Universidad de Alcalá

OCCURRENCE AND ENVIRONMENTAL FATE OF MICROPLASTICS AS EMERGING ANTHROPOGENIC POLLUTANTS

Doctoral Thesis
by

Carlos Edo Cuesta

Supervised by:
Roberto Rosal García
Francisca Fernández Piñas

Doctoral Program in
Hydrology and Water Resources Management

2023

Universidad de Alcalá

Occurrence and environmental fate of microplastics as emerging anthropogenic pollutants

Doctoral Program in **Hydrology and Water Resources Management**

Doctoral Thesis presented by:

Carlos Edo Cuesta

Supervised by:

Roberto Rosal García

Dpto. Química Analítica, Química Física e Ingeniería Química

Universidad de Alcalá

Francisca Fernández Piñas

Departamento de Biología
Universidad Autónoma de Madrid

Alcalá de Henares, 2023

Plastic is carbon," fossil fuels in another form, CIEL's president, Carroll Muffett, told me. Or, as the geographer Deirdre McKay phrases it, plastic is climate change, just in its solid state.

Rebecca Altman in How bad are plastics, really?

PREFACE

This dissertation is submitted for the degree of Doctor of Philosophy in the University of Alcalá, Madrid. The research presented in this Thesis was carried out at the Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering of Universidad de Alcalá and the Department of Biology of Universidad Autónoma de Madrid. It was developed during the period from October 2018 to February 2023 with Dr. Roberto Rosal, professor at Universidad de Alcalá and Dr. Francisca Fernández Piñas, professor at Universidad Autónoma de Madrid as supervisors.

The chapters developed for this Thesis are research published in several peer reviewed internationals journals with high impact factor, named Marine Pollution Bulletin, Environmental Pollution and Science of The Total Environment. The material generated has also been published in different media like radio or written press and used in some scientific conferences such as MICRO2022 and the Joint Annual Meeting of the Entomological Society of America (2022).

The author certifies that all the results presented in this work are original with exception of the data where due reference has been made to the work of others. It must be remarked that data and information presented in this work, or the related information has not been used to obtain a certification from another university.

FUNDING

This Thesis was supported by a pre-doctoral FPI grant (BES-2017-082031) from the Spanish Ministry of Science, Innovation and Universities. Financial support of this Thesis was provided by Spanish Government with the research plans CTM2016-74927-C2-1-R and PLEC2021-007693 and with the creation of the thematic network of Micro- and Nanoplastics in the Environment (RED2018-102345-T, EnviroPlaNet Network).

AGRADECIMIENTOS

Quiero empezar agradeciendo a mis supervisores Roberto y Paqui el permitirme tener la oportunidad de trabajar en este grupo de investigación (gracias a Paco también por ello). A Roberto, gracias por rescatarme del paro cuando ya estaba a punto de rendirme y olvidarme de la investigación. Contigo he aprendido muchísimo de cómo funciona "el sistema" y qué esperar de él. Ya de ciencia ni hablamos, ha sido un placer observar y aprender desde el otro lado de la mesa. Y a Paqui, gracias por tantos consejos e ideas, tanto en lo científico como en lo personal, sin duda me ayudaran en el futuro. Trabajando juntos hemos tenido la suerte de conseguir un gran botín de buenos resultados que me van a ayudar mucho en los siguientes pasos, de nuevo, gracias.

Como no acordarme del "Maiki" (Miguel para las formalidades). Hace tiempo te veía desde los ojos de un estudiante de máster al que intentabas enseñar los scripts de R (lo siento, sigo sin entenderlos, simplemente no se me da) sin saber que un tiempo después casi nos íbamos a convertir en "los del Río". Bromas aparte, te empeñaste en luchar por tu "microplásticos por tierra, mar y aire" y creo que ya lo tienes aquí. Mil gracias por dejarme formar parte de esas aventuras. He aprendido mucho de una de las mejores personas (e investigadores) que conozco. ¡Gracias por tantos buenos consejos!

También me tengo que acordar de tantos otros que he conocido estos años. Laura, la de turras que te he dado con los plastiquitos sin yo entender mucho lo que tu estabas haciendo, Irene, ¿cuántas horas hemos echado intentando criar a las innombrables?, Álvaro, la de veces que me habré acercado diciendo: Me pasa esto, siempre encontrabas una solución. Han sido muchas muchas horas de brainstorming entre todos, de probar experimentos y de contaros mis cosas. Gracias, sin vosotros esta tesis tampoco estaría escrita.

Tampoco puedo olvidarme de tantos otros compañeros de Alcalá o de la Autónoma, María, Georgiana, Carlos, Fer, Daniela, Mario, Yeray, Tamayo, Berta, Idoia, Sergio, Carmen, Chizoba, Virginia, Chloe, Sofía. A Ana por ayudarme con tantas gestiones, a Luis por aguantarme en el infrarrojo o a la gente de Faro, Tainá, David, Juliano, Gil, Joana, que hicieron de la estancia algo mucho más fácil. En fin, no me enrollo más que 4-5 años dan para conocer mucha gente buena y no acabo, gracias a todos.

No puedo terminar estas líneas sin nombrar a mi familia, los grandes culpables de esto. A mi padre Blas, a cuyo duro esfuerzo debo tanto el poder dedicarme cada día a lo que me gusta, su ejemplo y afán de perseverancia sin duda me han hecho luchar por llegar hasta aquí. A mi hermana Cristina, incansable todos estos años en esto de escucharme y animarme para seguir adelante con todo, si alguien se merece que culmine esto con éxito es ella. A mi tía Chelo, que, aunque desgraciadamente no ha llegado a ver esta tesis, es muy culpable de hacerme ser la persona que soy hoy, y por supuesto al resto de mi familia y amigos por todo su apoyo durante estos años, sin su paciencia y aliento no habría terminado esto.

¡Muchas gracias!

CONTENTS OF THIS DOCTORAL THESIS

INDEX

SUMMARY / RESUMEN

SUMMARY

Background

In the end of the 19th century, chemists started to create synthetic materials combining natural products like cellulose and some artificial compounds that showed impressive properties of plasticity and resistance. Later, in the beginning of the 20th century bakelite was introduced as the first full plastic material created (Chalmin, 2019). This was the beginning of an era in which gradually first, and massively in the end, plastic has been replacing different materials like paper, glass or metal among others. Numbers show that we have passed from a few tonnes generated in the 1950´s to a current production up to 400 million tonnes per year (OECD, 2022). Scientists estimate that in 70 years, more than 8.3 billion tonnes of plastic waste have been generated, from which about an 80 % has finished in landfills or dispersed in the environment (Geyer et al., 2017).

Plastic materials have multitude of uses because of their characteristics. From big objects used as containers, coatings, insulators to smaller size applications like clothes, cosmetics or components of abrasives. Plastics with their largest dimension between 5 centimetres (cm) and 1 micrometre (µm) are called microplastics (MPs). Materials created in this range of sizes are referred to as primary MPs. It is widely recognized that the plastics spread in nature, begin to suffer the effect of environmental stressors. The sun exposes them with ultraviolet radiation and climatic conditions contribute with water, air, and changes in temperature. All these factors produce photochemical and hydrolytic reactions, mechanical erosion and cracking leading the material to disintegrate in small pieces, which is the origin of the secondary MPs.

In common plastics, a huge variety of polymers are found. The purpose of the product determines the polymer selected. Next, a range of different additives (including plasticizers, flame retardants, UV protectors or colorants) are included to create the final product. Until the last two decades, plastic materials were considered almost inert. The paradigm has changed since the emergence of data showing the presence of plastic debris in different environmental compartments. Since then, plastic debris have been under scrutiny trying to assess their potential effects to the biota and to human health. The damages described to date are heterogenous ranging from “simple” mechanical

effects on the digestive tract to “complex” physicochemical effects when the polymer, the additives or both are released inside the organisms or their cells. Furthermore, plastics can act as vector for pathogens and hazardous chemical present in the environment. This vector role can be enhanced in aged plastics due to their larger surface and their different polarity compared to pristine counterparts.

The occurrence of microplastics in the environment has become a widely covered topic in the scientific literature. Researchers from all over the world have conducted thousands of studies trying to understand the dissemination of these materials in the environment. Unfortunately, the effort is shadowed by a lack of methodology standardisation that makes sometimes impossible data intercomparison. Although we know that MPs are present in all environments, the available data show such a dispersion that is still difficult to make a proper risk assessment. The global tendency indicates that studies on detection, quantification and identification of MPs will be probably declining in the next years. The recent trend in academia is to focus on nanoplastics (NPs), the smallest fraction of plastic that due to their size, are more easily internalized and are expected to cause damage in cellular compartments. In the search for solutions to plastic pollution, bio-based and biodegradable materials are increasingly produced and are expected to contribute to reducing the volume of plastic waste produced. The impact of bioplastics, the risk posed by NPs as well as the development of standard methodologies and reference materials for the continuous monitoring of MPs are expected to be key topic for the next years.

Objective

The main objective of the present thesis is the assessment of the presence of MPs in different environmental compartments together with their quantification and identification as well as their potential transfer between them. The approach will address the fate of plastic pollution from their origin in anthropogenic activities to the environmental compartment namely the atmosphere, soil, freshwater, and marine water.

Outline of this Thesis

The thesis structure is based on articles published prior to the PhD defence. Each article is a self-standing chapter, but as explained before, all of them were conducted with the idea of connectivity and final fitting into a single project. The summary of the chapters is presented below:

Chapter 1. General introduction. This chapter puts in context different aspects of the main topic studied during this thesis, namely plastic pollution in the environment. The history and evolution of plastics, the problem posed by plastic pollution and the future perspectives are presented. The objectives of the thesis are also detailed and linked to the content of the rest of chapters.

Objective. The main ideas on which this Doctoral Thesis has been based and the specific objectives to be met are presented.

Chapter 2. Occurrence and identification of microplastics along a beach in the biosphere reserve of Lanzarote (published in *Marine Pollution Bulletin*, 143, 220-227, 2019). The chapter presents the work conducted in a beach situated in La Graciosa, Canary Islands (Spain). This remote place situated in a protected area, is a witness of one of the greatest plastic depositions in the world. The sources of the plastics, the implementation of protocols for marine research on plastics and a detailed assessment of results is presented.

Chapter 3. Fate of microplastics in wastewater treatment plants and their environmental dispersion with effluent and sludge (published in *Environmental Pollution*, 259, 113837, 2020). This research focuses on the dissemination of microplastics in an inland environment. The wastewater industry is evaluated as a potential source for microplastics in riverine ecosystems and eventually leading to marine pollution. A completely new protocol is used to quantify the amount of microplastics both in treated water and in the sludge generated during wastewater treatment.

Chapter 4. Microplastics in sediments of artificially recharged lagoons: case study in a biosphere reserve (published in *Science of The Total Environment*, 729, 138824, 2020). Directly linked with the previous chapter, this work is intended to assess the

dissemination of microplastics in natural freshwater ecosystems. A series of endorheic lagoons artificially recharged with treated wastewater were selected. The use of sediments in which the accumulation of microplastic has been quantified is presented.

Chapter 5. Occurrence and transport of microplastics sampled within and above the planetary boundary layer (published in *Science of The Total Environment*, 761, 143213, 2020). The presence of microplastics in places with limited human pressure like Arctic or Antarctic are attributed to the capability of microplastics to travel through the atmosphere. In this chapter a protocol for sampling microplastics in high altitudes is developed and data are presented and modelled to shed light on how these materials travel through the atmosphere and reach distant locations.

Chapter 6. Honeybees as active samplers for microplastics (published in *Science of The Total Environment*, 767, 144481, 2021). Once the presence of microplastic has been certified in some of the most unexpectable locations and even at high altitudes, this research focuses on the monitoring of the MPs transmitted by air. The use of honeybees to detect the presence and dispersion of microplastics is presented. In this chapter bees from urban apiaries are used as bioindicators for the presence of MPs in different areas near a big city and their possible sources are discussed.

Chapter 7. Microplastics in organic compost: influence of waste collection system and composting methodology (published in *Science of The Total Environment*, 813, 151902, 2022). The recycling of household organic matter is part of the circular economy with the aim of reducing the load of wastes worldwide. The organic part of municipal wastes can be converted into compost to be used in agriculture. However, there is risk that poor waste management results in contaminated material with plastics that may end disseminated in agricultural fields. Here a protocol is presented to assess the amount of plastic that reach agricultural lands via compost. The possible presence of bioplastics in compost was assessed to compare their persistence in contrast to traditional plastics.

Chapter 8. General discussion. This chapter discusses in a unified manner the studies performed during the doctoral thesis and highlights the most important results obtained.

Conclusions. This final section lists the main conclusions from this doctoral thesis.

References

Chalmin, P. 2019. *The history of plastics: from the capitol to the tarpeian rock*. Field Actions Sci. Rep., 19 (2019), pp. 6-11.

Geyer R., Jambeck, J. and Lawender, K. 2017. *Production, use, and fate of all plastics ever made*. Science Advances, Vol. 3, Issue 7. DOI: 10.1126/sciadv.1700782

OECD. 2022. *Global Plastics Outlook: Policy Scenarios to 2060*. OECD Publishing, Paris, https://doi.org/10.1787/aa1edf33-en.

RESUMEN

Antecedentes

A finales del siglo XIX, los químicos comenzaron a crear materiales sintéticos combinando productos naturales como la celulosa y algunos compuestos artificiales que mostraban impresionantes propiedades de plasticidad y resistencia. Más tarde, a principios del siglo XX, se introdujo la baquelita como el primer material plástico completo creado (Chalmin, 2019). Este fue el comienzo de una era en la que paulatinamente primero y masivamente al final, el plástico ha ido reemplazando a diferentes materiales como el papel, el vidrio o el metal entre otros. Los números muestran que hemos pasado de unas pocas toneladas generadas en la década de 1950 a una producción actual de hasta 400 millones de toneladas por año (OECD, 2022). Los científicos estiman que en 70 años se han generado más de 8.300 millones de toneladas de residuos plásticos, de los cuales un 80% ha acabado en vertederos o se ha dispersado en el medio ambiente (Geyer et al., 2017).

Los materiales plásticos tienen multitud de usos por sus características. Desde grandes objetos utilizados como contenedores, revestimientos, aislantes hasta aplicaciones de pequeño tamaño como ropa, cosméticos o componentes de abrasivos. Los plásticos con una dimensión mayor entre 5 centímetros (cm) y 1 micrómetro (µm) se denominan microplásticos (MPs). Los materiales creados en este rango de tamaños se conocen como MPs primarios, pero estos no son los únicos. Es ampliamente reconocido que los plásticos esparcidos en la naturaleza comienzan a sufrir el efecto de los estresores ambientales. El sol los expone con radiación ultravioleta y las condiciones climáticas contribuyen con el agua, el aire y los cambios de temperatura. Todos estos factores producen reacciones fotoquímicas e hidrolíticas, erosión mecánica y rotura que llevan al material a desintegrarse en pequeños fragmentos, originando los MPs secundarios.

Entre los plásticos comunes encontramos una enorme variedad de polímeros. El propósito del producto determina el polímero seleccionado. A continuación, los fabricantes de compuestos incluyen una gama de diferentes aditivos (incluidos plastificantes, retardantes de llama, protectores UV o colorantes) para crear el producto final. Hasta las últimas dos décadas, los materiales plásticos se consideraban casi inertes.

El paradigma ha cambiado desde la aparición de datos que muestran la presencia de desechos plásticos en diferentes ambientes. Desde entonces, los desechos plásticos han estado bajo estudio tratando de evaluar sus efectos en la biota y la salud humana. Los daños descritos hasta la fecha son bastante diferentes, y van desde "simples" efectos mecánicos sobre el tracto digestivo hasta "complejos" efectos fisicoquímicos cuando el polímero, los aditivos o ambos se liberan en el interior de los organismos o sus células. Además, los plásticos pueden actuar como vectores de patógenos y sustancias químicas peligrosas presentes en el medio ambiente. Este papel de vector puede aumentar en plásticos envejecidos debido a su mayor superficie y su diferente polaridad en comparación con sus versiones prístinas.

La aparición de microplásticos en el medio ambiente se ha convertido en un tema ampliamente cubierto en la literatura científica. Investigadores de todo el mundo han realizado miles de estudios tratando de comprender la difusión de estos materiales en el medio ambiente. Desafortunadamente, el esfuerzo se ve ensombrecido por la falta de estandarización de la metodología que, en ocasiones, hace imposible la comparación de datos. Si bien sabemos que los MPs están presentes en todos los entornos, los datos disponibles muestran tal dispersión que aún es difícil realizar una evaluación de riesgos adecuada. La tendencia mundial indica que probablemente los estudios sobre detección, cuantificación e identificación de MPs estarán en declive en los próximos años. La tendencia actual de la ciencia es centrarse en los nanoplásticos (NPs), ya que, debido a su tamaño, se internalizan más fácilmente y se espera que causen daños en los compartimentos celulares. En la búsqueda de soluciones a la contaminación plástica, se producen cada vez más materiales biobasados y/o biodegradables de los que se espera que contribuyan a reducir el volumen de residuos plásticos producidos. Se espera que el impacto de los bioplásticos, el riesgo que representan las NP y el desarrollo de métodos estandarizados para el monitoreo continuo de las MP sean temas clave para los próximos años.

Objetivo

El objetivo principal de la presente tesis es la evaluación de la presencia, así como de la cuantificación e identificación de MPs en diferentes compartimentos ambientales y su

potencial transferencia entre ellos. El enfoque abordará el destino de la contaminación plástica desde su origen en actividades antropogénicas hasta diferentes compartimentos ambientales, a saber, la atmósfera, el suelo, el agua dulce y el agua marina.

Estructura de esta Tesis

La estructura de la tesis se basa en artículos publicados con anterioridad a la defensa de la misma. Cada artículo es un capítulo independiente, pero como se explicó anteriormente, todos se realizaron con la idea de conectividad y encaje final en un solo proyecto. El resumen de los capítulos se presenta a continuación:

Capítulo 1. Introducción general. Este capítulo pone en contexto diferentes aspectos del tema principal estudiado durante esta tesis, la contaminación plástica. Se presenta la historia y evolución de los plásticos, el problema que plantea la contaminación plástica y las perspectivas de futuro. También se detallan los objetivos de la tesis y se vinculan con el contenido del resto de capítulos.

Objetivo. Se presentan las ideas principales en las que se ha basado la presente Tesis Doctoral y los objetivos específicos a cumplir.

Capítulo 2. Occurrence and identification of microplastics along a beach in the biosphere reserve of Lanzarote (publicado en *Marine Pollution Bulletin*, 143, 220-227, 2019). El capítulo presenta el trabajo realizado en una playa situada en La Graciosa, Islas Canarias (España). Este remoto lugar situado en un área protegida, es testigo de una de las mayores deposiciones plásticas del mundo. Se presentan las fuentes de los plásticos, la implementación de protocolos para la investigación marina sobre los mismos y una evaluación detallada de los resultados.

Capítulo 3. Fate of microplastics in wastewater treatment plants and their environmental dispersion with effluent and sludge (publicado en *Environmental Pollution*, 259, 113837, 2020). Esta investigación se centra en la dispersión de microplásticos en un medio terrestre. La industria de las aguas residuales se evalúa como una fuente potencial de microplásticos en los ecosistemas fluviales y, eventualmente, de contaminación marina. Se utiliza un protocolo completamente

nuevo para cuantificar la cantidad de microplásticos tanto en el agua tratada como en los lodos generados durante el tratamiento de aguas residuales.

Capítulo 4. Microplastics in sediments of artificially recharged lagoons: case study in a biosphere reserve (publicado en *Science of The Total Environment*, 729, 138824, 2020). Directamente relacionado con el capítulo anterior, este trabajo tiene como objetivo evaluar la diseminación de microplásticos en ecosistemas naturales de agua dulce. Se seleccionaron una serie de lagunas endorreicas recargadas artificialmente con aguas tratadas. Se presenta el uso de sedimentos para determinar la acumulación de microplásticos.

Capítulo 5. Occurrence and transport of microplastics sampled within and above the planetary boundary layer (publicado en *Science of The Total Environment*, 761, 143213, 2020). La presencia de microplásticos en lugares con presión humana limitada como el Ártico o la Antártida se atribuye a la capacidad de los microplásticos para viajar a través de la atmósfera. En este capítulo se desarrolla un protocolo para el muestreo de microplásticos en altitudes elevadas y se presentan datos que se han modelizado para arrojar luz sobre cómo estos materiales viajan a través de la atmósfera y llegan a lugares distantes.

Capítulo 6. Honeybees as active samplers for microplastics (publicado en *Science of The Total Environment*, 767, 144481, 2021). Una vez certificada la presencia de microplásticos en algunos de los lugares más inesperados e incluso a gran altura, esta investigación se centra en el seguimiento de los MPs transmitidos por vía aérea. Se presenta el uso de abejas para detectar la presencia y dispersión de MPs en aire. En este capítulo se utilizan abejas de colmenares urbanos como bioindicadores de la presencia de MPs en diferentes áreas cercanas a una gran ciudad y se discuten sus posibles fuentes.

Capítulo 7. Microplastics in organic compost: influence of waste collection system and composting methodology (publicado en *Science of The Total Environment*, 813, 151902, 2022). El reciclaje de materia orgánica doméstica forma parte de la economía circular con el objetivo de reducir la carga de residuos a nivel mundial. La parte orgánica de los residuos municipales se puede convertir en compost para su uso en la agricultura.

Sin embargo, existe el riesgo de que una mala gestión de los residuos resulte en un material contaminado con plásticos que puedan terminar diseminados en los campos agrícolas. Aquí se presenta un protocolo para evaluar la cantidad de plástico que llega a las tierras agrícolas a través del compost. Se evaluó la posible presencia de bioplásticos en el compost para comparar su persistencia frente a los plásticos tradicionales.

Capítulo 8. Discusión general. En este capítulo se analizan de forma unificada los estudios realizados durante la tesis doctoral y se destacan los resultados más importantes obtenidos.

Conclusiones. En este apartado final se recogen las principales conclusiones de esta tesis doctoral.

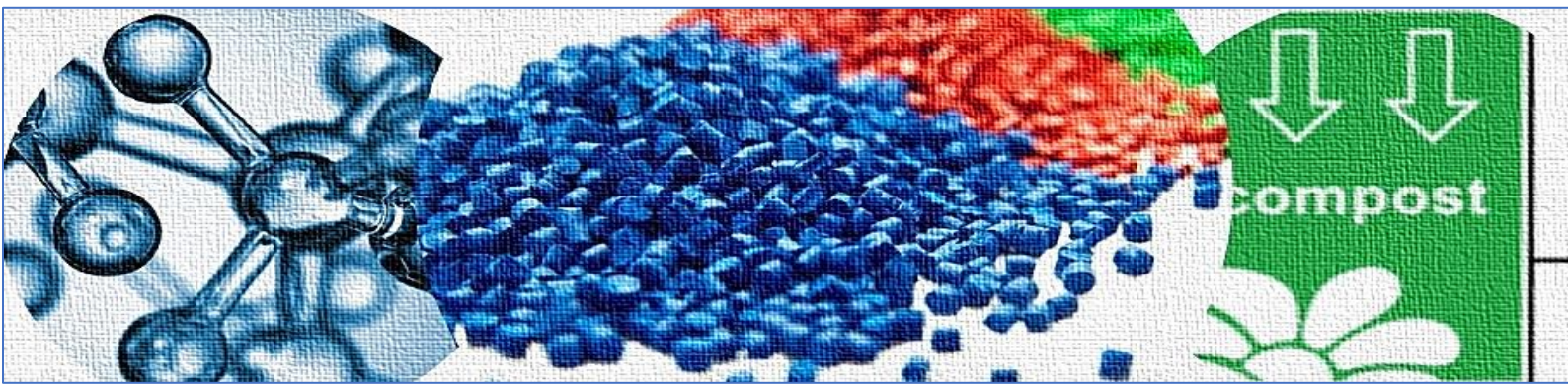
Referencias

Chalmin, P. 2019. *The history of plastics: from the capitol to the tarpeian rock*. Field Actions Sci. Rep., 19 (2019), pp. 6-11.

Geyer R., Jambeck, J. and Lawender, K. 2017. *Production, use, and fate of all plastics ever made*. Science Advances, Vol. 3, Issue 7. DOI: 10.1126/sciadv.1700782

OECD. 2022. *Global Plastics Outlook: Policy Scenarios to 2060*. OECD Publishing, Paris, https://doi.org/10.1787/aa1edf33-en.

CHAPTER 1. GENERAL INTRODUCTION



1.1. History of plastics

Life in the beginning of the twentieth century was totally different from the current one. Traditional raw materials consisted of wood, paper, glass, or metal, materials that in most cases were intended to last long periods and/or to be reusable. For that society it was possible to attain a reasonable equilibrium between consumption and the residues generated. With population growth, such equilibrium disappeared, and resources became scarce. The use of natural materials resulted in resource overexploitation. The history of technology uses the example of ivory in the beginning of the era of plastics. Ivory was so scarce and expensive that industry was forced to develop a cheaper material with similar performance but lower impact and cost. The material developed, known as "celluloid" failed as substitute for ivory, but allowed the development of photography and filmmaking industry. This is just an example, but the advent of synthetic polymers completely changed the paradigm on the use of raw materials and was the starting point of the plastic industry (Friedel, 1983; Altman, 2021).

The history of plastic can be tracked down to the early years of the nineteenth century with the discovery of rubber by Charles Goodyear, a process that allowed creating the first semi-synthetic polymer with true industrial applications (Fig 1.1). However, it was not until the following century when the development of synthetic polymers started taking advantages of the advances of organic chemistry during the preceding decades. Bakelite was the first fully synthetic polymer followed by polyethylene and polypropylene, polystyrene and polyamides that appeared all of them before the II World War (Geyer, 2020). The same materials have been in the base of the plastics industry essentially until now, when a new paradigm appeared with the advent of bioplastics (Mastrolia et al., 2022), either biobased or compostable plastics (Fig. 1.2.).

Polyethylene and polypropylene summarize the virtues of plastics as useful materials. They are malleable, resistant, cheap and allow the creation of a wide variety of products (Crespy et al., 2008). Certainly, these products have change life in all senses allowing price reduction and many new applications. Almost everything in our daily routine is related to polyolefins, which together with polystyrene, account for more than 56% of the plastic demand (PlasticsEurope, 2021). Plastic commodities played a leading role in

the industrial revolution of the twentieth century by providing materials that could be moulded to a diversity of forms, with advantages in weight reduction, increase in isolation capability and a wide variety of other useful properties. Plastics allowed the development of companies, industries and boosted the economy as a whole (Amato, 2013).

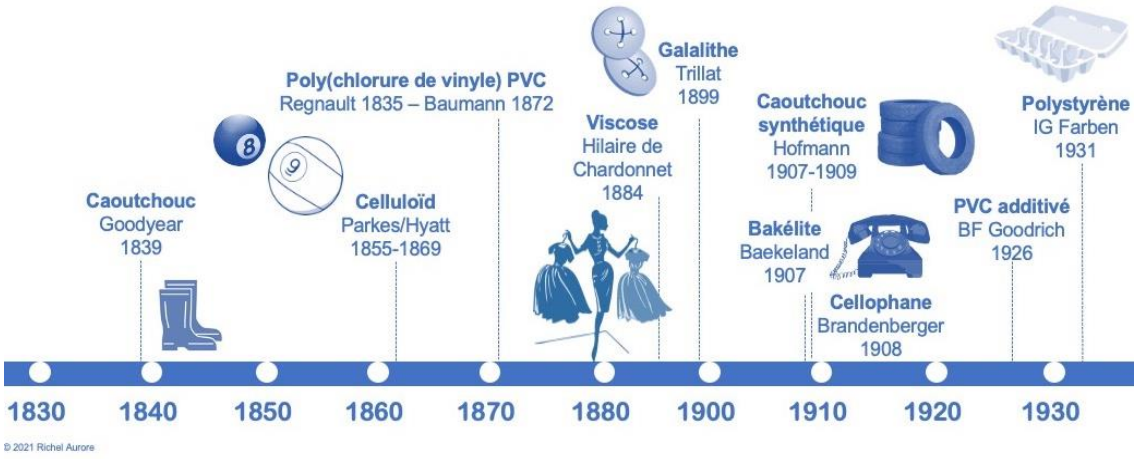


Figure 1.1. Chronogram with important milestones for the industry of plastics in the 19th and 20th centuries (Chem4us, 2021).

One of the best examples comes from the food industry, for which plastic improved self-life and changed the way fresh products are transported and marketed with impact in population diet and health. Besides, modern healthcare would have been impossible without plastic technology that allowed the production of many goods, from medical devices to innovative artificial organs. Even the future of the planet is in the hands of plastic with the development of lightweight materials made of plastic for energy industry in solar panels or wind turbines (Gómez and Rima, 2019).

It is important to differentiate between polymers and plastics. Polymers are the main component of plastics and can also be of natural origin like cellulose, cotton, silk, or starch, among others (ECHA, 2012). The real improvement was the development of synthetic polymers from petroleum raw materials. In fact, plastic goods largely substituted those made from natural polymers, as in the case of synthetic clothes. From the 1950s onwards, the plastic industry boosted its production from a few tonnes of plastics to more than 360 million tonnes in 2019 (Su et al., 2022). This figure probably represents an underestimation since the data related to the production of polyester or

acrylic fibres are not clear (PlasticsEurope, 2021). The figures say that the plastic production accumulated so far reached almost 10^{10} tonnes (Geyer et al., 2017). Besides plastic pollution, such a huge amount of plastic makes it difficult to ignore its possible influence in global warming due to the massive mobilisation of carbon (Ford et al., 2022).

1.2. The problem with plastics

The massive consumption of plastics has run in parallel with data showing the presence of plastic debris in the environment and with the problems associated to plastic waste management. In the last fifty years, an enormous increment in the generation of plastic waste has been observed. Until 2015, It has been estimated that the total amount of plastic waste generated could reach 5000 million tonnes (Geyer et al., 2017). Discarded plastic has three different management options: landfill disposal, incineration, or recycling. Over the years, landfills have been the main sink for plastics, with an important fraction of uncontrolled disposal of domestic and industrial wastes, particularly in certain regions of the world. Then incineration appeared as a good solution because it uses plastic as raw material to produce energy, although for plastic of fossil origin, it contributes to a net emission of carbon dioxide to the atmosphere (in Europe, during 2020, 42% of plastic was incinerated for energy recovery (Plastics Europe, 2021)). The recycling of plastic waste has increased greatly during the last decades allowing plastics a second life until their final disposal. Recycling represents nowadays more than one third of the post-consumer plastic treatment in the European Union plus United Kingdom, Switzerland, and Norway (PlasticsEurope, 2021). Apart from landfilling, recycling and incineration, the uncontrolled dispersion of plastic in the environment has become a major problem and a cause for concern. We have got used to see natural places covered with plastic bags, cigarette butts or more recently disposable face masks (Aragaw et al., 2022; De la Torre et al., 2022). All of them are subject to environmental stressors such as ultraviolet radiation, water or mechanical abrasion that break polymers backbones generating smaller and smaller plastic fragments (Singh and Sharma, 2008)

Since 2004, when the article coining the term microplastic (MP) appeared (Thompson et al., 2004), scientists have focused on the study of the presence of plastic materials in the environment and their consequences. More than 2000 scientific papers are published

every year on that topic, accounting for 6608 articles in the 1961-2021 interval (Jenkins et al., 2022). During these years, researchers from all over the world have explored ***marine and coastal waters*** in all the regions including Arctic and Antarctic oceans; ***riverine waters*** including the industrial and sewage waters from ***wastewater treatment plants (WWTP)*** that generally discharge into rivers; the ***atmosphere*** at different heights and finally, ***soils***. ***Biota***, targeted as a target for MP pollution, has been widely explored using organisms from different trophic levels. Table 1.1 summarizes the main environmental compartments in which plastics have been found and the type of study performed.

Table 1.1. Main compartments studied for the presence of MPs and examples of the different topics explored (Source: WOS).

Compartment	Research done
Marine	Detection in surface and coastal waters; detection in marine and coastal sediments; studies on MPs drifted by currents; studies on sorption and desorption of chemicals on MPs
Riverine	Detection in Rivers, lagoons and reservoirs; studies of MPs distribution fluxes; effects of dams in MPs distribution
Wastewater	Detection in wastewater treatment plants, in water bodies recharged with sewage water; in studies of WWTP remediation technologies
Air/Atmosphere	Studies of outdoor dry and wet deposition; indoor deposition; high altitude detection; bioindicators for MPs detection;
Soils	Detection of MPs in agricultural soils; detection in industrial soils; in soils amended with sewage water; studies on bioplastics degradation; profile studies
Consumption	Detection of MPs in drinking water and in food; studies of food containers resistance
Biota	Detection of MPs in different tissues; toxic effects of MPs and NPs by ingestion; toxicity of leachates from plastic items; studies of MPs as vectors of microorganisms.

All this effort has contributed to a certain, but still insufficient degree of standardization in descriptors like sizes and typologies. Many articles focus on bulk materials, the so-called meso- and macroplastics (above 5 cm and 25 mm respectively) (Rodríguez et al. 2020, Scopetani et al. 2021, Cowger et al. 2022, Ledieu et al. 2022, Liro et al. 2022), but the most studied fraction is by far the one constituted by MPs, which are defined as

materials with their largest dimension in the 1 µm - 5 mm range (Galgani et al., 2013; GESAMP, 2016). MPs designed in that specific size for certain purposes (i.e., components of cosmetics) are called ***Primary Microplastics***, while those originated in the breakup of larger plastic items are referred to as ***Secondary Microplastics***. The main difference between them refers to their original source.

In MPs research studies, a classification of MPs in different typologies depending on the shape and morphology is necessary to shed light on their potential origin (Free et al. 2014). Different authors classify plastic using sometimes ambiguous typologies. ***Fragments*** are irregular particles, foams, flakes or even plastic pellets, at times retaining the initial form of plastic products (Fig 1.2 (A-F)); ***films*** are flat particles with one dimension much smaller than the other two (Fig 1.2 (L-M)); ***fibres*** or ***filaments*** (Fig 1.2 (G-K)), also called threads or lines, are particles with one dimension larger than the other two and differentiated based on the bigger thickness and rigidity of filaments compared to fibres. Finally, some authors characterize ***microspheres*** or also called spheres or beads (Fig 1.2 (N-O)), to refer to particles with rounded and spherical shape (Hartmann et al. 2019). Figure 1.2 shows photographic examples of these typologies found in environmental samples.

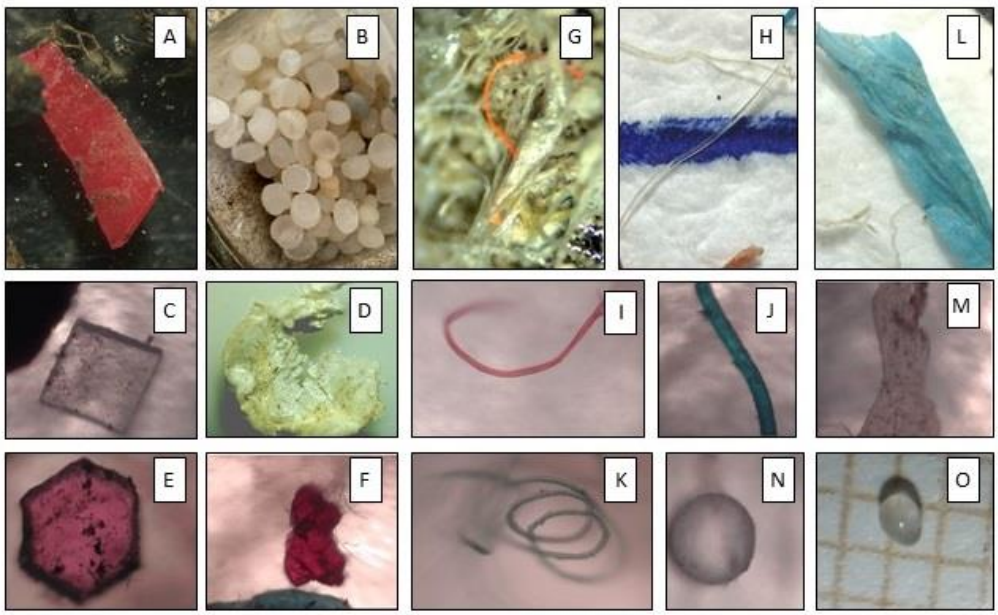


Figure 1.2. Photographs of MPs found in samples from the articles of these Thesis showing the different typologies found: Fragments (A-F), fibres and filaments (G-K), films (L-M) and microspheres (N-O).

A major turning point was the evidence that plastics are not completely inert and their accumulation in the environment not only constitutes an aesthetic problem. The scientific data accumulated so far, growing evidence that conventional plastics are a threat to living organisms (EU, 2018). Once in the environment, due to their specifical characteristics, the smaller plastic fragments can be responsible for several problems to the co-occurring biota: physical damage caused to organisms like the mechanical blockage of the digestive tract of organisms (Hoang and Mitten, 2022) or even, if particles are small enough, internalization and migration to different tissues (Campanale et al., 2020). Besides, plastics can be carriers of other organisms potentially pathogenic (González-Pleiter et al., 2020) or transport contaminants sorbed from the environment (Jiménez-Skrzypek et al., 2021) as well as chemical introduced during their manufacture (Paluselli et al., 2019). The consequences of the exposure to the small plastic fragments are still unclear, but the precautionary principle forces to take actions even if a complete risk assessment has not been satisfactorily performed yet.

The release of chemical additives is a key issue when studying MPs. These additives make each product unique and adapted for its final use. This topic is on the spotlight since the variety of additives is huge, and many are protected by secrecy, making their exact composition unknown (Campanale et al. 2020). Additives can be broadly classified into several groups, the main of which are plasticizers, fillers, UV stabilizers, antioxidants, lubricants, flame retardants and dyes (Table 1.2). ***Plasticizers*** are molecules inserted between the polymeric chains and with relatively high molecular mobility. This feature reduces the attraction forces between polymer macromolecules and gives plasticity and resistance to the final product (Marturano et al. 2017). ***Fillers*** consist of materials designed to increase the strength of final the product, avoid its contraction, and boosting its mechanical properties (Andrady and Rajapakse, 2016). ***UV stabilizers*** are compounds that reduce the chemical damage caused to the polymeric chains by UV radiation, including that of solar light (Hansen et al. 2015). Polymer degradation in the presence of oxygen is limited by the presence of ***Antioxidants***, additives used to avoid this phenomenon, especially during melt processing (SpecialChem, 2022). ***Lubricants*** are substances aimed at facilitating plastic flow during processing, reducing breakage and friction in the equipments used for it (Seemuth P,

2022). ***Flame retardants*** are compounds included in the plastics that are normally not bonded to the polymeric chains that protect the material in case of fire events or that slow down combustions avoiding emergency situations. Normally, flame retardants are designed to be released in contact with fire, reducing flame temperature or facilitating carbonization (Dufton P., 2018). Finally, ***Dyes*** are the most recognizable plastic additives. They are compounds, either organic or inorganic, that give the selected colour to the final product. Transparent ones are based on soluble compounds and opaque colours come from insoluble ones (Sastri V., 2010). The main additives used for plastic formulation are shown in Table 1.2.

Apart from the most common ones, the list of additives in the market is endless and include other types like antistatic agents, clarifiers, and dispersing agents, among others. Clearly, many of these additives may have ecological impact by themselves and can be responsible for the contamination of water, air and soils when leached from plastics (Campanale et al., 2020). Such compounds have been associated to alterations in humans and other mammals. This is the case of bisphenol-A, typically used as plasticizer that has been proved to act as endocrine disruptor in many studies (Xu et al., 2013; Faheem and Bhandari, 2021). In many cases, the reproductive system has been affected by additives including ovarian disorders (Takeuchi et al., 2004) and testosterone alterations (Galloway et al., 2010). Alterations in the immune and carcinogenic effects have also been described (Martin et al. 2010; Alabi et al., 2019).

Table 1.2. Main components of the most common plastic additives.

Type of Additive	Chemicals included in their structure
Fillers	Clay, silica, glass, chalk, talc, asbestos, alumina, rutile, carbon black and carbon nanotubes
Plasticizer	Phthalates; Non-phthalates (phosphoric esters, citrated, adipates or sebacates, trimellitates and benzoates) or biobased (glycerol, polyols and vegetable oils)
UV Stabilizer	HALS (Hindered amine light stabilizers); Cadmium, barium or lead salts
Antioxidants	Phenols and aromatic amines
Dyes	Inorganic pigments (heavy metals) or organic pigments (azo dyes, phthalocyanines, anthraquinone chromophores)
Lubricants	Calcium or magnesium stearates
Flame Retardant	Chlorine, bromine, phosphorus, or aluminium hydroxide

Clearly, one of the most dangerous problems related to plastics are related to chemical compounds that they may release to the environment. The list of additives is increasing at the same time as technology evolves, and many of them are proprietary formulations the exact composition of which is unknown. More research is needed including surveillance programs to guarantee that products reaching the market do not harm the environment.

1.3. A new Era for plastics

As explained before, recycling has become the second most important post-consumer management strategy for plastics. Used plastics can be used as raw materials to produce new plastic objects, or even for their conversion into fuel (Mani et al. 2009; Bezergianni et al. 2017) or other chemical products (Roy et al. 2021). Regulations are becoming stricter in order to boost plastic recovery, closing the lifecycle of plastics, and avoiding its spreading into the environment.

Public awareness and marketing strategies are evolving towards an increased environmental sensitivity. Companies have incentives to invest in sustainable products and strategies, sometimes with controversial results. Trying to use plastics in a greener way, recycled plastics have been created to generate new items with the same or different characteristics from the old ones. The idea of reusing is certainly appealing, but not always entirely satisfactory. One example is the polyethylene terephthalate (PET) recycling industry. The traditional recipients for beverages are recyclable, but in recent years, fancy companies converting them into textiles appeared with a feeling of green business (CMFoundation, 2022). The reality is that the conversion of polyester into fabrics breaks the cycle because textiles cannot be recycled, and their fibres become unrecoverable. From a recyclable material a non-recyclable one is obtained, which, otherwise, is responsible for synthetic fibre pollution, one of the most extended types of MP pollution in the environment (Athey and Erdle, 2022).

The pressure of regulatory measures intended to reduce plastic waste, triggered the creation of alternative materials. An example is the ban to single use plastics, established by different regulations worldwide i.e. directive EU 2019/904 (EU, 2019). An alternative for single use objects is the use of bioplastics. These artificial polymers are made from

natural raw materials and perform like traditional plastics (Fig. 1.3). The advent of ***biobased materials*** from natural feedstocks, as an alternative for those of fossil origin, is helping to reduce greenhouse gas (GHG) emissions since they only release the carbon previously fixed from the atmosphere and not that retained in petroleum. From biomass raw materials such as sugar cane or corn, either conventional plastics like polyethylene or biodegradable alternatives can be produced (Molina-Besch and Kszleri, 2022). The difference is the ***biodegradability*** of the product. A material is biodegradable if it allows microorganisms to convert it into natural substances such as water, carbon dioxide (CO_2) or other nutrients (Karak et al. 2016). Thus, bioplastics like polylactic acid (PLA), polyhydroxyalkanoates (PHAs) and others have the ability to be removed from the environment by natural degradation processes.

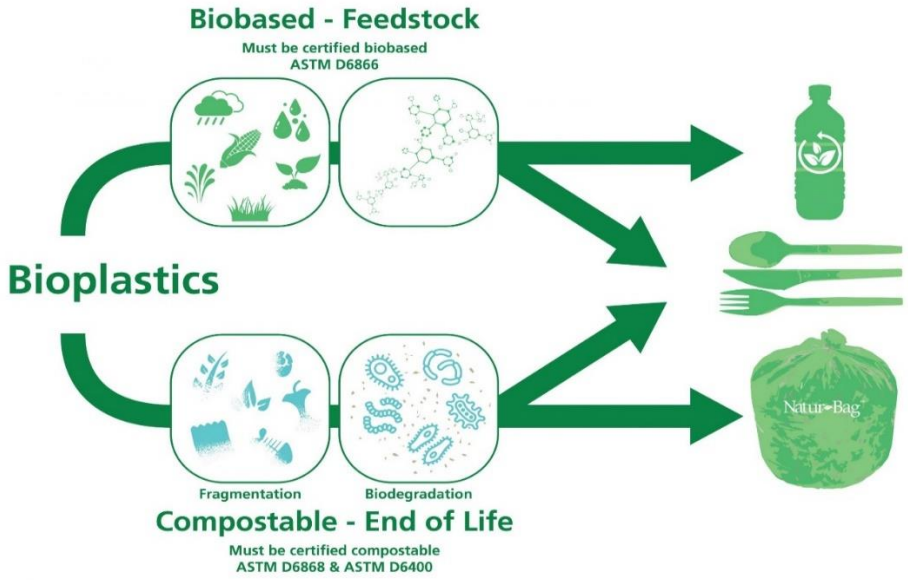


Figure 1.3. Source and fate of bioplastics, scheme of industrial possibilities (Naturbag, 2022).

Compostable materials are a subcategory of bioplastics that go one step beyond in the necessity to increase the circularity of plastic materials. Traditional plastics are consumed and discarded, generating plastic pollution if mismanaged. The idea behind bioplastics is to create materials with the capacity of disappear into the environment also providing nutrients to soil capable to restart the cycle. Many of these materials come from starch and other vegetal sources and guarantee their complete microbial decomposition, but only under certain conditions, like those of industrial composting plants.

We are seeing a clear increase in the usage of these materials, mainly as bags, or wrapping and containers in food industry, but also as agricultural plastics, in uses like mulching. A problem arising from these materials is the almost absolute lack of information regarding their toxicity. Even materials fulfilling all regulations can create a risk due to the dissemination of small fragments with still unknown effect towards environmental organisms, but that proved toxic in certain cases (González-Pleiter *et al*., 2019; Uribe-Echeverria and Beiras, 2022). The necessity to guarantee the safety of both bio- and compostable materials that arrive to the environment is also derived from the fact that they share some additives with conventional plastics besides including new ones. There is a need for monitoring the new bioplastic materials reaching the market to ensure the absence of negative impacts to the environment.

1.4. References

Alabi O, Ologbonjaye K, Awosolu O, E A. 2019. *Public and Environmental Health Effects of Plastic Wastes Disposal: A Review*. Journal of Toxicology and Risk Assessment; 5.

Altman R. *The myth of historical bio-based plastics*. Science 2021; 373: 47-49.

Amato JA. 2013. *Plastic: A Toxic Love Story. By Susan Freinkel* (Boston: Houghton Mifflin Harcourt, 2011. 324 pp.). Journal of Social History; 46: 811-814.

Andrady A, Rajapakse N. 2016. *Additives and Chemicals in Plastics. Hazardous Chemicals Associated with Plastics in the Marine Environment* in Handbook of Environmental Chemistry. SPRINGER, 2016. 978-3-319-95566-7. https://doi.org/10.1007/698_2016_124

Aragaw TA, De-la-Torre GE, Teshager AA. 2022. *Personal protective equipment (PPE) pollution driven by the COVID-19 pandemic along the shoreline of Lake Tana, Bahir Dar, Ethiopia.* 2022. Science of The Total Environment; 820: 153261.

Athey SN, Erdle LM. 2022. *Are We Underestimating Anthropogenic Microfibre Pollution? A Critical Review of Occurrence, Methods, and Reporting*. 2022; 41: 822-837.

Bezergianni S, Dimitriadis A, Faussone GC, Karonis D. 2017. *Alternative Diesel from Waste Plastics*. 2017. Energies 10(11):12. English. doi:10.3390/en10111750. Cited in: Pubmed; PMID WOS:000417046500069.

Campanale C, Massarelli C, Savino I, Locaputo V, Uricchio VF. 2020. A *Detailed Review Study on Potential Effects of Microplastics and Additives of Concern on Human Health*. Int J Environ Res Public Health 2020; 17.

Chem4us. 2021. "*Plastics and bioplastics: a 200-year history of research and development*." Retrieved september 2022, from http://www.chem4us.be/plastics-and-bioplastics-a-200-year-history-of-research-and-development/

CMFoundation. 2022. *Licence to greenwash. How certification schemes and voluntary initiatives are fuelling fossil fashion.* Changing Markets Foundation.

Cowger W, Gray A, Brownlee S, Hapich H, Deshpande A, Waldschläger K. 2022. *Estimating floating macroplastic flux in the Santa Ana River, California*. Journal of Hydrology: Regional Studies; 44: 101264.

Crespy, D., Bozonnet, M., & Meier, M. 2008. *100 Years of Bakelite, the Material of a 1000 Uses*. 47(18), 3322-3328. doi: https://doi.org/10.1002/anie.200704281.

De-la-Torre GE, Dioses-Salinas DC, Pizarro-Ortega CI, Fernández Severini MD, Forero López Ana D, Mansilla R, et al. 2022. *Binational survey of personal protective equipment (PPE) pollution driven by the COVID-19 pandemic in coastal environments: Abundance, distribution, and analytical characterization*. Journal of Hazardous Materials; 426: 128070.

Dufton, P.W. 1998. *Functional Additives for the Plastics Industry: A Report from Rapra's Industry Analysis Group*; CRC Press: Shrewsbury, UK.

ECHA, European Chemicals Agency. 2012. Guidance on polymers and monomers. Section 2.2 and Section 3.2.1.3. Retrieved from: https://echa.europa.eu/documents/10162/23036412/polymers_en.pdf/9a74545f-05be-4e10-8555-4d7cf051bbed

EU, European Commission. 2018. *A European Strategy for Plastics in a Circular Economy*. COM/2018/028.

EU, European Comission. 2019. *Single-use plastics: new rules for reporting on the reduction of single-use plastic food containers adopted.* EU 2019/904.

Ford HV, Jones NH, Davies AJ, Godley BJ, Jambeck JR, Napper IE, Suckling CC, Williams GJ, Woodall LC, Koldewey HJ. 2022. *The fundamental links between climate change and marine plastic pollution*. Science of The Total Environment; 806:150392. doi: https://doi.org/10.1016/j.scitotenv.2021.150392.

Free CM, Jensen OP, Mason SA, Eriksen M, Williamson NJ, Boldgiv B. 2014. *High-levels of microplastic pollution in a large, remote, mountain lake*. Marine Pollution Bulletin; 85: 156-163

Faheem M, Bhandari RK. 2021. *Detrimental Effects of Bisphenol Compounds on Physiology and Reproduction in Fish: A Literature Review*. Environmental Toxicology and Pharmacology. 2021/01/01/; 81:103497. doi: https://doi.org/10.1016/j.etap.2020.103497.

Friedel R. 1983. *Pioneer Plastic: The Making and Selling of Celluloid.* Univ. of Wisconsin Press.

Galgani F., Hanke G., Werner S., Oosterbaan L., Nilsson P., Fleet, D., et al. 2013. *Guidance on Monitoring of Marine Litter in European Seas, in EUR – Scientific and Technical Research Series*. ISSN 1831-9424 (Online), eds G. Hanke, S. Werner, F. Galgani, J. M. Veiga, and M. Ferreira (Luxembourg: Publications Office of the European Union).

Galloway T, Cipelli R, Guralnik J, Ferrucci L, Bandinelli S, Corsi AM, Money C, McCormack P, Melzer D. 2010. *Daily bisphenol A excretion and associations with sex hormone concentrations: results from the InCHIANTI adult population study.* Environmental health perspectives. Nov;118(11):1603-8. eng. Epub 2010/08/28. doi:10.1289/ehp.1002367. Cited in: Pubmed; PMID 20797929.

GESAMP. 2016. *Sources, Fate and Effects of Microplastics in the Marine Environment: Part Two of a Global Assessment.* IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, p. 220. Report Studies GESAMP No 93.

Geyer R, Jambeck JR, Law KL. 2017. *Production, use, and fate of all plastics ever made*. 3: e1700782.

Geyer R. 2020. Chapter 2 - *Production, use, and fate of synthetic polymers*. In: Letcher TM, editor. Plastic Waste and Recycling. Academic Press, 2020, pp. 13-32.

Gómez F, Rimma S. 2019. *Plastics and the Environment. Setting the facts straight on plastics*. Retrieved from https://www.weforum.org/agenda/2019/10/plastics-what-are-they-explainer/. World Economic Forum.

González-Pleiter M, Tamayo-Belda M, Pulido-Reyes G, Amariei G, Leganés F, Rosal R, et al. 2019. *Secondary nanoplastics released from a biodegradable microplastic severely impact freshwater environments*. Environmental Science: Nano; 6: 1382-1392.

González-Pleiter M, Edo C, Casero-Chamorro MC, Aguilera Á, González-Toril E, Wierzchos J, et al. 2020. *Viable Microorganisms on Fibers Collected within and beyond the Planetary Boundary Layer*. Environmental Science & Technology Letters 7: 819-825.

Hartmann NB, Hüffer T, Thompson RC, Hassellöv M, Verschoor A, Daugaard AE, Rist S, Karlsson T, Brennholt N, Cole M, Herrling MP, Hess MC, Ivleva NP, Lusher AL, Wagner M. 2019. *Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris*. Environmental Science & Technology. 2019/02/05;53(3):1039-1047. doi:10.1021/acs.est.8b05297.

Hansen, E.; Nilsson, N.H.; Lithner, D.; Lassen, C. 2013. *Hazardous Substances in Plastic Materials*; Klima- ogforurensningsdirektoratet: Vejle, Denmark.

Hoang TC, Mitten S. 2022. *Microplastic accumulation in the gastrointestinal tracts of nestling and adult migratory birds*. Science of The Total Environment; 838: 155827.

Jenkins T, Persaud BD, Cowger W, Szigeti K, Roche DG, Clary E, et al. 2022. *Current State of Microplastic Pollution Research Data: Trends in Availability and Sources of Open Data*. 2022; 10.

Jiménez-Skrzypek G, Hernández-Sánchez C, Ortega-Zamora C, González-Sálamo J, González-Curbelo MÁ, Hernández-Borges J. 2021. *Microplastic-adsorbed organic contaminants: Analytical methods and occurrence.* TrAC Trends in Analytical Chemistry; 136: 116186.

Karak N. 2016. *15 - Biopolymers for paints and surface coatings*. In: Pacheco-Torgal F, Ivanov V, Karak N, Jonkers H, editors. Biopolymers and Biotech Admixtures for Eco-Efficient Construction Materials. Woodhead Publishing; p. 333-368.

Ledieu L, Tramoy R, Mabilais D, Ricordel S, Verdier L, Tassin B, et al. 2022. Macroplastic transfer dynamics in the Loire estuary: Similarities and specificities with macrotidal estuaries. Marine Pollution Bulletin; 182: 114019.

Liro M, Mikuś P, Wyżga B. 2022. *First insight into the macroplastic storage in a mountain river: The role of in-river vegetation cover, wood jams and channel morphology*. Science of The Total Environment; 838: 156354.

Mani M, Subash C, Nagarajan G. 2009. *Performance, emission and combustion characteristics of a DI diesel engine using waste plastic oil*. Appl Therm Eng. 29(13):2738-2744. English. doi:10.1016/j.applthermaleng.2009.01.007. Cited in: Pubmed; PMID WOS:000267326200019.

Martin LB, Hopkins WA, Mydlarz LD, Rohr JR. 2010. *The effects of anthropogenic global changes on immune functions and disease resistance*. Annals of the new york academy of sciences; 1195(1):129-148. doi:https://doi.org/10.1111/j.1749-6632.2010.05454.x.

Marturano V, Cerruti P, Ambrogi V. 2017. *Polymer additives*. Physical Sciences Review; 2.

Mastrolia, C., D. Giaquinto, C. Gatz, M. N. Pervez, S. W. Hasan, T. Zarra, C.-W. Li, V. Belgiorno and V. Naddeo. 2022. *Plastic Pollution: Are Bioplastics the Right Solution?.* Water. 14(22): 3596.

Molina-Besch K, Keszleri H. 2023. *Exploring the industrial perspective on biobased plastics in food packaging applications – Insights from Sweden*. Sustainable Production and Consumption; 2023/01/01/;35:72-84. doi:https://doi.org/10.1016/j.spc.2022.10.018.

Naturbag. "*Bioplastics 101*". 2022. Retrieved September, 2022, from https://naturbag.com/bioplastics101/

Paluselli A, Fauvelle V, Galgani F, Sempéré R. 2019. *Phthalate Release from Plastic Fragments and Degradation in Seawater*. Environmental Science & Technology. 2019/01/02;53(1):166-175. doi:10.1021/acs.est.8b05083.

PlasticsEurope. 2021. *Plastics - the Facts 2021*. *An analysis of European plastics production, demand and waste data*.

Rodríguez C, Fossatti M, Carrizo D, Sánchez-García L, Teixeira de Mello F, Weinstein F, et al. 2020. *Mesoplastics and large microplastics along a use gradient on the Uruguay Atlantic coast: Types, sources, fates, and chemical loads.* Science of The Total Environment; 721: 137734

Roy PS, Garnier G, Allais F, Saito K. 2021. *Strategic Approach Towards Plastic Waste Valorization: Challenges and Promising Chemical Upcycling Possibilities*. CHEMSUSCHEM 5;14(19):4007-4027. English. doi:10.1002/cssc.202100904. Cited in: Pubmed; PMID WOS:000670536200001.

Scopetani C, Chelazzi D, Martellini T, Pellinen J, Ugolini A, Sarti C, et al. 2021. *Occurrence and characterization of microplastic and mesoplastic pollution in the Migliarino San Rossore, Massaciuccoli Nature Park (Italy*). Marine Pollution Bulletin; 171: 112712.

Sastri VR. 2010. Chapter 5 - *Polymer Additives Used to Enhance Material Properties for Medical Device Applications*. In: Sastri VR, editor. Plastics in Medical Devices. Boston: William Andrew Publishing;. p. 55-72.

Seemuth P. 2022. *Lubricants for Polymers*. SpecialChem TMSP, editor. November 2022. Available from: https://polymer-additives.specialchem.com/selection-guide/lubricants#PaulSeemuth.

Singh B, Sharma N. 2008. *Mechanistic implications of plastic degradation*. Polymer Degradation and Stability; 93: 561-584

SpecialChem. 2022. *Antioxidants Stabilizers Selection for Polyolefins (PP, PE).* SpecialChem TMSP, editor. November 2022. Available from: https://polymer-additives.specialchem.com/selection-guide/antioxidants-stabilizers-selection-for-polyolefins-pp-pe

Su L, Xiong X, Zhang Y, Wu C, Xu X, Sun C, et al. 2022. *Global transportation of plastics and microplastics: A critical review of pathways and influences*. Science of The Total Environment; 831: 154884.

Takeuchi T, Tsutsumi O, Ikezuki Y, Takai Y, Taketani Y. 2004. *Positive Relationship between Androgen and the Endocrine Disruptor, Bisphenol A, in Normal Women and Women with Ovarian Dysfunction*. Endocrine Journal;51(2):165-169. doi:10.1507/endocrj.51.165.

Thompson, R.C., Olsen, Y., Mitchell, R., Davis, A., Rowland, S., John, A., McGonigle, D. and Russell, A. 2004. *Lost at sea: where is all the plastic?* Science 304, 5672, 838-838.

Uribe-Echeverría T, Beiras R. 2022. *Acute toxicity of bioplastic leachates to Paracentrotus lividus sea urchin larvae.* Marine Environmental Research; 2022/04/01/;176:105605. doi:https://doi.org/10.1016/j.marenvres.2022.105605.

Xu H, Yang M, Qiu W, Pan C, Wu M. 2013. *The impact of endocrine-disrupting chemicals on oxidative stress and innate immune response in zebrafish embryos*. Environmental Technology and Toxicology; 32(8):1793-1799. doi: https://doi.org/10.1002/etc.2245.

WOS, 2022. *Web of science.* Clarivate. Retrieved: November 2022. url: https://www.webofscience.com/wos/woscc/basic-search

OBJECTIVES

The aim of this Doctoral Thesis is to establish an **exhaustive characterization of the source, and chemical identification of the MPs that reach our ecosystems.** The characterization of MPs that reach the less studied environments is a need in view of the relative scarcity of data compared with marine studies. The interconnection of different environments is key to understand the missing aspects concerning the fate of MPs, which is a prerequisite to perform any risk assessment. The present Doctoral Thesis emphasizes this connectivity by studying the arrival of plastic materials to soil or through air or wastewater, among other links.

Moreover, the characterization of MPs is essential to allow regulators to establish limits in newly produced regulations. Thanks to the large number of articles published, the scientific community has become aware of the knowledge gaps existing in MPs research, one of the main being the lack of methodological standardization for characterization procedures. Different matrices require different extraction procedures, and different protocols lead to results difficult to compare even for the same substrate or compartment. This makes difficult to understand the magnitude of the problem and to establish the aforementioned regulations. This is the second objective of this Doctoral Thesis: to establish a common protocol for all matrices that may allow result comparison.

In this Doctoral Thesis, the main characterization technique used was Fourier-Transformed Infrared Spectroscopy (FTIR), but others like Raman or optical microscopy, allowed the characterization of MPs. Overall, they joint use of those techniques is expected to yield a full picture of the variety of the materials that constitute plastic debris in the environmental compartments studied. All that information is intended to pave the way to a better understanding of the risks associated to plastic litter and to help the administrations to take decisions for their reduction or their correct management in order to improve future environmental quality and avoid their effect on biota.

The **specific objectives** of this Doctoral Thesis are:

1. The characterization of the plastics that arrive in coastal environments and to expand that research to other compartments such as freshwater with the study of effluents from wastewater treatment plants (WWTPs) or soils, by studying the sludge generated in WWTPs or/and the compost from municipal organic wastes that are used as an agricultural amendment.
2. Increase the information available on the presence of MPs in the atmosphere, the less studied compartment, and their dispersion by establishing a novel high-altitude detection system and/or by using honeybees as bioindicators of their presence in aerial ecosystems.
3. Establish a common experimental procedure along all the Doctoral Thesis that allow the intercomparison of results and to allow the standardization of protocols. The protocols follow the next steps: (1) Pre-treatment, (2) Sample assessment by means of optical evaluation and (3) polymer spectroscopical identification.
4. Regarding identification, the development of a complete database of spectra from different polymers is expected, generating knowledge about spectroscopy and that helps at gaining experience in the spectroscopic analysis of plastic polymers and will be used in the different research to perform during the doctoral Thesis.
5. Contribute to the improvement of quality assurance vs quality control protocols (QA/QC). To this, different protocols will be sought to guarantee the cleanliness of all the materials to be used during the processing of MPs samples and avoid the cross-contamination caused by the sampler which can be the cause of false positives.
6. Generate knowledge about the polymers found in the different environments, both plastic and man-modified natural materials such as cellulose or cotton and serve as a guide for the traceability of their source or the establishment of control plans for the waste originated of both traditional materials and new materials in development.

CHAPTER 2. OCCURRENCE AND IDENTIFICATION OF MICROPLASTICS ALONG A BEACH IN THE BIOSPHERE RESERVE OF LANZAROTE

2.1. Introduction

The pollution of marine environment with microplastics is a global threat that poses one of the most serious environmental problems for aquatic ecosystems (Cole et al., 2011; Chae and An, 2017). Fragments lower than 5 mm are commonly defined as microplastics in line with the NOAA definition, which turned into an international standard (Gago et al., 2016). No lower size boundary is clearly defined despite its potential relevance (Gigault et al., 2018). The boundary between categories is commonly established based on the size opening of the sieves used for sampling or sorting. Accordingly, plastics with two dimensions smaller than mesh openings are eventually slip through the mesh and may get missed or counted in the category immediately lower. It has been argued that this phenomenon contributes to significant differences in mass and particle counts (Everaert et al., 2018). Concerning the chemical nature of plastic debris, the most used plastics are the most commonly found among sorted microplastics (Imhof et al., 2017). The higher occurrence corresponds to polyethylene (PE) and polypropylene (PP) together with polystyrene (PS), the latter probably overrepresented in debris because of its major use as packaging material. Polyethylene terephthalate (PET), polyvinyl chloride (PVC), and other synthetic fibres are usually reported in lower amounts. The high-volume usage of PE together with its floatability makes it the material with higher likelihood of being recovered from marine litter (Hidalgo-Ruz et al., 2012).

Worldwide plastic production amounted to 348 million tonnes in 2017. In 2016, the more recent year available, the amount of plastic wastes collected through official schemes in the EU (plus Norway and Switzerland) amounted to 27.1 million tonnes, representing less than half of the total plastics production in the same countries (PlasticsEurope, 2018). The balance corresponds to goods still in use and non-collected waste, eventually ending up in the environment, particularly in oceans, which act as the final sink of most plastic debris. Accordingly, a high amount of plastics is being reported in seas and oceans as floating fragments. Eriksen et al. (2014) estimated a total number of 5.25 trillion (5.25×10^{12}) plastic particles in the world's oceans weighing one quarter million tonnes. Noteworthy, the observed amount of lower size microplastics is much lower than expected, which may imply the existence of efficient mechanisms that remove small plastic particles from the ocean surface (Eriksen et al., 2014). It has been

suggested that coastal areas constitute a sink of plastics buried in beaches and marshes (Herrera et al., 2018). Another explanation is that deep-sea sediments accumulate microplastics (Woodall et al., 2014). Concerning environmental fate, the fragmentation to lower sizes is a well-known fact eventually making plastic debris undetectable to current sampling methods (Koelmans et al., 2015).

The obvious environmental risk associated to microplastics refers to the mechanical damage due to plastic ingested by marine organisms (Li et al., 2018). Large plastic debris, classified as mesoplastics or macroplastics, can produce damage to wildlife and fisheries (Kühn et al., 2015). Besides, their impact in touristic activities is apparent causing an important aesthetic issue, with economic losses due to the cost of cleaning and the reduction of visitors in touristic coastal areas (Jang et al., 2014). Other risk factor associated to plastic debris in the marine environment is the leaching of plastics additives. Plastic fragments may also pose a chemical risk due to the adsorption of hydrophobic pollutants on their surface (Avio et al., 2017). This issue is controversial as it has been argued that environmental concentrations are much lower than those required for plastics to behave as a vector of anthropogenic pollutants (Koelmans et al., 2016). Plastic debris exert biohazard due to its role in the spreading of microbial pathogens implicated in outbreaks for a variety of wildlife forms (Lamb et al., 2018). It has also been shown that microplastics in environments co-polluted with metals and antibiotics may develop co-selection of metal-driven antibiotic resistances, which is also an emerging threat to human health (Yang et al., 2019).

The Canary Islands are bathed by the Canary Current, which is a wind-driven surface current associated to the North Atlantic Gyre responsible for a high level of plastic pollution in the beaches of the Northern Islands (Baztan et al., 2014). Lanzarote and its Northern minor islands constituting the Chinijo Archipelago, are a highly protected area, declared Biosphere Reserve by UNESCO in 1993. Despite being a highly protected area, their beaches are highly polluted by microplastics, with average mass surface concentration of 23.7 g/m^2 (annual maximum 125 g/m^2) and an average of 1656 pieces/m^2 (1 mm < size < 5 mm) (Herrera et al., 2018). In this work, the systematic quantification and characterization of plastic debris collected at several locations along Ámbar Beach in La Graciosa island is reported. The purpose was to perform a thorough

particle size and chemical composition assessment to increase the knowledge about the plastic pollution that arrive at shorelines and evaluate its impact on a remote protected area.

2.2. Experimental section

2.2.1. Area of study

The sampling was carried out during the first week of September 2018 along the sandy beach Ámbar locally known as Lambra (Canary Islands, Spain). This beach is in the North of La Graciosa Island, the largest island of the Chinijo archipelago which, together with Lanzarote Island, constitute UNESCO Biosphere Reserve since 1993. Ámbar beach presents a total coastline length of 600 m, with intercalary stretches of white sand and black rocks along the shoreline (Fig. 2.1). Ámbar beach is oriented towards N-NE being one of the first locations in La Graciosa affected by the predominant winds and the ocean Canary Current, which runs in parallel to the African coast in SW direction until reaching the islands. La Graciosa (29 km^2) has a small population of < 800 people concentrated in two villages. Ámbar is an isolated beach characterized by low tourist pressure and limited waste accumulation not significantly increased by tourism or fishing activities. Nevertheless, there is evident deposition of plastic debris over the sand at different heights depending on tide levels, which tend to appear mixed up with wave-driven algae.

2.2.2. Sampling and sorting

Sampling was performed along the lowest high tide line due to the high amount of organic matter (mainly algae) deposited by waves along the highest tide line (Baztan et al., 2014). Sampling points A to D (Fig. 2.1) cover a linear extension of 90 m in the centre of the beach. All sampling points were located below the 5 m contour line and represented different hydrodynamic conditions. Whereas points A and B were open to the sea, C and D were protected from the waves by a line or rocks. A grade of 1.5-1.7 m existed between points C-D and the lowest point A due to the slope of the beach. Accordingly, points C and D were protected in a relatively quiet area even during high tides. Figs. S2.1 and S2.2 (Annex - Supplementary material, SM) show aerial images of Ámbar beach indicating sampling points and the directions of sea entry during high tides.

Finally, according to the Spanish State Meteorological Agency (AEMET), the meteorological conditions during sampling and the period immediately before were stable with absence (< 2.5 mm) of precipitations and any abnormal winds.

The exact location of each sampling point was: A: 29°16'44.9"N 13°29'44.0"W, B: 29°16'45.6"N 13°29'44.8"W, C: 29°16'46.3"N 13°29'45.7"W and D: 29°16'46.7"N 13°29'46.3"W (Fig. 2.1, S2.1 and S2.2 (SM)).

Sampling was performed in 1 m × 2 m rectangles comprising free sandy and algae covered zones with a sampling depth of 1 cm (Baztan et al., 2014; Herrera et al., 2018). The sampling recommendations of the Technical Subgroup on Marine Litter (TSG-ML) were followed as ex- posed in the Guidance on Monitoring of Marine Litter in European Seas (Hanke et al., 2013). The samples, consisting of sand and debris, were sieved using a 5 mm opening sieve. Sieved samples were then separated by density in a stainless-steel bucket, using sea water. Plastic debris were then collected using a 1 mm opening size sieve. All collected microplastic debris was dried and preserved in glass bottles for further analysis.

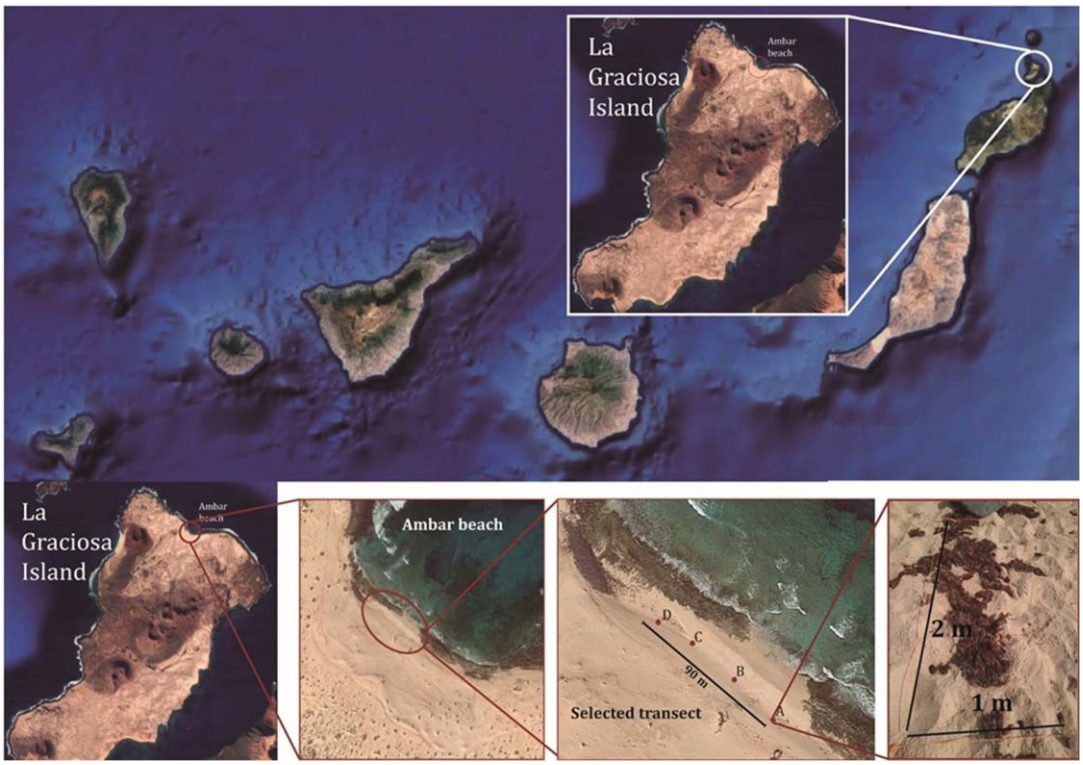


Figure 2.1. Localization of the study area in La Graciosa island, in the North part of the Canary Islands (Spain).

Samples were carefully inspected with a stereo microscope Motic SMZ140 Series. All particles supposed to be microplastics were separated from tar balls and algal structures, counted, and organized by type and colour. A selection based on colours and typologies wider than usual has been performed trying to gain a more detailed description (Hidalgo-Ruz et al., 2012). For all typologies and sampling points, the total amount of microplastics was weighed. After that, size measurement was performed by exhaustively photographing all plastic debris and processing images by means of the ImageJ software. Projected area, perimeter, length and width were recorded for every microplastic. Random subsamples from every colour and type were separated for polymer identification.

2.2.3. Analytical methods

The chemical composition of microplastics was assessed by means of Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) and Raman spectroscopy. ATR-FTIR spectra were obtained in a Thermo-Scientific Nicolet iS10 apparatus with a Smart iTR-Diamond ATR module. The associate software was OMNIC version 9.1.26 (Thermo Fisher Scientific Inc., Massachusetts, USA). Spectra were taken in the 4000–800 cm^{-1} range with a resolution of 4 cm^{-1} (data spacing of 0.483 cm^{-1}) using 32 scans. Between samples, the ATR-crystal was cleaned with isopropanol and background signal updated. Raman spectra were obtained using a Thermo Scientific DXR Raman Microscope with Omnic for dispersive Raman software version 8 (Thermo Fisher Scientific). Samples were observed using 10×, 20×, and 50× objectives. Measurements were performed using a 780 nm laser with a power range from 1 to 10 mW with a 400 lines mm^{-1} grating. The power was selected depending on the fluorescence produced by each particle (7-8 mW generally yielded good quality spectra). Spectral range selected was 3100 to 200 cm^{-1}, resolution 1.92 (spectral data spacing 0.964 cm^{-1}) and the number of repetitions and the duration of acquisition time was adjusted for every sample depending on signal-to-noise ratio and the quality of spectra. Both in FTIR and Raman studies, a minimum of three spectra were taken per particle in three random points. Polymer identification was performed by statistically comparing (Pearson correlation) the obtained spectra with a library created with pure polymers acquired from Sigma-Aldrich and Goodfellow as well as using the spectral libraries included in Omnic Spectra software. The minimum

matching for positive identification was set at 80% as recommended elsewhere (Rios-Mendoza et al., 2018). Scanning Electron Microscopy (SEM) was also used to visualize gold-covered plastic debris in a Philips XL30-FEG apparatus.

2.3. Results and discussion

In this work, the coastal line of Ámbar beach was sampled in four specific points (identified as A, B, C and D) as indicated before. The total weight of plastic particles collected was 290 g, which makes an average of 36.3 g/m^2 for the whole sampled surface, in line with results reported before (Baztan et al., 2014; Herrera et al., 2018). It is interesting to note the high variability observed along the beach. Moving from point A-B to D, the number of particles increased by almost a factor of 20 (Fig. 2.2A). The results for the four sampled points were, expressed in mass units: (A) 8.5 g/m^2, (B) 13.3 g/m^2, (C) 19.8 g/m^2, and (D) 103.4 g/m^2. The accumulation of plastic particles in point D clearly indicated their preferential deposition in the most protected area of the beach.

Microplastic particles, separated from sand and organic matter, were classified by shape into seven categories: Fragments, pellets, moulded particles, foams, filaments, microbeads and films. The categories were taken from the literature with the inclusion of “moulded particles” as a subcategory of plastic fragments that did not completely lose their original shape (Hidalgo-Ruz et al., 2012). Besides, and for every category, they were separated into twelve colours, namely, black, blue, brown, green, grey, orange, pink, purple, red, translucent, white and yellow. Figs. S2.3 and S2.4 (SM) show examples of the different colours and typologies. Shape distribution yielded 87% fragments, 9% pellets, 1.4% filaments and < 1% for the other categories (Fig. 2.2).

Most plastic particles were fragments or secondary microplastics product of the disaggregation of larger materials into smaller pieces (Bonanno and Orlando-Bonaca, 2018). On the contrary, Antunes et al., studied debris in Portuguese coasts and found that pellets were dominant (79%) with foams being also an important part of the sampled materials (Antunes et al., 2018). Plastic pellets are usually associated to industrial activity, which is far from the remote area sampled in this work (Domènech et al., 2019). The almost absence of foams in Ámbar beach could be explained because of

the lower intensity of fishing activities. La Graciosa Island is almost uninhabited, and fishing is limited to traditional fishing according to its character of marine reserve.

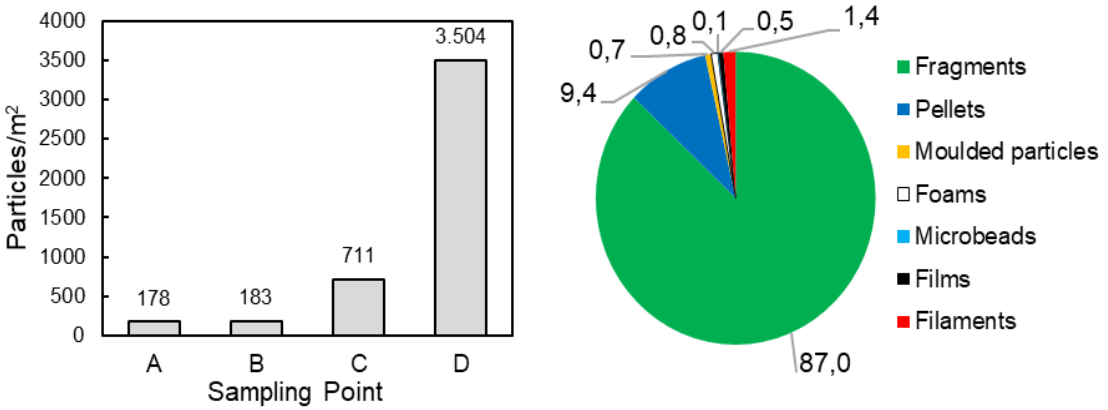


Figure 2.2. Variability of plastic particles along the shoreline (A) and number percent global distribution among typologies (B).

The Spanish Ministry of Environment attributed the contribution of fishing to no > 2% of the total marine pollution (MAGRAMA, 2018). The marine pollution in the Canary Islands has a diffuse origin and that from local sources can be preferentially attributed to tourism, which is the main economic activity of the region, but touristic pressure in the Chinijo reserve is very low. Another difference with other literature sources was the presence of fibers, which was very limited, amounting only to 0.2% in number. Whitmire et al. stated that fibers dominated in majority of sampling points in a study performed in USA, with beads being also frequent (Whitmire et al., 2017). Globally, our work shows the kind of diffuse pollution expected in remote areas far from the main sources of human activity and the capacity of microplastics to diffuse around the globe.

The dimensions of all particles were measured using ImageJ software. Our study recovered a total number of 9149 plastic particles between 1 mm and 5 mm opening size sieves. All of them were photographed, length, width, and perimeter measured, and projected area calculated. They were finally classified in the six size categories indicated in Fig. 2.3. It is interesting to note that 5 mm nominal mesh opening was compatible with the passing of a considerable number of particles with projected area diameter > 5 mm. In our case, 29.8% of the total number of particles passing through 5 mm sieves, therefore classified as microplastics, presented projected area diameter > 5 mm. Projected area diameter, defined as the diameter of a circle with the same projected

area as the particle, was chosen as the most representative dimension for size classification. Clearly is an orientation-dependent measure that refers to the preferential stable orientation of the particle and its use can be controversial in case of highly anisometric particles. Fig. S2.5 (SM) compares particle width with projected area diameter for all the particles measured in this study. Fig. 2.3 also shows that the abundance of particles decreased with decreasing size. The fraction ranging 4–5 mm was more abundant (23.1%) than the smaller categories: 3–4 mm (20.2%), 2–3 mm (17.9%), 1–2 mm (8.8%), and < 1 mm (0.2%). These results were compatible with the disaggregation of homogeneous particles producing smaller fragments without complete disaggregation of the parent particle. Similar results were previously reported for plastics from Famara beach in the neighbouring island of Lanzarote, Canary Islands (CEDEX, 2018).

Our results showed the occurrence of many asymmetric particles, which are those falling below the parity line in the graph shown in Fig. S2.5 (SM). The plot shows that many particles had projected area diameter > 5 mm and could be considered as meso-debris according to their largest dimension. Conventionally, however, size cutoff is based on mesh size opening without explicitly considering the non-sphericity of plastic particles in line with the generally accepted definitions of TSG-ML and NOAA (Gago et al., 2016). The fraction < 1 mm was not sampled, but an important amount of particles < 1 mm was clearly observed in situ during sampling. Although discarded, this small fraction, consisting of fragments with size like sand grains may pose an important threat to the environment (Anderson et al., 2016). It is interesting to note that the asymmetry of sampled plastic particles increased when moving to points further to the sea entrance. Fig. S2.6 (SM) shows the tendency to lower projected circularity for fragments sampled in points C and D. This result may be explained by a preferential accumulation of more irregular fragments in the most protected part of the beach due to its specific hydrodynamic conditions.

Microplastics have also been sorted by colour (Fig. S2.7, SM). The analysis showed that both transparent and opaque particles were almost equally collected (about 50%). CEDEX sampling showed that amber, white and transparent particles were the most frequently found in debris collected from beaches (CEDEX, 2017, 2018). Colour

distribution is influenced by the presence of pellets as they constitute most of the particles without specific colouration. However, a much higher proportion of clear colours was found (translucent, 50%; white 22%), which was not explained by the abundance of pellets (> 90% of which were translucent). A probable reason is that translucent fragments lost colour because of bleaching due to ageing. A relationship between colour and age was found before, indicating that opaque materials become translucent upon degradation (Fanini and Bozzeda, 2018). Turner and Holmes assessed the palette of colours for plastic pellets found in Maltese beaches (Turner and Holmes, 2011). They concluded that weathering and photooxidation resulted in the production of secondary quinoidal compounds that impart a yellowish colour. The fraction of yellowish plastics in our sampling was relatively small, probably meaning a further stage of ageing processes. Blue and green colours were the following most commonly found (10% and 5%, respectively) with other colours in lower frequencies. Colour characterization is important because some species of seabirds and some marine organisms select their preys depending on colour (Mattsson et al., 2015; Veiga et al., 2016).

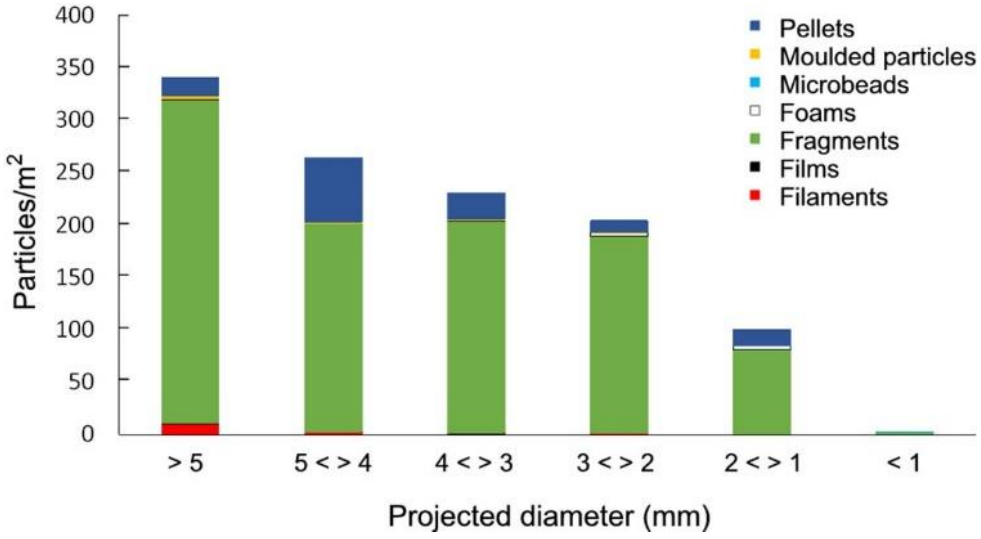


Figure 2.3. Size frequency and typology of the different microplastic particles as an average of all sampling points.

A representative subsample was prepared with plastics from every sampling point and typology to perform chemical identification by Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) and Raman spectroscopy. They are vibrational spectroscopy techniques allowing non-destructive analysis in a fast and reliable way.

Both techniques are generally applied to identify the chemical nature of microplastics in environmental samples. Their combination was necessary for the characterization of the smaller fractions and the coloured samples (Käppler et al., 2016; Strungaru et al., 2019). The number of plastic particles analysed was 711, representing 7.8% of the total number of particles recovered. Raman microscopy was used to identify the smaller sized particles and fragile particles, which showed tendency to break in smaller pieces. ATR-FTIR was used for larger particles or for particles with less tendency to break (Cabernard et al., 2018). Both techniques resulted in good quality spectra (Fig. 2.4), the use of Raman was more difficult because of its sensitivity to additives sometimes complicated the assessment of polymer characteristic bands. One example is shown in Fig. S2.8 (SM) in which cooper phthalocyanine, a blue colorant typically used in plastics, was identified (Caggiani et al., 2016; Ribeiro-Claro et al., 2017). The presence of additives is a subject topic of controversy as their leaking is a well-known cause for environmental concern (Koelmans et al., 2016; Whitmire et al., 2017).

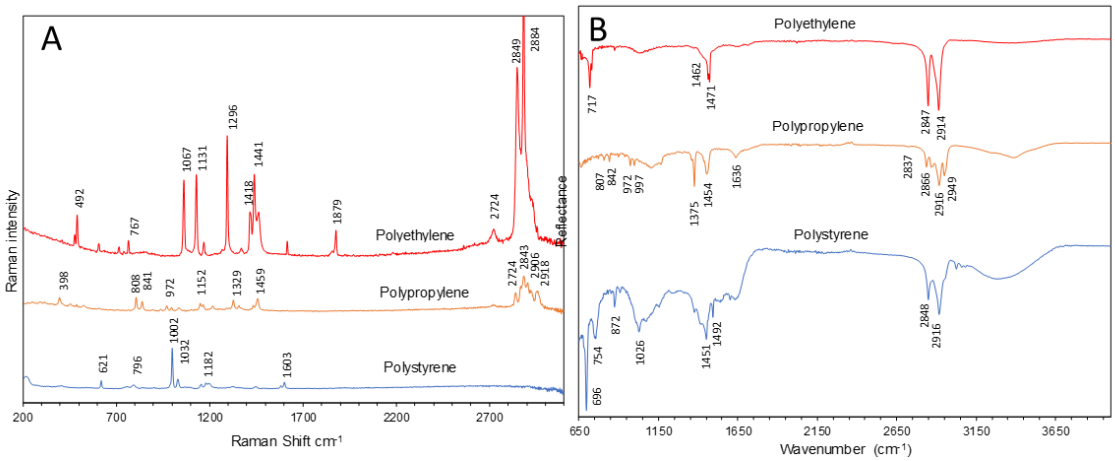


Figure 2.4. Raman (A) and ATR-FTIR (B) representative spectra of different sampled microplastic fragments identified as PE, PP and PS.

The results of chemical analyses are summarized in Fig. 2.5. The most frequent polymer found in samples was PE, which corresponded to 63% (n = 445) of the particles. PP was the second representative with 32% (n = 226). PS minority occurred in only a 3% (n = 22) of the plastics. This result agreed with the fact that these three polymers account for roughly 90% of the 348 million tonnes of plastics produced annually (Mattsson et al., 2015; PlasticsEurope, 2018). PS was found mainly as forms of small size displaying the characteristic spongy structure of PS packaging material (Crawford and Quinn, 2017).

Besides, PS appeared underrepresented in comparison with other sampling campaigns (Antunes et al., 2018; CEDEX, 2018; MAGRAMA, 2018). PE clearly dominated fragments and pellets, in coincidence with other's findings (Turner and Holmes, 2011). In our sampling 20% of pellets were PP. White particles preferentially consisted of PP (48% of white particles were PP), but translucent particles were dominated by PE (66%). PS was found mainly in white colour according to its main use. Concerning the 2% of remaining particles, polyurethane, methacrylate and some copolymers (polyethylene-polypropylene, polypropylene-polyethylene and polyvinyl acetate-polyethylene) were identified. The characterization and significance of this minor fraction was a difficult task because of the chemical changes due to ageing and the scarcity of references.

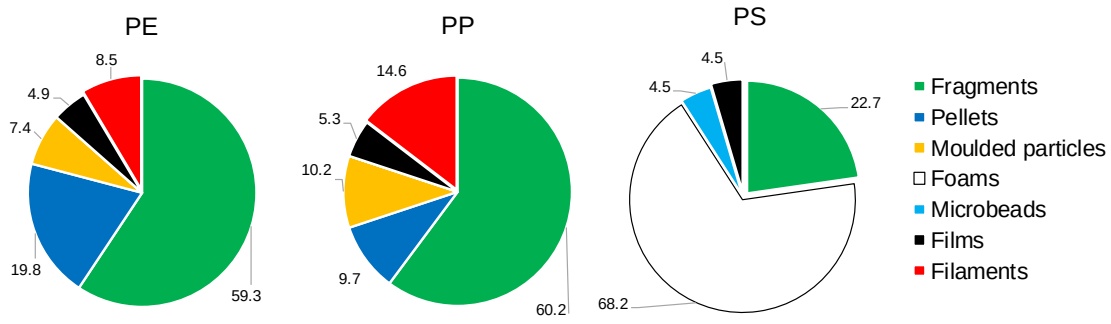


Figure 2.5. Percent distribution of plastic typology by polymer nature.

It is a well-known fact that many organisms, including commercially important fish species, are exposed to buoyant, low-density plastics, which include PE, PP and PS (Cole et al., 2011). The main polymers detected in this work have a density ranging low enough to make them buoyant. Higher density polymers, usual in land-based wastes such as PET or PVC, were not represented in our samples suggesting sinking or association to biota before reaching the coast. The density of marine debris has been recognized as a major driver for their environmental fate (Rochman, 2018; Song et al., 2018). It is interesting to compare the ratio PE/PP obtained in our sampling with production figures. In 2017 the European (PlasticsEurope, EU28 + NO/CH) plastic demand by type was 29.8% for PE (high and low density) and 19.3% for PP, which corresponded to a PE/PP ratio of 1.54, lower than the ratio PE/PP 1.96 (in number) obtained in this work. The figures for 2006 (PlasticsEurope) were similar, 29% PE and 19% PP for PE/PP ratio 1.5. PE/PP production ratio was higher during the eighties and nineties (1.7-1.8) due to the increasing demand for PP (Geyer et al., 2017).

Other studies found PE/PP ratios above production figures. Pan et al. reported PE/PP 1.61 in the Northwestern Pacific Ocean sampled using surface manta trawl with mesh size of ~330 µm. Modelling studies showed that plastic particles released to the marine environment may stay near coastal regions for years or decades (Lebreton et al., 2012). It may happen that certain conditions favour the preferential sampling of very old plastic particles, with historical PE/PP ratios, but the most probable cause for the bias in PE/PP ratio is that the hydrodynamic characteristics of the beach led to the accumulation of the more aged fragments in the more protected parts. The lower proportion of PP would be the consequence of its lower stability with respect to PE due to the presence of tertiary carbon atoms in the backbone, which are more prone to abiotic attack than the secondary carbons of PE (Gewert et al., 2015). Fig. 2.6 shows the ratio PE/PP for the four areas sampled in this work displaying a clear tendency towards higher PE/PP ratios for points C and D, which were those more distant from the opening in the rocks that connected the beach with the sea.

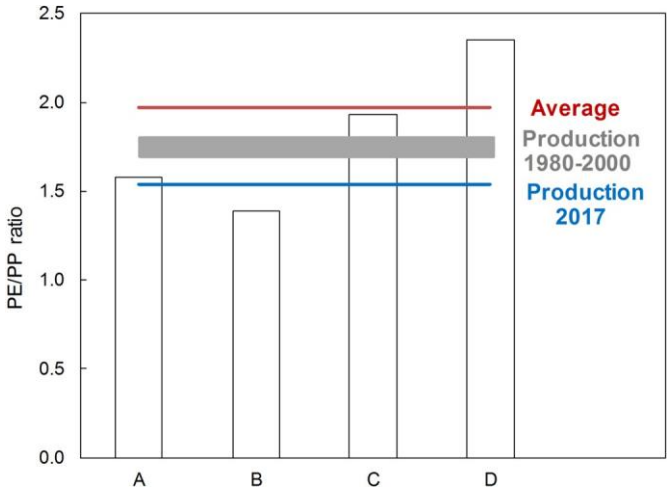


Figure 2.6. Relative abundance of PE and PP in the points sampled in this work compared to production ratios.

Sampled particles were clearly affected by environmental elements like photobleaching, and sand erosion. Visually, the samples obtained in this study could be described of soft consistence PE particles, while PP appeared more as brittle fragments, films and filaments. Fragments varied from pure smooth and flat surfaces to granulated or cracked surfaces. SEM images of aged particles show characteristic cracks, protrusions, and depositions covering their surface (Fig. 2.7). Detailed SEM images comparing samples of PE, PP and PS particles compared to new, pristine, pellets are shown in Fig. S2.9 (SM).

In this work, the characteristics defining microplastic (1 mm < > 5 mm) particles were established along a coastal line of an area of high biological importance and low human impact. The high amount of microplastic debris collected and measured, highlights the magnitude of global plastic pollution. The relevance of having precise estimations of microplastics in beaches comes from the fact that coastal lines are one of the most important points of contact of anthropogenic heterogeneous materials with wildlife (Coppock et al., 2017). It is to be noticed that cleaning plastic litter on Ámbar beach is systematically performed since 2006 on a weekly basis, but only for macroplastics that can be manually collected. Volunteer groups do the best to recover minor fragments during extra summer campaigns, but due to the difficulty of separating them from sand, microplastics are not collected and once produced, remain unaffected by cleaning operations.

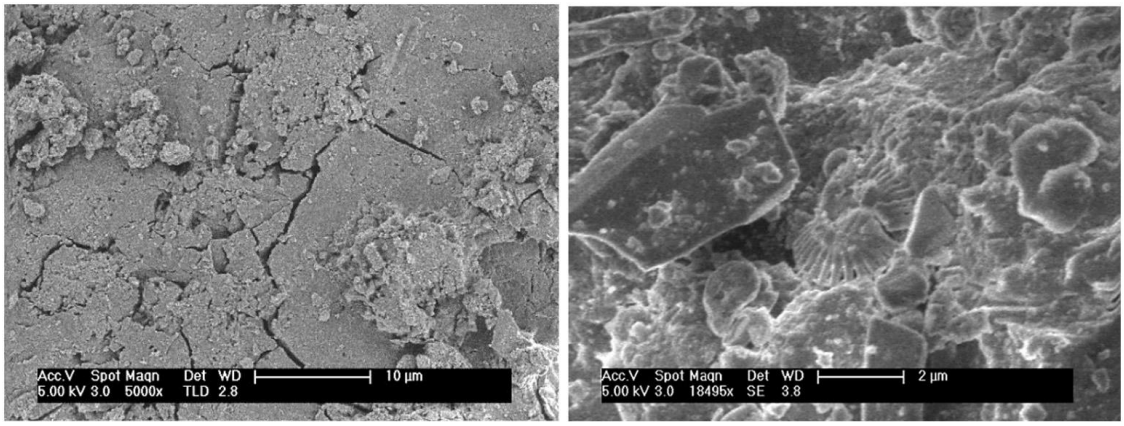


Figure 2.7. SEM images of sampled PE fragments.

2.4. Conclusions

In this study, the sandy beach Ámbar was sampled. Ámbar beach is in a remote area in almost desertic island belonging to the Chinijo archipelago in the Canary Islands. An average density of 36.3 g/m^2 of microplastics in the 1-5 mm range was obtained, with a large variability along the 90 m of the beach sampled (from 8.5 g/m^2 to 103.4 g/ m^2). No relationship was found between microplastics and local activities. The Canary Current, a wind-driven surface current part of the North Atlantic Gyre was deemed responsible of the high level of plastic pollution.

The total number of sampled plastic particles was 9149, in a distribution dominated by fragments (87%) and pellets (9%), with minor amounts of filaments, foams, moulded fragments and films. The average projected area diameter of sampled particles was 4.2 mm, explained by the large amount of microplastics with their larger dimension > 5 mm. Sampled plastics showed a high proportion of clear colours (translucent or white) that was not explained by the abundance of pellets, which was attributed to the bleaching of coloured plastics due to ageing.

Raman and FTIR spectroscopy were used for the chemical identification of polymers. A total of 711 analyses showed that PE was the most frequently found polymer (63%) followed by PP (32%) and PS (3%). PE dominated most categories, particularly fragments and pellets, with high proportion of PP in moulded fragments, filaments and films. PS was found as white fragile foams according to its use as packaging material. We also measured a significant increase in the PE/PP ratio in the higher and more protected parts of the beach, suggesting the preferential accumulation of the more aged fragments in calmed parts of the beach.

2.5. References

Anderson, J.C., Park, B.J., Palace, V.P., 2016. *Microplastics in aquatic environments: implications for Canadian ecosystems*. Environ. Pollut. 218, 269–280.

Antunes, J., Frias, J., Sobral, P., 2018. *Microplastics on the Portuguese coast*. Mar. Pollut. Bull. 131, 294–302.

Avio, C.G., Gorbi, S., Regoli, F., 2017. *Plastics and microplastics in the oceans: from emerging pollutants to emerged threat.* Mar. Environ. Res. 128, 2–11.

Baztan, J., Carrasco, A., Chouinard, O., Cleaud, M., Gabaldon, J.E., Huck, T., Jaffrès, L., Jorgensen, B., Miguelez, A., Paillard, C., Vanderlinden, J.P., 2014. *Protected areas in the Atlantic facing the hazards of micro-plastic pollution: first diagnosis of three is- lands in the Canary Current*. Mar. Pollut. Bull. 80, 302–311.

Bonanno, G., Orlando-Bonaca, M., 2018. *Ten inconvenient questions about plastics in the sea*. Environ. Sci. Pol. 85, 146–154.

Cabernard, L., Roscher, L., Lorenz, C., Gerdts, G., Primpke, S., 2018. *Comparison of Raman and Fourier Transform Infrared Spectroscopy for the quantification of microplastics in the aquatic environment*. Environ. Sci. Technol. 52, 13279–13288.

Caggiani, M.C., Cosentino, A., Mangone, A., 2016. *Pigments Checker version 3.0, a handy set for conservation scientists: a free online Raman spectra database*. Microchem. J. 129, 123–132.

CEDEX, 2017. *Asistencia técnica en las tareas de implantación de la directiva marco de la estrategia marina. Diseño, desarrollo y ejecución de los programas de seguimiento. Programa de seguimiento de micropartículas en playas (bm-6)* - 2016. Ministerio de Fomento. Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente. Gobierno de España.

CEDEX, 2018. *Apoyo técnico en las estrategias marinas. Informe específico. Programa de seguimiento de micropartículas en playas (BM-6)* - 2017.

Chae, Y., An, Y.J., 2017. *Effects of micro- and nanoplastics on aquatic ecosystems: current research trends and perspectives*. Mar. Pollut. Bull. 124, 624–632.

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

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

Crawford, C.B., Quinn, B., 2017. 5 - *Microplastics, standardisation and spatial distribution.* In: Crawford, C.B., Quinn, B. (Eds.), Microplastic Pollutants. Elsevier, pp. 101–130.

Domènech, F., Aznar, F.J., Raga, J.A., Tomás, J., 2019. *Two decades of monitoring in marine debris ingestion in loggerhead sea turtle, Caretta caretta, from the western Mediterranean.* Environ. Pollut. 244, 367–378.

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

Everaert, G., Van Cauwenberghe, L., De Rijcke, M., Koelmans, A.A., Mees, J., Vandegehuchte, M., Janssen, C.R., 2018. *Risk assessment of microplastics in the ocean: modelling approach and first conclusions*. Environ. Pollut. 242, 1930–1938.

Fanini, L., Bozzeda, F., 2018. *Dynamics of plastic resin pellets deposition on a microtidal sandy beach: informative variables and potential integration into sandy beach studies*. Ecol. Indic. 89, 309–316.

Gago, J., Galgani, F., Maes, T., Thompson, R.C., 2016. *Microplastics in seawater: recommendations from the Marine Strategy Framework Directive implementation process*. Front. Mar. Sci. 3.

Gewert, B., Plassmann, M.M., MacLeod, M., 2015. *Pathways for degradation of plastic polymers floating in the marine environment.* Environ. Sci.: Processes Impacts 17, 1513–1521.

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

Gigault, J., Halle, A.T., Baudrimont, M., Pascal, P.Y., Gauffre, F., Phi, T.L., El Hadri, H., Grassl, B., Reynaud, S., 2018. *Current opinion: what is a nanoplastic?* Environ. Pollut. 235, 1030–1034.

Hanke, G., Galgani, F., Werner, S., Oosterbaan, L., Nilsson, P., Fleet, D., Kinsey, S., Thompson, R., Palatinus, A., van Franeker, J.A., Vlachogianni, T., Scoullos, M., Veiga, J.M., Matiddi, M., Alcaro, L., Maes, T., Korpinen, S., Budziak, A., Leslie, H., Gago, J., Liebzeit, G., 2013. *Guidance on Monitoring of Marine Litter in European Seas*. Publications Office of the European Union.

Herrera, A., Asensio, M., Martínez, I., Santana, A., Packard, T., Gómez, M., 2018. *Microplastic and tar pollution on three Canary Islands beaches: an annual study.* Mar. Pollut. Bull. 129, 494–502.

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

Imhof, H.K., Sigl, R., Brauer, E., Feyl, S., Giesemann, P., Klink, S., Leupolz, K., Löder, M.G.J., Löschel, L.A., Missun, J., Muszynski, S., Ramsperger, A.F.R.M., Schrank, I., Speck, S., Steibl, S., Trotter, B., Winter, I., Laforsch, C., 2017. *Spatial and temporal variation of macro-, meso- and microplastic abundance on a remote coral island of the Maldives, Indian Ocean*. Mar. Pollut. Bull. 116, 340–347.

Jang, Y.C., Hong, S., Lee, J., Lee, M.J., Shim, W.J., 2014. *Estimation of lost tourism revenue in Geoje Island from the 2011 marine debris pollution event in South Korea*. Mar. Pollut. Bull. 81, 49–54.

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

Koelmans, A.A., Besseling, E., Shim, W.J., 2015. *Nanoplastics in the aquatic environment.* Critical review. In: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer International Publishing, Cham, pp. 325–340.

Koelmans, A.A., Bakir, A., Burton, G.A., Janssen, C.R., 2016. *Microplastic as a vector for chemicals in the aquatic environment: critical review and model-supported reinterpretation of empirical studies*. Environ. Sci. Technol. 50, 3315–3326.

Kühn, S., Bravo-Rebolledo, E.L., van Franeker, J.A., 2015. *Deleterious effects of litter on marine life.* In: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer International Publishing, Cham, pp. 75–116.

Lamb, J.B., Willis, B.L., Fiorenza, E.A., Couch, C.S., Howard, R., Rader, D.N., True, J.D., Kelly, L.A., Ahmad, A., Jompa, J., Harvell, C.D., 2018. *Plastic waste associated with disease on coral reefs.* Science 359, 460–462.

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

Li, J., Liu, H., Chen, J.P., 2018. *Microplastics in freshwater systems: a review on occurrence, environmental effects, and methods for microplastics detection*. Water Res. 137, 362–374.

MAGRAMA, 2018. *Programa de seguimiento de basuras marinas en playas. Informe de resutados 2017*. Subdirección General para la Protección del Mar. Dirección General de Sostenibilidad de la Costa y del Mar. Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente. Gobierno de España, pp. 332.

Mattsson, K., Hansson, L.A., Cedervall, T., 2015. *Nano-plastics in the aquatic environment*. Environ. Sci.: Processes Impacts 17, 1712–1721.

PlasticsEurope, 2018. Plastics – *The Facts 2018: An Analysis of European Plastics Production, Demand and Waste Data.* PlasticsEurope: Association of Plastics Manufacturers, Brussels.

Ribeiro-Claro, P., Nolasco, M.M., Araújo, C., 2017. *Chapter 5 - characterization of microplastics by Raman spectroscopy.* In: Rocha-Santos, T.A.P., Duarte, A.C. (Eds.), Comprehensive Analytical Chemistry. Elsevier, pp. 119–151.

Rios-Mendoza, L.M., Karapanagioti, H., Álvarez, N.R., 2018. M*icro(nanoplastics) in the marine environment: current knowledge and gaps.* Curr. Opin. Environ. Sci. Health 1, 47–51.

Rochman, C.M., 2018. *Microplastics research—from sink to source*. Science 360, 28. Song, Y.K., Hong, S.H., Eo, S., Jang, M., Han, G.M., Isobe, A., Shim, W.J., 2018.

Song, Y.K., Hong, S.H., Eo, S., Jang, M., Han, G.M., Isobe, A., Shim, W.J., 2018. *Horizontal and vertical distribution of microplastics in Korean coastal waters*. Environ. Sci. Technol. 52, 12188–12197.

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

Turner, A., Holmes, L., 2011. *Occurrence, distribution and characteristics of beached plastic production pellets on the island of Malta (central Mediterranean*). Mar. Pollut. Bull. 62, 377–381.

Veiga, J.M., Fleet, D., Kinsey, S., Nilsson, P., Vlachogianni, T., Werner, S., Galgani, F., Thompson, R.C., Dagevos, J., Gago, J., Sobral, P., Cronin, R., 2016. *Identifying Sources of Marine Litter. MSFD GES TG Marine Litter - Thematic Report*. JRC Technical Reportpp. 43.

Whitmire, S.L., Van Bloem, S.J., Toline, C.A., 2017. *Quantification of Microplastics on National Park Beaches.* National Oceanic and Atmospheric Administration, pp. 27.

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

Yang, Y., Liu, G., Song, W., Ye, C., Lin, H., Li, Z., Liu, W., 2019. *Plastics in the marine environment are reservoirs for antibiotic and metal resistance genes*. Environ. Int. 123, 79–86.

2.6. Supplementary material of Chapter 2

Contents

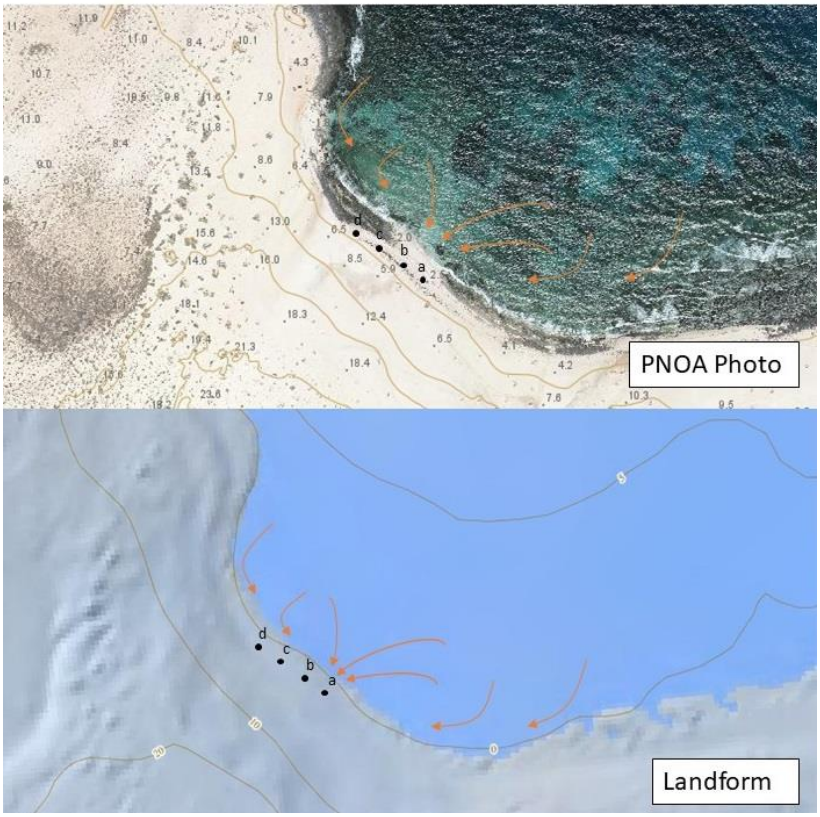


Figure S2.1. Ortophotograph (Plan Nacional de Ortofotografía Aérea, PNOA) of the sampling area showing contour curves and point heights. (Source IBERPIX, http://www.ign.es/iberpix2/visor/).

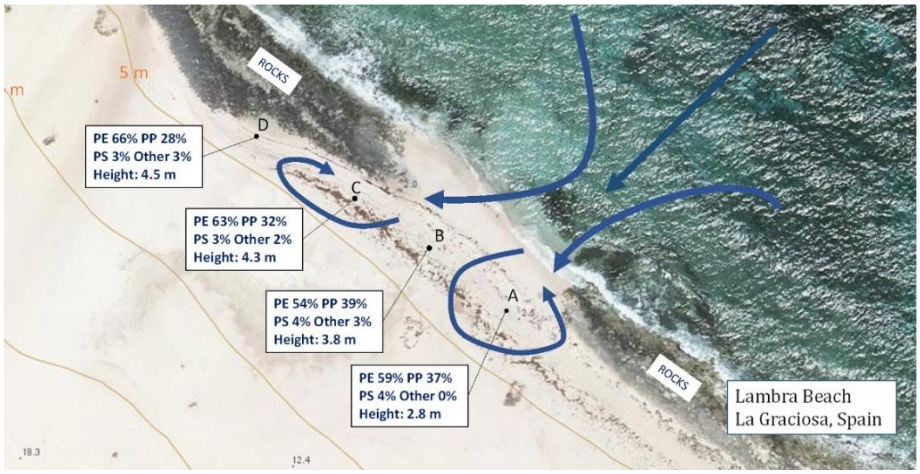


Figure S2.2. Detailed aerial image of Ámbar beach showing the directions of sea entry during high tides, sampling point heights, and the relative abundance of different plastics (Source VISOR IDECANARIAS https://visor.grafcan.es/visorweb/#).

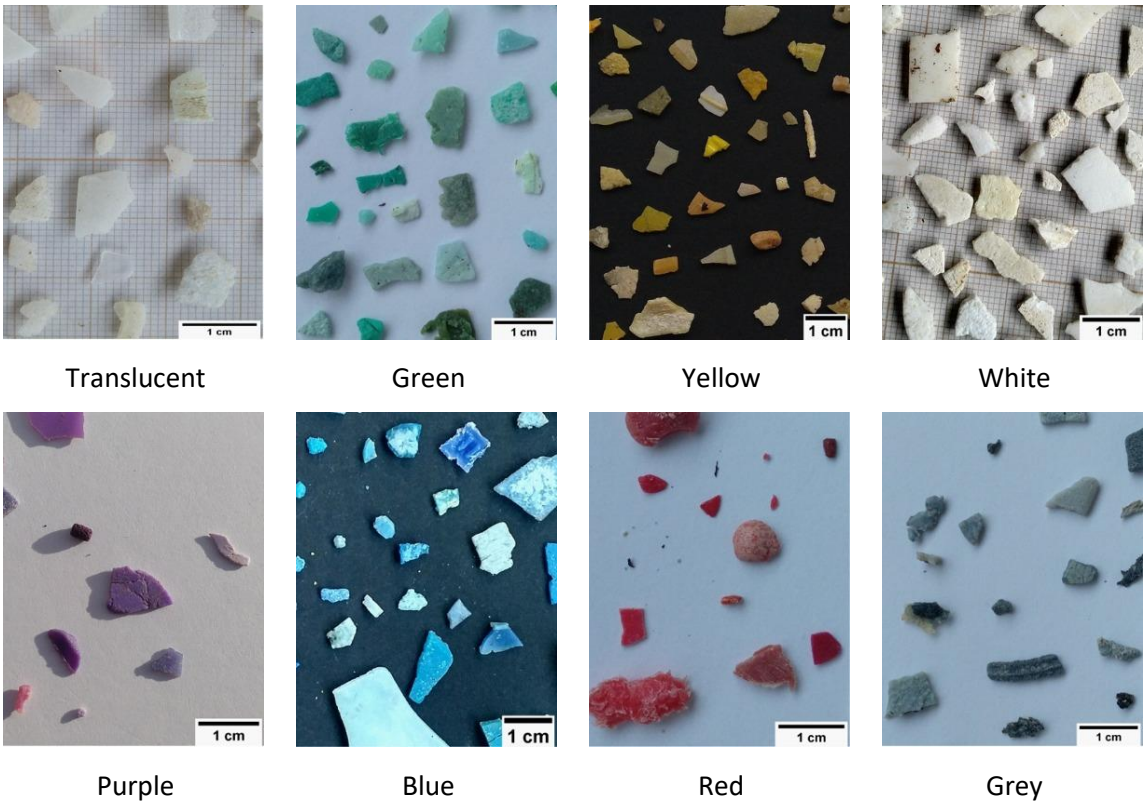


Figure S2.3. Plastic fragments by colour.

Figure S2.4. Examples of different plastics typologies.

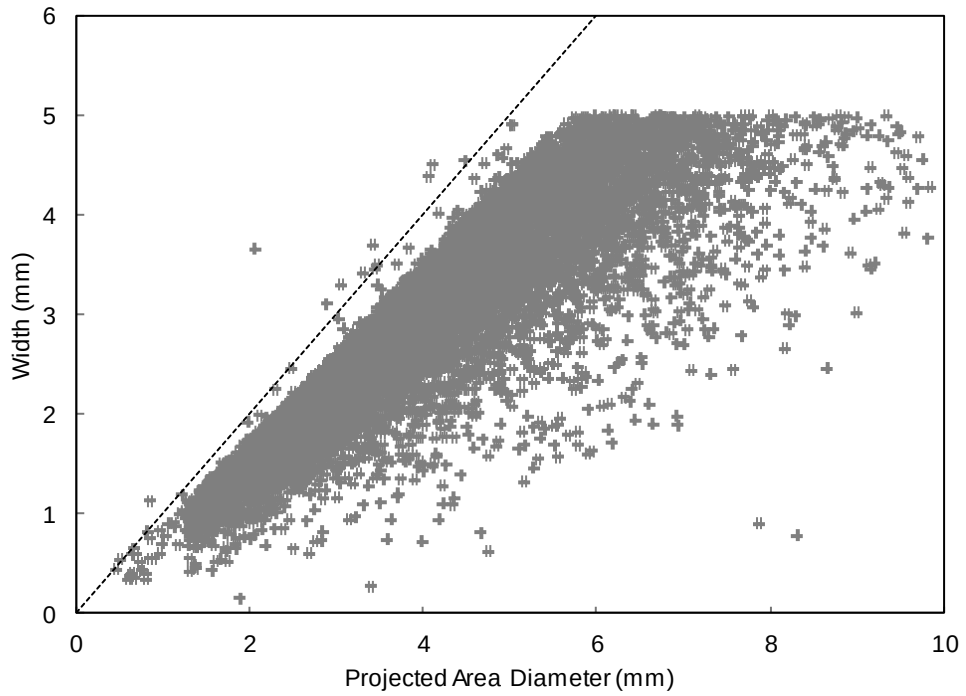


Figure S2.5. Comparison between particle width (directly from particle images) with projected area diameter calculated as the diameter of the circle with the same area as the particle.

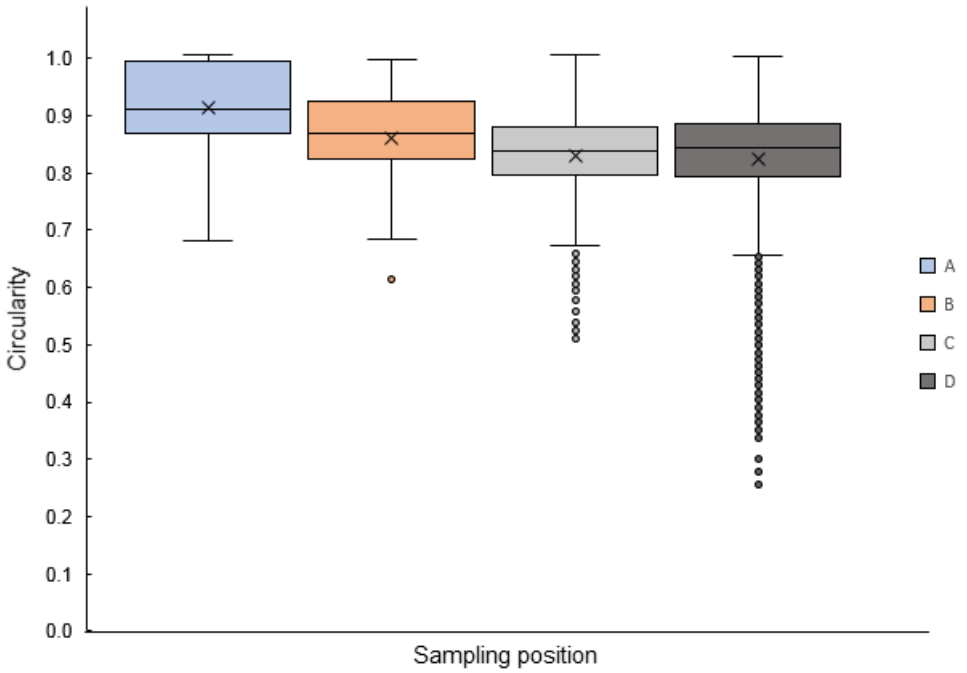


Figure S2.6. Circularity for plastic particles sampled indicating average, median and outliers.

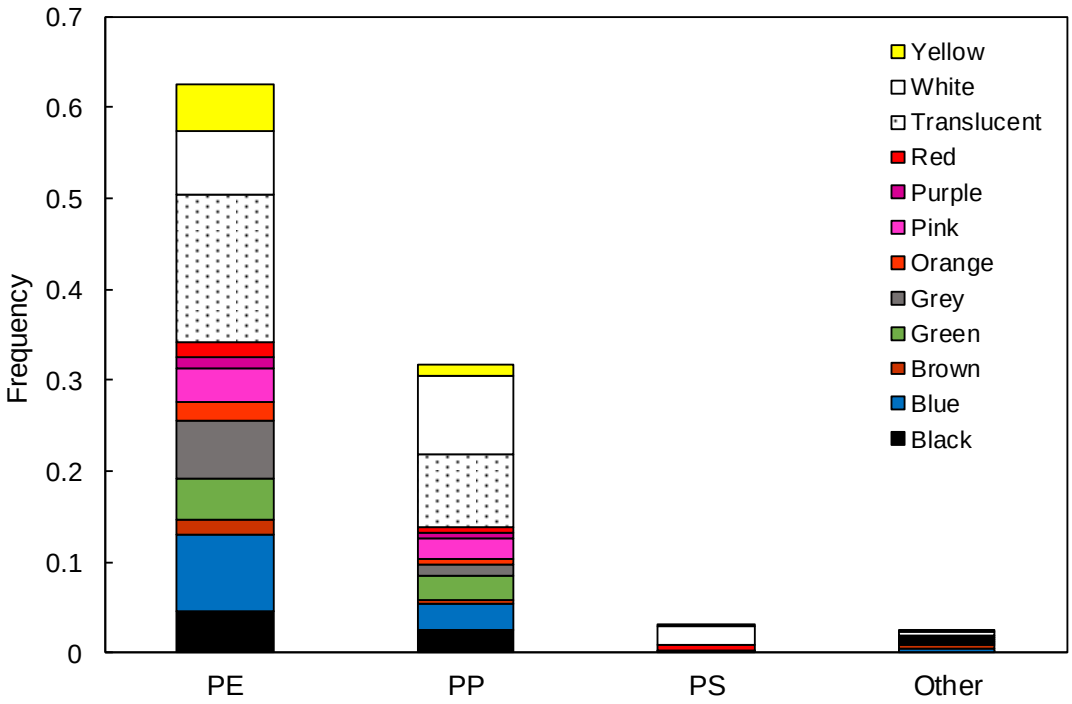


Figure S2.7. Classification of plastic particles by colour and chemical nature.

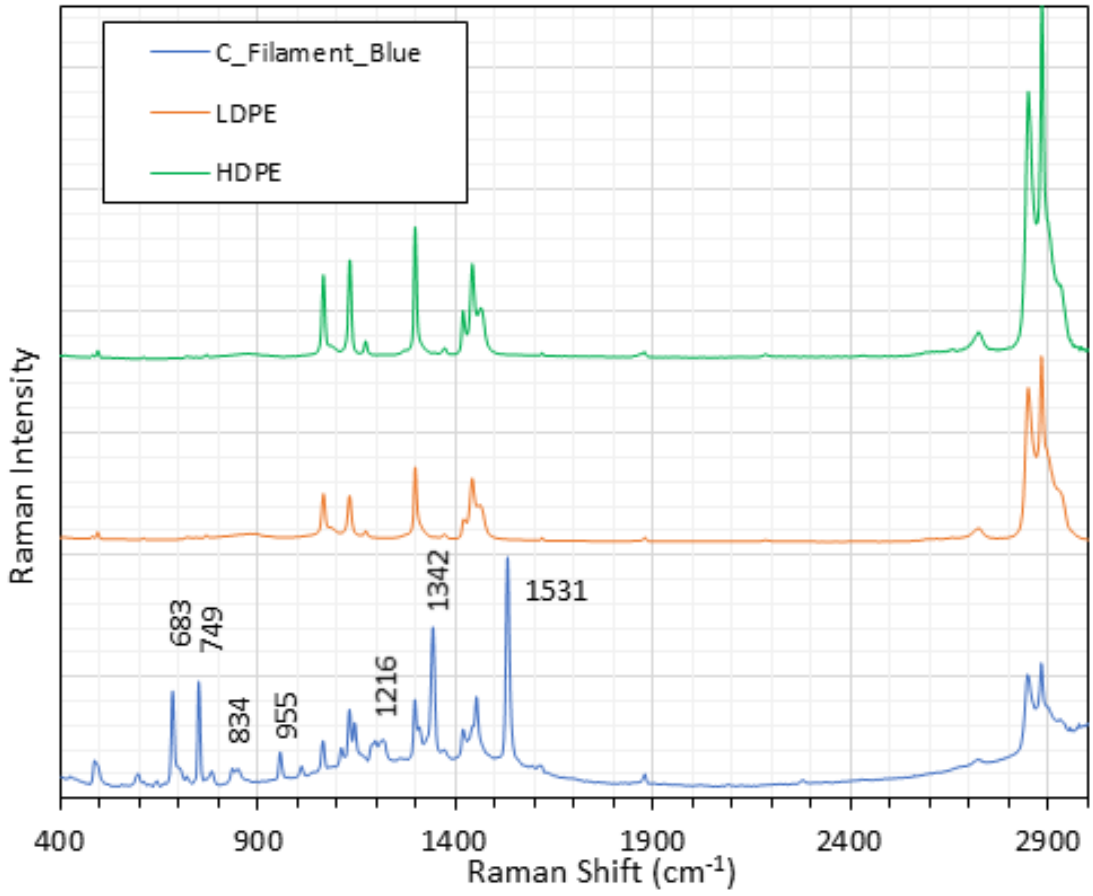


Figure S2.8. Comparison images between Raman spectra (785 nm) from a blue filament and PE spectra. Spectra changes have been identified as belonging to cooper phthalocyanine (88 % similarity from Lens Raman library database).

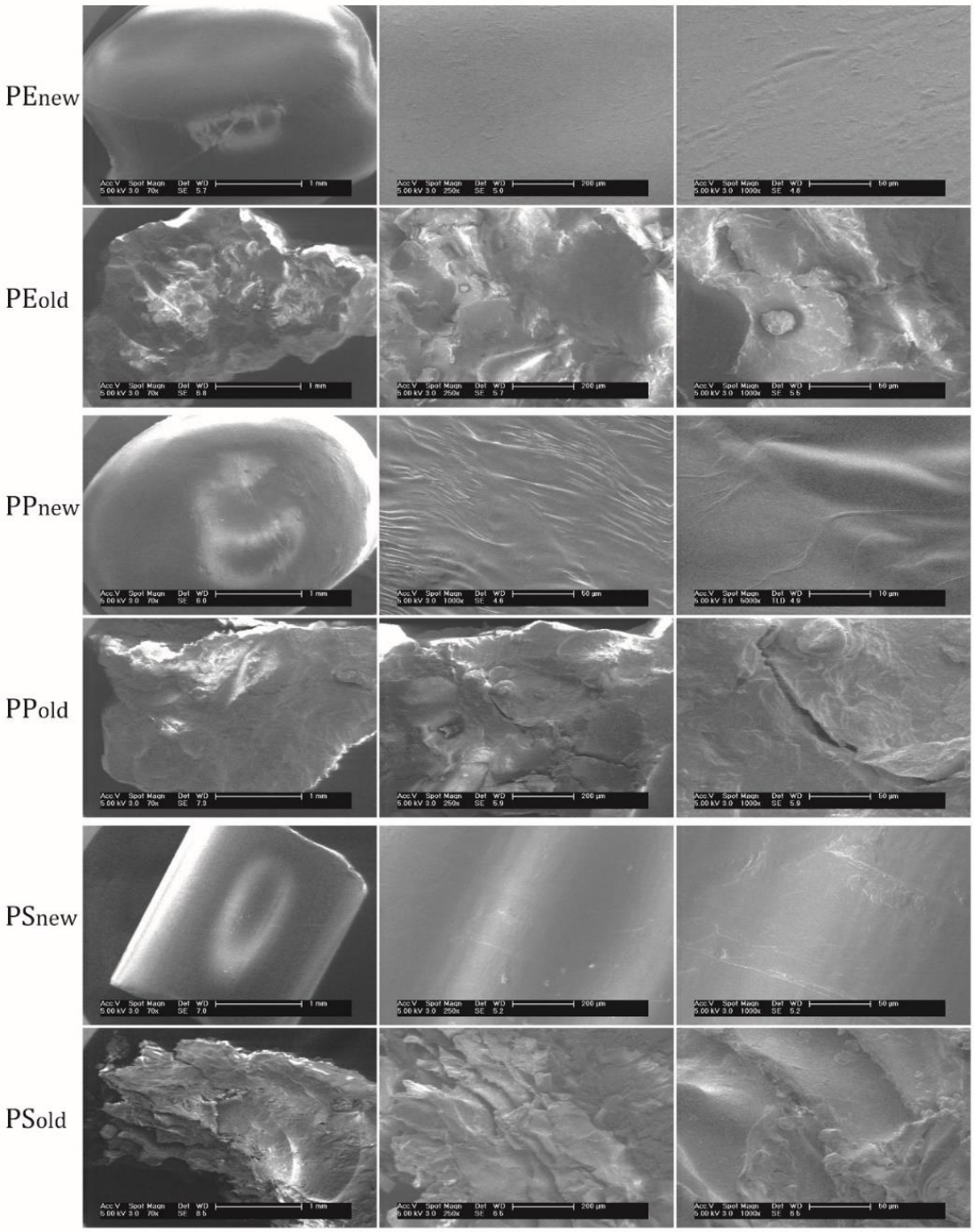


Figure S2.9. SEM images of sampled plastic particles (aged) compared with pristine (new) pellets of the same composition.

CHAPTER 3. FATE OF MICROPLASTICS IN WASTEWATER TREATMENT PLANTS AND THEIR ENVIRONMENTAL DISPERSION WITH EFFLUENT AND SLUDGE

3.1. Introduction

Microplastics are in the spotlight and a subject topic of continuous press releases reporting their presence in the most diverse environments (Zhang et al., 2019). Initially considered a local, and mostly aesthetic issue, scientists have now recognized plastic pollution as a major global pollution threat, and a key priority for research (Napper and Thompson, 2019). Plastic pollution has been largely studied in marine environments where plastic debris are ubiquitous in surface water and sediments (Clark et al., 2016; Ling et al., 2017). The presence of microplastics has also been reported in essentially all freshwater ecosystems (Dris et al., 2018; Li et al., 2018a). In addition to aqueous environments, the presence of microplastics has been reported in agricultural soils with potential risks for food chains (Corradini et al., 2019; Ng et al., 2018). Finally, both indoor and outdoor air have been proved to bear microplastics, mostly fragments or fibres, which may travel long distances transported by winds (Dris et al., 2016; Gasperi et al., 2018). The experimental evidence forced to consider microplastics as a new type of emerging contaminant potentially threating environment and human health. The issue reached government authorities and the European Parliament recently issued a resolution proposal (TA/2019/0071) stressing the need for addressing microplastics pollution in the context of wastewater treatment.

It is well-known that most plastic debris originate from land sources essentially due to improper waste management (Andrady, 2011; Yan et al., 2019). Even considering the marine environment, it has been estimated that 80% of plastic debris originate inlands (Li et al., 2016). In Europe, 64 million tonnes of new plastics were marketed for new uses, but only 8.4 million tonnes (13%) were recycled in 2017, the rest constituting a potential pollution source (PlasticsEurope, 2018). Once released, plastics undergo complex degradation processes leading to their progressive disintegration into smaller pieces (Eerkes-Medrano and Thompson, 2018; González et al., 2016). Due to the marine origin of plastic debris research, there is an almost consensus between researchers in using NOAA guidelines to classify plastics. Plastic fragments below 5 mm are commonly defined as microplastics in what turned to be an international standard (Edo et al., 2019; Gago et al., 2016). The lower size limit is not clearly established as the boundary with nanoplastics is still unclear. Gigault et al. proposed to define nanoplastics as fragments

<1000 nm with colloidal behaviour if coming from the degradation of larger particles (Gigault et al., 2018). Although the experimental evidence is limited, it is generally considered that lower sizes, including the smallest fractions of microplastics and nanoplastics may constitute a major threat for the environment (Andrady, 2011; González-Pleiter et al., 2019). It has been estimated that environmental samples contain much less small microplastics than expected, suggesting a possible accumulation in the biota (GESAMP, 2016). Additionally, small particles can cross lung or gastrointestinal epithelia and translocate to different tissues, although the experimental evidence for it is still limited (Ribeiro et al., 2019).

Plastics reach the environment through point sources or diffuse pollution. Diffuse or non-point sources include escapes from industrial plastic production facilities, runoffs from urban, agricultural or industrial areas, and atmospheric deposition (Vermeiren et al., 2016). It has been estimated that rivers transport between 1.15 and 2.41 million tonnes of plastic to worldwide oceans every year (Lebreton et al., 2017). Wastewater treatment plants (WWTP) have been identified as an important point source for microplastics emission, particularly regarding fibres (Browne et al., 2011). The sources of plastic debris reaching WWTP are cosmetics and personal care products, the wearing of plastic products like textiles, and car tyres or road paints. Through domestic wastewater or drainage systems, microplastics reach WWTP and may end up either discharged into waterbodies or dispersed with sludge (Ngo et al., 2019). Some studies showed that microplastic removal rates in WWTP are high, typically over 95%, but even if most micro- plastics are removed with sludge the remaining fraction still represents a huge amount (Lv et al., 2019; Sun et al., 2019). Moreover, the sludge produced in WWTP is frequently reused in agriculture as soil amendment because of its good properties as fertilizer (Gherghel et al., 2019). Both water and sludge reuse practises, although responding to the concept of circular economy, reintroduce microplastics into the environment and may constitute an important environmental threat (Gatidou et al., 2019). Overall, there is still a considerable knowledge gap about the role of WWTP in the cycle life of small plastic particles and fibres and a debate exists trying to elucidate the extent to which water discharges and sludge management and use contribute to the accumulation of microplastics in environmental compartments (Carr et al., 2016).

This work aims at shedding light on the fate of microplastics in a conventional wastewater treatment facility operating under Anaerobic-Anoxic-Oxic (A2O) technology. Samples were taken from the outlet of primary and secondary settlers and from sludge as well as from the pellets of heat-dried sludge marketed as soil amendment. We assessed the presence of microplastics in wastewater and sludge, compared the results with previous reports, and discussed the potential risks of microplastics to soil and freshwater ecosystems. In this work, we paid attention to manufactured natural polymers, a type of anthropogenic pollution with important similitudes with plastic microfibres. We also studied sludge, which wet or in the form of heat-dried pellets, constitute a way for microplastics dispersion into the environment that could require more stringent regulatory measures.

3.2. Materials and methods

3.2.1. Wastewater and sludge

Sampling was conducted during three different days in three different months during the Spring of 2019 at a WWTP located near Madrid (Spain). The installation is designed to treat 45000 m^3/day and consists of a primary clarifier followed by A2O biotreatment. The effluent discharges to the Henares River in the Tagus basin. The mixed sludge from the clarifiers is dewatered prior to anaerobic digestion to produce biogas used to generate electricity and heat in the WWTP. Digested sludge is further heat-dried to 300 °C in a rotary drier and sold for agricultural use, mainly in neighbouring areas. During the sampling period, the average flow rate of un- treated wastewater reaching the plant was 28 400 m^3/day. In the same period, the plant generated an average of 851000 m^3 of treated wastewater and 560 t of stabilized sludge per month. Heat- dried sludge is marketed in small pellets with a diameter of about 5 mm. The analysis of pellets yielded 92.4% dry matter and 60.8% organic matter. Heat treatment eliminates all biological activity so that no colony forming bacteria were detected in dry sludge. The content of metals allows its use as fertilizer up to 5 t per hectare and year according to the current local regulations.

3.2.2. Sampling

Water samples were directly collected from settler effluents, immediately transported to the laboratory and filtered through a sequence of three stainless steel meshes with 25 µm, 104 µm and 375 µm, opening sizes. Filters were then placed in glass beakers and put in contact with H_2O_2 (33% w/v) at 50 °C for 20-24 h to remove organic matter and prevent microorganism growth after which they were rinsed with Milli-Q water to remove residual H_2O_2 (Gies et al., 2018). The digestion time was chosen as optimum to ensure the complete removal of organic matter in the most difficult samples without affecting the integrity of microplastics. The samples were filtered through the same sequence of 375-104-25 µm meshes. All filters were dried and stored in previously cleaned glass Petri dishes prior to use. Wet sludge was collected from the anaerobic digestor. Dry sludge pellets, as marketed for soil amendment, were collected from the storage facilities. Samples of wet sludge and dry pellets (1 g) were treated with 30 mL H_2O_2 (33%) at 50 °C as indicated before. After H_2O_2 treatment the suspension was diluted with NaCl, 1.2 kg/L, kept under stirring for 24 h and allowed settling for another 24 h, after which, both supernatant and sediment, without any loss or particles during the process, were inspected filtered and inspected as indicated before.

Glass material was used whenever possible, and controls were taken before and during sampling. Two-litre Pyrex glass bottles were used to collect water from the primary settler. For the outlet of the secondary settler, 25 L white high-density polyethylene containers were used. All recipients were thoroughly cleaned with 10% HCl at least three times. Glass beakers, glass Petri dishes and steel tweezers were also cleaned in the same way to ensure the absence of plastic contamination. All material was covered with aluminium foils until use. The filtering system consisted of a Millipore Stainless 47 mm pressure holder. The stainless steel filters used were cleaned and heated to 500 °C prior to their use to remove all possible rests of organic matter. The integrity of steel mesh was checked by optical microscopy.

Controls to assess possible cross-contamination were performed by rinsing all used material and glassware elements with Milli-Q water, which was subsequently filtered through 25 µm opening size meshed and checked for the possible presence of microplastics. During sampling, filtering, observation, and measurement tasks, open

mesh filters were kept open to quantify possible contamination from the surrounding environment. Clothing was also controlled by using non-typical bright colours like blue or orange preferable 100% cotton.

3.2.3. Analytical procedure

Visual inspection and the counting of microparticles were performed using a stereomicroscope Euromex-Edublue equipped with USB digital camera and ImageFocus 4 software. The whole set of particles included plastic materials, natural fibres with evidence of anthropogenic process and natural materials as well as a residual category of non-identified fragments of fibres. Based on visual evaluation, a subsample of each typology was selected and derived for identification by means of Fourier-transform infrared spectroscopy (FTIR). FTIR spectra were recorded using a micro-FTIR a Perkin-Elmer Spotlight 200 Spectrum Two apparatus with mercury cadmium telluride (MCT) detector, which allowed high sensitivity measurements in the mid-infrared region. Samples were placed on KBr pellets and measuring parameters for the micro-transmission mode were as follows: spot 50 µm, 20 scans, resolution 8 cm^{-1}, spectral range 4000-550 cm^{-1}. The spectra were compared in the Omnic 9 software obtained from Thermo Scientific with a built-in database and with reference spectra created on purpose during this study. Positive matching between samples and database or standards was assessed when a minimum of 70% similitude was obtained.

3.2.4. Statistics

Confidence intervals (CI) were computed at 95% level with at least three replicates for each typology. For FTIR identification, Pearson correlation was used to assess matching between samples and database or standards.

3.3. Results

3.3.1. Occurrence of fragments and fibres in wastewater and sludge

According to typology, microparticles were first divided into fragments (small particles, films or beads) and fibres. For the purpose of our study, we defined fibres as microparticles with cylindrical shape and length to diameter ratio >3 according to the definition of ECHA proposal to restrict intentionally added microplastics (Agency, 2019).

The samples showed a diversity of plastic fragments of different shapes identified as secondary plastics. There were also many white or transparent fibres, further identified as cellulosic material and abundant coloured fibres. Fig. 3.1 shows a selection of fragments recovered from wastewater and sludge. A significant feature of these samples is the wide variety of colours, consequence of their anthropogenic origin. A total of 14 different colours were found, and as explained below, some were clearly identified as the product of dyeing natural fibres during manufacturing processes. This represents a wider range compared with other reported results (Bayo et al., 2020; Liu et al., 2019; Talvitie et al., 2017).

Microparticles were sorted in three size categories by means of steel meshes of 25 µm, 104 µm, and 375 µm size opening as follows: 25-104 µm; 104-375 µm and >375 µm, < 5 mm). Both in primary and secondary effluents, size distributions were dominated by lower sizes: 54% (25-104 µm), 34% (104-375 µm), and 12% (>375 µm, < 5 mm) for the primary and 48%, 28% and 23% respectively for the effluent of A2O settler. The results indicate that most microparticles corresponded with the smallest measured fraction. In the primary effluent fragment length (larger dimension as measured from microscopy images) ranged from 53 µm to 2100 µm (0.21 mm) whereas width (second dimension from projected images) was in the 18-900 µm range. Projected sizes in fibres range from 104 to 4000 µm (length) and 5-34 µm (width). In the secondary effluent, fragment length was in the 41-2890 µm range, while width varied from 34 to 1230 µm (0.33 mm). Size for fibres ranged from 144 to 1824 µm (length) and 8-89 µm (width). Fragments in wet sludge were in the 36-377 µm length range and 22-36 µm width range, like those found in heat-dried sludge use as soil amendment (29-533 µm length and 11-369 µm width). Fibres in wet sludge were in the 213-4716 µm (length range) and 5-34 µm (width range), while the figures for heat-dried sludge were 71-2224 µm (length range) and 7-58 µm (width range). Fig. 3.2 summarizes these results with relative abundances calculated for the larger dimension. Aspect ratio defined as the ratio between length and width for projected images for fragments was 2.0 and 1.9 for primary and secondary effluents, and 1.7 and 2.1 for wet and heat-dried sludge, respectively. For fibres the average values were 59 and 58 for wastewater (primary and secondary) and 101 and 46 for sludge (wet

and heat-dried). This difference made it possible to unambiguously classify particles as fragments or fibres.

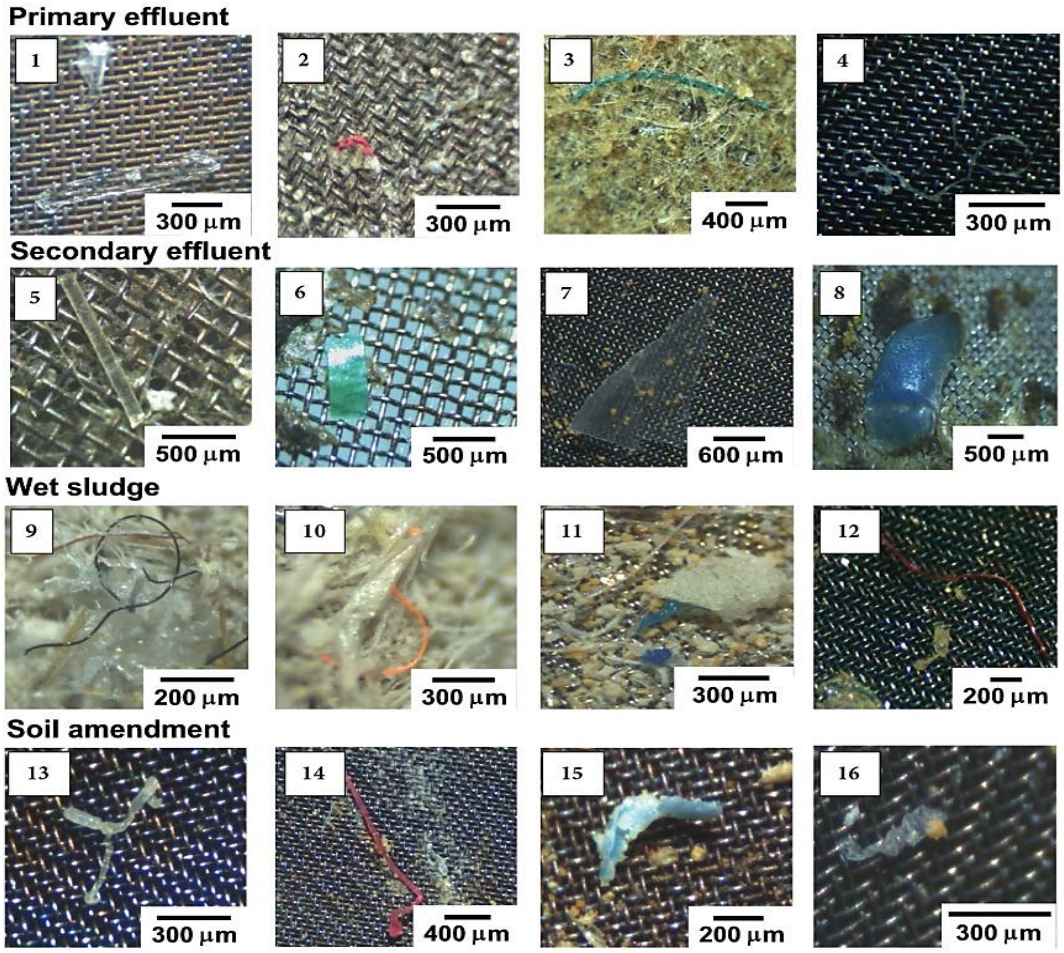


Figure 3.1. Microplastic particles from visual sorting (before FTIR analysis). 1-4: Samples from primary effluent (1: Transparent fragment and film, 2: Red fragment, 3: Blue fibre, 4: Transparent fibre); 5-8: Samples from secondary effluent (5: Transparent filament, 6: Green fragment, 7: Transparent film, 8: Blue fragment); 9-12: Samples from wet sludge (9: Black and red fibres on a white mass of cellulose fibres; 10: Orange fibre, 11: Blue fragments, 12: Red fibre); 13-16: Samples from soil amendment (13: Transparent fibre, 14: Red fibre, 15: Blue fragment, 16: Transparent fragment).

Particle and fibre counting in the effluent of the primary settler yielded 451 ± 106 microparticles (fragments and fibres)/L, the error indicating the standard deviation among samples. Clear (white and transparent) fragments and fibres represented 60% and 28% of the total amount of microparticles, while coloured fragments and fibres represented 9% and 3% respectively. The effluent from the secondary settler showed less fragments and fibres, with total amount of 26 ± 14 microparticles/L, which

corresponded to 94% removal efficiency in the secondary settler. They mainly consisted of clear (56%) and coloured fragments (24%), while fibres (15% clear, 5% coloured) were in lower amounts. WWTP sludge (mixed from primary and secondary settlers) showed an average of 314 ± 145 microparticles per gram of dry matter.

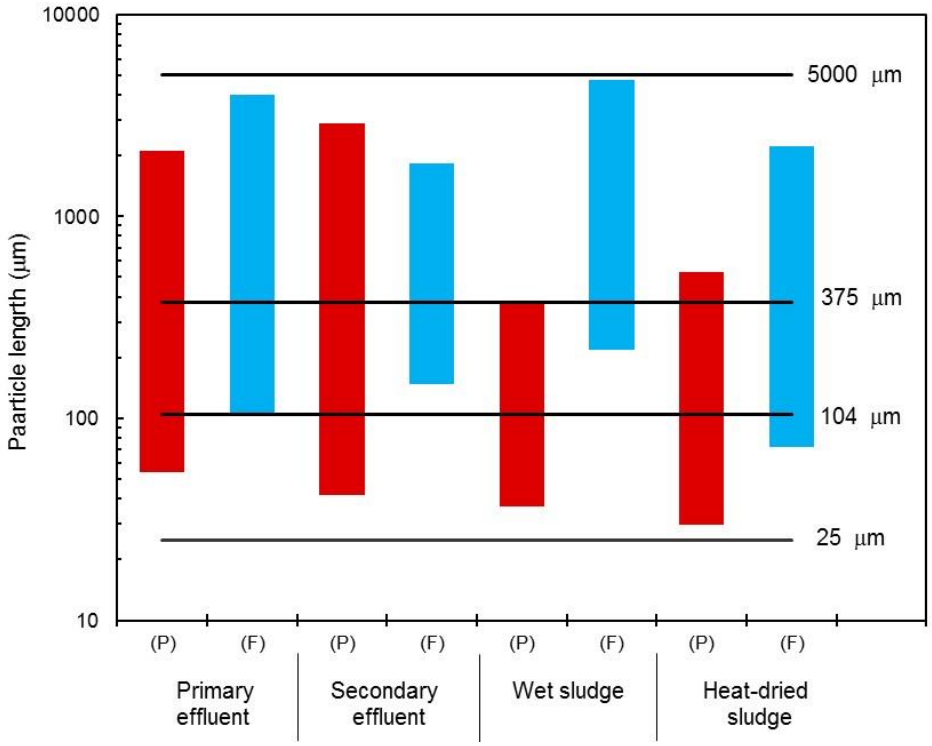


Figure 3.2. Range of sizes sampled (length, µm) in primary and secondary effluents and in wet and heat-dried sludge. P = particles (Red), F = fibres (blue).

In contrast to wastewater, mixed sludge was dominated by fibres both clear (white or transparent) and coloured. With respect to the total amount of microparticles, clear fibres represented 47%, clear fragments 31%, coloured fibres 15%, and coloured fragments 7%. Heat-dried pellets used as soil amendment carried a total amount of microparticulate particles (fragments and fibres) of 302 ± 83 microparticles per gram of amendment, very similar to WWTP sludge. Its distribution yielded clear fibres (67%), coloured fibres (17%), white fragments (11%) and coloured fragments (5%).

3.3.2. Micro-FTIR identification.

A subsample of 172 microparticles from wastewater and sludge were carefully inspected by micro-FTIR. The identification revealed plastic materials (n=77), natural substances with evidence of anthropogenic manufacturing processes (n=27), natural materials

(mainly cellulose, n=25), and non-identified substances (n=43). Manufactured natural polymers refer to materials based on natural constituents like cotton or wool that display evidence of having been manufactured to modify their properties, notably the presence of dyes. Non-identified materials refer spectra clearly showing non-plastic materials or with correlation matching <70%. Fig. S3.1 (Supplementary Material, SM) shows particle distribution among these categories FTIR characterization. In wastewater, both from primary and secondary settlers, the results were similar, with a percentage of plastics representing 35-40% of the total amount of microparticles analysed. In sludge, either wet sludge or heat- processed pellets, the percentage of microplastic particles identified raised to about 60%, with a considerably lower percentage of particles not identified with enough evidence.

A total of 12 different anthropogenic polymers and groups of polymers were identified in the samples which are listed together in Fig. S3.1 (SM). Among identified microplastic particles, 51% were fragments (and 49% fibres). In case of manufactured natural polymers 62% were fibres. The main polymers found in the primary effluent were, in decreasing occurrence: polyester fibres, polyethylene (PE), dyed cotton, polypropylene (PP) and cellophane fibres. In the secondary effluent, PE outnumbered dyed cotton, polyester fibres identified as PET, PP, and cellophane. Polyester fibres prevailed in sludge followed by acrylic fibres, PE, dyed cotton and PP. Other polymers identified were polymethyl methacrylate (PMMA), polycaprolactone (PCL), polyurethane (PU), and polystyrene (PS). The density of the polymers identified is indicated in Table S3.1 (SM). Most of them correspond to buoyant particles or are manufactured as foams with lower density than pure polymers.

Fig. 3.3 shows typical IR spectra of some sorted plastic materials, namely a PE fragment, a PP filament and a polyester fibre, together with the standards used for identification (coincident peaks are highlighted for the sake of clarity; spectra from other sampled polymers are shown in Fig. S3.2 (SM).

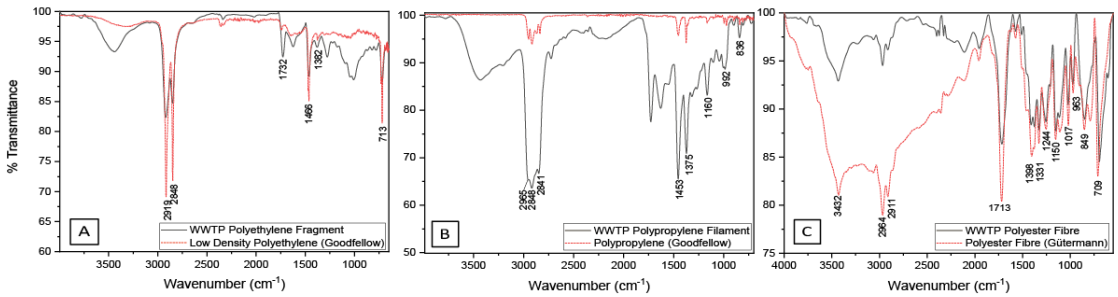


Figure 3.3. Infrared spectra of environmental samples and their corresponding reference standards for polyethylene (A), polypropylene (B), and a polyester fibre (C).

3.4. Discussion

Particle counting and the results of the identification of plastics and manufactured natural materials were combined to calculate the amount of plastics and all artificial materials in wastewater and sludge (Table 3.1). The total concentration of microplastic particles was 171 ± 42 particles/L in the primary effluent that got reduced to 10.7 ± 5.2 particles/L at the outlet of the secondary settler (coincident in this case with WWTP final effluent). The microplastic particles in sludge amounted to 133 ± 59 particles/g (of dry matter), not significantly different from the figure obtained in heat-dried sludge used as soil amendment. Overall, FTIR analyses confirmed the presence of the most common plastic materials including PE, PP, and polyesters and acrylic fibres as well as natural manufactured fibres in line with data published elsewhere (Magni et al., 2019; Zambrano et al., 2019). The variability observed in literature data is not generally high and can be mostly interpreted in terms of sociodemographic variables (Liu et al., 2019). The presence of low-density polymers, like PE in sludge samples agrees with data reported elsewhere (Mahon et al., 2017; Mintenig et al., 2017). The reason may be that microplastics get trapped into flocs favoured by their lost polarity and higher sorption potential, which favours their partitioning to the sediment phase.

Manufactured natural polymers were identified in all cases, although in lower amounts than microplastic particles. It is interesting to note the difficulty to accurately identifying certain fragments or fibres as natural or manufactured. Fig. S3.3 (SM) shows the spectra of three samples identified as cellulose. The FTIR spectra shows the typical bands from cellulose-based materials. Spectra are similar and the most common bands for all spectra are the broad band at about 3600-3200 cm^{-1} that corresponds to the OH

stretching vibration, the absorption at 2900 cm^{-1} due to the C-H stretching of alkyl groups, and the intense absorption at 1000-1080 cm^{-1} that corresponds to C-O stretching vibration (Reddy et al., 2016). Once computed manufactured natural materials as anthropogenic litter, the total quantity of microparticles discharged by the WWTP amounted to 12.8 ± 6.3 particles/L with the effluent and 183 ± 84 particles/g with sludge.

Table 3.1. Concentration of artificial microparticles in the samples.

	Primary Effluent	Secondary Effluent	WWTP Sludge	Soil Amendment
	particles/L	particles/L	particles/g	particles/g
Plastic Particles	171 ± 43	10.7 ± 5.2	133 ± 59	101 ± 19
Manufactured Natural Materials	66 ± 28	2.1 ± 6.3	49 ± 26	64 ± 20
Total Anthropogenic Particles	236 ± 66	12.8 ± 6.3	183 ± 84	165 ± 37

The role of WWTP in contributing to river and marine pollution has been studied in the past and identified as a potential major driver of plastic pollution in aquatic environments (Mourgkogiannis et al., 2018). Some authors reported removal efficiencies for WWTP of up to 98-99% for particles in the tens of micrometre range (Gies et al., 2018; Lares et al., 2018; Murphy et al., 2016; Ziajahromi et al., 2017). Other authors reported lower removal rates (Liu et al., 2019; Talvitie et al., 2017). Differences in sampling points and size ranges make it difficult to accurately compare results. A summary of recently reported data is shown in Table 3.2, which indicates the values concentrations of microplastics in raw wastewater, effluents from primary and secondary settlers and WWTP final effluent. Efficiencies are reported for the whole plant and, in brackets, comparing primary and discharged effluent. The results of removal efficiency obtained in the present study (93.7%) compared the outlet the primary settler and the final discharged effluent and were reasonably aligned with other published data, particularly when the range of sample sizes is similar (Ziajahromi et al., 2017). Noteworthy, there is considerable dispersion in the reported results for the removal of microplastic particles in the primary screening and clarification stages, which range from 20 to 40% to >99%. Table 3.2 shows reported concentrations in the final effluent, which range from <1 particle/L to 28.4 particles/L with our figure, 10.7 particles/L, in between. The fact that microplastics are not completely retained with sludge, results in

Table 3.2. Overview of previous studies used for comparison with this work.

Reference	Daily flowrate, m^3, (Population served/equivalent)	Size rage sampled, lower, upper	Microplastics, particles/L	Removal efficiency, %[e]	MP discharge, particles/day	Type of facility	Location and discharge
Murphy et al. (2016)	2.6×10^5 (650 000)	11 μm 65 μm	15.70 ± 5.23[a, b, c, d, e and f] 3.40 ± 0.28[b] 0.25 ± 0.04[d]	98.4 (92.6)	6.5×10^7	Primary and secondary treatments	River Clyde, Scotland
Mason et al. (2016)	ranges: 2.35×10^3 −3.82×10^5 (3500 −1 400 000)	125 μm 335 μm (and higher)	n.a.	n.a.	5×10^4 −1.5×10^7 Average: $(4.4 \pm 2.1) \times 10^6$	17 facilities not identified due to confidentiality, some including advanced filtration granular or biological	Discharges in San Francisco Bay, Lake Michigan and several lakes in New York area
Talvitie et al. (2017)	2.7×10^5 (800 000)	10 μm >300 μm	<0.651[d]	65-94[f] (>99[f])	1.7×10^6 −1.4×10^8	Pretreatment, activated sludge and denitrifying biological filter	Gulf of Finland, Baltic Sea, Finland
Ziajahromi et al. (2017)	3.08×10^5 (1 227 150) 1.7×10^4 (67 130) 6.1×10^4 (150 870)	25 μm 500 μm (and higher)	1.5−2.2[a] 0.21−0.28[d]	>99 (>90)	3.6×10^6 −4.6×10^8	3 WWTP, (1) only primary treatment, (2) primary, secondary and disinfection and (3) with tertiary membrane treatment	Discharges to ocean and to an urban river in Sidney, Australia
Lares et al. (2018)	10^4	250 μm 5 mm	57.6 ± 12.4[a] 0.6 ± 0.2[b] 1.0 ± 0.4[d]	98.3 (−)	1.0×10^7	WWTP with primary, secondary with activated sludge, and disinfection	Mikkeli, Finland
Gies et al. (2018)	4.9×10^5 (1 300 000)	>1 μm	31.1 ± 6.7[a] 2.6 ± 1.4[b] 0.5 ± 0.2[d]	98.3 (80.8)	8.2×10^7	Primary and secondary treatment & seasonal chlorination	Vancouver, British Columbia discharging to Fraser River near the Strait of Georgia
Magni et al. (2019)	4.0×10^5 (1 200 000)	10 μm 5 mm	2.5 ± 0.3[a] 0.9 ± 0.3[c] 0.4 ± 0.1[d]	84 (n.a.)	1.6×10^8	WWTP with primary, secondary and tertiary treatments (sand filter and disinfection)	WWTP located in Northern Italy; no details about discharge
Yang et al. (2019)	10^6 (2 400 000)	50 μm 5 mm	12.03 ± 1.29[a] 0.59 ± 0.22[d]	95.2 ± 1.6 (72 ± 12)	$5.9 \pm 2.2 \times 10^8$	Primary and secondary treatments, A2O, membrane treatment, and disinfection.	Gaobeidian treatment plant in Beijing, discharges to Tonghui River
Liu et al. (2019)	2.0×10^4	20 μm 5 mm	79.9 ± 9.3[a] 47.4 ± 7.0[b] 34.1 ± 9.4[c] 28.4 ± 7.0[d]	64.4 (40.1)	5.7×10^8	Primary and secondary treatments plus chlorination	Wuhan City, discharges into the Yangtze River via effluent pipe
Bayo et al. (2020)	3.5×10^5 (210 000)	0.45 μm 5 mm	12.43 ± 2.70[a] 9.73 ± 3.04[b] 3.21 ± 0.50[c] 1.23 ± 0.15[d]	90.1 (87.4)	6.7×10^6	Primary and secondary activates sludge process plus chlorine disinfection	Cartagena, Spain, discharging to Mediterranean Sea
This study	4.5×10^4 (300 000)	25 μm 5 mm	171 ± 43[b] 10.7 ± 5.2[d]	-(93.7)	3.0×10^8	Primary and A2O (Anaerobic, Anoxic, Oxic) biotreatment.	Plant located near Madrid and discharging to Henares River, tributary to Tagus River

n.a.: not available.
[a] Influent.
[b] Primary effluent.
[c] After secondary settler.
[d] Final effluent.
[e] Removal efficiency from primary effluent to discharge.
[f] Includes all microlitter (not only microplastics).

considerable emissions amounting to figures in the range of 10^6-10^8 particles emitted per day and per WWTP. Considering the average flow of raw wastewater during the sampling period (28 400 m^3/day), our data indicated a discharge of about 300 million microplastic particles (>25 µm) per day to the Henares River. This value is comparable to other in which the particle size range was similar (Liu et al., 2019; Talvitie et al., 2017; Yang et al., 2019). Considering the historical average flow of Henares River, about 10 m^3/s, the discharge we measured (one of the hundreds of WWTP discharging to Tagus basin, 154 only in Madrid) represented a contribution of 350 particles/m^3 of microplastics.

Microplastic particles concentrate in the sludge recovered from clarifiers. Therefore, any uses different from incineration inevitably result in their dissemination into the environment (Weithmann et al., 2018a). Our study showed a concentration of 133 ± 59 microplastics per gram of dry sludge and 101 ± 19 microplastics per gram in the heat-dried sludge used as soil amendment. It is noteworthy that processing sludge at temperatures reaching 300 °C did not significantly alter microplastic particles.

A summary of recent research can be found in Table 3.3 that shows considerable variability among authors, with concentrations in sewage sludge ranging from a few to several hundred of particles per gram of dry sludge. Such high variations could be attributed to differences in the efficiency of the mechanisms involved in microplastics removal, essentially the skimming of floating low-density debris and their capture into settling flocs (Carr et al., 2016; Gatidou et al., 2019).

When sludge, either wet or heat-dried (biologically inactivated) is improperly managed or used as soil amendment, microplastics find a route towards the environment. Table 3.3 shows the estimated number of plastic microparticles potentially emitted by different WWTP though sludge. It has been estimated that 86% of the 8 x 10^6 tons of sludge generated in China become released into the environment representing the emission of 1.6 x 10^{14} microplastic particles/year (Li et al., 2018b). Our results showed that the WWTP would emit 8 x 10^{11} plastic particles per year, within the broad range limited by the values of Mintenig et al. (2017) and Magni et al. (2019) for German and Italian WWTP respectively (Table 3.3). The spreading of microplastics into agricultural

soils as fertilizer is a cause for concern (Weithmann et al., 2018b). Microplastics can be found in agricultural soils that had undergone sludge applications in the past showing their persistence (Corradini et al., 2019). The production of sludge pellets in the area of Madrid accounts for roughly 100 000 t/year, all of them marketed for use in agriculture, mostly in neighbouring places and spread over a surface of about 14200 ha (Comunidad de Madrid, 2018). Our results showed that more than 10^{13} microplastic particles are disseminated every year in agricultural soils only in Madrid, where the use of sludge is limited to 5 t/ha per year (dry sludge).

Table 3.3. Some recent studies reporting microplastics emission with WWTP sludge.

Reference	Source of data	Population served	Size sampled	MPs (particles/g)	MPs emmited per year and WWTP
Mintenig et al. (2017)	6 WWTP - Germany	-	<500 µm	1 - 24	1.24×10^9 - 5.67×10^9
Mahon et al. (2017)	7 WWTP - Ireland	6500 - 2400000	250 - 4000 µm	4.2 - 15	-
Lares et al. (2018)	1 WWTP - Finland	55000	250 µm - 5 mm	170.9 ± 28.7	No data
Lusher et al. (2017)	8 WWTP - Norway	18150 - 615000	> 50 µm	1.7 - 19.8	2.2×10^9 - 2.8×10^{11}
Li et al. (2018b)	28 WWTP - China	51900 - 7050000	37 µm - 5 mm	1.60 - 56.4	-
Gies et al. (2018)	1 WWTP - Canada	1300000	> 1 µm	14.9 ± 6.3 (primary); 4.4 ± 2.8 (secondary)	1.64×10^{12}
Magni et al. (2019)	1 WWTP - Italy	1200000	10 µm - 5 mm	113 ± 57	1.24×10^{12}
Liu et al. (2019)	1 WWTP - China	-	20 µm - 5 mm	240 ± 31	-

Our results also showed that the size of particles in sludge was smaller than in wastewater effluent with almost all particles below 375 µm. Additionally, there was a predominant presence of fibres in sludge (31% and 20% of the anthropogenic particles in wastewater from primary and secondary settlers, and 62% and 84% in wet sludge and heat dried sludge, respectively). The higher amount of fibres in sludge has been reported before and even proposed as indicators of historical spreading of wastewater sludge (Corradini et al., 2019). Zubris and Richards, (2005) showed that fibers from sludge were detectable in soil even many years after application with the same characteristics they had when applied. The presence of fibres in influents and effluents of WWTP has been

extensively documented and mainly corresponds to the laundering of synthetic fibres (Zambrano et al., 2019). Other studies gave lower values, indicating a large seasonal variability. Browne et al. (2011), estimated > 1900 fibres/wash with potentially increasing up to three orders of magnitude in winter due to the higher usage of washing machines (Browne et al., 2011). It has been pointed out that washing procedures are subjected to culture habits, therefore influencing the amount of fibres that reach environmental compartments. The number of washing cycles per week, the different use of detergents, washing temperature, volume of water used, and type of clothes strongly influence the number of fibres released (Cesa et al., 2017; Kelly et al., 2019).

Together with synthetic fibres, there also exist a large variety of fibres from natural polymers including cellulose derivatives or wool, which have been processed up to a certain extent and, therefore, their release to the environment constitutes another kind of anthropogenic pollution. There are many types of manufacturing processes involved such as dying or bleaching or the blending with additives granting better mechanical properties, flame retardancy, and light stabilization among others (O'Brien et al., 2015). Manufactured natural polymers are not plastic materials, but in view of their anthropogenic character and the presence of additives, they should be considered for their possible risk if delivered into the environment. Cellulose fibres, for example, detach in huge quantities from toilet paper and may contain diverse substances like softeners (sometimes made of silicone derivatives), perfumes or metals like copper, magnesium or zinc, all of them added to improve certain properties of the final product (Abildgaard et al., 2003). Another risk associated with bleached fibres products is the presence of dioxins produced during manufacturing and that can be released during use and from detached fibres (Keenan et al., 1989). Besides, the obvious presence of a plethora of dyes is a well-known fact (Biermann and Wiggins, 2018).

The occurrence of microplastics in the environment is reasonably documented, and there is growing evidence that they interact with many organisms. However, the extent to which they pose an ecotoxicological threat is controversial and a subject topic of active research (de Souza et al., 2018). Several groups studied the environmental impact of plastic microparticles to different aquatic invertebrates by means of acute and chronic toxicity tests. The concentrations that proved toxic or statistically significant

effects were typically many orders of magnitude above environmentally relevant levels. Table S3.2 (SM) details some studies reporting median effects (LC_{50}, EC_{50}) or LOEC for microplastic particles > 1 µm to aquatic invertebrates. The reported values range from 7.1 x 10^4 particles/L (10-day mortality of *Hyalella azteca*) to 4.4 x 10^8 particles/L (120-h mortality of Daphnia magna adults), which are 4-to-8 orders of magnitude above usual concentrations in the effluents of WWTP (Table 3.2). Even using the conservative factor of 1000, applied for risk assessment if only limited data are available, no evidence of toxic risk can be appreciated. Concerning primary producers, most works did not find EC_{50} values due to the high concentrations required to induce toxic responses except if exposed to very low sizes (Prata et al., 2019; Wang et al., 2019b). Finally, no effect of PE and PS microplastics to the earthworm *Eisenia foetida* has been reported except for concentrations as high as 20% (w/w in soil) (Wang et al., 2019a).

It has been suggested that human health could be threatened by microplastics because they are known to accumulate in certain wild or aquaculture species of fish and shellfish. The concern refers to physical toxicity, and to additives or adsorbed chemicals. However, there is still insufficient information to assess the exposure of humans to microplastics via food with estimations ranging from tens to tens of thousands of particles ingested per year and an almost absolute lack of toxicological and epidemiological data (Smith et al., 2018). There is an urgent need for assessing the risk of anthropogenic plastics including key aspects like the production of secondary nanoparticles due to ageing and the translocation of small plastic particles to food chains to accurately assess such risk. There is also a need for standardization in sizes and other methodological details that make results fully comparable among studies. Sufficiently comparable to at least allow precise estimates of the global plastics cycle and to perform sound risk assessment calculations. Clearly, microplastics escape in considerable amount to current wastewater treatment practices. Some specific sources of pollution like domestic microfibers, synthetic or anthropogenically modified, could be reduced in origin by introducing changes in washing machines. Concerning WWTP, attention should be paid to enhance technologies limiting the emission of microplastics with the effluent and, overall, on the use of sludge as soil amendment.

3.5. Conclusions

This work evaluated the presence of microplastics through the different steps of a WWTP including heat-dried sludge used as soil amendment. Our results showed that the efficiency of the WWTP in removing microplastics was high, with a removal rate of 93.7% between primary settler and final effluent. The quantification of the particles released with the effluent yielded 12.8 ± 6.3 items/L including manufactured natural fibres, while sludge contained 183 ± 84 items/g (wet sludge) and 165 ± 37 items/g (heat-dried sludge).

FTIR identification revealed the existence of PE, PP, polyester and acrylic fibres and an important amount of natural fibres with evidence of anthropogenic processing.

Size distributions were dominated by the smaller particles, in the 25-104 µm range, which represented 54% and 48% of primary and secondary effluents. Fibres represented 31% and 20% of the anthropogenic particles in primary and secondary effluents and 62% and 84% in wet sludge and heat dried sludge, respectively.

Our results showed that despite the high efficiency of conventional facilities, a huge number of particles escaped through the discharge of treated wastewater. The WWTP we studied releases about 300 million microplastic particles per day to the Henares River representing an approximate load of microplastics of 350 particles/m^3. WWTP sludge contributes to microplastics pollution with 8 x 10^{11} plastic particles per year. Dried sludge used as soil amendment in the area of Madrid (100000 t/year) would disseminate 10^{13} microplastic particles per year in agricultural soils. There is no direct evidence that exposure concentration of microplastics due to WWTP effluent discharge and wastewater or sludge reuse results in direct toxicity to soil or aquatic organisms. However, the huge amount of debris released and the possibility of fragmentation to non-sampled sizes, with possible translocation to food chains, makes further research necessary.

3.6. References

Abildgaard, A., Hagen-Mikkelsen, S., Stuer-Lauridsen, F., 2003. *Survey of Chemical Substances in Consumer Products*. Survey of Chemical Substances in Paper Handkerchiefs and Toilet Paper. Danish Environmental Protection Agency.

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

Bayo, J., Olmos, S., Lopez-Castellanos, J., 2020. *Microplastics in an urban wastewater treatment plant: the influence of physicochemical parameters and environmental factors*. Chemosphere 238, 124593.

Biermann, T.W., Wiggins, K.G., 2018. *Colour analysis of fibres*. In: Robertson, J., Roux, C., Wiggins, G.K. (Eds.), Forensic Examination of Fibres, third ed. CRC Press, pp. 180-242.

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

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

Cesa, F.S., Turra, A., Baruque-Ramos, J., 2017. *Synthetic fibres as microplastics in the marine environment: a review from textile perspective with a focus on domestic washings*. Sci. Total Environ. 598, 1116-1129.

Clark, J.R., Cole, M., Lindeque, P.K., Fileman, E., Blackford, J., Lewis, C., Lenton, T.M., Galloway, T.S., 2016. *Marine microplastic debris: a targeted plan for understanding and quantifying interactions with marine life.* Front. Ecol. Environ. 14, 317-324.

Comunidad-de-Madrid, 2018. *Plan de Gestión de Lodos de Depuración de las Aguas Residuales 2017/2024*. In: Territorio, C.d.M.A.y.O.d. (Ed.), Estrategia de Gestión Sostenible de los Residuos de la Comunidad de Madrid (2017/2024), Madrid, Spain.

Corradini, F., Bartholomeus, H., Huerta-Lwanga, E., Gertsen, H., Geissen, V., 2019. *Predicting soil microplastic concentration using vis-NIR spectroscopy*. Sci. Total Environ. 650, 922-932.

de Souza, A.A., Kloas, W., Zarfl, C., Hempel, S., Rillig, M.C., 2018. *Microplastics as an emerging threat to terrestrial ecosystems*. Glob. Chang. Biol. 24, 1405-1416.

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

Dris, R., Imhof, H.K., Loder, M.G.J., Gasperi, J., Laforsch, C., Tassin, B., 2018. *Microplastic contamination in freshwater systems: methodological challenges, occurrence and sources.* In: Zeng, E.Y. (Ed.), Microplastic Contamination in Aquatic Environments. Elsevier, pp. 51-93.

Edo, C., Tamayo-Belda, M., Martínez-Campos, S., Martín-Betancor, K., González Pleiter, M., Pulido-Reyes, G., García-Ruiz, C., Zapata, F., Leganés, F., Fernández Pinas, F., Rosal, R., 2019. *Occurrence and identification of microplastics along a beach in the biosphere reserve of Lanzarote.* Mar. Pollut. Bull. 143, 220-227.

ECHA, 2019. *Restriction proposal for intentionally added microplastics in the EU - annex XV - intentionally added microplastics*. In: Agency, E.C. (Ed.), Agency, European Chemicals. ECHA, Helsinki. ECHA/NR/19/28.

Eerkes-Medrano, D., Thompson, R., 2018. *Occurrence, fate, and effect of microplastics in freshwater systems.* In: Zeng, E.Y. (Ed.), Microplastic Contamination in Aquatic Environments. Elsevier, pp. 95-132.

Gago, J., Galgani, F., Maes, T., Thompson, R.C., 2016. *Microplastics in seawater: recommendations from the marine strategy framework directive implementation process.* Front. Mar. Sci. 3.

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

Gatidou, G., Arvaniti, O.S., Stasinakis, A.S., 2019. *Review on the occurrence and fate of microplastics in sewage treatment plants*. J. Hazard Mater. 367, 504-512.

GESAMP, 2016. *Sources, Fate and Effects of Microplastics in the Marine Environment: Part Two of a Global Assessment.* IMO/FAO/UNESCO-IOC/UNIDO/WMO/ IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, p. 220. Report Studies GESAMP No 93.

Gherghel, A., Teodosiu, C., De Gisi, S., 2019. *A review on wastewater sludge valorisation and its challenges in the context of circular economy.* J. Clean. Prod. 228, 244-263.

Gies, E.A., LeNoble, J.L., Noel, M., Etemadifar, A., Bishay, F., Hall, E.R., Ross, P.S., 2018. *Retention of microplastics in a major secondary wastewater treatment plant in Vancouver, Canada*. Mar. Pollut. Bull. 133, 553-561.

Gigault, J., Halle, A.t., Baudrimont, M., Pascal, P.-Y., Gauffre, F., Phi, T.-L., El Hadri, H., Grassl, B., Reynaud, S., 2018. *Current opinion: what is a nanoplastic?* Environ. Pollut. 235, 1030-1034.

Gonzalez-Pleiter, M., Tamayo-Belda, M., Pulido-Reyes, G., Amariei, G., Leganés, F., Rosal, R., Fernández-Pinas, F., 2019. *Secondary nanoplastics released from a biodegradable microplastic severely impact freshwater environments.* Environ. Sci.: Nano 6, 1382-1392.

Gonzalez, D., Hanke, G., Tweehuysen, G., Bellert, B., Holzhauer, M., Palatinus, A., Hohenblum, P., Oosterbaan, L., 2016. *Riverine Litter Monitoring-Options and Recommendations* (MSFD GES TG Marine Litter-Thematic Report, JRC Technical Report).

Keenan, R.E., Sauer, M.M., Lawrence, F.H., 1989. *Assessment of potential health risks from dermal exposure to dioxin in paper products.* Chemosphere 19, 877-882.

Kelly, M.R., Lant, N.J., Kurr, M., Burgess, J.G., 2019. *Importance of Water-Volume on the Release of Microplastic Fibres from Laundry*. Environmental Science & Technology.

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

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

Li, J., Liu, H., Chen, J.P., 2018a. *Microplastics in freshwater systems: a review on occurrence, environmental effects, and methods for microplastics detection*. Water Res. 137, 362-374.

Li, W.C., Tse, H.F., Fok, L., 2016. *Plastic waste in the marine environment: a review of sources, occurrence and effects*. Sci. Total Environ. 566-567, 333-349.

Li, X., Chen, L., Mei, Q., Dong, B., Dai, X., Ding, G., Zeng, E.Y., 2018b. *Microplastics in sewage sludge from the wastewater treatment plants in China.* Water Res. 142, 75-85.

Ling, S.D., Sinclair, M., Levi, C.J., Reeves, S.E., Edgar, G.J., 2017. *Ubiquity of microplastics in coastal seafloor sediments*. Mar. Pollut. Bull. 121, 104-110.

Liu, X., Yuan, W., Di, M., Li, Z., Wang, J., 2019. *Transfer and fate of microplastics during the conventional activated sludge process in one wastewater treatment plant of China*. Chem. Eng. J. 362, 176-182.

Lusher, A., Hurley, R., Vogelsang, C., Nizetto, L., Olsen, M., 2017. *Mapping Microplastic in Sludge.* Norwegian Institute for Water Research (NIVA).

Lv, X., Dong, Q., Zuo, Z., Liu, Y., Huang, X., Wu, W.-M., 2019. *Microplastics in a municipal wastewater treatment plant: fate, dynamic distribution, removal efficiencies, and control strategies*. J. Clean. Prod. 225, 579-586.

Magni, S., Binelli, A., Pittura, L., Avio, C.G., Della Torre, C., Parenti, C.C., Gorbi, S., Regoli, F., 2019. *The fate of microplastics in an Italian wastewater treatment plant*. Sci. Total Environ. 652, 602-610.

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

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

Mintenig, S.M., Int-Veen, I., Loder, M.G.J., Primpke, S., Gerdts, G., 2017. *Identification of microplastic in effluents of wastewater treatment plants using focal plane array-based micro-Fourier-transform infrared imaging*. Water Res. 108, 365-372.

Mourgkogiannis, N., Kalavrouziotis, I.K., Karapanagioti, H.K., 2018. *Questionnaire based survey to managers of 101 wastewater treatment plants in Greece confirms their potential as plastic marine litter sources*. Mar. Pollut. Bull. 133, 822-827.

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

Napper, I.E., Thompson, R.C., 2019. *Marine plastic pollution: other than microplastic.* In: Letcher, T.M., Vallero, D.A. (Eds.), Waste. A Handbook for Management, second ed. Academic Press, pp. 425-442.

Ng, E.L., Huerta Lwanga, E., Eldridge, S.M., Johnston, P., Hu, H.W., Geissen, V., Chen, D., 2018. *An overview of microplastic and nanoplastic pollution in agroecosystems*. Sci. Total Environ. 627, 1377-1388.

Ngo, P.L., Pramanik, B.K., Shah, K., Roychand, R., 2019. *Pathway, classification and removal efficiency of microplastics in wastewater treatment plants*. Environ. Pollut. 255, 113326.

O'Brien, J.W., Thai, P.K., Brandsma, S.H., Leonards, P.E.G., Ort, C., Mueller, J.F., 2015. *Wastewater analysis of Census day samples to investigate per capita input of organophosphorus flame retardants and plasticizers into wastewater*. Chemosphere 138, 328-334.

PlasticsEurope, 2018. *Plastics the Facts 2018: an Analysis of European Plastics Production, Demand and Waste Data*. PlasticsEurope: Association of Plastics Manufacturers, Brussels.

Prata, J.C., da Costa, J.P., Lopes, I., Duarte, A.C., Rocha-Santos, T., 2019. *Effects of microplastics on microalgae populations: a critical review*. Sci. Total Environ. 665, 400-405.

Reddy, K.O., Maheswari, C.U., Dhlamini, M.S., Kommula, V.P., 2016. *Exploration on the characteristics of cellulose microfibres from Palmyra palm fruits*. Int. J. Polym. Anal. Charact. 21, 286-295.

Ribeiro, F., O'Brien, J.W., Galloway, T., Thomas, K.V., 2019. *Accumulation and fate of nano- and micro-plastics and associated contaminants in organisms*. Trac. Trends Anal. Chem. 111, 139-147.

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

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

Talvitie, J., Mikola, A., Setal € a, O., Heinonen, M., Koistinen, A., 2017. *How well is microlitter purified from wastewater? e a detailed study on the stepwise removal of microlitter in a tertiary level wastewater treatment plant.* Water Res. 109, 164-172.

Vermeiren, P., Munoz, C.C., Ikejima, K., 2016. *Sources and sinks of plastic debris in estuaries: a conceptual model integrating biological, physical and chemical distribution mechanisms*. Mar. Pollut. Bull. 113, 7-16.

Wang, J., Coffin, S., Sun, C., Schlenk, D., Gan, J., 2019a. *Negligible effects of microplastics on animal fitness and HOC bioaccumulation in earthworm Eisenia fetida in soil*. Environ. Pollut. 249, 776-784.

Wang, W., Gao, H., Jin, S., Li, R., Na, G., 2019b. *The ecotoxicological effects of microplastics on aquatic food web, from primary producer to human: a review*. Ecotoxicol. Environ. Saf. 173, 110-117.

Weithmann, N., Moller, J.N., L € oder, M.G.J., Piehl, S., Laforsch, C., Freitag, R., 2018a. *Organic fertilizer as a vehicle for the entry of microplastic into the environment*. Sci. Adv. 4 eaap8060-eaap8060.

Yan, M., Nie, H., Xu, K., He, Y., Hu, Y., Huang, Y., Wang, J., 2019. *Microplastic abundance, distribution and composition in the pearl river along Guangzhou city and pearl river estuary, China.* Chemosphere 217, 879-886.

Yang, L., Li, K., Cui, S., Kang, Y., An, L., Lei, K., 2019. *Removal of microplastics in municipal sewage from China's largest water reclamation plant.* Water Res. 155, 175-181.

Zambrano, M.C., Pawlak, J.J., Daystar, J., Ankeny, M., Cheng, J.J., Venditti, R.A., 2019. *Microfibres generated from the laundering of cotton, rayon and polyester based fabrics and their aquatic biodegradation*. Mar. Pollut. Bull. 142, 394-407.

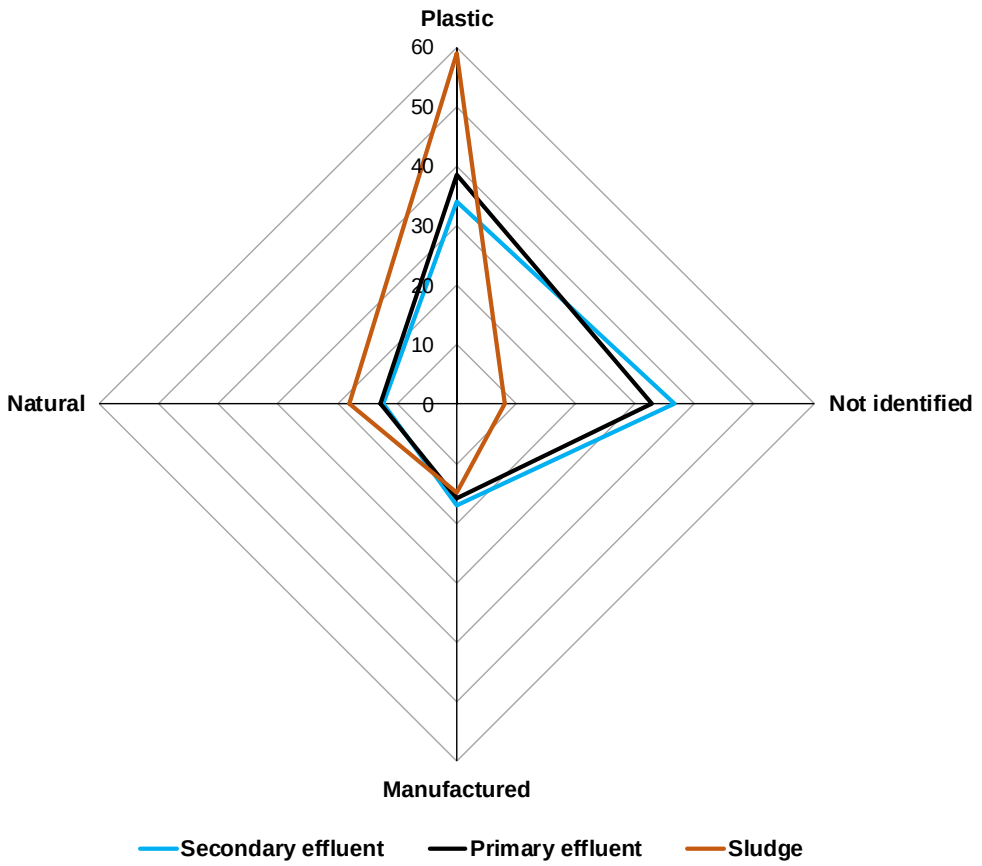
Zhang, Y., Gao, T., Kang, S., Sillanpää, M., 2019. *Importance of atmospheric transport for microplastics deposited in remote areas.* Environ. Pollut. 254, 112953.

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

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

3.7. Supplementary material of Chapter 3

Contents



Polymers identified by micro-FTIR

Cellulose polymers (cellophane, rayon/viscose, cellulose acetate, cotton)

Polymethyl methacrylate

Polyamide fibres

Polycaprolactone

Polyester fibres and resins

Polyethylene

Polyethylene terephthalate

Polypropylene

Polystyrene

Polyurethanes

Poly(vinyl acetate) and copolymers

Poly(vinyl chloride)

Figure S3.1. Distribution of materials present in the different samples after FTIR analyses. "Manufactured" refers to natural substances with evidence of anthropogenic modification and list of main polymers identified by micro-FTIR.

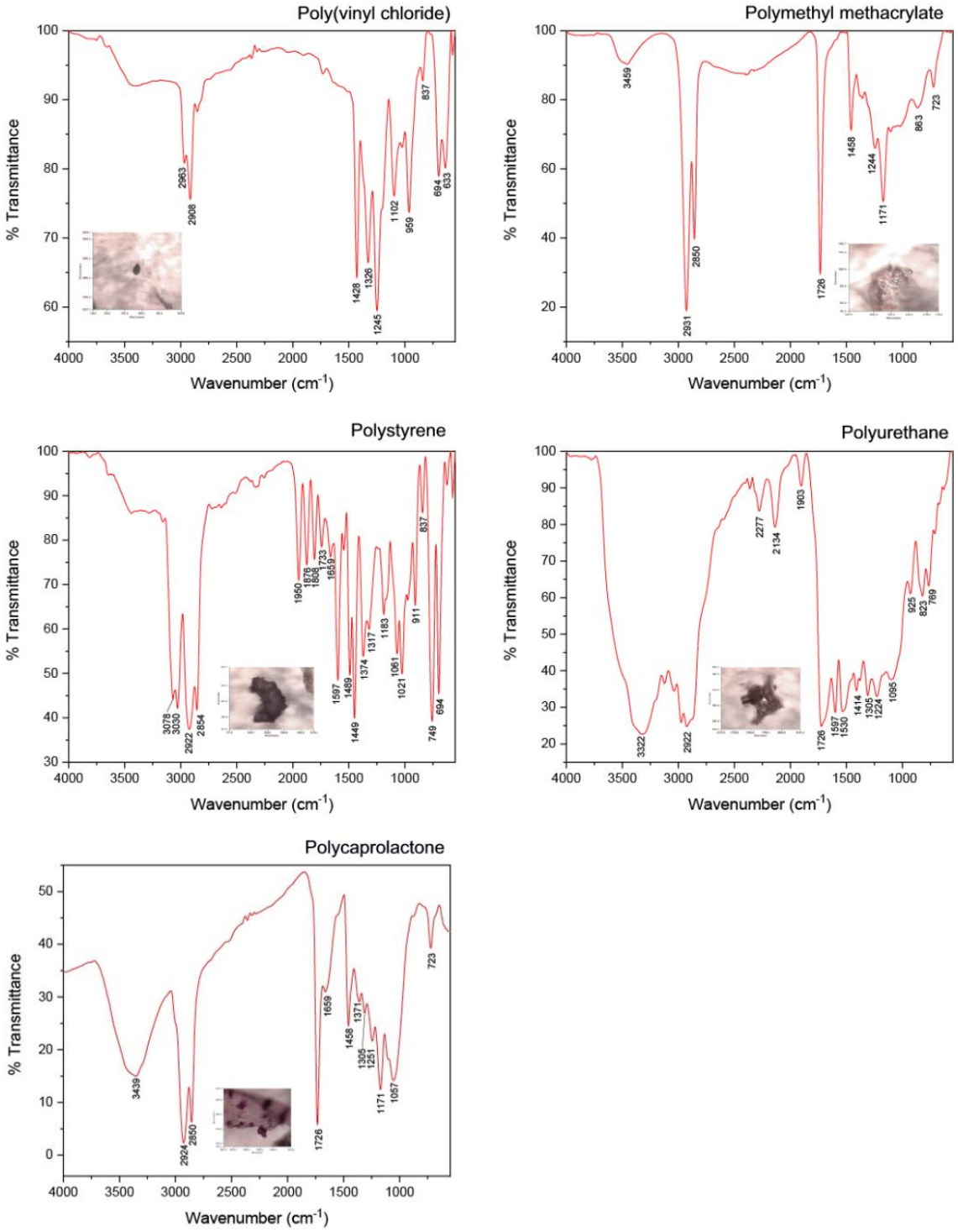


Figure S3.2. Infrared spectra of samples of poly(vinyl chloride), polymethyl methacrylate, polystyrene, polyurethane and polycaprolactone.

Table S3.1. Density of sampled polymers

Polymer type	**Abbreviation**	**Density range (g/cm^3)**
Polyethylene	PE	0.87-0.97
Polypropylene	PP	0.85-0.88
Polyethylene-terephtalate	PET	1.33-1.48
Poly(methyl methacrylate)	PMMA	1.12-1.17
Polyurethane	PU	0.87-1.42
Polystyrene	PS	0.96-1.05
Polycaprolactone	PCL	1.10-1.15
Polyamide	PA	1.13-1.41
Poly(vinyl acetate)	PVA	1.19-1.20
Poly(vinyl chloride)	PVC	1.38-1.39

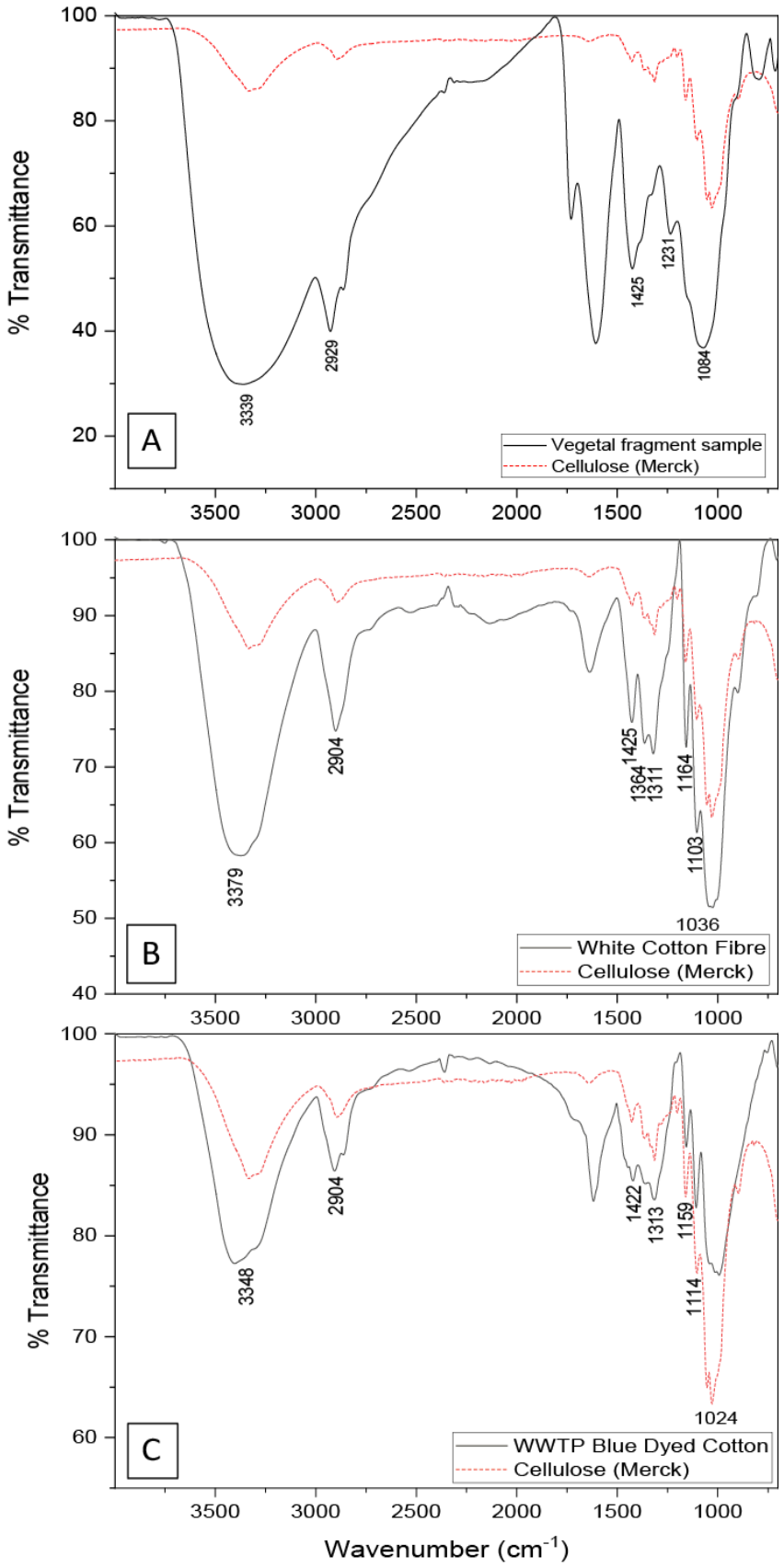


Figure S3.3. Infrared spectra of cellulose-based particles. A. Vegetal fragment. B. White cotton fibre. C. Blue-dyed cotton fibre.

Table S3.2. Toxicological data reported in the literature for microplastic particles > 1 mm.

Reference	Polymer	Test organism	Endpoint	LC/EC_{50} or LOEC, item/L
(Au, Bruce et al. 2015)	10-27 μm PE microparticles	*Hyalella azteca*	10-day mortality	4.6 x 10^7
(Au, Bruce et al. 2015)	20-74 μm PP microfibres	*Hyalella azteca*	10-day mortality	7.1 x 10^4
(Rehse, Kloas et al. 2016)	1-4 μm PE microspheres	*Daphnia magna*	96 h immobilization	1.3 x 10^8
(Lee, Shim et al. 2013)	6 μm PS microbeads	*Tigriopus japonicus*	Fecundity	2.1 x 10^5 (LOEC)
(Cole, Lindeque et al. 2013)	7.3 μm PS microbeads	*Centropages typicus*	Algal ingestion rate	7 x 10^5 (LOEC)
(Kaposi, Mos et al. 2014)	10−45 μm PE microspheres	*Tripneustes gratilla.*	Reduction of larvae body width	3 x 10^5 (LOEC)
(Eltemsah and Bøhn 2019)	6 μm PS microbeads	*Daphnia magna*	120-h mortality in juveniles	2.9 x 10^8
(Eltemsah and Bøhn 2019)	6 μm PS microbeads	*Daphnia magna*	120-h mortality in adults	4.4 x 10^8
(Ogonowski, Schür et al. 2016)	< 63 μm	*Daphnia magna*	Reproductive output	8.6 x 10^4

References (Table S3.2)

Au, S. Y., et al. (2015). "*Responses of Hyalella azteca to acute and chronic microplastic exposures*." Environmental Toxicology and Chemistry **34**(11): 2564-2572.

Cole, M., et al. (2013). "*Microplastic Ingestion by Zooplankton*." Environmental Science & Technology **47**(12): 6646-6655.

Eltemsah, Y. S. and T. Bøhn (2019). "A*cute and chronic effects of polystyrene microplastics on juvenile and adult Daphnia magna*." Environmental Pollution **254**: 112919.

Kaposi, K. L., et al. (2014). "I*ngestion of microplastic has limited impact on a marine larva*." Environmental Science & Technology **48**(3): 1638-1645.

Lee, K. W., et al. (2013). "*Size-dependent effects of micro polystyrene particles in the marine copepod Tigriopus japonicus.*" Environmental Science & Technology **47**(19): 11278-11283.

Ogonowski, M., et al. (2016). "*The effects of natural and anthropogenic microparticles on individual fitness in Daphnia magna.*" PLOS ONE **11**(5): e0155063.

Rehse, S., et al. (2016). "*Short-term exposure with high concentrations of pristine microplastic particles leads to immobilisation of Daphnia magna.*" Chemosphere **153**: 91-99.

CHAPTER 4. MICROPLASTICS IN SEDIMENTS OF ARTIFICIALLY RECHARGED LAGOONS: CASE STUDY IN A BIOSPHERE RESERVE

4.1. Introduction

Worldwide plastic production in 2018 amounted to 359 million tonnes according to PlasticsEurope (PlasticsEurope, 2019). From the same source, in Europe (EU plus Norway and Switzerland) 29.1 million tonnes were collected as post-consumer waste through official schemes, equivalent to 47% of the amount of plastics produced in the same countries; still 25% plastic post-consumer waste was sent to landfill and an undefined amount ended up in the environment. The origin of plastic waste disseminated into environmental compartments is diverse. Plastic debris reach the environment due to inadequate disposal practices including open landfills, wastewater discharges, or wind transport of airborne fragments (van Emmerik et al., 2019). It is accepted that most plastic waste ends up in oceans with estimated input in the order of 10 million tonnes every year (Jambeck et al., 2015). According to the 2016 report by the World Economic Forum, plastics in world's oceans will outweigh fish by 2050 if the projections for plastics production follows the estimated current trends (World Economic Forum, 2016). Concerning other compartments, it is well-known the presence of plastic wastes in terrestrial and freshwater environments (Blettler et al., 2018; Guo et al., 2020). Atmospheric fallout has also been recognized as a source of microplastics, particularly for fibres and urban environments (Dris et al., 2016).

Microplastics are a new class of persistent pollutants defined as plastic particles with size <5 mm with a lower boundary of 1 µm below which they are considered nanoplastics (Gago et al., 2016; Gigault et al., 2018). Some microplastics result from the degradation of bigger particles including textiles or tire wearing, while other have been specifically designed in small sizes for uses in cosmetics or blast cleaning (Godoy et al., 2019). Microplastics, and supposedly their nanosized fragments, are ubiquitous pollutants, found in all possible environments (Farady, 2019; Peeken et al., 2018; Zhang et al., 2020c). The effect of nanoplastics is essentially unknown, but their potential toxicity has already been demonstrated (González-Pleiter et al., 2019). Concerning inland ecosystems, there are three major sources of microplastic pollution: atmospheric deposition, including untreated stormwater collection and runoff, the discharge of wastewater treatment plants (WWTP) to freshwater environments, and the use of sewage sludge as fertilizer in agricultural soils (Edo et al., 2020; Klein and Fischer, 2019).

The sources and distribution of microplastics through atmospheric transport are still poorly known. On the contrary, there is a growing body of evidence about the role of WWTP in spreading microplastics (Carr et al., 2016; Lares et al., 2018). Even if many microplastics are removed with sludge, WWTP effluents still contain sufficient amount of microplastics to pose an environmental threat (Edo et al., 2020).

Once released into the environment, microplastics may interact with biota causing potential toxic effects (de Souza et al., 2018). Microplastics have been associated with chemical toxicity, either due to their capacity to act as vehicle for other pollutants or to the release of substances included in their formulation as additives (Wang et al., 2018). Microplastics have been found in a plethora or organisms, mainly from marine environments. However, acute exposure tests with different species, including their sensitive early life stages, did not result in significant toxic effects even at the highest environmental concentration (Beiras et al., 2018). Toxic concentrations in standard tests are typically several orders of magnitude above concentrations found in polluted environments, like wastewater effluents (Edo et al., 2020). However, few data are available concerning chronic exposures and sub-lethal effects (Jaikumar et al., 2019). There is an important research gap on the accumulation of microplastics within web chains, which includes humans. It has been estimated that the ingestion of microplastics via food may range from tens to tens of thousands of particles per year (Smith et al., 2018). The fragmentation of microplastics is known to give rise to particles <1 µm, usually classified as nanoplastics (Gigault et al., 2018). The exposure to nanoplastics may result in their accumulation in tissues and synergistic effects in the interaction with other toxicants (Lee et al., 2019). Nanoplastics have been associated with different effects such as reduced growth or alterations in reproductive patterns (Zhang et al., 2020b).

One key characteristic of wetlands is their capacity to act as sinks for some nutrients and to remove pollutants from agricultural runoff (Tournebize et al., 2017). Lagoon systems, engineered as constructed wetlands, are in wide use as a low-cost method to purify wastewater from small communities (Wu et al., 2015). Additionally, in some lagoons, authorities allow direct discharge of effluents from WWTP to cope with low water levels originated by aquifer overexploitation for intensive agriculture. Little is known, however, on the fate of many pollutants including regulated chemicals and

contaminants of emerging concern, that accumulate in lagoons (Gorito et al., 2017). For example, the use of reclaimed water alters the fate of nutrients through modification of natural cycles of drying-flooding in semiarid sites (Corrales-González et al., 2019).

In this work, we studied the presence of microplastics in lagoons representative from the extensive network of natural wetlands called "La Mancha Húmeda", declared Biosphere Reserve by UNESCO. We studied lagoons that receive wastewater inputs to maintain water level and compared them with non-artificially recharged lagoons. The amount and type of microplastics found in sediments were monitored and the results were compared with other studies.

4.2. Material and methods

4.2.1. Location

The lagoons selected for this study are located in Toledo province, in the wetland area called "La Mancha Húmeda" (Castilla-La Mancha, Spain) (Fig. 4.1). It is an area of approximately 4000 km^2, including transition zones, which plays an important role for biodiversity protection, aquifer recharge, sediment retention, flooding control and carbon sink, among others. Six lagoons were selected and sampled. Three of them, namely El Longar, ELG[1], Larga de Villacañas, LVC[2], and Laguna Grande de Quero, GQR[3] receive wastewater. (Tables S4.1 and S4.2 Supplementary Material, SM), show information related to discharges, water inputs and land use). The other three, Laguna Chica de Villafranca, CVF[4], Laguna del Altillo Chica (LAC[5]) and La Albardiosa, LAB[6], only receive water by rainfall and runoff and were used as control.

The six selected lagoons are part of a wide set of endorheic water bodies located in the Biosphere Reserve "La Mancha Húmeda". The landscape is flat, with predominance of agricultural lands spotted by endorheic lagoons fed by runoff and aquifer upwellings. Untouched lagoons are seasonal, with elevated salinity, even five times higher than seawater. These wetlands have a high ecological value, acting as breeding ground for migratory and aquatic birds. They are also colonised by a large number of endemic or endangered halophilic and aquatic plants (Cirujano and Medina, 2014). It is a highly protected area declared UNESCO Biosphere Reserve in 1981. Parts of it are listed under several protection figures including the Ramsar List of Wetlands of International

Importance, one Specially Protected Bird Area, and two National Parks, among others. However, all the area is highly menaced by aquifer over-exploitation for irrigation, which led groundwater level so low that natural replenishment by rainfall is insufficient. In this context, some lagoons receive treated wastewater in part in an attempt to maintain some water level and also because they are the natural sink for nearby communities. It is important to note that they are endorheic lagoons, connected with the underground aquifer, but without visible outlet.

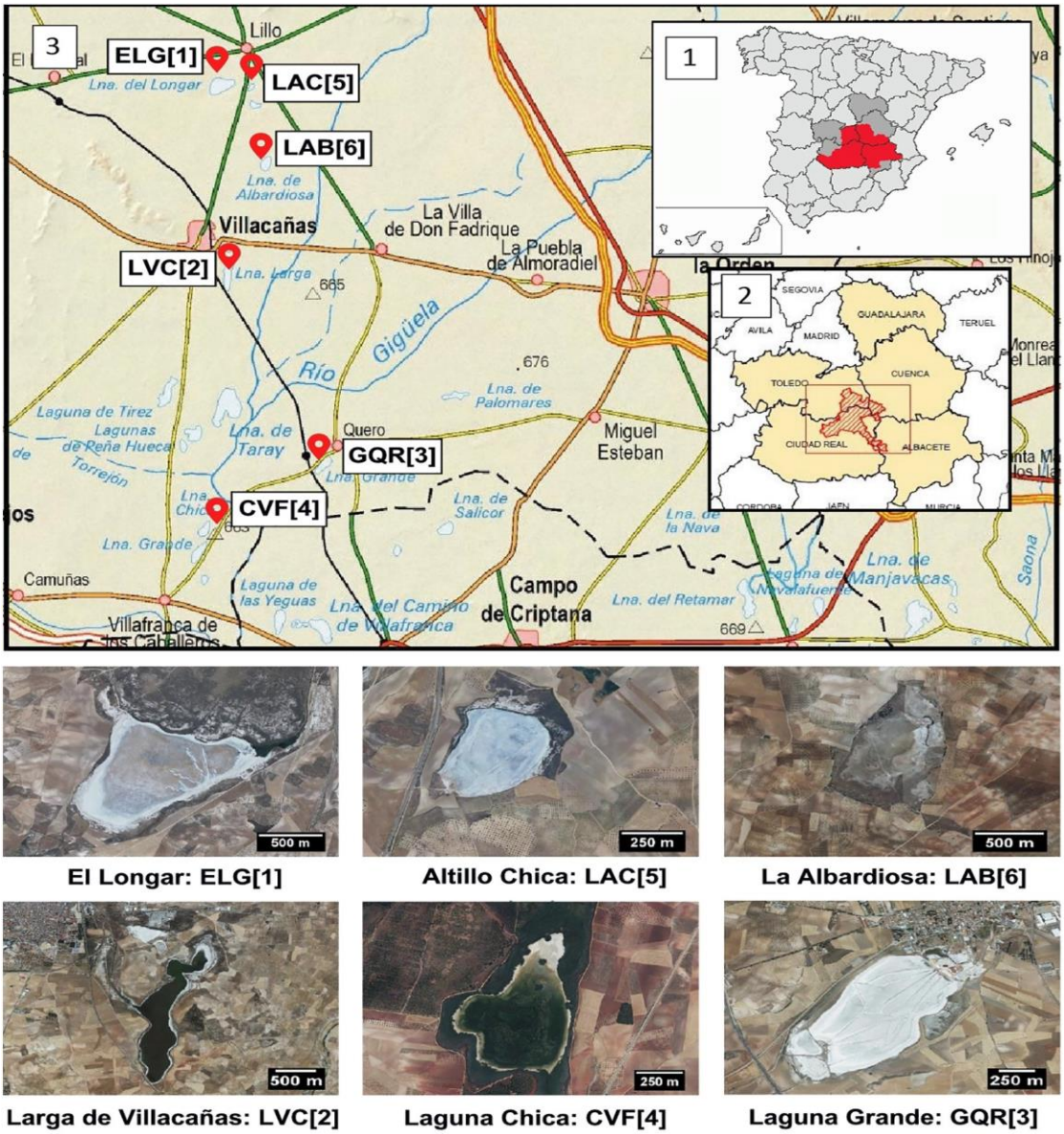


Figure 4.1. Map and main characteristics of the study zone and aerial view of sampling points. 1. Spain map with "La Mancha" natural zone red marked. 2. Castilla-La Mancha administrative region. 3. Location of sampling points and aerial view of the six lagoons. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Additional information related to the sampled lagoons is in the table 4.1 and in Fig. S4.1 (SM).

Table 4.1. Additional information of the sampled lagoons.

ID	Name and Location	Surface (ha)	Datum	Zone	UTM X	UTM Y	Discharge
ELG[1]	El Longar (Lillo)	96	ETRS89	30	472249,85	4394837,59	WWTP Lillo
LVC[2]	Laguna Larga (Villacañas)	84	ETRS89	30	472669,74	4383902,02	WWTP Villacañas
GQR[3]	Laguna Grande (Quero)	72	ETRS89	30	478114,83	4372345,71	Untreated discharge
CVF[4]	Laguna Chica (Villafranca de los Caballeros	37	ETRS89	30	471333,61	4368219,27	-
LAC[5]	Altillo Chica (Lillo)	15	ETRS89	30	473969,83	4394805,7	-
LAB[6]	La Albardiosa (Lillo)	32	ETRS89	30	474763,98	4390292,34	-

On sampling, LAC[5] and LAB[6] were completely dried with a considerable number of macroplastics of agricultural origin, most of them easily recognized as the usual green plant protectors. GQR[3] is surrounded by Quero village (1006 inhab.). Despite been upstream to the WWTP, there was a wastewater drainage coming from a collector without evidence of any treatments. A green filter made of macrophytes existed at the water discharge to LVC[2]. This lagoon showed a high amount of macrolitter inside and around the lagoon and also presented bad smell with high amount of black sediments, different from the rest of sampled locations. ELG[1] was almost dried except for the contribution of a WWTP discharge and showed many debris along the sediment line. CVF[4] was visually the most unaffected lagoon, surrounded by canes and without macrolitter. Fig. S4.1 (SM) shows pictures of the sampling points and some of the evidence of anthropogenic pollution.

4.2.2. Sampling

Sediment samples were collected using 1 L high density polyethylene (HDPE) bottles. Sediments were collected with a stainless-steel sediment collector cleaned with ultrapure water between samples. All sampling material was covered with aluminium foils to prevent particle deposition. Except for HDPE bottles in field sampling, plastic material was avoided, and only glassware was used in laboratory manipulation. To prevent cross contamination, all materials were carefully cleaned with ultrapure water and clothes worn by manipulators were controlled during sampling avoiding synthetic textiles and using cotton in bright colours whenever possible. To ensure absence of cross-contamination HDPE bottles were cleaned several times with ultrapure water and the resulting liquid examined for possible rests of plastic material. Both bottles and lids were added to a reference micro-FTIR database to check for possible coincidences. We did not detect any plastic debris from these bottles and lids during validation or in any of the samples.

Sampling was performed in May 2019, in a sunny day without wind (<10 km/h). Three different areas separated at least by 2 m were chosen close to the entry of the stream feeding each lagoon. For each sampling zone, a surface of 1 m × 1 m was selected and sediment from the first 2 cm was collected directly into the bottles. The minimum volume recovered was 500 mL. During sampling, an additional bottle was kept opened close to the sampling point as control for air deposition or contamination. All samples were covered with aluminium foil, capped, and stored in the freezer to avoid microbial growth. Once in laboratory, samples were dried under vacuum at 60 °C to remove water without affecting the plastics present in the sample and then frozen until subsequent analysis.

The microplastics obtained from sediments were compared with samples taken from wet deposition and from the effluent of a WWTP. Wet deposition samples were obtained in the rain events that took place during the month of July in nearby area. This was the first rain event that took place after lagoon sampling. For it, glass recipients were kept opened during rainfall with an additional one set close to the sampling point but protected from rain as contamination control. Samples from treated wastewater

were collected in Spring 2019 from the effluent of the secondary settler of a WWTP located in the same region. Details can be found elsewhere (Edo et al., 2020).

4.2.3. Recovery of microplastics

For microplastic extraction, dried sediment samples (5 g) were treated with 25 mL of H_2O_2 (33% w/v) to remove organic matter and left in oven (60 °C) for 20-24 h. This procedure, selected after different trials, removed enough organic matter to make microplastic counting feasible (Edo et al., 2020; Helcoski et al., 2020). A sodium chloride hypersaline solution (1.2 g/mL) was used to separate plastics by density. Several authors proposed NaCl solution as cheap and safe method for separating materials from sediments preferred over other salts like $ZnCl_2$ or NaI (Bayo et al., 2020; Cannas et al., 2017; Masura et al., 2015). Samples were magnetically stirred for 15 min and stored overnight at 4 °C to complete density separation. The supernatant was filtered through 25 µm stainless-steel mesh and dried at 60 °C. The 25 µm lower boundary was chosen in view of the spatial resolution of micro-FTIR spectroscopy, which is limited to 10-20 µm (Araujo et al., 2018). The rest of the sediment was also dried and evaluated in order to count the particles that could have been settled with the sediment due to their higher density or attached to other particles. These represented between 10 and 30% of the total number of suspected anthropogenic litter. Therefore, both supernatant and sediment, without any loss of particles during the process, were inspected. All particles >5 mm discarded. Processed samples were kept in glass Petri dishes until analyses. Throughout sample handling, clean 25 µm stainless-steel meshes were kept in open Petri dishes near the samples to control possible contamination during laboratory procedures. The same process was performed with rainfall samples and with minor modifications with the samples from the secondary WWTP effluent. Additional details can be found elsewhere (Edo et al., 2020).

4.2.4. Analytical procedure

Particle counting was performed with a Euromex-Edublue stereomicroscope fitted with ImageFocus 4 camera software. ImageJ software was used to measure particles. Polymer identification was performed by Micro-Fourier Transform Infrared Spectroscopy (micro-FTIR) using a Perkin-Elmer Spotlight 200 Spectrum Two apparatus

equipped with an MCT detector. This equipment uses Fourier-Transformed infrared spectroscopy (mid-infrared region) to obtain spectra that are compared with existing databases. Particles were placed individually with a zircon microneedle over potassium bromide (KBr) discs. The equipment operated in transmission mode with 8 cm^{-1} resolution and spectral range 4000-550 cm^{-1}. The amount of microplastics per gram of sediment in the first 2 cm of sediment was calculated by multiplying the counting of microparticles with the percentages of microparticles identified as microplastics using micro-FTIR. Control samples both from field and laboratory were examined under the stereomicroscope and micro-FTIR and compared with the clothes worn by the personnel. Particles and fibres similar in colour and shape with those in controls were subtracted from the counting.

The spectra from lagoon samples were compared with materials previously collected from wet deposition and with material recovered from another WWTP in the same region. All samples were equally treated with 33% H_2O_2 to remove organic matter present and avoid microbial growth, cleaned with ultrapure water, dried and stored until FTIR analysis. A total of 445 spectrums were studied with five different models. 190 spectrums from lagoons, 172 from WWTP and 83 from rain deposition. The spectra were randomly selected and in those from lagoons we ensured the same proportion in all of them. A group-to-group comparison was performed to assess the regions of the spectrum responsible for the difference among groups.

4.2.5. Statistics

Statistical methods were used to compare the FTIR spectra of microparticles recovered samples from lagoons with those from rainfall and wastewater. FTIR spectra for all samples were obtained under the same conditions and procedures. The identification of polymer type was performed with OMNIC 9 software obtained from Thermo Scientific. A minimum percentage of 60% was selected as matching as stated elsewhere (Liu et al., 2019). Matching system uses Pearson correlation to compare recorded spectra with databases. The obtained infrared spectra were processed with the multivariate tool SIMCA 15 (Sartorius Stedim Data Analytics, Umeå, Sweden). All spectra were smoothed and normalized with both rubber band baseline correction and standard

normal variate (SNV) methods, respectively. These methods allow a better comparison of the spectra while minimizing the differences in the light dispersion produced by the various particle sizes. To discriminate between groups (lagoons-wet deposition-wastewater) and to highlight differences among them, orthogonal partial least squares discrimination analysis (OPLS-DA) was performed. This method uses PCA methodologies to reduce the dimension of the data set al.lowing better correlations (Silva et al., 2017). Model fitting was assessed using R^2Y and Q^2 parameters. Hotelling's T^2 test was performed for hypothesis testing to recognise any possible outliers (Bylesjö et al., 2006).

4.3. Results

4.3.1. Abundance and morphology of microparticles

Fig. 4.2 shows the results of sampling for microparticles in the 25 µm- 5 mm range. Fig. 4.2A shows the histogram with microplastic sizes for all samples. Fig. 4.2B shows the typology and concentration in microparticles per gram of dry sediment in all sampled lagoons. The term microparticle refers here to fragments, filaments, films or fibres with possible anthropogenic origin either separated with flotation using the hypersaline solution or identified in the sediment from hypersaline flotation. Microparticles with clear natural origin, such as mineral particles or vegetal fragments, were not included. Therefore, and in what follows, the term microparticles refer to suspected small anthropogenic litter. Fig. 4.2B refers to concentration of microparticles (microplastics and non-microplastic fraction). The maximum concentration of microparticles was found in GQR[3] with 36.3 ± 7.7 particles/g followed by LVC[2] with of 28.9 ± 7.0 particles/g. The samples from the other four lagoons contained much less microparticles, with<10 particles/g of suspected anthropogenic litter, the lower figures recorded in samples from CVF [4] with 2.9 ± 1.2 particles/g (Fig. 4.2B). According to typology the majority of microparticles were fibres followed by fragments. Fibres represented between 50 and 65% of microparticles in all lagoons except LAB [6], in which fragments (53%) were the predominant typology. Films and filaments were in all cases less abundant, with occurrence <10% among all recovered microparticles. Using micro-FTIR, microparticles could be classified as microplastics and a non-microplastic fraction as shown in Fig. 4.2C, while Fig. 4.2D shows the typology distribution of microparticles identified as microplastics. Size distribution was calculated from

projected area diameter recorded from microscopic measurements (Fig. 4.2A). The median of size distribution for all microparticles was 86.4 µm (first and third quartiles 49.4 and 140.4 µm, respectively).

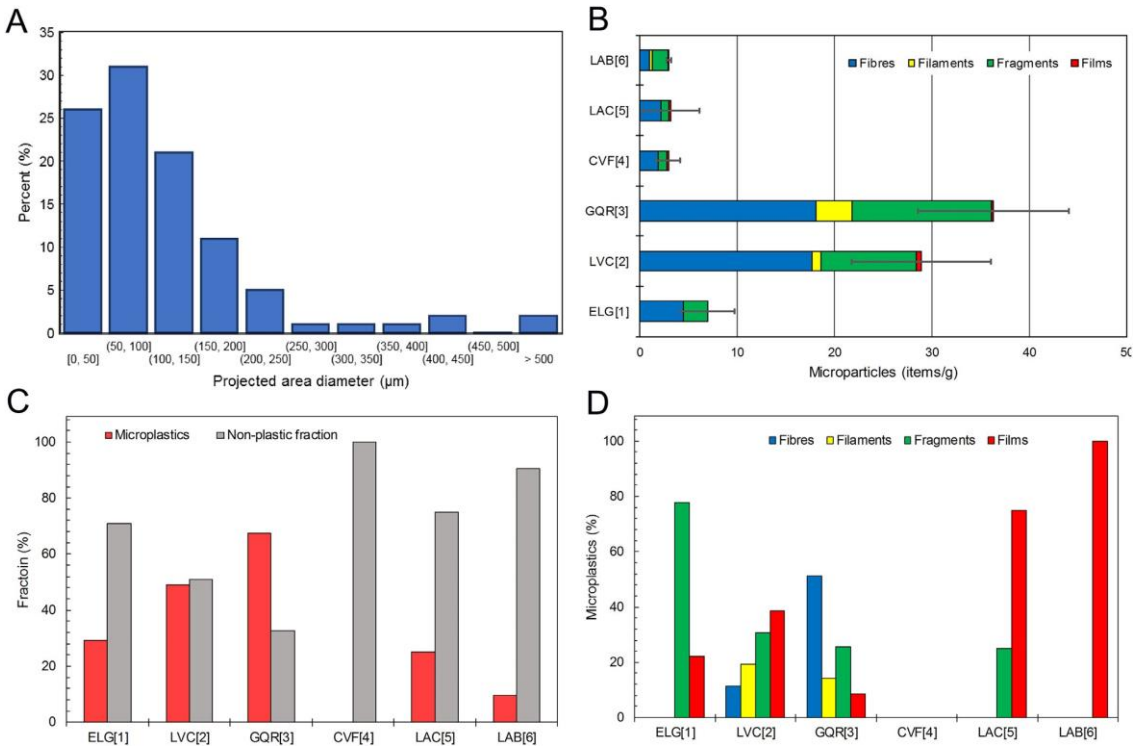


Figure 4.2. Projected area diameter histogram for microplastics identified in all samples (A). Microparticles (including microplastic and non-microplastic fraction) per gram of sediment according to typology (B). Fraction of microplastics (and non-microplastics) in all sampled lagoons according to micro-FTIR analyses (C). Fraction of microplastics within each typology as identified by micro-FTIR (D). (Legends refer to lagoons: El Longar: ELG(1); Larga de Villacañas: LVC(2); Laguna Grande de Quero: GQR(3); Laguna Chica de Villafranca: CVF(4); Laguna del Altillo Chica: LAC(5); La Albardiosa: LAB(6).)

4.3.2. Microplastics in sediments

A subsample of 190 microparticles was analysed by means of micro-FTIR, which represented 15% (Table S4.3, SM) of the total number of microparticles suspected to be anthropogenic. The use of a subsample was due to the impossibility of sampling the full population. In this case, the subsampling of the full population represented a theoretical accuracy of 6.3%, derived as shown elsewhere (Kedzierski et al., 2019).

The total number of microparticles identified as microplastic was 76 representing 40% of the potentially anthropogenic microparticles. The maximum number of microplastics

were found in the subsample from GQR[3] (35 out of 52, or 67%). As indicated before, this lagoon does not receive authorized discharge from any WWTP, but there is at least one obvious emission of untreated wastewater. An important number of microplastics were also found in ELG[1] and LVC[2], which are lagoons receiving treated wastewater from Lillo and Villacañas WWTP respectively.

In ELG[1] 9 out of 31 (29%) microparticles were identified as microplastic, whereas the same figures from LVC[2] were 26 out of 53 (49%). Microplastics were much less abundant in the three lagoons that do not receive wastewater. Only 4 and 2 microplastics were found in LAC[5] and LAB[6] respectively, whereas none of the 15 microparticles analyses from CVF[4] were plastics. Table S4.3 (SM) summarizes the results from micro-FTIR analyses.

In all cases, the most frequent material found in samples was cellulose, both natural, with probable origin in vegetal tissues, and anthropogenic, as part of textile fabrics or other manufactured items. These microparticles, listed in Table S4.3 (SM) as "anthropogenic" mainly consisted of fibres (>80%) identified as cotton/cellulose that could be classified as anthropogenic litter because of their non-natural colours.

Particles or fibres of natural materials like wool or cellulose derivatives may evidence anthropogenic origin due to their non-natural colour or the presence of other industrial additives. They comprise a category of anthropogenic litter including natural materials that underwent industrial processing and bear artificial additives like dyes, light stabilizers, and other chemicals used as part of their composition of for their manufacturing (González-Pleiter et al., 2020). Most microparticles identified as microplastics (92%) were found in the three lagoons receiving wastewater, namely ELG[1], LVC[2], GQR[3]. Noticeably, the highest amount (35 out of 52 microparticles analysed) corresponded to the samples taken from GQR[3], a lagoon suffering from non-treated wastewater discharge. Within the plastic fraction, 11 different types of polymers were found, namely polyethylene (PE), polypropylene (PP), polyester fibres (Pe), acrylic fibres (Ac), polystyrene (PS), polyvinyl chloride (PVC), polyvinyl fluoride (PVF), polytetrafluoroethylene (PTFE), polyamide (PA), polyurethane (PU), and polystyrene-acrylic blend (PS-Ac). Only six microplastic particles were found in lagoons without

wastewater discharge: LAC[5] with three PE films and one PP fragment, and LAB[6] with two films of PE and PVC. Polymer variety was higher in LVC[2] and GQR[3], with eight different polymers found in each lagoon. Fig. 4.3 shows the distribution of identified polymers in the three lagoons receiving wastewater. The most frequently found polymers, which accounted for 77% of the total number of microplastics were the polyolefins PE and PP, polyester (Pe), and acrylic (Ac). Figs. S4.2 and S4.3 (SM) show infrared spectra from representative materials found in samples.

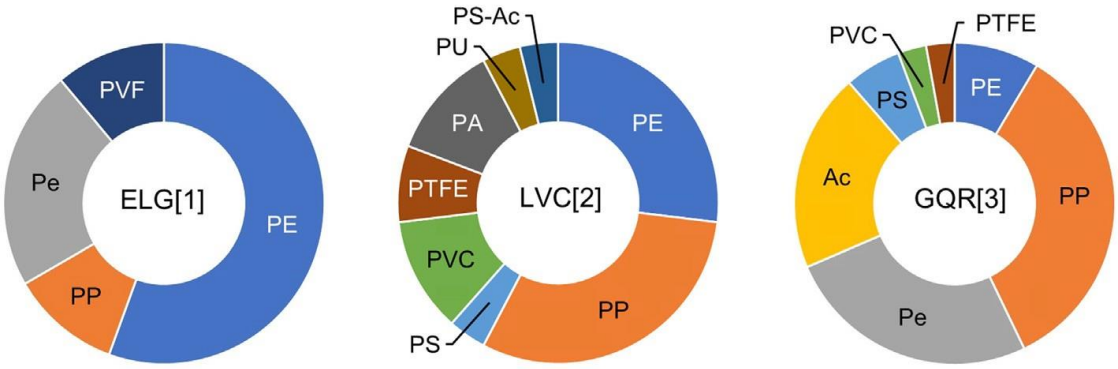


Figure 4.3. Polymer distribution per sample in lagoons ELG(1), LVC(2) and GQR(3). (PE: polyethylene; PP: polypropylene; Pe: polyester fibres; Ac: acrylic fibres (Ac); PS: polystyrene (PS); PVC: polyvinyl chloride; PVF: polyvinyl fluoride; PTFE: polytetrafluoroethylene (PTFE); PA: polyamide; PU: polyurethane; PS-Ac: polystyrene-acrylic blend.)

Images of representative microplastics are shown in Fig. S4.4 (SM). Significant differences in typology were also observed in samples from different lagoons. Films were the main microplastic shape found in non-artificially recharged lagoons LAC[5] and LAB[6], whereas in those receiving wastewater discharges fibres were the dominant typology (Fig. 4.2B). The presence of fibres in wastewater, mainly originated in domestic wastewater machines has been reported elsewhere and is a tracer of anthropogenic pollution (Napper and Thompson, 2016). In GQR[3], fibres accounted for almost 50% of microplastics.

4.3.3. Discrimination among spectra from different sources: Lagoons, wet deposition and wastewater effluent.

445 FTIR spectra were used to feed five different models. 190 (76 microplastics) corresponded to samples spectra taken from different lagoons, 172 (77 microplastics)

from WWTP effluent, and 83 (35 microplastics) were microparticles recovered from wet deposition. Wet deposition plastics essentially corresponded to fragments (60%) and fibres (35%). The median value for the size of these plastics was 360 µm for length, 39 µm for width, and 133 µm for projected area diameter. The more abundant polymers obtained from wet deposition were polyester and acrylic fibres (19% and 4% respectively), while cotton-cellulose fibres accounted for 26% of the total number of items. Details concerning WWTP effluent are available elsewhere (Edo et al., 2020). Briefly, the set used for this study mainly consisted of fibres (49%) and fragments (43%). Among them, the more abundant were polyester fibres (17%) followed by PE (9%), PP (6%) and acrylic fibres (5%). Besides, 28% of the total number of items were identified as cotton-cellulose. The median size of these particles was 181 µm. Accordingly, the three sets were comparable both in size and composition. Some spectra considered as outliers were removed during pre-screening. For each model, several group-to-group comparisons were performed, and contribution plots were used to identify the bands associated with the main differences.

The spectra were statistically compared using OPLS-DA after baseline correction. This method uses multivariate PCA to represent potentially correlated variables with linearly uncorrelated principal components. Model 1 used all spectra taken from lagoons, WWTP and wet deposition and was split into two Model 1-C with samples from non-artificially recharged lagoons (CVF[4], LAC[5] and LAB[6]) and Model 1-R with spectra from lagoons receiving wastewater (ELG[1], LVC[2] and GQR[3]). Model 2 used only the microparticles positively identified as microplastics and also comprised two Models 2-C and 2- R with the same background as Model 1. Model 3 compared non-plastic materials and Models 4 and 5 compared specific microplastics in different sets (polyester and acrylic respectively). None of the models used any Y orthogonal component. Table 4.2 shows all details including the parameters R^2X, R^2Y and Q^2. In order to avoid model overfitting, the final number of components was based on the auto-fitting cross-validation setting as suggested by OPLS-DA software. The number of model components prioritised class discrimination of each dataset.

The results from all models suggested the presence of intrinsic properties in FTIR spectra that allowed the discrimination among the three data groups. In all the analyses

performed, the data points representing polymers from different origins grouped closely with certain overlapping between them. OPLS-DA analysis was quantitatively assessed using the explained variation (R^2) of each principal component. In this work, the most appropriated models resulted from to the use of all materials at once.

Table 4.2. Parameters obtained from the different models calculated with orthogonal partial least squares-discrimination analysis OPLS-DA.

Id	Model Name	Number of Spectra	Descriptive Components	Orthogonal Component (x)	R^2X	R^2Y	Q^2
1	All microparticles in all Lagoons, WWTP and wet depositions	428	2	9	0.80	0.67	0.50
1-C	All microparticles in Lagoons CVF[4], LAC[5] and LAB[6], WWTP and wet depositions	296	2	5	0.72	0.39	0.21
1-R	All microparticles in Lagoons ELG[4], LVC[2] and GQR[3], WWTP and wet depositions	378	2	10	0.82	0.70	0.54
2	MPs in all lagoons, WWTP and wet depositions	178	2	3	0.61	0.33	0.10
2-C	MPs in Lagoons CVF[4], LAC[5] and LAB[6], WWTP and wet depositions	131	2	5	0.72	0.53	0.20
2-R	MPs in Lagoons ELG[4], LVC[2] and GQR[3], WWTP and wet depositions	178	2	6	0.74	0.49	0.19
3	All non-plastics microparticles in all lagoons, WWTP and wet deposition	251	2	3	0.68	0.33	0.20
4	Polyester in all lagoons, WWTP and wet deposition	54	3	4	0.75	0.69	0.12
5	Acrylics in all lagoons, WWTP and wet deposition	19	3	1	0.74	0.63	0.06

The best model for diagnosing differences between groups was Model 1-R with both the best explanation (R^2 = 82%) and the highest predictability (Q^2 = 54%). In general, predictability was <20% in all cases except for Models 1 and 1-R. The removal of the non-plastic spectra reduced the explanation and predictability of the remaining models (2, 3, 4, and 5) meaning that the only groups exhibiting significant differences appeared when comparing microparticles in lagoons undergoing wastewater discharge with particles from WWTP effluent and wet deposition.

Visually, the spectra from lagoons slightly overlapped with the other two groups. Nonetheless, they appeared always closer to WWTP effluent than to wet deposition samples. Fig. 4.4 shows the S-Plot of the model 1-R for all the materials in lagoons ELG[1], LVC[2] and GQR[3]. S-plots for the rest of the models are given in Figs. S4.5 and S4.6 (SM).

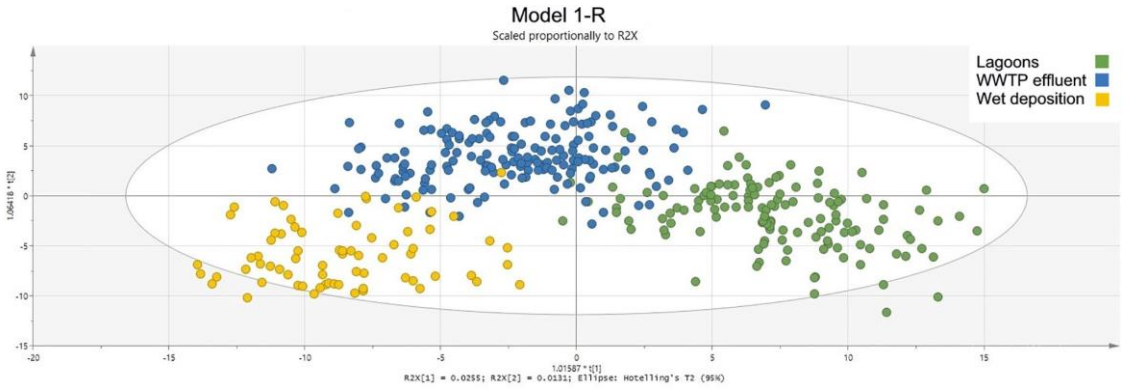


Figure 4.4. Scatter-Plot for model 1-R (details in Table 1) with data inside Hotelling's bubble (T2 test).

The differences between samples were highlighted by performing group comparisons from spectra with different sources, which allowed identifying the contributions of each group to the FTIR spectra. Fig. 4.5 shows the differences in spectral regions among samples from lagoons, WWTP effluent and wet deposition as obtained from the application of Models 1 (including all microparticles) and 2 (only microplastics).

In Model 1-C non-artificially recharged lagoons were differentiated by an intense carbonyl vibration (C=O), a C-O stretching, and C=C bending vibrations present in the ~1700 cm^{-1}, 1300-1100 and 900-700 cm^{-1} regions, respectively (Fleming and Williams, 2020). The materials in recharged lagoons (Model 1-R) displayed intense vibrations in the 3600-3000 cm^{-1} region. They would correspond to O-H, N-H, and aromatic C-H stretching and were found in lagoons at the same level as in WWTP effluent and wet deposition. FTIR in recharged lagoons showed vibrations in the 2500-2000 cm^{-1} intermediate region, which would correspond to vibrations in double and triple bonds that were no present in WWTP effluent or wet deposition samples. There were also differences in carbonyl region in samples from lagoons receiving wastewater discharge (R). The samples in lagoons showed an important absence of peaks close to ~1500 cm^{-1}, especially in recharged lagoons because of the difference with wet deposition and

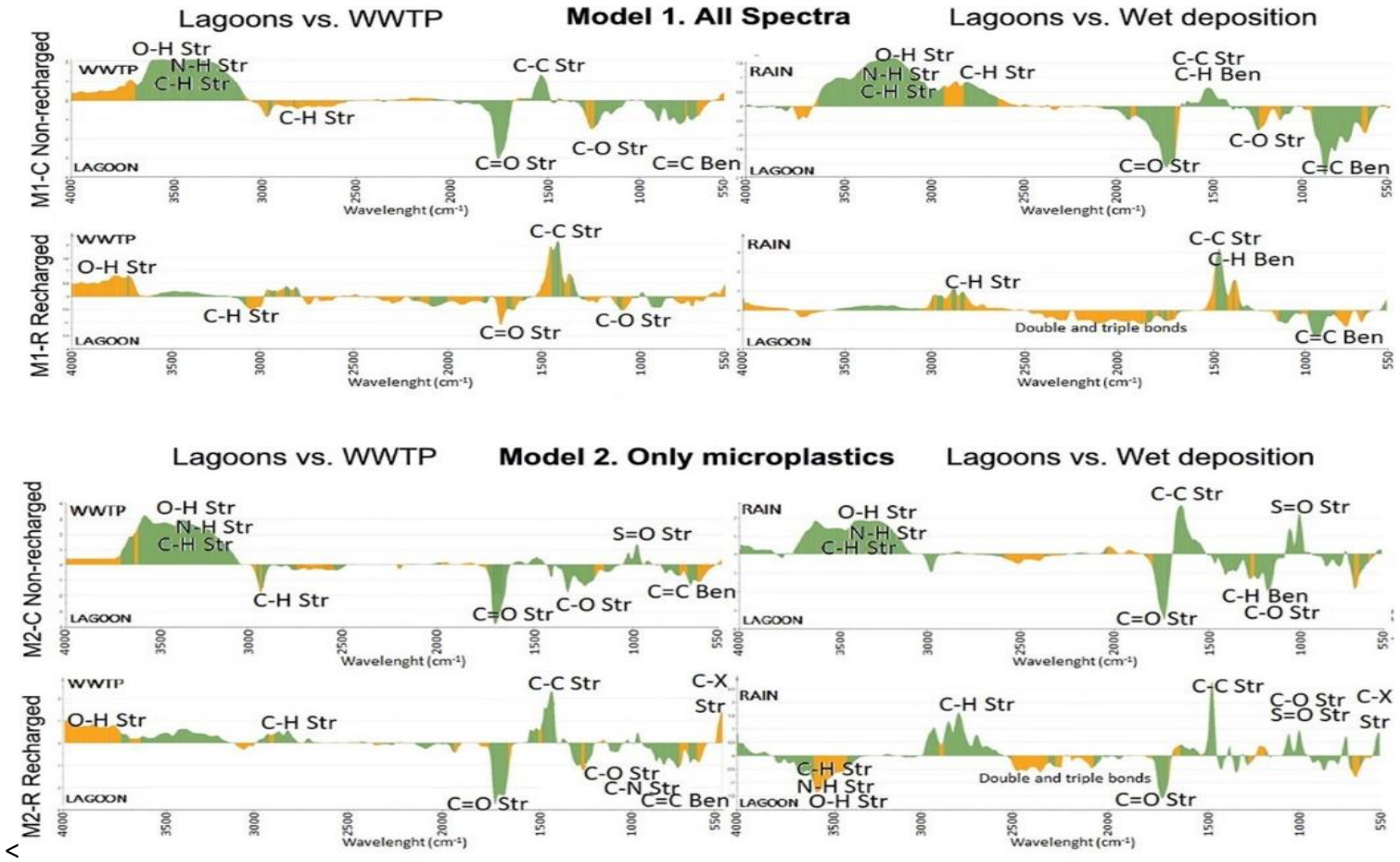


Figure 4.5. Contribution plot for comparing samples from different source. Inside each model the upper bands are non-artificially recharged lagoons (C) and the lower samples from recharged lagoons (R). Left panels: Lagoons vs. WWTP effluent; right panel Lagoons vs. Wet deposition. Str: Stretching vibration. Ben: Bending vibration. Orange represents variables outside three std. dev. range. All parameters are normalized (SNV).

WWTP samples. Samples from WWTP effluent and wet deposition showed bands in the 580-560 cm^{-1}, region that could be attributed to C-X bonds.

The results for Model 2 (Fig. 4.5, lower panels), that only considered microplastics, yielded similar results. Both in control and wastewater-receiving lagoons, differences in the stretching vibration of C=O (~1700 cm^{-1}) and C-O (~1160 cm^{-1}) were clearly identified. Control lagoons showed a lack of bands in the characteristic C-H, N-H, O-H vibration region (3600-3000 cm^{-1}) that were present in recharged lagoons. On the contrary, these vibrations were abundant when comparing the recharged lagoons against wet deposition samples. The aliphatic C-H chains close to 2900 cm-1 were present only in non-artificially recharged lagoons (Fleming and Williams, 2020). Other bonds absent in plastics from the lagoons were the bands at 1500 cm^{-1} or close to the 1000 cm^{-1} (possibly S=O or C-O stretching) that were present in particles from WWTP effluent and wet deposition. The band from halogenated carbons (~550 cm^{-1}) was also found in samples from WWTP effluent and wet deposition in contrast to lagoon samples.

4.4. Discussion

For a long time, the wetlands in La Mancha were threatened by the risk of disappearance. Until the middle of 20th century these natural spaces remained almost intact providing water for agriculture and shelter for different animals, especially birds. After the 50's, and due to the increasing demand for agricultural land, an important part of these wetlands was put in production under intensive agricultural schemes. Irrigated areas increased from historical 200-300 km^2 to 1300-1400 km^2 in the early nineties (Fornés et al., 2000). Most wetlands became eventually polluted with pesticides, industrial chemicals or untreated wastewater, and sometimes, even total desiccation happened because of aquifer overexploitation (Álvarez-Cobelas et al., 2010).

The area was declared Biosphere Reserve by UNESCO in 1981 in view of its high ecological value. Long after that, the ecological situation of many lagoons and aquifers is poor. Several protection figures and preservation plans showed limited success due to the disregard of the administrations involved. In this context, some wetlands, formerly temporary, became permanent due to the continuous supply of wastewater inflows from nearby WWTP.

This artificial recharge offered an apparent solution against the desiccation caused by the overexploitation of groundwater reservoirs. Consequently, recharged lagoons suffer from a continuous supply of pollutants, including microplastics that accumulate due to their endorheic character.

GQR[3] showed the highest concentration of microplastics with 24.4 ± 5.2 particles/g followed by LVC[2] with 14.2 ± 3.5 particles/g. Much lower levels were found in ELG[1], despite this lagoon receives a discharge of treated wastewater, and non-artificially recharged lagoons, in which the concentration of microplastics was <1 particle/g in CVF[4], LAC[5] and LAB[6]. Table 4.3. shows these values put in context with other data from literature.

Previous works have established the occurrence of microplastics in sediments of rivers, lakes and marine ecosystems. Scheurer and Bigalke reported concentrations up to 593 microparticles of plastic per kilogram of sediment in Swiss floodplain areas (Scheurer and Bigalke, 2018). Fuller and Gautam studied contaminated soils in Australia and found higher values that reached 67.5 g/kg (results in particle number not given) (Fuller and Gautam, 2016). Regarding wetlands, the study of Ziajahromi et al. revealed up to 595 microplastic particles/kg in an Australian constructed wetland arranged to treat storm runoff waters (Ziajahromi et al., 2020). Towsend et al. reported somewhat lower figures for sediments of a set of 20 urban wetlands near Melbourne, Australia (Townsend et al., 2019). River sediments were reported to contain different loads of microplastics with higher values in the thousands of particles per kilogram range (Klein et al., 2015; Peng et al., 2017; Zhang et al., 2020a).

Our results showed that endorheic lagoons receiving wastewater, even if treated according to current standards, may result in high concentration of microplastics in sediments, at least one order of magnitude higher that the highest values reported elsewhere. Assuming the usual values for the density of dry sediments, the extrapolation of our data to the microplastics per unit surface would yield values over 10^4 microplastics/m^2 (Verstraeten and Poesen, 2001).

Table 4.3. *Microplastics in sediments. Our data in the context of other author's findings.*

Sampling point	Size range	Concentration of microplastics	Reference
Laguna Grande (Quero, Spain) - GQR[3]	25 µm–5 mm	24.4 ± 5.2 particles/g	This work
Laguna Larga (Villacañas, Spain) - LVC[2]	25 µm–5 mm	14.2 ± 3.5 particles/g	This work
El Longar (Lillo, Spain) - ELG[1]	25 µm–5 mm	2.0 ± 0.8 particles/g	This work
Swiss foodplain soils (29 sites)	125 µm–5 mm	593 particles/kg 55.5 mg/kg	(Scheurer and Bigalke, 2018)
Soils from an industrial area in Australia (17 samples)	~30 µm–5 mm	300–67,500 mg/kg	(Fuller and Gautam, 2016)
Constructed wetland in Australia	N25 µm	595 ± 120 particles/kg (inlet) 320 ± 42 particles/kg (outlet)	(Ziajahromi et al., 2020)
Sediments from 20 urban wetlands in Australia	35 µm–1 mm	2–147 particles/kg (average 47 particles/kg)	(Townsend et al., 2019)
Urban section of Qin River, Guangxi, China	25 µm–5 mm	Up to 97 particles/kg	(Zhang et al., 2020a)
River shore sediments in the Rhine-Main area, Germany	63 µm–5 mm	21.8–932 mg/kg 228–3763 particles/kg	(Klein et al., 2015)
Sediments in Changjiang Estuary, China	46.8 µm–5		
mm particles/kg 2017)	20–340 (Peng et al.,		

It has to be considered that our samples were taken in points near the inlet of wastewater discharges in wastewater-receiving lagoons. It is reasonable to assume that non-flooded areas would have concentrations of microplastics closer to non-wastewater receiving lagoons. Also, it is conceivable that a large number of microplastics <25 μm may exist, which are generally outside the capacity of current identification techniques. Overall, we demonstrated that microplastic concentration may reach very high values. Higher than those reported before for any kind of sediment elsewhere. It is important to note that the concentration of pollutants in a given area is the balance between inflow and outflow and in this case, the lagoons are endorheic and do not discharge to any other stream or water body. On the contrary, microplastics accumulate in sediments and their concentration is expected to continuously increase with time.

The ecological risk of microplastics in sediments is difficult to assess. There are knowledge gaps that include a lack of standardized quantification methods and scattered data for the concentration of microplastics in most environmental compartments. Peng et al. found an average abundance of microplastics in river sediments of 802 particles/kg and suggested that their chemical composition may result in environmental risk associated to the presence of phenoxy resins, produced from bisphenol A and usually cured with isocyanates (Peng et al., 2018). Other studies suggest that microplastics act as a vector for other pollutants like metals (Akhbarizadeh et al., 2017). Recent studies indicate that long-term exposure to microplastics may impact sediment biota even at environmentally relevant concentrations by affecting sublethal endpoints such as energy reserves (Bour et al., 2018).

Our work demonstrated the presence of at least 11 different types of microplastics in the sediments of lagoons receiving wastewater discharges. The predominant polymers were those in most common use like PE and PP, which account for 90% of polymers in materials used in daily routine (PlasticsEurope, 2019). A minor fraction of the polymers identified in this work corresponded to those with higher density like PVC, PU or PVF that tend to sink and tend to appear in sediment samplings (Huang et al., 2020; Sun et al., 2019). Besides, many fibres were found, essentially polyester and acrylic fibres, which are typical residues from domestic washing machines (Napper and Thompson, 2016). The occurrence of fibres in wastewater has been reported elsewhere (Bayo et al.,

2019; Zambrano et al., 2019). An additional cause for concern is that natural fibres, like cotton or wool, when industrially processed, contain potentially harmful additives that may end up in the environment (Cesa et al., 2017). These chemicals include dyes, fire retardants, softening additives and many others and constitute a source of anthropogenic pollution somehow comparable to microplastics. Fragments, also usual in WWTP effluents, are common in the sediments from wastewater-fed lagoons and wetlands (Townsend et al., 2019; Zhang and Liu, 2018). The presence of relatively high amount of films in LVC [2], might be influenced by the green filter located immediately before sampling points. Laminated plastics are common in the construction of these filtering systems and their occurrence in downstream ecosystems has sometimes been reported (Ziajahromi et al., 2020). Microplastics found in non-artificially recharged lagoons were mostly dominated by films, probably materials generated elsewhere and transported by wind (Zhang et al., 2019).

In this work we use OPLS-DA to compare microparticles/microplastics from three different sources. The rationale was to assess the origin of the anthropogenic pollutants found in the sediments of recharged lagoons. Several studies highlighted the importance of atmospheric transport and wet or dry deposition in the spreading of anthropogenic materials to different environments (Klein and Fischer, 2019; Wright et al., 2019). Moreover, the presence of wastewater discharges in some lagoons would explain the similarities between collected microplastics with samples taken from other WWTP. The real situation is somewhat more complex due to the ageing of plastic materials deposited in natural environments during prolonged periods. Photolytic, photo-oxidative and thermo-oxidative reactions are responsible of accelerating polymer degradation and modify FTIR spectra with an increase in oxygenated moieties including those containing carbonyl, carboxyl of hydroxyl groups (Andrady, 2017; Prata et al., 2020). Another effect complicating the analysis is the colonization of debris materials by different organisms when disposed in a biotic medium for prolonged periods (Arias-Andres et al., 2018). The plastic fraction from lagoons receiving wastewater showed bands corresponding to O-H, N-H and C-H bonds similar to those found in wastewater samples and absent from control lagoons and wet deposition samples. Wet deposition samples differ from recharged lagoons in specific vibrations in the C-H region. Non-

artificially recharged lagoons clearly differed from the rest of samples because of the absence of vibrations in the 3600-3000 cm^{-1} region, which probably indicates a different origin for microplastics found in non-artificially recharged lagoons. Because of abundance and composition, the most probable origin of microplastics in lagoons receiving wastewater is wastewater discharge itself.

This work demonstrated that wastewater discharges in inland water bodies, particularly in endorheic lagoons, result in the accumulation of organic pollutants in high amounts. The occurrence of microplastics have never been reported in La Mancha wetlands, but the impact associated to wastewater recharge has been associated to changes in nutrient cycles due to the alteration of natural drying-flooding cycles (Corrales-González et al., 2019). Overall, our work showed that current wastewater treatment is not enough to avoid the accumulation of microplastic pollutants in the sensitive environment of endorheic lagoons. It is to be stressed that these lagoons do not discharge to other external bodies of water and, therefore, pollutants may accumulate in large amounts. The data available indicate the need to establish criteria concerning the quality of wastewater used to recharge lagoons and to decide if this is a sustainable practice compatible with preserving the natural status and biodiversity of protected areas.

4.5. Conclusions

This work studied the presence of microplastics in six lagoons from the extensive network of wetlands called “La Mancha Húmeda”, declared Biosphere Reserve by UNESCO. It was found that lagoons receiving wastewater effluents displayed very high concentration of microplastics in sediments with concentrations reaching the order of tens of microplastics (25 µm-5 mm) per gram.

In contrast to lagoons receiving wastewater, non-artificially recharged lagoons, that kept their natural drying and flooding cycle, showed much less microplastics, with films being the dominant shape. In lagoons receiving wastewater, fibres were the dominant typology, which can be attributed to domestic wastewater discharges.

Chemical analyses performed by micro-FTIR showed that the main materials in anthropogenic microlitter were the polyolefins polyethylene and polypropylene, and polyester and acrylic fibres. Up to 11 different polymer types were found in lagoons

receiving wastewater. Statistical analysis of FTIR spectra confirmed similarity with samples taken from WWTP effluent rather than from wet deposition samples.

Our results showed that wastewater recharge is not a suitable practice to maintain water levels in endorheic lagoons as it leads to the accumulation of microplastics in very high amounts. This is due to the closed or terminal character of endorheic basins.

4.6. References

Akhbarizadeh, R., Moore, F., Keshavarzi, B., Moeinpour, A., 2017. *Microplastics and potentially toxic elements in coastal sediments of Iran's main oil terminal (Khark Island*). Environ. Pollut. 220, 720–731.

Álvarez-Cobelas, M., Sánchez-Carrillo, S., Cirujano, S., Angeler, D.G., 2010. *A story of the wetland water quality deterioration: salinization, pollution, eutrophication and siltation*. In: Sánchez-Carrillo, S., Angeler, D. (Eds.), Ecology of Threatened Semi-Arid Wet- lands. Springer, New York.

Andrady, A.L., 2017. *The plastic in microplastics: a review*. Mar. Pollut. Bull. 119, 12–22.

Araujo, C.F., Nolasco, M.M., Ribeiro, A.M.P., Ribeiro-Claro, P.J.A., 2018. *Identification of microplastics using Raman spectroscopy: latest developments and future prospects*. Water Res. 142, 426–440.

Arias-Andres, M., Kettner, M.T., Miki, T., Grossart, H.-P., 2018. *Microplastics: new substrates for heterotrophic activity contribute to altering organic matter cycles in aquatic ecosystems*. Sci. Total Environ. 635, 1152–1159.

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

Bayo, J., Olmos, S., López-Castellanos, J., 2020. *Microplastics in an urban wastewater treatment plant: the influence of physicochemical parameters and environmental factors*. Chemosphere 238, 124593.

Beiras, R., Bellas, J., Cachot, J., Cormier, B., Cousin, X., Engwall, M., et al., 2018. *Ingestion and contact with polyethylene microplastics does not cause acute toxicity on marine zooplankton*. J. Hazard. Mater. 360, 452–460.

Blettler, M.C.M., Abrial, E., Khan, F.R., Sivri, N., Espinola, L.A., 2018. *Freshwater plastic pollution: recognizing research biases and identifying knowledge gaps*. Water Res. 143, 416–424.

Bour, A., Haarr, A., Keiter, S., Hylland, K., 2018. *Environmentally relevant microplastic exposure affects sediment-dwelling bivalves.* Environ. Pollut. 236, 652–660.

Bylesjö, M., Rantalainen, M., Cloarec, O., Nicholson, J.K., Holmes, E., Trygg, J., 2006. *OPLS discriminant analysis: combining the strengths of PLS-DA and SIMCA classification*. Journal of Chemometrics: A Journal of the Chemometrics Society 20, 341–351.

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

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

Cesa, F.S., Turra, A., Baruque-Ramos, J., 2017. *Synthetic fibres as microplastics in the marine environment: a review from textile perspective with a focus on domestic washings*. Sci. Total Environ. 598, 1116–1129.

Cirujano, S., Medina, L., 2014. *Plantas acuáticas de las lagunas y humedales de Castilla-La Mancha*. Editorial Liber Factory.

Corrales-González, M., Rochera, C., Picazo, A., Camacho, A., 2019. *Effect of wastewater management on phosphorus content and sedimentary fractionation in Mediterranean saline lakes.* Sci. Total Environ. 668, 350–361.

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

Edo, C., González-Pleiter, M., Leganés, F., Fernández-Piñas, F., Rosal, R., 2020. *Fate of microplastics in wastewater treatment plants and their environmental dispersion with effluent and sludge.* Environ. Pollut.259, 113837.

van Emmerik, T., Tramoy, R., van Calcar, C., Alligant, S., Treilles, R., Tassin, B., et al., 2019. *Seine plastic debris transport tenfolded during increased river discharge*. Front. Mar. Sci. 6.

Farady, S.E., 2019. *Microplastics as a new, ubiquitous pollutant: strategies to anticipate management and advise seafood consumers.* Mar. Policy 104, 103–107.

Fleming, I., Williams, D.H., 2020. *Spectroscopic Methods in Organic Chemistry*. Springer Int. Pub.

Fornés, J., Rodríguez, J.A., Hernández, N., Llamas, M.R., 2000. *Possible solutions to avoid conflicts between water resources development and wetland conservation in the "La Mancha Húmeda" biosphere reserve (Spain).* Physics and Chemistry of the Earth, Part B: Hydrology, Oceans and Atmosphere 25, 623–627.

Fuller, S., Gautam, A., 2016. *A procedure for measuring microplastics using pressurized fluid extraction.* Environmental Science & Technology 50, 5774–5780.

Gago, J., Galgani, F., Maes, T., Thompson, R.C., 2016. *Microplastics in seawater: recommendations from the marine strategy framework directive implementation process*. Front. Mar. Sci. 3.

Gigault, J., Halle, At, Baudrimont, M., Pascal, P.-Y., Gauffre, F., Phi, T.-L., et al., 2018. *Current opinion: what is a nanoplastic?* Environ. Pollut. 235, 1030–1034.

Godoy, V., Martín-Lara, M.A., Calero, M., Blázquez, G., 2019. *Physical-chemical characterization of microplastics present in some exfoliating products from Spain.* Mar. Pollut. Bull. 139, 91–99.

González-Pleiter, M., Tamayo-Belda, M., Pulido-Reyes, G., Amariei, G., Leganés, F., Rosal, R., et al., 2019. *Secondary nanoplastics released from a biodegradable microplastic severely impact freshwater environments.* Environmental Science: Nano 6, 1382–1392.

González-Pleiter, M., Velázquez, D., Edo, C., Carretero, O., Gago, J., Barón-Sola, Á., et al., 2020. *Fibres spreading worldwide: microplastics and other anthropogenic litter in an Arctic freshwater lake*. Sci. Total Environ. 722, 137904. https://doi.org/10.1016/j. scitotenv.2020.137904.

Gorito, A.M., Ribeiro, A.R., Almeida, C.M.R., Silva, A.M.T., 2017. *A review on the application of constructed wetlands for the removal of priority substances and contaminants of emerging concern listed in recently launched EU legislation*. Environ. Pollut. 227, 428–443.

Guo, J.-J., Huang, X.-P., Xiang, L., Wang, Y.-Z., Li, Y.-W., Li, H., et al., 2020. *Source, migration and toxicology of microplastics in soil.* Environ. Int. 137, 105263.

Helcoski, R., Yonkos, L.T., Sanchez, A., Baldwin, A.H., 2020. *Wetland soil microplastics are negatively related to vegetation cover and stem density.* Environ. Pollut. 256, 113391.

Huang, J.-S., Koongolla, J.B., Li, H.-X., Lin, L., Pan, Y.-F., Liu, S., et al., 2020. *Microplastic accumulation in fish from Zhanjiang mangrove wetland, South China.* Sci. Total Environ. 708, 134839.

Jaikumar, G., Brun, N.R., Vijver, M.G., Bosker, T., 2019. *Reproductive toxicity of primary and secondary microplastics to three cladocerans during chronic exposure*. Environ. Pollut. 249, 638–646.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., et al., 2015. *Plastic waste inputs from land into the ocean.* Science 347, 768–771.

Kedzierski, M., Villain, J., Falcou-Préfol, M., Kerros, M.E., Henry, M., Pedrotti, M.L., et al., 2019. *Microplastics in Mediterranean Sea: a protocol to robustly assess contamination characteristics.* PLoS One 14, e0212088.

Klein, M., Fischer, E.K., 2019. *Microplastic abundance in atmospheric deposition within the Metropolitan area of Hamburg,* Germany. Sci. Total Environ. 685, 96–103.

Klein, S., Worch, E., Knepper, T.P., 2015. *Occurrence and spatial distribution of microplastics in river shore sediments of the Rhine-Main area in Germany*. Environmental Science & Technology 49, 6070–6076.

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

Lee, W.S., Cho, H.-J., Kim, E., Huh, Y.H., Kim, H.-J., Kim, B., et al., 2019. *Bioaccumulation of polystyrene nanoplastics and their effect on the toxicity of Au ions in zebrafish embryos*. Nanoscale 11, 3173–3185.

Liu, K., Wang, X., Wei, N., Song, Z., Li, D., 2019. *Accurate quantification and transport estimation of suspended atmospheric microplastics in megacities: implications for human health*. Environ. Int. 132, 105127.

Masura, J., Baker, J., Foster, G., Arthur, C., 2015. *Laboratory methods for the analysis of microplastics in the marine environment: recommendations for quantifying syn- thetic particles in waters and sediments*. NOAA Technical Memorandum NOS- OR&R-48. Silver Spring.

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

Peeken, I., Primpke, S., Beyer, B., Gütermann, J., Katlein, C., Krumpen, T., et al., 2018. *Arctic sea ice is an important temporal sink and means of transport for microplastic*. Nat. Commun. 9, 1505.

Peng, G., Zhu, B., Yang, D., Su, L., Shi, H., Li, D., 2017. *Microplastics in sediments of the Changjiang Estuary, China*. Environ. Pollut. 225, 283–290.

Peng, G., Xu, P., Zhu, B., Bai, M., Li, D., 2018. *Microplastics in freshwater river sediments in Shanghai, China: a case study of risk assessment in mega-cities*. Environ. Pollut. 234, 448–456.

PlasticsEurope, 2019. *Plastics – The Facts 2019: An Analysis of European Plastics Production, Demand and Waste Data*. PlasticsEurope: Association of Plastics Manufacturers, Brussels.

Prata, J.C., Reis, V., Paço, A., Martins, P., Cruz, A., da Costa, J.P., et al., 2020. *Effects of spatial and seasonal factors on the characteristics and carbonyl index of (micro)plastics in a sandy beach in Aveiro, Portugal*. Sci. Total Environ. 709, 135892.

Scheurer, M., Bigalke, M., 2018. *Microplastics in Swiss floodplain soils.* Environmental Science & Technology 52, 3591–3598.

Silva, A.F.T., Sarraguça, M.C., Ribeiro, P.R., Santos, A.O., De Beer, T., Lopes, J.A., 2017. *Statistical process control of cocrystallization processes: a comparison between OPLS and PLS*. Int. J. Pharm. 520, 29–38.

Smith, M., Love, D.C., Rochman, C.M., Neff, R.A., 2018. *Microplastics in seafood and the implications for human health.* Current Environmental Health Reports 5, 375–386.

de Souza, A.A., Kloas, W., Zarfl, C., Hempel, S., Rillig, M.C., 2018. *Microplastics as an emerging threat to terrestrial ecosystems.* Glob. Chang. Biol. 24, 1405–1416.

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

Tournebize, J., Chaumont, C., Mander, Ü., 2017. *Implications for constructed wetlands to mitigate nitrate and pesticide pollution in agricultural drained watersheds*. Ecol. Eng. 103, 415–425.

Townsend, K.R., Lu, H.-C., Sharley, D.J., Pettigrove, V., 2019. *Associations between microplastic pollution and land use in urban wetland sediments*. Environ. Sci. Pollut. Res. 26, 22551–22561.

Verstraeten, G., Poesen, J., 2001. *Variability of dry sediment bulk density between and within retention ponds and its impact on the calculation of sediment yields*. Earth Surf. Process. Landf. 26, 375–394.

Wang, F., Wong, C.S., Chen, D., Lu, X., Wang, F., Zeng, E.Y., 2018. *Interaction of toxic chemicals with microplastics: a critical review*. Water Res. 139, 208–219.

World Economic Forum, 2016. *The New Plastics Economy — rethinking the future of plastics*. World Economic Forum EMFaMC.

Wright, S.L., Ulke, J., Font, A., Chan, K.L.A., Kelly, F.J., 2019. *Atmospheric microplastic deposition in an urban environment and an evaluation of transport*. Environ. Int. 105411.

Wu, H., Zhang, J., Ngo, H.H., Guo, W., Hu, Z., Liang, S., et al., 2015. *A review on the sustainability of constructed wetlands for wastewater treatment: design and operation.* Bioresour. Technol. 175, 594–601.

Zambrano, M.C., Pawlak, J.J., Daystar, J., Ankeny, M., Cheng, J.J., Venditti, R.A., 2019. *Microfibres generated from the laundering of cotton, rayon and polyester based fabrics and their aquatic biodegradation*. Mar. Pollut. Bull. 142, 394–407.

Zhang, G.S., Liu, Y.F., 2018. *The distribution of microplastics in soil aggregate fractions in southwestern China.* Sci. Total Environ. 642, 12–20.

Zhang, L., Liu, J., Xie, Y., Zhong, S., Yang, B., Lu, D., et al., 2020a. *Distribution of microplastics in surface water and sediments of Qin river in Beibu Gulf, China*. Sci. Total Environ. 708, 135176.

Zhang, W., Liu, Z., Tang, S., Li, D., Jiang, Q., Zhang, T., 2020b. *Transcriptional response provides insights into the effect of chronic polystyrene nanoplastic exposure on Daphnia pulex.* Chemosphere 238, 124563.

Zhang, Y., Gao, T., Kang, S., Sillanpää, M., 2019. *Importance of atmospheric transport for microplastics deposited in remote areas*. Environ. Pollut. 254, 112953.

Zhang, Y., Kang, S., Allen, S., Allen, D., Gao, T., Sillanpää, M., 2020c. *Atmospheric microplastics: a review on current status and perspectives.* Earth Sci. Rev., 103118

Ziajahromi, S., Drapper, D., Hornbuckle, A., Rintoul, L., Leusch, F.D.L., 2020. *Microplastic pollution in a stormwater floating treatment wetland: detection of tyre particles in sediment*. Sci. Total Environ. 713, 136356.

4.7. Supplementary material of Chapter 4

Contents

Table S4.1. List of annual authorised discharges to ELG[1] and LVC[2]. Source: Ministry of Agriculture, Food and Environment of Spain.

Location	Origin	Max Vol (m^3)	UTM	Coordinate X	Coordinate Y	Receiving Medium	Inhabitants	Lagoon	River
Lillo	Lillo WWTP	207685	30T	473649	4395945	Cat II	2000-9999 IE	ELG[1]	
Villacañas	Villacañas WWTP	929962	30T	474547	4380114	Cat I	10000 IE	LVC[2]	Riánsares
			30T	472737	4382619				

Cat I. Drinking water production, places destined to recreative activities, suitable for salmonids, special protection zones, protected areas, sensitive zones and groundwaters

Cat II. Suitable for cyprinids, suitable for molluscs farming and public use for certain recreative activities

Table S4.2. Water inputs, precipitations and use of soil in sampled lagoons.

ID	Water inputs	Average annual precipitation (mm)	Land use in surrounding areas
ELG[1]	Precipitation, runoff and wastewater	360	Pastures, dry farming, woody crops, arable crops, and urban green zones
LVC[2]	Precipitation, runoff and wastewater	500	Bare soils, woody crops, leaf forest, arable crops, and pastures
GQR[3]	Precipitation, runoff and wastewater	393	Arable crops, pastures, salt mines, and other crops
CVF[4]	Precipitation, runoff, Cigüela River and aquifer 20	390	Pastures, arable crops, vineyards, conifers, and other crops
LAC[5]	Precipitation and runoff	393	Dry farming, olive groves, combined crops, and bare soils
LAB[6]	Precipitation and runoff	360	Pastures, arable crops, woody crops, and vineyards

Table S4.3. Additional information concerning micro-FTIR analyses.

Sampling Point	Sampled microparticles	Analysed	MPs	Anthropogenic*	Other non-plastic microparticles	MPs (%)	Different polymer types
ELG[1]	139	31	9	3	19	29.0	4
LVC[2]	433	53	26	5	22	49.1	8
GQR[3]	544	52	35	3	14	67.3	8
CVF[4]	44	17	-	1	16	-	-
LAC[5]	48	16	4	5	7	25.0	2
LAB[6]	45	21	2	-	19	9.5	2

* Particles of natural materials like wool or cellulose with evidence of industrial origin

Figure S4.1. Photographs of sampling points and visual evidences of anthropogenic pollution.

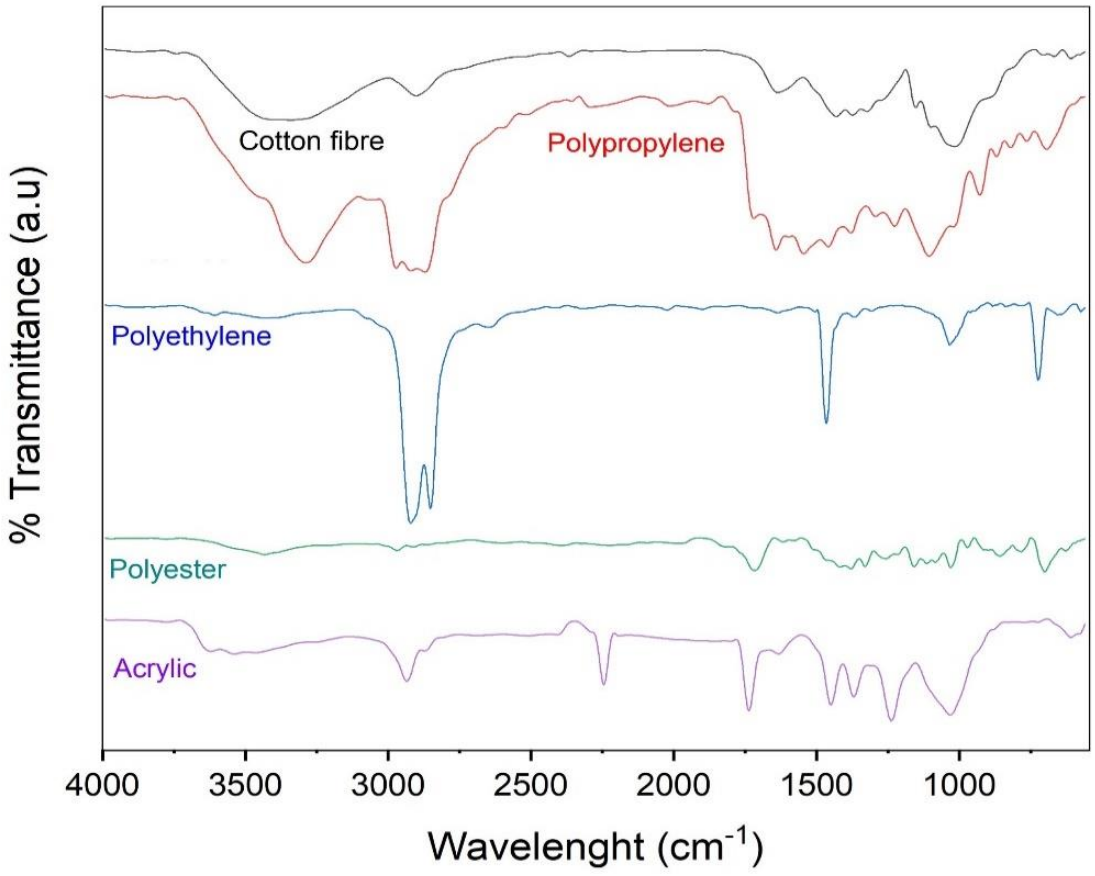


Figure S4.2. Examples of micro-FTIR spectra for the more abundant polymers in sediment samples.

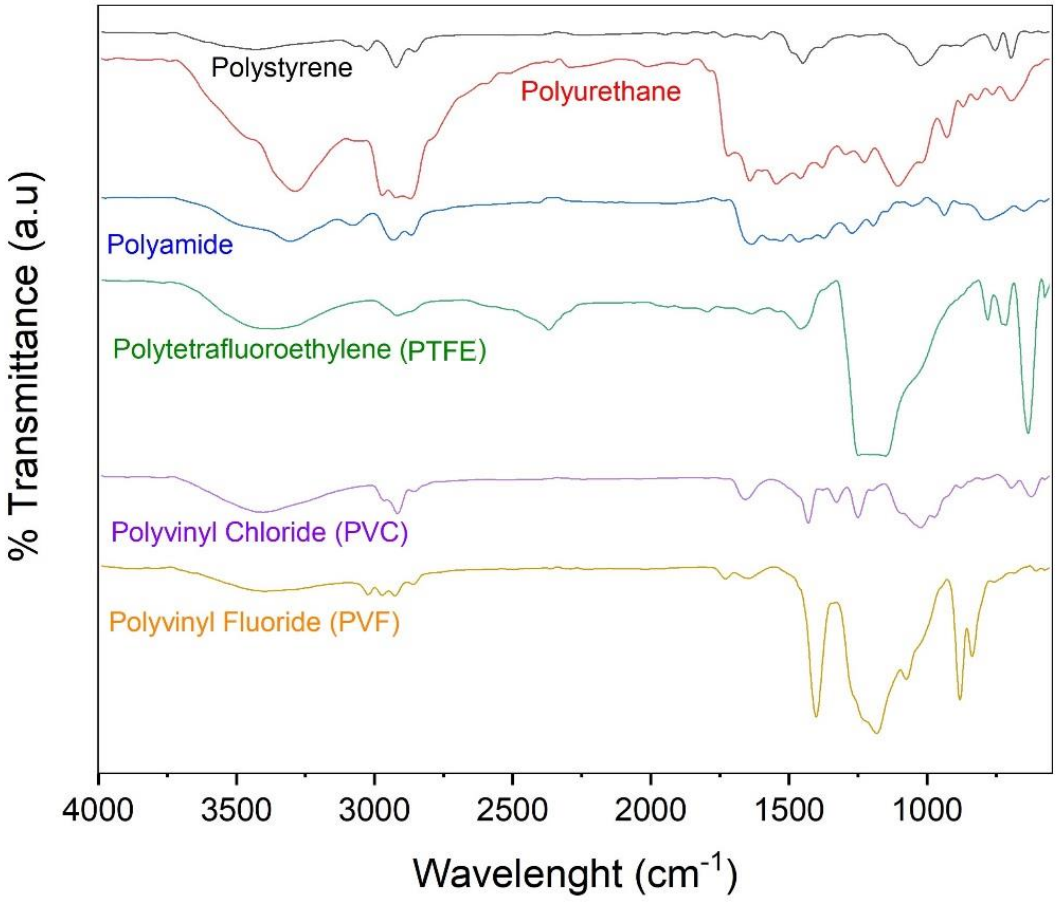


Figure S4.3. Examples of micro-FTIR spectra for the less abundant polymers in sediment samples.

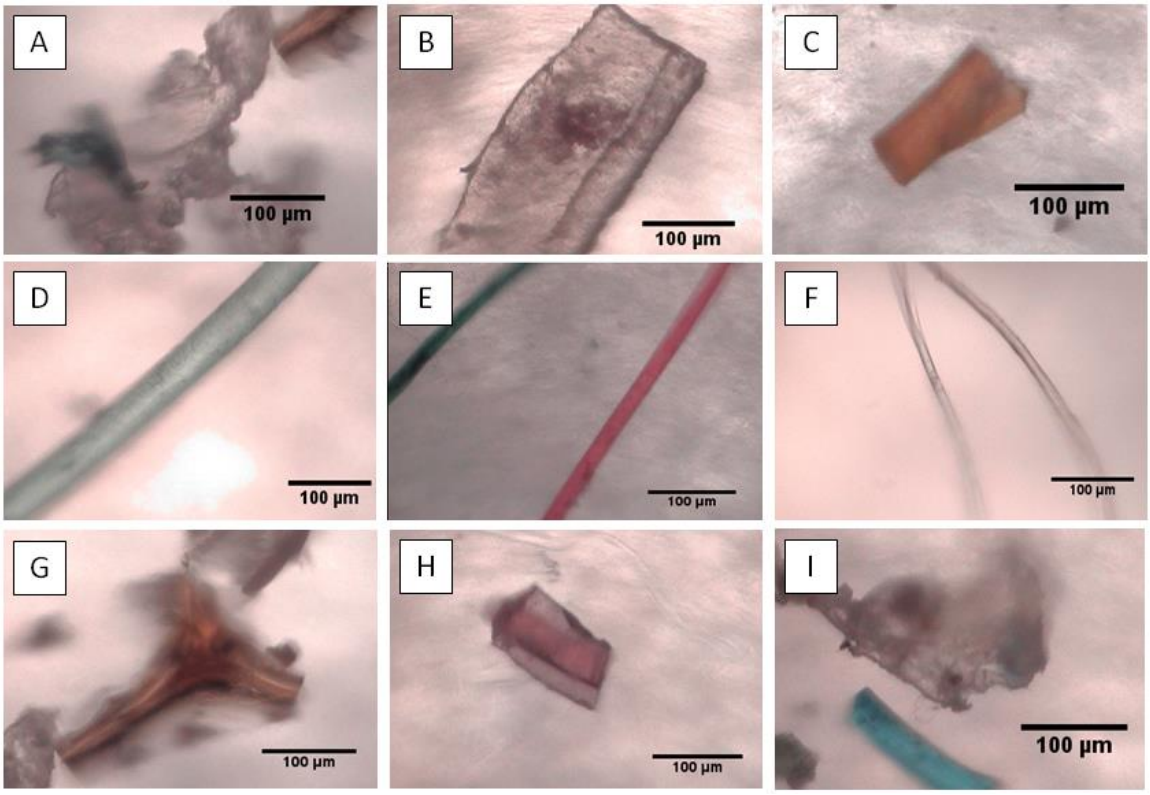


Figure S4.4. Microplastic particles. A-PFTE film (LVC[2]), B-PE fragment (ELG[1]), C-PP fragment (GQR[3]), D-PP filament (LVC[2]), E-Acrylic fibres (GQR[3]), F-Polyester fibres (GQR[3]), G-PU fragment (LVC[2]), H-PP fragment (GQR[3]) and I-PS film and PP filament (GQR[3]).

Figure S4.5. Score scatter plots of OPLS-DA developed Models 1 (1, 1-C and 1-R) and 2 (2, 2-C and 2-R) as indicated in Table 1.

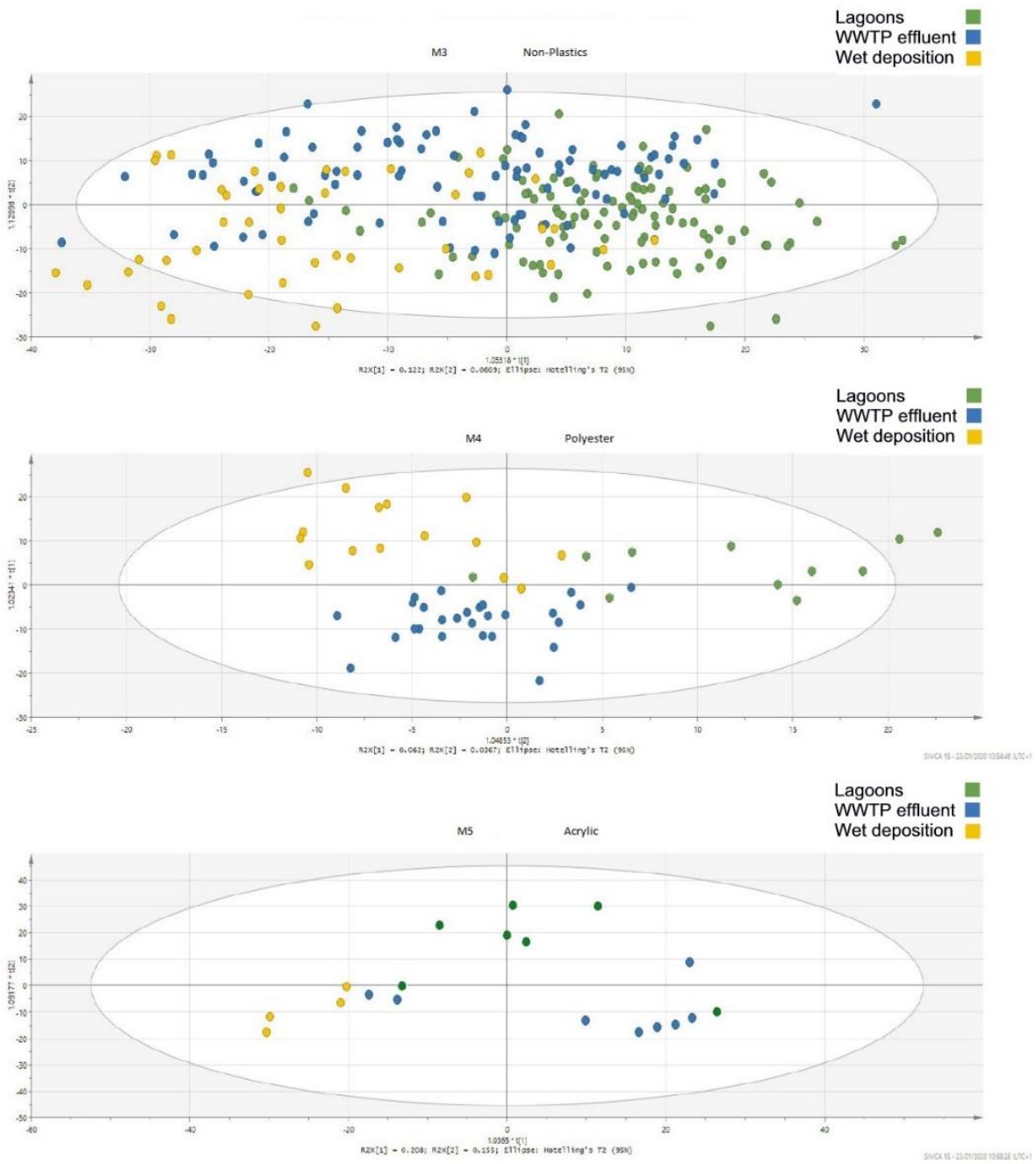


Figure S4.6. Score scatter plots of OPLS-DA developed Models 3, 4 and 5 as indicated in Table 1.

CHAPTER 5. OCCURRENCE AND TRANSPORT OF MICROPLASTICS SAMPLED WITHIN AND ABOVE THE PLANETARY BOUNDARY LAYER

5.1. Introduction

The uncontrolled release of plastics to the environment is a cause for global concern. Plastic pollution is an obvious consequence of the unproper management of plastic wastes, but it is also produced by the incidental abrasion and wearing of different goods (Bomgardner, 2017; Karbalaei et al., 2018; Knight et al., 2020). As a legacy from the marine origin of this research field, the term microplastic (MPs) refers to microparticles made of a polymeric matrix with their largest dimension ranging from 1 µm to 5 mm (Frias and Nash, 2019; GESAMP, 2019). MPs can easily move among ecosystems and cause hazardous effects to many organisms including humans due to their small size and persistence (Sharma and Chatterjee, 2017). The harmful effect of MPs strongly depends on their size. While larger particles may cause physical impacts like internal abrasions and blockages, smaller particles may translocate to internal tissues potentially accumulating in the food webs (Chang et al., 2020; Wang et al., 2021). Besides, chemical associated to plastics, like additives included during manufacture, non-intentionally added substances, or pollutants retained from the environment, are an additional cause for concern due to the possible damage to the environment or human health (Fred-Ahmadu et al., 2020).

The fate of MPs depends on the interconnection of the environmental compartments. From all the environmental compartments, the atmosphere is the least studied regarding the occurrence and spatial distribution of MPs. It has been suggested that atmospheric transport may play a significant role in the spreading of plastic pollution worldwide (Allen et al., 2019; Ganguly and Ariya, 2019; Zhang et al., 2019). Specifically, atmospheric transport would be responsible for the findings of MPs in areas far away from the main sources of pollution (Bergmann et al., 2019; Free et al., 2014; González-Pleiter et al., 2020b). Until now, the presence of MPs in the atmosphere has only been demonstrated through indirect deposition studies or sampling at ground or near to ground level (Dris et al., 2016; Klein and Fischer, 2019; Stanton et al., 2019). The highest concentrations have been reported in urban areas with concentrations generally in the order of a few MPs m^{-3} (Abbasi et al., 2019; Cai et al., 2017; Dris et al., 2017; Dris et al., 2015; Dris et al., 2016; Kaya et al., 2018; Klein and Fischer, 2019; Liu et al., 2019a; Liu et al., 2019c; Zhou et al., 2017). The size of airborne MPs varies from a few microns to the

millimetre range with median values in the hundreds of microns range. Micro-Raman (µRaman) and micro-Fourier Transform Infrared Spectroscopy (µFTIR) have been used to identify the atmospheric MPs with the finding of more than a dozen different polymers (Cai et al., 2017; Dris et al., 2016; Liu et al., 2019b). Airborne anthropogenic material not also includes MPs, but other artificial substances like extruded cellulose and many natural microparticles that underwent industrial processing such as industrially processed cotton or wool and that may result in similar environmental concerns (Stanton et al., 2019).

The sources and fate of atmospheric MPs remain poorly understood. Despite their many potential origins, no clear evidence has been reported to date. As a new area of atmospheric science, the available data are still limited in the field. Specifically, the way MPs become dispersed and transported into the atmosphere and the factors influencing their deposition have not been fully clarified yet. It has been suggested that films and fragments are probably derived from the disintegration of larger plastic goods like plastic bags and packaging materials, among other probable origins like building materials, industrial emissions, agriculture and particles released from waste incineration and emissions from the wear and tear of car tires (Kole et al., 2017; Liu et al., 2019b; Wright et al., 2020). Airborne MPs are usually dominated by fibres that can be attributed to the wearing of textiles, either natural or man-made. Finally, it should be noted that little is known about the movement of MPs in the atmosphere and the extent to which MPs can be transported with atmospheric air masses. The data available on-air mass trajectory analysis combined with atmospheric deposition studies, suggest that the fate and dispersion of airborne MPs strongly depend on atmospheric conditions such as wind speed and direction, the occurrence of precipitations and particle size (Chen et al., 2019; Enyoh et al., 2019; Gasperi et al., 2018).

So far, the occurrence of MPs in the atmosphere has been studied at ground level or a few metres above ground level. Our hypothesis is that MPs are present at high altitude, even above the Planetary Boundary Layer (PBL). MPs are released mainly from urban areas, where they reach higher concentration, get to the atmosphere and are eventually transported by winds long distances before being deposited. Here, we investigated the occurrence, spatial distribution, shape, and chemical composition of MPs directly

sampled in aircrafts flying up to ~3500 m above the sea level (a.s.l.) or ~2800 m above ground level (a.g.l.) over a high-density urban area (Madrid, Spain), a low-density urban area (Guadalajara, Spain), and rural and sub-rural areas in Central Spain. Furthermore, simulations were performed using the HYbrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) model in order to evaluate the atmospheric transport and deposition of MPs. This study provided the first direct evidence of the occurrence of MPs at high altitude in the atmosphere and showed that the atmosphere is an important compartment for the environmental distribution of MPs.

5.2. Materials and methods

5.2.1. Sample collection

Samples were obtained during three different flights of a CASA C-212 turboprop-powered cargo aircraft from the Spanish National Institute of Aerospace Technology in according to previous work. Incoming air was filtered using 25-µm size opening stainless steel meshes fitted into Whatman™ filter holders, which were directly connected to air intake openings as shown in Fig. 5.1. Air sampling lines were located at the leading edge of the airplane (Fig. 5.1A) and into the air intake on both sides (Fig. 5.1B) of the engines in a way that potential collection of debris produced by the engines, propeller, spinner and aircraft fairing was avoided. This procedure allowed collecting microparticles with an equivalent diameter down to 9.8 µm. Microparticles consisted of natural and artificial materials as well as synthetic polymers or MPs. Fig. S5.1 (Supplementary Material, SM) includes additional explanation on the nomenclature used in this work. Total airflow through the filters was measured using flowmeter 393 Series Float Style Rotameter (SKC, USA). Air output was measured from the filter directly using the flowmeter between three to six times during sampling.

The pressure drop between both parts of the filters was calculated using the conditions of the air outside the aircraft and yielded values in the 3.1-3.7 kPa range. This means that a certain mass of almost stagnant air existed inside the filtration line and, therefore, turbulence should not affect the filtration procedure.

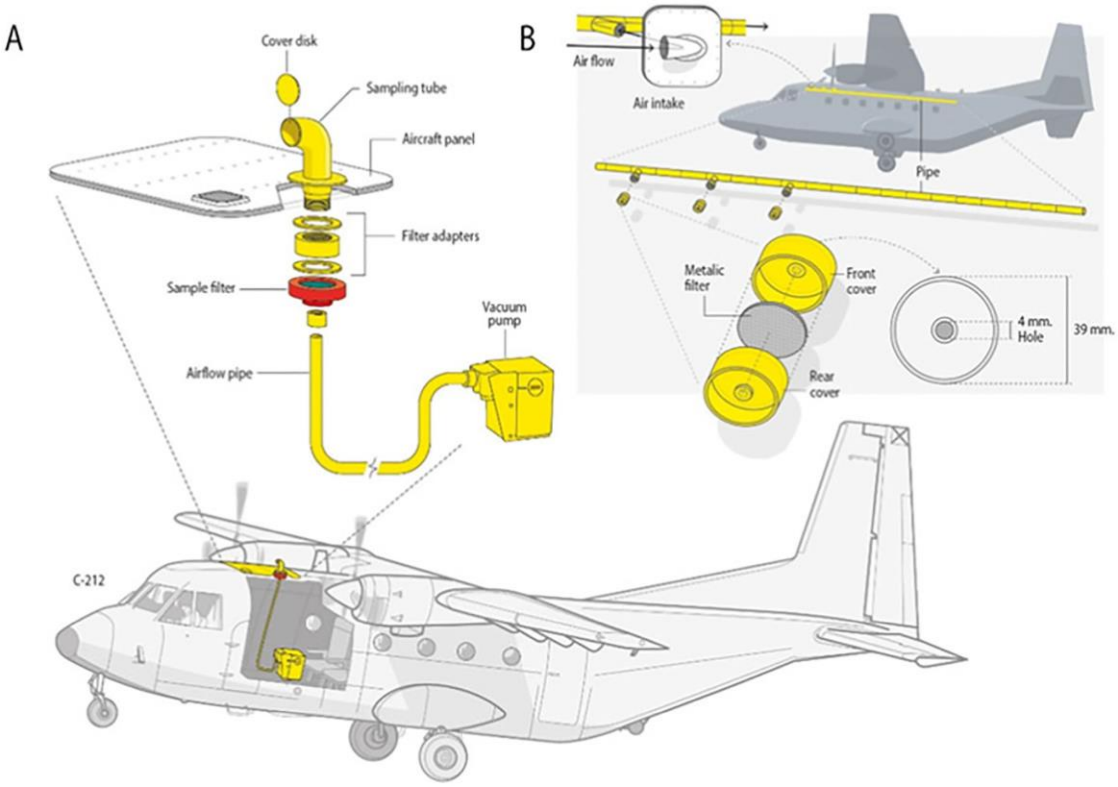


Figure 5.1. Aircraft scheme showing the location of air sampling lines. A: stainless steel filter holder inserted into the air intake, which opens at the leading edge of the airplane. B: filter holders inserted into the air intake on both sides of the aircraft.

5.2.2. Study area

The sampling campaigns consisted of three daytime flights that took place in the morning, approximately from 9 AM to 1 PM. Aircraft trajectories and sampling points were recorded for each flight and are shown in Fig. S5.2 (SM). In all flights, the aircraft took off from Torrejón Military Base. Flight 1 essentially flew all the time over rural areas. Flight 2 collected samples over the cities of Alcalá de Henares and Guadalajara and from Guadalajara to Valladolid flying over both rural and sub-urban areas. Flight 3 flew over Central Madrid (a high population density area: 5266 inhabitants km^{-2}) and Guadalajara (low-density area: 357 inhabitants km^{-2}). It is important to note that the flight over Central Madrid was particularly complex due its highly restricted airspace. The total volume of air filtered was 8780 L taken between 701 m a.s.l. (the minimum altitude recorded on a flight) and 3496 m a.s.l. (the maximum altitude recorded on a flight; see additional details in Table S5.1,(Supplementary material, SM). In general, the average altitude of the flights was above planetary boundary layer (PBL), which ranges

from 1.7 ± 0.5 km a.s.l. for the south of Spain (Granados-Muñoz et al., 2012) and between 0.5 and 1.5 km a.s.l. for the north of Spain (Banks et al., 2015). Thus, it can be considered that most of the microparticles were sampled above the PBL.

5.2.3. Quantification and identification of microparticles

Microparticles were defined as particles smaller than 5 mm along their largest dimension. Collected microparticles were measured and classified into fibres (microparticles with length/width ratio > 4) or fragments (microparticles with length/width ratio < 4) using a stereomicroscope Euromex-Edublue equipped with USB digital camera and ImageFocus 4 software. To avoid contamination, image acquisition was directly performed on the 25-µm stainless steel filters placed into their closed Petri dishes. A randomly distributed subsample of microparticles that included fibres and fragments of each filter in each area was selected for chemical identification (details are given in Table S5.1, (SM). In total, one third of all the microparticles collected were analysed by µFTIR using a Perkin-Elmer Spotlight 200 Spectrum Two apparatus with mercury cadmium telluride detector, which allowed high sensitivity measurements in the mid-infrared region. Microparticles were placed on a KBr matrix, which was used as a slide. The measuring parameters for the micro-transmission mode were spot 50 µm, 64 scans, resolution 8 cm^{-1}, spectral range 4000-550 cm^{-1}. The microparticles identified by µFTIR were larger than 10 µm. It has to be considered that 10 µm (at 1000 cm^{-1}) is the diffraction limit of IR spectroscopy, beyond which it is very difficult to obtain clear spectra (Primpke et al., 2017). The spectra were compared with a built-in database or with reference spectra specifically created for this study. A 65% matching was considered enough for positive identification according to the previous studies (Liu et al., 2019b). In specific cases, particularly for distinguishing between polyamides and wool/silk, a case-by-case study was undertaken.

Microparticles were classified in four classes based on their chemical nature: MPs, natural (natural fragments and natural fibres, such as cellulose, wool, cotton and linen with natural colours typical of each polymer such as white or grey), artificial (fibres of extruded cellulose, or natural fibres with non-natural colours or with evidence of anthropogenic processing), and unclassified (microparticles were labelled as

unclassified due to their low matching with standard spectra <65%). The concentration of each microparticle class in the atmosphere was calculated based on the proportion of microparticles identified in the subsample and the total flow through the steel meshes as determined from air flowmeters.

5.2.4. Prevention of procedural contamination.

To avoid sample contamination several measures were taken. All metal, steel and glass material were carefully cleaned with Milli-Q water, wrapped with aluminium foil and heated to 300 °C for 4 h. This procedure removed all possible rests of possibly interfering fibres and other organic substances from glassware and steel filters. The use of any plastic material was avoided. To account for possible contamination during sample collection, procedural blanks (25 µm steel meshes exposed to same experimental conditions except air filtration during the flights) and control blanks (Petri dishes with 25 µm steel meshes, which were kept open during sampling inside the aircraft to identify possible contamination from indoor air) were carried out. Possible contamination during quantification and identification of the samples was assessed by procedural blanks (opening Petri dishes with 25 µm steel meshes) to evaluate the possible contamination from the surrounding environment. All procedural blanks and controls were used during quantification and identification. Microparticles similar in chemical composition to those found in samples were subtracted from the total counting. Clothing was controlled throughout the whole process. During laboratory manipulation, the clothing of people manipulating samples was controlled by using non-typical bright colours like yellow, orange or purple, 100% cotton in all cases and with the provision that such colours would be excluded from the total counting if found. Further details are provided elsewhere (González-Pleiter et al., 2020a).

5.2.5. Model for atmospheric transport and deposition of microplastics

Atmospheric transport and deposition simulations were performed considering an initial unitary release at the median altitude of flight above Madrid. The simulations were performed using the HYbrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) model (Draxler and Rolph, 2010; Stein et al., 2015), developed by the National Oceanic and Atmospheric Administration (NOAA) Air Resources Laboratory (Rolph et al., 2017).

This model is widely used by the atmospheric sciences community for determining atmospheric transport and dispersion of pollutants including MPs (Allen et al., 2019; Aneja et al., 2006; Kallos et al., 2007; Reche et al., 2018). The Global Data Assimilation System (GDAS) meteorological data were used to feed HYSPLIT model mimicking the samples acquired during the flight. For simulations, the equivalent diameter of MPs (fibres and fragments) found above Madrid were calculated and deposition was parameterized (further details in Table S5.2 and supplementary section 1, SM).

5.3. Results

5.3.1. Occurrence and characterization of microparticles

A total set of 323 microparticles was found in the samples taken during the flights over high-density urban, low-density urban, sub-rural and rural areas of Central Spain (Table S5.1, SM). According to shape, microparticles were primarily classified into fragments and fibres. For the sake of clarity, a classification of the terms used in this work is given in Fig. S5.1 (SM). The dominant shape of microparticles found above sub-rural and rural areas were fibres, which represented up to 84% of the microparticles, while in flights over urban areas, fragments represented up to 67% of the microparticles. Equivalent diameters were calculated from recorded micrographs. For fragments, projected area diameter was used, while for fibres the equivalent diameter was defined as the aerodynamic diameter as calculated from the Harris-Fraser equation that describes fibre volume in terms of prolate spheroids (Gonda and Abd El Khalik, 1985) (see details in Table S5.2, SM). The majority of collected microparticles (59.6%) had equivalent diameters in the 10–70 µm range (Fig. 5.2). Some microparticles with equivalent diameter smaller than mesh opening size (25 µm) were collected, most probably because of their aspect ratio and orientation. Fibres ranged from 84 to 1709 µm length (average 662 µm, median 675 µm) and 4-97 µm width (average 25.4 µm, median 20 µm). Fragments ranged from 42 to 815 µm length (average of 204 µm, median 142 µm) and in 17-408 µm width (average 103 µm, median 72 µm). Overall, the volume concentration of microparticles ranged from 65.4 microparticles m^{-3} collected above urban areas to 13.8 microparticles m^{-3} in samples taken above rural zones. The highest abundance of microparticles was observed when sampling above Central Madrid, a highly populated area (about 3.2 million inhabitants). Lower values (39.4 microparticles m^{-3}) were

observed in the atmosphere above Guadalajara (city with about 86 000 inhabitants) and the lowest in flights above sub-urban and rural areas. Fig. 5.2 breaks up microparticles in terms of class as explained below. The category corresponding to smaller sizes (< 30 µm) was not the most populated one because it included particles or fibres that depending on their orientation can be retained or not.

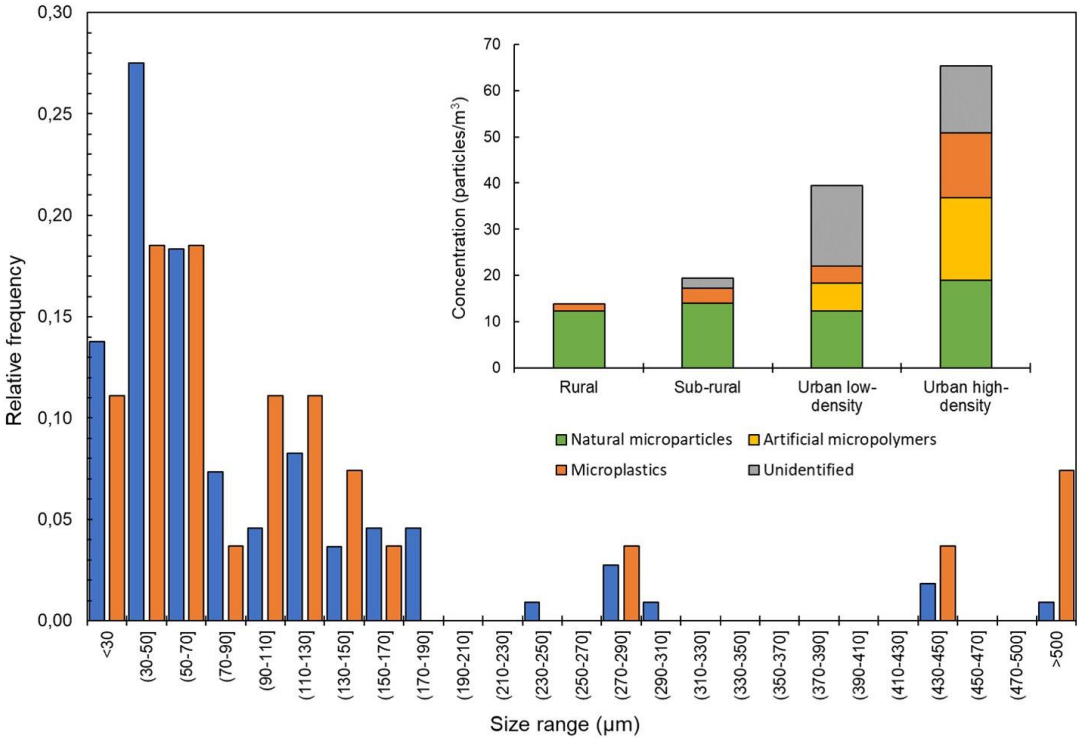
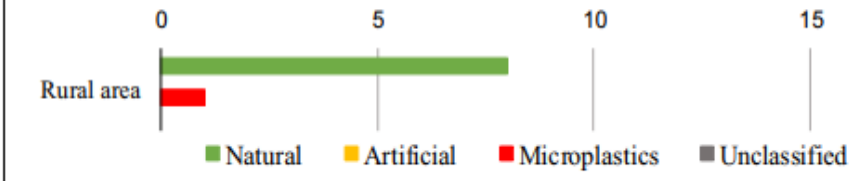


Figure 5.2. Size distribution based on equivalent diameter for the three flights. Blue bars for all microparticles, orange for MPs. Inset: volume concentration for the different class of microparticles: natural microparticles, artificial microparticles, MPs and unidentified microparticles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

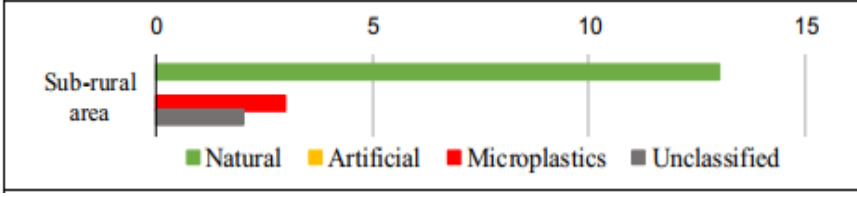
A subsample of 113 microparticles, one third of the total amount retained in the filters was analysed by µFTIR to elucidate their chemical composition (see additional details in Table S5.1, SM). The results of the analyses allowed discriminating among natural microparticles (mostly cellulose or wool), artificial materials (which are all fibres and therefore defined in ISO/TR 11827 Textiles - Composition testing - Identification of fibres) and MPs (synthetic polymers). Size distribution of MPs and the concentration of all classes of microparticles in the four tested locations are shown in Fig. 5.2. The full set of results is summarized in Table 5.1.

Table 5.1. Microparticle classes found in samples collected from flights. The graphs inside show absolute frequency by class.

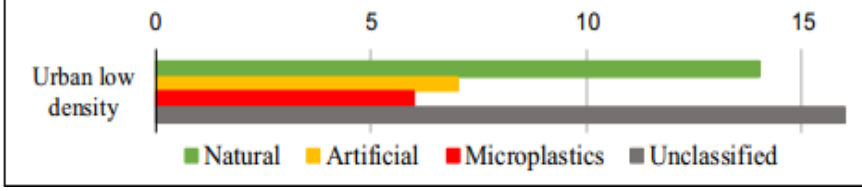
Rural area					
Class	**Shape**	**Number**	**Colour**	**Material and matching**	**Remarks**
Natural	Fragment	5	White	Cellulose (matching 50-60%)	
	Fibre	3	White	Cellulose-cotton (> 75%)	
Artificial	-				
Microplastics	Fibre	1	White	Polyester (> 85%)	Synthetic fibre
Unclassified	-				



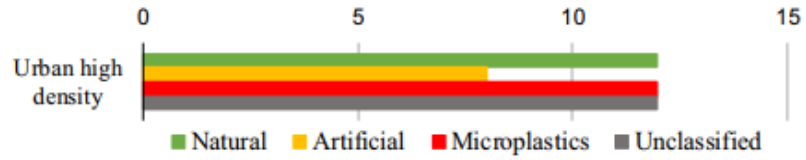
Sub-rural area					
Class	**Shape**	**Number**	**Colour**	**Material and matching**	**Remarks**
Natural	Fragment	1	White	Wool (> 85%)	
	Fragment	1	Black	Wool (> 80%)	
	Fragment	1	Green	Cellulose-cotton (> 65%)	
	Fibre	4	Translucid	Cellulose-cotton (matching > 65%)	
	Fibre	4	White	Cellulose-cotton (matching from > 65% to > 80%)	
	Fibre	1	White	Wool (> 60%)	
	Fibre	1	Black	Cotton (> 90%)	
Artificial	-				
Microplastics	Fibre	1	Red	Polyester (> 60%)	Synthetic fibre
	Fibre	1	White	Acrylic (> 75%)	Synthetic fibre
	Fibre	1	Green	Polyamide (>75%)	Synthetic fibre
Unclassified	Fragment	2	Black	Insufficient evidence	



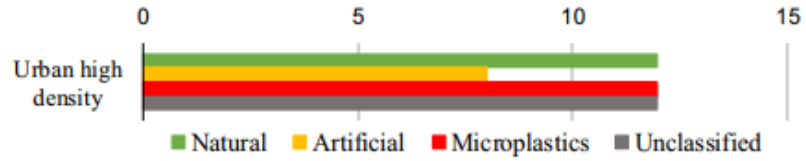
Urban low-density					
Class	**Shape**	**Number**	**Colour**	**Material and matching**	**Remarks**
Natural	Fragment	2	White	Cellulose-cotton (> 80%)	
	Fragment	2	White	Wool (> 70%)	
	Fibre	4	White	Cellulose-cotton (matching from > 65% to > 85%)	
	Fibre	1	Black	Cellulose-cotton (> 85%)	
	Fibre	1	Black	Wool (> 65%)	
	Fibre	1	White	Wool (> 65%)	
	Fibre	1	Transparent	Cellulose-cotton (> 75%)	
	Fibre	1	White-reddish	Cellulose-cotton (> 85%)	
	Fibre	1	Brown	Cellulose-cotton (> 70%)	
Artificial	Fibre	3	Blue	Cellulose-cotton (> 80%)	Possible denim fibre
	Fibre	1	Blue	Wool (> 65%)	Possible anthropogenic fibre
	Fibre	1	White-reddish	Wool (> 65%)	Possible anthropogenic fibre
	Fibre	1	Dark	Viscose-regenerated cellulose (> 65%)	Possible artificial fibre
	Fibre	1	Brown	Cellophane-regenerated cellulose (> 80%)	Possible artificial fibre
Microplastics	Fragment	1	Black	Polyurethane (>75% polyether urethane)	Elastomer
	Fragment	1	Grey	Polystyrene (> 95%)	Possible packaging material
	Fragment	3	White	Polyamide (> 75%)	Synthetic microparticle
	Fibre	1	Blue	Acrylic (> 80%)	Synthetic fibre
Unclassified	Fragment	4	Black	Insufficient evidence	
	Fragment	1	Brown	Insufficient evidence	
	Fibre	8	Black	Insufficient evidence	
	Fibre	2	White	Insufficient evidence	



Urban high-density					
Class	**Shape**	**Number**	**Colour**	**Material and matching**	**Remarks**
Natural	Fragment	3	White	Cellulose-cotton (> 75%)	
	Fibre	4	White	Cellulose-cotton (matching from > 75% to > 85%)	
	Fibre	2	Transparent	Cellulose-cotton (> 80%)	
	Fibre	1	Black	Cellulose-cotton (> 85%)	
	Fibre	2	Brown	Cellulose-cotton (> 80%)	
Artificial	Fibre	1	Red	Viscose-regenerated cellulose (> 70%)	Possible artificial fibre
	Fibre	1	Dark	Cellulose-cotton (> 85%)	Possible artificial fibre
	Fibre	4	Blue	Cellulose-cotton (matching from > 80% to > 85%)	Possible denim fibre
	Fibre	2	White	Viscose-regenerated cellulose (> 75%)	Possible artificial fibre
Microplastics	Fragment	3	White	Polyamide (> 80%)	Synthetic
					microparticle
	Fragment	1	Brown	Polyurethane (> 65%)	Synthetic foam
	Fragment	1	Transparent	Polyester (> 75%)	Plastic fragment
	Fragment	1	White	Polybutadiene (> 65%)	Synthetic elastomer
	Fibre	1	White	Polyester (> 75%)	Synthetic fibre
	Fibre	2	White	Polyamide (> 80%)	Synthetic fibre
	Fibre	3	White	Polyethylene/polypropylene (from > 65% to > 85%)	Synthetic fibres
Unclassified	Fragment	7	Black	Insufficient evidence	
	Fragment	3	Brown	Insufficient evidence	
	Fibre	1	Blue	Insufficient evidence	
	Fibre	1	White	Insufficient evidence	



					microparticle
	Fragment	1	Brown	Polyurethane (> 65%)	Synthetic foam
	Fragment	1	Transparent	Polyester (> 75%)	Plastic fragment
	Fragment	1	White	Polybutadiene (> 65%)	Synthetic elastomer
	Fibre	1	White	Polyester (> 75%)	Synthetic fibre
	Fibre	2	White	Polyamide (> 80%)	Synthetic fibre
	Fibre	3	White	Polyethylene/polypropylene (from > 65% to > 85%)	Synthetic fibres
Unclassified	Fragment	7	Black	Insufficient evidence	
	Fragment	3	Brown	Insufficient evidence	
	Fibre	1	Blue	Insufficient evidence	
	Fibre	1	White	Insufficient evidence	



The identification of sampled microparticles showed the presence of MPs, natural and artificial microparticles as well as a residual class of unclassified microparticles in different proportions. Natural microparticles predominated over rural areas, while MPs were present in all samples and were abundant in urban areas. The proportion of natural microparticles was higher above rural (89.1%) and sub-rural samples (72.3%) rather than in those taken over urban areas (31.4% and 29.0%). The concentration of MPs ranged from 13.9 MPs m^{-3} (Madrid) to 1.5 MPs m^{-3} (rural areas) as shown in the inset of Fig. 5.2. Seven different MPs types were identified in the samples (Table 5.1). The highest diversity was found in flights above Madrid, while the majority of MPs identified over rural and sub-urban areas were polyester, polyamide and acrylic fibres, which accounted for >60% of the identified MPs. Polyurethane, polystyrene, polybutadiene and polyolefins were also found in flights above the urban areas of Guadalajara and Madrid.

Artificial microparticles included fibres from extruded textiles like rayon and were only found in urban areas. Besides regenerated cellulose, natural fibres with non-natural colour were also classified as artificial fibres because of the evidence of industrial processing. The rationale is that natural fibres undergoing industrial processes are not environmentally neutral as they contain additive like dyes, flame retardants or light stabilizers, among others (O'Brien et al., 2015). Fig. 5.3 shows the FTIR spectra of four microparticles, three MPs and one artificial fibre together with their respective standards.

The matching between FTIR spectra and standards was >65% for all synthetic and artificial polymers, higher than the minimum percentage of 60% recommended elsewhere (Liu et al., 2019a). Special care was taken for assigning fibres to polyamide. It is not always easy to distinguish between synthetic polyamide and natural silk or wool because FTIR spectra look similar, and the shift of absorbance maxima are usually too small. The main difference is the width of the band at 3000-3500 cm^{-1}, due to the stretching vibration of N-H and O-H (adsorbed water), which is broader for natural products. Besides C=O stretching and C–N–H bending bands at ~1640 cm^{-1} and ~1530 cm^{-1}, respectively are sharper in synthetic materials. Generally, these differences are enough for discriminating between natural and synthetic materials, when working in

reflectance mode, but results are less concluding when analysing thin or degraded fibres from environmental samples in transmission mode (Peets et al., 2019). In this work, we attributed to natural materials (wool) spectra with matching <75% with synthetic polyamide standards and with the presence of strong bands in the 3000-3500 cm^{-1} region. Conversely, matching >75% and spectra without broad N-H stretching bands were considered evidence of synthetic polyamide. Fig. S5.3 (SM) shows spectra of two white fibres identified one as wool (not silk because of the absence of the characteristic silk band ~1710 cm^{-1}) and the other as synthetic polyamide.

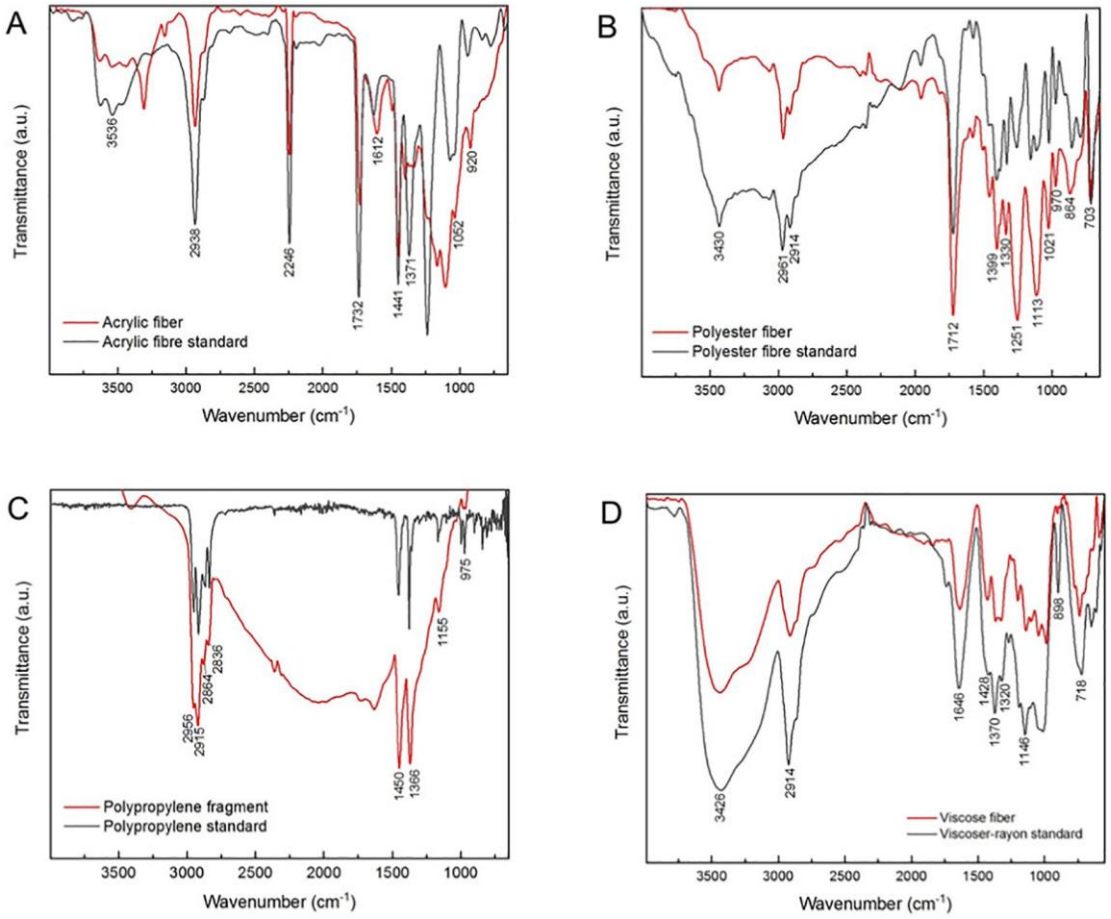


Figure 5.3. μFTIR spectra of one acrylic fibre (A), one polyester fibre (B), one polypropylene fragment (C) and one viscose fibre (D) accompanied by their corresponding standards.

5.3.2. Atmospheric transport and deposition of microplastics

Fig. 5.4 shows the deposition pattern for the representative MPs found for the flight above Madrid, a high-density urban area. The simulations performed for Madrid indicated that MPs were transported ~400 km reaching the north of Spain after 24 h

(Fig. 5.4B). A significant fraction of the simulated MPs was dispersed far away from their source location reaching Central Europe and, eventually, the south of United Kingdom, France and Belgium, more than 1000 km away from their point of sampling, supposedly close to their source (Fig. 5.4C and D).

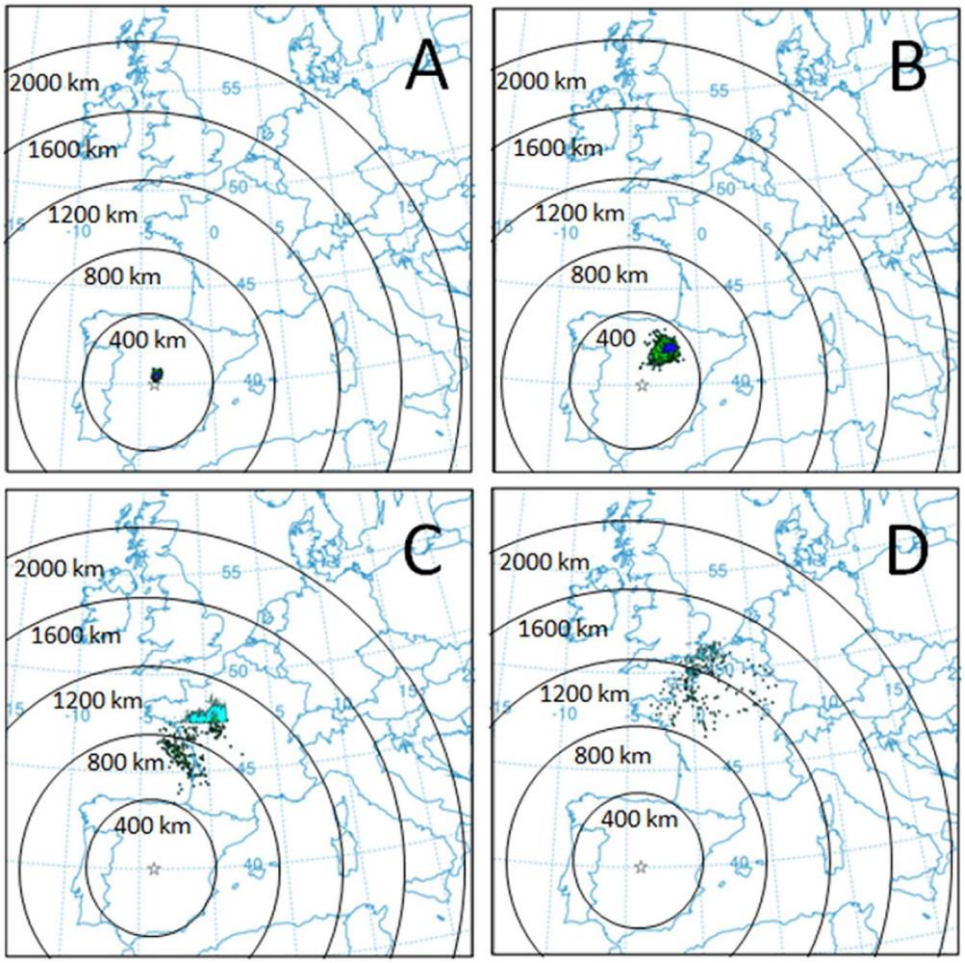


Figure 5.4. Simulation of deposition pattern of MPs collected during the flight above the high-density area of Madrid using NOAA HYSPLIT model. The results correspond to a representative size of sampled MPs. Simulations were performed for 1 h (A), 12 h (B), 24 h (C) and 36 h (D) with an initial mass release calculated based on MPs concentration measured in the sampling area at the altitude, day, and time of sampling and assuming that MPs were homogeneously distributed in the sampled region of Central Madrid (further details in Table S2, Supplementary Material, SM). The colours represent the order of magnitude of deposition values (yellow: > 1.0×10^{2} MPs/m^2; blue 1.0×10^{1} MPs/m^2; green: 1.0×10^{0} MPs/m^2; turquoise: > 1.0×10^{-1} MPs/m^2). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

According to the simulations, a fraction of MPs remained in the atmosphere after 36 h (Table S5.3, SM), allowing them to reach very distant places. Likewise, this implies that

the MPs collected in our flights cannot be strictly allocated in origin. In this sense, considering the south-southeasterly winds recorded during the flight above urban areas, it is probable that the Guadalajara sample was influenced by emissions from Madrid urban area.

5.4. Discussion

Our results showed that the concentration of microparticles tended to decrease when moving away from urban areas (Fig. 5.2). This result agreed with previous studies performed at ground level in different places (Liu et al., 2019b). Interestingly, the shape, size and chemical composition of microparticles also changed depending on the sampling area. Concerning shape, literature studies differ. Fragments were the dominant shape of microparticles recovered from urban areas and were in average larger than those collected over rural and sub-rural areas. On the contrary, fibres were the dominant shape in rural and sub-rural samples. The most probable explanation is that the origin of a significant fraction of the collected particles is in the highly populated area of Madrid. It is important to note that the flight above Madrid overflew the centre of the city over its main avenue. The literature results on the shape of particles found in the atmospheric fallout is controversial. Some studies carried out in or in the neighbourhood of populated cities showed that the MPs were mainly fibres (Cai et al., 2017; Dris et al., 2015). However, a study reporting the concentration of MPs in the atmospheric deposition from the metropolitan region of Hamburg showed fragments dominating as compared to fibres (Klein and Fischer, 2019). The differences can be attributed to local emission sources such as highways, the presence of forests or different meteorological conditions. It is important to consider that all data available to date correspond to atmospheric precipitation and not to direct observations in the atmosphere. Overall, the results suggested that densely populated cities are an important source of microparticles and may significantly contribute to the pollution due to anthropogenic substances in the atmospheric compartment. In fact, compared to air sampled in Shanghai, a higher concentration of microparticles was observed in Madrid, probably due to the higher population density of Madrid: Shanghai: 2059 inhabitants/km^2, Madrid: 5266 inhabitants/km^2 (Liu et al., 2019a). Higher surface temperature might cause microparticles emitted near ground level to rise from surface

and to reach high altitude in the atmosphere, eventually going beyond the PBL (Klein and Fischer, 2019; Liu et al., 2019a).

The simulations performed allowed calculating the rate of deposition of MPs. This calculation can be performed based on the total number of MPs estimated for the sampled area of Central Madrid (84 km^2 as shown in Fig. S5.4, SM), the sampled height (1500-2500 m a.g.l.) and the average MPs concentration (13.9 MPs m^{-3}; see details in the Table S5.1, SM). Using the median size of MPs, assuming homogeneous distribution within the sampled region, and multiplying the total concentration of MPs by the sampled area above the Central Madrid, the number of MPs could be roughly estimated as 11.6 × 10^{11} MPs between 1500 and 2500 m a.g.l. (see details in Table S5.2, SM). Predicted deposition values yielded cumulative deposition in the 100-117 MPs m^{-2} range for the first 24 h, which corresponded to the yellowish spots of Fig. 5.4. One tenth of these values corresponded to blue spots and one hundredth to green-coloured spots. The simulation resulted in expected deposition rates in the 0.1–10 MP m^{-2} day^{-1} range for the Bay of Biscay solely attributed to the MPs sampled over Madrid 24 h before (Fig. 5.4C). A comparison with literature data obtained from ground-level samplings can be performed. A selection of relevant results of deposition rates observed at ground level is given in Table 5.2. The literature data point towards deposition rates for MPs in the order of the hundreds of MPs per square metre and day without clear difference between urban, rural and even remote areas. It should be noted that all previous deposition studies measured MPs deposited or collected near the ground, whereas this work evaluated the fate of MPs directly sampled at high altitude at a given place and time. We analysed the trajectory of MPs and predicted their deposition rate in the first approach to this kind reported up to date. It is important to note that ground and altitude sampling represent two different and complementary approaches, the latter opening a new area in a field with very limited data. In fact, the data reported here are the first direct sampling of MPs in the atmosphere.

Based on our findings, MPs can be transported and dispersed hundreds and even thousands of km from their initial release location until they are finally deposited. These results can help explain how MPs may reach remote areas where there are not

significant anthropogenic activities in the vicinity (Allen et al., 2019; Ambrosini et al., 2019; Free et al., 2014; Zhang et al., 2016; Zhang et al., 2019).

Table 5.2. Literature data for the deposition rate of microparticles/microplastics.

Place	Shape	Size (µm)	Polymer type	Deposition Rate	Reference
Paris, urban	Mostly fibres (90%)	100- 5000	No chemical Identification performed	29 - 280 (average: 118) *	Dris et al., 2015
Paris, urban	Mostly fibres	50 - 5000	Natural fibres (50%), synthetic fibres (12%), synthetic polymers (17%)	2 -350 (average: 110 ± 96 and 53 ± 38; two sites) *	Dris et al., 2016
China, urban	All forms	<200 - 4200	Synthetic polymers	(Average: 31 ± 8 - 43± 4) **	Cai et al., 2017
Pyrenees, remote area	All forms	<25 -3000 (<50 - 600 square root of projected area	Synthetic polymers	(Average: 365 ± 69) *	Allen et al., 2019
Hamburg, urban & periurban	Mostly fragments	<63 - 5000	Synthetic polymers (77%)	136.5 - 512.0 **	Klein and Fischer, 2019

* Microparticles m^{-2} day^{-1}; ** MPs m^{-2} day^{-1}

So far, very few studies have focused on the atmospheric transport of MPs. Strictly speaking, ground-level sampling does not allow to unambiguously determine their origin. However, their most probable source are densely populated areas. In this work, we demonstrated that atmospheric transport may play a significant role in the long-range transport of small MPs, supporting the hypothesis that MPs can move between distant areas and countries in a few days, at least for MPs with size not larger than the tens of microns. Due to their low concentration in the atmosphere, and the difficulty to filtrate a high volume of air during flight time, the number of particles collected in this work was not high. However, this is the first time MPs are directly collected from the atmosphere at high altitude, thereby proving their presence even above the PBL. Further research would be needed to clarify the role of the atmosphere as a dispersion pathway of MPs by studying different areas, time periods, and altitudes, and aircraft missions would be a valuable tool for it.

5.5. Conclusions

In this ground-breaking study, we obtained direct evidence of the presence of MPs in the atmosphere at high altitude. We used aircrafts to collect samples above planetary boundary layer and detected higher concentration of microparticles and MPs when flying above densely populated areas. Seven types of synthetic polymers, either as fibres or as fragments, extruded textiles and industrially processed fibres were identified, and their concentration calculated. Our findings demonstrated for the first time the assumption that MPs are present in the atmosphere hundreds of metres above ground level. Atmospheric transport and deposition simulations using our results, indicated that urban areas could be sources of MPs, which may eventually end up in distant areas. This work shed light on the atmospheric long-range transport of MPs showing how they can constitute a global pollution issue.

5.6. References

Abbasi, S., Keshavarzi, B., Moore, F., Turner, A., Kelly, F.J., Dominguez, A.O., et al., 2019. *Distribution and potential health impacts of microplastics and microrubbers in air and street dusts from Asaluyeh County, Iran*. Environ. Pollut. 244, 153–164.

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

Ambrosini, R., Azzoni, R.S., Pittino, F., Diolaiuti, G., Franzetti, A., Parolini, M., 2019. *First evidence of microplastic contamination in the supraglacial debris of an alpine glacier*. Environ. Pollut. 253, 297–301.

Aneja, V.P., Wang, B., Tong, D.Q., Kimball, H., Steger, J., 2006. *Characterization of major chemical components of fine particulate matter in North Carolina.* J. Air Waste Man- age. Assoc. 56, 1099–1107.

Banks, R.F., Tiana-Alsina, J., Rocadenbosch, F., et al., 2015. *Performance evaluation of the boundary-layer height from lidar and the weather research and forecasting model at an urban coastal site in the North-East Iberian Peninsula*. Boundary Layer Meteorol. 157, 265–292.

Bergmann M, Mützel S, Primpke S, Tekman MB, Trachsel J, Gerdts G. *White and wonderful? Microplastics prevail in snow from the Alps to the Arctic*. Sci. Adv. 2019; 5: eaax1157.

Bomgardner, M.M., 2017. *The great lint migration*. Chem. Eng. News 95, 16–17.

Cai, L., Wang, J., Peng, J., Tan, Z., Zhan, Z., Tan, X., et al., 2017. *Characteristic of microplastics in the atmospheric fallout from Dongguan city, China: preliminary research and first evidence.* Environ. Sci. Pollut. Res. 24, 24928–24935.

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

Chen, G., Feng, Q., Wang, J., 2019. *Mini-review of microplastics in the atmosphere and their risks to humans*. Sci. Total Environ. 135504.

Draxler R, Rolph G. *HYSPLIT (HYbrid Single-Particle Lagrangian Integrated Trajectory) model access via NOAA ARL READY website (http://ready.arl.noaa.gov/HYSPLIT.php*). NOAA Air Resources Laboratory. Silver Spring, MD 2010; 25.

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

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

Dris, R., Gasperi, J., Mirande, C., Mandin, C., Guerrouache, M., Langlois, V., et al., 2017. *A first overview of textile fibres, including microplastics, in indoor and outdoor environments*. Environ. Pollut. 221, 453–458.

Enyoh, C.E., Verla, A.W., Verla, E.N., Ibe, F.C., Amaobi, C.E., 2019. *Airborne microplastics: a review study on method for analysis, occurrence, movement and risks.* Environ. Monit. Assess. 191, 668.

Fred-Ahmadu, O.H., Bhagwat, G., Oluyoye, I., Benson, N.U., Ayejuyo, O.O., Palanisami, T., 2020. *Interaction of chemical contaminants with microplastics: principles and perspectives*. Sci. Total Environ. 706, 135978.

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

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

Ganguly, M., Ariya, P.A., 2019. *Ice nucleation of model nanoplastics and microplastics: a novel synthetic protocol and the influence of particle capping at diverse atmospheric environments*. ACS Earth Space Chem. 3, 1729–1739.

Gasperi, J., Wright, S.L., Dris, R., Collard, F., Mandin, C., Guerrouache, M., et al., 2018. *Microplastics in air: are we breathing it in?* Curr. Opin. Environ. Sci. Health 1, 1–5.

GESAMP. *Guidelines for the monitoring and assessment of plastic litter in the ocean.* In: Kershaw PJ, Turra A, Galgani F, editors. Rep. Stud. GESAMP No. 99. IMO/FAO/ UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP/ISA Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, 2019, pp. 130.

Gonda, I., Abd El Khalik, A.F., 1985. *On the calculation of aerodynamic diameters of fibres*. Aerosol Sci. Technol. 4, 233–238.

González-Pleiter, M., Edo, C., Casero-Chamorro, M.C., Aguilera, Á., González-Toril, E., Wierzchos, J., et al., 2020a. *Viable microorganisms on fibres collected within and be- yond the planetary boundary layer*. Environ. Sci. Technol. Letters https://doi.org/ 10.1021/acs.estlett.0c00667.

González-Pleiter, M., Velázquez, D., Edo, C., Carretero, O., Gago, J., Barón-Sola, Á., et al., 2020b. *Fibres spreading worldwide: microplastics and other anthropogenic litter in an Arctic freshwater lake*. Sci. Total Environ. 722, 137904.

Granados-Muñoz, M.J., Navas-Guzmán, F., Bravo-Aranda, J.A., Guerrero-Rascado, J.L., Lyamani, H., Fernández-Gálvez, J., Alados-Arboledas, L., 2012. *Automatic determination of the planetary boundary layer height using lidar: one-year analysis over south-eastern Spain*. J. Geophys. Res. Atmos. 117, D18208.

Kallos, G., Astitha, M., Katsafados, P., Spyrou, C., 2007. *Long-range transport of anthropogenically and naturally produced particulate matter in the Mediterranean and North Atlantic: current state of knowledge*. J. Appl. Meteorol. Climatol. 46, 1230–1251.

Karbalaei, S., Hanachi, P., Walker, T.R., Cole, M., 2018. *Occurrence, sources, human health impacts and mitigation of microplastic pollution.* Environ. Sci. Pollut. Res. 25, 36046–36063.

Kaya, A.T., Yurtsever, M., Bayraktar, S.Ç., 2018. *Ubiquitous exposure to microfibre pollution in the air*. Eur. Phys. J. Plus 133, 488.

Klein, M., Fischer, E.K., 2019. *Microplastic abundance in atmospheric deposition within the metropolitan area of Hamburg*. Germany. Sci. Total Environ. 685, 96–103.

Knight, L.J., Parker-Jurd, F.N.F., Al-Sid-Cheikh, M., Thompson, R.C., 2020. *Tyre wear particles: an abundant yet widely unreported microplastic?* Environ. Sci. Pollut. Res. 27, 18345–18354.

Kole, P.J., Löhr, A.J., Van Belleghem, F.G.A.J., Ragas, A.M.J., 2017. *Wear and tear of Tyres: a stealthy source of microplastics in the environment.* Int. J. Environ. Res. Public Health 14, 1265.

Liu, K., Wang, X., Fang, T., Xu, P., Zhu, L., Li, D., 2019a. *Source and potential risk assessment of suspended atmospheric microplastics in Shanghai.* Sci. Total Environ. 675, 462–471.

Liu, K., Wang, X., Wei, N., Song, Z., Li, D., 2019b. *Accurate quantification and transport estimation of suspended atmospheric microplastics in megacities: implications for human health.* Environ. Int. 132, 105127.

Liu, K., Wu, T., Wang, X., Song, Z., Zong, C., Wei, N., et al., 2019c. *Consistent transport of terrestrial microplastics to the ocean through atmosphere.* Environ. Sci. Technol. 53, 10612–10619.

O'Brien, J.W., Thai, P.K., Brandsma, S.H., Leonards, P.E.G., Ort, C., Mueller, J.F., 2015. *Wastewater analysis of census day samples to investigate per capita input of organophosphorus flame retardants and plasticizers into wastewater*. Chemosphere 138, 328–334.

Peets, P., Kaupmees, K., Vahur, S., Leito, I., 2019. *Reflectance FT-IR spectroscopy as a viable option for textile fibre identification*. Heritage Sci. 7, 93.

Primpke, S., Lorenz, C., Rascher-Friesenhausen, R., Gerdts, G., 2017. *An automated approach for microplastics analysis using focal plane array (FPA) FTIR microscopy and image analysis*. Anal. Methods 9, 1499–1511.

Reche, I., D'Orta, G., Mladenov, N., Winget, D.M., Suttle, C.A., 2018. *Deposition rates of viruses and bacteria above the atmospheric boundary layer*. ISME J. 12, 1154.

Rolph, G., Stein, A., Stunder, B., 2017. *Real-time environmental applications and display system: READY*. Environ. Model. Softw. 95, 210–228.

Sharma, S., Chatterjee, S., 2017. *Microplastic pollution, a threat to marine ecosystem and human health: a short review*. Environ. Sci. Pollut. Res. 24, 21530–21547.

Stanton, T., Johnson, M., Nathanail, P., MacNaughtan, W., Gomes, R.L., 2019. *Freshwater and airborne textile fibre populations are dominated by 'natural', not microplastic, fibres*. Sci. Total Environ. 666, 377–389.

Stein, A., Draxler, R.R., Rolph, G.D., Stunder, B.J., Cohen, M., Ngan, F., 2015. *NOAA's HYSPLIT atmospheric transport and dispersion modeling system.* Bull. Am. Meteorol. Soc. 96, 2059–2077.

Wang, L., Wu, W.-M., Bolan, N.S., Tsang, D.C.W., Li, Y., Qin, M., et al., 2021. *Environmental fate, toxicity and risk management strategies of nanoplastics in the environment: current status and future perspectives.* J. Hazard. Mater. 401, 123415.

Wright, S.L., Ulke, J., Font, A., Chan, K.L.A., Kelly, F.J., 2020. *Atmospheric microplastic deposition in an urban environment and an evaluation of transport.* Environ. Int. 136, 105411.

Zhang, K., Su, J., Xiong, X., Wu, X., Wu, C., Liu, J., 2016. *Microplastic pollution of lakeshore sediments from remote lakes in Tibet plateau, China.* Environ. Pollut. 219, 450–455.

Zhang, Y., Gao, T., Kang, S., Sillanpää, M., 2019. *Importance of atmospheric transport for microplastics deposited in remote areas.* Environ. Pollut. 254, 112953.

Zhou, Q., Tian, C., Luo, Y., 2017. *Various forms and deposition fluxes of microplastics identified in the coastal urban atmosphere*. Chin. Sci. Bull. 62, 3902–3910.

5.7. Supplementary material of Chapter 5

Contents

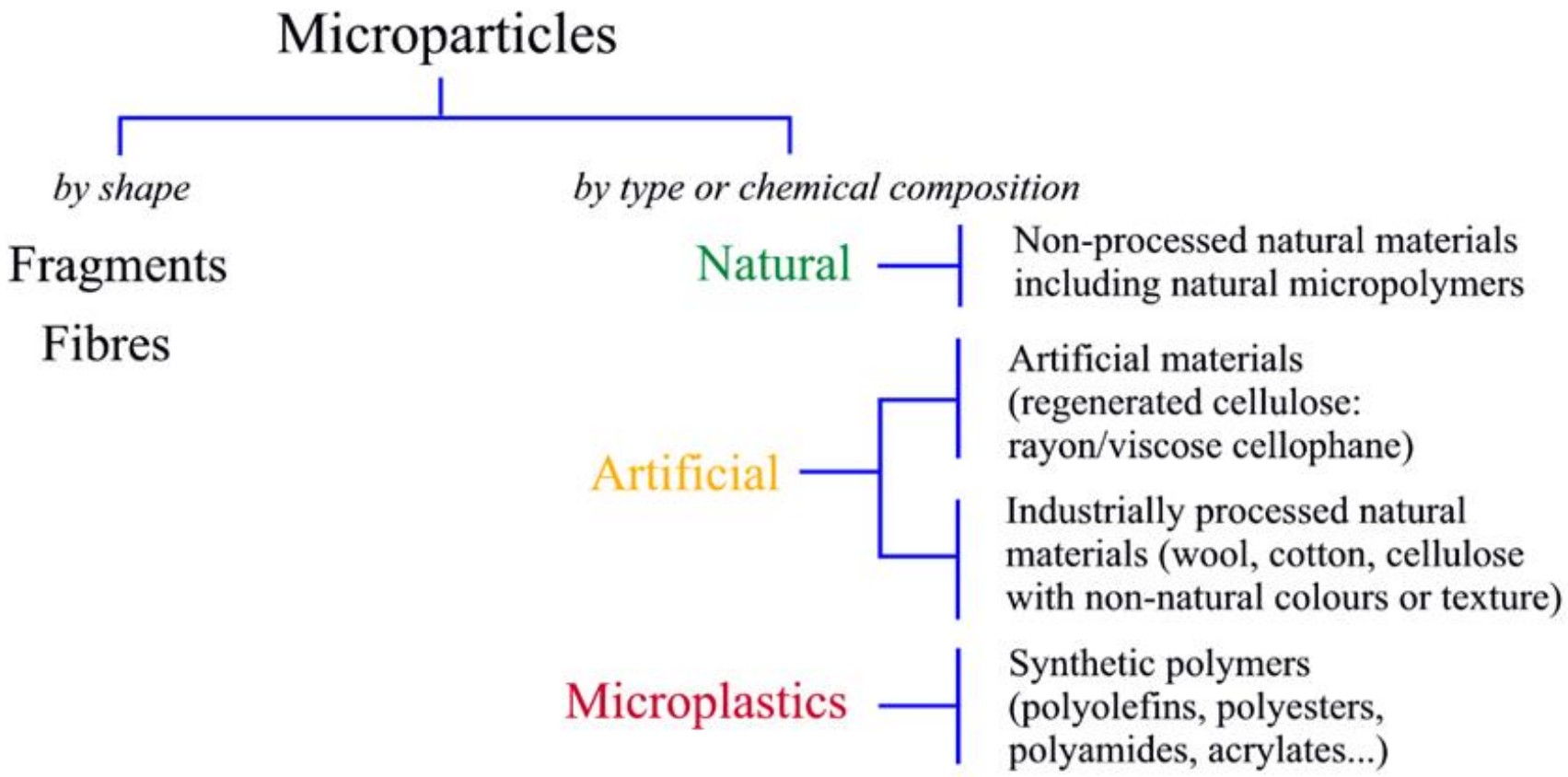


Figure S5.1. Scheme explaining the nomenclature used in this work.

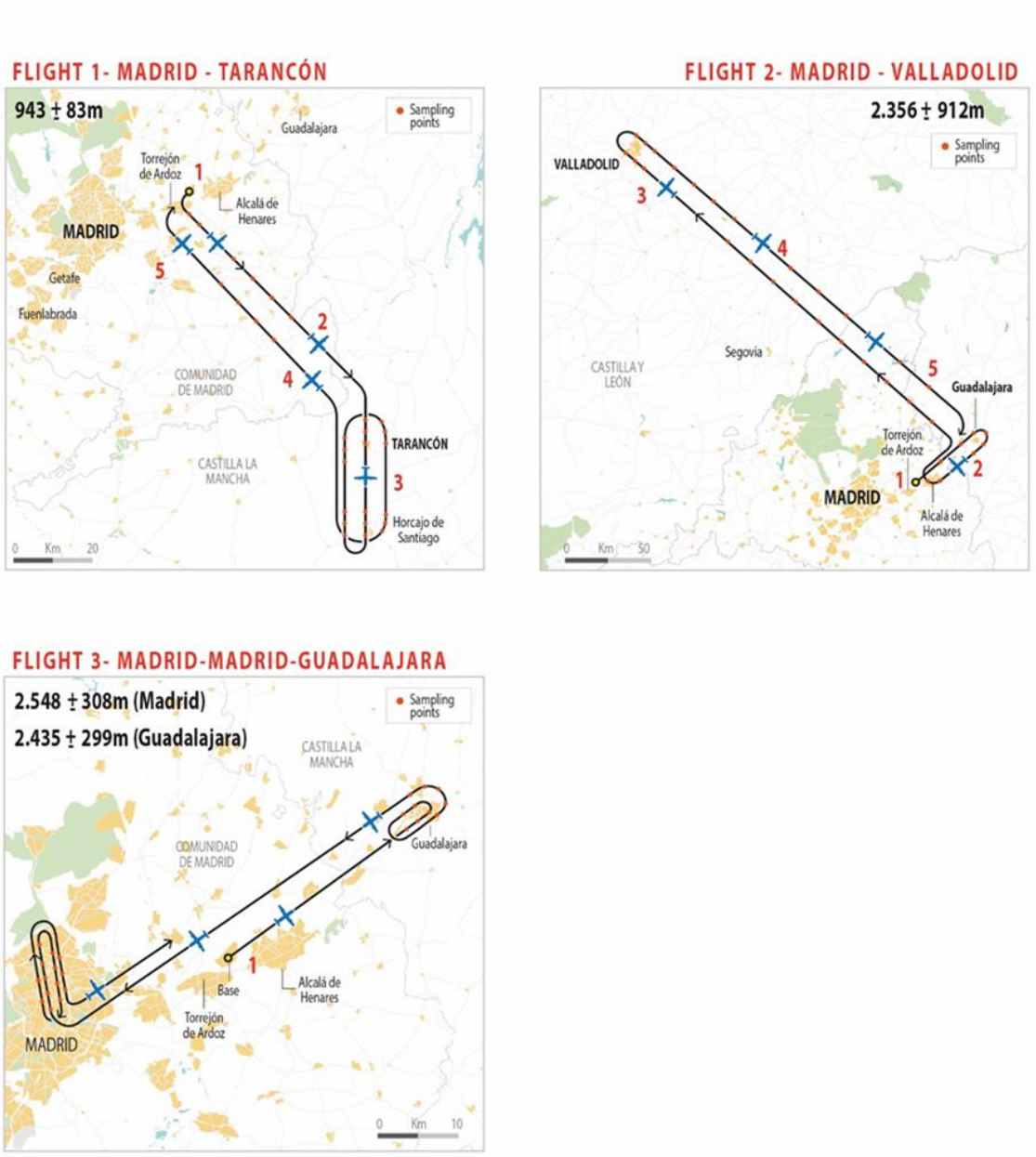


Figure S5.2. Flight information. Trajectories, sampling points, locations and average altitudes (a.s.l.) of the flights in which air samples were collected on April 25, 2018 (Flight 1), May 23, 2019 (Flight 2) and June 17, 2019 (Flight 3).

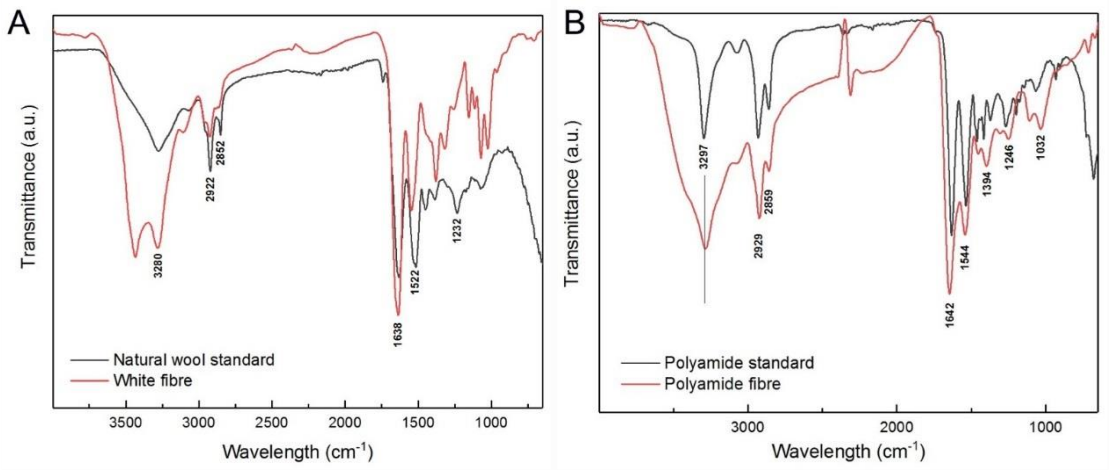


Figure S5.3. µFTIR spectra of fibres identified as wool (A) and synthetic polyamide (B) together with their corresponding standards.

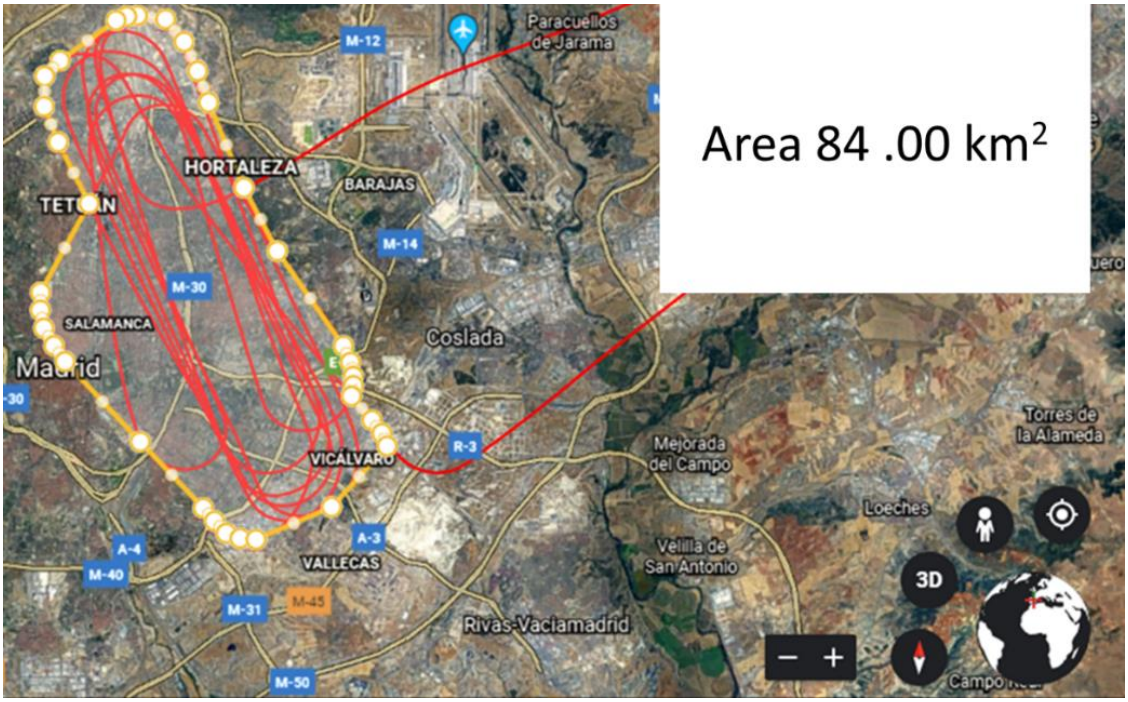


Figure S5.4. Estimation of the area of Madrid (high-density urban area) overflown during sampling using Google Earth. Red line indicates flight trajectory. Yellow line indicates the sampled area.

Table S5.1. Details on results. (Mean absolute deviation between brackets.)

	Flight 1	**Flight 2**	**Flight 3**			
Area	Rural	Sub-rural	Low-density urban		High-density urban	
Samples						
Collectors location	top	top	top	side	top	side
Number of collectors	1	1	1	6	1	6
Average altitude (m) a.s.l.	943 ± 83	2356 ± 912	2435 ± 299		2549 ± 308	
Median altitude (m) a.s.l.	941	2771	2216		2800	
Total of volume of air filtered (m^3)	1.30	2.52	2.48		2.68	
Volume of air filtered per filter (m^3)	1.30	2.52	0.75	0.29 (0.07)	0.75	0.32 (0.08)
Total of microparticles	18	49	96		160	
Microparticles per filter	18	49	17	13.2 (6.9)	21	23.6 (6.7)
Total fibres	12	41	46		53	
Fibres per filter	12	41	8	6.2 (4.5)	8	7.4 (3.7)
Total fragments	6	8	50		107	
Fragments per filter	6	8	9	7.0 (3.6)	13	16.2 (6.3)
Average of equivalent diameter (µm)	45.7 (16.6)	67.7 (40.8)	104.2 (78.0)		85.5 (56.0)	
Median of equivalent diameter (µm)	41.2	41.3	68.3		58.0	
Microparticles analysed by µFTIR	9	18	42		44	
Microparticles analysed per filter	9	18	16	4.3 (1.6)	18	4.8 (1.0)
[Microparticles/m^3]	13.8	19.5	39.4 (15.5)		65.4 (15.3)	

Natural microparticles analysed by µFTIR	8	13	14		12	
[Natural microparticles/m^3]	12.3	14.1	12.4 (11.7)		19.0 (8.0)	
Artificial microparticles analysed by µFTIR	0	0	7		8	
[Artificial microparticles/m^3]	0	0	5.9 (4.5)		17.9 (10.5)	
Microplastics analysed by µFTIR	1	3	6		12	
[Microplastics/m^3]	1.5	3.2	3.7 (2.7)		13.9 (8.7)	
Unidentified particles after µFTIR	0	2	15		12	
[Unidentified/m^3]	0	2.2	17.4 (7.8)		14.6 (8.2)	
Control + Procedural blank						
Microparticles per filter	3	6	3	2.5 (0.8)	3	2.5 (0.8)
Fibres per filter	1	3	1	1.3 (0.8)	1	1.3 (0.8)
Fragments per filter	2	3	2	1.2 (0.8)	2	1.2 (0.8)
Natural microparticles per filter	1	3	2	1.0 (0.7)	2	1.0 (0.7)
Artificial microparticles per filter	0	1	0	0	0	0
Microplastics per filter	0	0	0	0.2 (0.3)	0	0.2 (0.3)
Unidentified per filter	2	2	1	1.3 (0.8)	1	1.3 (0.8)

Table S5.2. Parameters used in the simulations performed using the HYbrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) model.

HYSPLIT Dispersion model*	
Meteorological data	GDAS1
Date	17/6/2019
Time	9 (UTC +2)
Latitude	40.4167
Longitude	-3.70325
Initial released (mass)[1]	1167600000000
Release time (h)[2]	1
Median altitude of sampling (m) MSL	2800
Ground level (m) MSL	665
Equivalent diameter of representative MPs (µm) found above Madrid[3]	35
Concentration (mass m^{-3}) averaged between	250[4] and 2800[5]

[1] It is an estimation assuming that MP were homogeneously distributed in the sampled region of Central Madrid. By multiplying the total concentration of microplastics by the volume of air above the sampled area of the Central Madrid, the number of MP could be roughly estimated.

[MPs] x Volume of air above Central Madrid = 1167600000000 MPs above area sampled between 1500 and 2500 metres above ground level.

> [MPs] = Concentration of microplastics found above Madrid including both top and side filters 13.9 MPs/m^3 (see Table S1).
>
> Volume of air over Central Madrid = It is an estimation of the volume of air between 1500 and 2500 metres above area sampled of the Central Madrid (84 km^2, see details in the Figure S3) as results 84 km^3

[2] Point release similar to sampling time

[3] The aerodynamic diameters of the MPs were computed by Henn (1996)

[4] European buildings generally do not exceed 250 metres (Pietrzak, J., 2014. Development of high-rise buildings in Europe in the 20th and 21st centuries. Challenges of Modern Technology, 5.)

[5] Median altitude of sampling

HYPERLINK https://www.ready.noaa.gov/HYSPLIT.php

References

Henn, A.R. Calculation of the Stokes and Aerodynamic Equivalent Diameters of a Short Reinforcing Fibre, Part. Syst. Charact, 1996; 13: 249-253.

Table S5.3. Percentage of microparticles found in flight 3 (over Madrid) that remained in the atmosphere after 12, 24 and 36 h. Three scenarios have been simulated as a function of particle size (see Materials and Methods 2.5 and Supplementary Section 1) for the flight: small MP, median MP, large MP and a representative size of sampled MPs.

Flight	Simulation time	Representative MPs	Small MP size case	Median MP size case	Large MP size case
3	t = 12 h	63.7%	97.6%	27.4%	14.5%
	t = 24 h	22.9%	82.8%	1.5%	0.1%
	t = 36 h	8.1%	68.6%	0.1%	0.0%

Supplementary Information: Model for atmospheric deposition of microplastics.

The simulations were performed for 36 h with an initial release in the sampling area (Madrid) integrated during a period < 1h (similar to sampling time) at the day and time of sampling (17/6/2019; UTC +2). Representative MPs size was simulated for flight 3 (equivalent diameter ~ 35 µm). In addition, given the strong dependence on the deposition process by the particles size, three additional cases covering a wide range of particle sizes were considered for this flight: (i) small MP size case (equivalent diameter ~ 10 µm) , (ii) median MP size case (equivalent diameter ~ 58 µm), and (iii) large MP size case (equivalent diameter ~ 90 µm). It should be noted that the most of collected MPs (> 50%) had equivalent diameters in the 10-90 µm range being the majority between 10 and 50 (Fig. 2). The gross size distribution found above Madrid can be allocated within the simulated range. The fibre equivalent diameter was computed by Henn (1996) and wet deposition was parameterized by means of in-cloud and below-cloud loss rates of $8 \cdot 10^{-5}$ s^{-1} (Rolph et al., 2017). Particle density is assumed to be 1.1 g/cm^3. The particle deposition velocity was set to a value representative of the particle density, size and modelled altitude.

References

Henn, A.R. Calculation of the Stokes and Aerodynamic Equivalent Diameters of a Short Reinforcing Fibre, Part. Syst. Charact, 1996; 13: 249-253.

Rolph G, Stein A, Stunder B. Real-time environmental applications and display sYstem: READY. Environ. Model. Softw. 2017; 95: 210-228.

CHAPTER 6. HONEYBEES AS ACTIVE SAMPLERS FOR MICROPLASTICS

6.1. Introduction

Microplastics (MPs) are defined as plastic particles with sizes ranging from 1 µm to 5 mm in their larger dimension (GESAMP, 2016). The definition is somewhat arbitrary and despite the prefix "micro", the size of MPs expands into the millimetre range due to practical and historical reasons (GESAMP, 2019). Below the lower boundary of 1 µm, plastic particles are referred to as nanoplastics, even though the definition is not coincident the usual size range of nanosized particles (Gigault et al., 2018). According to their origin, MPs can be primary or secondary. Primary MPs have been manufactured with their specific size for cosmetic or industrial purposes, while secondary microplastics come from the degradation of larger particles upon the effect of photochemical oxidation, hydrolysis and mechanical forces (GESAMP, 2019). Fibres produced from synthetic polymers are considered MPs, but artificial fibres include extruded cellulose or industrially processed natural fibres like cotton or wool, which can also be considered anthropogenic pollutants. These materials may contain additives and other chemicals and have received much less attention (Henry et al., 2019). MPs have been reported in all compartments, including apparently pristine environments in remote areas and are a global cause for concern due to their mobility and ubiquity and to the lack of knowledge about important aspects related to their fate and risk (Enyoh et al., 2019; Evangeliou et al., 2020; González-Pleiter et al., 2020b; Horton and Barnes, 2020; Li et al., 2018).

The atmospheric transport of MPs is still poorly known. The sources of airborne MPs are the disintegration of larger plastic products like building or packaging materials or point sources like industrial emissions (Wright et al., 2020). Urban sources are generally dominant with an important contribution of fibres produced during the wearing of synthetic textiles (Liu et al., 2019a). The presence of MPs in the atmosphere is a new field of research and the available data are still very limited. The sources and fate of atmospheric MPs are poorly known because of their many potential origins, their low concentrations, and the difficulty of sampling. The dispersion and transport of MPs and the factors influencing their chemical and mechanical transformation are complex and still not fully understood (Zhang et al., 2020). The occurrence of airborne MPs has been studied at ground level using active or passive collectors or by measuring their ground

deposition rate, but the available data are limited and difficult to interpret due to methodological issues, the rapid atmospheric mixing and the occurrence of unpredictable deposition events. The only broad study available showed deposition rates >100 MP m^{-2} day^{-1} in remote areas of North America. Air mass trajectory calculations and population metrics suggested an urban origin for wet-deposited MPs, while in the absence of precipitation, MPs might travel very large distances (Brahney et al., 2020). Overall, the data available in the literature showed deposition rates reaching values in the order of hundreds of MPs per square metre and day (Cai et al., 2017; Klein and Fischer, 2019).

Concerning their risk, MPs have been associated to chemical toxicity due to the release of additives, non-intentionally added substances, and pollutants retained from the environment (Hahladakis et al., 2018; Wang et al., 2018). While relatively large MPs can produce physical harm, small debris may cause the blockage of the intestines of small animals and, for sufficiently small particles, translocation and transfer through the food webs, thereby originating true toxic effects. The accumulation in tissues and translocation are phenomena described for plastics in the range of hundreds and tens of nanometres respectively (Sendra et al., 2020a; Shen et al., 2019). It has been reported that small particles could cause damage at cellular and molecular levels. Including immunotoxicity and genotoxicity (Ballesteros et al., 2020; Sendra et al., 2020b). An additional cause for concern is the possible migration of MPs to packaged food (Kedzierski et al., 2020). In fact, the presence of MPs in food is well documented with estimations of annual MP intake in order of tens of thousands of particles (Cox et al., 2019).

Honeybees (Apis mellifera) or honeybee products have been used as bioindicators for different pollutants (Devillers and Pham-Delègue, 2002). Their advantages include sensitivity to toxic substances, large flying capacity, including inaccessible places and high reproduction rate. The wide-range activity of honeybees, whose foraging range reaches several kilometres and the existence of tens of thousands female worker bees per colony make them useful as active samplers (Bargańska et al., 2016; Murcia-Morales et al., 2020). Monitored pollutants include products specifically used for beekeepers to control pests, as well as different kinds of environmental pollutants gathered by

honeybees during their foraging flights. Honeybees interact with essentially all elements in their environment and bring back pollutants to their hive, where they eventually accumulate and are transferred to honey, beebread and beeswax. The occurrence of distribution of pesticide residues in several beekeeping matrices including live honeybees, beeswax and pollen has been reported elsewhere (Calatayud-Vernich et al., 2018; Murcia-Morales et al., 2020). Honeybee colonies have also been explored as active samplers for heavy metals (Dżugan et al., 2018; Gajger et al., 2019; Zarić et al., 2017). It has also been showed that honeybees can gather airborne particulate matter that concentrates in specific parts of their bodies like the edge of wings and the head (Negri et al., 2015).

In this work, we tested the hypothesis that worker bees can take MPs from their foraging area, potentially acting as biosamplers of MP pollution. The research responds to the need for implementing wide geographical monitoring of airborne MPs to establish their distribution as new group of persistent anthropogenic pollutants (Bujnicki et al., 2019).

6.2. Materials and methods

6.2.1. Sampling

The sampling was conducted with the collaboration of the Danish association of beekeepers (Danmarks Biavlerforening). Nineteen different urban apiaries from Copenhagen (9) and other areas (10) in Denmark, were selected to test the interaction between environmental MPs and honeybees. The complete list of apiaries and their location is shown in Fig. 6.1 and Table 6.1.

The protocol for sampling honeybees was developed and delivered to the different beekeepers based on the recommendations for the analysis of MPs in biota samples stated elsewhere (Hermsen et al., 2018). The same sampling method was used in all locations and the honeybees were taken from the same place of the hive in all cases. Samples were taken in the early spring season in Danish beekeeping. This is a period in which colonies are building up and nectar flow has just started.

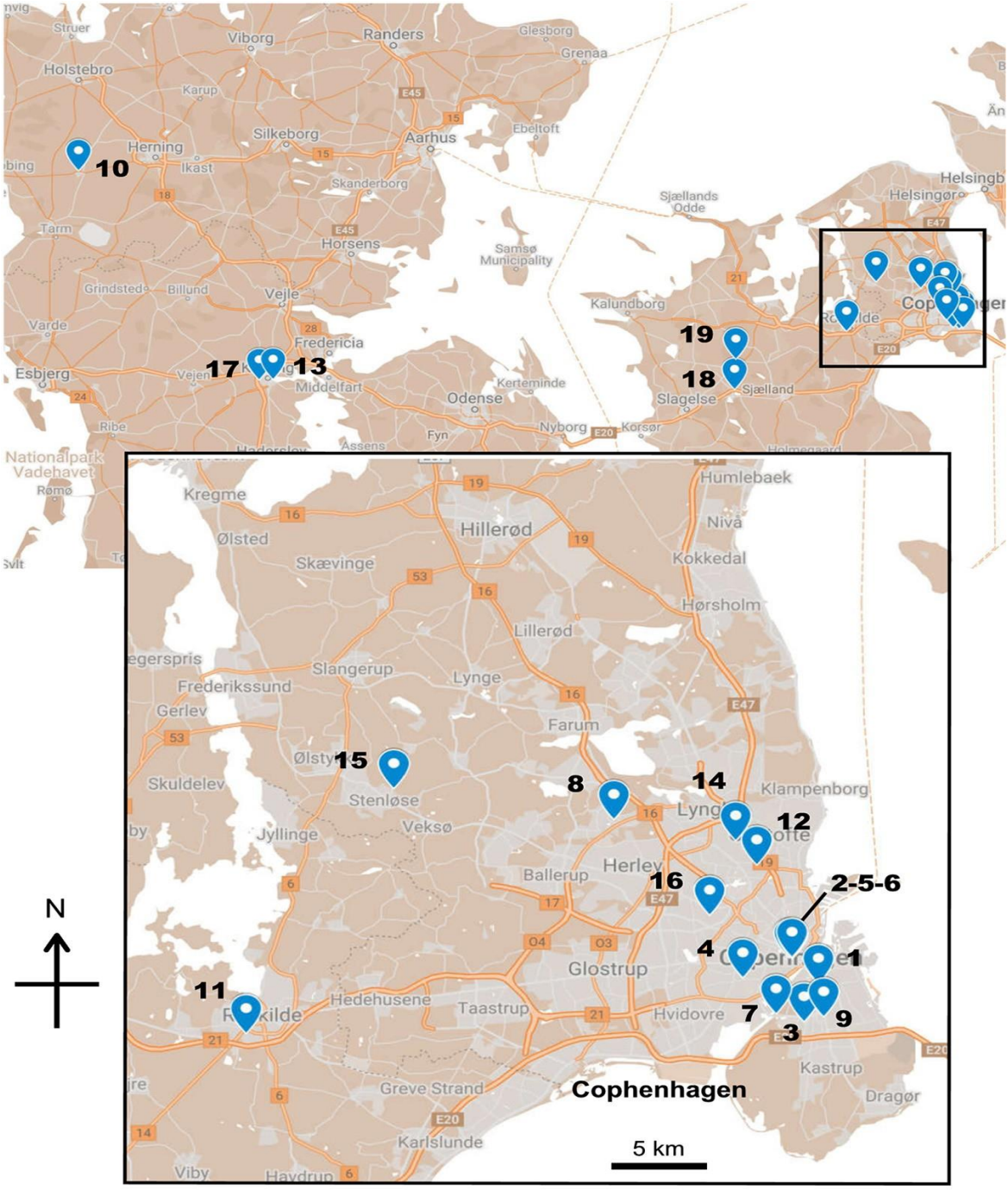


Figure 6.1. Location of sampling points.

Table 6.1. Site description of the different apiaries (provided by Danish Beekeeper Association).

12	Colonies	Location	Site description
1	3	Sundholm, Copenhagen	Heavy urban, with just few green areas
2	2	Dansk Industri, Copenhagen	Heavy urban, with just few green areas
3	3	Bella Centret, Copenhagen	Heavy urban, but green areas in the surroundings
4	3	Valby, Copenhagen	Heavy urban, but green areas in the surroundings, like railroads with flowers
5	3	Tivoli, Copenhagen	Heavy urban, with just few green areas. But a green Tivoli garden
6	2	Majors House, Copenhagen	Heavy urban, with just few green areas
7	3	Kalvebod, Copenhagen	Heavy urban, but green areas in the surroundings, like railroads with flowers
8	2	Værløse, Copenhagen	Suburban, lot of gardens in the surroundings
9	2	Westi, Copenhagen	Heavy urban, but green areas in the surroundings, like railroads with flowers
10	2	Flydtkjær, Videbæk	Country side, small village
11	2	Germuth, Roskilde	Suburban, green areas and gardens
12	2	Branner, Hellerup	Suburban, lot of gardens in the surroundings
13	2	Korsholm, Kolding	City, green areas and gardens
14	2	Andersen, Gentofte	Suburban, gardens in the surroundings
15	2	Ask Laurberg, Stenløse	Country side, small village
16	1	Holm-Petersen, Brønshøj	Urban, gardens
17	2	Wichmann-Hansen, Kolding	City, green areas and gardens
18	3	Sorb	Country side, small village
19	2	Stenlille	Country side, small village

Samples were taken close to the brood area of the colony, where bees fees larvae and start to store nectar. Honeybees were directly caught in the interior of 50 mL glass jars. Jars were labelled and stored in zip lock bags before being frozen. They were frozen directly into the jars without solution to avoid plastic degradation, organic decomposition and microbial growth, and were transported at soon as possible to the laboratory. The direction and intensity of wind during sampling days was recorded and shown in Fig. S6.1 (Supplementary material, SM). A minimum of two samples was recommended per apiary to have at least one replicate. For technical reasons, replicates, although from the same apiary, belonged to different hives. For each replicate, at least 50 honeybees were taken per sample with a minimum of 120 honeybees per apiary. A total number of 4187 honeybees was analysed.

6.2.2. Laboratory procedures

At the laboratory, the samples were defrosted and put inside beakers filled with 150 mL of ultrapure water and 50 mL ethanol. All honeybees in the same sample were washed together, with a minimum of 120 bees per sample. The mixture water-ethanol was chosen due to its capacity to detach particles from the body of the bees. After 15 min of gentle stirring, the liquid was filtered using 47 mm, 25 µm stainless steel filters in a Millipore stainless steel pressure holder system. Afterwards, their bodies were placed on the same filters and thoroughly washed to remove all possible particles. This procedure allowed recovering the material attached to the body of the honeybees without affecting their integrity. After this procedure, the filters were treated with 33% H_2O_2 at 60 °C for 24 h to digest the remains of organic matter. Most materials coming from insect bodies were destroyed using this procedure. Finally, filters were dried at 60 °C, placed into glass Petri dishes and sealed to avoid contamination during visual inspection and particle count.

6.2.3. Quantification and identification of microplastics

MPs samples, kept inside closed glass Petri dishes to avoid contamination, were observed using a stereomicroscope Euromex-Edublue equipped with camera and ImageFocus 4 software. All particles measuring <5 mm along their lager dimension were photographed and classified by morphological characteristics: size, shape, and colour.

When reporting shape, researchers in the field of plastic litter use categories like fragments or films for irregular shaped particles, and fibres and filaments for high aspect ratio debris as well as other specific categories that vary somehow among research groups. In our case, we classified microplastics into fragments, films, fibres, and filaments with the characteristics detailed below (Frias and Nash, 2019; Lusher et al., 2017). Fragments were defined as particles with irregular shape and edges, with possible origin in the fragmentation of larger particles. Films are also irregular, but thinner than fragments and with flexible aspect. Fibres and filaments are characterized because their larger dimension (length) is considerably higher than the second projected area dimension (width or diameter). For the purpose of this work, we considered fibres or filaments those microparticles with aspect ratio (length/width) > 4; otherwise, they were classified as fragments or films. Filaments differentiate from fibres because they have the same thickness along their length and show sharp ends (Magni et al., 2019). We computed the projected dimensions of all microparticles using micrographs and the image analysis program ImageJ. The equivalent diameter was calculated for particles and films as projected area diameter, and for fibres and filaments as aerodynamic diameter calculated as follows (Prodi et al., 1982):

$$D = \frac{3W}{2}\sqrt{\frac{\rho}{\frac{0.385}{\ln(2\beta)-0.5}+\frac{1.23}{\ln(2\beta)+0.5}}}$$

where β is the aspect ratio based on projected dimensions (length/width or L/W), W the width of diameter of the fibre or filament and ρ its relative density (taken as 1.000).

Photographs were processed with ImageJ software for obtaining projected particle length and width. All microparticles suspected of being MPs were analysed using micro–Fourier Transform Infrared Spectroscopy (micro-FTIR). For it, particles were deposited on KBr disks and measured in transmission mode using a Perkin-Elmer Spotlight 200 Spectrum two apparatus with mercury cadmium telluride detector that allows obtaining optimum results in the mid-infrared region. The conditions for the analyses were: 50 µm spot size, a minimum of 20 scans, resolution of 8 cm^{-1} and a spectral range 550-4000 cm^{-1}. Resultant spectra were processed through OMNIC 9 software and compared with

existent databases and with our own spectra. Matching >70% was considered enough for positive identification of plastic materials (Liu et al., 2019b). In some cases, spectra with noisy signals and matching >65% were judged satisfactory based on the identification of representative bands as explained below and in Supplementary material.

6.2.4. Prevention of procedural contamination

The measures taken to avoid sample contamination included field and laboratory procedures. For the collection of honeybees, only one person was involved wearing controlled clothes from non-synthetic materials and placed against the wind. Plastic materials such as synthetic polyester or acrylic goods were avoided, and no plastic material was used in hives. Nitrile gloves and metal tweezers were used if needed. Glass material was used to store the samples, previously cleaned carefully with pure water. During laboratory manipulation, only glass and steel material was used previously cleaned with ultra-pure water (filtered through a 0.22 µm filter, particle and bacteria free) a minimum of three times. Glass beakers were always covered with aluminium foil using specifically designed metallic cages. Prior to use, all laboratory materials were wrapped with aluminium foil and heated to 450 °C for 4 h to remove all possible contamination from fibres or other potentially interfering materials. The clothes worn by laboratory personnel were 100% cotton with non-typical colours. During all field and laboratory sample manipulation steps, Petri dishes were kept open with glass fibre filters in order to identify possible contamination from the environment. Plastics like those found in procedural controls were not considered.

6.2.5. Statistics

Pearson correlation was used to assess matching between samples and database or standards. A one-way ANOVA coupled with Tukey's HSD (honestly significant difference) post-hoc test was performed for comparison of means. The p-value for statistically significant difference was 0.05.

6.3. Results and discussion

By inspecting the whole set of sealed filters, we selected 125 microparticles <5 mm along their larger dimension of putative anthropogenic origin, which were individually studied using micro-FTIR. We only excluded particles of clearly natural origin. Fig. 6.2 shows size distribution and the relative abundance of fragments, films, fibres and filaments among MPs. The dominant shapes of MPs were fragments (52%) followed by fibres (38%) with lower amounts of filaments and films. Clearly, fibres and filaments displayed lower equivalent size because of their small diameter or lower projected dimension, which was in the 10.5-69.9 µm range. The average equivalent diameter was 64 ± 39 µm for fibres and 234 ± 156 µm for fragments, the intervals corresponding to standard deviations.

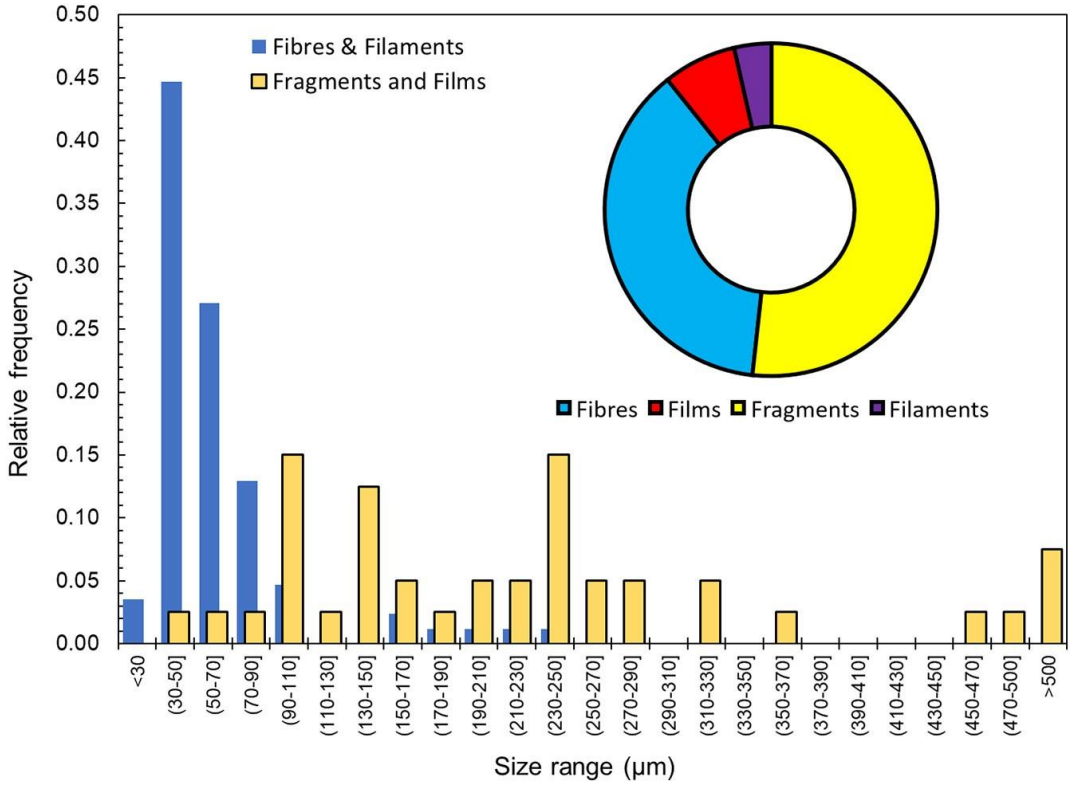


Figure 6.2. Size distribution of equivalent diameter. Blue bars for the sum of fibres and filaments, and orange for the sum of fragments and films. The inset shows the distribution of the different classes of microparticles: fibres, filaments, fragments and films. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The data on size distribution and shape are difficult to compare with literature data because atmospheric transport of MPs is new research area with still very limited data.

Specifically, concerning shape, there is no agreement in the available literature and fibre and fragments are found dominant depending on the source (Cai et al., 2017; Klein and Fischer, 2019).

All microparticles were analysed by micro-FTIR as described above resulting in 56 microparticles positively identified as MPs (21 fibres, 2 filaments, 29 fragments and 4 films). The MPs identified were polyester (PL), polyethylene (PE), polyvinyl chloride (PVC), polyurethane (PU), epoxy resin (EP), polyvinyl acetate (PVA), polyacrylonitrile (PAN), polyoxymethylene (POM), polypropylene (PP), polystyrene (PS), polysulfone (PSU), polytetrafluoroethylene (PTFE) and polyamide (PA). The absolute abundance of MPs is shown in Fig. 6.3. Clearly PL, >80% of which were fibres and filaments, was dominant, followed by PE and PVC, which were mostly fragments and films.

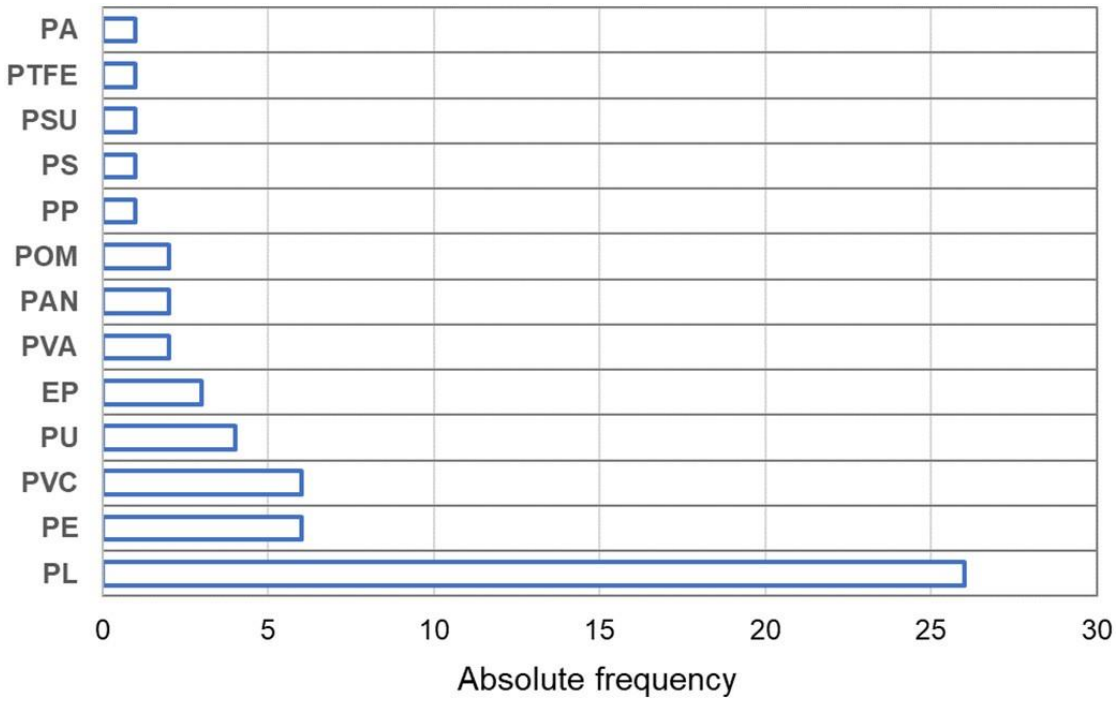


Figure 6.3. Chemical composition of MPs sampled in honeybees. PL: Polyester; PE: Polyethylene; PVC: Polyvinyl chloride; PU: Polyurethane; EP: Epoxy resin; PVA: Polyvinyl acetate; PAN: Polyacrylonitrile; POM: Polyoxymethylene; PP: Polypropylene; PS: Polystyrene; PSU: Polysulfone; PTFE: Polytetrafluoroethylene; PA: Polyamide

The identification of sampled microparticles showed, besides MPs, the presence of cotton, wax, vegetal debris, and different parts of honeybee bodies together with some

particles identified with insufficient evidence. Besides, we found 30 cotton fibres with non-natural colours, which included 19 blue, 7 black, and 1 red, apart from several more white, transparent and grey. In controls, we identified only one blue cotton fibre, possible from textile origin. Overall, the results indicated the presence of natural fibres of non-natural colours that can also be considered a tracer of anthropogenic pollution (González-Pleiter et al., 2020a). Natural fibres with evidence of industrial origin result in environmental concerns due to their content of dyes and other chemicals and are a class of ubiquitous airborne anthropogenic pollutants that received limited attention so far (Stanton et al., 2019).

Fig. 6.4 shows the FTIR spectra of six representative MPs; two fragments, 1 film, 2 fibres and 1 filament. The FTIR spectra of fibres and fragments showed the typical bands of the materials identified. The main features are as follows. The broad band centred at 3350 cm^{-1} and the absorption at 1720 cm^{-1} corresponded to the N-H stretching vibration and the stretching band of -C=O in the urethane bond (Fig. 6.4A). The characteristics bands at 2914 cm^{-1}, 2847 cm^{-1}, 1460 cm^{-1}, and 715 cm^{-1} of PE (Fig. 6.4B). The bands of C=O vibration at 1715 cm^{-1}, the stretching of the aromatic ring at 1410 cm^{-1} and the carboxylic anhydride from PL at 1021 cm^{-1} (Fig. 6.4C). The features of PVC (Fig. 6.4D) include the C-H stretching bands at 2850-2920 cm^{-1}, and the typical small shoulder at from C-Cl stretching at 840 cm^{-1}. The blue fibre of Fig. 6.4E could be identified as PAN based on the CΞN stretching band at 2240 cm^{-1}, and the aliphatic bands from methylene C-H stretching. The bands of EP that appear in Fig. 6.3F are the C-O-C epoxy vibration at 920 cm^{-1}, and the characteristic O-H stretching in the 3500-3200 cm^{-1} range. Other details about the identification are provided as Supplementary material. FTIR standards are provided in Fig. S6.2 (SM). Fig. 6.4 also shows the micrographs of the same fibres and fragments. For comparison, the typical hair length in honeybees is about 1 mm (Roquer-Beni et al., 2020).

The results reporting the abundance of MPs on honeybees are shown in Fig. 6.5 for all the locations studied in this work. Fig. 6.5A shows the number of MP per 100 honeybees for the different apiaries. The average for all apiaries is also indicated as a dashed line.

Fig. 6.5B shows the boxplot of MPs relative to the number of sampled honeybees for locations grouped into urban (a), suburban (b) and rural areas (c).

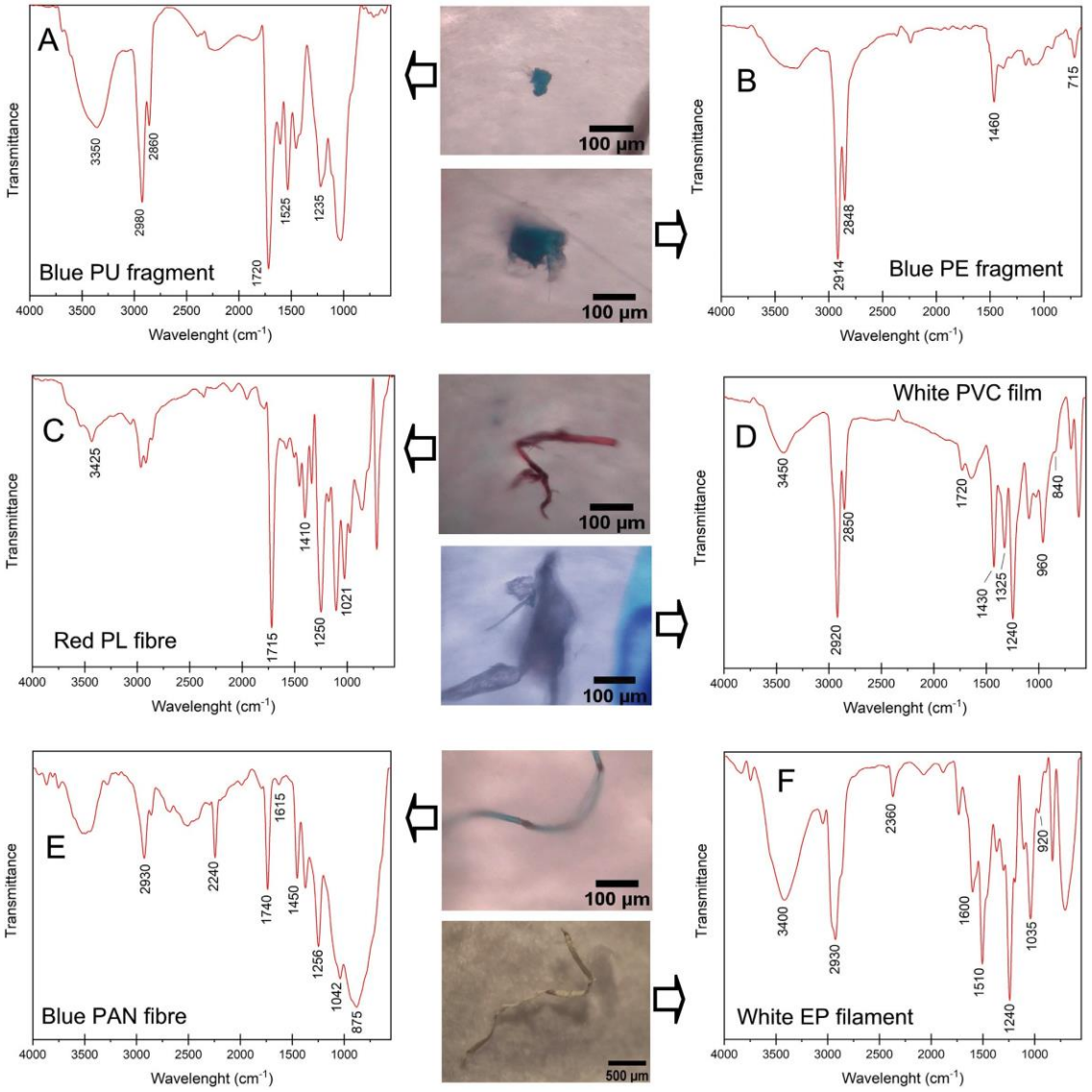


Figure 6.4. Micro-FTIR spectra and representative images of some of the MPs found in this work. (A) blue PU fragment, (B) blue PE fragment, (C) red PL fibre, (D) white PVC fragment, (E) blue PAN fibre and (F) white EP fragment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Our work showed the maximum concentration of MPs in the centre of Copenhagen (Location 1). The limited overall variability could be in part attributed to the fact that all points marked a (urban) were separated by less than 4.3 km, which is inside the foraging radius of Apis mellifera, reported as 5–6 km, with 50% of the workers foraging 6 km and 10% more than 9.5 km from their hive (Beekman and Ratnieks, 2000). Locations 18 & 19 were separated 6.5 km from the city and, noteworthy, locations 13 & 17 and 10 were

separated from the centre of Copenhagen by 20 and 25 km respectively. However, in all cases there were population nucleus nearby. For example, location 10 is a rural place, but surrounded by several towns including Herning, with 47,000 inhabitants. In all cases except location 16, a minimum of two neighbouring hives were sampled. The results showed that deviations from hives in the same apiary did not differ more than 60% (minimum 2%, 35% in average) expressed as MPs/bee, which supports the strength of the sampling procedure. Another reason for the relatively homogeneous concentrations obtained may be the homogenization produced by the wind dispersion of microplastics over large areas as explained below.

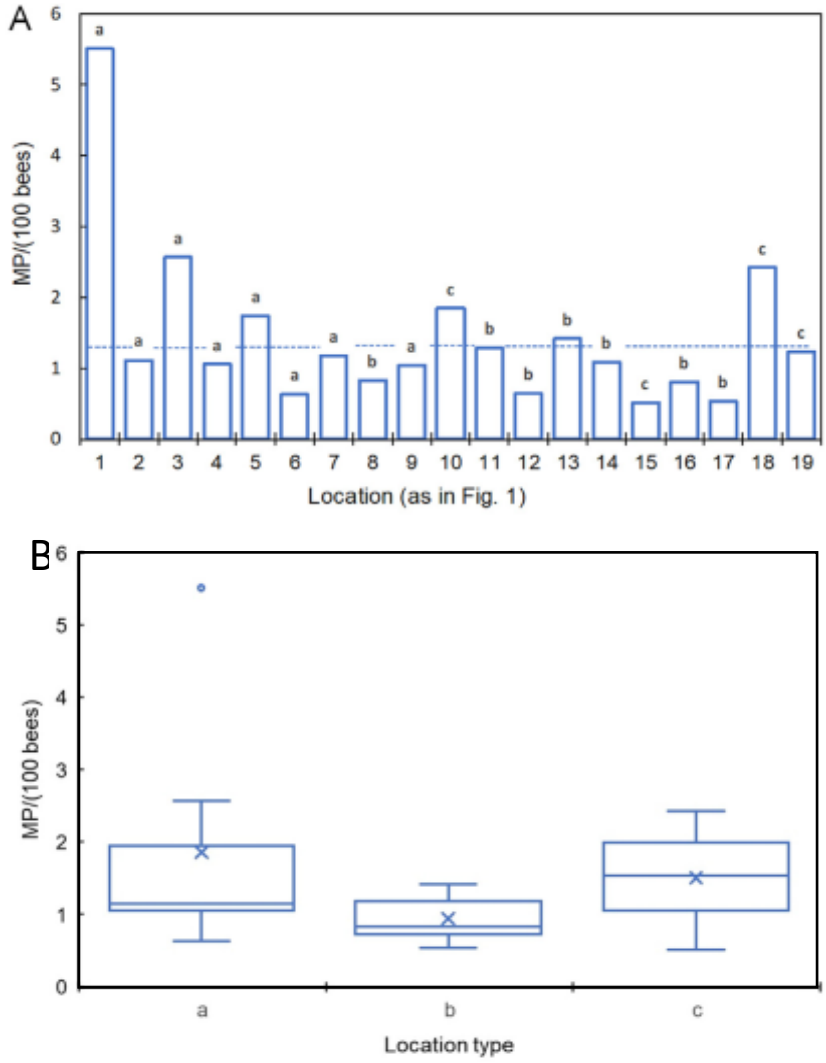


Figure 6.5. Relative abundance of MP per insect for all sampling points as indicated in Fig. 1. Urban zones with high population (a), suburban areas (b) and rural zones (c). In Panel B the abundance is represented for each type of zone as boxplot with crosses representing the average values. The dashed line in Fig. 5A represents the average for all apiaries.

Our results can be interpreted in the light of other studies on the atmospheric deposition of MPs. The few data available point to an abundance of MPs decreasing when moving away from urban areas (González-Pleiter et al., 2020a; Liu et al., 2019a). Besides, it has been recently shown that plastic fragments and small fibres can be transported by wind across long distances before being deposited even in very distant places (Brahney et al., 2020). Brahney et al. (2020) studied wet and dry deposition of MPs in remote areas of the United States. Their analysis of air mass trajectories suggested that urban centres are one of the main sources for at least wet-deposited MPs and that small plastic debris can be transported long distances before being deposited. Recently, the first direct evidence of the presence of MPs at high altitude showed a higher concentration in the air above cities in comparison with rural areas (González-Pleiter et al., 2020a). Overall, the available data show that MPs are emitted in densely populated areas and reach high altitude, which allows their transportation by wind to distant places.

The data available on the atmospheric precipitation of MPs in a Chinese city (Dongguan) showed an average deposition rate in the 31 ± 8 to 43 ± 4 MP m^{-2} day^{-1} range for MPs between 200 and 4200 µm (Cai et al., 2017). Allen et al. (2019) reported, for a remote area in the Pyrenees, a deposition rate of 365 ± 69 MP m^{-2} day^{-1} for 25-3000 µm MPs. In an urban and peri-urban study in Hamburg, Germany, Klein and Fisher reported deposition rates in the 136.5-512.0 MP m^{-2} day^{-1} range for sizes <63 µm and up to 5000 µm (Klein and Fischer, 2019). Accordingly, and despite the methodological differences among studies that make comparisons difficult, the literature data point towards deposition rates from tens to hundreds of MPs per square metre and day without important differences between urban, rural and less populated areas.

The fact that MPs can be dispersed long distances that may reach thousands of kilometres away from their initial point of release, explain that MPs reach remote areas and also that samples taken from rural areas, apparently safe from most of the emissions associated to human activity reported similar values to samples taken inside densely populated cities. In our case, the samples recovered from honeybees in rural areas near Copenhagen contained relatively high number of MPs, not too different from those hiving in central Copenhagen. This finding is also consistent with the westward winds, dominant during the sampling period as shown in Fig. S6.1 (SM). Using honeybees

as active MP samplers opens a new research pathway to compare sampling in environments with different practices and urban/industrial pressure. They offer the possibility to compare with dry and wet deposition campaigns or other types of research on the occurrence of MPs. Honeybees are particularly adapted to transport particulate material because of their morphological structures and their grooming behaviour for pollen collection, which, together with their foraging range and worldwide distribution, make them ideal as living samplers for environmental monitoring. When flying, their bodies become positively charged with static electricity, so that when the bee lands on a flower, the pollen particles stick to their static-charged hair and the same happens with other microparticles in their environment (Negri et al., 2015). Honeybees may complement other sampling systems in common use for monitoring air quality. It is important to note that very limited research has been performed so far on the atmospheric transport of MPs. Another feature of our research refers to the translocation of MP pollution to honeybee products like honey or beeswax, which could raise concerns about human health. Once, we demonstrated that honeybees may act as collectors for MP pollution, further studies could involve the use of in-hive passive samplers capable to collect MPs from a large number of honeybees in a less intrusive way. Further research is also needed on the occurrence of MPs in honeybee products and to assess the possible use of honeybees or other active samplers to assess human exposure to microplastics.

6.4. Conclusions

Honeybees were collected and processed from nineteen different apiaries from the centre of Copenhagen and other locations including suburban and rural areas. Our results showed the presence of MPs in all locations. The highest load corresponded to apiaries located in Copenhagen. Nevertheless, honeybees from suburban and rural areas also bore a considerable number of MPs adhered to their bodies. The presence of urban settlements inside the foraging range of honeybees, and the wind dispersion of MPs are the probable reasons explaining the presence of MPs in honeybees from rural hives.

Micro-FTIR analysis confirmed the presence of thirteen synthetic polymers, the most frequently of which was polyester. Fragments followed by fibres were the dominant shapes. Industrially processed cotton fibres were also frequently found. The results proved the presence of MPs and other anthropogenic materials adhered to the body of the honeybees, allowing their use as active samplers. This work demonstrates for the first time the possibility of using honeybees (Apis mellifera) as bioindicator for the presence of MPs in the environment.

6.5. References

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

Ballesteros, S., Domenech, J., Barguilla, I., Cortés, C., Marcos, R., Hernández, A., 2020. *Genotoxic and immunomodulatory effects in human white blood cells after ex vivo exposure to polystyrene nanoplastics*. Environ. Sci. Nano 7, 3431–3446.

Bargańska, Ż., Ślebioda, M., Namieśnik, J., 2016. *Honeybees and their products: bioindicators of environmental contamination.* Crit. Rev. Environ. Sci. Technol. 46, 235–248.

Beekman, M., Ratnieks, F.L.W., 2000. *Long-range foraging by the honeybee, Apis mellifera L*. Funct. Ecol. 14, 490–496.

Brahney, J., Hallerud, M., Heim, E., Hahnenberger, M., Sukumaran, S., 2020. *Plastic rain in protected areas of the United States*. Science 368, 1257–1260.

Bujnicki J, Dykstra P, Fortunato E, Grobert N, Heuer R, Keskitalo C, Nurse P. *Environmental and Health Risks of Microplastic Pollution. Scientific Opinion 6/2019.* European Commission, Directorate-General for Research and Innovation, Group of Chief Scientific Advisors, Brussels, 2019.

Cai, L., Wang, J., Peng, J., Tan, Z., Zhan, Z., Tan, X., Chen, Q., 2017. *Characteristic of microplastics in the atmospheric fallout from Dongguan city, China: preliminary research and first evidence*. Environ. Sci. Pollut. Res. 24, 24928–24935.

Calatayud-Vernich, P., Calatayud, F., Simó, E., Picó, Y., 2018. *Pesticide residues in honeybees, pollen and beeswax: assessing beehive exposure*. Environ. Pollut. 241, 106–114.

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

Devillers, J., Pham-Delègue, M.-H., 2002. *Honeybees. CRC Press, Estimating the Environmental Impact of Chemicals.*

Dżugan, M., Wesołowska, M., Zaguła, G., Kaczmarski, M., Czernicka, M., Puchalski, C., 2018. *Honeybees (Apis mellifera) as a biological barrier for contamination of honey by environmental toxic metals.* Environ. Monit. Assess. 190, 101.

Enyoh, C.E., Verla, A.W., Verla, E.N., Ibe, F.C., Amaobi, C.E., 2019. *Airborne microplastics: a review study on method for analysis, occurrence, movement and risks.* Environ. Monit. Assess. 191, 668.

Evangeliou, N., Grythe, H., Klimont, Z., Heyes, C., Eckhardt, S., Lopez-Aparicio, S., Stohl, A., 2020. *Atmospheric transport is a major pathway of microplastics to remote regions*. Nat. Commun. 11, 3381.

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

Gajger, I.T., Kosanović, M., Oreščanin, V., Kos, S., Bilandžić, N., 2019. *Mineral content in honeybee wax combs as a measurement of the impact of environmental factors*. Bull. Environ. Contam. Toxicol. 103, 697–703.

GESAMP. *Sources, fate and effects of microplastic in the marine environment: part two of a global assessment*. In: Kershaw PJ, Rochman CM, editors. Rep. Stud. GESAMP No. 93. (IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP) Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, 2016, pp. 220.

GESAMP. *Guidelines for the monitoring and assessment of plastic litter in the ocean.* In: Kershaw PJ, Turra A, Galgani F, editors. Rep. Stud. GESAMP No. 99. IMO/FAO/ UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP/ISA Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, 2019, pp. 130.

Gigault, J., Halle, At, Baudrimont, M., Pascal, P.-Y., Gauffre, F., Phi, T.-L., El Hadri, H., Grassl, B., Reynaud, S., 2018. *Current opinion: what is a nanoplastic?* Environ. Pollut. 235, 1030–1034.

González-Pleiter, M., Edo, C., Aguilera, Á., Viúdez-Moreiras, D., Pulido-Reyes, G., González- Toril, E., Osuna, S., de Diego-Castilla, G., Leganés, F., Fernández-Piñas, F., Rosal, R., 2020a. *Occurrence and transport of microplastics sampled within and above the planetary boundary layer*. Sci. Total Environ. 143213.

González-Pleiter, M., Velázquez, D., Edo, C., Carretero, O., Gago, J., Barón-Sola, Á., Hernández, L.E., Yousef, I., Quesada, A., Leganés, F., Rosal, R., Fernández-Piñas, F., 2020b. *Fibres spreading worldwide: microplastics and other anthropogenic litter in an Arctic freshwater lake*. Sci. Total Environ. 722, 137904.

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

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

Hermsen, E., Mintenig, S.M., Besseling, E., Koelmans, A.A., 2018. *Quality criteria for the analysis of microplastic in biota samples: a critical review.* Environ. Sci. Technol. 52, 10230–10240.

Horton, A.A., Barnes, D.K.A., 2020. *Microplastic pollution in a rapidly changing world: implications for remote and vulnerable marine ecosystems*. Sci. Total Environ. 738, 140349.

Kedzierski, M., Lechat, B., Sire, O., Le Maguer, G., Le Tilly, V., Bruzaud, S., 2020. *Microplastic contamination of packaged meat: occurrence and associated risks.* Food Packag. Shelf Life 24, 100489.

Klein, M., Fischer, E.K., 2019. *Microplastic abundance in atmospheric deposition within the metropolitan area of Hamburg, Germany*. Sci. Total Environ. 685, 96–103.

Li, J., Liu, H., Paul, Chen J., 2018. *Microplastics in freshwater systems: a review on occurrence, environmental effects, and methods for microplastics detection.* Water Res. 137, 362–374.

Liu, K., Wang, X., Fang, T., Xu, P., Zhu, L., Li, D., 2019a. *Source and potential risk assessment of suspended atmospheric microplastics in Shanghai*. Sci. Total Environ. 675, 462–471.

6.6. Supplementary material of Chapter 6

Contents

Supplementary Information on the analytical determination of microplastics. (Referred to Fig. 6.4 in the body of the article). The FTIR spectrum of PU blue fragment (Fig. 6.4A) displayed the typical broad band centred at 3350 cm^{-1} that corresponds to the N-H stretching vibration of the urethane bonds. The bands in the 2920-2860 cm^{-1} region are associated to vibrational modes of the -CH_2 groups and the absorption at 1720 cm^{-1} corresponds is the characteristic stretching band of the -C=O group of the urethane bond. The characteristic bands of the CN and C-O-C groups appeared at 1525 cm^{-1} and 1235 cm^{-1}, while the region just above 1000 cm-1 displayed bands attributed to the N–CO–O and other typical absorption of polyurethanes (Demétrio-da-Silva, et al. 2013). The blue fragment of Fig. 6.4B was clearly identified as polyethylene (PE) in view of the presence of its four characteristics bands at 2914 cm^{-1}, 2847 cm^{-1}, 1460 cm^{-1}, and 715 cm^{-1} (Gulmine et al., 2002). The red polyester (PL) fibre (Fig. 6.4C) showed the characteristic bands of C=O vibration at 1715 cm^{-1}, the stretching of aromatic ring at 1410 cm^{-1} and the carboxylic anhydride at 1021 cm^{-1}. The white film (of Fig. 6.4D) showed the characteristic features of polyvinyl chloride (PVC), which include the bands of C-H stretching at 2850-2920 cm^{-1}, CH_2 deformation at 1325 cm^{-1}, CH rocking at 1240 cm^{-1}, trans-CH wagging at 960 cm^{-1} and the typical small shoulder at from C-Cl stretching at 840 cm^{-1}. The carbonyl stretching at 1720 cm^{-1} may indicate ageing through dehydrochlorination and oxidation processes or the presence of stabilizers like phthalates. The band at 3300-3500 corresponds to a hydrated fragment. The blue fibre of Fig. 6.4E could be identified as PAN based on the bands at 2240 cm^{-1}, which corresponded to the C≡N stretching, and the aliphatic bands at 2930 cm^{-1} and 1450 cm^{-1} from methylene C–H stretching. The weak absorption at 1615 cm^{-1} could be attributed to C=C probably from a butadiene copolymer (Ju et al., 2013). Finally, the white filament of Fig. 6.4F was attributed to epoxy resin (EP). The C–O–C from the epoxy group appeared at 920 cm^{-1}. The band at 2930 cm^{-1} corresponded to methylene C–H stretching and the characteristic O–H stretching band was clearly observed in the 3500–3200 cm^{-1} range. The stretching of benzene ring appeared at 1600 cm^{-1}, and 1510 cm^{-1} and the absorptions at 1240 cm^{-1} and 1035 cm^{-1} corresponded to the C–O stretching in the backbone of the polymer. Reference spectra are shown in Fig. S6.2 (SM).

Demétrio-da-Silva V, dos Santos LM, Subda SM, Ligabue R, Seferin M, Carone CLP, Einloft S. Synthesis and characterization of polyurethane/titanium dioxide nanocomposites obtained by in situ polymerization. Polym. Bull. **2013**; 70: 1819-1833.

Gulmine JV, Janissek PR, Heise HM, Akcelrud L. Polyethylene characterization by FTIR. Polymer Testing **2002**; 21: 557-563.

Ju A, Guang S, Xu H. Effect of comonomer structure on the stabilization and spinnability of polyacrylonitrile copolymers. Carbon **2013**; 54: 323-335.

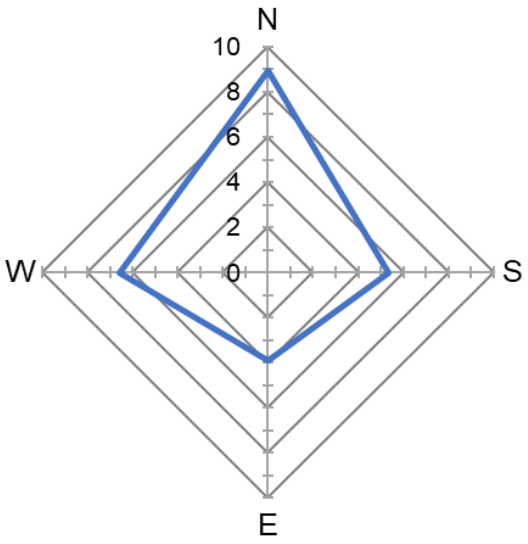


Figure S6.1. Compass rose for the average winds during daylight hours on sampling days. The scale is the average wind velocity in km/h (data from the meteorological observatory at Copenhagen Airport)

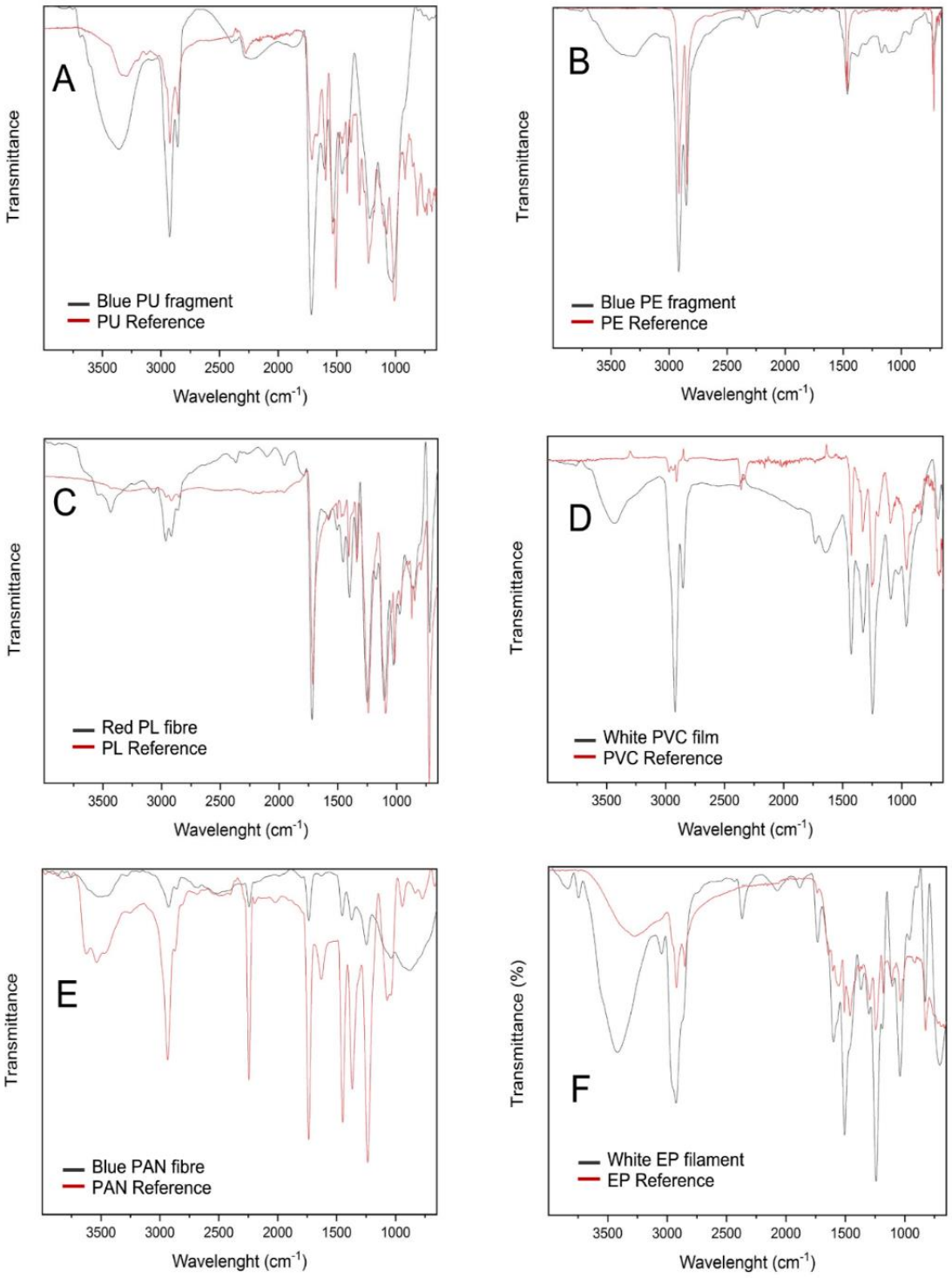


Figure S6.2. Reference spectra for the microplastics identified in Fig. 5.4 in the body of the article.

CHAPTER 7. MICROPLASTICS IN ORGANIC COMPOST: INFLUENCE OF WASTE COLLECTION SYSTEM AND COMPOSTING METHODOLOGY

7.1. Introduction

Plastics were introduced by the middle of the 20th century and progressively gained a privileged position in our lives due to their outstanding properties, which allowed the rapid substitution of other materials and a plethora of new uses associated to the demands of our modern society.

The current worldwide production of plastic is estimated in 368 million tonnes with main uses in packaging and building & construction, which represent together 60% of the plastic demand in the EU plus United Kingdom, Norway and Switzerland (PlasticsEurope, 2020). For the same geographical unit, the amount of plastic waste collected roughly represents half of the total amount produced and still 7.2 million tonnes of plastics are sent to landfills (PlasticsEurope, 2020). Improper waste management and the wearing of plastic goods during use lead to the accumulation of plastic debris in all environmental compartments. The problem is far from new. The early findings of plastic debris in the ocean can be tracked back to the 1970s, but during the last decade the concern about the dissemination of small and very small plastic fragments attracted substantial attention from researchers and even from the general public (Ryan, 2015). Small plastics are termed microplastics (MPs) if their larger dimension is <5 mm with a lower boundary of 1 µm below which, plastic particles produced from the fragmentation of larger debris are usually classified as nanoplastics (NPs) (GESAMP, 2019; Gigault et al., 2018). Although a considerable body of evidence exists on the occurrence of large plastic debris, the data on the smaller fractions of MPs are still scarce due to the difficulties associated to their separation and analysis (Xu et al., 2020).

Plastic debris enter the environment through different ways. Atmospheric dissemination has recently received attention as small airborne debris, mainly consisting of synthetic fibres, may travel long distances from their source (González-Pleiter et al., 2021). The role of wastewater treatment plants is also well-known. Domestic and industrial wastewaters contain a large number of fibres, MPs used in personal care products, tyre wear debris and other MPs that are not completely removed in treatment plants (Ali et al., 2021; Liu et al., 2021). The typical removal efficiency of current wastewater treatment process is >90% meaning that most MPs contained in raw

wastewater accumulate in sludge (Xu et al., 2021). In fact, the use of wastewater sludge as soil amendment could be a contributor to MPs dissemination into soil, and from it to other environmental compartments (Edo et al., 2020a; Gao et al., 2020). Besides, agricultural practices like mulching may result in involuntary dissemination of plastic debris to the environment. However, the information available on the sources, fate and effects of MPs in soil are scarce (Yuanqiao et al., 2020). Only limited data are available due to the reduced number of studies accomplished so far and because of the lack of acute effects on biota, which complicates risk assessment (Cheng et al., 2021; Wang et al., 2019; Zhu et al., 2018). For the case of fibres, the information is even more scarce (Selonen et al., 2020). Besides, MPs release dangerous additives, sorb other pollutants and their fragmentation is known to produce smaller and more concerning fragments including NPs (Bueno-Ferrer et al., 2010; Guo et al., 2020; Tagg and Labrenz, 2018).

Apart from sludge and concerning agricultural practices, digestates and compost produced from the Organic Fraction of Municipal Solid Wastes (OFMSW) are used throughout the world as soil amendment due to their content in stabilized organic matter and nutrients (Carabassa et al., 2020). The current legal scenario for the waste management sector forces improvements in selective collection, total amount of collected OFMSW and recycling procedures (Laso et al., 2019). The Directive 2018/851 makes the collection of OFMSW mandatory for all member states from 2023. Together with organic wastes, there is the possibility of collecting other types of wastes with similar biodegradability and compostability, which includes compostable bioplastic materials certified according to the EN13432. The implementation of adequate waste management policies aimed to promote the efficient separate collection of OFMSW, diverting it out of landfills, constitutes a key element in this new framework. The total generation of municipal solid waste ranges from 0.11 to 4.54 (average 0.74) kg per person per day (Kaza et al., 2018), from which the OFMSW represents approximately 40% by weight. Therefore, the efficiency of separate collection systems, the biological treatment technology applied, and the requirements for the quality of compost are key drivers for sustainable waste management. In terms of OFMSW collection, different strategies can be implemented, ranging from the street bin containers to personalized door-to-door collection systems with different impacts on product quality. The collected

OFMSW can be treated in composting or in combined anaerobic/aerobic treatment facilities with a variety of different layouts and technologies.

In this work, the presence of plastic debris in samples of composted OFMSW recovered and treated in five different industrial facilities (four composting plants and one using anaerobic digestion followed by composting) was studied. The purpose was to identify whether the collection and treatment systems affect the concentration of MPs in final refined compost. In this framework the efforts were mainly focused to quantify the number of plastic particles contained in the final OFMSW compost, their typology and polymer composition, with emphasis on the fraction <1000 µm. Special attention was paid to compostable biopolymers due to their role as a tool to promote a high-quality collection of OFMSW especially in door-to-door collection systems.

7.2. Experimental section

7.2.1. Materials

Compost samples were obtained from five different composting facilities located in the northeast of Spain. The samples were taken in five consecutive months in 2021 (from February to June) and consisted of two replicates of about 200 g each per selected plant. All samples were collected after the refining operation and following the procedure to avoid contamination described below. Samples were stored in sealed aluminium bags for their transportation to the laboratory.

7.2.2. OFMSW facilities and collection systems

The facilities were selected based on the different technologies used and the diverse strategies followed to collect organic wastes. Their main characteristics are listed in Table 7.1. The OFMSW collection systems taken into consideration in this study were based on different combinations of street bin and door-to-door collection. In street bin collection, containers for organic waste are located at curbside and are periodically washed out and the collected organic waste transferred to the composting plant. This system does not permit any control concerning the disposal quality and does not guarantee that citizens use compostable bags. In door-to-door collection systems, citizens place twice a week their organic waste (small volume, usually 7–10 L) in specific places from where it is collected by dedicated trucks. In this collection system, the use

of compostable bags is encouraged or mandatory. In all cases, the waste collected is a mixture of domestic and commercial activities (including restaurants), with higher intensity for the later in more densely populated areas. Once in the plant, the OFMSW undergo a sequence of pre-treatment steps with the purpose of removing all non-compostable or oversized materials. The intensity of pretreatment systems is directly linked to the need of removing non-compostable materials, which is more or less stringent depending on the biological technology adopted to treat the OFMSW. Usually, a pretreatment step is optional in composting, but it is a requirement in anaerobic treatment followed by composting (Plant P2 in our study). In plants P1–P4 the OFMSW are mixed and sieved (80 mm) and a magnetic separator is used to remove ferromagnetic metals. P5 is somewhat special because it serves a small community with high citizenship commitment, which includes the exclusive use of compostable bags besides door-to-door collection. The composting technologies used in the different plants are conventional and consist of open windrows, aerated static piles, and in-vessel tunnels.

Table 7.1. Summary of the main characteristics of composting plants and input materials

Plant	P1	P2	P3	P4	P5
Capacity (tonnes/yr.)	20000	45000	12500	8000	750
Collecting systems	15 % door-to-door; 85 % compost containers	25 % door-to-door; 75 % compost containers	30 % door-to-door; 70 % compost containers	35 % door-to-door; 65 % compost containers	100 % door-to-door
Impurities (%)	18	6	10	7	3
Pretreatment	Sieve and magnetic separator	Sieve, magnetic separator, pulper and grit removal	Sieve and magnetic separator	Sieve and magnetic separator	No pre-treatment
Composting technology	Windrows	Anaerobic digestion + tunnel composting	Aerated static pile	Tunnel + windrows	Aerated static pile
Bulking agent	Local sources (65 %) and agricultural wastes (35 %)	Wood manufacturing companies	Local recycling centres (60 %) and other public bodies	Local public bodies (75 %) and private companies (25 %)	Local recycling centres
Population density (inhab./km^2)	90	4200	1900	2000	20

Table 7.1 shows the amount of non-compostable materials as determined by periodic inspection of 200–250 kg of OFMSW at the entrance of each plant (6–74 independent samples depending on plant size). The (mass) percentage of non-compostable materials listed in Table 7.1 corresponds to the 4th term of 2020 and the first term of 2021, which are the reference periods for the final compost sampled in this work. A detailed characterization of the non-compostable fraction is included as Supplementary material in Table S7.1. Another important factor is the addition of a vegetal fraction as bulking agent to facilitate aeration, and balancing carbon-to-nitrogen ratio (Adhikari et al., 2008). As shown in Table 7.1, bulking agents have different origins, from local wood processing companies to pruning and garden wastes.

7.2.3. Microplastics separation

A total amount of 60 g (30 g/sample in two replicates) was processed for each composting plant and sampling month (a total of 300 g of evaluated material per plant at the end of the study). Compost samples were first processed using size separation with stainless-steel sieves into three different fractions: >3.55 mm (large), 1.00–3.55 mm (medium) and <1.00 mm (small). All samples >1.00 mm were separated in batches of about 10 g each, immersed in ultrapure water and sonicated using an ultrasound disperser (BioBlock Scientific, France) operating at 500 W for 30 s with the aim of separating aggregates of plastics with other particles. Subsequently the samples were filtered through 375 µm stainless steel filters and dried at 60 °C.

The fraction containing particles <1 mm was separated in batches of about 5 g, put in contact with 30 mL of hydrogen peroxide (30% w/v) and kept covered in oven at 60 °C overnight to remove the organic matter that could interfere with visual examination and spectroscopic characterization. Samples were then filtered through 25 µm stainless steel meshes and washed with deionized water to remove residual hydrogen peroxide. The resultant material was put in contact with $ZnCl_2$ solution (concentration 700 g/L, density 1.70 ± 0.05 g/cm^3) to perform a density separation. The suspensions were allowed to settle at least for 1 h and the supernatant filtered again through 25 µm stainless steel filters. The sediment was discarded, and the samples dried at 60 °C before visual inspection.

The methodology used, based in a combination of sieving, oxidative treatment, and density separation used a protocol adapted from similar ones reported elsewhere for water and soil samples (Möller et al., 2021; M.O. Rodrigues et al., 2020). The method as described here yielded high recovery rates (>95% for plastics >500 µm) in line with previously published results (Kang et al., 2020). We did not observe degradation of plastic fragments that could be attributed to oxidative treatment or corrosion due to $ZnCl_2$ and that could impair polymer identification. Besides, as stated below, a quality check performed for bioplastics also showed recovery rates >98% for fragments >500 µm.

7.2.4. Microplastics analysis and classification

All suspected plastic particles were picked up with metal tweezers or a needle, depending on their size, and stored in closed glass containers until spectroscopic characterization. Then they were identified, photographed, and measured using a Euromex-Edublue stereomicroscope equipped with Image Focus software. Additionally, potential plastics were classified into four typologies: fragments, films, fibres, and filaments. Fragments were particles with irregular shape, while films corresponded to particles with one dimension significantly lower than the other. Fibres and filaments had one dimension (length) considerably higher than the other two (Rosal, 2021). In what follows, we considered fibres or filaments particles with length/width > 4 and differentiated filaments from fibres because filaments displayed the same thickness along their length and presented sharp ends (Magni et al., 2019). Fig. S7.1 (Supplementary material, SM) shows a scheme of the steps followed for the separation and identification of plastic particles. The quantitative results were expressed as number of plastic particles per unite mass of compost (dry weight, DW basis).

The chemical identification of polymer type was performed using Fourier Transformed Infrared Spectroscopy (FTIR). In detail, for particles >1 mm Attenuated Total Reflectance (ATR-FTIR) spectra were recorded in a Thermo-Scientific Nicolet iS10 equipped with Smart iTR-Diamond. Particles <1 mm were put on KBr discs and analysed using a PerkinElmer Spotlight 200 Spectrum Two micro-FTIR apparatus equipped with an MCT detector. In both cases 32 scans were used with 8 cm^{-1} spectral resolution and 4000–550 cm^{-1} spectral range. The obtained spectra were compared with the databases

existing in software Omnic 9 (Thermo Scientific) and with our own databases. Pearson correlation was used with a minimum of 65% matching for positive identification as stated elsewhere, except in some specific cases for which a case-by-case study was performed (González-Pleiter et al., 2021; Liu et al., 2019).

7.2.5. Bioplastics determination

The applicability of the procedure reported above to biodegradable and compostable polymers was assessed as follows. Four materials made of compostable bioplastics certified by the European Standard EN-13432 were selected: two commercial bags from local markets, one wrapping film from a commercial food package, and specimens from the material Mater-Bi, supplied by Novamont S.p.A. The biopolymers were cut into small pieces <1 mm, carefully mixed with compost at concentrations of 10 particles/g and separated using the procedure outlined before for the smaller fraction (oxidation with H_2O_2 at 60 °C, filtration and flotation using $ZnCl_2$). The fragments previously added were recovered with efficiency >98%, and correctly identified using FTIR, thereby showing that (micro)bioplastics were resistant to the chemicals used for the separation and that the method of ZnCl2 flotation displayed efficiencies comparable with those reported elsewhere for conventional plastics (Rodrigues et al., 2020). This validation procedure was repeated three times. The infrared spectra of the commercial biodegradable materials used in this work are included in Fig. S7.2 (SM) together with the identification of the main peaks. The absorptions corresponding to starch-based materials are clearly observed and attributed to thermoplastic starch, which is widely used for the manufacture of commercial biodegradable plastics in compounds with different copolymers and fillers.

7.2.6. Contamination control

Several measures were taken to avoid the potential contamination of samples with plastic materials during sampling and laboratory handling. Sample collection was performed by a single person, which used non plastic tools. The samples were quickly introduced in close aluminium bags, labelled, and shipped to the laboratory. All material used were previously cleaned carefully with pure water. During laboratory handling only glass and steel material was used which was previously cleaned with ultrapure water at

least three times. All solutions used for filtering and density separation were prepared with ultrapure water and filtered through 0.45 µm. Glassware was also cleaned with ultrapure water and then heated at 450 °C for 4 h. Clean materials were always covered with aluminium foil also heated to 450 °C for 4 h to remove all possible contamination with potentially interfering materials. In addition, laboratory clothes were made of cotton. During laboratory manipulation, contamination controls consisted of 47 mm Petri dishes containing glass fibre filters, which were kept open near the workplace during all manipulation procedures. Most of the particles (30) that appeared in control filters were white cellulose fibres. The particles identified as plastics in procedural controls were one red polyamide fibre, one yellow acrylic fibre, three polyester fibres (two black and one transparent), and one red polysiloxane filament. The fibres or filaments with similar colour and typology found in the samples that corresponded to the controls with plastic were discarded and not included in the total number of plastic particles given below.

7.3. Results and discussion

7.3.1. Abundance of plastic litter

The average total amount of compost processed per each plant was 297.9 ± 10.1 g during the five-month study with an average of 59.6 ± 1.3 g of compost per plant per month. The visual inspection of samples led to a total number of 10563 particles classified as potential impurities from which a sub-fraction of 1532 particles was analysed by ATR-FTIR or micro-FTIR depending on their size. The subsample size (15%) was calculated to allow a maximum error < 2.5% in the estimation of composition with 95% confidence intervals, as shown elsewhere (Kedzierski et al., 2019). FTIR analyses identified 1357 particles consisting of synthetic polymers distributed among all typologies, which represented >85% of the particles analysed. Overall, fibres represented the dominant shape (42.7%) followed by fragments (31.2%), films (22.1%) and filaments (3.9%). Fragments and films were treated together as well as fibres and filaments because of the methodology used to calculate equivalent diameters from projected images. For fragments and films, the equivalent diameter was that of the circle with the same projected area. For fibres and filaments, the equivalent diameter used was the aerodynamic diameter calculated for the average density of the most common

polymers (Rosal, 2021). Fig. 7.1 shows the relative frequency of all plastic litter detected (Fig. 7.1A) and for the lower size fraction (Fig. 7.1B, <5000 µm). The same data disaggregated for the different plants are presented in Fig. S7.3 (SM).

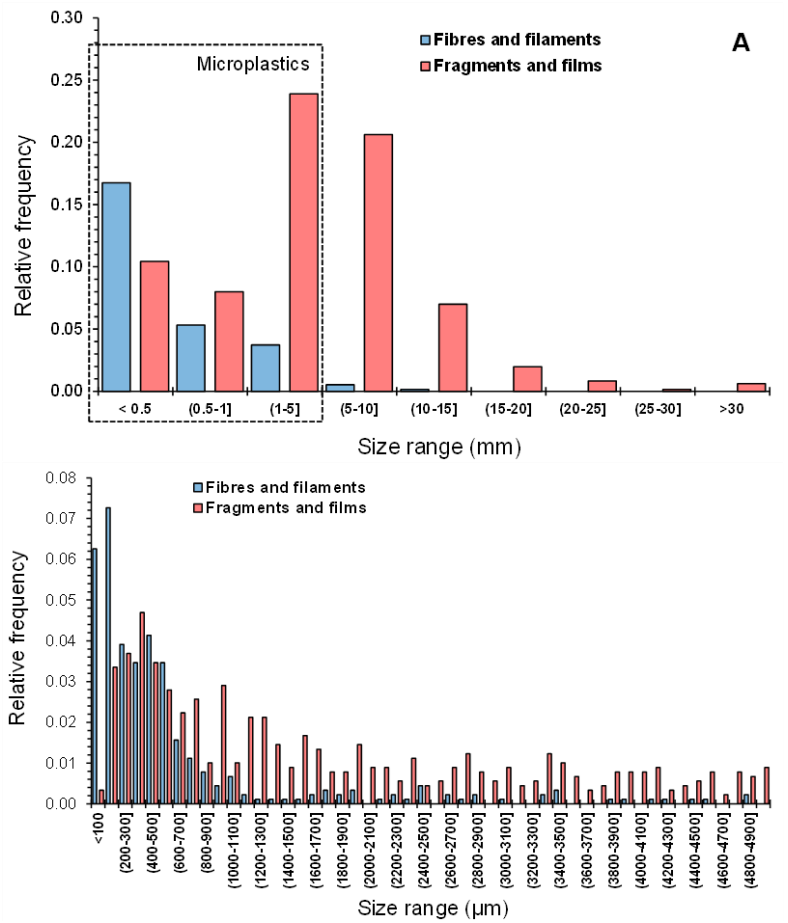


Figure 7.1. (A) Size distribution for the plastics sampled in this work (all plants, all samples) and (B) for the MPs (all plants, all samples, < 5 mm). Size was calculated as equivalent diameter as indicated in the text.

The size distribution of plastic impurities was markedly dependent on their typology. While almost all fibres and filaments (97.4%) were below 5 mm, only 57.6% of particles and films were below that threshold. Plastic particles with equivalent diameter < 2 mm represented 89.6% and 37.7% of the total amount of fibres-filaments and fragments-films respectively. These cutoffs are relevant because impurities in compost, which include metals, glass, and plastics, with size from 2 mm to 5 mm are legally limited in a number of standard methods for the assessment of compost quality.

Sometimes this is done by establishing a maximum concentration for the sum of impurities with a given particle size, while some regulations specify individual limits for

films and other plastic materials (Saveyn and Eder, 2014). Fig. 7.1 shows that most plastic particles corresponded to the smaller size ranges with clear prevalence of MPs (equivalent diameters <5 mm). For the case of fibres, the most abundant sizes were in the range of several hundreds of microns or below.

7.3.2. Chemical composition of plastics in compost

Chemical analyses (ATR-FTIR and micro-FTIR) allowed the identification of 16 different synthetic polymers or groups of polymers in 1322 particles. From the other 210 inspected particles that were not identified as plastics, 62 corresponded to cellulose-cotton and vegetal debris, and the rest to materials whose composition could not be spectroscopically assessed. About 40% of the cellulose-based materials displayed non-natural colours (black, red, blue, orange) evidencing some type of anthropogenic processing. This type of materials might be classified as pollutants as the industrial origin behind their non-natural colours suppose the presence of dyes and other industrial additives (Edo et al., 2020b).

The most abundant polymer was polyethylene (PE) followed by polystyrene (PS), polyester (PES), polypropylene (PP), polyvinyl chloride (PVC), and acrylic polymers (ACR, which include polymethylmethacrylate and acrylic fibres and films). These six polymers account for 96.7% of the plastic materials found and were the only ones exceeding 1% of the total number of 1322 particles identified as plastics. PE was predominant in all plants and throughout the sampling campaign. Besides, samples from plants P1, P2, and P3 displayed a considerable amount of PS, and also PP and PES. Fig. 7.2 shows the fraction of the different polymers identified in all compost samples throughout the period under study.

Apart from the most abundant plastics, other synthetic polymers were occasionally found. These were polyamide, polyurethane, alkyd resins, acrylonitrile butadiene styrene, polysulfone, polycarbonate, polyvinyl acetate, epoxy resins, silicone, ethylene-vinyl acetate and polyacrylonitrile, which, altogether accounted for the remaining 3.3% of the specimens identified as synthetic polymers. Among the minor polymers, polyamide and polyurethane and alkyd resins, exceeded 0.5%, which corresponded to

less than six particles for type and per sample. No particles that could be attributed to biopolymers were found in any of the samples.

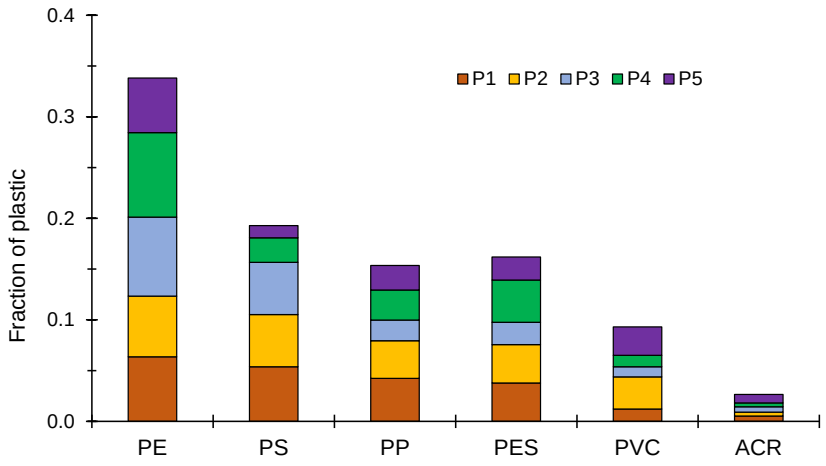


Figure 7.2. Main synthetic polymers found and their relative frequency (in unit fraction) for the different plants studied. PE: polyethylene; PS: polystyrene; (PES) polyester; (PP) polypropylene; (PVC) polyvinyl chloride; (ACR) acrylic polymers.

Polymer composition differed considerably among typologies. Fig. 7.3 shows the different materials found in the form of fragments, films, fibres, and filaments. Fragments were dominated by PS and films by PE, which corresponds with the major uses of those polymers in packaging. Most fibres were PES, PP, and acrylic, while filaments displayed a variety of polymers dominated by PP, PE, and PVC. PVC appeared occasionally as green colour filaments, probably due to its use as industrial yarns. PVC was also found as transparent films, which was attributed to its massive use for food wrapping. In the present study, PS was barely found in its expanded foam form. On the contrary, most of the fragments found corresponded to the material widely used in the food industry as yogurt container, fruit carrier, or other similar containers as a consequence of their proven food safety (Gelbke et al., 2019). Most fibres were PES, PP, and acrylic, typical polymers used in textiles for clothing and other industries. Overall, the composition of the plastic debris found in this work corresponded to the usual polymer found in packaging materials like bags or food containers and were consistent with data reported elsewhere (Gui et al., 2021). The correspondence between typology and polymer type can be visualized in the PCA plot given in Fig. S7.4 (SM). Besides, some images of the plastic particles samples in this work are shown in Fig. S7.5 (SM).

Fig. 7.4 shows the total concentration of plastic particles and MPs are expressed in items per mass of dry compost. The results showed a considerable reproducibility with limited

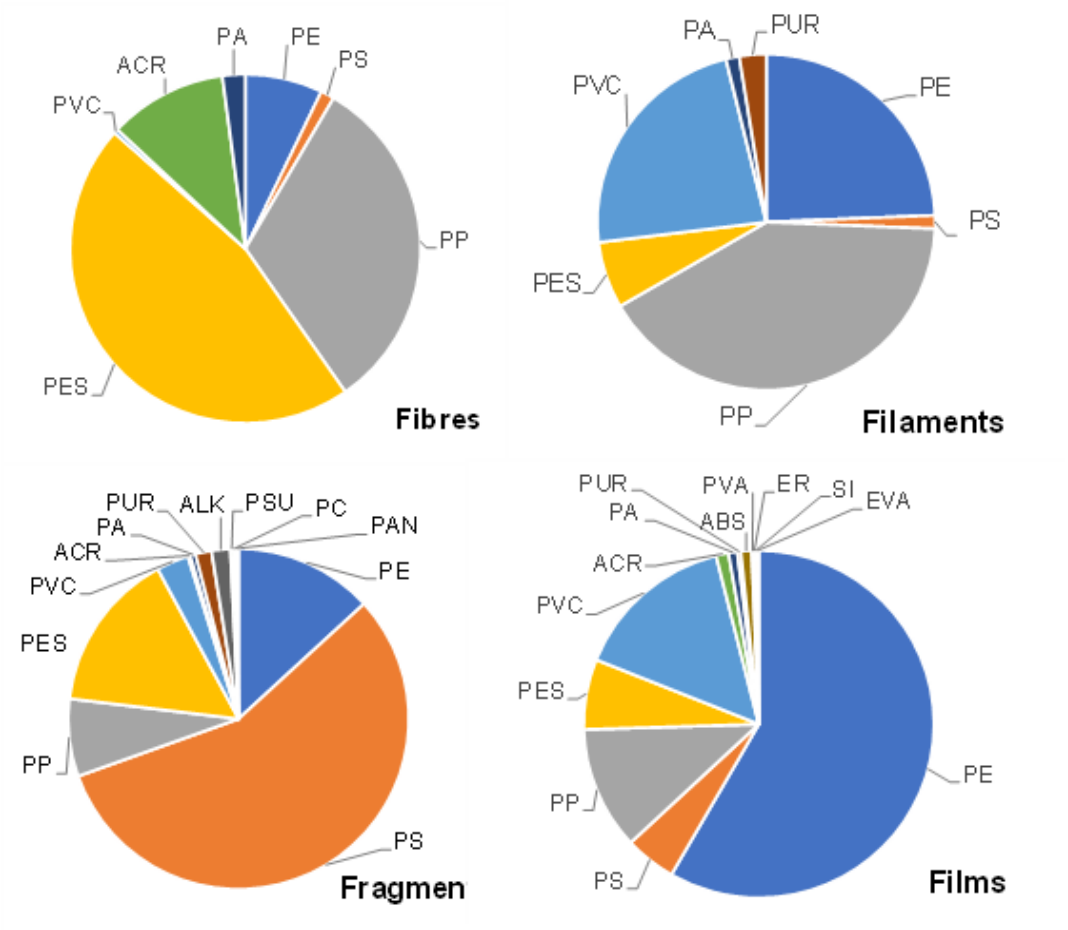


Figure 7.3. Composition of plastic particles by typology. PA: polyamide; PUR: polyurethane; ALK: alkyd resins; ABS: acrylonitrile butadiene styrene; PSU: polysulfone; PC: polycarbonate; PVA: polyvinyl acetate; ER: epoxy resin; SI: silicone; EVA: ethylene-vinyl acetate; and PAN: polyacrylonitrile.

changes along the sampling period as evidenced by the relatively small differences between maximum and minimum values for plastic concentration along the five month sampling period. Plants P1 and P2 consistently displayed a concentration of plastics in the range of 20–35 particles/g of DW; P3 and P4 in the range of 10–20 particles/g of DW, while P5 was the one with the lowest plastic content, as low as 8–12 particles/g of DW (average 9.5 ± 1.9 particles/g of DW). The overall fraction of MPs (with respect to the total number or plastic particles) was in the 63–74% range, representing 6.4–19.7 MPs/g DW. The results showed that the majority of plastic impurities consisted of small particles, below the 5 mm threshold. The concentration data is aggregated per month and facility are shown in Fig. S7.6 (SM).

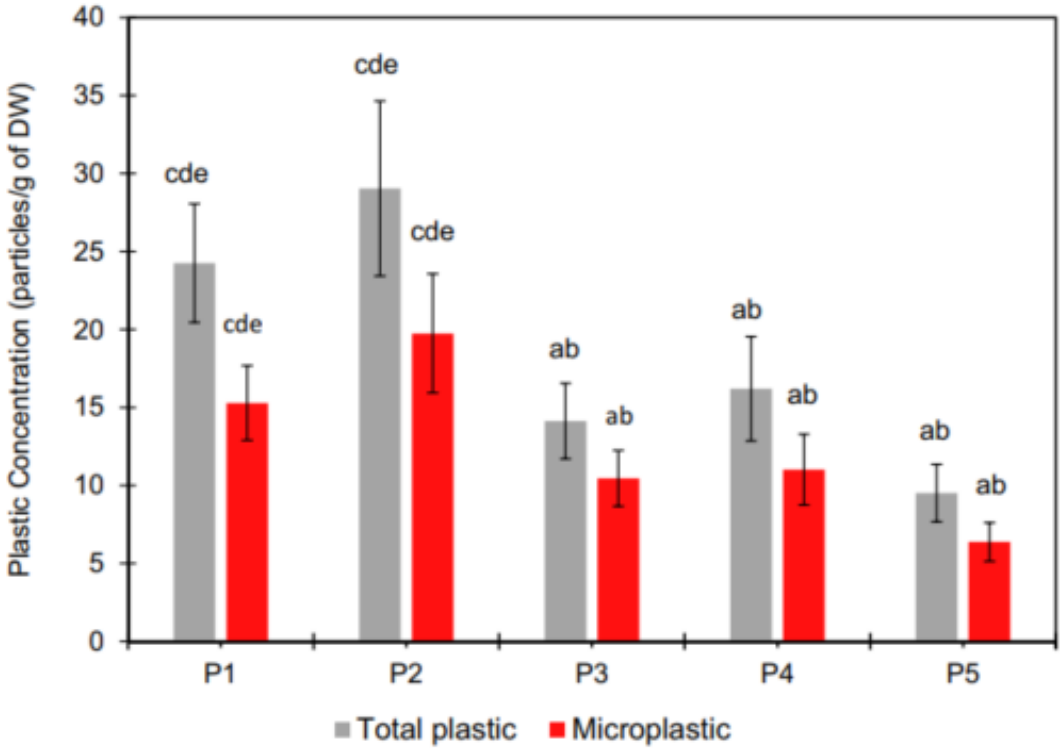


Figure 7.4. Concentration of plastic particles per g of compost (DW, dry weight). Error bars represent plus/minus one standard deviation for the five samples taken from each plant. The dark part of the bar represents the concentration of MPs (< 5 mm).

As indicated before, the results showed fibres were the main typology followed by fragments and films (Fig. S7.3 (SM)). The data showed that fibres and filaments were predominant in plants P2 and P5 (>50%), while fragments and films slightly outnumbered fibres and filaments in plants P1 and P3. The data on the different typologies showed a consistent tendency to increase in the number of fibres (from 6.3% in M1, to 10.4% in M6) along with a reduction in the number of fragments during the same period (8.0% to 5.0%). The data, split into months, plants and for these two main typologies are shown in Fig. S7.7 (SM). This tendency was not clearly accompanied by a change in the fraction of the different polymers as shown by Fig. S7.8 (SM), which represents the fraction of PES in fibres and of PS in fragments and might be due to seasonal variations.

Different studies have reported the presence of MPs in soil environments because of the use of wastewater sludge as fertilizer (Edo et al., 2020a; Li et al., 2018). The data

available suggest that wastewater sludge could release tens of thousands of MPs per kg of dry soil when dispersed in agricultural lands (Zhang and Liu, 2018).

The use of compost obtained from biowaste composting processes for soil amendment is another way of entry for MPs to the environment, which deserved much less attention.

MPs have been reported in compost samples from several countries up to thousands of items per kg (van Schothorst et al., 2021; Vithanage et al., 2021). Weithmann et al. (2018) demonstrated that fertilizers from composting facilities contained 70-122 MPs/kg of dry product in plants using aerobic and anaerobic treatment of OFMSW respectively. The study, however, was limited to particles >1 mm (Weithmann et al., 2018). Another recent study on the presence of MPs in compost from organic rural domestic wastes found an average abundance (0.05–5 mm) of 2.4 ± 0.4 MPs/g of dry weight compost (Gui et al., 2021). Our results showed a concentration of plastic particles in the 10-30 particles/g of dry weight compost, mostly <5 mm (about 5–20 MPs/g), and 30% of them <1 mm, which is a reasonably good agreement with Gui's findings in spite of the different origin of the raw biowaste.

Even if the presence of plastic in compost is well documented, the risk posed to agricultural soil and interconnected environments is still poorly known. MPs in compost are suspected to act as a carrier of toxic chemicals including metals, to influence the carbon cycle, t o alter microbial distribution and to produce negative effects to soil biota. Besides, as strange materials in soil, they will possibly induce changes in soil physical, chemical, and biological properties. However, most of the existing studies on the environmental impact of plastic debris performed so far showed no risk or very limited risk for short term exposure (Selonen et al., 2020).

Judy et al. (2019) demonstrated that municipal wastes containing plastic had no effect to wheat seedling and biomass production as well as to earthworm growth, reproduction, and mortality even after up to 9 months of exposure (Judy et al., 2019). However, other researchers found significant effects, particularly when studying smaller plastic particles. Kim et al. exposed the nematode *Caenorhabditis elegans* to submicron PS particles and found significant offspring decrease for concentrations of 10 mg/kg of

soil, which intensified in clay-rich soils (Kim et al., 2020). The higher plant *Vicia faba* suffered changes in enzymatic (catalase, superoxide dismutase, peroxidase) activity when exposed to PS MPs (5 µm or smaller) and growth reduction when treated with 100 nm PS-NPs (Jiang et al., 2019). In sum, the long-term impact of plastics is essentially unknown, especially referring to the effect and potential accumulation in tissues of small size MPs and NPs.

Rodrigues et al. demonstrated that separated OFMSW collection systems could play a key role to control the contamination with non-compostable materials in biowaste processing plants (L.C. Rodrigues et al., 2020). The contamination of OFMSW with non-compostable materials results in a high impact on compost quality in terms of the concentration of plastics and other debris, and this is true despite the plant engineering efforts adopted to remove them. It is desirable that the organic materials generated at home are completely free of non-biodegradable and compostable materials (i.e., conventional plastic, glass, textiles, metals). It is a well-known fact that door-to-door collection, rather than publicly available compost containers reduces the amount of impurities in compost and rejection rates in composting plants (Malamis et al., 2017). Our study showed lower rejection rates in plants with higher rate of door-to-door collection compared with combined collection systems that make use of street bin dedicated containers as shown in Table 7.1.

Our results allowed some insight into the actions that may help to control the presence of plastic impurities in OFMSW compost. Table S7.2 (SM) shows the covariance matrix relating the concentration of plastics with other quantitative variables that represent the differences among plants. Most of the variance was explained by plant capacity, which in turn displayed significant autocorrelation with population density, but the fraction of door-to-door collection and the fraction of impurities were also significant (p-value < 0.05) to explain variability. Instead, the sampling month was not significantly explaining variance and was removed from the analysis. The PCA plot (Fig. S7.9, SM) expresses this information as a set of new variables, the principal components (PC). PC1 explains 73.7% of variance (68.7% considering only fibres) and is mainly influenced by plant capacity and door-do-door collection fraction. Besides Plant capacity and door-to-door collection are negatively correlated. PC2 explains 8.2% (12.8% for fibres) of

varianceand is mainly influenced by the presence of impurities and population density, which are uncorrelated. The effect of plant size and population density are probably explained by the sociodemographic characteristics of the population served, where more OFMSW are expected to be accompanied by higher plastic waste due to different lifestyle (Shittu, 2020).

It is widely accepted that more careful collection schemes and more stringent selection at the plant entrance, lead to lower plastic contents in the final product. However, our results showed that even for plants in less populated places with rigorous collection policies, plastic impurities, in particular small MPs, were still present. Plant P5 would disseminate 4.8×10^9 MPs/yr and the five plants studied in this work would contribute to MP pollution with a total load of 1.4×10^{12} MPs/yr altogether. Besides, it is important to note that composting conditions combine temperature, humidity, and an aerobic environment, which are known to trigger plastic ageing and fragmentation as a consequence of mechanical stress, oxidation and abiotic disintegration (Gui et al., 2021). As it has been shown, the plastic fragments spread into the environment will continue to undergo ageing and fragmentation process to produce smaller fragments including NPs (Sorasan et al., 2021).

The analyses performed in this work demonstrated that bioplastics were completely absent from all compost samples analysed. In the specific case of P5, all bags accepted in door-to-door collection should be compostable (consideration supported also by the very low amount of impurities rejected by the process; see Table 7.1). The data provided by the analyses at the entrance of all plants (Table S7.1, SM) indicated that compostable bags were found in most plants, reaching ~90% of all bags in plant P5. However, we could not find any fragment of biodegradable bags or other biodegradable polymers in spite the efforts made in that direction. The case of P2 was special as it represented the only plant with an anaerobic digestion process followed by post-digestate composting. Fig. 7.4 shows that P2 compost was the one with highest concentration of plastic debris, which could be explained by the pretreatment technology applied in this plant before OFMSW digestion. Anaerobic digesters are sensitive to improper materials so a high-performance pretreatment is required to avoid failures in the digestion phase. These systems could be responsible for the mechanical fragmentation of plastics entering with

OFMSW, which would appear later as contaminants in the final product. This explanation would require additional studies to be confirmed.

Our results highlighted the presence of plastic impurities in compost from OFMSW. The results from non-compostable waste shown in Table S7.1 (SM) has been made publicly available by the Agència de Residus de Catalunya (https://sdr.arc.cat/). The data in the additional information to each characterization file showed that most non-classified waste corresponded to a few types of waste. The most common are facemasks, coffee capsules, kitchen scourers and cleaning cloths, drug blisters, rests of food packaging and a variety of different plastic-containing objects including electronic devices. The presence of such macrowastes gives a clue on the origin of the small plastic debris obtained in this work, which can be mostly attributed to an improper waste management and separate collection, although certain contribution of in-plant contamination could exist (for example, due to atmospheric deposition) or cross-contamination through the use of bulking agents containing plastics. Measures should be taken to avoid the presence of MPs into the final compost in order to limit as much as possible their dispersion to soils. This goal could be achieved by limiting the use of non-compostable plastics in domestic uses like food packaging in favour of bioplastics. Another strategy could be the design of environmentally friendly plastic goods, easy to sort and classify at home by the end-users.

7.4. Conclusions

The presence of plastic debris in compost obtained from OFMSW was studied by analysing samples of final compost taken over a five month period from five different composting facilities representative of different collection systems, rates of impurities, and technology. From our study we concluded that: (1) The total concentration of plastic particles was in the 10–30 items/g of DW range; (2) The concentration of MPs was in the 5–20 items/g of DW; (3) Fibres were predominant and were mostly in the lower size range (25% < 500 µm); (4) The plastic materials found were polyethylene, polystyrene, polyester, polypropylene, polyvinyl chloride, and acrylic polymers in that order of abundance; (5) Smaller plants, with door-to-door collection schemes produced compost with less plastic impurities; and (6) Compostable bioplastics are completely absent from

compost even if there was evidence that they arrived to the composing plants at least as biodegradable plastic bags.

7.5. References

Adhikari, B.K., Barrington, S., Martinez, J., King, S., 2008. *Characterization of food waste and bulking agents for composting*. Waste Manag. 28, 795–804.

Ali, I., Ding, T., Peng, C., Naz, I., Sun, H., Li, J., et al., 2021. *Micro- and nanoplastics in wastewater treatment plants: occurrence, removal, fate, impacts and remediation technologies – a critical review.* Chem. Eng. J. 423, 130205.

Bueno-Ferrer, C., Garrigós, M.C., Jiménez, A., 2010. *Characterization and thermal stability of poly(vinyl chloride) plasticized with epoxidized soybean oil for food packaging.* Polym. Degrad. Stab. 95, 2207–2212.

Carabassa, V., Domene, X., Alcañiz, J.M., 2020. *Soil restoration using compost-like-outputs and digestates from non-source-separated urban waste as organic amendments: limitations and opportunities.* J. Environ. Manage. 255, 109909. https://doi.org/10.1016/j. jenvman.2019.109909.

Cheng, Y., Song, W., Tian, H., Zhang, K., Li, B., Du, Z., et al., 2021. *The effects of high-density polyethylene* and polypropylene microplastics on the soil and earthworm Metaphire guillelmi gut microbiota. Chemosphere 267, 129219.

Edo, C., González-Pleiter, M., Leganés, F., Fernández-Piñas, F., Rosal, R., 2020a. *Fate of microplastics in wastewater treatment plants and their environmental dispersion with effluent and sludge*. Environ. Pollut. 259, 113837.

Edo, C., González-Pleiter, M., Tamayo-Belda, M., Ortega-Ojeda, F.E., Leganés, F., FernándezPiñas, F., et al., 2020b. *Microplastics in sediments of artificially recharged lagoons: case study in a biosphere reserve*. Sci. Total Environ. 729, 138824.

Gao, D., Li, X.-Y., Liu, H.-T., 2020. *Source, occurrence, migration and potential environmental risk of microplastics in sewage sludge and during sludge amendment to soil*. Sci. Total Environ. 742, 140355.

Gelbke, H.-P., Banton, M., Block, C., Dawkins, G., Eisert, R., Leibold, E., et al., 2019. *Risk assessment for migration of styrene oligomers into food from polystyrene food containers.* Food Chem. Toxicol. 124, 151–167.

GESAMP, 2019. *Guidelines for the monitoring and assessment of plastic litter in the ocean*. In: Kershaw, P.J., Turra, A., Galgani, F. (Eds.), IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP/ISA Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, p. 130.

Gigault, J., Halle, A.T., Baudrimont, M., Pascal, P.-Y., Gauffre, F., Phi, T.-L., et al., 2018. *Current opinion: what is a nanoplastic?* Environ. Pollut. 235, 1030–1034.

González-Pleiter, M., Edo, C., Aguilera, Á., Viúdez-Moreiras, D., Pulido-Reyes, G., GonzálezToril, E., et al., 2021. *Occurrence and transport of microplastics sampled within and above the planetary boundary layer*. Sci. Total Environ. 761, 143213.

Gui, J., Sun, Y., Wang, J., Chen, X., Zhang, S., Wu, D., 2021. *Microplastics in composting of rural domestic waste: abundance, characteristics, and release from the surface of macroplastics.* Environ. Pollut. 274, 116553.

Guo, J.-J., Huang, X.-P., Xiang, L., Wang, Y.-Z., Li, Y.-W., Li, H., et al., 2020. *Source, migration and toxicology of microplastics in soil.* Environ. Int. 137, 105263.

Jiang, X., Chen, H., Liao, Y., Ye, Z., Li, M., Klobučar, G., 2019. *Ecotoxicity and genotoxicity of polystyrene microplastics on higher plant Vicia faba*. Environ. Pollut. 250, 831–838.

Judy, J.D., Williams, M., Gregg, A., Oliver, D., Kumar, A., Kookana, R., et al., 2019. *Microplastics in municipal mixed-waste organic outputs induce minimal short to long-term toxicity in key terrestrial biota*. Environ. Pollut. 252, 522–531.

Kang, P., Ji, B., Zhao, Y., Wei, T., 2020. *How can we trace microplastics in wastewater treatment plants: a review of the current knowledge on their analysis approaches*. Sci. Total Environ. 745, 140943.

Kaza, S., Yao, L.C., Bhada-Tata, P., Van Woerden, F., 2018. *What a W2.0: A Global Snapshot of Solid Waste Management to 2050*. Urban Development. World Bank, Washington, DC.

Kedzierski, M., Villain, J., Falcou-Préfol, M., Kerros, M.E., Henry, M., Pedrotti, M.L., et al., 2019. *Microplastics in Mediterranean Sea: a protocol to robustly assess contamination characteristics*. PLOS ONE 14, e0212088.

Kim, S.W., Kim, D., Jeong, S.-W., An, Y.-J., 2020. *Size-dependent effects of polystyrene plastic particles on the nematode Caenorhabditis elegans as related to soil physicochemical properties*. Environ. Pollut. 258, 113740.

Laso, J., García-Herrero, I., Margallo, M., Bala, A., Fullana-i-Palmer, P., Irabien, A., et al., 2019. *LCA-nased comparison of two organic fraction municipal solid waste collection systems in historical centres in Spain*. Energies 12.

Li, X., Chen, L., Mei, Q., Dong, B., Dai, X., Ding, G., et al., 2018. *Microplastics in sewage sludge from the wastewater treatment plants in China*. Water Res. 142, 75–85.

Liu, K., Wang, X., Wei, N., Song, Z., Li, D., 2019. *Accurate quantification and transport estimation of suspended atmospheric microplastics in megacities: implications for human health*. Environ. Int. 132, 105127.

Liu, W., Zhang, J., Liu, H., Guo, X., Zhang, X., Yao, X., et al., 2021. *A review of the removal of microplastics in global wastewater treatment plants: characteristics and mechanisms*. Environ. Int. 146, 106277.

Magni, S., Binelli, A., Pittura, L., Avio, C.G., Della Torre, C., Parenti, C.C., et al., 2019. *The fate of microplastics in an italian wastewater treatment plant*. Sci. Total Environ. 652, 602–610.

Malamis, D., Bourka, A., Stamatopoulou, E., Moustakas, K., Skiadi, O., Loizidou, M., 2017. *Study and assessment of segregated biowaste composting: the case study of Attica municipalities.* J. Environ. Manag. 203, 664–669.

Möller, J.N., Heisel, I., Satzger, A., Vizsolyi, E.C., Oster, S.D.J., Agarwal, S., et al., 2021. *Tackling the challenge of extracting microplastics from soils: a protocol to purify soil samples for spectroscopic analysis.* Environ. Toxicol. Chem. https://doi.org/10.1002/etc.5024.

PlasticsEurope, 2020. *Plastics – The Facts 2019: An Analysis of European Plastics Production, Demand and Waste Data*. PlasticsEurope: Association of Plastics Manufacturer, Brussels.

Rodrigues, L.C., Puig-Ventosa, I., López, M., Martínez, F.X., Ruiz, A.G., Bertrán, T.G., 2020a. *The impact of improper materials in biowaste on the quality of compost.* J. Clean. Prod. 251, 119601.

Rodrigues, M.O., Gonçalves, A.M.M., Gonçalves, F.J.M., Abrantes, N., 2020b. *Improving cost-efficiency for MPs density separation by zinc chloride reuse.* MethodsX 7, 100785.

Rosal, R., 2021. *Morphological description of microplastic particles for environmental fate studies.* Mar. Pollut. Bull. 171, 112716.

Ryan, P.G., 2015. *A brief history of marine litter research.* In: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer International Publishing, Cham, pp. 1–25.

Saveyn, H., Eder, P., 2014. *End-of-Waste Criteria for Biodegradable Waste Subjected to Biological Treatment (Compost & Digestate): Technical Proposals.* JRC Scientific and Policy Reports, Publications Office of the European Union, Luxembourg.

Selonen, S., Dolar, A., Jemec Kokalj, A., Skalar, T., Parramon Dolcet, L., Hurley, R., et al., 2020. *Exploring the impacts of plastics in soil – the effects of polyester textile fibres on soil invertebrates*. Sci. Total Environ. 700, 134451.

Shittu, O., 2020. *Emerging sustainability concerns and policy implications of urban household consumption: a systematic literature review.* J. Clean. Prod. 246, 119034.

Sorasan, C., Edo, C., González-Pleiter, M., Fernández-Piñas, F., Leganés, F., Rodríguez, A., et al., 2021. *Generation of nanoplastics during the photoageing of low-density polyethylene*. Environ. Pollut. 289, 117919.

Tagg, A.S., Labrenz, M., 2018. *Closing microplastic pathways before they open: a model approach.* Environ. Sci. Technol. 52, 3340–3341.

van Schothorst, B., Beriot, N., Huerta Lwanga, E., Geissen, V., 2021. *Sources of light density microplastic related to two agricultural practices: the use of compost and plastic mulch*. Environments 8.

Vithanage, M., Ramanayaka, S., Hasinthara, S., Navaratne, A., 2021. *Compost as a carrier for microplastics and plastic-bound toxic metals into agroecosystems.* Curr. Opin. Environ. Sci. Health 24, 100297.

Wang, J., Liu, X., Li, Y., Powell, T., Wang, X., Wang, G., et al., 2019. *Microplastics as contaminants in the soil environment: a mini-review.* Sci. Total Environ. 691, 848–857.

Weithmann, N., Möller, J.N., MGJ, Löder, Piehl, S., Laforsch, C., Freitag, R., 2018. *Organic fertilizer as a vehicle for the entry of microplastic into the environment.* Sci. Adv. (4), eaap8060

Xu, Z., Sui, Q., Li, A., Sun, M., Zhang, L., Lyu, S., et al., 2020. *How to detect small microplastics (20–100 µm) in freshwater, municipal wastewaters and landfill leachates? A trial from sampling to identification*. Sci. Total Environ. 733, 139218.

Xu, X., Zhang, L., Jian, Y., Xue, Y., Gao, Y., Peng, M., et al., 2021. *Influence of wastewater treatment process on pollution characteristics and fate of microplastics*. Mar. Pollut. Bull. 169, 112448.

Yuanqiao, L., Caixia, Z., Changrong, Y., Lili, M., Qi, L., Zhen, L., et al., 2020. *Effects of agricultural plastic film residues on transportation and distribution of water and nitrate in soil*. Chemosphere 242, 125131.

Zhang, G.S., Liu, Y.F., 2018. *The distribution of microplastics in soil aggregate fractions in southwestern China*. Sci. Total Environ. 642, 12–20.

Zhu, D., Chen, Q.-L., An, X.-L., Yang, X.-R., Christie, P., Ke, X., et al., 2018. *Exposure of soil collembolans to microplastics perturbs their gut microbiota and alters their isotopic composition. Soil* Biol. Biochem. 116, 302–310

7.6. Supplementary material of Chapter 7

Contents

Table S7.1. Detailed characterization (in wt.%) of non-compostable materials identified at the entrance of all plants (Source: Agència de Residus de Catalunya).

Figure S7.1. Scheme of the steps followed for the separation and identification of plastic particles.

Figure S7.2. ATR-FTIR spectra of the bioplastics used as references in this work. MaterBi was supplied by Novamont and the remaining #1, #2 and #3 were certified materials obtained from local markets.

Figure S7.3. Size distribution for the plastics recovered from the different plants studied (P1 to P5, details in Table 1). Size was calculated as equivalent diameter as indicated in the text.

Figure S7.4. PCA plot for the relationship between typology and polymer composition.

Figure S7.5. Micrographs of some plastic particles sampled in this work.

Figure S7.6. Concentration of plastic particles per g of compost (DW, dry weight). Error bars represent plus/minus one standard deviation for the five samples taken from each plant. M1 to M5 represents months during the sampling period (February-June 2021).

Figure S7.7. Concentration of fibres (A) and fragments (B) for the different plants during the sampling period. M1 to M5 represent months during the period February-June 2021.

Figure S7.8. Unit fraction of PS in fragments (A) and PES in fibres (B) for the different plants during the sampling period. M1 to M5 represent months during the period February-June 2021.

Table S7.2. Covariance matrix for the quantitative variables included in this study.

Figure S7.9. PCA plot for the independent variables studied in this work.

Table S7.1. Detailed characterization (in wt.%) of non-compostable materials identified at the entrance of all plants (Source: Agència de Residus de Catalunya).

	Samples		Glass	Paper & cardboard	Plastic items	Plastic bags*	Metals	Textiles	Total
P1	54		0.66	0.74	5.13	1.70	0.52	2.15	14.43
P2	74		0.23	0.41	1.13	1.07	0.11	0.67	4.43
P3	60		0.60	1.12	1.66	0.83	0.33	1.17	8.32
P4	10		0.99	1.01	2.93	0.91	0.52	1.90	10.03
P5	6		-	0.25	0.25	-	0.06	0.23	1.65

* Estimation of the presence of compostable bags: P1 10-15%, P2 ~10 %, P3 15-20%, P4 very low, P5 ~90 %

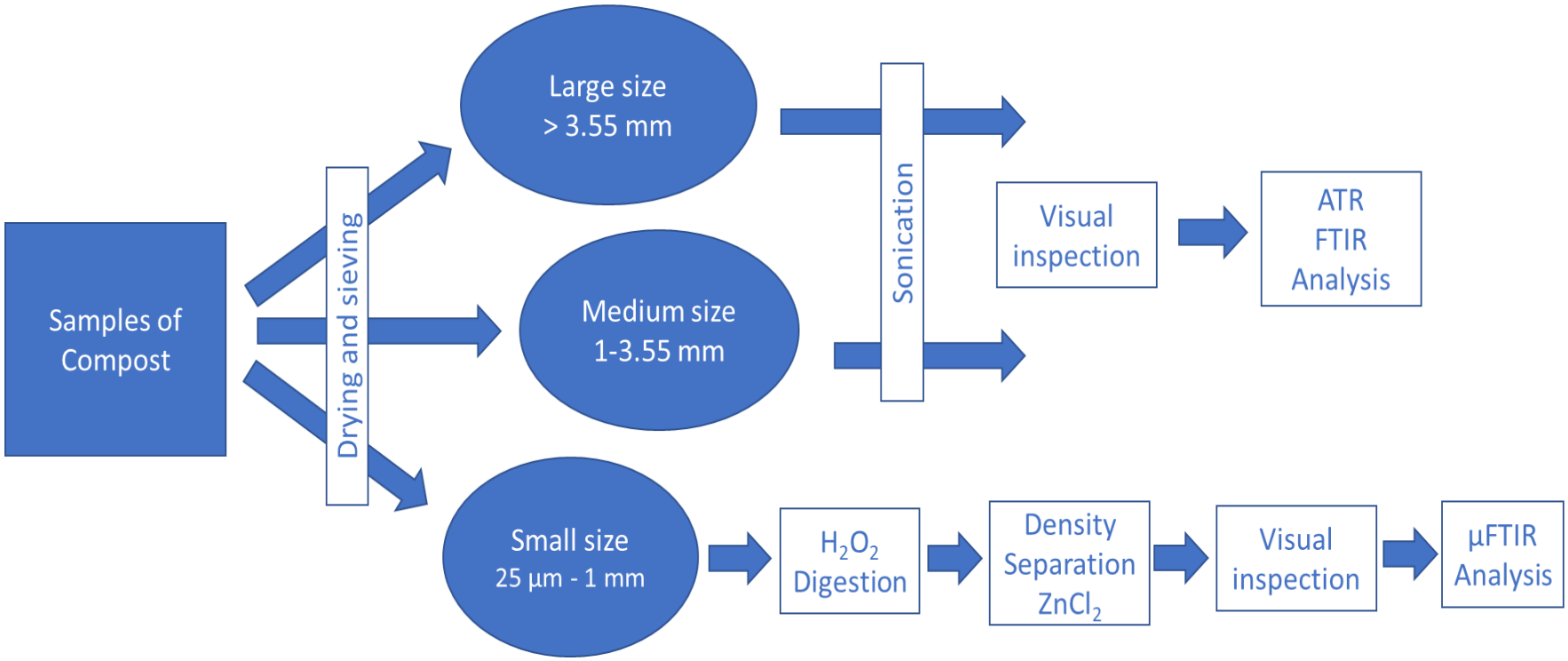


Figure S7.1. Scheme of the steps followed for the separation and identification of plastic particles.

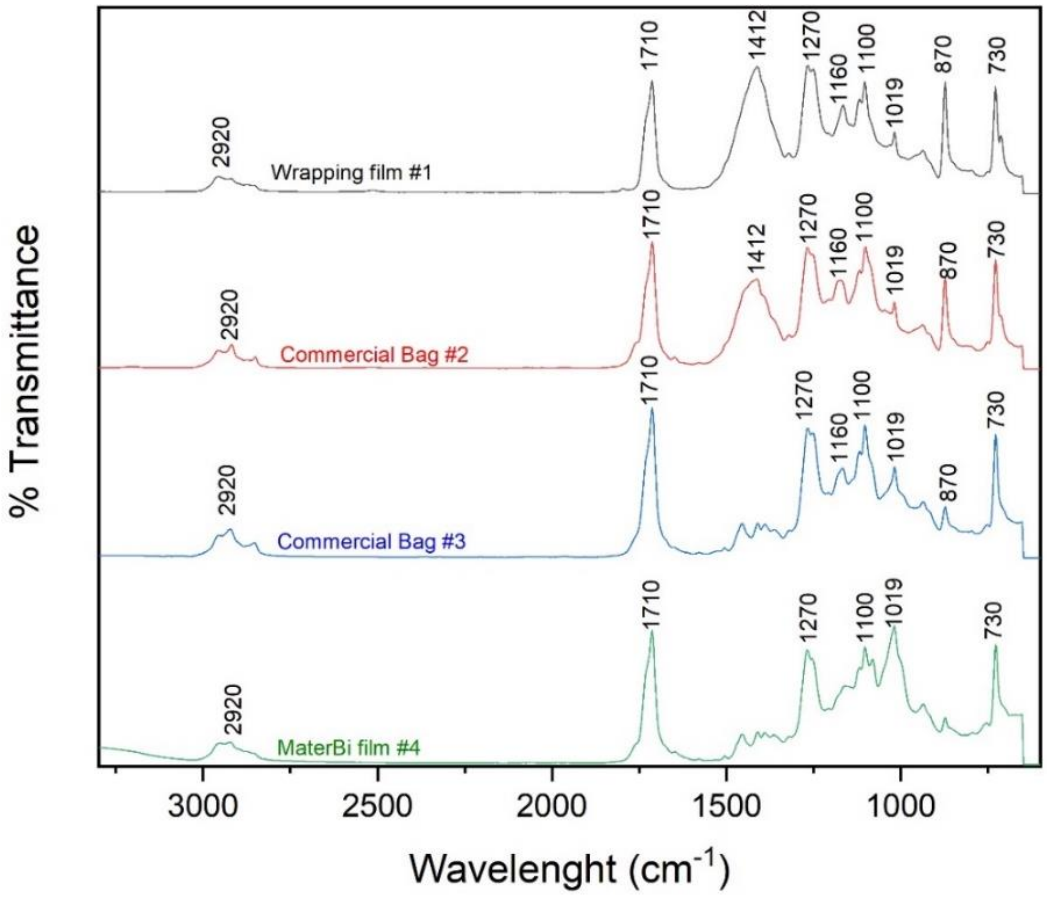


Figure S7.2. ATR-FTIR spectra of the bioplastics used as references in this work. MaterBi was supplied by Novamont and the remaining #1, #2 and #3 were certified materials obtained from local markets.

The peaks corresponding to starch-based materials are clear observed and attributed to thermoplastic starch, which is widely used for the manufacture of commercial biodegradable plastics in compounds with different copolymers and fillers. Accordingly, the spectra of all materials shared some common features. The bands in the 2970-2850 cm^{-1} region corresponded to the asymmetric and symmetric stretching of C–H aliphatic alkyl chain bonds. The carbonyl peak (C=O stretching) was clearly observed at 1710 cm^{-1}. The broad band at 1412 cm^{-1}, predominant in two of the samples (#1 and #2 in Fig. S2), was attributed to the bending and wagging deformation of C-H bonds. The stretching vibration in 1160, 1100, 1019 and 870 cm^{-1} corresponded to C–O stretching vibration, C–C deformation, and the out-of-plane bending vibrations of C–H bonds. The bands at 1270 cm^{-1} and 730 cm^{-1} could be attributed to C–O stretching of ester bonds, and the out of plane deformation of the aromatic ring, probably from PBAT (Nainggolan et al., 2013; Aldas et al., 2020).

Aldas M, Ferri JM, Lopez-Martinez J, Samper MD, Arrieta MP. *Effect of pine resin derivatives on the structural, thermal, and mechanical properties of Mater-Bi type bioplastic*. J. Appl. Polym. Sci. 2020; 137: 48236.

Nainggolan H, Gea S, Bilotti E, Peijs T, Hutagalung SD. *Mechanical and thermal properties of bacterial-cellulose-fibre-reinforced Mater-Bi(®) bionano-composite*. Beilstein J Nanotechnol 2013; 4: 325-9.

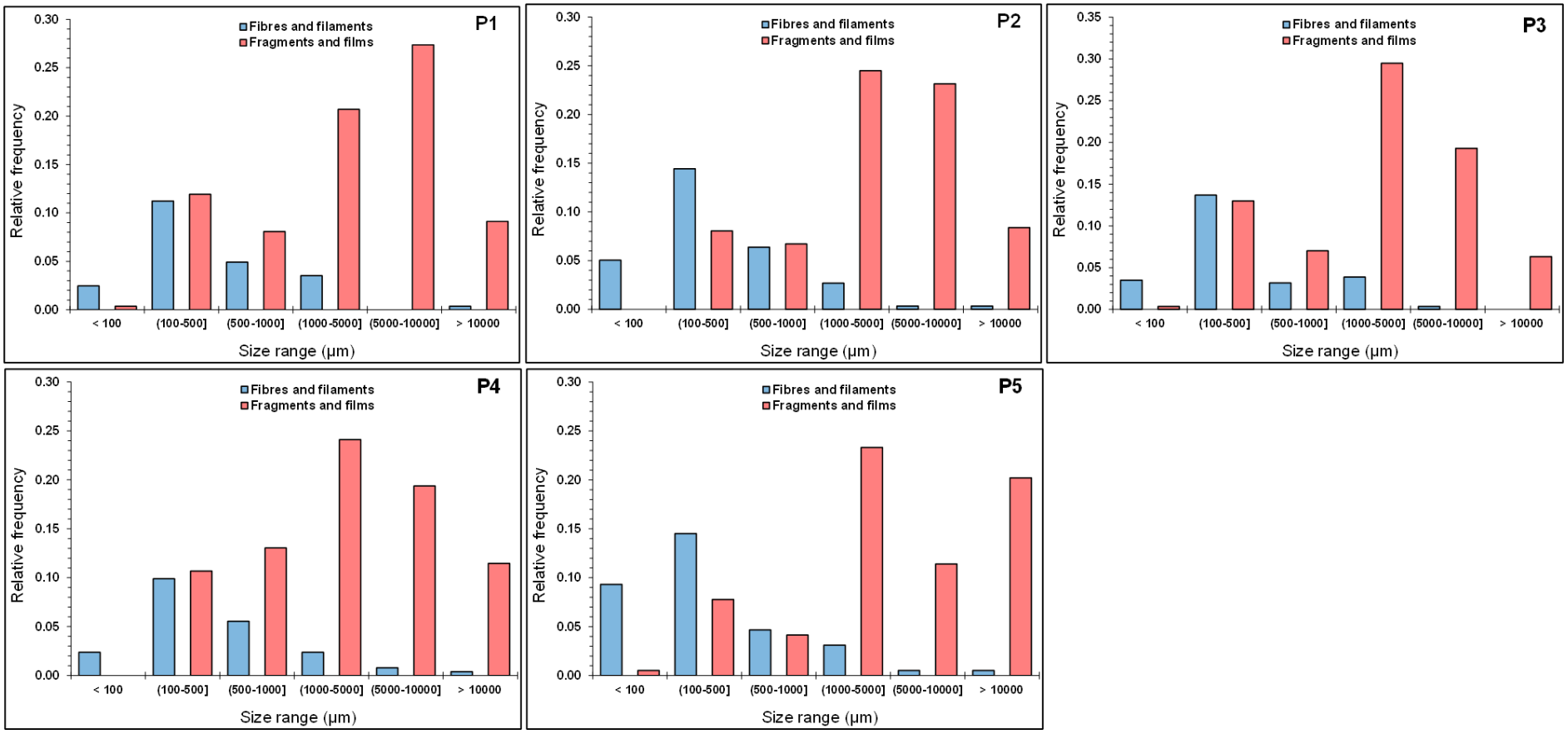


Figure S7.3. Size distribution for the plastics recovered from the different plants studied (P1 to P5, details in Table 2). Size was calculated as equivalent diameter as indicated in the text.

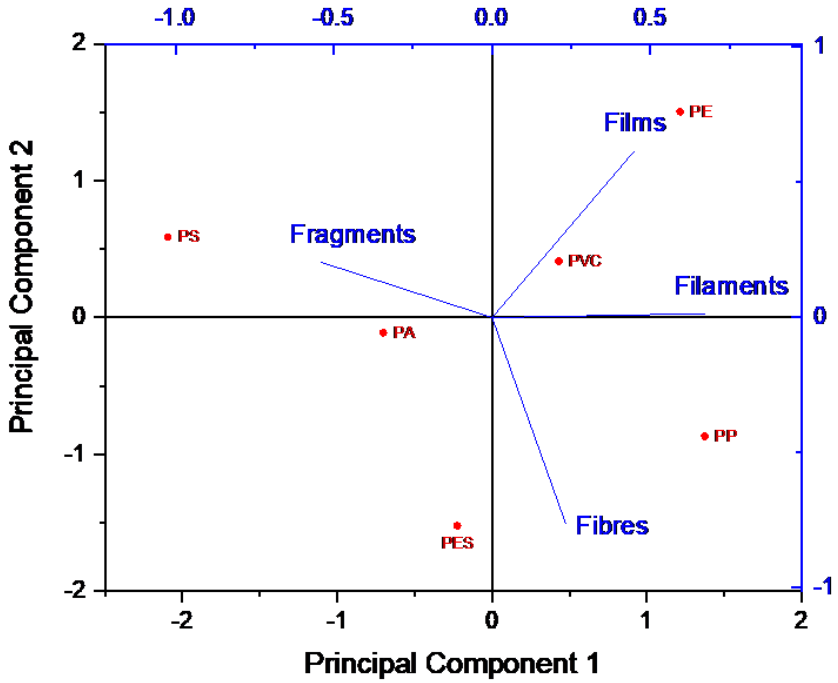


Figure S7.4. PCA plot for the relationship between typology and polymer composition.

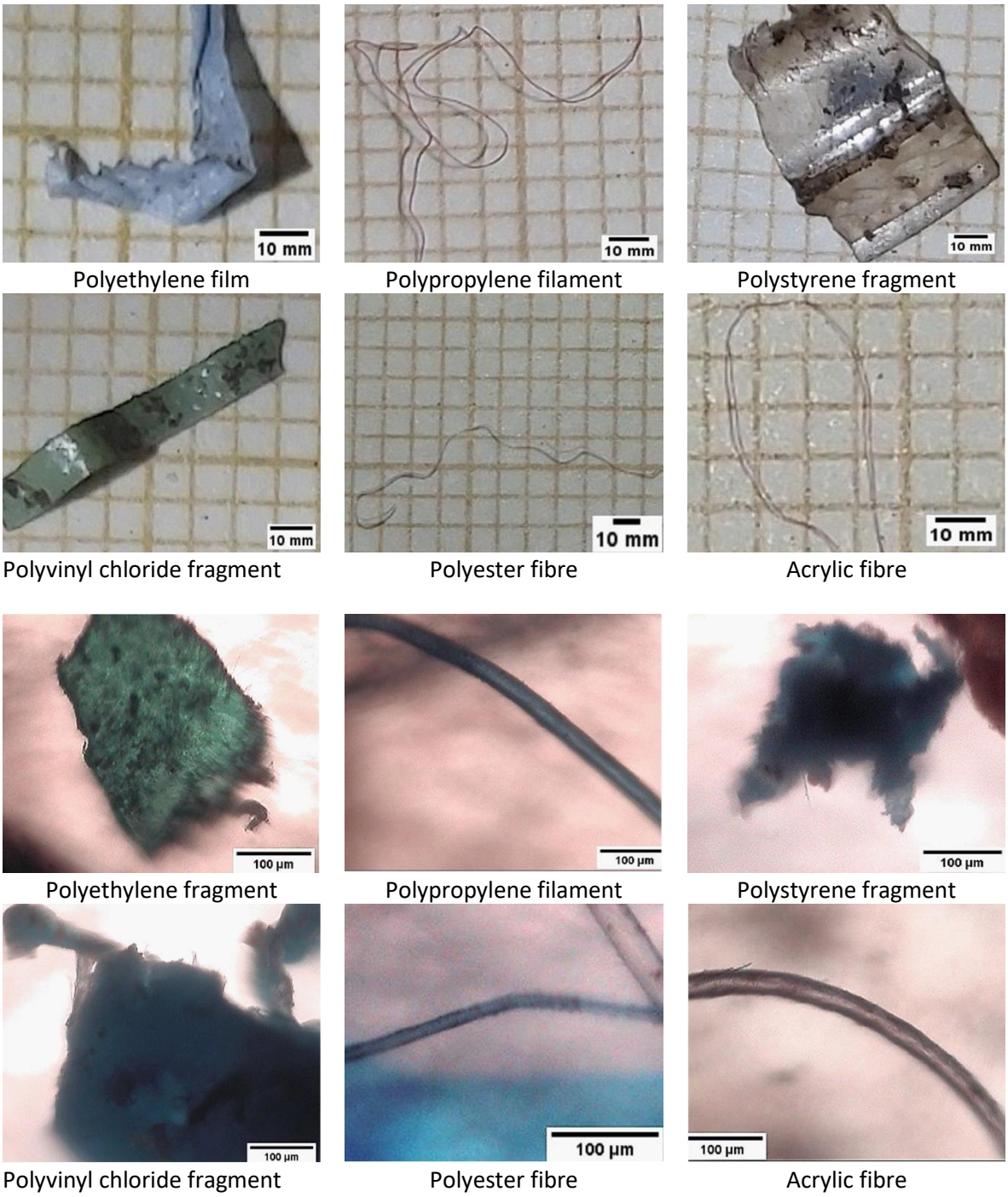


Figure S7.5. Micrographs of some plastic particles sampled in this work.

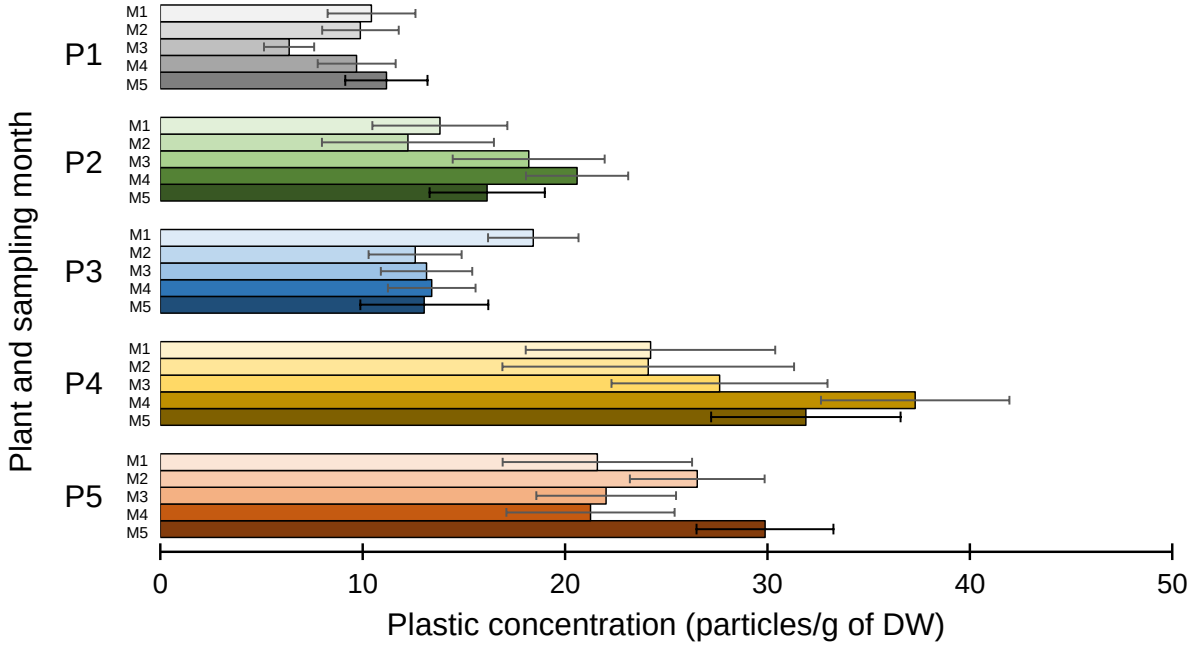


Figure S7.6. Concentration of plastic particles per g of compost (DW, dry weight). Error bars represent plus/minus one standard deviation for the five samples taken from each plant. M1 to M5 represents months during the sampling period (February-June 2021).

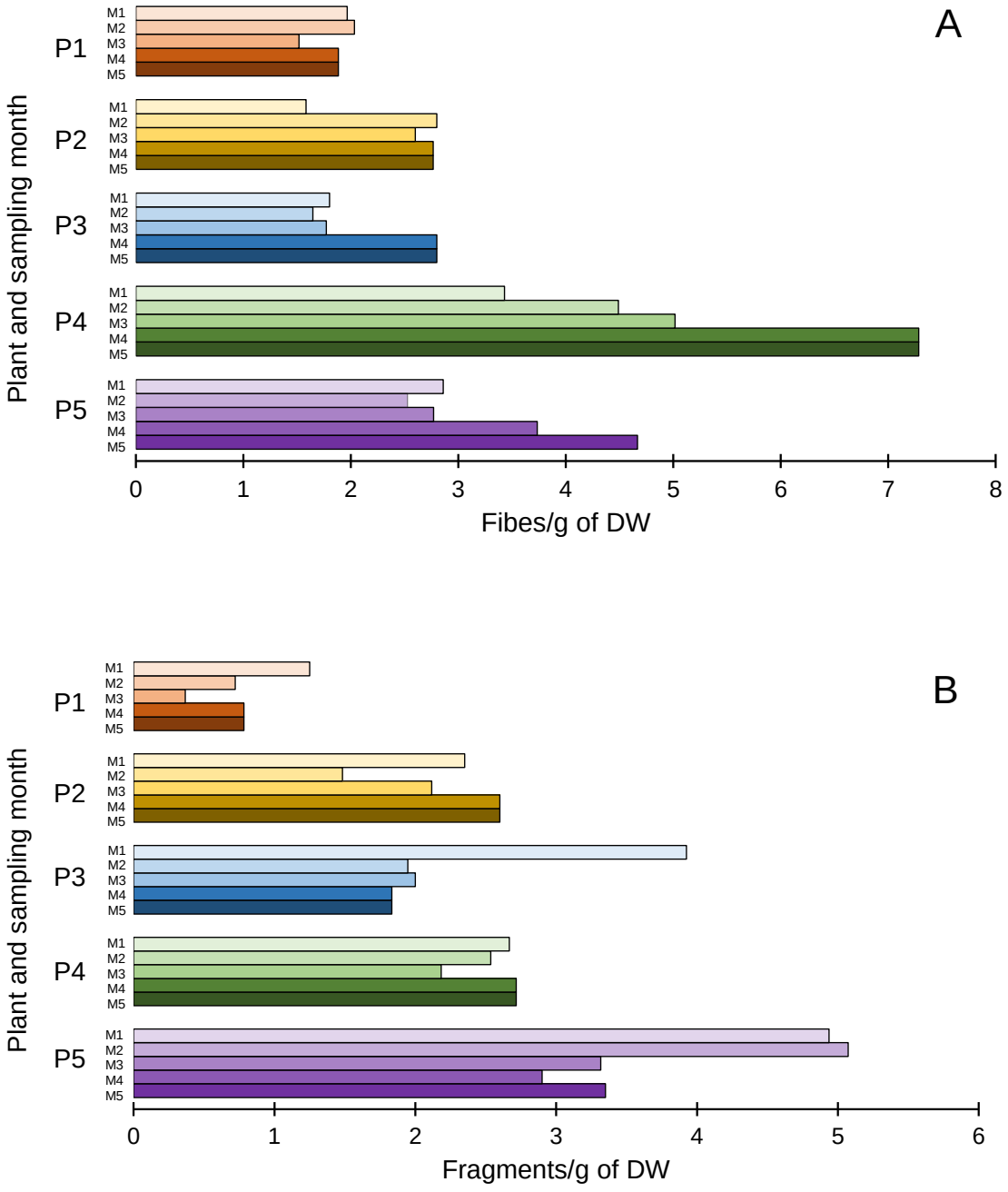


Figure S7.7. Concentration of fibres (A) and fragments (B) for the different plants during the sampling period. M1 to M5 represent months during the period February-June 2021.

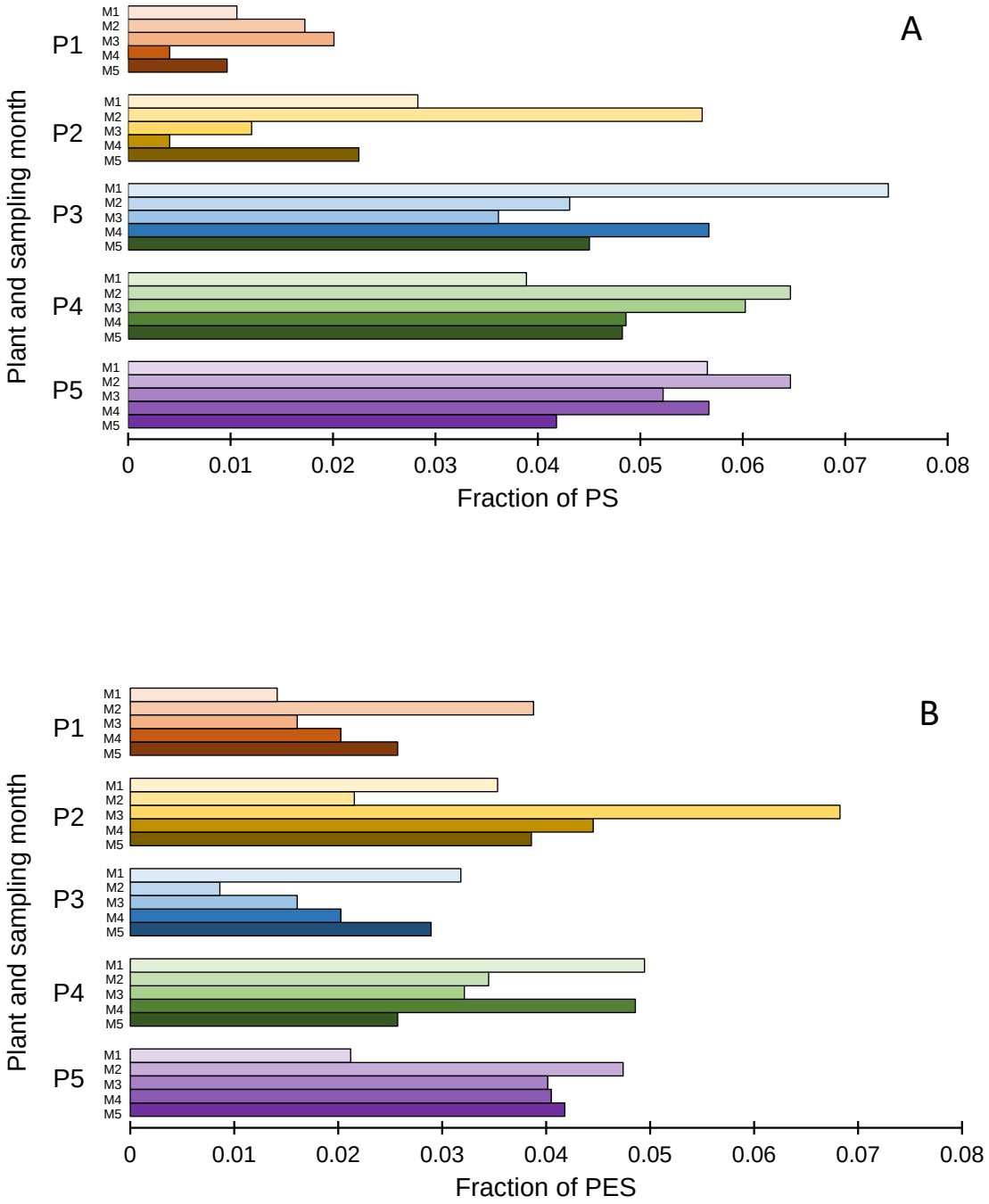


Figure S7.8. Unit fraction of PS in fragments (A) and PES in fibres (B) for the different plants during the sampling period. M1 to M5 represent months during the period February-June 2021.

Table S7.2. Correlation matrix for the quantitative variables included in this study.

(all plastics)

	Plastic (particles/g)	*Capacity (tonnes/yr)*	*Door-to-door (fraction)*	*Rejection (fraction)*	*Population (inhab./km²)*
Plastic	1				
Capacity	0.84246	1			
Door-to-door	-0.66939	-0.60043	1		
Rejection	0.37480	0.13939	-0.69715	1	
Population	0.51383	0.76160	-0.43647	-0.31511	1

(only fibres and filaments)

	Plastic (particles/g)	*Capacity (tonnes/yr)*	*Door-to-door (fraction)*	*Rejection (fraction)*	*Population (inhab./km²)*
Plastic	1				
Capacity	0.97445	1			
Door-to-door	-0.52459	-0.60043	1		
Rejection	0.16817	0.13939	-0.69715	1	
Population	0.63732	0.76160	-0.43647	-0.31511	1

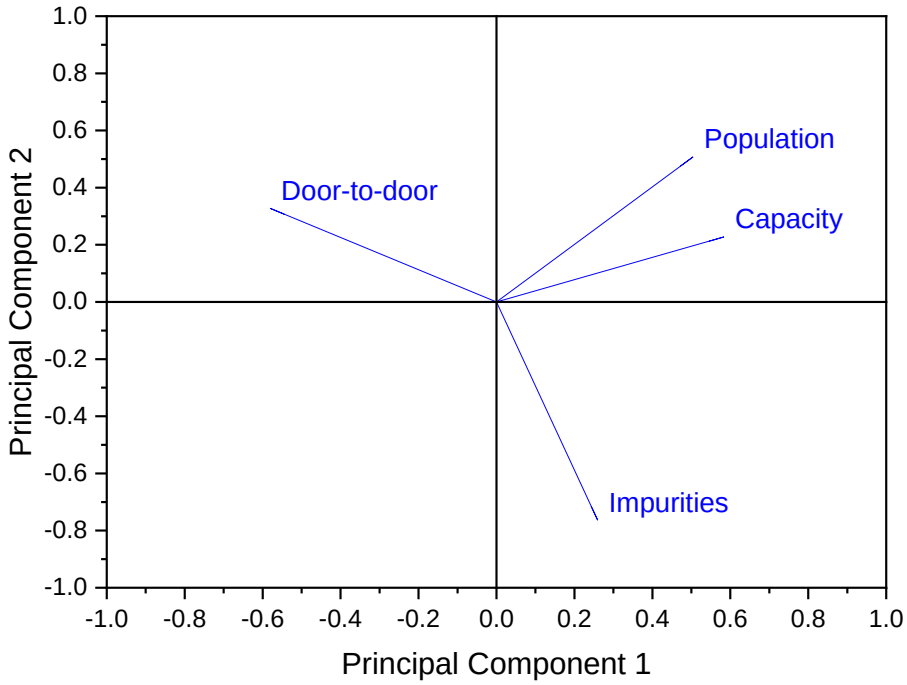


Fig. S7.9. PCA plot for the independent variables studied in this work.

CHAPTER 8. GENERAL DISCUSSION

8.1. General discussion

The title of this Thesis "*Occurrence and environmental fate of microplastics as emerging anthropogenic pollutants*" summarizes the end towards it was aimed at: the use of available techniques for describing and quantifying the plastic materials present in the environment. To do this, different compartments were studied and the plastic debris in them evaluated by different methods ranging from conventional spectroscopic technologies to the use of sentinel species as biomarkers for this new type of anthropogenic pollution. The evaluation of the ways of entry of plastic pollution into the environment, the transfer of plastic among compartments, and the fate of biomaterials have also been dealt with in this Doctoral Thesis.

In recent years, the scientific research on microplastics (MPs) has shown exponential global growth. The issue reached the public opinion and in Spain, this word even became one of the most used neologisms (FUNDEURAE, 2018). The abundance of news, images or debates related to big masses of plastics observed in the oceans quickly became popular as people expressed concerns on the origin of those plastics and the real extent of the problem. The answer was worrying because the litter spread in oceans and observed in garbage patches has its origin in diffuse spills or discharges occurring in many parts of the world. The findings of plastic litter quickly overcame marine ecosystems and got extended to the rest of environmental compartments, even though the marine one is the most explored media so far.

In **Chapter 2**, a coastal marine ecosystem was investigated. In the context of a series of clean-up activities carried out by non-governmental organizations (NGOs), a scientific sampling campaign was performed to evaluate the extent of the plastic pollution in a protected region of the Canary Islands. The results showed that the selected location was a hotspot for plastic litter arriving from the North Atlantic Subtropical Gyre. The results evidenced the importance of marine currents in MPs transportation, specifically in places exposed to currents driving plastic from accumulation spots. The plastics recovered from the sampled beach arrived in a variety of conditions, from a few relatively new to a majority of aged and brittle fragments. Ageing signals have been previously documented by other authors (Álvarez-Hernández et al., 2019; Fanini and Bozzeda, 2018; Sorasan et al., 2022) and indicate that oceanic gyres accumulate plastics

with different weathering subject to currents that can move them to distant places during which they undergo changes upon the action of environmental stressors.

The results showed that beaches can accumulate plastic pollution given that marine conditions favour their arrival from accumulation hotspots. The research included in **Chapter 2** answers the questions on the main origin of plastic reaching beaches in the north of the Canary Islands. After analysing the characteristics of the sampling site, the activities developed in the neighbourhood and the characteristics of sampled plastics, it could be determined that that main problem was a current-driven diffuse pollution, with very limited contribution of local activities. The research protocol developed for the research presented in **Chapter 2** focused on larger MPs excluding particle sizes below 1 mm due to operational restrictions. The presence of smaller particles was the subject of the other studies presented in the rest of chapters of this document, which were specifically designed for that purpose.

In **Chapter 3** the research moved to inland territory and to a known point source for plastic pollution. The study was performed in a conventional wastewater treatment plant (WWTP), which is one of the well-known contributors to MP pollution to receiving bodies. The presence of microplastic fibres, one of the most common synthetic materials that arrive to those plants was widely confirmed and quantified, most probably attributed to the laundering of synthetic clothes (Bayo et al., 2016; Browne et al., 2011; Zambrano et al., 2019). Besides, the study showed that a large number of MPs are retained in the sludge, which is further dried and used for the fertilization of agricultural soils (Gherghel et al., 2019). The dispersion of plastic in agricultural soils was later explored in **Chapter 7** because of the use of compost from organic municipal waste as soil amendment. The reuse of municipal wastes satisfies the needs of circular economy but has the drawback of allowing the dispersion into the environment of plastic pollutants even if careful management strategies like the door-to-door collection are applied.

WWTPs have also been explored afterwards to explore their possible impact in sensitive receiving environments. Freshwater ecosystems are of great importance for a multitude of organisms and represent the direct entry of pollution from inland water into the seas and oceans (Sarkar et al., 2021). As shown in **Chapter 3**, the existing wastewater

treatment technologies present a great effectiveness in the removal of MPs, reaching over 98 % in some cases (Lares et al., 2018; Murphy et al., 2016; Ziajahromi et al., 2017). Even if treated wastewater is generally clean, the large volume of treated water still leaves a considerable number of plastics in discharged streams, in this study about 300 million MPs per day, but the problem is much more important in smaller populations in which wastewater treatment facilities are poorer or insufficiently attended.

Chapter 4 focuses on the study of sediments from endorheic lagoons artificially recharged with wastewater. The MPs contained in the discharge from WWTPs gets confined in endorheic lagoons and accumulated in their sediments. The concentration of MPs in the sediments from lagoons located in a protected wetland area was evaluated. Treated wastewater is used in that area to artificially maintain the level of water in lagoons that would be temporary otherwise. Despite the lower quality of water, the lagoons host a variety of fauna, mainly birds that take advantage of the permanent presence of water. However, artificially recharged lagoons showed high values of MPs accumulated in sediments compared to the temporary non-recharged ones showing that artificial recharge with wastewater can lead to negative impacts, at least with the technologies currently available. The reduction of MPs in the environment requires further efforts to provide high quality treated water and a better monitoring by the administrations involved in the preservation of the environment.

Chapter 4 showed the presence of MPs also in non-artificially recharged lagoons. although in considerably lower extent compared to artificially recharges ones. The presence of MPs in those of relatively wild and unconnected ecosystems can be explained by diffuse pollution from non-point sources, including atmospheric transportation. The comparison between MPs in lagoon sediments and those from WWTPs showed that the most probable origin of the MPs found and described in **Chapter 4** was the atmospheric deposition. **Chapter 5** focused on the evaluation of this type of MP pollution through direct measurements using aircraft sampling. Is has already been described that MPs can be transported by air currents and reach distant places (Allen et al., 2019; Zhang et al., 2019). The techniques followed in the past allow obtaining indirect data on MPs attributed to atmospheric deposition by surface sampling (Dris et al., 2015) but were unable to unambiguously confirm and quantify their

presence in the atmosphere, especially at the high altitude necessary for long-range migration. **Chapter 5** presents a new sampling methodology that allows the direct measurement of MPs in air at different altitudes and to model their transportation to other places by atmospheric currents. A series of flights performed using military aircrafts at high altitudes flying over rural and urban areas allowed an unambiguous quantification of the concentration of MPs present at high altitude and to assess their capacity to reach distant places, even thousands of konometers in just a few hours. This work highlights the capacity of MPs to reach remote places with little human presence like those described in Antarctica (González-Pleiter et al., 2020), the Arctic (Hamilton et al., 2022; Ross et al., 2021), or certain mountain areas like the Great Himalayas (Napper et al., 2020).

The findings described in **Chapter 5** evidenced that all environments are or may be affected by the atmospheric deposition of plastic pollution (Brahney et al., 2020; Klein and Fischer, 2019; Purwiyanto et al., 2022; Truong et al., 2021). As indicated in **Chapter 1**, plastics can become a risk for humans and other living beings by inhalation (Baeza-Martínez et al., 2022). **Chapter 6** is directly connected to **Chapter 5** and explores the use of an insect as bioindicator of plastic pollution in the atmospheric compartment. The selected animal, honeybee (*Apis mellifera*), constitute a perfect example of animal that shares habitat with the humans and whose apiaries can be deployed as control stations. In the chapter, urban apiaries in different areas of the city of Copenhagen were selected to monitor the existing pollution. These insects are capable of flying long distances, covering several kilometres around their hives. In those areas and when flying, bees collect suspended particles and take them to the hives. Back to the apiaries, the bees keep the proofs of plastic pollution in the area attached by electrostatic attraction. In this work urban, suburban, and rural places were compared confirming that less plastic pollution was found in rural environments, consisting with a predominant urban origin of atmospheric plastic pollution (Evangeliou et al., 2022).

As indicated before, this Doctoral Thesis has established a clear connection between agriculture and MPs (Fig. 8.1). **Chapter 3** presented evidence of this plastic pollution reaching agricultural fields due to the use of WWTP sludge as organic amendment. This is not the only contribution bringing plastic pollution to soils. The organic fraction of

municipal waste is easily converted in agricultural compost with benefits in terms of circular economy because of the possibility of converting a waste into a resource. **Chapter 7** studies the presence of plastic remains in the compost generated from the separated collecting of municipal organic residues after their processing in plants with different technologies and collection schemes. The results from **Chapter 7** indicate that more densely populated areas generate organic wastes with higher amount of plastic contamination that in turn, result in compost with more plastic, which amounted to 5 - 20 particles/g of dry weight, with predominance of fibres. The European regulation establishes a stringent limitation for municipal residues arriving in landfills and the need to establish separate collection schemes for organic waste (Directive 2008/98/EC as amended by Directive EU 2018/851). As a result a high amount of compost from organic municipal waste is expected to be produced (Laso et al., 2019). It is important to assess its quality in terms of plastic pollution and to establish the better ways of processing to avoid or limit the dissemination of MPs into the environment. The results showed that smaller plants, with door-to-door collection produce compost with less plastic, in the lower range of the interval shown above. No compostable plastics were found in any case, confirming that they can be a suitable alternative to traditional plastics in applications that can be processed by industrial composting to ensure that no remains of plastics reach agricultural fields from municipal organic wastes. From the results obtained in this work, it was concluded that properly operated composting plants were efficient removing compostable plastics, but compost still contained a considerable amount of plastic particles, predominantly in fibre form and consisting mainly of the polymers polyethylene, polystyrene, polyester, polypropylene, polyvinyl chloride, and acrylic polymers in that order of abundance. Other authors demonstrated that domestic composting technologies are far from meeting the quality requirements of industrial plants like those studied in this work (Mateos-Cárdenas, 2022). This is important for companies that introduce new formulations in their novel products and regulators that are currently promoting home composting without clear evidence on the impact of composted products containing plastic to the environment.

The studies presented in this Doctoral Thesis show the interconnectivity of the different environmental compartments. MPs have been detected in all environments, water, air

and soil, and the evidence of transportation between them are clearly established. In their receiving environment plastics may disintegrate and impact the biota and even the humans (Baeza-Martínez et al., 2022; Leslie et al., 2022; Ragusa et al., 2021; Schwabl et al., 2019).

The works carried out during this Doctoral Thesis allowed estimating the amounts of MPs for different size ranges. The figures showed concentrations > 100 g/m^2 in a beach obtained from La Graciosa, a volcanic island in the Canary Islands Archipelago (**Chapter 2**), and inputs to the marine environment from the effluent of wastewater treatment plants that may reach 300 million MPs/m^3 (**Chapter 3**). In the atmospheric compartment, direct sampling allowed estimating a total number of MPs above Central Madrid of about 10^{12} particles between 1500 and 2500 m above ground level, with predicted deposition rates > 100 MPs m^2/day in nearby areas (**Chapter 5**). In the case of agricultural soils, the sources of MPs studied were the reuse of sewage sludge (**Chapter 3**) and the use of compost from organic municipal waste (**Chapter 8**), which altogether yielded values in the order of 10^4-10^5 MPs per kilogram of amendment dispersed into soils. It has been stated that terrestrial ecosystems could harbour much more plastic than marine ecosystems due to their relatively low mobility (Horton et al., 2017). This is an issue that requires further studies. Figure 8.1 summarizes the main estimates obtained in this Doctoral Thesis with the main interconnections between compartments.

As shown along this work, spectroscopy has proved to be a powerful tool for the detection of MPs. Fourier-Transform Infrared Spectroscopy (FTIR) was used in the studies described in Chapters **3-4-5-6 and 7** and resulted the most cost-effective way of identifying plastics in all environmental compartments. The technique has low pre-treatment requirements and does not affect sample integrity. The information provided by their spectra in the mid-infrared is accurate and comparable with commercial and in-house databases. If adequately handled, it may also provide information about the degree of ageing of samples and may give clues on their possible origin (Sorasan et al., 2022). In **Chapter 2,** Raman spectroscopy was also used. Coupled to a microscope, micro-Raman, the second most used spectroscopic technique for plastics identification,

proved useful especially for the detection of the additives included in plastic formulation.

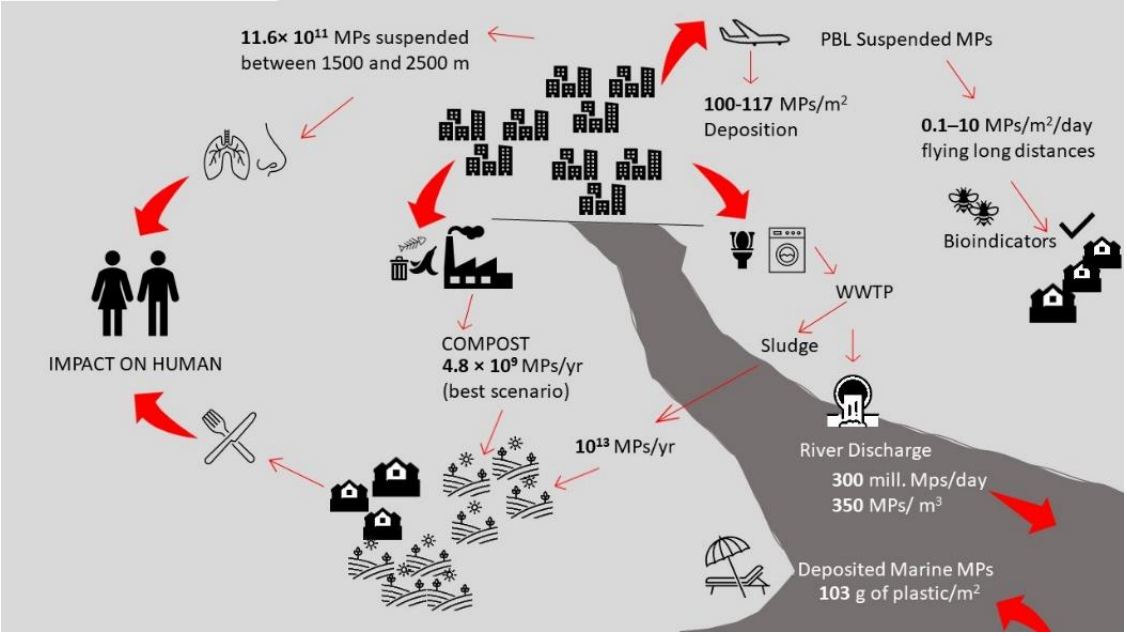


Figure 8.1. Schematic representation of the amounts of MPs obtained in the different works of this Doctoral Thesis and their potential mobility and fate.

Throughout this Doctoral Thesis, emphasis has been placed on identifying and quantifying the plastic materials that reach our ecosystems. In most cases, polyethylene, polypropylene and polystyrene are the main polymers identified, which is consistent with their wide number of uses (Sharma et al., 2021). These materials are quite stable, and the numerous works performed to date do not clarify their potential effects to the environment. More work is necessary for it, but their wide distribution as anthropogenic pollutants has been perfectly established. With the development of protocols for the analysis of small MPs, the quantification of smaller size debris has been made possible in a range of matrices. Other polymers have been identified, either as particles or fibres that include polyester and acrylic fibres from the synthetic fabrics used in many clothes, remains of polyvinyl chloride or polyurethane from domestic or industrial origin and many more. Some of them have dangerous additives (Bueno-Ferrer et al., 2010) like the per- and polyfluoroalkyl non-stick compounds (PFAS) liberated by Teflon™ that become public concern in the last decades (Sajid and Ilyas, 2017; Glüge et al., 2020) or the stabilizer Di-(2-ethylhexyl)-phthalates (DEHP) liberated by PVC after long uses (Latini et al., 2010). Furthermore, some represent controversial paradigms like the reuse of

polyethylene terephthalate or other polyesters to create fibres for new fancy textiles. With this new shape, the material becomes unrecyclable and the risk that plastic ends up in the environment is larger in fibre than in bulk form, thereby increasing the risk of plastic contamination.

The risks associated with MPs are largely dependent on their size, which is key to understand the potential damage that could induce to living beings. In **Chapters 3 and 5** it has been shown that fibres display a variety of lengths throughout all the MP range (5000-100 µm) although fragments are usually smaller (3000-40 µm). In all the protocols used in this Doctoral Thesis, the cut-off of 25 microns has been used as the lower size for separations, although in some cases, smaller particles and fibres could be identified. The use of the projected diameter as well as other parameters, was found useful to compare the particles found in a sample (Rosal, 2021). For the results shown in **Chapters 4 and 6** it has been determined that the average projected diameter corresponds to about 100 µm for fibres and between 100 and 300 µm in the case of fragments. These sizes are much smaller than those described in **Chapter 2**, but it has to be taken into account that in that specific work the size cut-off was limited to 1 mm due to sampling requirements. More recent publications on marine plastics confirm the existence of a large quantity of materials considerably below millimetre sizes (Expósito et al., 2021; Villanova-Solano et al., 2022). This is in line with the conclusions of **Chapter 7**, in which it has been shown that as size decreases, the number concentration of MPs increases. In the near future, science is going to focus on submicron particles, which correspond to the definition of nanoplastics, already detected in some environments, although with very fragmentary and scarce data (Davranche et al., 2020; Materić et al., 2022). The damage to the biota exerted by such small size materials is expected to be high due to their possible internalization (Wang et al., 2021).

The additives from polymers are another cause for concern. They are already the subject topic of intense research. Today, there is still much secrecy concerning the composition of many additives that eventually leak into the environment as plastics become exposed to stressors. As discussed in other chapters, these chemical compounds can produce diverse biological effects ranging from the well-studied endocrine disruption (Jobling et al., 1995) to carcinogenic problems (Hauser and Calafat, 2005; Hu et al., 2022). Along

this Doctoral Thesis, not only plastics, but also natural material like cellulose and cotton have been detected with non-natural colours, which evidenced their non-natural origin and an industrial processing that most probably introduced other additives in their formulation to become fibres for fabrics or objects for different uses. In parallel with the detection of MPs it is recommended that this kind of pollutants is closely controlled due to the easiness they have to pass to the environment. The required techniques may include Raman spectroscopy but also other equipment like chromatographic separations coupled with mass-spectrometry (Materić et al., 2022).

The role of the administrations is essential to limit the spreading of MP pollution in the environment. The results presented in **Chapters 2 and 4** represent clear pictures of this situation: MPs arriving everywhere, in this case in the less desirable places, protected areas and natural reserves. Other works showed that natural spaces are endangered by plastics from atmospheric deposition (Brahney et al., 2020). In this context, results in **Chapters 5 and 6** explain how the dispersion of MPs through the air may constitutes a serious problem for the environment. As seen before, places like Antarctica or the Arctic also receive plastic pollution from atmospheric dispersion. The implications of the spreading of anthropogenic pollution have been largely discussed. The development of systematic monitoring campaigns and early warning systems as performed with other particles in suspension could change the paradigm about this relatively overlooked compartment and on the sources of emission including the manufacturing and use of synthetic fibres.

New materials were tested in the works described in **Chapter 7** with the inclusion of compostable bags of diverse origin in the testing programme. The results were promising since composting stations showed a total capacity of removal, while the analytical procedures preserved their integrity. The development of new safer materials should be a priority for companies and administrations with the aim of providing goods that do not contribute to plastic pollution. Currently, biodegradable materials must comply with certain regulations like those established in the European standard EN-13432 (AENOR, 2001) that forces biodegradable materials to comply with strict composting requirements. This type of demand needs to be expanded to other type of materials to ensure they cannot accumulate in certain environmental compartments or

that they can be used without spreading plastic pollution. In this regard, it is important to note that there are risks associated with chemicals included in newly developed materials as explained elsewhere (Psarouthakis, Z., 2022). This Doctoral Thesis shows that the fate of plastic is difficult to predict, and it is clear that not all bioplastics are going to be properly treated under controlled conditions. For those that end-up disseminated into the environment, more research and regulation is needed.

As a conclusion, MPs have been widely detected and quantified in almost all possible environmental compartments. The concentration and nature of the sampled plastics was clearly linked to human activities and wastes. It is mandatory to ensure a sustained reduction in plastic waste production, to limit the use of single use plastics and to increase the life of plastic products in circulation. Plastics are wonderful materials, with low energy content and many irreplaceable uses, but their end-of-life phase must be properly managed not to miss their benefits. The development of new materials that gradually replace traditional plastics will be key in the near future for the reduction of global warming and the dependency of fossil fuels, but their entire lifecycle has to be considered as in the case of conventional materials. Finally, monitoring systems capable of tracking plastic dispersed into the environment should be firmly established to allow quick response actions and to detect misfunctions.

8.2. References

AENOR. 2001. *UNE-EN 13432:2001. Requirements for packaging recoverable through composting and biodegradation. Test scheme and evaluation criteria for the final acceptance of packaging*.

Allen S, Allen D, Phoenix VR, Le Roux G, Durántez Jiménez P, Simonneau A, et al. 2019. *Atmospheric transport and deposition of microplastics in a remote mountain catchment.* Nature Geoscience; 12: 339-344.

Álvarez-Hernández C, Cairós C, López-Darias J, Mazzetti E, Hernández-Sánchez C, González-Sálamo J, et al. 2019. *Microplastic debris in beaches of Tenerife (Canary Islands, Spain).* Marine Pollution Bulletin; 146: 26-32.

Baeza-Martínez C, Olmos S, González-Pleiter M, López-Castellanos J, García-Pachón E, Masiá-Canuto M, et al. 2022. *First evidence of microplastics isolated in European citizens' lower airway.* Journal of Hazardous Materials; 438: 129439.

Bayo J, Olmos S, López-Castellanos J, Alcolea A. 2016. *Microplastics and microfibers in the sludge of a municipal wastewater treatment plant*. International Journal of Sustainable Development and Planning; 11: 812-821.

Brahney J, Hallerud M, Heim E, Hahnenberger M, Sukumaran S. 2020. *Plastic rain in protected areas of the United States*; 368: 1257-1260.

Browne MA, Crump P, Niven SJ, Teuten E, Tonkin A, Galloway T, et al. 2011. *Accumulation of Microplastic on Shorelines Woldwide: Sources and Sinks*. Environmental Science & Technology; 45: 9175-9179.

Bueno-Ferrer C, Garrigós MC, Jiménez A. 2010. *Characterization and thermal stability of poly(vinyl chloride) plasticized with epoxidized soybean oil for food packaging. Polymer Degradation and Stability*; 95: 2207-2212.

Davranche M, Lory C, Le Juge C, Blancho F, Dia A, Grassl B, et al. 2020. *Nanoplastics on the coast exposed to the North Atlantic Gyre: Evidence and traceability*; 20: 100262.

Dris R, Gasperi J, Rocher V, Saad M, Renault N, Tassin BJEC. 2015. *Microplastic contamination in an urban area: a case study in Greater Paris*; 12: 592-599.

Evangeliou N, Tichý O, Eckhardt S, Zwaaftink CG, Brahney J. 2022. *Sources and fate of atmospheric microplastics revealed from inverse and dispersion modelling: From global emissions to deposition*. Journal of Hazardous Materials; 432: 128585.

Expósito N, Rovira J, Sierra J, Folch J, Schuhmacher M. 2021. *Microplastics levels, size, morphology and composition in marine water, sediments and sand beaches. Case study of Tarragona coast (western Mediterranean)*. Science of The Total Environment; 786: 147453.

Fanini L, Bozzeda F. 2018. *Dynamics of plastic resin pellets deposition on a microtidal sandy beach: Informative variables and potential integration into sandy beach studies*. Ecological Indicators; 89: 309-316.

FUNDEURAE. 2018. *Microplástico, palabra del año 2018 para la Fundéu.* BBVA.

Gherghel A, Teodosiu C, De Gisi S. 2019. *A review on wastewater sludge valorisation and its challenges in the context of circular economy*. Journal of Cleaner Production; 228: 244-263.

Glüge J, Scheringer M, Cousins IT, DeWitt JC, Goldenman G, Herzke D, Lohmann R, Ng CA, Trier X, Wang Z. 2020. *An overview of the uses of per- and polyfluoroalkyl substances (PFAS)* [10.1039/D0EM00291G]. Environmental Science: Processes & Impacts; 22(12):2345-2373. doi:10.1039/D0EM00291G.

González-Pleiter M, Edo C, Velázquez D, Casero-Chamorro MC, Leganés F, Quesada A, et al. 2020. *First detection of microplastics in the freshwater of an Antarctic Specially Protected Area.* Marine Pollution Bulletin; 161: 111811.

Hamilton BM, Jantunen L, Bergmann M, Vorkamp K, Aherne J, Magnusson K, et al. 2022. *Microplastics in the atmosphere and cryosphere in the circumpolar North: a case for multicompartment monitoring*. .

Hauser R, Calafat AM. 2005. *PHTHALATES AND HUMAN HEALTH*. Occupational and Environmental Medicine; 62: 806.

Horton AA, Walton A, Spurgeon DJ, Lahive E, Svendsen C. 2017. *Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities*. Science of The Total Environment; 586: 127-141.

Hu X, Yu Q, Gatheru Waigi M, Ling W, Qin C, Wang J, et al. 2022. *Microplastics-sorbed phenanthrene and its derivatives are highly bioaccessible and may induce human cancer risks.* Environment International; 168: 107459.

Jobling S, Reynolds T, White R, Parker MG, Sumpter JP. 1995. *A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic*; 103: 582-587.

Klein M, Fischer EK. 2019. *Microplastic abundance in atmospheric deposition within the Metropolitan area of Hamburg, Germany.* Science of The Total Environment; 685: 96-103.

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

Laso J, García-Herrero I, Margallo M, Bala A, Fullana-i-Palmer P, Irabien A, et al. 2019. *LCA-Based Comparison of Two Organic Fraction Municipal Solid Waste Collection Systems in Historical Centres in Spain.* Energies. 12, 2019.

Latini G, Ferri M, Chiellini F. 2010. *Materials Degradation in PVC Medical Devices, DEHP Leaching and Neonatal Outcomes.* Current Medicinal Chemistry; 17(26):2979-2989. doi:http://dx.doi.org/10.2174/092986710792064992.

Leslie HA, van Velzen MJM, Brandsma SH, Vethaak AD, Garcia-Vallejo JJ, Lamoree MH. 2022. *Discovery and quantification of plastic particle pollution in human blood.* Environment International; 163: 107199.

Mateos–Cárdenas A. 2022. *Fate of petroleum-based and plant-based teabags exposed to environmental soil conditions for one year*. Front. Bioeng. Biotechnol., 06. Sec. Bioprocess Engineering. Doi: https://doi.org/10.3389/fbioe.2022.966685.

Materić D, Kjær HA, Vallelonga P, Tison J-L, Röckmann T, Holzinger R 2022. *Nanoplastics measurements in Northern and Southern polar ice*. Environmental Research; 208: 112741.

Murphy F, Ewins C, Carbonnier F, Quinn B. 2016. *Wastewater Treatment Works (WwTW) as a Source of Microplastics in the Aquatic Environment*. Environmental Science & Technology; 50: 5800-5808.

Napper IE, Davies BFR, Clifford H, Elvin S, Koldewey HJ, Mayewski PA, et al. 2020. *Reaching New Heights in Plastic Pollution—Preliminary Findings of Microplastics on Mount Everest.* One Earth; 3: 621-630.

Psarouthakis, Z. 2022. *Sustainable plastic causing unsustainable damage: Impacts of petroleum and biopolymer microplastic exposure to a rocky reef fish (Forsterygion capito)*. (Thesis, Master of Science). University of Otago. Retrieved from http://hdl.handle.net/10523/13717

Purwiyanto AIS, Prartono T, Riani E, Naulita Y, Cordova MR, Koropitan AF. 2022. *The deposition of atmospheric microplastics in Jakarta-Indonesia: The coastal urban area.* Marine Pollution Bulletin; 174: 113195.

Ragusa A, Svelato A, Santacroce C, Catalano P, Notarstefano V, Carnevali O, et al. 2021. *Plasticenta: First evidence of microplastics in human placenta.* Environment International; 146: 106274.

Rosal R. 2021. *Morphological description of microplastic particles for environmental fate studies.* Marine Pollution Bulletin; 171: 112716.

Ross PS, Chastain S, Vassilenko E, Etemadifar A, Zimmermann S, Quesnel S-A, et al. 2021. *Pervasive distribution of polyester fibres in the Arctic Ocean is driven by Atlantic inputs.* Nature Communications; 12: 106.

Sajid M, Ilyas M. 2017. *PTFE-coated non-stick cookware and toxicity concerns: a perspective.* Environmental Science and Pollution Research; 2017/10/01;24(30):23436-23440. doi: 10.1007/s11356-017-0095-y.

Sarkar DJ, Das Sarkar S, Mukherjee S, Das BK. 2021. *Impact and Fate of Microplastics in the Riverine Ecosystem.* In: Kumar M, Snow DD, Honda R, Mukherjee S, editors. Contaminants in Drinking and Wastewater Sources: Challenges and Reigning Technologies. Springer Singapore, Singapore, pp. 95-115.

Schwabl P, Köppel S, Königshofer P, Bucsics T, Trauner M, Reiberger T, et al. 2019. *Detection of Various Microplastics in Human Stool.* Annals of Internal Medicine; 171: 453-457.

Sharma S, Basu S, Shetti NP, Nadagouda MN, Aminabhavi TM. 2021. *Microplastics in the environment: Occurrence, perils, and eradication*. Chemical Engineering Journal; 408: 127317.

Sorasan C, Edo C, González-Pleiter M, Fernández-Piñas F, Leganés F, Rodríguez A, et al. 2022. *Ageing and fragmentation of marine microplastics.* Science of The Total Environment; 827: 154438.

Truong T-N-S, Strady E, Kieu-Le T-C, Tran Q-V, Le T-M-T, Thuong Q-T. 2021. *Microplastic in atmospheric fallouts of a developing Southeast Asian megacity under tropical climate.* Chemosphere; 272: 129874.

Villanova-Solano C, Díaz-Peña FJ, Hernández-Sánchez C, González-Sálamo J, González-Pleiter M, Vega-Moreno D, et al. 2022. *Microplastic pollution in sublittoral coastal sediments of a North Atlantic island: The case of La Palma (Canary Islands, Spain)*. Chemosphere; 288: 132530.

Wang L, Wu W-M, Bolan NS, Tsang DCW, Li Y, Qin M, et al. 2021. *Environmental fate, toxicity and risk management strategies of nanoplastics in the environment: Current status and future perspectives.* Journal of Hazardous Materials; 401: 123415.

Zambrano MC, Pawlak JJ, Daystar J, Ankeny M, Cheng JJ, Venditti RA. 2019. *Microfibers generated from the laundering of cotton, rayon and polyester based fabrics and their aquatic biodegradation.* Marine Pollution Bulletin; 142: 394-407.

Zhang Y, Gao T, Kang S, Sillanpää M. 2019. *Importance of atmospheric transport for microplastics deposited in remote areas.* Environmental Pollution; 254: 112953.

Ziajahromi S, Neale PA, Rintoul L, Leusch FDL. 2017. *Wastewater treatment plants as a pathway for microplastics: Development of a new approach to sample wastewater-based microplastics*. Water Research; 112: 93-99.

CONCLUSIONS

Conclusions

- Microplastics have been found in all the compartments evaluated, namely marine and inland waters, air, soils, and composted municipal waste. The data obtained confirmed their status as emerging pollutant of high concern.
- The different studies carried out demonstrated the interconnection of environmental compartments. The presence of diffuse sources of microplastics and their mobility explain the transfer of microplastic between environments.
- The use of different spectroscopic tools, especially mid-infrared microscopy proved great usefulness in the identification of microplastics in environmental samples. The use of the same methodology allowed obtaining results comparable for the different compartments studied.
- The use of honeybees as bioindicators for plastic pollution could be useful to help plastic monitoring in urban and suburban environments, particularly concerning fibres.
- The results showed predominance of plastics based on polyethylene, polypropylene, and polystyrene. In addition, polyester and acrylic fibres were widely identified in all compartments revealing the need for controlling the spreading of synthetic fibres and their proper management after use.
- The use of biodegradable or compostable materials demonstrated a feasible alternative to reduce the arrival of plastic to soil via compost. However, these materials must be managed in the same way as traditional plastics to prevent their accumulation in the environment.

Conclusiones

- Se han encontrado microplásticos en todos los compartimentos evaluados, a saber, aguas marinas y continentales, aire, suelos y residuos municipales compostados. Los datos obtenidos confirmaron su estatus como contaminante emergente de alta preocupación.
- Los diferentes estudios realizados demostraron la interconexión de los compartimentos ambientales. La presencia de fuentes difusas de microplásticos y su movilidad explican la transferencia de microplásticos entre ambientes.
- El uso de diferentes herramientas espectroscópicas, especialmente la microscopía de infrarrojo medio, resultó de gran utilidad en la identificación de microplásticos en muestras ambientales. El uso de la misma metodología permitió obtener resultados comparables para los diferentes compartimentos estudiados.
- El uso de abejas melíferas como bioindicadores de la contaminación plástica podría ser útil para ayudar a monitorear el plástico en entornos urbanos y suburbanos, particularmente en lo que respecta a las fibras.
- Los resultados mostraron predominio de plásticos a base de polietileno, polipropileno y poliestireno. Además, las fibras de poliéster y acrílico fueron ampliamente identificadas en todos los compartimentos, lo que revela la necesidad de controlar la dispersión de las fibras sintéticas y su adecuado manejo después de su uso.
- El uso de materiales biodegradables o compostables demostró una alternativa factible para reducir la llegada de plástico al suelo vía compost. No obstante, estos materiales deben gestionarse de la misma forma que los plásticos tradicionales para evitar su acumulación en el medio ambiente.

LIST OF ABBREVIATIONS

A2O Anaerobic-Anoxic-Oxic (Technology)

a.g.l. Above Ground Level

a.s.l. Above Sea Level

ATR Attenuated Total Reflection (FTIR)

CI Confidence Intervals

CVF Chica de Villafranca Lagoon (Chapter 4)

DEHP Di-(2-ethylhexyl)-phthalates

DW Dry Weight

ELG El Longar Lagoon (Chapter 3)

EP Epoxy Resin

FTIR Fourier Transformed Infrarred Spectroscopy

GDAS Global Data Assimilation System Meteorological Data

GQR Laguna Grande de Quero (Chapter 4)

HYSPLIT HYbrid Single-Particle Lagrangian Integrated Trajectory

LAB La Albardiosa Lagoon (Chapter 4)

LAC Altillo Chica Lagoon (Chapter 4)

LVC Larga de Viñacañas Lagoon (Chapter 4)

MCT Mercury-Cadmium-Telluride Detector (FTIR)

MP/MPs Microplastic/Microplastics

NGOs Non-governmental organizations

NP/NPs Nanoplastic/Nanoplastics

NOAA National Oceanic and Atmospheric Administration

OFMSW Organic Fraction of Municipal Solid Wastes

OPLS-DA Orthogonal Partial Least Squares Discrimination Analysis

PA Polyamide

PAN Polyacrylonitrile

PBL Planetary Boundary Layer

PCA Principal Component Analysis

PCL	Polycaprolactone
PE	Polyethylene
PET	Polyethylene Terephthalate
PFAS	Per- and polyfluoroalkyl compounds
PHA/PHAs	Polyhydroxyalkanoates
PL/PES	Polyester
PLA	Polylactic Acid
PMMA	Polymethyl Methacrylate
POM	Polyoxymethylene
PP	Polypropylene
PS	Polystyrene
PSU	Polysulfone
PTFE	Polytetrafluoroethylene
PU	Polyurethane
PVA	Polyvinyl Acetate
PVC	Polyvinyl Chloride
PVF	Polyvinyl Fluoride
SEM	Scanning Electron Microscopy
SM	Supplementary Material
SUP/SUPs	Single Use Plastic/Plastics
TSG-ML	Technical subgroup on Marine Litter
WWTP	Wastewater treatment plant

LIST OF PUBLICATIONS

1. Edo, C., Tamayo-Belda, M., Martínez-Campos, S., Martín-Betancor, K., González-Pleiter, M., Pulido-Reyes, G., García-Ruiz, C., Zapata, F., Leganés, F., Fernández-Piñas, F. and Rosal, R. 2019. ***Occurrence and identification of microplastics along a beach in the Biosphere Reserve of Lanzarote***. Marine Pollution Bulletin, 143, 220-227.
2. Edo, C., González-Pleiter, M., Leganés, F., Fernández-Piñas, F. and Rosal R. 2020. ***Fate of microplastics in wastewater treatment plants and their environmental dispersion with effluent and sludge***. Environmental Pollution, 259, 113837.
3. González-Pleiter, M., Velázquez, D., Edo, C., Carretero, O., Gago, J., Barón-Sola, A., Hernández, L.E., Yousef, I., Quesada, A., Leganés, F., Rosal, R. and Fernández-Piñas, F. 2020. *Fibers spreading worldwide: Microplastics and other anthropogenic litter in an Arctic freshwater lake*. Science of The Total Environment, 722, 137904.
4. González-Pleiter, M., Edo, C., Casero-Chamorro, M.C., Aguilera, A., González-Toril, E., Wierzchos, J., Leganés, F., Fernández-Piñas, F. and Rosal, R. 2020. *Viable Microorganisms on Fibers Collected within and beyond the Planetary Boundary Layer***.** Environmental Science & Technology Letters, 7-11, 819-825.
5. Edo, C., González-Pleiter, M., Tamayo-Belda, M., Ortega-Ojeda, F.E., Leganés, F., Fernández-Piñas, F. and Rosal, R. 2020. ***Microplastics in sediments of artificially recharged lagoons: Case study in a Biosphere Reserve***. Science of The Total Environment, 729, 138824.
6. González-Pleiter, M.*, Edo, C.*, Velázquez, D., Casero-Chamorro, M.C., Leganés F., Quesada, A., Fernández-Piñas, F. and Rosal, R. 2020. *First detection of microplastics in the freshwater of an Antarctic Specially Protected Area*. Marine Pollution Bulletin, 161, 111811. *Equal Contribution.
7. González-Pleiter, M.*, Edo, C.*, Aguilera, A., Viúdez-Moreiras, D., Pulido-Reyes, G., González-Toril, E., Osuna, S., de Diego-Castilla, G., Leganés, F., Fernández-Piñas, F. and Rosal, R. 2021. ***Occurrence and transport of microplastics sampled within and above the planetary boundary layer***. Science of The Total Environment, 761, 143213. *Equal Contribution.
8. Edo, C., Fernández-Alba, A.R., Vejsnæs, F., van der Steen, J., Fernández-Piñas, F. and Rosal, R. 2021. ***Honeybees as active samplers for microplastics*****.** Science of The Total Environment, 767, 144481.
9. González-Pleiter, M., Lacerot, G., Edo, C., Lozoya, J.P., Leganés F., Fernández-Piñas, F., Rosal, R. and Teixeira-de-Mello, F. 2021. *A pilot study about microplastics and mesoplastics in an Antarctic glacier*. The Cryosphere, 15-6, 2531-2539.
10. Sorasan, C., Edo, C., González-Pleiter, M., Fernández-Piñas, F., Leganés, F., Rodríguez, A. and Rosal, R. *Generation of nanoplastics during the photoageing of low-density polyethylene***.** Environmental Pollution, 289, 117919, 2021.

11. Edo, C., Fernández-Piñas, F. and Rosal, R. 2022. ***Microplastics in organic compost: Influence of waste collection system and composting methodology*.** Science of The Total Environment, 813, 151902.
12. Sorasan, C., Edo, C., González-Pleiter, M., Fernández-Piñas, F., Leganés, F., Rodríguez, A. and Rosal, R. 2022. *Ageing and fragmentation of marine microplastics*. Science of The Total Environment, 827, 154438.

“PROTECTING OUR PLANET IS A MORAL IMPERATIVE”

Johan Rockström

Universidad de Alcalá