

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



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



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

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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Α

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

В

BMD ₁₀	Benchmark Dose at 10%
BW	Body Weight
BPA	Bisphenol A
ВВЬ	Butyl Benzyl Phthalate
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

Ε

μ-FTIR

F

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

Micro-Fourier Transform Infrared Spectroscopy

KOH	Potassium Hydroxide	Ν	
K.S.O.	Disodium Persulfate	Mg	Magnesium
		MRM	Multiple Reaction Monitoring
IC	Ion Chromatography	m/z	Mass-To-Charge Ratio
ICP-MS	Inductively Coupled Plasma – Mass Spectrometry	MALDI-MS	Matrix-Assisted Laser Desorption/Ionisation coupled
IC _x	Concentration required to cause x% of inhibition	MPLs	Microplastics
INC	Intergovernmental Negotiating Committee	MeOH	Methanol
ISO	International Organisation for Standardisation	MNPLs Mt	Micro and Nanoplastics or Micro(nano)plastics Metric Tonnes
НСН	Hexachlorocyclohexene	Μ	
H ₂ H ₂ O ₂ HQs HI HNO ₃ HILIC HRMS HYSPLIT	Hydrogen Hydrogen Peroxide Hazard Quotients Hazard Index Nitric Acid Hidrophilic Interaction Liquid Chromatography High Resolution Mass Spectrometry Hybrid Single-Particle Lagrangian Integrated Trajectory	LOAEL LC _x LDIR LC LC x LC LRMS LAGRANTO LOG k _{o/w}	Lowest Observed Adverse Effect Level Concentration required to cause x% of lethal re- sponse Laser Direct Infrared Spectroscopy Liquid Chromatography Comprehensive Two-Dimensional LC Low Resolution Mass Spectrometry Lagrangian Analysis Tool Logarithm Octanol-Water Partition Coefficient
HD-PE	High-Density Polyethylene	LCA LRAT LD ₅₀	Life Cycle Assessment Long-Range Atmospheric Transport Dose that is lethal to 50% in an organism
GIT	Gastrointestinal Tract	LD-PE	Low-Density Polyethylene

NPLs

Nanoplastics

NOM	Natural Organic Matter	PGA
NOAEL	No Observed Adverse Effect Level	РНВ
NaCl	Sodium Chloride	PHAs
NaOH	Sodium Hydroxide	PCL
ΝΤΑ	Nanoparticle Tracking Analysis	PEA
NTS	Non-Target Screening	PBAT
NORMAN	Network of reference laboratories, research centres	PBS
	and related organisations for monitoring of emerging	PTT
	environmental substances	PA
0		POPs
_		PCPs
OCDE	Organisation for Economic Cooperation and	PM _{2.5}
0	Oxygen	Py-GC-MS
02 •0H	Hydroxide Radical	
Öll		PLEX
Ρ		PUF
•		PCBs
PP	Polypropylene	PFASs
PS	Polystyrene	PAHs
PBD	Polybutadiene	PFOA
PI	Polyisoprene	PFBS
PET	Polyethylene Terephthalate	PFHXS
PVC	Polyvinyl Chloride	PFOS
PUR	Polyurethane	PBDES
PC	Polycarbonate	0
PDMS	Polydimethylsiloxanes	
РММА	Polymethylmethacrylate	qQq
PTFE	Polytetrafluoroethylene	q-IT
PBT	Polybutylene Terephthalate	q-Orbitrap
PLA	Polylactic Acid	q-TOF

GA	Polyglycolic Acid
ΗB	Polyhydroxybutyrate
lAs	Polyhydroxyalkanoates
CL	Polycaprolactone
A	Polyesteramides
BAT	Polybutylene Adipate Terephthalate
BS	Polybutylene Succinate
т	Polytrimethylene Terephthalate
A	Plastic Additive(s)
OPs	Persistent Organic Pollutants
CPs	Personal Care Products
M _{2.5}	Particulate Matter less than 2.5 µm
/-GC-MS	Pyrolysis coupled to Gas Chromatography and Mass Spectrometry
.EX	Plastic Explore
JF	Polyurethane Foam
CBs	Polychlorinated Biphenyls
ASs	Perfluoroalkyl Substances
AHs	Polycyclic Aromatic Hydrocarbons
ŌA	Perfluorooctanoic
BS	Perfluorobutanesulfonate
HxS	Perfluorohexasulfonate
OS	Perfluorooctanesulfonate
BDEs	Polybrominated Diphenyl Ethers

	Triple Quadrupole
	Quadrupole-Ion trap
bitrap	Quadrupole-Orbitrap
F	Quadrupole-Time Of Flight

QSAR	Quantitative Structure-Activity Relationship	11
QA/QC	Quality Assurance/Quality Control	0
R		UN UNEA
REACH	Registration, Evaluation, Authorisation, and restriction of Chemicals	UV UAE
ROS	Reactive Oxygen Species	ULW
RNS	Reactive Nitrogen Species	\ /
RNA	Ribonucleic Acid	V
RC ₅₀	Concentration required to cause 50% of the response	VOCs
µ-Raman	Micro-Raman Spectroscopy	v/v
RP	Reversed Phase	W
S		WWTPs
SDGs	Sustainable Development Goals	WHO
SERS	Surface Enhanced Raman Scattering	WW
SFC	Supercritical Fluid Chromatography	7
SML	Surface Microlayer	4
SIM	Selected Ion Monitoring	ZnCl ₂
Т		
TGA	Thermogravimetry	
TED-GC-MS	Thermal Extraction Desorption coupled with GC and MS	

Time Of Flight

Half-Life Time

Decreasing Time

United Nations
United Nations Environmental Assembly
Ultraviolet
Ultrasonic-Assisted Extraction
Underlying Seawater at 5 m Depth
Volatile Organic Compounds
Volume per Volume
Wastewater Treatment Plants
World Health Organisation
Wet Weight
Wet Weight
Zinc (II) Chloride

TOF

T_{1/2}

 \mathbf{T}_{D}

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 scientific 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.

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 MN-PLs 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 n°1: 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, <u>https://doi.org/10.1016/j.jhazmat.2020.124022</u>

- Publication n°2: 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, <u>https://doi.org/10.3389/fmars.2021.697424</u>
- **Publication n°3**: 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, <u>https://doi.org/10.1016/j.jhazmat.2023.131036</u>
- **Publication n°4**: 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 n°5: 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, <u>https://doi.org/10.3390/toxics8030059</u>

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 n°6: 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, <u>https://doi.org/10.1016/j.watres.2022.118645</u>
- Publication n°7: 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, <u>https://doi.org/10.1016/j.chemosphere.2023.140106</u>
- **Publication n°8**: 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.

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



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

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).

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) -[C₃Hɕ]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) -[C2H4]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) -[C2H4]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 ₈ H ₈] _n -	1.04	Significant stiffness, low thermal conductivity, resistance to chemicals.	Building insulation, packaging, egg trays, spectacle frames, etc.
Polybutadiene (PBD) -[C₄H6]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) -[C₅Hଃ]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 ₈ O₄] _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) -[C₂H₃Cl]ո-	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 _x HyO2N2]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)	1.20-1.22 (PC),	Dependent on resin or	Optical fibres (PBT), hub
Polydimethylsiloxanes (PDMS)	1.07 (ABS)	mix of them.	cups (ABS), roofing sheets (PC), touchscreens
Polymethylmethacrylate (PMMA)			(PIMMA), telecommunications (PTFE), aerospace,
Acrylonitrile Butadiene Styrene (ABS)			medical implants, surgical devices, membranes,
Polytetrafluoroethylene (PTFE)			seals, paintings, coatings, etc.
Polybutylene Terephthalate (PBT)			

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) -[C₃H₄O₂]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 ₂ H ₂ O ₂] _n -	Bio-based and partially biodegradable from monomers of biomass	Paints and coatings, adhesives, sealants, artificial suede, synthetic leather.
Polyhydroxy butyrate (PHB) -[C₄H6O₂]₀-	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 ₆ H 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:

- <u>Functional:</u> 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.,
- <u>Reinforcements:</u> 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 ubiguity, 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-

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, CO₂, N₂, 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.



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 plas-

tics 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 \cdot 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 SO₄⁻, 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 abi-

otic 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 $\longrightarrow R_i$. (2) $R_i + O_2 \rightarrow R_iOO$. (3) $R_iOO + RH \rightarrow R_iOOH + R$.

Propagation:

(1) $\mathbb{R} + \mathbb{O}_2 \longrightarrow \mathbb{ROO}$ (2) $\mathbb{ROO} + \mathbb{RH} \longrightarrow \mathbb{ROOH} + \mathbb{R}$

Termination:

(1) $R \cdot + R \cdot \longrightarrow R \cdot R$ (2) $R \cdot + ROO \cdot \longrightarrow ROOR$

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 Spainscale 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 μ m – 5 mm), and nanoplastics (NPLs, < 1 μ m); 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 ubiguitous 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 rivercoastal 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



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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 O₂ and water molecules, in order to generate other ROS-like ¹O₂ and O₂^{*-} 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 $\cdot OH$ 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 Fe²⁺, thus favouring the initiation of the Fenton reaction by the generation of a large amount of \cdot 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 \cdot 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, CO₂, and H₂O 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·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 μ m) 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 μ m). 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



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 – <i>Oryza sativa</i>	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 – <i>Skeletonema</i> <i>costatum</i>	Decreased algae density; Decrease in photosynthetic rate	PVC (1 μm; 20, 100, 200 mg·L ⁻¹)	(Zhang et al., 2017)
Plant – <i>Cucumis sativus</i>	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 - <i>Gallus domesticus</i>	Myocardial pyroptosis; Inflammatory cell infiltration; Mitochondrial lesions; Energy metabolism disorders	PS (5 μm; 1, 10, 100 mg·L ⁻¹)	(Zhang et al., 2022b)
Fly – <i>Hermetia ilucens</i>	Gut inflammation; Oxidative stress; Faecal hardening	PVC (200 nm; 20 μg∙Kg⁻¹)	(Xu et al., 2023)
Frog – <i>Physalaemus cuvieri</i>	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 – <i>Carassius</i> <i>auratus</i>	Genotoxicity effects by an increase in erythrocyte nuclear abnormalities	PS (44 nm; 15, 100 ng∙g⁻ ¹)	(Brandts et al., 2022)
Zebrafish – <i>Danio rerio</i>	afish – <i>Danio rerio</i> Spontaneous tail coiling, increase of the heart rate, MNPLs accumulation on the chorion surface, and potential morphological changes in interocular distance		(de Souza Teodoro et al., 2024)
Mussel – <i>Myt.</i> galloprovincialis	Reduction of AChE activity, nuclear distortions, and DNA damage	PE (1-100 μm; 1,000 mg·L ⁻¹)	(Avio et al., 2015)
Mussel – <i>Mytilus</i> <i>galloprovincialis</i> 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_{ro}), 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% (BMD₁₀) 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 RC₅₀, 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 MN-PLs 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 μ m, here we focus on particles of MNPLs from 1 nm to the smallest size fraction of MPLs (1-25 μ m) 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 $(pH \sim 2)$ 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 μ m might be able to interact with the intestinal epithelium tissues, and while only plastic particles up to 1.5 μ m 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 μ m (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 μ m, 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.



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 μ m 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 μ m 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:

$$HQ = \frac{EDI}{ADI}$$
 (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:

$$EDI = \frac{DI \cdot Ce}{BW}$$
 (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:

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

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 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 MN-PLs. 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 shortterm 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, Fe²⁺ (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 hypersaa line dissolutions made from NaCl or ZnCl₂ (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-al-kaline leaching, or a mix of both. As a rule, H_2O_2 with or without a strong acid (e.g. HNO₃) 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 strong-ly 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.





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-



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

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-



for various guatic compartments, B) Box and whisker plots (or boxplots) showing plastic particles less than 5 mm 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 MN-PLs 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·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 cen-tred 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 MN-PLs 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 Km² 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

Km², 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 n°1**) and the Mar Menor lagoon (Murcia, Spain; **Publication n°2**) 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 n°3), and in water and biota of remote areas (Publication n°4). 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 n°1: 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, <u>https://doi.org/10.1016/j.jhazmat.2020.124022</u>
- Publication n°2: 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, <u>https://doi.org/10.3389/fmars.2021.697424</u>
- **Publication n°3:** 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, <u>https://doi.org/10.1016/j.jhazmat.2023.131036</u>
- **Publication n°4:** 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

ARTICLE INFO

Keywords: Nanoplastics Microplastics Size exclusion chromatography HRMS Quantification Suspect screening 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), polyteylene (PE), polysbutadiene (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 particulate 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 plution 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 μ m, 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 he 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

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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 Cromatograffa (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 ($\geq 99.7\%$ 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 km², 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 M. Llorca et al.

Table 1

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

				Summer 20	018		Winter-spr	ing 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): \pm 0.15 °C.

^b pH SD: ± 0.02.

 $^{\rm c}\,$ Salinity SD: \pm 2% of the lecture.

were transported to the laboratory under cold conditions where they were kept at 4 $\,^\circ C$ before treatment.

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 um 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 APCTM XT45 1.7 μ m 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 μ L.

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 chromatograph equipped with Purospher® STAR RP-18 endcapped column (5 μ m, 2 \times 125 mm) from Merck. Toluene was used as mobile phase under isocratic mode at 0.5 mL/min. Injection volume was set at 20 μ L, and the total chromatography 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 losses 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 cocontaminants 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 PB 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

ng/L)

ulated

200

100



identified through $\mu\text{FT-IR}$ were PE and PP which have a contribution of 16% and 8%, respectively among the total polymers identified by the authors

1st sampling campaig

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 (Grav 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 northwestern sub-basin area (Tuscany, Italy) (Baini et al., 2018) or Turkish area (Gijven 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.

2nd sampling campaig

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

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

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

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 M. Llorca et al.

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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 1,2,3benzotriazole 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

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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 salvcylamide 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 adicarb 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

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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.

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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 oreanic 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

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





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
	1	Poly(methyl methacrylate)	PMMA	[C5H8O2]n	100.05243
	2	Polyacrylamide	PAM	[C₃H₅ON]n	101.035103
Acrylic Polymers	3	Poly(methyl acrylate)	PMA	[C4H6O2]n	86.03678
Activiter of ymers	4	Poly(ethyl methacrylate)	PEMA	[C5H10O2]n	102.06808
	5	Poly(2-hydroxyethyl methacrylate)	poly-HEMA	[C5H10O3]n	118.062995
Alkyd resins	6			[C14H18O8]n	314.10017
Aminoplasts	7	Melamine formaldehyde	MF	[C4H6N6]n	138.065394
Cellulose	8	Cellulose acetate	CAc	[C10H14O7]n	246.073955
	9	Methyl cellulose	MC	[C9H16O5]n	204.099775
	10	Ethyl cellulose	EC	[C11H22O5]n	234.146725
	11	Hydroxyethyl cellulose	HEC	[C ₁₀ H ₂₀ O ₈] _n	268.11582
	12	Nitrocellulose	NC	[C6H8O9N2]n	252.022983
Coumarone-indene-	13	Poly(indene-co- coumarone)		[C ₁₇ H ₁₄ O] _n	234.104465
resins	14	Poly(methylstyrene-co- indene)		[C ₂₆ H ₄₆] _n	358.35995
F	15	Diglycidyl ether of bisphenol-A	DGEBA	[C ₁₈ H ₂₀ O ₃] _n	284.141245
Epoxy resins	16	Diglycidyl ether of bisphenol F	DGEBF	[C16H16O3]n	256.109945
	17	Polytetrafluoroethylene - TEFLON	PTFE	[C ₂ F ₄] _n	99.993612
Fluoropolymers	18	Polyvinylfluoride	PVF	[C ₂ H ₃ F] _n	46.021878
	19	Polyvinylidene fluoride	PVDF	$[C_2H_2F_2]_n$	64.012456
Polyacetals	20	Polyoxymethylene	POM	[CH ₂ O]n	30.010565
Polyacetylenes	21	Polyacetylene / polyethyne		$[C_2H_2]_n$	26.01565
Polyacrylics	22	Polyacrylic acid	PAA	[C3H4O2]n	72.02113
Polyalkylenes	23	Polyalkylene Glycol		[C ₂ H ₄ O] _n	44.026215
Polyamic acids	24	Polyamic acid	PAA	[C ₃₀ H ₁₈ O ₈ N ₂] _n	534.106318
	25	Alyphatic polyamide	Nylon 6	[C6H11ON]n	113.084064
Polyamides	26	Alyphatic polyamide	Nylon 66	$[C_{12}H_{22}O_2N_2]_n$	226.168128
	27	Polyphthalamide	PPA	[C ₁₄ H ₁₈ O ₂ N 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 ₂ N ₂ S] _n	452.15585
	32	Dicyclopentadiene based Benzoxazine		[C ₃₈ H ₃₇ O ₂ N ₂] _n	553.285503
Polybenzoxazoles	33	Polybenzoxaxole	PBO	[C14H6O2N2]n	244.121178
	34	Poly(bisphenol A carbonate)	РС	[C16H16O3]n	256.109945
Polycarbonates	35	Poly(propylene carbonate)	PPC	$[C_4H_6O_4[C_2H_4O]_m]_n$	162.052825
	36	Poly(cyclohexene propylene carbonate)	PCHPC	[C7H10O3]n	142.062995
Polycarbosilanes	37	Branched polycarbosilane		[C ₁₈ H ₃₂ Si ₃] _n	332.181184
	38	Bisphenol A Dicyanate		[C ₁₇ H ₁₄ O ₂ N ₂] _n	278.105528
Polycyanurates	39	Bisphenol E Dicyanate		$[C_{16}H_{12}O_2N_2]_n$	264.089878
rolycyanaraces	40	Tetramethyl Bisphenol F Dicyanate		[C ₁₉ H ₁₈ O ₂ N ₂] _n	306.136828
	41	Poly(1,4-butadiene)	PBD	[C ₄ H ₆] _n	54.04695
Polydienes	42	Polyisoprene (cis and trans)	PI	[C5H8]n	68.0626
	43	Polychloroprene	CR	[C₄H₅CI]n	88.007978
	44	Polyethylene terephthalate	PET, PETE	[C10H8O4]n	192.04226
	45	Polybutylene terephthalate	PBT, PBAT	[C ₁₂ H ₁₂ O ₄] _n	220.07356
	46	Polytrimethylene terephthalate	PTT	[C11H10O4]n	206.05791
Polyesters	47	Polyethylene naphthalate	PEN	[C14H10O4]n	242.05791
	48	Poly(propylene adipate)	PPA	[C9H14O4]n	186.08921
	49	Polylactic acid	PLA	[C3H4O2]n	72.02113
	50	Poly(ether ketone)	PEK	[C ₁₃ H ₈ O ₂]n	196.05243
Polyetheretherketones	51	Poly(ether ketone ketone)	PEKK	[C19H12O3]n	288.078645
	52	Poly(ether ether ketone)	PEEK	[C ₂₀ H ₁₂ O ₃] _n	300.078645
		Polyethylene glycol,	PEG,		
Polyethers	53	Polyethylene oxide,	PEO,	$[C_2H_2O]_n$	42.010565
		Polyoxyethylene	POE		

		Polypropylene glycoL,	PPG,		
	54	Polypropylene oxid,	PPOX,	[C3H6O]n	58.041865
		Polyoxypropylene	POP		
		Polytetrsamethylene	PTMG,		
	55	glycol, polytetramethylene ether glycol,	PTMEG,	[C4H8O]n	72.057515
		Polytetrahydrofuran	PTHF		
Polyimidazoles	56	Poly(4-vinyl imidazole)		[C5H6N2]n	94.053098
Polyimides	57	Poly(ether)imide	PEI	$[C_{37}H_{24}O_6N_2]_n$	592.163438
Polyketones	58	Polyketone	РК	[C ₃ H ₄ O] _n	56.026215
	59	Polyethylene	PE	$[C_2H_2]_n$	26.01565
Dalvala Gua	60	Polypropylene	PP	[C3H6]n	42.04695
Polyoletins	61	Polyisobutylene	PIB	[C4H8]n	56.0626
	62	Polybutylene	PB-1	[C4H8]n	56.0626
		Polyoxadiazole,			
Polyoxadiazoles	63	poly(oxadiazole-hydrazide) copolymer	POD	[C ₈ H ₄ ON ₂] _n	144.032363
Polyoxyalkylenes	64	Polyoxyethylene	POE	$[C_2H_4O]_n$	44.026215
Polyoxymethylenes	65	Polyoxymethylene	POM	[CH ₂ O] _n	30.010565
Delveverbendenes	66	Poly(p-phenylene oxide),	DDE		120 057515
Polyoxyphenylenes	66	poly(p-phenylene ether)	PPE	[C8H8O]n	120.057515
Polyphenyls	67	Poly(p-phenylene)	РРР	[C ₆ H ₄] _n	76.0313
Polypyrroles	68	Polypyrrole	РРу	[C8H6N2]n	130.053098
	69	Polysilane		[SiH ₂] _n	29.992578
	70	Poly(dimethyl silane)		[C ₂ H ₆ Si] _n	58.023878
Polysilanes	71	Poly(methyl phenyl silane)		[C7H8Si]n	120.039528
	72	Poly(trimethyl phenyl silane)		[C9H14Si2]	178.063406
Polysilazanes	73	Perhydropolysilazane		[H2Si-NH]n	45.003477
Polysiloxanes	74	Polydimethylsiloxane	PDMS	[C ₂ H ₆ SiO] _n	74.018793
	75	Polystyrene	PS	[C ₈ H ₈] _n	104.0626
Dalvetringer -	76	Poly(4-methoxystyrene)		[C9H10O]n	134.073165
Polystyrenes	77	Poly(4-methylstyrene)		[C9H10]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
Delveulfere -		Delveulfana	PSF,		442 422002
Polysullones	80	Polysullone	PSU	[C27H22O4S]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 ₄ S] _n	400.076932
	83	Thiokol	Thiokol	$[C_2H_4S_4]_n$	155.919588
	84	Polypropylene sulfide		[c3H6S]n	233.879382
Polythioethers	85	Poly(ethylene sulfide)	TR	$[C_2H_4S]_n$	60.003372
	86	Poly(phenylene sulfide)	PPS	[C ₆ H ₄ S] _n	108.003372
Polythiomethylenes	87	Polytrimethylene terephthalate	PTT	[C ₁₁ H ₁₀ O ₄] _n	206.05791
	88	Tolylene-2,4-diisocyanate Tolylene-2,6-diisocyanate	2,4-TDI 2,6-TDI	$[C_9H_6O_2N_2]_n$	174.042928
Polyurethanes Polyvinyl acetals	89	1,4-Phenylene diisocyanate	MDI	[C15H10O2N2]n	250.074228
	90	Isophorone diisocyanate	IPDI	[C ₁₂ H ₁₈ O ₂ N ₂] _n	222.136828
	91	Hexamethylene diisocyanate	HDI	[C ₈ H ₁₂ O ₂ N ₂] _n	168.089878
	92	Polyvinyl acetate	PVAC	[C ₄ H ₆ O ₂] _n	86.03678
Polyvinyl acetals	93	Copolymer ethylene vinyl acetate	EVA	$[C_2H_4]_n$ - $[C_4H_6O_2]_m$	114.06808
	94	Polyvinyl alcohol	PVA or PVOH	[C ₂ H ₄ O] _n	44.026215
Polyvinyl butyrals	95	Poly(vinyl butyral)		[C ₈ H ₁₃ O ₂] _n	141.091555
Polyvinyl formals	96	Poly(vinyl formal)		[C ₅ H ₈ O ₂] _n	100.05243
	97	Poly(vinyl chloride)	PVC	[C ₂ H ₃ Cl] _n	61.992328
Visual solutions	98	Poly(vinylidene chloride)	PVDC	[C ₂ H ₂ Cl ₂] _n	95.953356
vinyi polymers	99	Poly(vinyl bromide)		[C ₂ H ₃ Br] _n	105.941811
	100	Poly(vinylphosphonic acid)	PVPA	[C ₂ H ₅ O ₃ P] _n	107.997633

Addite Circle Circle<	Name	Formula	MM	$\log K_{ow}^*$			1st ca	mpaig	-				2n	d can	npaign	_		Í	
Maile Maile <th< th=""><th></th><th></th><th></th><th></th><th>z</th><th>2 9 5</th><th>8</th><th>11 01</th><th>13 15</th><th>12 14</th><th>с І 91</th><th>τ ε 7</th><th>ç</th><th>L</th><th>01 6</th><th>EI II</th><th>12 14</th><th>91</th><th>Uses or sources</th></th<>					z	2 9 5	8	11 01	13 15	12 14	с І 91	τ ε 7	ç	L	01 6	EI II	12 14	91	Uses or sources
Miller Citity Citity<								Plastic	additi	ves				1					
Base Citic Citic <thc< td=""><td>Aniline</td><td>C6H7N</td><td>93.05743</td><td>0.9*</td><td>H</td><td></td><td>хх</td><td></td><td></td><td>х</td><td></td><td></td><td>×</td><td>x</td><td>хх</td><td>x</td><td>L</td><td>x F</td><td>Plastics and dyes; precursor of polyurethane and polyaniline.</td></thc<>	Aniline	C6H7N	93.05743	0.9*	H		хх			х			×	x	хх	x	L	x F	Plastics and dyes; precursor of polyurethane and polyaniline.
Bit Bit Interplay Interplay<	Benzene	$C_6 H_6$	78.04678	2.13*	ХХ	х х	XX	х х	х х	хх	x x x	X X X	X	x x	хх	хх	х х	у х	Gasoline, production of styrene, nylon fibers, and in other plastics
Bender bende bende bende bende bende and the solution of	Bis(4-methyl-2-pentanyl) hydrogen phosphate	C ₁₂ H ₂₇ O ₄ P	266.1647	3.59**									x					_	Homopolymer or copolymers of styrene and other esters
Considemention State	Bisphenol AP	C ₂₀ H ₁₈ O ₂	290.13068	4.57**	x		x	×	x				×				хх	Ē	Plastic precursor (used as a substitute of Bisphenol A)
I = M = M = M = M = M = M = M = M = M =	or 6,6-bis(p-methoxyphenyl)fulvene			or 5.37**												_			
3 effely interfaciency of introductionC in	Butyl acrylate	$C_7 H_{12} O_2$	128.08336	2.36*	x						x x		Î	2		H	×	Ē	Paints, sealants, coatings, adhesives, fuel, textiles, plastics, caulk
3-ter-Buy/phenol CuBito 131+ 1 <th1< th=""> 1 1 1</th1<>	n-Butyl methacrylate	C ₈ H ₁₄ O ₂	142.0995	2.88*	H	×			х			x				H	H		Production of polymers of methacrylate among others
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	2-tert-Butylphenol	C ₁₀ H ₁₄ O	150.10382	3.31*	×	×	x	×		x	×	~	×	× ×				X I	Antioxidant and stabilizer, fire prevention materials
Displayer C Hu, O. SiO351 0.64* N <td>2,6-di-tert-Butylphenol</td> <td>C₁₄ H₂₂ O</td> <td>206.16603</td> <td>4.92*</td> <td>×</td> <td>×</td> <td>×</td> <td>×</td> <td></td> <td>×</td> <td>×</td> <td></td> <td></td> <td>F</td> <td></td> <td>\vdash</td> <td>F</td> <td>Ĺ</td> <td>UV stabilizers and antioxidants</td>	2,6-di-tert-Butylphenol	C ₁₄ H ₂₂ O	206.16603	4.92*	×	×	×	×		×	×			F		\vdash	F	Ĺ	UV stabilizers and antioxidants
Coloremant Colorem	Butyrolactone	C4 H6 O2	86.03651	-0.64*				×			×	×	×	хх	x		ŀ	x	Batteries, solvent in lotions and some polymers
	Cyclohexanone	C ₆ H ₁₀ O	98.07283	0.81^{*}	х	х	x	х	х	x	X X	X X J	X >	хх	хх	хх	х	x	Precursor of nylon. Additive in paints, dyes, oils, waxes, nubbers
Decompositional Chip.0 Cl:21456 Cl:P3135 9.2% N	ô-Decal actone	C ₁₀ H ₁₈ O ₂	170.13007	2.72*	ХХ	х х	XX	х х	х х	хх	×	X X X	X	x x	x	хх	_	x I	Biopolymer manufacture, food and agricultural industries
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Decanoic acid	C ₁₀ H ₂₀ O ₂	172.14566	4.09*	хх			×	×	×	×				хх		x	1	Manufacture of esters, rubber, dyes, plastics, food additives
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Diisononyl hexahydrophthalate	C ₂₆ H ₄₈ O ₄	424.35328	9.82*		x	×			×					х	×		-	Increase soft flexible qualities of plastics
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Dibutyl Phthalate	C16 H22 O4	278.1518	4.5*		x		×		x					x			x	Increase soft flexible qualities of plastics
	Dimethyl Phthalate	C ₁₀ H ₁₀ O ₄	194.0571	1.6^{*}	X X 2	x x x	×	х х	х х	x	x x x	_	~	×	x	x x	×	x	Increase the flexibility of plastics and rubber
$I-Hydroxyveclolosy1 Phenyl Ketone C_1H_{10} (2) (2) (2) (2) (2) (2) (2) (2) (2) (2)$	2-Ethylhexanoic acid	C ₈ H ₁₆ O ₂	72.05711	2.64*		x x	XX	x x		x	×	x x	×	×	хх	×	x	xI	Production of PVB, polyolesters, PVC stabilizer among others
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	1-Hydroxycyclohexyl Phenyl Ketone	C ₁₃ H ₁₆ O ₂	204.11414	1.78^{**}	хх		×	×	×		××	х х	×	××	x x	××	×	×	Used in polymers, plastics, dyes among others
$I_{12} I_{12} $	Indene	$C_9 H_8$	116.0623	2.92*	×	×	×	×	х	×	x	XXX	×	×	хх	x	хх	x	Production of indene/coumarone thermoplastic resins
$MonOcArthylder (MEH) [C, hE). O_{4} (E, O_{1} (E, H). O_{1} (E, H). O_{1} (E, H) (E, H) (E, H). O_{1} (E, H) (E,$	Lauryl peroxide	C ₂₄ H ₅₀ O ₂	370.37926	11.53**			×									Ţ			Plastic, rubber and paint modifier
Mondic aniplicity(c)(b)(c) <th< td=""><td>Mono(2-ethylhexyl) phthalate (MEHP)</td><td>C16 H22 O4</td><td>278.15022</td><td>4.80^{**}</td><td>X X 3</td><td>x x x</td><td>XX</td><td>хх</td><td>ХХ</td><td>хх</td><td>x x</td><td></td><td>×</td><td>×</td><td>×</td><td></td><td>×</td><td>x l</td><td>Increase soft flexible qualities of plastics</td></th<>	Mono(2-ethylhexyl) phthalate (MEHP)	C16 H22 O4	278.15022	4.80^{**}	X X 3	x x x	XX	хх	ХХ	хх	x x		×	×	×		×	x l	Increase soft flexible qualities of plastics
Nonmote add $(c_1 H_3 O_1)$ $(S_1 H_3 O_2)$ $(S_1 H_3 O_1)$ $(S_1 H_3 O_1$	Nadic Anhydride	C ₉ H ₈ O ₃	164.04676	0.528**							×		×	_	×	Ţ	×	7	Additive in plastics
4-Monylphenol Cl ₃ H ₂₄ O 2.01.815 5.76 ^a x x	Nonanoic acid	C ₉ H ₁₈ O ₂	158.13008	3.42*	x	×	×		x	×	×				хх	×		x	Preparation of plasticizers and lacquers
$\label{eq:action} Particle in pairts or Inbrients for inbrients ike in pairts or Inbrients of the first or $	4-Nonylphenol	C ₁₅ H ₂₄ O	220.1815	5.76*	хх		×	×		×	x x x	X X X		~			×	x	Production of TNPP; antioxidant in polymers
Benzotrizole $C_{1}H_{3}$ [19.04S2] 1.44° X [X X X X X X X X X X X X X X X X X X						Ű	Other &	npplica	tions li	ke in p	aints c	or lubr	icant	s					
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Benzotriazole	C ₆ H ₅ N ₃	119.04822	1.44*	x x	x x x	x x	x x	x x	x x	× ×	x x y	×	×	× ×	×	x	-	Corrosion inhibitor in paints among others
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	1,3-Bis(2-hydrazinoethyl)-5-isopropyl- 2,4-imidazolidinedione	C ₁₀ H ₂₂ N ₆ O ₂	258.18164	-2.07**		x	x x	×	x x	x x	x	x x x	×	×	x x	x	х х	-	Colorants, dyes, or pigments, for food and inks
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Dimethylsilanone	$C_2 H_6 O Si$	74.01858	2.6/4.25*	ХХ	x				x					хх	×	×	Ľ	Generated from decomposition of polydimethylsiloxanes
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Dimethylsilanediol	C ₂ H ₈ O ₂ Si	92.02904	0.91^{**}	х х х	хх	x	х	х	хх	x x x	X X J	X >	хх	хх	хх	х	Ĭ	Generated from decomposition of polydimethylsiloxanes
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	2-Dodecylsuccinic acid	C ₁₆ H ₃₀ O ₄	286.21287	5.06^{**}	_	х		х	х х	хх	x x x	X X X	X	x x	хх	хх	х х	-	Corrosion inhibitor and anti-scaling agents
(E.E)-2-4-Hepadienal (C. Hi_0 O) (10)/0721 (1.5)/(1.5)/(1.5) (1.5)/(1.5)/(1.5) (1.5)/(1.	(Fluoroethynyl)(triisopropyl)silane	C ₁₁ H ₂₁ F Si	200.14019	6.08**	_		x	хх	х	х							х	× 、	Generated from decomposition of polydimethylsiloxanes
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	(E,E)-2,4-Heptadienal	$C_7 H_{10} O$	110.07271	1.59^{**}	_		х х	×			x	X X X	×	×	х	x x	хх	x	Used in oil, petroleum, fuel oils, drilling oils among others
Heptadecaredioic acidC ₁ , H ₂ , O ₄ 300.228375.58**1xx	Heptanoic acid	C ₇ H ₁₄ O ₂	130.09904	2.42*	х	x x	XX	хх	х х	x	x x x	XX	×	×	хх	×	х х	x X	Used in oil, petroleum, fuel oils, drilling oils among others
HydroxyberzoritazoleCa Ha, O135.04386 60.9^{9**} <td>Heptadecanedioic acid</td> <td>C₁₇ H₃₂ O₄</td> <td>300.22837</td> <td>5.58**</td> <td></td> <td>×</td> <td>×</td> <td>×</td> <td>×</td> <td>x</td> <td>x x x</td> <td>x x</td> <td>×</td> <td>×</td> <td>x x</td> <td>×</td> <td>x</td> <td></td> <td>Surfactant in lubricant for engines, brake fluids, oils among others</td>	Heptadecanedioic acid	C ₁₇ H ₃₂ O ₄	300.22837	5.58**		×	×	×	×	x	x x x	x x	×	×	x x	×	x		Surfactant in lubricant for engines, brake fluids, oils among others
C ₁₅ Hendecenoic cicl C ₁₅ H ₉ O, 245,2235 6.2 ^{2**} x x x x x x x x x x x x x x x x x x	Hydroxybenzotriazole	C ₆ H ₅ N ₃ O	135.04386	0.69**		x x	×		×	×		×	×	~	x	×	x	-	Derivative of benzotriazole, used as corrosion inhibitor
$(\pm)-1-Phenylethanol C_8 H_{90} 0 122.0728 1.42^* pt relation (1) petroleum, find oils petro$	Pentadecanoic acid	C ₁₅ H ₃₀ O ₂	242.22325	6.62**	X X >	x x x	XX	хх	хх	x	x x x	x x	×	×	x x	×	x	x l	In products like toys, and lubricants for engines, brake fluids, etc.
Phthalimide C ₈ H ₅ N O ₂ 147,03156 1.15 [*] x x x x x x x x x	(±)-1-Phenylethanol	C ₈ H ₁₀ O	122.0728	1.42*			x	х		×	x	х х	×	×	×	×	хх	x X	Used in oil, petroleum, fuel oils, drilling oils among others
	Phthalimide	C ₈ H ₅ N O ₂	147.03156	1.15*	х	×	×	x		x	хх	х		×	x	×	x	x	Precursor to azo dyes (in plastic for their colouring properties).

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Tratecylic acid C ₁₃ H ₅₀ O ₅ 24, 1921 8 Tyrstool C ₄ H ₀ O ₅ 24, 1921 8 Ubquintome Q4 C ₅ H ₁₀ O ₅ 24, 3067 63 Ubquintome Q4 C ₅ H ₁₀ O ₅ 188, 6763 Ubquintome Q4 C ₅ H ₁₀ O ₅ 188, 6763 Ubquintome Q4 C ₅ H ₁₀ O ₅ 188, 6761 7 1-(6-Methoxy-2-mphthyl) ethanone C ₁₁ H ₁₂ O ₅ 200, 08336 2-lsoproylphtenyl hydroperoxide C ₁ H ₁₀ O ₅ 200, 08336 2-lsoproylphtenyl hydroperoxide C ₁ H ₂₀ O ₅ 137, 04701 Ethyl accute C ₁ H ₂₀ O ₅ 137, 04701 Photoxyberzamide C ₁ H ₂₀ O ₅ 137, 04701 Photoxyberzamide C ₁ H ₂₀ O ₅ 137, 04701 Photoxoberzamide C ₁ H ₂₀ O ₅ 137, 04701 Photoxoberzamide C ₁ H ₂₀ O ₅ 137, 04701 Al. C ₁₀ H ₁₀ O ₁ 166, 07757	18 5.56** x 63 0.62**	X X X X X X				XXX	x Food	l additive, soaps, cosmetics, detergents, lubricants, softening
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Z-stopropy/pneny/inytroperoxuee C + Hi, O; 123,0470 H-Hydroxybenzamide C + Hi, N O; 137,04701 Ethyl acetate C + Hi, N O; 137,04701 Brhylaepteroxuee C + Hi, O; 137,04701 Phenalter C + Hi, O; 136,08819 Mesitol C + Hi, O; 136,08819 Addromb.endere C + Hi, O; 136,08819	06 2.81**					X	It is a	t potential impurity in commercial preparations of naproxen
Tructure C111/C2 12.5/07/01 Ethyl actile C4 H ₂ O 136.08819 Mestiol C6 H ₁₀ O 136.08819 Phenalene C1 H ₁₀ O 166.07757 Aldiumb rotiona C1 H ₁₀ O 166.07757	3.22**	,			×		11 red	roduct burned as waste
Metal (1997) Metal (2014) Phenalene (2014) Aldiambaviana (2014) Aldiambaviana (2014) (v 0.235 v	× ×		~ ~	× × ×		v Solve	III IIagianees and odour agents of
Phenalene C.3 H10 166.07757 Al Jicords ovina C. H., N. O. S. 133.0556	19 2.73*	x x x x x x	x x x x x x		x x x x x x	x	x x Solve	ent also used in PPCPs and cleaners.
Aldioneh ovima C. H., N.O.S. 133-0558	57 4.17**		X	XX			Forme	ed during the combustion of fossil fuels
Aldicark ovine C, H.: N O S 133 0558			Pestic	sides				
	8 1.21**			x x		x	Carba	amate insecticide mainly used as nematicide in the past.
Bifenazate C ₁₇ H ₂₀ N ₂ O ₃ 300.14758	58 3.4*			х		х	Insect	ticide, acaricide. Non-classified
Chlorpyrifos C9H11Cl3NO3PS 348.92573	73 4.96*	x			x	×	Orgar	nophosphate insecticide, acaricide and miticide
EPTC / Torbin C ₉ H ₁₉ N O S 189.11809	09 3.21*	x		х	х	х х	Thioc	carbamate herbicide
2-Hexylcyclopentanone C ₁₁ H ₂₀ O 168.15072	72 3.35** x	x x x x	XXXXX	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 ₅ 212.067/9		X	X X	x	X X X	X X X	Plant	growth regulator
Phoxim C ₁₂ H ₁₅ N ₂ O ₃ PS 298.05266	66 4.39*	X X	X X X X	x x x	X X X X	×	x x Orgar	nophosphate insecticide
			Pharmac	seuticals				
Azelaic acid C ₉ H ₁₆ O ₄ 188.10406	06 1.57*	x x		хх	x x	X X X	x Used	in dermatological treatments.
Benzenesulfonamide C ₆ H ₇ N O ₂ S 157.01922	22 0.31* x	X X X X	XXXXXX	X X X X X	X X X X X	X X X X	x x Anti-8	asthmatic, bronchodilators, expectorants, antitussive agents.
N-Butylbenzenesulfonamide C10H15NO2S 213.08132	32 2.1**	x	x x	x x x	x x x x x	x x x	x x Anti-8	asthmatic, urinary tract, bladder, problems in kidneys, and in

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

Chlorzoxazone	C ₇ H ₄ Cl N O ₂	168.99245	1.6^{*}	L								\vdash			$\left \right $	х		\vdash		Muscle relaxer and pain release.	
Ditiocarb	C ₅ H ₁₁ N S ₂	149.03308	1.81^{**}	хх				×			×	×	×	х	$\left \right $	×	×	-	×	x antiviral agents, and main metabolite of DISULFIRAM	
17α-Estradiol	C ₁₈ H ₂₄ O ₂	272.17962	4.13^{**}	x						×		×	×					-		It is an endogenous steroidal estrogen	
Ethacrynic Acid	C ₁₃ H ₁₂ Cl ₂ O ₄	302.0112	3.3*	хх	×		×			×	×	×			$\left \right $	×	x x	-		Used to treat edema	
Fenbufen	C ₁₆ H ₁₄ O ₃	254.09312	3.2*	х	×					L		H	H		╘			x		Nonsteroidal anti-inflammatory drug.	
Hexestrol	C ₁₈ H ₂₂ O ₂	270.16068	4.98**	х		Ē	x x	х	х			×	X		x		×	×		It is a synthetic estrogen used as a hormonal antineopla	stic agent.
Mesterolone	C ₂₀ H ₃₂ O ₂	304.23882	4.24**	х	x		х	х			х	х х	х		х	х	х	-		x Androgen and anabolic steroid (AAS) medication	
9-Phenanthrol	C ₁₄ H ₁₀ O	194.07195	3.94**	L		x	хх			L		H	H		╘			\square		It is used to modulate vascular and smooth muscle cont	ractility.
Salicylamide	C ₇ H ₇ N O ₂	137.04717	1.28^{*}	хх			××	x		×		×		×	x	×		-		x analgesic and antipyretic properties like aspirin	
Sulfaphenazole	C ₁₅ H ₁₄ N ₄ O ₂ S	314.08394	1.52*	х х	x		х	х		х	х	х	хх	K X		X	хх	H		It is a sulfonamide antibacterial	
										Other	r.s										
1-Methylamino-1-(3,4-	C ₁₁ H ₁₅ N O ₂	193.10961	2.26^{**}		×		x	х	х х	x	×	×	×	×	хх		×	хх	×	x Drug isomer of ecstasy	
Methylenedioxyphenyl)Propane									_			_						_			

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 \pm 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.

doi: 10.3389/fmars.2021.697424 Keywords: microplastics, nanoplastics, LC-HRMS, suspected screening, additives

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MPLs/NPLs Seawater Mar Menor Lagoon

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 landbased 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 μ m 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 μ m, 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 μ m 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

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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 (Cerdanvola del Vallès, Barcelona, Spain) and polypropylene (PP) (MW~1,200 Da) supplied by American Polymer Standards Corporation (Ohio,

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United States), polyvinyl chloride (PVC) was obtained from IDAEA-CSIC (Barcelona, Spain) laboratories to perform the Sigma-Aldrich (Steinheim, Germany), and polyamides were analytical process. based on *ɛ*-Caprolactam, and the analytical standard (analytical grade) was obtained from Supelco.

Toluene HPLC solvent with >99.7% purity and HPLC-water were acquired from I.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 μ m GF/F were purchased from

Whatman (Maidstone, United Kingdom).

Analytical Standards

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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 (Nvlon), 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 $1 \mu g/L$ 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

2

MPLs/NPLs Seawater Mar Menor Lanoon

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 um 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

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

				Summer p	eriod					Winter pe	eriod			
Code	Area	Coordinates	Date	Seawater T (°C)	Daily T (°C)*	pН	Salinity (ppt)	O ₂ (mg/L)	Date	Seawater T (°C)	Daily T (°C)*	pН	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

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

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

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 μ m, 2 \times 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.

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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 polymer standards based on equivalent concentration (Schirinzi et al., 2019).

Tentative Identification of Plastic Additives

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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 ca. 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).

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Suspect profiles were tentatively identified using repetitive ass losses with a mass error below 3 ppm (identification infidence level 4) and they were subsequently filtered by mparing with a polymers' homemade library (level 3). the tentative identification of polymers (level 2) was carried to by using Kendrick Mass Defect (KMD) analysis. The

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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 (C₂H₆OSi)_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 μ m 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 $\mu g/mL$ 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.

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2,5

0,5

10000 8000 6000

2000

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

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,

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4

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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.

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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

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

including urbanization, tourism and fishing (Bayo et al., 2020).

9

2

3

4

5

PP

6

7

8

Summer 2018

Winter 2019

9



Summer 2018

Winter 2019

ΡE



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

Sample code (place)			Equivalent	concentral	tion (µg/L) \pm SD			Detected	at confidence level 2
	[PE] _{eq}	[PS] _{eq}	[PI] _{eq}	[PBD] _{eq}	[PP] _{eq}	[PVC] _{eq}	[PA]*	PNIPAm	Polysiloxanes
			1:	st sampling	period (Summe	r)			
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< td=""><td>n.d.</td><td>n.d.</td><td>0.0038 ± 0.0008</td><td>YES</td><td>YES</td></loq<>	n.d.	n.d.	0.0038 ± 0.0008	YES	YES
3 (Los Alcázares)	1.08 ± 0.09	n.d.	n.d.	n.d.	$5,\!153\pm20$	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.	$9,303\pm366$	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.	$6{,}386\pm408$	n.d.	0.0040 ± 0.0010	YES	YES
			2	nd samplin	g period (Winter)			
1 (Lo Pagán)	0.97 ± 0.03	n.d.	n.d.	n.d.	$6{,}276\pm649$	n.d.	0.0041 ± 0.0006	YES	YES
2 (Las	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.	$5,\!964\pm729$	n.d.	0.0041 ± 0.0014	YES	YES
4 (Canal del Estacio)	2 ± 1	n.d.	n.d.	n.d.	$7{,}591\pm202$	n.d.	0.0041 ± 0.0009	YES	YES
5 (La Manga)	1.04 ± 0.04	n.d.	n.d.	n.d.	$6{,}009\pm174$	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.	$5,734\pm689$	n.d.	0.0040 ± 0.0010	YES	YES
8 (Los Nietos)	< LOQ	n.d.	n.d.	n.d.	$6{,}309\pm397$	n.d.	0.0043 ± 0.0008	YES	YES
9	0.121 ± 0.02	n.d.	n.d.	n.d.	8.744 + 433	n.d.	0.0042 ± 0.0016	YES	YES

7

(Isla del Barón)

*[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

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 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),

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throughout the year.

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1st Sampling campaign (summer)

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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 distearvl 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 (ethylhexhyl 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

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2.1

2.2

2.3

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

2.4

2.5

■ Food additives & Flavor ■ PCPs ■ Pesticides ■ Pharamceuticals

2.6

2.7

2.8

2.9

40

35

30

25

20

15

10

40

35

30

25

20

15

11

12

1.3

1.4

1.5

■ Food additives & Flavor ■ PCPs ■ Pesticides ■ Pharamceuticals

2on Sampling campaign (winter)

1.6

1.7

18

19

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Other Compounds

used during the production of thermoplastic resins.

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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-ditert-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

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(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

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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.

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not have a clear interpretation but is related to some phthalates

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

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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

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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

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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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Katerina Savva

Víctor M. León

Esteban Abad

Marinella Farré

Frontiers in Marine Science, 2021, 8, 697424

Tables S1, S2 Figures S1, S2, S3

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	Other		UV filt				Plastic																			
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	Exact mass		119.04835	135.04326	133.06422	225.17218	124.08852	312.13512	390.27701	278.15121	222.08842	194.05791	390.27701	250.12010	278.15146	222.08899	132.07872	258.18232	370.30760	258.18294	230.15139	314.24539	270.25560	213.08235	255.25547	194.11542
	Formulae		CeH5N3	C ₆ H ₅ N ₃ O	C ₇ H ₇ N ₃	C13H23NO2	C8H12O	C19H20O4	C24H38O4	C16H22O4	C ₁₂ H ₁₄ O ₄	C10H10O4	C ₂₄ H ₃₈ O ₄	C ₁₄ H ₁₈ O ₄	C ₁₆ H ₂₂ O ₄	C ₁₂ H ₁₄ O ₄	C ₆ H ₁₂ O ₃	C14H26O4	C22H42O4	C14H26O4	C ₁₂ H ₂₂ O ₄	C ₁₈ H ₃₄ O ₄	C17H34O2	C ₁₀ H ₁₅ NO ₂ S	C ₁₆ H ₃₃ NO	C ₈ H ₁₈ O ₅
	Compound		1,2,3-Benzotriazole	1-Hydroxybenzotriazole	Tolytriazole	2,2,6,6-Tetramethyl-4-piperidyl methacrylate	1-Ethinylcyclohexanol	Benzyl butyl phthalate	Bis(2-ethylhexyl) phthalate	Dibutyl phthalate	Diethyl phthalate	Dimethyl phthalate	Dioctyl phthalate (DNOP)	Dipropyl phthalate	Mono(2-ethylhexyl) phthalate (MEHP)	Monobutyl phthalate	Butyl glycolate	Dibutyl adipate	Heptyl nonyl adipate	Diethyl sebacate	Dimethyl sebacate	Dibutyl sebacate	Isopropyl myristate	N-butylbenzenesulfonamide	Palmitamide	Tetraethylene glycol

Table S1 Plastic additives identified at confidence level 2.

			Antioxidant						Coating sealant and Surface	ueaunents		Curing agent	Retarder cross-links	lubricant	Flame retardant			Colourant		Intermediate in the manufacture	of perfumes, lubricants, greases,	rubber, dyes, plastics, food	additives and pharmaceuticals	Intermediate for flavourings,	periorites, priarritaceutoals, uyes, plastics, adricultural chemicals:	cleaning agent for a variety of	industrial applications, in	chemical and biodegradable	copolymer feedstock	Used in the preparation of	plasticisers and lacquers. Its	
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402.22413	382.34425	182.07104	110.03695	236.17729	300.20851	358.29055	234.16198	278.18789	150.10447	287.24577	104.01128	197.09566	122.03686	242.22458	210.10205	166.07634	224.08085	156.07873	hem also us	172.14633				74.00039						158.13068		
C20H34O8	C ₂₄ H ₄₆ O ₃	C ₆ H ₁₅ O ₄ P	C ₆ H ₆ O ₂	C ₁₅ H ₂₄ O ₂	C20H28O2	C ₂₁ H ₄₂ O ₂ S	C ₁₅ H ₂₂ O ₂	C17H26O3	C ₁₀ H ₁₄ O	C ₁₆ H ₃₃ NO ₃	C3H4O4	C ₁₂ H ₁₁ N ₃	C7H6O2	C ₁₅ H ₃₀ O ₂	C ₈ H ₁₉ O ₄ P	C ₆ H ₁₅ O ₃ P	C ₈ H ₁₇ O ₅ P	C8H12O3	monomers (some of t	C10H20O2			(C2H2O3						C ₉ H ₁₈ 0 ₂		
Tributyl citrate acetate	2-Butoxyethyl-9-octadecenoate	Triethylphospate	1,3-Benzenediol	2,6-Di-tert-butyl-4-methoxyphenol	Dehydroabietic acid	Distearyl 3,3'-thiodipropionate	IRGANOX 3114	3-(3,5-di-tert-butyl-4- hydroxyphenyl)propanoic acid	4-Tert-butylphenol	N, N-Bis(2-hydroxyethyl) dodecanamide	Malonic acid	Aniline	Benzoic acid	Pentadecanoic acid	Dibutyl phosphate	Diethyl ethyl phosphonate	Triethylphosphonoacetate	Tetrahydrofurfuryl acrylate	Intermediates in polymers synthesis or I	Decanoic acid (Capric)			:	Glyoxylic acid						Nonanoic acid		

1

																	used in conjunction with glyphosate.
	C12HFCl3O2	287.95093	×	×	×	×	×	×		×	×	×	×	×	×	×	X Antiseptic, preservative and disinfectant used in healthcare and other consumer products (cosmetics, household cleaning products, plastic materials, toys and paints).
	C10H18O2	170.13068	×	×	×		×	×	×								It naturally occurs in fruit and milk products and has applications in food, polymer, and agricultural industries.
ŧ.	C18H34O4	314.24539	× ×	×	×	×					^	~	×	×			Plasticiser in production of cellulose acetate butyrate, cellulose acetate propionate, ethyl cellulose, polyvinyl butyral, polyvinyl chloride, polystyrene, and many synthetic rubbers and other plastics.
	C2H2O4	89.99543	××	×	×	×	××	×	×	×		×	×	×	×	×	X Polyester monomers
decenoic acid	C10H18O3	186.12559	× ×	×	×		× ×	×	×								Reactant used in the synthesis of copolymers of acrylamide and sodium acryloyloxydecanoate.
	C3H4O4	104.01128	× ×			×	×		×	×	×	×	×	×	×		X Precursor to polyesters and coating agent
octadecenoic acid	C ₁₈ H ₃₄ O ₃	298.25030			×		×							×			Synthesis of sebacic acid which is used in the synthesis of nylons.
ol acetate	C ₈ H ₁₆ O ₃	160.11009	×									×			×		Used in the manufacture of polyvinyl acetate latex.
acrylate (2-EHA)	C7H12O3	144.07869				×		×	×		×			×	×	×	X Base monomer for the
																	preparation of acrytate adhesives; and copolymer for monomers of vinyl acetate, methyl acrytate, and styrene to modify.nolymer properties.
ic acid	C ₈ H ₁₆ O ₂	144.11508	×	×	×	×	×	×	×	×	×	×	×	×	×	×	 Production of polytinylibutyral plasticizers and synthetic lubricants, polyolesters, and polyvinylichoride stabilisers. In cosmetics, 2-EHA is used to produce emollients
uorohexane	C ₆ H ₁₀ F ₄	158.0.7135												×			Emulsion polymerisation process for producing fluoroelastomers.
ioxythiophene (EDOT)	C ₆ H ₆ O ₂ S	142.00881			×		××			×	×	×	×	×	×	×	X Precursor of the polymer PEDOT

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3,5-Di-tert-butylbenzaldehyde	C ₁₅ H ₂₂ O	218.16611			-													\times	Used in the synthesis of some	
Azelaic acid	CaH ₁₆ O ₄	188.10496	×	×	×						×	×			×	×	×	X	polymers Svnthesis of Nvlon 6.9 with	
			<	<	、 、						<	``````````````````````````````````````	< ,		:	<	<		hexamethylenediamine	
Benzene	CeHe	78.04682							×										Component of gasoline, and used in the production of	
																			ethylbenzene, a precursor to stvrene	
Benzyl vinyl ether	C ₉ H ₁₀ O	134.07277														\times		-	Monomer in the polymerisation of	
						_													organic polymers polyvinyl ethers.	
Butyl acrylate	C7H12O2	128.08342				×	×											-	Used in paints, sealants,	
						_													coatings, adhesives, fuel, textiles, plastics, and caulk.	
Cyclohexanone	C6H10O	98.07302							×									-	Precursor of nylon among other	
																		_	uses.	
Dimethylsilanediol	C ₂ H ₈ O ₂ Si	92.02936		•	×													•/	Silicone	
Indene	C9H8	116.06231	×	×	×	×	×	×	×	×	×	×	×	×	×	\times	\times	\times	Used in the production of	
						_													indene/coumarone	
					-						;				;				thermoplastics.	
N,N'-Bis(2-hydroxyethyl)terephthalamide	C ₁₂ H ₁₆ N ₂ O ₄	252.11177		_	~	~					×	×			×				Intermediate in the production of	
N-Methylpyrrolidone	C ₅ H ₉ NO	99.06810			Ê	×	×	×	×		t					\times			Used as a solvent for engineering	
																		_	polymers and coating resins.	
Phenol	C ₆ H ₆ O	94.04203					×			×			×	×	×	×	×		The major uses of phenol involve	
																			its conversion to precursors for	
Sebacic acid	C10H18O4	202.12048			Ê								-		×			×	Used in industry to synthesise the	
				_	_	_						_						_	monomer for nylon 610,	
				_	_	_						_							plasticisers, lubricants, hydraulic	
																		-	fluids, cosmetics, candles, etc.	
Suberic acid	C ₈ H ₁₄ O ₄	174.08912		×	_	_					×		×			×	×	×	Used in drug syntheses and	
													_					-	plastics manufacture.	
Tripropylene glycol	C ₉ H ₂₀ O ₄	192.13616	×		×	_			×	×	×	×	×	×	×	×	×	×	Raw material for the production	
				_	_	_								_				-	of polymers such as polyesters	
				_	_	_								_					and alkyd resins, as a plasticiser	
																			in polymers such as PVC.	
Phthalic acid	C ₈ H ₆ O ₄	166.02611			~	×	×	×	×	×			×	×		×	×	•,	Synthesis	
Phthalic anhydride	C ₈ H ₄ O ₃	148.01557	×	×	×	×	×	×	×	×	×	×	×	×	×	\times	×	×	Synthesis	
				-	-	-				1							Ĩ.	Í.		

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

					$\left \right $	$\left \right $	$\left \right $							Ī	ľ	ľ	ľ	ľ
1,2,3-Benzenetriol	C ₆ H ₆ O ₃	126.03034				_	_	_				×						
2,2'-(Dodecylimino)diethanol	C ₁₆ H ₃₅ NO ₂	273.26604	×			×	\sim	~		×	×	×	×	×	×	×	×	×
2-[2-(Dodecyloxy)ethoxy]ethanol	C ₁₆ H ₃₄ O ₃	274.25009	×		×	×	\sim	×	×	×	×	×	×	×	×	×	×	×
2-Hydroxyethil laurate	C14H28O3	244.20342									×		×	×			×	×
4-Hexadecylmorpholine	C ₂₀ H ₄₁ NO	311.31792	×															
Ethylhexyl ethylhexanoate	C ₁₆ H ₃₂ O ₂	256.24023	×	×	×	×	\sim	×	×	×	×	×	×			×	×	
Lauramide	C ₁₂ H ₂₅ NO	199.19302	×		×	×	\sim	×	×	×	×	×	×	×	×		×	×
Lauric acid	C ₁₂ H ₂₄ O ₂	200.17769	×							×								
Myristamide	C ₁₄ H ₂₉ NO	227.22448													×			1
N,N-Bis(2-hydroxyethyl)-9,12- octadecadienamide	C22H41NO3	367.30759						×										
N,N-Bis(2-hydroxyethyl)dodecanamide	C ₁₆ H ₃₃ NO ₃	287.24577					^	×										
Tridecylic acid	C13H26O2	214.19328					~											
PCPs and other uses	-	-												1	1			
4-Tert-butylcyclohexyl acetate	C ₁₂ H ₂₂ O ₂	198.16188				-		-								×		
4-Dodecylbenzenesulfonic acid	C18H30O3S2	326.19166			×													1
Benzaldehyde	C7H6O	106.04166				×	\sim	×	×	×							×	
Lauryldimethylamine oxide	C ₁₄ H ₃₁ NO	229.23999														×		
Myristic acid	C14H28O2	228.20893			×	×	\sim	×	×	×	×	×	×	×		×	×	×
Stearic acid	C18H36O2	284.27153	×	×	×	×	\sim	×			×		×	×	×	×	×	×
Toluidine	C ₇ H ₉ N	107.07328							×									
Undecanoic acid	C11H22O2	186.16191	×	×	×	×	\sim	×	×			×	×	×	×	×	×	×
Vanillin	C ₈ H ₈ O ₃	152.0474				×					×	×	×	×	×	×	×	×
Pesticides																		
1-Hydroxy-2-oxo-1-phenylhydrazine	C ₆ H ₆ N ₂ O ₂	138.04309			-	-						×						
1-Nonanoic acid	C9H18O2	158.13068														×		
Benzenesulfonamide	C ₆ H ₇ NO ₂ S	157.01975				×										×		

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Chapter 4

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Difenopenten-ethyl	C ₂₀ H ₁₉ F ₃ O ₄	380.12197																	×
Pharmaceuticals																			
(4-Isobutylphenyl)acetic acid (also known as ibufenac)	C ₁₂ H ₁₆ O ₂	192.11463															×		
1,2,4-Triazine-3-carboxylic acid	C4H3N3O2	125.02266	×		×			×											
17α-Estradiol	C ₁₈ H ₂₄ O ₂	272.17763				×	×					×							
1-Benzosuberone	C11H12O	160.08843					-			<u> </u>	×	×	×	×	×	×	×	×	\times
1-Hydroxy-4-methoxybenzene	C7H8O2	124.05261		×		×	×	×	×	×	×	×		×	×	×	×	×	\times
2-(3H)-Furanone	C20H22O6	358.14055									×	×	×	×	×	×	×	×	×
5,5-Diethylbarbituric acid	C ₈ H ₁₂ N ₂ O ₃	184.08684				×	×				×	×	×	×					
Aceclidine	C ₉ H ₁₅ NO ₂	169.10979	×	×		×	×	×	×	×	×	×			×	×		×	\times
Butyrophenone	C10H12O	148.08835															×		
D-(-)-Tartaric acid	C4H6O6	150.01703				×		×						×	×		×	×	
Ethosuximide	C7H11NO2	141.07868							×										
L-(+)-Tartaric acid	C4H6O6	150.01696			×														
Linoleic acid	C ₁₈ H ₃₂ O ₂	280.24025					×			×									
Phthalamide	C ₈ H ₅ NO ₂	147.03215		×		×	×	×	×	×	×	×	×	×	×	×	×	×	\times
Terodiline	C ₂₀ H ₂₇ N	281.21361					×	×	~			×							×
Valpromide	C ₈ H ₁₇ NO	143.13067				×	×	×	×	×							×		
Other uses related to pharamacuticals																			
Nonivamide	C ₁₇ H ₂₇ NO ₃	293.19834									×	×	×	×	×	×	×	×	×
Food additive and flavouring agents																			
2,3-Butadione	C4H6O2	86.03666					-	-	×	_	_								
2,4-Heptadienal	C7H10O	110.07292				×	×	×	×								×		
2-Isobutoxynaphthalene	C14H16O	200.11960											×	×	×	×	×	×	×
2-Methylpropyl-3-oxobutanoate (Isobutyl acetoacetate)	C8H14O3	158.09429	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
3-Octanone	C ₈ H ₁₆ O	128.12014				×	×			×					×			×	×

× × × ×

128.12014 115.04530

×

C₅H₉NS

Butyl isothiocyanate

Dibutyl succinate	C ₁₂ H ₂₂ O ₄	230.15156		×		×													
Dibutyldithiocarbamic acid	C ₉ H ₁₉ NS ₂	205.09589			×													×	
Ethylbenzoate	C ₉ H ₁₀ O ₂	150.06813		×			-		-										
Heptanoic acid	C7H14O2	130.09959	×				-							×			×	\times	\times
Hexanoic acid	C ₆ H ₁₂ O ₂	116.08382				Ê		×		×		<u> </u>		×	×		\times	\times	\times
Indole	C5H10O2	102.06822						-				_				×			
Nonanal	C ₉ H ₁₈ O ₂	142.13575				×		-	-	_		<u> </u>			×	×		\times	\times
Pyruvic acid	C ₃ H ₄ O ₃	88.01614				Ê		×	×	×				×	\times	×	\times		\times
Docosahexaenoic acid	C22H32O2	328.24023				×	⊢	-	_			×	×	×					
Ethyl acetate	C4H8O2	88.05226						-	×				_						
Compounds related to other types of sy	nthesis																		
3,3,3-Trifluoro-2,2-dimethylpropanoic acid	C5H7O2F3	156.04019	×		-	-	-	-											
4-Hexadecylaniline	C22H39N	317.30761				+		-	-	_		<u> </u>			×				
Valeric acid	C5H10O2	102.06821			-							<u> </u>		×					\times
Natural products		_																	
(3α,5β,7α)-3,7-Dihydroxy-12-oxocholan- 24-oic acid	C24H38O5	406.27089	×			<u> </u>													
Abietic acid	C20H30O2	302.22444	×		×											×			
Arachidic acid	C ₂₀ H ₄₀ O ₂	312.30260			×		-												
β-Estradiol	C ₁₈ H ₂₄ O ₂	272.17702	×	×	×	×	Ĉ	×	×	×	×	×	×	×	\times	×	\times	\times	\times
Other tentatively identified compounds									-										
1-(1,3-Benzodioxol-5-yl)-N-methyl-2- propanamine (MDMA)	C11H15NO2	193.10975							×	×							×		
2-Hexylciclopentanone	C11H20O	168.15142				×				×						×		×	
Diisopropyl methylphosphonate	C7H17O3P	180.09175	×	×							×	_		×		×			
Nonadecylic acid	C ₁₉ H ₃₈ O ₂	298.28658										_		×				\times	



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.



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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1. Introduction

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R3wtitddranata?

y 33 Ctesanosin Guintuen uo

l grutgtpints Me previrpintp enHqR epFtkspneutfinentfine, dnH apnet u (f sg, tpnt umutpintf eprRs pnt/ utiQS $_{\rm D}$ dBh rdtkspnetfi utH2PdHH gptEar pmgutanetpintH2mengtHmpme(gg pmgut gHpfntf nepmgutfinent uRMmqRngpsvtfine, dnHB) ff enSc(pmsvtngmQR epnetnatmrdtkspnetfi ut rsm gnHfiqdtD. (Tinat (pmg gnst gHmfNKRspe ungrH mper (gB) apnet platp(nitpisRngmfi utrnssmpHH gHplntf enrnHRmtRspe ungrH mper (gB) apnet platp(nitpisRngmfi utrnssmpHH gHplntf enrnHRmtRsp ingrL (thinspe rpntfi):) xhotfiqdtD. (Tinat pisRngmt anet D. (gB) apnet platp(nitpisRngmfi utrnssmpHH gHplntf enrnHRmtRi utenf m piHpfi cnB I dninSpe rpntfi nemtpingtrn(MgnHi gHtRug, t t, ngpmtz $_{61}$ upen (lipInt PnSR(mfi ut HRupHplnt) (Tint g swinplntfi nsv(neumapht S z QTuH I MntxDratpintutRff nepg, tggane(pmglunfi utpintfinsv(net rs unutpint pr gtMintSpe rpnHi dffi qdtpisRngmB

I dnint, dpilf epinatur rdtQS $_{\rm D,t}$ kspnetfi utrsm gnHifi qdtrnsHt(npd gnst pntent, nPnIn5pneg sine, gotrn(fnRgHit gHpingtplenmänsHn5pe rpiHMvt : x) htfi qdtD. (TnatpnsRngmanetD. (qgtm rdtp(mpntHcunsPnf nsv(neut gHpiRuta resp pntsm rdg, inate, gottRutHcgfs uprtf eprsmißi dotf ent rsm gg, fil qdt(npd gnst gHm5pe rpngtfi qdtpnsRngmt (ut pt 5 G d*g, t plntnsRrdH pngtnat HHppPnutcgtfs uprut gHt(gq(d*g, tnplnetf npngp st ne, gott uneMiH ngt plnt menunst fnnsB I dnt pnsRngmt n5pe rpt filment rngrngp piHt MPm20. µTt gHtfinentpe grameeniHpntTEtPc suf gHpdnt kg stPnsR(mtfi ut HkupnHtptD(Ttpntrd e rpmedFntplntf enksntnatplnt fs uprt HHppPnutg

I dninplnetplennån, dpltf epitnatm rdtkspnetfinentRunHtanetplntµAN Ørt gHxhS t g swindtanetne, gort gHinsm(ngp str eMigtil ElhEot g swindt gHanetgene, gortogut g swindtennf mrpPnswhphrd e rpneuntS z Qlut gHt plntenuptnatplntQS $_{\rm D}$ BI dnis uptqR epietfi utenunePnHtanetplntgene, gort g swintnat(np sub

y3g3 Mtdqz til ait7ndıfi-aDre Saturf Dat lænunfifgfcrdtaostitenuniDo

S npmensn, α strngHprogultuRrdt ut cetpn(fne pRenktficgHuf miht gHt Hænrpnglt gHte Hc pngltfinent(muRenHrngpgRnRuswHRez, tplntngprent u (f sg., treRantRug, tplnt(npmensn, α stup progtnætplntudd Bi dntH pnuh rnneHg pnutis ppRHnhsng, qRHndt uffinsst utficgHHænrpnghficgHuf miht gHt mit fne pRentærte mrdt u (f sg., tup pngtixxdit entuR((eanHt gt I Mntx6B

I dnt M r 3 fi eHt pe var pneonat nat eet (unnat finnent upRHonHt Rucg, t pdnt VwMedt xcg, snt Q eprismt T, e g, c gt Qpnje pnHt I e var pnewt iVüxQTO ot (nHnst aen(t pdnt z png st l rm gort gHt) p(nuf dneort) H(goape pngt iz l)) obNnetanRetH with M r 3 fi eHt pe var pneonatfinnentr srRs pnHtH cswt pt DJ dt: IEt ptD. t gHtO. (t MnPntum tsnPnsti BuBbhtfidordt entf enungpnHt gtN; BxDBt

y3Q3 1 ta-DnDof Eve nutdDoorf Dhot loTvfiRvo

$$\label{eq:linear} \begin{split} & \text{NnetpliniQS}_{D\ t}\text{cgne, gcrt(np surd erpnecu progli & & & \text{dramrdikspretfi ut rd+H; mpnHRucg, t t6/DtVNVz1_7(C 5pRentcgt tin9ngtem rpnetanet@0 dt fidsm(qp qcg, tpdmpn(fne pRent pfl, t'EBI dnghpdnm5pe rpfi ut+em+t anet7 dt gHemAthunsPnHrgtDfl/DV_6 / AVES _@unsRpngBNg sm5pe rpfi ment g swnHMt4QHHrpPnswEnRfsnHQs (t trnRfsnHtpnt)pn(crth(aungt xf mrpenurnf wt gHS _ utxf mrpen(npewi0EQ4) hx1S xoBi$$

Qtpdmtr unhategne, gortmytt g sweat^pl_jf epinatmrdikspnetfi ufi pneA unsPnHrgt6. (Trum snHrQLEtMppsmti@dt pt\$.t°EdtfemrnHnHrMvt gt Rspe ungorAucupnHnn5pe rpmgti: x) hotanetD0 (gBildnt qRnnRutn5pe rpt finent g swnHrMvtsqRdHongtrden (m, efdwti@t/%) I hyxt@EA 3IS t gongtrnsR(gt gH%) I hyxt@fotrngHRrpPqxtHnpmrpneotanetuRsf d pnHt gep pmHt gHtunsRWsntrdsnedHunguB

xoscrugin5d-initixd 6 of gHr eMng pmtiEl $\frac{6}{7}$ dtfinentogHenrpswtHipneA (gnHtMuplinin feor starpneur) st5-DB/fl =) s₆l 7b7-50 s₆l 7t= xd 6t gHt DB0-5tE t= El $\frac{6}{7}$ ^A gHt (ute pmut; 7DUBi dintrdn(σ strn(fngngptinatpdnt Q6 _{D.1}finent, enRfnHt uti otreRup stnet(ogne stiuR(tnat) s₆l 7bt d₆tEl $\frac{6}{7}$ th E hNnhi jt gHtS, o4iMt(egntrn(fngngptiuR(tnatEs^A gHz o4irot ne, gott gHzmin pp str eMng4iHotumrngH ewtgne, gott menusutixO bipdit uR(tnat) $\frac{6}{6}$ gHz 1 $\frac{2}{7}$ OB

) t^pl_{jt}f nepngtmatm rdtkspnetfi utRunHpntmP sR pntpdml ElhEtpdenR, dt t pdnet(sAnf por stpe gu(ouumgt ffen rdtHnuredMiHtggt;7Uttml fsnwcg, t t xRgunpts Mne pnewtun(cAngpgRnRutl ElhEtr eMngt menunst g swumeB

nahafitinaardawahfintariako450 (2023) 131036

y 3w3 endif 6/PGJ at loCRA at ta DnDo

Netm rdt kşmelt tf en PanRut g swuntplenR, dt gtnf pr st(orenurnf mi iL%yt(gpmeg pmg stTTEliy HgneliQ) i: gqniHxp pmudpitHipmrphilf en Angritinatf eprsnut gHikMenutHif nuppiHf i uf næne(nHBI dngliHipmrphilt S Qutfi nænt g swunH Rug, t tµAN (Ø tuf mrpn(npmt "z ornspitQD. tS [" il dne(niVadnetxrongpikrits gt-nuhle) it gqniHxp pmudinf ne pg, tRgHnet tuf mrp ste g, nitnat\$20–@...r($^{-Dx}$ gHRug, t tuf mrp stemnsRpmgtnat (@r($^{-Dx}$ gH&Qut guluAN (Ø tg swuntfi ut ff sonHpntPnetawpindif emngrint natS Qut fnsv(neutrn(fnuppigt)60 H

S z QTut gHtplmtemptratQS $_{\rm D,t}f$ dvuor strd e rpneu progltogtpne(utnat ufnit gHtpl fnhfi utr eenihnRptRug, txhS tplenR, dt tur ggog, tnæurpengt (orenurnfnti-xS &. DNt-hl TtTpHtiln3vnlt- f gotæn(tplntxrougpkrt gHt mrdgnsn, or st gqmtaphnt gPmeupwtatK ernsng tiEEd : KolkKnanent phnt g swochu (f snufi nemt eMngAn prihpnt(fenPmiqhnetrngHrpPqwW

y 303 1 ta DhDyf Eosfa e ni Dat los at Dardot ll rumn Dyf Eo A_p : 6A) Do

I dnt g swunnaf nsw(neunaphmS z Qlufi utr eenHnRptMvRug, tufnt mfrSkungtsqRdHrden(m, e f dwrnRfsmHf fqdtdc, dænnsRpngt(ut uf mrpen(npewtiTEixhEoAVyS xoBi I dntrden(m, e f dcrunß progti ut rdonPnH Rug, t g) rqRpwt TEti% pneuts CsaneHtS) h: x) ot uwpf h mqRdf nHfiqtl gt HP grnHff nsw(netrden(m, e f dwrlß) QEtrnSk(gt i) rqRpwt) QETM [I @bhDE2 µ(bhD. () dBNnetmrdt g sopr steRgb6. µTt natpdntpnSkngnin5pe rptfinentgwnpHfigdtD. tX tpnSkngnt utpdnt(nMsmt f d unit fine3g, t gt anre prt rngHpmgut gH pt t 9nfit e pnt at 0. B0 (T(q ^{-D}Bi I dm TEt uwpf(ti utrnRf snHt prt 1] A5 rpPn dwAedt qR HeRf nsml eMpe ft(uttuf mpen(npeuti I dne, ntNachnetxranghrksgt rug, t gt npe prdfinetgeng gt gt i f neae(ntNachnetxranghrksgt ur gt iNxct (nHmi e fi, 00...-7... ot fi qdt tdc, dtennsRpmg ta D20..t N%VS Bi dntnfp(st e (npeutnapht ngtHgueR(ngtr gtMhumgtgtnRet fenPenRutpRHt, 51)⁵

I dnt g swaatnatfs uprt HHpPmtnatplntS z QTuffi utr eenHnRptMt VQTEAVyS xhuf f snveg, tplnt) rqRpvtTEtmqRffnHtgtplatr unitfiqdt QRenuf dne®&xl) yt yQDU mgH4 ffnHt g svpr st rnsR(gt i0 μ (h 6 \times D60 ((dzen(ts ner3Bi dntgvrpmgtPnsR(ntfi ut6. μ Tnat(npd gnsrt n5pe rplt gHzplntrden(p, ef dctunf e pngtfi utr eenHzRqtRug, t t Mg ewt (nMsmtfd unt nat i) d (npd gnst gHzikatfi pneBi I dnt up epg, t , e Hngpffi utb. Xt i) dzerd7. ut gHpdngtgrem uniHem rdg, fl. tX ii) d pt DJB((gBi dnt, e Hngpffi ut(gp gniHzed) (gtgtanre prtrngHznguti enMuRcsMe miHzgpstplntggrst, e HzgptIt(gp gniHzed) (gtgtglt(gtgtglt(gtgtglt) gtgtglt)))

I dntrden(p, efdcrtuwpn(tfi ttrnRfsnHtpntpdnt)h5 rpPnltmqRdA fniHtfiqdt gtnsmrpentfe vtongG pngtunRerntihx@tinfne pg, tcgtgn, pPnt gHf nuppPntongau pngt(nHmtcgtunf e pntcgvnrpnguBG pt rqRanpagt fi utfnæne(nHtcgtNRsstS xAH p tHnfngH gptS x^{6t}iNxAHS x⁶ct(nHnti*e fi, o* D. .-D0..ctfiqdt t(utenunsRpngtnatD2lb).tN%VS B

y3K3 Itutot to:DDot loDSDsndunloDdinn rcotssiftdWnDo

I dnipnp stongtrden(pn, e (tril Octnatm rdtu (f snin Mp ganHiMxtNat rqRaupungtRug, tplint[r scMRetil dne(ntNadnetxrongpkrotunapfi entfinent f enrnunHianetplintdingpkr pngt gHtqR gpkr pngtnaf nsw(neutogtplint S z QFuBiQtMenalt apnetf m 3tf or 3og, t gHt, enRf og, hplintl OEtrden(pn , e (tinstm rdtu (f snifi nentgpneen, pnHmPnew7. tlinMnePog, tplintrneA entf ngHg, tS xtuf mp tut(Rsp gmnRusvtgtgpmeP stnat0. G tiæn(t0..t pnt7... e fi oBQm 3utgtfidordtS xtuf mp tutnfinHtdn(nsn, nRutuneont fi nentussmrpHt tutRuf mpHf nsw(neutglintu (f snuff nentussmrpHt nukuf nghlingpngupvHfidngphmf m 3t em utgplintu (f snuff nentussmupd gpdemu pn (nutplint) 5d R(tf m 3t em togplint endpen pnHkmsHt glintem tursurpHt tut M rf. s, enRgHB VnfinPneH plint uRuf mpt fi nentussmupH

irngkHngrntdHngpkr pngtsnPnstOdBI dngh pdntuRuf mrpHt fenksnut finem pngp pPnswtdHngpknHtRug, tenf nppPnt(utsnunutfiqdt t(utpnsne grnt Masnfit7 ff(tidHngpkr pngt prngkHngrntsnPnst@B) apnefi eHit pdnt uRMnnRngpkspe pngtupnf fi utngHRrpHMwrn(f eangtfiqdt tsMe ewnat dn(nf Hnfit nsv(neutidHngpkr pngt prngkHngrntsnPnst7dBI dngt pdnt j ngHer3t(utHnanrpij S Got g swattfi utr eenHnRpit gHpintpngp pPnt dHngpkr pngt naf nsv(neut rdomPnH rngkHngrntsnPnst6bI fidnetn ngnt (ngn(netgnmHtptMhunptkeuphpdntSA Sataapdnt) S Gif snptatplntpngp pPnt dHngpkr pngt naf nsv(neut rdomPnH rngkHngrnt snPnst 6bI fidnetn ngnt (ngn(netgnmHtptMhunptkeuphpdntSA Sataapdnt) UtHnanrpHfidordtapdnt HænengrnMhpfi nngpdntgn(og sij S t gHpdninj S Bi dnt(uttf mrp sf m 3tt natplntf nsv(netrn(f nuHlmatpd pf(ngn(net ent s; gnHf e sanstpntpdntSA Satnstpdntj S Gtf snBil dntdHngpkr pngt gHrngke(pngtirngkHngrnt snPnst Dat finent ngswt rdomPnHt Mwt rn(f eangt fiqdt up gH eHitB I dnt rngke(nHtf nsv(nett finentpdngtqR gpknHtMtRug, tup gH eHitB I dnt snPnst pmt RePnt; 6Jh@dB

R3v t i t ddmut Baanaan

Nnetpdntpngp pcPntcHngpckr pongtnatpdntf enksntnatf s uport HHopPmutcgt pdntu (f smiltpdntrneemuf ngHzg, tNxAHFS x^{6t}uf mrpe tfinentf enrmunHtRug, t En(fnRgHt GarnPnenet 7BDt iI dne(nt Nadnet xronender bt x gt - numbt E) bt : gennHixp pnuoBil dnipngp pcPnicHngpckr pengtreepnec tfimeniM unHingtpclnunt Hnured Milt nsunfidment :72UB Nm DRent Hnumrongt in 5 per prog. t (uuArden(pn, e f dort f m 3uolt, enRf cg, t i, enRf cg, t nat am pRemut fi qpdt rneens pni-lienpngpongtpo(mtf enksmudt/HnrngPnsRpongti uuc, gog, t HHRrpt gHt cunpuf mt ggnp pengut put, en Rf mHt am pRemudt gHtrn(fn RgHt ggnp pengt finentr eemHnRptMxtRug, t tgngAp e, mmHtfine39nfitfiodtnf mgtH p M unut uRrdt utEdm(xfcHmet gHt(FEsnRHtanetpdmtupeRrpRe stogane(pongt gHt (utuf mrpe ltemif mrpcPnswBI dngltpdntemiRsputfi mentk spnenHtMvtrn(f ecg, t n5 rpt(uutsnuumutfiqpdt tpnsne grmtnat6ff(btenpngpongtpc(mtfiqpdt t pnsme $grmtnat \pm 6$ BOtX t(gltcunpnf or tk pti > fl. tX obtae , (ngp pongt gHtpdmt fenPonRusvt(ngpongniHitfe(npneultogtHHopongtpntpdmt(utfm3temunsRA pongt gHtemuf nguniBil dntuRuf mrpturenning og, tfi utf meane(nHt utanssnfiu/tpdnt fs uport HH-ppcPmutfineentkg sswtpngp pcPnswtrngke(nH-ppntsnPnst6bt(prdcg,t pdmtm5fmec(mgp stae, (mgp pongtufmrpe t gHtrn(f ecg, tpdm(tfiqpdt ndmemor stf propegutaen(todotscope pRent gHtngscentH p MumuBNg sswb rncgrcHngptrn(fnRgHutficpdtpdntP cs Mantup gH eHutfinnentrngke(mHt gHt cHngpcknHt ptsnPnstDt; 6Jb@@B

y 3 3 CuturDurdtaat ta DrDo

Qegrd stEn(fngngt) g swutiQE) afi ut ffsmiltputpintQS $_{\rm D,\,t}u$ (A fsmtpi ptfinentrnssmpilt renutpint) ps gprtl rm gtRug, t-S QtQentup A pupp stungfi enten(tx) xtQuppRpntQrBiE evitz Elt: gqniltxp pnuBi dnt xrnenut gHTn Hg, tfsnptfinent, ngne pniHMvnn(fsnvg, tpintanssnfig, t u qR gpp pPntP ec Mmu/tS z QtUtl E+hEtiI np stl e, gcrtE eMg411 Edit (gene stixQ ltp rminsn(ngpit gHt(egnt nenunsBi dntg9Rngrninat eet (unut ut tqR sp pPntP ec Msntfi ut HHnHtfiqdegt tfl0tX trngkHngrmt snPnsBi

y3p:3 x Stanuat DDS it and lo Stanuadf uifaa2x1 fixv0o

)t])1] Et fen, e ((mt fi ut ansanfinkt put fenPngpt gwt fnuutMant rngp (og pogten, elkg, tu (fsog, t gHt g swuntnatplint nenunstu (fsmiti ogrsRHg, tplintfenrmung, tnatOtknsHfenrmHRe stMs g3tkspneut gHRug, t up gH elkenamengrmt(pne sutnatQhhQQhQkhQKGhQO gHQLEt gHun(mt fs uprt HHpPmBQt HHpnghplint nenunstu (fsog, tuwupt (fit unf ne pikk fidngtpdinPmunstfi utgtpe gupt gHtfiqdta PnRe Manfi gHtpnt PndHrenuvkt rngp (og pogtaen(tpdintenum erdtPmunstaRggsminfd RutaR(muß

I dntfenågem p(ngptnatpdntQS $_{D,t}u$ (fsnutfi utr ecoHnRptqgt trsm gt enn(tqgt ts (qs et9nfi tr Mgnplufi m ecg, trnppngts Mrn plu gH sstpdnt nqRof (ngptpd pffi utRunHrngucupnHnatup qsmutupnmstnet, s utfi dngnPnet f nucMmB

I dntkeuptupni tnatpilntuRuf mrphilturenngeg, t ffen rdtegPnsPniltMs g3ut uRMpe rpngBi dntrn(fnRgHit gHtplntinsw(neutHngpknilt prngkHngrnt snPnstDfinentqR gpkniltMst gtnSpng st sdMe pngtrRePntnatD.tfncgpiltRug, tplntnSpe rpg, tusPngtfinene(nHanetmrdtfnsw(neutMb egg, tpl nt sogm expte g, mutaen(t B 0tpnt0. μ , T^{-Dt} gHaen(t0. tpt 0... μ , T^{-Dt}fiqdt y⁶¹>. Bl. Bi dnt, nnHf neae(gruntaptn(npdnHti ut enPonRussu ununtH

fiqdtuf c3ntn5f nec(ngpit gHtpintqR gpkr pngltsgm eqwlif enraanglt rA rRe rwht gHt cgupeR(ngp st iCI] ut gHt (npinHt iS TI] ut s(qut nat qR gpkr pngtfi mennP sR piHtpints pnetcgrsRHg, tpintknsHMs g3uBi dnt (pc5tnæmrtfi ut ununHtanetnPnetwf nsv(neBS nennPnettenrnPnecmtnat mrdtf nsv(netfinentr econHnRpBL sdH pngtf e (npneut entfenungpiHtgt I Mntx7/inatpdntxRff sn(ngp evS) pnec sturrpngB

F. gl Tuht T mnd di Tcu Tlion

. 3p3 1 rice t DDnDo

I dntMr3tpe vnrpneomtnatplint eet(unntanetmrdtnatplintu (f sg, tf nA enHit ulnfi trngpe upg, t cg9Rngrmt Hnf ngHg, t ngt plnt nrm got en ongt i Nc B DoBI dnt pfi ntu (f sg, tp gurn pt plnt gnepdingts ppRHint ulnfi t nrm got et (uttMr3tpe vnrpneomt8Qpmenupg, svhplmt eet(unntrenumH plntgnepdt) ps gprttRMenf or st, venht 3gnfi gtpt rrR(Rs pntplnts e, mpt uRea rntrngrngpe pngutnaS Qut 60UBI fi ninatplintu (f smtfi nentg9RngrmH Mvt eet(unntanet t z nepdt) æor binædhentx d e BI dntu (f smtgetplnt pnf or st gHtQpmpenf or sten ongut Pnt tfenHn(og gptnec, cgtæn(tplnt muphnRplAm upi gHtpRthplnt etpe PnssnHnPnetplntingR pnec s\penf or st) ps gprt unPne sH vut Maanent qtfi ut u (f snHBI dnt anRet unRplneg(nupt u (f snutunfit etc (unntfi eqt(csnHg Q9Rngrmtæn(txnRplt) (nær t i) e, ngp t gHKe Fost gH(egnnfi pneuB)

. 3/3 v Witchnir, tuf of Eo $A_{p:}$ here nut d'harf Dhat lo Tv fiRv commado

Qt N; B 6h QS $_{\rm D}$ h (gme st n(fngngpåt l ElhElt xO h gH (egnt neunst rngrngpe pngut M unH ngt plnt M r3fi eH pe varpreenut ent f enA ungphHQS $_{\rm D}$ t(utrngrngpe pngutiN; B64 at gHrdn(a strn(fnupngut iN; B6C at entuchnf gt gHuR((edfnHqt M Mrix@Bl dnQS $_{\rm D}$, rngrngpe A pngute g, mHMmfi mgtDDB $_{\rm H}$, ($^{-7t}$ i661D616.6. at gH0flB $_{\rm H}$, ($^{-7t}$ i, Olt . D16.6D686Reg, tplntu (f sg, tr (f c, ghplmentfi utgswingme gaVH wt iD/1D616.6. at fi dad temRspnH tg rngrn at plnt snfi mgt snPnsu nat QS $_{\rm D}$ t iD/1BD $_{\rm H}$, ($^{-7}$ drnguupptfi qdtfi mptHnf nutgmgut Png, g, t nenusuB

QetNc, B6Chpdmt(vnetrdmt or strn(f nuppengtnatpdmtQS D tpdenR, dtpdmt anRet(cgt menunst, enRf uti(cgme stat(ecgmbaxQ) tr gHtl E1hEctcutenfi nepmHBb xfmrdxr sswbtegtpdmtkeuptpfintH wutnatu (fseg, btpdnæntfi ut tfenP sngrmtnat (ecgmtum tufe wtrngrngepe pengti7Ε) @cgHt6Ε617 μ, ·(⁻⁷htemuf mrpcPnswoBt QEHnmiHitpdmtMr3fi eHtpe vmrpnecmutudnfit gtcg9Rmgrmtnat ce9nfiutaen(tpdmt nrm gBi) ptuRMinqRngptu (fscg, tup pangutixxt\$t gHtxxtJobtcgt HHopengtpnt pd mium tuf e wice 9 Rngr mitipd mi) æcr git Himiniepti-Ruptog peRuong utup g Hin Rptifi opd t tf m 3trngrngpe pengtnat77B fl µ, (^{-7t}ngt6\$1D616. 6Dlt suntrngunpgt fi epdtpdnt ceA(uutMr 3 tpe vnrpneemuti Nc, BDoBH dntu (f smrnssnrpnHingt. 01t . D16. 6DtemrneHnHndmudc, dmupt(ecgmum tufe wti7DB622 μ, (⁻⁷ot gHtl E1t hEtrngrng pe penguti 7 B) fl μ , (⁻⁷ oB Qtf eperRs eltpdntdc, dnuptrngrng pe A pengtP sRntfi utanethEti. B\$0 µ, (⁻⁷dtfidnæntpdntpe rnt(mp strngrngpe A pengutf emuR(Mswtr (mtaen(tmqR pnec st) aeer gtMn(uutMRegeg, tHRupBlQt pdnts uptu (fscg, tfncgput ptpdntunRpdnæg(nuptsnr pengultpdnt(ecgntum t ufe wt menunst femP csut fight emuf mrpt pat pdmt npdmet rn(fngng puBt hPngt pdnR, dtpdnt cet(uutHcHtd Pnt gtcg9Rngrntæn(txnRpdt) (mear bipdntficgHt uf militfi utens poPnswtdc, dtHRecg, tpdmuntf meanHultrngucupng ptfi qpd tpd cutdc, dt rngrngepe pengtnatum Aufewt menunsBryn, eHog, tpdmtxQ btpdmtpengHtfiut qRqmtrngup gpthe g, cg, tMnpfinnsytDBDt gHt7B5 μ , ($^{-7}$ ltfiqdtpdmt(5 5c(R(t rngrngpe pengtsnPnstrneenuf ngHeg, tpntpdntu (f sntp 3ngtngt. 01. D16. 6DB

I dntdc, dmutrngrngpe pngutnatl dtTdtyMt gHtxrt ut, nm, ngortpernt nsm(ngputngt6\$1D616.6Dt entens pnHtpntpdntfenungrntnatRffnetreRup st enr3Ame(og, t(ogne stf eprsmult(ogsvtgtansurtusor pmtid5bibl(or ult ansHif eult gHtgngA(krtrs wudtMkp suntgtu(sstemucp gpt rrnunevt (ogne sut6bibln5dHnt gHtfnufd pmuBkn(mjernnsm(ngptuRrdt uERht xMt gHtfgt ent gn(snRuswidc, dtgtpdinu (f sog, tH wtcg9RngrnHMvaphnt) ær gtEn upBil dutf pmegtr gtMnt pecMkpnHtpntplntnæmrptnatplntuRMt x d e gtMn(uu4Mkegog, tfsk(mb R30v t i t ddronut d3tooo



bi3. 2. RDf nrmetl riAntion ok wx 1s. 4 Q8 D t C S gene stil ElhEltxQ it gHt(egent nenunstrngrngpe pagutM unHngtpdmtMr3fi eHtpe vnrpneamBt

. 3 3 A rdifét fost. Dirdodfes
f Dirdf fallvhol ut urdatri L
fi nostiurd Satuno et unio

I dnt pfingpwt pe gunrpt u (fsnut rnPneg, t ffen5q(pnswt D@J...3 (t aen(tLc, ntixf cgdpntQRgp t) eng utiEdcsnutfinent g swunHanetS z QTuB S z QTuffinentHapprpHkgtOJK tnaphnu (fsnuBQnsw(neutnaQhBQABQAbgAbgAbgAbf gHT nswH(mpdwscsn5 gntiQ6 Gxdtfinentprpp pPnswtHapptnH prngkA HagrntsnPns6BQ Gxdt sunt3gnfigt utuscragnthfi utpdnt(nuptaenqRngpt fnsw(netfenungpt cgt m, dpnngt u (fsnut ifl.tX oB QGS xh ut pdnt (nupt add (fneg pdrs utnakspne, gcrffnsw(neutifinuunnut gtne, gcrAxdMr3Mgmt natAiEV7 $_{Qx}$ xcA oAenfi m pfRqpt gHtatanRgHtgtnSpnguPmRuntcgtrgHRupec st gHReM gt ff sr pnguB

I dnt (nut rn((ngt rd gt HuperMapngut fi nænt Mnfi nmgt 6...t gHt 7... G t (ng, tpdntne, gart nsw(næuBQhtQQtt gHQQft næntanRgHtgtanRet u (fsmti 6. tX dtfi dsmtQxtfi utanRgHtgtngswingntu (fsmtRv, B7tudnfi ut un(ntn5 (fsmtnatpdntRut mrturenngg, t ffen rdt ffsmtHanetS z QTuA f nsw(næuBI dmutennRspt, enntfi qdtpdnuntf RMsudnHtMxtI e gart mt sRgst pdntz nepdt) ps gprt rm gtRug, tµAy (gtt mrpenur nd wtrgtfi dardtQhtQQtt gHQQtti neuts nutpdnt(nutprn((ngt nsw(næut; DJl62ti@lt utpdnwt d Pntf nutprn) n Mspvt gHtpdRut rrR(Rs pntgtpdntRea rntnrm gB

I dnine, gartf nsw(neutiQhiQQiQit gHQQiqi pfi nemikeupingp pPnswt dingpk nik fi nemi rngke(niki Mit rn(f eangt fi qdi up gH eikin gH plni nnjRP sngptrngrngpe pngut fi nemi qR gpk niki Mit up gH eikin sigit gH plni nnjRP sngptrngrngpe pngut fi nemi qR gpk niki Mit up gH eikin sigit gH plni nnjRP sngptrngrngpe pngut fi nemi qR gpk niki Mit up gH eikin sigit gH plni nnjRP sngptrngrngpe pngut fi nemi qR gpk niki Mit up gH eikin sigit gH plni nnjRP sngptrngrngpe pngut fi nemi qR gpk niki Mit up gH eikin sigit gH plni nnjRP sngptrgr pngut fi nsw(neuti nghnj strngrngpe pngut af sw(neuti nghqli ps gpri fi nsw(neuti nemi nRgHqgt@2X tnapiniuRHmiku (f sniki I dat at plni keut pf (nt pl pt (utrngrngpe pngut at s z Qit d Pnt Mingt Henrpsw(muRenikanetplni pf nuf dnemir nPneg, t ts e, ninrm garen ong BI dnid, dnutrngrngpe pngut Misng, tpni Qdi fi qdirngrmg ping te g, g, t aen(t2B) 0tpni DDE@gs, ($^{-7}$ BI dat at tuRgNnjki (nikr strn(f ngngphi an Hf r 3, cg, t gH cgupintuRMprgRngngtas pnSI

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ym eHg, tplnt, snM stuf pc stHupedMpmgtnatS z QlutfnssRpngt gHt Mr3fi eHpe vurpneomt pD. (t BiBBr srRs puHtReg, tplntgnepdAnRpdt) ps gprtpe gurptiNc, BDpNc, BxDdtplntQS $_{\rm D,\,t}$ u (f snutfi qdtplntdq, dnupt rngpgptnatS z Qlufi nentplnum(nentcg9RngrnHMt cet(unutaen(tplnt xnRpdt) (neor gtrn upt gHfi dngtplnemfi ut gtd(fnep gprngpedMpmgtnat plntum Af e vt nenust gHfi GgHt cgtplntz nepdt) ps gprt1 rm gBI dnumt u (f snutf enuppHtrngprgptnatQhbQQt gHQOBQtrngpe upti(nuptu (f snut



bi3. F. Rh5 (fsnutnatfnswnpdwangnti) dufnswf enfwangntiKott gHtfnswupwengntiEctuRufnrpturenngrg, uB) MPnhtplintn5perprihlrden(pn, e(tæn(tpdntfnsw(netSxtur, gstanRgHcgtpdntSxtufnrpetnatpdntu(fsnu4 gHMnsnfihpdntpngppPntcHngpkrpngtMvjngHcr3t(utHnanrptijSGotgswucBQE)t, ngnepnHMvt-SQQentunaffienteen(tx) xt QuppRpntQrBiE ewhz Elt: gquiHxppnu0B

m H 1 NnRgH f nsw(neut rngrngpe prngut nat f nswnpdwengmt iQhdt f nswf enf wengmt iQdt f nswupwengmt iQkat gH f nswanf engmt iQdt æn(tSz QTut gt æMhegmt f eprRs pnt (prnetegtpilm) ps gprul rm gBt

envernmatS 7 OT					
rsnunet pnt Rupe	wI n3·s ^{−F}	w n3·s ^{-F}	ww n3·s ^{-F}	wM n3·s ^{−F}	ns elin3 tntion
suntan Ro Htroot	D6BIDt	< S Tl Gt	< S Tl Gt	< S Tl GQ	xxtDt
) m ann Bildmr	0DB2@t	< S T1 Gt	< S Tl Gt	< S Tl Gt	xxt6t
) ps gpu ur unu	< S T1 Gt	< S T1 Gt	DB17t	< S Tl Gt	xxt7t
fi dnent rngrngp	< S T1 Gt	< S T1 Gt	< S T1] t	DB@flt	xxt@
dç, dtrngrnype	< S Tl Gt	< S T1 Gt	< S Tl Gt	.H02t	xxt0t
uRMpenfor st, we	< S T1 Gt	< S T1 Gt	< S T1 Gt	< S Tl Gt	xxt\$t
S z OTumveord(< S T1] t	< S T1 Gt	< S Tl Gt	< S Tl Gt	xxt2t
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i grintegt pi	< S Tl Gt	< S Tl Gt	< S Tl Gt	< S Tl Gt	xxtflt
pne rpingut Mipr	< S Tl Gt	< S T1 Gt	< S Tl Gt	< S Tl Gt	xxtD. t
(nuf dnær tHmf	< S T1 Gt	< S T1 Gt	< S Tl Gt	< S Tl Gt	xxtDDt
finentmup:(pml	< S Tl Gt	< S T1 Gt	< S Tl Gt	< S Tl Gt	xxtD6t
	< S Tl Gt	< S T1 Gt	< S Tl Gt	< S Tl Gt	xxtD7t
$F_{DD} = C_{MNPLs}.V$	D7BD\$t	< S Tl Gt	< S Tl Gt	< S Tl Gt	xxtD@
	< S Tl Gt	. B Dt	S T1] t	< S Tl Gt	xxtD0t
fidment Esz Ort	< S Tl Gt	< S Tl Gt	< S Tl Gt	< S Tl Gt	xxtD\$t
io (⁻⁷ oh oH	< S Tl Gt	< S TI Gt	< S T1] t	< S Tl Gt	xxtD2t
i U ^D OPI oDr	< S 11 Gt	< S TI Gt	< S 11 Gt	7HoDt	xxtDJ t
	< S '11 Gt	< S TI Gt	< S fl Gt	DB 7t	xxtDtlt
dwHenr eMngutt	2B/0t	< 5 11 Gt	< 8 11 Gt	< 8 11 Gt	xxtb. t

< S Tl G4u; g sullinpurprihlRgHnetpdnt(npdnHtsc(qtnatHinpurprigtanetQhtQQttQxt gHtQQt

I dmS z Qlutrngringpe progutegtidintum Afe vit menunsutfi mentimp(pri-Hi p 3g, tegint rrnRgitpilntfnsw(metrngringpe progut gHtpilntunHR(trngA ringpe progut anRgHtgtpilnt p(nuf dimeetQS $_{\rm L}$ tu (f smit rrneHg, tpitpilnt ff en rdtf enungpriHnsunfi diment; \$UBy miRspit entudhfi gtegtI MaixOt gH N; BixGp enif mrpPnswltgtpilntuRf sm(ngp evit unrpmgBI dntf enungrint at S z Qlutegtpilntum tufe wifi utngeerdniHtgtrin (f ecungtpilpilntinPne sst pA (in dimertQS $_{\rm D}$, tu (f smit rineHg), tip film Qxit

u (fsg, tup pmgtDOtprt@fff(tiQ0tu (fsg, tup pmgt6dbl dntuf pc st f pmgtnatS z QTutqtum Af e vt nenunstiN; Bx6otatnMPonRusvtu(os etput pd pnatpn st nenunstigHrnguapngpfiqdtpdnt ff enghtupeng, tg9Rngrnt natum tuf e wtane(pmgt ut t(mrd ga(tHzAvg, tplnt pí nuf dmertnrReA engrntnatS z QTuta PnRemHt ptdc, dtfi gHuf mHibil dmu (fsmtrnssmrpHt rsnumet pn Rupe st Engmit dc, ds; dt plnt rngpedNgngtnat plnt rngprgng st ce9nfi urn(og, tæn(txnRpd) (mer gtrn upiEVc, dtsnPnstnatS z QTutin nen sunt angHt gt plntu (fsmt g9RngrnHt Mvt cet (umt æn(t plnt gnepdt) ps gprH l dnuttemNspt , emut fi qdt plnutt f RvkauhHt Mvt T PngHmeAT fi lt fi dnentrngrngpe pmgut natS Qut gt plnturn fi pmet uRea rntnrm gt udnfi mH dc, dtrngrngpe pmgut natS ng, tis uprut gt plntrngpe st gnepdt) ps gprt S QTutingerd(ngrgtpdnum Af e vt nenunst gHpdn(exgnt pí nu fdnemB

l grmtogt pånt p(nuf dnænit gnpånet 3mvt (m²d gau t Hæðeg, t pånt egA præ rpngut Mpfi mægt påntsnfi næt p(nuf dnænt gHufkea rntnrm gtat pånt pA (nuf dnært Hånt nupmg B) p(nuf dnært Hævt Hån nupmg t 9R5mtig, $(-^{-6}.H^{-D}at$ finæntnupt, milt rnæft 2004)

fidment $E_{S \ z \ Qt}$ ent pdmt rngr ng pe pengt nat $S \ z \ QT$ ut ogt pdmt menunst f d unt ig, (⁻⁷olt gHtL_{Gt} at pdmt Hmf nurpongt Pmsnr qpwt nat pdmt fs uport f eporsmut i(·H^{-D}oBL sRmutnatL_{Gt}d PmtMnngtHmpne(cgmHtanetfnswrwrsort en(port dwHenr eMngutfiqudt(nsmrRs etfing, dputggtpdmte g, miD\$\$-7..tG spnguBiNnet pdmuntrdm(or supdminrm gortL_{Gt} ent enRgHt. EDr($\cdot u^{-D}$ diffidosnipdmunt ent nat. By r(u/Dtrsnuntprtpdntrn up; D716DUB) utpdntfs upprutHnpne(cgnHitdment d Pnt t(nsmrRs etfing, dpts e, metpd gtpd ptnatQ) Vultifimt uuR(mHt tLGtnat . By r (·u^{_D}btfiderdteutegtpdmtRffmeteg, mtnatpd_ptnatum(ePnspesmtne, gert f nssRp gpuBI datat tsnfi neAngHtL_{Gt}P sRnht gHtpdRut tsnfi neAngHtmup(pnt nat Hmfnungengt 9R5mut nat SzQTult ut se, met fepersmut ficsst d Pmt se, met Hnf nuppingte prultimPingtMixt gtneHinetnat(, gqrRHinBiNgGtanetpdintuR(tnatpdint pe, mpmHtf nsw(meute g, mHtaen(t@pntD7b0..g, (⁻⁶.H^{-D}BtKwtpdmtmup(A $pmgtnat N_{GGt} \ gHt E_{S \ z \ TQt} cgtpdmtum \ Af \ e \ wt \ menunst \ gHt cgtpdmt(\ ecgmt \ pA$ (nuf dment gHtrn(f egg, tpdmuntemuRsput fi cpdt pdntf enPanRut fi ne3ult pdnt n5rd g, mtnatS z QTut Mnpfinnngtum fi pnætuRea rmt gHtpdmt(ecgmt p(nA uf dmentegtpdmt) ps gpertl rm gtd utMnngtqR gpckmHtanetpdmtkeuptpc(mBt

) t(RspcP ec put g swucuti utf meane(mHMwQE) bliderdtfi ut, mene puHat m55 fs egeg, tpdmt 22 tX t nat pdmt pnp st P ec grmt aen (tQS $_{\rm D,t}$ u (f smut Mwt \$t

R3ny tit ddranuat a8mm

qR gpcp pcPntur snHtP ec Manuti Nc, Br@BQctpdntr untnatpdntxrnenutf snHt t qR scp ppPntP ec Msnt ens pnHt pnt pdnt cg9Rngrnt nat cet (unut i) ps gport lrm gbt)æcr gtEn upAx de br gHtxnRpdt) (næcr gtEn upotfi ut HHmHBs Engend-lut rrneHog, tpnt) ps gportl rm gtiMsRnøt gHixnRpdt) (næor gtrn upt i, ennyot entgaptrsRuppenHtogtpfintunf e puHt, enRf ut pt tragk HugerntsnPastnat flOtX htfidcsntu (fsmutpd pt entcg9RngrmHtMvt)æcr gtrn upAx d e tiwnsA snfi ot enlutnæmr pePnswltr sRupment-It fi opdtenn fmr ptpnt Mnpdtnpdnet, en Rf uBI dout (mgutpd ptpdntfdwuornrdm(or strn(fnuopengtnatpdnt)ps gportlrmgt gHt pdntxnRpdt) (near gtrn uptQS _{D t}atua (s etfi apdtenuf mrptpntpdntrn (f nucA pengtnatpdmt) ær gtEn upAx d e tQS D btanetfiderdtpdeuts ppnæteutmuf mrc ssvt eordtogt(ogne st gHtpe rminsmi ngputMmr Ruminatdo, dmetP sRmitnatx d e gt HRuphI dmtux(cs ecpwtMmpfinngtu (fsmutcg9RngrnHtMwt cet(uumutnatpdmt) ps gportl rm gt gHtpdmtxnRpdt) (near gtrn uptaut uunrc pmHtfigadtpdmtogA 9 Rngr minatum Auf e wit menunst gHis z OTutf nssRmng Bil doutf en PoHmutaRendmet mPdHngrmtpd ptun(mtSzQTutanRgHtggtQS_{Dt}u(fsmut(wtrn(mtæn(t (pometn5rd g, mutaen(turn fi pnetuRea rnt gHundnt(ecgnt p(nuf dmentut ut Hour Round-It MhPmB

. 3g3 v Witt durin Diturf of Exe rdifs at Durd Dat lo $A_{p:o}L$ -qu6/PG7 at loCRA o

xn(ms e, mf eprsnufi mentHmprpHkgtplmkspneußi dmmf eprsnufi ment (gswikMenutpl pfi mentHempswt g swunHiMutpAN Gyt g swunB) s(mpi0.t X tratplmu (f snuff enneppHtrnsRsnunkMenut gHplatfi un5f mpiHugrmt plat(pnec statngntnaphmt(mpt MgH gptMnf nsv(neu gHtatRunHtgt (gwtgHkupc st ff sor pngutiRrdt utrn(f nupmitgnppg, hRd Inspnewi rn pg, uiff r3, qg, h gHf fnet, 6@Bi plmekiMenutpi pfi mentanRgHugtplm u (f snufi ment inswmpmedh uRrdt ut gtu (f sg, tup pngt0h gHtuscr pnt rn pg, kkMenutiRrdt utz nef sn5t\$.@ff mentHmprpHtgtu (f sg, tup pngfBB Qt HHpngBg Pke skMenufi ment snufHmprpHtgtu (f sg, tup pngtBB Qt HHpngBt gRe skMenufi ment snufHmprkmiHanetn5 (f snitfi nnstkMenut fi mentanRgHtgtpfintu (f smaten(tu (f sg, tup pngtiXxdJt gHtD BQt HHpmpthkMenužæ, (ngutnatCht gHCOCfi ment snufHmprkmHB

N; Bx7(narphinxRffsq(ngp evtQane(pngturpng)tudhi utun(mtn5A (fsmtnaµAN Q)tuf mrpe matpdnt g swinHu (fsmtBxhSt g swintudhi mH pd punu spinatrRMrtud fmfi ufenfngHae gptgplnhu (fsmtB utn5fmrmH N; B x@B S nemPnelt qg tun(mt u (fsmtI pd tenngrin nat,, en, put iN; Bx@ct gHug, smtRf meX (rengtf epr smtI N; Bx@kti nemtnMmePnHB Nietn5 (fsmtIxtD/Drng qgmH,, en, putnat MRRDDB6 µ(tiN; Bx@ dt rn(fnuHhar eMsgtf e(evt epr smt engBHD@ g(tiN; Bx@dtpd ras natrn(MRupngtf enrmumBx (fsmtaen(tH wtig9RngrmHM et(umt aen(tpdm)) (neor gtrn uptudhi nHmg, gmtn5d Rupr eMgBQtpdaten onglt dp th sintMmgtenf nepHHfenRusv* ngF smFA wtmt sh; GhtQthimHt p 3q; updaturngHt ufmfig, gtrem untgpfinturgpterspie pRemfenPHHt

rn(MkupngtogtxnRpdt) (nær t; OLBVnfinPneligtogtngpne/penf a stenn angult pintog9RngrninatMm(utkenutæn(txnRpdt) (nær t gHz) æar tid utMingt enf nepnHz; JlflUB ym eHg, t pint rn(Mkupngt fenrmunnt pi pirnRsHz Mnt g9RngrnHzMupintenum erdtPmunstzRggnsm5G RupzR(mitogtpiatr unhpint u (fsmtfinentrnssnrpHzngswtfidngtpintMn pffi utcgt(npmglt gHzplint æt d HgnptMingtogtrngp rpfi qdtpintudd fli utpintfi gHtfi utrn(g, tæn(tpint udd 'utMnfil utHinrexMhLgtpintm(npinHzB

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I Mant6tudnfi utpdntfs uport HHxpcPmutpd ptfi mentpngp pcPnswtcHngpck nHB Qs uport HHopPmutfinentfemungptogtDfltmatpdmt6.tpe gunrputupRHonHBil gswingmt natpdntu (fsmutæmminatne, gortfnsw(meulifi ut suntæmminatfs uport HHopePmut ixxt2oBI dnt(nuptHnpnrpnHt HHppPmutfinentfs uprameutuRrdt utfdpd s pnult fight(ngn/Rpvstfdpd s pntiS KQot/Mrg, tngntnatpdnt(nuptaentyRngpswtanRgHt iJ@X tnatpdntu (fsmuðbl pdnetrn(fnRgHutanRgHt ptdc, dtænqRngrwtfinent rmpvsApecAgAVRpvstrope pmtcgtflOtX tnatpdmtu (fsmilt gHtxRMneort gHt) Fns ort rd-lut cgtfl. tX tnatpdmtu (fsmulit Qut cut gnpmfinepdwtpd pt rmpwst pec/gAVRpwst rope protiEquen 9 n 5 t) A@ tout tfs upprounce tan et(gwtpwf mutnatfnswmupnet gHt rs uuck mi-lit ut tgng Afmeucup ngpt fs uper cunnebt fider dt aut Pnnewit (Rrdt Rumi-lit gnfi H wutogtMnM unHifs uperultuRrdt utf nsws rpert rdHiOT) oB) gnpdmetnat pdnt(nuptrn((ngtrn(fnRgHutfi utuRMneart rcHlt gHteputHnecP pmltpdmint Mong, tgne(sswtRund-lt utfs upgranneultsRMear gpultdwHe Rsart9RdHultrnuA (nparultir gHsmiltingrBQ:t HH:pongltuRMneart rdHatRunH:cgtpdntuwgpdmuatnat f nsw (d-Hnutsm Hg, tpntgwsngt gHt s3wHtemugut gHt ut gtogpne(nHc pntanet en(porult gpaunf parult gHtf og pog, t(pnec sulltxo(os eswlt Fns ort rol-lt



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- ;6U) ssnghtxBit) ssnghtGBitS nuditj BitTmtynR5ht* BitQdnngc5ht.By Bitxng3nht-BhBit6.6.B h5 (og pongtnatpdntnrm gt ut tunRerntanet p(nuf dnæcrt(orenfs uprußQT1 xtl z htD0t iOdtm 6762@\$Bdmf u/11Hndbe, 1D, B072D1vnReg, sB ngnB 6762@\$B
- ;7U Koerdhis BitE ewhy BitDiff\$Bihsn(ngp str eMngAM unHt (npdnHtanet(ngqnecg, t nrrRf ping stn5f nuRemutpintf epirRs pritHimunstin5d RupB) menunstxrdI mrdgnst60ti7dit 66D-6@DB
- ;@UE (fnutH tynrd btNB BatS epgnFbtxB BatE (fnubtL BQBatH tynrd bt*B BatHint) gHe Hnht-BKBat6. 6DBBS crents uportfnssRpangtcgtxnRpdnægt) ps gport(ecgmtfi pnæu/t enPanfi tnær ReengytpengHiltun Rermilit gHif neuf nr pPmilitxrati np sthg Paengt2J6hD@\$0@DB mf u/11Hndhe, 1D, BD, D\$1yBr cmmz PB5, 6DBD@\$
- ; OU E eHnunhtNBGBhtG Rgmeht) ETETBhtS epoguhtEEEBht6. D\$Bt) treeper st gHtrn(f e ptPmt ffe au stratf nswrwrsart en (martdwHenr eMngutagtumHa (manut gHtuRuf mgHmHt eparRs pnt(pnec staen(t ts e, mtxnRpdt) (near gtuRMpenf ar stmupR ewBihgPoengt OnssRpt6D(dt6Dfl-66flBdpf u/11Hndhe, 1D, B), D\$1vBrgPf nsl6, D\$B (d8 DDB
- ;\$U E u ult* BhS epgnFAL ens h) Bhynur smult-ETBbLos AEnup hS BhG rduh-Bh-o(ngnFhKBh 6. 6. Bhgeerd(ngptnatf ne9Rnen s3wstuRMp grmutegtpdntum AiRea rnt(crens wnet gHt um Auf e wt menunsutogtpdmtxnRpdmegtl rm gBhgPoengtQnssRpt6\$2Btdppf u/11HncBne, 1t D. HD. D\$1vBngPf nsH6, 6, HDD00D6B
- ; 2U E upenA (ngmFtt-Bt* ngF snFA* w trKBtQcF eentrS BtE u strQBtQcF eenA/sP enFttEBt G rdult-Bat6. D\$Bal e, gnf dnuf d pntmupnet9 (ntenp eH gput gHtfs upor & Greut og tpdmt , snM stnrm gort p(nuf dmenBihgPœngtxrctI mrdgnst0. ti 67dtD6J7D-D6J7flBidpf u/11t Bhe, 1D. BD. 6D1 ruBmupB\$M @ @@B
- ; JU Ednit/Bixm, skynEngd (mehS Bhídg, hTBhGg, hEBhíRd+n(hQBhGnMer3dh) Bh xmHs rmSh) B BhEnnit/Bh/Shi/Bhi waneh-Bh6, 66BEsnRHf enrmung, tgHfinmSang, t nog, t ænrptMm(uttMegeg, t menunstf enf meponutnPmetpdmtunRpdAm upmegt) ps gporB En((Roth endthoPrenot7tiDottD_flB
- ; flU EdnhtVBhxm, sAynFngd (mehtS Bhí d g, hTBhG g, hEBhí RdHní hQBhxmHs rnSt000h) Br Bhí d g, ht[BhNswgghEBh6. 66Bxm ung stP ec pengutegtkentrngHepengut ente(fnep gpt HecPnetiptplintprogHnat menunstnf pr stf enf mepuntnPnetplintunRplAn upnegt) ps gprB) pf nutEdn(tQdwtGarRut66tiD7dhJ2\$2-J2J0B
- ; D. U Edngh* BaiNng, ht] Bat% g, ht-Bat6. 6. BS cgc/enPonfi tnat(or enf s upprutog tpd nt p(nuf dment gHtpdnæteaßutpntdR(gullsrrdI np sthgPængt2.7htD700. @hdpf u/11Hndhe, 1t D. BD. D\$1vBr commy PH5, DflBD700, @B
- ; DDU G g, htEBatxm; sAynFngd (nehtS BatEdnhtVBatid g, htTBatNne(ngpthtQBatI wsneht-Bat GnMer3dt) BatQRet-RuhtxBat%ng, htQBtBatz ngmuh) Batxni-ksrn8tQath) BatEnnhtVBat ynHn(gght-BatíRd-In(htQBatVnfinsshtxBatV wfinnHat-Bat6.66BKcn(uutMRegog, t gHt ecgnt menunstf enrmurg, tnPmetpdmtunRpdm upt) ps gportl rm g/t tl hS turg, sn#f eporsmt g swuB) gf nutEdn(tQdwu666tiD@hf7Jfl-fl@6Bdpf u/1Hndhe, 1D BDfl@ rf A6A 17Jfl&.66B
- ; D6U HntV ght% BQBhtx grdnFA.dH sht) BhtE g suhtS BhtQ epwhtz BcBcBht6. DflBiNsn prg, t (or enfs uprut gHt ,, em. pntane(pngtogtplnt%mupnegtS nHppnee gmgtxmBS et QnssRptKRsstD@ h067-070Bdpf u/1Hndhe, 1D. BJ. D\$1vij ef ns/Rsib. DflB DB 07B ; D7U GnstLngphtxBtG rdult-Bt6. . 2B) p(nuf dnecrtnrrReengrnt gHtHnf nuppngtnat
- f nswrwrsart en(partdwHenr eMngutogtpdmtgnepdm uppenfar st gHuRMpenfar st) ps gpart l rm gBhgPœngtxrctI mrdgnst@DtiD\$dt0\$. J=0\$D7Bdpf u/11Hndbe, 1D. BD. 6DI ; D@U Gog, lt-BatxRghtEBatV nintEBati dng, htTBatG dtGBatTdntNBat6. 66Bt) p(nuf dneort(crenfs uporut
- gtpdintz nepdfi mupnegtQ rdkrtl rm g/tHupedMRpanglitunRernht gHtHnfi nuppngBixrdI np st hgPængtJ6flhD0@72Bdpf u/1Hndhe, 1D. BD. D\$1vBr qnpngPbb. 66B00@72B

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- xpndsh) Bat6. 6. B) p(nuf dneor tpe guf neptout t(vnetf pdfi wtnat(crenf s upor utpnt ent number onguliz ntEn((RgtDDtiDdt77JDBtdmf u/11Hndhe, 1D, B), 7J1u@D@\$2A 6, A D26. DATB ; D2U Nec ult-BQB' BTBlz udlty Bt6. DflBS crenfs upru/tkgHcg, t trngunguRutngtpdmtHnkgqpangB
- S etOnsRrtKRsstD7.1hD@_D@Bdrrf u/1Hpdbe 1D BD D\$1v8 ef ns/R846 D1BDB 660 ;DJU * , nlt-lbt* s, gdtNbtS milti lbtI dn(funglty EBt6. D\$BS crenfs uprutogtum fi pne/t emrn((mgH pongutæn(tpdmt(ecgmupe pm, wtæ (mfi ne3tHcemrpdPmtc(f smf mgp pongt
- fenrmuRNengrtS etvret7Bdmfu/1Hnche 1D E7 Iflig all DSB 6DfB ; DflU * c, Rspti-BithstV Hedi V Bitz, Rwnght KBit * e utstik Bity nfi ngr Fw8ht TBit Rang 3vdiz Bit Nng, htxBat% anugnehtS Bat6. 6DBz gnfs upor ut entgnepdnet(arenfs upor utgnetng, agmment-ht g gnf eprsmiliz ptz gnpmrdgnstD\$ti0dt0. D-0. 2Bdpf u/11Hndhe, 1D. B. 7J1
- ODOSOA 6DA LISAOB ;6. U * c, Rspht-BatV ssmitt) phtK RHec(ngphtS BatQ ur shtQBfüllat* RasenhtNBatQddt1 BWTBathst
- V HediVBt*e ushtKhtymvg RHixht6. DJBEReengtnf geng/fid ptat tg gnfs uprB hgPengtQnssRpt670hD. 7. -D. 7@hdprf u/11Hndhe, 1D. D. 1%hgPf nst6. DJB DB 6@h ;6DU * ngF'snFA* w ltKBatG rdult-Baty nur smult-ETBatE M ssmenlat* Bat- (/ mgnFatKBat6. D@Ba One9Rnen s3ws miHuRMip grmutogtodini, snM strenf or st gHuRMenf or stuRea rinterin guilt
- hgPœngtxrdI mrdgnst@ti66dtD7. 2\$-D7. J@tdpf u/11Hndhe, 1D. ED. 6D1mt0. 7@l. FB ;66U * ngF snFA* w trKBtNneg 'gHnFAQcgnuttS BEEBtS ne smitTBtS nv gnssnitTBt) M HithBt Or hKBaGR emitEB Bt-d nemFltKBaG rdult-Ba6, DSBVc dt n nuf dnem-nrm gt
- Frd g, mtnatum(cPns posmt en(portdwHenr eMnguBtz pt*mmurctflti\$obt@J_@@6B ;67U * ngF 'snFA' w htKibhNneg 'gHnFAQgnuhtS BEIbtS ne smihtTibtS nv gnssnhtTibt) M HithIbt Org hKBtGR emitEBS Bt- (nonEhKBtG rduit-Bt6 DSBVc dt n nuf dnemårm ot n5rd g, mtnatum(cPns psmt en(prtdwHenr eMnguBz pt*mnurctflti\$oht@J-@c6B dppf u/11Hndhe, 1D. BD. 7J1g, mn62D@B
- :6@U i (nshxBh) schz Bh- d g, cehi Bhxd dhxBhhsA mgHwh) Bh6, JBOd e(rmRmr st ur, gck r grmtnatrmssRsnumt tenPcmfi Bh5f emutQnsv(tTmpt6ti DDcht20J-22J Bdpf u/11HncB e, 1D. B7D@@In5femulfnsw(smpH6..JBil. B
- ;60U T filij BB6. U2BQs uprugtpilm(egningPeng(ngpB) ggRtynPtS etxrdfhiDdi 6. 0-66fBd.fjr u/11hndhe, ID BD/G1 ggRenPA egnA D JDSA S.@fB ;6\$U Thfi ath/BBT/ffi ath/Bbxrdfi eFbxBaB6. @munsf ehFkrpmg/t (mrd ga(ulj mjhHhl(m uRen(ngpL gHk (nHmB)) (nor gt*mf dvur st: gngB
- ;62U Tsner htS Bht) sP enFAS RgnFhtGBht) M snuhtS BhtynHe, RnFAS nF FitxBhtx gpnuhtTB/BS BTB
- S BatTmáght LBS Bate (f cssnhi-B) Bats epgmFA* n(mFhteBat) M HathBatN eenhats Bat6. 6. Ba Scients unrutests memory and strong s ngPœng(ngpt gHtdR(gtdm spdBI engHuthgPœngt) g stEdn(t62Bdppf u/11Hndhe, 1t D. BD. D\$1vBmrB5. 6. Bm. . fl. Bt
- :6JU Tsner htS BitLm AV meene ht) BitxrdeeczFdi* Bitx PP hti Bit) M HithBitN eenittS Bit6, 6DB xrennnycg, tnatuRuf mrpniHt(crentig gnotfs upprutogtpdmthMentGnsp tiS mHqpnee gm gt xm oB-tV F eHtS met@ @tD6@ 66Btdmf u/11Hndhe, 1D. BD. D\$1vB vd F(1366, 6, BD6@ 66B
- ; 6 flU Tng, ht EBS Batz ur enss htS B) BatL sMne, htQB) Bat6. D7 BE eMngtMs r3tPuBMs r3tr eMngt gHnplnet ceMaegmt(pnec surngp cgcg, tmsm(ngp str eMg,/Qdwur st gHrdn(a st HuppgrpmguBhgPengtQnsRptDJDh62D-6J\$Bdpf u/1Hndble, 1D, B), D\$1\B . D7B \$B . flB
- ;7. U S e3crlt) Bitz an(gHitEBitKed-linght-B/BitS FnRgcA neggnetiz Bit* neggnetiz-EEBit hec3unghS BitKnfinghtS Bit6. DJBGnRMantpenRMantgaptIntxnRpdtQ rdxrtuRMpenf ar st, venat Qrem unHtfs upprtcg, muppingtMitkudtcgtpdntnrm gort rrR(Rs pingtFngmBtS etQnssRpt KRsstD7\$h0@_0\$@kdmfu/11Hndbe 1D B) D\$1vK ef nsM846 DIB fB 7DB
- ;7DU] Rmenshi [Bit) s upRmvhi) Bity nHec, RmFlixBitQs g hiNBity RoFlixEBy BitEnpulitz BitS uu , Rmit* Bit QRc, til Bat6. . DBQS D. t gHtQS 6Bt0tunRernt ff nepng(ngptogtpdmtK ernsng t S mpenfnsep gt em htE p sngc htxf gtidppfu/11Hndbhe, 10B) p(nuthgPængt70ti7\$dt \$@ 2-\$@DflBdppf u/11Hndbe, 1D. BD. D\$1xD706/67D. i. Da . 7\$D/6B
- ;76U ymvg RHitxBt) wg eHit) Bit* e uushtKBt* c, Rspit-Bit6. 66Biz gnfs uppru/tNen(t(nHinst muc situations of sta mREReal for Ensend-Harmen entyret02hD D061R
- ;77U ynrd(gtEdnsum hS BrtVnnssneght Ba6. 6. Bi dnt, snM stnHwumwtnatf s uprtf nssRpmgB xrangrnt7\$Jti\$@l\$dtDDJ@DDJ0Bdpfu/11Hndhe, 1D. BD6\$1urangrnBM @@JB ;7@U x grd ult-BatKmeenv sMFbtz BatE M ssmenla* BatG rdult-BatN eenhatS BatK ermanittGBat6. D6Bat l rrReengrminat menunsAMnRgHitaRssnengmutogtpdmt(niHopmee gm gtum t p(nuf dmenik
- hgPængtxrdI mrdgnst@sti7ohD770-D7@Bdpf u/11Hndhe, 1D. BD. 6D1m6. . 20J(B :70 Uxrdcsamelt-BS BbtS msh TBh BbtOe ndmelti Bi Bbt) (enlty Bh Bbt*e uur ght, By Bbt6, DJ Btxm t uf e wt menuns/tfidment(ecgntMinsn, wt(mmput p(nuf dmecrtrdm(aupewB) ExtEngptxrct@
- iD6dtD\$D2-D\$67Btdpf u/11Hndhe, 1D. BD. 6D1 rurngpurd\$M.\$2@ :7\$11 xrdeexEdt* BNBtTsner bs Bixnerity BtS nw enbthBiK ernstitgBt) M HithBiN eerits Bt 6. DflBI e rmt g svucutnatf nswupvengent(orenf s upprutogtg pRe stfi pneuBEdm(nuf dment 67\$Bdppf u/11Hndhe, 1D. B. D\$1vBrdm(nuf dmembo. DflB 2B 06B
- ;72U xrdw(gu3dth HTBt-mnght-Bht* RsHnhty BtNnggnehtj Bty RahtS Btxcg, nehtV HQBt VnssngHnelt-Bt6. D@BOHngprawcg, tu(sst(nsnrRsnutPc tdc, dteminsRpingt(u uf mrpen(mpewtrn((Rgor pog, trngk HngrmBing Poeng txrotImrdgnst@ti@bb6.fl2-6.flJBi
- mf u/11Hndhe, 1D, BD, 6D1mt0, , 6D, 0B ;7JU xd g3hTHS BatVnfi nsshxBatEs e3mbi) BGBatNemap, hxBatKen38dnPu3c3dhtLBatj f RuppghtLBat S rz R, dyngheBhe (fnuhi Bu%nnHiyBho. DoBi e, got(print gHgngAmae rpnewt nenunstnPnetplinten(npntxnRpdm upQ rdkr/mrm got gHrn(MuungtunRermiB) p(nut Edm(tQdwttD6tiD0t002-02\$Btdpf u/11HncBne, 1D. BDDfl@ rf AD6A002/6. D6B
- ;7flU I netV ssnht) Bat-m ggm RhtTBatS epc, g rhtS Bat- eHinhah BatQniHengnhaKBatKe rdhtTBat * c, Rstt-Bt6. D2Bz gnfs uprtogtpdntz nepdt) ps gprtxRMpenf ar st* venBthgPaengtxrdt I mrdgnst0Dti67dtD7\$Jfl-D7\$fl2Btdpf u/11Hndbe, 1D. B). 6D1 ruBupP2M 7\$\$2B
- ;@UIe cgcrbtS BatNsnemati-B5 BatQg3 ubtObtQnHenppdtS BTBatTn(MeHaNBatKnReHight*Ba neu&wit* BitKnuithBity RHardhtü BitLeHati) Bit6.6.B) @Miegnt(areafs uportf eparsmut
- Homoneron Hitzetondonteon(nont(eczent of nufdonen Bi-tEn((Reth ; @DU I e cgcrhtS BhtNsnemult-BS BhtQcg3 ultObhQnHenppdtS BTBhtTn(MeHltNBhtKnReHcght*Bht * neu3wht* BhtKnuuhthBhty RHcrdhtüBhtLeHdt) Bht6.6.Bt) ceMnegnt(crenfsuportfeporsmut

R3w tit dammat Amarian

HnpmrpniHtgstpdnten(npnt(eggnt p(nuf dnænikEn((Rgth epdthgPængtDtiDdtD-flkt dmf u/11Hndhe, 1D, BD, 7J1u76@2A 6, A . . \$DAvB ; (6U : g, nehtOlatx 3htEBS Batx spnehtS Batí cm, nehtQBatQ p g.nghtS BatKvnegndins(htl Bat6. 6. Ba

Q9Rngrmtnalle, gort)rdHungtpdmtxRearmEn(fnuppngtnatxmtxfewt)menunsB-tQdwut Edm(t)tD6@i6dt@66-@6fBdgfuy11Hndbe,1D,B).6D1ruBfrBlMfl2D,B

: OTU L.m. AV meene b) BitTsner bS BitKneenssAGc Fbf Bity milling Hn AV uunsmed e(b) Bit) M HithBitLoss gRnP ItEBS BitN eenittS Bit6. 66BQnsw(neutnat(crentig gnotfs upprtogt In Runding-Itn fifi most nated and Mermona t (moon finger at em B% most 66 hDDIS@08 : (2021) I.m. AV meene b) BarTsner btS Batx PP bti BarTmingbtLBS Bat) M HtVns. HnbthBatN eenbarS Bat 6. 6DBxrennngog, t gHigR gpdxr pongtnat(crentig gnotfs upprut gHifs upprt HHppPmutogt pdmtum fi pnetnatS etS ngnetT, nngBNengptS etxrctJth\$fl2@@ddpf u/11Hndbe, 10 D B77.1fla(euB6 6DB6fl2@6@B

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4.2.3 Publication n°3 (Supplementary material)

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

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

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

Acronym
EP
EC
PAM
PA
PBD
PE
PET
PI
PP
PMDS
PS

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

MNPLs.
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ı parame
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Table S3 \

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

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

Chapter 4

a/ m ³										Samples										
- 5/	R1934	R1933	R1930	R1923	R1929	R1901	R1911	R1902	R1903	R1904	R1905	R1906	R1907	R1908	R1910	R1909	R1912	R1928	R1927	R1926
OC OC	1.16	0.56	143	0.48	1.00	1.41	0.68	0.72	0.44	1.18	0.53	27.6	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
lineral	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
no)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
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
arine	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
termined	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
g/ m ²	0.00	200	2.1	0 60	0.0	010	0.00	000	10.01	10.01	1001	0.00	110	0.07	0.00	10.01	10.01	010	0.00	0.04
Li Be	0.03	70.0	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
ве	< 0.01	20.0 < 0.01	31.14	< 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	74.01	48.50	< 0.01
n y	0.07	10.0 2	1.17	10.0 2	10.05	0.11	0.00	0.06	10.02	0.01	< 0.01	0.02	0.03	0.03	0.07	0.04	< 0.01	0.02	0.03	0.03
3C	20.0	10.0 >	174.22	62.63	7.37	28.05	17.06	10.06	1 07	10.0	1 31	24.48	236	1.54	1 56	1.65	1 85	20.0	2.53	2 47
	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
C.	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
Υ	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
0M0	2.94	10.0 <	10.0	100	17.0	4.02	90.9 00.0	76.0	10.0 <	1002	0.10	10.0 <	14.0	100	14.0	2.04	10.4	10.07	10.07	40.7
5	010	10.02	0.12	10.0	10.04	0.04	00.0	10.0	10.02	10.0 /	10.0 /	10.07	10.07	10.0	10.0	010	10.0 2	10.0 ~	10.0 2	10.0 2
Sh	010	010	0.12	010	0.08	0.15	0.17	170	010	0.10	0.10	0.05	0.0	0.11	0.10	0110	0.00	500	0.00	0.03
č	< 0.01	< 0.01	010	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
PN	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

0.02	< 0.01	0.03	0.01	0.04	0.01	0.01	< 0.01	0.01	< 0.01	0.12	< 0.01	0.03	< 0.01	0.06	< 0.01	0.04	0.04
< 0.01	< 0.01	0.02	< 0.01	0.02	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.18	< 0.01	0.02	< 0.01	0.11	< 0.01	0.03	< 0.01
0.02	< 0.01	0.03	< 0.01	0.04	0.01	0.02	< 0.01	0.01	< 0.01	0.17	< 0.01	0.02	< 0.01	0.03	< 0.01	0.03	0.06
0.06	< 0.01	0.08	0.02	0.14	0.02	0.05	0.01	0.02	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.21	< 0.01	0.06	0.24
0.02	< 0.01	0.03	0.01	0.03	< 0.01	0.02	< 0.01	0.01	< 0.01	0.35	< 0.01	0.01	< 0.01	0.27	< 0.01	0.06	< 0.01
0.03	< 0.01	0.05	0.01	0.07	0.01	0.03	< 0.01	0.02	< 0.01	0.26	< 0.01	0.01	< 0.01	0.20	< 0.01	0.06	0.13
0.02	< 0.01	0.03	0.01	0.01	0.01	0.01	< 0.01	0.02	< 0.01	0.40	< 0.01	0.02	< 0.01	0.15	< 0.01	0.06	0.04
0.04	< 0.01	0.04	0.01	0.06	0.01	0.04	< 0.01	0.02	< 0.01	0.41	< 0.01	< 0.01	< 0.01	0.09	< 0.01	0.07	0.10
< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.26	< 0.01	< 0.01	< 0.01	0.12	< 0.01	< 0.01	0.00
0.04	< 0.01	0.07	0.01	0.06	0.01	0.03	0.01	0.02	< 0.01	0.14	< 0.01	< 0.01	< 0.01	0.12	< 0.01	0.08	0.14
0.02	< 0.01	0.04	0.01	0.04	< 0.01	0.02	< 0.01	0.01	< 0.01	0.20	< 0.01	< 0.01	< 0.01	0.17	< 0.01	0.06	0.07
< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.29	< 0.01	< 0.01	< 0.01	0.12	< 0.01	0.02	< 0.01
0.06	0.01	0.08	0.02	0.12	0.01	0.06	0.01	0.04	< 0.01	0.48	< 0.01	0.03	< 0.01	0.64	0.05	0.15	0.19
0.07	0.01	0.11	0.02	0.12	0.02	0.05	0.01	0.04	< 0.01	0.41	< 0.01	0.02	< 0.01	06.0	0.06	0.17	0.21
0.08	0.01	0.08	0.02	0.09	0.02	0.04	0.01	0.04	< 0.01	0.43	< 0.01	0.03	< 0.01	1.28	< 0.01	0.17	0.16
0.03	< 0.01	0.05	< 0.01	0.06	0.01	0.02	< 0.01	0.01	< 0.01	0.20	< 0.01	0.06	< 0.01	0.44	< 0.01	0.08	0.03
0.14	0.03	0.12	0.02	0.13	0.02	0.06	0.01	0.05	0.01	0.23	< 0.01	0.11	< 0.01	0.95	< 0.01	0.21	0.14
0.36	0.07	0.30	0.04	0.25	0.04	0.13	0.02	0.10	0.02	0.30	< 0.01	0.08	0.03	1.69	< 0.01	0.50	0.12
< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.09	< 0.01	0.02	< 0.01	0.04	< 0.01	< 0.01	< 0.01
0.03	< 0.01	0.02	0.01	0.08	< 0.01	0.03	< 0.01	0.01	< 0.01	0.10	< 0.01	0.02	< 0.01	0.11	< 0.01	0.03	0.11
Sm	Eu	Gd	Tb	Dy	Но	Er	Tm	Yb	Lu	Hf	Та	W	IT	Pb	Bi	Th	n

PE	РР	PS	PBD	PI
0	0	0	0	3,345
0	0	0	0	13,412
0	501	0	0	0
386	0	0	0	0
149	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	3,411
0	0	4	0	0
0	0	0	0	0
0	0	0	0	0
833	0	0	0	0
267	0	0	0	0
0	0	0	0	2,035
	PE 0 0 0 0 0 0 386 149 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	PE PP 0 0 0 0 0 501 386 0 386 0 149 0 0 <td< td=""><td>PE PP PS 0 0 0 0 0 0 0 501 0 386 0 0 149 0 0 0 0</td><td>PEPPPSPBD00000000050100386000149000833000</td></td<>	PE PP PS 0 0 0 0 0 0 0 501 0 386 0 0 149 0 0 0 0	PEPPPSPBD00000000050100386000149000833000

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











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 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.

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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 tons¹.

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 communities²) 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 μ m - 5 mm) and nanoplastics (NPLs, 1 nm - 1 μ m)³ being able to interact more efficiently with the marine biogeochemical cycles once they achieve the micro- and nanoscales⁴ 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 sizes⁷. 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 μ m 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 community¹⁸.

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Thus, some studies have already been launched assessing plastic pollution in marine surface water compartments from North¹⁹ and South²⁰ Atlantic, Pacific²¹, Artic⁶ 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 particles²⁷. 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 μ g·L⁻¹, while highest concentrations belonged to PE and PP, reaching 8 and 6 μ g·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 Matei²⁸, the natural depolymerisation of wasted PET in marine environments is prone owing to the ubiquitous presence of metallic ions (Na⁺, Mg²⁺, Ca²⁺,K⁺), carbonic salts like NaHCO₃ and KHCO₃, as well as NaCl and CaCl₂ 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 ($\approx 0.05 \text{ m} \cdot \text{s}^{-1}$) 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 ($\approx 0.3 \text{ m} \cdot \text{s}^{-1}$) 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 μ g PE·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 μ g·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 areas³⁴.

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 μ g·g⁻¹, where the highest concentration was found in S-AtOc-SS 18 for *Cheilopogon melanurus* (1 μ g PI·g⁻¹, and 26 μ g PE·g⁻¹). For filter feeders such as *Physalia physalis*, MNPLs were found from 3 to 18 μ g·g⁻¹, while in case

of Antarctic krill were between 4-21 μ g·g⁻¹. Specifically, for *Sargassum* algae captured in N-AtOc-SS 9 through a Langmuir circulation, 2 μ g 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 penguins³⁷, but usually through qualitative extrapolations based on microscopic and spectroscopic approaches.

Discussion

The plastic pollution reported in this study (1-8 μ g·L⁻¹) across the oceanic transect assessed were lower compared to our previous studies reported in the Ebro Delta³⁸ and Mar Menor lagoon ³⁹, respectively. Both Spanish Mediterranean estuarine areas presented MNPLs-polymers concentrations which reached the 7 and 9,000 μ g·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 μ m, MNPLs that may be governed by advection and diffusion processes across the marine water body; and 335-1,000 μ m, 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 distances²⁹.

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 heteroaggregation 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 interface⁴¹.

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 ($\approx 1.38 \text{ g}\cdot\text{mL}^{-1}$) than seawater ($\approx 1-1.04 \text{ g}\cdot\text{mL}^{-1}$), whereas captured jellyfish (N-AtOc-SS 7, 9 $\mu\text{g}\cdot\text{g}^{-1}$) and krill (Ant-SS 2, 21 $\mu\text{g}\cdot\text{g}^{-1}$) 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 sequestration⁴³. 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 μ g·g⁻¹, **Table 3**) contributed at 85, 54, and 66 % of their diet, respectively³⁷.

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 aerosols⁴⁴. In our previous work²⁴, we assessed the airborne MNPLs polymeric composition in PM₁₀ 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 subestimated. 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 representative 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 μ m 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 μ L 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 μ L 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 HNO₃ 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-HNO₃ 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 APCTM 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·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 μ L 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 μ m) column. The chromatographic separation was achieved using helium as mobile phase with a flow rate of 1.049 mL·min⁻¹. Starting temperature was 60 °C in isocratic conditions during 2 min, then a first gradient of 10 °C·min⁻¹ was employed reaching 180 °C. A second gradient of 5 °C·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 μ L 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 μ m x 0.25 μ m) column and using helium as mobile phase at 1.049 mL·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·L⁻¹ with $R^2 > 0.98$. For PET, an individual stock standard solution (1,000 mg·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·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:

$$MLoD = 3.3 \frac{s}{b}$$
(1)
$$MLoQ = 10 \frac{s}{b}$$
(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:

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

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

$$M \ efficiency (\%) = ME \ ionisation \ \times R \ \times \ 100 (5)$$

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 μ m) 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	РР	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]	[S MNPLs]	Analysed by
N-AtOc-SS 1	1	< LOD	< LOQ	< LOD	1	< LOD	2	LC(SEC)-HRMS
N-AtOc-SS 2	< 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	Py-GC-MS						
N-AtOc-SS 7	8	< 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	Py-GC-MS						
N-AtOc-SS 11	< LOD	< LOD	< LOQ	< LOD	< LOD	< LOD	< LOQ	LC(SEC)-HRMS
Eq-AtOc-SS 12	< LOD	Py-GC-MS						
S-AtOc-SS 13	< LOD	< LOD	< LOQ	< LOD	< LOD	< LOD	< LOQ	LC(SEC)-HRMS
S-AtOc-SS 14	< LOD	Py-GC-MS						
S-AtOc-SS 15	< LOQ	< LOD	< LOD	< LOQ	< LOD	< LOD	< LOQ	LC(SEC)-HRMS
S-AtOc-SS 16	< 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	< LOQ	LC(SEC)-HRMS				
S-AtOc-SS 20	< LOD	Py-GC-MS						
S-AtOc-SS 21	< LOQ	< LOD	< LOQ	LC(SEC)-HRMS				
S-AtOc-SS 22	< LOQ	< LOQ	< LOD	< LOD	< LOD	< LOD	< LOQ	Py-GC-MS
S-AtOc-SS 23	< LOD	LC(SEC)-HRMS						
S-AtOc-SS 24	< LOD	Py-GC-MS						
Ant-SS 1	1	< LOQ	< LOD	< LOD	< LOD	< LOD	1	LC(SEC)-HRMS
Ant-SS 2	< LOD	Py-GC-MS						
Ant-SS 3	< LOD	< LOD	< LOD	< LOD	< LOQ	< LOD	< LOQ	LC(SEC)-HRMS
Ant-SS 4	< LOD	Py-GC-MS						
Ant-SS 5	7	< 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	< LOQ	LC(SEC)-HRMS				
Ant-SS 9	< 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: Lin	nit of Detection; Lo	Q: Limit of Quar	ntification.					

Table 3: Accumulated polymers from	MNPLs (> 7	'00 nm) i	in different	aquatic	biota
expressed in $\mu g \cdot g^{-1}$ by means of LC(SE	C)-HRMS.				

Sampling Station (SS)	Organism (gender)	[PE]	[PP]	[PS]	[PI]	[PBD]	[PET]	[S 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: Lin	nit of Detection; LoQ: Limit of	of Quantification	; LR: Linearity R	ange.				



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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Marie Le Bagousse

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Esteban Abad

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Julien Gigault

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To be submitted in Nature Communications

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

N-AtOc-SS 1	Date	Coordinates (Latitude, Longitude)	Water Intered (L)	Salinity (psu)	Water Temperature (°C)	S U ₂ (mg·L ⁻)		WINU Specu (III.)
	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	9.6
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

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	Date	Coordinates (Latitude, I ometerde)	Water filtered (L)	Salinity (psu)	Water Temperature (°C)	S O ₂ (mg·L ⁻¹)	Wind direction (°)	Wind speed $(m \cdot s^{-1})$
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	9.6
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 Spa	mish south far navig.	ation record.						

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 S3 Manta Trawl for MPLs (335-1,000 $\mu m)$ 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.

Water filtered (L)

101,491

N° Cycles

3,524

Sampling Station (SS)

N-AtOc-SS 3

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

Biota captured (Gender)

-

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, pyro-lysates 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 ₆ H ₆	3.54	-
	104	104.0619	104.0621	1.171	C ₈ H 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	C10H10		
	178	178.0778	178.0777	0.326	C ₁₄ H ₁₀	-	
	193	193.1013	193.1012	0.482	C ₁₅ H ₁₃	-	
Ci 70.1	208	208.1248	208.1247	0.663	C16H16	25.52	0.0.0
Styrene Trimer	91	91.0543	91.0542	0.255	C ₇ H ₇	25.53	Confirmation
	208	208.1247	208.1247	0.332	C II	-	
	512	512.18/5	512.18/5 DD	0.033	C ₂₄ Π ₂₄		
	01	81.0600	F F	0.285	CIL	2.55	0
2,4-dimetnyi-1-neptene	81	81.0099	81.0098	0.283	C H	5.55	Quantificatio
	01	01.0542	01.0542	0.085	C-H-	-	
	91	91.0342	95.0855	0.388	C-H	-	
	95	97.1012	97.1012	0.072	CaHua	-	
	109	109 1012	109 1012	0.064	CoHua	-	
	111	111 1168	111 1168	0.153	CoHus	1	
	126	126 1404	126 1403	0.538	CoH10	1	
n-nentane	41	41,0385	41,0386	2.256	Call	1.12	Confirmation
a pentane	43	43,0542	43,0542	1.552	C2H7		Communition
	55	55.0542	55.0542	0.058	C ₄ H ₂	1	
	57	57.0699	57.0699	0.405	C ₄ H ₉	1	
	69	69.0699	69.0699	0.190	C ₅ H ₀	1	
	71	71.0855	71.0855	0.043	C ₄ H ₁₁	1	
		1	PE	1			
1 decene	41	41.0385	41.0386	2 546	C.H.	12.30	Quantificatio
1-uecene	41	41.0565	41.0580	2.340	C H	12.50	Quantificatio
	4.5	55 0542	55.0542	0.240	C.H.	-	
	57	57.0700	57.0600	0.240	C H	-	
	67	67.0542	67.0542	0.403	C H	-	
	69	69.0699	69.0699	0.000	C ₃ H ₇	-	
	83	83.0855	83.0855	0.204	C/Hu	1	
	95	95.0856	95.0855	0.874	C ₈ H ₁₁	1	
	97	97 1012	97 1012	0.134	CaHaa	1	
	111	111 1169	111 1168	0.297	CeHu	1	
	125	125 1326	125 1325	0.583	CoH17	1	
	140	140.1560	140.1560	0.127	CioHao	1	
1-triacontene	111	111.1169	111.1168	0.747	C8H15	34.93	Confirmation
1 thatomene	125	125,1326	125,1325	1.142	C ₉ H ₁₇	51.55	commune
	153	153,1639	153,1638	0.867	CuHa	1	
n-C ₆	81	81.0699	81.0698	0.331	C6H9	-	Oualitative
	85	85,1012	85,1012	0.035	C6H13		Z
	1	1	PET	1			
Benzoic acid	77	77.0386	77.0386	0302	C.H.	8 20	1
Belizoic aciu	105	105 0335	105 0335	0.368	C-H-O	0.37	-
	103	103.0355	103.0333	0.500	Call On	1	
4-(vinyloxycarbonyl)	65	65.0386	65.0386	0.665	C/H ₆ O ₂	13.80	Confirmation
benzoic acid	76	76.0308	76.0308	0.767	C4H4	15.07	commation
	104	104 0257	104 0257	0.420	C ₂ H ₄ O	1	
	121	121 0285	121 0284	0.777	C7HeO2	1	
	149	149.0235	149.0233	1,473	C ₈ H ₄ O ₂	1	
Divinyl terephthalate	65	65.0386	65.0386	0.972	CeHe	13.15	Quantificatio
	76	76.0308	76.0308	0.898	C6H4	13.13	Quantinoutio
	91	91.0543	91.0542	0.914	C ₂ H ₂	1	
	104	104.0257	104.0257	0.228	C ₇ H ₄ O	1	
	132	132.0208	132.0206	1.432	C ₈ H ₄ O ₂	1	
	147	147.0442	147.0441	0.707	C ₉ H ₇ O ₂	1	
	175	175.0390	175.0390	0.003	C ₁₀ H ₇ O ₃	1	
			Ы				
Isoprene	53	53.0387	53.0386	1 381	C.H.	1.11	
Isoprene	55	55.0587	55.0580	1.381	C H	1.11	-
Isoprono Dimor	67	67.0542	67.0542	0.098	C ₅ H ₇	5 71	Quantificati
(Diprene)	0/	77 0294	77 0294	0.795	C 11	5./1	Quantificatio
(Diprene)	//	01.0542	01.0542	0.302	C-H-	-	
	91	91.0343	91.0342	0.303	C, H7	-	
	93	95.0699	95.0699	0.249	C H	-	
	107	107.0856	107.0855	0.309	C ₈ H ₁₁	-	
	121	121.1013	121.1012	0.083	C II	-	
	130	130.1248	130.1247	1.015	C10H16	1	L

Isoprene Dimer	136	136.1247	136.1247	0.646	$C_{10}H_{16}$	5.36	Qualitative
(Dipentene)							
Isoprene Dimer	136	136.1248	136.1247	1.087	$C_{10}H_{16}$	4.52	Qualitative
(1,4-dimethyl-4-							
vinylcyclohex-1-ene)							
Isoprene Trimer	105	105.0700	105.0699	1.172	C_8H_9	11.69	Confirmation
-	119	119.0857	119.0855	1.369	$C_{9}H_{11}$		
	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	C15H24		
			PBD				
Butadiene	53	53.0387	53.0386	1.570	C_4H_5	1.03	-
Butadiene Dimer	67	67.0543	67.0542	0.793	C_5H_7	2.77	Quantificatio
(4-vinylcyclohexene)	77	77.0386	77.0386	0.350	C ₆ H ₅		
	91	91.0542	91.0542	0.145	C_7H_7		
	93	93.0699	93.0699	0.463	C ₇ H ₉		
	108	108.0934	108.0934	0.259	$C_{8}H_{12}$		
Butadiene Trimer	105	105.0700	105.0699	0.696	C_8H_9	8.09	Confirmation
	119	119.0857	119.0855	1.033	$C_{9}H_{11}$		
	133	133.1013	133.1012	1.150	C10H13		
	147	147.1169	147.1168	0.768	C11H15		
	162	162.1403	162.1403	0.075	C12H18		

			I							
				Recovery	(n=3; %)	Precision (n=3) -	Repeatability (%)	ME ionisa	tion (%)*	
Polymer	Acronym	$MLoD \ (\mu g \cdot L^{-1})$	MLoQ ($\mu g \cdot L^{-1}$)	$5 \text{ mg} \cdot \text{L}^{-1}$	$500 \ \mu g \cdot L^{-1}$	Inter-Day	Intra-Day	$5 \text{ mg} \cdot \text{L}^{-1}$	$500 \ \mu g \cdot L^{-1}$	Method efficiency (%)
Polyethylene	PE	0.5	1	74	61	8	4	75	71	50
Polypropylene	dd	0.5	1	77	72	6	3	85	83	63
Polystyrene	Sd	3	10	83	76	22	9	87	78	66
Polyisoprene	Id	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	6	115	134	77
*ME ionisation: < 100 % -	- Suppression;	> 100 % - Enhance	nent.							

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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 n°1** and **n°2** 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 μ m in atmospheric PM₁₀ and surface marine water samples taken along the Atlantic ocean and Southern ocean during 2 oceanographic expeditions. **Publications n°3** and **n°4** demonstrate the worldwide ubiquity of plastics, including those plastics that come simultaneously from the micro and na-

noscale. 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 n°3** and **n°4**.

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 n°1** and **n°2**, 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·L⁻¹ to 9 mg·L⁻¹ for most common polymers such as PE, PS, PP, PI, PBD, and PVC. In **Publications n°1** and **n°2**, 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 N° 8 and N° 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 μ g·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·L⁻¹ to 2μ g·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 Kg⁻¹, were located near our sites N° 6, N° 7, and N° 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 µg·g⁻¹, where omnivorous fishes were those fishes with higher polymer concentrations in their GITs. The highest concentration found in surface water in **Publication n°1** 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' (N° 3) and between 'Rambla del Abujón' (N° 6) and 'Los Nietos' (N° 8) in **Publication n°2**. 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 n°1**, 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 n°1** and **Publication n°2** 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 μ g·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 (μ g·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 n°1**), 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 n°2**), 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 ($\mu g \cdot L^{-1}$) 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 ($\mu g \cdot L^{-1}$) 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 guite 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 n°3**), while in the underlying seawater at 5 m depth (ULW) they were in the range of 2 to 8 μ g·L⁻¹ (**Publication n°4**), 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 μ g·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 guantifiable 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 MN-PLs-polymers was also assessed in the Bellingshausen Sea (west Antarctic

peninsula, **Publication n°4**) with concentrations ranging from 1 (Ant-SS 1) to 7 μ g·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 ($31.71^{\circ}N$) and the South Atlantic ocean ($45.11^{\circ}S$) by a mass-quantitative assessment of the MNPLs-polymeric composition in the air phase, and the potential of regional sources and seaspray aerosols by a comprehensive characterisation of PM₁₀ 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 n°3**, 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 PM₁₀ composition, the estimation of the global marine atmospheric half-life ($T_{1/2}$) for the Σ 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_p), which was calculated by least squares adjustment to the concentrations of **Equation 4**:

$\ln C(MNPLs, air) = -Kd \cdot t + b$ (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 n°3**. 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	и и
South Pacific Ocean	Active	2019	0.0392	(Chen et al., 2023)
North Pacific Ocean	Active	2020	0.0361	и и
Southern Ocean	Active	2020	0.0330	и и
East Antarctica	Active	2020	0.0055	<u> </u>
South China Sea	Active	2020	0.0039	(Wang et al., 2021)



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 inthe 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, $4\frac{\dot{4}}{4}$ 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 n°4**, through the Manta Trawl sampling, several species that were captured, such as phytoplanktonic (algae) and zo-oplanktonic 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 μ m) 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 guadricauda 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 $\mu q \cdot q^{-1}$, 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 μ g·g⁻¹ and 4-21 $\mu q \cdot q^{-1}$, 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 μ g·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.

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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,



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

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-NH2 (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 n°5**:

 Publication n°5: 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, <u>https://doi.org/10.3390/toxics8030059</u>

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 μ m. 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 macroand 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 μ m were supplied by Cospheric (Santa Barbara, CA, USA); 10 μ m PS, supplied by Phosphorex (Hopkinton, MA, USA); and PET microspheres below 300 μ m 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 μ g/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 \geq 99% was purchased from Merck (Madrid, Spain).

2.2. Experimental Design

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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 μ g/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 μ g/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.

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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 μ m film thickness). Following the temperature program that can be seen elsewhere [14]. The injection of 1 μ L 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 μ A, 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:

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

where % Ads is the percentage of adsorption, $[A]_t$ is concentration of compound A remaining in sediments after 21 days, and $[A]_0$ 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 n	g/g	10 n	g/g	15 r	ng/g	20 r	ng/g	25 r	ng/g
		Mean	$\pm SD$								
	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
%Ads on PE	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
$\Sigma PCBs$ on PE (ng)		0.103		0.253		0.417		0.498		0.574	
	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
%Ads on PET	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
$\Sigma PCBs$ on PET (ng)		0.132		0.315		0.426		0.541		0.657	
	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
%Ads on PS	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 \geq PCB-28 > PCB-101 > PCB-153 \geq PCB-138

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> 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 dIPCB-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	0.03	CP1, PP	5.67
PCB-52	2,2',5,5'-Tetrachlorobiphenylortho, ortho, meta, meta	\$3-53	4CL, 2M	5.84
PCB-101	2,2',4,5,5'-Pentachlorobiphenylortho, ortho, para, meta, meta	53-Ç-	4CL, 2M	6.38
PCB-118	2,3',4,4',5-Pentachlorobiphenylortho, meta, para, para, meta	-252	CP1, 4CL, PP, 2M	6.74
PCB-138	2,2',3,4,4',5'-Hexachlorobiphenylortho, ortho, meta, para, para, meta	Net .	4CL, PP, 2M	6.83
PCB-153	2,2',4,4',5,5'-Hexachlorobiphenylortho, ortho, para, para, meta, meta	2,25	4CL, PP, 2M	6.92
PCB-180	2,2',3,4,4',5,5'-Heptachlorobiphenylorth ortho, meta, para, para, meta, meta	10, \$7.63-	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). 4C1: congeners that have a total of four or more chlorine substituties regardless of positions chlorinas 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

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the increase of the number of chlorine atoms, the final adsorption being ranked as follows: PCB-28 \approx 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 highand 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 μ m 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 Toxics 2020, 8, 59

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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):

$$=K_F C^n$$
 (2)

$$\log q = \log K_F + \frac{1}{n} \log c \tag{3}$$

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.



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

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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 $\Sigma PCBs$ on MPLs in the seawater/sediment system.

	$log [q] = log K_F + 1/n log [c]$	log K _F	K_F (ng/mg)	п
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 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.

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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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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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Figures S1, S2





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

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Figure S1. schematic flowchart of experimental design.



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

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A)

Adsorption isotherms on PS 16000 PCB-28 y = 1286.2x + 6.8121 R² = 0.9768 14000 PCB-52 y = 1125.6x - 303.4 R² = 0.9836 12000 2 10000 PCB-101 y = 637.12x + 14.26 R² = 0.9305 8000 PCB-153 y = 558.86x - 126.46 R² = 0.8718 6000 • PCB-138 y = 441.95x - 280.14 R² = 0.7245 4000 2000 PCB-180 y = 322.71x - 228.04 R² = 0.6025 •• 25 PCB 118 y = 211.72x + 172.42 R² = 0.6696 15 10 20 -2000 Conc PCBs on sediment (ng/g)

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B)



C)



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

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5.3 Discussion

Publication n°5, presented the key adsorption/desorption mechanisms between different types of microplastics (MPLs) sizes between 1 to 300 μ m and representative PCBs in simulated coastal (water-sediment) ecosystems.

As shown in **Publication n°5**, 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 μ m), PS (10 μ m), and PET (< 300 μ m), 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·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 \geq 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·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·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 n°5** 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 μ m) 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



Figure 5.2 Adsorption percentages of PCBs and PFASs onto PE and PS-MPLs microspheres in simulated seawater environments at 10 μ g·L⁻¹ after 21 and 49 days, respectively.

%, 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.

Furthermore, differences between freshwater and seawater and the ageing of MPLs have not been tested in **Publication n°5**. 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 n°5** 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 Σ PCBs (**Publication n°5**) and Σ PFASs (Llorca et al., 2018) in estuarine systems.

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

	K⊧ (ng·mg⁻¹)	N۴	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 ' $K_{\rm F}$ ' 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 ' $n_{\rm F}$ ' can range from 0 to 10, between 0-1, 1-2, and 2-10 indicat-

ing 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- Σ 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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5.4 References

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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 MN-PLs 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 n°6**), and from single-use plastic bottles (Publication n°7) 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·q⁻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 n°8**).

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 n°6: 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, <u>https://doi.org/10.1016/j.</u> watres.2022.118645
- **Publication n°7:** Vega-Herrera, A., Garcia-Torné, M., Borrell-Diaz, X., Abad, E., Llorca, M., Villanueva, C. M., Farré, M., 'Exposure to micro(na-no)plastics polymers in water stored in single-use plastic bottles', 2023, Chemosphere, <u>https://doi.org/10.1016/j.chemosphere.2023.140106</u>
- **Publication n°8:** 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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Marinella Farré

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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D SG o) dKi oLi i togi (cgai host odm c aodmoE dai goLchsi opz li oi aodribá Sk55mRO) i gi fcgi blaj i coudt ci tai gdhgst Bst Adč dai go cain RQ tog) i czaj i go) dt hlád (gi rsn st dgeo al hsi o) dKi ost hsudai hoa) daoa) i ost ai g dus dasct ocfo D SG oudt ci dhox cdhKi gi oi ffi ua et c4.sczaddt hol l n dt oj i dra) qfil dgi rd o i aodribd5kv0ko4) cla5kv0:oGregudodt hoCdggi ld5k5vc05 gdadoi aodribd5k5kmBO O) i i di ffi ua et caskst AccgAdt s n odgi qgi rdai hoec () e sudre(dgsauri dhdh V dAi a) daoudt oudl i ast / dn n dar.geori sct ble gsAst dast Afgen a) i q cai ta sdro cfoa) i sgo l gfdui blac ost ai gduadE sa) caj i cag li cag cag udodt hoCdggi ld5k5vc05 gdadoi aodribd5k5vmBo fii uct hne1ba) i (rd asuudt (gch lu i urct AVii gn oz Osusee os tu a) i ad lhhsski o dgi cacuc Kdri t ared.cl t hozca) i q cren i g dt hlåct ui ost ai gt dsi i hlåucl ho Li qgi ri d i hqcfar gudodt hoCdggi ld5k5vmBD cgi cKi gdba) i o) ehgc() cLsuseec fo D SG dt ha) i sgo l gfdui u) gdguai ga sau on dBi a) i n (cai t adrofsi ucg gcgo ca) i gg(crin adt a odt ho(da) cAit i Hell ga) i gdE cg i ts Aa) i sgoi t Ksget n i t adro dt hoj i dra) on (dua qCgi gi ai adribd5kv%tBo cgi cdai adribd5k5k;65kv%hBo

v gst Bst AoE daigo) d oLiit oshit as 1 i hod oct i ocfoa) i on dst o(cait asdro gclai ocfo)lndtoi0(clgioacon sugcVdthotdtc(rdasu opDySGmopSgdado i andr Holo K 5k: 6 so andr Holo K 55 mBD SG oi 0 (c 1 gi on) dans or daags L1 ai hoac on gst Bst Ao E daigo) d oLiitoi asn daihodao3Rq-wk8o(dgasuri 6(ig ct 6eidgopj) dt Ao i aodrRoj5k5knModra) cl A) o cn i o al hsi ost hsudai oa) daost odhKdt ui hoE dai go agidan itao fdusnsasi Hota)s o Kdrlion sA)ao Liodorc Eodoctio (dgasV uri 6(ig ct 6eidgop7sg aist oi addrHolbk5wnRowt oa)s o(gi Kscl o al heHol7sg aist o i aodmRod i i hoa) i ouct uit agdasct oc fo(dgasuri ost ohgst Bst AoE dai gofgcn odo) sA) V(igfcgn dtuiofiEihs) oagidan it ao(rdtaop7sg aistoiaodrRef5k5wnRoxto dt ca) i gogi uit ao al he HaiCinhoi ao dn Rup5k 5 wund i i hoDSG o(dgasuri ost owno dn (ri ocfov dt s) oad(oE daigo) cE st Ao sn srdgogi 1 ra oacoa) ciocfoa) io 7 sg ait o al heRO) i oi asn dasct ocfodual drouct uit asdasct os on l u) dhi Ldaiho dthoKdgsi oston dteorghig orfon dAtsalhiRO) i iohsffigitui on sA) adhi(itho ct oa) i o. 1 drsæoc foa) i oE dai godaoa) i oudau) n i t ao sai Hod o 1 gfdui oE dai go Aitigdmeo(gi ita o)sA)igoDSG oriKir oa)dtoAgclthEdaig.oa)ioae(iocfo al heosfletic gost adtuileta) i oft dre souct shi goad (dE dai gofgen o) el i) erh o E) igi q(s(i odthoad(oudtost/lituioa) iogi lra ocgo1l aca) ioEdaigoagidaihodao a) i oi t hoc foa) i o(cadLsrs dasct o(gcui :cLl aosao) cl rhoLi o) sA) rsA) ai hoa) dao a) i gi odgi oKdgsdaset oLi aE i i to. l dtasl udaset on i a) ch odt hodt dreasudro (gcui i ol ihRoO) s os odr codouridgost hsudasct ocfoa) i ot i i hofc go. I drsaeo d lgdt uiodt holdrsaeouct agcropWM6WInonstoa) ion ia) choa) daadgid ihoaco (gcKshioduul gdaiohdadHøst ocghigoacod i o)ln dtoi0(clgioa) gclA)o hest Bst AdE dai 9007 ci m dt o andrikter kw9 dKdt dD cl 95Bo andrikter k5wnROC dadai ke t cost aigt dasct dreeo) dgn ct s i hoAl shirst io) d dLiit odhc (aih Hadt hoa) id io cfohsffi gi t aon i a) ch odt ho(gcacucr o(gi Kdsrłóżd odr co) sA) rsA) ai hoLeodo gi ui t areol t hi gadBi t ost ai gt dasct drost ai grdLcgdacgeo al heopKdt oD cl gsBo iadnRo5k5wnBAD cgicKigBob) iotln Ligozfoalhsi octoa) io(gi ituiocfoD SG osto hgst Bst AoE dai gos o (dg i Roxt of dual of gcn o5kw\$lot ct reo580 al hsi o) dKi ogi V (cgai hoa) i ocuul ggit ui ocfoD SG ost ohgst Bst AoE dai go e ain opzlioi aodrRð 5k55mMadthoctreodofiEoalhsio)dKiofcul ihoctoa)io(giituiocfoDSGosto ad(oE daigofgcno)cli)crhopCinhoiaodrRob5w.ofi)itoiaodrRob5wnBoO)iosFiHo n cg() crcAeHolth to ln Ligoc fo(dgasuri odgion c areo ldt as lihoLeown dAst AHo n sugc uc (ekke go sFi of gduaset daset od ((ge du) i RA4) srikafe goa) i odt dre s oe fo (crenig Hoin c accfoa) i io al hsi o Eigioudggsi hocl ao Leon sugc VC cl gsigo agdt fcgn o stfgdgiho n sugcuc(eo puCOxTmbol Tdn dto (iuagcuc(eHol (egcre s VUd o I) gcn dac Agd() eVD d o fi(i uagcn i ageo p(egVUI 6D finblo cgo a) i gn cAgdKsn i agsuo OUD VUI 6D fiRo - cE i Ki glob a) i i o d((gcdu) i o dgi o rsn saihd.eog) i o sFior fog) i oʻdgasuri og) dagudt d.i odt dre i hladt host oAit i gdro udt t caoLiod((rsihofcgo(dgasuri oLircEowun ocfohsdniaigtoE)su)odgio (gcLdLreca) i on c andLl thdt anst dngst Bst AdE dai gRD cgicKigHonl i oacoa) i orcE o uctuitagdasctocfo(dgasuri Holdon stsnlnoKcrlniocfowkkkoGo)doLiitogiuV cn n it hi hoacogi hl ui oa) i ol t ui gadst ae ost oai u) t s. l i o l u) od ou COxTo pD stait sAciaddrild5kw9nBo cEiKigldAcchogi lrao)dKiodr coLiitocLadstiho st on drroKcrl n i ocfoPkoGol st Ao adst ri o ai i rol raig ocfowkoun opCinhoi addrRol 5k5wmRcO) ictiihofcgo) sA) o dn (ricKcrlni os odtosn (cgadtaorsn sadasctoaco ucthluaoalhsi ocfo)cli)crhoad(oEdaigoLiudliocfoa)idhsflulraeocfoa)io dn (riguerriuaset Ro

xtoln n dgeBohi (saicdt d gAitactiihcacd thig adtho)ln dtoi0(c lgio a) gclA) dngstBstAdEdaigfob)igiodgiosAtsludtadBtcErihAioAd(oa) danudtoLio

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(dgareadaagsLl aihoacon) iodt dne asudruu) dmit Ai Hoh) daadgion c are ogindaihoaco (dgasuni odaan) ion Eon suge Vidt con iaig Vudriop Staits Aoiandh Hoskwo-mbolitho dn con) isgore Eon ctuit agalaset op c) t ctoiandh Hosk Skmbol) igios od at iih de go ti Eodt dne asudrod ((ge du)) i oa) dao Ecl mhofcul oct o Dy SG o Esa) oge Ll ao dn (nst And ((ge du)) i Ro

xt ca) i o(gi i tao al helda) ion dst oc LJ uaski o Eigioacohi Kirc(odot i Eo dt dreasudro E cgB/CE oLd i hoct odo (cren i gVI (i uao ugi i tst Aod((gcdu) Já a) dass o'Agel thi hact os Fioi Ourl sct ou) gen dac Agd() eldi st Addt odh Kdt ui ho (cren i gou) gen dac Agd() eo ucri n to ucl (ri ho aco) sA) Vgi cri æst on d o (i uagen i ægeo E sa) odt odan c () i gsuo (gi l gio() casst st dæct o c l gui o l thi got i Adaski odt ho(c sæski osct s dæct oucr hsæct op. SGI pMSI miMSS xo p±mV TD finadt hot cgn drc() d i ou) gen dæc Agd() eo xGM G2 y ME& acri n to dt hoi ru age (geloact s dæct ocl gui ost o(c sæski of hot i Adaski on chi op. SGI o p. xGM m⁶ fop±mV TD finadts or ghi gæcod i of tho i Adaski on chi op. SGI o p. xGM m⁶ fop±mV TD finadts or ghi gæcod i of tho i Adaski on chi op. SGI o p. xGM m⁶ fop±mV TD finadts or ghi gæcod i of tho i Adaski on chi op. SGI o p. xGM m⁶ fop±mV TD finadts or ghi gæcod i of tho i Adaski on chi op. SGI o p. xGM m⁶ fop±mV TD finadts or ghi gæcod i and thoi dt asfed D y SG of cren i g o) d o Li i to Ld i hoct odt ost ugi d st Aouct 1 gn dæct ori Ki fråst ut hst Aoa) i o n ct cn i g ou) dgduai gg dæct of st AO⁷ i thgsuBDO d ovi fi uag7D v måt dre s Hø dt hou ci hol dt aslor dt ho l dt asl udæct d: st AO att hdgh Ro

O) s dt i E on i a) chleE) su) os d.d i hoct ocl gr(gi Kscl d((gcdu) opfiu) sgV st Esi auhtHobkw9mbit d cadffi uai hokea) i (dgssuri osFi oth hos ol sadLri ofcgaa) i o d i nitacfo(cren i gsucuct ui tagdasct ost chgst Bst AoE dai głdE sa) on st sV n l n o dn (ri osFi dKcrl ni ogi.l sgi nita cr6PoGRO) i i odgi othKdt adAicl o fcgoD y SG odt dre s ost ohgst Bst AoE dai gdd noa) i eocffi goucn (ri ni tadgeo st fcgn dasct oacoca) i god((gcdu) i oE) su) o cgoa) i ot l n Li gocfo(dgssuri o >Pµn RCst drefeba) i ot i E dKdrshdai hod((gcdu) o) d dLiit ol i hoæcoshi tasfeo d t l dt asfeo y SG ost dS5od(dE dai god l i) crhodn (ri osfio) i odgi ui ctd o D i agc(crsadt oMgi dopbD MrbbOcocl goBt cE ri hAi Hoia) s os oct i ocfoa) i ol g ao al hsi o(gcKshst Aohdadoct oD y SG oucn (c sasct ofgen o) cl i) crh oad(Ho E) su) o cgi (gi i tadasKi crfadE) cri osseRo

2. Mael ris 1 ntmh 11 ction

2/1/ Chemicals and reayents

Mt dreasudro adt hdgh α cfo(creLl adhsiti opD R4 KK vkqkov dmdt ho(creV s c(giti opD R4 KK vk3kov dmBt ig o(lgu) di hofgen o4 dai goffRMopli ighdV tecrddhiro, dmi løbdguirct dløfi(dst nBsCreia) eriti opS*nBd(cre ægiti opSfm dt ho(crefKstermo) regshi opS, I mpD R4 KK vovSkkov dmE ig ol l((rsi hoLeo Scren i gfiadt hdghdfii gKsui dJn L- qpD dst FløUi gn dt enhalt hd(cre(gc(eriti o pSSmpD R4 KK vS5kov dmE d ocLadst i hdgen dVn i gsudt 6Scren i gfiadt hdghdfii gKsui dJn L- qpD dst FløUi gn dt enhalt hd(cre(gc(eriti o pSSmpD R4 KK vS5kov dmE d ocLadst i hdgen dVn i gsudt 6Scren i gfiadt hdghdfii gKsui dJn L- qpD dst FløUi gn dt enhalt hd(cre(gc(eriti o pSSmpD R4 KK v55kov dmE d ocLadst i hdgen dVn i gsudt 6Scren i gfiadt hdgh o I cg(cgdasct opD i ta gbdQ- Md 2 tsi hoffadi i nBd d (gcrduadn odt hoa) i sgoucgV gi (ct hst Aoueuri odt holstyf5Va) er) i Oemn() a) drdai opx*- Smo adt hdgh o Eigi g(lgu) di hdgen dD i guBqov dgn adhadbU ign dt enbo

2 tri oca) igEs ioadai hlodmocfoa) iogidAita oEigiocfodt dreasudroAgdhiRo Corditiof - TQD MfQC, ®SC2 fibe() su) oE d'oi ihod on cLsriq() diohl gat Ao - SCI odt dre i HoEd ordu l sgihofgen oD iguBiole() igid on ia) dt crholdrui act sV agsrikolth de daigeEigio(l gu) dihofgen oCs u) igif) in sudropCcl A) Legel A) Ho 2 tsaihof'st Ahen mbo

Urd on suge 1 Lgiol naig oUC6CoEsa) odo(cgio sFioefok Rqkqun oEigioeLV adstih ofgen o4) dan dto6CoIqoD dsh actih Be2tsaih o7stAhen mindd hotsage Aito lihod ohgestAoAd oEsa) o99R99P; o(lgsæoEd ol((rsihoLeonAsgoCaslshio pbdguiretdHoff(dstmBo

th skishl dro acuBo adt hdgho crl asct ocfos * 1465 S146 Fil46 si465, I Halt hoS bvo pvkkkon AGCnast opi of CIVD ficAgdhi dč dai godt hozcrl it i ocrki ta dči gi of gi V (dgi hopvkon Accfo DS G o adt hdgho č d os (ig i host ovkon Gozcrl it i mindt ho a) iedči gi ol L i. litareol i hozcor Ladst opi oudrsLgdasct all gKi or foD SG of cgo . ldt asl udasct (lg(c i Rot odrmad i Ho(rd asvo crl asct dči gi du) si Kih Leo l st Andt d ragd ct sud.da) dč sa) opi dast ARO) s of gcui dč d odr cd i hofc godro Ksdr of gscgoaco aj i sgost Ji uset ost acoaj i ou) gen dac Agd() suo e ai n Ro Cego (credn shi q6S Mmind d) (gcrduadn odt hoa) i sgoueuri Halt hov *. So adt hdgho cV rl asct opvkkkon AGCn6 zi gi of gi (dgi hHzed st AndrsLgdasct all gKi Ro Cegi du) o (crein globdisLgdasct all gKi opvkoni Kir or foact uit agdasct mči gi of gi (dgi ho danač costi dgasce ugdt Ai d.i ači i to Rk Podt hoP AgAGO that h.ači i to Pokat ho wklkon AFGTb

A/Qeya-z errera et al/

2/2/ Sampliny

Od(dž dai go dn (ni dž i gi ou cmi uai hodaci du) of c adrou chi oc foa) i ob D MnpT = 35 mliot on) i of i go cho cfoMI AI ozoc Quaz Li go 5k 5k RM Vozc adroc fo690 dn (ni o E i gi ou cmi uai hotaoa) i oB sau) i t of dl ui aze for go Kola i oj cli) cmi Holth Moso dn (ni o E i gi ou cmi uai hotaoa) i oB sau) i t of dl ui aze for go Kola i oj cli) cmi Holth Moso dn (ni o E i gi ou cmi uai hotao di Li go Ard ol carai ca) dao E i gi or gi Vest i hot E sa) o ni a) dt crhols SGI VE dai goldui azt i Holth hon) i ond (dž dai go Cia ti do Cia to Bo SGI Ve Gai goldui azt i Holth hon) i ond (dž dai go Cia ti do Cia to Cia sa) a) o ni a) dt crhols SGI VE dai goldui azt i Holth hon) i ond (dž dai go Cia ti do Jo Sai Rb) i fogi o a) i o dn (ni ou cmi uasct Hola) i o ad (o E d ori faogi t t st Aofe go 5on st o aco dK csho u t ad n st daze t od custal hoE sa) ca) i ol g ao drs. I cañov I gst Ao dn (rst Alda) i o ad (dž i gi or (i ti hoac ofl mč dai gr(gi l gi RD cgi c Ki golos Lrdt BcE dai go dn V (ni dž i gi ou cmi uai hot st Aoa) i o dn i ad (cgi Qu) Ai ad) st Aoa SGI dž dai gaz di ro a) i dc. zaari ost on) i dB sau) i t oc fod Kot rt ai gi Ro

2/3/ Sample pre-treatment and efitraction preparation

PoGocfoi du) ohşet Bst AoE dai go dn (ri oE d ol g aolrai gi hoa) gcl A) odo adst ri Vai irosi Ki d£ sa) odon i) orfoškom Mo gi Kscl red£ d) i hodt ho) i dai ho dar3kkoʻl dc gadari d ac5-oj acci Kd(c gdai dLl gt orffo(cai t acdruct adn st dt a Ro O) i t Mo) i od n (ri d£ d olrai gi hodAst oa) gcl A) addk Rakoun oArd on sugcl Lgi l rai goUG6Coa) dac£ d o(gi Kscl rechgsi hodt hoadgi hRO) i olrai gr£ sa) of dgasuV l rdai on dai gschr£ d chgsi horKi gt sA) andar%koʻl d (cac cuct adt at£ i sA) and tho Bi (andao-5koʻl d t asrop) i oD SG 6y SG d ogatusect (q gcui hl gi Ro

Mt ol rægd et suld s ai hoi Oægduæset op£ fiM* mb E d o(i gfc gn i hofe goa) i o i Oægduæset ør foD y SG ofgen a) i d rai gdE sa) ovkon Gør fører li ti ofe govkon st øtt ho a) i oæ Lædst i hei OægduæsE d ægdt fi ggi hoæ eddPken Godn Li goArd «KsdrRO) s o dt dræ æudre(getu ih lgi dE d ggi (i dæi hea) gi oæsn i Mgt t dræ er. Lædst st Ar& Rom Go (i go I ræi gRO) i oi OægduæsE d oi Kd(e gdai hol t hi godot sæge Ai t o ai dn oE sa) o ai n (i gdal gi odæßk of I Mæ couct ui tægdai a) i o dn (ri d (æc ov RPm GRO) i ov RPo n Goi OægduætE d ægdt fi ggi høæt hok eggi ob i bøt øldet. Vksdrøfe gøldD y SG ol (i uzo ugi i t st Ardt dræ s Ro

Mrs. Ica oc fow PkquGoc foa) i oachi i tioi 0 agdua dEigioagdt figgi hoac oddGIVo Kosho E sa) ost i gao dthoi Kd(cgdaiho ac ohge ti olthigo t sagc Aito agidn Ro O) i e dEigiogiuct asal aihd (oac ow Pkou Gon i a) dt crVE daigo 90 MRo

Cst droi 0agdua o E i gi o (gi i gKi hodao $-5{\rm ko}^\circ I$ o l t asroa) i o dt dreso Leo - SCI —- TD filo

2/4/ Instrumental analLsis

MahclLriol (iuaougiitstAad((gcdu) dEd d ihaacad i on c aacfaa)io (cren ig oa) daoE igiofclt host ohgst Bst AoE daigHaLd i hoct o-SGI --- TD fiRo Csg are Holfc go(cren i godt dre i HolsFioiOurl sctou) gcn dac Agd() eol st Aodt o dhKdt ui ho(cren i gou) gcn dacAgd() eopIMSI moucrl n t opMu. I saedMSI oz O3Po wRqqun o(dgasuriosFindEsa) stodtoMu. Isaeo SGI o e ain ofgen o4 daig of eg(Ro pD srfc gh HatD MHat2 t sai hofiadai mhatE d ol i hofc goa) i ou) gcn dac Agd() suo i (dV gdasctol stAHold on cLsrio() diHolwkk; cacrlitiol thigos cugdasuoucthsasct Ro O) i o/ cE ogdai oE d odhJl ai hodaoPkkouG6n st odt hoa) i ost Ji uasct oKcrl n i oE d o 5kouGRO) i ou) gcn dac Agd() suo e ai n dE d oucl (ri hoac oddW* 0 duasKi o) e Lgsho . l dhgl (cri VQgLsagd(on d o (i uagen i ai gofgen oO) i gn coCs) i gofiusi t as l uo n fidt o cíHoll MHol2 tsaihofiadai nholi.ls((ihoEsa) odtodan c() igsuo(gi lgio () cacscts dasctopMSSxmo clguiHoc(igdastAostotiAdasKiodtho(c sasKiosctV s dasct on chi RO) i odu. I s sasct oE d of i gfc gn i host off moudt on chi on mO Pkk-8kkmonE sa) odogi crlasct ocfo8kHikkofl monE sha) odao) drfon d0sn l n o pC4 - DnBcO) icc (asn droMSS xo cl guiouct hsasct of Eigiod of cmcE @) ida) o Ad odao%kod14. Hotell OsrsdgeoAd odao8Pod14. Hoteï\vit t oïCodaowkkodthoa) i oud(smdgeo dtho(gcLioain (igdal gi oEigion dstadstihodao3kkoʻIRO) iofiVGit oʻTCoriKiro Ed o i andaowkko, odthodon dOsn l nost Ji uasctoasn i ocfo5kkon. Ro

Mo i uct hou) gen dac Agd() suo i (dgdasct ofe gon i a) dt er VE dai go9@oi 0V agdua d£ d du) si Ki hd st Ando xCxi dG2 y M® & dac n t qo8qun Ho5o× wPkon n mo fgen &) it en it i Oot uRpO eggdt ui Hoi MHo2 t sai diadai mhalt hodon cLsri (i) d i o urn (c i hoef adui art sagsri owkk; qoMnodt hoE dai goE so) dcRv, qoK6Kmafegn suo dushqobmRO) i dfeme Est AoAgdhsi t anct hasset dE i gi dd((rsi h@ adgast AdE sa) o wk; dMHo2 k; db:dgen o adgaac dPon st ddo1 g aost i dgast ugi d i oac dPk; dMHo2 k; db:oj inho uct adt afe gavon st odo i uct horst i dgast ugi d i oac dPk; dMHo4k; db: d i dac adgast Aouct hasset ost oeghi goac ogi V. I srsLgdai ca) i ouerl n t RO) i on cLsri o

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() dioy'cE opdaidE do5kkoµGon st^{-wo}dthoa) iost JiuaectoKcrln i dE dowkoµGoo xtoa) soudikoba) ion do (iuagen iaigoE doi.ls((ihdE sa) odtoiriuageV (gdeosets dasetop* fixmo el guikobE egBst Aolthigo(c sasKiodthotiAdasKio sets dasetouethasaet Ray sageAitoE doi hodio) ida) hoE ii(hodi hodiOssdgeeo Ad oday'cE ogdai oz fo% klok klok hoBrodR Ropdg.sagdgeol tsa mabgi (iuasKire Rovdado E igiodu lsgihost dimoudtoD fion chiopmo Wakk-wkk kundan dogi er daseto c foqk Rkk.kc24 - Dodthondon dOsn l nost Jiuasetoasni ice fowkkon. Ro

O) i o ac adroset o u) gen dae Agdn o pOxI moe Ladst i ho Leo fl m Vudt o n Cfimo du. Is sasct d st Aca) i oz udrsLl gopO) i gn coCs) i gofiusit as1 umo cfaE dgi oE i gi o (grui i hofe goa) i oshi tasludaset odt holl dtasludaset or foD v SG of eren i g o duucghst Aoacoa) ion ia) chcrc Aeoa) dao) do(gi Kscl reo Liit ohi ugsLiho n i AdV i ggi gdoi aodr##5k5woGrc gudoi aodr##5k5wnRoxt oLesi f##dfai go(i dBo (suBst Aodt hoAgel (st AHota) i oOxl ou) gen dacAgdn ocfoi du) o dn (ri oEd o st aigge AdaihoiKige o 8k oc LigKst Aoa) iouc ggi (cthst AdD fio (iuagdo sn lrV adt i cl reost ost ai gKdr oc foPkk n 6Fopfgcn oPkkoac o8Hkkkon 6FnBcO) s oEd o gi (i dai hofc goi du) o8ko casn i o(i gsch ost odmoa) i cacadrosct ou) gcn dac Agdn o pOxInRASidBHE) su) dD fio (iuagdo) cE i hod ogi (iasasKion dorc i HaEigio n dgBihod ol (iuaiho(crenig RO)itodolg aolragdasctoai(dEd dLd ihocto a) iostait sae HonE) itoa) io(idBodgidostoa) io dn (ni oEd oni oa) dtoa) giio asn i ordgAi goa) dt oa) i on dOsn l n o(i dBodgi dost oa) i oLrdt B oa) daoE i gi o uct shigihod oLduBAgclthRofil (iuaiho(gc1ri oEigioaitadasKireoshitasV 1 i h Holl st Aogi (i asas Ki on d orc i o E sa) odon d o acrigdt ui o Lirc E o 8o ((n o nshitasludasctori Kiro3n1600) itHollLi, litao1ragdasctocfoa) iohdadoEdo ucthluaihdLeoucn (dgs ctdEsa)odo) cn in dhiorsLgdgeocfo(cren ig opGrcgudo i andr Holos k 5 winder) daoudt dLiorsA) areo cri Lriost over litionri Kiro8 mBO) it Hoa) io 7 it hgsuBdDd ov if i uaop7Dv modt dre soEd oudggsi hoel andt hoa) i oait adasKio shi tasludasct ocfo(cren i g odaori Ki ro5oE d odu) si Ki hRoxt oCsAl gi ofiwlad cn i o i0dn (ri ocfoa)i0l (iuao ugiitst Ao(gcuihlgioa) daoEd of crrcEihHadthoso hi ugsLihostoa) iodLcKiHodgio) cEtRo

Ict shigst Aca) i cucn (ri0sæccfoa) i on d o (i uægdocfo(cren i g ladh li caco a) i cai u) t crc Aecocfoa) i oL rithi h cucn (c sæct lada) i os cac(i chs ægsLl æct lad dhhl ua hadit hon I ræ(riou) dgAi hadit hofgdAn i tæct oi gsi lad) i of D v odt dre s o E d cal((rsih ladd (gi Kscl rec(gc(c i h d.ecfida.ci and rQp5kw8nRO) i om O cLV adst i h oLeo-SQI — TD fioE i gi ouct Ki gai hoæco7 i thgsuBoD d op7 D moduV ucghst Acaco^{*}. Rowr@

$KM (ion) = \frac{m}{z} (ion). \frac{round (R/X)}{R/X}$	pwmo
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E) igioy cn st dro7it hgsuBoDd opy 7D modt hol cggiuaoy cn st dro7it hgsuBo Dd oply 7D modgiogi(gi it aihost o*. Rp5maco*. Rp3m@

KM(ion) = round(KM(ion))	p5mo
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 $\Delta \text{KMD}(\text{ion}) = \text{NKM}(\text{ion}) - \text{KM}(\text{ion})$ p8mm

Icggiuaihoycn stdro7ithgsuBond Ro

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CNKM(ion) = NKM(ion) - ceiling (NKM(ion) - m / z (ion)) p3mo
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E) igi c∩os ca) ion di oc fodogi (ida) t sakkit hoz os odo(c saski ostai Aigol ihod odo Ld iol t sacicgaa) ion dru Indac toor fo/D opset mBy cgn dmelok o – wofe godo adt hodgho 7D v odt dne s Root odhh saset Hao) iouct ui (acc forigduaect dro Ld iol t sakkat age V hl uiho Leo Ccl. li acdt hofidae op5kwq mBot ugi d i ca) iogi crl acet oc fo7D v o (rca Hod o Ed o ai aihost ocl go (gi Kscl o aihsi opfiu) sgst Fsoi acdr Hods Ksw 3: , iAdV iggi gdi acdr Hods Ksw c3xe guddi adr Hods Ksw Bo

O) iodt dre soc fo(crenig dueo. SGI — - TD foo) clrhduidhct iost oucn V Lst daect dE sa) o7 D vodt dre soacodu) si Kioa) ion st sn ln ctln Ligoc foshit V asludaect (cost a koist uioa) iogiait aect oasn iopTambledWSI oucrln tohi (ith oc to n criul rdgeE isA) appD 4 mahdt hot caoct o(crenigaae (iRo

O) igifcgiHaa) iouct Kig sctoc fouct uit agdasct os ogi. IsgihHabh Iicaacoa) io hsffigituiost oaign oc foD 4 dLiaEiitoa) io(cren igd ihod o adt hdghHadt ho aiu) tsudro(cren ig oa) daodgio(gi itaostoa) io dn (ri Ro

xt oʻki tigdh"oʻhi oʻdgi doc fon) ioʻ) s'A) i aqʻi dBoc fon) ion d oʻ(iuzgdoʻgc 1 niost o dn (ni oʻ) d d.ii ti d i hoʻc çoʻ(cren igoshi tasl udavct oʻC sa) chaffi gi tao D 4 Ro Oʻ) i ouct uit agdavct oc Ladst i hoLeon) i ouchsLgdavct oul gʻGi oʻ(cren ig_uo E d o uct Kiggi honcoi. 1 skidni taouct uit agdavct oduuc ghst Aonco". RpPn"bbE) i gi cay) i o i. 1 skidni taoficuze gopoʻ, mohi (i th oct on) i od 1 n Ligoc fon ct cn i g ona) davdgi o

A/Qeya-z errera et al/

(gi it acst ca) io adt hdghqm/mon/)_{std} dt host ca) io dn (riqm/mon/)_{sample} p°. Ro p%mROD) iou/mon dgi ca) iogcl t hih Kidni ioc Lackst ih d.echsKishst Aca) ion dor fo a) iojsA) i aqí id Boc foa) iq (cren igqí gc 1 rid.eca) ion ct cn ign dor^o. RogumBo

$[Polymer]eq = \frac{[Polymer]cc}{Feq}$	pPmo	
$Feq = \frac{(n.mon)sample}{(n.mon)std}$	p%mo	goh4l tC4 goh4Futn goh4iToe goh4eroe
$(n.mon) = \frac{(mass) \ highest \ peak}{mass \ monomer}$	pqm	goh4Tt4rl goh4pDin

Cst dme Haa) i ou ct 1 gn dasct opuct 1 hit ui ori Kirovan Ed od i i hoa) gcl A) o a) i oi Oaigt dmudnsLgdai oul gKi or fo(cren i go adt hdgh HoLd i hoet oi. 1 sK dnit ao uct uit agdasct of cgs & Hob Filde Storb by HobS Hait hoS, I ost or i AdasKi odt ho(c saski o sct s dasct ouct hasact odt hobS Modt hov *- Sost o(c saski odt hot i AdasKi occt V s dasct ouct hasact Ro

2/P/ Statistical analLsis

D Inask/dgsdaiodt dne son) gel A) of get us(dnoi en (ctitaoMt dne son, SIMm) E doud((nsihoacoa) iongst Bast AoE daigo dn (ni oli st Aoz GrioMDor Oluiro a das asudno cfael dgiofgen oMh st cfangsfolgs holtgalt uin bol eggindaset odin et A ask/dgsdLri o pfs*holfsxholfschut holbsbur maEigin lottaslihod ona) io.l dgi houe st i ocfona) isgo dt Ari opt3echbast A HoldLniofitum oft hoe Ligik dast Hold n(ni HoEigingin dasho co a) iok/dgsdLri optiou gin bo

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

Ocod i oa) i o(i gfc gn dt ui oc foa) i odt dreasudron i a) ch Hola) i orst i dgsael duul gdueHø(gius set Hødt host agl n it adropsGQW modt hon ia) chopD GQW n rsn sa ocfo.ldt asludasct o Eigiod i ih RoO) iost agln it adro.ldrsaeo(dV gdn i aig oEigioudrul rdaihd st AcaitoudrsLgdasctoul gKio(cst a ost oach iti) hsffigit æsdæst AcaE corst i dgsæ ogdt Ai ofgen dkik PoacoPkouA6Godt hodo i uet h udrsLgdasct oul gKi ofgcn oPkoacowkkkoµA6GRoxt agl n it adrorst i dgsaeodt h gdt Ai oEigioi asn daihd st Aorstidgogi Agi sct oE sa) oAc chogi l ra op T^{50} k F9mRoxt aqln it adro(gius sctopt oo⊨ PmMai0(gi ihod oa)ioqirdasKio adthdgh hi Ksdasct opT fiv; muc fost agdVhdeodt host ai gVhdeogi (i dadLsrsæoE d oi Kdrl daih Roxt ordmoud i Hon) i o(gi us set dEd orc Eigon) dt owk; oft ho5P; of egost agdV dt host aig Winde oi Kdrl das ct Hogi (i uas Kire RO) i ox GQ vo Ed oi asn dai hofgen a) iost Jiuasct ocfo igsdrohsd asct Holadgast Aofgen oa) iore Ei ao(est aocfoa) i udrsLgdasct oul gKid tasroa) io(idBoEigiot cadhiaiuaihRoO) itHaa) iorsn sa ocf . I dt as I udasct o Eigioi ad Lrs) i hod oa) i orc Ei ao (cst aost oa) i oudrs Lgdasct ul gKi HøE) sri oa) i on i a) chorsn sa ocfo. I dt as1 udasct opD GQWmoE i gi oudrul rdai houct shigst Aca) i ouctuit agdaset ofduae gRo

D dagsOoiffiuao Ed oiKdrl daihofcgoidu) odt dreaiHoLeoucn (dgst Aoa) i rc(i ofgen oudrsLgdaioulgKi ohs crKihostoacrlitioperKitaoiOagduaset m dthohgst Bst AdE daigopn dagsOmBo

Occit 1 gi ca) i o 1 drsze occfora) i cgi 1 ra MaBo(gcuih 1 gdrod.rdt B oE i gi c(i gV fcgnihol st Adv Gwarfo SCI UC dai gazgdt fi ggi hast ca) i o dnion dt ti gaz oE dai go uct adstig od aca) i oj cli or Gran) gi dKcrit ai gj Mast ca) i o dnion dt ti gaz oE dai go d(oE dai go dn (ri Robrit B oE i gi ci Ozagduai hodt hodt drei hofcrme E st Aca) i o dni (d gcuih 1 gi od oa) i ogi aor fora) i odn (ri Roviai uzi hod y SG ost ca) i d.rdt Bo gi (gi i tai hori ca) dt o5; or fora) i odi.Ll th dt ui or fOD y SG ost ca) i d.rdt Bo st ca) i o dn (ri Math the E d ouct shi gi hore chi di ArsAsLiri RMrgi uc Ki ge or foi du) ac (i or fof cren i ggE d oudgegi hori al adot or gali ogi of c c shi or c Argdhio c foi Ki ge ort dra di gst Androre fong) i o ai (or fong) i odi t dre saudrit gruih 1 gi Ro

Mmcc faa) ioi.ls(n it aaa) dadE d d ih ofc go dn (nioi Oagduaect odt houridt Vo l (ouct s aihoc fo adst ni o aiiroc go Ard HonE) it i Kigo (c sLni Holaco it l gio c (aan dmunidt st Andt hogi hl uaect or fot.du BAgel t houct adn st daect RD daigsdr o Eigi ouridt od uuc ghst Acacca) i (g g cac uc roa) dae E d (gi Kscl rechi ugsLi hHolaco dKc shouge ouct adn st daect oc foa) ion (ni Ro Mmo dn (ni on dt s(l rdaect o Eigi oudgesi hocl aost od ordn st dgod sgo/c E d Lit u) ost od ouridt ordLe gdac ge oacco gi hl ui odsgLeg ti ouct adn st daect RD ceigi c Kig dohl g st Aodn (ni on dt s(l rdaect Ho I ceigi cud sgat Houri at t daect RD ceigi c Kig dohl g st Aodn (ni on dt s(l rdaect Ho I ceigi cud sgat Houri at t (Houri at mission at the state t Houri at t sect Fob Water Research 220 (2022) 118645

					Sgius sctopn = Pmo		
	xGQWo pfAno	xGQWo pµA6Gmo	D GQWo pt A6Gmo	TiucKigeo ; o	xt agdVo hdeo	xt ai gVó hdeo	
goh4l tC4H nl	kRwko	kRwko	klk5o	%ino	wPo	wqo	
goh4Futmdil nl	kRwko	wBkko	kÆko	%Po	w3o	5ko	
goh4iToerl nl	kЊРо	k₽ko	kRwko	PPo	wPo	w9o	
goh4eroe4H nl	klkPo	kRwko	kBk50	Pqo	wqo	5wo	
goh4Tt4rl nl	kRwko	kRwko	kBk50	\$50	WWO	w3o	
goh4pDin4hf cChoridl	kÆPo	k₽ko	kRwko	Р‰	w5о	w9o	
goh4ms idl	k₩5Po	k₽ko	kRwko	%50	wPo	5ko	
AMkg	k₽ko	k₽ko	kRwko	q8o	w50	w9co	

RmFH 2

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k\$kk3o	<d gqv="" o<="" td=""><td>w\$\$qPo</td><td>w8\$w₽o</td><td><d gqv="" o<="" td=""><td>] * fio</td><td>4 i ao</td></d></td></d>	w\$\$qPo	w8\$w₽o	<d gqv="" o<="" td=""><td>] * fio</td><td>4 i ao</td></d>] * fio	4 i ao
k\$kkPo	qIko	<d gqv="" o<="" td=""><td><d gqv="" o<="" td=""><td>PP3Po</td><td>] * fio</td><td>*dao</td></d></td></d>	<d gqv="" o<="" td=""><td>PP3Po</td><td>] * fio</td><td>*dao</td></d>	PP3Po] * fio	*dao
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k\$kk90	5k5Bko	<d gqv="" o<="" td=""><td><d gqv="" o<="" td=""><td>93Po</td><td>] * fio</td><td>4 i ao</td></d></td></d>	<d gqv="" o<="" td=""><td>93Po</td><td>] * fio</td><td>4 i ao</td></d>	93 P o] * fio	4 i ao
k\$kwko	%3 H \$0	<d gqv="" o<="" td=""><td><d gqv="" o<="" td=""><td>w3qH8o</td><td>y Qo</td><td>4 i ao</td></d></td></d>	<d gqv="" o<="" td=""><td>w3qH8o</td><td>y Qo</td><td>4 i ao</td></d>	w3qH8o	y Qo	4 i ao
k\$kwwo	<d gqv="" o<="" td=""><td>3P9Rvo</td><td><d gqv="" o<="" td=""><td><d gqv="" o<="" td=""><td>] * fio</td><td>4 i ao</td></d></td></d></td></d>	3P9Rvo	<d gqv="" o<="" td=""><td><d gqv="" o<="" td=""><td>] * fio</td><td>4 i ao</td></d></td></d>	<d gqv="" o<="" td=""><td>] * fio</td><td>4 i ao</td></d>] * fio	4 i ao
k\$kw50	58B0	<d gqv="" o<="" td=""><td><d gqv="" o<="" td=""><td>5\$8R⁄ø</td><td>y Qo</td><td>4 i ao</td></d></td></d>	<d gqv="" o<="" td=""><td>5\$8R⁄ø</td><td>y Qo</td><td>4 i ao</td></d>	5\$8R⁄ø	y Qo	4 i ao
k\$kw8o	PwBo	<d gqv="" o<="" td=""><td><d gq="" o<="" td="" v=""><td>P\$Po</td><td>y Qo</td><td>*dao</td></d></td></d>	<d gq="" o<="" td="" v=""><td>P\$Po</td><td>y Qo</td><td>*dao</td></d>	P\$Po	y Qo	*dao
k\$kw3o	wqRBo	w3w\$P9o	wkk%790	w%æPo] * fio	4 i ao
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k\$kwqo	<d gqwo<="" td=""><td><d gqv="" o<="" td=""><td><d gqv="" o<="" td=""><td>553Bso</td><td>] * fio</td><td>4 i ao</td></d></td></d></td></d>	<d gqv="" o<="" td=""><td><d gqv="" o<="" td=""><td>553Bso</td><td>] * fio</td><td>4 i ao</td></d></td></d>	<d gqv="" o<="" td=""><td>553Bso</td><td>] * fio</td><td>4 i ao</td></d>	553Bso] * fio	4 i ao
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k\$kw9o	w5wP9o	wwk8Rqo	<d o<="" qv="" td=""><td><d gqv="" o<="" td=""><td>] * fio</td><td>*dao</td></d></td></d>	<d gqv="" o<="" td=""><td>] * fio</td><td>*dao</td></d>] * fio	*dao
k\$k5ko	99 P o	<d 0<="" gov="" td=""><td><d 0<="" gov="" td=""><td><d 0<="" gov="" td=""><td>] * fio</td><td>*dao</td></d></td></d></td></d>	<d 0<="" gov="" td=""><td><d 0<="" gov="" td=""><td>] * fio</td><td>*dao</td></d></td></d>	<d 0<="" gov="" td=""><td>] * fio</td><td>*dao</td></d>] * fio	*dao
k\$k5wo	8\$R⁄o	<d gov="" o<="" td=""><td><d gov="" o<="" td=""><td>wł5o</td><td>y Qo</td><td>4 i ao</td></d></td></d>	<d gov="" o<="" td=""><td>wł5o</td><td>y Qo</td><td>4 i ao</td></d>	wł5o	y Qo	4 i ao
k\$k550	<d 0<="" gov="" td=""><td>wq\$kP9o</td><td><d gov="" o<="" td=""><td><d 0<="" gov="" td=""><td>] * fio</td><td>4 i ao</td></d></td></d></td></d>	wq\$kP9o	<d gov="" o<="" td=""><td><d 0<="" gov="" td=""><td>] * fio</td><td>4 i ao</td></d></td></d>	<d 0<="" gov="" td=""><td>] * fio</td><td>4 i ao</td></d>] * fio	4 i ao
k\$k580	35雨	3% ako	<d gov="" o<="" td=""><td><d gov="" o<="" td=""><td>] * fio</td><td>4 i ao</td></d></td></d>	<d gov="" o<="" td=""><td>] * fio</td><td>4 i ao</td></d>] * fio	4 i ao
k\$k530	₩%#80	<d gov="" o<="" td=""><td><d gov="" o<="" td=""><td>8859F9o</td><td>] * fio</td><td>*dao</td></d></td></d>	<d gov="" o<="" td=""><td>8859F9o</td><td>] * fio</td><td>*dao</td></d>	8859F9o] * fio	*dao
k\$k5Po	88 R ⁄ø	wkakR⁄ø	<d gov="" o<="" td=""><td><d gov="" o<="" td=""><td>1 * fio</td><td>*dao</td></d></td></d>	<d gov="" o<="" td=""><td>1 * fio</td><td>*dao</td></d>	1 * fio	*dao
k\$k5%0	<d 0<="" gov="" td=""><td>a%98₽0</td><td><d gov="" o<="" td=""><td><d gov="" o<="" td=""><td>v Oo</td><td>*d ao</td></d></td></d></td></d>	a%98₽0	<d gov="" o<="" td=""><td><d gov="" o<="" td=""><td>v Oo</td><td>*d ao</td></d></td></d>	<d gov="" o<="" td=""><td>v Oo</td><td>*d ao</td></d>	v Oo	*d ao
k\$k500	35\$190	< D GOv o	< D GOv o	3P8\$P0	v Qo	*d ao
k\$k5\$o	P3HSo	w%5PB90	<d 0<="" gov="" td=""><td>wk3Bso</td><td>1 * fio</td><td>4 i ao</td></d>	wk3Bso	1 * fio	4 i ao
k\$k590	3PF90	9%MB0	< D GOv o	88aBko	1 * fio	4 i a0
k\$k8k0	< D GOV o	w83P190	<d gov="" o<="" td=""><td><d 0<="" gov="" td=""><td>1 * fio</td><td>*d ao</td></d></td></d>	<d 0<="" gov="" td=""><td>1 * fio</td><td>*d ao</td></d>	1 * fio	*d ao
k\$k8wp	w5180	559k Boo	< D GOV o	wRwn	1 * fio	*d ao
k\$k850	w8190	<d 0<="" gov="" td=""><td><d 0<="" gov="" td=""><td>aRPo</td><td>y Qo</td><td>*dao</td></d></td></d>	<d 0<="" gov="" td=""><td>aRPo</td><td>y Qo</td><td>*dao</td></d>	aRPo	y Qo	*dao
k\$k880	< D GOV 0	< D GOV O	< D GOV o	39150	1*fio	*d ao
k\$k830	w3190	9w85B60	< D GOv o	39%%] *fio	4 i an
k\$k8Po	<d cov="" o<="" td=""><td>ww8al50</td><td></td><td></td><td>y Oo</td><td>*d ao</td></d>	ww8al50			y Oo	*d ao
k\$k8%		a3a%#%		awkBio	y Qo	4 i 20
k\$k8ac	wPIPo	19997000 1998Rap	w\$9aBe	<d gowb<="" td=""><td>1*fin</td><td>4 i 20</td></d>	1*fin	4 i 20
k\$k8\$0				DIRO	y Oo	4 i 20
141900		<d gqv0<="" td=""><td></td><td><d comb<="" td=""><td>y Q0 1 * 6o</td><td>- 1 i 20</td></d></td></d>		<d comb<="" td=""><td>y Q0 1 * 6o</td><td>- 1 i 20</td></d>	y Q0 1 * 6o	- 1 i 20
k\$k3kc	D3Rio	<_D GQV 0	vak 8Pc	<d gqw0<="" td=""><td>] 10] *fio</td><td></td></d>] 10] *fio	
k¢k2un	¢ Buo		<d (ouro<="" td=""><td></td><td>y Oo</td><td>*d 20</td></d>		y Oo	*d 20
KØKOWO	φηνο	< D QQV 0			y Qu	on an

^N viaiuaihol tasrouct 1 hituioriKiro5Ro

b. 31 Tuht T mnd diTcuTTion

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

xt oOdLri owłów XQQW HóD QQW Hógi uc Kigsi Hódt host ai głódt host agd Whdeo gi (i dadLszsae dc gna) i odt dre s oc foD SG ost chryst Bst Aodgi oln n dgs i hRGT i V uc Kigsi oE i gi oc Ladst i hoE sa) olst al (rsudai Hóc Ladst st Aogi lra ofgen oPPo pSxmaec & S; qrSfin RUC ch dD GQW dE i gi oc Ladst i hofe gna) i oi ri usi hof (ren i g o st ohgst Bst AoE dai gogdt Ast Aofgen ok RS oa cok RS ot AGRoD dagsOoi ffi uaoE d o



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wix. 1. I ctuitægdæct or fon sugept dten úrd æsu op Dy SG most ot A6Godnet Ana) i ohegst Bst Ade dai gol ((reor fon) i obelgui ret do Diæge (ensedt of Mgi dop D Mmi o) (gi i hod oul n l relækio l n ost oa) i old i owkore Adgea) n suo udri Rovid d (ovi ti gdai hole of UccAri on d ("i gki gefgen of M() d Li aout uRpi dersfegt solheli Mred t sai offadai med

i asın dai hoLeoucn (dgst Aoa) io rc (i ocfo adt hdghoul gKi ohs crKi host o i 0ægduæct o crKi tao dt hon dægs0Vn dau) i houdrsLgdæct oul gKi Ro Ti I ra o uct I gn d (or cPk; crfoect ol ((gi sct dcgshgst Bst AdE dai gKO) i i gi I ra o) sA) rsA) ava) i o i in dægs0Vn dau) i hoadt hdghoudrsLgdæct oul gKi for I dt ædtakKi oft dre i Ro

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

Mol (iuaougiitst Acd((gcdu) dE d cd((rsihoacost Ki asAdaica) iq(cait V asdrocuul ggituiocfo(cren ig ocfoD y SG ost oa) iogdt Aiocfok Ryk—Skoµn o hsdn iaigst od(dE daigodn (ri oa) daE igiocrniuaihdaa) id85c(cadrochi o cfoa) idoD MKO) iogi lra ocfoa) so al heoait adasKi reschitaslihoqo(cren ig odao uct 1 hitu id Kiro5qs% MoSSMoS MoSb V MoSfadt hos v D finabat hos oborfaa) in o E igiocct 1 gn ih d_eo adt hdgh quct 1 hitu ici Kirovmq6 * MoSfab MoS Mo dthoSfinBA) sridcl go(cren ig dE igio(gi itast cdaoid actiocfoa) iodn (ri o dauctuitagdasct or Kiga) id0 CQWRp6 * MoSMoS V Mot hos Srimb Findt hos Nori Kir o E igiodret da da cda da contine con i a do continuation action of a dauctuitagdasct or Kiga) id0 CQWRp6 * MoSMoS V Mot hos Srimb Findt hos Nori Kir o E igiodret da da tree a) id0 CQWRp6 * MoSMoS V Mot hos Srimb Findt hos Nori Kir o s cí gi it a host ct AccGRot cOdLiri df Finda) i cu) dgduai gsFdasct co fedrava) i c(creV n igsuca) dst cfcl thost ca) i cut dre in hod n (ri os o) cE ih Ro

Qt reactioclaocfaa) i d350 dn (ri odt dre ih op 5178; måhshot ca(gi it adt eo (cren igoda l dt asadaski ouctuit agdasct RMr cloist oct reactio dn (ri Hafcl go hsffigit ao (cren ig o Eigioshit aslihodt ho. l dt aslih Royti Hode E dok goa) gio (cren ig o Eigioshit aslihodt ho. l dt aslih Royti Hode Rei dok goa) gio a) io dn (ri Hagi (iuaski re Rot o CsARovHagi) iohs agsLl asct or for (cren ig odt hoa) io c Ladstihouctuit agdasct ot di kige d54 (ouc roho) roho J ct Ho

O) ion c adfgi.litareofcltho(crenigoEd o5*Mohiaiuzihoottho ldtasliho daoqwodtho%); ocfoa) iodt dre iho dn (ri Moigi (iuzsKireRoSSo Ed odr co

dn (ri Hogi (iuasKireRoO) i iogi lra odAgiioEsa) oca) igo(giKscl ost V Ki asAdaset oc foD SG ost chast Bst AcE dai godt hoad (oE dai gleist dE) su) oS* odt ho SSoEigiofgi.litarechiaiuaihopDstaitsAciaodrRef5kw9:oSsKcBct BeciaodrRef 5kw\$nBS * odt hos So) dKi oddE shi ol dAi Host url hst Aq(dga or gq(s(i ost dagst Bst Ao E daigo e ain odthoKdgscl o) cl i E dgiosain Holdthoa) sofduaoudtoi O (rdsto a) isgo(gi it ui ost ohgst Bst AoE dai go e ain od ot cai host oD st ait sAoi aodrRo p5kw9nRovi (saica) io) sA) ofgi. litueocfoa) i ioaE co(crenig Hoa) ieocuul ggiho daoncEouctuitagdasct RoxtoP\$; ocfoa) io dn (ni oEsa) oS*Houctuitagdasct o EigidLincEdPkotA6GHoldtho\$k; ocfoa) iodn (ni o/gi itaihouctuitagdasct o Lirc Eowkkot A6GRont oCsAR5Hoa) i oLc Oodt hoE) s Bigo(rcao) c Eoa) i ogirdasKio dLlthdtui ocfodn (ri oAgel (ihoduucghst Aoacoa) iouctuitagdaset ogdt Aio fcgoa) iohsffigitao(crenig RO) iouctuitagdasct ocfoSSoEigiodrcoLircEoPko t A6Gofcgon c aocfoa) io dn (ri RoO) ioa) sehon ceiofei. litareo hiaiuaiho (cren i goE d oSxop35R9; nbbidt host odhhsasct oa) s o(cren i goE d ofcl t hodao) sA) i gouctuit agdasct ost o (iusluodn (ri ogidu) st Ac9op dn (riok\$Hit83nboling) pdn (ri ok\$Hk5% odt hok\$Hk8% nbholt ho&90, A6Gopdn (riok\$HkkqnbbSxoird acnigo dgiol ihoston dteoith Vt iod((rsudasct ogi.lsgstAoAcchogi srsituiHarcEo E daigo E innto) sA) gait srig agit Aa) Ho Ac choadu BHodtho) sA) g) cagait srig agitAa)RoCcgoa)sogid ctHoa)iordgAi acithVi iofcgo(cresc(gitiHoLeofdgHoso stonegi Roxtodhhsasct HaAln oSxVLd ihoucn (clth ordgiol ihostod LLigeLdth Ho LdLeoLcaariots((ri HolthoiOaglhiho)ci od oa)ciol ihostocnioad(HodrctAo Esa)oca) igol i RD cgic Kigłofsxos odr col ihosto idrdta odthodh) i ski RS by o Ed oshit aslihodt holdt aslihost of clgor foa) io 85 odt dre ihodn (ri op 91P; mBo D cgic Kigłobace d ofcl thodaouctuitagdasct oc Kigowou A6Goo dn (ri ok \$Hikk 3Ho k\$Htw3Hok\$Ht8qHok\$Ht3kmBtSbvosodrcol ihofcgoa)iofdLgsudasctocfoasgi Honco sn (gcKica) ion iu) dt sudro(gc(igasi ocfo6 filde goac dLid ihod odh) i sKicfe go S*R&MoEd odr cohiaiuaihodaoshitas1udasctoriKiro5osto8q; ocfoa)io.dn (ri Ro

Chapter 6



wix. 2. bc0adt ho4)s Biga(rcaRD sugept dt cm/rd asu qxD y SG mafelt host odn (ri oAgel (ihaduucghst Acacca) iouct uit agdaset ogdt Aidfegaa) idhsffigit aa(cren ig oi0(gi ihost ot A66GR0

- cEikïgłón jiouctu utagdasct oz fós McEigiót thigan jiób CQW kyk Rok da A6G mato o dmonajio dn (n. Rol Ma) cIA) o S, Ios ol i host o (s(i of dLgsudasct of cgo Edaigo agdt (cgaost chagst Bst A6E daigo I) (reo e ain IdLiudI i oz fosa ogi s adtu i oz co dLgd sct Hóst on) io (gi i tao al hect cticz foa) io dn (ni o) cEihouctu utagd V asct orkigan i o GQV 1400 ji i aec (i oz fot (si odigion cn (c i hor foa) io S, Ion dags00 pu) dgduaigs i hoLeosa o) sA) chi Agiio cfoucn (duasct odt hogc LI ati modt hodo n s0 oz fót (rd asus i gorde i g ost oa) io I gfdui odh cgLi hoac oa) io (cren i gsuon dags00 pu) dgduaigs i hoLeosa o) sA) chi Agiio cfoucn (duasct odt hogc LI ati modt hodo n s0 oz fót (rd asus i gorde i g ost oa) io I gfdui odh cgLi hoac oa) io (cren i gsuon dags00 Pu v li oz cE i dBd.ct h Hót nd zavadhhsæk (i on sA) adui or du) i hoac oa) ion dags00 crki go asni odt ho 'c E dE sa) chegt Bst A6E daig dE) sin on ca S, I ogin dst d tu) dt Aiho st oa) io (s(i HodK cshst Ao(cren i gsup dAn itadasct o (gcui i Rov S/S thia) ero) i o) coro () a) drdai o pr * . Smód chou) dgduai sa suo (rd asus i gol i hos to S, Io

- cEiKigda) i uctuit agdæct or f& MÆ i gi ol thiga) i oD GQ Wopk Rvk ut A6Gmato dmoa) i o dn (ri RvMra) cl A) oS, I os ol i host of (si ofdLgsudæct of cgoE dai go agdt (cgaost dhgst Bst AÆ dai gol ((reo e ain KLiudi i or foa qei s adtui dano) i o kei host on) i o kei li host of (si o al hect.ecf dgBo i adht Q5 thest obgi d gi i tao al hect cita fraaj i o dn (ri o) cEi hout tuit agdav ext or kiga) i oQ v RvDi i ae(i or foi s(i or foi s(i

O) i ouct uit agdasct orfo(cren ig orfoD y SG ofcl thoston) so al heogdt Aiho fgrn owot A6GaceOnµA6GaE sa) on ihsdt ofcdr i orfows9ofcgS*Holw&33ofcgSxHolw&%o fcgSbv Hohtho5q\$ta A6GafcgSxSHO) i idfdhi ordigiohsfl ul razocucn (dgi dE sa) o ca) igo al hsi ort on) iocuul ggi tuiocfoD SG ohgst Bst A6E daighdi ul on c ace fo a) in ogi (cgaihoa) iot In Ligorfof dgasuri ost aidhorfouct uit agdasct Ro cEV i Kighof sg aist of 7 sg aist oi achtHohsfsSown) di oi ach a daihoa) iort uit agdasct orfo



A/Qeya-z errera et al/

wix. b. Gedhst Ao+ fiuegi dLs(reappdLcKinndthofiuegi qdLineEnn(reaon dhidLeoSystus(droien (ctitadMtdnes qdSIMndthod((nsihoneand(dEdaigo dn (ni Riitagesh dEigio Aitigdaihod)ac adaudmedEsa) ddoPp; o(geLdLsssaecaecostud hica) io dn (ni ocfoidu) o(e adrouchiocfoa) iob dguiret doDiage(ensatt dMgidqbDDMnBo

• East (Obs) • West (Obs)

F1 (38,30%)

A/Qeya-z errera et al/

D y SG ofc goa) iot l n LigocfoD y SG o(dgasuri ofcl t host ohgst Bst AoE daigo pk-k1555D y SG 6Gm2 sa) dKdrl i odgel t hovk ot A6G40E) su) odgi orc E igaa) dt ot o cl go al he10- cE iKigtelosao) cl rhoLio) sA) rsA) ai hoa) daoca) igo al hsi ocfo hgst Bst AoE daigogi (cgai hodon l u) oj sA) igot l n Ligorfo(dgasuri op%46w\$Heltho 3qkcD SG 6Gmp5sKc18ct Bed andrHel5kw\$;c7 c l a) of andrHel5kw\$;cfi) gl aso andrHel 5k5KmHelthosfoa) i ogi l ra odgi ogqdt fcgn i hoac on d oKdrl i Hela) it ocl go Kdrl i oE cl rhoLiost oa) i on i hsl n ogdt Ai ocfouct ui t agdasct oa) daodgi ogi V (cgai h10- cE iKigtelosao osf1 ul raoacoucn (dgi ocl gogi l ra oE sa) oa) c i ocfo (gi Ksc1 o al hsi 10

xt oCsARovlaksacudt oLioiit ca) daca) i oE i aigt o(c adruuchi ocfoa) i ob D Mo (gi it acdo) sA) igsKalgsi ac ccfo(cren ig ca) dt ca) c i ost ca) i oi di aigt odgi dRoaco) cl rhoLion it ac ti hca) daca) i oE i aigt odgi dos ol ((rsi hoLeotoheset Bst Ao E dai gazgi dan it ac(rdt acov 4 OSma) daco crudai host ca) i on lt sus(drsæcu fofiaRo _cdt ovi (foE) c i oudau) n it actig dos odran) i oCrL gi AdadTsKi gobE) sri ca) i o i di aigt odgi doc foa) i ob D Mos ol ((rsi hoLeoat) i on lt sus(drsæcu fofiaRo i c or 4 OSIdE) c i oudau) n it acs odraa) i obi c ò dTsKi globUm ct sac get Ao al heo cforesai gost oLca) ogsKi g o Ed o(i gfc:gn i hohl get Aoct i cei dgost ofskw%-Skwqo pfu) sgost Fsoi aalrHobk/SkrhhdE) i gi Leoa) i o(rd asu(crml asct ca) dadE d ofcl t hoE d o rsA) ared; Ai i gost on) i ofscLgi AdagSKi goucn (dgi hac ca) i of c ogsKi globE) su) o s ot chuce/pht ui dE; sa) ocl gogi l ra lo

xt oCsAR0vMakacudt odr col.ioiit ca) dao) sA) igcuct uitagdasct of igioldt V aslihost oa) ioE i aigt o(c admuchi ocfob D Mol dgasul rdgneofcgoSxolthoSbov RoOco i0dn stiodt ecoagithodn ct A ao dn (nst Ao sai MoldoSI MoE d oudgesihocl ao pCsAR6mdthoBofduacg dE igiou) c it oz cuckigodmorfoa) ioKdgadLsszee qDOdLrio fishmbel) igioa) id g aa Ecfduacg oi0 (rdstiho%319); ocfoa) ioacadroKdgadLsszeRo Mo Idrasdaskiö KdgadLniMon (asko gairda hoacca) idnest Bist AdE daiget) su) os o

I ((rsi há.ediæ) oct ov (jat há.edi pol) da s g i ta hac di) da s to hac age / si o o I ((rsi há.ediæ) oct ov (jat há.ediæ) hagsdah i nöt age / sa s o oct o da s o da s

S. onchuTionT

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O) i of gen dge o'Acdroc foc l go al he d'E d'on c'hi Kirc (odoge Ll and tho it sæk ki o . I dt asadaski oft dre asudron i a) chloE sa) d'E) su) oac oft i on) i ou ct uit agdasct o c fc(cren ig ost dD y SC opst on) i ogdt Ai or fck Rgk – Skoµn most chogt Bst AdE dai gide l st Aadon st sn l n o dn (ri o SFi or for GRO) i on i a) cho) c E i hoa) i of gi i tui or fo S* Ho5 filo Stob by Ho5 SHo5 Molith Ko's D finst chogt Bst AdE dai go dn (ri oa) da E i gi o ucrri uzi hodano i i oad (RoO) i o ski sl udt and hKdt ad Ai oc foa) s on i a) cho so st o (ge Kshst Ao l dt as li hou ct uit agdasct oc fc(cren ig odaoa) i on drogdt Ai oc fo (dgasuri oE sa) oj sA) o i t sæksæ opD GQ WoLi aE i i to kRs5–kRvkot A6GmbO) i o n dst osm sædasct oc foa) s on i a) chos oa) da D y SG of dgasuri odgi ot caucel t ai hloi a) l on dBst Asædnsfl ul raæcoucn (dgi oa) i ogi l ra oc foa) s o al he dE sa) oa) c i oc foc caj igo al hsi. Ro

O cocl goBtcErihAiHoa) sosoa) iolg ao al heogi (cgastAoa) iouctuitagdasct o cfo(cren i gsuo(dgasuri ost oa) i ogdt Ai ocfok Rqk-5koun ost o) cl i) crhoad(o E dai of ME) su) os ogi (gi it adaski octodo E) criousae BoO) i ot i E oc (aso s i ho dt dreasudron i a) choEd od((rsi hoac odt ost Ki asAdasct ocfoa) i ocuul geit ui ocfo D v SG ost ca) i chest Bst AcE dai goc foa) i cb dgui rct doD i agc (creadt oMgi dHadt ho a) iouctuit agdasct of clthoEigiodaoa) iot A6Gori KirRoO) io al heogi lra o gi Kidrihoa) dao S*Hod SxHod Sby Hod SSHoldtho Sy D fice i gioa) ion caofgi, litareo hiaiuaihoDySGRoDcgicKigHonfclgocfoa)in oEigiodaouctuitagdasct ocKigo D GQWqqS*HdSxHdSbyHddthoSSmhddthoSxcEd qa)iq(crenigqfclthodao)sA)i ao uctuitægdæsct ostoctio.dn (rikoleidu) st Acolou A6GRO) i iojdæsul rdgo.dn (ri o udt dLiost /lituihoLeofgdAn it a ocfoa) ioBsau) it oad(Hahdn dAihofdluiacQVo gstAleforgo) c i ocfoa) ioad(Ro-cEiKigken) iouctuitagdasct of clthostologstBstAo E daigcEigiorcEH6Esa) on ihsdtodthon idtoKdrli ocfoa)ioln ocfoa)ioacadro (crenig on id lgihostoa) io dn (ri oLiaEiitowP\$8odthoPw3otA6GRoQlgo gilra oudttcaoLioucn (dgihoEsa) o(giKsclo alhsi Hol(gsn dgsne HoLiudlio n c accfoa) in odgio Ld i hoct our lt ast Ao(dgasuri Hodt hon c accfoa) in o

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i0dn stihordgAigs(dgasuriogdtAi RCstdmekola)ioucn (dgs ctoLiaEiitoEdaigo l ((rsihoLeon)ionEcor 4 OS or (igdastAoston)iobD Mbol) cEihotcosAtsludtao hsffigitui Ro

xao) cl hhd.i (c cst ai hocl ana) dana) i gi os ot conl ggi taohsgi uzaki odLcl ani kir o cfoD y SG ost chagst Bst AoE dai glob (dgaadmechl i cocca) i orduBoc fo adt hdghs i ho dt dreasudrod((gcdu) i oa) daodgi odLni oaco. l dt asfeo(cren i g ocfoa) i orcE o (dgasuri o sFi ogdt Ai dE sa) dt cl A) o i t sakksæl hdlt hoa) s od((gcdu) of gcKshi o ucn (rin i tadgeogcLl aost fcgn dasct oacoca) i godKdsrdLri od((gcdu) i Haco a) s di hRo

Al churntion o os el tinx Intl rl Tt

O) i coll a) cg chi undgi ca) daca) i eo) dKi ot coBt cEt cucn (i ast Aolt dt usdro st aigi a ocgo(ig ct drogindasct) s(ca) dacucl nho) dKi od((idginboacost / lituio a) i dE cgBogi (cgai host ca) s of d(igBo

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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

Albert Vega-Herrera

Marta Llorca

Xavier Borrell-Diaz

Paula E. Redondo-Hasselerharm

Esteban Abad

Cristina M. Villanueva

Marinella Farré

Water Research, 2022, 220, 118645

Figure S1 Tables S1, S2, S3



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

Variables	PE	PI	PBD	РР
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)*	С	Н	(CxHy)n
	PE	734.7384	52	105	26
	PP	1,464.5962	105	209	34
08001	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
	PBD	969.6010	72	108	18
	PI	1,230.0600	90	145	18
08004	PI	1,801.5976	132	212	26
	PI	2,278.0073	168	268	34
	PI	2,702.3190	199	318	40
	PE	843.8848	60	121	30
	PE	1,320,4040	94	189	47
08005	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
	PI	1.351.0752	99	159	20
	PI	1.895.5783	139	223	28
08007	PI	2,372,9927	174	279	35
	PI	2,508,1209	184	295	37
	PE	954.0210	68	136	34
	PE	1.866.0273	133	267	66
08008	PE	2.316.5010	165	331	82
	PE	2.708.8730	193	387	96
	PE	713.7182	51	102	25
	PE	1.744.5220	125	249	62
08009	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
00010	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
	PE	689.7159	49	99	24
08012	pp	1 475 5500	105	211	35
00012	pp	1,769,8776	126	253	42
	PE	1 276 3495	91	182	45
08013	PP	1,885 0841	135	269	44
00015	pp	2,389,6210	171	341	56
	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
08014	PI	2,498,1695	184	294	37
	pp	1.843 0237	132	263	43
	11	1.0 12.0227	104	200	10

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	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
	PE	869.9125	62	124	31
08017	PP	1,379.8775	99	197	32
	PP	2,389.6133	171	341	56
	PE	759.7957	54	109	27
08018	PP	1,180.2484	84	169	28
	PP	2,347.5442	168	335	55
	PE	999.8609	71	143	35
	PE	1,576.3666	113	225	56
08019	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
	PE	1,511.2924	108	216	53
08021	PP	2,347.5444	168	335	55
	PP	2,642.8739	189	378	62
	PI	1,392.2322	102	164	20
	PI	1,664.4728	122	196	24
08022	PI	2,278.0123	168	268	34
	PI	2,686.3187	198	316	40
	PE	678.6754	48	97	24
08023	PI	1,419.1419	104	167	21
	PI	1,691.3858	124	199	25
	PE	678.6780	48	97	24
	PE	1,756.9084	125	251	62
	PP	1,366.3239	98	195	32
08024	PP	1,927.1161	138	275	45
	PP	2,221.4312	159	317	52
	PP	2,599.8181	186	371	61
	PE	706.7065	50	101	25
	PI	941.6989	69	111	14
08025	PI	1,623.3251	119	191	24
	PI	2,371.9895	174	279	35
	PI	2,509.0958	184	295	37
	PI	863.7307	64	102	12
08026	PI	1,596.4202	117	188	23
	PI	2,550.2749	188	300	37
	PE	1,262.3320	90	180	45
	PP	1,180.2403	84	169	28
08027	PP	1,885.0915	135	269	44
	PP	2,515.7480	180	359	59
-	PE	822.5056	59	118	29
	PI	1,351.0743	99	159	20
08028	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

	PI	1,801.6050	132	212	26
08030	PI	2,209.9541	162	260	32
	PI	2,702.3135	199	318	40
	PE	706.7101	50	101	25
	PI	1,487.2128	109	175	22
08031	PI	1,759.4586	129	207	26
	PI	2,167,8334	159	255	32
	PP	2,136.3268	153	305	50
	PE	1.262.3007	90	180	45
08032	PP	1.306.3748	93	187	31
	pp	1.642.7282	117	235	39
	PP	1.843.0208	132	263	43
08033	pp	2.053.2468	147	293	48
	pp	2 683 8990	192	383	63
	PE	706.7098	50	101	25
	PI	1 324 1679	97	156	19
08034	PI	1 733 5346	127	204	25
0000	PI	2 225 9508	164	262	33
	pp	2,223.3300	147	202	48
	PI	873 6480	64	103	12
08035	PI	1 419 1671	104	167	20
00055	 	1 750 /803	120	207	20
	DI	2 208 0302	162	260	32
	DI	795 6674	59	94	11
	 	1 665 /80/	122	106	24
08036	DI	2 482 2202	122	202	24
00050	DD	1 054 1065	75	151	25
	DE	706 7112	50	101	25
		762 1112	57	85	14
08037		1 555 2709	114	192	22
08037		2 011 1020	114	297	23
		2,011.1950	144	267	4/
00020		2 500 9252	132	203	43
08038	PP	2,399.8332	180	3/1	45
08039	PE DE	2,595,0012	91	260	43
	PE	2,383.0913	183	309	<u>92</u>
		2,179.3900	130	311	22
00040	PE	629,6236	45	90	22
	PBD	599.4836	44	6/	11
08040	P1	1,419.1498	104	10/	21
	PI DE	1,827.5242	134	215	21
08041	PE	6/5./048	48	97	24
	PS	1,593.9412	123	123	15
	PS	2,010.1869	155	155	19
00040	99	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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Marinella Farré

Chemosphere, 2023, 343, 140106

https://doi.org/10.1016/j.chemosphere.2023.140106



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Ndomhhufued Ao gor ann fupomhhufuz noi g oh f pf hAoi Q g om faa mufoed oe fol Q s oi muor g n dfoud o/ 55@ oe fol Q om os r a nib Of nDraff growthor antipun gool g of Q os enforg Bi dfa) ouh dfu9 hToMor gregfunnfuedonfi h) ol muor gregs hoi nud Eono s i afub JONkos eh aautEoneffling Al Q g oDunt. b fQ) aQ c) a(omhur mfondhoDunt. b fQ) aQ c) a(or QfQnamfol g o nfus mf ho mmofQ os enforef dfumaa) oQmgs fi aques rei dhnofie goQi s mdoQ mafQTo

2 z gmaaA9 dhudEmoni EE nforQmfoDeffa hol mf gounomodedhol EauEuDa ogeif ofeo crenig ofeoV0 y*nRoo

1. Introduction

Ndog p dfo) mgnAog a zndfo ffiegfnoQnz oD dos mh ofeomm nnofQ o eppigg dp ondho ffipfnæfis upger annfupnot V y*n(ædofQ o dzugeds dfondho Qi s ndoQ mafQot: e as ndno fonalAa 5/PDfb*ui o fonalAa 5. 5fb*ægpno fonalAo . 5. 5D(TeV y*nong opamme9 houdfeofl eos mudopamm nTevgus ng) oV y*nong o fQen of Qnfo df gofQ o dzugeds dfoudors maanuF nof Qei EQfofQ ugodf dh ho in Annahon nedhme) one of Qen of en Fs df hourdfears mean FnoD) o dzuedb s dfnaar gep nn noni pQonnor Q) nupnaAppQ s upnaondhoDue æ Eupnach Egnhinfuedo rgep nn not: us o fonallAo 5.. (To

v fgus dfnao ffi pfnommepunf hd ufQdV y*nordpai h opQ s upnadnns mE no r gehip hoD) om hufuz mondofQ ugofiegs i an fued nofQmfopm do mmua) campQoD pmin o fQ) ong d mila) oDedh hofeofQ or ea) s gnotD) oz moldh go\$mmanoDedhn(T62 do fQ or fQ grQmthAnnhnegr fued opmr mpuf) or for ea) s gnofinpuatinf nodegEndupontho egEndupopedfms udmdfnor g n dfoudoni ggei dhudEo dzugeds dfnomppi s i amb fued ced or ngfupa cmpfuz ori gfinp nondhof gnd nr egfofe oDue fnut Hge UndoQegn of b fipf(dt*ægpno fonalle 5. 5m(TeV eg ez gehin de dO ugons mænn F As upgebondho dntder annfipnotV0y*n(opndoD ofgndnæpnf hofQgei EQofuni nAor gehip o r O) nuonachus nE Audi us s nfuedondhoDues nEdu9 pufuedofOgei EOofO ofgeb r QupopQmdotKgndhfno fonalAa 5.. (To

HO of DuBi uf) of the variation of the v geds dfondhoQis mdoQ nafQondhong opannnu9 hotel mhm) nonnopedfns udndfno efos gEudEcopedo gdotRSRn(To, el z gAorfO g on gonfuzzol del a hE oEmrno pedp gdudEofQ ugo dzugeds dfmaoa z anondhofQ os mudogei f noefioQi s mdo crenig chi cfectQ caus ufnfuednoudofQ ugondma) nunïtvi gudEctQ canufo) mgnAo nns radEondhorg lfg nfs dforgep hig nonfngf hofeoD onfndhnghun ho tOgi fuo fonalita 5. / (To, el z gAN y*nondina) nuncunos muda) oDmn hoedonr pb fgenperupof pQduBi nAonipQomnojieg np dp AoubiHNxonndhoubxMVMDo tOpQ)s ndnlup fonal 5/qAp 5. / (Appes Dud hd ufQos upgenper) diegor ngfupa o pei dfudETbI egofO n og mædn/ømrr gempO noni pOomor) gea) nulEnnondho QuEQhr gfiegs mdp oauBiuhopQges mfeEgmrQ) opeira hol ufQoV mmoOr pb fges fg) gtv) gb RbV QAa v*RbV Q(gt, gs mD mu g ongthox epOs mg/Aa 5, /fb L Enh gg gno fanal 5. /n(ang and na) fupnaam r genpQ nafQnfapnalar gezuh o dQndp hor gfegs mdp oudof gs noefioBi ndfufnfuz ondna) nunoefioV y*nBo Veg ez gAuddQ opmn oefidinder amfupnot0 y*n(Ad 1 onns radEondhonns ra o rg lfg mfs dfonfgmf Eu nopes Dud hol ufQoudnfgi s dfmaondma) nunoi nudEo f pQduBi nodefoaus uf hoD) or mgfupa nonuF omg od h hofeoez gpes of Q o as ufnfuednohi ofeofQ ugons maanuF Ava nnofQnulo/qus outdhuns f got-uEni afo fmall = 5 / (Tb)

vuffig dfonfihu noQmz og regf hofQ ceppigg dp ceftoVy*noudoDeffaho hgud1udEol mf got\$ a condhol gndFAa 5/qfbS g1 nbV hgndeo fonaiPa 5/Pfb vndereiaeno fonaiPo. 5. 5 fol- gfno fonaiPo. 5. 5 fol-ns Dudeo fonaiPo. 5. . fo , i ndEo fonalita 5. falons ndhgno fonalita 5. (To el z gamafQei EQos enfoefo fQ n onfi hu nol g ofiepi n hoedor mgfupa nogmdEudEofiges o/ofeo. 5ous AxfQ o Bindfu9pnfuedd mndDnn hædor ngfupa opei dfudEor gozeais o duf Bodones o pmn nAnfQ os manae fo mpQorea)s gol mao nfus mf hoped nuh gud Eomos motorQmr o fegonzeor mgfupa nAufO or go dfmE cefinuh dfu9 horea)s gnAundhofO ugoh db nufu nd Reco fonal 5/Pfb/i pomy areo fonal 5/Pfb/ nder ei aeno fonal 5, 5fb : ntl1ntlvE onthoKnD at 5.5(To0 ed fQ a nnAoutlofQ n opmn nAofQ oamploefo geDinfd nnoQmsr gnofQ opesrmgunedoD fl donfihunïbVeg ez gAoufo Qis mdo crenig and Dmn hædd Qn d)r me fip næpi an fuedn A fQ og niafnang o QuEQa) audji dp haD) of Q anuF ae far mgfupa nof QmfapmalaD os maig handhof Q o nns ra onuFordo mpQonfih) To u gndov ToReco fonaïopnapian f hofQnfordhuzuhi mano l Qeo eda) o hard lo Deffa hol mf go s mì o D o udE nfudEo P5/55550 V v*no

uf sn.) mg^{-/}AomosipQoQuEQ go crenig opesrmg hofeofQ o%5550 $Vy^{*}n$) ng^{-/o}fegotQen ol Qeceda) chgullofmr d mf gotReco fom i Ra 5/P(TiNilo ndefQ gonfih) Ay TOW ppng azeo fonaTBi ndfu9 hoD) opei dfudEoV y*nogndEudEo figes of Tkofeo/ 5 ous druns f groud Deffa hd mf gnondhof gnd nfiegs hofeos mmo i dufno nfus mf hopedp dfgmfuednæfip k; quEo^{-/}⁴Wi ppng zeo fonzillo 5/P(Toldo ndefO gonfih) Anfeormanno v*nAnnameE ons ei dfoefinns rad moe Bige hofeo g mpQch f pfmDa ons ei dfnct, i ndEo fonalAo 5.. (To

2 doiO or fO ground h Ar ann funomh h fuz, na mnOurl Ediges o Deffa note de gud 1 ul Eo l mf gondhofQ ugch fgus dfnao ffipfnofeoQis mdoQ mafQoQnz oD dog regf ho tMIQDngrFnh Qo fonalAo 5. 5fthmoQuzmtRenfmo fonalAo 5. /ftK p ggnb gg gno fonal 5. . (TeNdov no Overno Renfino fonal for or ef dfunaogun 1 ce fo Dunr Q de ao Mo tKyM(andhanes ar QfQnamf nd g amm nn hathnaQaznaRenfna fanaiRa 5./(To HO or g n dp oe final) ar O de anot Myn (Ar gi i egenal) aondhor ea) i i egenal) ao ni Dnfmdp notyI MQ(andhoegEnder Qenr Qnf o nf gnat2 ySn(d g ananeo zmab inf hot MI ODnguFmh Oo fonalPa 5. 5fbl gdnigh FbMgguDnno fonalPa 5. w(Tbl ego fQn og mnednAss eg ogeDinfondhon dnufuz onfihu nong od hhofeoman nnafQo ref dfunacefnQis mdocrenig dfeoV0y*ndfQgeiEQchgud1udEd mf gBo

NlofQuron dn ArfQ op dfgnæcEenæcefirfQuronfi h) ourofeonum nnoV 0 y*nofiges o 855ods deo 5ous ordonudEa o n dDeffa hd nf gndiges druffi g dfores s grungo nirrau gnoudoOrmudToyea)s gnoefioV0y*nol goBinudfu9 houdos munoi dufnAo l Oua or ann fupon hhufuz nor g n dford of O onns r and g of dfn fuz oh dfu9 ho ndhorguegafuFhofeonfus mfofQosenforefdfunæ)oQmgsfiæpesreidhnofego Ois mdoO mafOlioMos fOeheaeE) dDmn hoedo*Rh x V Oofieaeel hoD) omos mmbo Bindfufnfuz ondhoning pfonpg dudEomrggenpQ norgzueina) oh zaer ho D) œi goEgei r ofeomm mor ea) s gnomthor annfupomhhufuz nog nr pfuz a) oudo hgudlud Ed mf gd mmomrrau hot L Emby gg gmo fom al Ao 5.. (To

2. Materials and methods

2.G. Shemicals and reavents

vea) fO) a d otvS (ondma) fuonaon findhma hotV TS T /. 55 ov m(ol mnoni r b rau hoD) oyea) s goOfmathmghoO gzup o-s D, otV madFAo-gs mat) (Toyea) b rger) ad dtyy(dtVT\$T /...50vm(d mmoeDfmud hofges oMs gupmedovea)s go OfmilhnighnoRegregnfuedotV dfegA2, AX duf hoOfmil n(Aunthorea) rger) a d o f g r Of Qnanf ot y SH(d mnompBiug hoD) o-eehol azel oRns DghE o*us uf ho t. idfudEhedAssdEandh(To

yannfuponhhufuz nonfindhinghnoefiofgu fQ) aor Qenr Qinf ot HSy (AsDunkt, b fQb) aO c) af of g r Of On amf AD d Feu Dompuh A fig Di f) an Oenr Onf of HKv (A figur O d) ao r Qenr Qnf ot Hyy (Aofguntt. hpQæge fQ) a(or Qenr Qnf Aofguntt. hDi fec) fQ) a(o r Qenr Qmf Aonf ngupompuh Aor nas ufupompuh AoQ cms fQ) aop) pæfgunuæ cmd Ao huDif) aor QfQmamf otv Ky (Ao audea upompuh Ao hus fQ) aor QfQmamf otv V y (Ao hu fQ) aor Of Quanf ouv Sy (Acea uponpuh Andmr Of Quana d Ang cms fQ) adhunuae cmd Ao hus fQ) aonhur nf otv V M(Aoded ndeuponpuh AonF amponpuh Aos) gunfuponpuh Ao hump fed ompg) ans the Atomi geampfing Atohurger) a do Ea) peatohurger) a do Ea) peachus fQ) ac fQ gAo. A bhuttertbDi f) ar Q deaAo/A AvbD dFefgunFea Aor bo pg neaonathougEnateco. %kol g or i gpQnn hofQgei EQoV gp1otv ngs nfmhfAo gs md)(To

X da nnoefQ gl un onfmf hAmaacefiofQ og mE dfnd g oefig y*Rondma) fupnao Egnh TöHeai d o>PP18@origuf)oR, x2VMO2*L®y*XOAd QupQd mucin ho muonus eDua or Qmm dhi gud Eq. y*RoV 0 y*nbrea) s gond ma) n nAd muon pBiug ho figes of gp1Ad Q g mmos fQmdeaAmp fedufgua omdhd mf gd g origpQmm ho fges ol unpQ goRQ s upmaot*ei EQDegei EQAoXduf ho: utlEhes (To-ammo9D go 9 af gno-141 of region Foefio 855 ods (d) goeD fmid hofges of Onfs milov*Ro

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tV minnfed AoX duf ho: ulEhes (AonthodufgeE doi n honnohg) ulEoEnnol ufQo 2,4, MT: gs polxmer analxsis PPTPPk@origuf)d mmonirrauhoD)oMugo*uBiuh otKmgp ædmakormud(To

Nihuzuhi maonfep1onfmilhmahoneai fuednoefiy.Somilhovyot/555os E4*(d g o rg rng houdofeai d ondhoni Dn Bi dfa) oin hofegofQ opnauDgnfuedopigz no tRRn(ToOfmithmethnol g or g zuei na) one ai DualF houdofe ai d oi nud EonnoQ mfo ranfonfo, 5Rd ufQopednfmdfos efuedoñego oQND ego mpQoudhuzuhi maorea) s gAo RRnd g condEudEcrives o/aLEC*-/ofectors Ec*-/Theorg rmg ompCapedp db fgnfuedæftifQ dRRnAtiafgnmei dhnd mncin hofegdkos udTBMædRRnor eudfnd g o zegf c hd) fieg dO a v*Rh xV Omdna) nuffil egovSHAmdadhuzuhi naonfep1o nfndhnghoneai fuedot/555os Eo*-/(ol mnor g r ng houdohupQæges fQnd bo fgi i egemp fupompuhoPP7/ondhofQ onns or gep hi g oh npgD hofegofQ o egEnduporea)s gnol mnofeæel hofeorg r mg ofQ opegg nredhudEopnauDgnfuedo pigz To

Of nucl hunghnoe for ann fupon hufuz nol g or g r ng honfo/5550s Eo* -/ outo s fQndeaandhofQ dAthuaif hoidfuao57kos Eo*-/oudd mf gbs fQndeaaP7/ofiego fO o9dnacced9ss mfuedcefinfO n omhhufuz noutdomss ranct/nfoa z aceficced9b h dp (oudopmon of Qonfor Q) d g or g n dfoudd mf gonns ran To

2.2) amples

v gud lud Ed mf go Deffa mæfif O o 5 os enforer i angones s gounar Demidhnoido Or muld g ompBi ug hardog fmænfeg nadoKngp ædmild ego mpQdDgnathA% andho /5or annfupoDeffa noefio/Tkondho5Tko*Aog nr pfuz a) Aol g ompBi ug hToNdo HnDa of AfQ open metions ranAfQ of)r oefir amfupdDeffa nAfQ oDeffa opmro s mf gunaAd mf gæguEudAondhofQ ozeais oefið mf go9af g houdo mpQopmn Aong o nis s mguF hTBMs edEofQ o 5on a pf hoDgmdhnAykong opammu9 hommos ud gmao 1 nf gotV\$ (ondho9z onnofg mf holgud1udEd nf gotHv\$ (T6\$ mf goegeudd mno pO p1 hofOgei EOgef9 punza) og peEdun hogunfnæfis ud gnæd mf gnor gezuh hoD) o fQ oI eehoOnfi f) ohuzunuedofges ofQ oSi ger mtloRes s unnuedotammfoi rhmf o V mgpOo/8A0 5. w(ot Res s united A0 5. w(ToV ut grad mf gound pQ ngmpf gun hoD) o ntdoidh gEgeidhoegtEudontdhos ud gnaAofgnp o as dfnontdhoefQ gopes b reidhnopedp dfgnfuednAnnd annafnadfgudnuporigt) 162 dofQ oefQ goQndhAo fg mf holngud lud Ed mf gnopmato Qmz ond) of) r oe five g Eudonathong oni DUpf hofeo nifQegtFhorO) nupepOs upmarfg mfs dfnod p mmg) diegdOs deos forOo nfnDanQ hor efnDuaf) opQngnpf gnfupnotRes s uniedAo 55P(To

2,3,) ample pre&reatment

Ons rand g of grif of af g hoi nud Eononfmuda mbrif aonu z d uf Qomos n Qoefio . Sous Aorg zueina) of mnQ hondhoQ mf honfo%/55Rofiego oQofeonzeuhoref dfumao reaaifndfnibHQ dAbfQ onns ranol g o9af g hofQgei EQo\$ Qmfs md®&Eammo 9D go9af gno I 4I o855 ods og % 8 os s or g zuei na) dngu hondh fing h Til udnæ). Av - I 4I 69 af gnol g dagu hoez gduEQfonnfo, 5Roir ofe opednfind fol uEQfondhol r fo nfo-. 5Rod Eannoy fguhunO ni dfuafO dV 0 v*no cfgmpfuedTo

Iegorea)s go cfgmpfued A9 af gnol g oni Ds gE houdo/5os *oefiofeai d To HQ or mgfupi and os mf gunaol mno cfgmpf hoi nudEomdoi afgmmedupommnunf ho cfgmpfuedoDnfQtXQMS(dfego/50s udAnnthdQ o9dnao cfgmpfd mndgnthnfigg ho feanok5os *ans D gaEannazuna 168 mpQa9 af gal mno afgmpf hafQg afus nAunatho fQ chuffig dfo cfgmpfnd g opes Dud hTeHQ opes Dud ho cfgmpfd mno zmrb egní hoidh gadufgeE donf ns onfo%5Rofeopedp dfgní ofQ o cfgmpfoir ofeo/"lko s *161 udnao cfgnpfd muchuzuh hadfedfQg oma Biefnoefick 55qu*audo*Rozuman16 2 d d mnog n gz hofegofQ œgEnndupor ea)s gonndma) nunïEi egofQ onndma) nunœfio ySHAnndonauBiefoefik 55qu*ol mnozmregnnf hoidfuao k 5qu*ondhog pedn fufif ho l ufQa.k5qu*dhupQæges fQnd andho/@cefiafguj i egemp fupampuhTaMdefQ go ma Bi efol moi n hofegor amfinonthhifir, nondina) niñ MofOironna Aufol mo zmregnf hofechg)d mai dh ganadufgeE danfg ns anathag pednfufi f hair afeo k55qu*d mf gbs fQndeadP7/16iudnao cfgnpfnd g org n gz honfo-.5Roidfuao fQ ondma) nunTK\$ d ei ahoaul ofeor eudfoei forQmforQ os mudor annfuponthufuz no in hordor amfundiegs i anfuednome ordofO condE cefiQEOa) of eour merdEa) one ab i Da outofQ oneaz dfos ucoi n hofeog pednfufi f o9dnao cfgmpfnAol QupQouno dei EQofegodedbBi mdfufnfuz omdna) nunTo\$ omrrau hononi nr pfonpg dudEo mrrgempQoudoegh gcfeouh dfuf) of dfmfuz a) ofQ orge9a oefior ann fupomhhufuz no rg n dfodd nf gonfeg houdor annfupdDeffa ndiges dhuffi g dfdDgndhnAbDi fodefono Bindfufnfuz and na) nunce fifOs To

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IegoV0y*nbrea)s goBindfufnfuz ondma)n nAonuFo cpainuedoauBiuho pOges nfeEgnr Q) opes Dud hd ufQoQEQbg neai fuedos minor pfges fg) ot*Ro tOSR(b x V O(ol mno s ræ) hToHO or ea) s gon rmgmfuedol mnompOu z ho inudEofQoMpBiuf)qy*Ron)nfsofgeso\$nfgnoRegrTotVusfeghAWMAXdufho Ofmfn (oBiurrholut Qondombzmdphorea) s gopeaisdot MpBiuf)o My RoGH% ko /TSqus or mgfupa on F (Ai nud Edieai d onnos e Dua or Qnm d eglud Eadonepgnfupo pedhufuednomfonni el centi cefto Tkos * os ud^{-/o}ndhondordUpfuedozeai s cefto/50 μ*Τό*Ron) nf s di macapeira hofeonno, x V OoJbScmpfuz oQ) DguhoBinnhgirea bo 2 gDufgmr os minionr pfges f gofiges oHQ gs eol unQ goQpu dfu9 pot Ondo3en Ao RMAX duf hoOfmf n(Ao Biurr hol ufQandanfs enrQ guparg nnig ar Qefeauedb umfuedotMyyNoneigo oer gnfudEoudod Enfuz ondhorenufuz ouedunnfuedo pedhufuedníkv mímu g ompBi ug houdofi axonpmutoV Oxseh otfiges ok 55 of eov 555 o m4fi onfonos mnog neai fuedoefiow5/5550fi azol uhfOnonfoOmatios nous is o tI\$, V(T60) ufgeE dol mmonir rau hoD) o*udh otKmap ædmakoOrmud(on dhol mmo in homonomQ mfQAml routhomic using) dEmmonfoer fusing el ognf noefig 5A55As ntdho. 5ongDufgng) oi dufnotniñ T(Aog nr pfuz a) ToKefQorgeD oQ nf gondho pmruæng)of sr gmfig di g on fomfo%556 RAdi Qua oO* dmox Ioa z aomfo/55oLo ndhd ifOrms mus is artUnfieddis offo 550s nTo

v mfmompBi unifuedomthor gep mudEol mnopmggu hoei foD) oGpmaDi goz %T o neffl ng dHQ gs ed unQ goQpu dfu9p(THQ oni nr pfonpg dudEom r gempQdiego V0y*nhrea)s gnouh dfu9pnfuedol mnomppeghudEofeofQ or gep hig or g zub eina) dh z ær hondhomrrau houdoei goEgeirot*ægpno fom alfo. 5. /fbL Embb gg gno fomalia. 5. / Dao. 5. . fo- ngpunbilegd o fonalia. 5. w(Tokgu j) AofQ o th dftppnftedontdhoped9gs mftedoefbySAvyyontdhoySHoV0y*nbrea)s gno l g or gfiegs hoD) of Q o dhguploV mmov fipfot: V v (ondina) nuncta z ao (Ao ndhofQ os manhBi ndfu9panfuedoefafQ orea)s gnofQgei EQofQ o cf gdnaarRnoefo rea)s gonfindhinghn Aon ringmf a) Ao Dinn hoedo Biuz na dfopedp dfgmfuedno ta z ao/(Tol uEDO/onQel nones o cms ra noefiopQges mfeEgns nonthoV Oo nr pfgmæfiuh dfu9 horea)s gnoudofQonmsranomdma)n hTo

2 P · lastic additives analysis

I egor annafupomhhufuz non subBi malfufnafuz onnalma) n nAonnog z gn hoauBi uho pQges mfeEgmr Q) ol mno s r æ) hoi nudEonnovigen r Q g® &OHMx ox y b/ qo dho pmrr hopeais dotkous Ao ox /.kos s (ofiges of/gp176HQ os eDua or Qmn ol mno pes ren hæfinnp fedufgua of 55@otM(ondhol mf goudod Emfuz opedhufuednondho 1 mf gompuhu9 honfo517@otz4z(ofegs upompuhotK(ofegor enufuz ouedumfuedo s eh TöHQ dieæel udEdEgmbu dfopedhufuednol g om rau h70/5@dMoñegokos udAo ad mondpg mn ofeok5@dMndo8os udAfreael hoD) onroud mondpg mn ofeoP5@dMo udæd os eg os udandhaQ ahapednfmdfafego/as udføfQ dAh pg mm afeanfmgfudEo pedhufuedmando os udomatho/os eg os udofeog b BiuanDgmf ofQ opeais d'TôMoj el o emf cefti5T os *os ud^{-/o}l muo s r ae) hd ufOondoudUpfuedozeai s cefti⁄5ou*To OufgeE dd mnoin honnon an OnfQAnl r Aundhonicusung) cEnnonfoj el ognf noefo : 5Ax5Axmetho, 5cmi Axy, nr pfuz, a) To

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2,6, MT: gs&plastic additives prioritisation studx

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T. besults and discussion

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V enfondEa li n or amfupDeffa nong os nh œftySHd Qa AfQ opnrnong o s nh œftyShyfdfQ g fig AfQ n ong dQ os enfo cr pf horea)s gg nhi no feoD org n dfoudofQ n onns ra nibv rr tf œftbQnfAnnoni nr pfonpg dtdEo mr genpQi nnonr au hdeonm nnæfQ gr ef dfunaœi gp næftpedfns utnfuedo hi gdEdQ DeffadEægri gt9pnfiedor gep m mddQ opm œftdedbs uf gnao I mf griBHQ og ni afnoeftfQ oni nr pfonpg dtdEonQel hdQnfoeda) afQ o cb r pf horea)s gnotySHAbySAndhoyy(ol g org n dfomDez ofQ oas tdeftb Bi ndft9pnfiedTbHQ n orea)s gnol g oped9gs hondthoBi ndft9 hoD) o cf gdnaonfndhnghnopi gz nibHQ oad ngt0 gnutE ml g omm nn hotx` ⁰ > 5TP8(dfgs o'qLEd^{--/o}fedxs Ed^{--/o}ffegyyondhoySAl Qa dfgySHAbynHedEo figes dxqLEd^{--/o}fedxs Ed^{--/o}ffeDqpi gpt) dd== w(d mno crg m ho mofQ og antiz onfndhnghon) zufuedotx.Vr o@(oeftodfgnondhoudf gthn) o

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/ü∕1ko	% ko	< V *eJ o	/%0	k; 80
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/wii/Tko	usko	< V *evo	< V *evo	w8ko
/wi51ko	wk80	< V *evo	kwo	: /50
/0/41/10:0	< V *ev o	< V CV0	/Duo	, / 50 / Pup
/ 700 KO	< V evo	< V evo	/ FWO	/ FWO
////1ko	< V es o	< V 200	< V 200	< V ej 0
/ku/ku		< V 200	< V 200	< V evo
/ KUKO	V eso	< V evo	(10%)	(D0/c
/; U/ KO	< V*evo	< V*evo	/ P%0	/ P%0
/; UD IKO	< v ~evo	< v~evo	< v ~evo	< v "ev o
/80/ KO	< v ~eJ o	W8. 0	< v ~evo	W8. 0
/ 800 KO	< v ~evo	/. K/O	< v ~evo	/.K/O
/qu/ ko	< v ~eJ o	< v ~ev o	. q‰	. 4%0
/ qub ко	8K. 0	8/50	/P. 0	/; K%0
/ PU/ KO	< V *ev o	< V *ev o	wkwo	wkwo
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naor mgms f groefiofQ oznathmfuedoulpaihudEofQ o s fQehmoaus ufo
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g ni afnæfifQunnfi h) øHnDa o/(ordog anfræddfædQen æDfnud hordoQei n Qeaho A) fmrol mf goudoKmgp ædmopuf) onfi h) ot L Enh gg gmo fonzillo. 5. . (ol uf Qono s mtlozmai oefiowwPouEor go) mgAofQ oudE nfuedoefioV 0 v*nol mnonatEOfa) o nir guegofiegofmid mig Fo, el z gAmppeghud Ecfeor (O o V middls O fd) of nfAo DefQos ndozmai nopmddefoD opednuh g honuEdu9pmdfa) ohuffi g dfToHQ g b feg AufQ caz ancefiorea)s gnoudE nf hoD) chgud 1 ud Ecfmr cl mf goego Deffa ho l mf gong onus uang AmdhofO oeda) dhuffig dp noD fl donfi hu nong ofO of)r o eforea)s gnoudoDeffa hol mf gondhofmrol mf g10xilofmrol mf gAySondhoyyol g o fO os enfones s eda) diei dhorea) s gnAd Qua orea) unerg d otv Nondhorea) b Difnhud ctyKv(d g on aneofeidhoudones cefiofQ on ns ran To, el z gAsyNo nthoyKvd g ceda) ceppnnedmaa) ofei dhonfoQEQcpedp dfgnfuednAard1 hofeo pedfns udnfuedofies of Q oQei n Qeahofmr b9ffudEos nf gunaribHQ og regf ho a z anoddDefQdhgud1udEd mf gonfi hu nopmddD opednuh g hog amfuz a) ozel oudo pes r manedd if QuefO go nfis nfiednor i DanO hdD feg d'Onfoin dBi mafnfiz, o mrrgempQ no cfgmreanfudEofQ odis D goeforea)s gupor mgfupa moudonns ra nTo MonOei ahoD odef hofOnfofO ogndE oeffondma) nuno s rae) hoD) oei godez ao Bi mdfufnfuz omrrgempQoh z ær hofeoBi mdfuf) or ea) s gnoefioV 0 y*noudo f gs noefis manounog anfuz a) ons mæchi ofeofQ omdma) n goin houdofQ os mano nr pfges f gotJb2gDufgmr (ofQmfoeda) omæel nofQ ouh dfu9pmfuedomdho Bindfu9pnfuedceforea)s gnofijes ck55cfecx555cvmiic, el z gAvei gonfihu no ng os eg og maunfupomothonirregfofQ od hofeonnan na noQis moto crenig ofeo V0v*n1b

x p dfonfi hu nog z na hofQnfor annfupor ngfupa nonhnegD hoedofumi m s uEQfadhi p adj ms s mfuedondho enf guegdfgndnæpnfueddQgei EQdfumi nAo z do df gofeofQ op arnondho c gfov 0 Mohms mE oD) oecthnfuz orfg mo tQQgdFlo fomHa 5/8 fk0mz.no fomHa 5. v(Ti ego cms ra Ames œffiQ n o ffi pfmQnz omag mh) oD doh s edrigmi hadonQfQgei EQpQgedupo cr eni g o cr gus dfmfed0 y*hrea) s gtV nffmedo fomHa 5./8 fkMDngEQei u fomHa . 5./fkWQei o fomHa 5./fk0gndhfno fomHa 5.. (To

Niog EnglinafeotQ cegtEuloefbhgd1lutEol nf gudE nf hotfmroegoramfupo Deffa hd nf g(An z guaQ) is nidQ nafQgmlnxpei ahD omneput hoafQei EQO V 0 y*ncrea) s gaz anpnidefoD qoednih g honEdu9nntfa) chuffi g dfibilo pm cefhagallutEd nf gofeg houdr amfupDeffa nAl quado cr pfdQnfor mdo a z anoefbreai fuedang ozel dDi foreudfutEcei fonudedlid EaEDa QAEQqedb p dfgnfuedcefbr amfuponhhufuz nAl Qa cudchmrd nf gopei aho cr pfdQEQ go a z anoefbreQ go s gEutEcegor gunf dfoegEndupor eai fndfnor gehi p hoD) o Qi s nitonthhouthi nfgmaonpfuzutu nofQnfofQ) oQnz o npmr hofQ or gfud dfo pedfgeatib

yg zuei nonfi hu modoDeffa hol mf geE d gmaa) oudz nfuEmf hol/y*monges o/o feo 5qus cinudEos upgenper) dieæel hoD) omr pfgenper upomrrgempQ nAandho fQ og niafnd g o crg nn honnofQ odis D goefioV y*n uf s n^{-/o}r gozeais o 9 af ghTeNdones on fihunAngOn o9 Eignol goin hofeon fusn f of Oorea) b s g'nos minor gozeais o9af g hopednuh gudEonus huis onQmr ofegonaeafQ o r mgfupa nAvudpai hudEofignEs dfnondho9DD gnofeE fQ gotReco fonalAo. 5/Pfb v nder ei æno fonalle. 5. 5fb: nd1nduE ondhoKnD ae. 5. 5(Tol ego cms ra Ao Wipping according function of the second sec mthofQ onfus mf hopedp dfgmfuednol g q k; quEo*-/otWippng areo fomalA . 5/P(TaMnapmidaD on dAtfQ n apedp dfgmfuednal g as i pQaQuEQ gafQmtlaudo fQ org n dfonfih) THQ o crantlnfuedofegofQunhunpg rntlp) opntloD onffgiDif ho fechuffig dfog muedníð 9 gnf Audof Quronfih) Ad o zmainf or myfupa nol uf Qohub ms f gndD æl o 5qus AnnandQ or up nafQnfapmaldD on dd ufQad p1 ho) nannao ramfupofignEs dfnofQnfofinaachel dohi gudEofQ oer dudEcefiofQ oDeffa opnuto nuEdu9 pmdfa) dDumnog ni afnT60 pedhAmdoez g nfus mfuedohi ofeofO opei dfudEo efiodedhr annfupor ngfupa nToHQighAofQ o ggegol Q donaaofQ or ngfupa nong o pednuh g hol ufQontopes s edos mtlonQmr ToMtlho9dnaa) AufeoeDfmutlozeDi nfo g ni afnoD) opei dfudEor mgfupa nonmonteBi mdfufnfuz om r gempQAtfQ os udus is o ns ei dfæfði mf gæ pes s dh hanonDei fo 5550° at: e as naho forallo 5/Pnfb Vulf duEo fonal 5/P(A) Qua ofQ ons ei dfoefi9 af g hd nf gordofQuronfi hu no l mmos i pOozel gehi de dO ozel ozus uf noefith f pfue d d'Omfor Rb x V Odf pOb duBi ommaael nTo

3,2,) uspect screeniny of plastic additives

Moninr pfongg dudEomrngempQnimmomrnauhofeonnn mofQoeppigg dpoefo ramnfupomhhufuz mutyMondoDeffahol mf gonns ran 100 Mfefnanzefil/sjopes reidhno l gon a pf hommof dfmfuz op mulhuhmf mopednuh gudEofQo, x V Owr pfgmAn/Qo Chemosphere 343 (2023) 140106



Fig. 1. 4C Hefmaoninr pf hopes reidhnontthopCrantfuponthhufuz noth dfu9 ho f dfnfuz a) ofuteoaz ao. oef0ped9h dp Avpannnu9 honnofQ ugohuffig dfof)r nonttho crg nn honnor gp dfnE dt@(T6M(T6)

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Chapter 6

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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

27,00035-LU 27,02231-BA 27,02019-GE 27,02402-HU 08,721177-ES 08,721177-ES 27,00031-SG 27,00031-SG 27,00003-CU 27,00009-GR	1 2 2 2 2 1 3 3 2 2 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0	PET PET PET PET PET	PE		trom 0.5 L bottles		
27,02231-BA 27,00019-GE 27,02402-HU 08,721177-ES 27,00055-GE 27,00031-SG 27,00013-CU 27,00013-CU	2 3 6 6 8 8 10	PET PET PET PET		6,000	5,110		Treated Drinking Water
27,00019-GE 27,02402-HU 08,721177-ES 27,00055-GE 27,00031-SG 27,00013-CU 27,00013-CU	3 5 6 8 8 10	PET PET PET	PE	6,170	5,200	ı	Mineral Water
27,02402-HU 08,721177-ES 27,00055-GE 27,00031-SG 27,00013-CU 27,00009-GR	4 5 6 8 8 10	PET PET	PE-PP	6,185	5,250	ı	Mineral Water
08,721177-ES 27,00055-GE 27,00013-SG 27,00013-CU 27,0009-GR	5 6 8 10	PET	PE	6,115	5,105	ı	Mineral Water
27,00055-GE 27,00031-SG 27,00013-CU 27,0009-GR	6 7 8 9 10	ner	PE	6,030	5,150	ı	Treated Drinking Water
27,00031-SG 27,00013-CU 27,0009-GR	7 8 9 10	LDI	PE	6,150	5,165	ı	Mineral Water
27,00013-CU 27,00009-GR	8 6 01	PET	PE	6,120	5,145	ı	Mineral Water
27,00009-GR	9 10	PET	PE	6,235	5,050	ı	Mineral Water
	10	PET	PE-PP	6,080	5,260	ı	Mineral Water
27,01078-OR		PET	PE	6,180	5,180	ı	Mineral Water
27,00042-GE	11	PET	PE	5,960	5,115	ı	Mineral Water
462,525791-BE	12	PET	PE	6,090	5,080	ı	Mineral Water
27,01051-GE	13	PET	PE	6,175	5,060	ı	Mineral Water
27,00684-SO	14	PET	PE	6,060	5,055	I	Treated Drinking Water
27,01383-J	15	PET	PE	6,030	4,905	ı	Mineral Water
27,01376-V	16	PET	PE	6,200	5,410	I	Treated Drinking Water
27,02518-LE	17	PET	PE	6,100	5,080	I.	Mineral Water
502,607920-PG	18	pp-pET	PE	6,210	5,120	ı	Treated Drinking Water
797,080850-FR	19	PET	PE-PP	6,230	5,280	I.	Mineral Water
27,01376-V	20	PET	PE	6,140	5,640	ı	Mineral Water
Blank 1	Blank 1	GLASS	PE	I	ĸ	4,975	Ŧ
Blank 2	Blank 2	GLASS	PE	ı	1	5,055	ı

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

software.
<pre>import openpyxl as xl wb = xl.load_workbook('SuspectScreeningPlasticAdditives.xlsx') sheet = wb['Hojal']</pre>
row = 2 for row in range(2, sheet.max_row + 1):
for in range $(5, 47)$: cell = sheet.cell(row,i).value
If cell == ": pass
cell = str(cell) cell = cellreplace(cell, X') sheet.cell(row,i).value = cell
wb.save('SuspectScreeningPlasticAdditives.xlsx')
Script 2: Estimation of the detection frequency of compounds in the mineral waters analysed from the single-use plasti bottles.
<pre>import openpyxl as xl wb = xl.load_workbook('SuspectScreeningPlasticAdditives.xlsx') sheet = wb['Hoja1']</pre>
<pre>cell_title = sheet['AW1'] title = 'Detection frequency' cell_title.value = title</pre>
<pre>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:</pre>
-1 countra $+=1$ detection frequency/40 detection frequency/40

import openpyrl as xl import chemcalculator from chemcalculator.chemcalculator import compute_mass we = xl.load workbook('SuspectScreeningPlasticAdditives.tkx') sheet = wb['Hoja1'] i = 2 for row in range (i, sheet.max_row + 1): compost = sheet.cell(i, 2).value = compost2 ecompost2 = compost2 ishet cell(i, 2).value = compost2 itel = 'Haas' cell_ittle.value = title cell_ittle.value = title iftle = 'Baas' cell_ittle.value = title iftle = 'Baas' cell_ittle.value = title for row in range (i, sheet.max_row + 1): compost2 = sheet.cell(i, 3).value mass = compute_mass(compost2) mass = float(mass) sheet.cell(j, 4).value = mass MolecularWeight = sheet.cell(j, 3).value DeltaMass = sheat(mass) sheet.cell(j, 4).value = mass MolecularWeight = sheet.cell(j, 3).value DeltaMass = compute_mass(compost2) sheet.cell(j, 4).value = mass MolecularWeight = sheet.cell(j, 3).value DeltaMass = float(mass) sheet.cell(j, 4).value = mass MolecularWeight = sheet.cell(j, 3).value DeltaMass = float(mass) sheet.cell(j, 4).value = betaMass j = i = 1 wb save('SuspectScreeningPlasticAdditives.tkx')

wb. save (`SuspectScreeningPlasticAdditives.xlsx')

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

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



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	РР	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		
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	Natural compounds	
Embelin		
N-Acetyl-L-leucine		
Traumatin		
7-Pentofuranosyl-7H-pyrrolo[2,3-d]pyrimidin- 4-amine Nitrosomorpholine	Other uses	

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		
2,2'-(Hexadecylimino)diethanol	Used in PP synthesis	st is	
3,3'-(Phenylphosphinediyl)dipropanenitrile	Polymerisation catalyst		
D-Xylonic acid	Used in Nylon synthesis		
N-Methylmorpholine	N-Methylmorpholine Stabilizer		
Octadecenylsuccinic anhydride	Hydrolisis stabilizer		
Bis(2-ethoxyethyl) hydrogen phosphate	Production of transparent polythiourethane bodies	Stabilizers	
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		
Diethyl phthalate (DEP)	Phthalate		
Bis(2-ethylhexyl) phthalate	Phthalate	Plasticisers	
Diisobutylphthalate	Phthalate		
Bis(2-ethylhexyl)adipate	Adipate	1 Iusticisers	
N-Butylbenzenesulfonamide	Polymerisation of polyamides		
Tetramethylurea	Plasticiser		
4-Isopropylphenyl diphenyl phosphate	Flame-retardant		
Tris(3,4-dimethylphenyl) phosphate	Tris(3,4-dimethylphenyl) phosphate Flame-retardant		
Aminopropyl dihydrogen phosphate	Flame-retardant		
Anthraquinone	Intermediate	Colourant agents	
Ethylene palmitate	Lubricant		
Methyl 9,10,12,13-tetramethoxyoctadecanoate Lubricant			
N-Caprylyldiethanolamine	Lubricant	Lubricants	
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 $< 100 \mu m$, 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 intertidal 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 μ m. 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 μ m 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.

Experimental section Chemicals

Polyethylene terephthalate microspheres (PET; 300 μ m) were purchased from GoodFellow Cambridge Limited (Huntingdon, England), while polystyrene (PS; 10-63 μ m) and polyethylene (PE; 10-63 μ m) 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 (HNO₃ 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 μ m 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 (AcNH₄: 77.08 g·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 μ m) and PET (300 μ m) 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 μ m and < 0.9 μ m for PE, while for PET, 10-63 μ m).

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 (NO₂⁻, NO₃⁻), and dissolved O₂ 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 NO₂⁻ and NO₃⁻ concentrations were below detection limits, with ~25 % and 35 % of positive measures and mean concentrations of 0.4 and 3.8 mg·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 °C. 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 °C of these compounds is much higher than the exposure concentrations used in this experiment (solubility of PFOS at 20 °C 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 μ g·L⁻¹, while the mixture of PFASs (composed with PFOA, PFBS, PFHxS and PFOS) was 5 μ g·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 μ g·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 °C.

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 \pm 0.2), gastric phase (8 mL of gastric fluid for 2 h at pH 2.0 \pm 0.2) and intestinal phase (8 mL of duodenal fluid and 4 mL of bile fluid for 2 h at pH 7.0 \pm 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 μ m and digested using 50 mL of KOH 10 % (w/v) overnight at room temperature, followed by acid digestion with 40 mL HNO₃ 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 N₂ 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 NH4OH 0.1 % in methanol. After elution, the extracts were evaporated under a gentle N₂ 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 μ L 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 APCTM 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-ExactiveTM 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 C₁₈ analytical column (Luna 150 x 2.0 mm, 5 μ m) 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 μ L. 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-ddMS²) 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 μ m) 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 μ L. 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-ddMS² 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·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 μ g·L⁻¹ and from 50 to 1,000 μ g·L⁻¹ with R² > 0.95. Matrix effect and recoveries at 10, 1 and 0.1 μ g·L⁻¹ were assessed for each matrix and corrected. For PET, an individual stock standard solution (1,000 mg·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 $(1,000 \text{ mg} \cdot \text{L}^{-1})$ was made to perform a calibration curve ranging from 1 to $1,000 \mu \text{g} \cdot \text{L}^{-1}$ in LC grade water, while for PFASs, internal standards

were used for quantification normalisation and the calibration curves ranged from 0.01 to $100 \ \mu g \cdot L^{-1}$.

Results and discussion 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 $t_2 1.6 \pm 0.3 \ \mu g \cdot g^{-1}$, and $t_1 2.2 \pm 0.1$ and $t_2 1.5 \pm 0.4 \ \mu g \cdot g^{-1}$, 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 μ g·L⁻¹) of PE at 10-63 μ m, between 1-10 μ m and inferior to 0.9 μ m. 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 μ m vs those in the range of 1-10 μ m, and between 10-63 μ m vs those with sizes below 0.9 μ m 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 μ m) vs. PE-MPLs (< 0.9 μ m). 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:

$$BCF = \frac{Conc.biota(ng/Kg)}{Conc.water(ng/L)}$$
(eq. 1)

The accumulation of triclosan (TCS) in mussel tissues with a nominal water concentration of 1.5 μ g·L⁻¹, after ten days of exposure, was below the limit of detection in seawater (0.07 ng·g⁻¹ w.w.). Pirone et al.[40], also obtained low accumulation ratios when mussels were exposed to 1.0 μ g·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 μ m, was 148, 55 and 8 ng·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 μ g·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 (C₈), and the compound has a sulphonic acid. Comparing the BCF (**Figure 4 (b)**) of the two C₈ 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 (C₆) (BCF 43.3) was

significantly higher than for PFOA (C₈) 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·L⁻¹) in comparison to the PFHxS with 6,2 mg·L⁻¹. The high filtration capacity of *Mytilus galloprovincialis (*up to 8 L·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 (t₂, 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 cooccurrence 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], C8-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 μ g·L⁻¹ the mean concentration of PS accumulated was only 420 ng·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 =
$$\frac{[PFAS] digestion}{initial [PFAS]} \times 100$$
 (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 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 μ m diameter the reduction percentage in their retention compared with 10 μ m 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 μ m 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 μ m 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.

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a)



b)



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 μ g·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 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 n°6 and n°7**), and contaminated food (**Publication n°8**), 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 n°6** and **Publication n°7**, 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 MN-PLs-polymers detected at the average concentration in drinking water samples from household taps in the Barcelona Metropolitan Area (Publication n°6).

Household tap water					
	PE	PP	PBD	PI	
EDI (µg·Kg ⁻¹ BW ⁻¹ ·day ⁻¹)	0.5E-02	1.4E-02	4.0E-02	6.5E-02	
ADI (µg·Kg ⁻¹ BW ⁻¹ ·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 (µg·Kg ⁻¹ BW ⁻¹ ·day ⁻¹)	0.5E-02	1.6E-02	4.6E-02	7.5E-02	
ADI (µg·Kg ⁻¹ BW ⁻¹ ·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 n°7).

Bottled water					
	PE	PP	PET		
EDI (μg·Kg ⁻¹ BW ⁻¹ ·day ⁻¹)	0.6E-02	1.7E-02	1.6E-02		
ADI (µg·Kg ⁻¹ BW ⁻¹ ·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 (µg·Kg ⁻¹ BW ⁻¹ ·day ⁻¹)	0.7E-02	2.0E-02	1.8E-02		
ADI (µg·Kg ⁻¹ BW ⁻¹ ·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 guantification 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 n°7**, 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 n°8**, 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 n°8**, triclosan was accumulated in PE > $PS > PET (t_1)$, whereas it only was bioaccumulated once adsorbed onto PS microspheres (t₂). 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 n°8**, 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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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 n°1**), the Mar Menor lagoon (**Publication n°2**), the North and South Atlantic ocean (**Publication n°3**), as well as for drinking water bottled in single-use plastic bottles from different Spanish commercial brands (**Publication n°7**). 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·m⁻³) and in the surface marine seawater compartments (8 μ g·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 MN-PLs 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 μ g·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 μ g·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·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 μ g·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 μ g·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 μ g·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 μ m, 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.

Chapter 7

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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