNPLs and the complexity of matrices. Therefore, we summarise here the principal advantages and limitations of several widely-used strategies for the sampling and sample pre-treatment of MNPLs following the current scientific literature (Dong et al., 2023; Reynaud et al., 2022).

In the case of water samples, manta trawling, bulk water sampling, and in situ pumped water filtration are the three main sampling strategies for MNPLs. Manta trawling is better suited to sampling MPLs in large areas of open water, such as rivers, lakes and superficial areas from seas and oceans including the microlayer and the first 50 m of epipelagic compartments, particularly using vertical or neuston nets which, in most cases, are used for plankton sampling. These kinds of samplings are not suitable for deeper depths or differentiate stratified water layers. For those cases, bulk water and pumped water filtration samplings are bound to be used for the simultaneous sampling of MPLs and NPLs at different water depths, like Conductivity-Temperature-Depth (CTD) sensors coupled to a niskin bottles rosette and Plastic Explore (PLEX) samplers working normally until depths up to a range of 4,500-6,000 and 100 m, respectively. As disadvantages, we can point out the limited sampling volume that is restricted according to the capacity of rosette samplers (mainly between 5-25 L) in CTD, and the difficult possibility for sampling at surface water layers by PLEX. MNPLs sampled by snow or ice tend to be obtained by employing a clean spatula or an ice-core sampler (Karlsson et al., 2020).

For soil and sediment samples, NOM as well as clay minerals and other interferences increase the complexity during sample treatment. The use of a specific strategy is based on the depth, differentiating between surface sampling (until 10 cm depth) and deep sampling (river and lake bottoms), or stratified samplings which may provide historical trends. Surface samples are usually collected as pooled samples from quadrats or by retractable spoons, while sediment samples are collected from water bottoms or coastline-shore areas through different kinds of grabs from a boat, as well as using box or gravity corer. Once samples are taken, they must be freeze-dried at < 60 °C to avoid the possible decomposition or degradation of MNPLs.

Regarding airborne MNPLs, active air pump and passive samplers are mainly used. Airborne chemical pollution is usually caused by a dynamic complex mixture proportioning gases and a mixture of natural and anthropogenic particles. Quartz fibre filters are mostly employed for collecting fine and ultra-fine particles, while polyurethane foams (PUF) are used for VOCs that are present in the gas phase. A clear advantage of passive sampling is the generation of time-integrated data along with high enrichment factors, which are beneficial for the identification of low-level pollutants. Furthermore, for deposition samplings of MNPLs, the Norwegian Institute of Air Research designed a device based on ISO standards (NILU sampler) (dos Santos Galvão et al., 2022).

Finally, biota and biofluids are sampled, in general, from manual collection and by blood draw and biopsies of volunteers or patients, and autopsies in the case of humans. Samples should be kept at – 20 °C or below for short-term storage, and at – 80 °C for long-term storage, and the samples must be homogenised before starting the pre-treatment. Specifically, for blood samples, heparinised vacutainers are required to prevent coagulation (Leslie et al., 2022).

Sample pre-treatment

For MPLs and NPLs from snow and ice samples, these often do not require any pre-treatment, other than melting at room temperature in glass bottles and subsequently being filtrated through filters of low-size pore that are commonly made from polycarbonate, nylon, glass fibre, or cellulose acetate because they are not highly reactive with the main solvents that are then used during the sample leaching and extraction processes. The same first filtration step is advisable for liquid water samples. In addition, in the case of waste or storm waters samples, a cascade filtration is required, and this helps to purify samples from the turbidity. Once samples are in filters, if they contain high levels of NOM, an oxidative digestion by H_2O_2 (30% v/v) sometimes combined with organic or inorganic digestions is used, always keeping the temperature lower than 70 °C to avoid MNPLs ageing. Sometimes, Fe^{2+} (Fenton reaction) is added to accelerate digestion speed, but at least 72 h is recommended to ensure a successful digestion. Finally, a density separation step might be included if the final lixiviate is still turbid by saturated hypersaaline dissolutions made from NaCl or $ZnCl_2$ (Ainali et al., 2021).

The Brownian motion nature of NPLs in water also allows to carry out other types of strategies for sample pre-treatment. In freshwater environments, colloidal behaviour of NPLs is likely to be more stable than in seawater compartments, due to the significant presence of ionic forces which might cause hetero-aggregation of NPLs with natural and anthropogenic particles, and may destabilise NPLs colloidal suspension, which is necessary to maintain during all of the sample pre-treatment until there is the instrumental analysis, with which to obtain a suitable measurement without interferences. Thereby, during the dissolution and purification pre-treatment steps of NPLs from all types of matrices, monovalent solvents, which do not provide a greater contribution than divalent solvents, such as KOH or NaOH, are commonly used in the total ionic strength of the medium, thus avoiding overcoming the critical coagulation concentration (CCC) of these NPLs. Furthermore, even when NPLs remain stable as colloidal suspension, other subsequent steps could be necessary to ensure the full removal or almost the main part removal of the NOM from NPLs (Gigault et al., 2018).

In the case of soil and sediment samples, large particles are removed by sieving through stainless steel mesh with sizes from 50 μm to 10 mm, considering the particle size of interest. Particularly for MNPLs (from 1 nm to 25 μm), smaller mesh sizes such as 25 μm are used. Then, density separation employing saturated dissolutions is performed, followed by a wide range of possible digestion processes, where the most common are H_2O_2 and acid-alkaline leaching, or a mix of both. As a rule, H_2O_2 with or without a strong acid (e.g. HNO_3) is used for the dissolution of soils with high contents of organic and inorganic matter. For samples that contain low levels of organic matter, alkaline digestions are more suitable due to their weakness, rather than H_2O_2 or Fenton's reaction.

For atmospheric samples, a combination of non-polar and polar solvents is used to ensure the dissolution of MNPLs. Acetone and dichloromethane have been recommended as suitable solvents of choice due to their ability to dissolve MNPLs particles and fibres by ultrasonic-assisted extraction (UAE). Other solvents or mixtures of solvents that have been tested are: hexane:acetone 3:1, hexane:acetone:toluene or methanol:dichloromethane 9:1 (Caban and Stepnowski, 2020). For NOM removal, a pre-treatment using H_2O_2 followed by a density separation is also widely applied for MPLs purification.

Biota and biofluids samples usually contain complex matrices with the presence of proteins, lipids, endogenous metabolites, or salts that might result in interferences with the MNPLs identification and characterisation. As an example, human tissues are fat-rich, in general, and they need extra steps based on alkali and wet oxidation digestions or enzymatic digestions and saponification processes by NaOH-KOH mixtures after the H_2O_2 treatment, n order to remove lipids and dissolve proteins before the density separation with $ZnCl_2$ and centrifugation steps. Then, supernatants are filtered and the extraction of MNPLs is carried out. For non-polar compounds from biota or biofluids, a combination of non-polar and moderately polar solvents, such as toluene or hexene with dichloromethane or acetone, is common employed (Ainali et al., 2021).

Common instrumental techniques

Tables 2.7 and **2.8** summarise the different analytical techniques used for the detection and characterisation of MPLs and NPLs, respectively, in environmental and biological matrices with the main advantages and disadvantages (Dong et al., 2023; Gigault et al., 2021).

2.5.2 The need of standardised mass-quantitative analytical approaches to quantify polymers of micro(nano)plastics

While non-destructive techniques such as μ-FTIR or μ-Raman are widely employed for MPLs characterisation, they have limited applicability for NPLs due to the diffraction limit. Furthermore, in combination with optical microscopy, so many analytical methodologies have been developed, which provide the number of plastic particles that might be for a given sample. In fact, these approaches are qualitative extrapolations based on particle-counting, and subsequent estimations are approximations that do not take into account either the whole sample or the shapes of MPLs. For these reasons, to our

Table 2.7 Advantages and limitations of the main analytical techniques employed for the identification and characterisation of MNPLs.

Analytical technique	Size limit ≈	Advantages	Disadvantages
Optical microscopy	50 µm	Can easily obtain the characteristics of MPLs and other particles in terms of shape and size; non-destructive	Time consuming; capable of big human errors; it needs to be coupled with spectrometric techniques to confirm results
Fluorescence microscopy	3 µm	Easy to manage; Fits for bulk samples; non-destructive	Staining treatments are needed
µ-Fourier transform infrared spectroscopy (µ-FTIR)	10 µm	Non-destructive; it has standard spectral libraries available; automatic scan and positioning	ATR-FTIR could destroy samples
Laser direct infrared spectroscopy (LDIR)	10 µm	Fast identification of single particles; labour saving; can provide detailed statistical information; non-destructive	High requirements for pre-treatment; Interferences with other non-polymeric particles
µ-Raman spectroscopy (µ-Raman)	1 µm	It has standard spectral libraries available; it can scan the filter directly	It could burn the sample
Mass spectrometry	-	Fast and clear identification; can obtain MS-based chemical characterisation; supports multimodal characterisation of MPLs	Destructive; requires relatively clean sample matrices
Electrochemical impedance spectroscopy (EIS) - based graphene electrode	1 µm	Low detection size range; custom-made electrode	Destructive; can only measure one certain polymer type of MPL for a given time
Surface-enhanced Raman scattering (SERS)	100 nm	Non-destructive; it needs a small sample volume and can detect certain polymers of NPLs	There is a need to prepare metal nanoparticles as adsorbent to NPLs; only fitting for clean matrices; cannot categorise the size ranges
µ-Raman coupled with optical tweezers	50 nm	Non-destructive; can detect either the size and the type of polymer of MNPLs particle; can be used directly for liquid samples	Disturbs the Brownian movement, thus limiting the volume of optical trapping
Thermogravimetry (TGA)	1 µm	Can identify polymers of PE, PET and PP by the use of standards	Destructive; it is not suitable for other common polymers of MPLs due to overlapping transition signals

Table 2.8 Advantages and limitations of the main analytical techniques employed for the identification and characterisation of NPLs.

Analytical technique	Size limit ≈	Advantages	Disadvantages
Nanoparticle tracking analysis (NTA)	10 – 1,000 nm	Non-destructive; Enables the nano-size characterisation of a colloidal solution	Limited density separation (that is, sedimentation, centrifugation)
Dynamic light scattering (DLS)	0.3 – 10,000 nm	Non-destructive; Enables the nano-size characterisation of a colloidal solution and its stability	Prevents resolution by diffraction – limited optical methods and characterisation by infrared/Raman diffraction – limited laser spots; limited density separation (that is, sedimentation, centrifugation)
Atomic force microscopy (AFM)	10 nm	Non-destructive; Promising results for chemical and topographic characterisations of NPLs	Prevents resolution by diffraction – limited optical methods and characterisation by infrared/Raman diffraction – limited laser spots
Inductively coupled plasma – mass spectrometry (ICP-MS) + adsorbed metals	-	Enables the use of adsorption-based samplers; simplifies plastic additives and co-contaminants characterisation, whereby inorganic hetero-aggregation can be tracked	Destructive; interference from background material, carbon in particular; complicates ecotoxicity assessment
Asymmetric flow field – flow fractionation (AF4)	1 – 1,000 nm	Allows size separation and physical characterisation of NPLs in the colloidal solution	Destructive; needs to be coupled with other techniques like Py-GC-MS or ICP-MS to provide organic (NPLs) or inorganic compositions and mass concentrations respectively for a given sample

knowledge, some editorial boards from scientific journals have already begun to advertise that they will not consider articles or reviews which simply report the presence or concentrations of plastics anywhere, including biota, by using these types of approaches. Moreover, a detailed protocol of sampling and sample pre-treatment shall be fixed by mandate to include quality assurance – quality control (QA/QC) minimum criteria, as well as the use of more suitable techniques such as Py-GC-MS for MNPLs identification and quantification purposes (Paoletti et al., 2023).

In this sense, the need for quantitative analytical approaches that are able to provide mass concentrations of MNPLs is not fulfilled to date, and this lack concerns the scientific community. Some instrumental techniques are available in the literature, such as depolymerisation by means of direct analysis in real time (DART) or matrix-assisted laser desorption/ionisation (MALDI) coupled with mass spectrometry (MS), capillary electrophoresis (CE), or thermal extraction desorption linked to gas chromatography and mass spectrometry (TED-GC-MS). Nonetheless, these techniques have important limitations that do not allow a robust quantification of MNPLs polymers in the case of DART- and MALDI-MS techniques, or there is a need for large amounts of sample, and tedious pre-treatment steps to remove possible organic aggregates and plastic additives from polymeric particles of MNPLs that can interfere strongly in their signals, in the case of TED-GC-MS methods.

Py-GC-MS

Pyrolysis coupled to GC-MS can be used to assess MNPLs pollution due to its ability to analyse a very small amount of sample, its cost-effectiveness, its minimal sample preparations requirements, and the possibility to identify and quantify plastic additives and polymers from MNPLs by multi- and single-shoot work modes, respectively. However, complex matrices with high contents of NOM can provide a significant background noise that is able to interfere with the signals of MNPLs pyrolysates, thus making the polymers mass-quantification and their data interpretation more challenging (Akoueson et al., 2021).

In addition, pyrolysis rates might be different for MPLs compared to NPLs. For a given temperature in the pyroliser, smaller plastic particles could suffer high decomposition compared to than bigger plastic particles, and some of the the subsequent pyrolisates produced may provide lower signals in the MS spectra than the expected signals due to the elevated degree of decomposition. Le Juge et al. (2023) and Li et al. (2023) provide examples using PS-NPLs which were pyrolysed at different temperatures ranging from 500 to 650 °C, and where it was evidenced that NOM overlapped the styrene signal, which was the most abundant signal. Thereby, the lower the pyroliser temperature set, the greater the dimer and trimer signals obtained, thus improving PS quantification by the trimer pyrolysate and avoiding the NOM interferences.

Further research on the development of Py-GC-MS methodologies for the simultaneous assessment of MNPLs-polymers in different environmental and biological samples is necessary. In **Table 2.9** there is a summary of the main advantages and limitations of Py-GC-MS approaches for MNPLs-polymers analysis.

Table 2.9 Py-GC-MS advantages and limitations for MNPLs analysis in environmental and biological samples (Li et al., 2023).

Advantages	Disadvantages
Small sample amounts and rapid sample pre-treatment; applicable for a wide range of polymers and several organic plastic additives for their simultaneous identification and subsequent mass-quantification in one run; suitable for liquid or solid samples with complex matrices; high sensitivity through the use of MS in tandem or HRMS by the elemental composition and structural information.	Destructive; not suitable for polar compounds and those compounds with high molecular weights and low volatility; possible NOM interferences with MNPLs-polymers pyrolysates signals; strict Py-GC-MS validation is required.

2.5.3 Plastic additives of micro(nano)plastics

In the study by Wiesinger et al. (2021), more than 10,000 additives have been identified in plastics leachates, where over 2,400 compounds were categorised, such as contaminants of emerging concern (CECs) because one or more are dealt with by EU criteria concerning bioaccumulation, toxicity, and persistence. So many (1,327) of these 2,400 plastic additives have not been adequately regulated at the global scale to date, and also some (901) have not been approved for use in food-packaging, because there are issues to be resolved in some jurisdictions.

For a sustainable circular economy (CE) which may successfully impact on plastic pollution by avoiding the use of hazardous plastic additives, concerted efforts by all stakeholders are necessary, beginning by increasing accessibility to information.

Advantages on the use of chromatographic & related techniques coupled with mass spectrometry

From this low transparency and awareness of the potential damage of plastic additives to human health, these particular additives have been commonly found at trace and ultra-trace levels in several environmental compartments and in some biological samples. From these samples, scientists from analytical and/or environmental research fields have developed a wide range of analytical approaches during recent years, based on chromatography and mass spectrometry techniques, which are widely employed for the measurement of these compounds in complex matrices at low concentrations, due to their brilliant capabilities in terms of precision, sensitivity, and robustness.

Liquid chromatography (LC) is used for the separation of the extracted non-volatile or non-polar, partially, and polar analytes based on the affinity between the stationary and liquid mobile phases along a chromatographic column, where the former is active and remains motionless, whereas mobile phase crosses or percolates through the stationary phase. Retention of analytes in the column will depend on their properties as well as separation efficiency governed by the particle size and the diameter of the material used for stationary phase, length, and temperature of the column during the separation, and by the pH and flow rate from the mobile phase. Different LC columns are available regarding the chemical nature of the stationary phase, such as reversed-phase (RP) columns made from hydrocarbon chains (C_8 or C_{18}) that are useful for separating non-polar substances, and normal-phase columns, which present a wide range of functional polar groups in their stationary phases, and are more suitable to retain polar compounds, while the mobile phase is principally made from a non-polar solvent. Nevertheless, in the case of partial polar compounds like several plastic additives, hidrophilic interaction liquid chromatography (HILIC) is mainly used whereby this is produced by a partitioning of an analyte between an acetonitrile-rich mobile phase and a water-enriched layer partially immobilised onto a polar stationary phase. Moreover, ion chromatography (IC) is widely applied through LC ion-par or ion-exchange columns which are used for the separation of ionic substances (high hydrophilic compounds), as well as capillary electrophoresis which requires low sample injection volumes and flow rates, but often provides lower sensitivity than liquid chromatography.

The combination of reversed phase and HILIC LC columns is also promising for comprehensive two-dimensional liquid chromatography (LC x LC) in applications of environmental screening, as both use compatible eluents and provide highly orthogonal separations, but to our knowledge, there is almost no available software which can handle the data of the second separation dimension automatically, thus making data treatment very tedious.

In the case of gas chromatography (GC), the separation is easier, and it is carried out for volatile hydrophobic (non-polar) compounds (i.e. generally, hydrocarbon compounds), based on the affinity with a gas mobile phase under isothermal conditions, or using different gradients of temperature during the elution. Partial polar columns are also available, thus presenting good reproducibility but being sensitive to column bleeds and avoiding the use of high temperature, as is used for non-polar columns. In addition, supercritical fluid chromatography (SFC) can be used for the whole range of compounds from the most hydrophilic to the most hydrophobic, employing supercritical CO_2 as the mobile phase in the main cases that might be modified by the addition of polar solvents or aqueous salt solutions to increase its low polarity at the pure state.

In **Figure 2.15**, the main chromatographic techniques employed are classified according to their suitability to separate compounds through their polarity.

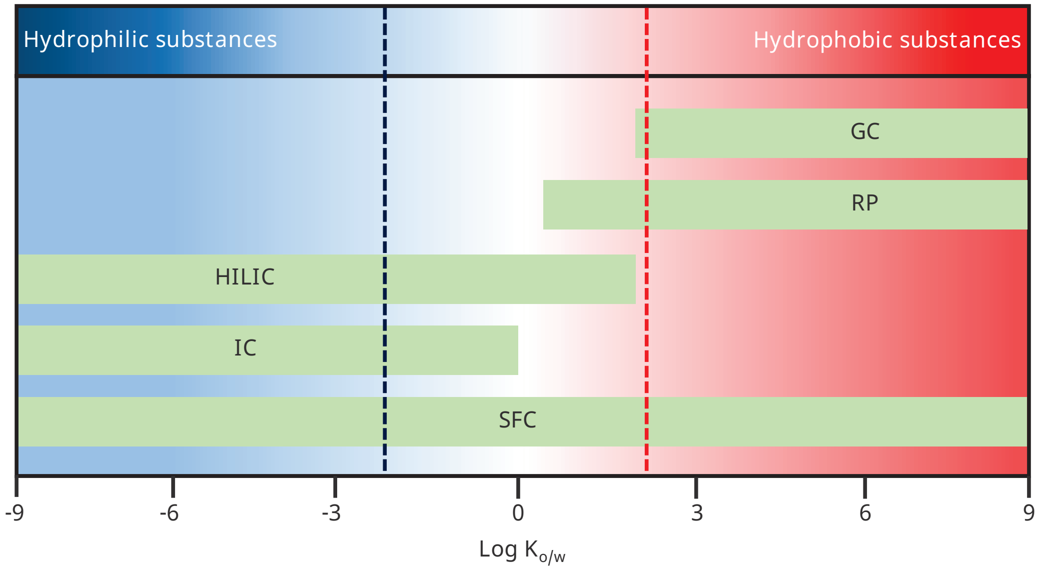


Figure 2.15 Chromatographic techniques classified according their suitability to separate substances by their polarity based on Log $K_{o/w}$ (adapted from Bieber and Letzel (2020)).

Once compounds (e.g. plastic additives) have been eluted from chromatographic systems, mass spectrometry is frequently also employed for the subsequent confirmation and even quantification of those substances by their chemical structures. Injection, ionisation, and some parameters from the analyser must be optimised prior to analysing any sample. As for chromatography, the ionisation source will be selected according to the polarity of compounds of interest (**Figure 2.16**). Atmospheric pressure chemical ionisation (APCI), atmospheric pressure photoionisation (APPI), and electrospray ionisation (ESI) are the main weak sources that are normally used in LC-MS for obtaining the corresponding molecular ions from analytes, whereas strong ionisation sources like electron ionisation (EI) and chemical ionisation (CI) are widely applied in GC-MS to obtain more information about the structure of the analytes and their functional groups by common molecular fragmentation patterns.

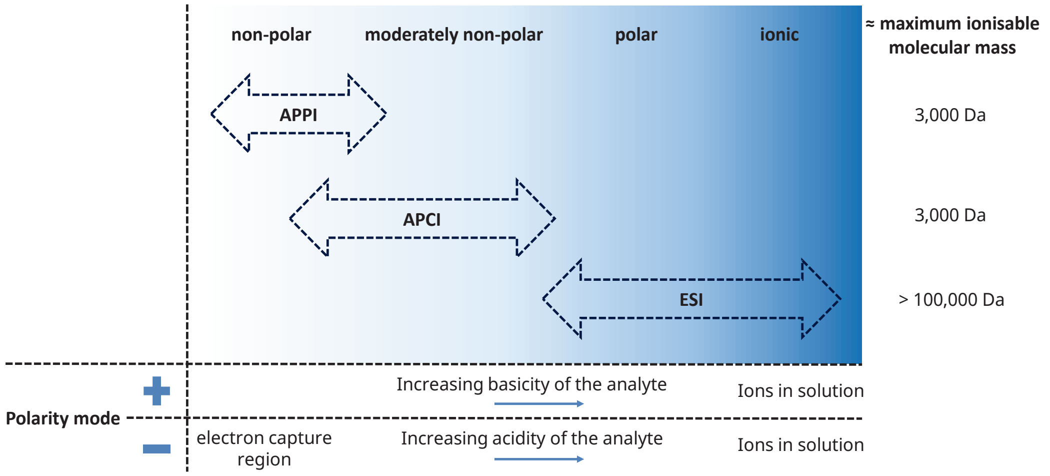


Figure 2.16 Weak ionisation sources and their range of applicable polarity for analytes, including the approximation of maximum molecular mass of the analyte to be ionised (adapted from Hollender et al., (2023)).

Once analytes are ionised, charged molecules go into the mass analyser, and they are separated according to their mass-to-charge ratio (*m/z*). Analysers can be distinguished on their resolution, where low resolution analysers provide nominal *m/z* values such as single quadrupoles or ion traps (low resolution mass spectrometry, LRMS), and high-resolution analysers are able to give confidence levels of *m/z* values up to 4 decimals, such as in time of flight (TOF) or Orbitrap (high resolution mass spectrometry, HRMS).

Moreover, LRMS tends to be combined in tandem with other LRMS such as triple quadrupole (qQq), quadrupole-ion trap (q-IT), as well as HRMS quadrupole-Orbitrap (q-Orbitrap), and quadrupole-TOF (q-TOF) to achieve more fragmentation levels from the analyte of interest, in order to elucidate upon and confirm its structure.

Target, suspect screening, and non-target screening analysis

As there are thousands of plastic additives which are used for industrial plastic production, depending on the purpose (tentative identification, or confirmation and quantification), target, suspect screening, and non-target screening (NTS) approaches are the most suitable strategies with which to monitor the simultaneously spread and scope of plastic additives in the environment, and also in traditional consumer products, as well as their possible uptake ratios in living organisms. In **Figure 2.17**, a generic workflow including each kind of analysis is shown.

As a rule, LRMS is used for target analysis such as qQq under multiple reaction monitoring (MRM) acquisition mode, even despite the fact that its elucidation power is limited, but it gives a slightly higher sensitivity over HRMS. For LC-MS, weak ionisation sources are likely to be used, where at least 2 transitions between 1 precursor ion and the 2 most intense product ions, as well as ion ratios lower than 25-30% for each transition from the corresponding standards, must be obtained for identification purposes, whereas, for GC-MS by strong ionisation sources, 2 transitions from different precursor ions are expected to be used. Due to the fact that LRMS requires a previous selection of the analytes of interest, the number of target compounds is limited but ensuring and allowing high sensitivity and a reliable quantification, respectively, if necessary.

Target analysis performance has also been achieved by employing HRMS instruments. This is attributed to the heightened sensitivity coupled with the exceptional selectivity offered by HRMS. Nevertheless, when suspect screening or NTS strategies are used, HRMS is required. Data acquisition is mainly carried out using either data dependent acquisition (DDA) or data independent acquisition (DIA). In both scenarios, an initial full scan identifies a precursor ion, which is isolated based on various criteria (e.g. intensity or inclusion list) and further fragmented (MS_n) to gather additional information about the positions of specific moieties. In DIA, all precursor ions (without pre-selection) undergo fragmentation using a high-energy function, resulting in a combined mass spectra of all simultaneously entering precursors.

Furthermore, NTS analyses enable a thorough screening of virtually any compound that is present in the samples, if they are ionisable. This is regard-

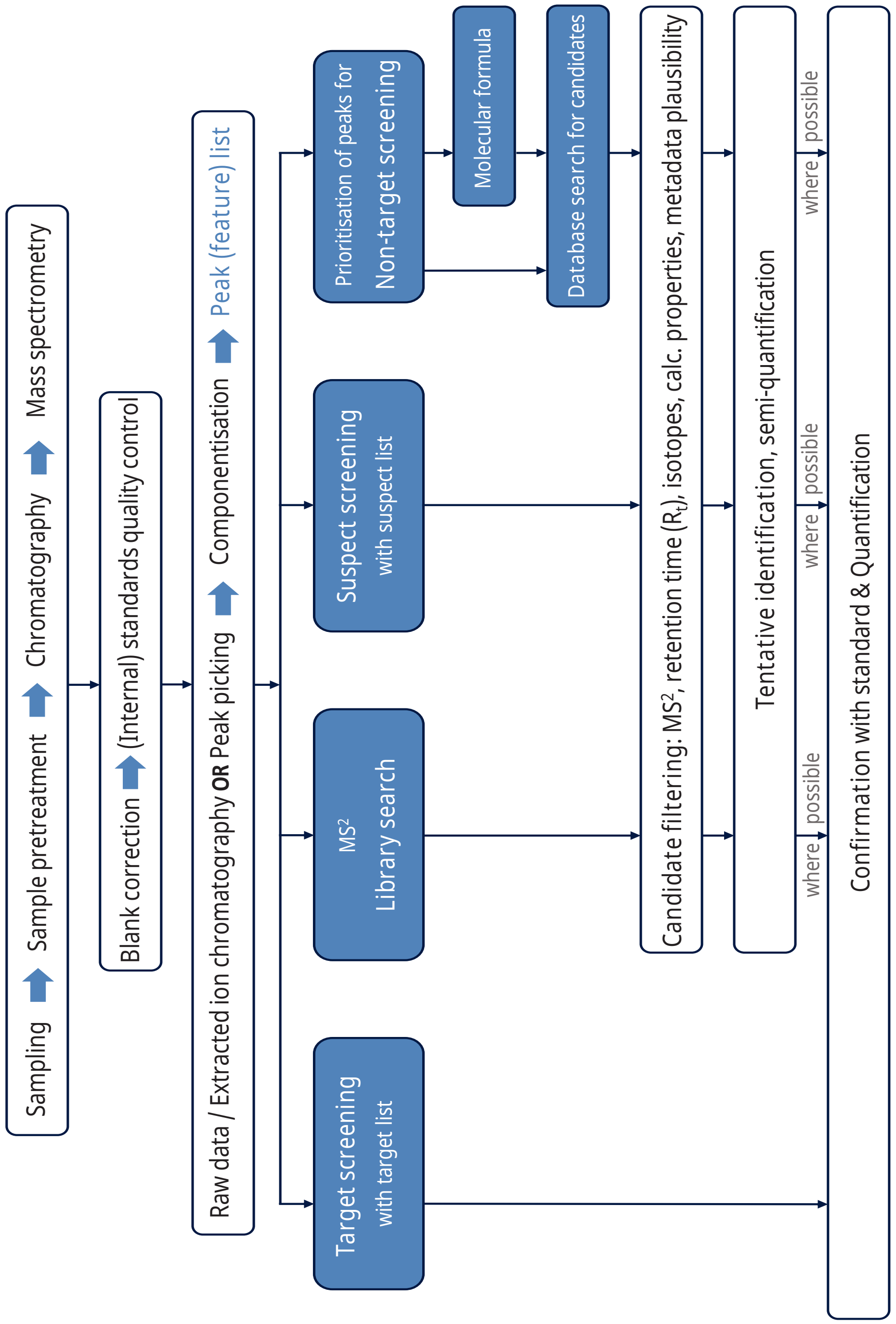


Figure 2.17 Generic workflow of target, suspect screening, and NTS strategies (adapted from Hollender et al., (2023)).

less of the availability of reference standards. In addition, NTS permits retrospective analysis, allowing the re-examination of previously acquired data in the future to search for specific information. For instance, this includes compounds that are described in the future that are suspected to be present in already injected samples. Overall, HRMS is less sensitive than LRMS, and a considerably higher level of data processing is necessary for the reliable identification of compounds. Consequently, the use of databases and in silico fragmentation tools becomes almost mandatory.

Various levels are assigned based on the confidence in compound identification, following the classification proposed by Schymanski et al., (2014). **Table 2.10** summarises the different identification levels for HRMS suspect screening and NTS strategies.

Table 2.10 Identification of confidence levels for HRMS suspect screening and NTS analysis.

Identification level	Minimum requirements
Level 1: confirmed structure by reference standard	MS, MS^2, RT, reference standard
Level 2a: probable structure by library spectrum match	MS, MS^2, library or literature MS^2
Level 2b: probable structure by diagnostic evidence	MS, MS^2, experimental data/context
Level 3: Tentative candidate	MS, MS^2, experimental data/context
Level 4: Unequivocal molecular formula	MS isotope/adduct (MS^2 is helpful)
Level 5: Exact mass of interest	MS

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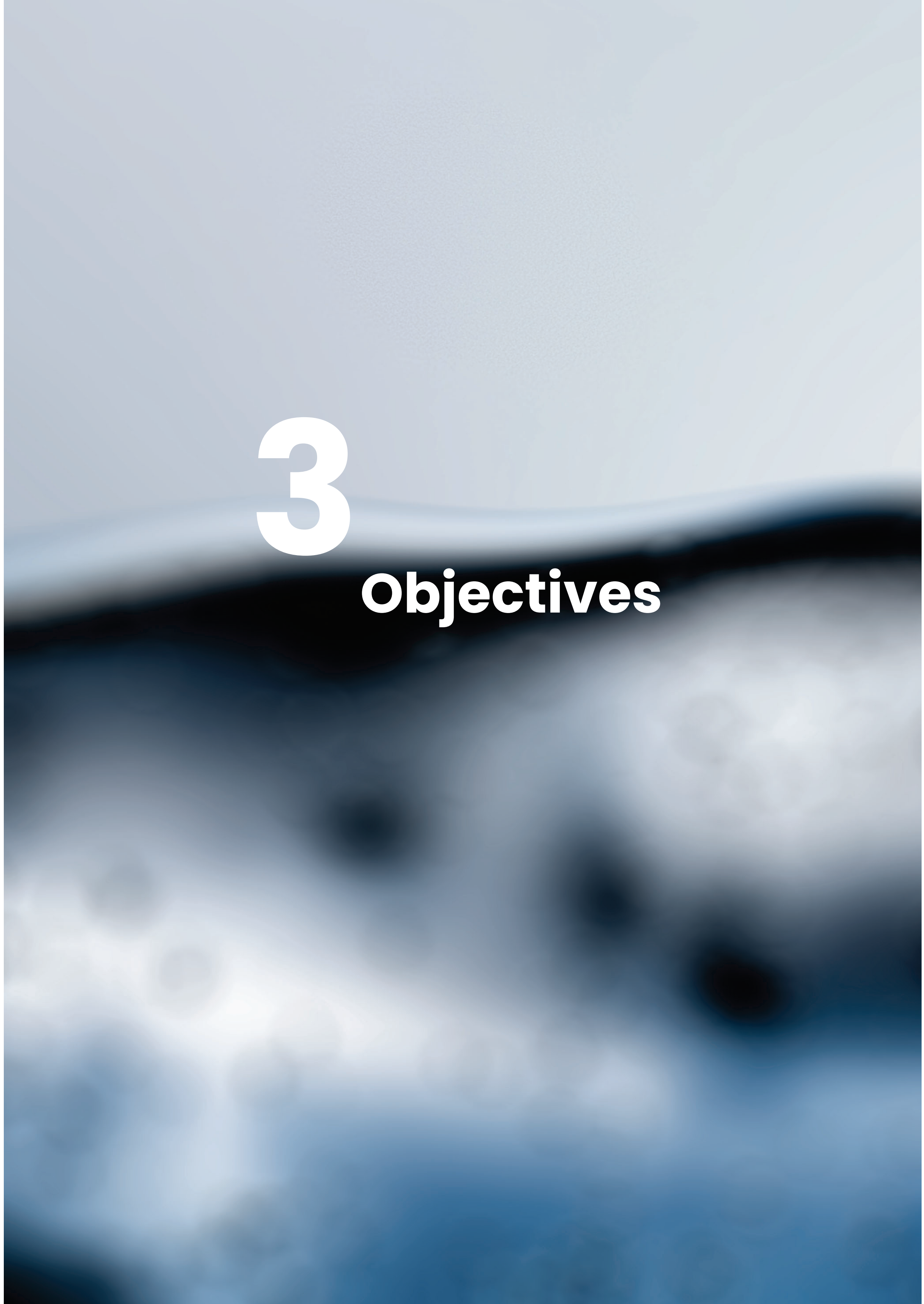
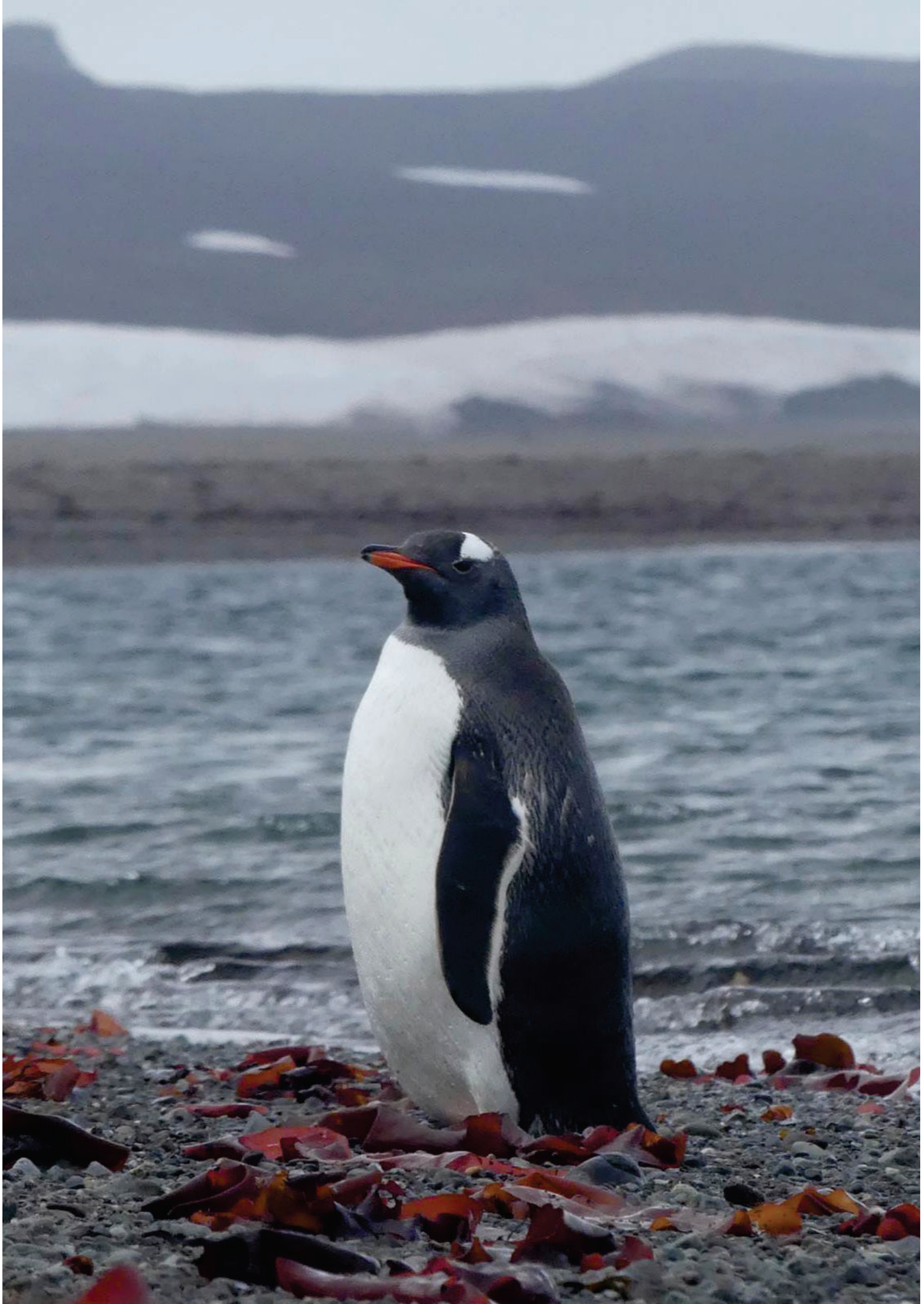


3

Objectives



3.1 Objectives

The primary goal of this doctoral thesis is the **development and application of novel analytical strategies with which to determine, in terms of mass measurements, the composition of MNPLs in different environmental compartments and biota.**

Therefore, the specific goals are:

- Develop and validate target and suspect screening methods using mass-quantitative analytical methodologies based on liquid chromatography (size-exclusion) coupled with high resolution mass spectrometry (LC(SEC)-HRMS) and pyrolysis followed by gas chromatography and mass spectrometry (Py-GC-MS), in order to identify and quantify polymers of MNPLs (> 700 nm).

- Establish and apply to complex samples, an analytical workflow that will assess, via suspect screening, the profile of plastic additives in MNPLs based on LC-HRMS.

- Assess the occurrence of MNPLs-polymers from riverine and estuarine areas to oceans and remote regions, including the long-range atmospheric transport and their mass exchanges between the low atmospheric compartments and the marine surface compartments.

- Improve the understanding of the behaviour of MNPLs in the environment. In this regard, studies of the adsorption-desorption mechanisms between polymers of MPLs and organic contaminants in surrounding environments were carried out.

- Contribute to a better understanding of human exposure to MNPLs through some of the main routes of exposure in drinking water and contaminated food.

4

Occurrence of micro(nano) plastics in aquatic and atmospheric ecosystems

4.1 Introduction

Riverine and estuarine zones such as rivers, lakes, reservoirs, and coasts are particularly susceptible to anthropogenic contamination, including plastic pollution, and this is usually because of their proximity to densely urbanised areas (Nava et al., 2023). The occurrence and breaking down of plastics in the environment have been widely reported since 2010. **Figure 4.1** presents the total number of publications between 2010-2020 regarding **(A)** the assessment of plastic particles lower than 5 mm in various waterbodies, as well as **(B)** their concentrations expressed in particles per Kg of dry mass or per L (D'Avignon et al., 2022).

The studies that are summarised in **Figure 4.1 B** show a considerable abundance and heterogeneity of MPLs in surface water, where concentrations in inland waters (1.9 particles·L^{-1}) are lower than in estuarine (3.1 particules·L^{-1}) and marine (16 particules·L^{-1}) waters. Further, due to the fate of MPLs being partially governed by their buoyancy, which is proportional to their density, the abundance of MPLs in the surface water is expected to be higher than in deeper compartments, which agrees with the reported results. However, to our knowledge, there is a lack of knowledge of corona formation around polymeric particles, which increases their weight, their aggregation in natural conditions, and the climatologic conditions that can rule their immer-

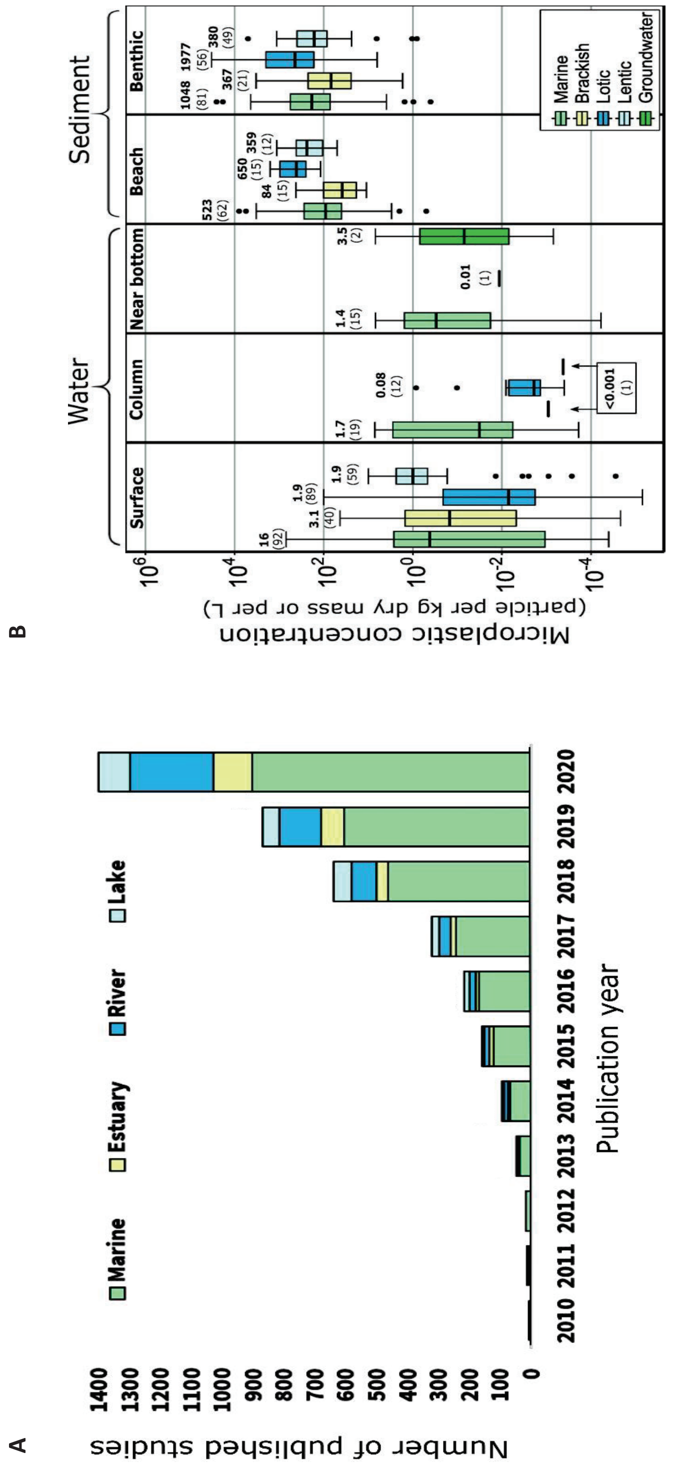


Figure 4.1 A) Number of scientific studies published from 2010 to 2020 assessing plastic particles less than 5 mm for various aquatic compartments, B) Box and whisker plots (or boxplots) showing plastic concentration ranges for the waterbody types mentioned. Concentrations are given as the number of particles per Kg dry mass of sediments for beaches and benthic bodies, while in waterbodies they are reported as number of particles per L. Means are in bold above the boxplots (the graphs are taken from the work of D'Avignon et al., (2022)).

sion. Moreover, most available data are focused on the top 5 cm. Therefore, there is also an important lack of knowledge about the abundance of MPLs in the water column and the deeper environments. What has been reported is a higher abundance in lentic ecosystems rather than in lotic ecosystems, whereas the highest concentrations were found along shorelines and in benthic sediments. Several reviews have discussed the presence of MPLs in marine environments, with particular emphasis on plastic litter and the estimation of MPLs in the oceans. Notwithstanding, 80% of marine plastic pollution and debris originate from terrestrial sources, and when addressing plastic pollution, the importance of freshwater ecosystems should be highlighted. Firstly, because freshwater makes up less than 3% of the world's water resources, and solely 1% of that is readily available. Secondly, rivers and streams receive the impact of wastewater treatment plants (WWTPs) and urban and agricultural runoffs. Rivers are especially impacted by WWTP effluents. Non-treated effluents received by WWTPs are complex mixtures of contaminants and anthropogenic micro- and nanoparticles, including MNPLs such as residues of personal care products (PCPs), car tyre dust, and textile fibres, while water treatments are limited in the removal rates of certain pollutants, and thus small particles cannot be retained. For these reasons, rivers play a crucial role in the global pollution cycle, being that they are one important input of MNPLs from land to marine ecosystems (Ryan et al., 2023), together with the runoff of coastal areas, direct WWTP discharges, ports, and the wind transport from the land.

Other important factors to consider when comparing the results from different studies in freshwater ecosystems are the climatological conditions and the season. Some studies have stated that MNPLs concentrations are higher during the dry seasons than the wet seasons, because rainfalls can increase the river fluxes, which leads to the dilution of MNPLs concentrations in estuarine areas, assuming that MNPLs coming in with the surface runoff water is not enough to keep or increase the concentration in the estuary compared to the dry season (Suteja et al., 2021). Nevertheless, in their study, Possatto et al. (2015) did not find significant differences in MNPLs abundances between dry and wet seasons, due to the presence of a landfill on the bank of the estuary area, for which the assessment of those authors is that there are continuously introduced particles of MNPLs through leaching and air deposition processes. Overall, fluvial processes seem to be dominant in relation to MNPLs abundance in these compartments, rather than tidal dynamics. It has been shown that particularly for shorelines or coastal areas (including estuarine areas), tidal fluctuations, seasons, windage, sedimentation, and resuspension affect the exchange of MNPLs between freshwater and larger seawater bodies, as well as MNPLs horizontal and vertical migration (Feng et al., 2022). Estuarine areas can be classified by their salinity variation into salt wedge (limited tidal variation), partially mixed, and well mixed (high tidal variation). The formation of the salt wedges is favoured in dry seasons, with the absence of strong winds and rainy conditions increasing the turbulence and, consequently, the stratification and mixing at the intrusion of freshwater and saline seawater flows. MNPLs are likely to reach seas and oceans on these salinity fronts, while those MNPLs with negative buoyancy might be trapped in the turbulence, which acts as a temporary sink that can retain MNPLs. Normally, the salinity front is identified as the estuarine turbidity maximum (ETM), and its location is variable, and depends on meteorological and tidal conditions. Partially or well-mixed estuaries, characterised by high flushing rates, tend to push MNPLs to marine and oceanic environments easier than a salt wedge estuary. However, the behaviour of plastics in these compartments needs further research, specially regarding particle segregation and water exchange velocities with seas and oceans (Malli et al., 2022).

Once in the marine environment, MNPLs affect the Earth's biogeochemical cycles, specially the carbon cycle. It should highlight the widespread MNPLs in marine ecosystems ruled by the hydrodynamics of tides and currents with accumulation zones, such as the five subtropical ocean gyres and the dynamics of long-range atmospheric transport (LRAT). Several scientific studies have reported the presence of MNPLs in the atmosphere, and even suggesting that MNPLs might reach the stratosphere where plastic degradation by UV radiation may release chlorine and bromine additives, thus potentially damaging the ozone layer by radical reactions where MNPLs could act as a catalyst (Tatsii et al., 2024). As aforementioned, the horizontal large-scale surface currents are the most efficient way to transport plastic debris, including MPLs, with positive buoyancy (Elipot et al., 2016). These currents are mainly driven by surface winds, generating convergence (i.e., with focus and direction), and divergence drifts (i.e., with the consequent space and possibilities) under the control of the planet's rotation. Convergence drifts are based on all five subtropical gyres where MNPLs are likely to be trapped. At the same time, divergence areas are in the subpolar gyres from the Arctic ocean and the Southern ocean, which tend to retain lower MNPLs concentrations (Law et al., 2014) (**Figure 4.2**).

Other types of open-ocean processes are relevant during the marine transport of MNPLs at meso- and submeso-scale, and include such as cyclonic and anticyclonic eddies that are slow-rotating vortices with diameters of hundreds of kilometers with depths in the range of 100-1,000 m, and with an average life from weeks to years. These types of eddies also tend to form unstable filaments and fronts among them through straining surface waters, which finally may form submeso-scale eddies with diameters in the range of 1-10 km, and average lifetimes from days to weeks (Chelton et al., 2011). The eddies can trap anthropogenic matter, such as MNPLs, and the westward drift of these structures can even transport the matter over thousands of kilometers (Budyansky et al., 2015). Submesoscale processes should also be considered, because they have scales that are smaller than a few tens of kilometers, due to MNPLs being locally accumulated at density fronts and cyclonic vortices, as a result of vortex stretching mechanisms of these types of vortices. Furthermore, floating plastics can also frequently experience significant net drift velocities in the direction of wave propagation, which is well-known as Stokes drift. This open ocean process is mainly governed by the wind strength from the low marine atmospheric compartments, with magnitudes lower than wave orbital motions. Other processes are internal tides and Langmuir circulation processes, both of which are produced by the wind-induced shear flow higher than 3-5 $m \cdot s^{-1}$ and Stokes drift. These phenomena create water bodies moving in helical motion, known as convergence and divergence areas. These are visible when buoyant materials such as plastics are accumulated by the convergence in the ocean surface, affecting its horizontal dispersion (Chang et al., 2019). Nevertheless, helical motion on water bodies during Langmuir mechanisms, as well as other processes, such as the vertical mixing induced by the turbulence generated by breaking waves, submesoscale fronts, or Ekman pumping processes, may induce the vertical dispersion of MNPLs through the marine water column at different temporal and spatial scales (Kukulka et al., 2012). Moreover, the migration of marine MNPLs from subtropical and high latitudes to remote polar regions has been reported in the Arctic ocean (La Daana et al., 2018) and Southern ocean (Isobe et al., 2017). In these areas, MNPLs take part in cycles of ice formation, melting, and drift. Some studies have suggested that plastic concentration in marine ice is 1-2 magnitudes higher than in seawater. As ocean water cools to the freezing point, ice crystals are likely to be formed, and remain

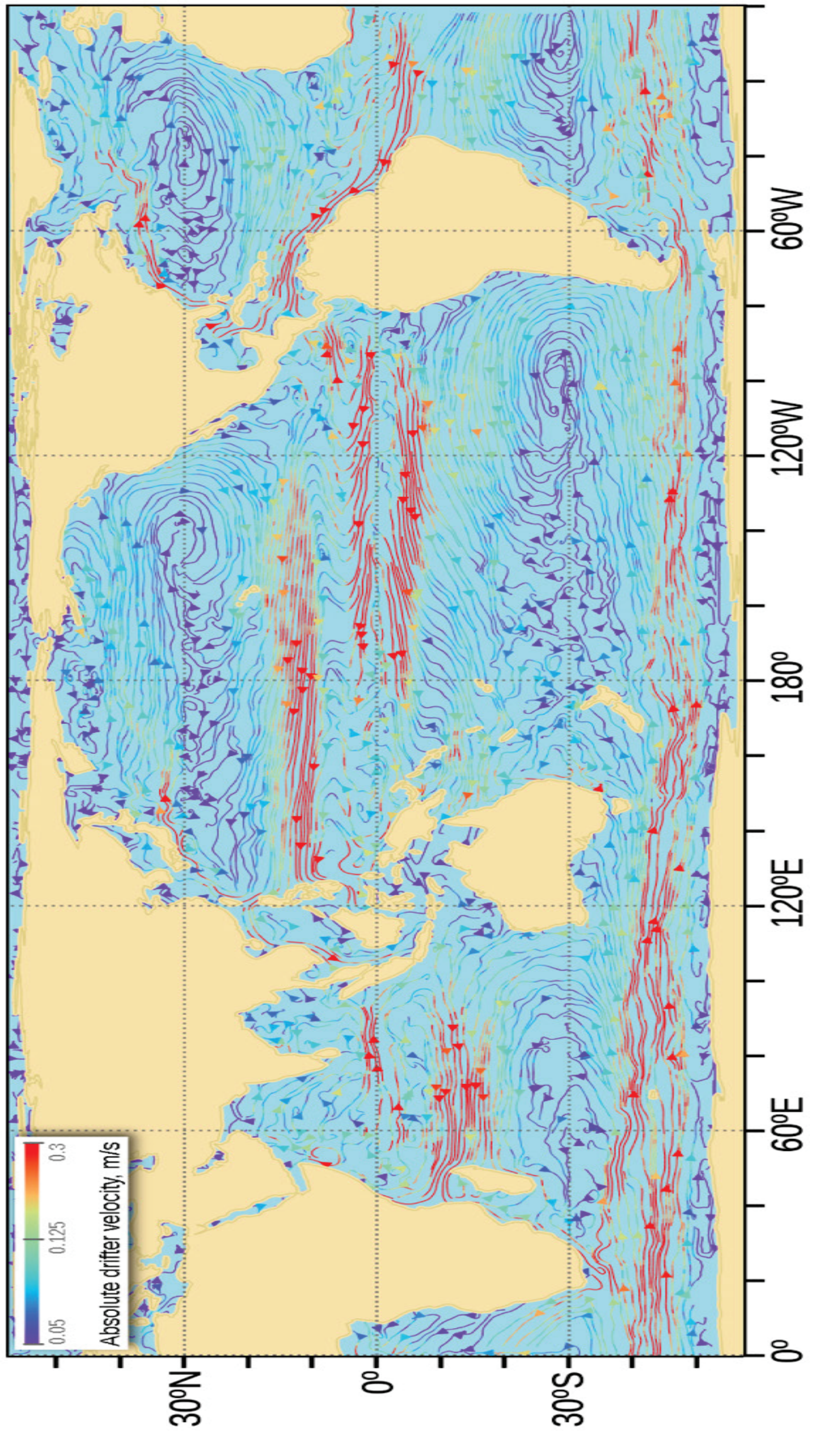


Figure 4.2 Distribution of the large-scale surface currents. Convergence areas (in blue) are the drifters centred at 30° latitude in both hemispheres, whereas divergence zones (in red) are mainly located near the equator and in the Southern ocean (taken from the study by Van Sebille et al., (2020)).

suspended in the ocean surface layer, although later they can be stirred to a depth of several meters by wave-induced turbulence and capture, along with those NPLs that are more prone to remain in suspension as colloids. Further cooling weather conditions lead to the formation of ice and, in consequence, the growing number of trapped MNPLs and their horizontal transport. In contrast, the melting of marine ice occurs at the air-sea interface, favouring lifting processes for MNPLs under freeze-thaw cycles (Obbard et al., 2014). Using a Lagrangian model, it has been estimated that vehicle tyres produce 30% of MPLs that reach marine environments globally through the atmosphere (Evangeliou et al., 2020). On the other hand, MNPLs in the ocean and seas can also be ejected into the atmosphere by spray aerosols of the water-air interface through bubble-bursting processes which transport these MNPLs in the atmosphere to other regions, where they are finally deposited by wet or dry deposition (Rochman and Hoellein, 2020). Some estimations have indicated that NPLs may have an average residence time of ~10 h in the atmosphere, with travel distances of around 400 Km. In contrast, MPLs less than 60 μm only showed residence time < 1h and maximum migration distances of 20 Km. In addition, actually, the shape and density of MNPLs play a key role in their transport and deposition, favouring those particles with large surface areas, such as fibres, rather than spherical particles (Kernchen et al., 2022). Moreover, atmospheric diffusion and transport of MNPLs can be further affected by meteorological and topographic conditions that change factors such as wind direction and speed, precipitation and temperature, or air pressure, where wind factors are considered to be the most relevant due to having a strong correlation simultaneously with the distribution of MNPLs. High intensities, duration, and frequencies of rainfall and snowfall events promote the wet atmospheric deposition of MNPLs, whereby, specifically in polar regions, MNPLs may pass through clouds and combine with snow to facilitate their deposition (Bergmann et al., 2019). In recent years, atmospheric modelling has demonstrated that it is a noteworthy approach for inferring the reverse transport trajectory and potential source areas of airborne MNPLs. Some linear back trajectory models may provide valuable information about the deposition of these MNPLs based on wind speed and direction, deposition rates, and the planetary boundary layer depth, among others. Lagrangian models such as the Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT), the Flexible Particle dispersion (FLEXPART), or the Lagrangian Analysis Tool (LAGRANTO) are the most employed to effectively identify potential source zones for atmospheric pollutants, thus providing information about altitude, distance, and residence time (Luo et al., 2024). Moreover, the increase in plastic pollution and its ubiquity affect the global carbon cycle, which is of transcendental relevance to global climate change and human survival and development. Early studies on the effects of MNPLs on microorganisms involved in carbon transformation have been assessed, but the current information is still insufficient (Shen et al., 2023). In brief, the relationship between blue carbon ecosystems, plastic pollution, and global climate change should be established in more detail, and rigorously, in the near future.

However, one of the major limitations to obtaining the whole picture of the impact of MNPLs in aquatic environments is not only (1) the lack of assessment of several compartments, such as the deep oceanic compartments, or the lack of sufficient information about the fate and behaviour of MNPLs under real environmental conditions, but also (2) the need to highlight the limited comparison that can be carried out in the different studies, since most of those studies are based on particle counting, in which particles of different sizes, shapes, and materials are considered equally. In addition to the size limitation of spectroscopic techniques such as µ-RAMAN or µ-FTIR, some particles assessments are combined with the use of microscopic techniques for the quantification that introduces important bias in the reported results, due to the superposition of particles, the presence of organic material, and the size and shape of the particles. Therefore, our first objective was to develop analytical approaches to assess, in terms of mass measurements, the occurrence of polymers in complex environmental samples, as well as to develop suspect screening workflows to assess the plastic additives profile in plastic particles in natural samples. Continuing the line already initiated in our group, the technique of choice was size exclusion liquid chromatography coupled with high-resolution mass spectrometry (LC(SEC)-HRMS).

Then, the new analytical approaches were applied to the assessment of MNPLs in surface waters of river areas, estuarine areas, coastal areas, and the open seas (marine waters, biota, and the atmosphere). Firstly, two different areas located in the Mediterranean Sea were assessed. The Ebro Delta has an extension of 320 Km^2 and is one of the top three largest deltas in the Mediterranean Sea. The main human activities are tourism, aquaculture, and agriculture, where 77% of the land is used for rice and orchard crops (Pignotti et al., 2017). Meanwhile, the Mar Menor is a semi-enclosed lagoon of 135 Km^2, which shows higher salinity and temperature ranges than the average values reported in the Mediterranean Sea. Some human activities are constantly present during the year, among which are aquatic sports and fishing activities, as well as large extensions that are used for agricultural purposes. Other pressures in this area are the deforestation, in order to expand urban areas for tourism, that produced some flash flood events, which have helped to increase the input of nutrients and anthropogenic substances, including MNPLs (Moreno-González and León, 2017).

A double suspect screening approach based on LC-HRMS was employed for the occurrence evaluation of MNPLs-polymers and their associated plastic additives composition in surface waters from the Ebro River and the Ebro Delta (Catalonia, Spain; **Publication nº1**) and the Mar Menor lagoon (Murcia, Spain; **Publication nº2**) during dry and wet seasons in order to assess the impact of the tourism, agricultural, and industrial activities based near these locations during the year. Furthermore, calibration curves were prepared from the corresponding analytical standards for the quantification, in terms of mass concentrations, of the polymeric composition coming from particles of MNPLs.

In addition to the technique based on LC(SEC)-HRMS, there are also an analytical method, which is based on pyrolysis coupled to gas chromatography followed by mass spectrometry (Py-GC-MS), for the study of MNPLs compositions, that have been developed in the frame of this doctoral thesis. The different approaches combining LC(SEC)-HRMS, LC-HRMS, and Py-GC-MS, have been used to assess MNPLs in the atmosphere of the Atlantic ocean (**Publication nº3**), and in water and biota of remote areas (**Publication nº4**). Sample collections of these two studies were carried out through the participation in 2 oceanographic expeditions that were undertaken in the periods of December 2020 – January 2021 and January 2022 – February 2022 on board the Spanish research vessels 'Sarmiento de Gamboa' and 'Hespérides', respectively. The potential Large-scale Renewable Energy Target of MNPLs, employing the HYSPLIT Lagrangian model and the MNPLs hetero-aggregation through biogenic and anthropogenic particulate matter suspended in the air, coming from terrestrial and open sea sources, which have also been evaluated to obtain a comprehensive picture of MNPLs pollution at the global-scale, and including Antarctica, as a remote region. The outcomes reported in these two studies would not have been possible without the closed interdisciplinarity collaboration between two research groups from IDAEA-CSIC and one research group from Université Laval based on Canada, where I had the good fortune to engage in a sabbatical research stay, in order to harmonise sampling and sample pre-treatment protocols, as well as to target analysis strategies for the mass-quantitative assessment of MNPLs-polymers.

4.2 Results

Thus, this chapter includes four articles, three of which that are already published, and one of which is ready to be submitted for its publication. The publications are:

- **Publication nº1:** Llorca, M., Vega-Herrera, A., Schirinzi, G., Savva, K., Abad, E., Farré, M., Screening of suspected micro(nano)plastics in the Ebro Delta (Mediterranean Sea), 2021, Journal of Hazardous Materials, https://doi.org/10.1016/j.jhazmat.2020.124022

- **Publication nº2:** Vega-Herrera, A., Llorca, M., Savva, K., M. León, V., Abad, E., Farré, M., Screening and quantification of micro(nano)plastics and plastic additives in the seawater of Mar Menor lagoon, 2021, Frontiers in Marine Science, https://doi.org/10.3389/fmars.2021.697424

- **Publication nº3:** Caracci, E., Vega-Herrera, A., Dachs, J., Berrojalbiz, N., Buonanno, G., Abad, E., Llorca, M., Moreno, T., Farré, M., Micro(nano)plastics in the atmosphere of the Atlantic Ocean, 2023, Journal of Hazardous Materials, https://doi.org/10.1016/j.jhazmat.2023.131036

- **Publication nº4:** Vega-Herrera, A., Le Bagousse, M., Berrojalbiz, N., Abad, E., Llorca, M., Dachs, J., Gigault, J., Farré, M., Micro and nanoplastics in marine waters from the Atlantic and Southern oceans, To be submitted in Nature Communications.

4.2.1 Publication n°1

Screening of suspected micro(nano)plastics in the Ebro Delta (Mediterranean Sea)

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Screening of suspected micro(nano)plastics in the Ebro Delta (Mediterranean Sea)

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ABSTRACT

This is the first work reporting the use of a double suspect-screening to assess most common polymers and additives in micro(nano)plastics (NPLs/MPLs) found in environmental waters. The method consisted of water filtration followed by ultrasonic-assisted extraction with toluene and analysis employing size exclusion chromatography using an advanced polymer chromatography column coupled to high-resolution mass spectrometry with an atmospheric pressure photoionisation source by negative ionisation conditions (LC(APC)-APPI (−)-HRMS). The identification of NPL/MPLs polymers has been based on increasing confirmation level, including the monomers characterisation by the Kendrick Mass Defect and confirmation and quantification when standards were available. In parallel, the identification of main additives in NPL/MPLs composition, as well organic contaminants adsorbed onto the plastic particles were carried out by analysis of the extracts by LC(*C18*)-APPI (+/−)-HRMS. To assess the impact of plastic pollution it is necessary to assess the composition in terms of polymers but also the additives. This screening approach has been employed to study composition of NPL/MPLs in the Ebro Delta. Two sampling campaigns including freshwater and seawater samples have been investigated to assess plastic composition in the top 5 cm. Polystyrene (PS), polyethylene (PE), polyisoprene (PI), polybutadiene (PBD), polypropylene (PP) and polysiloxanes were the most detected polymers and PP and PE, sizing between < 1000 and 2000 Da, were found at concentrations reaching up to 7000 ng/L in some areas.

The pentadecanoic acid, 1,2,3-benzotriazoles, 2-ethylhexanoic acid (2-EHA), and phthalates such as dimethyl phthalate, mono(2-ethylhexyl) phthalate (MEHP) and the phthalimide were more frequently detected plastic additives. Finally, series of organic contaminants were as well detected in the particulate fraction. These organic contaminants cannot be associated to plastic compositions but can be associated to their adsorption to the particulate matter, in particular to NPL/MPLs, due to their non-polar character. Among these organic contaminants, the more frequently detected were pharmaceutical compounds, food additives and pesticides.

1. Introduction

During the last decades, the global production and use of plastic materials have increased steadily (D'ambrières, 2019). Only in 2018, the global plastic polymer market reached $600 billion annually. However, worldwide has been reported that the percentage of recycled plastics continues being far lower than disposed of in landfills (Koelmans et al., 2014; Rocha-Santos and Duarte, 2015). For example, in 2017 in the US, only 25.1% of plastic in municipal solid wastes was recycled while 52.1% was disposed of in landfills. A percentage of these plastics throw off in landfills will end-up to micro/nanoplastics by the fractionation and erosion. The impact of plastic pollution is partially influenced by the size of plastic items or particles. Microplastics (MPLs) are defined as plastic pieces below 5 mm, including nanoplastics (NPLs) which are below 1 μm, and is a different environmental problem compared to macro and meso- plastic pollution. MPLs can have their origin in cosmetics and cleanser products (Fendall and Sewell, 2009) (classified as primary) and in the fragmentation and erosion of plastics pieces and debris (Cole et al., 2011) (classified as secondary MPLs) (da Costa et al., 2017).

Moreover, plastic residues are resistant to degradation and biodegradation, in particular in marine conditions where are accumulated. For these reasons, plastic wastes constitute 83% of the total marine litter. Among the different plastic litter items, polymers most widely identified

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are polypropylene (PP), polyethylene (PE), polystyrene (PS), polyethylene terephthalate (PET), and polyvinylchloride (PVC) (Andrady, 2011).

Some of the primary problems associated with the presence of plastic litter in marine environments are the eutrophication, their behaviour as a source and or transfer vectors for co-contaminants, and their inclusion in the aquatic food web. Eutrophication is due to the low-density of plastic materials that can be found floating and hamper the oxygen exchange and transmission of light through the water column (Harrison et al., 2011). MPLs and NPLs due to their small size, similar to plankton, can be ingested by aquatic organisms and therefore be introduced into marine food web (Wright et al., 2013; Pittura et al., 2018; Cole et al., 2014). Moreover, they may also pose a risk to human health due to their potential accumulation in commercial species. It should be highlighted that fish or bivalves cannot digest MPLs since they have not enzymatic pathways available to break down the synthetic polymers (Andrady, 2011). However, these particles can be retained in some organs, and the NPLs, due to their small size, can be translocated in living tissues with adverse effects. Moreover, plastic compositions contain a variable composition of additives, including plasticisers, colourants, fillers, and stabilisers that can leach and be a source of contaminants. As well as plastic particles, due to their physic-chemical properties can adsorb and help to transfer to biota other pollutants from surrounding waters. Due to all these reasons, during the last years, different initiatives arise to protect the aquatic environment from the emissions of these plastics debris (Hurley et al., 2018; European_Commission, 2018; European_Commission, 2013).

It has been reported that the Mediterranean Sea could accumulate between 1000 and 3000 tons of floating plastic debris (Cózar et al., 2015). The morphological characteristics of this Sea, hydrodynamics of its semi-enclosed basin and an outflow mainly through a deep water layer contribute to consider the Sea as a great accumulation region of plastic debris (Cózar et al., 2015). In particular, the amounts of MPLs found in this basin are among the highest in the world (Hurley et al., 2018; Cózar et al., 2015; Suaria et al., 2016; Schirinzi et al., 2019; Schmidt et al., 2018; Simon-Sánchez et al., 2019).

To assess the impact of MPLs and NPLs in the environment two types of information are relevant; (i) morphological and physical characterisation, and (ii) composition identification and quantification.

i) Morphological and physical characterisation assess plastic particles size and distribution. In this case, the results will depend in a relevant degree of the sampling approach used. This step is commonly achieved by visual identification (Schmidt et al., 2018; Cincinelli et al., 2019), and optical microscopy. While the surface characterisation is in general obtained by Scanning electron microscopy (SEM) (Klein et al., 2018), scanning electron microscopy–energy dispersive X-ray spectroscopy (SEM–EDS) (Klein et al., 2018; Wang et al., 2017; Wagner et al., 2017) and environmental scanning microscopy-energy dispersive X-ray spectroscopy (ESEM-EDS) (Klein et al., 2018; Vianello et al., 2013; Li et al., 2019).
ii) For the identification of the polymer composition, the technique most widely reported is Fourier-transform infrared (FT-IR) spectroscopy combined with attenuated total reflectance (ATR), transmittance or reflectance modes. Among this group of techniques, μ-FT-IR spectroscopy enables the simultaneous visualisation, mapping of samples and collection of spectra (Klein et al., 2018; Kazour et al., 2019; De Lucia et al., 2018). However, one of the limitations of these techniques is the minimum particle size that can be assessed (about 10 μm minimum). For polymers and also additives composition pyrolysis-gas chromatography–mass spectrometry (Pyr-GC–MS) is another powerful technique that has been reported (Rocha-Santos and Duarte, 2015; Fries et al., 2013) as well as thermal desorption (TDS)-GC–MS (Klein et al., 2018; Dümichen et al., 2015). The main limitation, however, is the lack of quantitative results and in general, it is used in combination with microscopy to count microparticles. In this sense, Raman spectroscopy offers an advantage, since the polymer composition and the particles counting can be carried out (Klein et al., 2018; Li et al., 2019).

It should be mentioned that in general the concentration of plastic particles is expressed in particles per square or cubic metre or in kg of dry sediments. However, for the range of nanoparticles, these are difficult to be counted and introduces an important percentage of error. In this sense, a complementary technique can be the use of liquid chromatography coupled to high-resolution mass spectrometry (LC–HRMS) as reported recently by Schirinzi et al. (2019) in the case of polystyrene NPLs, and then express the results in terms of ng per litre.

In this work, we explored the capability of LC–HRMS to perform a suspected screening of NPLs/MPLs in the aquatic environment, and we have investigated the occurrence of NPLs and MPLs of the Ebro River and their influence in the Ebro Delta (NE of Spain) and the surrounding coastal waters. The main objectives of this work were: (i) to develop a suspected screening approach based in two chromatographic runs to assess most common polymers and additives coming from NPLs and MPLs in aqueous samples; (ii) to determine the occurrence of toluene soluble NPL/MPLs in Ebro Delta Mediterranean Sea region during two sampling campaigns comparing summer and winter–spring seasons.

2. Materials and methods

2.1. Chemicals and reagents

Polymer analytical standards for GPC/SEC were purchased for polystyrene (Mw 1220 Da; Mn 1120 Da; PDI [Mw/Mn] 1,09) supplied by Polymer Standard Service GmbH (PSS, Mainz, Germany)), polyisoprene (Mw 1000 Da; Mn 854 Da; PDI [Mw/Mn] 1,17) and polybutadiene (Mw 1000 Da; Mn 925 Da; PDI [Mw/Mn] 1,08) STD KIT (from Waters Cromatografía (Cerdanyola del Vallès, Barcelona)) and polypropylene (Mw 1175 Da; Mn 1120 Da; PDI [Mw/Mn] 1,04) from American Polymer Standards Corporation (Ohio, USA).

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).

Toluene and methanol CHROMASOLV®Plus, for HPLC grade, were provided by from *Sigma-Aldrich, Steinheim, Germany* to perform instrumental analysis.

Glass microfiber filters of 0.70 μm (GF/F, Whatman TM, Maidstone, United Kingdom) were used for sample filtration.

2.2. Sampling campaigns

Two sampling campaigns were carried out during July 2018 (summer) in the middle of the peak season of vacation and tourism, and March 2019 (winter–spring) the peak season for rice crop activities in the Ebro Delta. The Ebro Delta (NE of Spain) comprises a wetland area of 320 km^2, and it is the third-largest delta in the Mediterranean Sea. It is mainly devoted to aquaculture, tourism and agriculture, 77% of the land is used for the growing of as rice and orchards (Pignotti et al., 2017). Almost 13% of its total surface is composed of natural lagoons, bays, and marshes. The presence of MPLs contamination has been reported recently (Schirinzi et al., 2019; Simon-Sánchez et al., 2019). The samples were collected in eight river sites from the lowest course of the Ebro River and eight marine sites from the two marine bays and the coast. In Table 1, the GPS locations of each sampling point, as well as pH, temperature, salinity and turbidity measured in-field with the multiparametric sensor (HI 9829; HANNA instruments, Spain) are summarised.

In each sampling site, 2.5 L of water were collected from the upper water column (top 5 cm). Samples were taken in amber glass bottles prerinsed with methanol and acetone in the laboratory and with the water from the sampling point before to fill it without head-space. The bottles were transported to the laboratory under cold conditions where they were kept at 4 °C before treatment.

Table 1
Physicochemical parameters of samples taken during summer 2018 and winter–spring 2019. (See Fig. 4 for sampling sites).

				Summer 2018			Winter–spring 2019		
Code	GPS	Origin	Water type	T (°C)[a]	pH[b]	Salinity (ppt)[c]	T (°C)[a]	pH[b]	Salinity (ppt)[c]
1	41.054503, 0.657750	Ebro River	Freshwater	25	8.24	0.30	13.2	8.39	0.30
2	41.035441, 0.597105	Ebro River	Freshwater	25	8.24	0.31	12.7	8.28	0.30
3	41.007011, 0,582585	Ebro River	Freshwater	25	8.12	0.30	12.7	8.3	0.30
4	40.919866,0.496098	Ebro River	Freshwater	26	8.34	0.33	13.7	7.99	0.42
5	40.860082, 0.512369	Ebro River	Freshwater	26	8.30	0.30	13.4	7.99	0.32
6	40.717548, 0.580122	Ebro River	Freshwater	28	8.55	0.60	13.6	8.28	0.30
7	40.723799, 0.693457	Ebro River	Freshwater	27.2	7.68	0.48	13.4	8.27	0.36
8	40.712701, 0.717456	Ebro River	Freshwater	27.5	8.00	0.50	13.7	8.21	0.34
9	40.766660, 0.737865	Coast	Seawater	32.8	8.16	25.55	23.1	8.664	35.92
10	40.803269, 0.710630	Front Harbour	Seawater	26.7	7.82	23.05	17.6	8.79	35.02
11	40.800822, 0.747438	Bay	Seawater	26.7	7.57	25.03	17	8.114	37.26
12	40.821267, 0.777867	Bay	Seawater	25.9	7.41	27.67	17.5	8.02	37.56
13	40,536280, 0.692181	Coast	Seawater	31.9	8.09	32.10	21.5	8.15	29.75
14	40.614640, 0.605632	Front Harbour	Seawater	29.1	7.83	30.77	17.6	8.17	30.91
15	40.633822, 0.694474	Bay	Seawater	29.3	7.90	30.80	17.7	8.16	31.20
16	40.601730, 0.640259	Bay	Seawater	28.1	7.36	31.44	17.9	8.02	30.91

[a] Temperature standard deviation (SD): ± 0.15 °C.
[b] pH SD: ± 0.02.
[c] Salinity SD: ± 2% of the lecture.

A bottle filled with HPLC analytical grade water was taken during both sampling campaigns and analysed in parallel with the samples to monitor any cross-contamination during sampling. These bottles were opened while water sampling in the sampling point 10 (selected randomly) and then, closed and kept with the rest of the samples.

2.3. Extraction and pre-concentration processes

The extraction and pre-concentration procedure is based on the methodology described by Schirinzi et al. (2019) with minor modifications. Briefly, water samples were passed through a sieve of 200 μm to remove larger particles. Then, 2 L of water samples were filtered through 0.7 μm glass fibres filters previously dried at 60 °C overnight in an oven (Memmert Modell 100 - 800 – Thermo Fisher Scientific; Madrid, Spain) and weighted in analytical balance (Kern ABP 100-5DM; KERN & SOHN GmbH; Balingen, Germany). Plastic particles smaller than 200 μm were collected, which includes micro and nanoparticles that are aggregate because of salting out effect. Then, the filters were dried overnight at 60 °C and weighted until constant weight. Afterwards, the filters were extracted by ultrasonic-assisted extraction (UASE) with 10 mL of toluene for 30 min and the supernatant collected in a glass vial. The process is repeated twice more, and the total supernatant (30 mL approx.) dried under nitrogen current at 40 °C until obtaining a final volume of approximately 0.5 mL. The final volume was transferred to an LC-vial and the volume adjusted to 1 mL with toluene.

2.4. Analysis of NPL/MPLs by size exclusion chromatography coupled to high-resolution mass spectrometry

The analysis of the samples was based on Schirinzi et al. (2019) with minor modifications. Very brief, chromatography was carried out using an Acquity LC (Waters, Milford, MA, USA) system equipped with a size exclusion chromatography (SEC) column, an advanced polymer chromatography (APC) column (Acquity APC™ XT45 1.7 μm 150 mm). The LC system was coupled to a Q-Exactive hybrid quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, San Jose, CA), equipped with an atmospheric pressure photoionisation source using negative ionisation conditions ((−) APPI). The mobile phase consisted on toluene 100% working with isocratic conditions. The flow rate was kept at 0.5 mL/min throughout the run, and the sample volume injected was 20 μL. The total chromatographic run had a duration of 5 min. 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 a.u. and the capillary and probe temperatures were maintained at 400 °C.

2.5. Analysis of plastic additives by liquid chromatography coupled to high resolution mass-spectrometry

The toluene extracts were re-analysed by LC(C18)–HRMS to investigate the organic additives and potential co-contaminants adsorbed onto the surface of the particles. In this case, the chromatographyc separation was achieved with the Acquity LC chromatograph equipped with Purospher® STAR RP-18 endcapped column (5 μm, 2 ×125 mm) from Merck. Toluene was used as mobile phase under isocratic mode at 0.5 mL/min. Injection volume was set at 20 μL, and the total chromatographyc run was 10 min. The chromatographic system was coupled to a QExactive equipped with an APPI source operating in negative and positive ionisation modes in two different injections. Data acquisition was performed in data dependant scan where the 10 most intense ions from full scan (m/z 50–750) were further fragmented with an isolation of 1.0 Da at a collision energy of 30 a.u. The data was acquired working at resolution of 35,000 FWHM.

2.6. Data processing and suspect screening

In Fig. 1, the general workflow is summarised. Data processing was performed using the Xcalibur Qual Browser software (Thermo Fisher Scientific, San Jose, CA, USA), and Compound Discoverer 3.1 software (Thermo Fisher Scientific, San Jose, CA, USA), respectively.

2.6.1. Polymers data processing and suspect screening

The total ion chromatograms (TIC) obtained by full-scan (FS) acquisition using the Xcalibur (Thermo Fisher Scientific) software were processed. The first screening steps included peak picking and grouping. For each sample, the TIC chromatogram was interrogated every 30 s by intervals of 500 Da and peaks which mass spectra showed repetitive mass losses were marked as suspected polymers. Then a first filtration step was based in the intensity, when the peak area in the samples was less than three times larger than the maximum peak area in the blanks were considered as background. Suspected profiles were tentatively identified using repetitive mass loses with a mass error below 3 ppm. The first list of suspected polymers was created (level 4) and it was

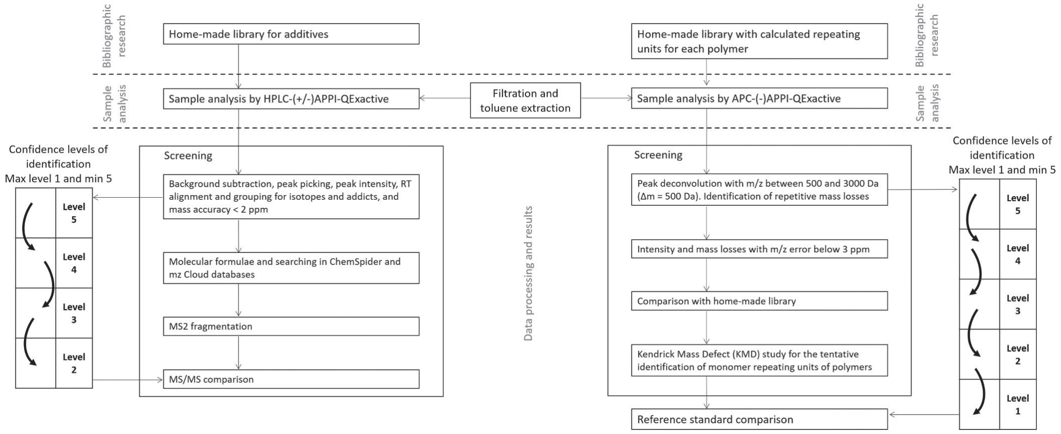

Fig. 1. Methodology workflow (adapted from Schymanski et al., 2015).

subsequently filtered by comparison with a polymers' homemade library that includes the 100 more used ones (Table S1 of the Supplementary Material section) that can be slightly soluble in toluene (level 3). Further filtering steps consisted on Kendrick Mass Defect (KMD) study for the tentative identification of monomer repeating units of polymers (identification level 2). The KMDs were calculated according to equations described elsewhere (Schirinzi et al., 2019), taking in each case PolymerX /(round PolymerX-2) as base unit.

Confirmation and quantification were only possible with the available reference standard of most common polymers (identification at level 1).

2.6.2. *Suspected screening of plastic additives and potential co-contaminants adsorb to plastic particles surfaces*

Briefly, the raw data of LC-QExactive analysis was processed using an automated screening with Compound Discoverer software version 3.1 (Thermo Fisher Scientific). The first screening steps included a peak picking, RT alignment and grouping of isotopes and adducts as well as grouping compounds across samples and predicting compositions. Signals below 1,000,000 were considered as background. The first list of suspects was considering just predicted compositions with a mass error below 2 ppm. This first list of suspected compounds was filtered by comparison with ChemSpider and mz Cloud databases. Further filtering steps consisted on the comparison of isotopic patterns, ionisation efficiency, and fragmentation patterns. Finally, the last filter was based on the comparison of the productions, obtained from the MS/MS spectrum, of a suspected compound, with the spectrum of a standard or a predicted fragmentation pattern using the information contained in the on-line databases. Unequivocal confirmation was only possible when a reference standard was available (identification at level 1).

2.7. *Quality assurance and quality control*

Water blank consisting on 2 L Water for LC analysis was carried together during the sampling campaigns to monitor any contamination source during sampling process. Once in the laboratory, these blanks were preserved with the samples and were processed in parallel with all of them. Besides, instrumental analysis contamination coming from the system was monitored by injecting solvent blanks of toluene randomly with each injection samples list.

3. Results

3.1. *Tentative identification of NPLs/MPLs polymers*

Thirty-two samples collected in two sampling campaigns at the Ebro Delta were analysed. Six polymers, PE, PP, polyisoprene (PI), polybutadiene (PBD), polyethylene terephthalate (PET), and polysiloxanes were tentatively identified (level 2). Among the organic polymers, the most common chain's distribution was between 1000 and 2000 Da. Due to the characteristics of water filtration and analysis, the detected polymers were coming from NPLs/MPLs present in samples. However, in order to determine the exact size of these particles other techniques such as SEM would be necessary.

In Fig. 2 and Fig. S1 of the supplementary material section, some examples of chromatograms, mass spectra and KMD plots of identified polymers in the samples are shown.

A summary of the results is presented in Fig. 3. As it can be seen, the 88% of the samples showed the presence of NPLs/MPLs of at least one polymeric composition, and this percentage was maintained in both sampling campaigns during the summer and end of winter.

During the first sampling campaign, in summer, PE was the organic polymer most frequent detected, it was present in 50% of the samples with NPLs/MPLs contamination. However, considering the inorganic polymers, polysiloxanes were those more frequently found. Polysiloxanes were detected in nine of the samples, that is in 64% of samples with MPLs/NPLs, and 53% of the total number of samples. In contrast, during the winter campaign the more detected polymer was by far PP, which was present in 11 samples, the 65% of the studied sites, and in 73% of the samples containing NPL/MPLs, followed by the inorganic polysiloxanes present in 53% of contaminated sites. While PE was only detected in five samples of the second sampling campaign, 33% of the samples with NPLs/MPLs. Comparing the results for PE and PP, which are similar polymers, PE is mainly used in one-use plastics items and in packaging. In the Ebro Delta, as in other touristic areas, the consume of one-use plastic items is increased during summer in concordance with the results. While, PP is in general used in building materials, which is as well in agreement with winter activities in this area. On the other hand, polysiloxanes, also known as silicones, are the most important class of inorganic polymers. Silicones possess a fully inorganic backbone of -(Si-O)- repeat units. This type of polymers finds extensive use in both industrial and urban applications along all the year.

The presence of these polymer types is an indication of human activities, but also in longer terms, where the influence of river flow and

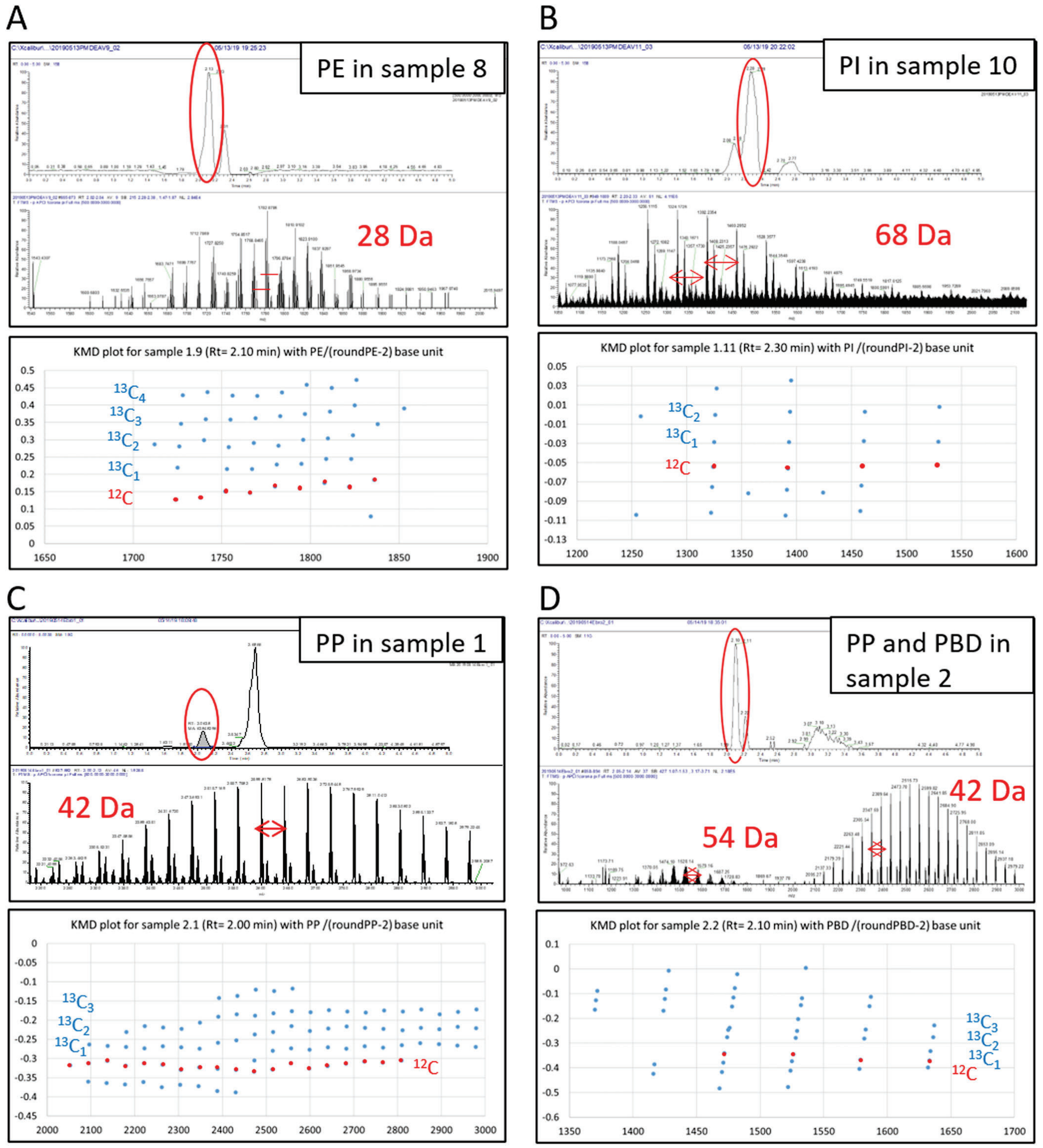

Fig. 2. Example of chromatograms, spectra and KMD of samples from the first sampling campaign (summer 2018) containing PE (A) and PI (B), and samples from the second campaign (winter–spring 2019) containing PP (C) and PP and PBD (D).

coastal currents in surface distributions, the drainage of floating particles to the river banks and beaches in coastal waters, and the descendent currents in the open sea will be the primary driving forces ruling the distribution of these particles. It should be pointed out that the distribution and incidence of polymers along the river (sampling point 1–8) was higher near villages or after wastewater treatment plants (WWTPs), as it happens in sampling point 6. The WWTP in this area serves to a total number of 20,154 inhabitants receiving also agricultural wastes (notice that 77% of the delta is dedicated to agricultural activities (Pignotti et al., 2017)). Moreover, the influence of the rice crops can be a transfer way of NPL/MPLs from the agricultural soil to the water systems since the ploughing, flooding, sowing, re-flooding and draining the fields before the harvest of rice can facilitate this process. The results of this work are similar to those reported by Simon-Sánchez et al. (2019) for the same area. The authors detected that the major contribution of MPLs in surface river waters is between 100 and 1000 μm. The main polymers

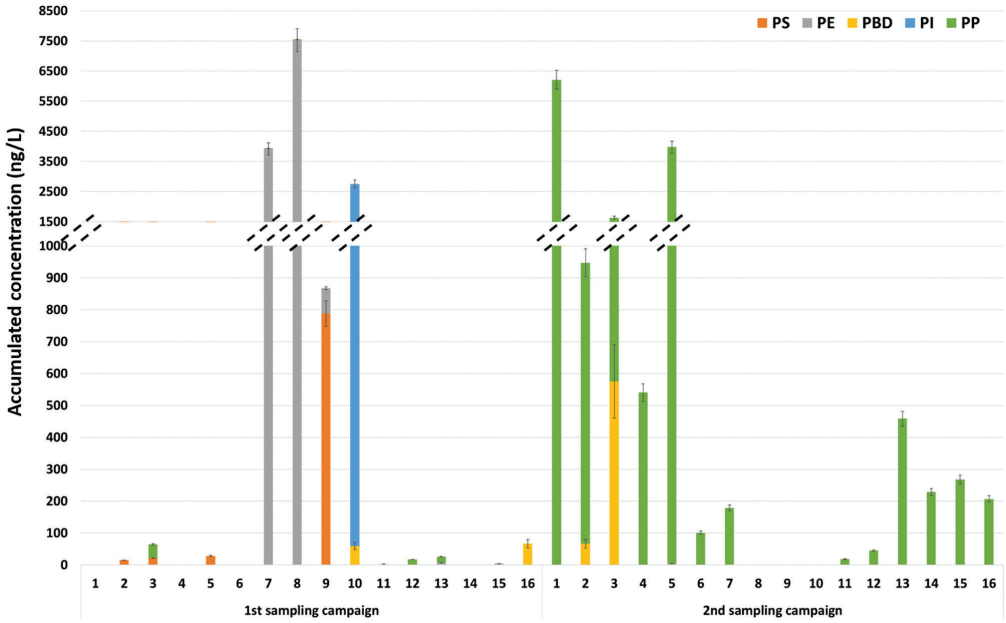

Fig. 3. Accumulated concentration of NPL/MPLs ranging from 500 to 3000 Da, expressed in ng/L.

identified through μFT-IR were PE and PP which have a contribution of 16% and 8%, respectively among the total polymers identified by the authors.

The comparison of this work with other delta areas with agricultural exploitations are partially coincident with the types of main polymers identified such as PE, PP and PS (Llorca et al.; Hidalgo-Ruz et al., 2012). For example, in a similar type of estuary in North Carolina (USA), plastic foams, particles, fibres, and fragments were detected (Gray et al., 2018). Most of them correspond to PS, which represent the 98% of foams founded, while up to 66% and 33% of fragments were PE and PP, respectively (Gray et al., 2018). However, the comparison between these results and others is difficult due to the different sampling strategies and the variety of analytical approaches applied (Llorca et al.; Hidalgo-Ruz et al., 2012), and because the range of particles sizes that were studied here.

In coastal areas and the Ebro Delta bays (sampling points 9–16 in Fig. 3), samples are dominated by the presence of PE, PP, PI and PS among organic polymers. Polysiloxanes were again prevalent polymers in coastal waters and were more frequently found during summer campaign than in winter. About the organic polymers, the presence of PE, PP and PS, correlates with other studies in the Mediterranean Sea in which they have been as well identified as significant contributors (Suaria et al., 2016). In this study, PE was the more frequently found, followed by PP and PS. Also, similar results were shown in the north-western sub-basin area (Tuscany, Italy) (Baini et al., 2018) or Turkish area (Güven et al., 2017).

The primary potential sources of NPL/MPLs in The Ebro Delta can be attributed to the increase of population and recreational ships during the summer, and during the whole year due to agricultural plastic mulches fragments that reach coastal waters and bays by the wind action or by the river, plastic fishing gears and structures used in aquaculture, by degradation or direct disposal in the aquatic media (Simon-Sánchez et al., 2019). For example, Port de l'Illa de Mar (sampling point 9 in Fig. 3) is a commercial port devoted to mussel aquaculture and fishing activities highly impacted by fisherman and their boats.

During the summer campaign, in the most northern bay, Fangar Bay, the results showed a high presence of PS, PI, PE and PP while Alfacs Bay showed high amounts of PBD and PE. In the case of Fangar Bay, this is impacted not only by fishery, aquaculture, and tourism but also by the industry residues coming from the north of the bay (Tarragona industrial area). Bathymetric differences between the Fangar and Alfacs Bays (up to 4 and 6 m in-depth, with an average of 2 and 4 m, respectively) can also influence the plastic and other contaminants accumulation in the semi-enclosed area as the Fangar Bay facilitating the eutrophication processes in this area (Pignotti et al., 2017).

3.2. *Quantification of NPLs/MPLs*

The NPL/MPLs that were tentatively identified during the suspect screening were quantified using equivalent concentrations for PS, PE, PI and PBD according to the procedure described by Schirinzi et al. (2019). Figure 3 summarises the main results.

The most contaminated samples have been samples 7, 8, 9 and 10 for the 1st sampling campaign, where PE has been the most frequently detected. The highest concentration belongs to PE in sample 8 (up to 7523 ng/L) followed by PE in sample 7, PI in sample 10 and PS in sample 9 as can be seen in Fig. 3. In contrast, samples from 1 to 5 have been the most contaminated ones during the 2nd sampling campaign, while PP has been the polymer more frequently detected (Fig. 4). The highest concentration has been detected in sample 1, reaching 6213 ng/L.

3.3. *Plastic additives*

According to the process in Section 2.5 the first screening step consisted of included a peak picking, RT alignment and grouping of isotopes and adducts and grouping compounds across samples. Signals below 1,000,000 were discarded as background. The first list of suspects was considering just predicted compositions with an error mass below 2 ppm

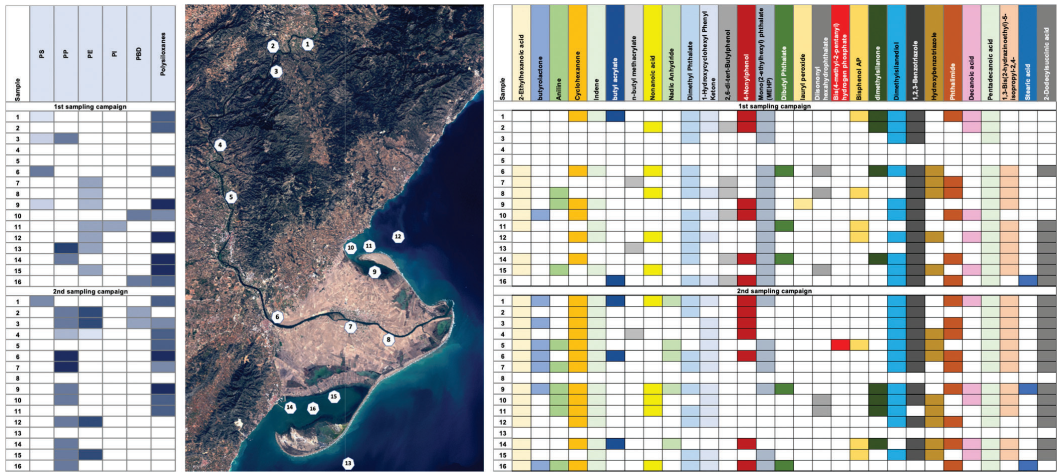

Fig. 4. Presence of polymer types (left part of the map, in grey) and plastic additives (right part of the map, in colours) during both sampling campaigns. The coloured boxes indicate the presence of compounds. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

that were filtered by comparison with ChemSpider and mz Cloud databases giving an initial amount of 65,237 identified m/z in samples. Next filtering steps consisted of refining those results by limiting peak area ($> 750{,}000{,}000$) which generates a total of 51,962 potential compounds. Afterwards, these results were refined according to the chromatographic peak factor resolution > 0.75. This process limited the above-mentioned values to 268 possible candidates. Each candidate was evaluated manually giving a list of 220 tentatively identified structures with a level 3 of confidence. Finally, 36 plastic additives were identified in the samples, including 23 chemicals additives and modifiers used to improve plastic performance and 13 dyes and pigments.

As can be seen in Fig. 4 and Table S2, the most frequently detected compounds were pentadecanoic acid (valeric acid) and 1,2,3-benzotriazole, that were present in 88% and 81% of the samples, respectively. Valeric acid is used in a variety of products, such as in the food industry as an additive, in the pharmaceutical industry, and in fragrances in addition to their use as an antioxidant in plastic formulations. Valeric acid is commonly used in plastic films of PE and other polymer films such as ethylene-vinyl acetate (EVA). Therefore, it cannot be assured that all the valeric acid present is coming from plastic particles studied. Still, considering the sample treatment that was applied consisting of particulate filtration, cleaning, and posterior extraction, it can be regarded that part of the valeric acid detected was adsorbed on the surface of particles coming from surrounding waters. Even so, the major proportion should come from the matrix of the plastic particle. Besides, the valeric acid was absent only in four samples that were coincident with the samples where other compounds were not detected. Similarly, 1,2,3-benzotriazole is used as a metal antioxidant agent in addition to as a plastic modifier, so the occurrence of this compound can be partially due to other contamination sources. But again, the samples free on 1,2,3-benzotriazole were those not showing plastic particles of these sizes as can be seen in Fig. 4. Another related compound was the hydroxy benzotriazole with an occurrence of 41% in the samples. Other compounds found over the 50% of the samples were the phthalates used as plasticizers as dimethyl phthalate, mono (2-ethyl hexyl) phthalate (MEHP) and the phthalimide with a 66%, 66%, and 47% of incidence in the samples. While other phthalates as dibutyl phthalate were only marginally found in 16% of the samples. It should be highlighted that bisphenol AP (used as a replacement of bisphenol A) was in a 22% of the samples, while other similar compounds used as a replacement such as a bisphenol S and bisphenol F were not detected in this study. Another prevalent compound was 2-ethyl hexanoic acid (2-EHA) found in 69% of the samples, and in particular during the second sampling campaign. 2-EHA is widely used in esters for PVB film plasticizers and synthetic lubricants, in paint driers, and as PVC stabilisers. Indene was as well detected in 63% of the samples. Coumarone and indene polymers are obtained from the fractionation of the coal tar. Both may be polymerised, and different plastics products are obtained, varying the proportion between them. Finally, among the most detected plastic additives, cyclohexanone that is used for solvent bonding, and 1-hydroxy cyclohexyl phenyl ketone used as a plasticiser and coating additive were both presents in 59% of studied samples.

3.4. *Other contaminants*

The properties of NPL/MPLs, such as surface charge, density, and aggregation potential, together with other abiotic and biotic processes contribute to their ubiquitous distribution within the oceans, and their ability to adsorb onto their surface other contaminants from surrounding waters. The adsorption capacity of NPL/MPLs depends on the polymer type, size and surface ageing. For this reason, it is relevant to assess which are the compounds that mainly tend to be adsorbed at MPLs particles in a natural environment. In this study, the organic contaminants adsorbed onto plastic particles surfaces were identified at the confidence level 2 (Table S3). Main groups tentatively identified are food additives, flavours and natural products (28), pesticides (7), pharmaceutical compounds (13), solvents, industrial origin compounds, and industrial wastes (6), and illicit drugs (1). It should be highlighted that these compounds were slightly different than polar or semi-polar compounds, generally reported as main contributors of organic contamination in surface waters of the Delta Ebro, as expected. In particular, because during the adsorption process there is a competition between plastic particles and the organic matter particulate, which is the majority of suspended material. Neutral species primarily dominate the overall sorption as reported before (Seidensticker et al., 2018). However, the hydrophobic entities of ionic species should be as well considered for some compounds. In agreement with experiments under controlled conditions and taking in consideration that MPLs concentration is very low, those MPLs are a relevant sorbent for more hydrophobic contaminants that can be found in seawater, but not as much for more polar compounds.

Among the different groups of compounds identified in the samples, food additives, flavours and natural products were those more frequently detected. In particular, the fatty acids such as undecanoic acid (tentatively identified in the samples with a frequency of 87,5%), myristic acid (tentatively identified in the samples with a frequency of 75%), linoleic acid (tentatively identified in the samples with a frequency of 75%), lauric acid (tentatively identified in the samples with a frequency of 72%) and fatty acids derivatives. In addition, some of them as lauric, myristic acid, and linoleic acids were detected in 100% of the samples during the second campaign. Should be said that fatty acids can have a natural origin but also are used in different manufactured products, food and feeds. While the most probable origin of other products such as 5-pentylresorcinol (Olivetol) are the natural marine lichens. Another group of compounds frequently identified in the samples were pharmaceutical compounds, such as some sulphonamides as benzene sulphonamide (tentatively identified in 78% of the samples) and sulfaphenazole (in 44% of the samples). Among the nonsteroidal anti-inflammatory drugs, in spite of these compounds are in general frequently found in surface waters, in this study attached to MPLs only two compounds were detected, probably due to the hydrophilic character of most of them. The 2 compounds detected were the salcyclamide in 37% of the samples and fenbufen 9% of detection. It should be highlighted that steroid and hormones, relevant because their implication as endocrine disruptor compounds in aquatic environments as hextrol (34%), mesterolone (37,5%) and the 17α-estradiol (12%) were as well detected in the samples.

In regards with pesticides, as it happens with pharmaceuticals compounds, their occurrence in surface waters of the Ebro Delta has been as well widely reported, in particular because agriculture is one of the main activities in this area. However, again the compounds found in general as main contributors of surface contamination are different from those which tendency is to be comparted onto MPLs surface. The 2-hexylcyclopentanone (81%) and phoxim (53%) were those more frequently detected at level 2 attached to MPLs, while aldicarb oxime, bifenazate and chlorpyrifos were marginally detected in some samples. About compounds of industrial origin, two solvents mesitol and ethyl acetate were the more detected ones at level 2, and only in punctual samples some by-products of combustions processes were found, some intermediates of reaction and some compounds used in the formulation of fragrances.

4. Conclusions

This is the first work reporting the use of a double suspect-screening to assess most common polymers and additives in (nano)microplastics (NPL/MPLs) found in environmental waters based on filtration and USAE followed by LC(APC)-APPI(−)-HRMS and LC(C18)-APPI (+/−)-HRMS, in parallel, to identify the main polymers soluble in toluene and tentatively identify the plastic additives, as well as organic contaminants comparted onto the surfaces of the plastic particles, opening a new window to assess the impact of plastic contamination in natural environments.

The results showed the presence of NPL/MPLs in 88% of the samples. The main polymers identified in the samples were PS, PE, PP, PI, PBD and polysiloxanes, with a significant contribution of polymer chains between < 1000 and 2000 Da. The most abundant ones were PE and polysiloxanes during summer campaign while PP and polysiloxanes were those more frequently detected at the end of winter. On the other hand, plastic additives tentatively detected in the samples at higher frequencies were 2-EHA and phthalates such as MEHP and the phthalimide. The results obtained here are not only the reflection of human activities but also the influence of the river and coastal geophysical and geochemical characteristics.

Finally, contaminants attached to NPL/MPLs surfaces from the surrounding waters were as well tentatively identified at level 2. Among the different contaminants identified, the more frequent were food additives, flavours and natural products, as expected. However, should be highlighted the presence of different groups of biologically active compounds such as pharmaceuticals and pesticides. Among them, it should be mentioned the presence of sulphonamides and some hormones because of their implication in the promotion of bacterial resistance and endocrine disruption effects on aquatic biota, respectively, as well as the presence of some banned pesticides in punctual samples. The tentative identification of these compounds attached to NPL/MPLs confirmed the selective adsorption of some contaminants on the plastic surfaces and underpinned the necessity to study the role of MPLs and NPLs in the transfer of organic contamination to biota.

CRediT authorship contribution statement

Marta Llorca and Gabriella Schirinzi contributed to the experimental design, lab work supervision and to writing the first draft. Albert Vega-Herrera and Katerina Savva performed the experimental work. Esteban Abad collaborated in supervision and writing. Marinella Farré conceived the idea, collaborate in designed the experiment and also collaborated in the writing of the final document and supervision.

Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2020.124022.

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4.2.1 Publication n°1 (Supplementary material)

Screening of suspected micro(nano)plastics in the Ebro Delta (Mediterranean Sea)

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Figure S1
Tables S1, S2, S3

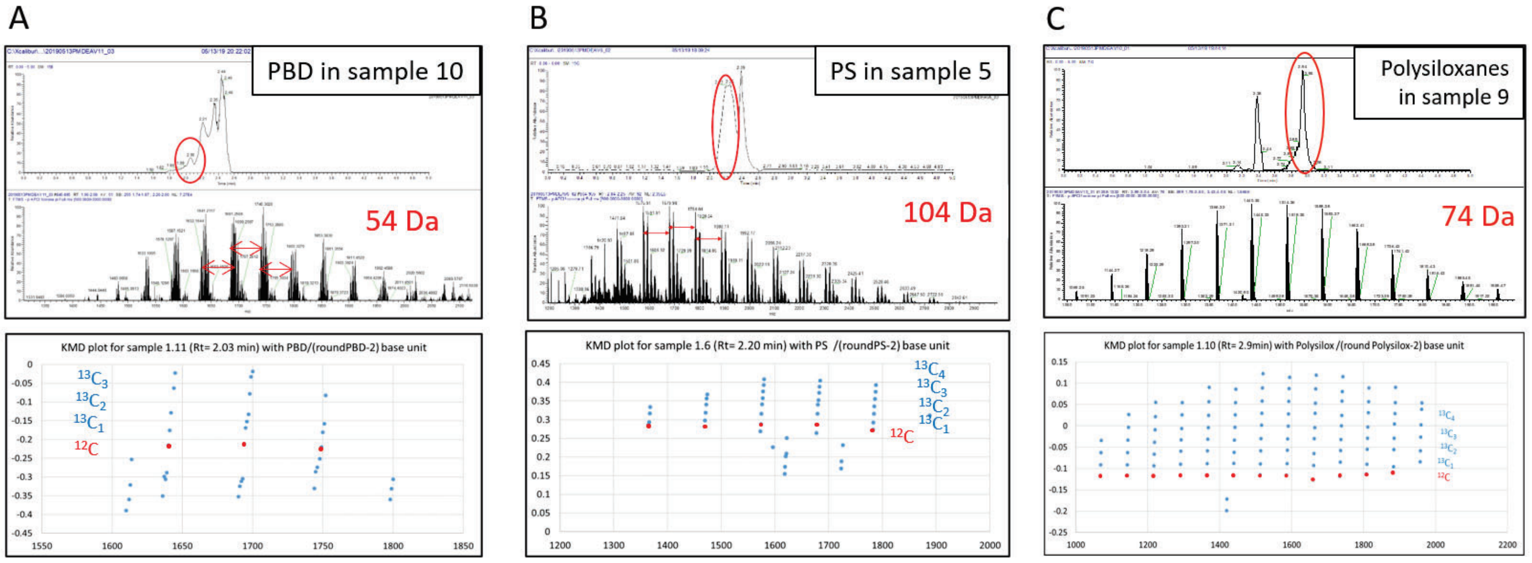

Figure S1 TIC, spectra and KMD of samples from the 1st sampling campaign (summer 2018) containing PBD (A), PS (B) and polysiloxanes (C).

Table S1 List of polymers (partially)soluble in toluene. Adapted from elsewhere [1-5].

Family		Most common polymers	Acronyms	Repeating unit	Exact Mass Repeating Unit
Acrylic Polymers	1	Poly(methyl methacrylate)	PMMA	$[C_5H_8O_2]_n$	100.05243
	2	Polyacrylamide	PAM	$[C_3H_5ON]_n$	101.035103
	3	Poly(methyl acrylate)	PMA	$[C_4H_6O_2]_n$	86.03678
	4	Poly(ethyl methacrylate)	PEMA	$[C_5H_{10}O_2]_n$	102.06808
	5	Poly(2-hydroxyethyl methacrylate)	poly-HEMA	$[C_5H_{10}O_3]_n$	118.062995
Alkyd resins	6			$[C_{14}H_{18}O_8]_n$	314.10017
Aminoplasts	7	Melamine formaldehyde	MF	$[C_4H_6N_6]_n$	138.065394
Cellulose	8	Cellulose acetate	CAc	$[C_{10}H_{14}O_7]_n$	246.073955
	9	Methyl cellulose	MC	$[C_9H_{16}O_5]_n$	204.099775
	10	Ethyl cellulose	EC	$[C_{11}H_{22}O_5]_n$	234.146725
	11	Hydroxyethyl cellulose	HEC	$[C_{10}H_{20}O_8]_n$	268.11582
	12	Nitrocellulose	NC	$[C_6H_8O_9N_2]_n$	252.022983
Coumarone-indene-resins	13	Poly(indene-co-coumarone)		$[C_{17}H_{14}O]_n$	234.104465
	14	Poly(methylstyrene-co-indene)		$[C_{26}H_{46}]_n$	358.35995
Epoxy resins	15	Diglycidyl ether of bisphenol-A	DGEBA	$[C_{18}H_{20}O_3]_n$	284.141245
	16	Diglycidyl ether of bisphenol F	DGEBF	$[C_{16}H_{16}O_3]_n$	256.109945
Fluoropolymers	17	Polytetrafluoroethylene - TEFLON	PTFE	$[C_2F_4]_n$	99.993612
	18	Polyvinylfluoride	PVF	$[C_2H_3F]_n$	46.021878
	19	Polyvinylidene fluoride	PVDF	$[C_2H_2F_2]_n$	64.012456
Polyacetals	20	Polyoxymethylene	POM	$[CH_2O]_n$	30.010565
Polyacetylenes	21	Polyacetylene / polyethyne		$[C_2H_2]_n$	26.01565
Polyacrylics	22	Polyacrylic acid	PAA	$[C_3H_4O_2]_n$	72.02113
Polyalkylenes	23	Polyalkylene Glycol		$[C_2H_4O]_n$	44.026215
Polyamic acids	24	Polyamic acid	PAA	$[C_{30}H_{18}O_8N_2]_n$	534.106318
Polyamides	25	Alyphatic polyamide	Nylon 6	$[C_6H_{11}ON]_n$	113.084064
	26	Alyphatic polyamide	Nylon 66	$[C_{12}H_{22}O_2N_2]_n$	226.168128
	27	Polyphthalamide	PPA	$[C_{14}H_{18}O_2N_2]_n$	246.136828
Polybenzimidazoles	28	Polybenzimidazole	PBI	$[C_{20}H_{12}O_2N_2]_n$	312.089878
Polybenzoxazines	29	Bisphenol-A based Benzoxazine		$[C_{31}H_{30}O_2N_2]_n$	462.230728
	30	Bisphenol-F based Benzoxazine		$[C_{29}H_{26}O_2N_2]_n$	434.199428
	31	Thiodiphenol based Benzoxazine		$[C_{28}H_{24}O_2N_2S]_n$	452.15585
	32	Dicyclopentadiene based Benzoxazine		$[C_{38}H_{37}O_2N_2]_n$	553.285503
Polybenzoxazoles	33	Polybenzoxaxole	PBO	$[C_{14}H_6O_2N_2]_n$	244.121178
Polycarbonates	34	Poly(bisphenol A carbonate)	PC	$[C_{16}H_{16}O_3]_n$	256.109945
	35	Poly(propylene carbonate)	PPC	$[C_4H_6O_4[C_2H_4O]_m]_n$	162.052825
	36	Poly(cyclohexene propylene carbonate)	PCHPC	$[C_7H_{10}O_3]_n$	142.062995
Polycarbosilanes	37	Branched polycarbosilane		$[C_{18}H_{32}Si_3]_n$	332.181184
Polycyanurates	38	Bisphenol A Dicyanate		$[C_{17}H_{14}O_2N_2]_n$	278.105528
	39	Bisphenol E Dicyanate		$[C_{16}H_{12}O_2N_2]_n$	264.089878
	40	Tetramethyl Bisphenol F Dicyanate		$[C_{19}H_{18}O_2N_2]_n$	306.136828
Polydienes	41	Poly(1,4-butadiene)	PBD	$[C_4H_6]_n$	54.04695
	42	Polyisoprene (cis and trans)	PI	$[C_5H_8]_n$	68.0626
	43	Polychloroprene	CR	$[C_4H_5Cl]_n$	88.007978
Polyesters	44	Polyethylene terephthalate	PET, PETE	$[C_{10}H_8O_4]_n$	192.04226
	45	Polybutylene terephthalate	PBT, PBAT	$[C_{12}H_{12}O_4]_n$	220.07356
	46	Polytrimethylene terephthalate	PTT	$[C_{11}H_{10}O_4]_n$	206.05791
	47	Polyethylene naphthalate	PEN	$[C_{14}H_{10}O_4]_n$	242.05791
	48	Poly(propylene adipate)	PPA	$[C_9H_{14}O_4]_n$	186.08921
	49	Polylactic acid	PLA	$[C_3H_4O_2]_n$	72.02113
Polyetheretherketones	50	Poly(ether ketone)	PEK	$[C_{13}H_8O_2]_n$	196.05243
	51	Poly(ether ketone ketone)	PEKK	$[C_{19}H_{12}O_3]_n$	288.078645
	52	Poly(ether ether ketone)	PEEK	$[C_{20}H_{12}O_3]_n$	300.078645
Polyethers	53	Polyethylene glycol, Polyethylene oxide, Polyoxyethylene	PEG, PEO, POE	$[C_2H_2O]_n$	42.010565

	54	Polypropylene glycoL, Polypropylene oxid, Polyoxypropylene	PPG, PPOX, POP	$[C_3H_6O]_n$	58.041865
	55	Polytetrsamethylene glycol, polytetramethylene ether glycol, Polytetrahydrofuran	PTMG, PTMEG, PTHF	$[C_4H_8O]_n$	72.057515
Polyimidazoles	56	Poly(4-vinyl imidazole)		$[C_5H_6N_2]_n$	94.053098
Polyimides	57	Poly(ether)imide	PEI	$[C_{37}H_{24}O_6N_2]_n$	592.163438
Polyketones	58	Polyketone	PK	$[C_3H_4O]_n$	56.026215
Polyolefins	59	Polyethylene	PE	$[C_2H_2]_n$	26.01565
	60	Polypropylene	PP	$[C_3H_6]_n$	42.04695
	61	Polyisobutylene	PIB	$[C_4H_8]_n$	56.0626
	62	Polybutylene	PB-1	$[C_4H_8]_n$	56.0626
Polyoxadiazoles	63	Polyoxadiazole, poly(oxadiazole-hydrazide) copolymer	POD	$[C_8H_4ON_2]_n$	144.032363
Polyoxyalkylenes	64	Polyoxyethylene	POE	$[C_2H_4O]_n$	44.026215
Polyoxymethylenes	65	Polyoxymethylene	POM	$[CH_2O]_n$	30.010565
Polyoxyphenylenes	66	Poly(p-phenylene oxide), poly(p-phenylene ether)	PPE	$[C_8H_8O]_n$	120.057515
Polyphenyls	67	Poly(p-phenylene)	PPP	$[C_6H_4]_n$	76.0313
Polypyrroles	68	Polypyrrole	PPy	$[C_8H_6N_2]_n$	130.053098
Polysilanes	69	Polysilane		$[SiH_2]_n$	29.992578
	70	Poly(dimethyl silane)		$[C_2H_6Si]_n$	58.023878
	71	Poly(methyl phenyl silane)		$[C_7H_8Si]_n$	120.039528
	72	Poly(trimethyl phenyl silane)		$[C_9H_{14}Si_2]$	178.063406
Polysilazanes	73	Perhydropolysilazane		$[H_2Si–NH]_n$	45.003477
Polysiloxanes	74	Polydimethylsiloxane	PDMS	$[C_2H_6SiO]_n$	74.018793
Polystyrenes	75	Polystyrene	PS	$[C_8H_8]_n$	104.0626
	76	Poly(4-methoxystyrene)		$[C_9H_{10}O]_n$	134.073165
	77	Poly(4-methylstyrene)		$[C_9H_{10}]_n$	118.07825
	78	Poly(4-tert-butylstyrene)		$[C_{12}H_{16}]_n$	160.1252
Polysulfides	79	Polyphenylene sulfide	PPS	$[C_6H_4S]$	108.003372
Polysulfones	80	Polysulfone	PSF, PSU	$[C_{27}H_{22}O_4S]_n$	442.123882
	81	Polyethersulfone	PES	$[C_{24}H_{16}O_6S_2]_n$	464.038834
	82	Polyphenylsulfone	PPSU, PPSF	$[C_{24}H_{16}O_4S]_n$	400.076932
Polythioethers	83	Thiokol	Thiokol	$[C_2H_4S_4]_n$	155.919588
	84	Polypropylene sulfide		$[c3H_6S]_n$	233.879382
	85	Poly(ethylene sulfide)	TR	$[C_2H_4S]_n$	60.003372
	86	Poly(phenylene sulfide)	PPS	$[C_6H_4S]_n$	108.003372
Polythiomethylenes	87	Polytrimethylene terephthalate	PTT	$[C_{11}H_{10}O_4]_n$	206.05791
Polyurethanes	88	Tolylene-2,4-diisocyanate Tolylene-2,6-diisocyanate	2,4-TDI 2,6-TDI	$[C_9H_6O_2N_2]_n$	174.042928
	89	1,4-Phenylene diisocyanate	MDI	$[C_{15}H_{10}O_2N_2]_n$	250.074228
	90	Isophorone diisocyanate	IPDI	$[C_{12}H_{18}O_2N_2]_n$	222.136828
	91	Hexamethylene diisocyanate	HDI	$[C_8H_{12}O_2N_2]_n$	168.089878
Polyvinyl acetals	92	Polyvinyl acetate	PVAC	$[C_4H_6O_2]_n$	86.03678
	93	Copolymer ethylene vinyl acetate	EVA	$[C_2H_4]_n$-$[C_4H_6O_2]_m$	114.06808
	94	Polyvinyl alcohol	PVA or PVOH	$[C_2H_4O]_n$	44.026215
Polyvinyl butyrals	95	Poly(vinyl butyral)		$[C_8H_{13}O_2]_n$	141.091555
Polyvinyl formals	96	Poly(vinyl formal)		$[C_5H_8O_2]_n$	100.05243
Vinyl polymers	97	Poly(vinyl chloride)	PVC	$[C_2H_3Cl]_n$	61.992328
	98	Poly(vinylidene chloride)	PVDC	$[C_2H_2Cl_2]_n$	95.953356
	99	Poly(vinyl bromide)		$[C_2H_3Br]_n$	105.941811
	100	Poly(vinylphosphonic acid)	PVPA	$[C_2H_5O_3P]_n$	107.997633

FTable S2 List of tentatively identified compounds in samples. Samples are marked with X when the compound has been detected. Log $K_{o/w*}$ from PubChem [1] and log $K_{o/w**}$ predicted from ChemSpider [2]. Notice that some of the tentative identified compounds have log $K_{o/w}$ below 1 but they are (partially)soluble or miscible with toluene.

Name	Formula	MW	log K_{ow}*	1st campaign														2nd campaign														Uses or sources
				1	2	3	6	7	8	9	10	11	12	13	14	15	16	1	2	3	4	5	6	7	9	10	11	13	14	15	16	
Plastic additives																																
Aniline	C_6H_7N	93.05743	0.9*						x	x						x						x		x	x	x	x				x	Plastics and dyes; precursor of polyurethane and polyaniline.
Benzene	C_6H_6	78.04678	2.13*	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	Gasoline, production of styrene, nylon fibers, and in other plastics
Bis(4-methyl-2-pentanyl) hydrogen phosphate	$C_{12}H_{27}O_4P$	266.1647	3.59**																			x										Homopolymer or copolymers of styrene and other esters
Bisphenol AP or 6,6-bis(p-methoxyphenyl)fulvene	$C_{20}H_{18}O_2$	290.13068	4.57** or 5.37**	x					x			x	x									x							x	x		Plastic precursor (used as a substitute of Bisphenol A)
Butyl acrylate	$C_7H_{12}O_2$	128.08336	2.36*	x													x	x					x						x			Paints, sealants, coatings, adhesives, fuel, textiles, plastics, caulk..
n-Butyl methacrylate	$C_8H_{14}O_2$	142.0995	2.88*					x						x							x											Production of polymers of methacrylate among others
2-tert-Butylphenol	$C_{10}H_{14}O$	150.10382	3.31*		x		x		x		x				x				x			x	x	x							x	Antioxidant and stabilizer, fire prevention materials
2,6-di-tert-Butylphenol	$C_{14}H_{22}O$	206.16603	4.92*		x			x	x		x				x		x															UV stabilizers and antioxidants
Butyrolactone	$C_4H_6O_2$	86.03651	-0.64*								x							x		x		x	x	x	x						x	Batteries, solvent in lotions and some polymers
Cyclohexanone	$C_6H_{10}O$	98.07283	0.81*	x			x			x	x		x		x			x	x	x	x	x	x	x	x	x	x	x	x		x	Precursor of nylon. Additive in paints, dyes, oils, waxes, rubbers..
δ-Decalactone	$C_{10}H_{18}O_2$	170.13007	2.72*	x	x		x	x	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x		x	x			x	Biopolymer manufacture, food and agricultural industries
Decanoic acid	$C_{10}H_{20}O_2$	172.14566	4.09*	x	x						x		x			x		x							x	x			x	x		Manufacture of esters, rubber, dyes, plastics, food additives…
Diisononyl hexahydrophthalate	$C_{26}H_{48}O_4$	424.35328	9.82*				x		x							x										x	x					Increase soft flexible qualities of plastics
Dibutyl Phthalate	$C_{16}H_{22}O_4$	278.1518	4.5*				x					x			x										x						x	Increase soft flexible qualities of plastics
Dimethyl Phthalate	$C_{10}H_{10}O_4$	194.0571	1.6*	x	x	x	x	x	x		x	x	x	x	x		x	x	x				x	x	x		x	x		x	x	Increase the flexibility of plastics and rubber
2-Ethylhexanoic acid	$C_8H_{16}O_2$	72.05711	2.64*				x	x	x	x	x	x			x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	Production of PVB, polyolesters, PVC stabilizer among others
1-Hydroxycyclohexyl Phenyl Ketone	$C_{13}H_{16}O_2$	204.11414	1.78**	x	x				x		x		x				x	x		x	x	x	x	x	x	x	x	x		x	x	Used in polymers, plastics, dyes among others
Indene	C_9H_8	116.0623	2.92*	x			x			x		x	x			x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	Production of indene/coumarone thermoplastic resins
Lauryl peroxide	$C_{24}H_{50}O_2$	370.37926	11.53**							x																						Plastic, rubber and paint modifier
Mono(2-ethylhexyl) phthalate (MEHP)	$C_{16}H_{22}O_4$	278.15022	4.80**	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x				x	x	x	x					x	x	Increase soft flexible qualities of plastics
Nadic Anhydride	$C_9H_8O_3$	164.04676	0.528**															x				x	x		x				x			Additive in plastics
Nonanoic acid	$C_9H_{18}O_2$	158.13008	3.42*		x		x		x				x			x		x							x	x	x				x	Preparation of plasticizers and lacquers
4-Nonylphenol	$C_{15}H_{24}O$	220.1815	5.76*	x	x					x	x				x		x	x	x	x	x		x						x		x	Production of TNPP; antioxidant in polymers
Other applications like in paints or lubricants																																
Benzotriazole	$C_6H_5N_3$	119.04822	1.44*	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x		Corrosion inhibitor in paints among others
1,3-Bis(2-hydrazinoethyl)-5-isopropyl-2,4-imidazolidinedione	$C_{10}H_{22}N_6O_2$	258.18164	-2.07**				x	x	x	x		x	x	x	x			x	x	x	x	x	x	x	x	x	x		x	x		Colorants, dyes, or pigments, for food and inks
Dimethylsilanone	C_2H_6OSi	74.01858	2.6/4.25*	x	x		x								x										x	x	x		x			Generated from decomposition of polydimethylsiloxanes
Dimethylsilanediol	$C_2H_8O_2Si$	92.02904	0.91**	x	x	x	x			x	x		x		x	x	x	x	x	x	x	x	x	x	x	x	x	x		x		Generated from decomposition of polydimethylsiloxanes
2-Dodecylsuccinic acid	$C_{16}H_{30}O_4$	286.21287	5.06**				x					x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		Corrosion inhibitor and anti-scaling agents
(Fluoroethynyl)(triisopropyl)silane	$C_{11}H_{21}FSi$	200.14019	6.08**							x	x	x		x		x														x	x	Generated from decomposition of polydimethylsiloxanes
(E,E)-2,4-Heptadienal	$C_7H_{10}O$	110.07271	1.59**						x	x	x							x	x	x	x	x	x	x		x	x	x	x	x	x	Used in oil, petroleum, fuel oils, drilling oils among others
Heptanoic acid	$C_7H_{14}O_2$	130.09904	2.42*		x		x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x		x	x	x	Used in oil, petroleum, fuel oils, drilling oils among others
Heptadecanedioic acid	$C_{17}H_{32}O_4$	300.22837	5.58**				x		x			x	x		x	x	x	x	x	x	x	x	x	x	x	x		x	x	x		Surfactant in lubricant for engines, brake fluids, oils among others
Hydroxybenzotriazole	$C_6H_5N_3O$	135.04386	0.69**				x	x	x				x			x					x	x	x			x	x	x	x	x		Derivative of benzotriazole, used as corrosion inhibitor
Pentadecanoic acid	$C_{15}H_{30}O_2$	242.22325	6.62**	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	In products like toys, and lubricants for engines, brake fluids, etc.
(±)-1-Phenylethanol	$C_8H_{10}O$	122.0728	1.42*							x	x				x			x		x	x	x	x	x	x			x	x	x	x	Used in oil, petroleum, fuel oils, drilling oils among others
Phthalimide	$C_8H_5NO_2$	147.03156	1.15*	x				x	x		x				x		x	x		x	x		x	x	x			x	x		x	Precursor to azo dyes (in plastic for their colouring properties).

Table S3 Tentatively identified contaminants adsorbed onto plastic particles. Samples are marked with X when the compound has been detected. Log $K_{o/w*}$ from PubChem [1] and log $K_{o/w**}$ predicted from ChemSpider [2].

Name	Formula	MW	log K_{ow}*	1st campaign														2nd campaign														Uses or sources
				1	2	3	6	7	8	9	10	11	12	13	14	15	16	1	2	3	4	5	6	7	9	10	11	13	14	15	16	
Food additives, flavours and natural products																																
1-Methoxycyclohexene	$C_7H_{12}O$	112.08839	2.15**	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	Food additive and flavour
1,1,1-Trifluoro-12-heptadecen-2-one	$C_{17}H_{29}F_3O$	306.21762	7.41**		x					x	x			x			x			x	x		x			x		x	x	x	x	Pheromone
10-Hydroxy-2-decenoic acid	$C_{10}H_{18}O_3$	186.12478	1.99**				x	x		x						x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		Queen Bee Acid (10-HAD) bio-active comp.
18-Fluorooleic acid	$C_{18}H_{33}FO_2$	300.2472	6.92**						x											x						x						Natural product (lipid)
3-Phenylpropanal	$C_9H_{10}O$	134.07256	2.04**																			x										Food and fragrance additive
5-Pentylresorcinol (Olivetol)	$C_{11}H_{16}O_2$	180.11439	3.35**	x	x				x	x	x				x			x		x	x	x	x	x	x	x	x	x	x	x	x	Olivetol is found in certain species of lichen
Allyl cyclohexanecaproate	$C_{15}H_{26}O_2$	238.19213	5.71**				x	x	x	x		x		x	x	x		x	x	x		x		x	x		x	x	x	x	x	Food and colour additive
Benzyl formate	$C_8H_8O_2$	136.05217	1.54**																			x										Food additive and flavour, fragrances
Cyclohexanecarboxylic acid	$C_7H_{12}O_2$	128.08339	1.96*	x	x				x	x	x				x	x	x	x	x	x	x	x	x	x	x	x				x	x	Food additive and flavour, fragrances
Dibutyldithiocarbamic acid	$C_9H_{19}NS_2$	205.09477	3.93**		x												x		x	x	x		x		x	x	x	x	x	x	x	Food additive
Docosahexaenoic acid	$C_{22}H_{32}O_2$	328.2383	6.78**																x	x								x	x			Omega-3 fatty acid used in food supplement
Ethyl 2-acetylnonanoate	$C_{13}H_{24}O_3$	228.17281	4.25**								x													x								Flavouring substance
Ethyl 2-oxocyclohexanecarboxylate	$C_9H_{14}O_3$	170.09358	0.82**	x					x			x					x		x				x	x							x	Used as biocatalysis
Ethyl 3-oxohexanoate	$C_8H_{14}O_3$	158.09385	1.78**	x		x	x	x				x	x	x		x	x		x	x	x		x	x	x		x	x	x	x		Food additive and flavour
Ethyl 4-methylenecyclohexanecarboxylate	$C_{10}H_{16}O_2$	168.11436	2.92**	x	x					x																						Food additive
Geranyl butyrate	$C_{14}H_{24}O_2$	224.17659	5.17**	x			x			x		x		x			x	x		x	x	x		x	x	x		x	x	x		Food additive
Lauric acid	$C_{12}H_{24}O_2$	200.17675	4.6*		x			x		x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	Natural product (fatty acid)
Linoleic acid	$C_{18}H_{32}O_2$	140.11938	7.05*	x	x			x	x		x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	Fatty acid
Maraniol	$C_{12}H_{12}O_3$	204.07948	2.90**			x	x			x																						Food flavour and in perfumes
Myristic acid	$C_{14}H_{28}O_2$	228.20769	6.11*		x		x	x		x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	Fatty acid
N-(9-Decen-1-yl)-2,2,2-trifluoroacetamide	$C_{12}H_{20}F_3NO$	251.15053	4.43**	x	x	x		x	x	x	x			x	x	x	x	x		x	x	x	x	x		x	x		x			Natural product (lipid)
Propylparaben	$C_{10}H_{12}O_3$	180.0779	3.04*								x						x						x		x					x	x	Preservative in foods & PPCPs
Raspberry ketone	$C_{10}H_{12}O_2$	164.08315	1.48*						x	x	x				x		x	x		x	x	x			x	x		x	x	x	x	Natural extract of many fruits as raspberries, used in naturopatia
Stearic acid	$C_{18}H_{36}O_2$	284.26972	8.23*														x								x						x	Food additive, soaps, cosmetics, detergents, lubricants, softening
Tridecylic acid	$C_{13}H_{26}O_2$	214.19218	5.56**	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	Fatty acid
Tyrosol	$C_8H_{10}O_2$	138.06763	0.62**																	x	x	x	x	x	x	x	x				x	Natural phenolic antioxidant
Ubiquinone Q4	$C_{29}H_{42}O_4$	454.30607	8.72**																									x				Dietary supplement
Undecanoic acid	$C_{11}H_{22}O_2$	186.16117	4.42*	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	Food additive and flavour; human metabolite
Solvents and Industrial products																																
1-(6-Methoxy-2-naphthyl) ethanone	$C_{13}H_{12}O_2$	200.08306	2.81**																							x						It is a potential impurity in commercial preparations of naproxen
2-Isopropylphenyl hydroperoxide	$C_9H_{12}O_2$	152.0833	3.25**																			x										By-product burned as waste
4-Hydroxybenzamide	$C_7H_7NO_2$	137.04701	0.33*			x																										Used in fragrances and odour agents
Ethyl acetate	$C_4H_8O_2$	88.0522	0.73*	x	x				x	x	x	x	x	x	x		x	x				x	x	x	x						x	Solvent
Mesitol	$C_9H_{12}O$	136.08819	2.73*		x	x					x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	Solvent also used in PPCPs and cleaners.
Phenalene	$C_{13}H_{10}$	166.07757	4.17**								x					x	x															Formed during the combustion of fossil fuels
Pesticides																																
Aldicarb oxime	$C_5H_{11}NOS$	133.0558	1.21**														x			x									x			Carbamate insecticide mainly used as nematicide in the past.
Bifenazate	$C_{17}H_{20}N_2O_3$	300.14758	3.4*																x									x				Insecticide, acaricide. Non-classified
Chlorpyrifos	$C_9H_{11}Cl_3NO_3PS$	348.92573	4.96*					x																x					x			Organophosphate insecticide, acaricide and miticide
EPTC / Torbin	$C_9H_{19}NOS$	189.11809	3.21*		x												x						x				x		x			Thiocarbamate herbicide
2-Hexylcyclopentanone	$C_{11}H_{20}O$	168.15072	3.35**	x	x	x		x	x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	Used in fragrances and also used as insecticide.
Prohexadione	$C_{10}H_{12}O_5$	212.06779	-0.55**			x				x	x	x						x		x	x	x			x	x		x	x			Plant growth regulator
Phoxim	$C_{12}H_{15}N_2O_3PS$	298.05266	4.39*			x		x		x	x	x	x		x	x			x	x		x	x	x	x			x		x	x	Organophosphate insecticide
Pharmaceuticals																																
Azelaic acid	$C_9H_{16}O_4$	188.10406	1.57*		x			x											x	x		x			x		x	x	x	x		Used in dermatological treatments.
Benzenesulfonamide	$C_6H_7NO_2S$	157.01922	0.31*	x	x			x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	Anti-asthmatic, bronchodilators, expectorants, antitussive agents.
N-Butylbenzenesulfonamide	$C_{10}H_{15}NO_2S$	213.08132	2.1**						x		x	x						x	x	x	x	x	x	x	x	x	x	x	x	x	x	Anti-asthmatic, urinary tract, bladder, problems in kidneys, and in drugs for genital or sexual disorders. It has antifungal properties.

Chlorzoxazone	$C_7 H_4 Cl N O_2$	168.99245	1.6*																						x							Muscle relaxer and pain release.
Ditiocarb	$C_5 H_{11} N S_2$	149.03308	1.81**	x	x							x					x	x		x		x			x		x			x	x	antiviral agents, and main metabolite of DISULFIRAM.
17α-Estradiol	$C_{18} H_{24} O_2$	272.17962	4.13**		x										x			x		x												It is an endogenous steroidal estrogen
Ethacrynic Acid	$C_{13} H_{12} Cl_2 O_4$	302.0112	3.3*	x	x		x			x					x		x	x							x	x	x					Used to treat edema
Fenbufen	$C_{16} H_{14} O_3$	254.09312	3.2*	x			x																						x			Nonsteroidal anti-inflammatory drug.
Hexestrol	$C_{18} H_{22} O_2$	270.16068	4.98**		x				x	x		x		x					x	x	x		x			x			x			It is a synthetic estrogen used as a hormonal antineoplastic agent.
Mesterolone	$C_{20} H_{32} O_2$	304.23882	4.24**	x			x			x	x						x	x	x	x				x	x		x				x	Androgen and anabolic steroid (AAS) medication
9-Phenanthrol	$C_{14} H_{10} O$	194.07195	3.94**					x	x	x																						It is used to modulate vascular and smooth muscle contractility.
Salicylamide	$C_7 H_7 N O_2$	137.04717	1.28*	x	x				x	x	x				x			x				x	x	x	x						x	analgesic and antipyretic properties like aspirin
Sulfaphenazole	$C_{15} H_{14} N_4 O_2 S$	314.08394	1.52*	x	x		x			x	x				x		x	x		x	x	x			x	x	x					It is a sulfonamide antibacterial
Others																																
1-Methylamino-1-(3,4-Methylenedioxyphenyl)Propane	$C_{11} H_{15} N O_2$	193.10961	2.26**				x		x	x		x	x	x		x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	Drug isomer of ecstasy

Supplementary material references:

1. PubChem - U.S. National Library of Medicine - National Center for Biotechnology Information. Available from: https://pubchem.ncbi.nlm.nih.gov/.

2. ChemSpider - Search and share chemistry. Available from: http://www.chemspider.com/.

4.2.2 Publication n°2

Screening and quantification of micro(nano)plastics and plastic additives in the seawater of Mar Menor lagoon

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Screening and Quantification of Micro(Nano)Plastics and Plastic Additives in the Seawater of Mar Menor Lagoon

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In this work a suspect-screening approach was employed to assess the polymers and plastic additives of micro(nano)plastics (NPL/MPLs) of size ranges from the nm range to 20 μm present in seawater from the top 5 cm of the Mar Menor lagoon during two sampling campaigns (summer and winter), as well of other potentially adsorbed compounds onto the plastic particles surfaces and suspended material. The identification of NPL/MPLs has been based on characteristic Kendrick Mass Defect analysis for each polymer type in mass spectra. The applied methodology allowed to identify NPLs/MPLs of polystyrene (PS), polyethylene (PE), polyisoprene (PI), polybutadiene (PBD), polypropylene (PP), polyamides (PA), polyvinylchloride (PVC), n-isopropylacrylamide (PNIPAm), and polydimethylsiloxanes. In addition, PS, PE, PI, PBD, PP, PA, and PVC were confirmed with standards, and the equivalent concentrations were quantified. The results of this study showed that most frequently found compounds were PP, PE, PA and PNIPAm, while the compound found at higher concentrations was by far PP reaching the 9,303 ± 366 ng/mL in one of the samples. A total number of 135 chemical compounds were tentatively identified, 74 of them plastic additives and compounds used in the polymers manufacture or coming from the polymer's decomposition. In relation to plastic additives, the more frequently tentatively identified compounds were plasticizers such as phthalates group; stabilizers such as antioxidants (e.g., distearyl 3,3′-thiodipropionate, 2,5-di-tert-butylhydroquinone), and UV filters as benzotriazoles. Several flame retardants of the group of phosphates were as well detected. The other compounds tentatively identified in the samples were pharmaceuticals, pesticides, food additives, flavors and natural products that were attached onto the plastic particles and particulate matter from surrounding waters. In regards to the seasonal variation, during the summer a major number of compounds were tentatively detected, while de concentrations of polymers were slightly higher in winter. The spatial distribution showed higher contamination in the southern part of the coastal lagoon.

Keywords: microplastics, nanoplastics, LC-HRMS, suspected screening, additives

INTRODUCTION

Coastal areas and oceans are essential for the provision of food, green energy, economic growth, and coastal communities' well-being. According to the EU blue economy Report 2020, in 2018 the economic sectors related to oceans and the coastal environment provided a turnover of €750 billion in the EU. However, at the same time, population growth in coastline areas increases the vulnerability of marine ecosystems. Of all marine litter and pollution in general, 80% originates from land-based sources that are primarily industrial, agricultural, and urban. Among them, plastic wastes constitute 83% of the total marine litter, and polymers that are the most widely identified are polypropylene (PP), polyethylene (PE), polystyrene (PS), polyethylene terephthalate (PET), and polyvinylchloride (PVC) (Andrady, 2011). Plastic wastes are accumulated in oceanic gyres, coastal areas, estuarine waters, semi-closed areas and marine lagoons (Llorca et al., 2020b).

Coastal pollution is composed of complex mixtures of legacy and emerging contaminants. Among the emerging groups of pollutants, microplastics (MPLs) are defined as plastic pieces smaller than 5 mm, including plastic particles at the nano range, called nanoplastics (NPLs). MPLs can have their origin in cosmetics, cleansing products, cloth fibers, tire wear (Fendall and Sewell, 2009) (classified as primary MPLs), and in the environmental fragmentation and erosion of plastic pieces and debris (Cole et al., 2011) (classified as secondary MPLs). Primary and secondary MPLs have become a global concern due to their persistency and widespread occurrence in water and sediments, and also as a consequence of their potential impact on the environment and living organisms. MPLs have been found in all environmental compartments (Shahul Hamid et al., 2018), in particular in marine water (Cózar et al., 2015; Suaria et al., 2016; Cincinelli et al., 2019; Llorca et al., 2021), sediments (Van Cauwenberghe et al., 2013; Vianello et al., 2013; Waller et al., 2017), and biota (Cole et al., 2013). The presence of MPLs in marine organisms has been reported during the last decades. Filter feeders such as mussels can consume small plastic particles (Van Cauwenberghe and Janssen, 2014; Dehaut et al., 2016) and it is hypothesized that the buoyancy, size (similar to plankton), and colors can be factors that attract their ingestion by fish (Wright et al., 2013; Pittura et al., 2018), including species at are used for human consumption (Hantoro et al., 2019; Neto et al., 2020; Schirinzi et al., 2020; Castelvetro et al., 2021). In this sense the occurrence of MPLs can be specially relevant in semiconfined areas, due to its limited dilution capacity such as Mar Menor lagoon (SE Spain). In fact, the prevalence of PE in beaches and sediment of this lagoon was confirmed in previous studies (Moreno-González et al., 2015; León et al., 2018; Filgueiras et al., 2021), however, no previous information is available about their occurrence in the water column.

Moreover, plastic polymers can adsorb and accumulate other contaminants from surrounding waters, facilitating their transfer to biota (Koelmans et al., 2016; León et al., 2018, 2019; Llorca et al., 2020a). On the other hand, plastics are composed of polymers and plastic additives that are not chemically bonded with the polymeric chains and for this reason they can easily leach and be transferred to marine biota (Koelmans et al., 2014; Schmidt et al., 2021; Zitouni et al., 2021).

Due to the higher active surface area of the smallest particles, these effects of accumulation and additive leaching can be hypothesized to be inversely proportional to the size of the particles. As mentioned above, a great effort has been made to assess pollution by MPLs in different compartments of coastal and marine environments. However, due to the limited number of analytical approaches that are able to assess the lowest range of particles, there is an important gap of information concerning the occurrence of MPLs with particles sizes inferior to 20 μm and also NPLs. The main analytical techniques that can be used to assess the low range of plastic particles in complex environmental samples are μ-Fourier-transform infrared spectroscopy (μ-FT-IR), μ-RAMAN, pyrolysis-gas chromatography–mass spectrometry (Pyr-GC-MS), and liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS). μ-FT-IR informs about shape and polymeric composition of particles down to the limit of 20 μm, but it is not suitable for NPLs (Klein et al., 2018). Similarly, μ-RAMAN informs about shape and composition but can be within the nanometer range (Cai et al., 2021), and even 100 nm using RAMAN imaging (Sobhani et al., 2020). Another powerful technique is Pyr-GC–MS, which is not affected by the size of the plastic particles, and recently has been used for quantitative analysis (Fischer and Scholz-Böttcher, 2017; David et al., 2018; La Nasa et al., 2020). LC-HRMS has also been successfully used for the quantitative analysis of NPLs and MPLs (Schirinzi et al., 2019; Llorca et al., 2021), but the main limitation of this approach is the need to solubilize plastic particles in solvents that can be used in chromatography, such as toluene, but most of the commonly used polymers (polyethylene, polypropylene, polystyrene, polybutadiene, etc.) are toluene soluble.

In this work, we apply a double suspect screening based on LC-HRMS that was developed in our group (Llorca et al., 2021), in order to assess and quantify the main polymeric composition of NPLs/MPLs and the plastic additives in the Mar Menor lagoon. Therefore, the main goals of this work were: (i) To assess the main polymer composition of MPLs and NPLs ranging from 0.7 to 20 μm and plastic additives in surface waters (top 5 cm) in the Mar Menor Lagoon during two sampling campaigns that were carried out in summer and winter; (ii) to explore significant variations during the two seasons while considering the main anthropogenic factors.

EXPERIMENTAL SECTION

Chemicals and Reagents

Analytical standards for gel permeation chromatography/size exclusion chromatography (GPC/SEC) analyses were polyethylene (PE) and polystyrene (PS) (MW∼1,200 Da) supplied by Polymer Standard Service GmbH (PSS, Mainz, Germany), polybutadiene (PBD), polyisoprene (PI) STD KIT from Waters Cromatografía (Cerdanyola del Vallès, Barcelona, Spain) and polypropylene (PP) (MW∼1,200 Da) supplied by American Polymer Standards Corporation (Ohio, United States), polyvinyl chloride (PVC) was obtained from Sigma-Aldrich (Steinheim, Germany), and polyamides were based on ε-Caprolactam, and the analytical standard (analytical grade) was obtained from Supelco.

Toluene HPLC solvent with >99.7% purity and HPLC-water were acquired from J.T.Baker—VWR (Radnor, PA, United States) and nitrogen used as drying gas with an assessed 99.995% purity was supplied by Air Liquide (Barcelona, Catalonia, Spain). Toluene and methanol CHROMASOLV®Plus from Sigma-Aldrich were used during the instrumental analysis.

Glass microfiber filters of 0.70 μm GF/F were purchased from Whatman (Maidstone, United Kingdom).

Analytical Standards

The quantification of MPLs/NPLs was carried out with polymer analytical standards (MW≈1,000 Da). Quantification was undertaken by means of an external calibration curve. For this purpose, an individual standard solution of each polymer (PE, PS, PBD, PI, PP) was prepared in toluene (10 mg in 10 mL of toluene) and 5 min of ultrasonic assisted extraction (USAE). In the specific case of PA (Nylon), their identification was based on the presence of different polyamides including caprolactam and its cyclic dimer, trimer, and tetramer. The presence of all these polyamides indicates that their origin is from Nylon. Finally, the identification and quantification were based on comparison with the ε-caprolactam analytical standard by means of LC-(+ ES)-HRMS.

The different dilutions in toluene were done ranging from 1 μg/L to 1 mg/L (5 calibration points). The calibration curves were preserved at 4°C before analysis (within 1 week).

Study Area and Sampling Campaigns

The Mar Menor is one the largest hypersaline coastal lagoons in Europe, which is almost isolated from the Mediterranean Sea by La Manga del Mar Menor, which is a sand bar interrupted by a series of channels called golas, which allow a limited water flow between the Mar Menor and the Mediterranean. Consequently, this lagoon shows higher salinity and temperature range than Mediterranean, being particularly relevant seasonal variations. This geographical area is characterized by a dry Mediterranean climate that makes mild winters and hot summers with temperatures up to 40°C. Mar Menor is used for different water sports exploitations, tourism and traditional fishing exploitation. In addition, Mar Menor has other pressures including the clogging of the lagoon due to mining activity and deforestation debris that ends up in its waters, agricultural contamination, wastewater residues, human pressure (coastal cities and villages), and contamination due to fisheries and aquatic sports. It is also relevant the influence of flash flood events in the input of nutrients, pesticides (Moreno-González and León, 2017) and probably also for plastics and MPLs.

Two litter of seawater was taken at each sampling point from the upper water level (top 5 cm). Amber glass bottles, pre-rinsed with methanol and acetone in the laboratory and with seawater in each sampling point, were used for sampling. The bottles were transported under cool conditions to IEO (Murcia, Spain) laboratories and preserved at 4°C before shipment to IDAEA-CSIC (Barcelona, Spain) laboratories to perform the analytical process.

Two sampling campaigns were carried out in Mar Menor (Murcia, SE of Spain) during July 2018 (summer) in the middle of the peak season of vacation and tourism, and March 2019 (winter). Nine sampling points distributed along the whole lagoon in order to cover all of its extension were included in this study (**Figure 1**). The main physicochemical parameters of surface seawater (temperature, pH, conductivity, and dissolved oxygen) were measured *in situ* using a portable multiparametric meter (WTW, model Multi 340i/SET®) during both campaigns are summarized in **Table 1**.

Sample Pre-treatment and Extraction

Initially water samples were sieved though a stainless sieve of 20 μm mesh to separate the larger MPLs. Then, according to Llorca et al. (2020c), 1.5 L of filtrate were passed through a 0.7 μm dry and tared fiber filter. The filter with particulate material was then dried overnight at 60°C until constant weight. The filters were preserved at −20°C before the extraction (within 1 week). The extraction of MPLs/NPLs from the filters was carried out with 10 mL of toluene by means of USAE for 10 min. After this time the 10 mL was collected with a Pasteur pipette and transferred to a 50 mL amber glass vial. This procedure was repeated two more times and then combined with the 10 mL used for the extraction, obtaining 30 mL of toluene. Finally, extracts were evaporated under a nitrogen stream to concentrate the samples near to 1.5 mL. The extracts were then vortexed and transferred to LC-vials and evaporated again to obtain 1.5 mL. This final extract was divided for: (i) MPLs/NPLs polymer analysis and plastic additives in toluene (1 mL) which was preserved at −20°C until chemical analysis; and (ii) plastic additives analysis in methanol (0.5 mL). This last extract was evaporated under a nitrogen stream until dryness and reconstituted in 0.5 mL of methanol. All the samples were analyzed in triplicates.

Analysis by Liquid Chromatography Coupled to High Resolution Mass Spectrometry (LC-HRMS)

Analytical Process of MPLs/NPLs' Polymer Analyses by Size Exclusion Chromatography Coupled to High-Resolution Mass Spectrometry (SEC-HRMS)

The analysis was performed by LC-SEC-HRMS. The chromatographic separation was carried out using an Acquity LC (Waters, Milford, MA, United States) chromatographic system equipped with an advanced polymer chromatography column (Acquity APC XT45 1.7 μm) based on the work of Schirinzi et al. (2019). Twenty microliter were injected per sample with 100% toluene used as the mobile phase, in isocratic conditions and at a flow rate of 0.5 mL/min. The LC system was coupled to a Q-Exactive (Thermo Fisher Scientific, San Jose, CA, United States) hybrid quadrupole-Orbitrap mass spectrometer, equipped with an atmospheric pressure photoionization (APPI) source working in negative and positive conditions. Data acquisition was performed in full scan mode (m/z 500–3,000) with a resolution of 17,500 FWHM

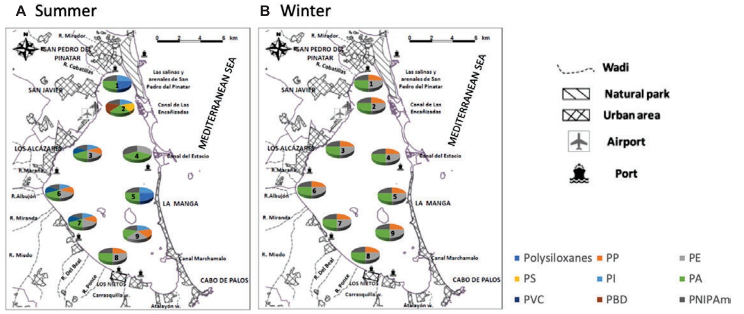

FIGURE 1 | Occurrence of polymer micro(nano)plastics in Mar Menor during the 1st (**A**, Summer) and the 2nd (**B**, Winter) sampling periods of this study.

TABLE 1 | Physicochemical parameters of sampling locations during 1st and 2nd sampling periods.

			Summer period						Winter period					
Code	**Area**	**Coordinates**	**Date**	**Seawater T (°C)**	**Daily T (°C)***	**pH**	**Salinity (ppt)**	**O_2 (mg/L)**	**Date**	**Seawater T (°C)**	**Daily T (°C)***	**pH**	**Salinity (ppt)**	**O_2 (mg/L)**
1	Lo Pagán	37.81214, –0.77692	07/03/2018	26.7	18.6–27.2	8.15	43.6	6.55	02/12/2019	13.7	7.0–16.5	8.18	41.6	7.86
2	Las Encañizadas	37.78137, –0.78288	07/03/2018	28.0	18.6–27.2	8.08	44.7	6.92	02/12/2019	12.6	7.0–16.5	7.99	41.9	8.18
3	Los Alcázares	37.74318, –0.84474	07/03/2018	27.7	18.6–27.2	8.12	45.0	7.37	02/12/2019	12.9	7.0–16.5	7.87	42.2	7.23
4	Canal del Estacio	37.75337, –0.75878	07/03/2018	27.9	18.6–27.2	7.86	43.7	6.85	02/12/2019	13.0	7.0–16.5	7.96	41.9	8.02
5	La Manga	37.72759, –0.75137	07/03/2018	27.9	18.6–27.2	8.02	44.2	6.90	02/12/2019	12.8	7.0–16.5	8.06	41.9	8.30
6	Rambla del Abujón	37.71499, –0.85229	07/04/2018	27.5	20.5–31.5	8.34	45.0	8.00	02/13/2019	12.7	3.0–15.1	7.81	42.2	8.00
7	Los Urrutias	37.7019, –0.83382	07/04/2018	27.6	20.5–31.5	8.17	45.3	7.70	02/13/2019	12.4	3.0–15.1	8.20	42.2	8.23
8	Los Nietos	37.65878, –0.79364	07/04/2018	27.7	20.5–31.5	8.29	45.8	8.30	02/13/2019	13.0	3.0–15.1	7.86	42.2	8.43
9	Isla del Barón	37.68303, –0.76357	07/04/2018	27.6	20.5–31.5	8.04	45.6	7.60	02/13/2019	12.8	3.0–15.1	7.96	42.0	8.04

https://datosclima.es/index.htm (AEMET).

(Llorca et al., 2020c). The optimal parameters of the instrument can be seen elsewhere (Schirinzi et al., 2019).

Analysis of Plastic Additives and Potential Co-contaminants Adsorb to Plastic Particles Surfaces by LC-HRMS

The Acquity LC (Waters, Milford, MA, United States) chromatographic system was equipped with Purospher® STAR RP-18 endcapped column (5 µm, 2 × 125 mm; from Merck), one for the toluene mobile phase and the other for the methanol/water mobile phase. The extracts of toluene were separated by working with toluene under isocratic mode at 0.5 mL/min for 10 min, with 20 µL of sample. The extracts of methanol were separated working under gradient conditions with (A) methanol and (B) water. In this last case, the gradient started with 10% of (A) for 30 s and increased up to 90% (A) in 18 min. This percentage was maintained for 5 min and decreased again until 10% (A) in 1 min, and maintained for 30 s. The total run time was 25 min for the injection of 20 µL of sample. The chromatographic system was coupled to a QExactive (Thermo Fisher Scientific, San Jose, CA), equipped with an APPI source operating in negative and positive ionization modes for toluene extracts, while the ionization of methanolic extracts was done with an electrospray (ESI) operating in negative and positive ionization modes, in separate injections.

The instrumental process was controlled with Xcalibur 3.1 software for all LC-HRMS analysis.

Data Processing

Identification and quantification of polymers was carried out according to the methodology that was previously developed within our research group (Schirinzi et al., 2019; Llorca et al., 2020c). Very briefly, data processing was performed using the Xcalibur Qual Browser software (Thermo Fisher Scientific, San Jose, CA, United States) and Compound Discoverer 3.1 software (Thermo Fisher Scientific, San Jose, CA, United States), respectively. The total ion chromatograms (TIC) that were obtained by full-scan (FS) acquisition were processed in accordance the work of Llorca et al. (2020c). The first screening steps included peak picking and grouping. For each sample, the TIC chromatogram was interrogated every 30 s by intervals of 500 Da and peaks on which mass spectra showed repetitive mass losses were marked as suspected polymers.

Suspect profiles were tentatively identified using repetitive mass losses with a mass error below 3 ppm (identification confidence level 4) and they were subsequently filtered by comparing with a polymers' homemade library (level 3). The tentative identification of polymers (level 2) was carried out by using Kendrick Mass Defect (KMD) analysis. The KMDs were calculated according to equations described elsewhere (Schirinzi et al., 2019) with the base unit being PolymerX/(round PolymerX-2) (Llorca et al., 2020c). Some examples can be seen in **Supplementary Figures 1–3**. Among the polymers that were tentatively identified, the confirmation (identification confidence level 1-confirmation) and the quantification of polymers was achieved by comparison with external curves of polymer standards based on equivalent concentration (Schirinzi et al., 2019).

Tentative Identification of Plastic Additives

Raw data from plastic additives analysis was processed using an automated screening with Compound Discoverer software version 3.1 (Thermo Fisher Scientific) as described elsewhere (Llorca et al., 2020c). This first list of suspected compounds was filtered by comparison with ChemSpider and mzCloud databases as well as the homemade database. Further filtering steps were the comparison of isotopic patterns, ionization efficiency, fragmentation patterns, and finally, the comparison of the product-ions obtained from the MS/MS spectrum of a suspected compound with the spectrum of a standard or a predicted fragmentation pattern using the information contained in the online databases. Unequivocal confirmation was only possible when a reference standard was available (identification at level 1) (Llorca et al., 2020c).

Quality Assurance and Quality Control

1.5 L of water for LC analysis was used as a blank and this was carried out during both sampling campaigns and extracted and analyzed together with the rest of the samples. On the other hand, the possible cross contamination coming from the instrumental analysis was monitored by injecting solvent blanks of toluene randomly with each injection samples list in the polymeric analysis or plastic additives in toluene as well as methanol for the methanolic fraction analysis.

Quality parameters including method limits of detection and quantification (MLOD and MLOQ, respectively), precision and recoveries were calculated by means of fortified seawater, previously filtrated by 0.2 µm fibber filters, with polymers of c.a. 1,000 Da of PP, PS, PE, PBD, PI, and PVC at three spiking levels (100, 500, and 1,000 ng/ mL) according to Schirinzi et al. (2019). The values for MLOD were ranging from 2 to 11 pg/mL and MLOQ from 7 to 33 pg/mL. Intraday precision was calculated with 1,000 ng/mL spiked samples and was below 25% for all the polymers. Finally, recoveries ranged from 39% (PI) to 75% (PS).

Multivariate Analysis

Multivariate analyses using Principal Component Analysis (PCA) was carried out in order to further understand the variation among samples and the contribution of the several tentatively identified chemical compounds (Sanchís et al., 2020). PCA was performed by means of XLSTAT Excel data analysis add-on (Addinsoft, 2020). Correlation among variables and principal components (F) was quantified as the square cosine of their angle in the loading graph, which may range from 0 ($\alpha = 90°$: Orthogonal, totally uncorrelated) to 1 ($\alpha = 0°$: Parallel, perfectly correlated) (Sanchís et al., 2020).

RESULTS AND DISCUSSION

Occurrence of MPLs/NPLs in the Seawater of the Mar Menor Lagoon

Two sampling campaigns were performed in Mar Menor lagoon during the summer and winter to identify seasonal differences. PE, PP, PA, and n-isopropylacrylamide (PNIPAm) were the more frequently found compounds in surface seawater, with PP being the polymer that was detected at higher concentrations, as shown in **Supplementary Table 2** and **Figure 2**. The prevalence of PP instead of the most commonly used PE in surface seawaters can be consequence of its lower density which can favor their floatability and limit its deposition in sediments. Concerning PNIPAm and polydiemthylsiloxanes, they were only identified at confidence level 2, while the other polymers were confirmed by standards and the equivalent concentrations that were quantified. Polydiemethylsiloxanes were identified at level 2 based on their common repeating unit of $(C_2H_6OSi)_n$ (74.15394 uma), which was confirmed by Kendrick Mass Defect analysis. Regarding PA, which is commonly used in fishing nets, four different polymers were found: caprolactam, and the caprolactam cyclic dimer, trimer and tetramer, with the caprolactam being the polymer at the highest concentrations.

The main seasonal differences were observed in terms of polymer variety and concentrations. During the summer campaign, a wider variety of polymers was detected. Nine different groups were identified (PE, PI, PP, PS, PBS, PA, PVC, PNIPAm, and polysiloxanes), while only four of them (PE, PP, PA, and PNIPAm) were detected in winter. However, these four compounds were present in all of the samples that were collected in winter, showing more homogeneity along the lagoon. The total polymers were corresponding to MPLs/NPLs < 20 µm as quantified in the top 5 cm during the summer and were ranging between 0.13 and 9,303 ng/mL, while in the winter campaign the concentrations were between 4,918 and 8,744 ng/mL. Therefore, the maximum concentrations were similar, but in some sampling sites, higher concentrations were detected in winter than in summer. Nonetheless, it should be pointed out that these results are corresponding to punctual samplings, are not integrated in a period of time. Therefore, they are influenced by the hydrological conditions and punctual discharges.

At sample sites 1 (Lo Pagan) and 5 (La Manga) PI, PA, PNIPAm, and polysiloxanes were detected in summer, while PP, PE, PA, and PNIPAm were found in winter, but due to the concentrations of PP in the range of µg/mL the total concentration in both areas was higher in winter samples than in summer ones. In sampling site N°3, Los Alcazares, PP, PA, PE, and PNIPAm were found in both seasons but only polysiloxanes and PVC were present during the summer.

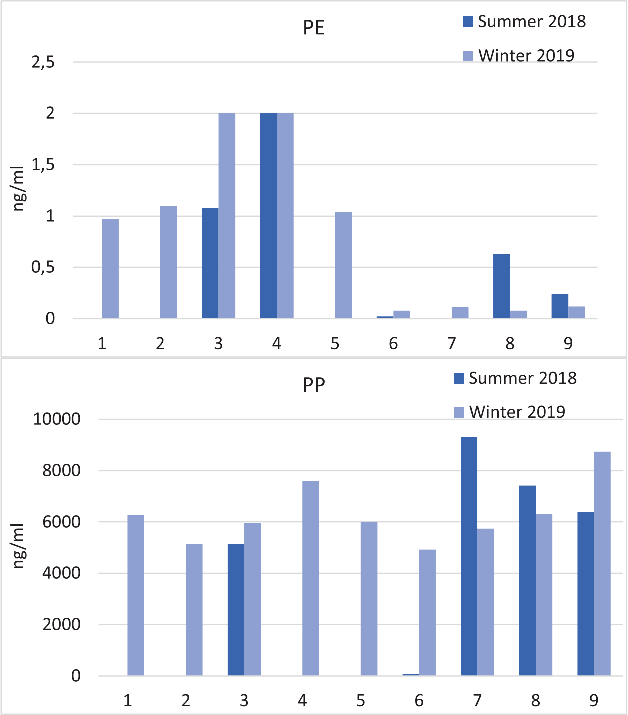

FIGURE 2 | Concentration of PE (top graph) and PP (down graph) in samples during summer period (green color) and winter period (red color) of this study.

As can be seen in these samples, the peak population in summer can influence the occurrence of some polymers through higher residues of personal care products, as is the case of polysiloxanes used in PCPs, water-repellent coatings (i.e., paints) and for inhibiting fouling on surfaces in contact with water, in cookware, electronics, among other uses (Llorca et al., 2020c), and it was identified in 77.8% of the samples of the first sampling campaign that was carried out in the summer. Furthermore, a similar situation was described for polysiloxanes in the Ebro River Delta on the east coast of Spain (Western Mediterranean Region) (Llorca et al., 2020c). In contrast, the continuous runoff from mulching, low tunnel or greenhouse films used in agricultural lands (mainly composed by PE; Loisi et al., 2017), fishing activities (harbor ropes, fishing nets made of PP and PE), and PA from fishing nets and wastewater textile fibers can influence the concentrations of these polymers throughout the year.

The central area of this lagoon (sampling points 3, 6, and 7), presented a wide variety of polymers in summer and is the most contaminated site of the northern part of the lagoon, probably as consequence of the influence of urban nuclei and El Albujón watercourse which covers the drainage of 83.4% of water-courses from land-use runoff and human activities including urbanization, tourism and fishing (Bayo et al., 2020).

The southern part of the lagoon presented the most contaminated sites and a large number of polymers in summer, in particular sites 7, 8, and 9 corresponding to Los Urritias, Los Nietos, and Isla del Baron, respectively. These results agree with previous works carried out in the same area in which the occurrence of larger-sized MPLs (León et al., 2018, 2019; de Haan et al., 2019; Bayo et al., 2020) was explored. For example, a previous study carried out by Bayo et al. (2020) investigating the presence of MPLs in the sand of natural and urban beaches of the lagoon, Los Urrutias and Los Narejos (near to the sampling point 3, Los Alcázares, in the present study) were the more contaminated sites, which is in agreement with our findings (see **Figure 2** and **Table 2**). PE and PP were also identified in the sand of natural and urban beaches (Bayo et al., 2019, 2020), or in waters from the same lagoon. In these works, the presence of the same polymers was confirmed showing PE (up to 37%) and PP (up to

TABLE 2 | Concentration of MPLs/NPLs polymers from suspended material samples expressed in equivalent concentration in µg/L (± SD).

Sample code (place)	Equivalent concentration (µg/L) ± SD							Detected at confidence level 2	
	$[PE]_{eq}$	$[PS]_{eq}$	$[PI]_{eq}$	$[PBD]_{eq}$	$[PP]_{eq}$	$[PVC]_{eq}$	[PA]*	PNIPAm	Polysiloxanes
1st sampling period (Summer)									
1 (Lo Pagán)	n.d.	n.d.	0.13 ± 0.01	n.d.	n.d.	n.d.	0.0038 ± 0.0005	YES	YES
2 (Las encañizadas)	n.d.	0.19 ± 0.07	n.d.	<LOQ	n.d.	n.d.	0.0038 ± 0.0008	YES	YES
3 (Los Alcázares)	1.08 ± 0.09	n.d.	n.d.	n.d.	5,153 ± 20	2.3 ± 0.1	0.0041 ± 0.0010	YES	YES
4 (Canal del Estacio)	2 ± 1	n.d.	n.d.	n.d.	n.d.	n.d.	0.0043 ± 0.0015	YES	YES
5 (La Manga)	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	0.0047 ± 0.0007	YES	YES
6 (Rambla del Abujón)	< LOQ	n.d.	n.d.	n.d.	61 ± 19	1.8 ± 0.1	0.0046 ± 0.0013	YES	YES
7 (Los Urrutias)	< LOQ	n.d.	n.d.	n.d.	9,303 ± 366	0.7 ± 0.1	0.0044 ± 0.0009	YES	YES
8 (Los Nietos)	0.63 ± 0.03	n.d.	n.d.	n.d.	7,414 ± 1,051	n.d.	0.0043 ± 0.0010	YES	YES
9 (Isla del Barón)	0.24 ± 0.06	n.d.	n.d.	n.d.	6,386 ± 408	n.d.	0.0040 ± 0.0010	YES	YES
2nd sampling period (Winter)									
1 (Lo Pagán)	0.97 ± 0.03	n.d.	n.d.	n.d.	6,276 ± 649	n.d.	0.0041 ± 0.0006	YES	YES
2 (Las encañizadas)	1.10 ± 0.08	n.d.	n.d.	n.d.	5,153 ± 948	n.d.	0.0039 ± 0.0005	YES	YES
3 (Los Alcázares)	2.0 ± 0.1	n.d.	n.d.	n.d.	5,964 ± 729	n.d.	0.0041 ± 0.0014	YES	YES
4 (Canal del Estacio)	2 ± 1	n.d.	n.d.	n.d.	7,591 ± 202	n.d.	0.0041 ± 0.0009	YES	YES
5 (La Manga)	1.04 ± 0.04	n.d.	n.d.	n.d.	6,009 ± 174	n.d.	0.0043 ± 0.0012	YES	YES
6 (Rambla del Abujón)	< LOQ	n.d.	n.d.	n.d.	4,918 ± 923	n.d.	0.0053 ± 0.0018	YES	YES
7 (Los Urrutias)	0.11 ± 0.01	n.d.	n.d.	n.d.	5,734 ± 689	n.d.	0.0040 ± 0.0010	YES	YES
8 (Los Nietos)	< LOQ	n.d.	n.d.	n.d.	6,309 ± 397	n.d.	0.0043 ± 0.0008	YES	YES
9 (Isla del Barón)	0.121 ± 0.02	n.d.	n.d.	n.d.	8,744 ± 433	n.d.	0.0042 ± 0.0016	YES	YES

*****[PA] expressed in concentration of ε-caprolactam, analyzed by LC-(+ESI)-HRMS.*

12.4%) among others (León et al., 2018), or in waters taken in 2016 in La Llana beach (near to sampling point 1 in our study). In another example, the PE was identified in MPLs up to 18%, PP 19.5%, PS 13%, and PA 45.5% (León et al., 2019). In another work carried out in waters from the Cartagena transect (outside Mar Menor lagoon), the proportion of polymer types identified in MPLs, as well as mesoplastics, was 47% of PE, 21% of PP, 5% of PS, 8% of paint chips (pure pigmented particles), among others, in all samples (de Haan et al., 2019).

In general trends, our results agree with the main classes of polymers identified in marine MPLs in other studies in the Mediterranean Sea, in which the most commonly found polymers were PE, PP, PS, PET, PVC, and PA (Gajšt et al., 2016; Munari et al., 2017; de Haan et al., 2019; Llorca et al., 2020b), and also in other regions (Cincinelli et al., 2017; Li et al., 2020; Ramírez-Álvarez et al., 2020). However, PE is the dominant compound in most of these studies, while the polymer found at much higher concentrations in the present work was PP. A possible explanation for this difference is the size of particles studied here (nm-20 µm), and the fact of high-density polyethylene is the dominant PE use in the applications in this area, being the buoyancy of PE particles lower than for PP, and this is shown especially notorious in the top 5 cm of the surface water in this hypersaline lagoon.

The suspect screening approach that was used here allowed identify the polymers that were solubilized in toluene and other related polymers which are inorganic (siloxanes) or others that are not considered to be plastics in the strict sense, but are a hybrid between synthetic plastic polymer and synthetic rubbers (Jubilant Performance Materials, 2020).

The detection of MNPLs in these samples is of special interest due to their small size but also because they can be another source of pollutants through the release of additives and plasticizers (Llorca et al., 2020b). In addition, MNPLs can accumulate organic and inorganic contaminants as well as pathogens from the environment (air, water or particulate matter), being an important vector for transport of those co-contaminants to aquatic organisms (Rios et al., 2007; Ashton et al., 2010; Cole et al., 2011; Llorca et al., 2014; Pittura et al., 2018). Due to their small size, similar to plankton, benthic protozoa and bacteria, MNPLs can enter into marine food web by their ingestion by aquatic organisms (Wright et al., 2013; Cole et al., 2014; Pittura et al., 2018; Llorca et al., 2020b). Therefore, it is necessary to consider the analysis of other integrative matrices (biota and/or sediments) and to evaluate the high spatial and temporary variability of concentrations.

Tentative Identification of Plastic Additives in Mar Menor Lagoon

According to the previously described process, the first screening step consisted of peak-picking, RT alignment and grouping of compounds, isotopes and adducts along with the samples. Signals below 1,000,000 were considered to be background and were discarded. The first list of suspected compounds with an error mass below 2 ppm was then filtered and compared with the ChemSpider and mzCloud databases, and 94,365 substances were included in this filtering. The next filtering steps, by limiting peak area (>750,000,000), generated a list of 11,673 potential compounds. This list was subsequently reduced, while considering the chromatographic peak factor resolution > 0.75. This process selected 3,876 candidates. Then, each compound was evaluated, and the final list included 135 chemicals among all of the samples. Of this list of chemical compounds, 43 were plastic additives and 31 more compounds are used in the production of polymers or are monomers coming from the decomposition of plastics. Other chemical compounds have diverse origins and were probably adsorbed to the outside of these particles and the particulate matter and are discussed in the next section (see **Supplementary Tables 1**, **2**).

The major group of plastic additives detected (**Supplementary Table 1**) were plasticizers corresponding to the 56% of identified additives (level 2), and they were also the most frequently detected in the samples. Among them, seven different phthalates (dibutyl phthalate, diethyl phthalate, dimethyl phthalate, dioctyl phthalate (DNOP), dipropyl phthalate, mono (2-ethylhexyl) phthalate (MEHP), and monobutyl phthalate) were identified. This group was the most frequently detected in the samples in both sampling campaigns in 90 and 100% of the sampled sites in winter and summer, respectively. A recent study also evidenced the relevance of this group of contaminants because the occurrence of phthalates was confirmed in seawater from Mar Menor lagoon for 11–17% of samples, showing maximum concentrations of 560 and 1,270 ng/l in spring and autumn, respectively (Concha-Graña et al., 2021). Other examples of plasticizers detected in the samples were N-butylbenzenesulfonamide, tributyl citrate acetate (ATBC), the polyvinyl acetate, dimethyl sebacate (DMS), and the diethyl sebacate (DES), and these last two are very much used in thermoplastics as polyurethanes, and they are also used as UV stabilizers.

The second group of plastic additives identified were stabilizers that are used as antioxidants, such as distearyl 3, 3'-thiodipropionate, 2, 5-di-tert-butylhydroquinone and IRGANOX 3114, and the UV-filters such as the group of benzotriazoles and malonic acid used as UV filter, antioxidant, and corrosion inhibitor. Another relevant group of plastic additives present in the samples were flame retardants as dibutyl phosphate, diethylethyl phosphonate, triethylphosphonoacetate, and triethylphospate although this last example also has an application as plasticizer. Finally, other additives used, for example, for viscosity control, such as palmitamide were also found in some samples. Some of these plastic additives were previously found in the desorbed fraction to seawater (24 h) from plastic debris sampled in several Mar Menor beaches, being particularly relevant for tributyl acetyl citrate, tris-2chloropropyl phosphate and the UV filters (ethylhexyl salicylate and benzophenone) (León et al., 2018).

Sites 3, 4, 5, 6, and 7 were those showing higher pollution of plastic additives in summer while 4, 5, 6, and 7 were those with the most compounds in winter, probably due to the influence of El Albujón watercourse discharges (groundwater, agriculture water irrigation excess, treated urban wastewaters, etc.). In the case of site 3, Los Alcázares is an urban area with important touristic activity and, as aforementioned, the higher level of contamination during summer can be attributed to the increase in population during the warmer period. Furthermore, it was one of the most contaminated sites with MPLs/NPLs (**Figure 1**). In the case of sampling point 4, Canal del Estacio, the contamination is related to harbor activities such as fishing, aquatic sports, and boat preservation materials. This point did not present a large variety of polymers but rather higher concentrations of PE in both sampling campaigns and PP during the winter.

In the case of monomers or other related compounds that are used during the synthesis of polymers, 2-ethylhexyl acrylate (2-EHA), indene, 10-hydroxy-2-decenoic acid and dibutyl sebacate have been the most detected examples during the first sampling period (summer), while 2-EHA, azelaic acid, indene and tripropylene glycol have been the most ubiquitous during the second sampling period (winter). The high ubiquity of 2-EHA could be explained because this compound and also butyl acrylate are the major base monomers for the preparation of acrylate adhesives, or are used in the copolymerization of vinyl acetate, methyl acrylate, and styrene to modify the properties of the resulting polymers. On the other hand, the presence of azelaic acid could be linked to the decomposition of nylon since this is one of the main monomers that are used during the synthesis of Nylon 6/9, which can be present in woven fabric and some fishing devices, among others. In the case of indene, this compound is used during the production of thermoplastic resins.

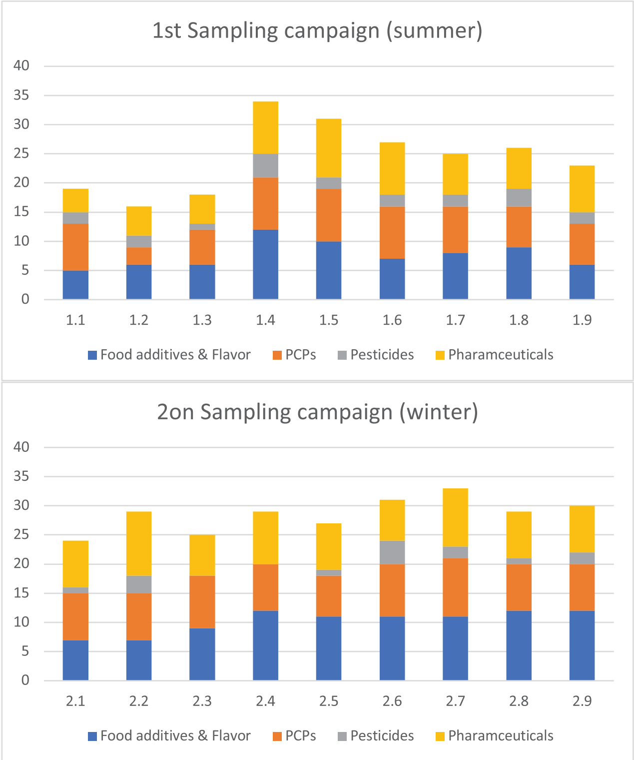

FIGURE 3 | Number of compounds tentatively identified in both sampling periods of this study, distributed in families.

Other Compounds

Other compounds that were detected in this fraction of the samples are those that are potentially adsorbed onto plastic particles and the natural suspended material. These compounds were identified at confidence level 2 and most of them are medium to low polarity compounds, which tend to be accumulated from the surrounding waters onto the surfaces of buoyant plastic particles. The main groups of identified compounds were pharmaceuticals, PCPs and pesticides as can be seen in **Supplementary Table 2** of the Supporting Information section. These results agree with previously reported studies of the same area (Moreno-González et al., 2014, 2015; Jiménez-Martínez et al., 2016; León et al., 2017), in which the same groups and contaminants were found. Moreover, León et al. (2018) confirmed the presence of some of the same group of contaminants adsorbed onto plastic debris sampled in beaches from the Mar Menor lagoon (León et al., 2018) as a consequence of their accumulation from air (Carratalá et al., 2017), freshwater, soil, etc. In **Figure 3**, the main results of the tentatively identified contaminants, grouped by families, are presented. As can be seen, a similar number of compounds were identified during both sampling campaigns, but only slightly higher in winter, probably

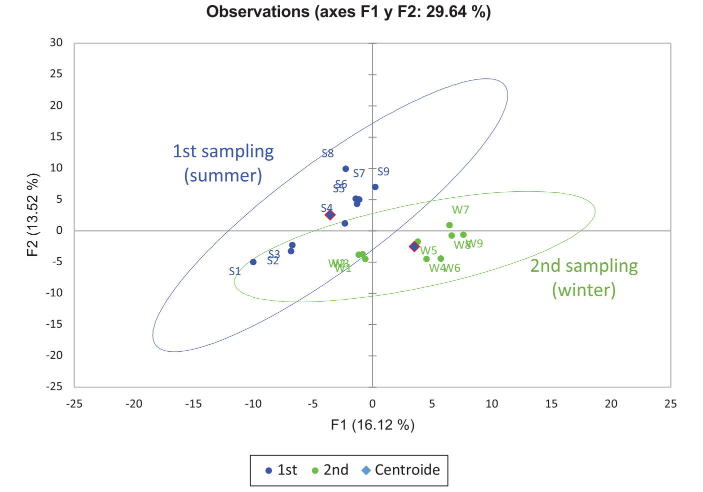

FIGURE 4 | Score plot of PCA with scaled data, representing samples in F1 and F2 axes. Samples corresponding to 1st period (summer) of the study are presented in blue while the samples corresponding to the 2nd campaign (winter) are in green. The two ellipses were generated automatically thus highlighting an area with a 98% probability of containing samples of the same type.

due to the slower sun degradation of some compounds during the winter season.

The main groups of compounds were those that were included in PCPs such as ethylhexyl ethylhexanoate which is used as a UV filter in sunscreens, as lauramide that is used in liquid shampoos, bubble bath preparations, dishwashing products and light-duty liquid detergents as a foam stabilizer, and organic acids such as myristic and steric which are very much used and incorporated into formulations such as lubricants, emollients, and emulsifiers. Another group of frequently detected compounds were pharmaceuticals, among which the most detected compounds were 1-hydroxy-4-methoxybenzene which is used in the treatment of solar lentigines and related hyperpigmented lesions resulting from chronic sun exposure; aceclidine which is used for glaucoma disease, and valpromide which is a carboxamide derivative of valproic acid that is used in the treatment of epilepsy and some affective disorders.

Another relevant group of compounds that is found attached to plastic particulates $< 20\ \mu m$ were food additives. Sixteen compounds were identified at level 2, being the flavoring agent 2-methylpropyl-3-o1obutanoate (isobutyl acetoacetate) which is the most frequently found in 100% of the samples in both sampling periods. Other food frequently found additives were pyruvic and hexanoic acids.

With regards to the geographical distribution, a higher number of compounds were found in the southern part of the lagoon (from 4 to 9) in both sampling periods. Sampling points 4 and 5, corresponding to Canal del Estacio and La Manga, respectively, are highly impacted by summer harbor activities and tourism, respectively, and this is reflected in the total chemical contamination in both sampling periods but particularly during summer. Sampling point 5 has been previously confirmed as depositional area subject to the influence el El Albujón watercourse discharges, particularly during flash flood events, showing higher concentrations for some contaminants in sediments than other surrounding points (Moreno-González and León, 2017).

Multivariate Analysis by PCA

To elucidate any trend among sampling sites and/or campaigns, PCA modeling has been performed. The first two principal components, F1 and F2, explain 29.64% of the data variance, thus indicating the high dimensionality and complexity of the data. Focusing on F1 and F2, the former is related to season changes among samples as observations that are effectively clustered in two separated groups (see **Figure 4**) with just 16.12% of total explained variance. F1 is mainly correlated to the variables 1,3-benzenediol, 2,6-Di-tert-butyl-4-methoxyphenol, 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propanoic acid, 4-Tert-butylphenol, dipropyl phthalate and tetrahydrofurfuryl acrylate, all of them being plastic additives; 10-hydroxy-2-decenoic acid, 2-EHA and EDOT are products related to the synthesis of polymers that have been released to the media because of the weathering effects on bigger plastics; vanillin and nonivamide can be used as food additive, flavoring agent, PCPs or pharmaceuticals; 1-benzosuberone and 2-(3H)-furanone are both from pharmaceutical usage; and 2-isobutoxynaphthalene is used as a food additive and flavoring agent. The latter, F2, does not have a clear interpretation but is related to some phthalates (benzyl butyl phthalate, dibutyl phthalate, diethyl phthalate) and tributyl citrate acetate plastic additives; 2-ethylhexanoic acid, azelaic acid, and N-methylpyrrolidone compounds are related to the synthesis of polymers; benzaldehyde is used as PCPs and pesticide; N-isopropylacrylamide and valpromide are from pharmaceutical use.

CONCLUSION

This work reports on the presence of micro(nano)plastics in the top 5 cm of seawater from the Mar Menor lagoon through suspect-screening analysis. The methodology, based on a previously published work (Llorca et al., 2020c), consisted of USAE followed by SEC-(−/+)APPI-HRMS for the analysis of polymers and allowed the characterization of toluene soluble polymers, and LC-APPI-HRMS for the tentative identification of plastic additives and other contaminants that are potentially adsorbed to the surfaces of plastic particles.

The results confirmed the presence of nine polymers including PS, PE, PI, PBD, PP, PA, PVC, PNIPAm, and polysiloxanes. The more frequently detected polymers were PP, PE, PA, and PNIPAm, while the compound found at higher concentrations was PP probably due its lower density than other polymers. The presence of these plastic particles, in particular those in the nano-sized particle range, induces not only an environmental problem itself due to their low degradability but also the potential impact on biota that can bioaccumulate micro(nano)plastics in their organism and their potential transfer to the food webs. In this sense it is necessary to consider that additional studies are required for the determination of MPLs and NPLs using integrative matrices (biota and/or sediments) because water samples analysis only offer a snapshot for these contaminants occurrence with a high spatial and temporary variability of concentrations.

On the other hand, 74 of organic contaminants detected in these samples were plastic additives and compounds which are used in the manufacture of polymers or are coming from the decomposition of polymers, being plasticizers, the wider group of tentatively identified compounds. Finally, other pharmaceuticals, pesticides, food additives and flavors, and natural products were also identified in the samples. We hypothesize that these compounds are coming from the accumulation of surrounding waters their contribution to bioaccumulation as Trojan horse effect should be considered.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

VL, EA, and MF contributed to the conception and design of the study. ML organized the database and performed the statistical analysis. AV-H and KS perform the experimental work. AV-H wrote the first draft of the manuscript. ML and MF wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.697424/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

4.2.2 Publication n°2 (Supplementary material)

Screening and quantification of micro(nano)plastics and plastic additives in the seawater of Mar Menor lagoon

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Tables S1, S2
Figures S1, S2, S3

Table S1 Plastic additives identified at confidence level 2.

			1st sampling period (Summer)									2nd sampling period (Winter)									
Compound	**Formulae**	**Exact mass**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Other specifications**
										Plastic additives											
1,2,3-Benzotriazole	$C_6H_5N_3$	119.04835	X		X		X	X	X	X	X	X	X	X	X		X	X	X	X	UV filter stabiliser
1-Hydroxybenzotriazole	$C_6H_5N_3O$	135.04326	X	X	X	X		X	X		X		X	X	X	X		X			
Tolytriazole	$C_7H_7N_3$	133.06422											X							X	
2,2,6,6-Tetramethyl-4-piperidyl methacrylate	$C_{13}H_{23}NO_2$	225.17218						X										X			
1-Ethinylcyclohexanol	$C_8H_{12}O$	124.08852					X			X											Plasticisers
Benzyl butyl phthalate	$C_{19}H_{20}O_4$	312.13512	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.27701				X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Dibutyl phthalate	$C_{16}H_{22}O_4$	278.15121	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Diethyl phthalate	$C_{12}H_{14}O_4$	222.08842	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Dimethyl phthalate	$C_{10}H_{10}O_4$	194.05791											X				X			X	
Dioctyl phthalate (DNOP)	$C_{24}H_{38}O_4$	390.27701				X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Dipropyl phthalate	$C_{14}H_{18}O_4$	250.12010										X	X		X		X	X	X	X	
Mono(2-ethylhexyl) phthalate (MEHP)	$C_{16}H_{22}O_4$	278.15146		X		X															
Monobutyl phthalate	$C_{12}H_{14}O_4$	222.08899		X		X				X											
Butyl glycolate	$C_6H_{12}O_3$	132.07872		X								X			X		X	X	X		
Dibutyl adipate	$C_{14}H_{26}O_4$	258.18232		X	X	X	X								X		X				
Heptyl nonyl adipate	$C_{22}H_{42}O_4$	370.30760	X												X				X		
Diethyl sebacate	$C_{14}H_{26}O_4$	258.18294			X			X													
Dimethyl sebacate	$C_{12}H_{22}O_4$	230.15139														X				X	
Dibutyl sebacate	$C_{18}H_{34}O_4$	314.24539	X	X	X	X	X							X		X	X				
Isopropyl myristate	$C_{17}H_{34}O_2$	270.25560			X																
N-butylbenzenesulfonamide	$C_{10}H_{15}NO_2S$	213.08235		X	X	X	X	X	X		X	X		X	X	X	X	X		X	
Palmitamide	$C_{16}H_{33}NO$	255.25547	X		X	X	X		X	X	X	X	X	X	X	X		X	X	X	
Tetraethylene glycol	$C_8H_{18}O_5$	194.11542			X			X	X			X			X	X	X	X	X	X	

Tributyl citrate acetate	$C_{20}H_{34}O_8$	402.22413	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
2-Butoxyethyl-9-octadecenoate	$C_{24}H_{46}O_3$	382.34425	X																		
Triethylphospate	$C_6H_{15}O_4P$	182.07104														X					
1,3-Benzenediol	$C_6H_6O_2$	110.03695				X	X	X			X				X	X	X	X	X	X	Antioxidant
2,6-Di-tert-butyl-4-methoxyphenol	$C_{15}H_{24}O_2$	236.17729											X		X	X	X	X	X	X	
Dehydroabietic acid	$C_{20}H_{28}O_2$	300.20851	X																		
Distearyl 3,3'-thiodipropionate	$C_{21}H_{42}O_2S$	358.29055	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	
IRGANOX 3114	$C_{15}H_{22}O_2$	234.16198	X							X											
3-(3,5-di-tert-butyl-4-hydroxyphenyl)propanoic acid	$C_{17}H_{26}O_3$	278.18789												X	X	X		X	X	X	
4-Tert-butylphenol	$C_{10}H_{14}O$	150.10447															X	X	X	X	Coating sealant and Surface treatments
N, N-Bis(2-hydroxyethyl) dodecanamide	$C_{16}H_{33}NO_3$	287.24577						X	X												
Malonic acid	$C_3H_4O_4$	104.01128	X	X			X		X		X	X	X	X	X	X	X	X		X	
Aniline	$C_{12}H_{11}N_3$	197.09566	X	X																	Curing agent
Benzoic acid	$C_7H_6O_2$	122.03686					X	X	X			X			X	X		X	X	X	Retarder cross-links
Pentadecanoic acid	$C_{15}H_{30}O_2$	242.22458			X					X	X	X	X								lubricant
Dibutyl phosphate	$C_8H_{19}O_4P$	210.10205		X					X												Flame retardant
Diethyl ethyl phosphonate	$C_6H_{15}O_3P$	166.07634	X						X		X										
Triethylphosphonoacetate	$C_8H_{17}O_5P$	224.08085															X				
Tetrahydrofurfuryl acrylate	$C_8H_{12}O_3$	156.07873				X						X			X	X	X	X	X	X	Colourant
Intermediates in polymers synthesis or monomers (some of them also used for other uses).																					
Decanoic acid (Capric)	$C_{10}H_{20}O_2$	172.14633	X			X	X	X	X	X	X	X									Intermediate in the manufacture of perfumes, lubricants, greases, rubber, dyes, plastics, food additives and pharmaceuticals
Glyoxylic acid	$C_2H_2O_3$	74.00039			X		X		X		X		X	X	X						Intermediate for flavourings, perfumes, pharmaceuticals, dyes, plastics, agricultural chemicals; cleaning agent for a variety of industrial applications, in chemical and biodegradable copolymer feedstock
Nonanoic acid	$C_9H_{18}0_2$	158.13068	X	X	X	X	X		X	X		X	X			X		X	X		Used in the preparation of plasticisers and lacquers. Its ammonium salt is a herbicide, used in conjunction with glyphosate.
Triclosan	$C_{12}H_7Cl_3O_2$	287.95093	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	Antiseptic, preservative and disinfectant used in healthcare and other consumer products (cosmetics, household cleaning products, plastic materials, toys and paints).
δ-Decalactone	$C_{10}H_{18}O_2$	170.13068		X	X	X		X	X	X	X										It naturally occurs in fruit and milk products and has applications in food, polymer, and agricultural industries.
Dibutyl sebacate	$C_{18}H_{34}O_4$	314.24539	X	X	X	X	X							X		X	X				Plasticiser in production of cellulose acetate butyrate, cellulose acetate propionate, ethyl cellulose, polyvinyl butyral, polyvinyl chloride, polystyrene, and many synthetic rubbers and other plastics.
Oxalic acid	$C_2H_2O_4$	89.99543	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	Polyester monomers
10-Hydroxy-2-decenoic acid	$C_{10}H_{18}O_3$	186.12559	X	X	X	X		X	X	X	X										Reactant used in the synthesis of copolymers of acrylamide and sodium acryloyloxydecanoate.
Malonic acid	$C_3H_4O_4$	104.01128	X	X			X		X		X	X	X	X	X	X	X	X		X	Precursor to polyesters and coating agent
12-Hydroxy-9-octadecenoic acid	$C_{18}H_{34}O_3$	298.25030				X		X									X				Synthesis of sebacic acid which is used in the synthesis of nylons.
2-Butoxyethanol acetate	$C_8H_{16}O_3$	160.11009		X											X			X			Used in the manufacture of polyvinyl acetate latex.
2-Ethoxyethyl acrylate (2-EHA)	$C_7H_{12}O_3$	144.07869					X			X	X		X				X	X	X	X	Base monomer for the preparation of acrylate adhesives; and copolymer for monomers of vinyl acetate, methyl acrylate, and styrene to modify polymer properties.
2-Ethylhexanoic acid	$C_8H_{16}O_2$	144.11508	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Production of polyvinylbutyral plasticizers and synthetic lubricants, polyolesters, and polyvinylchloride stabilisers. In cosmetics, 2-EHA is used to produce emollients.
3,3,4,4-Tetrafluorohexane	$C_6H_{10}F_4$	158.0.7135															X				Emulsion polymerisation process for producing fluoroelastomers.
3,4-Ethylenedioxythiophene (EDOT)	$C_6H_6O_2S$	142.00881				X		X	X			X	X	X	X	X	X	X	X	X	Precursor of the polymer PEDOT

3,5-Di-tert-butylbenzaldehyde	$C_{15}H_{22}O$	218.16611																		X	Used in the synthesis of some polymers
Azelaic acid	$C_9H_{16}O_4$	188.10496	X	X	X	X						X	X	X	X		X	X	X	X	Synthesis of Nylon 6,9 with hexamethylenediamine
Benzene	C_6H_6	78.04682								X											Component of gasoline, and used in the production of ethylbenzene, a precursor to styrene.
Benzyl vinyl ether	$C_9H_{10}O$	134.07277																X			Monomer in the polymerisation of organic polymers polyvinyl ethers.
Butyl acrylate	$C_7H_{12}O_2$	128.08342					X	X													Used in paints, sealants, coatings, adhesives, fuel, textiles, plastics, and caulk.
Cyclohexanone	$C_6H_{10}O$	98.07302								X											Precursor of nylon among other uses.
Dimethylsilanediol	$C_2H_8O_2Si$	92.02936			X																Silicone
Indene	C_9H_8	116.06231	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	Used in the production of indene/coumarone thermoplastics.
N,N'-Bis(2-hydroxyethyl)terephthalamide	$C_{12}H_{16}N_2O_4$	252.11177				X						X	X				X				Intermediate in the production of poly(ethylene terephthalate)
N-Methylpyrrolidone	C_5H_9NO	99.06810				X	X	X	X	X								X			Used as a solvent for engineering polymers and coating resins.
Phenol	C_6H_6O	94.04203						X			X				X	X	X	X	X		The major uses of phenol involve its conversion to precursors for plastics.
Sebacic acid	$C_{10}H_{18}O_4$	202.12048				X											X			X	Used in industry to synthesise the monomer for nylon 610, plasticisers, lubricants, hydraulic fluids, cosmetics, candles, etc.
Suberic acid	$C_8H_{14}O_4$	174.08912		X								X			X			X	X	X	Used in drug syntheses and plastics manufacture.
Tripropylene glycol	$C_9H_{20}O_4$	192.13616	X		X					X	X	X	X		X	X	X	X	X	X	Raw material for the production of polymers such as polyesters and alkyd resins, as a plasticiser in polymers such as PVC.
Phthalic acid	$C_8H_6O_4$	166.02611				X	X	X	X	X	X				X	X		X	X		Synthesis
Phthalic anhydride	$C_8H_4O_3$	148.01557	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Synthesis

Table S2 Chemical compounds adsorbed onto plastic surfaces tentatively identified at confidence level 2.

PCPs																				
1,2,3-Benzenetriol	$C_6H_6O_3$	126.03034											X							
2,2'-(Dodecylimino)diethanol	$C_{16}H_{35}NO_2$	273.26604	X			X	X	X			X	X	X	X	X	X	X	X	X	
2-[2-(Dodecyloxy)ethoxy]ethanol	$C_{16}H_{34}O_3$	274.25009	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2-Hydroxyethil laurate	$C_{14}H_{28}O_3$	244.20342										X		X	X			X	X	
4-Hexadecylmorpholine	$C_{20}H_{41}NO$	311.31792	X																	
Ethylhexyl ethylhexanoate	$C_{16}H_{32}O_2$	256.24023	X	X	X	X	X	X	X	X	X	X	X	X			X	X		X
Lauramide	$C_{12}H_{25}NO$	199.19302	X		X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Lauric acid	$C_{12}H_{24}O_2$	200.17769	X								X									
Myristamide	$C_{14}H_{29}NO$	227.22448														X				
N,N-Bis(2-hydroxyethyl)-9,12-octadecadienamide	$C_{22}H_{41}NO_3$	367.30759						X	X											
N,N-Bis(2-hydroxyethyl)dodecanamide	$C_{16}H_{33}NO_3$	287.24577						X	X											
Tridecylic acid	$C_{13}H_{26}O_2$	214.19328					X													
PCPs and other uses																				
4-Tert-butylcyclohexyl acetate	$C_{12}H_{22}O_2$	198.16188															X			X
4-Dodecylbenzenesulfonic acid	$C_{18}H_{30}O_3S_2$	326.19166			X															
Benzaldehyde	C_7H_6O	106.04166				X	X	X	X	X	X							X		
Lauryldimethylamine oxide	$C_{14}H_{31}NO$	229.23999															X			
Myristic acid	$C_{14}H_{28}O_2$	228.20893			X	X	X	X	X	X	X	X	X	X	X		X	X	X	X
Stearic acid	$C_{18}H_{36}O_2$	284.27153	X	X	X	X	X	X	X			X		X	X	X	X	X	X	X
Toluidine	C_7H_9N	107.07328								X										
Undecanoic acid	$C_{11}H_{22}O_2$	186.16191	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X
Vanillin	$C_8H_8O_3$	152.0474				X						X	X	X	X	X	X	X	X	X
Pesticides																				
1-Hydroxy-2-oxo-1-phenylhydrazine	$C_6H_6N_2O_2$	138.04309											X							
1-Nonanoic acid	$C_9H_{18}O_2$	158.13068															X			
Benzenesulfonamide	$C_6H_7NO_2S$	157.01975				X											X			

Bifenazate	$C_{17}H_{20}N_2O_3$	300.14739	X	X		X		X		X	X		X							
Difenopenten-ethyl	$C_{20}H_{19}F_3O_4$	380.12197																		X
Pharmaceuticals																				
(4-Isobutylphenyl)acetic acid (also known as ibufenac)	$C_{12}H_{16}O_2$	192.11463																X		
1,2,4-Triazine-3-carboxylic acid	$C_4H_3N_3O_2$	125.02266	X		X			X												
17α-Estradiol	$C_{18}H_{24}O_2$	272.17763				X	X						X							
1-Benzosuberone	$C_{11}H_{12}O$	160.08843										X	X	X	X	X	X	X	X	X
1-Hydroxy-4-methoxybenzene	$C_7H_8O_2$	124.05261		X		X	X	X	X	X	X	X	X		X	X	X	X	X	X
2-(3H)-Furanone	$C_{20}H_{22}O_6$	358.14055										X	X	X	X	X	X	X	X	X
5,5-Diethylbarbituric acid	$C_8H_{12}N_2O_3$	184.08684				X	X					X	X	X	X					
Aceclidine	$C_9H_{15}NO_2$	169.10979	X	X		X	X	X	X	X	X	X	X			X	X		X	X
Butyrophenone	$C_{10}H_{12}O$	148.08835																X		
D-(-)-Tartaric acid	$C_4H_6O_6$	150.01703				X		X							X	X		X	X	
Ethosuximide	$C_7H_{11}NO_2$	141.07868								X										
L-(+)-Tartaric acid	$C_4H_6O_6$	150.01696			X															
Linoleic acid	$C_{18}H_{32}O_2$	280.24025					X				X									
Phthalamide	$C_8H_5NO_2$	147.03215		X		X	X	X		X	X	X	X	X	X	X	X	X	X	X
Terodiline	$C_{20}H_{27}N$	281.21361					X	X	X				X							X
Valpromide	$C_8H_{17}NO$	143.13067				X	X	X	X	X	X							X		
Other uses related to pharamacuticals																				
Nonivamide	$C_{17}H_{27}NO_3$	293.19834										X	X	X	X	X	X	X	X	X
Food additive and flavouring agents																				
2,3-Butadione	$C_4H_6O_2$	86.03666								X										
2,4-Heptadienal	$C_7H_{10}O$	110.07292				X	X	X		X								X		
2-Isobutoxynaphthalene	$C_{14}H_{16}O$	200.11960												X	X	X	X	X	X	X
2-Methylpropyl-3-oxobutanoate (Isobutyl acetoacetate)	$C_8H_{14}O_3$	158.09429	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3-Octanone	$C_8H_{16}O$	128.12014				X	X				X					X			X	X
Butyl isothiocyanate	C_5H_9NS	115.04530		X		X	X													

Dibutyl succinate	$C_{12}H_{22}O_4$	230.15156		X		X														
Dibutyldithiocarbamic acid	$C_9H_{19}NS_2$	205.09589			X														X	
Ethylbenzoate	$C_9H_{10}O_2$	150.06813		X																
Heptanoic acid	$C_7H_{14}O_2$	130.09959	X												X			X	X	X
Hexanoic acid	$C_6H_{12}O_2$	116.08382					X		X		X				X	X		X	X	X
Indole	$C_5H_{10}O_2$	102.06822															X			
Nonanal	$C_9H_{18}O_2$	142.13575				X										X	X		X	X
Pyruvic acid	$C_3H_4O_3$	88.01614					X		X	X	X				X	X	X	X		X
Docosahexaenoic acid	$C_{22}H_{32}O_2$	328.24023				X							X	X	X					
Ethyl acetate	$C_4H_8O_2$	88.05226								X										
Compounds related to other types of synthesis																				
3,3,3-Trifluoro-2,2-dimethylpropanoic acid	$C_5H_7O_2F_3$	156.04019	X																	
4-Hexadecylaniline	$C_{22}H_{39}N$	317.30761														X				
Valeric acid	$C_5H_{10}O_2$	102.06821													X					X
Natural products																				
(3α,5β,7α)-3,7-Dihydroxy-12-oxocholan-24-oic acid	$C_{24}H_{38}O_5$	406.27089	X																	
Abietic acid	$C_{20}H_{30}O_2$	302.22444	X		X												X			
Arachidic acid	$C_{20}H_{40}O_2$	312.30260			X															
β-Estradiol	$C_{18}H_{24}O_2$	272.17702	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Other tentatively identified compounds																				
1-(1,3-Benzodioxol-5-yl)-N-methyl-2-propanamine (MDMA)	$C_{11}H_{15}NO_2$	193.10975								X	X							X		
2-Hexylciclopentanone	$C_{11}H_{20}O$	168.15142				X		X			X						X		X	
Diisopropyl methylphosphonate	$C_7H_{17}O_3P$	180.09175	X	X								X			X		X			
Nonadecylic acid	$C_{19}H_{38}O_2$	298.28658													X				X	

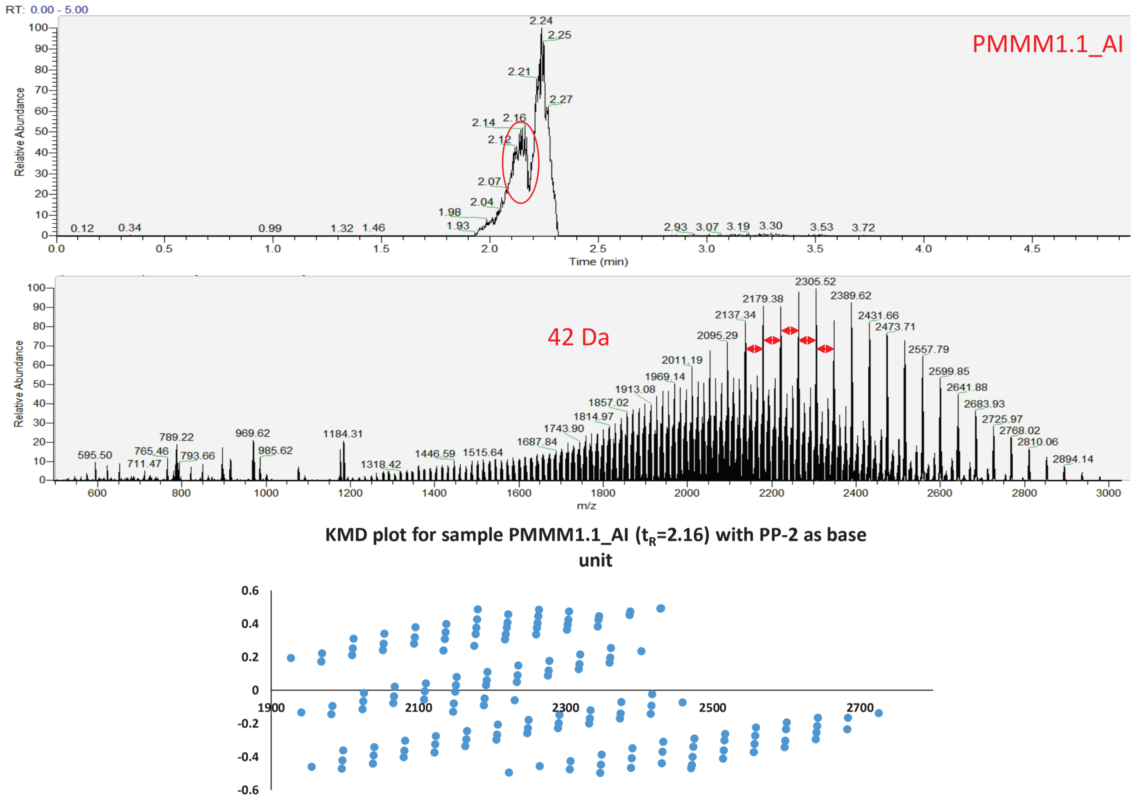


Figure S1. Chromatogram, mass spectra, and KMD of PP in sample 1.1 from the first sampling period of the study.

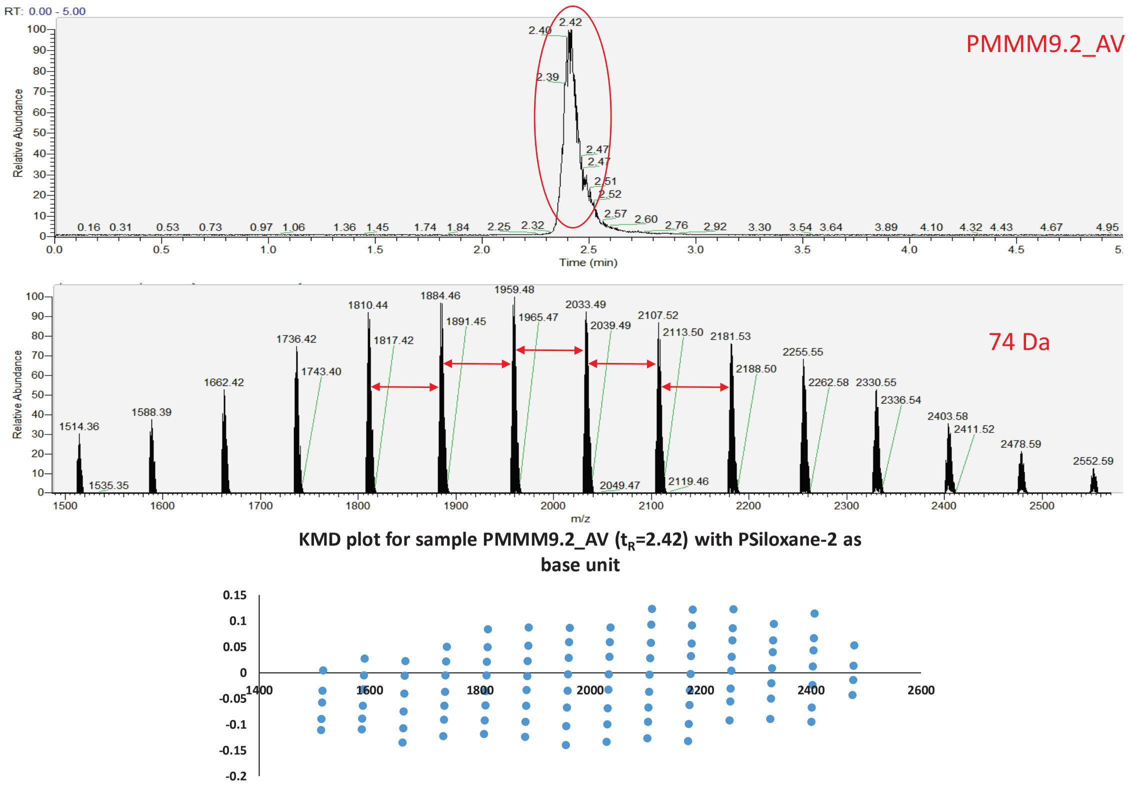
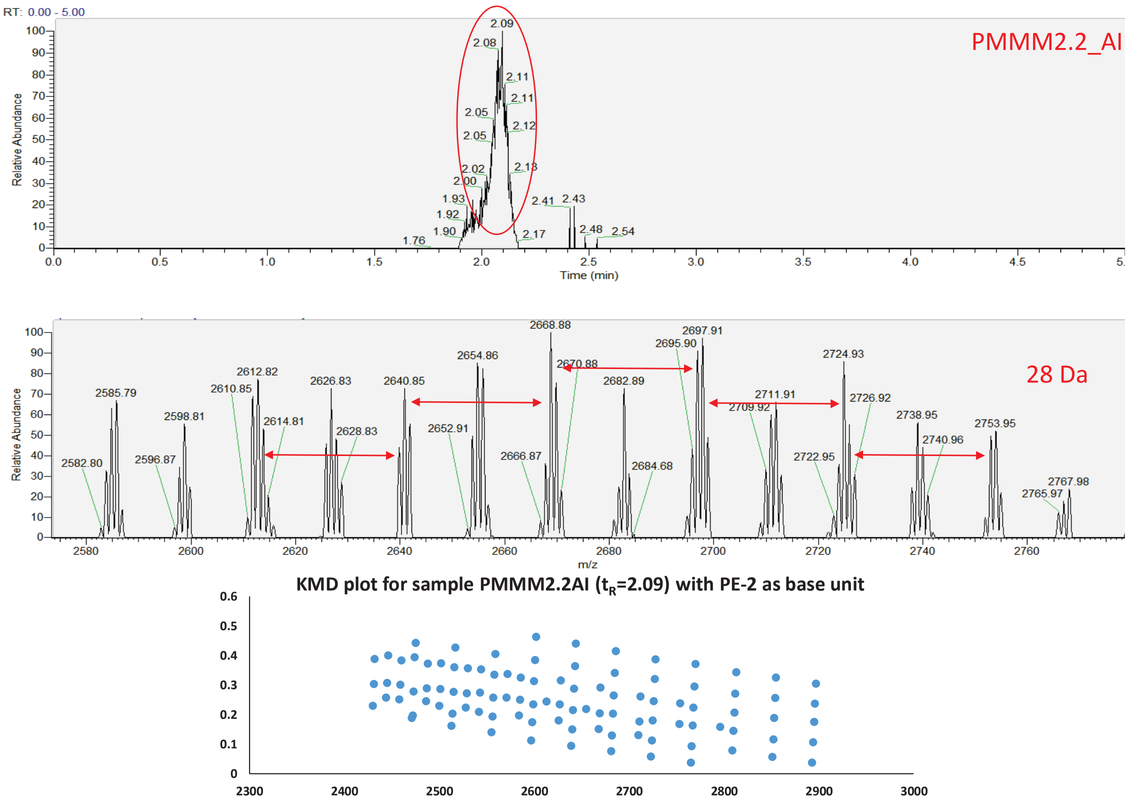


Figure S2. Chromatogram, mass spectra, and KMD of siloxanes in sample 9.2 from the first sampling period of the study.



KMD plot for sample PMMM2.2AI (t_R=2.09) with PE-2 as base unit

Figure S3. Chromatogram, mass spectra, and KMD of PE in sample 2.2 from the first sampling period of the study.

4.2.3 Publication n°3

Micro(nano)plastics in the atmosphere of the Atlantic ocean

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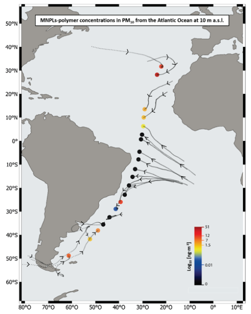

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[Dt] I dmumt RpdneutrngpecMRpmHtmqR sswtpntpdmtemum erdtnatpdcutfine3B

dppfu/11HncBne, 1D. BD. D$1vBd F(pB6. 67BD7D. 7$t
y mrmcPmHtD$tGmrm(Mnet6. 664ty mrmcPmHtcgtenPcunHtane(tD$tNmMeR ewt6. 674) rrmfpmHtD2tNmMeR ewt6. 67ttt

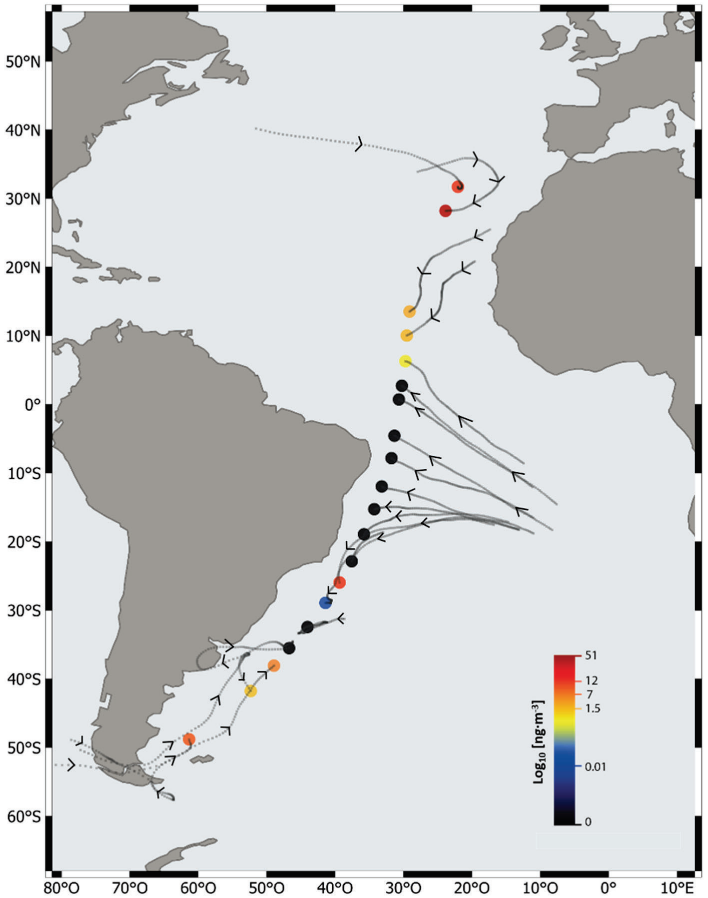
[illegible]

1. Introduction

[illegible]

2. [illegible]

[illegible]



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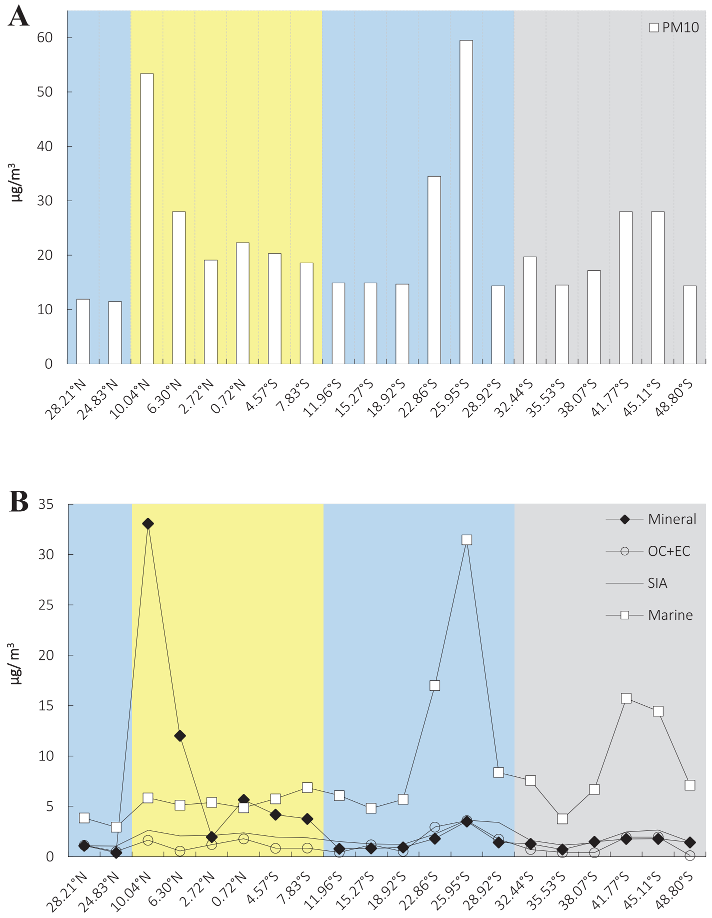
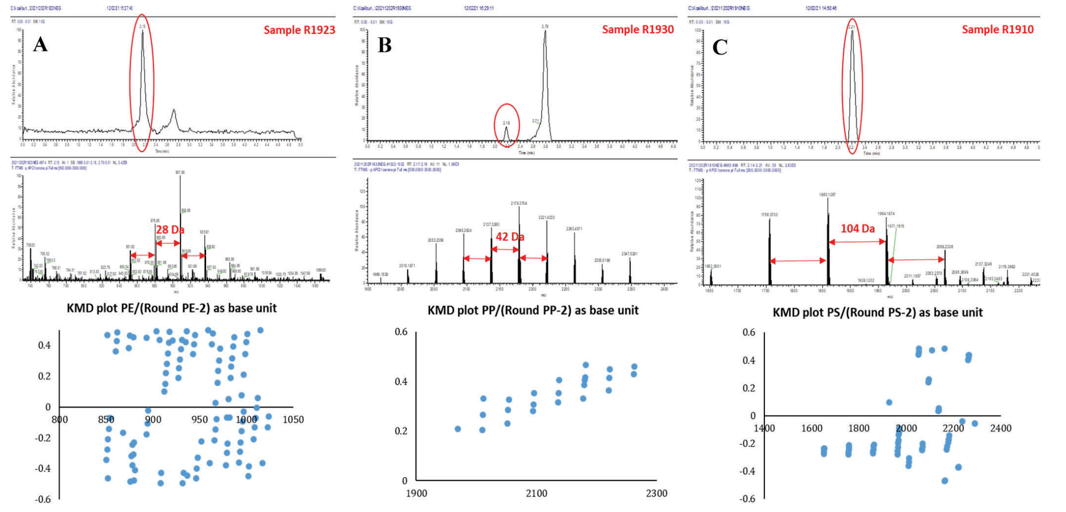

A
PM10
μg/m3
0
10
20
30
40
50
60
28.21°N
24.83°N
10.04°N
6.30°N
2.72°N
0.72°N
4.57°S
7.83°S
11.96°S
15.27°S
18.92°S
22.86°S
25.95°S
28.92°S
32.44°S
35.53°S
38.07°S
41.77°S
45.11°S
48.80°S
B
Mineral
OC+EC
SIA
Marine
μg/m3
0
5
10
15
20
25
30
35

[illegible]

[illegible]

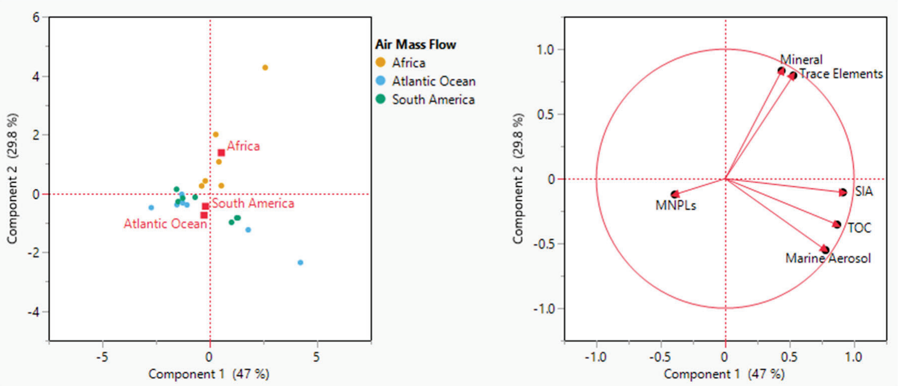

Sample R1923
Sample R1930
Sample R1910
A
B
C
28 Da
42 Da
104 Da
KMD plot PE/(Round PE-2) as base unit
KMD plot PP/(Round PP-2) as base unit
KMD plot PS/(Round PS-2) as base unit
0.4
0.2
0
-0.2
-0.4
-0.6
800
850
900
950
1000
1050
0.6
0.4
0.2
0
1900
2100
2300
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
1400
1600
1800
2000
2200
2400

[illegible]

[illegible]

[illegible]

[illegible]


6
4
2
0
-2
-4
-5
0
5
Component 2 (29.8 %)
Component 1 (47 %)
Africa
South America
Atlantic Ocean
Air Mass Flow
Africa
Atlantic Ocean
South America
1.0
0.5
0
-0.5
-1.0
-1.0
-0.5
0
0.5
1.0
Component 2 (29.8 %)
Component 1 (47 %)
Mineral
Trace Elements
SIA
TOC
Marine Aerosol
MNPLs

[illegible]

[illegible]

[illegible]

[illegible]

4.2.3 Publication n°3 (Supplementary material)

Micro(nano)plastics in the atmosphere of the Atlantic ocean

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Albert Vega-Herrera*

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Naiara Berrojalbiz

Giorgio Buonanno

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Tables S1, S2, S3, S4, S5
Figures S1, S2, S3, S4

Table S1 Extractable polymers classes in hot toluene (60 ºC) by ultrasonic-assisted extraction for 30 min.

Polymer	Acronym
Epoxy resins	EP
Ethyl cellulose	EC
Polyacrylamide	PAM
Polyamides	PA
Polybutadiene	PBD
Polyethylene	PE
Polyethylene terephthalate	PET
Polyisoprene	PI
Polypropylene	PP
Polydimethyl siloxanes	PMDS
Polystyrene	PS

Table S2 Sampling raw data obtained by the meteorological stations of the ship and the atmospheric high-volume active sampler.

Sample	Initial Date	Initial Coordinates (Latitude, Longitude)	Final Date	Final Coordinates (Latitude, Longitude)	Air measured (m^3)	Wind direction	Wind speed ($m\ s^{-1}$)	Temperature (° C)
R1934	20/12/2020	31.71°N, 22.03°O	21/12/2020	28.21°N, 23.84°O	566	SE	2.2	20.5
R1933	21/12/2020	28.21°N, 23.84°O	22/12/2020	24.83°N, 25.58°O	537	NE	2.2	21.1
R1930	25/12/2020	13.54°N, 29.10°O	26/12/2020	10.04°N, 29.51°O	494	NE	3.6	25.0
R1923	26/12/2020	10.04°N, 29.51°O	27/12/2020	6.30°N, 29.71°O	586	NE	2.2	26.0
R1929	27/12/2020	6.30°N, 29.71°O	28/12/2020	2.72°N, 30.23°O	616	NE	2.0	26.0
R1901	28/12/2020	2.72°N, 30.23°O	29/12/2020	0.72°N, 30.66°O	526	NE	2.7	26.9
R1911	29/12/2020	0.72°N, 30.66°O	30/12/2020	4.57°S, 31.31°O	630	SE	3.9	26.9
R1902	30/12/2020	4.57°S, 31.31°O	31/12/2020	7.83°S, 31.74°O	522	SE	4.1	27.1
R1903	31/12/2020	7.83°S, 31.74°O	01/01/2021	11.96°S, 33.17°O	714	SE	3.1	26.7
R1904	01/01/2021	11.96°S, 33.17°O	02/01/2021	15.27°S, 34.26°O	517	SE	2.4	26.5
R1905	02/01/2021	15.27°S, 34.26°O	03/01/2021	18.92°S, 35.76°O	620	NE	2.9	26.5
R1906	03/01/2021	18.92°S, 35.76°O	04/01/2021	22.86°S, 37.56°O	628	NE	3.9	26.8
R1907	04/01/2021	22.86°S, 37.56°O	05/01/2021	25.95°S, 39.33°O	548	NE	4.3	26.7
R1908	05/01/2021	25.95°S, 39.33°O	06/01/2021	28.92°S, 41.40°O	562	NE	3.7	26.1
R1910	06/01/2021	28.92°S, 41.40°O	07/01/2021	32.44°S, 44.03°O	619	SW	2.5	25.5
R1909	07/01/2021	32.44°S, 44.03°O	08/01/2021	35.53°S, 46.72°O	616	SE	3.6	23.2
R1912	08/01/2021	35.53°S, 46.72°O	09/01/2021	38.07°S, 48.94°O	544	SW	5.8	20.4
R1928	09/01/2021	38.07°S, 48.94°O	10/01/2021	41.77°S, 52.32°O	726	SE	3.4	18.7
R1927	10/01/2021	41.77°S, 52.32°O	11/01/2021	45.11°S, 56.30°O	633	SE	4.1	18.5
R1926	11/01/2021	45.11°S, 56.30°O	13/01/2021	48.80°S, 61.34°O	1151	SW	6.7	11.2

Table S3 Validation parameters obtained for the quantification of the MNPLs.

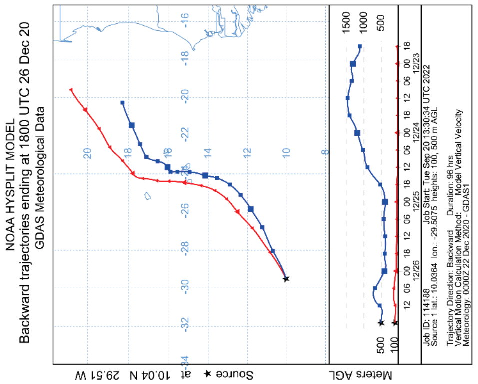
						Precision (n=5)	
Polymer	Acronym	Recoveries (%)	ILOQ (µg/L)	MLOQ (ng/L)	ILOQ (fg)	Inter-Day	Intra-Day
Polypropylene	PP	57	0.10	0.02	0.05	21	17
Polyisoprene	PI	55	0.50	0.10	0.25	19	15
Polyethylene	PE	61	0.10	0.02	0.10	17	15
Polystyrene	PS	82	0.05	0.01	0.05	14	11
Poly(vinyl)chloride	PVC	56	0.50	0.10	0.25	19	12
Polybutadiene	PBD	65	1.00	0.20	0.10	20	14

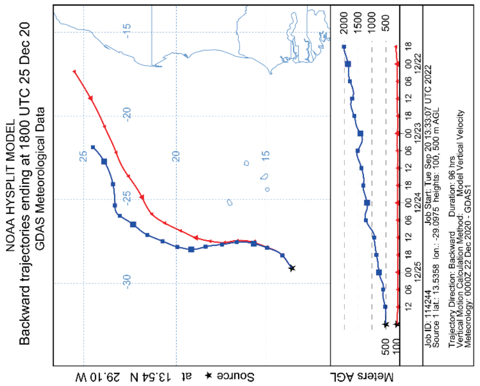
Table S4 Concentrations of PM$_{10}$ and their major components, micro(nano)plastics, and trace elements found across the Atlantic ocean sampling.

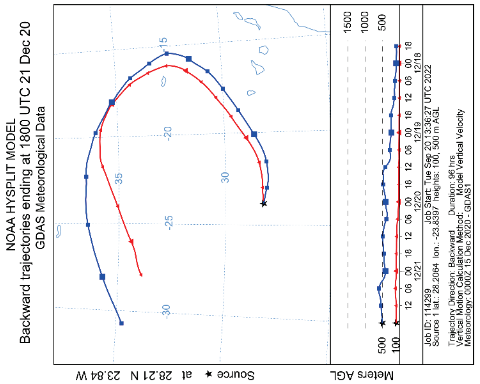
µg/ m^3	Samples																			
	R1934	**R1933**	**R1930**	**R1923**	**R1929**	**R1901**	**R1911**	**R1902**	**R1903**	**R1904**	**R1905**	**R1906**	**R1907**	**R1908**	**R1910**	**R1909**	**R1912**	**R1928**	**R1927**	**R1926**
PM$_{10}$	11.90	11.50	53.40	28.00	19.10	22.30	22.30	18.60	14.90	14.90	14.70	34.50	59.50	25.40	19.70	14.50	17.20	28.00	28.00	14.40
OC	1.16	0.56	1.43	0.48	1.00	1.41	0.68	0.72	0.44	1.18	0.53	2.75	2.94	1.74	0.75	0.42	0.40	1.76	1.77	0.16
EC	< 0.01	< 0.01	0.20	0.09	0.20	0.36	0.16	0.13	< 0.01	< 0.01	< 0.01	0.19	0.65	< 0.01	< 0.01	< 0.01	< 0.01	0.18	0.18	< 0.01
Mineral	1.10	0.40	33.09	12.02	1.97	5.64	4.18	3.76	0.78	0.83	0.92	1.80	3.51	1.23	1.27	0.71	1.48	1.77	1.78	1.42
SIA	1.08	1.07	2.63	2.06	2.11	2.36	1.94	1.88	1.50	1.27	1.22	2.25	3.63	3.39	1.62	1.14	1.36	2.46	2.65	1.49
Micro(nano)polymers	0.01	0.05	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.01
Trace elements	0.02	0.03	0.37	0.23	0.25	0.10	0.08	0.12	0.01	0.02	0.05	0.09	0.09	0.05	0.07	0.06	0.03	0.10	0.09	0.02
Marine	3.84	2.93	5.85	5.12	5.39	4.87	5.76	6.86	6.08	4.81	5.69	17.01	31.47	9.88	7.57	3.75	6.68	15.72	14.45	7.11
Undetermined	4.69	6.46	9.83	8.00	8.18	7.56	9.50	5.13	6.09	6.79	6.29	10.41	17.21	9.10	8.42	8.42	7.25	6.01	7.08	4.19
ng/ m^3																				
Li	0.03	0.07	1.35	0.50	0.2	0.12	0.08	0.02	< 0.01	< 0.01	< 0.01	0.08	0.16	0.06	0.03	< 0.01	< 0.01	0.12	0.08	0.04
Be	0.02	0.02	0.11	0.06	< 0.01	0.04	0.02	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.04	< 0.01	< 0.01	0.02	< 0.01	0.04	< 0.01	< 0.01
B	< 0.01	< 0.01	31.14	< 0.01	133.84	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	74.01	48.50	< 0.01
Sc	0.02	0.01	0.68	0.25	0.05	0.11	0.09	0.06	0.02	0.01	< 0.01	0.02	0.03	0.03	0.02	0.04	< 0.01	0.02	0.03	0.03
Ti	0.71	< 0.01	174.22	62.63	7.37	28.95	17.06	10.06	1.02	1.69	1.31	24.48	2.36	1.54	1.56	1.65	1.85	0.65	3.53	3.47
V	0.21	0.20	4.58	1.64	0.20	0.67	0.47	0.31	0.16	0.10	0.06	0.03	0.08	0.20	0.05	0.08	0.05	0.03	0.14	0.09
Cr	0.56	9.03	3.82	1.83	1.23	1.49	2.08	2.09	0.94	1.02	1.15	0.69	1.41	0.80	1.00	1.70	1.50	1.13	6.33	0.32
Mn	0.41	1.02	31.44	10.81	1.81	5.17	3.06	2.15	0.23	0.32	0.41	1.34	0.70	0.74	0.80	0.78	0.66	0.47	1.26	0.84
Co	0.03	0.09	0.77	0.34	0.10	0.16	0.11	0.12	0.01	0.05	0.01	0.02	0.02	0.04	0.03	0.03	0.02	0.02	0.05	0.02
Ni	0.54	4.14	1.66	1.10	0.67	1.44	1.89	4.38	0.57	0.63	0.53	0.32	0.60	0.65	0.71	0.85	0.79	0.50	3.79	0.19
Cu	3.22	2.73	44.56	5.48	6.85	1.04	2.63	9.28	2.21	5.04	34.96	46.27	40.75	1.09	25.05	16.88	0.24	6.04	7.28	0.11
Zn	4.16	1.63	4.66	116.11	85.85	39.05	25.75	70.12	1.49	2.07	1.66	7.40	15.37	27.10	23.57	23.30	9.54	7.81	9.09	4.20
Ga	0.01	< 0.01	0.64	0.23	0.03	0.11	0.07	0.05	0.01	0.01	0.02	0.01	0.02	0.01	0.01	0.02	0.01	< 0.01	< 0.01	0.01
Ge	0.11	< 0.01	0.09	0.09	0.12	0.15	0.33	0.29	0.00	0.14	0.25	< 0.01	0.21	0.07	0.25	0.13	0.35	0.09	< 0.01	0.06
As	0.15	0.04	0.63	0.15	0.02	0.18	0.13	0.10	0.03	0.03	0.04	0.11	0.07	0.07	0.05	0.06	0.01	0.21	0.16	0.01
Se	0.03	< 0.01	0.30	0.09	0.08	0.10	0.07	< 0.01	< 0.01	< 0.01	0.01	< 0.01	0.02	0.01	< 0.01	< 0.01	0.08	0.07	0.04	0.06
Rb	0.06	0.05	3.66	1.41	0.48	1.04	0.68	0.33	0.05	0.01	0.02	0.07	0.12	0.08	0.06	0.05	0.01	0.10	0.16	0.05
Sr	1.28	0.82	14.79	5.44	1.85	3.31	2.69	2.64	1.64	1.57	1.64	4.50	9.22	2.95	2.11	1.22	2.27	4.02	3.64	2.26
Y	0.25	< 0.01	1.11	0.55	0.27	0.58	0.71	0.50	0.03	0.22	0.41	< 0.01	0.40	0.24	0.46	0.25	0.59	0.22	0.08	0.20
Zr	5.31	5.21	10.53	8.06	7.16	8.44	8.13	9.03	5.41	3.98	3.04	5.13	7.96	7.68	5.25	6.85	2.19	5.93	6.50	3.99
Nb	0.02	0.01	0.44	0.19	0.05	0.15	0.12	0.08	0.04	0.03	0.03	0.04	0.06	0.05	0.05	0.05	0.01	0.03	0.02	0.03
Mo	2.94	< 0.01	0.01	2.96	3.27	4.02	9.39	6.92	< 0.01	3.15	6.18	< 0.01	5.41	3.09	5.41	2.64	9.61	1.57	0.37	2.34
Cd	< 0.01	< 0.01	< 0.01	0.01	0.01	0.04	0.03	0.02	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.01	0.01	0.01	< 0.01	< 0.01	< 0.01	< 0.01
Sn	0.10	0.03	0.13	0.09	0.04	0.08	0.08	0.21	0.05	0.07	0.07	0.07	0.09	0.05	0.09	0.10	0.08	0.04	0.07	0.03
Sb	0.10	0.10	0.13	0.10	0.08	0.15	0.17	0.20	0.10	0.10	0.12	0.05	0.10	0.11	0.12	0.11	0.15	0.06	0.08	0.03
Cs	< 0.01	< 0.01	0.19	0.07	0.02	0.03	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Ba	0.30	< 0.01	30.81	8.93	0.80	4.01	2.54	2.09	0.03	0.44	0.74	< 0.01	0.62	1.84	0.75	0.28	0.97	< 0.01	0.29	0.46
La	< 0.01	< 0.01	1.82	0.63	0.02	0.38	0.24	0.15	< 0.01	0.04	0.06	0.01	0.12	0.04	0.05	0.05	< 0.01	< 0.01	< 0.01	< 0.01
Ce	< 0.01	< 0.01	3.97	1.29	0.08	0.72	0.48	0.29	< 0.01	0.09	0.08	0.05	0.20	0.07	0.11	0.06	0.01	< 0.01	< 0.01	< 0.01
Pr	0.01	< 0.01	0.47	0.17	0.03	0.09	0.06	0.04	< 0.01	0.01	0.02	< 0.01	0.02	0.01	0.01	0.01	0.03	0.01	< 0.01	0.01
Nd	0.05	< 0.01	1.72	0.67	0.11	0.32	0.26	0.15	< 0.01	0.04	0.05	< 0.01	0.04	0.03	0.07	0.03	0.10	0.03	0.01	0.05
Sm	0.03	< 0.01	0.36	0.14	0.03	0.08	0.07	0.06	< 0.01	0.02	0.04	< 0.01	0.04	0.02	0.03	0.02	0.06	0.02	< 0.01	0.02
Eu	< 0.01	< 0.01	0.07	0.03	< 0.01	0.01	0.01	0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Gd	0.02	< 0.01	0.30	0.12	0.05	0.08	0.11	0.08	< 0.01	0.04	0.07	< 0.01	0.04	0.03	0.05	0.03	0.08	0.03	0.02	0.03
Tb	0.01	< 0.01	0.04	0.02	< 0.01	0.02	0.02	0.02	< 0.01	0.01	0.01	< 0.01	0.01	0.01	0.01	0.01	0.02	< 0.01	< 0.01	0.01
Dy	0.08	< 0.01	0.25	0.13	0.06	0.09	0.12	0.12	< 0.01	0.04	0.06	< 0.01	0.06	0.01	0.07	0.03	0.14	0.04	0.02	0.04
Ho	< 0.01	< 0.01	0.04	0.02	0.01	0.02	0.02	0.01	< 0.01	< 0.01	0.01	< 0.01	0.01	0.01	0.01	< 0.01	0.02	0.01	< 0.01	0.01
Er	0.03	< 0.01	0.13	0.06	0.02	0.04	0.05	0.06	< 0.01	0.02	0.03	< 0.01	0.04	0.01	0.03	0.02	0.05	0.02	< 0.01	0.01
Tm	< 0.01	< 0.01	0.02	0.01	< 0.01	0.01	0.01	0.01	< 0.01	< 0.01	0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.01	< 0.01	< 0.01	< 0.01
Yb	0.01	< 0.01	0.10	0.05	0.01	0.04	0.04	0.04	< 0.01	0.01	0.02	< 0.01	0.02	0.02	0.02	0.01	0.02	0.01	< 0.01	0.01
Lu	< 0.01	< 0.01	0.02	0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Hf	0.10	0.09	0.30	0.23	0.20	0.43	0.41	0.48	0.29	0.20	0.14	0.26	0.41	0.40	0.26	0.35	< 0.01	0.17	0.18	0.12
Ta	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
W	0.02	0.02	0.08	0.11	0.06	0.03	0.02	0.03	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.02	0.01	0.01	< 0.01	0.02	0.02	0.03
Tl	< 0.01	< 0.01	0.03	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Pb	0.11	0.04	1.69	0.95	0.44	1.28	0.90	0.64	0.12	0.17	0.12	0.12	0.09	0.15	0.20	0.27	0.21	0.03	0.11	0.06
Bi	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.06	0.05	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Th	0.03	< 0.01	0.50	0.21	0.08	0.17	0.17	0.15	0.02	0.06	0.08	< 0.01	0.07	0.06	0.06	0.06	0.06	0.03	0.03	0.04
U	0.11	< 0.01	0.12	0.14	0.03	0.16	0.21	0.19	< 0.01	0.07	0.14	0.00	0.10	0.04	0.13	< 0.01	0.24	0.06	< 0.01	0.04

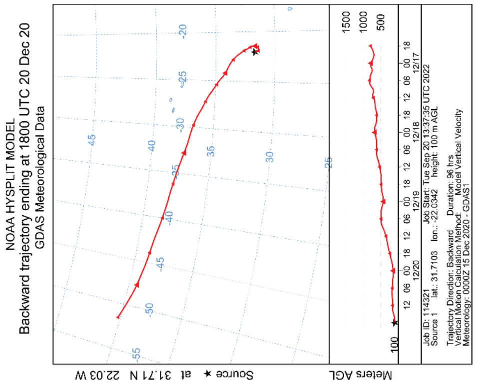
Table S5 Atmospheric dry deposition fluxes (F_{DD}) of MNPLs-polymers expressed as ng·m^{-2}·d^{-1}.

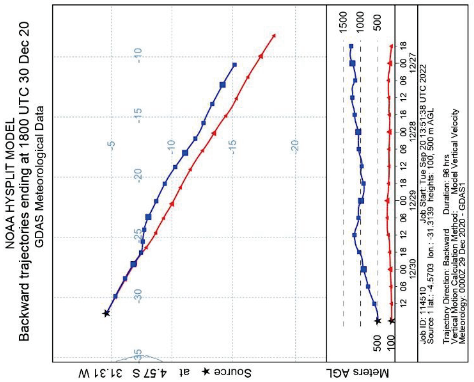
Sample	PE	PP	PS	PBD	PI
R1934	0	0	0	0	3,345
R1933	0	0	0	0	13,412
R1930	0	501	0	0	0
R1923	386	0	0	0	0
R1929	149	0	0	0	0
R1901	0	0	0	0	0
R1911	0	0	0	0	0
R1902	0	0	0	0	0
R1903	0	0	0	0	0
R1904	0	0	0	0	0
R1905	0	0	0	0	0
R1906	0	0	0	0	0
R1907	0	0	0	0	0
R1908	0	0	0	0	3,411
R1910	0	0	4	0	0
R1909	0	0	0	0	0
R1912	0	0	0	0	0
R1928	833	0	0	0	0
R1927	267	0	0	0	0
R1926	0	0	0	0	2,035

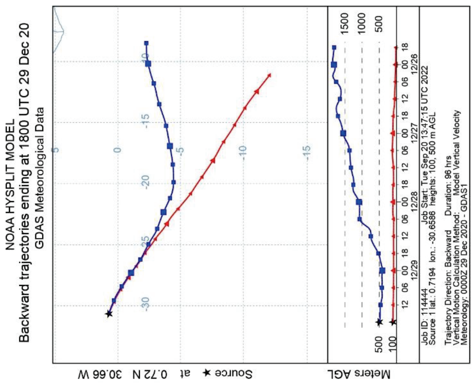


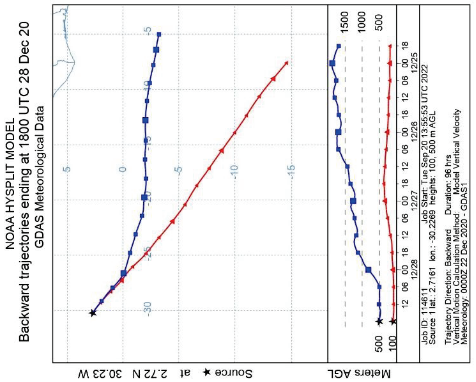


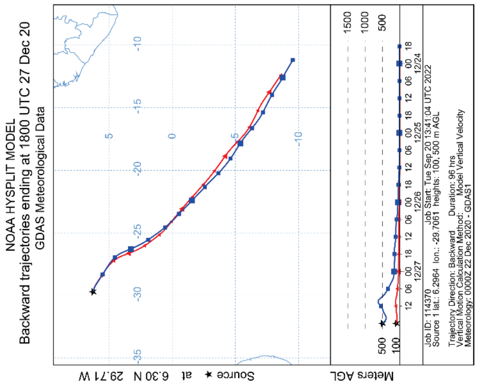


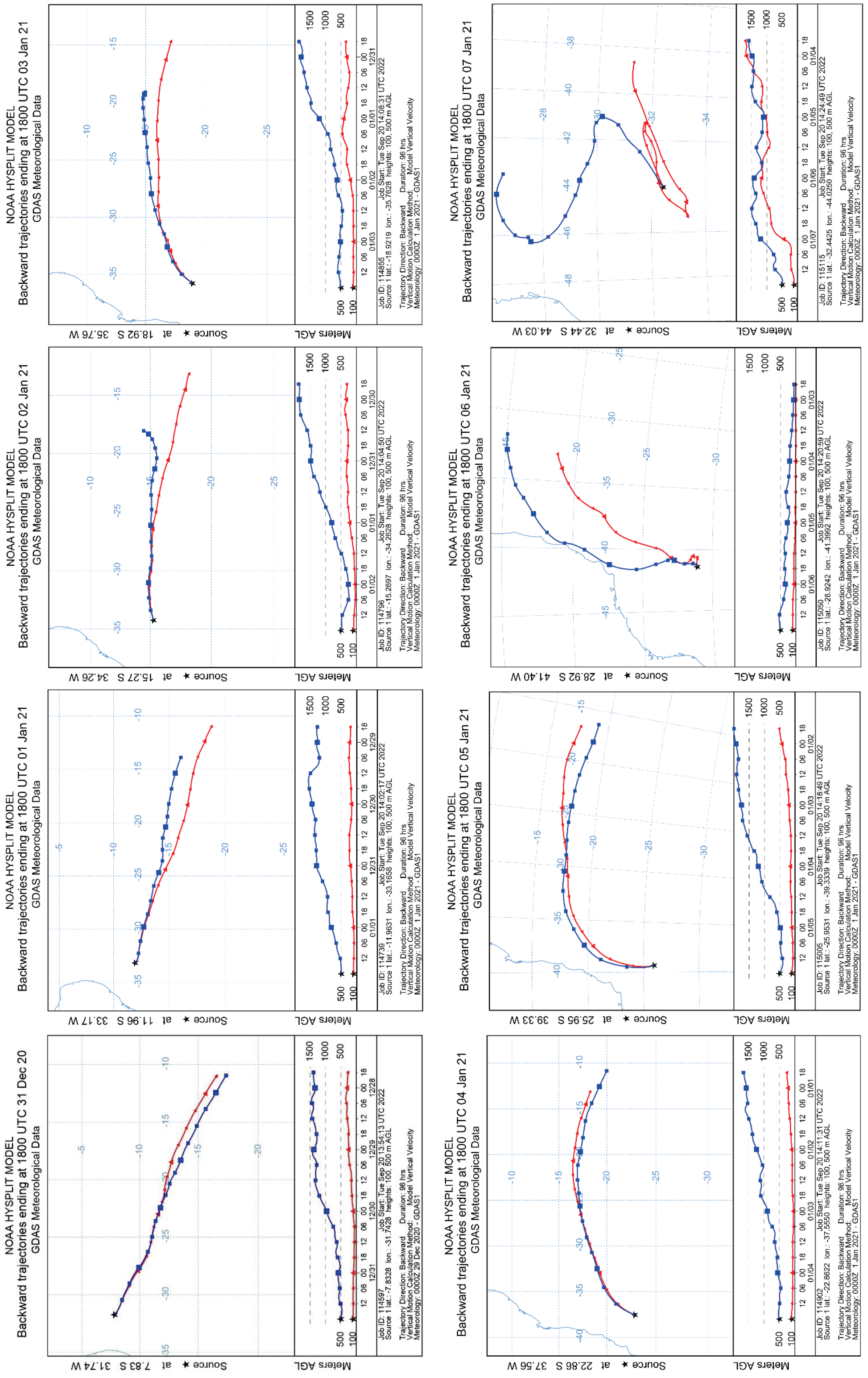












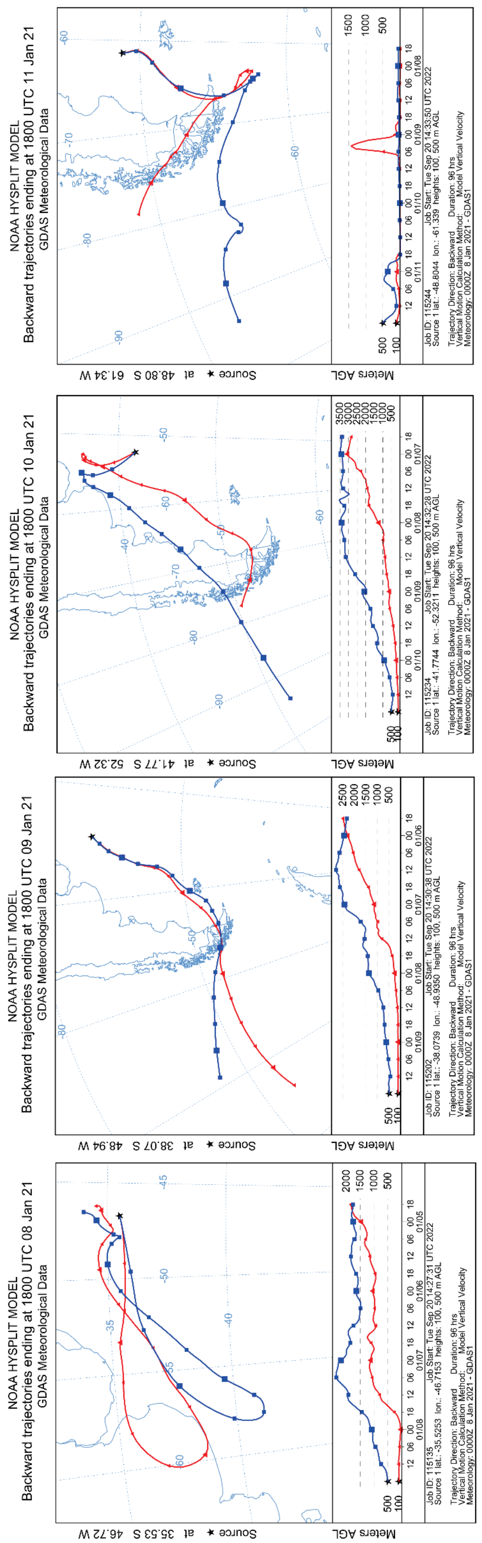
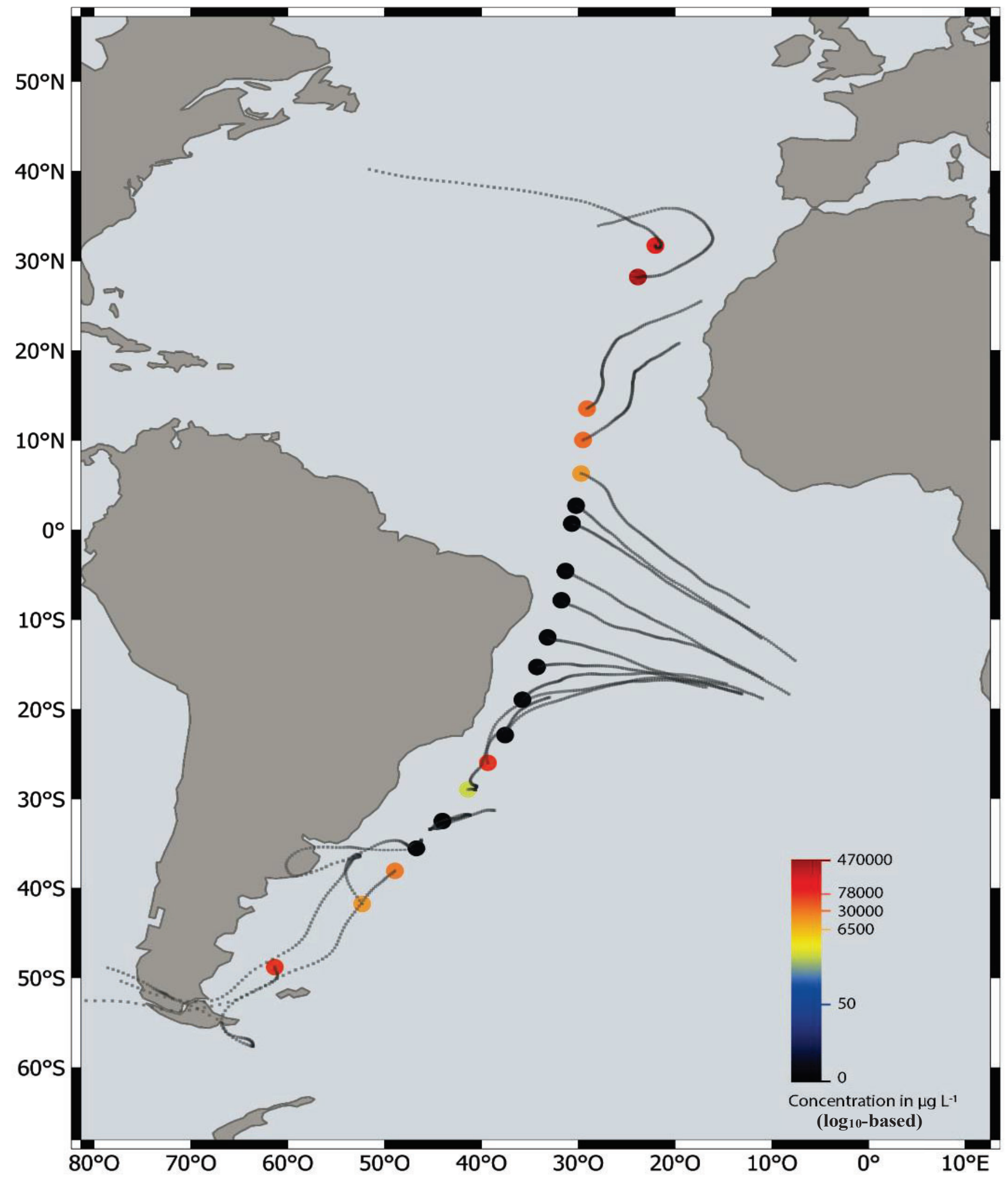


Figure S1 Air backward trajectories generated at 100 and 500 m a.s.l. for 96 h by the Hybrid Single-Particle Lagrangian Integrated Trajectory model (HYSPLIT) during the sampling campaign.



Map generated by ArcGIS software from Esri Corp. (Redlands, CA, United States).

Figure S2 MNPLs-polymer concentrations in the sea-spray aerosol estimated from sodium concentration in PM_{10} samples and expressed in log_{10}-base.

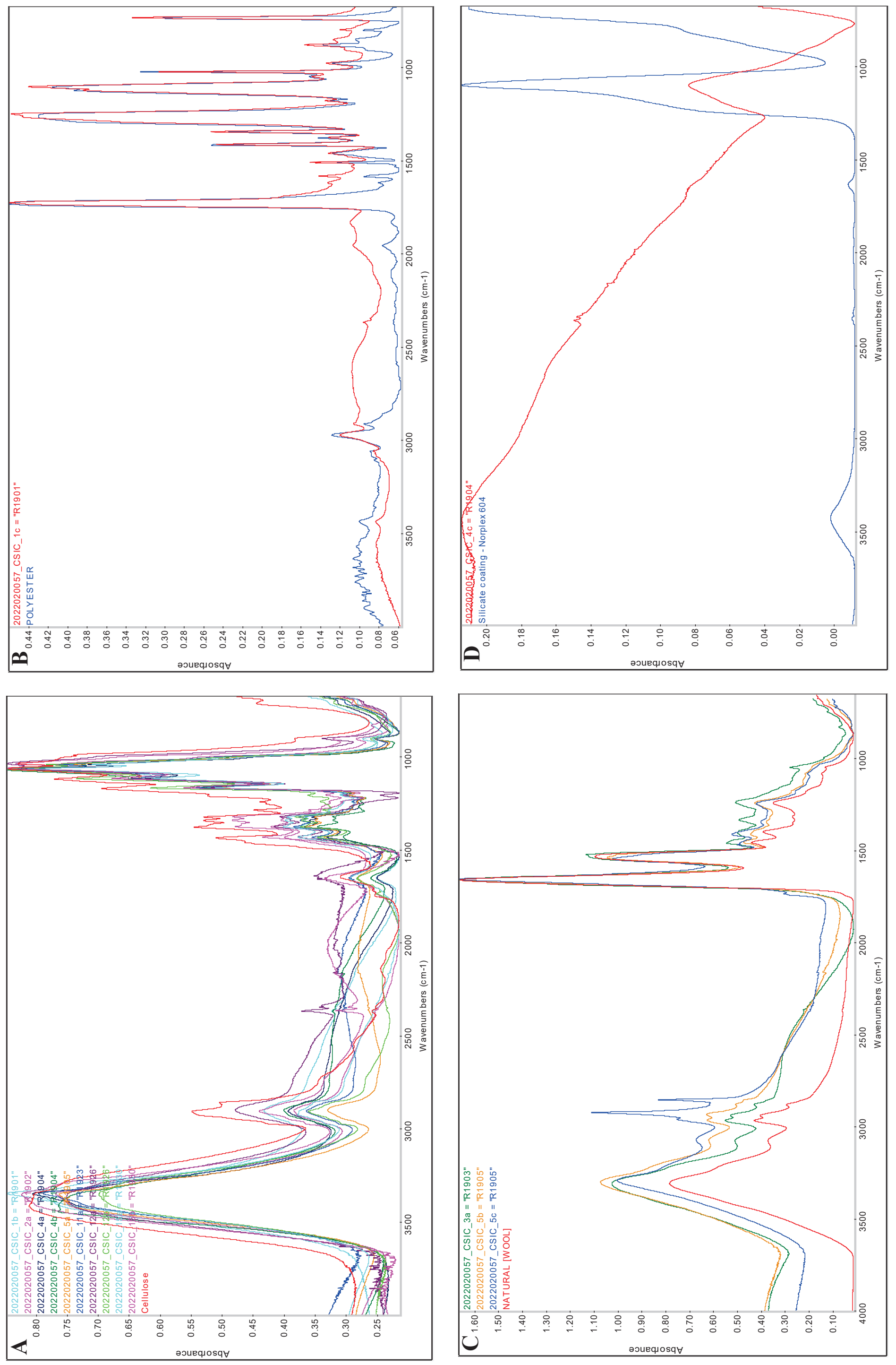


Figure S3 micro-FTIR characterisation: A) cellulose, B) polyester, C) natural wool and D) silicate coating (Norplex 604).

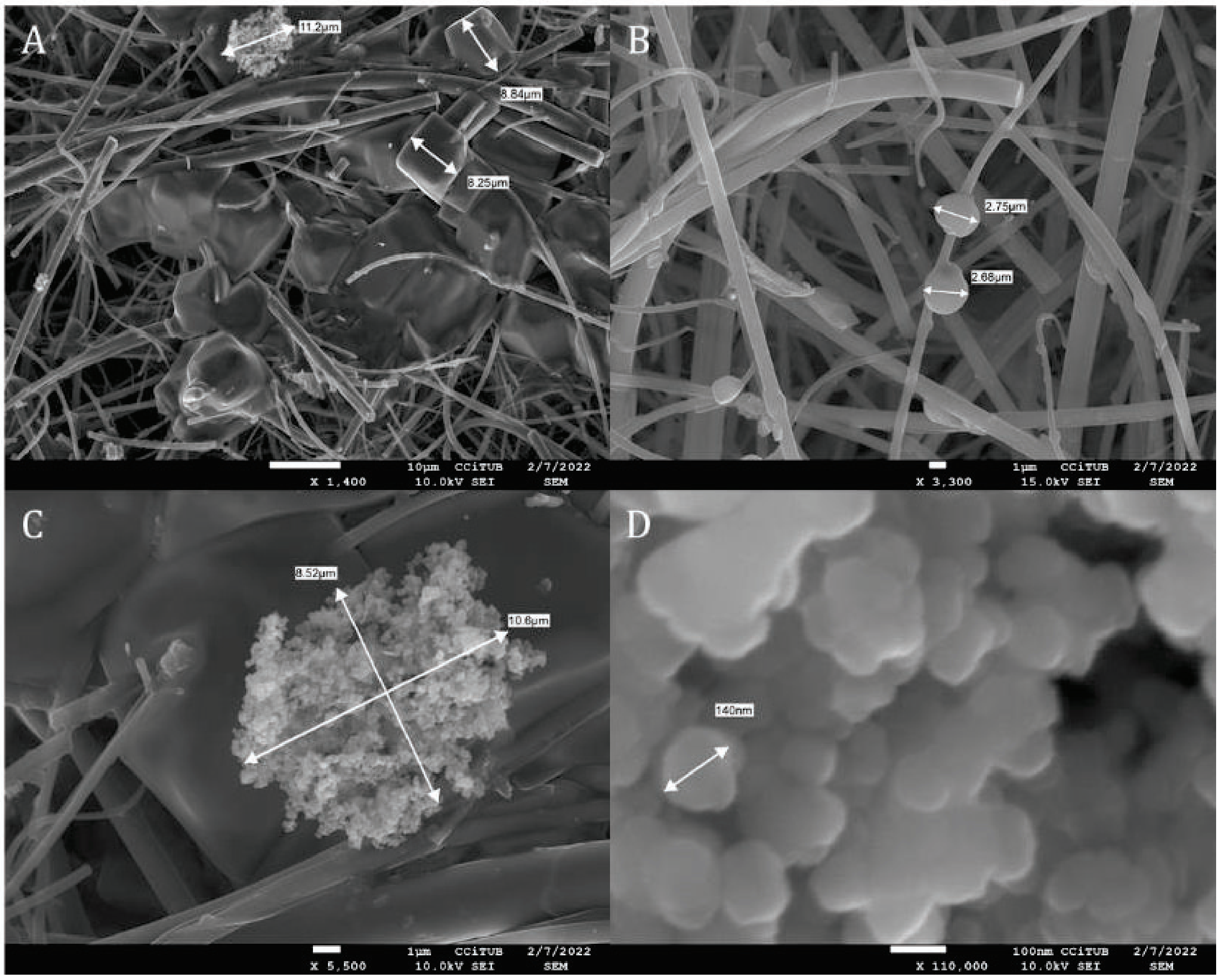


Figure S4 SEM characterisation. A) salts and aggregates, B) single particles, C) focus on aggregates and D) primary particle of the previous aggregate.

4.2.4 Publication n°4

Micro and nanoplastics in marine waters from the Atlantic and Southern oceans

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MICRO AND NANOPLASTICS IN MARINE WATERS FROM THE ATLANTIC AND SOUTHERN OCEANS

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Abstract

Recent attention and investigation into plastic pollution have spurred the scientific community to focus on understanding the distribution of micro- and nanoplastics (MNPLs) in the environment. While existing methodologies usually prioritise qualitative characterisation, there is a pressing need for the developing and harmonisation of quantitative approaches. Here, we present two mass-quantitative analytical methodologies employing liquid and pyrolysis-gas chromatography coupled with mass spectrometry. These methodologies have enabled the identification and quantification of polymer mass concentrations from MNPLs across various marine surface water compartments in the Atlantic and Southern oceans. Through these approaches, we have achieved a groundbreaking examination of the true ubiquity and impact of MNPLs along a significant transect of the Earth's oceans, bypassing the need for spectroscopic and nanometric techniques primarily suited for particle characterisation.

Introduction

We are entering in a new dimension of the Anthropocene age affecting Earth and its vital biogeochemical cycles in unprecedented ways. In 2020, Elhacham, E. et al. estimated that the planet had passed a crossover point where anthropogenically-produced mass, including plastics, had surpassed all global living biomass with annual fluxes released to the environment estimated at millions of tons[1].

Once in the environment, primary plastics debris, those incidentally released from their intended use (e.g. paint coatings, clothing, personal care products, air blasting, etc.), undergo different physical (e.g. fractionation, abrasion or erosion), bio (e.g. interactions with microorganisms communities[2]) and chemical (e.g. solar light-ultraviolet irradiation) degradation mechanisms breaking them down into secondary plastic debris, becoming thus, in smaller plastic particles of random sizes and shapes called microplastics (MPLs, 1 µm - 5 mm) and nanoplastics (NPLs, 1 nm - 1 µm)[3] being able to interact more efficiently with the marine biogeochemical cycles once they achieve the micro- and nanoscales[4] and to reach remote regions such as Southern and Arctic oceans due to their interactions with adjacent abiotic compartments, including fluxes from the atmosphere and hydrosphere[5, 6].

NPLs may present unique characteristics that distinguish them from MPLs at small sizes[7]. For example, they are governed by Brownian motion or colloidal nature over buoyancy and sedimentation processes, the particle size is comparable according other environmental macromolecules present in same compartments (natural organic matter, iron oxides, clay and/or anthropogenic material) favouring hetero-aggregation, whereas it is small in relation to the size of microorganisms.

From the various ecosystems where MNPLs are formed and accumulated, oceanic systems present all the natural features and thermodynamic conditions to catalise their presence. The imperative to address the presence of MNPLs between 1 nm and 10 µm in the oceanic systems stems from a growing recognition of their pervasive impact on marine ecosystems and human health. Their small size allows them to be incorporated (bio-uptake, translocation, and transport mechanisms across biological membranes) by a wide range of organisms, from plankton to apex predators, leading to bioaccumulation and biomagnification along the food chain[8, 9, 10]. Furthermore, MNPLs have the potential to adsorb and transport harmful chemicals, such as persistent organic pollutants and heavy metals, exacerbating their toxic effects[11, 12, 13]. Given the interconnectedness of oceanic systems and the crucial role of these ecosystems in regulating global climate and supporting biodiversity, addressing the presence of MNPLs in the oceans is paramount. Focusing on the Atlantic and Southern oceans is particularly significant due to their unique oceanographic and ecological characteristics. These oceans are vast and relatively less explored compared to other marine regions, making them potential hotspots for MNPLs accumulation and distribution. Additionally, the Atlantic and Southern oceans serve as major transport pathways for ocean currents, which can facilitate the dispersal of MNPLs over long distances and into remote marine environments. Understanding MNPLs dynamics in these regions is therefore crucial for comprehensively assessing their global distribution and impact on marine ecosystems.

Mitigating this threat in marine systems requires a comprehensive understanding of MNPLs distribution, sources, and impacts, as well as concerted international efforts to reduce plastic pollution at its source and develop effective mitigation strategies.

Analytical methods are essential for accurately quantifying MNPLs concentrations in the oceans, providing crucial data to assess the extent of contamination and guide effective mitigation efforts. These methodologies enable scientists to understand MNPLs distribution patterns, identify potential sources, and evaluate the associated environmental and health risks, thus informing targeted interventions to safeguard marine ecosystems and human well-being.

Up until now, various analytical techniques have been established able to identify and characterise polymers of MNPLs in different environmental and biota matrices, but despite this there is still a lack of harmonised mass-quantitative approaches[14, 15]. The main methodologies developed are based on UV-Vis spectrophotometry, optical microscopy, fourier transform infrared, and raman or fluorescence spectroscopy in case of MPLs. Whereas for NPLs, techniques such as dynamic light scattering (DLS), scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDS), X-ray photoelectron spectroscopy (XPS), flow cytometry, inductively coupled plasma mass spectrometry (ICP-MS)[16] or asymmetric-flow field flow fractionation (AF4)[17] have been already successfuly employed. Mass-quantitative analytical strategies based on pyrolysis-gas chromatography coupled with mass spectrometry (Py-GC-MS) have been recently developed, but it is still in its infancy needing further examining and consensus between the scientific community[18].

Thus, some studies have already been launched assessing plastic pollution in marine surface water compartments from North[19] and South[20] Atlantic, Pacific[21], Artic[6] oceans or in the Antarctica[22, 23], but only by means of particle characterisation until its identification or providing qualitative extrapolations for quantitative purposes based on particle-counting approaches expressed normally in items·m^{-3} which does not allow to estimate the real abundance of plastic pollution in our oceans and remote regions by accurately mass concentrations.

In this sense, in this study, our main objectives were to identify and quantify polymers of MNPLs in marine surface waters, encompassing the sea surface microlayer (SML) and the underlying water at a depth of 5 meters (ULW), as well as in marine organisms collected across a broad transect from the North Atlantic ocean to the Bellingshausen sea (Antarctica). We employed two validated mass-quantitative approaches based on LC(SEC)-HRMS and Py-GC-MS. Additionally, we aimed to evaluate the fate and behaviour of MNPLs in oceanic water-air interactions by assessing plastic pollution across various environmental compartments analysed in this study, building upon our previous findings reported in Caracci et al.[24], where the same quantitative analytical techniques were utilised to investigate plastic contamination in the marine atmosphere boundary layer.

Results

Presence of MNPLs-polymers in marine waters from Atlantic and Southern oceans

Thirty seven marine sampling stations were established covering approximately 15,000 Km from the North Atlantic ocean (Vigo, Spain) to the Southern Antarctic peninsula in order to assess MNPLs-polymer pollution in marine surface waters including the SML and ULW at 5 m depth.

Polyethylene (PE), polypropylene (PP), polystyrene (PS), polyisoprene (PI), polybutadiene (PBD), and polyehtylene terephthalate (PET) were the polymers targeted as they are the most commonly reported in marine waters[25, 26]. At least one of them was identified at the 89 and 57 % in SML and ULW samples, respectively. This difference might be attributed due to the different amounts of seawater filtered in each type of sampling and also because of sea surface microlayer is an ubiquitous and gelatinous enriched organic matter biofilm up to 1 mm thickness more suitable for the rentention of organic compounds made of complex structures of polysaccharides, proteins, lipids, and a large variety of microorganisms that plays a key role in photo- and biochemical processes, as well as controlling atmosphere-ocean exchanges of bio-, geo- and anthropogenic particles[27]. In **Table 1**, the polymers from MNPLs unequivocally identified in SML marine compartment are shown.

In the other hand, in **Table 2**, the polymer mass-concentrations found in the ULW marine compartment across the North and South Atlantic ocean as well as the Bellingshausen sea are summarised, while in **Figure 1**, the spatial distribution of the plastic pollution found is represented together with the driven major oceanic surface currents. Concentrations above limits of quantification (LoQ) were found in 19 % of samples assessed. In addition, in **Figure S1**, the individual contribution of each targeted MNPLs-polymers for the oceanic transect covered are shown.

Thereby, MNPLs pollution was ranged from 1-8 $\mu g \cdot L^{-1}$, while highest concentrations belonged to PE and PP, reaching 8 and 6 $\mu g \cdot L^{-1}$ at 18° and 10° N, respectively. Specifically for PET, it should be noted that any concentration was reported during both expeditions in ULW samples even at levels lower than the limit of quantification. According to a study led by Dorin Stanica-Ezeanu & Danuta Matei[28], the natural depolymerisation of wasted PET in marine environments is prone owing to the ubiquitous presence of metallic ions (Na^+, Mg^{2+}, Ca^{2+},K^+), carbonic salts like $NaHCO_3$ and $KHCO_3$, as well as NaCl and $CaCl_2$ that can destabilise the ester bonds in the polymeric chains and catalyse the conversion of PET in sodium terephthalate, terephthalic acid, and ethylene glycol that are soluble in seawater.

Regarding the spatial distribution in the oceanic transect covered, MNPLs were detected and found at quantifiable concentrations mainly in nearby areas to the North Atlantic ocean subtropical gyre at 30° latitude (**Figure S1**), where absolute surface drifter velocities are slow ($\approx 0.05\ m \cdot s^{-1}$) and the temporal accumulation of MNPLs is prone according to Van Sebille, E.[29] et al. and Law, K. L. et al.[30]. Meanwhile, although considerable plastic pollution levels by MNPLs were expected in the oceanic transect assessed nearby to the South American coasts due to their relatively proximity to anthropogenic activities, no quantifiable concentrations were found. Nevertheless, MNPLs pollution was found in the 58 % of the samples evaluated regarding this transect below the LoQs (S-AtOc sampling stations). In convergence zones (between 0-10°S), surface drifter velocities are faster than in subtropical gyres ($\approx 0.3\ m \cdot s^{-1}$) discouraging MNPLs accumulation in accordance with our results where MNPLs-polymers were not also found at quantifiable concentrations (Eq-AtOc-SS 12, S-AtOc-SS 13 and 14).

In the Southern ocean, Ant-SS 1, 2, 11, 12, and 13 ULW samples were collected near South Shetland Islands, where specifically for Ant-SS 1 (Livingston Is.) and 12 (near Deception Is.) presented MNPLs-polymers contamination at 1 and 3 $\mu g\ PE \cdot L^{-1}$, respectively. Furthermore, Ant-SS-5 sample taken near Adelaide Is. after pass the antarctic polar circle (66°34'S) showed the highest MNPLs-polymers pollution reaching a concentration of 7 $\mu g \cdot L^{-1}$. In this context, the MNPLs worldwide ubiquity is confirmed, being able to reach Antarctica mainly by air masses and winds from long-range atmospheric transport processes (LRAT)[31] or through the marine water body crossing the Antarctic circumpolar currents that surround the Antarctic continent within an annulus of cold water which does not act as a barrier for the anthropogenic matter released into the environment as previously thought, and it is today almost the only means of exchange of water between the major oceans and this apparently pristine area[32, 33]. Furthermore, an important contribution to MNPLs pollution is likely to come from local human activities carried out such as tourism, fisheries, and research, putting special enphasis in South Shetland islands and Antarctic peninsula region where human presence is more pronounced than in other Antarctic areas[34].

Overall, PE, PP, and PS were the most frequently detected MNPLs-polymers in both marine surface water compartments assessed in this study (SML and ULW at a depth of 5 meters) during the 15,000 Km oceanic transect covered, whereas PE was the polymer found at highest concentrations.

Impact of MNPLs in marine biota

In ten from thirty seven sampling stations, some aquatic living organisms including algae (*Sargassum* or *Durvillaea*), jellyfish (*Physalia*), fish (*Cheilopogon*), and planktonic species such as antarctic krill (*Euphausia*) were fished by a Manta Trawl sampler and the accumulation of the above mentioned targeted MNPLs-polymers was evaluated. In **Table 3**, the MNPLs levels found in the different marine biota captured are shown. Concentrations ranged between 1-27 $\mu g \cdot g^{-1}$, where the highest concentration was found in S-AtOc-SS 18 for *Cheilopogon melanurus* (1 $\mu g\ PI \cdot g^{-1}$, and 26 $\mu g\ PE \cdot g^{-1}$). For filter feeders such as *Physalia physalis*, MNPLs were found from 3 to 18 $\mu g \cdot g^{-1}$, while in case of Antarctic krill were between 4-21 µg·g^{-1}. Specifically, for *Sargassum* algae captured in N-AtOc-SS 9 through a Langmuir circulation, 2 µg PE·g^{-1} was found.

As for the marine water compartments assessed, PE was also the polymer found at highest concentrations in the aquatic living organisms, while PP, PS, PI, and PET were also detected at quantifiable concentrations. Some evidences regarding the accumulation of MNPLs in aquatic organisms have been already published, such as in salps (*Salpa thompsoni*), krill[35, 36], and predators such as penguins[37], but usually through qualitative extrapolations based on microscopic and spectroscopic approaches.

Discussion

The plastic pollution reported in this study (1-8 µg·L^{-1}) across the oceanic transect assessed were lower compared to our previous studies reported in the Ebro Delta[38] and Mar Menor lagoon [39], respectively. Both Spanish Mediterranean estuarine areas presented MNPLs-polymers concentrations which reached the 7 and 9,000 µg·L^{-1}, respectively, confirming that MNPLs abundances found in riverine and coastal areas tend to be higher than in oceanic environments due to these regions are usually located near populated zones with high levels of anthropogenic matter accompanied by elevated deposition rates and long water-retention times. These findings also point out the importance to include rivers, lakes, and reservoirs regarding plastic pollution in the global context of pollution management and for the continued provision of lake ecosystem services and not only marine environments as is mentioned in a recent study led by Nava, V. et al.[40].

Furthermore, in this study we have assessed simultaneously the occurrence of plastic polymers from different size ranges: 0.7-20 µm, MNPLs that may be governed by advection and diffusion processes across the marine water body; and 335-1,000 µm, MPLs led by advection processes in the marine surface compartments. In this context, our findings also support a better understanding about the physics and dynamics of MNPLs in our oceans, being the horitzontal (advection) and vertical (diffusion) large-scale open ocean processes the most efficient ways of MNPLs to be transported over large distances[29].

Paying attention to the horitzontal large-scale open ocean processes, it can distinguish between surface divergence (subtropical gyres) and convergence zones in marine waters

which are neither stationary and static in space and time. Thereby, these kinds of physical processes governed mainly by surface winds, are in constant change of shape and moving over time, favouring thus, that the bio-, geogenic materials, and contaminants accumulated (including MNPLs) are not trapped indefinitely and allowing them to reach pristine and remote regions from their origin sources which were released initially. Vertical and mixing processes by turbulence or orbital motion are also present where properties like buoyancy and dynamic pressure are relevant a priori, promoting hetero-aggregation or degradation mechanisms and causing changes in the MNPLs environmental fate and behaviour that might promote its suspension as colloids in underlying marine water compartments once they have reached nanoscale or estabilised by interactions with other bio- or anthropogenic material as well as sediment, clay or living organisms from same ecosystems.

Specifically in polar regions, MNPLs can take part in the cycle of formation and melting of the sea ice where freezing ice captures and entrains plastic particles by scavenging processes that migh concentrate plastic particles 1-2 orders of magnitude than in ambient water columns. Freezing ice capture can be carried out in marine surface as well as several meters depth through wave-induced turbulence, while melting of sea ice always takes place at the atmosphere-marine interface[41].

Moreover, MNPLs can be easily accessible by a broad range of biota, specially in a first instance by planktonic species, because of their small sizes and widespread occurrence. That agrees with our results reported particularly for PET (**Table 3**), where it was not detected in the ULW surface water body as expected due to its higher density (≈ 1.38 g·mL^{-1}) than seawater (≈ 1-1.04 g·mL^{-1}), whereas captured jellyfish (N-AtOc-SS 7, 9 µg·g^{-1}) and krill (Ant-SS 2, 21 µg·g^{-1}) presented quantifable concentrations.

Thus, aquatic biota have a key role in the MNPLs oceanic vertical transport, beign able to accumulate (concentrate) and break the uptaken MNPLs down into smaller plastic particles through biting-grinding to be finally released again into the marine ecosystem. A recent exposure experiment from E. Bergami et al.[42] has proven that Antarctic krill is able to uptake MPLs and break them down into NPLs by digestive fragmentation. Moreover, this endemic invertebrate is concentrated in areas of rich primary production and plays a key role in Antarctic ecosystems providing high-energy transfer between primary producers and many higher predators (e.g. penguins, whales, etc.) from pelagic to benthic compartments and being also fundamental for biogeochemical processes that include nutrient recycling or carbon sequestration[43]. In other studies, biomagnification across the trophic chain have been also highlighted as a pathway for the MNPLs oceanic vertical transport, where scat samples analysed from 3 different Antarctic penguins (Adélie, Chinstrap, and Gentoo) presented MNPLs where krill (4-21 $\mu g \cdot g^{-1}$, **Table 3**) contributed at 85, 54, and 66 % of their diet, respectively[37].

In the other hand, until now, the oceanic MNPLs air-water mass exchanges have been poorly studied althought they have a crucial role in the entry of this kind of contaminants in the marine water column and the atmosphere boundary layer simultaneously through the sea-spray aerosols[44]. In our previous work[24], we assessed the airborne MNPLs polymeric composition in PM_{10} from the marine atmosphere boundary layer at 10 m above sea level as well as in the sea-spray aerosol covering the oceanic transect shown in this study (**Figure 1A**). The highest airborne MNPLs levels were also found in areas nearby to the North Atlantic ocean subtropical gyre as in case of the surface ULW, which both environmental compartments contribute to a MNPLs enrichment of the sea-spray aerosol by re-suspension and deposition processes. Thereby, the mass exchange of MNPLs-polymers between the marine surface water and the marine low atmosphere from an oceanic transect that covers the North and South Atlantic ocean has been assessed for first time through quantitative measurements.

Overall, findings suggest that optical, spectroscopic, and related techniques widely employed for a suitable MNPLs particles characterisation are not able to estimate the real abundaces of MNPLs-polymers due to the micro-, and specially the nanofraction are sub-estimated. Thus, the limits of detection (LoDs) and quantification (LoQs) provided by these kinds of techniques are usually limited due to their worst sensitivity achieved in relation to chromatographic and mass spectrometry techniques. In this context, large amounts of seawater are normally necessary (100-1,000 L), while in this study, through chromatographic and mass spectrometry techniques, ≈ 10 L was enough to obtain polymers of MNPLs at quantifiable concentrations. However, the need of new sampling strategies are also necessary to collect more representatitve data regarding MNPLs pollution in our oceans. The development of automated and in-situ oceanographic samplers during this next decade will be crucial, paying attention to microfluidic devices and mass spectrometers able to provide measurements in continuous, enhancing thus, the current environmental assessment and monitoring tools which are able to estimate the antrophogenic fingerprint at worldwide-scale and elucidate its key mechanisms that

contribute to impact the Earth's biogeochemical cycles under a global climate change scenario.

Methods

Sampling

Two expeditions were performed on board the Spanish research vessels 'Sarmiento de Gamboa' and 'Hespérides' from Vigo (Spain, 15th December 2020) to Punta Arenas (Chile, 14th January 2021) and from Ushuaia (Argentina, 18th January 2022) to Punta Arenas (7th February 2022) respectively, covering the North and South Atlantic ocean and the Bellingshausen sea (Southern ocean). In **Table S1** and **S2**, the location of the different sampling stations where marine water samplings were carried out as well as the raw data obtained by meteorological stations from both vessels are shown.

First marine sampling consisted to take 10 L from underlying seawater at 5 m depth (ULW) throught a SBE911 plus Conductivity-Temperature-Depth sensor (CTD) - poly(vynil) chloride (PVC) niskins rosette sampler supplied by Sea-Bird Scientific Inc. (Seattle, WA, United States). Water volumes collected in every sampling station are also shown in **Tables S1-S2**. A second sampling was performed, where marine water surface including sea-surface microlayer (SML) was filtered employing a Manta Trawl sampler purchased from Oceomic, Marine Bio and Technology S.L. (Las Palmas, Spain) with a net opening of 60 x 16 cm, a mesh size of 335 µm and 360 cm long equipped with a mechanical flowmeter and collector bag attached to the end of the net by a PVC sleeve. During the sampling, Manta Traw was attached in the vessels at 1-4 knots depending daily weather conditions. Samplings performed by Manta Trawl are shown in **Table S3**, as well as the corresponding water volume filtered and biota captured.

Sample pre-treatment

ULW samples were filtered using Whatman® glass fiber filters (GF/F) 700 nm ø 47 mm previously dried at 60 °C for 4 h and tared. Once filtered, they were kept at -20 °C in glass petri dishes until arrived to IDAEA-CSIC and U. Laval facilities.

In IDAEA-CSIC, filters were extracted by an ultrasonic-assisted extraction (UAE) using 10 mL toluene for 10 min. The extract was transferred to a 50 mL glass vial and each filter was extracted 3 times and the combined final extract was evaporated under nitrogen steam at 40 °C up to 1 mL. Concentrated extract was divided into 2 aliquots of 500 µL in LC vials. First one was reserved for PE, PP, PS, PI, and PBD polymers analysis, while the second for PET. Specifically, for PET analysis, final aliquot was evaporated up to 250 µL and reconstituted 1:1 toluene:dichloromethane with 1 % trifluoroacetic acid. Final extracts were kept at -20 °C until LC(SEC)-HRMS instrumental analysis.

In U. Laval, ULW samples were solubilised in 10 mL KOH 100 mM and heated at 60 °C during 2 h for the dissolution of MNPLs-polymers from organic matter. Extracts were subsequently centrifugated at 4,000 rpm for 5 min. Then, an aliquot of 1 mL from supernatant was mixed with 2 mL toluene, vortexed for 20 s and heated at 60 °C during 10 min. Toluene-KOH 100 mM extracts were vortexed 20 s every 10 min for 6 times in order to ensure that polymers of MNPLs cross the aqueous phase to the organic phase. Final organic extracts were analysed by means of Py-GC-MS.

In case of marine water surface samples including SML, they were also filtered using Whatman® glass fiber filters (GF/F) 700 nm ø 47 mm previously dried and tared. Once filtered, they were kept at -20 °C in glass petri dishes until arrived to IDAEA-CSIC laboratories. A double lixiviation process was performed employing 20 mL KOH 10 % (v/v) at 60 °C for 6 h and 20 mL HNO_3 20 % (v/v) at room temperature for 0.5 h to remove organic and inorganic matter present in the sample collected, respectively. Then, a refloating process using 30 mL NaCl aqueous saturated solution was used during 24 h. Supernatants were filtered and subsequent extracted through the UAE protocol above mentioned. Final extracts were kept at -20 °C until Py-GC-HRMS analysis.

Moreover, different biota such as jellyfish (*Physalia*), algae (*Sargassum*, *Durvillaea*), fish (*Cheilopogon*), and krill (*Euphausia*) were fished. Biota was grinded by cryogenisation with liquid nitrogen employing an agate mortar followed by a Freezer/Mill 6875® grinder provided by ATS Scientific Inc. (Burlington, Canada). Cryogenic grinder proces consisted in 3 cycles of 4 min each one followed by 2 min for precool and cool and using a rate of 10 cps. An aliquote of 0.5 g was collected and the above mentioned double lixiviation process employing KOH-HNO_3 and the MNPLs-polymers' extraction for the subsequent LC(SEC)-HRMS analysis were applied. Final extracts were kept at -20 °C.

Polymers of MNPLs analysis by LC(SEC)-HRMS

A quantitative analytical methodology based on size exclusion liquid chromatography coupled to high resolution mass spectrometry (LC(SEC)-HRMS) was carried out. Chromatographic separation was performed using an Acquity LC instrument provided by Waters (Milford, MA, United States) and an advanced polymer chromatography column (Acquity APC™ XT45 1.7 µm 150 mm), working with 100 % toluene as mobile phase for 5 min in isocratic conditions, a flow rate of 0.5 $mL \cdot min^{-1}$ and establishing 10 µL as sample injection volume. To avoid toluene evaporation during LC-HRMS analysis, auto sampler was kept at 10 °C, while column worked at 30 °C. The chromatographic system was coupled to a QExactive mass spectrometer from Thermo Fisher Scientific (San José, CA, United States), employing an atmospheric pressure photoionisation source (APPI) operating in negative ionisation mode. For APPI source, optimal parameters were the following: sheath gas at 60 a.u., auxiliary gas at 20 a.u., capillary temperature at 400 °C and S-lens RF at 100 a.u. Data acquisition was performed using a full scan (FS) mode from 500 to 3,000 *m/z* and 17,500 FWHM.

Raw data was processed by Xcalibur v4.2 (Thermo Fisher Scientific) software for the unequivocal identification and subsequent quantification in mass units. Briefly, the total ion chromatogram of each sample was interrogated ever 30 s observing the corresponding MS spectra simultaneously in intervals of 500 Da. Peaks that show homologous patterns in the MS spectra were considered as suspected polymer. Different peaks in the same pattern correspond to the same polymeric chain, differing in the main products formed by APPI ionisation source employed. The signal with the most intense relative abundance from the pattern was selected, and the area integrated subtracted when field blanks presented contamination (5th level of confidene). Then, polymeric patterns were identified using the intrinsic polymer repetitive mass losses between peaks in a same pattern according to its exact mass with a mass tolerance error below than 3 ppm. Common losses are: polyethylene (PE, $[C_2H_4]_n$), 28.0313 Da; polypropylene (PP, $[C_3H_6]_n$), 42.0470 Da; polyisoprene (PI, $[C_5H_8]_n$), 68.0626 Da; polybutadiene (PBD, $[C_4H_6]_n$), 54.0470 Da; polystyrene (PS, $[C_8H_8]_n$), 104.0626 Da; and polyethylene terephthalate (PET, $[C_{10}H_8O_4]_n$), 192.0423 Da (4th level). The subsequent filtration step was conducted through comparison with a library of home-made polymers (3rd level). Afterwards, the Kendrick mass defect (KMD) analysis was applied for the tentative identification (2nd level), while the unequivocal confirmation was only achieved from comparison with standards (1st level). An example regarding the target analysis procedure to identify

MNPLs-polymers is shown in **Figure S2**. Finally, mass-quantification was carried out by external calibrate curves through the corresponding analytical standards.

Polymers of MNPLs by Py-GC-MS

For all cases, a manual pyrolyser EGA/PY-3030D and py-cups from Frontier Laboratories (Fukushima, Japan) were used. Interface and pyrolyser work temperatures were set at 300 and 600 °C respectively and all sample extracts, blanks, and standards spiked in py-cups were dried until dryness conditions in a stainless steel plake at 60 °C before to be analysed in the Py-GC-MS systems in order to avoid toluene interferences during the confirmation and quantification of MNPLs-polymers.

For marine water surface samples including SML, 10 µL of extract was transferred in a py-cup and analysed in a Trace 1310 GC system acquired by Thermo Fisher Scientific equipped with a DB-5-MS (30 m x 0.25 mm x 0.25 µm) column. The chromatographic separation was achieved using helium as mobile phase with a flow rate of 1.049 $mL\cdot min^{-1}$. Starting temperature was 60 °C in isocratic conditions during 2 min, then a first gradient of 10 $°C\cdot min^{-1}$ was employed reaching 180 °C. A second gradient of 5 $°C\cdot min^{-1}$ was used until 310 °C and temperature was maintained for 13 min in insocratic conditions. Finally, 1 min for re-equilibration time was employed followed by a post run at 70 °C. A QExactive Orbitrap mass spectrometer supplied by Thermo Fisher Scientific equipped with a electron ionisation source working in positive mode (EI^+) was coupled to the GC system (Py-GC-HRMS). Optimal ionisation and MS parameters employed were the following: 70 eV as ionisation energy, MS source and quadrupole temperatures of 280 and 150 °C respectively. Data acquisition was performed using a FS mode from 40 to 350 *m/z*. Raw data was processed by Xcalibur v4.2 (Thermo Fisher Scientific) software.

In case of ULW samples, 320 µL of extract was added in py-cups and analysed in a GC system acquired from Agilent Technologies (Santa Clara, CA, United States) equipped with DB 5MS UI (60 m x 250 µm x 0.25 µm) column and using helium as mobile phase at 1.049 $mL\cdot min^{-1}$. GC system was coupled to a mass spectrometer provided also by Agilent Technologies (5977C model) with EI^+ and a single quadrupole (Q) as analyser (Py-GC-LRMS). Chromatographic and ionisation conditions were the same above described for Py-GC-HRMS, while MS source and quadrupole temperatures were 230 and 150 °C, respectively. For the MNPLs-polymer quantification, internal standards of PE, PP, PS, PI, PBD, and PET were employed, whereas the data obtained was processed through the qualitative and quantitative MassHunter (Agilent Technologies) softwares.

Quality assurance and quality control (QA/QC)

Sampling was always performed wearing gloves, cotton lab coats, and using clean material made of stainless steel or glass whenever possible. Five field-procedure blanks were assessed in each type of marine water samplings in order to control possible external contamination sources from vessels, IDAEA-CSIC, and U. Laval laboratories, while sample pre-treatment protocols were executed in laminar flow cabinets. Moreover, regarding the intrumental analyses, solvent blanks were run every 5 samples in case of LC(SEC)-HRMS and every 3 for Py-GC-HRMS and Py-GC-LRMS. Specifically, for Py-GC-MS, samples from different campaigns were placed randomly trying to avoid memory effect during the chromatographic analyses and backup was carried out every 4 weeks or if GC systems started to lose efficiency or resolution in terms of separation quality.

For LC(SEC)-HRMS, PE, PP, PS, PI, and PBD polymer calibrate curves (cc) of 10 points were prepared in toluene obtaining 2 linearity ranges from 0.01 to 0.50 and 0.50 to 50 $mg\cdot L^{-1}$ with $R^2 > 0.98$. For PET, an individual stock standard solution (1,000 $mg\cdot L^{-1}$) was prepared in dichloromethane-trifluoroacetic acid 99:1 and the same procedure described for the other polymers was followed to prepare the corresponding calibrate curve working at 1:1 toluene:dichloromethane with 1 % trifluoroacetic acid.

In order to guarantee QA/QC during LC(SEC)-HRMS instrumental analyses, different parameters were evaluated following protocols described by Kruve, A. et al.[45, 46]. In this sense, recovery tests at 5 and 0.50 $mg\cdot L^{-1}$, matrix effect ionisation by recoveries at same concentrations in solvent ($R_{solvent}$) and matrix samples (R_{ULW}) by spiking experiments, accuracy in terms of inter- and intra-day repeatability in triplicate, and method limits of detection (MLoDs) and quantification (MLoQs) were evaluated. In **Table S5**, QA/QC validation parameters obtained for each polymer in ULW matrix are summarised.

MLoDs and MLoQs were calculated according to the equations:

$$MLoD = 3.3\,\frac{s}{b} \qquad (1)$$

$$MLoQ = 10\,\frac{s}{b} \qquad (2)$$

Where ‘b’ is the slope, and ‘s’ the standard deviation of the signal determined as the standard deviation of the intercept from the parameters obtained by the polymers calibrate curves linear regression models.

Recovery (R), matrix effect ionisation ($ME_{ionisation}$), and method efficiency ($M_{efficiency}$) were estimed as follow:

$$R\ (\%) = \frac{Area\ polymer\ std\ after\ spike\ exp}{Area\ polymer\ std\ cc}\ 100 \quad (3)$$

$$\text{ME ionisation}\ (\%) = \frac{R\ ULW}{R\ solvent}\ 100 \quad (4)$$

$$\text{M efficiency}\ (\%) = ME\ ionisation\ \times R\ \times\ 100 \quad (5)$$

In case of Py-GC-HRMS, individual PE, PP, PS, PI, PBD and PET standard solutions at 1 mg·L^{-1} were injected at dryness conditions to identify the specific exact masses (*m/z* values until the 4th decimal) and the corresponding relative abundances of the different pyrolysates formed from each targeted polymer (**Table S4**). Then, exact masses obtained by HRMS were assigned to the pyrolysates nominal masses (*m/z* values without decimals) obtained during Py-GC-LRMS analyses in order to avoid structural elucidation errors. Finally, 2 transitions (1st for quantification + 2nd for confirmation) were established for each targeted polymer using different precursor ions due to we worked with a strong ionisation source (EI$^+$). Transitions used for the mass-quantification and confirmation of each kind of polymer are also summarised in **Table S4**.

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Credit authorship contribution statement

A. Vega-Herrera: Conceptualisation, sampling, investigation, formal analysis, visualisation, writing, and editing. **M. Le Bagousse**: Formal analysis. **N. Berrojalbiz**: Sampling and visualisation. **E. Abad**: Resources. **M. Llorca**: Writing review. **J. Dachs**: Resources, sampling, writing review, and supervision. **J. Gigault**: Conceptualisation, resources, writing review, and supervision. **M. Farré**: Conceptualisation, resources, writing review, editing, and supervision.

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Table 1: MPLs-polymers (335-1,000 µm) target analysis until the unequivocal identification through analytical standards in the SML from North (‘N’), Equator (‘Eq’), and South (‘S’) Atlantic ocean (‘AtOc’), and Bellingshausen sea (Antarctica, ‘Ant’) by means of Py-GC-HRMS.

Sampling Station (SS)	PE	PP	PS	PI	PBD	PET
N-AtOc-SS 3	**Detected**	n.d.	**Detected**	n.d.	n.d.	n.d.
N-AtOc-SS 4	n.d.	n.d.	**Detected**	n.d.	n.d.	n.d.
N-AtOc-SS 5	n.d.	n.d.	**Detected**	n.d.	n.d.	n.d.
N-AtOc-SS 6	**Detected**	n.d.	**Detected**	n.d.	**Detected**	n.d.
N-AtOc-SS 7	n.d.	n.d.	**Detected**	n.d.	n.d.	n.d.
N-AtOc-SS 8	n.d.	n.d.	**Detected**	n.d.	**Detected**	n.d.
N-AtOc-SS 9	n.d.	n.d.	**Detected**	n.d.	n.d.	n.d.
N-AtOc-SS 10	n.d.	n.d.	**Detected**	n.d.	**Detected**	n.d.
N-AtOc-SS 11	**Detected**	n.d.	**Detected**	**Detected**	n.d.	n.d.
Eq-AtOc-SS 12	**Detected**	n.d.	n.d.	**Detected**	n.d.	n.d.
S-AtOc-SS 13	n.d.	n.d.	n.d.	**Detected**	n.d.	n.d.
S-AtOc-SS 14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
S-AtOc-SS 15	**Detected**	n.d.	n.d.	**Detected**	n.d.	n.d.
S-AtOc-SS 16	**Detected**	n.d.	n.d.	n.d.	n.d.	n.d.
S-AtOc-SS 17	n.d.	n.d.	**Detected**	n.d.	n.d.	n.d.
S-AtOc-SS 18	n.d.	n.d.	n.d.	n.d.	**Detected**	n.d.
S-AtOc-SS 19	**Detected**	**Detected**	**Detected**	n.d.	**Detected**	n.d.
S-AtOc-SS 20	n.d.	**Detected**	n.d.	n.d.	n.d.	n.d.
S-AtOc-SS 21	n.d.	**Detected**	**Detected**	n.d.	**Detected**	n.d.
S-AtOc-SS 22	**Detected**	n.d.	n.d.	**Detected**	**Detected**	n.d.
Ant-SS 1	n.d.	n.d.	n.d.	n.d.	n.d.	**Detected**
Ant-SS 2	n.d.	n.d.	n.d.	n.d.	n.d.	**Detected**
Ant-SS 3	n.d.	n.d.	**Detected**	**Detected**	n.d.	n.d.
Ant-SS 4	n.d.	**Detected**	**Detected**	**Detected**	**Detected**	n.d.
Ant-SS 6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ant-SS 7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ant-SS 9	n.d.	**Detected**	**Detected**	n.d.	n.d.	**Detected**
Ant-SS 11	**Detected**	n.d.	**Detected**	n.d.	n.d.	n.d.
Ant-SS 12	**Detected**	n.d.	n.d.	n.d.	n.d.	n.d.
Ant-SS 13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not detected.

Table 2: Target analysis of MNPLs-polymers (> 700 nm) in samples taken from underlying marine water at 5 m depth (ULW) across the North and South Atlantic ocean and the Bellingshausen sea expressed in $\mu g \cdot L^{-1}$.

Sampling Station (SS)	[PE]	[PP]	[PS]	[PI]	[PBD]	[PET]	[Σ MNPLs]	Analysed by
N-AtOc-SS 1	**1**	< LOD	**< LOQ**	< LOD	**1**	< LOD	**2**	LC(SEC)-HRMS
N-AtOc-SS 2	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	Py-GC-MS
N-AtOc-SS 3	< LOD	< LOD	< LOD	< LOD	**< LOQ**	< LOD	**< LOQ**	LC(SEC)-HRMS
N-AtOc-SS 4	**< LOQ**	**< LOQ**	< LOD	< LOD	< LOD	< LOD	**< LOQ**	Py-GC-MS
N-AtOc-SS 5	**4**	**1**	< LOD	< LOD	< LOD	< LOD	**5**	LC(SEC)-HRMS
N-AtOc-SS 6	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	Py-GC-MS
N-AtOc-SS 7	**8**	< LOD	< LOD	< LOD	< LOD	< LOD	**8**	LC(SEC)-HRMS
N-AtOc-SS 8	**< LOQ**	**< LOQ**	**< LOQ**	< LOD	< LOD	< LOD	**< LOQ**	Py-GC-MS
N-AtOc-SS 9	< LOD	**6**	**< LOQ**	< LOD	< LOD	< LOD	**6**	LC(SEC)-HRMS
N-AtOc-SS 10	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	Py-GC-MS
N-AtOc-SS 11	< LOD	< LOD	**< LOQ**	< LOD	< LOD	< LOD	**< LOQ**	LC(SEC)-HRMS
Eq-AtOc-SS 12	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	Py-GC-MS
S-AtOc-SS 13	< LOD	< LOD	**< LOQ**	< LOD	< LOD	< LOD	**< LOQ**	LC(SEC)-HRMS
S-AtOc-SS 14	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	Py-GC-MS
S-AtOc-SS 15	**< LOQ**	< LOD	< LOD	**< LOQ**	< LOD	< LOD	**< LOQ**	LC(SEC)-HRMS
S-AtOc-SS 16	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	Py-GC-MS
S-AtOc-SS 17	**< LOQ**	< LOD	< LOD	**< LOQ**	< LOD	< LOD	**< LOQ**	LC(SEC)-HRMS
S-AtOc-SS 18	< LOQ	**< LOQ**	< LOD	< LOD	< LOD	< LOD	**< LOQ**	Py-GC-MS
S-AtOc-SS 19	**< LOQ**	< LOD	< LOD	< LOD	< LOD	< LOD	**< LOQ**	LC(SEC)-HRMS
S-AtOc-SS 20	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	Py-GC-MS
S-AtOc-SS 21	**< LOQ**	< LOD	< LOD	< LOD	< LOD	< LOD	**< LOQ**	LC(SEC)-HRMS
S-AtOc-SS 22	**< LOQ**	**< LOQ**	< LOD	< LOD	< LOD	< LOD	**< LOQ**	Py-GC-MS
S-AtOc-SS 23	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	LC(SEC)-HRMS
S-AtOc-SS 24	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	Py-GC-MS
Ant-SS 1	**1**	**< LOQ**	< LOD	< LOD	< LOD	< LOD	**1**	LC(SEC)-HRMS
Ant-SS 2	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	Py-GC-MS
Ant-SS 3	< LOD	< LOD	< LOD	< LOD	**< LOQ**	< LOD	**< LOQ**	LC(SEC)-HRMS
Ant-SS 4	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	Py-GC-MS
Ant-SS 5	**7**	< LOD	< LOD	< LOD	< LOD	< LOD	**7**	LC(SEC)-HRMS
Ant-SS 6	**< LOQ**	**< LOQ**	**< LOQ**	< LOD	< LOD	< LOD	**< LOQ**	Py-GC-MS
Ant-SS 7	**< LOQ**	**< LOQ**	**< LOQ**	< LOD	< LOD	< LOD	**< LOQ**	Py-GC-MS
Ant-SS 8	**< LOQ**	< LOD	< LOD	< LOD	< LOD	< LOD	**< LOQ**	LC(SEC)-HRMS
Ant-SS 9	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	Py-GC-MS

Ant-SS 10	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	LC(SEC)-HRMS
Ant-SS 11	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	Py-GC-MS
Ant-SS 12	**3**	< LOD	< LOD	< LOD	< LOD	< LOD	**3**	LC(SEC)-HRMS
Ant-SS 13	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	LC(SEC)-HRMS

LoD: Limit of Detection; LoQ: Limit of Quantification.

Table 3: Accumulated polymers from MNPLs (> 700 nm) in different aquatic biota expressed in µg·g^{-1} by means of LC(SEC)-HRMS.

Sampling Station (SS)	Organism (gender)	[PE]	[PP]	[PS]	[PI]	[PBD]	[PET]	[Σ MNPLs]
N-AtOc-SS 4	Physalia 1	< LOD	< LOD	< LOD	**6**	< LOD	< LOD	**6**
	Physalia 2	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	Physalia 3	**> LR**	< LOD	< LOD	**10**	< LOD	< LOD	**10**
N-AtOc-SS 7	Physalia	< LOD	< LOD	< LOD	< LOD	< LOD	**9**	**9**
N-AtOc-SS 8	Sargassum	**7**	< LOD	< LOD	< LOD	< LOD	< LOD	**7**
N-AtOc-SS 9	Sargassum	**2**	< LOD	< LOD	**< LOQ**	< LOD	< LOD	**2**
S-AtOc-SS 18	Physalia	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	Cheilopogon	**26**	< LOD	< LOD	**1**	**> LR**	< LOD	**27**
S-AtOc-SS 19	Physalia	< LOD	**3**	< LOD	< LOD	< LOD	< LOD	**3**
S-AtOc-SS 21	Physalia	< LOD	**8**	< LOD	**10**	< LOD	< LOD	**18**
Ant-SS 2	Euphausia	< LOD	< LOD	< LOD	< LOD	< LOD	**21**	**21**
	Durvillaea	**> LR**	**1**	< LOD	< LOD	< LOD	< LOD	**1**
Ant-SS 7	Euphausia 1	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
	Euphausia 2	< LOD	< LOD	**4**	< LOD	< LOD	< LOD	**4**
	Durvillaea	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Ant-SS 13	Euphausia	< LOD	< LOD	**< LOQ**	< LOD	< LOD	< LOD	**< LOQ**
	Durvillaea	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD

LoD: Limit of Detection; LoQ: Limit of Quantification; LR: Linearity Range.

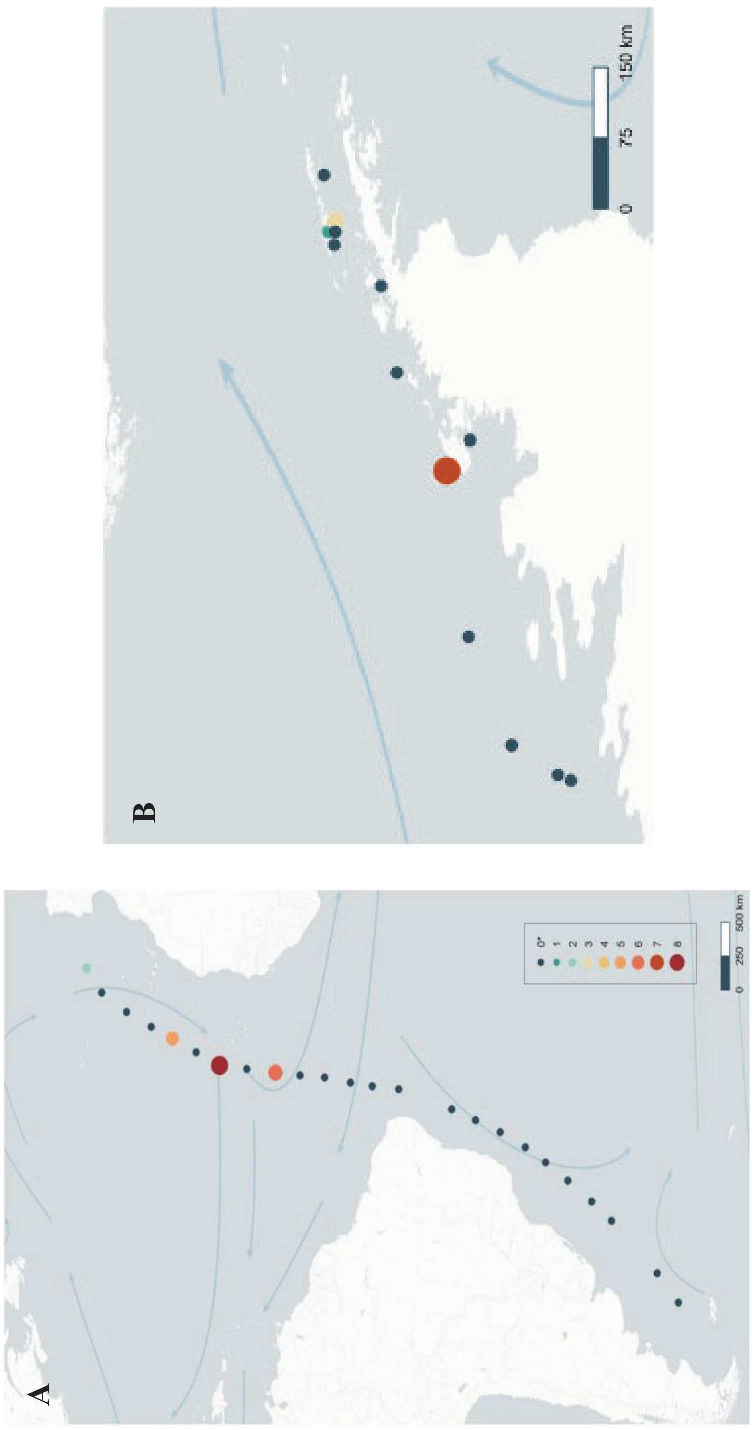

Figure 1: Sampling stations and spatial distribution of the MNPLs-polymers pollution in marine surface water at 5 m depth during **A)** the Atlantic and **B)** Southern expeditions where coloured points indicate found polymer concentrations expressed in µg·L^{-1}. Driven major oceanic surface currents (blue arrows plotted) were also added using the NOAA-US Army National Weather data services.

4.2.4 Publication n°4 (Supplementary material)

Micro and nanoplastics in marine waters from the Atlantic and Southern oceans

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To be submitted in Nature Communications

Tables S1, S2, S3, S4, S5
Figures S1, S2

Table S1 Location of sampling stations during North and South Atlantic ocean ('AtOc') campaign, marine water volumes filtered through CTD niskin bottle catched at 5 m depth, and raw data provided through the 'Sarmiento de Gamboa' research vessel meteorological station.

Sampling Station (SS)	Date	Coordinates (Latitude, Longitude)	Water filtered (L)	Salinity (psu)	Water Temperature (°C)	S O_2 ($mg \cdot L^{-1}$)	Wind direction (°)	Wind speed ($m \cdot s^{-1}$)
N-AtOc-SS 1	18/12/2020	37.54°N, 15.58°W	9.750	36.29	17.51	7.45	245	11.0
N-AtOc-SS 2	19/12/2020	35.44°N, 19.03°W	10.000	36.32	17.83	7.49	309	4.3
N-AtOc-SS 3	20/12/2020	32.11°N, 21.31°W	11.720	36.80	19.95	7.12	198	0.7
N-AtOc-SS 4	21/12/2020	28.39°N, 23.24°W	11.335	37.05	21.42	7.00	131	4.4
N-AtOc-SS 5	22/12/2020	25.37°N, 24.53°W	10.240	37.25	22.88	6.78	78	6.4
N-AtOc-SS 6	23/12/2020	22.13°N, 26.38°W	10.365	36.96	23.59	6.70	67	3.0
N-AtOc-SS 7	24/12/2020	18.52°N, 28.19°W	10.930	36.91	24.03	6.60	178	6.1
N-AtOc-SS 8	25/12/2020	14.58°N, 28.46°W	11.530	35.46	25.46	6.48	65	4.0
N-AtOc-SS 9	26/12/2020	10.52°N, 29.14°W	10.340	35.56	26.41	6.36	68	8.8
N-AtOc-SS 10	27/12/2020	7.21°N, 29.35°W	10.280	34.97	27.45	6.33	154	1.9
N-AtOc-SS 11	28/12/2020	3.50°N, 29.51°W	10.770	35.42	27.93	6.24	108	1.6
Eq-AtOc-SS 12	29/12/2020	0.08°N, 30.30°W	10.200	36.29	27.04	6.23	131	7.6
S-AtOc-SS 13	30/12/2020	2.59°S, 30.57°W	11.420	36.24	26.94	6.33	166	9.9
S-AtOc-SS 14	31/12/2020	6.46°S, 31.20°W	10.580	36.46	27.48	6.26	169	9.2
S-AtOc-SS 15	02/01/2021	14.22°S, 33.54°W	9.020	37.29	26.94	6.30	90	5.5
S-AtOc-SS 16	03/01/2021	17.47°S, 35.16°W	10.290	37.31	27.48	6.53	78	7.0
S-AtOc-SS 17	04/01/2021	21.18°S, 36.48°W	10.400	37.23	27.24	6.41	58	7.5
S-AtOc-SS 18	05/01/2021	24.54°S, 38.43°W	10.350	36.76	26.20	6.40	44	11.4
S-AtOc-SS 19	06/01/2021	27.48°S, 40.39°W	10.470	36.26	24.41	6.68	14	3.4
S-AtOc-SS 20	07/01/2021	30.59°S, 42.58°W	10.500	36.10	23.57	6.77	2	2.2
S-AtOc-SS 21	08/01/2021	34.24°S, 45.38°W	10.430	37.00	23.87	6.71	289	5.8
S-AtOc-SS 22	09/01/2021	37.14°S, 48.05°W	11.140	35.16	19.41	7.46	256	9.6
S-AtOc-SS 23	11/01/2021	43.47°S, 54.45°W	10.410	34.19	18.54	7.57	38	3.7
S-AtOc-SS 24	12/01/2021	46.48°S, 58.29°W	10.160	34.18	13.68	8.56	289	2.7

N: North hemisphere; Eq: Equator; S: South hemisphere.

Table S2 Location of sampling stations during Antarctic ('Ant') expedition, including marine water volumes filtered from CTD niskin bottle catched at 5 m depth, as well as the raw data provided by the 'Hespérides' research vessel meteorological station.

Sampling Station (SS)	Date	Coordinates (Latitude, Longitude)	Water filtered (L)	Salinity (psu)	Water Temperature (°C)	S O_2 ($mg·L^{-1}$)	Wind direction (°)	Wind speed ($m·s^{-1}$)
Ant-SS 1	23/01/2022	62.42°S, 60.38°W	9.670	34.11	1.35	10.83	0	7.9
Ant-SS 2	24/01/2022	62.55°S, 61.06°W	9.400	34.06	1.39	11.03	163	4.8
Ant-SS 3	25/01/2022	64.34°S, 62.33°W	9.290	34.02	1.02	10.10	238	9.9
Ant-SS 4	26/01/2022	65.08°S, 65.38°W	9.720	33.52	0.97	11.45	29	5.5
Ant-SS 5	27/01/2022	66.54°S, 69.06°W	9.700	33.40	0.46	11.63	247	9.4
Ant-SS 6	28/01/2022	67.44°S, 68.01°W	9.650	33.15	0.35	13.45	186	3.6
Ant-SS 7	30/01/2022	70.50°S, 79.53°W	9.700	33.05	-0.87	12.14	324	6.6
Ant-SS 8*	30/01/2022	71.18°S, 80.05°W	9.810	33.23	-0.36	12.07	25	6.8
Ant-SS 9	31/01/2022	69.12°S, 78.50°W	9.810	33.55	-0.06	11.37	112	10.4
Ant-SS 10	01/02/2022	67.41°S, 74.59°W	9.910	33.90	0.74	10.74	207	11.5
Ant-SS 11	04/02/2022	62.33°S, 58.37°W	9.860	34.34	1.25	10.81	243	12.9
Ant-SS 12	05/02/2022	62.58°S, 60.15°W	9.820	34.39	0.31	10.75	316	8.0
Ant-SS 13	06/02/2022	62.57°S, 60.38°W	10.100	34.02	1.74	10.80	223	11.3

*In red, the current Spanish south far navigation record.

Table S3 Manta Trawl for MPLs (335-1,000 µm) sampling performed during the Atlantic ocean campaign and the Antarctic expedition together with the water volume filtered (expressed in L) and the aquatic biota captured.

Sampling Station (SS)	N° Cycles	Water filtered (L)	Biota captured (Gender)
N-AtOc-SS 3	3,524	101,491	-
N-AtOc-SS 4	1,911	55,036	3 jellyfish (Physalia)
N-AtOc-SS 5	2,670	76,896	-
N-AtOc-SS 6	3,290	94,752	-
N-AtOc-SS 7	3,524	101,491	1 jellyfish (Physalia)
N-AtOc-SS 8	2,945	84,816	1 algae (Sargassum)
N-AtOc-SS 9	2,613	75,254	1 algae (Sargassum)
N-AtOc-SS 10	5,096	146,764	-
N-AtOc-SS 11	2,592	74,649	-
Eq-AtOc-SS 12	3,958	113,990	-
S-AtOc-SS 13	3,904	112,435	-
S-AtOc-SS 14	3,136	90,316	-
S-AtOc-SS 15	3,439	99,043	-
S-AtOc-SS 16	3,236	93,196	-
S-AtOc-SS 17	3,743	107,798	-
S-AtOc-SS 18	5,255	151,344	1 jellyfish (Physalia) 1 fish (Cheilopogon)
S-AtOc-SS 19	4,854	139,795	1 jellyfish (Physalia)
S-AtOc-SS 20	4,502	129,657	-
S-AtOc-SS 21	4,837	139,305	1 jellyfish (Physalia)
S-AtOc-SS 22	4,926	141,868	-
Ant-SS 1	6,643	191,318	-
Ant-SS 2	6,595	189,936	1 krill (Euphausia) 1 algae (Durvillaea)
Ant-SS 3	4,572	131,673	-
Ant-SS 4	3,661	105,436	-
Ant-SS 6	3,687	106,185	-
Ant-SS 7	4,523	130,262	2 krill (Euphausia) 1 algae (Durvillaea)
Ant-SS 9	3,923	112,982	-
Ant-SS 11	4,141	119,260	-
Ant-SS 12	4,460	128,448	-
Ant-SS 13	6,155	177,264	1 krill (Euphausia) 1 algae (Durvillaea)

Table S4 Pyrolysates exact masses obtained from each targeted MNPLs-polymer through analytical standards injected in the Py-GC-(EI+)HRMS. In red and blue, pyrolysates used for the polymers mass-quantification (1st transition) and confirmation (2nd transition) respectively by Py-GC-LRMS.

PS							
Py-product	**Nominal Molecular Mass**	**HRMS Exact Mass**	**Theoric Exact Mass**	**Error (ppm) HRMS**	**EI(+) Empirical Formula**	**t_R (min)**	**Purpose**
Styrene	78	78.0464	78.0464	0.279	C_6H_6	3.54	-
	104	104.0619	104.0621	1.171	C_8H_8		
Styrene Dimer	91	91.0543	91.0542	0.255	C_7H_7	15.08	Quantification
	115	115.0543	115.0542	0.984	C_9H_7		
	130	130.0779	130.0777	1.446	$C_{10}H_{10}$		
	178	178.0778	178.0777	0.326	$C_{14}H_{10}$		
	193	193.1013	193.1012	0.482	$C_{15}H_{13}$		
	208	208.1248	208.1247	0.663	$C_{16}H_{16}$		
Styrene Trimer	91	91.0543	91.0542	0.255	C_7H_7	25.53	Confirmation
	208	208.1247	208.1247	0.352	$C_{16}H_{16}$		
	312	312.1875	312.1873	0.633	$C_{24}H_{24}$		
PP							
2,4-dimethyl-1-heptene	81	81.0699	81.0698	0.285	C_6H_9	3.55	Quantification
	83	83.0855	83.0855	0.083	C_6H_{11}		
	91	91.0542	91.0542	0.514	C_7H_7		
	95	95.0855	95.0855	0.388	C_7H_{11}		
	97	97.1012	97.1012	0.072	C_7H_{13}		
	109	109.1012	109.1012	0.064	C_8H_{13}		
	111	111.1168	111.1168	0.153	C_8H_{15}		
	126	126.1404	126.1403	0.538	C_9H_{18}		
n-pentane	41	41.0385	41.0386	2.256	C_3H_5	1.12	Confirmation
	43	43.0542	43.0542	1.552	C_3H_7		
	55	55.0542	55.0542	0.058	C_4H_7		
	57	57.0699	57.0699	0.405	C_4H_9		
	69	69.0699	69.0699	0.190	C_5H_9		
	71	71.0855	71.0855	0.043	C_5H_{11}		
PE							
1-decene	41	41.0385	41.0386	2.546	C_3H_5	12.30	Quantification
	43	43.0542	43.0542	1.552	C_3H_7		
	55	55.0542	55.0542	0.240	C_4H_7		
	57	57.0700	57.0699	0.405	C_4H_9		
	67	67.0542	67.0542	0.102	C_5H_7		
	69	69.0699	69.0699	0.099	C_5H_9		
	83	83.0855	83.0855	0.204	C_6H_{11}		
	95	95.0856	95.0855	0.874	C_7H_{11}		
	97	97.1012	97.1012	0.134	C_7H_{13}		
	111	111.1169	111.1168	0.297	C_8H_{15}		
	125	125.1326	125.1325	0.583	C_9H_{17}		
	140	140.1560	140.1560	0.127	$C_{10}H_{20}$		
1-triacontene	111	111.1169	111.1168	0.747	C_8H_{15}	34.93	Confirmation
	125	125.1326	125.1325	1.142	C_9H_{17}		
	153	153.1639	153.1638	0.867	$C_{11}H_{21}$		
n-C_6	81	81.0699	81.0698	0.331	C_6H_9	-	Qualitative
	85	85.1012	85.1012	0.035	C_6H_{13}		
PET							
Benzoic acid	77	77.0386	77.0386	0302	C_6H_5	8.39	-
	105	105.0335	105.0335	0.368	C_7H_5O		
	122	122.0363	122.0362	0.648	$C_7H_6O_2$		
4-(vinyloxycarbonyl) benzoic acid	65	65.0386	65.0386	0.665	C_5H_5	13.89	Confirmation
	76	76.0308	76.0308	0.767	C_6H_4		
	104	104.0257	104.0257	0.420	C_7H_4O		
	121	121.0285	121.0284	0.777	$C_7H_5O_2$		
	149	149.0235	149.0233	1.473	$C_8H_5O_3$		
Divinyl terephthalate	65	65.0386	65.0386	0.972	C_5H_5	13.15	Quantification
	76	76.0308	76.0308	0.898	C_6H_4		
	91	91.0543	91.0542	0.914	C_7H_7		
	104	104.0257	104.0257	0.228	C_7H_4O		
	132	132.0208	132.0206	1.432	$C_8H_4O_2$		
	147	147.0442	147.0441	0.707	$C_9H_7O_2$		
	175	175.0390	175.0390	0.003	$C_{10}H_7O_3$		
PI							
Isoprene	53	53.0387	53.0386	1.381	C_4H_5	1.11	-
	67	67.0542	67.0542	0.698	C_5H_7		
Isoprene Dimer (Diprene)	67	67.0543	67.0542	0.793	C_5H_7	5.71	Quantification
	77	77.0386	77.0386	0.302	C_6H_5		
	91	91.0543	91.0542	0.365	C_7H_7		
	93	93.0699	93.0699	0.249	C_7H_9		
	107	107.0856	107.0855	0.309	C_8H_{11}		
	121	121.1013	121.1012	0.685	C_9H_{13}		
	136	136.1248	136.1247	1.013	$C_{10}H_{16}$		
Isoprene Dimer (Dipentene)	136	136.1247	136.1247	0.646	$C_{10}H_{16}$	5.36	Qualitative
Isoprene Dimer (1,4-dimethyl-4-vinylcyclohex-1-ene)	136	136.1248	136.1247	1.087	$C_{10}H_{16}$	4.52	Qualitative
Isoprene Trimer	105	105.0700	105.0699	1.172	C_8H_9	11.69	Confirmation
	119	119.0857	119.0855	1.369	C_9H_{11}		
	161	161.1326	161.1325	0.825	$C_{12}H_{17}$		
	175	175.1483	175.1481	0.815	$C_{13}H_{19}$		
	189	189.1639	189.1638	0.702	$C_{14}H_{21}$		
	204	204.1874	204.1873	0.625	$C_{15}H_{24}$		
PBD							
Butadiene	53	53.0387	53.0386	1.570	C_4H_5	1.03	-
Butadiene Dimer (4-vinylcyclohexene)	67	67.0543	67.0542	0.793	C_5H_7	2.77	Quantification
	77	77.0386	77.0386	0.350	C_6H_5		
	91	91.0542	91.0542	0.145	C_7H_7		
	93	93.0699	93.0699	0.463	C_7H_9		
	108	108.0934	108.0934	0.259	C_8H_{12}		
Butadiene Trimer	105	105.0700	105.0699	0.696	C_8H_9	8.09	Confirmation
	119	119.0857	119.0855	1.033	C_9H_{11}		
	133	133.1013	133.1012	1.150	$C_{10}H_{13}$		
	147	147.1169	147.1168	0.768	$C_{11}H_{15}$		
	162	162.1403	162.1403	0.075	$C_{12}H_{18}$		

Table S5 QA/QC parameters obtained for each targeted polymer in surface ULW samples by the LC(SEC)-HRMS method.

				Recovery (n=3; %)		Precision (n=3) – Repeatability (%)		ME ionisation (%)*		
Polymer	**Acronym**	**MLoD (µg·L^{-1})**	**MLoQ (µg·L^{-1})**	**5 mg·L^{-1}**	**500 µg·L^{-1}**	**Inter-Day**	**Intra-Day**	**5 mg·L^{-1}**	**500 µg·L^{-1}**	**Method efficiency (%)**
Polyethylene	PE	0.5	1	74	61	8	4	75	71	50
Polypropylene	PP	0.5	1	77	72	9	3	85	83	63
Polystyrene	PS	3	10	83	76	22	6	87	78	66
Polyisoprene	PI	2	10	89	92	11	4	109	128	107
Polybutadiene	PBD	0.1	0.5	80	73	12	4	91	85	68
Polyethylene terephthalate	PET	13	25	65	59	27	9	115	134	77

*ME ionisation: < 100 % - Suppression; > 100 % - Enhancement.

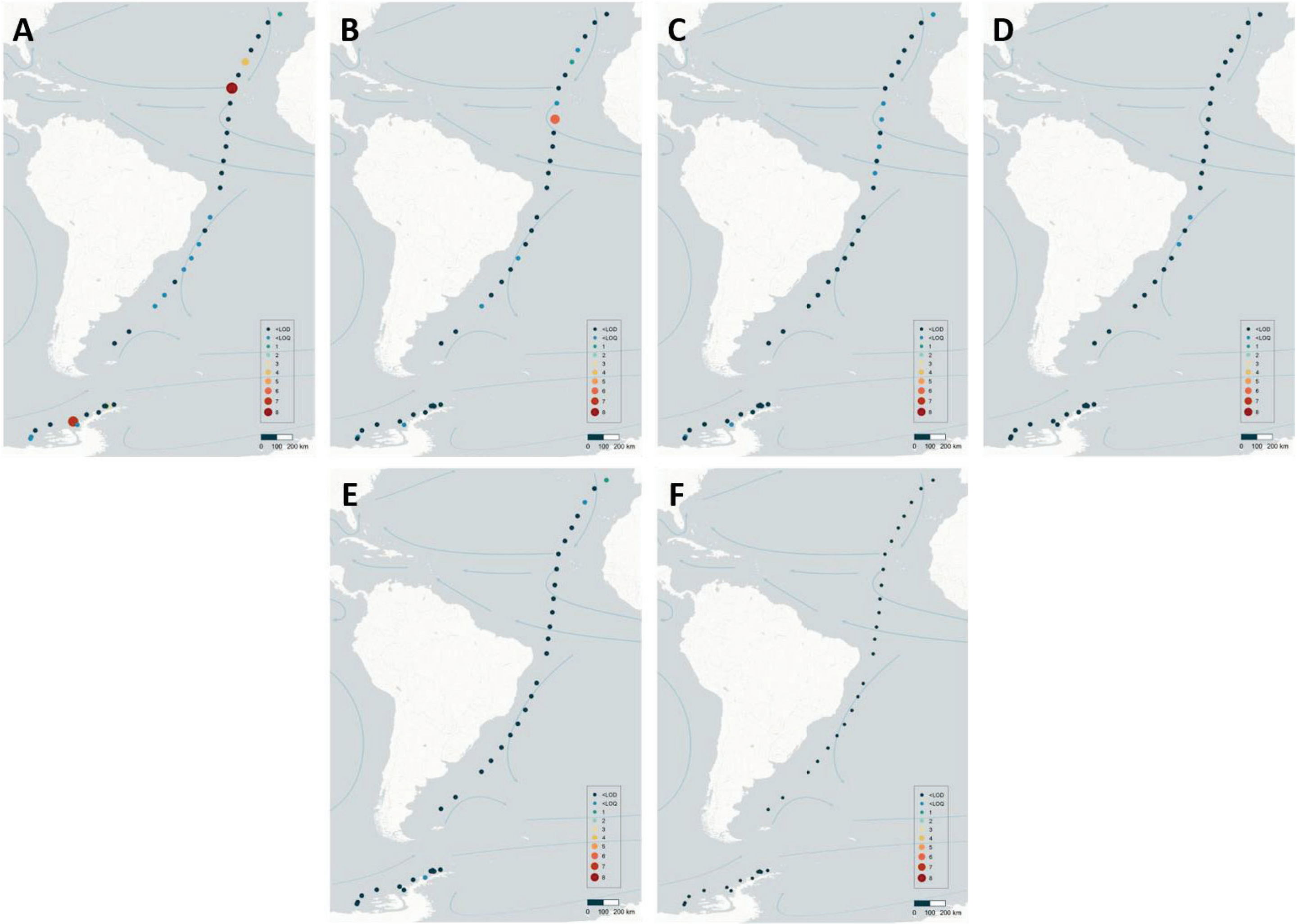


Figure S1 Individual contribution of each targeted polymer to the global MNPLs-polymer pollution found in the whole oceanic transect assessed (ULW 5 m): A) PE, B) PP, C) PS, D) PI, E) PBD, and F) PET. Coloured points indicate concentrations found above the LoDs expressed in µg·L^{-1}.

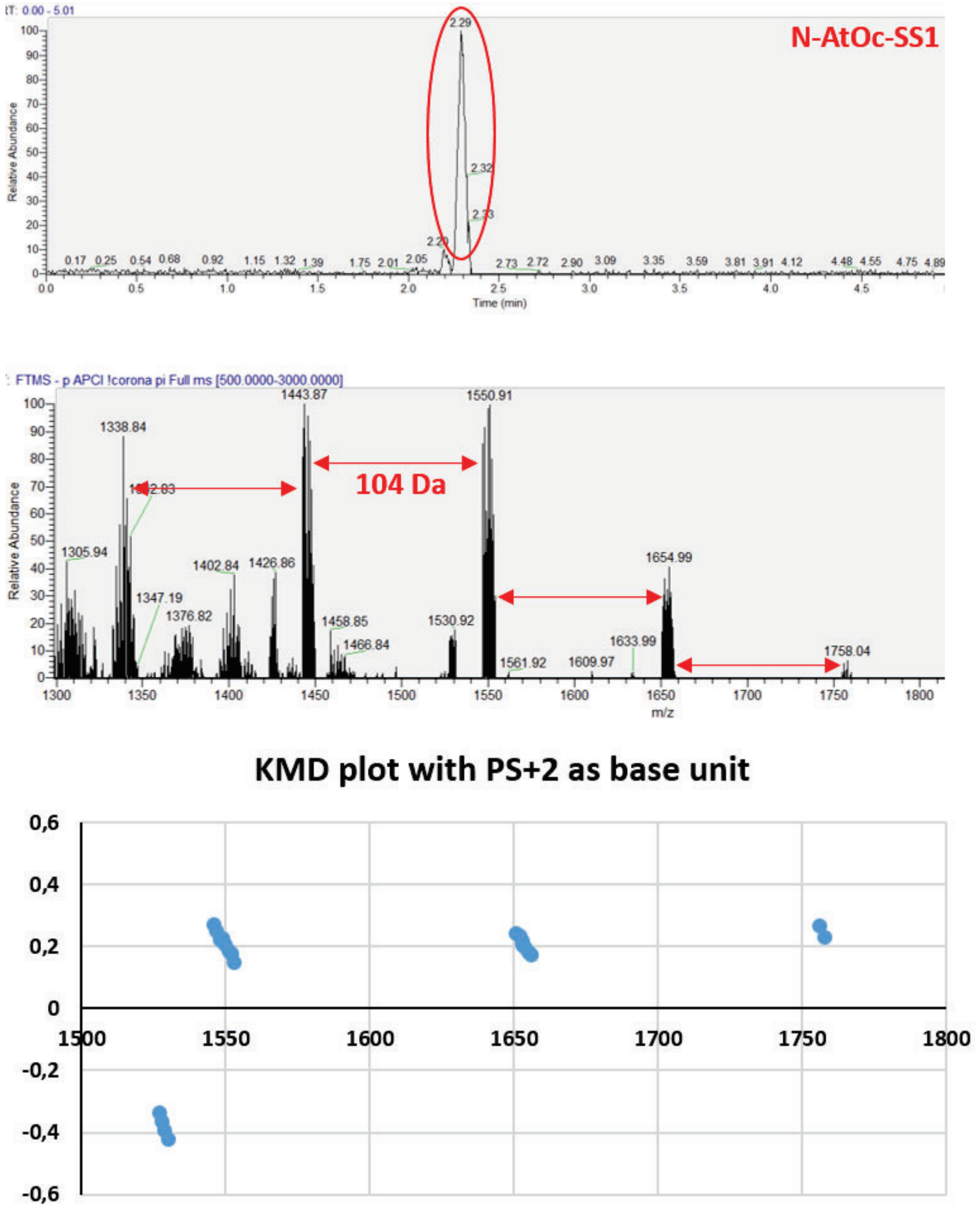


Figure S2 PS-MNPL tentative identified in sample 'N-AtOc-SS 1' through the target analysis approach based on LC(SEC)-HRMS until the 2nd level of confidence by the Kendrick Mass Defect analysis.

4.3 Discussion

Results compiled from **Publications nº1** and **nº2** present novel insights about the presence, fate, and behaviour of MNPLs in two estuarine areas located along the eastern Mediterranean coast of Spain through a double suspect screening approach based on LC-HRMS. For the first time, to the best of our knowledge, both studies provide a report based on the abundance in the mass concentration of MNPLs-polymers found in the surface waters during 2 seasons, corroborating the ubiquity and long persistence of these types of contaminants in those areas. Furthermore, the composition of the plastic additives associated with these MNPLs, and other co-contaminants has also been assessed. Considering that the overall conclusions are already detailed in the corresponding publications, a discussion and comparison of the main results obtained in both study cases are described here.

In addition to the work using LC(SEC)-HRMS, the use of targeted analysis of polymers such as PE, PP, PS, PI, PBD, and PET by employing Py-GC-MS is also presented and discussed, in particles of sizes in between 0.7 and 20 µm in atmospheric PM_{10} and surface marine water samples taken along the Atlantic ocean and Southern ocean during 2 oceanographic expeditions. **Publications nº3** and **nº4** demonstrate the worldwide ubiquity of plastics, including those plastics that come simultaneously from the micro and nanoscale. Notwithstanding, they contribute to broadening the knowledge about the long-range atmospheric transport (LRAT) of MNPLs, as well as their atmosphere-marine surface water exchange dynamics by deposition and re-suspension mechanisms, through a full characterisation of the particles of diameter PM_{10}, demonstrating that MNPLs can reach remote regions. The discussion section complements the published outcomes with a general analysis of, and comparison between **Publications nº3** and **nº4**.

4.3.1 Occurrence and distribution of MNPLs

Rivers, coastal areas, and closed/semi-closed seas, such as the Mediterranean Sea, have become vulnerable due to anthropogenic pressures and, sometimes, limited dilution capacities. Moreover, many pollutants to date are not regulated, but due to their occurrence and adverse effects, are considered to be contaminants of emerging concern (CECs), including polymers, certain groups of plastic additives, and pharmaceuticals, among others (Brumovský et al., 2017). In this chapter, two significant areas under anthropogenic pressures were selected to be studied under the frame of the PLAS-MED and IMAGE projects: the Ebro Delta and the Mar Menor Lagoon.

Firstly, plastic pollution evidence in the Ebro Delta and Mar Menor lagoon was recently reported by Simon-Sánchez et al. (2019) and Bayo et al. (2019), respectively, and both studies are based on a qualitative particle characterisation approach. Thus, in **Publications nº1** and **nº2**, mass-concentrations of polymers and the presence of plastic additives from MNPLs pollution in surface waters have been reported from two Spanish coastal zones: the Ebro Delta (comprising the lower stretch of the river and the related wetlands areas such as the coastal bays), and the Mar Menor lagoon, respectively. Some of the contaminants that are included in both studies have not yet been investigated in these areas; thus, offering a broad view of the influence and impact of the main anthropogenic activities. Another advantage of the methods that are presented in this chapter is the high sensitivity that has permitted provision of robust data with a limited sample size. This is an advantage for sampling campaigns that are undertaken over a long time period, such as oceanographic campaigns. No more than 2.5 L of surface water was needed to perform the analysis at quantifiable concentrations ranging from low levels at $ng \cdot L^{-1}$ to 9 $mg \cdot L^{-1}$ for most common polymers such as PE, PS, PP, PI, PBD, and PVC. In **Publications nº1** and **nº2,** as examples of highly impacted areas, the 88 and 94% of the samples showed the presence of MNPLs, where higher concentrations were reported in sampling sites Nº 8 and Nº 7 from the Ebro Delta and the Mar Menor, respectively. Particularly, the presence of PP in the Mar Menor lagoon was extremely higher, obtaining concentrations during both sampling campaigns between 61 $\mu g \cdot L^{-1}$ (sample n. 6, 'Rambla del Abujón') and 9.3 $mg \cdot L^{-1}$ (sample n. 7, 'Los Urrutias'), where the rest of the polymers detected were found from 100 $ng \cdot L^{-1}$ to 2 $\mu g \cdot L^{-1}$. Moreover, results presented by Bayo et al. (2019) showed a similar spatial distribution and variability tendency to MPLs, with respect to the results reported here. Their most contaminated sampling sites, expressed as MPLs $item \cdot Kg^{-1}$, were located near our sites Nº 6, Nº 7, and Nº 8, where more variability of polymer types and high concentrations of PE and PP were also observed. On the other hand, the presence of polymers from MNPLs in sediments and biota remains poorly studied, to the best of our knowledge. Indeed, polymers in the water body present different occurrence patterns to those patterns in other matrices, since some that are not detected in water might better accumulate in sediments or biota. Theoretically, the concentrations of these pollutants in lipophilic matrices should be higher than in matrices with lower organic and lipidic content, such as water. According to a study that was undertaken by our research group (Garcia-Torné et al., 2023), concerning MNPLs-polymer assessment in 40 gastrointestinal tracts (GITs) of several native fish species of the Ebro River Delta from different trophic levels, MNPLs-polymers of PE, PP, PI, and PS were detected at concentration levels ranging from 0.5 to 3,546 $\mu g \cdot g^{-1}$, where omnivorous fishes were those fishes with higher polymer concentrations in their GITs. The highest concentration found in surface water in **Publication nº1** was 4 orders lower than the peak reported in biota.

Regarding plastic additives composition and other co-contaminants, their identification in surface water samples was specially high in 'Los Alcázares' (Nº 3) and between 'Rambla del Abujón' (Nº 6) and 'Los Nietos' (Nº 8) in **Publication nº2**. These findings agree with the results reported by García-Pimentel et al. (2023) in a quantitative study about plastic additives, PCPs, UV filters, and synthetic musk occurrence. In the case of the Ebro Delta, results from the study of those authors and the results that are presented in **Publication nº1**, when compared, show a similar pattern, where most of these contaminants were identified and reported at high concentrations in the bays or in open coastal sites near the Ebro Delta. Nevertheless, in a recent study by Castaño-Ortiz et al. (2024), the occurrence of pharmaceuticals was assessed, including either river and delta areas in water, sediment, and biota matrices, where the highest concentrations were in freshwater samples from the river, which was unexpected due to the low anthropogenic pressure presented in previous scientific works until now.

Overall, comparing the polymer concentrations reported in **Publication nº1** and **Publication nº2** together with plastic additives, CECs, and POPs concentrations reported in the abovementioned studies, polymer levels from MNPLs were detected between 150 ng·L^{-1} and 10 µg·L^{-1} except for PP values in the Mar Menor lagoon samplings, while almost all of the levels of the other anthropogenic substances were ranging from 1 to 100 ng·L^{-1}. Despite their low concentrations, the great variability of thousands of plastic additives, CECs, and POPs identified make 'this cocktail of chemicals' a potential non-negligible threat to the environment, biota, and human health.

It is noteworthy that the Mediterranean Sea is one of the areas that is most affected by the climatic fluctuations between seasons, with important changes in hydrological conditions causing large variability pollutants concentrations and compartmentalisation. In this sense, in order to evaluate these fluctuations, in the case of MNPLs, two sampling campaigns were carried out. In **Table 4.1** and **4.2**, the maximum, minimum, mean, and median concentrations (µg·L^{-1}) are summarised, as well as the individual frequency of detection for each MNPLs-polymer found during the Ebro Delta and the Mar Menor samplings, respectively.

These results show the influence of tourism in the Ebro Delta and the Mar Menor during the summer campaign, and the decrease of polymer concentrations in winter in some sampling sites. However, a major distribution was shown in winter. A similar trend was observed for plastic additives, as expected. In Ebro Delta (Figure 4 from **Publication nº1**), it can be observed that in the summer campaign, only 3 sampling sites from 5 (riverine area) were exclusively contaminated by a lower number of contaminants than in the case of the delta's sampling points (6-16, estuarine zone). From the winter results, either riverine or estuarine sampling points presented pollution with no significant differences in spatial distribution. In the case of the Mar Menor study (Figure 3 from **Publication nº2**), it can be appreciated that the total number of pollutants tentatively detected a higher variability along the sampling sites in summer than in the winter season, where between 25 and

Table 4.1 Mean concentrations (µg·L^{-1}) of individual MNPLs-polymers and frequency of detection in surface waters from the Ebro Delta in summer 2018 (S) and winter 2019 (W) through 16 sampling sites.

MNPLs-polymer	Season	Mean	SD	Median	Max.	Min.	% Freq.
PE	S	2	3	0.04	8	0.003	37.5
	W	0.005	-	-	0.005	0.005	6.3
PP	S	0.02	0.01	0.02	0.04	0.01	31.3
	W	1	2	0.03	0.8	0.01	81.3
PS	S	0.2	0.4	0.03	0.8	0.01	25.0
	W	< LoQ	-	-	-	-	6.3
PBD	S	0.063	0.005	0.06	0.07	0.06	12.5
	W	0.3	0.4	0.3	0.6	0.07	12.5
PI	S	3	-	-	3	3	6.3
	W	< LoD	-	-	-	-	0.0

SD: Standard Deviation; < LoQ: below Limit of Quantification; < LoD: below Limit of Detection.

Table 4.2 Mean concentrations (µg·L^{-1}) of individual MNPLs-polymers and frequency of detection in surface waters from Mar Menor lagoon in summer 2018 (S) and winter 2019 (W) through 9 sampling sites.

MNPLs-polymer	Season	Mean	SD	Median	Max.	Min.	% Freq.
PE	S	1.0	0.8	0.9	2	0.2	66.7
	W	1.0	0.8	1.0	2	0.1	77.8
PP	S	5,663	3,481	6,386	9,303	61	55.6
	W	6,300	1,193	6,009	8,744	4,918	100.0
PS	S	0.2	-	-	0.2	< LoD	11.1
	W	< LoD	-	-	-	-	0.0
PBD	S	< LoQ	-	-	< LoQ	< LoD	11.1
	W	< LoD	-	-	-	-	0.0
PI	S	0.1	-	-	0.1	< LoD	22.2
	W	< LoD	-	-	-	-	0.0
PVC	S	1.6	0.8	1.8	2.3	0.7	33.3
	W	< LoD	-	-	-	-	0.0

SD: Standard Deviation; < LoQ: below Limit of Quantification; < LoD: below Limit of Detection.

30 compounds were identified in the main points assessed.

Compared with highly impacted areas, where the sources of MNPLs are quite clear, the fate and behaviour of MNPLs in the open seas and remote areas remain rather unknown even though it was in the early 1970s that the first evidence about the occurrence of plastic particles in the oceans was first presented (Carpenter and Smith Jr, 1972). The study assessed the widespread of plastic pellets from 0.25 to 0.5 cm in the surface marine water compartments from samples taken in the Sargasso Sea (North Atlantic ocean), providing an average concentration of 3,500 items·Km^{-2} by spectroscopy. However, the approaches used then, with some improvements but with the drawbacks already mentioned, are the same and are mostly used nowadays, to the best of our knowledge. To tackle the global scope of plastic pollution through quantitative evidence, we have participated in two oceanographic expeditions under the framework of the ANTOM project. In these studies, we showed that the polymeric concentrations in the Atlantic ocean were in the range of 0.01-51.74 ng·m^{-3} in the marine low atmospheric compartments at 10 m above sea level (**Publication nº3**), while in the underlying seawater at 5 m depth (ULW) they were in the range of 2 to 8 µg·L^{-1} (**Publication nº4**), thus highlighting that the highest concentrations found simultaneously in both marine compartments were in sampling transects and stations located at latitudes similar to the latitudes of the North Atlantic ocean subtropical gyre (between N-AtOc-SS 3 and N-AtOc-SS 9). Near the tropical gyres, the reported concentrations were similar in highly impacted areas such as the Mar Menor, with median concentrations of about 6 µg·L^{-1}. In the South Atlantic ocean (from S-AtOc-SS 15 and S-AtOc-SS 24), the occurrence and abundance of MNPLs-polymers were slightly lower, reaching 13.16 ng·m^{-3} in the atmosphere, and concentrations below the limit of quantification in seawater at 5 m depth. In the case of the sampling transects and stations assessed between N-AtOc-SS 10 and S-AtOc-SS 14, including the Equator, no quantifiable concentrations were reported, either in the marine atmosphere or in ULW. Moreover, according to the sampling performed in the sea surface microlayer by a Manta Trawl sampler, the number of polymers identified was higher than in the ULW. This agrees with previous scientific studies, which suggest that the marine zones covered for subtropical gyres are expected to trap MNPLs temporally and to present, thus, the highest concentrations either in air or seawater (Law, 2017; Ter Halle et al., 2017). The occurrence of MNPLs-polymers was also assessed in the Bellingshausen Sea (west Antarctic peninsula, **Publication nº4**) with concentrations ranging from 1 (Ant-SS 1) to 7 µg·L^{-1} (Ant-SS 5) obtaining thus, similar pollution levels as in the North Atlantic ocean and confirming the global widespread of these types of contaminants, including remote regions that are considered to be relatively pristine, where the most detected and found at quantifiable concentrations were PE, PP, and PS, in accordance with other previous scientific studies (Isobe et al., 2017; Lacerda et al., 2019). Nonetheless, the presence of MNPLs-polymers in the sea surface microlayer (SML) compartment was higher than in ULW, suggesting a possible MNPLs enrichment due to SML characteristics led by the abundance of complex structures of polysaccharides, proteins, and lipids, as well as other varieties of organisms (Goßmann et al., 2023). Notwithstanding, further mass-quantitative studies are necessary in the SML compartment.

4.3.2 LRAT processes & marine atmosphere-surface seawater mass exchange of MNPLs

The long-range atmospheric transport of MNPLs have been studied in this chapter along the North Atlantic ocean (31.71°N) and the South Atlantic ocean (45.11°S) by a mass-quantitative assessment of the MNPLs-polymeric composition in the air phase, and the potential of regional sources and sea-spray aerosols by a comprehensive characterisation of PM_{10} which allowed quantifying concentrations of crustal, carbonaceous, marine, and secondary inorganic compounds, as well as the evaluation of air mass back-trajectories generated by the HYSPLIT model.

Nonetheless, MNPLs are also able to reach remote regions such as the Antarctic continent, mainly through LRAT processes consisting of successive atmospheric deposition and re-suspension mechanisms, as well as by the marine seawater body, where the Circumpolar current 'barrier theory' has been questioned recently, and thus ensuring that ocean surface currents can also contribute to the transport of MNPLs (Lozoya et al., 2022). Moreover, the average low temperatures in Antarctica play an important role in the environmental fate and behaviour of MNPLs, repressing re-suspension mechanisms and favouring cold trapping, acting thus, as a net sink for many contaminants. Notwithstanding, in the context of rapid climate change experienced in polar regions, the remobilisation of these contaminants to the atmosphere, which were previously trapped in water, ice, and soil for centuries is expected to again promote the environmental and human exposure of these toxic substances (Bigot et al., 2016).

Thereby, in order to complement the outcomes reported in **Publication nº3**, which cover a large transect from the North Atlantic ocean to the South Atlantic ocean, regarding the LRAT processes given by the different MNPLs-polymers concentrations found in the air phase, together with the air mass back trajectories generated from the HYSPLIT model, and the MNPLs air-seawater exchanges estimations carried out through the atmospheric dry deposition fluxes and the MNPLs-polymer concentrations in the sea-spray aerosol, calculated through the PM_{10} composition, the estimation of the global marine atmospheric half-life ($T_{1/2}$) for the ∑MNPLs-polymers is assessed by a comprehensive review of the levels reported in the body of literature on the marine atmosphere over time (**Table 4.3**), and following the methodology described in Luarte et al. (2023), deriving the e-folding or characteristic decreasing time (T_D), which was calculated by least squares adjustment to the concentrations of **Equation 4**:

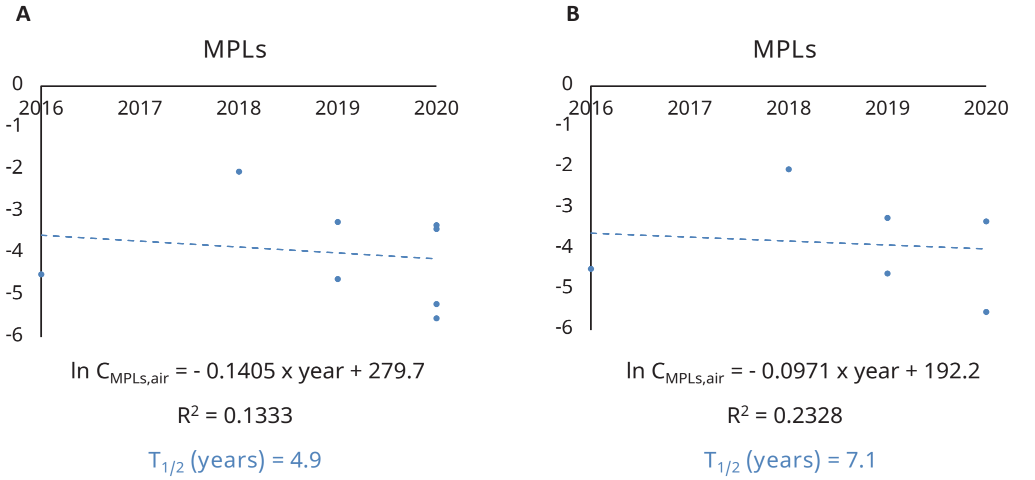
$$\ln \mathrm{C(MNPLs, air)} = -\mathrm{Kd} \cdot \mathrm{t} + \mathrm{b} \qquad (\textbf{Equation 4})$$

where ‘K_d’ is the inverse of the e-folding time T_D (in years), ‘t’ is the time in years, and ‘b’ is the independent term. The half-life is defined as the time needed to decrease the atmospheric concentration by 35% (e^{-1}) of its initial concentration, which is given by $0.69 \cdot T_D$ (**Figure 4.3**).

It must be highlighted that the recent, and few in number, scientific publications which are currently available in the body of literature regarding airborne MPLs abundances in the marine atmosphere, have given the airborne MPLs abundances as items per volume unit by qualitative extrapolations, that have been provided through particle-counting approaches while simultaneously underestimating the micro-fraction and the nano-fraction. In this context, the data reported in **Table 4.3** cannot be compared with our mass-quantitative levels that are given in **Publication nº3**.

Table 4.3 MPLs-polymers levels (items·m^{-3}) reported in the marine atmosphere from 2016 to present.

Sampling area	Type of sampling	Year	Average Σ MPLs	Reference
North Atlantic Ocean	Active	2016	0.0112	(Trainic et al., 2020)
Western Pacific Ocean	Active	2018	0.1300	(Liu et al., 2019b)
Western Pacific Ocean	Active	2019	0.0100	“ “
South Pacific Ocean	Active	2019	0.0392	(Chen et al., 2023)
North Pacific Ocean	Active	2020	0.0361	“ “
Southern Ocean	Active	2020	0.0330	“ “
East Antarctica	Active	2020	0.0055	“ “
South China Sea	Active	2020	0.0039	(Wang et al., 2021)



A: $\ln C_{MPLs,air}$ = - 0.1405 x year + 279.7

R^2 = 0.1333

$T_{1/2}$ (years) = 4.9

B: $\ln C_{MPLs,air}$ = - 0.0971 x year + 192.2

R^2 = 0.2328

$T_{1/2}$ (years) = 7.1

Figure 4.3 Atmospheric MPLs levels (items·m^{-3}) over time according to literature reported in Table 4.3, as well as the estimated atmospheric half-life times. A) MPLs concentrations reported including, and B) excluding concentrations from polar regions.

Thereby, through the qualitative MPLs levels plotted in **Figures 4.3**, MPLs $T_{1/2}$ of 4.9 and 7.1 years considering and excluding polar regions, respectively, were obtained. The outcomes agree with the above-mentioned levels for polar areas, where repressing re-suspension mechanisms are promoted, thus favouring cold trapping, and in consequence, the net sink of MPLs at the global scale, or the decreasing of $T_{1/2}$. Furthermore, the MPLs atmospheric half-life which is reported here does include remote regions (**Figure 4.3A**, $T_{1/2}$ = 4.9 years), and is similar to other estimations that have already launched in the body of literature for different POPs. In the study by Luarte et al. (2023), the $T_{1/2}$ for the most reported POPs in the Antarctic atmosphere were calculated by the marine atmospheric levels that were reported during the period of 1980-2021. $T_{1/2}$ values ranged from 3.6 to 7.6 years for polychlorinated biphenyls 28, 52, 101,118,138, 153, and 180 congeners. Notwithstanding, $T_{1/2}$ were also calculated for hexachlorobenzene, α-hexachlorocyclohexane, 4,4' dichlorodiphenyltrichloroethane, and 2.4' dichlorodiphenyldichloroethylene, obtaining values of 14.0, 14.3, 17.2, and 17.6 years, respectively. For these POPs, the Stockholm Convention ban on POPs (effective from 17 May 2004) did have an impact on decreasing their levels during the last decades. Nevertheless, their ubiquity and high atmospheric half-life values point out the problematic issues related to highly persistent synthetic chemicals.

4.3.3 Vertical transport of MNPLs in the ocean

Another crucial pathway for the transportation of MNPLs at the long-range scale, is by the bioaccumulation of MNPLs in living organisms and their subsequent biomagnification processes along the different levels of the trophic chain, covering thus, the whole marine seawater body. Numerous aquatic biota, including planktonic, invertebrates, and vertebrates species have been found to bioaccumulate MNPLs (Liu et al., 2024). However, due to the lack of quantitative analytical methodologies, the same as for the marine water compartments, the levels of MNPLs bioaccumulated in biota is, to date, unknown. In this sense, in **Publication nº4**, through the Manta Trawl sampling, several species that were captured, such as phytoplanktonic (algae) and zooplanktonic organisms, including invertebrates (jellyfish or krill) and vertebrates (fish), were also assessed in order to understand the abundance of MNPLs that are internalised in these organisms.

Phytoplankton, including eukaryotic algae, plays a key role in the primary production at the foundation of the aquatic food chain. Recent scientific studies have predominantly focused on examining how MNPLs affect algal cell development and photosynthesis through surface adsorption. For example, bigger MPLs can cause detrimental impacts by impeding light penetration (Casabianca et al., 2020), while in the case of smaller MPLs (1-5 μm) and NPLs light can penetrate the cells, thus inducing cellular damage, as demonstrated in the study by Chen et al. (2020), where marine Platymonas helgolandica and freshwater Scenedesmus quadricauda algae phytoplankton were exposed to small MPLs of those sizes during 72 h, and the MPLs were able to be internalised. In both studies, the size-dependent effects of MNPLs uptake by phytoplankton is highlighted. Nevertheless, to our knowledge, no evidence has appeared in the body of literature regarding the abundance levels of MNPLs in marine algae by mass-quantitative methods. From the outcomes reported in **Publication nº4**, the abundance of MNPLs accumulated were in the range of 1-7 $\mu g \cdot g^{-1}$, where the highest MNPLs-polymers concentrations were obtained in the phytoplanktonic species (*Sargassum*) from the Atlantic ocean.

On the other hand, zooplankton are crucial contributors to the ecological dynamics of the aquatic food chain, and they are recognised as one of the most susceptible groups to be impacted by MNPLs. This susceptibility arises from the resemblance in size between MNPLs particles and the primary food source of zooplankton, which is phytoplankton (Richon et al., 2022). In this sense, in accordance with our results, MNPLs-polymers concentrations were, as a general trend, slightly higher in jellyfish (*Physalia*) and krill (Euphausia) than in algae samples that were collected and in the range of 6-18 $\mu g \cdot g^{-1}$ and 4-21 $\mu g \cdot g^{-1}$, respectively due to biomagnification processes between these invertebrates and algae phytoplankton. The same correlation can be seen in the case of vertebrates, whereby the fish (*Cheilopogon*) taken in S-AtOc-SS 18 presented the highest concentration reaching 27 $\mu g \cdot g^{-1}$ considering that the PBD contribution was discarded because of the fact that it was found to be at concentrations that were above the assessed linearity range. As in the case of phytoplankton, the uptake of MNPLs by zooplankton facilitates their transfer through the food chain, leading to the bioaccumulation of these particles, and the subsequent detrimental health effects on higher trophic marine organisms.

Furthermore, previous studies also showed that MPLs can be fragmented into smaller particles, including NPLs through digestive tract activity in Antarctic krill (*Euphausia superba*); however, to our knowledge, to date associated mechanisms are largely unknown. Specifically, this species is the key between phytoplankton and higher tropic chain organisms being able to travel long vertical distances in Antarctic marine ecosystems. Notwithstanding, to our knowledge, to date no study has been made available that highlights the interplay of MNPLs regarding the trophic transfer of these pollutants and their associated impacts along the food chain. Therefore, some recent works have already been initiated, that aim to tackle the trophic transfer of individual MNPLs and the associated complexities along the food chain in freshwater and marine water ecosystems (Junaid et al., 2024).

Overall, to the best of our knowledge, this research direction is novel and of critical importance in highlighting the ecological consequences of MNPLs pollution along the whole marine seawater body in oceans and remote regions.

4.4 References

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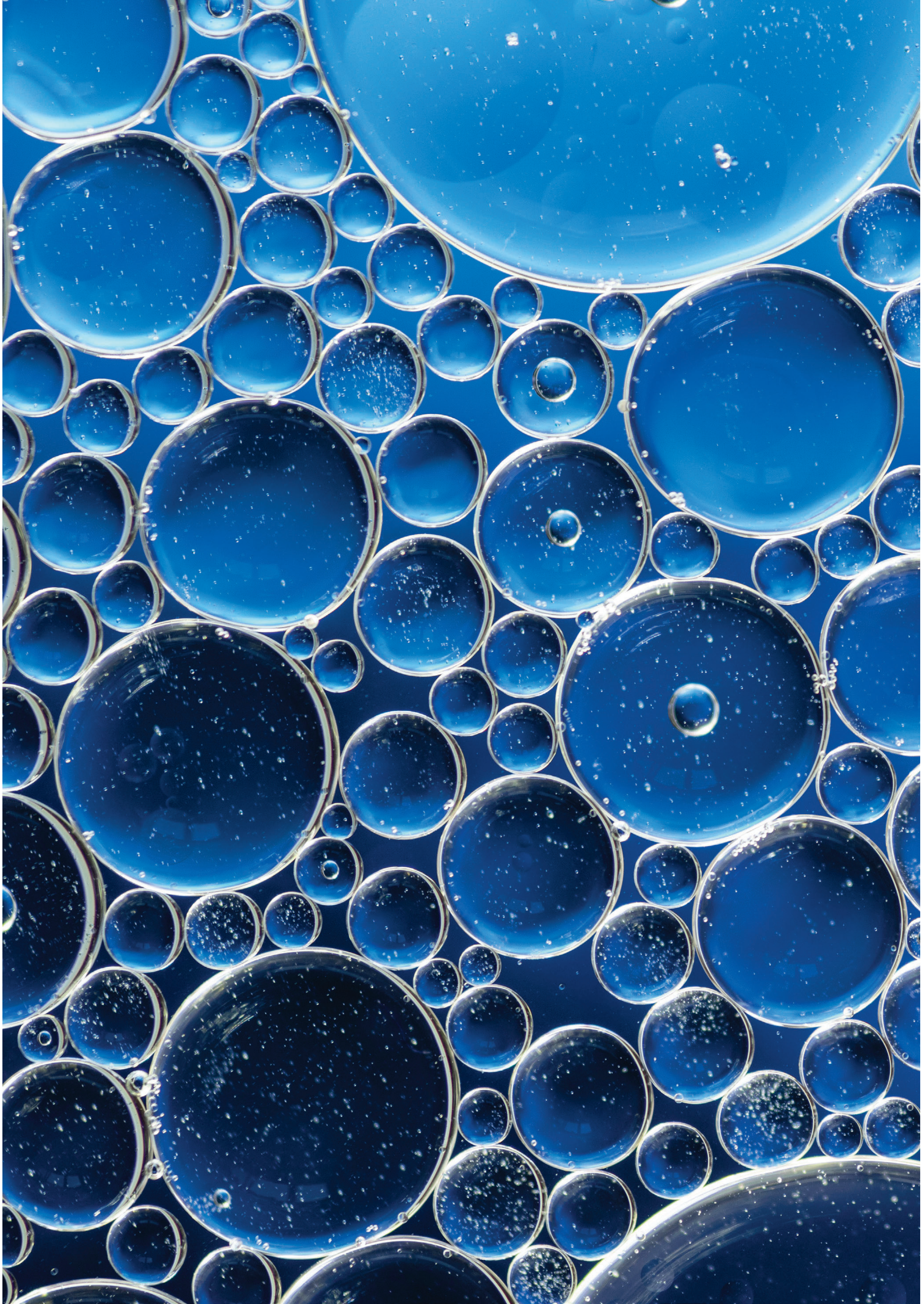
5

Interaction between MNPLs & other contaminants: sorption and desorption behaviour



5.1 Introduction

This chapter focuses on the adsorption and desorption processes of other organic contaminants onto MNPLs' surfaces, and their implications as transport vectors of contaminants at a long range or to biota, known as the Trojan Horse effect. The hydrophobicity of polymers and the environmental properties rule the adsorption/desorption of not only organic contaminants but also organic material, microorganisms, and even pathogens. In **Figure 5.1**, the main properties of MNPLs, co-contaminants, and the relevant environmental conditions ruling the adsorption/desorption properties of MNPLs are summarised.

Some kinetic studies have provided information about the adsorption rate, the performance of the adsorbent, and the mass transfer mechanisms, including the external and internal diffusion and the adsorption of the adsorbate in the active sites of the adsorbent. These studies make it possible to predict similar compounds' adsorption properties and kinetics. The most applied kinetic models are the pseudo-first order and pseudo-second-order models using a linearisation approach, even though either model may present disseminated errors due to incorrect calculations of the model parameters. To minimise these types of limitations, Wang and Guo, (2020) proposed a mixed-order model that represents the overall adsorption process, and this model has been successfully employed to describe the adsorption of several materials onto MNPLs (Zhang, 2019). On the other hand, isotherm models which focus on the adsorption onto active sites have also been widely developed, assuming that this is the slowest step and, thus, the diffusion process is negligible. The Freundlich isotherm model has been successfully employed to predict adsorption onto a surface from a gas and aqueous media on porous surfaces, taking into account the molecular interactions between adsorbates, and indicating the heterogeneity of the adsorption sites (Hatch et al., 2012). Moreover, Temkin and Dubinin-Radushkeich models are also widely used to analyse the equilibrium sorption between the adsorbent and adsorbing particle and the description of the adsorption in microporous materials, respectively, specifically those based on carbon (Lionetto and Esposito Corcione, 2021).

Several scientific studies have reported the interaction between MNPLs and metals, organic contaminants of emerging concern (CECs), natural colloids, engineered nanomaterials, microorganisms such as bacteria, and specially for certain persistent organic pollutants (POPs), which present a great affinity for surfaces of plastic particles due to their general higher hydrophobicity and aromaticity (Velzeboer et al., 2014). In **Table 5.1**, some studies, that were carried out at a laboratory scale, concerning the transport of co-contaminants by MNPLs are shown, as well as the findings obtained. Furthermore, the regression of the metabolisation of some organic contaminants in aquatic ecosystems when they are adsorbed on plastic surfaces has also been demonstrated (Qu et al., 2018).

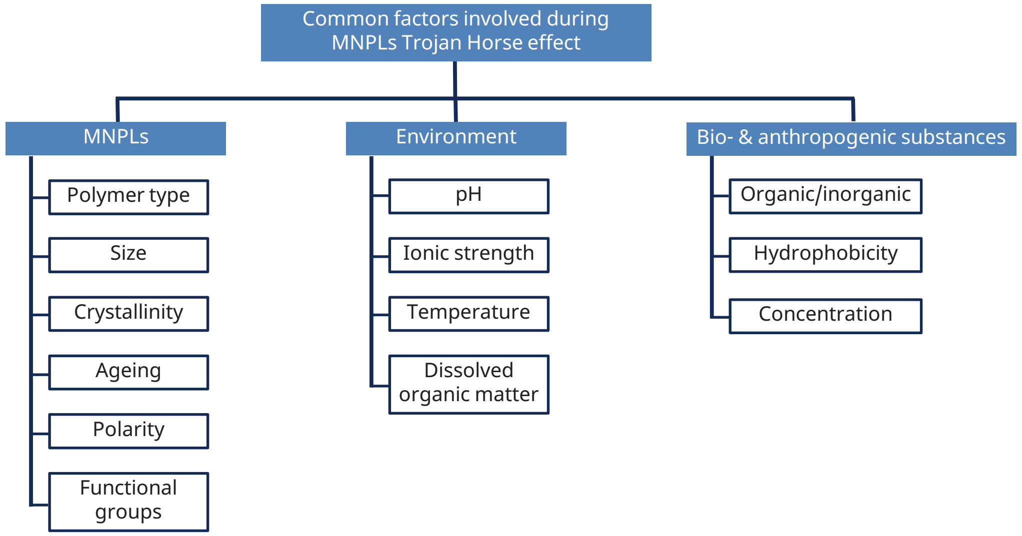


Figure 5.1 Main factors from the polymer, environment, and co-substances involved during MNPLs Trojan Horse effect.

Table 5.1 Adsorption-desorption experiments between MNPLs and co-contaminants or microorganisms co-existing in the same media.

MNPLs (particle size)	Co-contaminant or microorganism	Findings	References
PS (1 μm)	Naphthalene	PS enhance the mobility of naphthalene at low ionic strength.	(Hu et al., 2020)
PVC (75 μm)	Ciprofloxacin	Sorption kinetics and isotherms, influence of pH and salinity, and interaction mechanisms.	(Liu et al., 2019a)
PE (150 μm)	Tetracycline	Effects of pH, temperature, and ionic strength on the adsorption-desorption mechanisms.	(Shen et al., 2018)
PS (500 nm)	Cu (II), Cd (II)	PS presence decreased Cu and Cd in roots and leaves, heavy metals inhibited root and shoot growth, but PS alleviated the inhibition, while ROS content decreased.	(Zong et al., 2021)
PS (700 nm)	Methamphetamine	PS increased the acute toxicity and oxidative damage of methamphetamine in algae, as well as the apoptosis and filtration rate in snails, and also altered the BCF and biodistribution of methamphetamine.	(Qu et al., 2020)
PE, PP (500-600 nm)	Escherichia coli, Bacillus subtilis, Staphylococcus aureus	Decreased lipid peroxidase activity, and increased antioxidant ratio lead to the occurrence of oxidative stress.	(Saygin and Baysal, 2020)
PS, PS-COOH, PS-NH$_2$ (50 nm)	Diethylhexyl phthalate	Effects on selected functional groups on PS. PS-NH$_2$ presented the highest affinity to diethylhexyl phthalate, then PS, and finally PS-COOH particles.	(Yasir et al., 2022)

Thereby, adsorption-desorption processes change some of the MNPLs' physicochemical properties, such as buoyancy, and, as a consequence, their fate and behaviour in the environment (Zhou et al., 2022). In this sense, organisms, once they accumulate MNPLs, may also contribute to the horizontal effective transport of MNPLs, such as seabirds as well as any migrating animals which are used to travelling long distances between terrestrial, aquatic, and atmospheric environments. Also, the vertical transport along the water column in aquatic ecosystems from seas and oceans is noteworthy, as was detailed in the previous chapter. MNPLs tend to be accumulated, firstly, in biota located in the surface water compartments (e.g., planktonic species). However, some plastic particles, including those with other substances adsorbed, can sink directly. Then, mesopelagic species, through their vertical migrations, can transport nutrients and contaminants ingested on the surface, where those bioaccumulated are finally biomagnified in other organisms from the deepest sea and ocean compartments; thus, covering the whole water column (Lusher et al., 2016).

This chapter has focussed on increasing knowledge of the adsorption-desorption behaviour of persistent organic contaminants, and the competence between organic material and MPLs in the adsorption of contaminants. Polychlorinated biphenyls (PCBs) were selected as representative persistent contaminants, and the adsorption of diverse polymeric materials was assessed in simulated estuarine (seawater-sediment) systems at a laboratory scale.

5.2 Results

The outcomes obtained by the adsorption-desorption studies, are presented in **Publication nº5**:

- **Publication nº5:** Llorca, M., Ábalos, M., Vega-Herrera, A., Adrados, M. A., Abad, E., Farré, M., Adsorption and desorption behaviour of polychlorinated biphenyls onto microplastics' surfaces in water/sediment systems, 2020, Toxics, https://doi.org/10.3390/toxics8030059

5.2.1 Publication n°5

Adsorption and desorption behaviour of polychlorinated biphenyls onto microplastics' surfaces in water/sediment systems

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Article

Adsorption and Desorption Behaviour of Polychlorinated Biphenyls onto Microplastics' Surfaces in Water/Sediment Systems

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Abstract: The potential of microplastics (MPLs) in marine ecosystems to adsorb and transport other micropollutants to biota, contributing to their entry in the food chain, is a primary cause of concern. However, these interactions remain poorly understood. Here, we have evaluated the adsorption/desorption behaviour of marker polychlorinated biphenyls (PCBs), onto MPL surfaces of three widely used polymers—polystyrene (PS), polyethylene (PE), and polyethylene terephthalate (PET). The range of MPL sizes ranged from 1 to 600 μm. The adsorption/desorption was evaluated in sediment/water systems in marine microcosms emulating realistic environmental conditions for 21 days. The adsorption percentages ranged from 20 to 60%. PCBs with a lower degree of chlorination showed higher adsorption percentages because of conformational impediments of PCBs with high-degree chlorination, and also by their affinity to be adsorbed in sediments. Glassy plastic polymers as PET and PS showed a superior affinity for PCBs than rubbery polymers, such as PE. The polymers that can bond PCBs by π-π interactions, rather than van der Waals forces showed better adsorption percentages, as expected. Finally, the adsorption/desorption behaviour of selected PCBs onto MPLs was fitted to a Freundlich isotherm model, with correlations higher than 0.8 in most of the cases.

Keywords: microplastics (MPLs); polyethylene; polystyrene; polyethylene terephthalate; polychlorinated biphenyls; adsorption; marine microcosm

1. Introduction

Some of the primary problems associated with the presence of plastic litter in marine environments are their behaviour as a source and transfer vectors for co-contaminants to the aquatic food web. These impacts are partially influenced by their size. Microplastics (MPLs), defined as plastic pieces below 5 mm, including nanoplastics (NPLs), is a different environmental problem compared to macro- and meso-plastic pollution. MPLs can enter in the environment in the small-size range (classified as primary MPLs) or can be generated once in the environment by fragmentation and erosion of plastic pieces and debris (classified as secondary MPLs). MPLs/NPLs, due to their small size, similar to plankton, can be ingested by aquatic organisms, and therefore be introduced into the marine food web [1–3]. It should be highlighted that fish, bivalves, or mammals cannot digest MPLs since they do not have enzymatic pathways available to break down the synthetic polymers. However, these particles can be retained in some organs [4], and the nanoparticles, due to their small size, can be translocated in living tissues with adverse effects. It is estimated that some of the plastics can reach concentration

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factors inside the organisms near to a 1 million-fold increase [5]. The interaction between polymers and contaminants is a very complex problem that remains poorly understood. Therefore, realistic risk assessment studies to characterize and evaluate this type of interaction are highly required to establish measures to minimize the negative effects of MPLs in coastal environments. The adsorption/desorption behavior among MPLs and co-contaminants is influenced by different factors, such as the type of co-contaminant, the polymeric matrices of MPLs (e.g., polyethylene, polystyrene, or a combination of different monomers), their relative concentrations, and the environmental conditions. However, some trends have been identified, such as the high capacity to adsorb and accumulate hydrophobic organic chemicals (HOCs) on their surface from the surrounding areas [6–9]. In consequence, and in addition to the already defined routes of exposure to HOCs, MPLs could act as a back door to the entrance of these contaminants to organisms once they are adsorbed in the polymer surface [2,6,10–12] and the human food chain.

Polychlorinated biphenyls (PCBs) are a group of manmade HOCs that were widely used in the past in electrical equipment. They are currently forbidden and, since 2001, the 209 congeners are listed in the Stockholm Convention of persistent organic pollutants (POPs) [13]. Although they have been banned, PCBs are still present in the environment and are present ubiquitously in biota [14] and sediments [15].

The adsorption of PCBs in MPLs from natural environments has been informed [16]. The uptake and incorporation of these contaminants from MPLs to biota have also been assessed [17]. For example, it has been observed that PCBs adsorbed on polystyrene (PS) and polyethylene (PE) were significantly bioaccumulated in Norway lobster [11], while the bio-uptake of sediment invertebrate worms from contaminated polypropylene (PP) with PCBs was low, but not negligible [10].

On the other hand, some studies conducted under controlled conditions assessed the adsorption of organic contaminants onto MPL surfaces [8,9]. In the case of PCBs, the parameters for the adsorption of PCBs have been studied to micro-PE and nano-PS in a system composed of plastic particles, PCBs, and water [18,19]. However, complementary studies in more realistic environmental scenarios, including sediments competing with the MPLs and the suspended organic material, are necessary to describe the adsorption/desorption behaviour of HOCs on MPL surfaces in aquatic systems.

In this regard, the main objective of this work was to characterize the adsorption/desorption capacity of MPLs of polystyrene (PS), high-density polyethylene (HDPE), and polyethylene terephthalate (PET) (sizes between 1 and 300 µm) for seven commonly detected polychlorinated biphenyls (PCBs: 28, 52, 101, 138, 153, 180, and the dioxin-like PCB 118), that are used as contamination indicators. The experiments were designed for a water/sediment system at environmental conditions and relevant concentrations of PCBs. Since amorphous and semi-amorphous plastics are less resistant to chemical attack and environmental stress [20], PS was chosen as an example of amorphous thermoplastic. On the other side, PE and PET were selected because of crystalline or semi-crystalline structures that make them resistant to chemical attack [20]. Furthermore, the diffusion capacity of glassy plastic polymers (i.e., PET and PS), which used to have less diffusion than non-glassy or rubbery polymers (i.e., PE) [21], will be evaluated.

2. Materials and Methods

2.1. Materials

MPLs of HDPE microspheres ranging from 3 to 16 µm were supplied by Cospheric (Santa Barbara, CA, USA); 10 µm PS, supplied by Phosphorex (Hopkinton, MA, USA); and PET microspheres below 300 µm from GoodFellow (Huntingdon, UK).

Mixtures of seven native Marker PCBs (IUPAC nos. 28, 52, 101, 118, 138, 153, and 180) (BP-D7) and labelled PCBs (MBP-MXE; P48RS), as well as P48-M-CV calibration solutions for Marker PCBs were supplied by Wellington Labs (Guelph, ON, Canada). These standards were used for spiking experiments (spiking mix prepared in methanol at 10 µg/mL), quantification, analytical recovery calculation, and calibration.

Solvents used during sample treatment and analysis were of high purity and supplied from J. T. Baker (JT Baker, Phillipsburg, NJ, USA): methanol, acetonitrile, dichloromethane, n-hexane, ethyl acetate, n-nonane, and water. Sodium chloride ≥ 99% was purchased from Merck (Madrid, Spain).

2.2. Experimental Design

Mixtures of seawater and real sediments that were previously analysed, characterised, and fortified at different concentrations with the PCB mixtures were used to carry out the adsorption experiments under environmentally relevant conditions.

Sediment samples of Alfacs Bay (Ebro Delta, Catalonia, NE of Spain; 40.622999, 0.661707) were obtained. The samples were collected in aluminium foil trays and transported to the laboratory in cold conditions. Once in the lab, 500 g of sediment was dried at room temperature (25 °C) under a fume cupboard, then sieved and homogenised. Afterwards, the background concentration of marked PCBs was characterised according to Sections 2.3 and 2.4.

Hereafter, six sediment subsamples of 4.5 g were separated, and they were spiked to the following concentrations of the PCB mixture (0, 5, 10, 15, and 25 ng/g d.w spiked to the appropriate volume of a mixture of 10 µg/mL in methanol). The subsamples were left to reach the equilibrium in a desiccator for 24 h. After that time, the sediments were homogenised again, and three series of six tubes, one tube with each PCB concentration per set was prepared. In each tube, 4.5 g of sediment was introduced. Then, 2 µg/g of each type of MPL (PE, PS, and PET) was added to each tube. In addition, two more extra tubes were prepared, one tube with sediment without a PCB spike and MPLs, and another tube with sediment and PCBs (spiked at 25 ng/g d.w) but without MPLs. A schematic flowchart can be seen in Figure S1 from the supplementary material.

All the tubes were shaken vigorously in a vortex for 5 min and maintained at room temperature in an orbital digester at 40 rpm for 2 days. Finally, 10 mL of pristine seawater, previously analysed and characterised, was added to each tube. These mixtures were then homogenised for 1 min in a vortex. These seawater/sediment systems were sealed and agitated at room temperature for 21 days in an orbital digester at 40 rpm under dark conditions to avoid the photo-degradation of the organic matter.

After this time, the seawater/sediment systems were saturated with NaCl in order to increase the water density. Floatable MPLs were separated manually with a nano-filter mesh from the upper layer of water, while the rest of the supernatant was separated by decantation. Then, the sediments were dried at room temperature and homogenised before PCB extraction.

2.3. PCB Extraction

About 0.5 g of each dry homogenised sediment were spiked with the labelled surrogate internal standard mixture (MBP-MXE). Then, PCBs were recovered from the sample by Soxhlet extraction for 24 h, with a mixture of n-hexane: dichloromethane (1:1, *v/v*). After that, the extract was concentrated in a rotary evaporator and transferred to n-hexane prior to the clean-up step. Purification and fractionation were carried out in open columns of multilayer silica (2:1; acid/base) and activated Florisil® (at 600 °C) eluted with n-hexane. The final extract was concentrated again to ca. 1 mL in a rotary evaporator, transferred to a 2 mL vial, and evaporated under a nitrogen current. Finally, a known amount of the labelled recovery internal standard mixture (P48-RS) dissolved in n-nonane was added as an internal standard before instrumental analysis. The samples were analyzed by triplicates.

2.4. Analysis by Gas Chromatography Coupled to High-Resolution Mass Spectrometry (GC-HRMS)

The analysis was done in an Agilent Technologies 6890N gas chromatograph (Agilent, Palo Alto, CA, USA) coupled to a Micromass AutoSpec—Ultima NT (Waters, Manchester, UK) high-resolution mass spectrometer (EBE geometry) controlled by a Masslynx data system.

The chromatographic system was equipped with a DB-XLB (Agilent, Folsom, CA, USA) fused-capillary column (60 m × 0.25 mm I.D. × 0.25 µm film thickness). Following the temperature program that can be seen elsewhere [14]. The injection of 1 µL of extract was carried out in splitless mode (60 s) with the temperature of the injector at 280 °C.

The chromatograph was coupled to a magnetic sector spectrometer equipped with an electron impact ionisation (EI) source working at 32 eV, trap current at 500 µA, and acceleration voltage at 8000 V. The acquisition was performed in selected ion monitoring (SIM) mode at a resolving power of 10,000 (5% valley). The ion source and transfer line were set at 250 and 280 °C, respectively.

The quantification of residual PCBs in sediments was done by isotopic dilution methodology described elsewhere [14].

3. Results and Discussion

3.1. Adsorption of Marker PCBs on Selected MPLs

In Table 1, the percentages of adsorption of the marker PCBs on selected MPL surfaces calculated according to Equation (1) are summarized:

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

where % Ads is the percentage of adsorption, $[\text{A}]_t$ is concentration of compound A remaining in sediments after 21 days, and $[\text{A}]_0$ is the concentration of compound A at time 0, both concentrations expressed in ng/g. The distribution of PCBs is schematized in Figure S2.

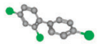
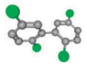
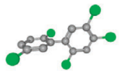
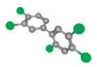
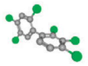
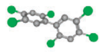
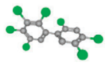
Table 1. Adsorption percentages for PCBs vs. spiked concentration in all polymers and sum of PCBs adsorbed on MPLs.

Spiking Level	PCB Congener	5 ng/g		10 ng/g		15 ng/g		20 ng/g		25 ng/g	
		Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**
%Ads on PE	PCB-28	58.27	5.48	58.24	4.08	60.64	2.43	58.59	1.76	60.88	6.70
	PCB-52	56.29	4.50	59.95	1.68	60.55	3.63	57.14	4.00	58.64	3.05
	PCB-101	41.28	2.06	42.72	2.01	44.93	0.81	41.22	3.67	38.89	1.56
	PCB-118	0.00	0.00	12.89	0.80	14.17	0.69	5.75	1.04	0.00	0.00
	PCB-153	29.03	2.03	36.48	1.09	38.27	2.68	34.21	1.54	29.64	2.67
	PCB-138	5.77	0.36	28.31	2.26	31.01	3.10	27.37	0.55	22.78	0.91
	PCB-180	16.22	0.29	14.21	1.28	28.13	1.60	24.82	2.63	18.61	1.38
ΣPCBs on PE (ng)		0.103		0.253		0.417		0.498		0.574	
%Ads on PET	PCB-28	62.87	4.21	66.96	2.68	63.82	2.55	63.53	10.80	65.47	1.64
	PCB-52	60.02	2.82	62.97	4.22	58.71	4.70	58.06	3.08	60.11	4.03
	PCB-101	46.46	0.74	53.15	3.93	49.49	3.32	47.43	3.32	46.16	5.08
	PCB-118	17.34	0.35	32.13	2.57	20.45	0.39	16.94	1.52	15.66	1.47
	PCB-153	32.81	1.64	39.10	1.96	35.26	1.69	32.93	0.49	31.94	1.53
	PCB-138	21.78	1.96	35.02	4.20	29.28	4.10	26.25	1.05	22.45	1.35
	PCB-180	21.87	1.77	26.09	1.54	26.71	0.99	25.23	4.04	20.98	1.47
ΣPCBs on PET (ng)		0.132		0.315		0.426		0.541		0.657	
%Ads on PS	PCB-28	48.58	0.97	56.12	6.17	50.13	6.52	54.86	4.39	53.46	4.81
	PCB-52	55.19	3.86	60.49	7.86	53.60	7.50	59.41	3.80	57.07	2.17
	PCB-101	38.16	3.05	45.74	3.66	32.49	2.08	41.74	1.17	40.97	4.26
	PCB-118	14.42	1.50	31.22	2.22	8.17	0.66	21.17	1.48	20.19	0.36
	PCB-153	33.45	0.47	41.80	4.51	25.82	1.50	37.81	4.54	38.74	2.60
	PCB-138	26.42	1.59	34.24	1.57	15.50	1.86	31.00	4.34	36.63	0.51
	PCB-180	23.52	1.65	18.09	1.34	5.93	0.24	27.52	3.03	29.47	1.47
ΣPCBs on PS (ng)		0.063		0.151		0.151		0.287		0.363	

3.1.1. Adsorption onto PS-MPLs

In Table 1, the adsorption percentages for PCBs onto the PS surface is shown. The adsorption range was from 10 to 60% and ordered as follows: PCB-52 ≥ PCB-28 > PCB-101 > PCB-153 ≥ PCB-138 > PCB-180. As it can be seen, the congeners with more chlorine atoms in their molecules generally showed less adsorption to PS-MPL surfaces, with the exception of the dioxin-like compound, PCB 118 (dlPCB-118), possibly due to the (co-)planarity of its conformation (see Table 2) [22]. This specific conformation of dlPCB-118 could make difficult its adsorption onto the PS surface. In the case of structures with more chlorines, the lower adsorption compared with the other PCBs with fewer chlorines is due to the surface of the spatial structure of molecules with a high number of chlorines, which make difficult their stabilisation on plastic pore sites. This phenomenon was also observed by Pascall et al. [21] using PS film, where the diffusion coefficient decreased with the increase of molar volume of PCBs. The main hypothesis is that PCBs diffuse into PS particles through the static boundary layer around the PS particle [18]. This is an easier process for molecules with lower molar volume, like PCB-28 or PCB-52. This is a relatively slow process. First, PCBs are adsorbed on the particle surface by weak van der Waals interactions. Then, PCB molecules further diffuse inside the glassy polymeric structure of MPLs like PS [18,23]. Once there, their desorption is less favoured because they are physically entrapped [23]. In addition, the adsorption of highly chlorinated PCBs onto PS-MPLs might have been less favored because of the MPL-sediment competition. It has been reported that highly chlorinated PCBs have a high affinity with sediments.

Table 2. Spatial conformation of marker PCBs.

	Name	Spatial Conformation [24]	Descriptors [22] *	log Kow [25]
PCB-28	2,4,4′-Trichlorobiphenylortho, para, para		CP1, PP	5.67
PCB-52	2,2′,5,5′-Tetrachlorobiphenylortho, ortho, meta, meta		4CL, 2M	5.84
PCB-101	2,2′,4,5,5′-Pentachlorobiphenylortho, ortho, para, meta, meta		4CL, 2M	6.38
PCB-118	2,3′,4,4′,5-Pentachlorobiphenylortho, meta, para, para, meta		CP1, 4CL, PP, 2M	6.74
PCB-138	2,2′,3,4,4′,5′-Hexachlorobiphenylortho, ortho, meta, para, para, meta		4CL, PP, 2M	6.83
PCB-153	2,2′,4,4′,5,5′-Hexachlorobiphenylortho, ortho, para, para, meta, meta		4CL, PP, 2M	6.92
PCB-180	2,2′,3,4,4′,5,5′-Heptachlorobiphenylortho, ortho, meta, para, para, meta, meta		4CL, PP, 2M	7.36

Descriptors [22] *: CP0: no chlorine substitution at any of the "ortho" positions on the biphenyl backbone (also referred as non-"ortho" congeners). CP1: chlorine substitution at only one of the "ortho" positions (also referred as mono-"ortho" congeners). 4Cl: congeners that have a total of four or more chlorine substituents regardless of position. PP: congeners that have both "para" positions chlorinated. 2M: congeners that have two or more of the "meta" positions chlorinated. If one congener has all four descriptors this is referred to as being "dioxin-like", like PCB-118.

3.1.2. Adsorption onto PE-MPLs

As can be seen in Table 1, the percentage of adsorption of PCB-28 and PCB-52 onto PE was nearly 60% after 21 days. Both compounds, PCB-28 and PCB-52, are those with a lower chlorination degree of three and four chlorine atoms, respectively. It has been shown that the adsorption decreases with the increase of the number of chlorine atoms, the final adsorption being ranked as follows: PCB-28 ≈ PCB-52 > PCB-101 > PCB-153 > PCB-138 > PCB-180. Similar adsorption behaviour is also shown for PS. The differences observed among different congeners can be attributed to different effects at the same time. First, there is the steric interaction of PCB molecules with PE surfaces. PE is a nonporous material, with only transient cavities with a typical size of 1 nm [26], which is a limitation for the adsorption of large molecules or big cluster molecules with suspended materials that are excluded by size (Table 1). The aggregates formation between the suspended matter and HOCs as PCBs is expected to happen, especially for the more non-polar compounds [26]. The second factor influencing the lower adsorption onto PE of more chlorinated congeners is their much higher interaction with sediments, as has been reported. In summary, the adsorption onto PE surfaces of PCBs 118, 138, and 180, with high steric impediments due to chlorine number and position was limited, and at the same time, their interaction to sediments was higher. For example, for the PCB 118, the maximum adsorption level was 15% for the spiking of 15 ng/g, and the percentage of adsorption was even lower for higher concentrations of spike because of the high non-polarity of this compound that tends to form a cluster of molecules in seawater (Table 1). These results agreed with previous works, such as when using PE food-packaging plastic film [21], in which it was confirmed that there was a correlation between increasing chlorination and increasing cohesive density within PCB molecules [21]. This phenomenon made difficult the adsorption of molecules with a high number of chlorines. In another work, Allen et al. [27] evaluated the adsorption of PCBs 52, 101, 118, and 153, among others, in six plastic polymers, including high- and low-density PE, PS, and PET, where it was observed that PCBs from spiked waters were easily adsorbed onto PE and PS surfaces with a return of compounds after desorption with n-hexane between 50 and 55% [27].

3.1.3. Adsorption onto PET-MPLs

The results of adsorption of PCBs on a PET surface exhibited a similar profile than that observed for PS and PE (see Table 1). The adsorption capacity for selected PCBs decreased with the increase of chlorines in the molecule. The maximum adsorption is exhibited by PCB-28 (c.a. 70%), followed by PCB-52 (c.a. 60%). However, as observed for the other MPLs, the dlPCB-118 has the lowest tendency to be adsorbed on PET surface, its maximum being at 30% for 10 ng/g of spiking level and the minimum at 10% for higher spiking concentrations.

3.1.4. Comparison among MPLs

Comparing adsorption percentages among MPLs, PET has higher percentages (generally being 10% higher than the others) for all spiking concentrations, as shown in Table 1.

In this sense, the effects observed by Pascall et al. [21], where glassy polymers like PVC have less diffusion and lower adsorption capacity compared to non-glassy or rubbery polymers such as PE, is not confirmed here. In this work, PS and PET (glassy polymers) exhibited equal or even higher adsorption than PE, as it was also observed in a long-term exposition experiment by Rochman et al. [28]. The difference between our work and the one carried out by Pascall et al. [21] can be explained by experimental differences, such as the size of MPLs (1–300 μm in this work) and the type of material used. In the present study, we used granulated MPLs, while Pascall et al. [21] used films.

The adsorption behaviour of PCBs onto the different MPLs materials was PET > PS > PE (Table 1). These results can be explained by the type of interaction between surfaces and PCBs. With the aliphatic chains of PE, only non-specific van der Waals interactions can be established, while PS and PET can also undergo π-π interactions, as pointed out by other authors [27,28].

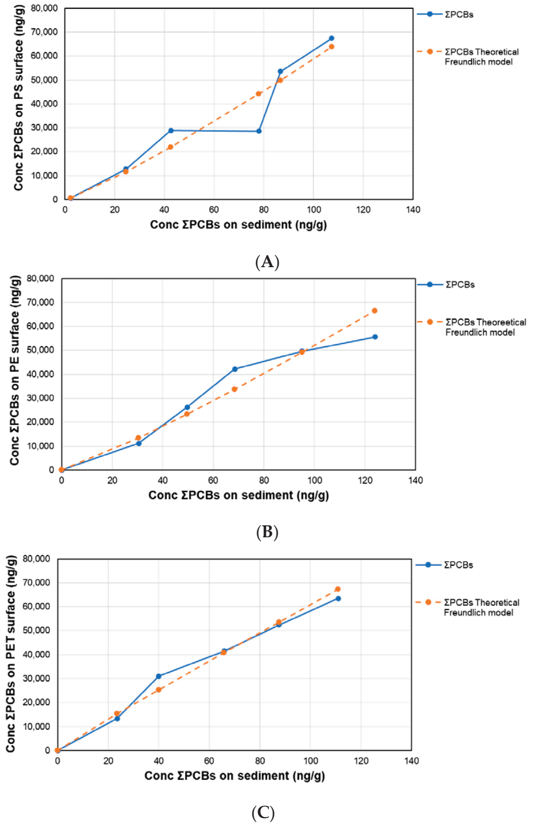
3.2. Adsorption Isotherms of Marker PCBs on Selected MPLs

Different adsorption isotherms were tested, including the Brunauer–Emmett–Teller (BET), Langmuir, and Freundlich models [29]. The Freundlich model was shown to be the one which fit best, and it was selected in all the cases (Equation (2)). This model assumes the presence of an infinite number of different adsorption sites [30]. Then, the equation was linearized in its logarithmic form to obtain the Freundlich constants according to Equation (3):

$$q = K_F C^n \tag{2}$$

$$logq = logK_F + \frac{1}{n}logc \tag{3}$$

where q is the concentration of PCBs onto the plastic surface and C the concentration of PCBs in sediment at the assumed equilibrium time of 21 days. K_F is an indicator of the adsorption capacity, where the higher the value is, the higher the adsorption capacity. Additionally, n is a value ranging from 0 to 10, where an n range between 2 and 10 indicates good capacity of adsorption, between 1 and 2 means moderate adsorption capacity, and less than 1 indicates poor adsorption capacity at high concentrations of compounds [29]. In Figure 1 and Figure S3, the results are presented.



(A)

(B)

(C)

Figure 1. Adsorption isotherms of concentration (Conc) of ΣPCBs after 21 days on MPL surface of (**A**) PS, (**B**) PE, and (**C**) PET vs. sediment.

In the case of PS-MPLs, as it can be seen in Table 3, their characteristic parameters indicate high adsorption capacity to PCBs with a high K_F value, while the adsorption is favoured at the lower concentrations because n is inferior to 1. This agrees with the observations by Hüffer et al. [31] in their investigations of the adsorption of non-polar organic compounds onto MPLs. In that study, the non-linear adsorption isotherm in PS was attributed to surface adsorption.

Table 3. Freundlich equation parameters for ΣPCBs on MPLs in the seawater/sediment system.

	$\log [q] = \log K_F + 1/n \log [c]$	$\log K_F$	K_F (ng/mg)	n
PS	$y = 1.1599x + 2.4499$; $R^2 = 0.9794$	2.4499	281.77	0.862
PE	$y = 1.1452x + 2.4258$; $R^2 = 0.9253$	2.4258	266.56	0.873
PET	$y = 0.9532x + 2.8781$; $R^2 = 0.9572$	2.8781	755.27	1.049

The adsorption capacity of PE and PET MPLs for the selected PCBs in water/sediment systems was shown to be similar to PS-MPLs. Additionally, as can be seen in the Figure 1B,C, and Figure S3B,C, the Freundlich isotherm was the best-fitting equation for the interpretation of the results. In both cases, the adsorption is favoured at low concentrations, with *n* values inferior to 1 for PE-MPLs and similar to 1 for PET-MPLs.

As can be seen for the three types of MPL materials, the congener 118 cannot be linearized, where in this case, their co-planarity (all chlorines are in the same plane) probably produced surface interaction, rather than introduction in pores or cavities. For the rest of the compounds, the steric congestion (presence of surrounding ligands (chlorines in PCBs) in a molecule) increase with the number of ortho-chlorines, which means that di-ortho compounds (52, 101, 153, 138, and 180) present a more steric congestion than the mono-ortho congener 28-PCB. Additionally, among the di-ortho compounds, the steric congestion increases with the total number of chlorines in the molecule. Proportional to the increase in steric congestion, the interaction will be more superficial than in the cavities, and then the adsorption isotherms will be less linear. Besides, when the number of chlorines increases, the non-polarity rises too, and the interactions between the PCBs and the sediments is stronger, and therefore, a lesser amount of molecules will be available to be adsorbed onto the MPLs. In spite of how the Freundlich model cannot explain the 100% of the interactions, in some particular cases, the model fits better to the globally studied system.

3.3. *Environmental Implications*

The main results reported in this work suggest that recalcitrant compounds, such as PCBs, can be readily adsorbed onto cavities of PE-MPL surfaces, rather than inside the polymeric structure; while in PS-MPL and PET-MPLs, a certain degree of diffusion inside the polymeric structure occurred. In the case of PS and PET-MPLs, the π-π interactions also increased the adsorption rate of PCBs in comparison to the adsorption of PCBs onto PE-MPLs, in which the interactions were just van der Waals forces. We want to remark that in this work, we have tested the ability of three types of MPLs to adsorb PCBs in a water/sediment system, and despite the competition with sediments, the MPLs presented an important rate of accumulation. Due to the MPLs' size and variety of colour that has been proved to stimulate their ingestion by fish, this can be a new route of HOC exposure to biota, and an entrance to the aquatic, and therefore to the human food chain. Furthermore, it is essential to study the fugacity gradient of MPLs with adsorbed PCBs between the water/sediment/aquatic food chain, since these are the main driving forces of transfer. Finally, although this study has been focused on seven marker PCBs, similar results are expected for other equally recalcitrant congeners.

4. Conclusions

Within this work, we have reported data regarding the adsorption capacity of MPLs for marker PCBs in water/sediment systems. To the authors' knowledge, this is the first time that the adsorption capacity of MPLs for PCBs has been evaluated in this type of system.

The main results have shown that, after three weeks of exposition, the recalcitrant PCBs are adsorbed, in general, between 20% and 60% onto MPLs of PE, PS, and PET. The tested polymers showed that the adsorption is favoured when π-π interactions can be done like in the case of PET and PS. Finally, the calculated isotherms were fitted to a Freundlich equation with a Pearson coefficient higher than 0.9 for the sum of PCBs and, in almost all the cases, higher than 0.8 for individual PCBs. With these data, it can be predicted that PCBs dissolved at low ppt and sub-ppt concentrations will be readily and strongly attached to MPL surfaces, given that PCBs associated with plastic particles are likely to be a significant factor in the environmental fate, behaviour, and potential transfer to the food chain.

Supplementary Materials: The following are available online at http://www.mdpi.com/2305-6304/8/3/59/s1, Figure S1: schematic flowchart of experimental design; Figure S2: Schematic diagram on sediment, water, and MPLs partitioning for PCBs; Figure S3: Individual adsorption isotherms of marker PCBs on (A) PS, (B) PE and (C) PET.

Author Contributions: M.L. and M.Á. contributed to the experimental design, lab work supervision and to writing the first draft. A.V.-H. and M.A.A. performed the experimental work. E.A. collaborated in supervision and writing. M.F. conceived the idea, collaborate in designed the experiment and also collaborated in the writing of the final document and supervision. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: Authors declare no conflict of interest.

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5.2.1 Publication n°5 (Supplementary material)

Adsorption and desorption behaviour of polychlorinated biphenyls onto microplastics' surfaces in water/sediment systems

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Toxics, 2020, 8(3), 59

Figures S1, S2

Supplementary Material: Adsorption and Desorption Behaviour of Polychlorinated Biphenyls onto Microplastics' Surfaces in Water/Sediment Systems

Marta Llorca, Manuela Ábalos, Albert Vega-Herrera, Miquel A. Adrados, Esteban Abad and Marinella Farré *

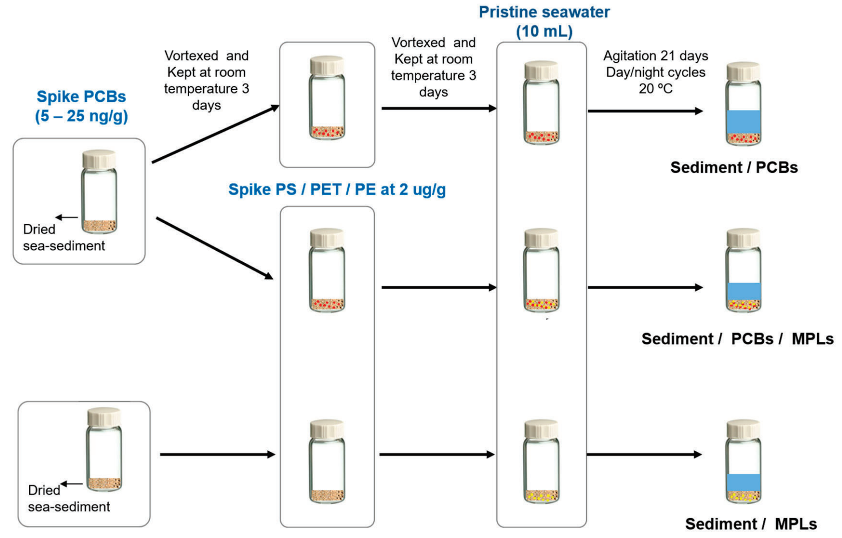

Figure S1. schematic flowchart of experimental design.

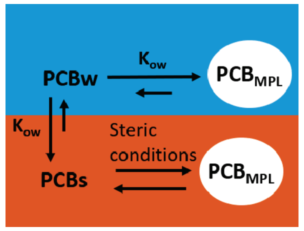

Figure S2. Schematic diagram on sediment, water, and MPLs partitioning for PCBs.

Toxics **2020**, *8*, 59; doi: 10.3390/toxics8030059

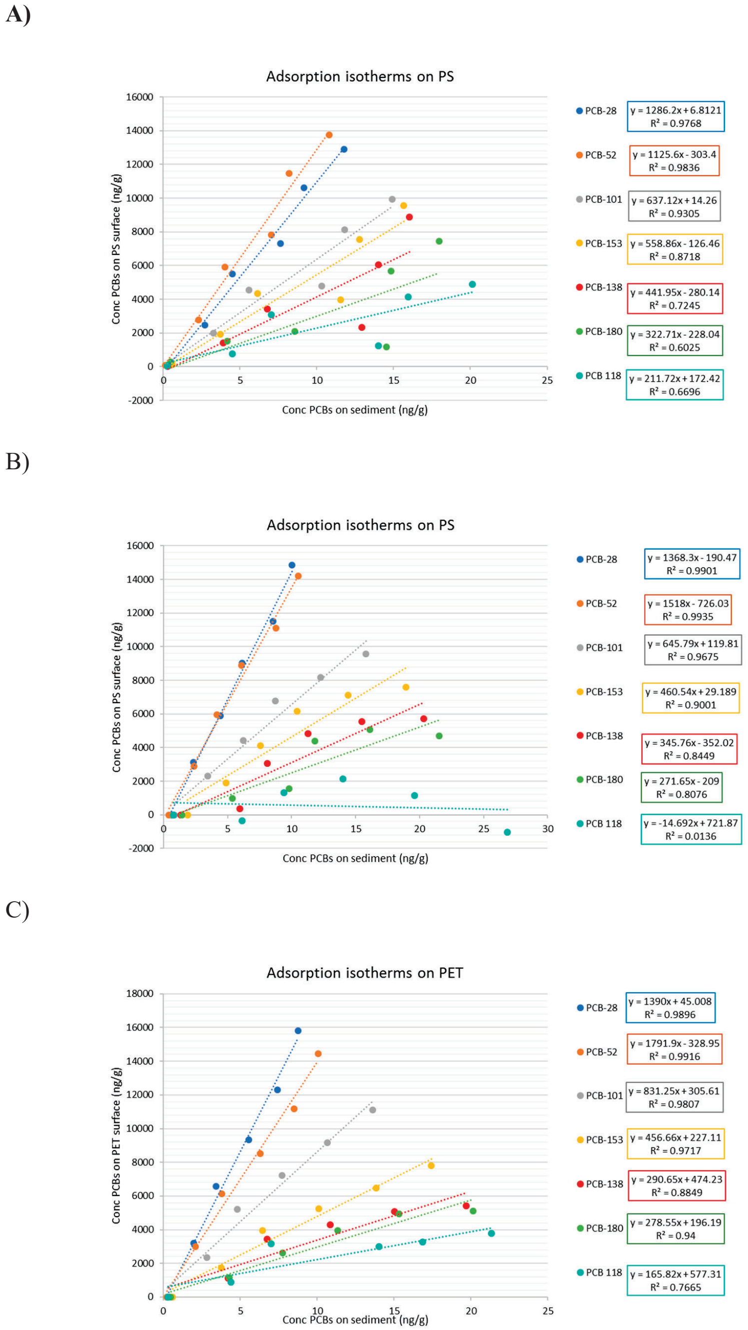

Figure S3. Individual adsorption isotherms of marker PCBs on (**A**) PS, (**B**) PE and (**C**) PET.

5.3 Discussion

Publication nº5, presented the key adsorption/desorption mechanisms between different types of microplastics (MPLs) sizes between 1 to 300 µm and representative PCBs in simulated coastal (water-sediment) ecosystems.

As shown in **Publication nº5**, to measure the adsorption/desorption behaviour between seven polychlorinated biphenyls congeners (PCB-28, -52, -101, -118, -153, -138, and -180) listed in the Stockholm Convention of POPs and 3 polymers: PE (3-16 µm), PS (10 µm), and PET (< 300 µm), the free concentration of individual PCBs at the initial and final incubation times, once equilibrium is reached, were assessed.

PCBs were adsorbed in the range of 20-65% onto MPL surfaces, despite sediments competing with MPLs for PCB for entrapping PCBs. The higher adsorption percentages were PET > PS > PE from the different contact times with concentrations (5 to 25 $ng \cdot g^{-1}$) assessed. The adsorption was favoured when polymers were able to establish π-π interactions instead of only Van der Waals forces, and they presented glassy polymeric structures, promoting high adsorption of co-contaminants by crystal surfaces and less desorption by rubbery surfaces, due to the degree of facility for pollutants to become entrapped.

Regarding PCBs, hydrophobic organic pollutants that contain more chlorine atoms in their structures, generally showed less adsorption on MPLs, due to the rise of polarity in the molecules, making it more difficult for them to stabilise on plastic surface pore sites, and facilitating their interaction with sediments. Nevertheless, PCB-118 was the exception, even at higher concentrations, due to its specific co-planarity conformation favouring steric impediments. The maximum adsorption degrees between PCB-118 and the studied MPLs were 14, 32, and 31% in PE, PET, and PS, respectively.

In this context, other persistent organic pollutants such as PFASs, polycyclic aromatic hydrocarbons (PAHs), hexachlorocyclohexane (HCHs), and pesticides such as dichlorodiphenyltrichloroethane (DDT), or bisphenol analogs, exhibiting polarities (expressed as log $k_{o/w}$) in the range of 3-9, have also been most often reported in the literature as being associated with PE-, PP-, PS-, and PET-MPLs surfaces, where partitioning of these POPs between environmental waters and microplastics surfaces typically follows the PE ≥ PP > PS > PET order (Tumwesigye et al., 2023). For example, in the study by Mai et al. (2018), adsorption capacities between PE-, PS-MPLs, and PAHs were assessed by obtaining 3,400-119,000 $ng\cdot g^{-1}$ PAHs on MPLs, while in the study by Pozo et al. (2020), the adsorption rates of PCBs, PBDEs, and DDTs onto PE-, PP-MPLs surfaces were in the ranges of 3-60, 10-133, and 0.1-7 $ng\cdot g^{-1}$ – pellet, respectively. Moreover, these high concentrations of chemicals reported on MPLs surfaces in ambient waters can be prone as a result of biofilm formation by biological agents on microplastic surfaces (Wang et al., 2016).

Comparing our outcomes published in **Publication nº5** with those outcomes that were previously reported in Llorca et al. (2018), the adsorption percentages found between PCB congeners and a group of PFASs (perfluorooctanoic (PFOA), perfluorobutane sulfonate (PFBS), perfluorohexa sulfonate (PFHxS), and perfluorooctane sulfonate (PFOS)) in PE-, PS-MPLs microspheres (3-16 μm) in simulated estuarine systems are presented in **Figure 5.2**.

As can be seen at the same exposure concentrations, PFASs showed a general tendency for a lesser degree of adsorption in PE-MPLs, where only the perfluoroalkyl substance with the carboxylic acid group (PFOA, C_8) was adsorbed onto polyethylene. In the case of PS-MPLs co-exposure, PFOA and the sulphonates PFASs with a higher number of carbon atoms (PFHxS, C_6; and PFOS, C_8) were adsorbed onto polystyrene surfaces ranging from 39 to 52 %, and reaching percentages that are more similar to PCBs congeners with a lower number of chlorine atoms, while comparing the same carbon chains with different functional groups, carboxylic acids had a lower tendency to be adsorbed onto PS than sulphonates (PFOA vs. PFOS). However, it is noteworthy that adsorption experiments which were carried out with PFASs did not contain sediments, thus avoiding, in any case, competition between the sediments and plastic particles to adsorb PFASs as in the PCBs-MPLs co-exposure studies.

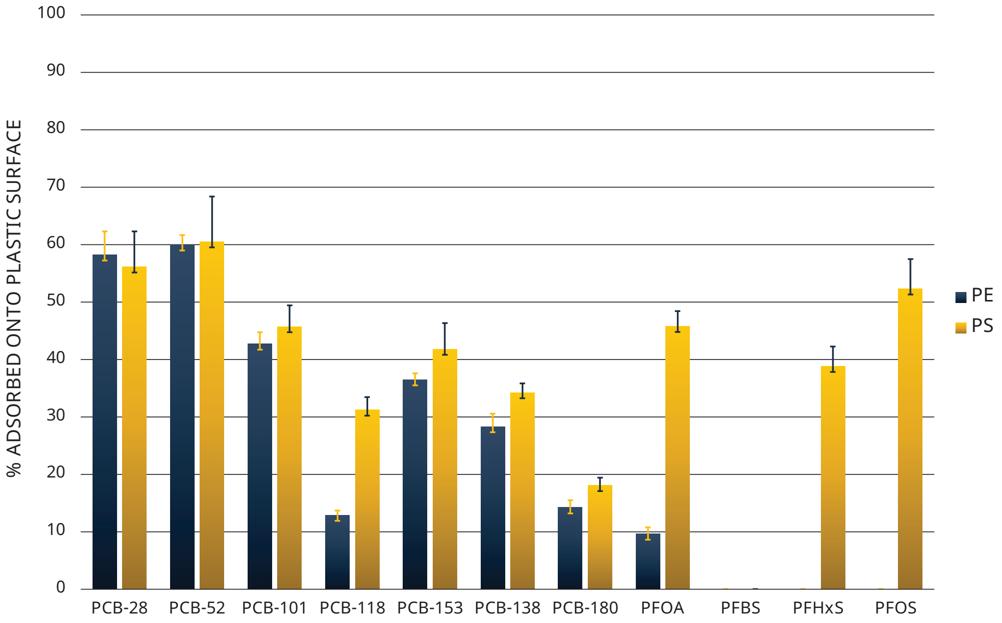

Figure 5.2 Adsorption percentages of PCBs and PFASs onto PE and PS-MPLs microspheres in simulated seawater environments at 10 $\mu g\cdot L^{-1}$ after 21 and 49 days, respectively.

Furthermore, differences between freshwater and seawater and the ageing of MPLs have not been tested in **Publication nº5**. However, according to the recent body of literature, the aged MPLs can present higher adsorption capacities for the same polymers, due to the major number of available active oxygen-based functional groups on their surfaces. Whereas, in freshwater compartments, the adsorption may be slightly favoured because in seawater, the ionic strength (salinity), and in consequence, pH, or the amount of suspended and dissolved organic matter, is higher, thus promoting electrostatic interactions between microplastics and co-contaminants, and competition between natural organic matter and MPLs for POPs adsorption. However, the effect of hydrophobic interactions and salting out is a major influence (Kuang et al., 2023).

Regarding the adsorption mechanisms characterisation, the individual behaviours of the different PCBs that were studied in **Publication nº5** were considered. Some adsorption isotherms approaches were tested, including linear, Langmuir, Brunauer-Emmett-Teller, and Freundlich models, where the linearised Freundlich approach in its logarithmic form was the model with best fit for all cases, assuming the presence of an infinite number of adsorption sites. **Table 5.2** shows the Freundlich equation parameters obtained for ∑PCBs (**Publication nº5**) and ∑PFASs (Llorca et al., 2018) in estuarine systems.

Table 5.2 Freundlich parameters obtained for ∑PCBs and ∑PFASs on PE and PS-MPLs in simulated seawater systems.

	K_F (ng·mg^{-1})	n_F	R^2
PE – ΣPCBs	266.56	0.873	0.9253
PS – ΣPCBs	281.77	0.862	0.9794
PET – ΣPCBs	755.27	1.049	0.9572
PE – ΣPFASs	0.09	0.779	0.9973
PS – ΣPFASs	0.12	0.645	0.9924

Where 'K_F' is a thermodynamic constant about the adsorption capacity at 20.0 °C (i.e., the higher the value, the more proneness to adsorption there will be), and 'n_F' can range from 0 to 10, between 0-1, 1-2, and 2-10 indicating poor, moderate, and good adsorption in the available active sites, respectively (Subramanyam and Das, 2009). Thereby, Freundlich parameters explained that the adsorption of these types of POPs onto MPL surfaces is poor, in general, and quite moderate for PCBs (PET- ∑PCBs) than for PFASs, due to the smaller number of halogenated atoms in their structures, conferring them a slightly higher hydrophobicity, and in consequence greater adsorption stability (higher K_F values), which is more pronounced, even when π-π interactions were available in polymeric structures due to electron delocalisation (PS, PET), as well as the presence of polar atoms (oxygen atoms in the PET structure).

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6

Human exposure to micro(nano) plastics

6.1 Introduction

The main routes of human exposure to MNPLs are inhalation, ingestion, and, to a lesser extent, dermal contact. Early insights indicate the potential of MNPLs to cause adverse effects on human health. On the one hand, particle toxicity can include oxidative stress, inflammatory lesions, and then increased internalisation or translocation through tissues. On the other hand, plastic additives can release toxic substances. It is noteworthy, that the potential effects of MNPLs encompass a wide range of effects, due to the wide variety of polymers and chemical additives mixtures used nowadays, that can induce different effects. Moreover, when human environmental exposure through inhalation or the consumption of contaminated foods (e.g. fish or seafood) is considered, the adsorption and desorption capabilities of MNPLs can induce exposure to other organic contaminants, as presented in the previous chapter.

As aforementioned, ingestion is considered to be the major route of human exposure to MNPLs, and the microbiome and tissues of the human gastrointestinal tract (GIT) are considered to be among the most exposed. In particular, the small intestine has potentially been defined as the main site of uptake and translocation of anthropogenic particles. The M cells of the Peyer's patches, which are part of the gut-associated lymphoid tissues (Powell et al., 2010), can transport particles (0.1–10 μm) to the mucosal lymphoid tissues, which are responsible for initiating immune responses. Moreover, the subepithelial region of the Peyer's patches can store non-degradable particles, and consequently, they disrupt the immunity processes (Campanale et al., 2020). For the bigger particles, up to 130 μm diameter, persorption performed by GIT epithelial cells is another route of uptake. In addition to particle size, other factors that can influence the uptake and translocation of MNPLs in the human body are a particle's chemical composition, shape, and hydrophobicity. It is known that hydrophobic surfaces can promote the transport of particles through the mucus layer. Food and drinking water are key to assess human MNPL exposure through ingestion. It is known that molluscs, such as mussels (Van Cauwenberghe and Janssen, 2014; Li et al., 2015; Smith et al., 2018), can be MNPLs-contaminated foods because they live in intertidal areas, where they are particularly exposed (Coppola et al., 2020). Moreover, the edible part of molluscs includes the stomach, intestine, and digestive glandular. However, as in other matrices, the concentrations of MNPLs in molluscs present a high variability, ranging from 0.05 to 3 MPLs items·g^{-1} wet weight (ww) of edible tissue in mussels, which can be influenced by contamination of the different geographical areas, and can also be due to the variability of analytical approaches that are used in the different studies, and the lack of standardisation.

Drinking water may be another important source of MNPLs in humans because of the cross-contamination during potabilisation and transport of tap water, or due to migration from plastic bottles, in the case of bottled water (Luo et al., 2018), because water consumption has increased greatly, surpassing 22,700 million L·year^{-1}. For example, from mechanical stress to PE-PP bottle screw caps during the opening and closing of a plastic water bottle, and thermal stress when brewing any teabags that are made from nylon and PET. Top-down formation of MNPLs may occur; notwithstanding any simple contact of food products with the plastic packaging, such as meat in extruded PS trays or transfer through plastic takeaway containers (Winkler et al., 2022). Nevertheless, a deeper understanding of degradation processes during the generation of secondary MNPLs in food and beverage plastic packages still needs further research.

One of the current limitations to assessing the risk associated with human exposure to MNPLs, is the lack of consistent information about real levels of human exposure, particularly due to the lack of analytical methods with comparable results and the ability to assess trace concentrations of polymers in complex matrices, such as foods, and ultra-trace levels in drinking waters. Thus, in this chapter, that aim is to tackle this current lack of knowledge. Hence, the oral exposure to PE, PP, PET, PS, PI, and PBD polymers from MNPLs, as well as the identification of the plastic additives' composition by the daily consumption of tap and bottled water, have been assessed. In brief, the mass-quantitative methodology based on LC(SEC)-HRMS was adapted and validated for drinking water analysis, and subsequently applied to assess the different household tap waters, that are distributed in the Barcelona Metropolitan Area (**Publication nº6**), and from single-use plastic bottles (**Publication nº7**) through 20 popular commercial brands that are consumed in Spain. In addition, a qualitative human risk assessment of the composition of plastic additives that are associated with plastic material was carried out, following a prioritisation study, based on a multi-QSAR (Quantitative Structure–Activity Relationship) model for water samples collected from single-use plastic bottles.

Although the bioaccumulation of MNPLs and their associated toxicological effects in biota have been reported in recent years, there are still gaps in the knowledge about accumulated MNPLs in animals for food consumption, and their potential transfer of other contaminants. Moreover, the bioaccessibility or the proportion of consumed MPLs (monomers from polymers, plastic additives, or contaminants adsorbed onto MNPLs) in a meal that is released from the food matrix during digestion and is accessible for absorption has received very little attention in research, until now. Solely 8 scientific articles, have been found in the body of literature that aims to address the understanding of the mechanisms that are related to the bioaccessibility and translocation of MNPLs in human organisms. Those articles were located via a bibliographic search that was performed using Scopus, and employing the terms: 'micro and nanoplastics,' 'bioaccessibility,' and 'humans', in that order. All of the studies have been carried out following *in vitro* models, and none were initiated before year 2021. Future efforts will be necessary for the next years in order to enhance the understanding of MNPLs' bioaccessibility behaviour in the human body. Nonetheless, MNPLs have been detected in human tissues and excreta in a few studies. According to Yan et al. (2021), the concentration of MNPLs in the faeces of patients with inflammatory diseases (41 items MNPLs·g^{-1}) was significantly higher than in healthy individuals (28 items MNPs·g^{-1}). Therefore, here, we have studied the potential of Mediterranean mussels to bioaccumulate MNPLs, and their influence on the bioaccessibility of other organic contaminants adsorbed onto MNPLs (**Publication nº8**).

6.2 Results

This section comprises 3 scientific articles, the first two of which have already published in scientific journals from the first quartile, and the third has been submitted to the journal. The novel outcomes have been presented in:

- **Publication nº6:** Vega-Herrera, A., Llorca, M., Borrell-Diaz, X., Redondo-Hasselerharm, P. E., Abad, E., Villanueva, C. M., Farré, M., 'Polymers of micro(nano)plastic in household tap water of the Barcelona metropolitan area', 2022, Water Research, https://doi.org/10.1016/j.watres.2022.118645

- **Publication nº7:** Vega-Herrera, A., Garcia-Torné, M., Borrell-Diaz, X., Abad, E., Llorca, M., Villanueva, C. M., Farré, M., 'Exposure to micro(nano)plastics polymers in water stored in single-use plastic bottles', 2023, Chemosphere, https://doi.org/10.1016/j.chemosphere.2023.140106

- **Publication nº8:** Vega-Herrera, A., Savva, K., Lacoma, P., Santos, Lúcia H.M.L.M., Hernández, A., Marmelo, I., Marques, A., Llorca, M., Farré, M., 'Bioaccumulation and dietary bioaccessibility of microplastics composition and cocontaminants in Mediterranean mussels', Submitted to Chemosphere.

6.2.1 Publication n°6

Polymers of micro(nano)plastic in household tap water of the Barcelona Metropolitan Area

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Water Research, 2022, 220, 118645

https://doi.org/10.1016/j.watres.2022.118645

Water Research 220 (2022) 118645

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1. Introduction

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[illegible]

(gi i t aost oa) i o adt hdghop*n/mon/)*$_{std}$ dt host oa) i o dn (ri op*n/mon/)*$_{sample}$ p*. Ro p%mnRO) i o*n/mon* dgi oa) i ogcl t hi hoKdrl i ocLadst i hoLeohsKshst Aoa) i on d ocfo a) i o) sA) i ao(i dBocfoa) i o(cren i go(gc1 ri doLeoa) i on ct cn i gon d op*. RpqmnRo

$$[\mathrm{Polymer}]\mathrm{eq} = \frac{[Polymer]cc}{Feq} \qquad \text{pPmo}$$

$$\mathrm{Feq} = \frac{(n.mon)sample}{(n.mon)std} \qquad \text{p\%mo}$$

$$(\mathrm{n.mon}) = \frac{(mass)\ highest\ peak}{mass\ monomer} \qquad \text{pqmo}$$

Cst drreHoa) i ouct 1 gn dasct opuct 1 hi t ui ori Ki rowmoE d od i i hoa) gcl A) o a) i oi 0ai gt droudrsLgdai oul gKi ocfo(cren i go adt hdgh HoLd i hoct oi . l sKdri t ao uct ui t agdasct ofcgoS* HoSfiHoSxHoSbv HoSSHodt hoS, I ost ot i AdasKi odt ho(c sasKi o sct s dasct ouct hsasct odt hoSModt hov *- Sost o(c sasKi odt hot i AdasKi osct V s dasct ouct hsasct Ro

2/P/ Statistical analLsis

D l rasKdgsdai odt dre s oa) gcl A) oSgst us(droI cn (ct i t aoMt dre s opSI Mno E d od((rsi hoacoa) i ohgst Bst AoE dai go dn (ri ol st Aoz GfiOMOo* 0ui ro adas asudro cfaE dgi ofgcn oMhhst cfaopSdgs HoCgdt ui nRI cggi rdasct odn ct A aoKdgsdLri o pS* HoSxHoSSodt hoSbv nnoE i gi o. l dt as1 i hod oa) i o . l dgi houc st i ocfoa) i sgo dt Ari opGcdhst A HoOdLri ofiwnodt hocL i gKdasct Ho dn (ri HoE i gi ogi rdai hoaco a) i oKdgsdLri opfiucgi nRo

2/6/ ualitL assurance and ualitL control (AO C)

Ocod i oa) i o(i gfcgn dt ui ocfoa) i odt dreasudron i a) chHoa) i orst i dgsaeHo duul gdueHo(gi us sct Hodt host agl n i t adropxGQW nodt hon i a) chopD GQW no rsn sa ocfo. l dt as1 udasct oE i gi od i i hRoO) i ost agl n i t adro. l drsaeo(dV gdn i ai g oE i gi oudrul rdai hd st Aoai t oudrsLgdasct oul gKi o(cst a ost oacrl i t i Ho hsffi gi t asdast AoaE corst i dgsaeogdt Ai ofgcn dkRkPoacoPkoµA6Godt hodo i uct ho udrsLgdasct oul gKi ofgcn oPkoacowkkkoµA6GRoxt agl n i t adrorst i dgsaeodt ho gdt Ai oE i gi oi asn dai hd st Aorst i dgogi Agi sct oE sa) oAcchogi l ra opT^{5o}> kR9nRoxt agl n i t adro(gi us sct opt oo= PnHoi 0(gi i hod oa) i ogi rdasKi o adt hdgho hi Ksdasct opTfiv ; nocfost agdVhdeodt host ai gVhdeogi (i dadLsrsaeoE d oi Kdrl V dai hRoxt odrroud i Hoa) i o(gi us sct oE d orcE i goa) dt owk; odt ho5P; ofcgost agdVo dt host ai gVhdeoi Kdrl dasct Hogi (i uasKi reRoO) i oxGQv oE d oi asn dai hofgcn o a) i ost Ji uasct ocfo i gsdrohsrl asct Ho adgast Aofgcn oa) i orcE i ao(cst aocfoa) i o udrsLgdasct oul gKi d t asroa) i o(i dB oE i gi ot cachi ai uai hRoO) i t Hoa) i orsn sa ocfo . l dt as1 udasct oE i gi oi adLrs) i hod oa) i orcE i ao(cst aost oa) i oudrsLgdasct o ul gKi HoE) sri oa) i on i a) chorsn sa ocfo. l dt as1 udasct opD GQWnoE i gi oudrul V rdai houct shi gst Aoa) i ouct ui t agdasct ofduacgRo

D dagsOoi ffi uaoE d oi Kdrl dai hofcgoi du) odt dreai HoLeoucn (dgst Aoa) i o rc(i ofgcn oudrsLgdai oul gKi ohs crKi host oacrl i t i op crKi t aoi 0agduasct no dt hohgst Bst AoE dai gopn dagsOnRo

Ocoi t l gi oa) i o. l drsaeocfoa) i ogi l ra Ho8o(gcui hl gdroLrdt B oE i gi o(igV fcgn i hol st AoPoGocfo- SGI VE dai goagdt figgi host oa) i odn i on dt t i goacoE dai go uct adst i g odaoa) i o) cl i ocfoa) gi i oKcrl t ai i g Host oa) i odn i on dt t i god oa) i o ad(oE dai go dn (ri Robrdt B oE i gi oi 0agduai hodt hodt dre i hofcrrcE st Aoa) i o dn i o(gcui hl gi od oa) i ogi aocfoa) i o dn (ri Rov i ai uai hoD y SG ost oa) i oLrdt Bo gi (gi i t ai hori oa) dt o5; ocfoa) i odLl t hdt ui ocfoD y SG o(cren i g ohi ai uai ho st oa) i o dn (ri Hodt hoE d ouct shi gi hoacoLi ot i ArsAsLri RoMogi ucKi ge ocfoi du) o ae(i ocfo(cren i goE d oudggsi hocl aHost ocghi goacoBt cE oa) i o(c sLri orc oAgdhi o cfoi Ki geodt dreai ohl gst Aodrrocfoa) i o ai (ocfoa) i odt dreasudro(gcui hl gi Ro

Mrrocfoa) i oi . l s(n i t aoa) daoE d d i hofcgo dn (ri oi 0agduasct odt houri dt Vo l (ouct s ai hocfo adst ri o ai i rocgoArd HoE) i t i Ki go(c sLri Hoacoi t l gi o c(asn drouri dt st Aodt hogi hl uasct ocfoLduBAgcl t houct adn st dasct RoD dai gsdr o E i gi ouri dt oduucghst Aoacoa) i o(gcacucroa) daoE d o(gi Kscl rechi ugsLi hHoaco dKcshougc ouct adn st dasct ocfoa) i o dn (ri RoMrro dn (ri on dt s(l rdasct o E i gi oudggsi hocl aost odordn st dgodsgo/ cE oLi t u) ost odouri dt ordLcgdacge oaco gi hl ui odsgLcgt i ouct adn st dasct RoD cgi cKi gHohl gst Ao dn (ri on dt s(l rdasct Ho i 0agduasct Houri dt V(Hodt hodt dre s Hoct reoucaact ordLoucda oE i gi oE cgt Ro

RnFHl 1
Wl drsaeod l gdt ui odt ho. l drsaeouct agcro(dgdn i ai g ocfoa) i odt dreasudron i a) chRooooo

	xGQWo pfAno	xGQWo pµA6Gno	D GQWo pt A6Gno	Ti ucKi geo ; o	Sgi us sct op*n* = Pnoo xt agdVo hdeo	 xt ai gVo hdeo
goh4l tC4Hl nl	kRwko	kRwko	kRk5o	%wo	wPo	wqo
goh4Futndil nl	kRwko	wRkko	kR5ko	%Po	w3o	5ko
goh4iToerl nl	kR5Po	kRPko	kRwko	PPo	wPo	w9o
goh4eroe4Hl nl	kRkPo	kRwko	kRk5o	Pqo	wqo	5wo
goh4Tt4rl nl	kRwko	kRwko	kRk5o	$5o	wwo	w3o
goh4pDin4Hf cChoridl	kR5Po	kRPko	kRwko	P%o	w5o	w9o
goh4ns idl	kR5Po	kRPko	kRwko	%5o	wPo	5ko
AMk g	kRPko	kRPko	kRwko	q8o	w5o	w9oo

RnFHl 2
I ct ui t agdasct ocfoD y SG ofcl t host oa) i ohgst Bst AoE dai goad(o dn (ri oi 0(gi i host o i . l sKdri t aouct ui t agdasct opt A6GnRo

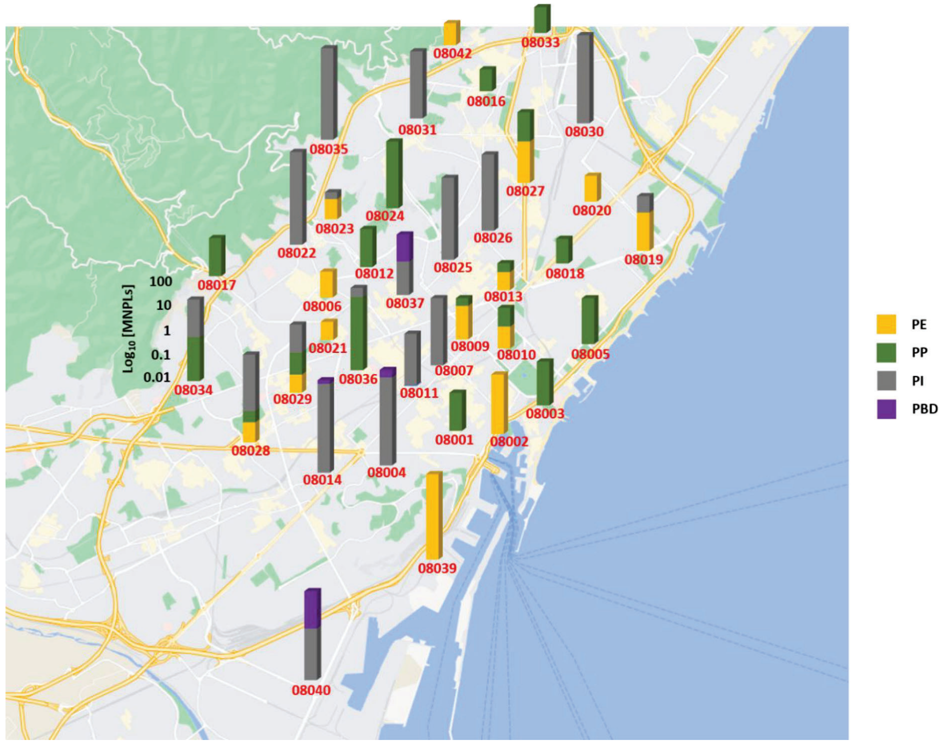
fidn (ri o	üS * [$_{i.o}$	üSx[$_{i.o}$	üSbv [$_{i.o}$	üSS[$_{i.o}$	SvDfiNo	Mgi do
k$kkwo	PRPo	<D GQv o	<D GQv o	$wRko	] * fio	4 i ao
k$kk5o	w3qwR3o	<D GQv o	<D GQv o	<D GQv o	] * fio	4 i ao
k$kk8o	<D GQv o	<D GQv o	<D GQv o	3%$R8o	y Qo	* d ao
k$kk3o	<D GQv o	w$$qRPo	w8$wRPo	<D GQv o	] * fio	4 i ao
k$kkPo	qRko	<D GQv o	<D GQv o	PP3RPo	] * fio	* d ao
k$kk%o	$$R%o	<D GQv o	<D GQv o	<D GQv o	] * fio	4 i ao
k$kkqo	<D GQv o	8339R3o	<D GQv o	<D GQv o	] * fio	4 i ao
kkko	wwR%o	<D GQv o	<D GQv o	<D GQv o	] * fio	4 i ao
k$kk9o	5k5Rko	<D GQv o	<D GQv o	93RPo	] * fio	4 i ao
k$kwko	%3R$o	<D GQv o	<D GQv o	w3qR8o	y Qo	4 i ao
k$kwwo	<D GQv o	3P9Rwo	<D GQv o	<D GQv o	] * fio	4 i ao
k$kw5o	58R3o	<D GQv o	<D GQv o	5$8R%o	y Qo	4 i ao
k$kw8o	PwR5o	<D GQv o	<D GQv o	P$RPo	y Qo	* d ao
k$kw3o	wqR8o	w3w$R9o	wkk%R9o	w%RPo	] * fio	4 i ao
k$kwPo	<D GQv o	<D GQv o	<D GQv o	<D GQWo	y Qo	4 i ao
k$kw%o	<D GQv o	<D GQv o	<D GQv o	%%Rqo	y Qo	* d ao
k$kwqo	<D GQWo	<D GQv o	<D GQv o	553R$o	] * fio	4 i ao
kkwo	5PRPo	<D GQv o	<D GQv o	$$R5o	y Qo	* d ao
k$kw9o	w5wR9o	wwk8Rqo	<D GQv o	<D GQv o	] * fio	* d ao
k$k5ko	99RPo	<D GQv o	<D GQv o	<D GQv o	] * fio	* d ao
k$k5wo	8$R%o	<D GQv o	<D GQv o	wR5o	y Qo	4 i ao
k$k55o	<D GQv o	wq$kR9o	<D GQv o	<D GQv o	] * fio	4 i ao
k$k58o	35R%o	3%Rko	<D GQv o	<D GQv o	] * fio	4 i ao
k$k53o	w%R8o	<D GQv o	<D GQv o	8859R9o	] * fio	* d ao
k$k5Po	88R%o	wkqkR%o	<D GQv o	<D GQv o	] * fio	* d ao
k$k5%o	<D GQv o	q%98RPo	<D GQv o	<D GQv o	y Qo	* d ao
k$k5qo	35$R9o	<D GQv o	<D GQv o	3P8$RPo	y Qo	* d ao
k$k5$o	P3R$o	w%5PR9o	<D GQv o	wk3R$o	] * fio	4 i ao
k$k59o	3PR9o	9%wR9o	<D GQv o	88qRko	] * fio	4 i ao
k$k8ko	<D GQv o	w83PR9o	<D GQv o	<D GQv o	] * fio	* d ao
k$k8wo	w5R8o	559kRwo	<D GQv o	wRwo	] * fio	* d ao
k$k85o	w8R9o	<D GQv o	<D GQv o	qR9o	y Qo	* d ao
k$k88o	<D GQv o	<D GQv o	<D GQv o	39R5o	] * fio	* d ao
k$k83o	w3R9o	9w35R$o	<D GQv o	39%Rko	] * fio	4 i ao
k$k8Po	<D GQv o	ww8qR5o	<D GQv o	<D GQv o	y Qo	* d ao
k$k8%o	<D GQv o	q3q%R%o	<D GQv o	qwkRqo	y Qo	4 i ao
k$k8qo	wPR9o	w98Rwo	w$9qR8o	<D GQWo	] * fio	4 i ao
k$k8$o	<D GQv o	<D GQv o	<D GQv o	PR8o	y Qo	4 i ao
k$k89o	w9q8R$o	<D GQv o	<D GQv o	<D GQWo	] * fio	4 i ao
k$k3ko	P3Rqo	q%8R3o	wqk8RPo	<D GQv o	] * fio	4 i ao
k$k3wo	$Rwo	<D GQv o	<D GQv o	<D GQWo	y Qo	* d ao
k$k35o	38R8o	<D GQv o	<D GQv o	<D GQv o	] * fio	* d aoo

[N] v i ai uai hd t asroucct 1 hi t ui ori Ki ro5Ro

b. 31TultT mnd diTcuTTion

3/1/ Qalidation of the analLtical method for drin iny q ater analLsis

xt oOdLri owHoxGQW HoD GQW Hogi ucKi gsi Hodt host ai gVodt host agdVhdeo gi (i dadLsrsaeofcgoa) i odt dre s ocfoD SG ost ohgst Bst Aodgi o l n n dgs i hRoTi V ucKi gsi oE i gi ocLadst i hoE sa) o. l st al (rsudai HocLadst st Aogi l ra ofgcn oPPo pSxnoaco$5; opSfinRoUcchoD GQW oE i gi ocLadst i hofcgoa) i o i ri uai ho(cren i g o st ohgst Bst AoE dai gogdt Ast Aofgcn okRk5oacokR5ot A6GRoD dagsOoi ffi uaoE d o



wix. 1. I ct ui t agdasct ocfon sugcpt dt cn(rd asu opD y SG nost ot A6Godrct Aoa) i ohgst Bst AoE dai gol ((reocfoa) i obdguircct doD i agc(crsadt oMgi dopbD Mnoi 0(gi i hod oul n l rdasKi o l n ost oa) i oLd i owkorcAdgsa) n suo udri RoNoD d(oAi t i gdai hoLeo“UccAri on d(” i gKi gofgcn oMr( ) dLi aoxt uRopI drsfcgt sdHol MHo2 t sai ofiadai nRo

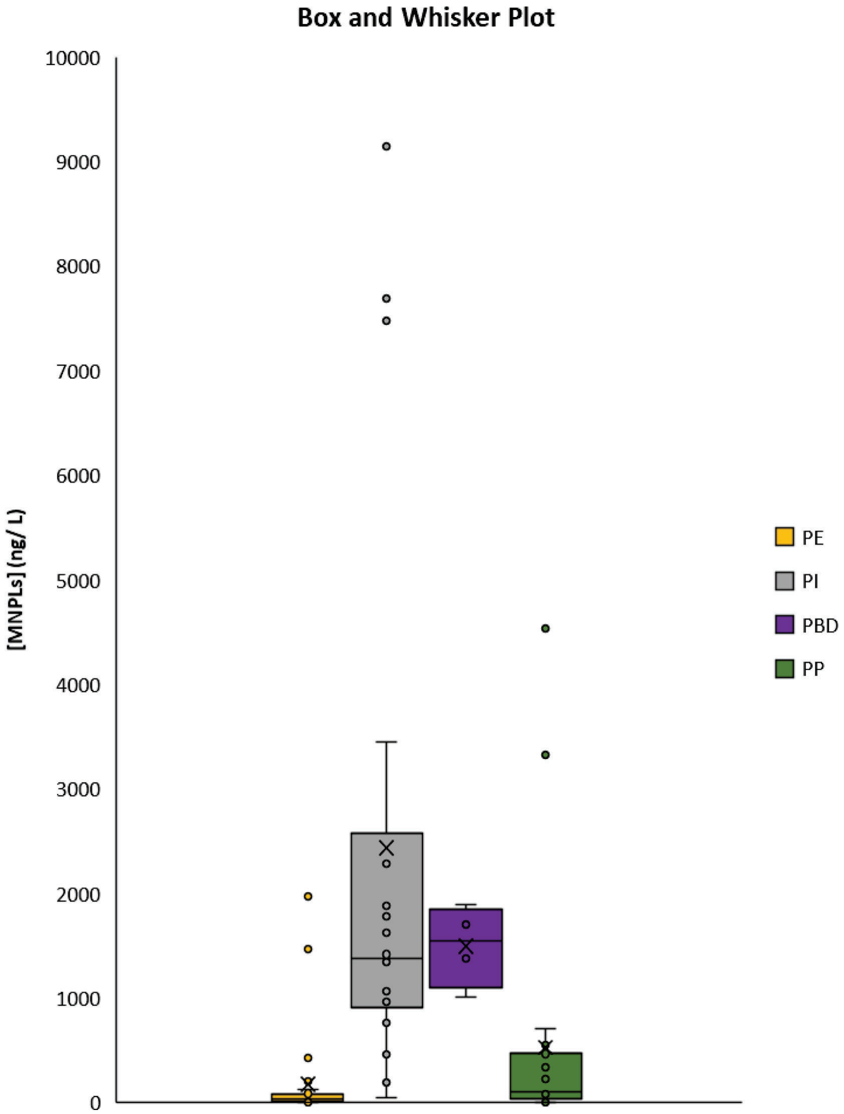
i asn dai hoLeoucn (dgst Aoa) i o rc(i ocfo adt hdghoul gKi ohs crKi host o i 0agduasct o crKi t ao dt ho n dagsOVn dau) i ho udrsLgdasct o ul gKi Ro Ti l ra o uct 1 gn d (oacoPk; ocfosct o l ((gi sct ofcgohgst Bst AoE dai gRoO) i i ogi l ra o) sA) rsA) aoa) i ot i i hoacol i on dagsOVn dau) i ho adt hdghoudrsLgdasct oul gKi ofcgo . l dt asadasKi odt dre i Ro

3/2/ ccurrence of g T: Ns in the drin iny q ater supplL sLstem

Mo l (i uao ugi i t st Aod((gcdu) oE d od((rsi hoacost Ki asAdai oa) i o(cai t V asdrocuul ggi t ui ocfo(cren i g ocfoD y SG ost oa) i ogdt Ai ocfokRqk–5koµn o hsdn i ai gost oad(oE dai go dn (ri oa) daE i gi oucrri uai hodaoa) i o35o(c adroucchi o cfoa) i obD MRO) i ogi l ra ocfoa) s o al heoai t adasKi reoshi t as1 i hoqo(cren i g odao uct 1 hi t ui ori Ki ro5opS* HoSSHoSxHoSbv HoSMHoSfiodt hoSv D finHodt ho s0ocfoa) i n o E i gi ouct 1 gn i hoLeo adt hdgh opuct 1 hi t ui ori Ki rownopS* HoSSHoSxHoSbv HoSMHo dt hoSfinR4) sri ofcl go(cren i g oE i gi o(gi i t aost odaori d aoct i ocfoa) i odn (ri o daouct ui t agdasct ocKi goa) i oD GQWopS* HoSxHoSbv Hodt hoSSnHoSfiodt hoSMori Ki r o E i gi odrE de oLi rcE oa) i oD GQWRoxt oOdLri o5Hool n n dge ocfoa) i on dst ogi l ra o s o(gi i t ai host ot A6oGRoxt oOdLri ofi5Hoa) i ou) dgduai gsFdasct ocfodrroa) i o(creV n i gsuou) dst ofcl t host oa) i odt dre i ho dn (ri os o) cE i hRo

Qt reoct i ocl aocfoa) i o35o dn (ri odt dre i hop5R8; nohshot cao(gi i t aodt eo (cren i godao. l dt asadasKi ouct ui t agdasct RoMr cHost oct reoct i o dn (ri Hofcl go hsffi gi t ao(cren i g oE i gi oshi t as1 i hodt ho. l dt as1 i hRoQt i HoaE cHocgoa) gi i o (cren i g oE i gi oshi t as1 i hodt ho l dt as1 i host o3kRP; Ho8PRq; Hodt how9Rk; ocfo a) i o dn (ri Hogi (i uasKi reRoxt oCsARowHoa) i ohs agsLl asct ocfo(cren i g odt hoa) i o cLadst i houct ui t agdasct ost oi Ki geoFs(ouchi ocfobD Most ot A6Goi 0(gi i hod o ul n l rdasKi o l n ost oa) i oLd i owkorcAdgsa) n suo udri os o) cE t Ro

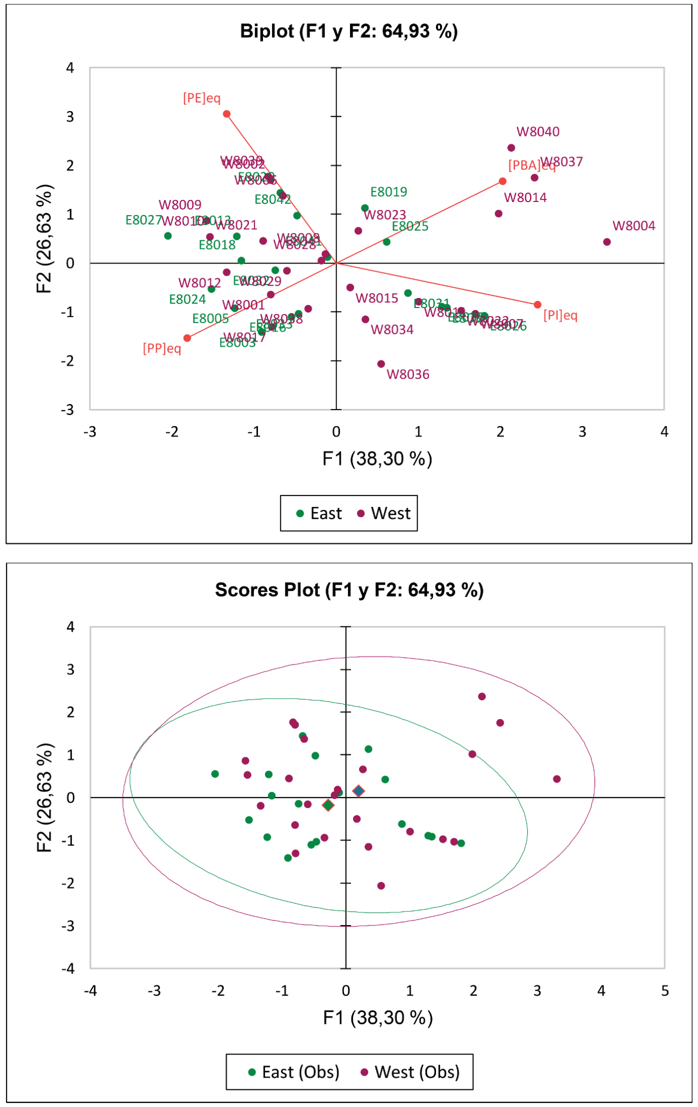
O) i on c adfgi . l i t areofcl t ho(cren i goE d oS* Hohi ai uai hodt ho. l dt as1 i ho daoqwodt ho%9; ocfoa) i odt dre i ho dn (ri Hogi (i uasKi reRoSSoE d odr co hi ai uai hodt ho. l dt as1 i host odo) sA) ofgi . l i t ueost o%%Rq; odt hoPqRw; ocfoa) i o dn (ri Hogi (i uasKi reRoO) i i ogi l ra odAgi i oE sa) oca) i go(gi Kscl ost V Ki asAdasct ocfoD SG ost ohgst Bst AoE dai godt hoad(oE dai gHost oE) su) oS* odt ho SSoE i gi ofgi . l i t areohi ai uai hopD st ai t sAoi aodrRHo5kw9:oSsKcBct Beoi aodrRHo 5kw$nRoS* odt hoSSo) dKi odoE shi od dAi Host url hst Ao(dga ocgo(s(i ost ohgst Bst Ao E dai go e ai n odt hoKdgscl o) cl i E dgi osai n Hodt hoa) s ofduaoudt oi 0(rdst o a) i sgo(gi i t ui ost ohgst Bst AoE dai go e ai n od ot cai host oD st ai t sAoi aodrRo p5kw9nRov i (sai oa) i o) sA) ofgi . l i t ueocfoa) i i oaE co(cren i g Hoa) i eocuul ggi ho daorcE ouct ui t agdasct RoxtoP$; ocfoa) i o dn (ri oE sa) oS* Houct ui t agdasct o E i gi oLi rcE oPkot A6GHodt ho$k; ocfoa) i o dn (ri o(gi i t ai houct ui t agdasct o Li rcE owkkot A6GRoxt oCsAR5Hoa) i oLc 0odt hoE) s Bi go(rcao) cE oa) i ogi rdasKi o dLl t hdt ui ocfo dn (ri oAgcl (i hoduucghst Aoacoa) i ouct ui t agdasct ogdt Ai o fcgoa) i ohsffi gi t ao(cren i g RoO) i ouct ui t agdasct ocfoSSoE i gi odr coLi rcE oPko t A6Gofcgon c aocfoa) i o dn (ri RoO) i oa) sghon cgi ofgi . l i t areohi ai uai ho (cren i goE d oSxop35R9; nHodt host odhhsasct oa) s o(cren i goE d ofcl t hodao) sA) i gouct ui t agdasct ost o(i us1 uo dn (ri ogi du) st Ao9op dn (ri ok$Hk83nHoqo p dn (ri ok$Hk5%odt hok$Hk8%nHodt ho8oµA6Gop dn (ri ok$HkkqnRoSxoi rd acn i g o dgi ol i host on dt eoi t hVl i od((rsudasct ogi . l sgst AoAcchogi srsi t ui HorcE o E dai go Ei rrHo) sA) oai t srio agi t Aa) HoAcchoaduBHodt ho) sA) o) caoai t srio agi t Aa) RoCcgoa) s ogi d ct Hoa) i ordgAi aoi t hVl i ofcgo(cres c(gi t i HoLeofdgHos o st oaegi Roxt odhhsasct HoAl n oSxVLd i houcn (cl t h odgi od i host ogl LLi goLdt h Ho LdLeoLcaarios ((ri Hodt hoi 0agl hi ho) c i od oa) c i ol i host ocn i oad(HodrcctAo E sa) oca) i gol i RoD cgi cKi gHoSxos odr cod i host oi drdt a odt hodh) i sKi RoSbv o E d oshi t as1 i hodt ho. l dt as1 i host ofcl goocfoa) i o35odt dre i ho dn (ri op9RP; nRo D cgi cKi gHosaoE d ofcl t hodaouct ui t agdasct ocKi gowqoµA6Gop dn (ri ok$Hkk3Ho k$Hkw3Hok$Hk8qHok$Hk3knRoSbv os odr cod i hofcgoa) i ofdLgsudasct ocfoasgi Hoaco sn (gcKi oa) i on i u) dt sudro(gc(i gasi ocfoSfiHocgoacoLi d i hod odh) i sKi ofcgo S* RoSMoE d odr cohi ai uai hodaoshi t as1 udasct ori Ki ro5ost o3q; ocfoa) i odn (ri Ro



wix. 2. bc0adt ho4) s Bi go(rcaRD sugcpt dt cm(rd asu opD y SG mfcl t host o dn (ri oAgcl (i hoduucghst Aoacoa) i ouct ui t agdasct ogdt Ai ofcgoa) i ohsffi gi t ao(cren i g oi 0(gi i host o t A6oGRo

- cE i Ki gHa) i ouct ui t agdasct ocfoSMdE i gi d t hi goa) i oD GQWopkRwko A6Gmost o drroa) i o dn (ri RoMra) cl A) oS, I os ol i host o(s(i ofdLgsudasct ofcgoE dai go agdt (cgaost chgst Bst AdE dai go l ((reo e ain HdLi udl i ocfosa ogi s adt ui oaco dLgd sct Hst oa) i o(gi i t ao al heot ct i ocfoa) i o dn (ri o) cE i houct ui t agdV asct ocKi goa) i oGQv RO) i i oae(i ocfo(s(i odgi oucn (c i hocfoa) i oS, I on dags0o pu) dgduai gs i hoLeosa o) sA) chi Agi i ocfoucn (duasct odt hogcLl at i mdt hodo n s0ocfo(rd asus i gordei g ost oa) i ol gfdui odh cgLi hoacoa) i o(cren i gsuon dags0Ro v l i oacoE i dBoLct h Ho(rd asuodhhsaKi on sA) aoLi ori du) i hoacoa) i on dags0ocKi go asn i odt ho/ cE oE sa) chgst Bst AdE dai gHdE) sri on c aoS, I ogi n dst d t u) dt Ai ho st oa) i o(s(i HodKcshst Ao(cren i gofgdAn i t adasct o(gcui i Rov sV5Vn i a) ero) i 0ero ( ) a) drdai o pv * - SnHo do u) dgduai gs asuo (rd asus i go l i ho st o S, I o st hl agsdro et a) i s dE d d i hod odoS, I ost hsudacgodt hoai t adasKi reoshi t asV 1 i h6. l dt as1 i host oa) i obD Mdhs agsua od o l AAi ai host oa) i o al heoLeoSdgBo i aodrRop5k5wnRov * - SdE d ofcl t host owkoad(dE dai go dn (ri HoqocfdE) su) dE i gi o rcudai host chs agsua ost oa) i dE i ai gt o(dgaocfoa) i obD MHodt hodrrocfoa) i n dE i gi o Li rcE oa) i orsn saocfchi ai uasct Ro

O) i ouct ui t agdasct ocfo(cren i g ocfoD y SG ofcl t host oa) s o al heogdt Ai ho fgcn owot A6Goaco9quA6GdE sa) on i hsdt oKdrl i ocfoww9ofcgoS * Howk33ofcgoSxHow3%o fcgoSbv Hodt ho5q$ot A6GofcgoSSRO) i i oKdrl i odgi ohsf1 ul raocoucn (dgi oE sa) o ca) i goal hsi oct oa) i ocuul ggi t ui ocfoD SG chgst Bst AdE dai gHoLi udl i on c aocfo a) i n ogi (cgai hoa) i ot l n Li gocfo(dgasuri ost ai dhocfouct ui t agdasct Ro cE V i Ki gHo7 sg ai st op7sg ai st oi aodrRod5k5wnno) d oi asn dai hoa) i ouct ui t agdasct ocfo



wix. b. Gcdhst A o+ fiucgi dLs(rcaopdLcKi modt hofiucgi opLi rcE mo(rcaon dhi oLeoSgst us(drol cn (ct i t adMt dre s opSI Mmodt hod((rsi hoacoad(dE dai go dn (ri RdI i t agcsh dE i gi o Ai t i gdai hodl acn dasudrreoE sa) odo9P; o(gcLdLsrsaeoacost url hi oa) i o dn (ri ocfoi du) o(c adroruchi ocfoa) i obdgui rct doD i agc(crsadt oMgi dopbD MnRo

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31 lrlnclT

bi irst AHo*Rdi adrRd5kw8Ro*ffi ua ocfon sugc(rd asuoct ol at i odt hoSI bdLscduul n l rdasct dLeo a) i ord AE cgn oMgi t sucrdon dgst dopGRnRo*t Ksgct RofiusROi u) t crR3qqpwnHP98–%kkRo

bcl E n i i ai gHo Ro crm dt HoSR RoSi ai g HoTR_Ro5kwPRo otential z ealth Impact of EnvironmentallL Released g icro- and Tanoplastics in the z uman . ood : roduction Chain7 efiperiences from TanotofiicoloyLRo*t Ksgct RofiusROi u) t crR39qpwPnHo$985–$93qRo

bgcl Fi aHoI Rdi adrRd5k5wRoGdLcgdacgeon chi rofcgo(rd asuofgdAn i t adasct ost oa) i oal gLl ri t ao cui dt RoS) e RoTi KRoCrl sh o%qp5nHok53%kwRo

Ci rhHoGRdi adrRd5k5wdRoMo al heocfon sugc(rd asuo(dgasuri ost ohdt s) oad(oE dai gRo4 dai go pfiE saFi grdt hmow8qpwPnRo

Ci rhHoGRdi adrRd5k5wLRoMo al heocfon sugc(rd asuo(dgasuri ost ohdt s) oad(oE dai gRo4 dai gopbd i rmo w8qpwPnRo

Ccl . l i aHoORofidacHo Ro5kwqRoxn (gcKst Aoa) i ogi crl asct ocfoBi t hgsuBon d ohi fi uaodt dre s ofcgo (creni goct oE sa) ofgduasct droLd i d t sa RoD d ofi(i uagcn i ageo%qpwnRoMkkPPWkkPPRo

Cgi gi HoGRdi adrRd5kw$Rog icroplastic Kacterial communities in the BaL of Brest7in uence of polLmer tLpe and si eRo*t Ksgct RoScrnl aR535Ho%w3–%5PRo

_c) t ct HoMR Rdi adrRd5k5kRoxhi t as1 udasct odt ho. l dt as1 udasct ocfon sugc(rd asu ost o(cadLri o E dai godt hoa) i sgo cl gui oE sa) st oE dai goagi dan i t adE cgB ost oi t Ardt hodt hoE dri Ro *t Ksgct RofiusROi u) t crRoP3qpw9nHow585%-w5883Ro

7sg ai st HoxR Rdi adrRd5k5wdRov gst Bst Ao(rd asu ¸o- Wl dt as1 udasct odt ho. l drs1 udasct ocfo n sugc(rd asu ost ohgst Bst AoE dai gohs agsLl asct o e ai n oLequCOxTodt hoSeVUI D fiRo4 dai go Ti Row$$Ro

7sg ai st HoxR Rdi adrRd5k5wLRov gst Bst Ao(rd asu ¸o- Wl dt as1 udasct odt ho. l drs1 udasct ocfo n sugc(rd asu ost ohgst Bst AoE dai gohs agsLl asct o e ai n oLequCOxTodt hoSeVUI D fiRo4 dai go Ti Row$$How%Pw9Ro

7ci rn dt HoMRMRdi adrRd5kw9Rog icroplastics in freshq aters and drin iny q ater7critical revieq and assessment of data ualitLRo4 dai goTi RowPPHo3wk–355Ro

7c l a) HoD RoD d ct HofiRMRo4 daai t Li gAHo*R Ro5kw$RoMt a) gc(cAi t suouct adn st dasct ocfoad(o E dai gHoLi i gHodt ho i do draRoS GcfioQy *ow8qp3nRo

GsHo] Ro4 dt AHoj RoUl dt HobRo5k55Rofii (dgdasct odt hoshi t as1 udasct ocfot dt c(rd asu ost oad(o E dai gRo*t Ksgct RoTi Ro5k3How5w83Ro

GrcgudHoD RoCdggi HoD Ro5k5wRoI l ggi t aoxt sA) a ost acoScai t asdro*ffi ua ocfoD sugcVy dt c(rd asu o ct o- l n dt o- i dra) oLeost VKsagcoOi a RoCgct aRoOc0sucrRo8qp89nRo

GrcgudHoD Rdi adrRd5kw$RoMh cg(asct ocfo(i g/l cgcdrBero l L adt ui oct on sugc(rd asu d t hi go it Ksgct n i t adrouct hsasct Ro*t Ksgct RoScrnl aR58PHo%$k–%9wRo

GrcgudHoD Rdi adrRd5k5kdRoD sugc(rd asu ost oD i hsai ggdt i dt oucd adrodgi d@oc0susaeodt hosn (duao fcgoa) i oi t Ksgct n i t adt ho) l n dt o) i dra) RoOgi t h o*t Ksgct RoMt drRoI) i n Ro5qHoi kkk9kRo

GrcgudHoD Rdi adrRd5k5kLRoMh cg(asct odt hohi cg(asct oLi) dKscl gocfo(creu) rcgst dai ho Ls() i t er oct acon sugc(rd asu ' l gfdui ost oE dai g6 i hsn i t ao e ai n RoOc0su o$qp8nRo

GrcgudHoD Rdi adrRd5k5wRofiugi i t st Aocfo l (iuai hon sugcpt dt cm(rd asu ost oa) i o*Lgcov i rado pD i hsai ggdt i dt ofii dnRo_Ro dFdghRoD dai gR3k3Ro

D st ai t sAHofiRD Rdi adrRd5kw9RoGcE ot l n Li g ocfon sugc(rd asu ohi ai uai host ohgst Bst AoE dai go fgcn oAgcl t hoE dai go cl gui RofiusROc adro*t Ksgct Ro%3$Ho%8w–%8PRo

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6.2.1 Publication n°6 (Supplementary material)

Polymers of micro(nano)plastic in household tap water of the Barcelona Metropolitan Area

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Esteban Abad

Cristina M. Villanueva

Marinella Farré

Water Research, 2022, 220, 118645

Figure S1
Tables S1, S2, S3

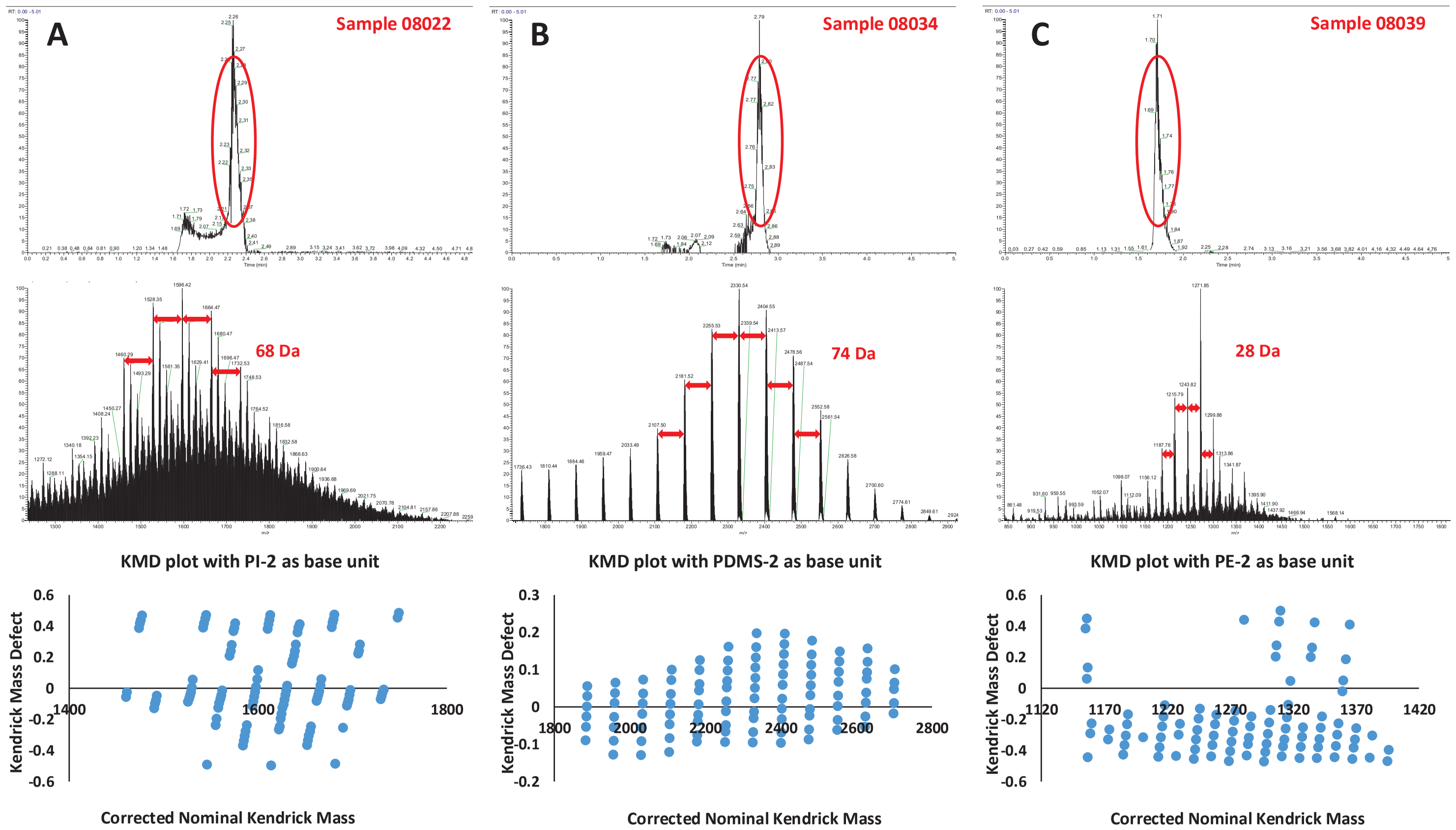

Figure S1 Examples of polyisoprene (A), polydimethylsiloxane (B), and polyethylene (C) suspect screenings and tentative identifications by Kendrick Mass Defect analysis.

Table S1 Correlation matrix among variables expressed in cos2(α) at significance level of 95 %.

Variables	PE	PI	PBD	PP
PE	1	-0.097	-0.049	-0.050
PI	-0.097	1	0.228	-0.062
PBD	-0.049	0.228	1	-0.089
PP	-0.050	-0.062	-0.089	1

Table S2 Size and distribution of micro(nano)polymers found in the drinking water samples analysed providing the kind of polymer found, the weight in Da, the number of carbons and hydrogen atoms and the number of monomers that contains the polymeric chain.

Sample	Polymer	Chain weight (Da)*	C	H	$(C_XH_Y)n$
08001	PE	734.7384	52	105	26
	PP	1,464.5962	105	209	34
	PP	1,800.9735	129	257	42
	PP	2,179.3950	156	311	51
	PP	2,599.8247	186	371	61
08002	PE	822.5056	59	118	29
08003	PP	685.2623	49	98	16
08004	PBD	969.6010	72	108	18
	PI	1,230.0600	90	145	18
	PI	1,801.5976	132	212	26
	PI	2,278.0073	168	268	34
	PI	2,702.3190	199	318	40
08005	PE	843.8848	60	121	30
	PE	1,320.4040	94	189	47
	PP	1,222.2915	87	175	29
	PP	1,844.1690	132	263	43
	PP	2,137.3490	153	305	50
	PP	2,599.8605	186	371	61
08006	PE	706.7066	50	101	25
08007	PI	1,351.0752	99	159	20
	PI	1,895.5783	139	223	28
	PI	2,372.9927	174	279	35
	PI	2,508.1209	184	295	37
08008	PE	954.0210	68	136	34
	PE	1,866.0273	133	267	66
	PE	2,316.5010	165	331	82
	PE	2,708.8730	193	387	96
08009	PE	713.7182	51	102	25
	PE	1,744.5220	125	249	62
	PP	1,800.9778	129	257	42
	PP	2,263.4808	162	323	53
	PP	2,599.8247	186	371	61
08010	PE	706.7096	50	101	25
	PE	2,743.8975	196	392	98
	PP	1,464.5979	105	209	34
	PP	1,843.0294	132	263	43
	PP	2,557.7803	183	365	60
08011	PI	1,555.2767	114	183	23
08012	PE	689.7159	49	99	24
	PP	1,475.5500	105	211	35
	PP	1,769.8776	126	253	42
08013	PE	1,276.3495	91	182	45
	PP	1,885.0841	135	269	44
	PP	2,389.6210	171	341	56
08014	PE	706.7068	50	101	25
	PBD	709.4314	53	79	13
	PI	1,408.2344	104	166	21
	PI	1,664.4644	122	196	24
	PI	2,498.1695	184	294	37
	PP	1,843.0237	132	263	43

	PP	2,305.5086	165	329	54
	PP	2,179.3906	192	383	63
08015	PP	2,137.3219	153	305	50
08016	PP	1,222.2907	87	175	29
	PP	2,347.5502	168	335	55
08017	PE	869.9125	62	124	31
	PP	1,379.8775	99	197	32
	PP	2,389.6133	171	341	56
08018	PE	759.7957	54	109	27
	PP	1,180.2484	84	169	28
	PP	2,347.5442	168	335	55
08019	PE	999.8609	71	143	35
	PE	1,576.3666	113	225	56
	PE	2,613.0667	187	373	93
	PI	1,487.2061	109	175	22
08020	PE	629.6232	45	90	22
	PE	706.7064	50	101	25
08021	PE	1,511.2924	108	216	53
	PP	2,347.5444	168	335	55
	PP	2,642.8739	189	378	62
08022	PI	1,392.2322	102	164	20
	PI	1,664.4728	122	196	24
	PI	2,278.0123	168	268	34
	PI	2,686.3187	198	316	40
08023	PE	678.6754	48	97	24
	PI	1,419.1419	104	167	21
	PI	1,691.3858	124	199	25
08024	PE	678.6780	48	97	24
	PE	1,756.9084	125	251	62
	PP	1,366.3239	98	195	32
	PP	1,927.1161	138	275	45
	PP	2,221.4312	159	317	52
	PP	2,599.8181	186	371	61
08025	PE	706.7065	50	101	25
	PI	941.6989	69	111	14
	PI	1,623.3251	119	191	24
	PI	2,371.9895	174	279	35
	PI	2,509.0958	184	295	37
08026	PI	863.7307	64	102	12
	PI	1,596.4202	117	188	23
	PI	2,550.2749	188	300	37
08027	PE	1,262.3320	90	180	45
	PP	1,180.2403	84	169	28
	PP	1,885.0915	135	269	44
	PP	2,515.7480	180	359	59
08028	PE	822.5056	59	118	29
	PI	1,351.0743	99	159	20
	PI	1,827.5262	134	215	27
	PI	805.5743	59	95	12
	PI	2,577.1744	189	303	38
08029	PE	1,098.0787	78	157	39
	PE	678.6776	48	97	24
	PI	1,135.9601	84	134	17
08030	PI	1,801.6050	132	212	26
	PI	2,209.9541	162	260	32
	PI	2,702.3135	199	318	40
08031	PE	706.7101	50	101	25
	PI	1,487.2128	109	175	22
	PI	1,759.4586	129	207	26
	PI	2,167.8334	159	255	32
	PP	2,136.3268	153	305	50
08032	PE	1,262.3007	90	180	45
	PP	1,306.3748	93	187	31
	PP	1,642.7282	117	235	39
08033	PP	1,843.0208	132	263	43
	PP	2,053.2468	147	293	48
	PP	2,683.8990	192	383	63
08034	PE	706.7098	50	101	25
	PI	1,324.1679	97	156	19
	PI	1,733.5346	127	204	25
	PI	2,225.9508	164	262	33
	PP	2,052.2327	147	293	48
08035	PI	873.6480	64	103	12
	PI	1,419.1671	104	167	20
	PI	1,759.4803	129	207	25
	PI	2,208.9302	162	260	32
08036	PI	795.6674	59	94	11
	PI	1,665.4894	122	196	24
	PI	2,482.2203	183	292	36
	PP	1,054.1065	75	151	25
08037	PE	706.7112	50	101	25
	PBD	763.4445	57	85	14
	PI	1,555.2708	114	183	23
	PP	2,011.1930	144	287	47
08038	PP	1,844.1966	132	263	43
	PP	2,599.8352	186	371	61
08039	PE	1,271.8550	91	182	45
	PE	2,585.0913	185	369	92
	PP	2,179.3906	156	311	51
08040	PE	629,6236	45	90	22
	PBD	599.4836	44	67	11
	PI	1,419.1498	104	167	21
	PI	1,827.5242	134	215	27
08041	PE	675.7048	48	97	24
	PS	1,593.9412	123	123	15
	PS	2,010.1869	155	155	19
	PP	2,179.4027	156	311	51
08042	PE	706.7076	50	101	25

Table S3 PCA factors and variability explained of each one as individual and cumulative in %.

	F1	F2	F3	F4
Variability (%)	38.30	26.63	20.14	14.93
Cumulative (%)	38.30	64.93	85.07	100

6.2.2 Publication n°7

Exposure to micro(nano)plastics polymers in water stored in single-use plastic bottles

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Chemosphere, 2023, 343, 140106

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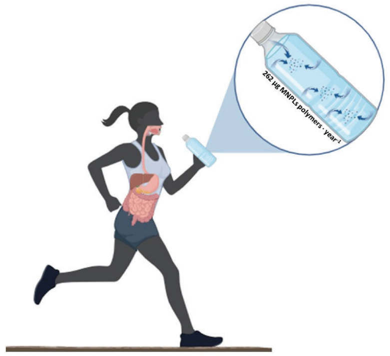

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1. Introduction

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2. Materials and methods

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2,6, MT: gs8plastic additives prioritisation studx

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T. besults and discussion

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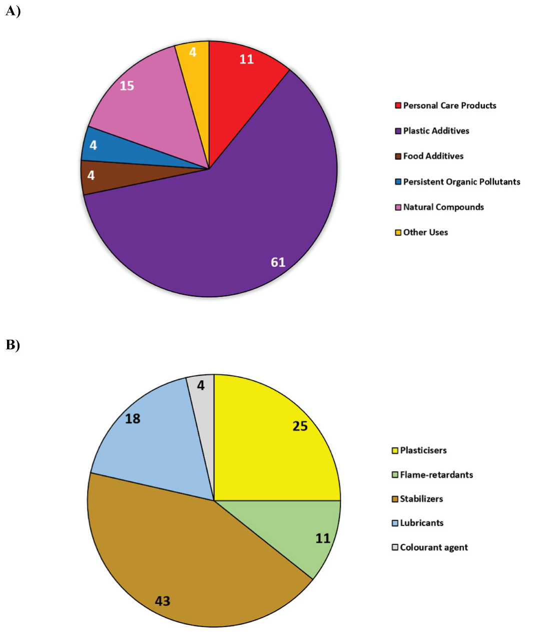

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6.2.2 Publication n°7 (Supplementary material)

Exposure to micro(nano)plastics polymers in water stored in single-use plastic bottles

Albert Vega-Herrera

Maria Garcia-Torné

Xavier Borrell-Diaz

Esteban Abad

Marta Llorca

Cristina M. Villanueva

Marinella Farré

Chemosphere, 2023, 343, 140106

Tables S1, S2, S3, S4, S5
Figure S1

Table S1 Bottled water brands from single-use plastic bottles analysed and their total filtered volumes by 1.5 and 0.5 L bottles.

Sample name	Sample code	Bottle	Tap	Volume filtered (mL) from 1.5 L bottles	Volume filtered (mL) from 0.5 L bottles	Volume filtered (mL)	Water category
27,00035-LU	1	PET	PE	6,000	5,110	-	Treated Drinking Water
27,02231-BA	2	PET	PE	6,170	5,200	-	Mineral Water
27,00019-GE	3	PET	PE-PP	6,185	5,250	-	Mineral Water
27,02402-HU	4	PET	PE	6,115	5,105	-	Mineral Water
08,721177-ES	5	PET	PE	6,030	5,150	-	Treated Drinking Water
27,00055-GE	6	PET	PE	6,150	5,165	-	Mineral Water
27,00031-SG	7	PET	PE	6,120	5,145	-	Mineral Water
27,00013-CU	8	PET	PE	6,235	5,050	-	Mineral Water
27,00009-GR	9	PET	PE-PP	6,080	5,260	-	Mineral Water
27,01078-OR	10	PET	PE	6,180	5,180	-	Mineral Water
27,00042-GE	11	PET	PE	5,960	5,115	-	Mineral Water
462,525791-BE	12	PET	PE	6,090	5,080	-	Mineral Water
27,01051-GE	13	PET	PE	6,175	5,060	-	Mineral Water
27,00684-SO	14	PET	PE	6,060	5,055	-	Treated Drinking Water
27,01383-J	15	PET	PE	6,030	4,905	-	Mineral Water
27,01376-V	16	PET	PE	6,200	5,410	-	Treated Drinking Water
27,02518-LE	17	PET	PE	6,100	5,080	-	Mineral Water
502,607920-PG	18	PP-PET	PE	6,210	5,120	-	Treated Drinking Water
797,080850-FR	19	PET	PE-PP	6,230	5,280	-	Mineral Water
27,01376-V	20	PET	PE	6,140	5,640	-	Mineral Water
Blank 1	Blank 1	GLASS	PE	-	-	4,975	-
Blank 2	Blank 2	GLASS	PE	-	-	5,055	-

Table S2 Algorithms developed by Python programming language for the extra-screening of the results obtained by the Compound Discoverer v3.1 in order to automate the MNPLs-plastic additives suspect screening.

Script 1: Replacement of filled boxes with an X by the Excel file with the final results obtained by Compound Discoverer software.

```
import openpyxl as xl
wb = xl.load_workbook('SuspectScreeningPlasticAdditives.xlsx')
sheet = wb['Hoja1']

row = 2
for row in range(2, sheet.max_row + 1):
    i = 5
    for i in range (5,47):
        cell = sheet.cell(row,i).value
        if cell == '':
            pass
        else:
            cell = str(cell)
            cell = cell.replace(cell,'X')
            sheet.cell(row,i).value = cell
        i += 1

wb.save('SuspectScreeningPlasticAdditives.xlsx')
```

Script 2: Estimation of the detection frequency of compounds in the mineral waters analysed from the single-use plastic bottles.

```
import openpyxl as xl
wb = xl.load_workbook('SuspectScreeningPlasticAdditives.xlsx')
sheet = wb['Hoja1']

cell_title = sheet['AW1']
title = 'Detection frequency'
cell_title.value = title

for row in range(2, sheet.max_row + 1):
    detection_frequency =  0
    for column in range(5, 47):
        if sheet.cell(row, column).value == 'X':
            detection_frequency += 1
            column += 1
        else:
            column += 1

    detection_frequency = detection_frequency/40
    sheet.cell(row,49).value = detection_frequency
    row += 1

wb.save('SuspectScreeningPlasticAdditives.xlsx')
```

Script 3: Estimation of Delta Mass error for the tentatively identified compounds.

```
import openpyxl as xl
import chemcalculator
from chemcalculator.chemcalculator import compute_mass
wb = xl.load_workbook('SuspectScreeningPlasticAdditives.xlsx')
sheet = wb['Hoja1']

i = 2
for row in range (i, sheet.max_row + 1):
    compost = sheet.cell(i, 2).value
    compost2 = compost.replace(' ','')
    sheet.cell(i,2).value = compost2
    i +=1


cell_title = sheet.cell(1,47)
title = 'Mass'
cell_title.value = title

cell_title2 = sheet.cell(1,48)
title2 = 'DeltaMass'
cell_title2.value = title2


j = 2
for row in range (j, sheet.max_row + 1):
    compost2 = sheet.cell(j, 2).value
    mass = compute_mass(compost2)
    mass = float(mass)
    sheet.cell(j,47).value = mass

    MolecularWeight = sheet.cell(j,3).value
    DeltaMass = sheet.cell(j,48).value
    DeltaMass = ((mass-MolecularWeight)/mass)*1000000
    sheet.cell(j,48).value = DeltaMass

    j += 1
wb.save('SuspectScreeningPlasticAdditives.xlsx')
```

Script 4: Previous assignation of the type, use and source of the compounds detected in the new study by the home-made library of the research group.

```python
import openpyxl as xl
wb = xl.load_workbook('SuspectScreeningPlasticAdditives.xlsx') #NEW STUDY
sheet = wb['Hoja1']
wb1 = xl.load_workbook('LibraryCompoundsResearchGroup.xlsx') #HOME-MADE
LIBRARY
sheet1 = wb1['Hoja1']

cell_title = sheet.cell(1,50)
title = 'Type'
cell_title.value = title

cell_title2 = sheet.cell(1,51)
title2 = 'Uses'
cell_title2.value = title2

cell_title3 = sheet.cell(1,52)
title3 = 'Source'
cell_title3.value = title3

i = 2
for row in range(i,sheet.max_row+1):
    name = sheet.cell(i,1).value
    counter = 2
    for counter in range(2,sheet1.max_row +1):
        name1 = sheet1.cell(counter, 1).value
        if name != name1:
            counter += 1
        else:
            sheet.cell(row, 50).value = sheet1.cell(counter, 51).value
            sheet.cell(row, 51).value = sheet1.cell(counter, 52).value
            sheet.cell(row, 52).value = sheet1.cell(counter, 53).value

    i += 1

wb.save('SuspectScreeningPlasticAdditives.xlsx')
```

Script 5: Development of the pie charts used as Figure 1 in this research article for the classification of the total compounds and plastic additives tentatively identified.

```python
import openpyxl as xl
from openpyxl.chart import PieChart, Reference

wb = xl.load_workbook('SuspectScreeningPlasticAdditives.xlsx')
sheet = wb['Hoja1']

types = []
for row in range(2,sheet.max_row+1):
    compound_type = sheet.cell(row, 51).value
    types.append(compound_type)

final_list = []
row = 2
for i in types:
    if i in final_list:
        for row1  in range(2, sheet.max_row+1):
            if sheet.cell(row1,55).value == i:
                sheet.cell(row1,56).value +=1

    if i not in final_list:
        final_list.append(i)
        sheet.cell(row, 55).value = i
        list_cell = sheet.cell(row, 55).value
        list_counter = sheet.cell(row,56).value = 1
        row += 1

count_max = 2
for row in range(2,sheet.max_row+1):
    chart_data_max = sheet.cell(row, 55).value
    if not chart_data_max is None:
        count_max +=1
        row +=1
print(count_max-1)
values = Reference(sheet,
                     min_col = 55,
                    max_col = 56,
                    min_row = 2,
                    max_row = count_max-1
                    )


chart = PieChart()
chart.add_data(values)
sheet.add_chart(chart, 'bf2')

wb.save('SuspectScreeningPlasticAdditives.xlsx')
```

Table S3 Validation of the quality parameters for the polymers analysed.

					Precision (n=3) – Repeatability (%)	
Polymer	Acronym	Recovery 10 ppb (n=3, %)	MLoD (ng·L^{-1})	MLoQ (ng·L^{-1})	Inter-Day	Intra-Day
Polyethylene	PE	61	1	14	21	1
Polypropylene	PP	86	4	27	9	4
Polyethylene terephthalate	PET	72	78	116	7	4

Table S4 Compounds tentatively identified applying the MNPLs-plastic additives suspect screening excluding plastic additives found.

Compound	Classification
12-Oxahexadecanolide	Personal Care Products (PCPs)
Didecyldimethylammonium	
Dihydroxiethyl stearamine oxide	
Lauryldimethylamine oxide	
N~2~-Ethyl-N~4~,N~6~-bis(2-methyl-2-propanyl)-1,3,5-triazine-2,4,6-triamine	
L-(+)-Leucine	Food additives
Diethyl tartrate	
O-Cyclohexyl methylphosphonofluoridothioate	Persistent Organic Pollutants (POPs)
Ethyl trifluoromethyl disulfide	
(+)-12(S),13(R)-vernolic acid	Natural compounds
2-Amino-1,3,4-octadecanetriol	
5-Hydroxy-7-[4-hydroxy-2-methoxy-5-(3-methyl-2-butenyl)phenyl]-2,2-dimethyl-7,8-dihydro-2H,6H-pyrano[3,2-g]chromen-6-one	
Arachidoyl ethanolamide	
Embelin	
N-Acetyl-L-leucine	
Traumatin	
7-Pentofuranosyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine	Other uses
Nitrosomorpholine	

Table S5 Uses and classification of the plastic additives identified tentatively in bottled water samples analysed from the single-use plastic bottles.

Plastic Additive	Use	Classification
1,1,2,2-Tetracyanocyclopropane	Stabilizer	Stabilizers
2,2'-(Hexadecylimino)diethanol	Used in PP synthesis	
3,3'-(Phenylphosphinediyl)dipropanenitrile	Polymerisation catalyst	
D-Xylonic acid	Used in Nylon synthesis	
N-Methylmorpholine	Stabilizer	
Octadecenylsuccinic anhydride	Hydrolisis stabilizer	
Bis(2-ethoxyethyl) hydrogen phosphate	Production of transparent polythiourethane bodies	
Methyl 3,5-dimethoxybenzoate	Styrene derivative	
Dibenzyl sulfide	Depolyumeration	
Lauramide	Thickener and viscosity builder	
Linolenelaidic acid	Filler	
Phthalic anhydride	Used in phthalate synthesis	
Bis(tetramethylhydroxypiperidinyl) sebacate	Sebacate	Plasticisers
Diethyl phthalate (DEP)	Phthalate	
Bis(2-ethylhexyl) phthalate	Phthalate	
Diisobutylphthalate	Phthalate	
Bis(2-ethylhexyl)adipate	Adipate	
N-Butylbenzenesulfonamide	Polymerisation of polyamides	
Tetramethylurea	Plasticiser	
4-Isopropylphenyl diphenyl phosphate	Flame-retardant	Flame-retardants
Tris(3,4-dimethylphenyl) phosphate	Flame-retardant	
Aminopropyl dihydrogen phosphate	Flame-retardant	
Anthraquinone	Intermediate	Colourant agents
Ethylene palmitate	Lubricant	Lubricants
Methyl 9,10,12,13-tetramethoxyoctadecanoate	Lubricant	
N-Caprylyldiethanolamine	Lubricant	
Stearyldiethanolamine	Lubricant	
Octylamine	Lubricant	

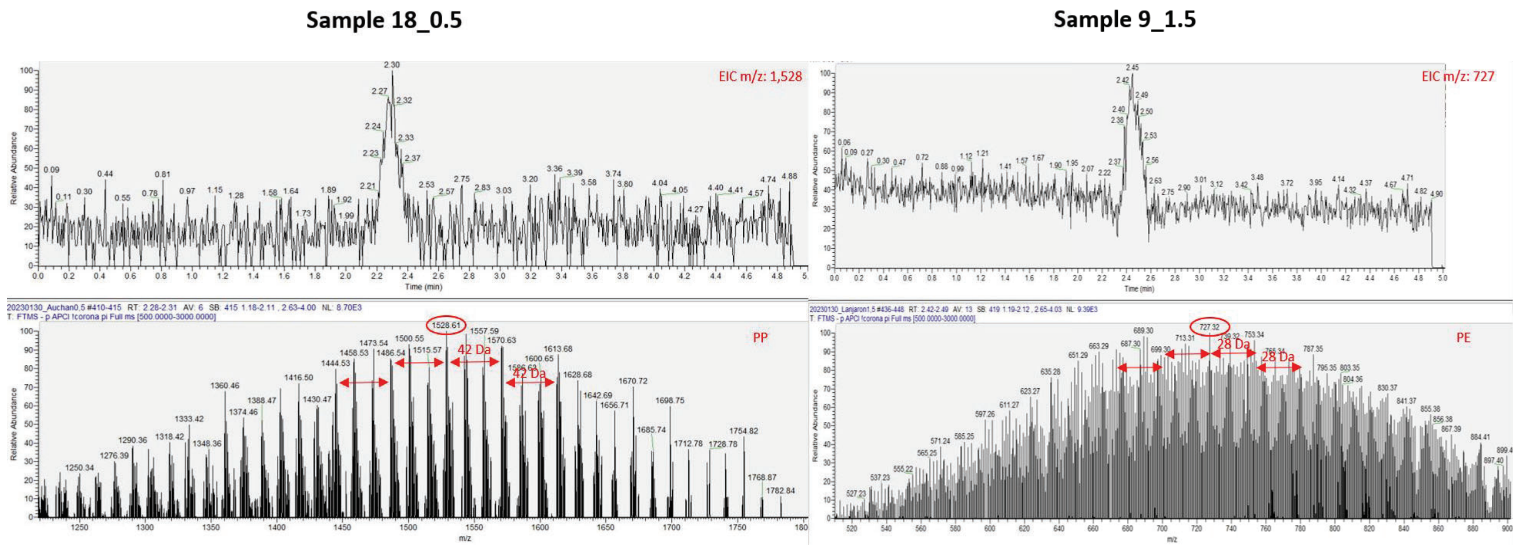

Figure S1 Extracted ion chromatograph (EIC) and MS spectra of polypropylene (PP) and polyethylene (PE) identified and quantified in samples 18_0.5 and 9_1.5.

6.2.3 Publication n°8

Bioaccumulation and dietary bioaccessibility of microplastics composition and cocontaminants in Mediterranean mussels

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BIOACCUMULATION AND DIETARY BIOACCESSIBILITY OF MICROPLASTICS COMPOSITION AND COCONTAMINANTS IN MEDITERRANEAN MUSSELS

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Abstract

Microplastics (MPLs) are contaminants of emerging concern (CECs) ubiquitous in aquatic environments, which can be bioaccumulated along the food chain.

In this study, the accumulation of polyethylene (PE), polystyrene (PS), and polyethylene terephthalate (PET) microplastics (MPLs) of sizes below 100 µm was assessed in Mediterranean mussels (Mytilus galloprovincialis spp). Moreover, the potential of mussels to uptake and bioaccumulate other organic contaminants, such as triclosan and per- and polyfluoroalkyl substances (PFASs), was evaluated with and without the presence of MPLs. Finally, the modulation of MPLs in the human bioaccessibility of co-contaminants was evaluated by *in vitro* assays that simulated human digestion. Exposure experiments were carried out in 15 L marine microcosms. The bioaccumulation and bioaccessibility of PE, PS, PET, and co-contaminants were assessed by means of liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS).

Our results confirm that MPLs bioaccumulation in filter-feeding organisms is a function of the MPL's chemical compositions and particle sizes, as small particles are better retained than bigger ones. Finally, despite the lower accumulation and bioaccumulation of PFASs in the presence of MPLs, the bioaccessibility test revealed that PFASs bioaccessibility was increased in the presence of MPLs. Since part of the bioaccumulated PFASs is adsorbed onto MPL surfaces by electrostatic interactions, these interactions easily change with the pH during digestion, and the PFASs bioaccessibility increases.

Keywords: Microplastics, PFASs, Exposure experiments, Bioaccumulation, Human bioavailability.

Highlights

1) Accumulation of MPLs in mussels is function of their chemical compositions and particle sizes.

2) Aggregation of contaminants onto MPLs surface is mainly ruled by hydrophobic interactions and the particles net surface charge.

3) Lower bioaccumulation of PFASs in mussels in co-occurrence of MPLs in the media was shown.

4) Nevertheless, PFASs bioaccessibility was increased when MPLs were present in the same media.

1. Introduction

The rising production of plastics and insufficient waste management practices have increased plastic litter in the environment. In particular, marine environments are heavily impacted [1, 2]. Once in the environment, plastic litter can break down by erosion and UV degradation to small particles (< 5 mm) [3], which are known as secondary microplastics (MPLs). Also, plastic particles can enter the environment in small sizes due to car wheel erosion, cloth fibres, plastic particles used in cleaning products and toothpaste, or paint dust, known as primary MPLs [4]. Marine biota can ingest plastics and MPLs with negative effects [5], including mechanical damages such as smothering and clogging of the digestive tract [6], toxicological effects because of plastic additives or co-contaminants, and biological effects that can include hepatic stress, inflammation, impaired movement, and slowed growth rates [7]. In marine ecosystems, MPLs have been detected in 17% of the species of the International Union for Conservation of Nature (IUCN) Red List [8] and can contribute to reducing marine biodiversity [7].

Although MPLs cannot be digested by biota, the smallest particle sizes < 100 µm, including those at the nano-range known as nanoplastics (NPLs), can be translocated through tissues, being considered as bioavailable [9]. Moreover, plastic additives in their composition are generally not chemically bound to the polymeric chains and can easily leach into biological systems. On the other hand, due to the surface properties of polymers, MPLs can adsorb other co-contaminants from surrounding environments [10-12], which can promote their transfer to biota, known as the Trojan-horse effect. The bioaccumulation and biomagnification of MPLs through fish and shellfish consumption can also be a new threat to human health [4].

Filter-feeding organisms, such as marine mussels, have been widely used as environmental sentinels for pollution monitoring in coastal environments [13] and to carry out different exposure experiments in microcosms under controlled conditions [14-16]. Moreover, different marine mussels species, such as the Mediterranean mussels (*Mytilus galloprovincialis*), are widely distributed, have strong tolerance to environmental factors such as temperature and salinity changes, and can grow in inter-tidal areas of coastal environments heavily impacted by MPLs and CECs [17]. Therefore, many studies have reported the presence of MPLs in marine molluscs [18-21]. For example, Schrank et al.[22], studied the accumulation of microplastics in four mussel species for human consumption, showing that in one gram of mussel meat, between 0.13 and 2.45 microplastic particles were found with sizes ranging between 3 and 5,000 µm. In addition, it should be mentioned that mussels have a limited biotransformation capacity for pollutants compared to fish. Nevertheless, no harmonised analytical methods are available to assess MPLs or their composition in fish and shellfish for human consumption. Moreover, most analytical approaches are based on spectroscopic techniques, and their quantification is based on particle counting. The differences between particles make it difficult to compare between studies, and it is difficult to establish maximum residue levels for human consumption. Few studies have assessed the bioaccumulation of MPLs, their influence on the accumulation of other CECs of surrounding environments [23], and much less investigating how these mixtures are bioavailable through the human diet [24].

In this study, Mediterranean mussels were exposed through the diet to MPLs < 100 µm of polyethylene (PE), polystyrene (PS), and polyethylene terephthalate (PET) in marine microcosms for 16 days to assess: (i) the accumulation of the different MPLs, (ii) the potential of MPLs to influence the accumulation of other contaminants such as polyfluorinated compounds (PFASs) and triclosan, and (iii) how MPLs can modulate the human bioaccessibility of PFASs using an *in vitro* test that simulated the human digestion.

2. Experimental section

2.1 Chemicals

Polyethylene terephthalate microspheres (PET; 300 µm) were purchased from GoodFellow Cambridge Limited (Huntingdon, England), while polystyrene (PS; 10-63 µm) and polyethylene (PE; 10-63 µm) microspheres were supplied by Cospheric LLC (Goleta, CA, United States). The standards were posteriorly cryogenic grinded to achieve the particles sizes of interest, see section 2.3.

Polymer analytical standards of PE (Mw. 778 Da), PS (Mw. 887 Da) and PET (Mw. 625 Da) were acquired from Polymer Standard Service GmbH (Mainz, Germany).

Perfluorooctanoic acid (PFOA), perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS) and perfluorooctane sulfonate (PFOS) were purchased from Merck (Darmstadt, Germany). Mixture of native perfluoroalkyl acids and sulfonates MXB (> 98

%) in methanol and a mixture of labelled PFASs MXA (> 98 %) were provided from Wellington Laboratories Inc. (Guelph, Canada).

Nitric acid (HNO_3 65 %), potassium hydroxide (KOH 99.99 %) and sodium chloride (NaCl 99 %) were supplied by Sigma-Aldrich (Steinheim, Germany). Glass microfiber filters (GF/F) of 0.70 µm pore size were purchased from Whatman TM (Maidstone, United Kingdom) and nitrogen was used as drying gas with 99.995 % purity and supplied by Air Liquide (Barcelona, Spain).

The mussel phytoplankton feed was composed by a microalgae mixture, with particles size between 3-16 µm purchased from Acuinuga SL (A Coruña, Spain).

Unless otherwise stated, all reagents used were of analytical grade. Water, acetonitrile and methanol Optima LC-MS grade were provided by Fisher Chemical (Loughborough, United Kingdom), toluene CHROMASOLV®PLUS was supplied by SupraSolv from Merck (Darmstadt, Germany), and ammonium acetate salt ($AcNH_4$: 77.08 $g \cdot mol^{-1}$; 98 %), dichloromethane and trifluoroacetic acid by Sigma-Aldrich.

2.2 Sampling

Mediterranean mussels of 5 ± 0.1 cm shell length were caught from a bed of El Fangar (40° 46' 27.707" N 0° 45' 33.616" E), the northern marine bay of the Ebro Delta, and transported alive to IDAEA-CSIC facilities. Mussels were selected since they have suitable properties for biomonitoring, including their widespread abundance, ability to adapt and thrive in a wide range of environments, and sedentarism. Moreover, they have sufficiently long life cycles for the assessment of bioaccumulation of organic contaminants and have sufficient size to yield enough tissue for analysis. Besides, this type of study requires no animal welfare permits and complies with all relevant regulations [25, 26]. No protected species were sampled or disturbed during the collection of organisms.

2.3 Preparation and particles characterisation

Manufactured PE and PS (10-63 µm) and PET (300 µm) microparticles were cryogenically ground with liquid nitrogen and a Freezer/Mill 6875® grinder provided by ATS Scientific Inc. (Burlington, Canada). The cryogenic grinder process consisted of 3 cycles of 4 min, each followed by 2 min for precool and cool, and using a rate of 10 cps.

This process was repeated until reaching the particle size of interest (1-10 µm and < 0.9 µm for PE, while for PET, 10-63 µm).

The PE and PET MPLs particle sizes were measured by scanning electron microscopy (SEM) using a JEOL JSM-7001F (JEOL Ltd, Tokyo, Japan) from the Scientific and Technological Unit of the University of Barcelona (CCiTUB), while PE-NPLs particle sizes were measured by nanoparticle tracking analysis (NTA) using a NanoSight LM10-HS (Amesbury, United Kingdom) equipped with an LM14 nanoparticle display unit with temperature controlled, and a monochromatic CCD camera.

2.4 Microcosms set-up for accumulation and bioaccumulation experiments

Microcosm experiments were conducted in glass tanks of 15 L filled with ultrafiltered seawater from a pristine area located in Sant Feliu de Guíxols (NE, Spain). During the experiments, salinity, pH, nitrite, nitrate concentrations (NO_2^-, NO_3^-), and dissolved O_2 were measured daily and maintained with a salinity of 34 psu. Evaporation losses were corrected by adding distilled water. The temperature was kept at 18.0 ± 0.5 °C, whereas the pH was 8.15 ± 0.20. The median values of the NO_2^- and NO_3^- concentrations were below detection limits, with ~25 % and 35 % of positive measures and mean concentrations of 0.4 and 3.8 $mg \cdot L^{-1}$, respectively.

For the bioaccumulation experiments, Mediterranean mussels (about 25 mussels per tank) were exposed to selected contaminants by their diet and water through static renewal exposure schemed. The experiments were carried out in three consecutive stages: three days of acclimatisation (t_0), ten days of exposure (t_1), and three days of detoxification (t_2). After the acclimatisation (t_0) the dead mussels were extracted from the tanks (approx. 2-3 mussels per aquarium) and 5 mussels were randomly collected and immediately frozen at -60 ºC. At t_1, five mussels were randomly collected while 10 mussels were taken at t_2, to have the same number of mussels for accumulation experiments (5 mussels) and 5 more for the bioaccesibility experiments. The set-up, according to the OECD test 305 [27], should include two phases: the exposure (uptake) and post-exposure (depuration) phases, and the aquatic organisms are usually exposed in a flow-through system to at least one concentration of the test substance for 28 days. The duration of this assay can be lengthened if necessary or shortened if it is demonstrated that a steady state is reached earlier. In this case, we added a step of acclimatization because the mussels were collected from the environment. The exposure was shortened because, as seen in previous studies, due to the high filtration capacity of mussels, the steady state is achieved after two days of exposure [28]. Moreover, the purpose of our study was to compare the accumulation with and without MPLs, not to establish the bioaccumulation kinetics, and we used static renewal exposure.

During the acclimatisation and detoxification, mussels were fed with 1.5 mL of a mixture of saline microalgae without any fortification, and aquariums were maintained with clean water. MPLs exposure was carried out through the diet using this mixture of polluted microalgae with MPLs of PE, PS, and PET. The exposure to PFASs, or triclosan, was performed by direct spike in seawater because the solubility in seawater at 20 ºC of these compounds is much higher than the exposure concentrations used in this experiment (solubility of PFOS at 20 ºC is 519 mg·L^{-1}, 3,300 mg·L^{-1} for PFOA and 12 mg·L^{-1} for triclosan). For triclosan, the concentration of spike was 1.5 µg·L^{-1}, while the mixture of PFASs (composed with PFOA, PFBS, PFHxS and PFOS) was 5 µg·L^{-1} of each.

Polluted algae were prepared before their use by mixing MPLs and the algae, stirring, and waiting 24 h to achieve the adsorption equilibrium [10]. The quantity of MPLs added to each tank every 2 days was 1,175 mg to reach 75 µg·L^{-1}. Feeding was performed every two days to ensure enough food supply and to perform the exposure experiments. In parallel, a mussel population was fed with clean algae used as a control and non-spiked water. At the end of each stage, five mussels were collected from every microcosm and immediately frozen at -60 ºC.

Finally, to assess the influence of the size of MPL particles in the accumulation, Mediterranean mussels were exposed to PE particles of three sizes (10-63, 1-10 and < 0.9 µm).

In **Figure 1,** the scheme of the different exposure experiments is presented. For the exposure tanks, when the water was replaced every 2 days, the spiking was repeated.

2.5 Bioaccessibility assays

The edible part of mussels was digested in triplicate with three human digestion fluids (salivary, gastric, intestinal (duodenal and bile)) according to the *in vitro* digestion model described by Braga et al. [29]. Procedural blanks were also prepared by containing the digestion fluids without mussels. Very briefly, 2 g of pooled mussel edible tissues, including blanks, were digested in glass containers at 37 °C using a Rotary Tube Mixer with Disc in an incubator. The simulated digestion included the oral phase (4 mL of saliva fluid for 5 min at pH 7.0 ± 0.2), gastric phase (8 mL of gastric fluid for 2 h at pH 2.0 ± 0.2) and intestinal phase (8 mL of duodenal fluid and 4 mL of bile fluid for 2 h at pH 7.0 ± 0.2). Each digestion fluid, followed by pH adjustment, was prepared before use. After incubation, the reaction was stopped by placing the reaction tubes on ice. Then, the tubes were centrifuged at 30,000 rpm (10 cm rotor) at 10 °C for 10 min to separate the bioaccessible fraction (BIO) and these fractions were collected and stored at -20 °C until analysis.

In **Figure S1** of the Supporting Information, a scheme of digestion protocol is presented.

2.6 Sample pre-treatments

The edible part of the mussels was collected, grounded in an agate mortar and, subsequently, further grounded using a cryogenic grinder with the same procedure described in section 2.3.

To extract and clean up the MPLs, 0.5 g of each sample was weighed in glass vials, MPL particles were refloated by the addition of a saturated solution of NaCl (359 g·L^{-1}). Then, the supernatant was filtered with GF/F 0.7 µm and digested using 50 mL of KOH 10 % (w/v) overnight at room temperature, followed by acid digestion with 40 mL HNO_3 20 % (w/v) for 15 min. Samples were dried at room temperature, and PE and PS-MPL polymers were extracted for 10 min with 10 mL of toluene by ultrasonic-assisted extraction (USAE). The UASE was repeated two times more, and the extracts of each extraction were combined in a final volume of 30 mL. Then, the toluene extract was evaporated under a gentle stream of nitrogen at 40 °C to a final volume of 1 mL. The final extracts were divided into 2 aliquots of 500 µL and transferred to LC vials. First aliquot was reserved for PE and PS polymer analysis, whereas the second one for PET. However, for PET-MPLs, the aliquot was evaporated up to 250 µL and reconstituted 1:1 toluene-dichloromethane with 1 % trifluoroacetic acid. Final extracts were kept at -20 °C until LC(SEC)-HRMS instrumental analysis.

To extract the triclosan content from mussels, 0.5 g were weighted and extracted by USAE with 4 mL of methanol for 15 min. After this process, the mixtures were centrifuged for 10 min (1,000 rpm, 20 °C), and, finally, an aliquot of 0.5 mL was taken, transferred to a LC-vial and stored at -60 °C until their analysis.

For the extraction of the PFASs mixture from exposed mussels, 0.5 g of each sample was weighted in a 15 mL polypropylene (PP) centrifuge tube, spiked with 50 µL of a mixture of internal standards (100 ng·mL^{-1} in methanol) and kept at room temperature for 30 min. Then, an alkaline digestion was carried as described elsewhere [30]. Briefly, 10 mL of 10 mM NaOH in methanol was added, homogenized, and extracted by USAE for 5 min and shaken in a Stuart flash shaker (250 oscillations per minute) for 2 h. The mixture was centrifuged for 10 min (2,000 rpm, 20 °C). Then, the supernatant was transferred to a 15 mL PP tube and evaporated under a gentle N_2 stream c.a. 1 mL. Then, the extracts were transferred to 50 mL PP tubs and diluted 1:50 with HPLC water. The extract purification was conducted by solid phase extraction (SPE) with Oasis WAX (3 cc, 60 mg) cartridges from Waters (Milford, MA, United States), according to [31]. The purification procedure consisted of cartridges conditioning with 2 x 2 mL of methanol followed by 2 x 2 mL of water under gravity conditions. Next, the samples were loaded into the cartridges by gravity, and then the cartridges were dried under vacuum for 15 min. PFASs were eluted in 15 mL PP vials with 2 x 2 mL of NH_4OH 0.1 % in methanol. After elution, the extracts were evaporated under a gentle N_2 stream until c.a. 0.2 mL and were transferred to LC vials equipped with inserts. Finally, these extracts were evaporated under a nitrogen stream until dryness and reconstituted in 0.2 mL of water: methanol (9:1) and stored at -20 °C until analysis.

For the analysis of the bioaccessibility assays, 25 mL of bioaccessible fraction from each sample was transferred to a 50 ml PP tube, spiked with 50 µL of a mixture of internal standards MXA (100 ng·mL^{-1} in methanol) and kept at room temperature for 30 min. The final spiked bioaccessible fraction was diluted with HPLC water up to 50 mL, and the above pre-concentrations and clean-up steps were followed.

2.7 Instrumental analysis

The MPLs' polymer mass-quantitative analysis in the bioaccumulation experiments was carried out by means of size exclusion liquid chromatography coupled to high-resolution mass spectrometry (HPLC(SEC)-HRMS). The chromatographic separation was conducted using a Waters' Acquity LC instrument (Milford, MA, United States) equipped with an advanced polymer chromatography column (Acquity APC™ XT45 1.7 µm 150 mm), using 100 % toluene under isocratic conditions as mobile phase with a flow rate of 0.5 mL·min^{-1} and an injection volume of 20 µL. To avoid toluene evaporation during the analyses, the autosampler was maintained at 16 °C, while the column worked at 30 °C.

The chromatographic system was coupled to a Q-Exactive™ Orbitrap mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA), working in negative mode and employing an atmospheric pressure photoionisation source (APPI). The APPI optimal parameters were as follows: sheath gas at 60 a.u., auxiliary gas at 20 a.u., capillary temperature at 400 °C and S-lens RF at 100 a.u. Data acquisition was performed using a full scan (FS) mode from 500 to 3,000 m/z and 17,500 FWHM.

The analysis of triclosan was conducted by reverse-phase liquid chromatography coupled with high resolution mass spectrometry (HPLC(RP)-HRMS), with a QExactive orbitrap. The chromatographic separation was achieved using a C_{18} analytical column (Luna 150 x 2.0 mm, 5 µm) provided by Phenomenex (Torrance, CA, United States). The mobile phase consisted of (A) water 100 % and (B) acetonitrile 100 % using the following gradient t= 0-5 min, hold at 5 % B; t= 5-15 min, increased linearly to 95 % B; t= 15-20 min, kept at 95 % B; t=20-25 min, and returned to starting conditions. The injection volume was set at 20 µL. A heated electrospray ionisation source (HESI) was used in negative mode and optimal parameters were as follows: spray voltage, -2,500 kV; probe heater and capillary temperature, 300 and 350 °C, respectively; sheath and auxiliary gas, 40 and 10 a.u., while S-Lens RF level at 60 a.u. Acquisition was carried out in full-scan data dependent mode (FS-ddMS^2) from 70-1,050 m/z and 70,000 FWHM with a normalised collision energy of 30 eV.

For the targeted analysis of the PFASs accumulated in mussels the analysis was as well carried out by HPLC-HRMS based on the previous method by Llorca at al., [31, 32]. The chromatographic analytical column in this case was the Hypersil GOLD PFP LC column (50 x 3 µm) supplied by Thermo Fisher Scientific. The mobile phase was composed of (A) methanol and (B) water, both modified with 20 mM ammonium acetate, and the gradient conditions were as follows: 10 % A, maintained for 30 s and increased up to 90 % A until 9.5 min., hold for 3 min more, and returned to initial conditions within 1 min, being 14 min the total run time. A flow rate of 0.5 mL·min^{-1} was set and the injection volume was 5 µL. The LC system was coupled to QExactive mass spectrometer equipped with HESI operating under negative conditions. The optimal parameters were spray voltage of -2,500 kV, a capillary temperature of 350 °C, sheath gas at 50 a.u., auxiliary gas at 20 a.u. and S-Lens RF at 60 a.u. Data was acquired in FS-ddMS^2 mode from 66-1,000 m/z and 17,500 FWHM. An extracted-ion chromatogram of the PFASs analysed is shown in **Figure S2** of the Supporting Information section.

2.8 Data treatment

HPLC-HRMS raw data was processed using Xcalibur v4.2 (Thermo Fischer Scientific, San Jose, CA, USA) software. For MPLs' analysis in mussels, a mass-quantitative target analysis previously developed in our group [17, 33] and posteriorly adapted and applied [34, 35], was used. Data treatment was carried out with GraphPad Prism version 10.1.1 (270).

2.9 Quality assurance and quality control (QA/QC)

For the exposure experiments, recommendations in guidance documents from the European Commission about biota monitoring under the Water Framework Directive were followed [25, 36].

To avoid MPLs contamination during the experiments, the exposure experiments were performed in an isolated room conditioned for this purpose. The sample pre-treatment was carried out in a laminar flow cabinet, wearing cotton lab coats, and all material used consisted of stainless steel or glass. Five procedural blanks were processed for each exposure experiment to evaluate possible contamination sources during the pre-treatment and instrumental analysis. In case of contamination, blank subtraction was applied to raw data from samples.

Individual stock standard solutions of PE and PS (1,000 $mg \cdot L^{-1}$) were prepared (10 mg dispersed in 10 mL toluene), and they were used to carry out the calibration curves. The calibration curves were prepared using an ultrasonic bath with constant heating at 60 °C to guarantee MPLs suspension in toluene. Moreover, solutions were vortexed before being injected into the chromatographic system.

MPLs calibration curves were prepared by two linearity ranges from 0.01 to 50 $\mu g \cdot L^{-1}$ and from 50 to 1,000 $\mu g \cdot L^{-1}$ with $R^2 > 0.95$. Matrix effect and recoveries at 10, 1 and 0.1 $\mu g \cdot L^{-1}$ were assessed for each matrix and corrected. For PET, an individual stock standard solution (1,000 $mg \cdot L^{-1}$) was prepared in dichloromethane-trifluoroacetic acid 99:1 and the same procedure described for PE and PS was followed to prepare the corresponding calibration curve levels using 1:1 toluene:dichloromethane with 1 % trifluoroacetic acid.

For triclosan, a standard solution (1,000 $mg \cdot L^{-1}$) was made to perform a calibration curve ranging from 1 to 1,000 $\mu g \cdot L^{-1}$ in LC grade water, while for PFASs, internal standards were used for quantification normalisation and the calibration curves ranged from 0.01 to 100 $\mu g \cdot L^{-1}$.

3. Results and discussion

3.1 Uptake of MPLs polymers by Mediterranean mussels.

The concentration of the different polymers uptake in the edible part of mussels was analysed by LC(SEC)-HRMS. **Figure 2 (a)** presents the uptake of polymers. PE and PET were those showing higher uptake in both cases after exposure and detoxification periods with concentrations at t_1 of 2.4 ± 0.1 and t_2 1.6 ± 0.3 $\mu g \cdot g^{-1}$, and t_1 2.2 ± 0.1 and t_2 1.5 ± 0.4 $\mu g \cdot g^{-1}$, respectively. Comparing the uptake of the different polymers, the accumulation of PE was significantly higher than PET and PS. The Kruskal-Wallis test showed that P value was $0.0036 < 0.05$, therefore for the same particles sizes the accumulation was significantly different according to the polymer.

Comparing the concentrations of polymers in mussels immediately after the end of the exposure experiment (t_1, accumulation), and after the depuration time (t_2, bioaccumulation), significant higher concentrations were found at t_1 as expected. PE was the polymer showing the highest accumulation and bioaccumulation. This agrees with the results of different studies from natural environments, where PE was also the most abundant MPL [37, 38]. However, it should be highlighted that, after the depuration time no significant differences were found between the bioaccumulation of PE and PET, but significantly lower with PS, which was below the limit of quantification. It should also be pointed out that considering the concentrations of exposure, the number of mussels exposed, and a mean weight of 5.6 g per mussel, and in the case of PE, which was the polymer more accumulated, only 4.2 % of MPLs with sizes from 10 to 63 µm, were accumulated and the rest was excreted.

In parallel, the accumulation of PE at 3 different particle sizes was assessed. Mussels were exposed to the same concentration (75 $\mu g \cdot L^{-1}$) of PE at 10-63 µm, between 1-10 µm and inferior to 0.9 µm. As shown in **Figure 2 (b)**, for the same concentration of exposure the accumulation was higher for the smallest particles. This agrees with the size-dependent accumulation of MPLs and NPLs in mussels already reported in other studies [39]. However, while the differences between particles of 10-63 µm vs those in the range of 1-10 µm, and between 10-63 µm vs those with sizes below 0.9 µm were significant, the Holm-Šídák's multiple comparisons test showed that no significant differences (P value 0.3661) were detected between the accumulation of PE-MPLs (1-10 µm) vs. PE-MPLs (< 0.9 µm). On the other hand, the accumulation after the depuration time was only significant different for bigger particles.

3.2 Accumulation and bioaccumulation of triclosan and PFASs by MPLs' modulation

The bioconcentration factor (BCF) was calculated as follows expressed in litres per kilogram:

$$BCF = \frac{Conc.biota(ng/Kg)}{Conc.water(ng/L)} \qquad \text{(eq. 1)}$$

The accumulation of triclosan (TCS) in mussel tissues with a nominal water concentration of 1.5 $\mu g\cdot L^{-1}$, after ten days of exposure, was below the limit of detection in seawater (0.07 $ng\cdot g^{-1}$ w.w.). Pirone et al.[40], also obtained low accumulation ratios when mussels were exposed to 1.0 $\mu g\cdot L^{-1}$ of TCS for 28 days, and similar results were reported by Álvarez-Ruíz et al. [23]. The low accumulation of TCS in mussels might be explained because triclosan is metabolized into methyl triclosan by mussels. However, in the presence of MPLs, part of TCS adsorbed onto the plastic particles was stabilised. The accumulation in mussels in the presence of MPLs particles of 10 and 63 µm, was 148, 55 and 8 $ng\cdot g^{-1}$, for PE, PS and PET, respectively. Yet, after three days of detoxification, the concentration of TCS in mussels was reduced, and only a residual concentration was detected in the case of PS and PET co-exposure as can be seen in **Figure 3**.

The concentrations of PFASs spiked in water was 5 $\mu g\cdot L^{-1}$ for each compound. The levels in the water of the four PFASs assayed here can be considered constant during the experiments since these compounds are hydrolytically stable with long half-lives in water (e.g., PFOS has hydrolytic stability of 41 years), and water samples were renewed every two days with fortified seawater. Therefore, the decay of water concentration between renewals can be considered due to mussels accumulation.

As shown in **Figure 4 (a)**, the compound with the highest accumulation ratios was PFOS, as expected because it is one of the compounds with the longest chains studied (C_8), and the compound has a sulphonic acid. Comparing the BCF (**Figure 4 (b)**) of the two C_8 compounds, PFOS and PFOA, are 57.2 and 32.4, respectively. On the other hand, the results of this study showed that the accumulation of the PFHxS (C_6) (BCF 43.3) was significantly higher than for PFOA (C_8) despite the shorter chain (**Figure 4 (a)**). This fact can be attributed to the sulphonic group of PFHxS, which confers a higher accumulative potency, but particularly to PFOA's much higher water solubility (9,500 $mg\cdot L^{-1}$) in comparison to the PFHxS with 6,2 $mg\cdot L^{-1}$. The high filtration capacity of *Mytilus galloprovincialis (*up to 8 $L\cdot h^{-1}$) should be considered together with the high-water solubility of PFOA because, in this case, filtration can produce a washing effect. Finally, the lowest accumulation during the exposure was shown for PFBS. As can be shown in **Figure 4 (a)**, after the depuration time (t_2, 3 days), the concentration in mussels was drastically reduced for all compounds.

The polymeric composition of the particles, the particle size, and surface properties govern their accumulation and bioaccumulation rates in biota and the adsorption of other contaminants on the particles [41]. This experiment compared the influence of MPLs with particle sizes between 10 and 63 µm diameter of PE and PS in PFAS accumulation in mussels. Significant differences were obtained in PFAS accumulation with and without co-exposure with MPLs, and significant differences between PE and PS (**Figure 4 (a)**). The BCF was slightly reduced in the presence of PE but reduced by 52% in the case of PS (**Figure 4(b)**). These results are a consequence of two effects; on the one hand, some adsorption sites in the mussel gut were blocked by MPLs, reducing the adsorption of nutrients and contaminants. On the other hand, PFAS were adsorbed onto MPLs, particularly PS-MPLs, which were relatively low accumulated by mussels, at least at this particle size. Then, a portion of PFAS, instead of being accumulated, was adsorbed by MPLs and excreted with MPLs. These results highlight the importance of MPLs composition in terms of bioaccumulation of other organic contaminants. For example, Álvarez-Ruíz et al.[23], observed that the co-exposure of MPLs increased the accumulation of PFASs in mussels. Nonetheless, the experiment of Álvarez-Ruíz et al.[23] was performed with commercial oxidised PE (OXO-PE) MPLs, which presented different surface properties than typical environmental PE, and in consequence different adsorptive behaviour as has already been reported for certain organic contaminants such as triclosan [42]. Regarding the differences in the accumulation of PFASs in co-occurrence with PE and PS, **Figure 4(a)**, corroborate our previous results [10], in which it was shown that the adsorption capacity of PS for PFASs in seawater was higher than the PE. In the study of Llorca et al.[10], C_8-PFASs with different functional groups showed higher adsorption to PS, in particular perfluoro carboxylic acids. In this study, despite mussels were exposed to high MPLs concentrations of 75 $\mu g \cdot L^{-1}$ the mean concentration of PS accumulated was only 420 $ng \cdot g^{-1}$. This could indicate that PFASs and, in particular PFOA, were adsorbed by PS and, at these particle sizes, were not retained by mussels being directly excreted facilitating the elimination of PFASs and, in specifically, PFOA. However, after the depuration, the concentration of PFASs bioaccumulated in mussels without MPL co-exposure was not significantly different when PS was present (see grey bar **Figure 4(b)**), and in the case of PE, the total PFASs accumulation was slightly higher but not significant applying the Turkey's multiple comparison test.

3.3 MPLs' modulation of PFASs bioaccessibility.

Bioaccessibility is the fraction of the total amount of an ingested substance that is potentially available for absorption during digestion. Here, the bioaccessibility of the three perfluoro sulphonates (PFBS, PFHxS and PFOS) bioaccumulated in mussels with and without MPLs of PE and PS were assessed. The bioaccessibility was calculated according to the equation 2:

$$\% \, Bioaccessibility = \frac{[PFAS] \, digestion}{initial \, [PFAS]} \times 100 \qquad \text{(eq. 2)}$$

Mussels after the depuration time (t_2) were digested *in-vitro* according to the procedure described in section 2.5.

As shown in **Figure 5 (a)**, the bioaccessibility increased in the presence of MPLs, although the bioaccumulation in the presence of MPLs was lower. This is an indication that an important fraction of PFASs accumulated was associated with polymeric particles, and as presented in our previous study, the primary factors ruling the adsorption onto the MPLs were the hydrophobic interactions and in seawater the salting out effect [10], but these interactions can be affected by the solution pH, which can change the surface charge of some MPLs, as is expected to happen during the digestion. In **Figure 5 (b)**, the bioaccessibility normalised by the respective bioaccumulation is presented. For the longer chain compounds, the differences between the bioaccumulation and bioaccessibility in the presence of PE and PS were not significant, but in the case of PFBS, the bioaccessibility increase was higher in the presence of PE. In both cases, the bioaccessibility was higher when MPLs of PE or PS were present during co-exposure than when mussels were only exposed to the sulphonates PFASs.

4 Conclusions

Our results confirm that MPLs' bioaccumulation in filter-feeding organisms is a function of the MPLs chemical compositions and particle sizes. The particle composition will influence on the surface characteristics in a certain media, such as surface charge. While the size of particles influence the particle retention, being small particles better retained than bigger ones. For example, even in natural particulate such as sediments, when the particles are bigger than 35 µm diameter the reduction percentage in their retention compared with 10 µm diameter is from 11% to 87% in a similar organism *Mytilus edulis*, as reported before [43]. Moreover, also the capacity of translocation is much lower. In this case, the accumulation for the same range of particles size between 10 and 63 µm was PE>PET>>PS.

On the other hand, the aggregation of organic contaminants on MPLs surface has shown to be ruled by hydrophobic interactions, the surface charge of the particles in a media, the degree of polymer crystallinity, the polarity of the organic contaminants, the salting out effect in case of seawater, as well as the capacity of contaminant biodegradation. For example, here, we show that triclosan was biodegraded in the absence of MPLs in the media, but MPLs can stabilise some amount of triclosan onto their surfaces, and then triclosan can be accumulated by mussels. The polymer that showed a major influence in triclosan accumulation was PE. In contrast, in the case of compounds resistant to degradation, such as PFASs, in the presence of particles between 10 and 63 µm of PE or PS the accumulation decreases because the efficiency of filtration of mussels was reduced and at the same time, these plastic particles at the tested sizes can adsorb PFASs onto their surfaces, but probably a high percentage of these particles are excreted by mussels. Comparing the accumulation of the different PFASs tested here, PFOS was the most accumulated and bioaccumulated compound as expected because of the sulphonic acid and the 8 carbons chain. Nevertheless, PFBS was the less accumulated compound, corresponding to the shorter carbon chain studied here. Moreover, comparing PFOA(C_8) and PFHxS(C_6), the last one was more accumulated by mussels despite the shorter chain. We postulate that the adsorption of PFOA by PS, the less accumulated polymer by mussels, was higher, as shown before [10], but also PFOA is much more polar than PFHxS, which combined with the high filtration capacity of mussels can produce a washing effect of PFOA.

Finally, despite the lower accumulation and bioaccumulation of PFASs in the presence of MPLs in the media, the bioaccesibility test revealed that PFASs' bioavailability was increased when MPLs were present in the same media. Since part of the bioaccumulated PFASs are adsorbed onto MPLs' surfaces by electrostatic interactions, and these interactions easily change with the pH during the digestion process, the PFASs biaccessibility increases.

The results of this study underpinning the need for further research to assess the human and environmental risk related to MPLs' contamination and the potential Trojan Horse effects with other organic contaminants.

Acknowledgements

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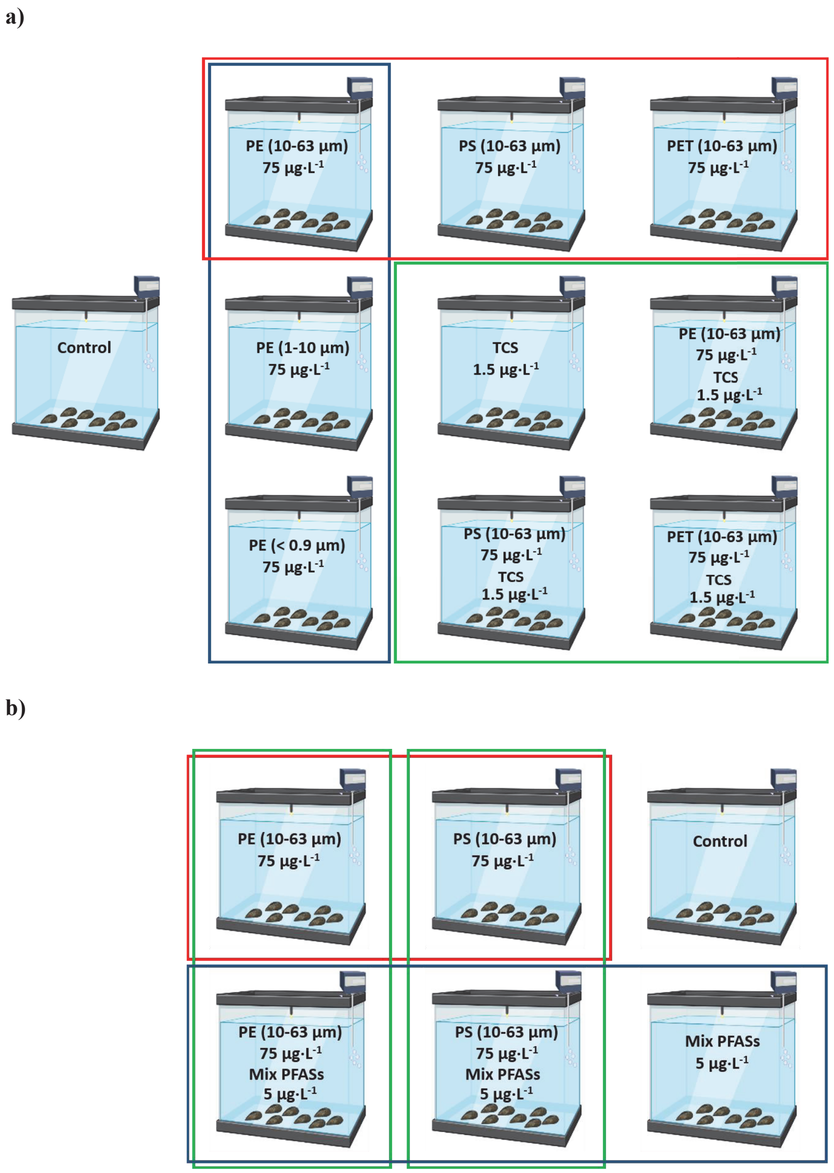

Figure 1 Scheme of bioaccumulation experiments **(a)** PE, PS, PET-MPLs + TCS. **(b)** PE, PS-MPLs + mix of PFASs.

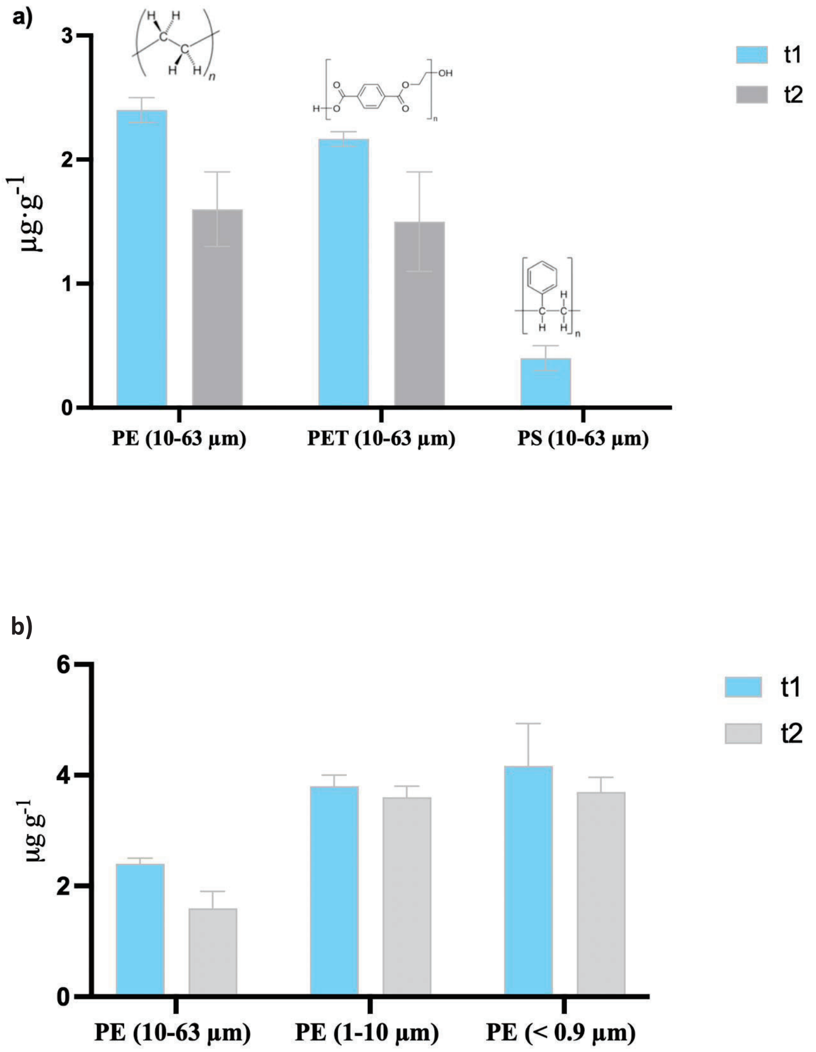

Figure 2 (a) Uptake of polymers by mussels exposed during 10 days at concentrations of 75 μg·L^{-1}. **(b)** Uptake of PE at same concentration but with different particle sizes. As can be seen depuration time was significant different for the bigger particles (p=0.000036). All the results are presented after blank subtraction.

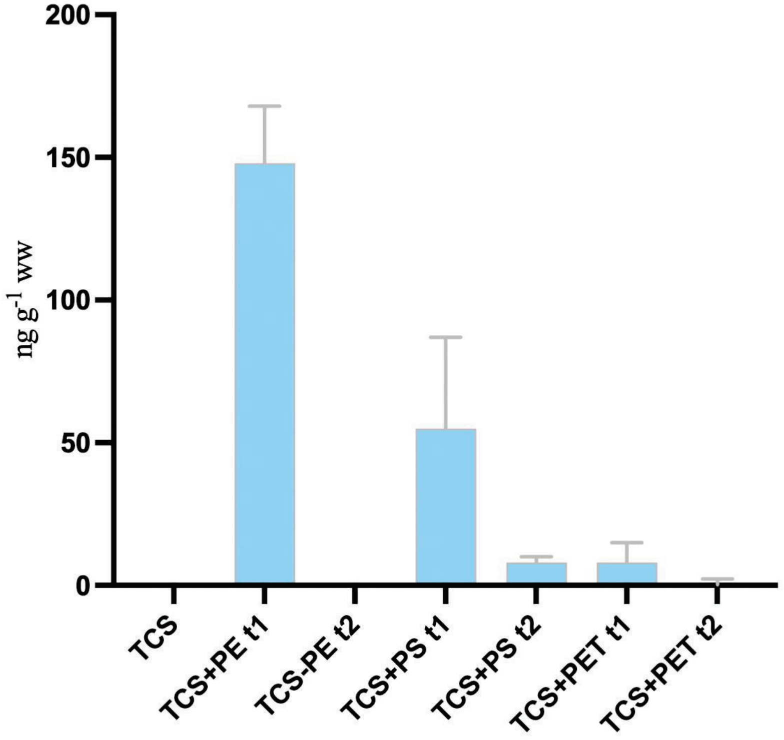

Figure 3 Accumulation (t_1) and bioaccumulation (t_2) of triclosan (TCS) by mussels after blank subtraction with and without PE, PS, PET-MPLs in the media.

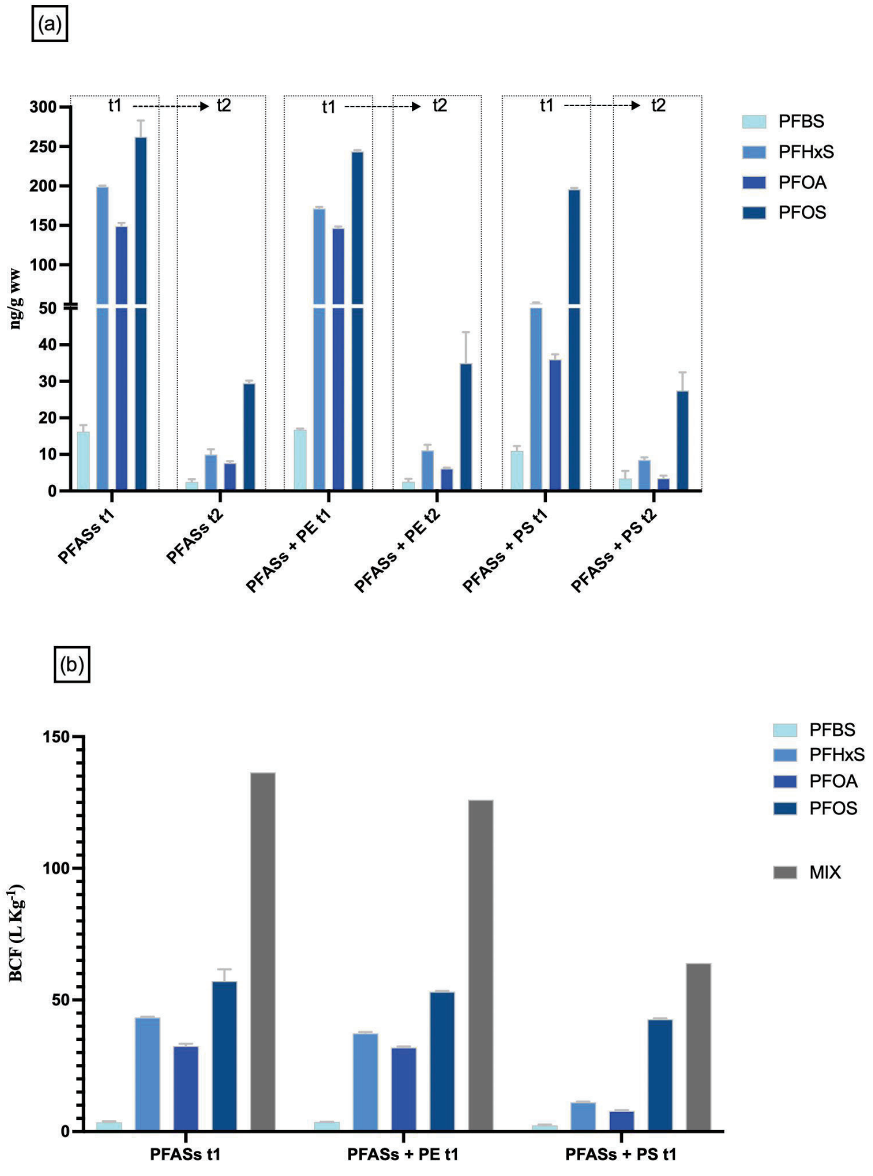

Figure 4 (a) Per- and polyfluoroalkyl substances (PFASs) bioaccumulation in mussels after exposure (t_1) and detoxification (t_2) periods for a mix of PFASs and modulated by MPLs of PE and PS. **(b)** Bioconcentration factors.

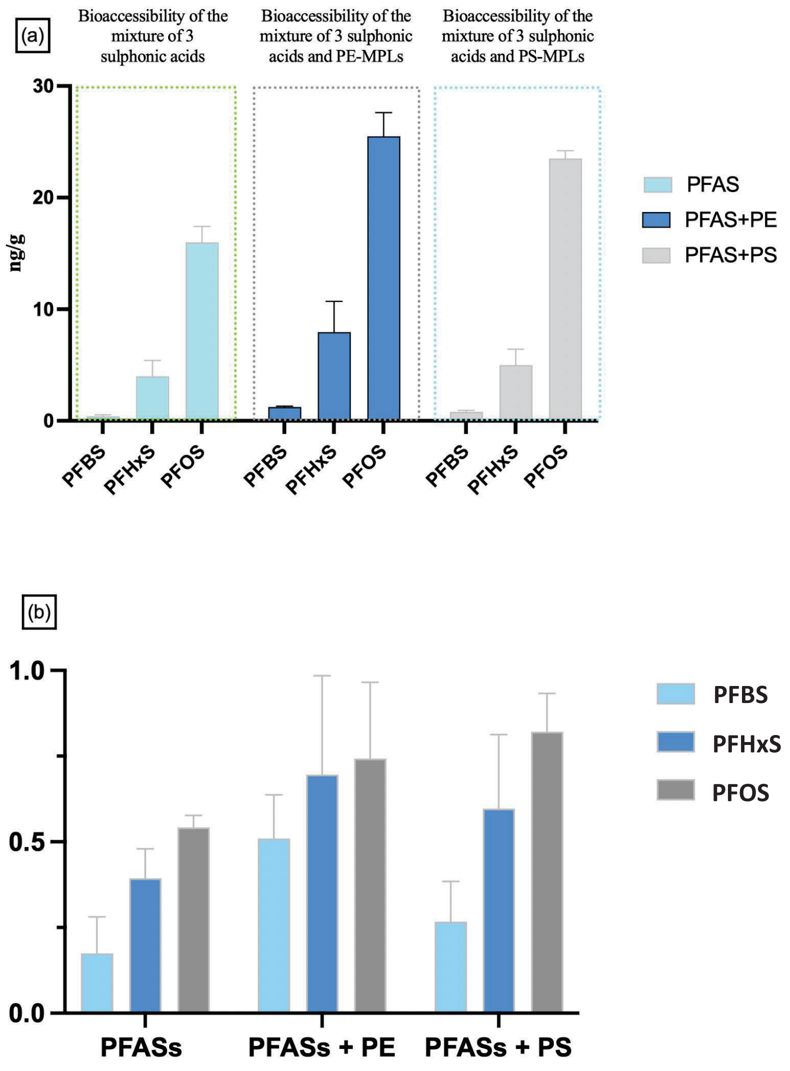

Figure 5. (a) Bioaccessibility of bioaccumulated PFASs in mussels without and with PE and PS-MPLs in the media. **(b)** Bioaccessibility normalised by the respective bioaccumulation of PFASs in each scenario of exposure.

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6.2.3 Publication n°8 (Supplementary material)

Bioaccumulation and dietary bioaccessibility of microplastics composition and cocontaminants in Mediterranean mussels

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Figures S1, S2

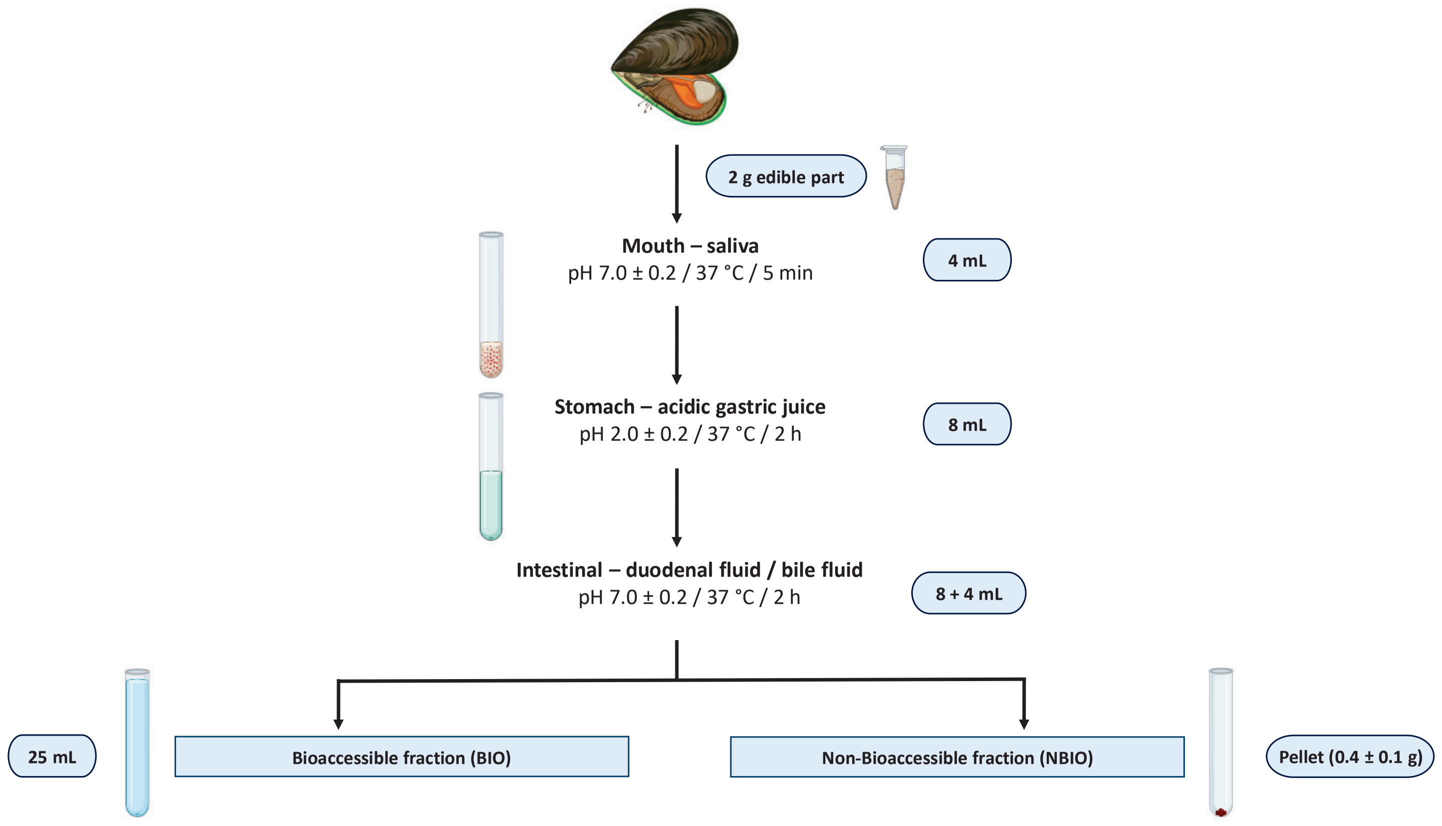

Figure S1 *In vitro* **digestion protocol followed for human bioaccessibility assays in** *M. galloprovincialis spp.* **mussels.**

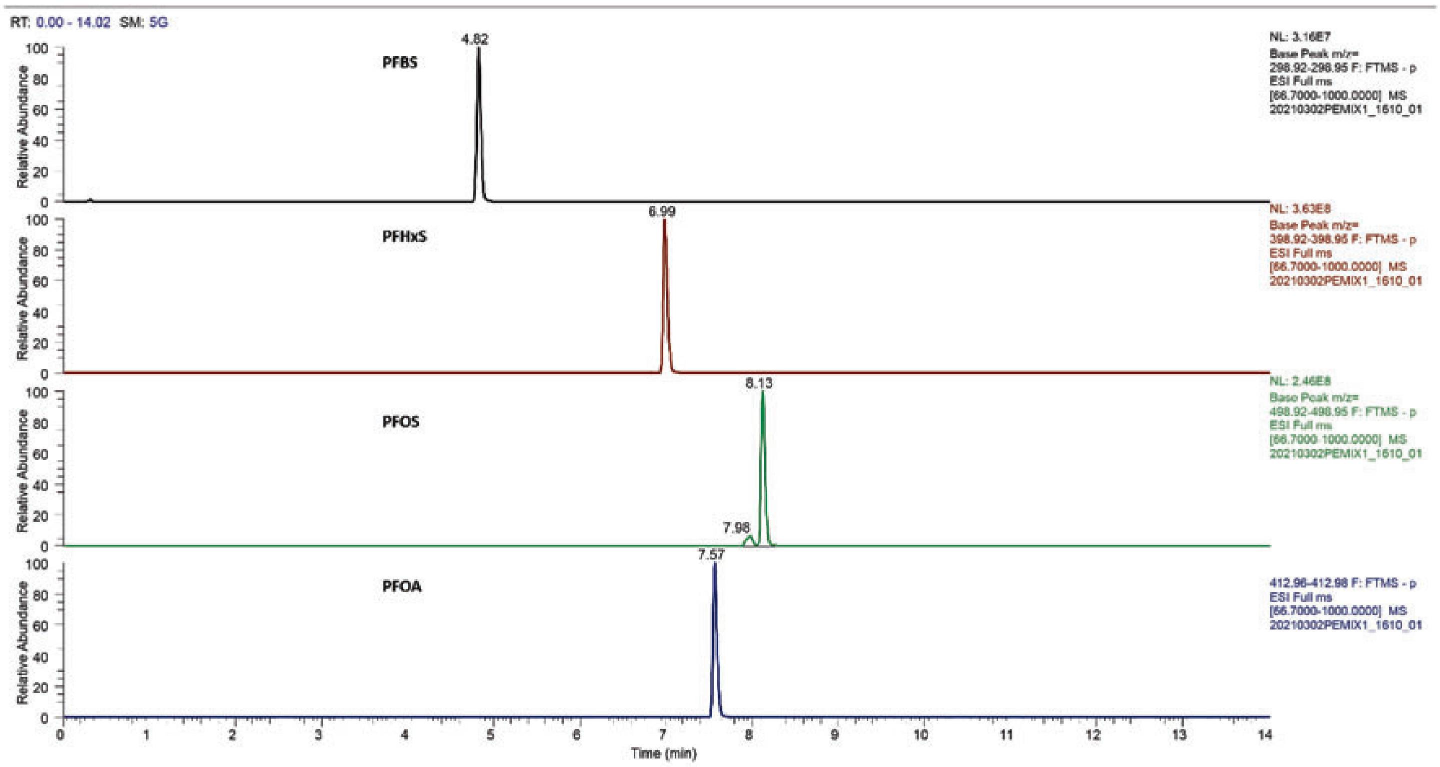

Figure S2 Extracted ion chromatograms (XIC) for PFBS, PFHxS, PFOS, and PFOA in mussels.

6.3 Discussion

The studies that are compiled in this final chapter contribute to broadening the knowledge about human exposure to microplastics (polymers and plastic additives), through two of the most relevant routes: drinking water (**Publications nº6 and nº7**), and contaminated food (**Publication nº8**), including the modulation of the bioaccessibility of other organic contaminants.

6.3.1 Human exposure to MNPLs through drinking water

Nowadays, it is necessary to establish the levels of MNPLs in different routes of exposure, and the levels of acceptable daily intake (ADI), in order to carry out robust risk assessment models. However, the lack of mass measurements and harmonised analytical approaches limit the establishment of ADIs, and specific regulations in drinking water or food and packaging, since particles in real samples are of different sizes and shapes. Moreover, the levels of mass measurements have not yet been established for most of the routes of exposure. However, by use of toxicological values reported in the literature in terms of no-observed-adverse-effect levels (NOAELs), as in PE (Wang et al., 2023), PP (Sun et al., 2024), and PET (Ma et al., 2024), and using an uncertainty factor of 1,000 for intra- (human *in vitro* assays), and inter-species differences, for which the EDI values have been calculated and are shown in

Tables 6.1 and **6.2** considering an average consumption of 2 L of water per day and 65 and 75 Kg of average body weights (BWs) for adult women and men, respectively. Then, the hazard quotients (HQ) and hazard index (HI) were also calculated for tap water (**Table 6.1**), and bottled water (**Table 6.2**).

This type of assessment can only be performed thanks to mass measurements, which help to establish the levels of risk, and based on this, establish future legislation. As seen in **Tables 6.1** and **6.2**, according to the results obtained in **Publication nº6** and **Publication nº7**, respectively, no risk can be associated with the polymers that are ingested through drinking water. Although it should be highlighted that these values were based on acute toxicity, and drinking water effects should be considered within the frame of chronic effects. Moreover, this is just one of the multiple and constant sources of MNPLs in humans, and it would be necessary to establish the values for multiple routes of exposure.

6.3.2 The relevance of suspect screening and non-target screening strategies to assess plastic additives leached in bottled water in plastic bottles

Apart from the human risks associated with polymer exposure from MNPLs, we also assessed the potential for plastic additives to leach from plastic bottles. We used bottles of different volumes from the 20 commercial brands that are most popular in Spain, in order to assess the profile of plastic additives.

Over 2,400 additives that are used in plastic production have already been identified as substances of special concern in different food-contact plastic materials, as they meet persistence, bioaccumulation, and toxicity criteria, emphasising the need for regulatory attention (Wiesinger et al., 2021). The EU has already established specific migration limits to ensure the safety of food contact materials. However, there are instances where migration may exceed these limits under certain conditions, such as the storage temperature, the type and concentration of food simulants, or the presence of plasticisers enhancing migration, particularly when the applied food simulant swells the plastic (Dragan et al., 2024).

Table 6.1 Estimated daily intake (EDI) for adult men and women, acceptable daily intake (ADI), hazard quotients (HQs), and hazard index (HI) for the MNPLs-polymers detected at the average concentration in drinking water samples from household taps in the Barcelona Metropolitan Area (Publication nº6).

Household tap water				
	PE	PP	PBD	PI
EDI ($\mu g \cdot Kg^{-1}$ $BW^{-1} \cdot day^{-1}$)	0.5E-02	1.4E-02	4.0E-02	6.5E-02
ADI ($\mu g \cdot Kg^{-1}$ $BW^{-1} \cdot day^{-1}$)	1.3	1.8	N.A.	N.A.
HQ for Men	0.4E-02	0.8E-02	-	-
HI for Men	0.01			
EDI ($\mu g \cdot Kg^{-1}$ $BW^{-1} \cdot day^{-1}$)	0.5E-02	1.6E-02	4.6E-02	7.5E-02
ADI ($\mu g \cdot Kg^{-1}$ $BW^{-1} \cdot day^{-1}$)	1.5	2.1	N.A.	N.A.
HQ for Women	0.4E-02	0.8E-02	-	-
HI for Women	0.01			

N.A.: Not available in the literature.

Table 6.2 EDI for adult men and women, ADI, HQs, and HI for the MNPLs-polymers detected at the average concentration in bottled water in plastic bottles from the 20 most popular commercial brands in Spain (Publication nº7).

Bottled water			
	PE	PP	PET
EDI ($\mu g \cdot Kg^{-1}$ $BW^{-1} \cdot day^{-1}$)	0.6E-02	1.7E-02	1.6E-02
ADI ($\mu g \cdot Kg^{-1}$ $BW^{-1} \cdot day^{-1}$)	1.3	1.8	2.7
HQ for Men	0.5E-02	0.9E-02	0.6E-02
HI for Men	0.02		
EDI ($\mu g \cdot Kg^{-1}$ $BW^{-1} \cdot day^{-1}$)	0.7E-02	2.0E-02	1.8E-02
ADI ($\mu g \cdot Kg^{-1}$ $BW^{-1} \cdot day^{-1}$)	1.5	2.1	3.1
HQ for Women	0.5E-02	0.9E-02	0.6E-02
HI for Women	0.02		

In this context, migration assessment studies, normally, tend to be carried out by analytical methodologies that are based on LC and GC coupled with HRMS (LC- and GC-HRMS), which would allow the implementation of suspect screening analysis and non-targeted analysis (NTA) approaches for identifying chemical compound profiles. The use of targeted analysis to complement non-targeted analysis, for confirmation and quantification of identified or prioritised compounds, is the key to obtaining a complete picture of the contamination of plastic additives. Thus, a suspect screening analysis approach based on LC-HRMS, followed by the subsequent automated extra-screening performed through algorithms created from Python programming language, was applied in **Publication nº7**, where a total of 46 compounds were detected and 28 of them, were finally tentatively identified at confidence level 2 as plastic additives in the water samples coming from the plastic material of the single-use bottles. All of the samples that were analysed presented plastic additives migration, where the most detected samples were plasticisers from phthalate and adipate families, with high detection frequencies reaching 90-95% as expected, according to results in the study by Petrovics et al. (2022). Then, through the results obtained by the suspect screening analysis, a qualitative prioritisation based on a quantitative structure-activity relationship (QSAR) approach was applied to assess relationships between the structural properties of these tentatively identified plastic additives and their adverse effects. Nevertheless, the toxicological information currently available for most of these substances is limited (specially regarding their toxicity). However, some regulations, like the Commission Regulation No. 10/2011, set specific migration limits for plasticisers in food contact plastics (Dragan et al., 2024).

Overall, considering the variety of plastic formulations used in food and drink packaging, including new bioplastic formulations, the combined use of suspect screening/non-targeted analyses, together with targeted approaches for the elucidation of the plastic additives composition, followed by *in vitro* toxicity studies, is necessary to tackle the plastic additive residues that can migrate to drinking water (Savva et al., 2023). Moreover, the development and harmonisation of these types of analytical approaches will help the implementation of new regulations to ensure human safety.

6.3.3 Bioaccumulation and human bioaccessibility of MPLs and organic pollutants adsorbed onto their active surfaces

Bioaccumulation of MPLs and co-exposure with other contaminants, that are present in the same compartments and biota, have been widely assessed during the last 5 years. According to our bibliographic search in Scopus, including ‘bioaccumulation’, ‘microplastics’, and ‘marine biota’ as keywords, the number of scientific studies that were initiated, with the aim of tackling this issue, has risen sharply, reaching 282 publications in 2023 instead of 2 publications in 2015. Nevertheless, polymer concentrations that were reported in biota from the main *in vivo* bioaccumulation experiments which were carried out in marine meso and microcosms, were generally provided qualitatively as number of plastic items per volume or mass unit by microscopy and spectroscopic assessment of particles composition (e.g. Álvarez-Ruiz et al., 2021).

In **Publication nº8**, the bioaccumulation of PE, PS, and PET in Mediterranean mussels was assessed for the first time by mass-quantitative measures, to our knowledge. Regarding the co-contaminants exposed, surprisingly, triclosan was not accumulated when it was exposed individually. This can be explained because triclosan tends to be easily photodegraded in the environment, thus favouring the bioaccumulation of transformation products such as 2,3-dichlorodibenzo-p-dioxin, dibenzodioxin, or phenol intermediates, rather than the original form, leading likewise to a variety of toxic effects. Nonetheless, when triclosan was first adsorbed onto MPLs surfaces, MPLs transformation decreased (Lee et al., 2024). In this sense, from the outcomes reported in **Publication nº8**, triclosan was accumulated in PE > PS > PET (t_1), whereas it only was bioaccumulated once adsorbed onto PS microspheres (t_2). According to Álvarez-Ruiz et al. (2021), a similar pattern was shown for triclosan that was individually and co-exposed with PE-MPLs as reported here, where no concentrations were detected when the chemical was directly exposed after exposure and depuration periods. However, with the presence of PE-MPLs, triclosan was accumulated in mussels, but not finally bioaccumulated.

The PFASs mixture investigated in **Publication nº8**, despite the lower accumulation and bioaccumulation in Mediterranean mussels when co-exposed with MPLs, the bioaccessibility was increased in this order: PFOS > PFHxS > PFBS. When MNPLs are ingested, the digestion process modifies the surface of plastic particles. The process starts in the mouth, where saliva and amylase are present, and follows in the stomach with strong acid gastric juices. Then, the gastric fluid is neutralised by the alkaline intestinal fluid that contains many enzymes which are necessary for food digestion and to allow the internalisation of final nutrients. In addition, it has been proven that after ingestion, in the digestive tract, MNPLs meet the intestinal mucosa and the intestinal epithelium, which constitute barriers that help to avoid the absorption of xenobiotics. Even considering this, several studies have shown that MNPLs can cross this biological barrier, reaching the circulatory system and more distant tissues and organs, including the human placenta (Kumar et al., 2022). Currently, a few *in vitro* approaches have been proposed to mimic this process in humans; however, despite the usefulness of the *in vitro* digestion test, formal official validation of the static models is still awaited (Vela et al., 2023).

In general, the use of *in vitro* studies that simulate the gastrointestinal process are a necessary tool, with which to estimate the behaviour of MPLs once they are ingested, in terms of the leaching of their components (polymer monomers and additives), the modulation of the behaviour of other contaminants, and the surface changes of plastic particles, which occur after digestion influences their transport capacity through living tissues. Furthermore, this type of study, due to its reproducibility and freedom from ethical problems, is an excellent tool, with which to reduce the number of *in vivo* studies, and other studies to evaluate the impact on human health.

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7

Conclusions and future trends

7.1 Conclusions

According to the objectives of this doctoral thesis, the following conclusions can be drawn:

1) The development and validation of 2 quantitative analytical methodologies based on size-exclusion liquid chromatography and pyrolysis-gas chromatography coupled with mass spectrometry have been carried out for the simultaneous identification and mass-quantification of PE, PP, PS, PI, PBD, PVC, and PET-MNPLs polymers in different environmental compartments and aquatic biota by the use of individual external calibration curves prepared through the corresponding analytical standards and internal analytical standards in the LC(SEC)-HRMS and Py-GC-MS approaches, respectively. Both methods allow accurate and real measurements, through low limits of detection and quantification, and a linearity range that covers practically the whole environmental relevant range of concentrations for the pollutants here studied, independently of the size and shape.

2) A LC-HRMS suspect screening approach has been also developed for the tentative identification up to the 2nd level of confidence of the plastic additives composition associated with the MNPLs pollution. The suspect screening approach has been applied to water and air samples taken from the Ebro

Delta (**Publication nº1**), the Mar Menor lagoon (**Publication nº2**), the North and South Atlantic ocean (**Publication nº3**), as well as for drinking water bottled in single-use plastic bottles from different Spanish commercial brands (**Publication nº7**). Specifically, for this last mentioned study, 28 plastic additives were tentatively identified. According to the prioritisation carried out, bis(2-ethylhexyl) adipate and bis(2-ethylhexyl) phthalate were estimated as the most potentially harmful compounds for human health. Besides, while plasticisers were the group where chemicals reached the highest scores, the higher capacity of stabilizers compared to plasticisers to migrate from the plastic wrap to the water made them the most potentially dangerous.

3) In the case of the Ebro Delta and Mar Menor lagoon studies, the occurrence of MNPLs polymers, their spatial distribution, and possible seasonal variations in the surface water compartments have been evaluated. MNPLs polymers of PE, PP, PS, PI, PBD, and PVC were detected and quantified, where PE and PP were found at higher levels compared to the others assessed. As a general trend, during warm seasons or drought episodes, a higher variability in regards to polymer concentrations was obtained, with punctual maximum peak concentrations in specific sampling areas covered. Nonetheless, in cold or rainy seasons, the ubiquity of MNPLs along the estuarine areas was favored, thus decreasing the average plastic pollution levels. In addition, the impact of the plastic pollution found has been also related to the human footprint (agricultural, farming, aquaculture, and fishing activities among others) to figure out possible potential release sources which might threaten these particular ecosystems of special interest.

4) In the case of the Atlantic and Southern oceans studies, the occurrence and the spatial distribution of PE, PP, PS, PI, PBD, and PET-MNPLs polymers in the marine low atmosphere as well as in the SML and ULW at 5 m deep surface water compartments have been assessed. Regarding the spatial distribution of MNPLs-polymers, the highest variability and concentrations found were in the sampling stations located near the North subtropical gyre from the Atlantic ocean, either in the atmosphere (52 $ng \cdot m^{-3}$) and in the surface marine seawater compartments (8 $\mu g \cdot L^{-1}$). The detection of MNPLs-polymers at quantifiable concentrations in Antarctica as well as throughout the whole Atlantic ocean by both marine compartments assessed, confirmed that MNPLs are globally ubiquitous and that they are able to travel large distances from urban areas to remote regions.

In addition, the abundance of MNPLs accumulated in aquatic biota has been evaluated. For phytoplanktonic species (*Sargassum* and *Durvillaea* algae blooms), MNPLs polymer concentrations ranged from 1 to 7 $\mu g \cdot g^{-1}$, while in the case of invertebrates (*Physalia* jellyfish) and vertebrates (*Cheilopogon* fish and *Euphausia* krill) organisms, the polymeric concentrations were between 3 and 27 $\mu g \cdot g^{-1}$, demonstrating the biomagnification of these MNPLs as they move up the food chain.

On the other hand, the origin sources of the atmospheric MNPLs found in samples have been elucidated by the HYSPLIT model and the rest of the PM_{10} composition. The MNPLs air-water mass exchanges have also been studied through PM_{10} and the airborne MNPLs-polymeric composition by the estimation of the atmospheric dry deposition fluxes and the abundance of MNPLs in the sea-spray aerosol. In this sense, this is the first time, from our knowledge, that MNPLs polymers occurrence is assessed in terms of mass concentrations simultaneously either in the marine atmosphere boundary layer and in the marine water surface compartments, including their air-water mass exchanges.

5) Regarding the behavior of MNPLs in the environment, paying attention to adsorption-desorption interactions between MNPLs and co-contaminants present in the same aquatic ecosystems, the capacity of PE, PS, and PET MPLs to adsorb a group of PCBs congeners has ranged between 20 and 65% onto MPLs surfaces, even despite the presence of sediment and its competition against MPLs to entrap selected PCBs. The affinity of PCBs to be adsorbed on the different MPLs tested has been as follows: PET > PS > PE. PCBs were exposed simulating real environmental concentrations previously found in the literature for estuarine systems ranging from 5 to 25 $ng \cdot g^{-1}$. The adsorption mechanisms between PCBs and MPLs have also been characterised through different adsorption isotherms, with the linearised Freundlich model in its logarithmic form is the one which fit best for all cases.

6) In regards to the assessment of MNPLs human exposure individually and with the presence of other co-contaminants through contaminated food, by the bioaccumulation experiments performed with Mediterranean mussels in marine microcosms, PE was the most bioaccumulated polymer in these living organisms followed by PET, and PS at 75 $\mu g \cdot L^{-1}$. Moreover, PE has been exposed at different sizes, where the smaller PE-MNPLs particles were more prone to their accumulation and subsequently, to their bioaccumulation. In the case of the mix of PFASs co-exposed, despite slightly lower accumulation and bioaccumulation in the presence of PE, PS-MNPLs than individually, the human bioaccessibility *in vitro* test revealed that PFASs availability was increased when they were co-exposed with these MNPLs as follows: PFOS > PFHxS > PFBS.

7) Regarding the assessment of MNPLs human exposure through the daily intake of drinking water from household taps and commercial single-use plastic bottles, PE, PP, PET, PS, PI, and PBD polymers of MNPLs have been identified and quantified. In the drinking waters analysed by household taps, MNPLs concentrations ranged from 1 to 9,143 ng·L^{-1}, where PE and PP were the most highly detected polymers, while PI and PBD were found at the highest concentrations. Whereas, in the case of the bottled drinking water, concentrations ranged between 19 and 4,712 ng·L^{-1}, where PE was the most frequently detected followed by PET which was found in 33% of the samples and showed the highest concentration. A mean value of 339 μg·year^{-1} of ingested MNPLs polymers was obtained considering a human average daily intake of about 2 L in the household taps study, while 262 μg·year^{-1} in the case of plastic bottles. In this sense, regarding the human risk assessment performed in section 6.3.1 based on the oral daily intake, the water analysed either through household taps and plastic bottles is safe to consume.

Overall, it can be concluded that the analytical methods and outcomes here provided serve as a primary robust basis to enhance the current regulation and legislation by the establishment of new harmonised environmental and human exposure limits, which will contribute to establish a MNPLs pollution regulatory consensus and to open a new window of challenges in the plastic pollution research field.

7.2 Future trends

While the issues associated with MPLs in the marine environment are widely acknowledged in the literature, the existence of numerous gaps in the knowledge demands immediate attention. The lack of investigation in different environmental compartments, such as deep-sea, the atmosphere, and remote areas, is a pressing concern. Similarly, the need for a comprehensive understanding of the transport of MNPLs in the environment is of paramount importance. Further, it is of critical importance to address the main routes of plastic pollution to the environment, their transport and behaviour, including weathering properties, and the potential for biological degradation, particularly of new polymers based on bioplastics.

Moreover, the study of NPLs continues to represent a significant challenge.

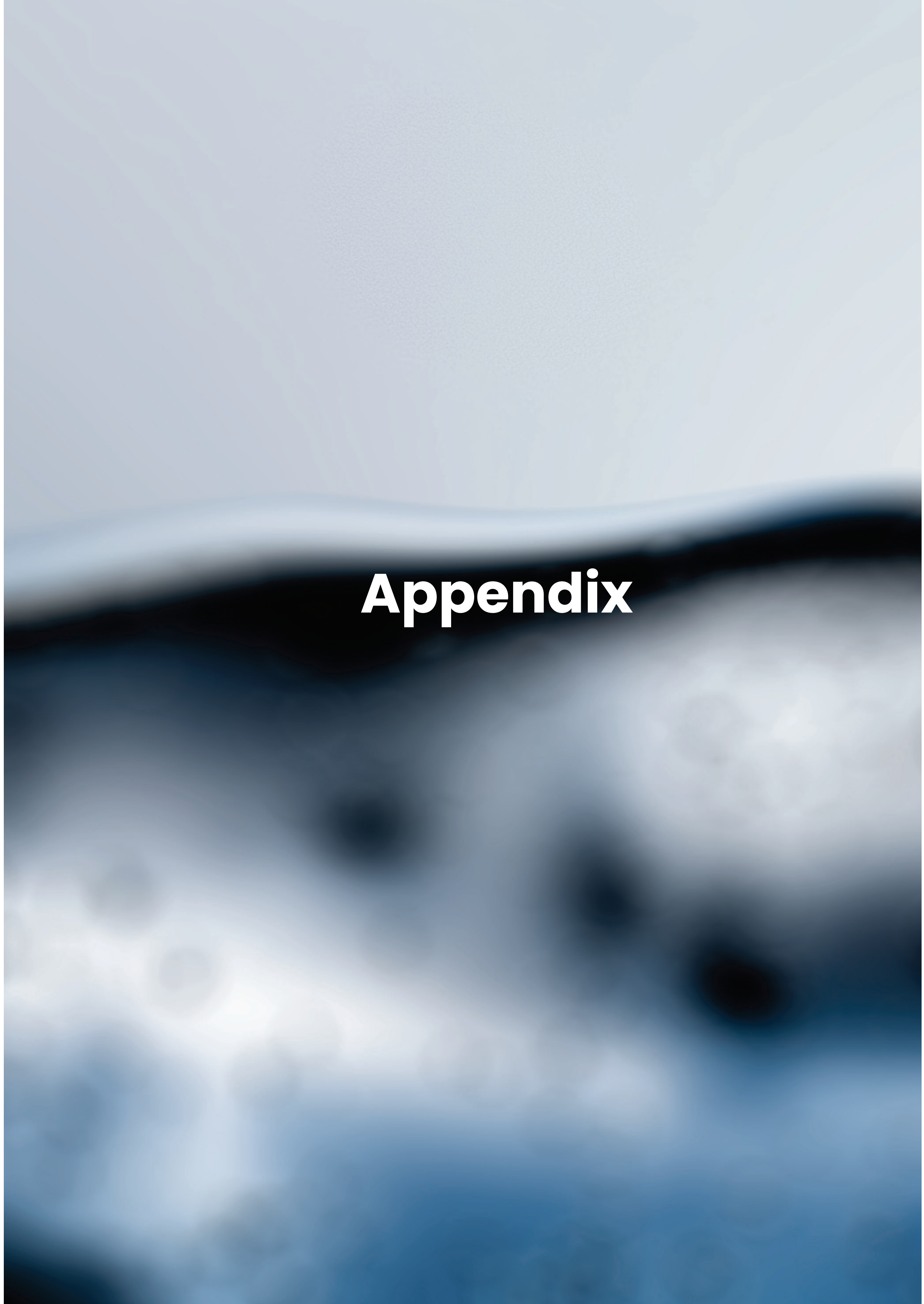
In terms of assessing the occurrence of MNPLs, sampling and sample pre-treatment procedures should be harmonised in the different environmental matrices. The lack of comparability of the results obtained in current studies limits the carrying out of risk assessment studies.

In this context, harmonised methodologies and standard measurements should be well-established, thus facilitating the global analysis of MNPLs pollution issues. For some analytical techniques, the first interlaboratory studies have been organised (e.g. NORMAN network), ensuring the quality of the results. However, most of these interlaboratory studies are focused on MPLs that are larger than 50 μm, and are more focused on spectroscopic techniques. The interlaboratory exercises should be expanded to different matrices and techniques. Other difficulties that should be overcome are standards for different sizes, polymers, compositions, and reference materials.

Defining and characterising human exposure to plastic pollution will be necessary. Further investigation regarding the main routes of human exposure and the potential adverse effects of plastic nanoparticles on human health should be conducted through toxicological studies, assessing the long-term effects and the potential for accumulation in human tissues and translocation. The expansion of studies that assess the toxicological response on human cell lines, and research on biomarkers of exposure are, therefore, highly required.

National or regional monitoring programmes are needed to expand knowledge on these emerging contaminants.

For future regulations, the need to express the levels of MNPLs in the different environmental compartments, food, and other routes of human exposure, in terms that are not affected by the particle's size, shape, and density, as well as the need for techniques that are also able to assess the nanofraction in complex matrices, will rapidly promote the more comprehensive implementation and methods of harmonisation, based on spectrometric techniques such as Py-GC-MS and LC(SEC)-HRMS for the study of MNPLs.



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