MICROPLASTICS: AN EMERGING CONTAMINANT IN ENVIRONMENTAL COMPARTMENTS - A COMPREHENSIVE ANALYSIS OF DISTRIBUTION AND ENVIRONMENTAL IMPACT

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UNIVERSITAT ROVIRA i VIRGILI

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DOCTORAL THESIS

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FAIG CONSTAR que aquest treball, titulat "**Microplastics: An Emerging Contaminant in Environmental Compartments - A Comprehensive Analysis of Distribution and Environmental Impact**", que presenta **Lara Dronjak** per a l'obtenció del títol de Doctor, ha estat realitzat sota la meva direcció al Departament d´ Enginyeria Quimica d'aquesta universitat.

HAGO CONSTAR que el presente trabajo, titulado "**Microplastics: An Emerging Contaminant in Environmental Compartments - A Comprehensive Analysis of Distribution and Environmental Impact**", que presenta **Lara Dronjak** para la obtención del título de Doctor, ha sido realizado bajo mi dirección en el Departamento de Ingeneria Quimica de esta universidad.

I STATE that the present study, entitled "**Microplastics: An Emerging Contaminant in Environmental Compartments - A Comprehensive Analysis of Distribution and Environmental Impact**", presented by **Lara Dronjak** for the award of the degree of Doctor, has been carried out under my supervision at the Chemical Engineering of this university.

Tarragona, 24th of July, 2023

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With heartfelt appreciation,

Lara

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I dedicate my thesis to my sister Jelena, and my parents Ivana and Dejan

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Abbreviations

DWTP: Drinking water treatment plant

MPs: Microplastics

NPs: Nanoplastics

PA: Polyamide (Nylon)

PC: Polycarbonate

PE: Polyethylene

PES: Polyester

PET: Polyethylene Terephthalate

PMMA: Polymethyl Methacrylate

PP: Polypropylene

PS: Polystyrene

PTFE: Polytetrafluoroethylene

PVC: Polyvinyl Chloride

WWTP: Wastewater treatment plant

Table of Contents

UNIVERSITAT ROVIRA I VIRGILI
MICROPLASTICS: AN EMERGING CONTAMINANT IN ENVIRONMENTAL COMPARTMENTS - A COMPREHENSIVE ANALYSIS OF DISTRIBUTION AND ENVIRONMENTAL IMPACT
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Summary

Microplastic (MP) pollution is a widespread global issue, as it is found and identified in various natural ecosystems, including water, soil, and air. This has become a major concern for both the scientific community and the general public. MPs are defined as any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1 µm to 5 mm, of either primary or secondary manufacturing origin, which are insoluble in water. MPs enter the natural environment through a variety of pathways, including release from Wastewater Treatment Plants (WWTPs), urban runoff from streets and urban areas, transportation via rivers and streams from various sources like urban runoff, agricultural fields, and industrial areas, atmospheric deposition from air transport into water bodies and terrestrial environments, direct introduction through coastal activities like boating and fishing, industrial discharges from plastic-using and producing industries, improper waste disposal, littering, illegal dumping, and the land application of sewage sludge and organic waste amendments as fertilizers that introduce MPs into the soil, potentially reaching water bodies through runoff and leaching. These multiple routes contribute to the widespread distribution of MPs in the environment, necessitating comprehensive efforts to address and mitigate their impact on ecosystems.

One of the main routes through which MPs enter the natural environment is via wastewater treatment plants (WWTPs). MPs have been detected in both the influent and effluent of WWTPs worldwide. WWTPs play a vital role in cleaning water flow, however they are not specifically designed to remove MPs from wastewater streams. The release of plastic waste into wastewater can facilitate its uptake by biofilm-forming microorganisms, which can travel across the ocean and become a vector for the spread of potential pathogens or of antibiotic resistance genes on a global scale. Despite high removal efficiency, with most cases exceeding 95%, a significant number of MPs still enter the natural environment through effluent discharge. MPs are potential carriers of toxic chemicals due to their large specific surface area and strong hydrophobicity.

Numerous heavy metals, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), phthalates, pesticides, and nano-scale adsorbents have been detected on the surface of MPs. Therefore, a comprehensive case study was conducted on a WWTP in Catalonia, Spain (Chapter 1), focusing on the concentration, size, morphology, and composition of MPs in various treatment units in water and sludge lines. The study revealed significant reductions in MPs concentrations as water underwent treatment, with an overall removal efficiency exceeding 96%. The most commonly detected polymers in different units of the WWTP included polyethylene (PE), synthetic cellulose, polypropylene (PP), polyvinyl chloride (PVC), polyethylene-polypropylene (PE-PP), polyethylene-co-ethyl acrylate (PEEA), polyamide (PA), acrylamide, and polyester (PES). It is noteworthy that several MPs are still present in the effluent of the plant, and it was estimated that 5.1×10^{10} MP/year will ultimately find their way into receiving water body, particularly river in the studied area.

A crucial aspect that demands attention is that the rivers serve as an influent source for drinking water treatment plants (DWTPs). DWTPs ensure the quality and safety of drinking water and currently there are no legislative restrictions on the abundance of this new emerging pollutant. Only a few studies investigated MPs based on full-scale DWTP and Directive (EU) 2020/2184 of the European Parliament and of the Council acknowledges the issue of MPs in drinking water and directs the European Commission to investigate and evaluate their presence and potential impacts to safeguard public health and ensure the quality of water intended for human consumption. Thus, the second chapter of the thesis focuses on investigating the presence of MPs in a full-scale DWTP located in Catalonia, Spain (Chapter 2). In general, the operation of a DWTP with respect to the reduction of MPs was effective and with elevated removal efficiency. Clarifiers together with the sand filtration unit had the greatest impact on MPs reduction, >99%. However, an increase in MPs concentration, in the DWTP effluent, was observed and the final removal efficiency was 98.3% which is still higher than the values reported for other European drinking water treatment plants.

On the other hand, MPs that are removed during the treatment process in both WWTPs and DWTPs tend to accumulate in the sludge. The Spanish regulatory decree AAA/1072/2013, which governs the use of sewage sludge in agriculture, did not initially consider MPs from sewage sludge as a recognized pollutant, as the accumulation of these contaminants in agricultural land was not yet suspected at that time. The deposition of MPs on European agricultural soils is estimated to be in the range of 63,000 to 430,000 tons annually, primarily attributed to sludge application. MPs present in agricultural soils pose a significant concern as they have the potential to be ingested by soil organisms, entering the food chain, and posing a risk to human health. Consequently, we conducted third holistic assessment on MPs present in sewage sludge and other organic waste amendments utilized as fertilizers in agricultural practices in Catalonia, Spain (Chapter 3). The study encompassed seven distinct organic amendments, including sewage sludge, horse manure, two composts and two digestates from agri-food industries and selectively separated municipal organic waste, and a biostabilized product from non-selectively separated municipal organic waste. The study revealed notable micro and macro plastic concentrations in the analyzed samples, with levels ranging from 7137 to 482943 MPs/kg of dry weight, depending on the origin and treatment of organic amendments. These findings highlight that the application of organic amendments to soil serves as a significant pathway for the introduction of MPs into the natural environment, particularly terrestrial.

Our research in Catalonia, Spain, has provided valuable insights into the presence and impact of MPs in WWTPs, DWTPs, and organic waste amendments. To gain a better understanding of the behavior of these particles, we extended our investigation to marine sediments in the San Francisco Bay (The Bay) area (Chapter 4). Marine sediments gained recognition as a final reservoir for MPs accumulation. Even MPs that are initially buoyant will eventually sink over time due to accumulation of organic and inorganic material. The investigation encompassed both spatial and temporal trends of MPs distribution in the Bay. This was achieved by analyzing surface sediment grab samples and core samples collected from various locations within the

Bay. The highly urbanized nature of the Bay area further highlights the importance of this research, as it exhibits elevated MPs concentrations compared to other marine sediments worldwide. Addressing this widespread issue of MPs in the environment requires a multi-stage approach, including improved waste management, reduced plastic use, enhanced wastewater treatment, and increased awareness to mitigate the impact of MPs pollution on the environment.

In conclusion, the multi-faceted issue of microplastic pollution necessitates a multi-pronged approach including improved waste management, reduced plastic consumption, advanced wastewater treatment, and heightened public awareness. As we move forward, future research should delve into the ecological and health impacts of MPs, explore innovative remediation strategies, and establish standardized methods for MPs analysis. Collaborative efforts involving scientists, policymakers, industries, and the public are pivotal to effectively combatting this global concern.

Resumen

La contaminación por microplásticos (MP) es un problema muy extendido globalmente, ya que se puede encontrar e identificar en varios ecosistemas naturales, incluyendo agua, suelo y aire. Esto se ha convertido en una preocupación importante tanto para la comunidad científica como para el público en general. Los MP se definen como cualquier partícula sólida sintética o matriz polimérica, con forma regular o irregular y un tamaño que varía entre 1 µm y 5 mm, de origen manufacturado primario o secundario, e insoluble en agua. Los MP llegan al entorno natural a través de diversas vías, como pueden ser las Plantas de Tratamiento de Aguas Residuales (PTAR), por transporte a través de ríos y corrientes desde diversas fuentes como la escorrentía urbana, de campos agrícolas y áreas industriales, por deposición atmosférica a cuerpos de agua y entornos terrestres, por introducción directa a través de actividades costeras como navegación y pesca, por descargas industriales de industrias que utilizan y producen plásticos, por disposición inadecuada de desechos, por arrojo ilegal, y por la aplicación terrestre de lodos de alcantarillado y enmiendas orgánicas como fertilizantes que introducen MPs en el suelo, alcanzando potencialmente cuerpos de agua a través del escurrimiento y la lixiviación. Estas múltiples vías contribuyen a la distribución generalizada de MPs en el entorno, lo que requiere integrar diferentes enfoques para abordar y mitigar su impacto en los ecosistemas.

Una de las principales vías por las cuales los MPs ingresan al entorno natural es a través de las plantas de tratamiento de aguas residuales (PTAR). Los MPs se han detectado tanto en el influente como en el efluente de las PTAR en todo el mundo. Si bien las PTAR desempeñan un papel vital en la limpieza del agua, no están específicamente diseñadas para eliminar los MPs de los flujos de aguas residuales. La liberación de desechos plásticos en estas aguas puede facilitar su captura por microorganismos que forman biopelículas, los cuales pueden viajar a través del océano y convertirse en vectores para la propagación de posibles patógenos o genes de resistencia a antibióticos a escala global. A pesar de la alta eficiencia de eliminación, en la mayoría de los casos superando el 95%, un número significativo de MPs aún ingresan al entorno natural a través del efluente. Los MPs son portadores potenciales de productos químicos tóxicos debido a su gran área superficial específica y su fuerte hidrofobicidad. Se han detectado numerosos metales pesados, bifenilos policlorados (PCB), hidrocarburos aromáticos policíclicos (HAP), ftalatos, pesticidas y adsorbentes a nanoescala en la superficie de los MPs. Por lo tanto, se realizó un estudio en una PTAR en Cataluña, España (Capítulo 1), centrado en la concentración, tamaño, morfología y composición de los MPs en varias unidades de tratamiento en líneas de agua y lodos. El estudio reveló reducciones significativas en las concentraciones de MPs a medida que el agua se sometía a tratamiento, con una eficiencia de eliminación total que

superaba el 96%. Los polímeros más comúnmente detectados en diferentes unidades de la PTAR incluyeron polietileno (PE), celulosa sintética, polipropileno (PP), cloruro de polivinilo (PVC), polietileno-polipropileno (PE-PP), polietileno-co-etil acrilato (PEEA), poliamida (PA), acrilamida y poliéster (PES). Cabe destacar que el 4% de MPs que no son eliminados, estimados en 5,1 x 1010 MP/año, finalmente llegarán a los cuerpos de agua receptores, particularmente los ríos en el área estudiada.

Un aspecto crucial que requiere atención es que los ríos sirven como fuente de influencia para las plantas de tratamiento de agua potable (PTAP). Las PTAP aseguran la calidad y seguridad del agua potable y actualmente no existen restricciones legislativas sobre la abundancia de este nuevo contaminante emergente. Solo unos pocos estudios investigaron los MPs en PTAP a escala completa y la Directiva (UE) 2020/2184 del Parlamento Europeo y del Consejo reconoce el problema de los MPs en el agua potable y dirige a la Comisión Europea a investigar y evaluar su presencia e impactos potenciales para salvaguardar la salud pública y garantizar la calidad del agua destinada al consumo humano. Por lo tanto, el segundo capítulo de la tesis se centra en investigar la presencia de MPs en una PTAP a escala completa ubicada en Cataluña, España (Capítulo 2). En general, la operación de una PTAP con respecto a la reducción de MPs fue efectiva y con una alta eficiencia de eliminación. Los clarificadores junto con la unidad de filtración de arena tuvieron el mayor impacto en la reducción de MPs, >99%. Sin embargo, se observó un aumento en la concentración de MPs en el efluente de la PTAP, y la eficiencia de eliminación final fue del 98.3%, lo cual sigue siendo superior a los valores informados para otras PTAP europeas.

Por otro lado, los MPs que se eliminan durante el proceso de tratamiento tanto en las PTAR como en las PTAP tienden a acumularse en los lodos. El decreto reglamentario español AAA/1072/2013, que regula el uso de lodos de alcantarillado en la agricultura, inicialmente no consideró a los MPs de los lodos de alcantarillado como un contaminante reconocido, ya que la acumulación de estos contaminantes en suelos agrícolas no se sospechaba en ese momento. La deposición de MPs en suelos agrícolas europeos se estima en el rango de 63.000 a 430.000 toneladas anuales, atribuidas principalmente a la aplicación de lodos. Los MPs presentes en suelos agrícolas representan una preocupación significativa, ya que tienen el potencial de ser ingeridos por organismos del suelo, ingresar en la cadena alimentaria y representar un riesgo para la salud humana. En consecuencia, realizamos una tercera evaluación integral de los MPs presentes en lodos de alcantarillado y otras enmiendas orgánicas utilizadas como fertilizantes en prácticas agrícolas en Cataluña, España (Capítulo 3). El estudio abarcó siete enmiendas orgánicas distintas, incluidos lodos de alcantarillado, estiércol de caballo, dos compost y dos

digestatos de industrias agroalimentarias y desechos orgánicos municipales selectivamente separados, y un producto biestabilizado de desechos orgánicos municipales no separados selectivamente. El estudio reveló concentraciones notables de micro y macro plasticos en las muestras analizadas, con niveles que oscilan entre los 7.137 y los 482.943 MPs/kg de peso seco, dependiendo del origen y tratamiento de las enmiendas orgánicas. Estos hallazgos destacan que la aplicación de enmiendas orgánicas al suelo sirve como una vía significativa para la introducción de MPs en el entorno natural, especialmente en el terrestre.

Nuestra investigación en Cataluña, España, ha proporcionado información valiosa sobre la presencia e impacto de los MPs en PTAR, PTAP y enmiendas orgánicas. Para comprender mejor el comportamiento de estas partículas, extendimos nuestra investigación a sedimentos marinos en el área de la Bahía de San Francisco (La Bahía) (Capítulo 4). Los sedimentos marinos han ganado reconocimiento como un reservorio final para la acumulación de MPs. Incluso los MPs que son inicialmente flotantes eventualmente se hunden con el tiempo debido a la acumulación de material orgánico e inorgánico. La investigación abarcó tanto las tendencias espaciales como temporales de la distribución de MPs en la Bahía. Esto se logró mediante el análisis de muestras de sedimentos superficiales y muestras de núcleos recolectados en diversas ubicaciones dentro de la Bahía. La naturaleza altamente urbanizada del área de la Bahía resalta aún más la importancia de esta investigación, ya que exhibe concentraciones elevadas de MPs en comparación con otros sedimentos marinos en todo el mundo. Abordar este problema generalizado de los MPs en el entorno requiere un enfoque multifacético, que incluye mejorar la gestión de residuos, la reducción del uso de plásticos, el tratamiento avanzado de aguas residuales y una mayor conciencia para mitigar el impacto de la contaminación por MPs en el medio ambiente.

En conclusión, el problema multifacético de la contaminación por microplásticos requiere un enfoque integral que incluya una gestión de residuos mejorada, reducción del consumo de plásticos, tratamiento avanzado de aguas residuales y mayor conciencia pública. A medida que avanzamos, la investigación futura debe profundizar en los impactos ecológicos y de salud de los MPs, explorar estrategias de remediación innovadoras y establecer métodos estandarizados para el análisis de MPs. Los esfuerzos de colaboración entre científicos, políticos, industrias y el público son fundamentales para combatir eficazmente esta preocupación global.

Resum

La contaminació per microplàstics (MP) és un problema àmpliament estès a nivell mundial, ja que es pot trobar i identificar en diversos ecosistemes naturals, incloent aigua, sòl i aire. Això s'ha convertit en una preocupació rellevant tant per a la comunitat científica com per al públic en general. Els MP es defineixen com qualsevol partícula sòlida sintètica o matriu polimèrica, amb forma regular o irregular i una grandària que varia entre 1 µm i 5 mm, d'origen manufacturat primari o secundari, i insolubles en aigua. Els MP arriben a l'entorn natural a través de diverses vies, com les Plantes de Tractament d'Aigües residuals (PTAR), pel transport a través de rius i corrents des de diverses fonts, com l'escolament urbà, dels camps agrícoles i àrees industrials, per deposició atmosfèrica als cossos d'aigua i entorns terrestres, per introducció directa a través d'activitats costaneres com la navegació i la pesca, per descàrregues industrials d'indústries que utilitzen i produeixen plàstics, per la disposició inadequada de residus, per llançament il·legal, i per l'aplicació terrestre de fangs de claveguera i esmenes orgàniques com a fertilitzants que introdueixen MPs al sòl, arribant potencialment als cossos d'aigua mitjançant l'escorrentia i la lixiviació. Aquestes múltiples vies contribueixen a la distribució generalitzada de MPs en l'entorn, el que requereix integrar diferents enfocaments per abordar i mitigar el seu impacte en els ecosistemes.

Una de les principals vies per les quals els MPs ingressen a l'entorn natural és a través de les plantes de tractament d'aigües residuals (PTAR). Els MPs s'han detectat tant en l'influent com en l'efluent de les PTAR a tot el món. Tot i que les PTAR juguen un paper vital en la neteja de l'aigua, no estan específicament dissenyades per eliminar els MPs dels fluxos d'aigües residuals. La lliberació de residus plàstics en aquestes aigües pot facilitar la seva captura per microorganismes que formen biofilms, els quals poden viatjar a través de l'oceà i esdevenir vectors per a la propagació de possibles patògens o gens de resistència als antibiòtics a escala global. Malgrat l'alta eficiència d'eliminació, en la majoria dels casos superant el 95%, un nombre significatiu de MPs encara ingressa a l'entorn natural a través de l'efluent. Els MPs són portadors potencials de productes químics tòxics a causa de la seva gran àrea superficial específica i la seva forta hidrofòbia. S'han detectat nombrosos metalls pesants, bifenils policlorats (PCB), hidrocarburs aromàtics policíclics (HAP), ftalats, pesticides i adsorbents a nanoescala a la superfície dels MPs. Per tant, es va realitzar un estudi en una PTAR a Catalunya, Espanya (Capítol 1), centrat en la concentració, grandària, morfologia i composició dels MPs en diverses unitats de tractament en línies d'aigua i fangs. L'estudi va revelar reduccions significatives en les concentracions de MPs a mesura que l'aigua es sotmetia a tractament, amb una eficiència d'eliminació total que superava el 96%. Els polímers més comúment detectats en diferents

unitats de la PTAR van incloure polietilè (PE), cel·lulosa sintètica, polipropilè (PP), clorur de polivinil (PVC), polietilè-polipropilè (PE-PP), polietilè-co-etil acrilat (PEEA), poliamida (PA), acrilamida i polièster (PES). Cal destacar que el 4% de MPs que no són eliminats, estimats en 5,1 x 1010 MPs/any, finalment arribaran als cossos d'aigua receptors, particularment els rius a l'àrea estudiada.

Un aspecte crucial que requereix atenció és que els rius serveixen com a font d'influència per a les plantes de tractament d'aigua potable (PTAP). Les PTAP asseguren la qualitat i seguretat de l'aigua potable i actualment no existeixen restriccions legislatives sobre l'abundància d'aquest nou contaminant emergent. Només uns pocs estudis van investigar els MPs en PTAP a escala completa i la Directiva (UE) 2020/2184 del Parlament Europeu i del Consell reconeix el problema dels MPs a l'aigua potable i dirigeix la Comissió Europea a investigar i avaluar-ne la presència i els impactes potencials per salvaguardar la salut pública i garantir la qualitat de l'aigua destinada al consum humà. Per tant, el segon capítol de la tesi es centra en investigar la presència de MPs en una PTAP a escala completa situada a Catalunya, Espanya (Capítol 2). En general, l'operació d'una PTAP pel que fa a la reducció de MPs va ser efectiva i amb una alta eficiència d'eliminació. Els clarificadors juntament amb la unitat de filtració de sorra van tenir el major impacte en la reducció de MPs, >99%. No obstant això, es va observar un augment en la concentració de MPs a l'efluent de la PTAP, i l'eficiència d'eliminació final va ser del 98,3%, la qual cosa segueix sent superior als valors informats per a altres PTAP europees.

D'altra banda, els MPs que s'eliminen durant el procés de tractament tant a les PTAR com a les PTAP tendeixen a acumular-se als fangs. El decret reglamentari espanyol AAA/1072/2013, que regula l'ús de fangs de claveguera a l'agricultura, inicialment no va considerar els MPs dels fangs de claveguera com a contaminants reconeguts, ja que l'acumulació d'aquests contaminants en sòls agrícoles no es sospitava en aquell moment. La deposició de MPs en sòls agrícoles europeus s'estima en el rang de 63.000 a 430.000 tones anuals, atribuïdes principalment a l'aplicació de fangs. Els MPs presents en sòls agrícoles representen una preocupació significativa, ja que tenen el potencial de ser ingerits per organismes del sòl, entrar a la cadena alimentària i representar un risc per a la salut humana. En conseqüència, es va realitzar una tercera avaluació integral dels MPs presents en fangs de claveguera i altres esmenes orgàniques utilitzades com a fertilitzants en pràctiques agrícoles a Catalunya, Espanya (Capítol 3). L'estudi va abastar set esmenes orgàniques diferents, inclosos fangs de claveguera, estronc de cavall, dos compostos i dos digestats d'indústries agroalimentàries i residus orgànics municipals selectivament separats, i un producte bieestabilitzat de residus orgànics municipals no separats selectivament. L'estudi va revelar concentracions notables de micro i macroplàstics a les mostres analitzades, amb nivells

que oscil·len entre els 7.137 i els 482.943 MPs/kg de pes sec, depenent de l'origen i tractament de les esmenes orgàniques. Aquests resultats destaquen que l'aplicació d'esmenes orgàniques al sòl serveix com una via significativa per a la introducció de MPs en l'entorn natural, especialment en el terrestre.

La nostra investigació a Catalunya, Espanya, ha proporcionat informació valuosa sobre la presència i l'impacte dels MPs a PTAR, PTAP i esmenes orgàniques. Per comprendre millor el comportament d'aquestes partícules, vam estendre la nostra investigació als sediments marins a l'àrea de la Badia de San Franciso (La Badia) (Capítol 4). Els sediments marins han guanyat reconeixement com a reservori final per a l'acumulació de MPs. Fins i tot els MPs que són inicialment flotants finalment s'enfonsen amb el temps a causa de l'acumulació de material orgànic i inorgànic. La investigació va abastar tant les tendències espacials com temporals de la distribució de MPs a la Badia. Això es va aconseguir mitjançant l'anàlisi de mostres de sediments superficials i mostres de nuclis recollits en diverses ubicacions dins de la Badia. La naturalesa altament urbanitzada de l'àrea de la Badia destaca encara més la importància d'aquesta investigació, ja que exhibeix concentracions elevades de MPs en comparació amb altres sediments marins arreu del món. Abordar aquest problema generalitzat dels MPs a l'entorn requereix un enfocament multifacètic, que inclou millorar la gestió de residus, la reducció de l'ús de plàstics, el tractament avançat d'aigües residuals i una major consciència per a mitigar l'impacte de la contaminació per MPs en el medi ambient.

En conclusió, el problema multifacètic de la contaminació per microplàstics requereix un enfocament integral que inclogui una millora en la gestió de residus, la reducció del consum de plàstics, el tractament avançat d'aigües residuals i una major consciència pública. A mesura que avancem, la investigació futura ha d'aprofundir en els impactes ecològics i de salut dels MPs, explorar estratègies de remediament innovadores i establir mètodes estandarditzats per a l'anàlisi de MPs. Els esforços de col·laboració entre científics, polítics, indústries i el públic són fonamentals per a combatre eficaçment aquesta preocupació global.

Introduction

Historical Perspectives on Microplastics and Terminology

The initial investigation into the consequences of extensive plastic production dates back to 1972 when Carpenter and Smith published their study on plastic pollution in the surface of the Sargasso Sea and reported an average of 3500 particles/km², many of which ranged in size between 0.25 and 0.5 cm. These findings marked a pivotal starting point for studying plastics in the environment. The term "microplastic" was first used by Ryan (1988) during a study of plastic pollution on South African beaches. However, it was only after Thompson et al. (2004) used the term in their research article "Lost at sea - Where is all the plastic at?" published in Science Magazine that it gained widespread acceptance in the scientific community. In 2009, Arthur et al. introduced an upper size limit to the MPs defining them as “plastic particles smaller than 5 mm” and in 2019, Frias and Nash put forth a consensus on the definition of microplastics, which takes into account not only size and origin but also incorporates key physical and chemical defining properties:

“Microplastics are any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1 µm to 5 mm, of either primary or secondary manufacturing origin, which are insoluble in water”.

Understanding Microplastics: Classification and Characteristics

Origins of microplastics

MPs are categorized either as primary or secondary based on their sources of origin. Primary MPs are intentionally designed and manufactured for specific uses, while secondary MPs result from the degradation of larger plastic items (Cole et al., 2011). Primary MPs are used in various applications, such as personal care products, air-blasting media, drug delivery in medicine (Zitko and Hanlon, 1991; Gregory, 1996; Patel et al., 2009), and virgin plastic production pellets, although some researchers have debated their inclusion in this category (Andrady, 2011; Costa et al., 2010).

The main known primary MPs sources are reported and classified in Figure 1, based on data recently published from Denmark (Lassen et al., 2015), Sweden (Magnuson et al., 2016), Norway (Sundt et al., 2014), and Germany (Essel et al., 2015).

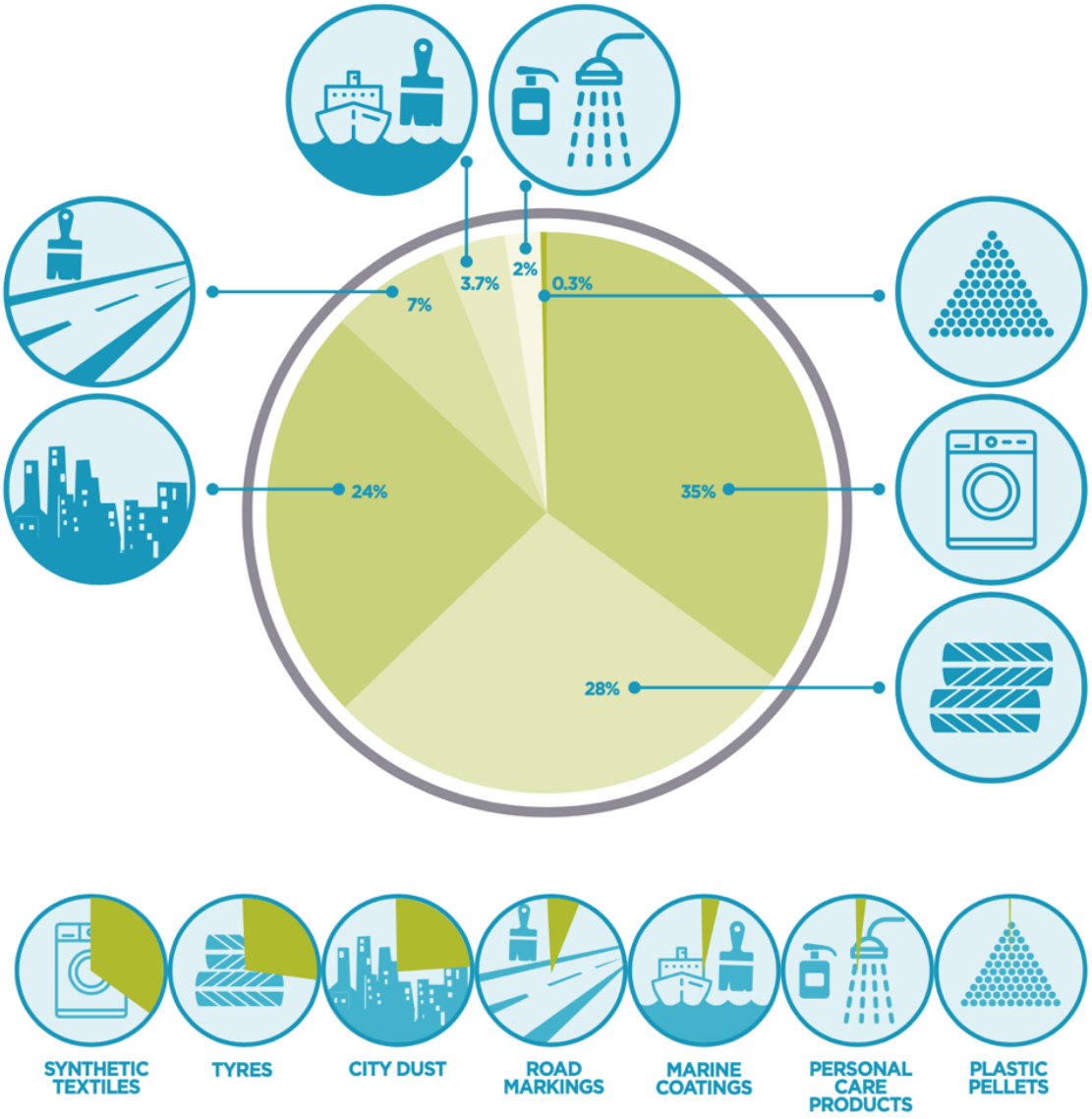


Figure 1 Global releases of primary MPs to the world oceans, by source (in %)

Source: Boucher, J., & Friot, D. (2017)

Size of microplastics

The commonly accepted upper size limit for microplastics is 5 mm. The lower size limit for MPs was established at 1 µm according to Frias and Nash (2019), however due to technological challenges, the current practical range for identification lies between 20 and 100 µm using micro-Fourier Transformed Infrared Spectroscopy (µ-FTIR) (Frias et al., 2018); however, technological advancements are expected to eventually decrease this limit to 1 µm. The overall term of MPs is composed of small MPs (smaller than 1 mm) and large MPs (1-5 mm), to differentiate between two commonly used definitions for MPs (Figure 2).

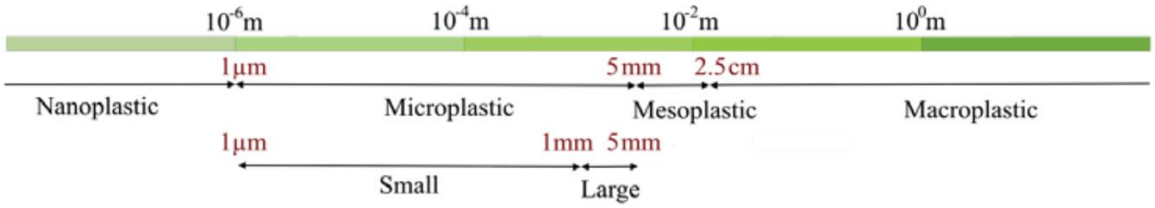


Figure 2 Size of plastics. Suggestion for plastics nomenclature based on size, as proposed by the European MSFD technical subgroup on Marine Litter (MSFD GES Technical Subgroup on Marine Litter, 2013).

Morphology of microplastics

Microplastics are categorized also based on their shapes, which can offer insights into their potential sources (Free et al., 2014; McCormick et al., 2014; Rochman et al., 2019). In the present study, we use the following categories:

- **Fragments**: Irregularly shaped particles, possibly originating from the degradation of larger plastic debris
- **Pellets:** Spherical particles, intentionally produced microbeads used in consumer goods and pre-production plastic materials
- **Films**: Thin planes, may come from the breakdown of film-like debris, such as plastic bags and wraps
- **Fibers**: Thin fibrous particles, potentially originating from textiles, fishing gear, and cigarette filters
- **Fiber balls**: Accumulation of several fibers

Chemical composition of microplastics

Microplastics can exhibit diverse polymer compositions. These materials, such as polyethene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyurethanes, polyethylene terephthalate (PET), polyester (PES), and others, are considered "virgin plastics" and are responsible for around 90% of the global plastic production (Plastics Europe, 2018; Andrady and Neal 2009; Espinosa et al., 2016). Widely used polymers like PET, PP, PE, PES, PVC, PS, polyamide (PA), and nylon find frequent applications in manufacturing various plastic products and processing food items (Sutton and Sedlak, 2017).

Common polymers are assigned specific recycling numbers that indicate their type, facilitating recycling processes (Figure 3). These numbers not only help in sorting and processing recyclable materials efficiently but also promote environmentally conscious practices by encouraging proper disposal and reprocessing of plastic waste.

Figure 3 Main plastic resin types, their application and recycling number

Source: MacArthur et al. (2016)

A New Emerging Contaminant in the Environment

Microplastics have emerged as a significant ecological issue across various environmental compartments. Scientific studies have reported their presence in the most remote locations of the Earth, including the deepest oceans, highest mountains, and polar regions (Walkinshaw et al., 2020). The focus of recent research has been on identifying and mapping the distribution of MPs within a range of ecosystems such as terrestrial soils, freshwater bodies, the atmosphere,

and marine environments. Figure 4 summarizes main sources and pathways of MPs to aquatic environment.

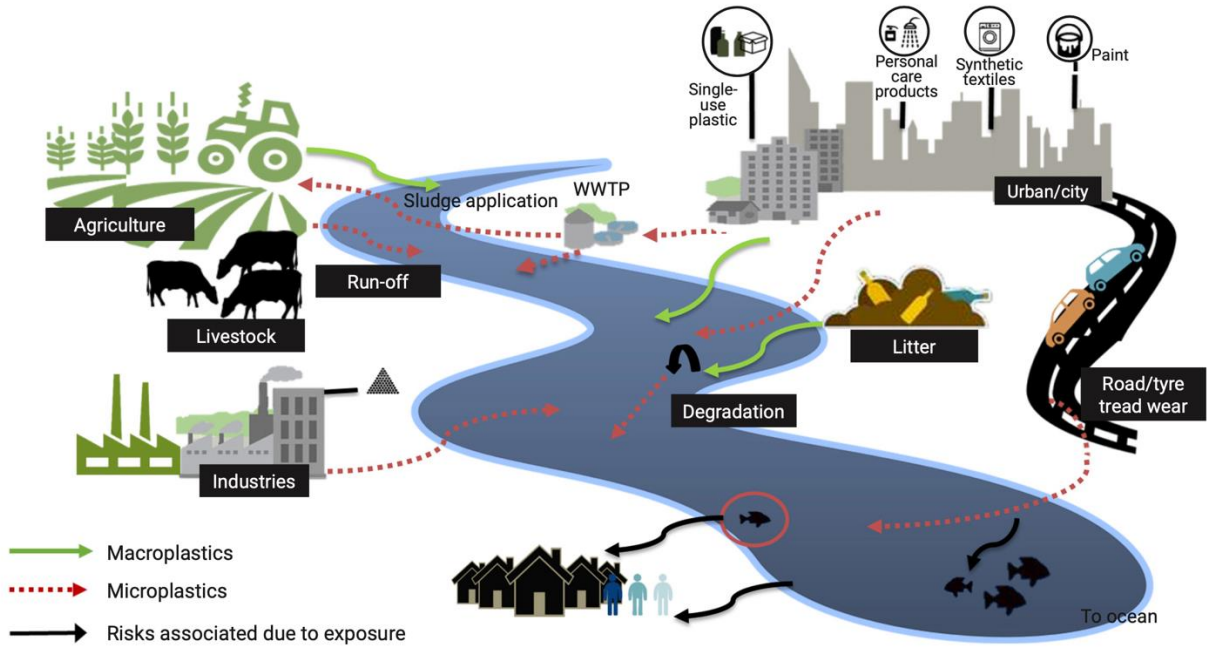


Figure 4 Main sources and pathways of macroplastics and microplastics to water

Source: Nikiema et al. (2020)

Microplastics in freshwater

Microplastic contamination in freshwater ecosystems poses a significant concern, especially given that freshwater serves as a crucial source of drinking water for human populations (Novotna et al., 2019). The physical characteristics of MPs, such as their shape, can influence their distribution within river systems, from surface water to the sediments. Various factors, including the size of the water body, wind patterns, and particle density, impact the movement and transport of MPs in freshwater environments (Eriksen et al., 2013; Fischer et al., 2016; Free et al., 2014). Recent research has highlighted the prevalence of MPs in diverse freshwater settings, including lakes, ponds, rivers, streams, and even sediments (Table X). European studies have uncovered the infiltration of MPs into rivers and lakes. For instance, Dris et al. (2015) reported MPs contamination in the French river Seine. Similarly, MPs have been detected in water surfaces and sediment of various water bodies in different countries, including Italy's Lake Chiusi and Lake Bolsena (Fischer et al., 2016), the surface waters of Flemish rivers in Belgium (Slootmaekers et al., 2019), and the sediments of the Kelvin River in the United Kingdom (Blair et al., 2019).

MPs tend to accumulate in sediment, making them a primary sink for MPs within river systems. Consequently, their concentrations in sediments are significantly higher than in water (Wang et al., 2017). Beyond their physical presence, MPs serve as carriers for adsorbing or absorbing toxic substances, effectively transporting pollutants. Hydrophobic persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), as well as heavy metals, can be absorbed and concentrated by MPs due to their chemical structure (Talvitie et al., 2017; Amrutha and Warrier, 2020). Additionally, MPs have been found to accumulate microorganisms (Bondelind et al., 2020).

Microplastics in atmosphere

Microplastics have infiltrated the atmosphere through various pathways, primarily originating from sources like synthetic textiles, the erosion of synthetic rubber tires, and urban dust (Prata, 2018). Additional contributors include building materials, industrial emissions, plastic fragments from household items, resuspended particles, landfills, traffic emissions, waste incineration, synthetic particles in horticultural soils, and the use of sewage sludge as fertilizer (Dris et al., 2016, 2017; Liebezeit and Liebezeit, 2014). Once released into the air, diverse forms of MPs undergo continuous transportation and eventual settling into soil or sediment. There, they may undergo translocation, bioaccumulation, and accumulation in trophic levels (Klein and Fischer, 2019).

In order to understand the distribution of MPs, researchers have delved into the comparison between indoor and outdoor concentrations. Notably, Zhang et al. (2020d) undertook an investigation revealing higher MPs levels within indoor environments when comparing with outdoor levels. Likewise, Dris et al. (2017) found that MPs concentrations within a Paris apartment were notably higher, between 1 and 60 fibers/m^3, exceeding outdoor concentrations of 0.3–1.5 fibers/m^3. These differences in MPs presence indoors can be attributed to heightened indoor sources and a lower removal of particles through dispersion processes compared to outdoor environments.

Moreover, shedding light on the far-reaching impact of MPs, a recent study conducted by Allen et al. (2019) underscored the transport of atmospheric MPs from urban settings to other remote areas with no anthropogenic activity. They observed an approximate deposition rate of 366 particles/m^2/day of MPs in remote mountainous areas like the French Pyrenees.

Microplastics in soil

The presence and dispersion of MPs in soil constitute a significant environmental concern, with soil serving as the second-largest depository for these pollutants (Horton et al., 2017; Zhang and Liu, 2018; Rillig, 2012). Different sources, including industrial emissions, residential plastic waste, sewage sludge, atmospheric deposition, and wastewater irrigation, contribute to direct and indirect contamination of soil ecosystems (Zhou et al., 2020a; Blasing and Amelung, 2018). Once integrated into soil environments, MPs undergo natural degradation processes and accumulate within plants, soil organisms, and biodiversity (Chae and An, 2018; de Souza Machado et al., 2018). Moreover, MPs act as carriers, facilitating the transfer of various soil pollutants and toxins to biota and living organisms (He et al., 2018).

Agricultural soil surfaces exposed to MPs can play a significant role in their dispersion to the atmosphere or nearby water bodies through runoff mechanisms. MPs present in floodplains can enter aquatic systems through heavy rains and floods (O'Connor et al., 2019; Gao et al., 2021). Recent studies estimated that from 63 to 430 thousand tons of MPs are transported annually from farmland to ocean or surface water in Europe and 44–300 thousand tonnes in North America (Guo et al., 2020).

Microplastics in marine environment

MPs have emerged as pervasive marine pollutants of global concern (Shim and Thompson, 2015). Global models estimating plastic waste input into oceans through rivers reveal an alarming 1.15–2.41 million tons annually, with the top 20 polluting rivers mainly situated in Asia, accounting for 67% of the total (Lebreton et al., 2017). MPs from sources like cosmetics, pellets, and air-blasting media are reaching rivers through domestic and industrial drainage systems (Sharma and Chatterjee, 2017). Wastewater treatment plant's (WWTPs) discharges containing MPs are realized directly into oceans or rivers contributing to the quantity of MPs in the environment (Sun et al., 2019).

Human activities including tourism, fishing, shipping, and marine industries introduce enormous quantities of plastics and MPs into coastal oceans (Cole et al., 2011). An estimated 5 trillion plastic pieces are floating in the world's oceans, spanning from the Arctic to the Antarctic (Isobe et al., 2015; Matsuguma et al., 2017). Despite their prevalence, understanding the distribution and fate of MPs in the ocean remains challenging due to their complex behavior upon entering the environment. Studies have indicated the presence of MPs across various marine

compartments, ranging from surface waters to deep-sea environments (Desforges et al., 2013), sediments (Matsuguma et al., 2017), and even freshwater systems (Sarkar et al., 2019).

Additionally, MPs suspended in the atmosphere become distributed within marine air and the ocean's surface. The dynamics of wind movement can transport atmospheric MPs pollution from terrestrial ecosystems to the marine environment, adding to the complexity of their dispersion (Rose et al., 2023).

MPs effect on humans

MPs have emerged as a concern due to their potential health impacts on humans. The primary entry points for MPs into the human body include ingestion, inhalation, and dermal contact. Ingestion is the predominant pathway, with an estimated intake of 39–52 thousand MPs per person annually (Ragusa et al., 2022). These particles can be directly introduced through the atmosphere, drinking water, and sea salt, or indirectly through the food chain. Once consumed, only MPs that are sufficiently small or have formed a biocompatible surface "corona" can traverse the intestinal mucus to reach intestinal cells. Subsequently, MPs can be internalized, entering the circulatory system and potentially depositing in organs like the gut, liver, and kidneys.

Inhalation represents another route of exposure, with an estimated daily intake of 272 MPs through this pathway. Inhaled MPs, particularly those deposited in the lungs, can lead to inflammation. Smaller MPs might even enter the circulation or lymphatic system through interactions with macrophages, posing additional respiratory complications. While dermal contact with MPs is generally less concerning, it's noteworthy that MPs smaller than 100 nm have the potential to cross the skin barrier (Gautam et al., 2022b).

The health implications of MPs internalization and accumulation in the human body remain a significant concern. Despite uncertainties, various studies on animal models (da Silva Brito et al., 2022), marine organisms (Wang et al., 2020), and human cell lines (Gautam et al., 2022a; Yong et al., 2020) have indicated potential negative consequences. Recent research even links MPs to male fertility issues and sperm quality deterioration (D'Angelo and Meccariello, 2021). Although the exact health impacts of MPs on humans are not fully understood, studies in animals have highlighted their translocation to different organs, leading to adverse effects (Ramsperger et al., 2023). Crucial factors influencing their toxicity include physical and chemical properties, concentrations, and the presence of microbial films (Sajid et al., 2023).

MPs have been detected in diverse human samples, ranging from stool and placenta to organs like the liver and lungs. These findings underscore the importance of understanding MPs' potential effects and devising strategies to mitigate their impact on human health (Figure 5).

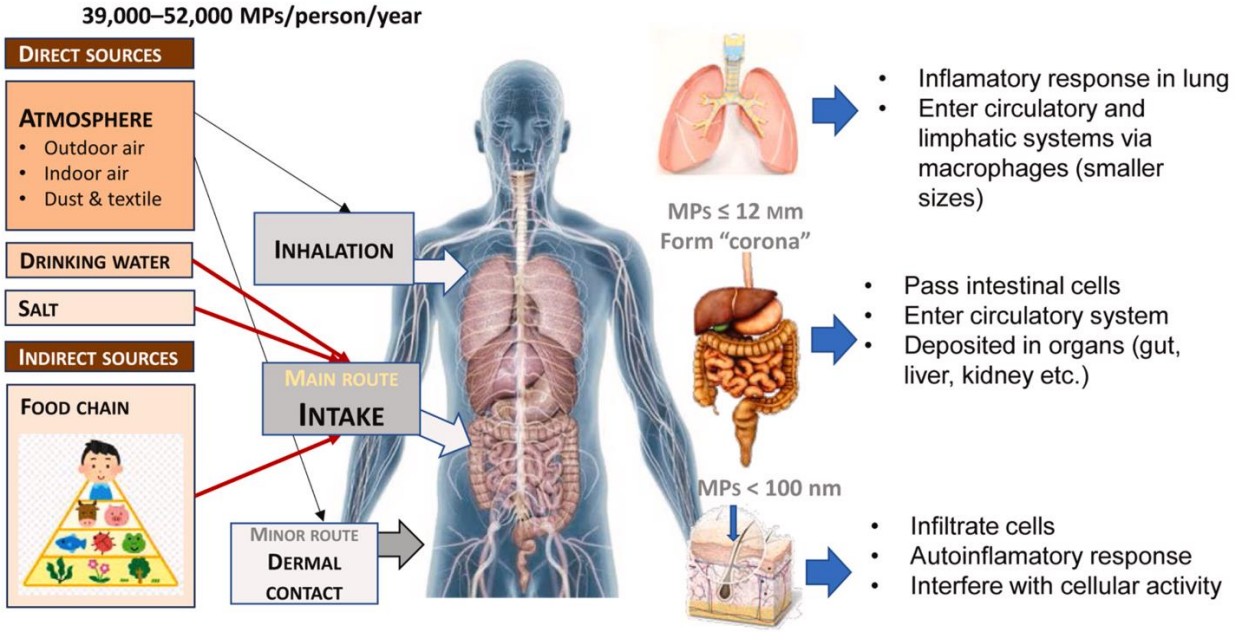


Figure 5 Potential pathways and routes of exposure to MPs/NPs and potential toxic effects on humans

Source: Barceló et al. (2023)

Hypothesis and objectives

The study's hypothesis posits that microplastic (MP) pollution within the natural environment predominantly arises from various terrestrial sources. Among these sources, Wastewater Treatment Plants (WWTPs) play a significant role, as these treatment facilities face challenges in fully eliminating MPs due to their small size, low density, and resistance to biodegradation.

The overarching objective of this research is to conduct a comprehensive investigation into the origin, levels, and impact of MPs in the coastal region of Catalonia, Spain, aiming to enhance water body quality, refine management practices, and contribute to the preservation and restoration of the aquatic environment. Additionally, a supplementary study was undertaken to analyze sediments from the San Francisco Bay, acknowledging their role as a recognized final repository for MPs.

Wastewater treatment plant:

- To characterize MPs in each wastewater treatment unit, including water and sludge lines and to calculate removal rates to identify the most effective treatment unit for MPs removal. Furthermore, the estimation of annual MPs discharge into the environment through this route will be calculated.

Drinking water treatment plant:

- To characterize MPs in various treatment units of a freshwater supplied DWTP, including water and sludge lines, to calculate removal efficiency to identify the treatment unit with the highest impact on MPs removal and to estimate the intake of MPs in drinking water from this source.

Organic waste amendments:

- To characterize MPs in seven commonly used organic waste amendments for agricultural soils and soil restoration practices, to estimate the annual release of MPs into the terrestrial environment through soil application and to assess the potential risks associated with the use of organic waste amendments.

Marine sediments:

- To characterize MPs in sediment samples from the San Francisco Bay, to examine the spatial and temporal distribution patterns of MPs in sediment across different sub-regions of the Bay.

Methodology and materials

Up to date, there is no standardized method for MPs identification except the effort of scientific community. However, there are still some general rules in MPs methodology that consist of MPs sampling, pretreatment process and identification. Given the absence of a standardized methodology for microplastics investigation, the research design involved the adaptation of protocols tailored to the specific nature and requirements of each sample type.

Sampling of Microplastics

Sampling methods are crucial for accurately assessing the presence and distribution of MPs in different environmental matrices. In this study, a rigorous approach was employed to collect samples from various sources, including WWTP, DWTP, organic waste amendments, and marine sediments. The sampling procedures were tailored to each specific context to ensure representative and reliable results.

- Collection of water samples within wastewater treatment plants and drinking water treatment plants

In water environments, such as WWTPs and DWTPs, different sampling techniques were employed.

1. Automatic Sampling Systems: The utilization of the Autosampler ISCO 3700 alongside in-house autosamplers situated within the WWTP

In case of WWTP, composite samples were collected over 24-hour periods to account for variations and fluctuations utilizing the automatic sampling systems for water line samples.

Figure 6 Wastewater treatment plant autosamplers

2. Specialized Stainless-Steel Filter Holder: Employed exclusively for DWTP scenarios

In case of DWTP water was filtered through an in-line stainless filter holder containing a PTFE filter (Sartorius, 10 µm pore size) connected to a tap. This sampling approach allows us to sample relatively large volumes of water, nearly 1m^3 in the effluent of the plant, avoiding sample contamination, during large periods of time (8 h).

Figure 7 Sampling of water from drinking water treatment plant's taps

3. Stainless-steel sieves for filtration of high water volumes

In both cases, where it was not possible to collect water samples with the application of primary sampling techniques, water was filtered through a set of stainless-steel sieves with different mesh size, on site.

Figure 8 Stainless-steel sieves for filtration of drinking water treatment plant influent

- Collection of soil and sludge samples within wastewater treatment plant, drinking water treatment plant and organic waste amendments

The grab sampling method, a common technique in environmental sampling, was employed for the collection of soil and sludge samples across diverse operational settings, encompassing WWTP, DWTP, and the organic waste amendments. This methodological approach involves the direct and instantaneous retrieval of representative samples from specific points within the targeted matrices.

- Collection of sediment samples from the San Francisco Bay area

Surface sediment grab samples were collected from various regions of the San Francisco Bay using a stainless-steel scoop to collect samples from the center of the Van Veen grab.

Figure 9

Laboratory Pretreatment Protocol

Upon sample collection, a laboratory pretreatment protocol was employed to extract microplastics (MPs) from the collected samples. This involved procedures such as density separation, digestion, and filtration to isolate the MPs from the matrix of the sample.

In the present study, pretreatment protocols were developed in order to extract MPs and remove organic and inorganic matter. These protocols based on nature and requirements of the samples. In general, protocols consist of advanced oxidation, alkaline and enzymatic digestion, in order to remove organic matter, and density separation in order to remove inorganic matter. These processes have been used widely by other authors that have been investigated MPs in different environmental samples.

In the context of isolating MPs from environmental samples, a robust pre-treatment protocol is essential to eliminate interfering organic and inorganic materials. Several diverse methods have been employed for MPs identification, including alkaline digestion, advanced oxidation, enzymatic digestion, and density separation (Dehaut et al., 2016; Löder et al., 2017; Masura et al., 2015; Tagg et al., 2017). Establishment of an effective pre-treatment procedure to ensure accurate MPs analysis was one of the aims of the thesis.

One method utilized to remove organic matter is Fenton's reagent. Masura et al. (2015) recommended this technique for MPs identification in marine water. The rapid oxidation reaction between Fenton's reagent and organic compounds leads to the breakdown of organics into carboxylic acids, aldehydes, carbon dioxide, and water, while leaving MPs unaffected (Masura et al., 2015; Tagg et al., 2017). However, for samples with substantial organic content, catalytic oxidation alone may be insufficient. Enzymatic digestion, as proposed by Löder et al. (2017), emerges as an effective approach to selectively remove organic matter such as proteins, lipids, and carbohydrates without impacting MPs. In contrast, alkaline treatment is more invasive and necessitates careful handling to prevent damage to MPs. The use of KOH solution, as demonstrated by Kühn et al. (2017) and recommended by Dehaut et al. (2016), exhibited minimal impact on most polymers except cellulose acetate. Another oxidizing agent, 33% hydrogen peroxide, has also been explored to degrade organic materials while causing minimal polymer degradation (Nuelle et al., 2014; Qiu et al., 2016; Zhao et al., 2017).

For the removal of inorganic matter, density separation emerged as a successful strategy. This method relies on exploiting the different densities of MPs and surrounding materials. Notably, MPs with higher densities, such as polyvinyl chloride (PVC) and polyethylene terephthalate (PET) (Prata et al., 2019), can be effectively separated by adjusting the density of the salt solution (Ziajahromi et al., 2017). A solution of zinc chloride with a density of 1.8 g/mL was selected, and the extraction process was repeated thrice on the same filter to ensure thorough separation. Table 2 provides an overview of the treatment processes utilized in this study.

Table 2 Pre-treatment processes for microplastics extraction

	REAGENT	CHEMICAL FORMULA	PROCESS CONDITIONS
1	Fenton	$H_2O_2 + Fe^{2+}$	Add 25 ml of H_2O_2 33% and 4-10 drops of $FeSO_4 * H_2O$ (0.05M) (or until a yellowish colour appear) slowly and simultaneously in a sterilized plastic tube and sonicate at 40 ºC for 10 min. Leave it for 24 h.
2	Hydrogen peroxide	H_2O_2	Add 50 ml H_2O_2 33% and sonicate at 40 ºC for 10 min. Leave it for 24 h.
3	Potassium hydroxide	KOH	Add 50 ml of KOH (2M) and sonicate at 40 ºC for 10 min. Leave it for 48 h.
4	Cellulase	$(C_6H_{12}O_6)_n$	Add 100 ml of cellulase, 230 ml of $NaC_2H_3O_2$ (0.2M) and 100 ml of CH_3COOH (0.02M) to maintain the pH= 5. Apply intermittent agitation at 40ºC for 2-3 days.
5	Chitinase		Add chitinase + $NaC_2H_3O_2$ (0.2 M) + CH_3COOH (0.2 M) to maintain the pH= 5, Apply intermittent agitation at 40ºC for 2-3 days.
6	Zinc chloride	$ZnCl_2$	Add 50 ml of $ZnCl_2$ (1.8 g/mL or 1.9 g/mL) and sonicate at 30-40 ºC for 10 min. Leave it for 4-12 h. Centrifuge the sample with $ZnCl_2$ at 3000 rpm for 1 min and filter the solution through a PTFE filter 5 µm. Repeat the same process 3 times in the same filter. Clean the filter with HNO_3 (0.02M).
7	Sodium chloride	NaCl	Add 50 ml of NaCl (1.2 g/mL), and sonicate at 40 ºC for 10 min, leave it for 24 h separation and filter the solution through a PTFE filter 5 µm

Spectroscopical Confirmation

Following the pretreatment protocol, the isolated microplastics (MPs) were subjected to spectroscopical confirmation to verify their identity. Techniques such as Fourier-transform infrared spectroscopy (FTIR) and Raman spectroscopy were utilized to determine the polymer composition of the identified microplastics.

Fourier Transform Infrared (FTIR) and Raman Spectroscopy in Microplastics Research

Fourier Transform Infrared (FTIR) and Raman spectroscopy have emerged as promising tools in the field of MPs research, offering non-destructive and insightful methods for identifying and

characterizing MPs in various environmental matrices. These spectroscopic techniques provide unique molecular and structural information that aids in the identification of MPs, assessment of polymer types, and even the potential additives and contaminants present on the surfaces of these particles.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy operates on the principle of measuring the absorption and transmission of infrared light by molecules. When applied to MPs research, FTIR is capable of identifying the chemical composition of polymers present in MPs. By analyzing the characteristic absorption bands of different functional groups within polymers, FTIR spectra can provide valuable information about the polymer types, allowing researchers to distinguish between various plastic materials. This technique also enables the detection of potential additives, such as plasticizers, stabilizers, and flame retardants, which contribute to the unique spectral signatures of MPs. FTIR spectroscopy is particularly advantageous in identifying weathered and degraded MPs, shedding light on the environmental fate of these particles (Primpke et al., 2020; Ye et al., 2022).

Raman Spectroscopy

Raman spectroscopy relies on the inelastic scattering of monochromatic light, resulting in vibrational transitions within molecules. In the context of MPs research, Raman spectroscopy offers high spatial resolution, making it suitable for identifying and characterizing individual MPs. Raman spectra provide information about the molecular structure and composition MPs, allowing researchers to differentiate between polymer types, even in complex mixtures. Raman spectroscopy is also sensitive to the crystallinity and orientation of polymer chains, offering insights into the mechanical properties and potential degradation of MPs. Moreover, the technique's non-destructive nature enables subsequent analyses on the same particle, making it valuable for comprehensive studies of MPs in environmental samples (Primpke et al., 2020; Ye et al., 2022).

Applications in Microplastics Research

FTIR and Raman spectroscopy have found widespread applications in microplastics research across various environmental matrices, including water, sediment, soil, and biota. These techniques have been employed to quantify MPs concentrations, characterize size distributions, and identify sources and transport pathways of MPs. Additionally, FTIR and Raman spectroscopy

have played a pivotal role in investigating the interactions between MPs and other environmental components, such as organic matter and pollutants (Ye et al., 2022).

Challenges and Future Directions

While FTIR and Raman spectroscopy offer robust methodologies for MPs analysis, challenges remain, including the detection of smaller particles and the differentiation of highly similar polymers. Future research efforts may focus on the development of advanced data analysis techniques, machine learning algorithms, and the integration of these spectroscopic methods with other analytical approaches to enhance the accuracy and efficiency of MPs characterization.

Data Analysis

Quantitative analysis of microplastics (MPs) involved assessing parameters such as size, morphology, concentration, and polymer composition. Statistical analysis was performed to determine removal rates, distribution patterns, and potential risks associated with the presence of MPs in the investigated samples.

The acquired data underwent analysis to assess the dynamics of MPs distribution and potential environmental implications. Basic mathematical operations, including calculations of removal rates, percentages, and standard deviations, were employed to unravel the fate of MPs within the studied systems. Removal rates were determined by quantifying the reduction in MPs concentrations at different treatment stages, providing insights into the efficiency of wastewater and drinking water treatment processes. Standard deviations were employed to characterize the variability and dispersion of data points, enabling a better understanding of the precision and reliability of the experimental outcomes.

Furthermore, additional mathematical operations were employed to extrapolate the amount of MPs that could potentially enter the natural environment through diverse routes. This approach facilitated a holistic assessment of the potential impacts of MPs on ecosystems and served as a foundation for informing future management strategies.

Quality Control

To ensure the accuracy and reliability of the results, quality control measures were implemented throughout the study. These included the use of blank samples, replication of analyses, and comparison with reference materials.

In the pursuit of accurate and reliable findings, a rigorous quality control framework was applied consistently across all aspects of the research encompassing microplastics in wastewater treatment plants, drinking water treatment plants, organic waste amendments, and marine sediments. These measures aimed to minimize contamination, ensure method accuracy, and uphold the integrity of the results. Key quality control measures included:

- **Pre-sampling Preparation**: Prior to sample collection, all equipment, instruments, and materials underwent thorough cleaning using a combination of 70% ethanol and ultrapure water, eliminating potential sources of contamination.
- **Reagent Purity**: Reagents were prepared exclusively using analytical-grade chemicals and filtered ultrapure water to maintain sample purity and prevent introduction of impurities.
- **Personnel Attire**: Laboratory personnel exclusively wore cotton lab coats during sample handling and analysis to mitigate the risk of introducing foreign particles.
- **Sample Pre-treatment**: All sample pre-treatment procedures were meticulously carried out within extraction hoods to prevent contamination from airborne particles and ensure the reliability of results.
- **Blank and Control Samples**: Procedural blank samples were collected both in the field and laboratory settings to account for background contamination. Controls were employed during sample collection to capture airborne fibers and quantify their potential influence.
- **Deposition Rate Calculation**: To address airborne fiber contamination, deposition rates of airborne fibers were calculated and factored into the sample processing methodologies.
- **Recovery Rate Experiments**: Recovery rate experiments were conducted using fluorescent microspheres to evaluate the accuracy of the analytical method and confirm its reliability in quantifying microplastics.
- **Seasonal Variability Consideration**: Seasonal variations were accounted for through sampling campaigns conducted during different seasons, enabling the assessment of potential fluctuations in MPs loads.
- **Duplicate Sampling Techniques**: Duplicate sampling techniques were employed to ensure consistency and verify the reliability of the results obtained across different samples and treatment units.

By adhering to these quality control measures, the research aimed to ensure that the obtained results accurately reflect the presence and impact of MPs across diverse environmental contexts, enhancing the overall credibility and validity of the findings.

Literature

Allen, S., Allen, D., Phoenix, V.R., Le, R.G., Jimenez, P.D., Simonneau, A., Binet, S., Galop, D. (2019). Atmospheric transport and deposition of microplastics in a remote mountain catchment. Nature Geoscience, 12, 339–344. https://doi.org/10.1038/s41561-019-0335-5.

Amrutha, K., & Warrier, A.K. (2020). The first report on the source to-sink characterization of microplastic pollution from a riverine environment in tropical India. Science of the Total Environment, 739, 140377.

Andrady, A. L. (2011). Microplastics in the marine environment. Marine pollution bulletin, 62(8), 1596-1605.

Andrady, A.L., & Neal, M.A. (2009). Applications and societal benefits of plastics. Philosophical Transactions of the Royal Society B: Biological Sciences, 364(1526), 1977-1984.

Arthur, C., Baker, J. E., & Bamford, H. A. (2009). Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris, September 9-11, 2008, University of Washington Tacoma, Tacoma, WA, USA.

Barceló, D., Picó, Y., & Alfarhan, A. H. (2023). Microplastics: Detection in human samples, cell line studies, and health impacts. Environmental toxicology and pharmacology, 104204.

Blair, R.M., Waldron, S., Phoenix, V.R., Gauchotte-Lindsay, C. (2019). Microscopy and elemental analysis characterisation of microplastics in sediment of a freshwater urban river in Scotland, UK. Environmental Science and Pollution Research, 26(12), 12491–12504. https://doi.org/10.1007/s11356-019-04678-1.

Blasing, M., & Amelung, W. (2018). Plastics in soil: analytical methods and possible sources. Science of the Total Environment, 612, 422–435.

Bondelind, M., Sokolova1, E., Nguyen, A., Karlsson, D., Karlsson, A., Bjorklund, K. (2020). Hydrodynamic modelling of traffic-related microplastics discharged with stormwater into the gota river in Sweden. Environmental Science and Pollution Research, 27, 24218–24230.

Boucher, J., & Friot, D. (2017). Primary microplastics in the oceans: a global evaluation of sources (Vol. 10). Gland, Switzerland: Iucn.

Carpenter, E. J., & Smith Jr, K. L. (1972). Plastics on the Sargasso Sea surface. Science, 175(4027), 1240-1241.

Chae, Y., & An, Y.J. (2018). Current research trends on plastic pollution and ecological impacts on the soil ecosystem: a review. Environmental Pollution, 240, 387–395.

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

Costa, M. F., Ivar do Sul, J. A., Silva-Cavalcanti, J. S., Araújo, M. C. B., Spengler, Â., & Tourinho, P. S. (2010). On the importance of size of plastic fragments and pellets on the strandline: a snapshot of a Brazilian beach. Environmental monitoring and assessment, 168, 299-304.

D'Angelo, S., & Meccariello, R. (2021). Microplastics: a threat for male fertility. International Journal of Environmental Research and Public Health, 18(5), 2392. https://doi.org/10.3390/IJERPH18052392.

da Silva Brito, W.A., Mutter, F., Wende, K., Cecchini, A.L., Schmidt, A., Bekeschus, S. (2022). Consequences of nano and microplastic exposure in rodent models: the known and unknown. Particle and Fibre Toxicology, 1–24. https://doi.org/10.1186/S12989-022-00473-Y.

de Souza Machado, A.A., Lau, C.W., Till, J., Kloas, W., Lehmann, A., Becker, R., & Rillig, M. C. (2018). Impacts of microplastics on the soil biophysical environment. Environmental Science & Technology, 52(17), 9656–9665. https://doi.org/10.1021/acs.est.8b01308.

Dehaut, A., Cassone, A. L., Frère, L., Hermabessiere, L., Himber, C., Rinnert, E., Rivière, G., Lambert, C., Soudant, P., Huvet, A., Duflos, G., & Paul-Pont, I. (2016). Microplastics in seafood: Benchmark protocol for their extraction and characterization. Environmental Pollution, 215, 223–233. https://doi.org/10.1016/J.ENVPOL.2016.05.018

Desforges, J.W., Galbraith, M., Dangerfield, N., & Ross, P.S. (2013). Widespread distribution of microplastics in surface water seawater in the North-East Pacific Ocean. Marine Pollution Bulletin, 79, 94–99.

Dris, R., Gasperi, J., Mirande, C., Mandin, C., Guerrouache, M., Langlois, V., & Tassin, B. (2017). A first overview of textile fibers, including microplastics, in indoor and outdoor environments. Environmental Pollution, 221, 453–458.

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. Environmental Chemistry, 12(5), 592–599. https://doi.org/10.1071/EN14167.

Dris, R., Gasperi, J., Saad, M., Mirande, C., & Tassin, B. (2016). Synthetic fibers in atmospheric fallout: a source of microplastics in the environment. Marine Pollution Bulletin, 104(1–2), 290–293. https://doi.org/10.1016/j.marpolbul.2016.01.006.

Eriksen, M., Mason, S., Wilson, S., Box, C., Zellers, A., Edwards, W., & Amato, S. (2013). Microplastic pollution in the surface waters of the Laurentian Great Lakes. Marine Pollution Bulletin, 77(1–2), 177–182.

Espinosa, C., Esteban, M.A., & Cuesta, A. (2016). Microplastics in aquatic environments and their toxicological implications for fish. In S. Soloneski & M.L. Larramendy (Eds.), Toxicology - New Aspects to This Scientific Conundrum (pp. 113–145). IntechOpen. https://doi.org/10.5772/64815.

Essel, R, Engel, L, Carus, M, and Ahrens, RH (2015) Sources of microplastics relevant to marine protection in Germany, Publication as pdf : www umweltbundesamt de/ publikationen/sources-of-microplastics-relevant-to-marine, 45pp

Fischer, E.K., Paglialonga, L., Czech, E., & Tamminga, M. (2016). Microplastic pollution in lakes and lake shoreline sediments–a case study on Lake Bolsena and Lake Chiusi (central Italy). Environmental Pollution, 213, 648–657.

Fischer, E.K., Paglialonga, L., Czech, E., Tamminga, M., 2016. Microplastic pollution in lakes and lake shoreline sediments–a case study on Lake Bolsena and Lake Chiusi (central Italy). Environ. Pollut. 213, 648–657.

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. Marine Pollution Bulletin, 85(1), 156–163.

Frias, J. P., & Nash, R. (2019). Microplastics: Finding a consensus on the definition. Marine pollution bulletin, 138, 145-147.

Frias, J., Pagter, E., Nash, R., O'Connor, I., Carretero, O., Filgueiras, A., ... & Gerdts, G. (2018). Standardised protocol for monitoring microplastics in sediments. Deliverable 4.2.

Gao, J., Pan, S., Li, P., Wang, L., Hou, R., Wu, W.M., & Hou, D. (2021). Vertical migration of microplastics in porous media: multiple controlling factors under wet-dry cycling. Journal of Hazardous Materials, 419, 126413.

Gautam, R., Jo, J.H., Acharya, M., Maharjan, A., Lee, D.E., Pramod, P.B., Kim, C.Y., Kim, K.S., Kim, H.A., & Heo, Y. (2022). Evaluation of potential toxicity of polyethylene microplastics on human derived cell lines. Science of the Total Environment, 838, 156089. https://doi.org/10.1016/j.scitotenv.2022.156089.

Gregory, M. R. (1996). Plastic 'scrubbers' in hand cleansers: a further (and minor) source for marine pollution identified. Marine pollution bulletin, 32(12), 867-871.

Guo, J.J., Huang, X.P., Xiang, L., Wang, Y.Z., Li, Y.W., Li, H., & Wong, M.H. (2020). Source, migration and toxicology of microplastics in soil. Environmental International, 137, 105263.

He, D., Luo, Y., Lu, S., Liu, M., Song, Y., & Lei, L. (2018). Microplastics in soils: analytical methods, pollution characteristics and ecological risks. Trends in Analytical Chemistry, 109, 163–172.

Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., & Svendsen, C. (2017). Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. Science of the Total Environment, 586, 127–141.

Isobe, A., Uchida, K., Tokai, T., & Iwasaki, S. (2015). East Asian Seas: a hot spot for pelagic microplastics. Marine Pollution Bulletin, 101, 618–623.

Klein, M., & Fischer, E.K. (2019). Microplastic abundance in atmospheric deposition within the Metropolitan area of Hamburg, Germany. Science of the Total Environment, 685, 96–103. https://doi.org/10.1016/j.scitotenv.2019.05.405.

Kühn, S., van Werven, B., van Oyen, A., Meijboom, A., Bravo Rebolledo, E. L., & van Franeker, J. A. (2017). The use of potassium hydroxide (KOH) solution as a suitable approach to isolate plastics ingested by marine organisms. Marine Pollution Bulletin, 115(1–2), 86–90. https://doi.org/10.1016/j.marpolbul.2016.11.034

Lassen, C , Foss Hansen, S , Magnusson, K , Noren, F , Bloch Hartmann, NI, Rehne Jensen, P, Gisel Nielsen, T, and Brinch, A (2015) Microplastics : Occurrence, effects and sources of releases to the environment in Denmark (The Danish Environmental Protection Agency)

Lebreton, L. C., Van Der Zwet, J., Damsteeg, J. W., Slat, B., Andrady, A., & Reisser, J. (2017). River plastic emissions to the world's oceans. Nature communications, 8(1), 15611.

Liebezeit, G., & Liebezeit, E. (2014). Synthetic particles as contaminants in German beers. Food Additives & Contaminants: Part A, 31(9), 1574-1578.

Löder, M. G. J., Imhof, H. K., Ladehoff, M., Löschel, L. A., Lorenz, C., Mintenig, S., Piehl, S., Primpke, S., Schrank, I., Laforsch, C., & Gerdts, G. (2017). Enzymatic Purification of Microplastics in Environmental Samples. Environmental Science and Technology, 51(24). https://doi.org/10.1021/acs.est.7b03055

MacArthur, D. E., Waughray, D., & Stuchtey, M. R. (2016). The new plastics economy, rethinking the future of plastics. In World Economic Forum. London, UK: Ellen MacArthur Foundation and McKinsey & Company.

Magnuson, K , Eliason, K , Frane, A , Haikonen, K , Hulten, J , Olshammar, M , Stadmark, J , and Voisin, A (2016) Swedish sources and pathways for microplastics to the marine environment wwwivlse/webdav/files/Rapporter/C183pdf

Masura, J., Baker, J., Foster, G., & Arthur, C. (2015). Laboratory methods for the analysis of microplastics in themarine environment: Recommendations for quantifying synthet- ic particles in waters and sediments. NOAA Technical Memorandum NOS-OR&R-48. NOAA Technical Memorandum, July.

Matsuguma, Y., Takada, H., Kumata, H., Kanke, H., Sakurai, S., Suzuki, T., & Newman, B. (2017). Microplastics in sediment cores from Asia and Africa as indicators of temporal trends in plastic pollution. Archives of Environmental Contamination and Toxicology, 73(2), 230–239.

McCormick, A., Hoellein, T. J., Mason, S. A., Schluep, J., & Kelly, J. J. (2014). Microplastic is an abundant and distinct microbial habitat in an urban river. Environmental science & technology, 48(20), 11863-11871.

Nikiema, J., Mateo-Sagasta, J., Asiedu, Z., Saad, D., & Lamizana, B. (2020). Water pollution by plastics and microplastics: a review of technical solutions from source to sea.

Novotna, K., Cermakova, L., Pivokonska, L., Cajthaml, T., & Pivokonsky, M. (2019). Microplastics in drinking water treatment–Current knowledge and research needs. Science of the Total Environment, 667, 730–740.

Nuelle, M. T., Dekiff, J. H., Remy, D., & Fries, E. (2014). A new analytical approach for monitoring microplastics in marine sediments. Environmental Pollution, 184. https://doi.org/10.1016/j.envpol.2013.07.027

O'Connor, D., Pan, S., Shen, Z., Song, Y., Jin, Y., Wu, W.M., & Hou, D. (2019). Microplastics undergo accelerated vertical migration in sand soil due to small size and wet-dry cycles. Environmental Pollution, 249, 527–534.

Patel, M. M., Goyal, B. R., Bhadada, S. V., Bhatt, J. S., & Amin, A. F. (2009). Getting into the brain: approaches to enhance brain drug delivery. CNS drugs, 23, 35-58.

Plastics Europe. (2018). Plastics - the facts 2018. An analysis of European plastics production, demand and waste data 16. Retrieved from https://www.plasticseurope.org/application/files/6315/4510/9658/Plastics_the_facts_2018_AF_web.pdf.

Prata, J. C., da Costa, J. P., Duarte, A. C., & Rocha-Santos, T. (2019). Methods for sampling and detection of microplastics in water and sediment: A critical review. In TrAC - Trends in Analytical Chemistry (Vol. 110). https://doi.org/10.1016/j.trac.2018.10.029

Prata, J.C. (2018). Airborne microplastics: consequences to human health. Environmental Pollution, 234, 115–126.

Primpke, S., Christiansen, S. H., Cowger, W., De Frond, H., Deshpande, A., Fischer, M., ... & Wiggin, K. J. (2020). Critical assessment of analytical methods for the harmonized and cost-efficient analysis of microplastics. Applied Spectroscopy, 74(9), 1012-1047.

Qiu, Q., Tan, Z., Wang, J., Peng, J., Li, M., & Zhan, Z. (2016). Extraction, enumeration and identification methods for monitoring microplastics in the environment. In Estuarine, Coastal and Shelf Science (Vol. 176). https://doi.org/10.1016/j.ecss.2016.04.012

Ragusa, A., Notarstefano, V., Svelato, A., Belloni, A., Gioacchini, G., Blondeel, C., ... & Giorgini, E. (2022). Raman microspectroscopy detection and characterisation of microplastics in human breastmilk. Polymers, 14(13), 2700. https://doi.org/10.3390/POLYM14132700.

Ramsperger, A.F.R.M., Bergamaschi, E., Panizzolo, M., Fenoglio, I., Barbero, F., Peters, R., et al. (2023). Nano- and microplastics: a comprehensive review on their exposure routes, translocation, and fate in humans. NanoImpact, 29, 100441. https://doi.org/10.1016/j.impact.2022.100441.

Rillig, M.C. (2012). Microplastic in terrestrial ecosystems and the soil. Environmental Science & Technology, 46. River plastic emissions to the world's oceans. Nat. Commun. 8 (1), 1–10.

Rochman, C. M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Bucci, K., ... & Hung, C. (2019). Rethinking microplastics as a diverse contaminant suite. Environmental toxicology and chemistry, 38(4), 703-711.

Rose, P. K., Jain, M., Kataria, N., Sahoo, P. K., Garg, V. K., & Yadav, A. (2023). Microplastics in multimedia environment: A systematic review on its fate, transport, quantification, health risk, and remedial measures. Groundwater for Sustainable Development, 100889.

Ryan, P. G. (1988). The characteristics and distribution of plastic particles at the sea-surface off the southwestern Cape Province, South Africa. Marine Environmental Research, 25(4), 249-273.

Sajid, M., Ihsanullah, I., Tariq Khan, M., & Baig, N. (2023). Nanomaterials-based adsorbents for remediation of microplastics and nanoplastics in aqueous media: a review. Separation and Purification Technology, 305, 122453. https://doi.org/10.1016/J.SEPPUR.2022.122453.

Sarkar, D.J., Sarkar, S.D., Das, B.K., Manna, R.K., Behera, B.K., & Samanta, S. (2019). Spatial distribution of meso and microplastics in the sediments of river Ganga at eastern India. Science of the Total Environment, 694, 133712.

Sharma, S., & Chatterjee, S. (2017). Microplastic pollution, a threat to marine ecosystem and human health: a short review. Environmental Science and Pollution Research, 24(27), 21530–21547.

Shim, W.J., & Thomposon, R.C. (2015). Microplastics in the ocean. Archives of Environmental Contamination and Toxicology, 69(3), 265–268.

Slootmaekers, B., Carteny, C.C., Belpaire, C., Saverwyns, S., Fremout, W., Blust, R., & Bervoets, L. (2019). Microplastic contamination in gudgeons (Gobio gobio) from Flemish rivers (Belgium). Environmental Pollution, 244, 675–684. https://doi.org/10.1016/j.envpol.2018.09.136.

Sun, J., Dai, X., Wang, Q., van Loosdrecht, M.C., & Ni, B.J. (2019). Microplastics in wastewater treatment plants: detection, occurrence and removal. Water Research, 152, 21–37. https://doi.org/10.1016/j.watres.2018.12.050.

Sundt, P , Schulze, P -E , and Syversen, F (2014) Sources of microplastic- pollution to the marine environment (MEPEX) www miljodirektoratet no/Documents/publikasjoner/M321/ M321 pdf

Sutton, R., & Sedlak, M. (2017). Microplastic monitoring and science strategy for San Francisco Bay. California: SFEI Contribution.

Tagg, A. S., Harrison, J. P., Ju-Nam, Y., Sapp, M., Bradley, E. L., Sinclair, C. J., & Ojeda, J. J. (2017). Fenton's reagent for the rapid and efficient isolation of microplastics from wastewater. Chemical Communications, 53(2), 372–375. https://doi.org/10.1039/c6cc08798a

Talvitie, J., Mikola, A., Koistinen, A., & Setala, O. (2017). Solutions to microplastic pollution–Removal of microplastics from wastewater effluent with advanced wastewater treatment technologies. Water Research, 123, 401–407. https://doi.org/10.1016/j.watres.2017.07.005.

Thompson, R. C., Olsen, Y., Mitchell, R. P., Davis, A., Rowland, S. J., John, A. W., ... & Russell, A. E. (2004). Lost at sea: where is all the plastic?. Science, 304(5672), 838-838. Total Environ. 575, 1369–1374. https://doi.org/10.1016/j.scitotenv.2016.09.213.

Walkinshaw, C., Lindeque, P.K., Thompson, R., Tolhurst, T., & Cole, M. (2020). Microplastics and seafood: lower trophic organisms at highest risk of contamination. Ecotoxicology and Environmental Safety, 190, 110066. https://doi.org/10.1016/j.ecoenv.2019.110066.

Wang, W., Ge, J., & Yu, X. (2020). Bioavailability and toxicity of microplastics to fish species: a review. Ecotoxicology and Environmental Safety, 189, 109913. https://doi.org/10.1016/J.ECOENV.2019.109913.

Wang, W., Ndungu, A.W., Li, Z., & Wang, J. (2017). Microplastics pollution in inland waters of China: a case study in urban surface waters of Wuhan, China. Science of the Total Environment, 575, 1369–1374. https://doi.org/10.1016/j.scitotenv.2016.09.213.

Ye, Y., Yu, K., & Zhao, Y. (2022). The development and application of advanced analytical methods in microplastics contamination detection: A critical review. Science of The Total Environment, 818, 151851.

Yong, C.Q.Y., Valiyaveettil, S., & Tang, B.L. (2020). Toxicity of microplastics and nanoplastics in mammalian systems. International Journal of Environmental Research and Public Health, 17, 1509. https://doi.org/10.3390/IJERPH17051509.

Zhang, G.S., & Liu, Y.F. (2018). The distribution of microplastics in soil aggregate fractions in southwestern China. Science of the Total Environment, 642, 12–20.

Zhang, Q., Zhao, Y., Du, F., Cai, H., & Wang, G., & Shi, H. (2020). Microplastic fallout in different indoor environments. Environmental Science and Technology, 54(11), 6530–6539. https://doi.org/10.1021/acs.est.0c00087.

Zhao, S., Danley, M., Ward, J. E., Li, D., & Mincer, T. J. (2017). An approach for extraction, characterization and quantitation of microplastic in natural marine snow using Raman microscopy. Analytical Methods, 9(9). https://doi.org/10.1039/c6ay02302a

Zhou, B., Wang, J., Zhang, H., Shi, H., Fei, Y., Huang, S., Tong, Y., Wen, D., Luo, Y., & Barcelo, D. (2020). Microplastics in agricultural soils on the coastal plain of Hangzhou Bay, east China: multiple sources other than plastic mulching film. Journal of Hazardous Materials, 388, 121814.

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 Research, 112. https://doi.org/10.1016/j.watres.2017.01.042

Zitko, V., & Hanlon, M. J. (1991). Another source of pollution by plastics: skin cleaners with plastic scrubbers. Marine Pollution Bulletin, 22(1), 41-42.

Chapter 1

Chapter 1. Tracing the fate of microplastics in wastewater treatment plant: A multi-stage analysis of treatment units and sludge

Abstract

Microplastic (MP) pollution is ubiquitous in the environment presenting a global problem for both scientists and the general public. One of the major pathways of MPs entering the natural environment is through wastewater treatment plants (WWTPs). Once MPs reach the natural environment, they are posing threat to aquatic ecosystems and public health. The aim of this study is to investigate the concentration, morphology, and composition of MPs in different treatment units of a WWTP. Sampling included different points across WWTP in the water and sludge lines. Pre-treatment of the samples consists of advanced Fenton oxidation, and alkaline and enzymatic digestion followed by density separation. Once the particles were isolated, their morphology and size were studied using a stereoscopic and optical microscope followed by final confirmation with ATR-FTIR and micro-FTIR spectroscopy. Microplastic particle concentrations exhibit significant reductions as water undergoes treatment in the WWTP. For summer sampling, concentrations decreased from 351 MPs/L (influent) to 35 MPs/L (primary clarifier), 32 MPs/L (biological reactor), and 13 MPs/L (2.3 MPs/L) (secondary clarifier). Similarly, winter sampling showed reductions from 403 MPs/L (influent) to 159 MPs/L (primary clarifier), 178 MPs/L (biological reactor), and 26 MPs/L (5.6 MPs/L) (secondary clarifier). Removal efficiency of WWTP is high and exceeds 96%. The most abundant morphology is fibers followed by fragments and films. Polymers such as PE, synthetic cellulose, PP, PVC, PE-PP, PEEA, PA, acrylamide, and PES are widely detected in different units of WWTP. The number of MPs that are avoided from being emitted into the environment through direct water discharge was estimated to be 9.1 x 10^{12} MPs/year. Removed MPs tend to accumulate in the sludge that is used for agricultural purposes although it should be managed as waste properly, avoiding the transition of MPs pollutants to terrestrial ecosystems adding to the number of MPs that will inevitably end up in receiving water bodies through direct WWTP effluent discharge that was set in 5.1 x 10^{10} MPs/year in the studied WWTP.

Key words: microplastic pollution, wastewater treatment plant, sludge, removal efficiency

Highlights

- Microplastics were characterized in water and sludge lines of a WWTP in two seasonal campaigns.
- High removal rates (96-99%) were established.
- Influent and effluent MPs levels range from 365 to 1058 and from 32 to 178 MPs/L
- Microplastics in dry sludge levels range from 51,450 to 133,000 items/$kg_{d.w.}$.
- Wastewater treatment plant avoid emission of $9.1 \cdot 10^{12}$ microplastics a year.

1. Introduction

Microplastic (MP) pollution is a global problem since it is present and detected in all-natural ecosystems (water, soil, and air), representing a great concern for both scientists and the general public. MP particles are synthetic, non-biodegradable with a diameter size below 5 mm. They can be described as primary MPs if they are manufactured directly or secondary MPs formed as a result of larger particle breaking due to various environmental stressors such as sunlight, water, and wind (Kurt et al., 2022).

One of the major pathways of MPs entering the natural environment is through wastewater treatment plants (WWTPs). MPs are found both in the influent and the effluent of wastewater treatment plants all around the world. Personal care products (PCPs) contain MPs such as polyethylene (PE), polypropylene (PP), and polystyrene (PS), and can be directly discharged into wastewater through human activities (Mintenig et al., 2017; Prata, 2018). Also, synthetic clothing made of polyester (PES) and nylon, might release thousands of fibers into wastewater during the washing processes (Napper & Thompson, 2016). Various processes can be distinguished in a WWTP and each treatment step has a specific objective for the overall purpose of cleaning the water flow. These systems are not currently designed for the targeted MPs removal from wastewater streams (Sol et al., 2020). Although the removal efficiency of the WWTP is relatively high and in most cases exceeds 95% (Lares et al., 2018; Mintenig et al., 2017; Murphy et al., 2016; Ziajahromi et al., 2021), the number of MPs that reach natural environment cannot be ignored. The release of plastic waste into wastewater can facilitate its uptake by biofilm-forming microorganisms, which can travel across the ocean and become a vector for the spread of potential pathogens or of antibiotic resistance genes on a global scale (Rist et al., 2018; Zagui et al., 2022). A recent study by Shen et al. (2021) discovered that MP particles can act as an important protective umbrella for bacteria during wastewater disinfection processes. Furthermore, MPs are potential carriers of toxic chemicals due to their large specific surface area and strong hydrophobicity. Numerous heavy metals including Zn, Cu, Pb, Ag (Ashton et al., 2010), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), phthalates, pesticides (Rodrigues et al., 2019; Xu et al., 2019), and some nano-scale adsorbents, such as TiO_2 (Fries et al., 2013) have been detected on the surface of MPs.

On the other hand, MP particles that are removed during the treatment process tend to accumulate in the sludge. Stabilized sludge is usually used as a fertilizer for agricultural purposes and transferred to terrestrial ecosystems given that it has several benefits for soil structure, water retention, soil nutrients, organic matter concentration, and soil microbial communities.

Reusing sludges contributes to the circular economy, however, it leads to the reintroduction of MPs into the environment and may pose a potential environmental threat. MPs from the soil can be ingested by the terrestrial biota, entering the food chain, and posing a risk to human health (Gatidou et al., 2019a). The risk that MPs pose to human health could be related to size. MPs smaller than 110 µm can pass through the portal vein, which carries blood from the intestine, pancreas, and spleen to the liver (WHO, 2019). Furthermore, MPs smaller than 20 µm can enter organs such as the liver and kidneys, while those larger than 150 µm are not adsorbed by the human body (WHO, 2019). Recently, MPs have been detected in the human placenta (Ragusa et al., 2021), human blood (Leslie et al., 2022), lung tissue (Jenner et al., 2022), and human breastmilk (Ragusa et al., 2022).

Understanding how MPs are removed is necessary to engineer and improve their removal capacities and to optimize the conditions that can affect the removal performance. For this reason, the present study is investigating the abundance of MPs in a full-scale study trying to understand the fate and behavior of this emergent contaminant. To the best of our knowledge present work is one of the most complete studies regrading MPs levels in the different treatment units of a WWTP including thickened and dry sludge and centrifugation water in a WWTP located in a Mediterranean Sea basin country such as Spain. The aim of this study was to examine the concentration, morphology, and composition of MP particles ranging in size from 5 mm to 20 µm in each wastewater treatment unit. Moreover, removal rates are calculated to determine which treatment unit has the greatest impact on MPs removal and, finally, to estimate the levels of MPs that will reach the environment, through this route, on an annual basis.

2. Materials and methods

2.1. Wastewater treatment plant

The studied WWTP is located close to Barcelona city with a design flow of 43,000 m^3/day, a population of 358,000 equivalent inhabitants, and the production of dry sludge of 944 kg/h. It consists of the pre-treatment units (sieving, grit remover, degreaser), primary treatment (clarifier) and secondary treatment (biological reactor), thickener (gravity thickener, floatation), stabilization (anaerobic stabilization), dehydration (centrifuge), and electricity generation (cogeneration) (Figure 1). The plant is receiving wastewater from domestic houses. The sewage sludge is used in agricultural applications while the final effluent is disposed of into the river.

The retentions times in both sampling periods were 0.36, 1.72, 6.33 and 6.23 h in the pre-treatment, primary clarifier, biological reactor, and secondary clarifier, respectively. Water influent were 38,000 and 36,000 m^3/day in Winter and Summer campaign, respectively.

2.2. Microplastic sampling

Sampling points at the WWTP included: Influent (raw wastewater), pre-treatment, primary clarifier, biological reactor, and secondary clarifier (WWTP effluent) in the water line and thickened sludge, dry sludge, and water from the centrifugation processes in the sludge line (Figure 1). Sampling was carried out in two campaigns during the summer (September 2020) and winter (March 2021) period. Composite samples of 24 hours were collected in the water line (influent of WWTP, pre-treatment units, biological reactor, secondary clarifier and effluent of WWTP) automatically and sequentially: 300 mL were sampled every 20 minutes in the influent; 250 mL every 15 min at the effluent of pre-treatment unit and biological reactor; 300 mL every 15 min was sampled at the effluent of secondary clarifier and WWTP effluent). The retention times of each operation were not considered given that the treatment plant was operating stably without significant alterations and the organic matter removal efficiency was constant.

At the effluent of primary clarifier, the automatic sampling equipment was damaged, and for this reason 20 L of the sample was taken manually every 4 hours without taking night hours into account. For the water line, collected sample volumes were 2 L from the influent, 5 L from the pre-treatment, 20 L from the primary clarifier, and 25 L from the biological reactor and effluent. For the sludge line, 2 L of thickened sludge, 10 L of water from the centrifugation process, and 2 kg of dry sludge were sampled. Additionally, samples of secondary clarifier were taken also by filtering the large volume of 400 L through a set of stainless steel sieves (*Cisa Sieving Technologies, 200 mm diameter x 50 mm height*) with mesh sizes: 20 µm, 45 µm, 125 µm, 0.5 mm, and 1 mm. Samples were immediately transported to the laboratory for further analysis. Samples were then homogenized and the subsamples were taken for further analysis considering the complexity and impurities of the matrix (Supplementary data Table S1).

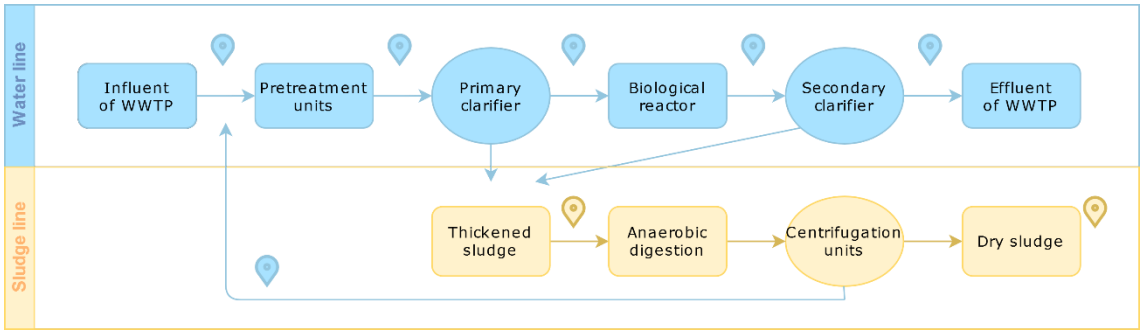


Figure 1. Flow diagram of different treatment plant units and sampling points for water and sludge line (blue sampling points are for water line and yellow for sludge line)

2.3. Microplastic analysis

2.3.1. Pre-treatment

For sample processing, the following chemicals and materials were used: absolute ethanol (Scharlau, >99.9%), iron (II) sulfate heptahydrate (Sigma-Aldrich, >99.0%), hydrogen peroxide (PamReac AppliChem ITW Reagents, 30%), PTFE- filter (Sartorius, 5 µm pore size), zinc chloride (Acros Organics, >98.0%), nitric acid (PamReac AppliChem ITW Reagents, >69.0%), potassium hydroxide (Scharlau, >90.0%), and cellulase TXL (ASA Spezialenzyme GmbH).

The samples underwent pre-treatment in order to eliminate organic and inorganic materials and isolate MPs. Although there is no standardized method for MPs identification to date, different approaches such as acid and alkaline digestion, oxidation, enzymatic digestion, and density separation can be applied (Dehaut et al., 2016; Löder et al., 2017; Masura et al., 2015; Tagg et al., 2017). For the purposes of this study, four versions of the same protocol were developed based on the nature and requirements of the sample (Figure 2). Protocol version I was adapted for secondary clarifier, protocol version II for thickened sludge, biological reactor, and water from centrifugation units, protocol version III for dry sludge, and protocol version IV for water samples coming from influent of WWTP, pre-treatment and primary clarifier.

Briefly, each sample, except dry sludge, was firstly sieved through stainless steel sieves (*Cisa Sieving Technologies, 200 mm diameter x 50 mm height*) with mesh sizes: 20 µm, 45 µm, 125 µm, 0.5 mm, and 1 mm. A portion (100g) of dry sludge was taken and mixed with ultrapure water first and then sieved. Each fraction was filtered through vacuum filter equipment on a 5 µm PTFE filter. In the first step, all the samples were treated with Fenton's reagent in order to remove organic matter from the sample. After the Fenton's reagent was applied, sonication of the sample at 40 °C for 10 minutes was performed and the sample was left for 24 hours and filtered again through a 5 µm PTFE filter (Masura et al., 2015). Masura et al. (2015) also suggested this method for MPs identification in marine water. The oxidation reaction between Fenton's reagent and organic compounds occurs within a short frame-time, in which organic compounds break down to carboxylic acids, aldehydes, carbon dioxide, and water without affecting MPs (Masura et al., 2015; Tagg et al., 2017). Since the wastewater samples have a high amount of organic matter, usually catalytic oxidation alone is not enough. Another approach that can successfully remove organic matter such as proteins, lipids, and carbohydrates and at the same time does not affect MPs is enzymatic digestion (Löder et al., 2017). In all protocols except

protocol I, the sample is treated with cellulase in an acidic buffer solution, to maintain the pH around 5 and left for 2-3 days, at 40 °C with intermittent agitation. As for protocol I, alkaline digestion was performed with 2 M potassium hydroxide, sonicated at 40 °C for 10 minutes, left for 1-2 days, and filtrated through a 5 µm PTFE filter. Alkaline treatment is more invasive than enzymatic digestion, and for that reason requires extra concern in order not to damage MPs. Usage of KOH solution showed in a study by Kühn et al. (2017) that most of the polymers were left unaffected (except for cellulose acetate). Furthermore, Dehaut et al. (2016) also recommended using KOH for MPs investigation. In the third step, all the samples were treated in the same way applying another oxidizing agent, 33% hydrogen peroxide, that can break down organic materials with little to no polymer degradation (Nuelle et al., 2014; Qiu et al., 2016; Zhao et al., 2017). Samples were sonicated at 40 °C for 10 min, left for 24 h, and filtered again. Additional steps were performed for some protocols such as alkaline digestion for protocol II, advanced Fenton's oxidation for protocol III, and Fenton's oxidation followed by alkaline digestion for protocol IV, in the same way, described above. The last step consists of density separation, for all protocols, in order to remove inorganic material from the samples. To separate all the MPs, the ones with a higher density such as polyvinyl chloride (PVC) and polyethylene terephthalate (PET) (Prata et al., 2019) should be considered and the density of the salt solution should be higher (Ziajahromi et al., 2017). Thereby, in this work, a solution of zinc chloride 1.8 g/mL was chosen, and the sample was sonicated at 40 °C for 10 min and left for 4-12 h. The samples with zinc chloride were centrifuged at 3000 rpm for 1 min and the supernatant was filtered through a 5 µm PTFE filter. The extraction process was repeated three times on the same filter. The filter was washed with 0.02 M nitric acid and stored in a clean petri dish for visual inspection and spectroscopic confirmation. Previous filters were also examined in case some MP particles were left behind.

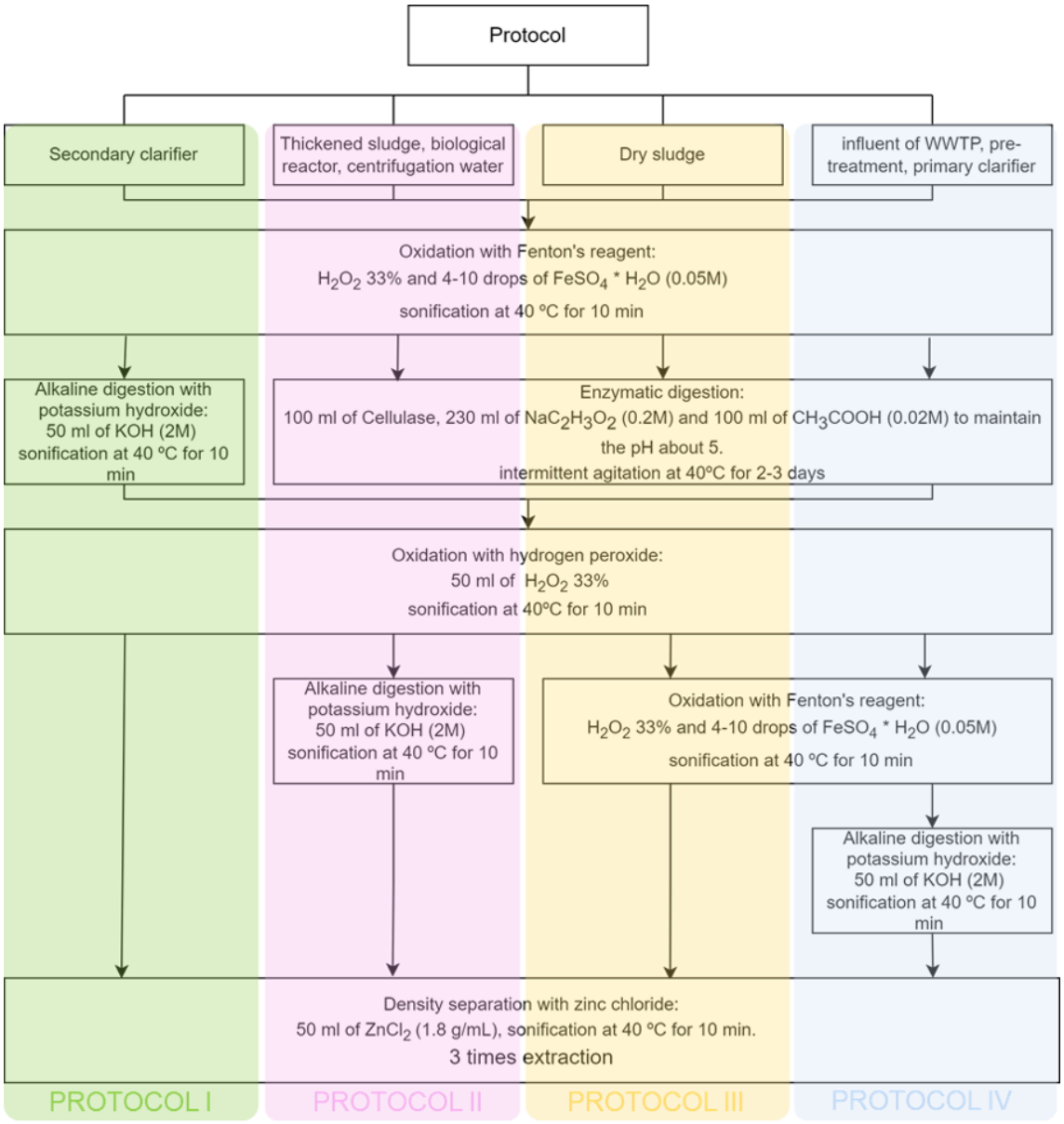


Figure 2. Pre-treatment protocol for microplastic identification. Protocol I was adapted for secondary clarifier, protocol II for thickened sludge, biological reactor, and water from centrifugation units, protocol III for dry sludge, protocol IV for water samples coming from influent of WWTP, pre-treatment and primary clarifier.

2.3.2. Microplastic identification

After purification of the samples, the particles were visually sorted and quantified using a *LEICA MZ10* spectroscopic microscope with a *FLEXACAM C1* camera and *Olympus CX41* optical microscope. All MPs particles are divided into three most prevalent shape categories: fragments, films, and fibers (Bayo et al., 2020; Hidayaturrahman & Lee, 2019; Lares et al., 2018).

Visual identification of the particles was followed by spectroscopic confirmation. Samples with particle sizes larger than 0.5 mm were identified with IR Perkin Elmer Frontier/ ATR diamond/

detector DTGS with the accumulation of 16 scans, spectral resolution 4 cm^{-1}, and spectral range from 4000 to 230 cm^{-1}. Spectra were also collected using a Cary 630 portable spectrometer (*Agilent Technologies Spain SL, Madrid, Spain)* with MicroLab PC software (*Agilent Technologies SL, Madrid, Spain*) in mid-infrared (MIR) region (4000-800 cm^{-1}) with 8 cm^{-1} resolution and 128 scans, equipped with a single bounce ATR diamond crystal accessory and a deuterated triglycine sulfate (DTGS) detector. Background scans were taken before each sample measurement to avoid ambient noise. For particles below 0.5 mm, subsamples were extracted onto calcium fluoride slides for mapping. *Thermo Scientific Nicolet iN 10* microscope was used to create infrared maps. This ultrafast mapping microscope has high spectral resolution and is coupled to MCT detector with pixel aperture 25 x 25 µm. Spectra was recorded at a spectral resolution of 4 cm^{-1}, in transmission mode, in the range of 715-4000 cm^{-1}

The spectra were analyzed with the *OMNIC Spectra MCS Software* and the identification was obtained by comparing unknown spectra with databases that can be found in the Supplementary information. The standard criteria of ≥ 70% between the sample and reference spectra were followed as described in Dronjak et al. 2022.

2.3.3. Quality control

Currently, there are no widely accepted methods for sampling, laboratory analysis, quality assurance/quality control (QA/QC), or reporting of the results of MPs in the environment, which has been widely acknowledged as a significant challenge for the field (Zheng et al., 2019). In the present study, a rigorous approach was applied for both sample collection and analysis by determining background contamination. Blank samples were applied in the field as well as in the laboratory. Moreover, analytical method recovery was evaluated by spiking the sample with fluorescent polyethylene microspheres (*Cospheric Inc., California, USA*) of different diameter sizes, and colors. The same type of microspheres was used in a previous study by Expósito et al. (2022) which followed a strict quality control protocol.

Prior to use, all tools and materials were cleaned with 70% ethanol and ultrapure water that had been filtered through a nitrocellulose filter with a pore size 0.45 µm (GF/F Whatman). Only analytical grade chemicals were used and reagents were prepared with filtered ultrapure water. Only cotton lab coats were worn during the sampling and analysis of the samples in the laboratory. All sample pre-treatment procedures were carried out in the extraction hoods.

For more reliable assessments, this work considers several strategies: taking the composite samples within 24 h, in order to eliminate daily variations of the load of MPs; studying the seasonal variations of the load of MPs by sampling in two campaigns during summer and winter

seasons; sampling high volumes of different treatment units; comparing the results of two different sampling techniques of the secondary clarifier (WWTP effluent) with auto-sampling, and in situ filtration; and performing recovery rate tests in order to confirm the methodology of the sample pre-treatment.

3. Results and discussion

3.1. Analytical method recovery evaluation

Recovery test was performed by spiking the sample (thickened sludge, 500 mL) with fluorescent polyethylene (PE) microspheres (*Cospheric innovations, CA, USA*) and repeating the process three times (Table 1). Average triplicate recovery was 75% for spiked MPs with a diameter size of 53-63 µm, 74% for spiked MPs with a diameter size of 125-150 µm, 70% for the particles with a diameter size of 250-300 µm and 100% for the particles with diameter sizes 450-500 µm indicating acceptable laboratory performance. Additional information on each data set can be found in the Supplementary Data (Table S2).

Table 1. Recovery tests for the different particle size ranges

Microplastic type	**Particle size [µm]**	**Recovery [%]**
Fluorescent Pink Polyethylene	53-63	75
Fluorescent Violet Polyethylene	125-150	74
Fluorescent Orange Polyethylene	250-300	70
Fluorescent Red Polyethylene	450-500	100

Furthermore, background contamination was determined by analyzing field and laboratory blanks. An open petri dish was placed while sampling and filtering effluent water for 9 h and while processing the samples in the laboratory. Only three fibers were found in the field blank and four fibers were in the laboratory blank. Based on the blank results, and taking into account the nature of the sample analyzed, it can be concluded that the cross-contamination of the sample was very limited. In addition, blank samples were pre-treated and analyzed together with the samples.

3.2. Abundance of microplastic particles in different treatment units of WWTP

Microplastics were detected in each WWTP unit, from influent to effluent as well as in sludge. Table 2 presents levels of MPs per liter (of water samples) and per kg (of dry sludge), and morphology (films, fibers, and fragments), ranging in size from 20 µm to 5 mm, for each treatment unit (water and sludge line), and for both sampling campaign (summer and winter). All MPs found in the WWTP were below 2 mm.

Table 2. Levels and morphology of MPs in different treatment units of WWTP

		Summer				Winter			
Section	**Sample**	**Levels**	**Morphology [%]**			**Levels**	**Morphology [%]**		
			Films	**Frag.**	**Fib.**		**Films**	**Frag.**	**Fib.**
Water line	Influent	369	10	42	48	1058	19	15	66
	Pre-treatment	351	39	28	33	403	23	5	72
	Primary clarifier	35	1	9	90	159	26	16	58
	Biological reactor	32	2	28	70	178	20	15	65
	Secondary clarifier (composite)	13	5	16	79	26	26	8	66
	Secondary clarifier (filtered)	2.3	4	47	48	5.6	16	22	63
Sludge line	Thickened sludge	4,356	48	11	41	10,344	20	13	67
	Centrifugation water	416	4	66	30	444	12	54	34
	Dry sludge (MPs/kg $_{dw}$)	51,450	12	42	46	133,200	31	18	51

All levels in MPs/L except for Dry sludge (MPs/kg d.w.) Frag.: Fragments; Fib.: Fibers

During the summer and winter campaigns, the concentration of MP particles (from 20 µm to 2 mm) in the influent of WWTP was 369 MPs/ L and 1058 MPs/ L, respectively. It can be observed that the winter period has almost a three times higher abundance of MPs compared to summer. One of the possible causes could be the precipitation that was recorded on the day and seven days before the summer sampling, which was 28.1 mm (available data from the Meteorological Service of Catalonia). Differences in water consumption during these periods should also be taken into account. However, both concentrations of MPs in the water entering the plant are within the range of values reported by Sun et al. (2019) and by Liu et al. (2021) which reviewed 72 and 38 WWTPs worldwide, indicating values between 15 and 10,000 MPs/L and between 0.28 and 31,400 MPs/L, respectively. Another study showed similar values in an urban WWTP located in Cadiz (Spain) with a concentration of 645 MPs/L in the influent (Franco et al., 2021). Schell et al. (2021) investigated untreated wastewater from five WWTPs, in central Spain, in MPs size ranges from 5 mm to 55 µm. All of the WWTPs were urban, while three also received industrial wastes, and reported concentrations ranged between 850 and 11,550 MPs/m^3, which is much higher than the results in the present study.

Concentration of MPs entering the WWTP decreases very significantly as the water is treated in the different units shown in Figure 3; i.e., 351, 35, 32, and 13 MPs/ L (2.3 MPs/ L) for summer sampling and 403, 159, 178, and 26 MPs/ L (5.6 MPs/ L) for the winter sampling period. Removal rates are as follows: 5%, 91%, 91%, 96% (99%), and 62%, 85%, 83%, and 98% (99%), for the summer and winter periods, respectively. An extensive difference was observed in MPs removal rate during the pre-treatment process, since this unit was inoperative during the summer sampling period, indicating that it has a large impact on MPs removal. In the next stage, the presence of the primary clarifier facilitates the sedimentation of MP particles increasing the removal rate by an additional 23% calculated for the winter period when both units were

operative. The following unit, the biological reactor, showed no significant effect on the reduction of MPs, on the contrary, in the winter period, it contributed to an increase of 2%. A study by Blair et al. (2019) indicated that after the preliminary, primary, secondary, and tertiary treatment processes in a WWTP located in the UK, the overall abundance decreased by 6%, 68%, 92%, and 96%, respectively.

The variations observed in the concentration of secondary clarifier (WWTP effluent) from 13 MPs/L (24 h composite), to 2.3 MPs/L (filtered) and from 26 MPs/L (24 h composite), to 5.6 MPs/L (filtered), for the summer and winter sampling respectively, are due to the different sampling techniques. Composite samples within 24 h were collected by autosampler including day and night hours, while the filtered sample was collected by sieving the effluent water through a set of stainless-steel sieves with different mesh sizes for a duration of 9 h, allowing a large volume of water to be filtered (400 L). Perhaps, the more reliable value is the one obtained by filtering a larger volume (400 L) although filtering of the effluent water did not cover the night hours. Nevertheless, removal rates have exceeded 96% in all effluent samples and are considered relatively high with a maximum of 99% for both sampling periods. Similar results are published in different studies that studied the removal of MPs in European WWTPs (Franco et al., 2021; Lares et al., 2018; Rasmussen et al., 2021).

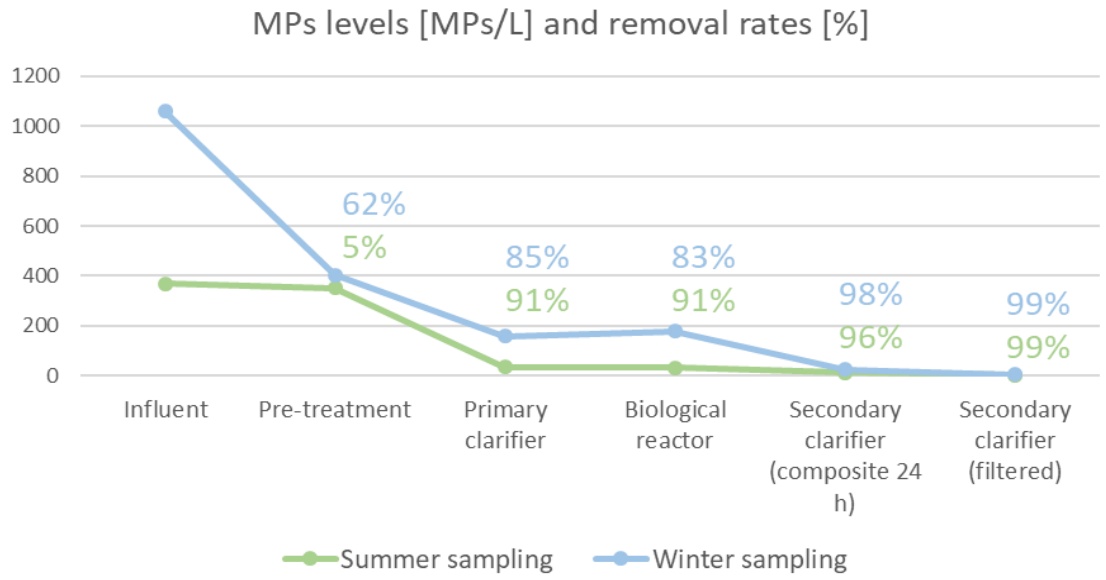


Figure 3. Concentration of microplastic particles (MPs/L) per each treatment unit of WWTP for summer and winter periods and removal rates [%] in the water line

MPs removal rates from five municipal and 2 industrial WWTPs located in Cadiz (Spain) ranged between 78-97% having mostly primary and secondary treatment (4 WWTP) while the rest (3 WWTP) having only secondary (Franco et al., 2020).

Besides overall removal rate calculations, the present study investigated the removal of certain MPs morphology types or size fractions (Table 3).

Table 3 Removal efficiency of different morphology types and size fractions for summer and winter period

Removal efficiency (RE%) summer season					
	fragments	**fibers**	**films**		
RE%	98.60	94.00	98.31		
	0.045-0.02mm	**0.125-0.045mm**	**0.5-0.125mm**	**1-0.5mm**	**2-1mm**
RE%	95.90	98.62	99.47	94.00	/
Removal efficiency (RE%) winter season					
	fragments	**fibers**	**films**		
RE%	98.72	97.52	96.56		
	0.045-0.02mm	**0.125-0.045mm**	**0.5-0.125mm**	**1-0.5mm**	**2-1mm**
RE%	91.77	93.25	98.94	98.08	95.60

These findings suggest that the removal rates of MPs in the wastewater treatment plant can vary depending on both the morphology and size fraction of the particles, as well as the sampling season. While fragments and films generally showed high removal rates, fibers exhibited slightly lower removal rates, especially during the winter season. Additionally, the efficacy of removal varied across different size fractions, with some size ranges showing high removal rates while others faced challenges.

Further studies are needed to explore the underlying factors influencing these variations and to optimize wastewater treatment processes for improved removal of MPs across different morphologies and size fractions, considering the specific challenges posed by different seasons.

Microplastic particles that are removed during wastewater treatment processes tend to accumulate in the sludge (Mahon et al., 2017) from primary and secondary clarifiers. The thickened sludge concentrations were 4,356 and 10,344 MPs/L and in the dry sludge 51,450 and 133,200 MPs/kg for the summer and winter sampling periods, respectively. Water from the centrifugation unit contained MPs very similar in both sampling periods with concentrations of 416 and 444 MPs/L. In many developed regions, sewage sludge is used as a fertilizer for agricultural purposes due to its economic advantages (Nizzetto et al., 2016). Reusing both water and sludge contributes to the circular economy, however, it leads to the reintroduction of MPs into the environment and may pose a potential environmental threat (Gatidou et al., 2019b). When sludge containing MPs is applied to the soil it can be ingested by the terrestrial biota, entering the food chain, and posing a risk to human health.

3.3. Morphology and size distribution of microplastic particles

During visual sorting, particles were divided into the most abundant shapes of MPs: fibers, fragments, and films (Bayo et al., 2020; Hidayaturrahman & Lee, 2019; Lares et al., 2018). It is

important to consider the morphology of MPs in the WWTPs since it is closely related and affects its removal efficiency (McCormick et al., 2014). Moreover, the shape of the particles is impacting the interaction between MPs and other contaminants or microorganisms in wastewater (Wang et al., 2018). Figure 4 presents the morphology distribution of MP particles for different treatment units and for both sampling periods.

The most dominant type of MPs in the WWTP is fibers, followed by fragments and films. Other studies reported that similar morphology such as fibers, pellets, fragments, and films were the most widely detected MPs in wastewater, and their abundances were 91%, 70%, 65%, and 21%, respectively (Bayo et al., 2020; Hidayaturrahman & Lee, 2019; Lares et al., 2018). Pellets were found on rear occasions in the WWTP, for example, in the summer sampling period, from the total number of MPs, they occupy only 0.2%. Their concentration is negligible, compared to other MPs morphology types. Fragments and pellets can originate from cosmetics and personal care products, such as toothpaste, masks, and soaps (Carr et al., 2016) while the films are from plastic packing bags (Kazour et al., 2019). Synthetic fibers are released during washing processes and washing machines are considered an important source of releasing MPs into the environment. Browne et al. (2011) estimated that from washing a single piece of clothing, more than 1900 fibers could be discharged into the sewage system. Due to different physicochemical treatments in WWTPs, fibers can form homo/hetero aggregates such as fiber balls, which are often detected in different treatment units. Since it is challenging to divide this formation and quantify each fiber separately, they are counted as a single fiber item leading to an underestimation of the total number of fibers. Schell et al. (2021) also discovered these fiber clumps in 5 WWTPs located in central Spain, although on rare occasions. This type of morphology is widely discovered in the marine environment too. In the study by Expósito et al. (2021) fiber balls were observed on the bottom of sediments associated with sediment grains and organic matter (seagrass, calcareous, and algae remains). Furthermore, due to their morphological characteristics, fibers are the most difficult particles to remove from WWTP due to their high length-to-width ratio (Ngo et al., 2019).

Figure 4. Morphology of microplastic particles in different treatment units of WWTP for both sampling seasons

Several examples of different MP particles detected in WWTP can be seen in Figure 5.

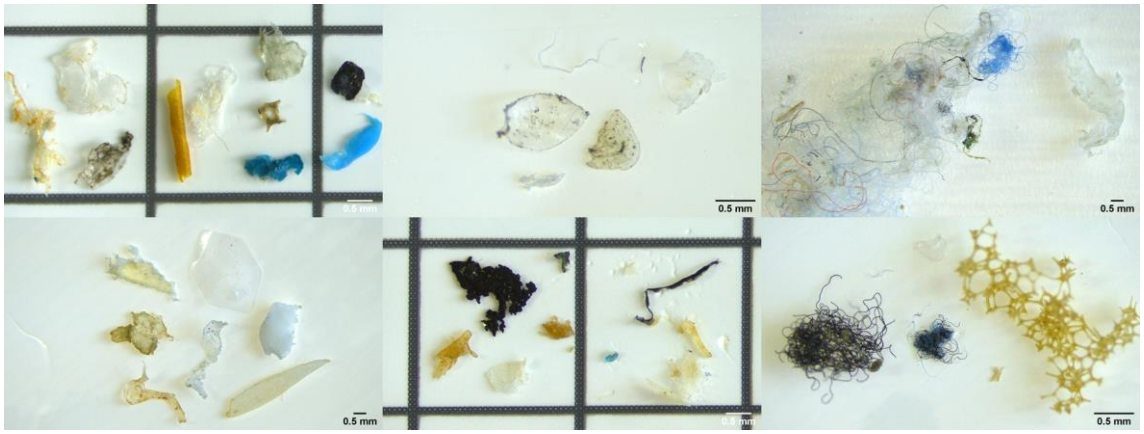


Figure 5. Commonly detected microplastic particles in WWTP including films, fragments, fibers, and fiber balls

The size distribution of MPs across the WWTP was also studied. The size range of MP particles was from 5 mm to 0.02 mm, divided into 5 sub-ranges: 2-1; 1-0.5; 0.5-0.125; 0.125-0.045, and 0.045-0.02 mm. Particles above 2 mm were not detected. With respect to these sub-ranges, no clear trends can be followed regarding MPs size removal. The same findings were reported by

Franco et al. (2020) indicating that no preferential removal of MPs occurred in the WWTP with respect to size.

However, the following seasonal variations were detected: In the water line, the most abundant particle sizes were 0.125-0.045, and 0.5-0.125 mm for summer and winter sampling, respectively. For the sludge line, in the summer period, the most abundant particle size ranged from 2-1 mm, and in the winter period from 0.5 to 0.125 mm. Considering both sampling periods, the majority of the particles were smaller MPs below 0.5 mm (Figure S1 in Supplementary data).

3.4. Composition of microplastic particles in different units of WWTP

After visual identification of the suspected MP particles, photos were recorded with *Leica FLEXACAM C1, IC90 E, ICC50 W/E* camera, and final confirmation was performed with FTIR spectroscopic technique. Since the identification of all suspected particles is very time-consuming, a subsample was prepared for further analysis including different morphologies and sizes. Particles visible to the human eye were extracted and identified manually with ATR-FTIR, while the rest of the particles were prepared on calcium fluoride slides for mapping and identified with micro-FTIR. Additional information on the maps can be found in Supplementary data.

A wide range of polymers was detected in different treatment units of WWTP (Figure 6). The most abundant polymers in the WWTP according to the conducted composition analyses were polyethylene (PE) (8-79%), synthetic cellulose (0-26%), polypropylene (PP) (0-39%), polyvinyl chloride (PVC) (0-17%), polyethylene-polypropylene copolymer (PE-PP) (0-21%), synthetic polymeric resin (0-91%), polyamide (PA) (0-21%), polyacrylonitrile (PAN) (0-13%) and polyester (PES) (0-12%) taking into account both sampling period separately. Although the spectral match of the polymer resin was about 70%, in the present study is classified as a synthetic polymer resin due to the need for further research. Other detected polymers, with an abundance of less than 10% are polyethylene terephthalate (PET), polycarbonate (PC), additives, polystyrene (PS), polyurethane (PU), Teflon (PTFE), ethylene vinyl alcohol (EVOH), and ethylene vinyl acetate copolymer (EVOH/EVA), and polyisobutadiene (PIB) for each sampling period (Table S2).

The most common materials found in the influent and effluent of 72 WWTPs worldwide, were PES (28-89%), PE (4-51%), PET (4-35%), and PA (3-30%), and in less extent acrylate, alkyd, PP, PS, PU, PS acrylic, polyvinyl alcohol (PVAL) and polylactide (PLA) with the highest abundance between 5-27% (Sun et al., 2019).

The results are also in accordance with the statistics of the most widely produced plastics in the world: PE 36%, PP 21%, and PVC 12% followed by PET, PU, and PS (less than 10% each) (Geyer et al., 2017), which explains their abundance in the WWTP analyzed in a present study.

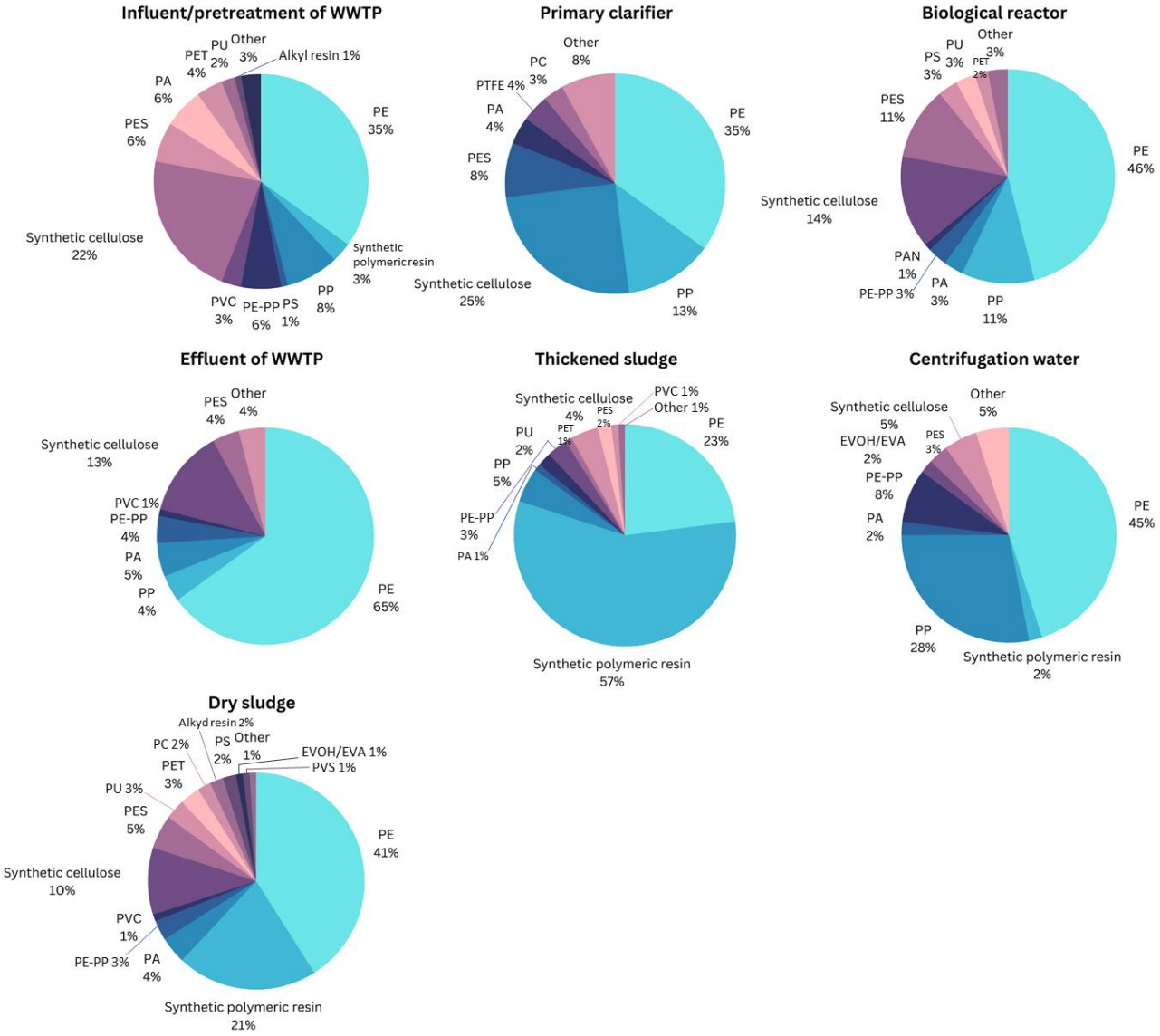


Figure 6. Composition of microplastics in different units of WWTP including summer and winter sampling periods.

The future polymer prioritization and methodology standardization can be made based on the most common polymers in WWTPs instead of all presented particles. The material information of MPs detected in WWTPs indicated that the majority of MPs in wastewater originated from human daily life (Sun et al., 2019).

Considering the amount of wastewater treated annually ($12.8 \cdot 10^6$ m^3/year) and the average number of MPs removed per litter for both sampling periods (709.55 MPs/L), it can be concluded that $9.1 \cdot 10^{12}$ MPs/year MPs are avoided from being emitted into the aquatic environment. The

removal efficiency of the WWTP is high, leading to the accumulation of MPs in the sludge. Current sludge management policy allows its usage for agricultural purposes, so the total annual number of MPs that would reach terrestrial ecosystems if all sludge were recycled in agricultural soils would be $7.6 \cdot 10^{11}$ MPs/year. For this reason, sludge should be managed as a waste properly, avoiding the transition of MPs pollutants to terrestrial ecosystems adding to the number of MPs that will inevitably end up in receiving water bodies through water discharge of WWTP which in the present case study is set at $5.1 \cdot 10^{10}$ MPs/year.

4. Conclusions

Based on the obtained results, it can be concluded that wastewater treatment plants play an important role when it comes to the fate of MP particles coming from households. The different treatment units in the water line exert a significant effect in reducing the MPs load with a maximum of 99%. The highest impact on MPs removal from wastewater has pre-treatment together with a primary clarifier while the secondary treatment showed no significant effect on the reduction of MPs. The most abundant morphology is fibers that are coming from synthetic materials during washing processes, followed by fragments and films. A wide range of polymers was detected including PE, synthetic cellulose, PP, PVC, PE-PP, PEEA, PA, PAN, and PES. Although WWTP can remove the majority of MPs from wastewater, these particles tend to accumulate in the sludge that is used for agricultural purposes. Sludge should be managed properly, as a waste, avoiding the transition of MPs pollutants to terrestrial ecosystems.

References

Ashton, K., Holmes, L., & Turner, A. (2010). Association of metals with plastic production pellets in the marine environment. *Marine Pollution Bulletin*, *60*(11), 2050–2055. https://doi.org/10.1016/J.MARPOLBUL.2010.07.014

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*. https://doi.org/10.1016/j.chemosphere.2019.124593

Blair, R. M., Waldron, S., & Gauchotte-Lindsay, C. (2019). Average daily flow of microplastics through a tertiary wastewater treatment plant over a ten-month period. *Water Research*, *163*, 114909. https://doi.org/10.1016/J.WATRES.2019.114909

Browne, M. A., Crump, P., Niven, S. J., Teuten, E., Tonkin, A., Galloway, T., & Thompson, R. (2011). Accumulation of microplastic on shorelines woldwide: Sources and sinks. *Environmental Science and Technology*, *45*(21), 9175–9179. https://doi.org/10.1021/es201811s

Carr, S. A., Liu, J., & Tesoro, A. G. (2016). Transport and fate of microplastic particles in wastewater treatment plants. *Water Research*, *91*, 174–182. https://doi.org/10.1016/J.WATRES.2016.01.002

Dehaut, A., Cassone, A. L., Frère, L., Hermabessiere, L., Himber, C., Rinnert, E., Rivière, G., Lambert, C., Soudant, P., Huvet, A., Duflos, G., & Paul-Pont, I. (2016). Microplastics in seafood: Benchmark protocol for their extraction and characterization. *Environmental Pollution*, *215*, 223–233. https://doi.org/10.1016/J.ENVPOL.2016.05.018

Dronjak, L., Exposito, N., Rovira, J., Florencio, K., Emiliano, P., Corzo, B., Schuhmacher, M., Valero, F., & Sierra, J. (2022). Screening of microplastics in water and sludge lines of a drinking water treatment plant in Catalonia, Spain. *Water Research*, *225*, 119185. https://doi.org/10.1016/J.WATRES.2022.119185

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*. https://doi.org/10.1016/j.scitotenv.2021.147453

Franco, A. A., Arellano, J. M., Albendín, G., Rodríguez-Barroso, R., Quiroga, J. M., & Coello, M. D. (2021). Microplastic pollution in wastewater treatment plants in the city of Cádiz:

Abundance, removal efficiency and presence in receiving water body. *Science of the Total Environment*, *776*. https://doi.org/10.1016/j.scitotenv.2021.145795

Franco, A. A., Arellano, J. M., Albendín, G., Rodríguez-Barroso, R., Zahedi, S., Quiroga, J. M., & Coello, M. D. (2020). Mapping microplastics in Cadiz (Spain): Occurrence of microplastics in municipal and industrial wastewaters. *Journal of Water Process Engineering*, *38*, 101596. https://doi.org/10.1016/J.JWPE.2020.101596

Fries, E., Dekiff, J. H., Willmeyer, J., Nuelle, M. T., Ebert, M., & Remy, D. (2013). Identification of polymer types and additives in marine microplastic particles using pyrolysis-GC/MS and scanning electron microscopy. *Environmental Sciences: Processes and Impacts*, *15*(10), 1949–1956. https://doi.org/10.1039/c3em00214d

Gatidou, G., Arvaniti, O. S., & Stasinakis, A. S. (2019a). Review on the occurrence and fate of microplastics in Sewage Treatment Plants. *Journal of Hazardous Materials*, *367*, 504–512. https://doi.org/10.1016/J.JHAZMAT.2018.12.081

Gatidou, G., Arvaniti, O. S., & Stasinakis, A. S. (2019b). Review on the occurrence and fate of microplastics in Sewage Treatment Plants. *Journal of Hazardous Materials*, *367*, 504–512. https://doi.org/10.1016/J.JHAZMAT.2018.12.081

Geyer, R., Jambeck, J. R., & Law, K. L. (2017). *Production, use, and fate of all plastics ever made*. https://www.science.org

Hidayaturrahman, H., & Lee, T. G. (2019). A study on characteristics of microplastic in wastewater of South Korea: Identification, quantification, and fate of microplastics during treatment process. *Marine Pollution Bulletin*, *146*. https://doi.org/10.1016/j.marpolbul.2019.06.071

Jenner, L. C., Rotchell, J. M., Bennett, R. T., Cowen, M., Tentzeris, V., & Sadofsky, L. R. (2022). Detection of microplastics in human lung tissue using µFTIR spectroscopy. *Science of The Total Environment*, *831*, 154907. https://doi.org/10.1016/J.SCITOTENV.2022.154907

Kazour, M., Terki, S., Rabhi, K., Jemaa, S., Khalaf, G., & Amara, R. (2019). Sources of microplastics pollution in the marine environment: Importance of wastewater treatment plant and coastal landfill. *Marine Pollution Bulletin*, *146*, 608–618. https://doi.org/10.1016/J.MARPOLBUL.2019.06.066

Kühn, S., van Werven, B., van Oyen, A., Meijboom, A., Bravo Rebolledo, E. L., & van Franeker, J. A. (2017). The use of potassium hydroxide (KOH) solution as a suitable approach to isolate

plastics ingested by marine organisms. *Marine Pollution Bulletin*, *115*(1–2), 86–90. https://doi.org/10.1016/j.marpolbul.2016.11.034

Kurt, Z., Özdemir, I., & James R., A. M. (2022). Effectiveness of microplastics removal in wastewater treatment plants: A critical analysis of wastewater treatment processes. *Journal of Environmental Chemical Engineering*, *10*(3). https://doi.org/10.1016/j.jece.2022.107831

Lares, M., Ncibi, M. C., Sillanpää, M., & Sillanpää, M. (2018). Occurrence, identification and removal of microplastic particles and fibers in conventional activated sludge process and advanced MBR technology. *Water Research*, *133*. https://doi.org/10.1016/j.watres.2018.01.049

Leslie, H. A., van Velzen, M. J. M., Brandsma, S. H., Vethaak, A. D., Garcia-Vallejo, J. J., & Lamoree, M. H. (2022). Discovery and quantification of plastic particle pollution in human blood. *Environment International*, *163*, 107199. https://doi.org/10.1016/J.ENVINT.2022.107199

Liu, W., Zhang, J., Liu, H., Guo, X., Zhang, X., Yao, X., Cao, Z., & Zhang, T. (2021). A review of the removal of microplastics in global wastewater treatment plants: Characteristics and mechanisms. *Environment International*, *146*, 106277. https://doi.org/10.1016/J.ENVINT.2020.106277

Löder, M. G. J., Imhof, H. K., Ladehoff, M., Löschel, L. A., Lorenz, C., Mintenig, S., Piehl, S., Primpke, S., Schrank, I., Laforsch, C., & Gerdts, G. (2017). Enzymatic Purification of Microplastics in Environmental Samples. *Environmental Science and Technology*, *51*(24). https://doi.org/10.1021/acs.est.7b03055

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. *Environmental Science and Technology*, *51*(2), 810–818. https://doi.org/10.1021/acs.est.6b04048

Masura, J., Baker, J., Foster, G., & Arthur, C. (2015). Laboratory methods for the analysis of microplastics in themarine environment: Recommendations for quantifying synthet- ic particles in waters and sediments. NOAA Technical Memorandum NOS-OR&R-48. *NOAA Technical Memorandum*, *July*.

McCormick, A., Hoellein, T. J., Mason, S. A., Schluep, J., & Kelly, J. J. (2014). Microplastic is an abundant and distinct microbial habitat in an urban river. *Environmental Science and Technology*, *48*(20), 11863–11871. https://doi.org/10.1021/es503610r

Mintenig, S. M., Int-Veen, I., Löder, M. G. J., Primpke, S., & Gerdts, G. (2017). Identification of microplastic in effluents of waste water treatment plants using focal plane array-based

micro-Fourier-transform infrared imaging. *Water Research*, *108*, 365–372. https://doi.org/10.1016/J.WATRES.2016.11.015

Murphy, F., Ewins, C., Carbonnier, F., & Quinn, B. (2016). Wastewater Treatment Works (WwTW) as a Source of Microplastics in the Aquatic Environment. *Environmental Science and Technology*, *50*(11), 5800–5808. https://doi.org/10.1021/acs.est.5b05416

Napper, I. E., & Thompson, R. C. (2016). Release of synthetic microplastic plastic fibres from domestic washing machines: Effects of fabric type and washing conditions. *Marine Pollution Bulletin*, *112*(1–2). https://doi.org/10.1016/j.marpolbul.2016.09.025

Ngo, P. L., Pramanik, B. K., Shah, K., & Roychand, R. (2019). Pathway, classification and removal efficiency of microplastics in wastewater treatment plants. *Environmental Pollution*, *255*, 113326. https://doi.org/10.1016/J.ENVPOL.2019.113326

Nizzetto, L., Bussi, G., Futter, M. N., Butterfield, D., & Whitehead, P. G. (2016). A theoretical assessment of microplastic transport in river catchments and their retention by soils and river sediments. *Environmental Science: Processes and Impacts*, *18*(8), 1050–1059. https://doi.org/10.1039/c6em00206d

Nuelle, M. T., Dekiff, J. H., Remy, D., & Fries, E. (2014). A new analytical approach for monitoring microplastics in marine sediments. *Environmental Pollution*, *184*. https://doi.org/10.1016/j.envpol.2013.07.027

Prata, J. C. (2018). Airborne microplastics: Consequences to human health? In *Environmental Pollution* (Vol. 234). https://doi.org/10.1016/j.envpol.2017.11.043

Prata, J. C., da Costa, J. P., Duarte, A. C., & Rocha-Santos, T. (2019). Methods for sampling and detection of microplastics in water and sediment: A critical review. In *TrAC - Trends in Analytical Chemistry* (Vol. 110). https://doi.org/10.1016/j.trac.2018.10.029

Qiu, Q., Tan, Z., Wang, J., Peng, J., Li, M., & Zhan, Z. (2016). Extraction, enumeration and identification methods for monitoring microplastics in the environment. In *Estuarine, Coastal and Shelf Science* (Vol. 176). https://doi.org/10.1016/j.ecss.2016.04.012

Ragusa, A., Notarstefano, V., Svelato, A., Belloni, A., Gioacchini, G., Blondeel, C., Zucchelli, E., De Luca, C., D'avino, S., Gulotta, A., Carnevali, O., & Giorgini, E. (2022). Raman Microspectroscopy Detection and Characterisation of Microplastics in Human Breastmilk. *Polymers*, *14*(13). https://doi.org/10.3390/polym14132700

Ragusa, A., Svelato, A., Santacroce, C., Catalano, P., Notarstefano, V., Carnevali, O., Papa, F., Rongioletti, M. C. A., Baiocco, F., Draghi, S., D'Amore, E., Rinaldo, D., Matta, M., & Giorgini, E. (2021). Plasticenta: First evidence of microplastics in human placenta. *Environment International*, *146*, 106274. https://doi.org/10.1016/J.ENVINT.2020.106274

Rasmussen, L. A., Iordachescu, L., Tumlin, S., & Vollertsen, J. (2021). A complete mass balance for plastics in a wastewater treatment plant - Macroplastics contributes more than microplastics. *Water Research*, *201*, 117307. https://doi.org/10.1016/J.WATRES.2021.117307

Rist, S., Carney Almroth, B., Hartmann, N. B., & Karlsson, T. M. (2018). A critical perspective on early communications concerning human health aspects of microplastics. In *Science of the Total Environment* (Vol. 626). https://doi.org/10.1016/j.scitotenv.2018.01.092

Rodrigues, M. O., Abrantes, N., Gonçalves, F. J. M., Nogueira, H., Marques, J. C., & Gonçalves, A. M. M. (2019). Impacts of plastic products used in daily life on the environment and human health: What is known? In *Environmental Toxicology and Pharmacology* (Vol. 72). https://doi.org/10.1016/j.etap.2019.103239

Schell, T., Hurley, R., Nizzetto, L., Rico, A., & Vighi, M. (2021). Spatio-temporal distribution of microplastics in a Mediterranean river catchment: The importance of wastewater as an environmental pathway. *Journal of Hazardous Materials*, *420*, 126481. https://doi.org/10.1016/J.JHAZMAT.2021.126481

Shen, M., Zeng, Z., Wen, X., Ren, X., Zeng, G., Zhang, Y., & Xiao, R. (2021). Presence of microplastics in drinking water from freshwater sources: the investigation in Changsha, China. *Environmental Science and Pollution Research*, *28*(31). https://doi.org/10.1007/s11356-021-13769-x

Sol, D., Laca, A., Laca, A., & Díaz, M. (2020). Approaching the environmental problem of microplastics: Importance of WWTP treatments. *Science of The Total Environment*, *740*, 140016. https://doi.org/10.1016/J.SCITOTENV.2020.140016

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 Research*, *152*, 21–37. https://doi.org/10.1016/J.WATRES.2018.12.050

Tagg, A. S., Harrison, J. P., Ju-Nam, Y., Sapp, M., Bradley, E. L., Sinclair, C. J., & Ojeda, J. J. (2017). Fenton's reagent for the rapid and efficient isolation of microplastics from wastewater. *Chemical Communications*, *53*(2), 372–375. https://doi.org/10.1039/c6cc08798a

Wang, W., Yuan, W., Chen, Y., & Wang, J. (2018). Microplastics in surface waters of Dongting Lake and Hong Lake, China. *Science of The Total Environment*, *633*, 539–545. https://doi.org/10.1016/J.SCITOTENV.2018.03.211

WHO. (2019). *Microplastics in drinking-water* (WHO 2019).

Xu, P., Ge, W., Chai, C., Zhang, Y., Jiang, T., & Xia, B. (2019). Sorption of polybrominated diphenyl ethers by microplastics. *Marine Pollution Bulletin*, *145*. https://doi.org/10.1016/j.marpolbul.2019.05.050

Zhao, S., Danley, M., Ward, J. E., Li, D., & Mincer, T. J. (2017). An approach for extraction, characterization and quantitation of microplastic in natural marine snow using Raman microscopy. *Analytical Methods*, *9*(9). https://doi.org/10.1039/c6ay02302a

Zheng, Y., Li, J., Cao, W., Liu, X., Jiang, F., Ding, J., Yin, X., & Sun, C. (2019). Distribution characteristics of microplastics in the seawater and sediment: A case study in Jiaozhou Bay, China. *Science of The Total Environment*, *674*, 27–35. https://doi.org/10.1016/J.SCITOTENV.2019.04.008

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 Research*, *112*. https://doi.org/10.1016/j.watres.2017.01.042

Ziajahromi, S., Neale, P. A., Telles Silveira, I., Chua, A., & Leusch, F. D. L. (2021). An audit of microplastic abundance throughout three Australian wastewater treatment plants. *Chemosphere*, *263*, 128294. https://doi.org/10.1016/J.CHEMOSPHERE.2020.128294

Chapter 2. Screening of microplastics in water and sludge lines of a drinking water treatment plant in Catalonia, Spain

Abstract

Microplastics (MPs) are emerging pollutants detected everywhere in the environment, with the potential to harm living organisms. The present study investigated the concentration, morphology, and composition of MPs, between 20 µm and 5 mm, in a drinking water treatment plant (DWTP) located close to Barcelona (Catalonia, NE Spain). The sampling included different units of the DWTP, from influent to effluent as well as sludge line. Sampling strategy, filtration, allows sampling of large volumes of water avoiding sample contamination, and during 8 hours in order to increase the representativeness of MPs collected. The pre-treatment of the samples consisted of advanced oxidation with Fenton's reagent and hydrogen peroxide, followed by density separation of the particles with zinc chloride solution. Visual identification was performed with an optical and stereoscopic microscope with final Fourier-transform infrared spectroscopic (FTIR) confirmation. MPs were found in all DWTP samples, with concentrations from 4.23 ± 1.26 MPs/L to 0.075 ± 0.019 MPs/L in the influent and effluent of the plant, respectively. The overall removal efficiency of the plant was 98.3%. The most dominant morphology was fibers followed by fragments and films. Twenty-two different polymer types were identified and synthetic cellulose, polyester, polyamide, polypropylene, polyethylene, polyurethane, and polyacrylonitrile were the most common. Although MPs could be incorporated from the distribution network, MPs intake from drinking water from this DWTP was not an important route compared to fish and seafood ingestion.

Keywords: Plastic pollution, drinking water, sludge, removal ratios

1. Introduction

Microplastics (MPs) are defined as an emergent pollutant that is ubiquitously detected in the natural environment (marine and freshwater, soil and air) (Franco et al., 2021) posing a threat to the health of living organisms (Alimi et al., 2018; Chen et al., 2020; Waring et al., 2018). MPs are synthetic, non-biodegradable polymers with a diameter size of less than 5 mm. Divided by origin, they can be either primary (directly manufactured microbeads and fibers), or secondary (breakdown of larger plastic). Primary MPs come mainly from personal care products and textile fibers, while secondary ones are from weathering, photolysis, and decomposition of discarded plastics in the environment (Expósito et al., 2021; Ziajahromi et al., 2017). MPs may act as vectors for environmental pollutants, hazardous chemical additives, and pathogen microorganisms which have the potential to be spread through the food chain (Prata, 2018; Rist et al., 2018; Rodrigues et al., 2019).

Food and water intake have been identified as a route of exposure to MPs. They have been detected in numerous samples of commercially available seafood, bottled water, tap water, honey, and salt (Iñiguez et al., 2017; Liebezeit and Liebezeit, 2015; Mason et al., 2018; Mintenig et al., 2019; Pivokonsky et al., 2018; Tanaka and Takada, 2016; Yang et al., 2015). To date, toxicological studies on the possible effects of MPs on human health have not been fully established (Revel et al., 2018; WHO, 2019; Wright and Kelly, 2017) mainly due to challenges in the experimental design and determining the actual MPs concentration to which organisms are exposed. However, a recent study found that MPs could alter human microbiota (Tamargo et al., 2022). It is worth noting that, in addition to ingestion, inhalation of MPs present in the air, as well as dermal contact, are also routes of exposure that must be considered (Prata et al., 2020).

MPs pollution receiving considerable attention is also evidenced by the number of publications that have drastically increased from 2011 until now (Sol et al., 2020). Additionally, MPs have been mentioned in the European drinking water directive (2020/2184) to be included in the risk assessment of the DWTP and their supply system. However, there is a lack of published data addressing the problem of MPs contamination in drinking water treatment plants (DWTP) (Wang et al., 2020). Drinking water treatment plants ensure the quality and safety of drinking water, thus it is urgent to investigate the fate of MPs in different treatment processes. Currently, there are no legislative restrictions on the abundance of MPs in drinking water (Novotna et al., 2019; Wang et al., 2020). Only a few studies investigated MPs based on full-scale DWTP. Pivokonský et al. (2020) investigated the occurrence and fate of microplastic particles, up to 1 µm, at two

different DWTPs (with advanced and conventional treatment systems), within one river catchment in the Czech Republic, and discovered a significant difference between them: DWTP containing flocculation, sedimentation, sand filtration, and granular activated carbon filtration units had MPs removal efficiency higher than 80%, unlike the other DWTP with flocculation and sand filtration units, that had MPs removal rate of only 40%. Other studies by Wang et al. (2020) and Shen et al. (2021) also reported a high removal rate of DWTPs located in China, both greater than 85%. Furthermore, characterization of MPs in eight DWTPs located in the UK showed that is possible to eliminate over 99.99% of MPs from their source water (river, groundwater, or an upland reservoir) leading to its accumulation in dry sludge (Johnson et al., 2020). Although MP's removal rates in DWTP are high, several MPs are still found in the effluents of DWTP. Investigating MPs in a size range of 20 µm to 5000 µm, Mintenig et al. (2019) reported that the concentration of MPs in the German DWTP effluent was 0.007 MPs/L while Dalmau-Soler et al. (2021) reported the concentration of 0.06 ± 0.04 MPs/L, in the effluent of Spanish DWTP, for the same size range. This study performed by Dalmau-Soler et al., (2021) investigated the presence of microplastics along the same river basin as in the present study, however different drinking water treatment plant was observed. The microplastic methodology is similar in both studies allowing better comparison of the results such as identification method (FTIR spectroscopy), analyzed size (20µm to 5 mm), and units used to report the MPs levels (MPs/L). Furthermore, although the plant is different, the treatments in both cases are similar where the plant from the previous study consists of clarifiers, sand filtration, ozonation, GAC filtration and ultrafiltration, and reverse osmosis. Some studies have also found that during the water treatment in the plant, the number of MPs increases, such as a recent study that reported the reduction of overall removal from 70 to 52% in a Clearwell (Cherniak et al., 2022). Information about the presents of MPs in the effluents of DWTP cannot be ignored, since the treated water is intended for the local population.

The aim of the present study is to investigate the concentrations, morphology, and composition of MPs in different treatment units of a river freshwater supplied DWTP including both, water and sludge lines. Removal efficiency was calculated to determine which treatment unit had the greatest impact on MPs removal and finally, to estimate the intake of MPs by drinking water from this source.

2. Materials and methods

2.1. Drinking water treatment plant characteristics

The studied DWTP of Llobregat river is located near Barcelona (NE Spain) and has a treatment capacity of 3.2 m^3/s. The DWTP consists of the treatment units depicted in Figure 1. Briefly, the first treatment is water roughing in which the surface river water (pH 8) passes through bars that prevent large materials, suspended in the water, from entering the plant. The water then flows through three grit channels. Potassium permanganate is added (0.5-1.5 mg/L) for the pre-oxidation of organic and inorganic compounds. In the next step, the water enters the mixing chambers, where pH is adjusted (7.4-7.7) with carbon dioxide, and chemicals such as coagulants and flocculants (Aluminium Polychloride 25mg/L and PolyDADMAC 0.8mg/L) were added for turbidity removal. The water then goes to eight clarifiers (Pulsator®), where the flocs present in the water are sedimented and the excess sludge is discharged. After clarification, oxidation with chloride dioxide is performed. The next process is filtration in two steps in twelve sand filters (70 cm thickness). Three of them are filled with two layers of sand (0.8-1.6 mm and 1.4-2.5mm grain size) and the other nine are filled with one layer (1-2mm grain size) together with sand Filtralite® material. This step is used not only for physical filtration but also for biofiltration. Sand filtration processes were followed by a granular activated carbon (GAC) filtration process, 15 filters of 150 cm thickness, Norit GAC1240, where part of the dissolved organic matter is adsorbed (organochlorides). Part of the treated water with the conventional treatment undergoes microfiltration with 10 µm polypropylene cartridge filters and reversible electrodialysis (EDR), which removes a high percentage of salt content in treated water. Then water is remineralized with calcium hydroxide in solution and carbon dioxide injection. After that, the remineralized water is mixed with the water non-treated with microfiltration, and EDR units. Final disinfection is done with a sodium hypochlorite solution, and water is stored in two tanks of 213,000 m^3 before the distribution.

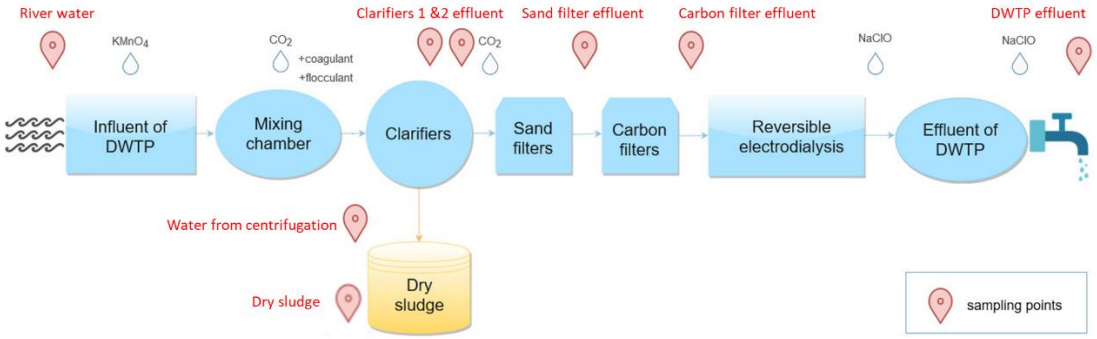


Figure 1. Flow diagram of the drinking water treatment plant (DWTP) units and sampling points

2.2 Sampling methodology

Microplastic samples from the 6 water line sampling points were obtained by filtration from DWTP in May 2021 for 8 hours. A total of 360L (3 replicates of 120L each) of river water (influent) from the field inside the plant for 8 hours were filtered through a set of stainless-steel sieves (Cisa Sieving Technologies, 200 mm diameter x 50 mm height) with mesh size: 20, 50, 100, 500 and 1000 µm. To avoid contamination, the other water line samples were taken from the taps of the DWTP laboratory, from the effluents of treatment units (clarifier lines 1 (n=7) and 2 (n=7), sand filter (n=3), and activated carbon filter unit (n=3)) and the effluent of the plant (n=7). Water was filtered through an in-line stainless filter holder containing a PTFE filter (*Sartorius*, 10 µm pore size) connected to a tap for 8 hours. The total volumes of water sampling were 29L, 28L, 267L, 600L, and 965L for clarifier line 1 effluent, clarifier line 2 effluent, sand filter effluent, carbon filter effluent, and effluent of the plant, respectively. Water volume filtered varied from one treatment unit's effluent to another according to the expected MPs levels (Mintenig et al., 2019; Pivokonsky et al., 2018). Stainless steel sieves covered in aluminum foil and PTFE filters stored in glass Petri dishes were immediately transferred to the laboratory for further analysis. This sampling approach allows us to sample relatively large volumes of water, near $1m^3$ in the effluent of the plant, avoiding sample contamination and during large periods (8 hours). More information regarding sampled volume and the replicates are summarized in the supplementary data. Finally, dry sludge (300 g) (n=1) and water from centrifugation process (5 L) (n=1) were taken from the operation unit of the sludge line, in glass bottles pre-cleaned with filtered water. Glass bottles were covered with aluminum foil to prevent contamination from the cap.

2.3. Analysis of samples in the laboratory

In order to perform pre-treatment of the samples, the following chemicals and materials were used: absolute ethanol (*Scharlau*, >99.9%), iron (II) sulfate heptahydrate (*Sigma-Aldrich*, >99.0%), hydrogen peroxide (*PamReac AppliChem ITW Reagents*, 30%), PTFE- filter (*Sartorius*, 5 and 10 µm pore size), and zinc chloride (*Acros Organics*, >98.0%).

Nowadays there are no standard procedures for measuring MPs in drinking water treatment plants (Conesa and Ortuño et al., 2022). Pre-treatment of the samples was performed in order to extract MPs and remove all organic and inorganic matter to avoid interference. Three versions of the same protocol are established in the laboratory based on the requirements and nature of the samples (from highest organic matter content in dry sludge or centrifugated water to lowest organic matter content in water from clarifiers, sand/carbon filters, or effluent). In general, the protocol consists of oxidation to remove organic matter. Oxidation with Fenton's reagent was recommended by US National Oceanic and Atmospheric Administration (NOAA) for MPs analysis in marine water (Masura et al., 2015) and other authors have also used it as a purifying step in microplastic identification from different water samples, sediments and organisms (Erni-Cassola et al., 2017; Karami et al., 2016). Hydrogen peroxide is another oxidizing agent that can digest organic matter with little or no polymer degradation (Nuelle et al., 2014; Qiu et al., 2016; Zhao et al., 2017). In order to remove inorganic matter from the samples, density separation was applied using a salt solution (Ziajahromi et al., 2017). In the present work, zinc chloride solution (density: 1.8 g/mL) was chosen because its density is suitable for successful extraction of high-density microplastics such as polyvinyl chloride (PVC) (density: 1.16-1.58 g/cm^3) and polyethylene terephthalate (PET) (density: 1.37-1.45 g/cm^3) (Prata et al., 2019a).

Pre-treatment methodology is summarized in Figure 2. Briefly, the influent of DWTP was filtrated through stainless-steel sieves (Cisa Sieving Technologies, 200 mm diameter x 50 mm height) with mesh sizes of 20, 50, 100, 500, and 1000 µm. Each fraction was filtered through vacuum filter equipment on a 10 µm PTFE filter, treated with Fenton's reagent, and sonicated for 10 min at 40 °C. The suspension was left for 24 h and was filtered again through a 10 µm PTFE filter. Density separation with zinc chloride solution (1.8 g/mL) was performed next. The sample was sonicated at 40 °C for 10 min and left for 4-12 h. Furthermore, it was centrifugated at 3000 rpm for 1 min and the supernatant was filtered through a 10 µm PTFE filter. The process was repeated three times on the same filter. The quantity of organic and inorganic matter, in the sieves with particle size ≥50 µm was not significantly high to interfere with MPs identification and for this reason, visual inspection was performed immediately and the oxidation process for

organic matter removal was not carried out. In contrast, for particles between 20 and 50 µm, the PTFE filters were treated with hydrogen peroxide 33-35% for 24 h.

Samples of dry sludge and water from sludge centrifugation units were filtrated through stainless-steel sieves with mesh sizes of 20, 50, 100, 500, and 1000 µm in the laboratory and dried at 40-45°C for 3-4 days, and the weight of dry material was recorded. The methodology for processing was elaborated with a combination of steps and different sequences based in Mintening et al.,(2017), Prata et al., (2019b), Ben-David et al., (2021), Ziajahromi et al., (2021), Elkhatib and Vinka Craver (2020) methodologies. In this study Fenton's solution was applied for 24 h due to high organic matter content.

For clarifier lines 1 and 2, sand and carbon filters as well as treated water units of DWTP, due to low organic and inorganic particle content on the PTFE filters, were directly inspected taking into account MPs size equal to or larger than 20 µm.

Visual identification was performed for all filters of treatment units and after purification of the river and sludge samples with a stereoscopic microscope LEICA MZ10 with 1 µm resolution and 80 x magnification coupled to FLEXACAM C1 camera and docked screen of 32 cm, light projected from above and below to get good image of surface structure and appearance, in order to sort the particles according to their most common shapes: fibers, films and fragments (Bayo et al., 2020; Hidayaturrahman and Lee, 2019; Lares et al., 2018) as well as to quantify them. Particle shapes between 20 and 50 µm were then confirmed with an Olympus CX41 optical microscope at 40 and 50X magnification and 400 and 500 X total magnification. All particles identified were extracted from the filters and were taken to analysis for composition

In order to determine the size of the MPs, ImageJ software was used, previously calibrated with *Olympus Objective Micrometer* reference (10 µm). Fiber length was registered and for fragments the mean between maximum and minimum diameter was registered.

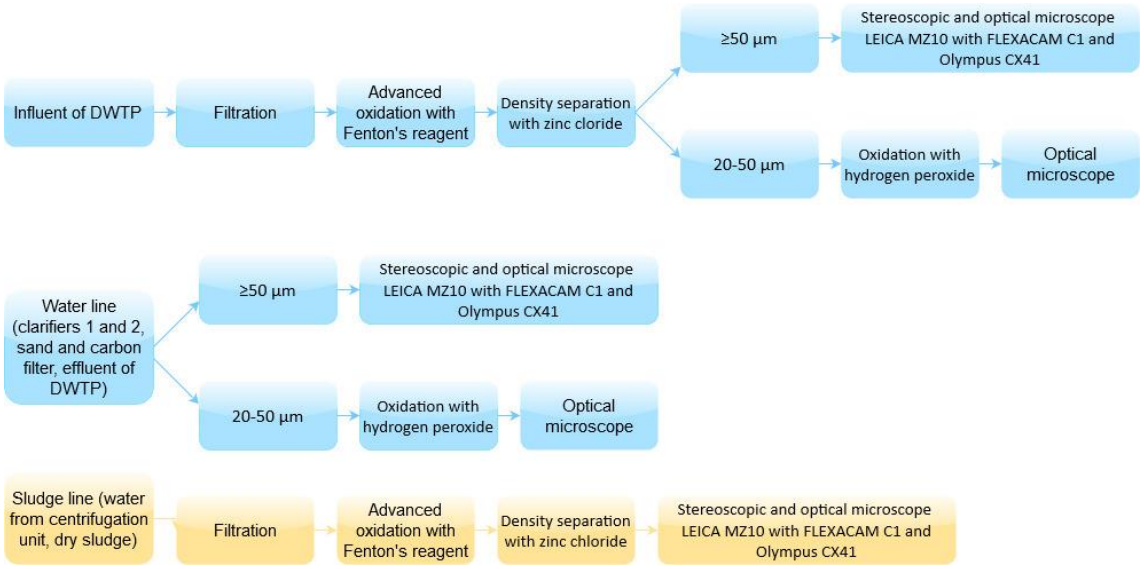


Figure 2. Pre-treatment protocols for microplastic identification and visual inspection

2.4. Qualitative analysis

After visual identification, MPs composition was analyzed by µFTIR and ATR-FTIR spectroscopic techniques. Only for river, water from sludge centrifugation and sludge samples, of the total number of particles between 20 and 500 µm, sub-samples were extracted on calcium fluoride slides for maps elaboration due high quantity of possible plastic particles. For subsample, particles were chosen according to morphology, color, and appearance for every filter section, until completing the entire filter area to ensure representativeness. For other samples (clarifiers, sand, carbon filter, and effluent), all particles detected were analyzed for their composition.

Particles larger than 500 µm were analyzed by Thermo Scientific GladiATR Highest Performance NICOLET Diamond ATR-FTIR spectrometer with OMNIC™ Paradigm Software. The measurements were performed in reflection mode in the range of 400–4000 cm^{-1} with 16 scans at a spectral resolution of 4 cm^{-1}. The background was done before analysis and every 6 samples. Infra-red maps over calcium fluoride slides were performed for particles from 20 to 500 µm by µFTIR analysis or ultrafast mapping microscope Thermo Scientific Nicolet™ iN™10 with MCT detector with pixel aperture 25x25 µm in transmission mode, in the range of 715-4000 cm^{-1} with 4 scans at a spectral resolution of 4 cm^{-1}. The spectra were analyzed with the OMNIC Specta MCS Software and the identification was obtained by comparing unknown spectra with databases such as HR Nicolet Sampler Library, Hummel Polymer Sample Library, Polymer Laminate Films, Wizard Library as well as an own library that was generated with more than 80 spectra.

For all particles isolated from the treatment unit's samples such as clarifiers 1 and 2, sand filter, activated carbon filter, and treated water, all particles ≥20 µm were placed over calcium fluoride slides for µFTIR analysis using Leica stereoscopy microscope (resolution of 1 µm).

To avoid the loss of MPs, for sludge, water from sludge centrifugation and river samples, in the PTFE filters containing particle sizes from 20 to 50 µm, all particles were retired from the filter for composition analysis, then for missing particles recruitment, the filter was washed vigorously with hydrogen peroxide 33-35% for 24 h and filtered on siliceous filters (1cm^2, pore size 5-6 µm wide, square shape, 12 µm pore distance, membrane thickness 500 µm). The entire filter was checked first for some single particle presence with microscopy µFTIR and then µFTIR maps were elaborated. For µ-FTIR maps, an area of 3 mm^2 was established randomly over siliceous filters considering interference absence and accurate IR spectra generation, in that area, a spectrum was made every 25 µm with a total of more than 15000 spectra for analysis with Omnic Picta Software and libraries selected as HR Nicolet Sampler Library, Hummel Polymer Sample Library, Polymer Laminate Films, Wizard Library. The µ-FTIR maps were obtained by reflection, four scan accumulations, the spectral resolution of 4 $cm^{-1,}$ and spectral range of 4000-715 cm^{-1}.

Similarly, all PTFE filters from all treatment units of DWTP were also treated with hydrogen peroxide 33-35% for 24h and filtered over siliceous filters. All siliceous filters were analyzed as above mentioned.

Match spectra greater than or equal to 70% similarity to the reference spectra were accepted. Unidentified spectra were compared to two own libraries elaborated with more common plastics in the market and weathered fragments, films, and fibers from environmental samples applying the same criteria for reference spectra acceptance. Furthermore, particles were analyzed according to their characteristic absorption band of each polymer chemical grouping bonds according to Campbell et al., (2000) and Coates (2000), for frequencies ranging mostly from 3650-500 cm^{-1}, 1480-1430 cm^{-1} for C-C aromatic ring stretching, 1790-1700 cm^{-1} for double binding C-O stretching, 2980-2780 cm^{-1} for C-H stretching of aliphatic and 3150-3030 cm^{-1} of aromatics. For aliphatic organohalogen, natural and synthetic cellulose identification, frequencies from 1150 to 550 cm^{-1}, 1200-1000 cm^{-1}, 1500-1200 cm^{-1} (C-H and C-O-H bending modes), 2900-2820 cm^{-1} (C-H stretching modes) and from 3600 to 3000 cm^{-1} (O-H stretching modes) were also evaluated. The particles identified as plastic were added to the final quantification. If the particle still could not be identified, it was considered to be a definite unidentified category.

2.5. Quality control

To avoid cross-contamination, ultrapure water was filtered through a 0.45 µm pore size filter (*GF/F Whatman*, 47 mm diameter, nitrocellulose). All equipment and materials were washed with filtered ultrapure water and 70% ethanol before sampling. All reagents were prepared with filtered ultrapure water. During the analysis of the samples in the laboratory, only laboratory coats made of cotton were worn. The extraction hoods were used during all sample pre-treatment processes. Moreover, procedural blank samples were collected in the field as well as in the laboratory and the corresponding corrections were applied to the results.

Controls were applied during river sampling activities and consisted of two open Petri dishes, 90 cm in diameter, to collect possible airborne fibers. The results were 8 fibers (3 black, 2 red, and 3 blue) and 6 fibers (3 black, 2 blue, and 1 red) in control 1 and 2, respectively. In consequence, the maximum fibers value of 8 samples were removed of the final quantification result of the various PTFE filters analyzed from every sieve size used in river water filtration. This selection was in a randomly way, choosing several filters and removing the fibers according only by color and length. Inside the laboratory of DWTP, for catchment samples, were located two controls near of sampling pipelines that representing the outlet of each treatment unit, two 60 mm petri dish was placed open near the sampling area while activity was developed. The results were 0 particles in both controls.

For accurate results and due to the sample processing site in laboratory is not entirely free from fibers a deposition rate of airbone fibers was calculated prior to sample processing. This rate was calculated for the laboratory workspace where the samples were processed. The procedure was four open petri dishes (60 cm in diameter) with PTFE filters were placed into similar distance in laboratory and covering the entire area for a month, after that the fibers deposited were quantified and referred by filter area and time with a result of 0.04 fibers/cm^2/day. Considering a PFTE filter area of 17.4 cm^2, 1 fiber was randomly subtracted from each analyzed filter needed organic matter or turbidity removals. Finally, in the procedural blank, MPs (fibers, films, or fragments) were not found.

For the detection of particles lost through the different steps of the sample treatment, a recovery test (n=3) was conducted per sample batch, spiking a mix of colored polyethylene spheres with diameters from 53 to 500 µm (Cospheric Inc., California, USA) in water and sludge samples. The recovery rates in sludge were 75 and 91 % for 53–63 µm, 74 and 95 % for 125–150 µm, 70 and 95 % for 250–300 µm, and 100 and 100 % for 425–500 µm for sludge and water samples. No degradation signs (i.e. mechanical damages, fragmentations, or chemical attacks)

were observed on spheres. The methods applied in the present work fulfill almost all minimum requirements established by Schymanski et al. (2021).

3. Results and discussion

3.1. Abundance and removal of microplastic particles in the water line of DWTP

Microplastic particles were detected in all water samples, from influent to effluent. Table 1 presents the MPs concentrations, removal efficiency, and sizes for each treatment unit in the water line. Due to the morphological characteristics of the fibers and their large length/width ratio, they were not categorized in size fractions, yet their size can be found in Supplementary data.

Table 1. Levels (MPs/L), size distribution and removal efficiency of MPs in the DWTP.

Unit	*n*	Concentration [MPs/L]	Removal efficiency [%]	Size (µm)*				
				2000-1000	1000-500	500-100	100-50	50-20
DWTP influent	3	4.230±1.263	-	6%	13%	43%	30%	8%
Clarifier line 1	7	1.577±0.696	62.7	<1%	30%	50%	20%	<1%
Clarifier line 2	7	1.388±0.543	67.2	<1%	<1%	50%	25%	25%
Sand filter	3	0.038±0.025	99.1	<1%	<1%	67%	33%	<1%
Carbon filter	3	0.032±0.013	99.2					
DWTP Effluent	7	0.075±0.019	98.3	<1%	<1%	39%	46%	15%

*Size was analysed for films and fragments. Concentration expressed as mean standard ±deviation

The concentration of MPs in the DWTP influent (raw water) was 4.23 ± 1.26 MPs/L. The most abundant MPs concentration was between 500 and 100 µm (43%) followed by a fraction of 100-50 µm (30%) considering films and fragments. These results are lower than the results published by Pivokonský et al. (2020) whose concentration, for raw water, was 1296 MPs/L and 23 MPs/L, for advanced and conventional treatment, in the DWTP located in the Czech Republic, respectively. The different results are mainly caused by different size ranges, as the previous study investigate MPs up to 1 µm. Another study by Mintenig et al. (2019) determined concentrations of MPs, larger than 20 µm, in raw water, that ranged from 0 to 0.007 MPs/L, although raw water refers to groundwater extraction at least 30 m deep, in Germany. Other studies conducted in European rivers found MPs mean levels of 12, 42, and 5.53 MPs/m^3, in Rhone, Tet, and Elbe river, respectively (Constant et al., 2020). However, influent water cannot be representative of river water, and water from the Mediterranean river, here studied, are not comparable to the rivers of northern or central Europe. Only a recent study (Dalmau-Soler et al.,

2021) was found regarding the same river that feeds the DWTP here studied, with similar MPs levels. Along the river basin, concentration was ranging from non-detected to 3.6 MPs/L while the mean concentration in the influent of DWTP was 0.96 ± 0.46 MPs/L. Still, data on the presents of MPs in water in Mediterranean rivers, is very scarce (Guerranti et al., 2020). However, the differences between methodologies, lack of standardization, and size ranges of analyzed MPs make difficult these comparisons.

The overall MPs removal rate was 98.3%, from 4.23 ± 1.26 MPs/L, in raw water, to 0.075 ± 0.019 MPs/L in the effluent. Considering each treatment unit, clarifier eliminated the most of MPs, between 63 and 67%, depending on the water line studied. The most abundant size fraction for both clarifiers was between 500 and 100 µm considering films and fragments. Subsequently, the sand filter increases the removal efficiency up to 99% where the concentration of 0.038 ± 0.025 MPs/L was found in the effluent of this treatment unit. Previous research by Dalmau-Soler et al. (2021) and Li et al. (2020) also reported that the first treatment stage (coagulation/sedimentation in clarifiers and sand filtration) had the highest impact on MPs removal, investigating full-scale DWTPs, as well as some lab-scale studies by Ma et al. (2019). Furthermore, a recent study by Cherniak et al., 2022 found that coagulation, flocculation, and sedimentation accounted for the highest removal (70 %), however negligible additional removal was observed in the filtration unit (<1%).

The next treatment unit, the activated carbon filter, does not increase significantly the removal efficiency with a level of 0.032 ± 0.025 MPs/L in their effluent. By contrast, after the microfiltration, electrodialysis, and remineralization stage, an increase of MPs content, from 0.032 to 0.075 ± 0.019 MPs/L, was observed. Wang et al. (2020) observed similar behavior in the effluent of the ozonation unit, leading to an increase in small and fibrous MPs. The reason may be that the MPs in this operating unit broke down due to the shear force of the water flow (Horton et al., 2017). Johnson et al. (2020) noted that there is a possibility of MPs generated within DWTP itself, while Dalmau-Soler et al. (2021) performed migration tests to check this theory, and the results suggested that MPs can be released from DWTP when the materials are old; however, this probably does not occur at normal DWTP working conditions. Our speculation was that remineralization of the treated water with calcium hydroxide and post-chlorination with sodium hypochlorite probably added particles to the effluent water. These MPs pollution could come from solid reagents ($Ca(OH)_2$) packaged with plastic, chlorination, or pollution coming from the resuspension of storage tanks, however more studies are needed to elucidate this. Nevertheless, the removal efficiency of DWTP, in this study (98.3%), is higher than the ones observed in other European plants that ranged from 70 to 90% (Pivokonsky et al., 2018;

Pivokonský et al., 2020). Other work performed in a pilot station established a removal rate of MPs fibers and fragments in 81 and 89%, respectively, in absence of coagulant and 96 and 97%, respectively, in presence of coagulant (Negrete Velasco et al., 2022). When comparing different water treatment plants in the same river basin, the removal rate reported was similar with an overall value of 93% (Dalmau-Soler et al., 2021). Certainly, direct comparison between the studies should be taken with caution due to differences in DWTP technology, MP sampling, sample pre-treatment, analytical identification methods to differences related to spatial variations and sampling location (Novotna et al., 2019).

Regarding the DWTP effluent, the concentration was 0.075 ± 0.019 MPs/L (fraction between 100 and 50 µm most abundant considering films and fragments), which is higher by one order of magnitude than the average value determined in a German DWTP that catch underground water (Mintenig et al., 2019) of 0.007 MPs/ L. Potable water from eight DWTPs within the UK had similar results, typically less than 0.002 MPs/L, accounted for MPs ≥ 25 µm (Johnson et al., 2020) and also similar to effluent water of Spanish DWTP located in the same river basin (Dalmau-Soler et al., 2021), with MPs levels ranging from 0.03 to 0.11 MP/L. On the contrary, these values were much lower than the values reported by Wang et al. (2020) of 930 ± 71 MPs/ L in the effluent of one of the largest advanced drinking water plant located in China. The typical capacity of this plant is 120 million m^3/day compared to the treatment plant in our study, with a treatment capacity of only 276,480 m^3/day. A recent study investigated tap water samples from different developed countries including France, Germany, Japan, and the United States, showing much higher results than the ones obtained in the present study, with the MPs concentration ranging from 1.9 to 225 MPs/L, and the overall mean concentration of 39 ± 44 MPs/L (size range: 19.2 µm to 4200 µm) (Mukotaka et al., 2021). Kosuth et al. (2018) also investigated MPs in the tap water from 14 different countries in Europe, America, Asia, and Africa with the concentration ranging from 0 to 61 MPs/L and the overall mean of 5.45 MPs/L (size range: 100 to 5000 µm). The highest concentration of 61 MPs/L was found in the USA, while the four lowest means were from European Union countries: non-detected; 0.91; 1.82; 1.83 MPs/L for Italy, Germany, France, and Ireland, respectively. However, it should take into account the low volumes (0.5 L) of water analyzed by this study (Kosuth et al., 2018). In some cases, MPs concentration could vary from the effluent of DWTP to tap water, since the household pipes made from durable plastic (PVC, PE, and PA) could contribute to the increase of MPs in the tap water over time (Mintenig et al., 2019; Tong et al., 2020), however, Shen et al. (2021) reported no difference between these two samples.

The morphology of MPs has also been investigated and visually categorized into three main shape types: fragments, films, and fibers (Bayo et al., 2020; Hidayaturrahman and Lee, 2019; Lares et al., 2018). Figure 3 shows that fiber morphology was the most prevalent in DWTP influent, similarly reported by other authors (Cherniak et al., 2022; Dalmau-Soler et al., 2021; Sarkar et al., 2021; Wang et al., 2020).

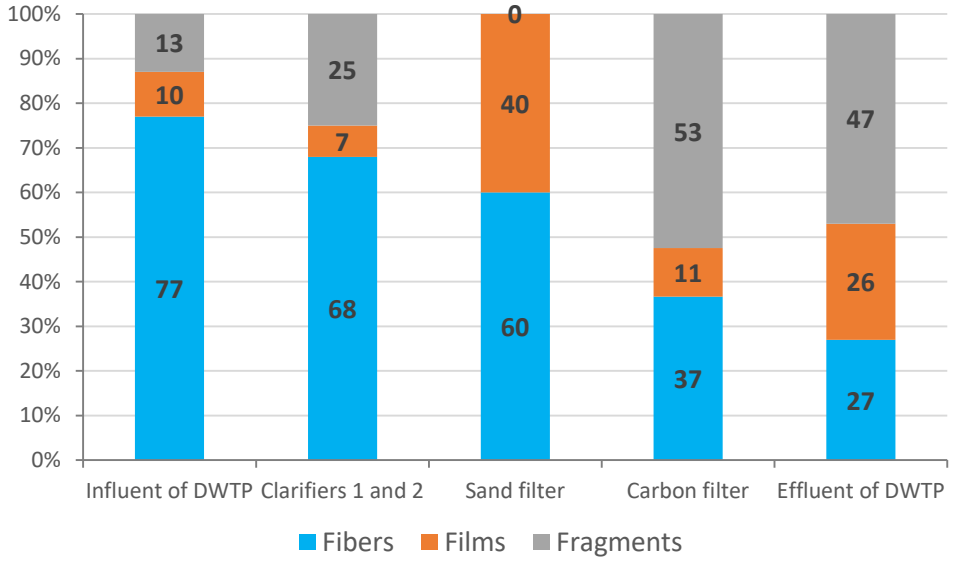


Figure 3. Microplastics morphology for different treatment units of drinking water treatment plant (DWTP).

The proportion of fibers decreased while treatment advanced from 77% (MPs/L) in raw water to 27% (MPs/L) in the effluent. Several explanations are present regarding the better removal of fibers, especially in the first treatment steps. Fibers are more likely to form flocs and settle (Katrivesis et al., 2019) since they are usually heavier polymers such as polyamide (PA) or nylon (density 1.15 g/cm3), polyester (PES) (density 1.38 g/cm^3) and cellulose acetate (CA) (density 1.30 g/cm^3) and their chemical structure can facilitate attachment to coagulant flocs such as carbonyl (C=O) chemical groups of PES or PET (Xue et al., 2022), also PA fibers can be ionized depending on pH media and interact with coagulants and flocculants. Furthermore, the surface of MPs has an impact on its removal, so the rougher the surface, the better the removal (Shahi et al., 2020). It is most likely, that these synthetic fibers, in the water environments, are released from washing machines (Napper and Thompson, 2016). Fragments and films are microplastics that remain in the effluent water and are more difficult to remove during the treatment process with an abundance of 47 and 26%, respectively. Figure 4 shows different morphology types detected in the influent and effluent of DWTP.

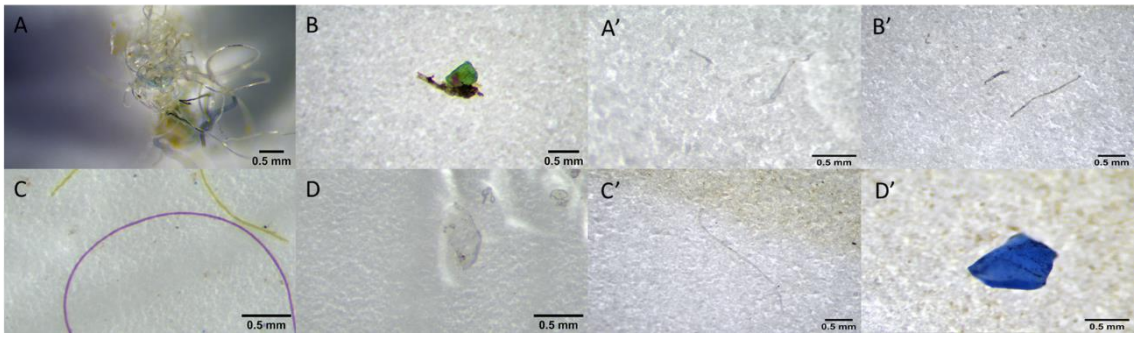


Figure 4. MPs detected in influent: A. group of fibers B. fragment C. individual fibers D. films and effluent of DWTP: A'. film and fiber B'. fibers C'. fiber D'. fragment with 50-80 x magnification

3.2. Abundance of microplastic particles in the sludge line of DWTP

Microplastic removed from the water, during the treatment process, tends to accumulate in the sludge (Conesa and Ortuño, 2022). Dry sludge and water from sludge centrifugation unit MPs concentration are depicted in Table 2.

Table 2. Concentration of MPs and size distribution in the sludge line of the DWTP.

Unit	Concentration [MPs/L] [MPs/$kg_{w.w.}$]	Size (µm)*				
		2000-1000	1000-500	500-100	100-50	50-20
Water from centrifugation	194	52%	<1%	7%	13%	28%
Dry Sludge	14,360	24%	10%	32%	26%	8%
*Size was analyzed for films, fragments and fibers						

Water from the sludge centrifugation unit had a MPs concentration of 194 MPs/L dominated by particles of sizes ranging from 2000 to 1000 µm (Figure 5A). Fibers were more than 75% of MPs detected in water from the sludge centrifugation unit. The concentration in the dry sludge was 14,360 MPs/$kg_{w.w.}$ (or 35,680 MPs/kg_{dw}). The highest concentration of particles was between 500 and 100µm, followed by a fraction of 100-50 µm (Figure 5B). Fibers were, again, the most abundant morphology in general, however, it can be observed that their concentration decreases from larger size ranges (2000-1000 µm) to the smallest (50-20 µm), where film and fragment type increases significantly. High fiber content was in line with high removal efficiency from treated water. Another study also collected sludge produced during the water treatment process, at four DWTPs in the UK. The results varied extremely from non-detected to 86,000 MPs/g $_{dw}$. The authors commented that more samples are necessary to understand such variations (Johnson et al., 2020).

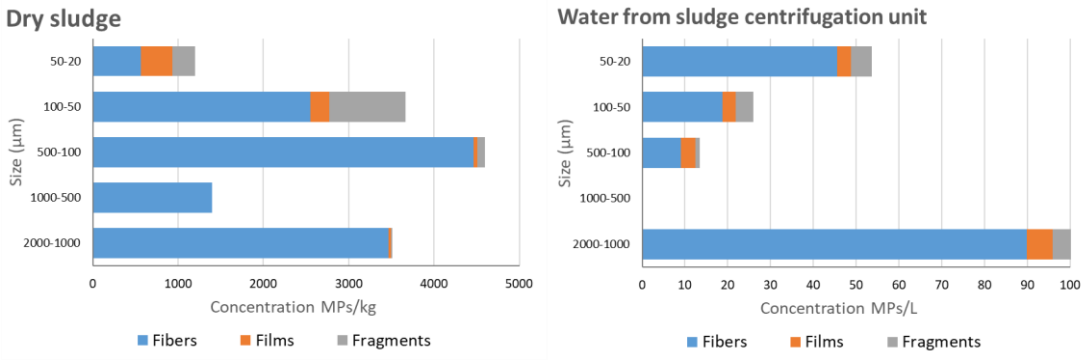


Figure 5. Microplastics concentration and morphology for each size in dry sludge and in water from sludge centrifugation unit

Taking into account the results obtained in our study, we suggest that both dry sludge and water from sludge centrifugation should be properly managed as waste, avoiding agricultural uses or its return to the river, in order not to transmit contamination to terrestrial ecosystems. The implementation of new technologies that enable the removal of MPs from sludge as well as from environmental samples is definitely needed in order to move forward towards a circular economy for plastic waste and to protect the environment (Sol et al., 2020).

3.3. Composition of microplastic particles

Each sample, after visual inspection and particle quantification, was photographed with Leica FLEXACAM C1, IC90 E, ICC50 W/E camera and subject to final confirmation with FTIR spectroscopic technique. For this purpose, MPs maps were elaborated. In Figure 6 can be seen µFTIR map for the influent of DWTP.

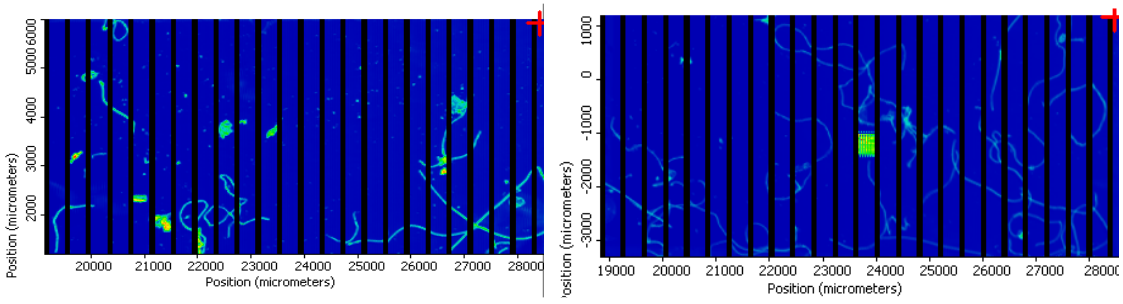


Figure 6. microFTIR maps for influent of DWTP

A total of 345 particles were confirmed and 22 different polymers were identified; however, the most common were: synthetic-cellulose, PES, polypropylene (PP), PA, and polyethylene (PE) (Figure 7). In addition, PET and polystyrene (PS) were also found. Pivokonský et al. (2020) reported similar results for the most commonly observed MPs in almost every sample: CA, PET, PVC, PE, and PP. Synthetic cellulose (rayon, cellulose acetate) was found in all samples with a

high abundance from 24 to 53%. Although some authors, in previous studies, reported it as a non-plastic particle (Wang et al., 2020; Yu et al., 2016) in the present study it was considered a synthetic polymer and was included in the quantification of the microplastics. One of the reasons was that after pre-treatment of the samples, cellulose did not degrade, which should happen in the case of natural material, indicating synthetic origin. All of these polymers are commonly used materials. Cellulose, PES, and PA are synthetic fibers used in the textile industry; PE is used for reusable bags, trays, containers, toys, and household utensils, while PP is used for food packaging, pipes, car parts, etc. (PlasticEurope, 2021).

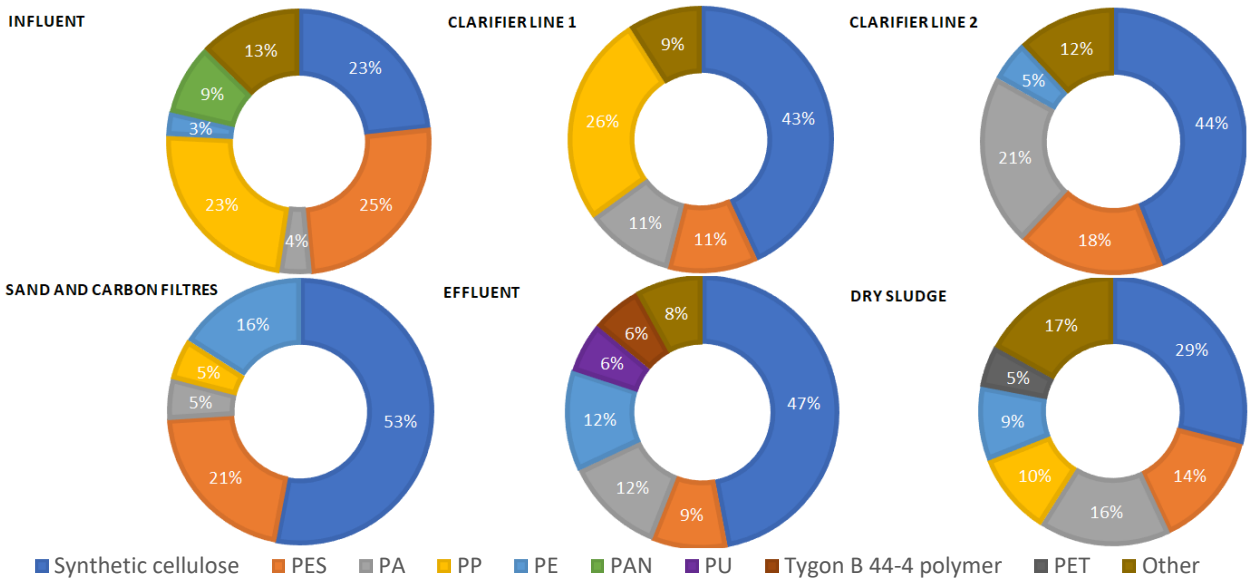


Figure 7. Composition of microplastic (films, fragments and fibers) from different units of the drinking water treatment plant. PES (Polyester); PA: Polyamide; PP: polypropylene; PE: polyethylene; PAN: polyacrylonitrile*; PU: polyurethane; PET: Polyethylene terephthalate.*

Other polymers detected in this study were: polyurethane (PU), PET, polyacrylonitrile (PAN), Tygon B 44-4 polymer (a new generation polymer with high content of PVC and silicon, but without phthalates), with abundance >5 % (which are included in Figure 7) and polycarbonate (PC), PS, PVC, polybutadiene (PBD), polyisobutene (PIB), polymethyl methacrylate (PMMA), polyvinyl acetate (PVA), polyvinyl stearate, PP-PE copolymer, epoxy resin, alkyl-resin, ethylene vinyl alcohol/ethylene vinyl acetate (EVOH/EVA), fluoroelastomer (synthetic rubber) with abundance <5 % (the proportions of materials with low abundance in DWTP units <5 % are denoted as “other”).

Considering MPs composition and morphology, fibers in the water line were made of synthetic cellulose, PES, PA, PAN, and to a lesser extent of PMMA, while fragments and films are mainly made of PP, PE, PA, and to a lesser extent of PU, polyvinyl stearate, PP-PE, and PET. For dry sludge, besides the main fiber composition (synthetic cellulose, PES, and PA), fibers made of PAN, PE-PP copolymer, and PP were also detected to a lesser extent. Fragments and films in sludge were made of PE, PP, PA, and to a lesser extent PET, PVC and PU.

Some polymers that are detected in the effluent of DWTP were not found in the influent, opening the possibility of MPs generated within DWTP itself. However, to confirm this statement, more proofs are needed. Due to the Covid-19 pandemic and the global use of disposable masks that are consisting of polymers such as PES, PP, and PS, in the following years is expected a significant increase in its concentration in freshwater sources (Xu & Ren, 2021).

According to the present results, assuming a water consumption per capita of around 1.0 L/day (Martínez et al., 2017), all tap water coming from this DWTP, and no extra incorporation of MP in distribution pipelines, the annual intake of MP through drinking tap water was set at 27 MP/person/year. This MPs intake through drinking water is negligible compared with another study that set the annual ingestion of MPs, via food and beverages, between 39,000 to 52,000 items/year (Cox et al., 2019). Most probably, seafood is one of the top three contributors to MPs human consumption, especially in the countries like Japan, with a much greater dietary proportion of seafood, with the highest estimation of 104.2 g/day, leading to daily consumption of 154 MPs (Cox et al., 2019; Micha et al., 2015). Another study (Catarino et al., 2018) indicated that MPs ingestion by a human via wild mussels is minimal compared to exposure via household fibers fallout during a meal. They predicted a concentration of 123 MPs/year/capita in the UK for MPs ingestion by humans via consumption of mussels and it can go to 4620 MPs/year/capita in countries with higher shellfish consumption such as France, Belgium, and Spain. By comparison, fiber exposure during a meal via dust fallout in a household was estimated to be 13,731- 68,415 MPs/year/capita.

4. Conclusions

Microplastic particles are detected in all samples within DWTP but their concentration varies significantly. In general, the operation of a drinking water treatment plant with respect to the reduction of microplastic particles is effective and with elevated removal efficiency. Clarifiers together with the sand filtration unit had the greatest impact on MPs reduction, >99%. However, an increase in microplastic particles, in the DWTP effluent, was observed and the final removal efficiency was 98.3 % which is still higher than the values reported for other European drinking

water treatment plants. It should be noted that direct comparison between the papers is challenging, since to date, there is no standardized analytical method for MPs identification.

As observed in the case of wastewater treatment plants, microplastic particles that are removed during treatment processes tend to accumulate in the sludge, especially synthetic fibers that are coming from washing machines. Both sludge and the centrifuged water must be properly managed as waste, avoiding agricultural uses or its return to the river, not to transmit contamination to terrestrial and aquatic ecosystems. New technologies should be implemented that enable the MPs removal from the sludge.

References

Alimi, O.S., Farner Budarz, J., Hernandez, L.M., Tufenkji, N. (2018). Microplastics and Nanoplastics in Aquatic Environments: Aggregation, Deposition, and Enhanced Contaminant Transport. Environmental Science and Technology, 52(4), 1704-1724. https://doi.org/10.1021/acs.est.7b05559.

Ben-David, E.A., Habibi, M., Haddad, E., Hasanin, M., Angel, D.L., Booth, A.M., Sabbah, I. (2021). Microplastic distributions in a domestic wastewater treatment plant: Removal efficiency, seasonal variation and influence of sampling technique. Science of the Total Environment, 752, 141880. https://doi.org/10.1016/j.scitotenv.2020.141880.

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. https://doi.org/10.1016/j.chemosphere.2019.124593.

Catarino, A.I., Macchia, V., Sanderson, W.G., Thompson, R.C., Henry, T.B. (2018). Low levels of microplastics (MP) in wild mussels indicate that MP ingestion by humans is minimal compared to exposure via household fibres fallout during a meal. Environmental Pollution, 237, 675-684. https://doi.org/10.1016/j.envpol.2018.02.069.

Campbell, D., Pethrick, R.A., White, J.R. (2000). Polymer Characterization (Physical Techniques). Second Edition, Stanley Thornes LTD.

Chen, G., Feng, Q., Wang, J. (2020). Mini-review of microplastics in the atmosphere and their risks to humans. Science of the Total Environment, 703, 135504. https://doi.org/10.1016/j.scitotenv.2019.135504.

Cherniak, S.L., Almuhtaram, H., McKie, M.J., Hermabessiere, L., Yuan, C., Rochman, C.M., Andrews, R.C. (2022). Conventional and biological treatment for the removal of microplastics from drinking water. Chemosphere, 288, 132587. https://doi.org/10.1016/j.chemosphere.2021.132587.

Coates, J. (2000). Interpretation of Infrared Spectra, A Practical Approach in Encyclopedia of Analytical Chemistry, R.A. Meyers (Ed.), John Wiley & Sons Ltd.

Conesa, J.A., Ortuño, N. (2022). Reuse of Water Contaminated by Microplastics, the Effectiveness of Filtration Processes: A Review. Energies, 15, 2432. https://doi.org/10.3390/en15072432.

Constant, M., Ludwig, W., Kerhervé, P., Sola, J., Charrière, B., Sanchez-Vidal, A., Canals, M., Heussner, S. (2020). Microplastic fluxes in a large and a small Mediterranean river catchment: The Têt and the Rhône, Northwestern Mediterranean Sea. Science of the Total Environment, 716, 136984. https://doi.org/10.1016/j.scitotenv.2020.136984.

Cox, K.D., Covernton, G.A., Davies, H.L., Dower, J.F., Juanes, F., Dudas, S.E. (2019). Human Consumption of Microplastics. Environmental Science and Technology, 53(12), 7068-7074. https://doi.org/10.1021/acs.est.9b01517.

Dalmau-Soler, J., Ballesteros-Cano, R., Ma, R., Boleda, R., Paraira, M., Ferrer, N., Lacorte, S. (2021). Microplastics from headwaters to tap water: Occurrence and removal in a drinking water treatment plant in Barcelona Metropolitan area (Catalonia, NE Spain). Environmental Science and Pollution Research, 28, 59462-59472. https://doi.org/10.1007/s11356-021-13220-1.

Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption (recast). Official Journal of the European Union, L 435, 1-62.

Elkhatib, D., Oyanedel-Craver, V. (2020). A Critical Review of Extraction and Identification Methods of Microplastics in Wastewater and Drinking Water. Environmental Science and Technology, 54(12), 7037-7049. https://doi.org/10.1021/acs.est.9b06672.

Erni-Cassola, G., Gibson, M.I., Thompson, R.C., Christie-Oleza, J.A. (2017). Lost, but Found with Nile Red: A Novel Method for Detecting and Quantifying Small Microplastics (1 mm to 20 μm) in Environmental Samples. Environmental Science and Technology, 51(23), 13641-13648. https://doi.org/10.1021/acs.est.7b04512.

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. https://doi.org/10.1016/j.scitotenv.2021.147453.

Franco, A.A., Arellano, J.M., Albendín, G., Rodríguez-Barroso, R., Quiroga, J.M., Coello, M.D. (2021). Microplastic pollution in wastewater treatment plants in the city of Cádiz: Abundance, removal efficiency and presence in receiving water body. Science of the Total Environment, 776, 145795. https://doi.org/10.1016/j.scitotenv.2021.145795.

Guerranti, C., Perra, G., Martellini, T., Giari, L., Cincinelli, A. (2020). Knowledge about microplastic in Mediterranean tributary river ecosystems: Lack of data and research needs on such a crucial marine pollution source. Journal of Marine Science and Engineering, 8(3), 216. https://doi.org/10.3390/jmse8030216.

Hidayaturrahman, H., Lee, T.G. (2019). A study on characteristics of microplastic in wastewater of South Korea: Identification, quantification, and fate. Environmental Pollution, 250, 447-455. https://doi.org/10.1016/j.envpol.2019.04.142.

Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C. (2017). Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. Science of the Total Environment, 586, 127-141. https://doi.org/10.1016/j.scitotenv.2017.01.190.

Iñiguez, M.E., Conesa, J.A., Fullana, A. (2017). Microplastics in Spanish Table Salt. Scientific Reports, 7(1), 1-7. https://doi.org/10.1038/s41598-017-09128-x.

Johnson, A.C., Ball, H., Cross, R., Horton, A.A., Jürgens, M.D., Read, D.S., Vollertsen, J., Svendsen, C. (2020). Identification and Quantification of Microplastics in Potable Water and Their Sources within Water Treatment Works in England and Wales. Environmental Science and Technology, 54(19), 12326-12334. https://doi.org/10.1021/acs.est.0c03211.

Karami, A., Romano, N., Galloway, T., Hamzah, H. (2016). Virgin microplastics cause toxicity and modulate the impacts of phenanthrene on biomarker responses in African catfish (Clarias gariepinus). Environmental Research, 151, 58-70. https://doi.org/10.1016/j.envres.2016.07.024.

Katrivesis, F.K., Karela, A.D., Papadakis, V.G., Paraskeva, C.A. (2019). Revisiting of coagulation-flocculation processes in the production of potable water. Journal of Water Process Engineering, 27, 193-204. https://doi.org/10.1016/j.jwpe.2018.12.007.

Kosuth, M., Mason, S.A., Wattenberg, E. v. (2018). Anthropogenic contamination of tap water, beer, and sea salt. PLoS ONE, 13, 1-18. https://doi.org/10.1371/journal.pone.0194970.

Lares, M., Ncibi, M.C., Sillanpää, M., Sillanpää, Markus. (2018). Occurrence, identification and removal of microplastic particles and fibers in conventional activated sludge process and advanced MBR technology. Water Research, 133, 236-246. https://doi.org/10.1016/j.watres.2018.01.049.

Liebezeit, G., Liebezeit, E. (2015). Origin of synthetic particles in honeys. Polish Journal of Food and Nutrition Sciences, 65, 1574-1578. https://doi.org/10.1515/pjfns-2015-0025.

Li, Y., Li, W., Jarvis, P., Zhou, W., Zhang, J., Chen, J., Tan, Q., Tian, Y. (2020). Occurrence, removal and potential threats associated with microplastics in drinking water sources. Journal of Environmental Chemical Engineering, 8, 104527. https://doi.org/10.1016/j.jece.2020.104527.

Ma, B., Xue, W., Hu, C., Liu, H., Qu, J., Li, L. (2019). Characteristics of microplastic removal via coagulation and ultrafiltration during drinking water treatment. Chemical Engineering Journal, 359, 159-167. https://doi.org/10.1016/j.cej.2018.11.155.

Martínez, M.A., Rovira, J., Sharma, R.P., Nadal, M., Schuhmacher, M., Kumar, V. (2017). Prenatal exposure estimation of BPA and DEHP using integrated external and internal dosimetry: A case study. Environmental Research, 158, 566-575. https://doi.org/10.1016/j.envres.2017.07.016.

Mason, S.A., Welch, V.G., Neratko, J. (2018). Synthetic Polymer Contamination in Bottled Water. Frontiers in Chemistry, 6, 1-11. https://doi.org/10.3389/fchem.2018.00407.

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

Micha, R., Khatibzadeh, S., Shi, P., Andrews, K.G., Engell, R.E., Mozaffarian, D. (2015). Global, regional and national consumption of major food groups in 1990 and 2010: A systematic analysis including 266 country-specific nutrition surveys worldwide. BMJ Open, 5(9), e008705. https://doi.org/10.1136/bmjopen-2015-008705.

Mintenig, S.M., Int-Veen, I., Löder, M.G.J., Primpke, S., Gerdts, G. (2017). Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging. Water Research, 108, 365-372. https://doi.org/10.1016/j.watres.2016.11.015.

Mintenig, S.M., Löder, M.G.J., Primpke, S., Gerdts, G. (2019). Low numbers of microplastics detected in drinking water from ground water sources. Science of the Total Environment, 648, 631–635. https://doi.org/10.1016/j.scitotenv.2018.08.178.

Mukotaka, A., Kataoka, T., Nihei, Y. (2021). Rapid analytical method for characterization and quantification of microplastics in tap water using a Fourier-transform infrared microscope. Science of the Total Environment, 790, 148231. https://doi.org/10.1016/j.scitotenv.2021.148231.

Napper, I.E., Thompson, R.C. (2016). Release of synthetic microplastic plastic fibres from domestic washing machines: Effects of fabric type and washing conditions. Marine Pollution Bulletin, 112, 39-45. https://doi.org/10.1016/j.marpolbul.2016.09.025.

Negrete Velasco, A., Ramseier Gentile, S., Zimmermann, S., Stoll, S. (2022). Contamination and Removal Efficiency of Microplastics and Synthetic Fibres in a Conventional Drinking Water Treatment Plant. Frontiers in Water, 4, 835451. https://doi.org/10.3389/frwa.2022.835451.

Novotna, K., Cermakova, L., Pivokonska, L., Cajthaml, T., Pivokonsky, M. (2019). Microplastics in drinking water treatment – Current knowledge and research needs. Science of the Total Environment, 667, 730-740. https://doi.org/10.1016/j.scitotenv.2019.02.431.

Nuelle, M.T., Dekiff, J.H., Remy, D., Fries, E. (2014). A new analytical approach for monitoring microplastics in marine sediments. Environmental Pollution, 184, 161-169. https://doi.org/10.1016/j.envpol.2013.07.027.

Pivokonsky, M., Cermakova, L., Novotna, K., Peer, P., Cajthaml, T., Janda, V. (2018). Occurrence of microplastics in raw and treated drinking water. Science of the Total Environment, 643, 1644–1651. https://doi.org/10.1016/j.scitotenv.2018.08.102.

Pivokonský, M., Pivokonská, L., Novotná, K., Čermáková, L., Klimtová, M. (2020). Occurrence and fate of microplastics at two different drinking water treatment plants within a river catchment. Science of the Total Environment, 741, 140236. https://doi.org/10.1016/j.scitotenv.2020.140236.

PlasticEurope. (2021). Plastics - the Facts 2021. An analysis of European plastics production, demand and waste data. Available from: https://plasticseurope.org/knowledge-hub/plastics-the-facts-2021/. Last accessed: 22/03/2022.

Prata, J.C. (2018). Airborne microplastics: Consequences to human health? Environmental Pollution, 234, 115-126. https://doi.org/10.1016/j.envpol.2017.11.043.

Prata, J.C., da Costa, J.P., Duarte, A.C., Rocha-Santos, T. (2019a). Methods for sampling and detection of microplastics in water and sediment: A critical review. TrAC - Trends in Analytical Chemistry, 110, 150-159. https://doi.org/10.1016/j.trac.2018.10.029.

Prata, J.C., da Costa, J.P., Girão, A.V., Lopes, I., Duarte, A.C., Rocha-Santos, T. (2019b). Identifying a quick and efficient method of removing organic matter without damaging microplastic samples. Science of the Total Environment, 686, 131-139. https://doi.org/10.1016/j.scitotenv.2019.05.456.

Prata, J.C., da Costa, J.P., Lopes, I., Duarte, A.C., Rocha-Santos, T. (2020). Environmental exposure to microplastics: An overview on possible human health effects. Science of the Total Environment, 702, 134455. https://doi.org/10.1016/j.scitotenv.2019.134455.

Qiu, Q., Tan, Z., Wang, J., Peng, J., Li, M., Zhan, Z. (2016). Extraction, enumeration and identification methods for monitoring microplastics in the environment. Estuarine, Coastal and Shelf Science, 176, 102-109. https://doi.org/10.1016/j.ecss.2016.04.012.

Revel, M., Châtel, A., Mouneyrac, C. (2018). Micro(nano)plastics: A threat to human health? Current Opinion in Environmental Science and Health, 1, 17-23. https://doi.org/10.1016/j.coesh.2017.10.003.

Rist, S., Carney Almroth, B., Hartmann, N.B., Karlsson, T.M. (2018). A critical perspective on early communications concerning human health aspects of microplastics. Science of the Total Environment, 626, 720-726. https://doi.org/10.1016/j.scitotenv.2018.01.092.

Rodrigues, M.O., Abrantes, N., Gonçalves, F.J.M., Nogueira, H., Marques, J.C., Gonçalves, A.M.M. (2019). Impacts of plastic products used in daily life on the environment and human health: What is known? Environmental Toxicology and Pharmacology, 72, 103239. https://doi.org/10.1016/j.etap.2019.103239.

Sarkar, D.J., das Sarkar, S., Das, B.K., Praharaj, J.K., Mahajan, D.K., Purokait, B., Mohanty, T.R., Mohanty, D., Gogoi, P., Kumar V, S., Behera, B.K., Manna, R.K., Samanta, S. (2021). Microplastics removal efficiency of drinking water treatment plant with pulse clarifier. Journal of Hazardous Materials, 413, 125347. https://doi.org/10.1016/j.jhazmat.2021.125347.

Schymanski, D., Oßmann, B.E., Benismail, N., Boukerma, K., Dallmann, G., von der Esch, E., Fischer, D., Fischer, F., Gilliland, D., Glas, K., Hofmann, T., Käppler, A., Lacorte, S., Marco, J., Rakwe, M.E., Weisser, J., Witzig, C., Zumbülte, N., Ivleva, N.P. (2021). Analysis of microplastics in drinking water and other clean water samples with micro-Raman and micro-infrared spectroscopy: minimum requirements and best practice guidelines. Analytical and Bioanalytical Chemistry, 413, 5969–5994. https://doi.org/10.1007/s00216-021-03498-y.

Shahi, N.K., Maeng, M., Kim, D., Dockko, S. (2020). Removal behavior of microplastics using alum coagulant and its enhancement using polyamine-coated sand. Process Safety and Environmental Protection, 141, 9-17. https://doi.org/10.1016/j.psep.2020.05.020.

Shen, M., Zeng, Z., Wen, X., Ren, X., Zeng, G., Zhang, Y., Xiao, R. (2021). Presence of microplastics in drinking water from freshwater sources: the investigation in Changsha, China. Environmental Science and Pollution Research, 28(31), 42313-42324. https://doi.org/10.1007/s11356-021-13769-x.

Sol, D., Laca, Amanda, Laca, Adriana, Díaz, M. (2020). Approaching the environmental problem of microplastics: Importance of WWTP treatments. Science of the Total Environment, 740, 140016. https://doi.org/10.1016/j.scitotenv.2020.140016.

Tamargo, A., Molinero, N., Reinosa, J.J., Alcolea-Rodriguez, V., Portela, R., Bañares, M.A., Fernández, J.F., Moreno-Arribas, M.V. (2022). PET microplastics affect human gut microbiota communities during simulated gastrointestinal digestion, first evidence of plausible polymer biodegradation during human digestion. Scientific Reports, 12(1), 1-15. https://doi.org/10.1038/s41598-021-04489-w.

Tanaka, K., Takada, H. (2016). Microplastic fragments and microbeads in digestive tracts of planktivorous fish from urban coastal waters. Scientific Reports, 6(1), 1-8. https://doi.org/10.1038/srep34351.

Tong, H., Jiang, Q., Hu, X., Zhong, X. (2020). Occurrence and identification of microplastics in tap water from China. Chemosphere, 252, 126493. https://doi.org/10.1016/j.chemosphere.2020.126493.

Wang, Z., Lin, T., Chen, W. (2020). Occurrence and removal of microplastics in an advanced drinking water treatment plant (ADWTP). Science of the Total Environment, 700, 134520. https://doi.org/10.1016/j.scitotenv.2019.134520.

Waring, R.H., Harris, R.M., Mitchell, S.C. (2018). Plastic contamination of the food chain: A threat to human health? Maturitas, 115, 64-68. https://doi.org/10.1016/j.maturitas.2018.06.010.

WHO. (2019). Microplastics in Drinking-Water. Available from: https://www.who.int/water_sanitation_health/publications/information-sheet190822.pdf. Last Accessed: 22/03/2022.

Wright, S.L., Kelly, F.J. (2017). Plastic and Human Health: A Micro Issue? Environmental Science and Technology, 51(12), 6634-6647. https://doi.org/10.1021/acs.est.7b00423.

Xu, E.G., Ren, Z.J. (2021). Preventing masks from becoming the next plastic problem. Frontiers of Environmental Science and Engineering, 15(6), 125. https://doi.org/10.1007/s11783-021-1413-7.

Xue, J., Samaei, S.H.-A., Chen, J., Doucet, A., Ng, K.T.W. (2022). What have we known so far about microplastics in drinking water treatment? A timely review. Frontiers of Environmental Science & Engineering, 16(5), 58. https://doi.org/10.1007/s11783-021-1492-5.

Yang, D., Shi, H., Li, L., Li, J., Jabeen, K., Kolandhasamy, P. (2015). Microplastic Pollution in Table Salts from China. Environmental Science and Technology, 49(22), 13622-13627. https://doi.org/10.1021/acs.est.5b03163.

Yu, X., Peng, J., Wang, J., Wang, K., Bao, S. (2016). Occurrence of microplastics in the beach sand of the Chinese inner sea: The Bohai Sea. Environmental Pollution, 214, 722-730. https://doi.org/10.1016/j.envpol.2016.04.080.

Zhao, S., Danley, M., Ward, J.E., Li, D., Mincer, T.J. (2017). An approach for extraction, characterization and quantitation of microplastic in natural marine snow using Raman microscopy. Analytical Methods, 9(9), 1470-1478. https://doi.org/10.1039/c6ay02302a.

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 Research, 112, 93-99. https://doi.org/10.1016/j.watres.2017.01.042.

Ziajahromi, S., Neale, P.A., Telles Silveira, I., Chua, A., Leusch, F.D.L. (2021). An audit of microplastic abundance throughout three Australian wastewater treatment plants. Chemosphere, 263, 128294. https://doi.org/10.1016/j.chemosphere.2020.128294.

Chapter 3

Chapter 3. Evaluating Micro and Macro Plastic Environmental Hazards: An In-Depth Analysis of Organic Waste Amendments

Abstract

Microplastics (MPs) are a growing concern as they are increasingly detected in various environmental compartments. One significant pathway of MPs entry into terrestrial ecosystems is through the application of organic waste as soil amendments. This study aims to investigate the size, morphology, and composition of MPs in different types of treated organic waste commonly used in agricultural soils and soil restoration. A total of seven (n=7) organic amendments, including sewage sludge, horse manure, two composts and two digestates from agri-food industries and selectively separated municipal organic waste, and a biostabilized product from non-selectively separated municipal organic waste, were analyzed. The samples underwent pretreatment steps including advanced Fenton oxidation, alkaline and enzymatic digestion, and density separation, followed by spectroscopic confirmation. The results revealed high plastic concentrations ranging from 7137 to 482943 items/kg of dry weight, depending on its origin and treatment. The most prevalent MPs morphologies were fragments, followed by fibers and films.

Among the particles, the most prevalent polymers observed were PE, PP, PET, PVC, and PS, whereas PES constituted the primary composition of the fibers. The present study underscores the substantial presence of MPs in organic waste amendments and when they are applied to soil can be ingested by terrestrial organisms, entering the food chain and posing risks to human health. Sewage sludge samples exhibited the highest MPs concentrations, while horse manure showed the lowest.

Keywords

Organic waste amendments, soil, microplastics, contaminants, fertilizers

1. Introduction

Microplastics have emerged as a significant contaminant of concern, and their ubiquitous presence in the environment has raised concerns regarding their potential risks to human and environmental health (Lambert and Wagner, 2018). Microplastics (MPs) are plastic particles that are smaller than 5 mm in size (Lusher et al., 2015). There are two main groups of MPs based on their origin: primary and secondary. Primary MPs are intentionally produced as small plastic pellets for various industrial applications, including household products, cosmetics, electronics, transportation, and more (Carrasco Silva et al., 2021). Secondary MPs, on the other hand, are larger plastic items that undergo fragmentation and reduction in size over time due to environmental conditions and exposure (Carrasco Silva et al., 2021).

Microplastics have gained increased attention from the public, water companies, and regulatory bodies. Of particular concern is the role of wastewater treatment plants (WWTPs) as a crucial pathway for microplastic pollutants to enter terrestrial environments (Sun et al., 2019; Karapanagioti and Kalavrouziotis, 2019). These microplastics find their way into the environment through various means, including discharge with final effluent, release from untreated sewage via combined sewer overflows (Woodward et al., 2021), or recycling of sewage sludge (a by-product of wastewater treatment) for agricultural land application. The Spanish regulatory decree AAA/1072/2013, which governs the use of sewage sludge in agriculture, did not initially consider microplastics (MPs) from sewage sludge as a recognized pollutant, as the accumulation of these contaminants in agricultural land was not yet suspected at that time (Sakali et al 2022).

Although MPs have been extensively studied in marine and freshwater ecosystems, there is still a significant knowledge gap when it comes to understanding the characteristics and fate of MPs in agroecosystems (Rashti et al 2023). In agroecosystems, MPs primarily originate from various sources, including irrigation with wastewater, the application of biosolids and compost to enhance soil quality, the presence of residual plastic films used as surface mulch, atmospheric deposition, and the transportation of MPs from landfills through wind and water (Nizzetto et al., 2016; Steinmetz et al., 2016; Rillig et al., 2017a). However, it's important to note that the effects of microplastic contamination on the soil environment are highly dependent on several factors. Soil properties, such as texture, structure, and organic matter content, play a crucial role in how

microplastics interact with the soil. Additionally, the characteristics of the plastic itself, including pigments, dyes, thermal stabilizers, photo-stabilizers, and antioxidants, also influence its behavior and potential impacts in the soil environment. Furthermore, the size and concentration of microplastic particles further contribute to their potential effects (Rashti et al 2023).

Organic waste amendments (OWAs) are materials that are added to soil to improve its physical properties and fertility. They are typically composed of organic matter derived from biomass and/or living beings, such as manure compost, biosolids, municipal solid wastes, wood chips, biochar, animal manure, straw, husk, geotextile, and sewage manure.

These wastes are typically applied to soils that have low levels of organic matter. Characteristically OWAs have high amounts of essential nutrients such as carbon, nitrogen, phosphorous, and potassium and other benefits that are deemed useful to soils. OWAs are an important part of the EU's idea of the circular economy because it takes nutrient-rich wastes and repurposes them into a variety of industrial and agricultural processes and reduces the amount of waste that ends up in landfills. This eventually allows for the soils that are low in organic material to be usable again in agricultural and industrial practices (Dadashi et al., 2019; Oldfield et al.,2018; Picariello et al.,2021).

Recent findings by Sun et al. (2019) indicate that a majority of microplastics, including fragments, accumulate in sewage sludge at concentrations 15 times higher than in effluent. The application of sewage sludge as fertilizer shows significant variation. According to experts, in North America and Europe, approximately 50% of sewage sludge is reused, while in Spain, 65% is reprocessed. (Van den Berg et al., 2020; Roig et al., 2012). The deposition of microplastics on European agricultural soils is estimated to be in the range of 63,000 to 430,000 tons annually, primarily attributed to sludge application (Nizzetto et al., 2016). Studies have shown that agricultural fields with a history of biosolid application exhibit notably higher concentrations of microplastics compared to fields that have not received biosolids (van den Berg et al., 2020). For this reason, soils are recognized as a significant environmental reservoir for microplastics, as highlighted by Hurley et al (2018a).

Microplastics present in agricultural soils pose a significant concern as they have the potential to be ingested by soil organisms, particularly earthworms, which play a vital role as ecosystem engineers (Rilling, 2012). The ingestion of microplastics by earthworms has implications not only for their own well-being but also for the overall functioning of the soil ecosystem. Earthworms contribute to essential soil functions, including nutrient cycling, fertility, and the maintenance of optimal physical, chemical, and biological properties in agricultural lands. Additionally,

earthworms actively interact with plastic particles in the soil, participating in their uptake, breakdown, and distribution throughout the soil profile (Huerta Lwanga et al., 2017a; Rillig et al., 2017b; Kwak and An, 2021). The ingestion of microplastics by earthworms can lead to various adverse effects, including oxidative stress, internal abrasions, obstruction of their digestive systems, and interference with important biological functions such as energy reserves and biomass reduction (Huerta Lwanga et al., 2016; Jiang et al., 2020).

The objective of the present study was to investigate the size, morphology, concentration, and composition of microplastics in seven distinct organic waste amendments commonly utilized in agricultural soils and soil restoration practices and to establish a threshold for potential harm when utilizing sludge for land application, while also maximizing the value of sludge to promote a circular economy. By conducting this research, we aimed to contribute to the understanding of microplastic pollution in agricultural systems and assess the potential risks associated with the application of organic waste amendments. This study provides valuable insights for the development of targeted mitigation strategies and sustainable waste management practices and is the first of its kind in Catalonia, Spain.

2. Materials and methods

2.1. Organic waste amendment samples

In a present study, seven (n=7) differently treated organic waste amendments were analyzed on the presence of MPs: Compost from selectively separated municipal wastes (MW-C), digestate from selectively separated municipal wastes (MW-D), biostabilized waste from non-selectively separated municipal organic wastes (MW-B), digestate from agri-food industry (AF-D), compost from agri-food industry (AF-C), horse manure (HM), and sewage sludge from wastewater treatment plant (WW-SS). In Table 1 is summarized the sample types, origin, components and treatment process.

Table 1 Summary table of the sample types, origins, components, and processes

Organic waste amendment	Origin	Component	Treatment process
Biostabilized waste (BM)	ECOPARC 2 (in Monstcada I Reixac)	municipal wastes	mechanical and biological processes
Municipal Compost (MC)	ECOPARC 2 (in Monstcada I Reixac)	municipal wastes	mechanical and biological processes
Agri-food Compost (AC)	Fumanya	Majority raw materials (90% of the total): Expired or unsuitable materials for consumption; Sludge agri-food industry (meat slaughterhouses); Sludge treatment plant in the agri-food industry (without meat); Animal tissue remains; Manure; Bin sludge; Pruning and gardening remains; Others (10%)	Piled with gore-tex closure, with probe on top (42 days), 3 m piles; 2-3 months ripening on piles flipped with shovel (weekly), 3 m piles
Digestate (DW)	ECOPARC 4 (in Hostalets de Pierola)	municipal wastes	mechanical and biological processes
Agri-food Digestate (ADW)	Apergas	Majority raw materials: Slurry and cow manure (50%); Sludge agri-food industry (45%); Others (5%)	Anaerobic digestion for 40 days. At 36- 38ºC
Horse manure (HM)	Fumanya	horse manure	n/a
Sewer Sludge (SS)	WWTP. El Prat del Llobergat	sewage sludge	n/a

2.2. Analysis of the samples in the laboratory

Chemicals used for the pretreatment of the samples included: absolute ethanol (Scharlau, >99.9%), iron (II) sulfate heptahydrate (Sigma-Aldrich, >99.0%), hydrogen peroxide (PamReac AppliChem ITW Reagents, 30%), PTFE-filter (Sartorius, 5 µm pore size), zinc chloride (Acros Organics, >98.0%), nitric acid (PamReac AppliChem ITW Reagents, >69.0%), potassium hydroxide (Scharlau, >90.0%), and cellulase TXL (ASA Spezialenzyme GmbH).

Each sample, weighing 100 g, was separated into 50 g duplicates and subsequently mixed with ultrapure 95water. The wet sieving process was then conducted using stainless steel sieves with varying mesh sizes: 0.02, 0.045, 0.1 mm. However, for larger size fractions: 0.5, 1, 2, and 5 mm, 95water mixing was omitted, and instead, dry sieving was conducted.

The fraction of samples larger than 0.5 mm was analyzed through visual inspection using a binocular microscope (32x magnification). The suspected plastic particles (thermoplastics) are

tested for their reaction to heat (550ºC) and checked if they float in a solution of $ZnCl_2$ (density of 1.8 g/mL). Subsequently, the isolated plastics are analyzed using infrared spectroscopy with a Perkin Elmer Frontier instrument equipped with an ATR (Attenuated Total Reflectance) accessory, using Spectrum software for 16 scans within a working range of 400-4000 cm^{-1} and a spectral resolution of 4 cm^{-1}.

The fractions of samples smaller than 0.5 mm undergo different treatments to digest the organic matter in the sample. This includes Fenton oxidation, alkaline hydrolysis, and enzymatic treatment with cellulase adapting a methodology for sewage sludge by Dronjak et al. (2023). Furthermore, these treatment methods are widely employed in microplastics research by other studies (Dronjak et al., 2022; Exposito et al., 2021; Exposito et al., 2022). Subsequently, the microplastics are separated by density through successive extractions using salt solutions of NaCl (density 1.2 g/mL) and $ZnCl_2$ (density 1.8 g/mL). Detailed pre-treatment protocol is explained bellow.

After the wet sieving of the samples, each size fraction was taken to an oven and allowed to dry for a week at 50°C. After the drying is completed the weight of the dried OA was taken. For the further analysis a subsample was taken due to high MPs abundance among the samples. In order to remove organic matter from the samples, the Fenton process was applied (33% H_2O_2 and Fe (II) SO4 (0.02M)). The mixture is covered and sonicated at 40°C for 10 minutes and left for 24 hours. After the 24-hour time period, the samples were taken and filtered through 10 µm PTFE filters. The process continues with enzymatic digestion. Samples were treated with cellulase at optimal pH 5 (buffer solution: CH_3COONa and CH_3COOH). The mixture was then covered and placed into a hot water bath at 40°C at 80 RPM. The mixture was left in the hot water bath for a week. After the samples were processed, they were filtered again through 10 µm PTFE filter. Additional oxidation was performed using 33% hydrogen peroxide solution and it was allowed to process for 24 hours. After 24 hours the samples were removed and once again the samples were washed using ultrapure water and filtered. In order to remove inorganic matter, two subsequently density separation was applied, with sodium chloride (density 1.2 g/mL), allowing the light weight MPs to be extracted, and zinc chloride (density 1.8 g/mL), allowing the rest of the MPs to be separated. Extraction was applied three times for each solution, to ensure that all the MPs were removed. For the very dirty samples, additional step was applied with alkaline solution. The samples were treated with potassium hydroxide, sonicated at 40°C and left for 24 h. After that, samples were ready for visual inspection. The isolated microplastics are then observed under a LEICA MZ10 stereoscopic microscope (80x magnification) equipped with a

Flexcam C1 Olympus CX41 camera with a resolution of 1 µm. Fragments, films, and fibers are distinguished in the observations.

The isolated microplastics are fixed on a CaF_2 slide and analyzed using infrared spectroscopy (IR) through mapping with a Thermo Scientific Nicolet IM10 microscope. The 97pixel size is set at 25x25 µm, and the analysis includes 4 scans within a working range of 715-4000 cm^{-1} and a spectral resolution of 4 cm^{-1}.

The spectra are processed using the OMNICTM Spectra software and compared with various commercial libraries such as the Nicolet Sampler Library, Hummel Polymer Sample Library, Polymer Laminate Films, and KnowItAll-Willey, as well as a library developed by the research group itself. Plastics polymers are considered when they show a match of over 70% with the reference library. Additionally, for each spectrum, the presence of characteristic bands identifying the main functional groups of the identified polymers is verified.

2.3. Quality control

In a present study, a rigorous approach was adopted to ensure accurate results and to avoid cross contamination. Prior to use, all instruments and materials underwent a cleaning process using 70% ethanol and ultrapure water filtered through a nitrocellulose filter with a pore size of 0.45 µm (GF/F Whatman). Reagents were prepared exclusively with analytical grade chemicals and filtered ultrapure water. Throughout the sampling and analysis in the laboratory, researchers wore cotton lab coats, and all sample pre-treatment procedures were strictly performed within extraction hoods.

Analytical method recovery was assessed by spiking the samples with fluorescent polyethylene microspheres of different sizes and colors from a trusted source (Cospheric Inc., California, USA), previously done in study by Dronjak et al. (2023). The sludge sample was spiked with fluorescent polyethylene (PE) microspheres from Cospheric Innovations, USA and the results showed that the average triplicate recovery rates for different sizes of spiked MPs were 75% for 53-63 µm, 74% for 125-150 µm, 70% for 250-300 µm, and 100% for 450-500 µm diameter microspheres, indicating acceptable laboratory performance in detecting and quantifying microplastics in the sludge samples.

3. Results and discussion

3.1. Occurrence of micro and macro plastics in organic waste amendments

The study presents the quantitative analysis of micro and macro plastic particles within various organic waste amendments. Two replicates were conducted for each sample and statistical measures of variability were incorporated to enhance accuracy. It should be noted that certain samples exhibit high standard deviations and coefficients of variation, indicating the complexity associated with the heterogeneous distribution of MPs. The units are expressed as the number of plastic items per kilogram of dry sample.

The analysis of 98plastic pollution in organic98 waste amendments is crucial for understanding the extent of contamination and potential environmental implications. In this study, we investigated the occurrence of micro and macro plastics9898 in seven (n=7) 98differently treated organic98 waste amendments, including compost (MW-C), and digestate (MW-D) from municipal waste, biostabilized organic98 waste amendment from municipal waste (MW-B), digestate (AF-D) and compost (AF-C) from the 98agri-food industry, 98horse manure 98organic waste amendment (HM), and sewage sludge from a wastewater treatment plant (WW-SW).

The organic98 waste amendment samples were analyzed for both microplastics and macro plastics. Microplastics were defined within the size range of 0.02 mm to 5 mm, while macro plastics referred to particles from 5 mm extending up to a few 98centimeters in size. During the analysis, only a few macro plastics were discovered, and their total concentration accounted for less than 1% when compared to the smaller size fraction. However, it is important to note that even though the concentration of macro plastics is low compared to microplastics when considering the number of particles, the mass of the macro plastics is, in fact, higher than that of microplastics which could have implications in terms of potential harm and the capacity of macro plastics to act as carriers for other chemicals and pollutants on their 98surface98. Therefore, both microplastics and macro plastics need to be thoroughly evaluated to comprehensively understand the potential risks they pose to the 98environment and living organisms.

Our results revealed varying levels of 98micro and macro plastic98 contamination among the different9898 98organic waste amendments. Sewage sludge from the wastewater treatment plant (WW-SS) showed the highest concentration of plastic98 pollution with a remarkable 551,695 items/kg of dry weight (d.w.). It was closely followed by digestate samples from municipal wastes (MW-D) at 372,119 items/kg d.w. and 98agri-food waste digestate (AF-D) at

254,073 items/kg d.w. Municipal waste compost (MW-C) and 99agri-food compost (AF-C) displayed similar results, both with concentrations around 148,389 and 141,701 items/kg d.w., respectively. Biostabilized waste from municipal sources (MW-B) exhibited lower 99plastic99 concentrations at 122,845 items/kg d.w. Finally, 99horse manure (HM) had the lowest concentration among the samples, with 6,774 items/kg d.w. (Table 2).

Table 2. Occurrence of micro and macro plastics in organic99 waste amendments, 99standard deviation, and 99coefficient of variation

Organic waste amendment	*Abbreviation*	*Concentration [Items/kg d.w.]*			*SD [Item/kg d.w.]*	*CV [%]*
		Replica 1	*Replica 2*	*Mean*		
Compost from Municipal Waste	MW-C	130093	166686	148389	25875	17
Digestate from Municipal Waste	MW-D	472326	271913	372119	141713	38
Biostabilized from Municipal Waste	MW-B	99073	146617	122845	33619	27
Digestate from Agri-food Industry	AF-D	159821	348325	254073	133293	52
Compost from Agri-food Industry	AF-C	100286	183116	141701	58570	41
Horse Manure	HM	7137	6410	6774	514	8
Sewage Sludge from Wastewater Treatment Plant	WW-SS	482943	620448	551695	97231	18

The 99coefficient of variation (CV) was used to assess the variability in 99plastic concentrations among the samples. HM (8%), MW-C (17%), and WW-SS (18%) samples exhibited relatively low to moderate variability, indicating relatively consistent plastic99 99distribution. MW-D and MW-B samples had moderate CVs (38% and 27% respectively), suggesting some variability in 99plastic concentrations, due to the heterogeneous 99distribution of such particles. AF-C and AF-D had the highest CVs (41% and 52% respectively), indicating significant variability of pollutant. The importance of calculating CV is emphasized as it provides insights into 99plastic99 variability, informing waste management strategies and risk assessments. Despite variations, the study underscores the presence of micro and macro plastics in all samples, highlighting the need for effective mitigation strategies and continued monitoring of 99plastic99 contamination in 99organic waste amendments.

In the northeastern region of Spain, Edo et al. (2022) conducted a study investigating 100plastic100 debris occurrence in refined compost from five facilities processing the Composted Organic Fraction of Municipal Solid Waste (OFMSW). The selected facilities represented 100different collection 100systems, impurity levels, and 100technologies. Their results revealed an overall concentration of 100plastic particles in the compost ranging from 10,000 to 30,000 items/kg d.w. Moreover, the concentration of microplastics in the compost varied from 5,000 to 20,000 items/kg d.w. These findings indicate considerably lower concentrations of MPs compared to both types of compost from our current study.

In a separate study conducted in the southwest of Spain, the occurrence of MPs (larger than 100 µm) in sludge samples from four urban wastewater treatment plants (WWTPs) representing conventional treatment technologies was investigated. The MP concentrations in sludge samples ranged from 96,000 to 769,000 MPs/kg d.w. Based on the total sewage sludge production in Spain, and the fact that it is used for agricultural purposes, the average amount of MPs derived from sewage sludge that could enter the soil was estimated to be 2.80×10^{14} MPs/year (Sakali et al 2022).

In another recent study by Rashti et al. (2023), high concentrations of MPs were reported in three biosolid samples collected from major WWTPs in Queensland, Australia. The MP concentrations in these samples ranged from 55,400 to 73,800 MPs/kg d.w.

A comprehensive review study by Porterfield et al. (2023) examined microplastics in composts, digestates, and food wastes. They found that the abundance of 100microplastics in composts and digestates varied widely across 100the100100 studies. Plastic levels in composts ranged from 12 (Braun et al., 2021) to 82,800 items/kg (Huerta-Lwanga et al., 2021), while in digestates, levels ranged from 70 to 1670 MPs/kg (O'Brien, 2019; Weithmann et al., 2018), with one study reporting 3.87×10^{7} items/kg (Meixner et al., 2020). The variability observed was significant, spanning several orders of magnitude, highlighting the inconsistent and diverse presence of 100microplastics100 in these organic materials. Furthermore, other factors may contribute to this variability, including the diversity in sampling locations, the methodologies used for sampling and analysis, and the reporting styles adopted by the researchers.

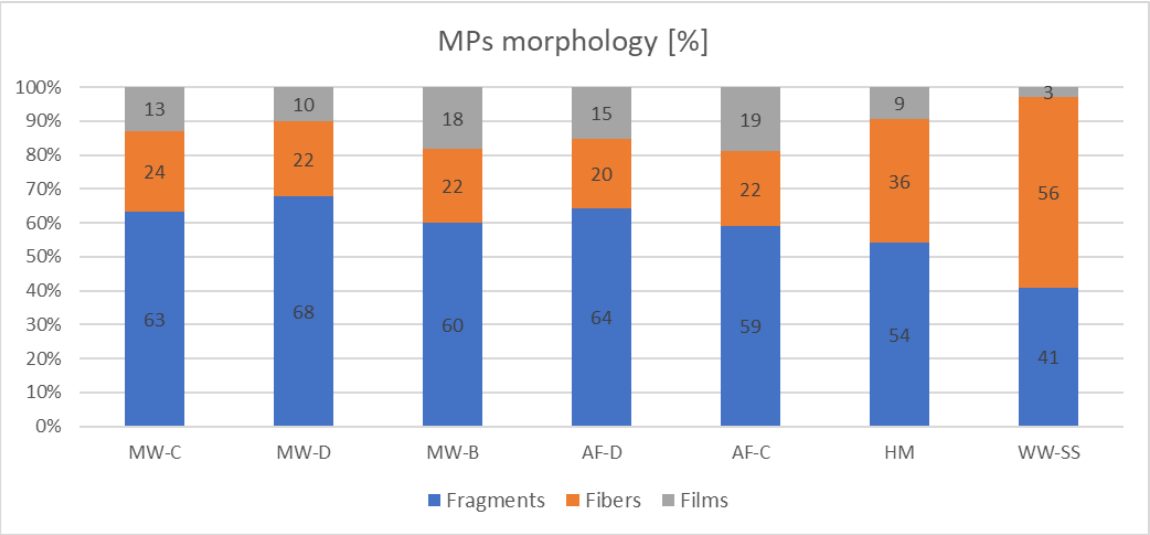


Figure 1. Morphology of microplastics in 101different organic101 waste amendment samples

When examining the morphology of microplastics in the 101organic101 waste amendments as shown in Figure 1, it was observed that fragmented MPs were predominant across most samples, except for sewage sludge (WW-SS). Notably, WW-SS demonstrated a different101101 composition, mostly comprised of 101fibers (56%), followed by fragments (41%), and films (3%). In contrast, the other six types of OWAs consistently exhibited a high presence of 101microplastic101 fragments, from 54% to 68%. Fibers represented the next prominent category, constituting 20% to 56% of the composition, while films exhibited a range of 3% to 19% of the total.

The findings from our sewage sludge analysis align with our previous study, revealing that f101ibers 101are101 the most prevalent morphology in the sludge, followed by fragments and films. These synthetic f101ibers are commonly released during washing processes, and washing 101machines101101 are recognized as significant contributors to the release of microplastics (MPs) into the 101environment. Browne et al. (2011) estimated that washing a single piece of clothing could result in the discharge of over 1900 fibers into the sewage sludge. Lares et al. (2018) also who found synthetic fibers to be the most abundant MPs type in sludge samples collected with an abundance of 94% in the activated sludge samples.

In another investigation carried out by Harley-Nyang et al. (2022), on microplastics in sewage sludge and biosolids, it was found that the overall number of particles exceeded the number of 101fibers. Among the total confirmed microplastic count, approximately 42.5% were identified as 101fibers, while the remaining 57.5% were classified as particles.

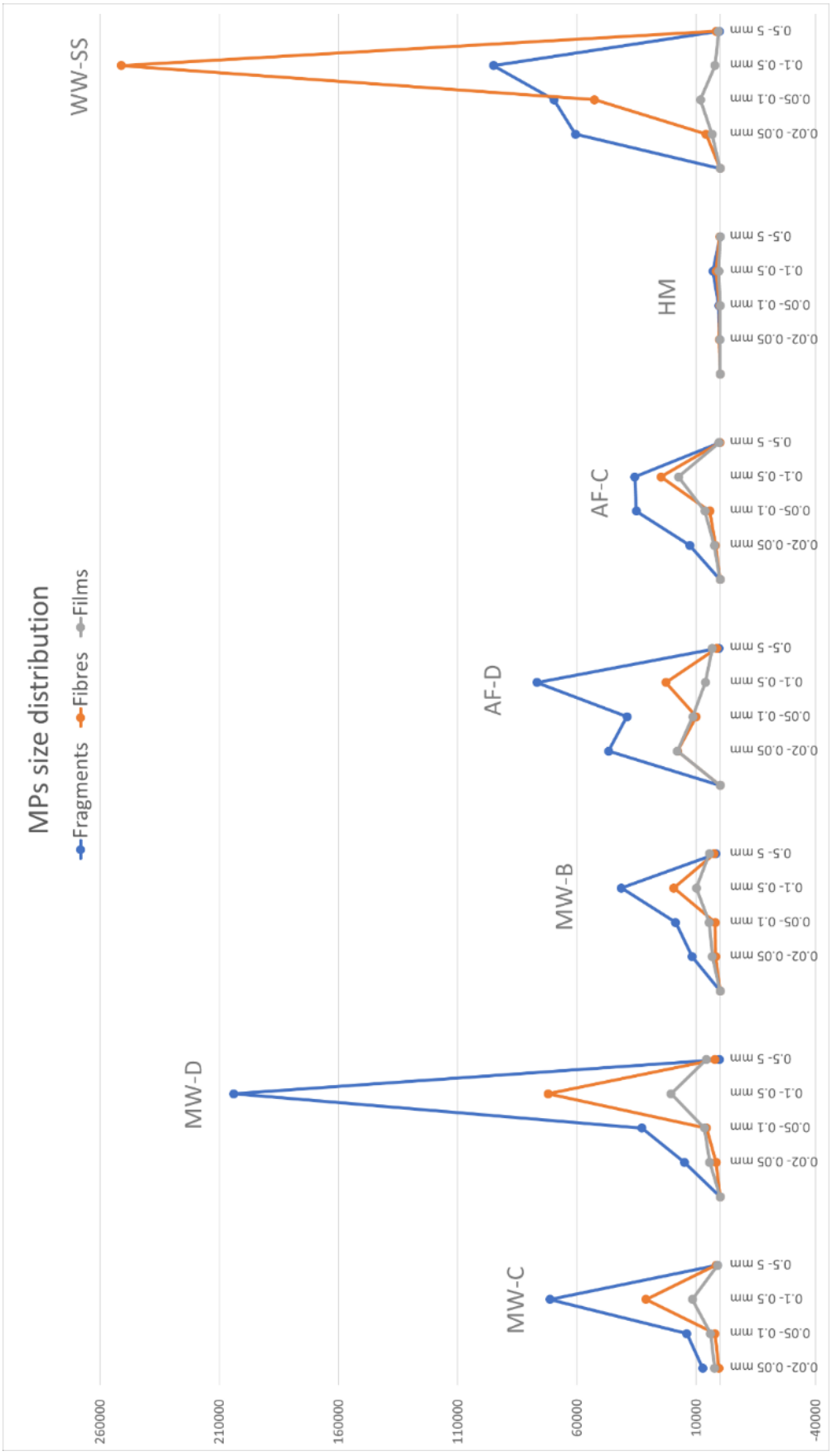


Figure 2. Size 102distribution of microplastics in 102organic waste amendments

The microplastic particles were categorized into four distinct size ranges: 0.02-0.05 mm, 0.05-0.1 mm, 0.1-0.5 mm, and 0.5-5 mm. The results indicate that the smaller size fractions, particularly those below 0.5 mm, were dominant across all 103organic103 waste amendment samples. Notably, the size range of 0.1-0.5 mm consistently displayed the highest concentration of microplastics among all the samples. Combining the findings, Harley-Nyang et al. (2022) found that a significant portion of microplastics, across 103different103103 locations, fell within the 0.1 to 0.5 mm range, while exploring sizes from 0.05 to 5 mm. In parallel, Sakali et al. (2022) reported a comparable predominant size, 103observing it to be within the 0.1 to 0.335 mm range for the particles. Both studies focused on the analysis of sludge samples obtained from WWTPs, with different103103 treatment processes.

3.2.Composition of microplastics in organic waste amendments

The chemical composition of the suspected MPs was determined using a microFTIR spectroscopic mapping technique. Subsamples of each OAs were extracted and placed on calcium fluoride slides, taking into account variations in size ranges and morphologies to ensure the most representative subsample was prepared (Figure 5). The polymer composition was then categorized into two distinct groups: MPs particles, including films and fragments, and 103fibers.

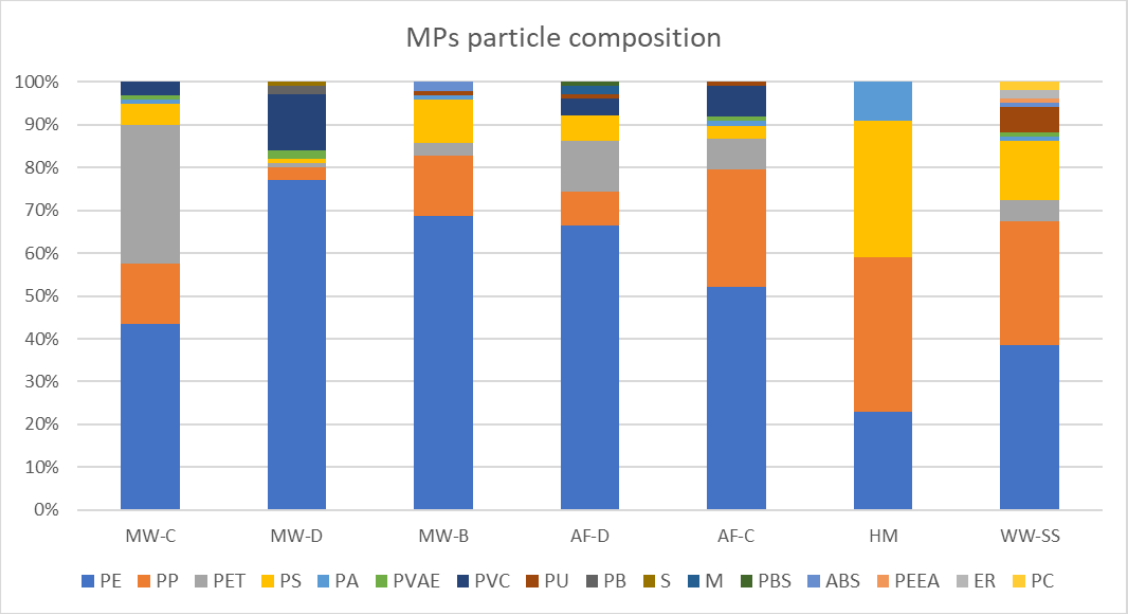


Figure 3. Microplastics composition including fragments and films

Polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS), polyamide (PA), polyvinyl acetate ethylene (PVAE), polyvinyl chloride (PVC), polyurethane (PU), polybutene (PB), silicone (S), melamine (M), polybutene-styrene (PBS), acrylonitrile butadiene styrene (ABS), polyethylene ethyl acrylate (PEEA), epoxy resin (ER), polycarbonate (PC)

A total of 16 polymers were identified in the composition of fragments and films (Figure 3). Among the samples, polyethylene (PE) was the most prevalent, ranging from 23% to 77%, followed by polypropylene (PP) (3% to 36%), polystyrene (PS) (1-32%), polyethylene terephthalate (PET) (0% to 32%), and polyvinyl chloride (PVC) (0% to 13%). Additionally, various other polymers, such as polyamide (PA), polyvinyl acetate ethylene (PVAE), polyurethane (PU), polybutene (PB), silicone (S), melamine (M), polybutene-styrene (PBS), acrylonitrile butadiene styrene (ABS), polyethylene ethyl acrylate (PEEA), epoxy resin (ER), and polycarbonate (PC), were found in concentrations less than 10% among the samples.

These findings align well with global statistics on the production of plastics. The most prevalent plastics worldwide are PE (36%), PP (21%), and PVC (12%), as reported by Geyer et al. (2017). In a recent review study by Porterfield and et (2023) who investigated microplastics in composts, digestates, and food wastes reported that the most frequently identified polymers included PE, PP, and PS. These polymers are also the most common plastics used in food packaging (Ncube et al., 2020).

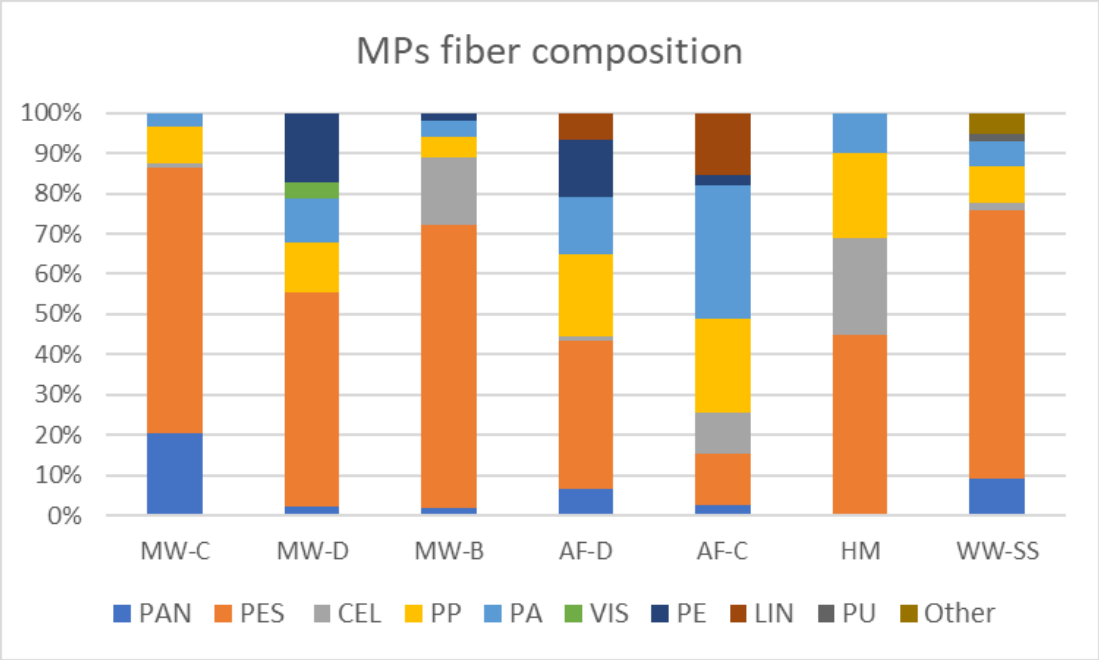


Figure 4. Microplastics composition including 104fibers

Polyacrylonitrile (PAN), polyester (PES), synthetic cellulose (CEL), polypropylene (PP), viscose (VIS), polyethylene (PE), linen (LIN), and polyurethane (PU).

The analysis of fiber composition revealed that a total of 8 polymers were detected, with one of them being a natural origin (Figure 4). Polyester (PES) emerged as the most abundant polymer,

ranging from 15% to 71% among the samples. Following this, polyamide (PA) constituted 3% to 39%, polypropylene (PP) (5-27%), and synthetic cellulose (CEL) (0-24%), polyacrylonitrile (PAN) (0-21%), linen (LIN) (0-18), and polyethylene (PE) (0-17%). In contrast, polyurethane (PU) and viscose (VIS) exhibited concentrations lower than 10% among the samples.

Figure 5. Micro-FTIR maps for polymer identification (1 cm x 1 cm square)

3.3.Environmental risk assessment

Given the potential concern of a substantial presence of MPs in the organic amendments, it becomes imperative to conduct a risk assessment to identify and implement best practices for risk mitigation. To accomplish this, the nitrogen level guideline will serve as the benchmark to ascertain the maximum allowable annual application of each OA to agricultural soils. The EU standard (Directive 96/676) for the amount of nitrogen that can be applied to one hectare of land is 170 kg of N per year.

To determine the maximum allowable dose for each organic amendment applied to agricultural soils, a predicted no-effect concentration (PNEC) mean was obtained from literature as 82,000 particles/kg (Tunali et al., 2023) in soil. However, other authors (Qiu et al., 2022) estimated a Hazardous Concentration of 5% (HC5) at 224 MP/kg. The HC5 value is used to derive the PNEC by dividing it with assessment factors ranging from 1 to 5. Consequently, the PNEC according to these authors appears significantly lower than the value obtained by Tunali et al. (2023).

The following formula was then used to evaluate the risk characterization ratios (RCRs) for OAs with the measured environmental concentration distribution (PEC):

$$RCR = PEC/PNEC$$

In order to calculate the RCR, it is necessary to estimate the Predicted Environmental Concentration of each OA. To calculate the RCR, relevant data such as nitrogen content, nitrogen content per ton of OA, maximum OA tonnage permissible for the area, soil density (1.4 g/cm3), and depth (0.2m) were required. These parameters are listed in Table 3, providing valuable insights for assessing the potential environmental impact of OAs.

Table 3 Risk Assessment Parameters for Organic Amendments including total nitrogen produced, nitrogen content per ton of OA, the maximum tonnage of OA permissible for a specific area, microplastic (MP) content per kilogram, the total quantity of MPs added to the area in a given year, and the resulting soil concentration.

Organic Amendment	OA Total N (%) dw	OA kg N/t	Max t of OA added	OA MPs/kg	MP added to soil per year	C soil (MP/kg*year)
MW-B	2.09	20.9	8.4	113030	9.5.E+08	338
MW-C	2.36	23.6	7.4	144487	1.1.E+09	383
AF-C	3.11	31.1	5.6	140901	7.9.E+08	283
MW-D	3.89	38.9	4.5	362785	1.6.E+09	583
AF-D	3.10	31	5.6	248622	1.4.E+09	501
HM	0.55	5.5	31.8	6544	2.1.E+08	74
WW-SS	5.21	52.1	3.4	549042	1.8.E+09	659

Table 3 presents essential parameters to assess the potential environmental impact of applying Organic Amendments (OAs) to agricultural soils. It includes factors like total nitrogen content, microplastic quantities, permissible OA tonnage, and resulting soil concentrations. These parameters aid in making informed decisions for sustainable agricultural practices.

Based on the data presented in Table 1, the maximum total dry weight percentage of nitrogen produced through OAs would be 5.21%, specifically for the most heavily polluted OA, WW-SS.

This corresponds to 52.9 kg of nitrogen per ton of WW-SS. Considering this value, the maximum amount of WW-SS that could be applied to a specific plot of land would lead to the introduction of approximately 1.8 billion microplastics to the area, resulting in a soil concentration of 659 MPs/kg*year.

Table 4 Calculations of Risk Characterization Ratios (RCRs) based on literature data and predictions indicating the number of years for the safe application of Organic Amendments (OAs).

Organic Amendment	RCR (Qiu et al., 2022)	Number years safe application	RCR (Tunali et al., 2023)	Number years safe application
BW	1.51	0.7	0.004	242.6
MC	1.71	0.6	0.005	214.3
AC	1.26	0.8	0.003	289.6
DW	2.60	0.4	0.007	140.7
ADW	2.24	0.4	0.006	163.6
HM	0.33	3.0	0.001	1102.7
SS	2.94	0.3	0.008	124.5

In the Table 4, the RCR for the highest group, WW-SS is 0.008, based on this value the MP OA could be applied with limited impact for 124.5 years. This predicted value is based on the implementation of a mean PNEC that excluded the highest observed no-effect concentration (HONEC) and took into consideration the MP size range of 10-4000 µm (Tunali et al., 2023). When the HONEC was taken into consideration, the mean PNEC dropped to 3,300. The HONEC is an important value to look at or take into consideration due to the fact that it provides information for assessing the safe level of exposure. Furthermore, the study revealed that MP shape significantly influences mass-based toxicity, with fibers and films exhibiting higher toxicity compared to spheres and fragments. PVC was identified as the MP type with the highest potential to carry the most toxicity.

On the other hand, taking into consideration estimations based on the Qiu et al. (2022) study, the highest RCR would be 2.94 and would only allow for safe application for 0.3 years of application. Qiu et al. (2022) reviewed 38 studies on the impact of MPs on soil physicochemical properties and biota. Higher species sensitivity was observed in invertebrates, particularly nematodes, which experienced oxidative stress and gene expression issues due to MP exposure.

The Risk Characterization Ratios (RCR) presented by Qiu et al. (2022) and Tunali et al. (2023) showed considerable variation in the number of years that each OA could be safely applied. However, it's essential to note that this variation is primarily attributed to the overall lack of

research in this field. As more studies are conducted in the future, greater consistency can be expected, allowing for more robust guidelines to be established. In the meantime, the current findings can serve as valuable reference points and preliminary guidelines for assessing the safe application of OAs.

4. Conclusion

Organic waste amendments play a significant role in introducing microplastics to terrestrial environments. Our study examined seven differently treated organic waste amendments and found varying concentrations of micro and macro plastic particles, ranging from 6,774 items/kg d.w. to 551,695 items/kg d.w. While the number of macro plastic particles among the samples was not significantly high compared to micro plastics, the presence of macro plastics demands serious attention due to their larger masses and greater surface area, allowing them to absorb various pollutants.

The most prevalent morphology observed was fragments, followed by fibers and film. Spectroscopic analysis revealed that the dominant polymers in the fragments and films were PE, PP, PS, PET, and PVC, while fibers were predominantly composed of PES.

To address the high microplastics pollution associated with organic waste amendments, several measures can be taken. Source separation and pre-treatment can help reduce microplastic contamination in waste streams. Enhanced sorting, awareness campaigns, and education can promote responsible disposal practices. Research, monitoring, and stringent regulations are essential for understanding and controlling microplastics pollution. Exploring innovative technologies and promoting sustainable plastic use and recycling are crucial steps. Collaboration among stakeholders is vital for developing comprehensive solutions to mitigate microplastics pollution in organic waste amendments. Implementing these strategies will contribute to a healthier environment and more sustainable waste management practices.

Literature

Assured Biosolids Limitied. Assured Biosolids Limited (ABL) Biosolids Assurance Scheme (BAS) Position Statement on the potential impact of microplastics on biosolids recycling to agricultural land. Available at: https://assuredbiosolids.co.uk/wp-content/uploads/2018/07/M icroplastics-Position-Statement-27th-July-2018.pdf, 2018.

Braun, M., Mail, M., Heyse, R., & Amelung, W. (2021). Plastic in compost: prevalence and potential input into agricultural and horticultural soils. Science of The Total Environment, 760, 143335.

Browne, M. A., Crump, P., Niven, S. J., Teuten, E., Tonkin, A., Galloway, T., & Thompson, R. (2011). Accumulation of microplastic on shorelines woldwide: sources and sinks. Environmental science & technology, 45(21), 9175-9179.

Carrasco Silva, G., Galleguillos Madrid, F. M., Hernández, D., Pincheira, G., Peralta, A. K., Urrestarazu Gavilán, M., ... & Fuentes Peñailillo, F. (2021). Microplastics and Their Effect in Horticultural Crops: Food Safety and Plant Stress.

Dadashi, S., Sepanlou, M. G., & Mirnia, S. K. (2019). Influence organic compost compounds on soil chemical and physical properties. International Journal of Human Capital in Urban Management, 4(1).

Dronjak, L., Exposito, N., Rovira, J., Florencio, K., Emiliano, P., Corzo, B., ... & Sierra, J. (2022). Screening of microplastics in water and sludge lines of a drinking water treatment plant in Catalonia, Spain. Water Research, 225, 119185.

Dronjak, L., Exposito, N., Sierra, J., Schuhmacher, M., Florencio, K., Corzo, B., & Rovira, J. (2023). Tracing the fate of microplastic in wastewater treatment plant: A multi-stage analysis of treatment units and sludge. Environmental Pollution, 122072.

Edo, C., Fernández-Piñas, F., & Rosal, R. (2022). Microplastics identification and quantification in the composted organic fraction of municipal solid waste. Science of The Total Environment, 813, 151902.

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.

Expósito, N., Rovira, J., Sierra, J., Gimenez, G., Domingo, J. L., & Schuhmacher, M. (2022). Levels of microplastics and their characteristics in molluscs from North-West Mediterranean Sea: Human intake. Marine Pollution Bulletin, 181, 113843.

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

Harley-Nyang, D., Memon, F. A., Jones, N., & Galloway, T. (2022). Investigation and analysis of microplastics in sewage sludge and biosolids: A case study from one wastewater treatment works in the UK. Science of the Total Environment, 823, 153735.

Huerta Lwanga, E., Gertsen, H., Gooren, H., Peters, P., Salánki, T., Van Der Ploeg, M., ... & Geissen, V. (2016). Microplastics in the terrestrial ecosystem: implications for Lumbricus terrestris (Oligochaeta, Lumbricidae). Environmental science & technology, 50(5), 2685-2691.

Huerta Lwanga, E., Mendoza Vega, J., Ku Quej, V., Chi, J. D. L. A., Sanchez del Cid, L., Chi, C., ... & Geissen, V. (2017). Field evidence for transfer of plastic debris along a terrestrial food chain. Scientific reports, 7(1), 14071.

Jiang, X., Chang, Y., Zhang, T., Qiao, Y., Klobučar, G., & Li, M. (2020). Toxicological effects of polystyrene microplastics on earthworm (Eisenia fetida). Environmental Pollution, 259, 113896.

Karapanagioti, H. K., & Kalavrouziotis, I. K. (Eds.). (2019). Microplastics in water and wastewater. Iwa Publishing.

Kwak, J. I., & An, Y. J. (2021). Microplastic digestion generates fragmented nanoplastics in soils and damages earthworm spermatogenesis and coelomocyte viability. Journal of Hazardous Materials, 402, 124034.

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

Lusher, A. (2015). Microplastics in the marine environment: distribution, interactions and effects. Marine anthropogenic litter, 245-307.

Lusher, A. L., Hurley, R., Vogelsang, C., Nizzetto, L., & Olsen, M. (2017). Mapping microplastics in sludge.

Lwanga, E. H., Gertsen, H., Gooren, H., Peters, P., Salánki, T., van der Ploeg, M., ... & Geissen, V. (2017). Incorporation of microplastics from litter into burrows of Lumbricus terrestris. Environmental Pollution, 220, 523-531.

Meixner, K., Kubiczek, M., & Fritz, I. (2020). Microplastic in soil–current status in Europe with special focus on method tests with Austrian samples. AIMS Environmental Science, 7(2).

Ncube, L. K., Ude, A. U., Ogunmuyiwa, E. N., Zulkifli, R., & Beas, I. N. (2020). Environmental impact of food packaging materials: A review of contemporary development from conventional plastics to polylactic acid based materials. Materials, 13(21), 4994.

Nicholson, F. A. (2018). Biosolids to Market: A Strategic Proposal to Explore the Threats to Biosolids to Land: Now and in the Future. UK Water Industry Research Limited..

Nizzetto, L., Futter, M., & Langaas, S. (2016). Are agricultural soils dumps for microplastics of urban origin?.

O'Brien, B. J. (2019). Physicochemical properties of residuals from anaerobic digestion of dairy manure and food waste: Nutrient cycling implications and opportunities for edible mushroom cultivation. The University of Vermont and State Agricultural College.

Oldfield, E. E., Wood, S. A., & Bradford, M. A. (2018). Direct effects of soil organic matter on productivity mirror those observed with organic amendments. Plant and soil, 423, 363-373.

Picariello, E., Pucci, L., Carotenuto, M., Libralato, G., Lofrano, G., & Baldantoni, D. (2020). Compost and sewage sludge for the improvement of soil chemical and biological quality of Mediterranean agroecosystems. Sustainability 13: 26.

Porterfield, K. K., Hobson, S. A., Neher, D. A., Niles, M. T., & Roy, E. D. (2023). Microplastics in composts, digestates, and food wastes: A review. Journal of Environmental Quality, 52(2), 225-240.

Qiu, Y., Zhou, S., Zhang, C., Zhou, Y., & Qin, W. (2022). Soil microplastic characteristics and the effects on soil properties and biota: A systematic review and meta-analysis. Environmental Pollution, 120183.

Rashti, M. R., Hintz, J., Esfandbod, M., Bahadori, M., Lan, Z., & Chen, C. (2023). Detecting microplastics in organic-rich materials and their potential risks to earthworms in agroecosystems. Waste Management, 166, 96-103.

Rillig, M. C. (2012). Microplastic in terrestrial ecosystems and the soil?.

Rillig, M. C., Ingraffia, R., & de Souza Machado, A. A. (2017). Microplastic incorporation into soil in agroecosystems. Frontiers in plant science, 8, 1805.

Rillig, M. C., Ziersch, L., & Hempel, S. (2017). Microplastic transport in soil by earthworms. Scientific reports, 7(1), 1362.

Roig, N., Sierra, J., Martí, E., Nadal, M., Schuhmacher, M., & Domingo, J. L. (2012). Long-term amendment of Spanish soils with sewage sludge: Effects on soil functioning. Agriculture, ecosystems & environment, 158, 41-48.

Sakali, A., Coello, D., Brigui, J., Albendín, G., Arellano, J., Quiroga, J. M., & Rodríguez-Barroso, R. (2022). Annual estimates of microplastics in municipal sludge treatment plants in southern Spain. Journal of Water Process Engineering, 49, 102956.

Steinmetz, Z., Wollmann, C., Schaefer, M., Buchmann, C., David, J., Tröger, J., ... & Schaumann, G. E. (2016). Plastic mulching in agriculture. Trading short-term agronomic benefits for long-term soil degradation?. Science of the total environment, 550, 690-705.

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

Tunali, M., Adam, V., & Nowack, B. (2023). Probabilistic environmental risk assessment of microplastics in soils. Geoderma, 430, 116315

UK Water Industry Research, 2019. Sink to river - river to tap: review of potential risks from microplastics.[Online] Available at: https://ukwir.org/sink-to-river-river-to-tap-reviewof-potential-risks-from-microplastics.

Van den Berg, P., Huerta-Lwanga, E., Corradini, F., & Geissen, V. (2020). Sewage sludge application as a vehicle for microplastics in eastern Spanish agricultural soils. Environmental Pollution, 261, 114198.

Wagner, M., & Lambert, S. (2018). Freshwater microplastics: emerging environmental contaminants? (p. 303). Springer Nature.

Weithmann, N., Möller, J. N., Löder, M. G., Piehl, S., Laforsch, C., & Freitag, R. (2018). Organic fertilizer as a vehicle for the entry of microplastic into the environment. Science advances, 4(4), eaap8060.

Woodward, J., Li, J., Rothwell, J., & Hurley, R. (2021). Acute riverine microplastic contamination due to avoidable releases of untreated wastewater. Nature Sustainability, 4(9), 793-802.

Chapter 4

Chapter 4. Spatial and Temporal Trends of Microplastics Accumulation in the Sediments of the San Francisco Bay Area

Abstract

San Francisco Bay's sediment is currently monitored for a variety of contaminants; however, data regarding microplastics (MPs) are still scarce. MPs occurrence in sediment samples has gained recognition as a reservoir for MPs accumulation. Moreover, Bay sediment is also an important matrix for monitoring because sediment tends to accumulate certain contaminants and act as a source of contaminants to the Bay food web. This study analyzed MPs ranging from 25 µm to 5 mm in surface sediment grab samples (n=8) and two sediment core samples (n=2 cores analyzed as 11 samples from different depths). Findings provide evaluation of MPs levels from different regions of the Bay, as well as an indication of temporal trends. Sample preparation included treatment using advanced Fenton's oxidation and alkaline digestion, followed by enzymatic digestion and density separation with $ZnCl_2$ in order to remove organic and inorganic matter, respectively. Finally, samples were subjected to spectroscopic (µFTIR) confirmation. MPs levels detected in Bay surface grab samples ranged from 2.1 to 11.9 MPs/g dry weight (n=8), with a mean value of 6.2 MPs/g. The most abundant morphology was fibers, followed by fragments and films. The sample with the highest MPs level was found in the subembayment referred to as the Lower South Bay, which is expected due to the heavy influence of wastewater treatment plant discharge and urban runoff in this subembayment, as well as lower exchange rates with the rest of the Bay and ocean compared to other parts of the Bay. The lowest MPs level was measured in two samples that were collected where two major rivers enter the Bay. In the sediment cores (n=11 core slices), an increasing trend was observed from bottom to top layers with the concentration ranging from 1.2 to 5.5 MPs/g. Both surface grab samples and cores contained a mixture of polymers, and the most abundant ones that could be identified via spectroscopy included polyethylene (PE), polypropylene (PP), polyester (PES), synthetic cellulose, and polyamide (PA). In addition, rubbery black particles that could not be definitively identified but were likely from tire wear were frequently observed.

Keywords: microplastics pollution, marine sediments, core, San Francisco Bay

Highlights

- San Francisco Bay‘s surface sediment (n=8) and sediment cores (n=2) were analyzed for microplastics (MPs)
- Sediment microplastics concentration: mean 6.2 MPs/g dry weight
- Highest concentration observed in Lower South Bay
- Cores showed maximum microplastic accumulation at the surface layer
- Fibers were the most abundant morphology among the samples
- Polymers such as polyethylene, polypropylene, polyester, synthetic cellulose, and polyamide were identified

1. Introduction

Microplastics (MPs), defined as plastic particles measuring less than 5 mm, have become a prominent environmental issue worldwide. MPs are not a conventional chemical contaminant; rather, they are a diverse mixture of anthropogenic debris consisting of various sizes, polymers, chemical additives, and adsorbed pollutants (Rochman et al., 2019). They are composed of synthetic, insoluble compounds, and particles can be categorized based on distinct morphologies, including fibers, films, foams, fragments, and pellets (Hartmann et al., 2019). A comprehensive monitoring of MPs contamination involves the physical-chemical characterization of various parameters such as size, shape, color, and polymer type. This characterization process requires appropriate sample preparation and subsequent analysis utilizing suitable analytical methods (Morgado et al., 2023).

MPs can enter aquatic environments through various pathways including atmospheric deposition (Dris et al., 2016), effluent from wastewater treatment plants (Ziajahromi et al., 2017, Zhu et al, 2021), urban runoff (Werbowski et al., 2021) and illegal dumping (Horton & Dixon, 2018). In particular, the improper management of waste as well as particles released from synthetic items, such as tires, in outdoor urban areas have the potential to be dislodged from the landscape due to precipitation and subsequently carried through stormwater runoff to nearby water bodies (Werbowski et al., 2021).

While MPs presence in aquatic environments has received considerable attention, their occurrence in sediment samples has gained recognition as a reservoir for MPs accumulation (Eo et al., 2021; Expósito et al., 2021; Goswami et al., 2021). Given the persistent nature of MPs, they tend to accumulate extensively within sediments, potentially increasing the ecological risks associated with their presence (Niu et al., 2021). Consequently, there is a need to better understand the MPs accumulation in sediments. Conducting ecological risk assessments and pollution source analyses of MPs are vital steps in effectively managing and controlling MPs pollution in sedimentary environments (Al Nahian et al., 2022; Deme et al., 2022).

MPs can be ingested or absorbed by various marine organisms and have the potential to bioaccumulate, particularly in higher trophic level organisms (Capillo et al., 2020; Prokić et al., 2019). The effects of MPs depend on the organism and size and type of particle (Thornton Hampton et al., 2022), and can range from physical blockage, entanglement, and abrasion to the disruption of feeding and respiratory systems (Expósito et al., 2022). Long-term effects observed

in laboratory studies include gene regulation changes, immune responses, behavioral alterations, and developmental abnormalities in fish and mussels (Expósito et al., 2022). By 2050, according to the most pessimistic projections, the quantity of MPs is expected to surpass that of fish, indicating a potentially significant impact on the environment (Ellen MacArthur Foundation, 2016).

The San Francisco Bay Area is a dynamic and highly urbanized coastal region renowned for its ecological diversity and economic significance. However, alongside its numerous benefits, this area also faces environmental challenges, including pollution from diverse sources. Among the emerging contaminants of concern, MPs have gained considerable attention and have been monitored in surface water, fish, sediment, stormwater runoff, treated wastewater (Zhu et al., 2021), and mussels (Klasios et al., 2021). Although multiple San Francisco Bay matrices have been subject to monitoring, there are still significant knowledge gaps in understanding the geographic and temporal trends in MPs concentrations, particularly in the sediment. Addressing these knowledge gaps may inform and be used to evaluate the effectiveness of plastic mitigation strategies over time. Spatial and temporal information enables policymakers and environmental managers to develop targeted measures for reducing MPs pollution.

The aim of the present study was to investigate the concentrations, size, morphology, and chemical composition of MPs in sediment samples from the San Francisco Bay (hereinafter Bay). This study leveraged archived sediment samples from two different sampling events. First, archived surface grab samples that were collected from various locations within the Bay were analyzed to examine the spatial distribution patterns of MPs in sediment in different sub-regions of the Bay. Second, archived sediment cores were analyzed to investigate the temporal distribution of MPs in Bay sediment for the first time.

2. Materials and methods

2.1.Sampling of microplastics and sample preparation

San Francisco Bay is situated between the Sacramento and San Joaquin rivers to the north and the Pacific Ocean to the west, with more than 7 million inhabitants within the watershed. It serves as the drainage basin for approximately 40% of California (Sutton et al., 2016). Spanning across a varied terrain, the region consists of urban areas, agricultural landscapes, recreational parks, as well as undeveloped areas (Sedlak et al., 2017b). The discharge of effluent from 42 wastewater treatment plants (WWTPs) can have an impact on its ecosystem (Sutton et al., 2016). For the purposes of this study, the different Bay sub-regions from which microplastics

were analyzed are divided into four regions: Lower South Bay (LSB), South Bay, Central Bay (CB), and North Bay (NB) (Figure 1).

2.1.1. Collection of Surface Sediment Grab Samples for Evaluating Geographic Distribution of Microplastics in Bay Sediment

Sediment samples from the Bay were collected during the summer of 2018 through the Regional Monitoring Program for Water Quality in San Francisco Bay, which implements a rigorous probabilistic study design for sediment sampling to gather accurate representation of ambient sediment samples throughout the Bay for contaminants monitoring. From these archived sediments a total of eight surface grab samples (n=8) (Figure 1, blue sampling points) were selected for MPs sample analysis, with the selection distributed among different regions as follows: LSB (n=2), SB (n=2), CB (n=2), NB (n=2). The sediment sampling process involved utilizing a clean stainless-steel scoop to collect samples from the center of the Van Veen grab, specifically avoiding the sides. Care was taken to collect only the uppermost layer of sediment, approximately 5 to 10 mm in depth, which was then carefully transferred to sample jars.

2.1.2. Collection of Sediment Core Samples for Evaluating Temporal Trends in Microplastics in Bay Sediment

Two sediment cores (Figure 1, red sampling points) were collected during the summer of 2020 in the South Bay subregion known as Steinberger Slough in order to evaluate sediment concentration profiles to provide information about temporal trends. This area is a complex of sloughs and channels and is a priority area for monitoring legacy contaminants, such as PCBs. One of the sediment core samples is located at the outlet of Pulgas Creek (Core 1), and therefore most impacted by urban stormwater runoff, while the second core sample (Core 2) is 2,666 meters away and less directly influenced by Pulgas Creek discharges.

Figure 1 Sampling points in the San Francisco Bay area.

To process the collected sediment cores, a slicing technique was employed in the laboratory. Typically, cores are sectioned while frozen; however, this method was inconvenient for this study due to the plastic tubes in which the core samples were stored, as cutting could potentially introduce sample contamination. Hence, an alternative approach was adopted. The sediment core was extruded using a pre-cleaned customized tool made out of plastic plug wrapped in aluminum to avoid any cross-contamination, that was the same diameter as the tool.

Each sediment core was subsequently analyzed by dividing it into discrete sections of 5 cm. For Core 1, the total core length was 35 cm, resulting in seven prepared samples (n=7): 0-5 cm, 5-10 cm, 10-15 cm, 15-20 cm, 20-25 cm, 25-30 cm, and 30-35 cm. Regarding Core 2, the total core length measured 33 cm, and three samples were prepared (n=3): two surface samples (0-5 cm and 5-10 cm) and one bottom sample (28-33 cm). The bottom layer was divided into two samples and analyzed as laboratory duplicate to ensure the reliability of the results. The bottom layer of Core 2 is identified as potentially predating the 1900s, based on observed historical trends in terms of PCBs. Considering its age, this layer is not expected to contain a significant amount of MPs. Hence, it serves as a valuable reference sample for validating the methods employed in this study. Consequently, the analysis focused specifically on the bottom layer of Core 2 to validate the method employed.

2.2.Pretreatment protocol

Prior to sample pretreatment and analysis, the organic matter content was assessed following Loss on Ignition (LOI) method, which involved heating the sediment sample in a muffle furnace (550-600°C) to measure the weight loss due to the combustion of organic matter and to select the most suitable pre-treatment method for MPs extraction. The organic matter content was found to be below 1%. Therefore, a density separation step was conducted initially to eliminate inorganic matter from the sediment samples. Approximately 100 g of wet weight (ww) was analyzed for each sample. To account for moisture content, the humidity of each sample was determined by analyzing a 10-gram aliquot. The total sample mass analyzed is provided in *Supplementary data (Table S2)*. The results are calculated based on the dry weight (dw) of the samples.

The following chemicals and materials were used for MPs pretreatment: absolute ethanol (Scharlau, >99.9%), iron (II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) (Thermo Scientific, 98.0%), hydrogen peroxide (H_2O_2) (Thermo Scientific,35.0%), PTFE- filter (Sartorius, 5 µm pore size), zinc chloride ($ZnCl_2$) (Thermo Scientific, 98.0%), potassium hydroxide (KOH) (Thermo Scientific, 85.0%), chitinase (ASA Spezialenzyme GmbH), acetic acid (CH_3COOH) (Sigma-Aldrich, 99.8%), and sodium acetate ($NaC_2H_3O_2$) (Scharlau, 99.5%).

All sediment samples underwent pretreatment as part of the experimental protocol (Figure 2). The protocol for the MPs extraction was based on the nature and requirements of the samples. Considering the previous detection of high-density MPs in the Bay, such as tire wear particles (Werbowski et al., 2021), a density extraction method specifically designed to capture these particles was implemented using $ZnCl_2$ salt solution. Density was set to 1.9 g/cm^3 based on the recommendations of the previous study, to ensure that all high-density MPs were effectively extracted and separated from the sample (Klöckner et al., 2019). In order to eliminate organic matter from the sample, various established methods were employed, including advanced Fenton's oxidation, alkaline digestion, and enzymatic digestion (Expósito et al., 2021; Kühn et al., 2017; Löder et al., 2017; Masura et al., 2015). These techniques have been widely utilized in MPs research to effectively remove organic components and enhance the analysis of MPs.

To begin, each sample was homogenized and its mass was measured. An aliquot was then taken to determine the sample's humidity. Density separation was conducted next, allowing for the separation of components. This separation process was carried out over a 24-hour period. Following this, the supernatant was carefully separated into a new Erlenmeyer flask, and an additional $ZnCl_2$ solution was introduced for a second round of separation. The extraction

procedure was performed three times for each sample. The resulting supernatants were collected and sieved using stainless-steel sieves with mesh sizes of 0.5 mm and 25 µm. This division allowed for the separation of particles into two fractions: particles larger than 0.5 mm and particles below 0.5 mm. To remove organic matter, advanced Fenton oxidation was employed (hydrogen peroxide and iron (II) sulfate heptahydrate). The portion retained by the sieves underwent filtration using vacuum-filtration equipment and a 5 µm PTFE filter. The filtered samples were subjected to 10 minutes of agitation at 40°C and left to react for 24 hours. After 24 hours, samples were filtered through a 5 µm PTFE filter, and alkaline digestion with KOH was performed next by immersing the filter in a solution for 24 hours with 10 minutes of agitation. After this step, the sample was filtered once again using a 5 µm PTFE filter and placed in a clean petri dish for further analysis.

A further density separation step was conducted using a sodium chloride solution with a density of 1.2 g/ml. This separation process was carried out over a 24-hour period. The purpose of this second-density separation was to divide the already concentrated MPs into two groups based on their density, thereby facilitating the quantification process. The final step of the protocol involved enzymatic digestion using a chitinase solution in an acetic buffer solution (pH=5) at 40°C and agitation. The reaction took place for 48 hours and the solution was filtered through a 10 µm PTFE filter. Following this, visual inspection was carried out utilizing optical and stereoscopic microscopes to examine the MPs.

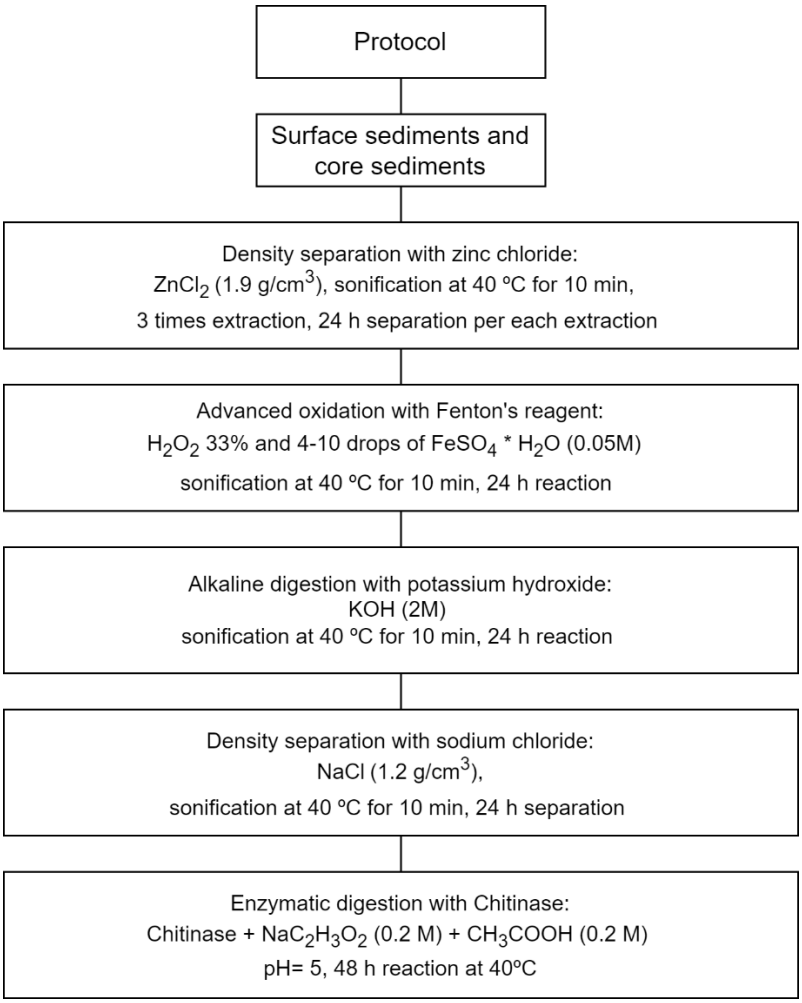


Figure 2 Pretreatment protocol for microplastics extraction

1.1. Quantitative and qualitative analysis of microplastics

Following sample pretreatment, the particles were visually sorted and quantified utilizing advanced microscopy techniques. A LEICA MZ10 spectroscopic microscope equipped with a FLEXACAM C1 camera and an Olympus CX41 optical microscope were employed for this purpose. Suspected MPs were categorized into two size fractions: above and below 0.5 mm, and further classified into three predominant shape categories: films, fragments, and fibers (Bayo et al., 2020; Hidayaturrahman & Lee, 2019; Lares et al., 2018).

Particle analysis below 0.5 mm was conducted on calcium fluoride slides using infrared (IR) mapping techniques (Supplementary data, Figure S3). Thermo Scientific Nicolet™ iN™10, equipped with an MCT detector and a pixel aperture of 25x25 µm in transmission mode was used. The IR spectra were obtained over a range of 715-4000 cm^{-1} with a spectral resolution of 4 cm^{-1}, utilizing 4 scans. Particles larger than 0.5 mm were analyzed by infrared spectroscopy with a Perkin Elmer Frontier™ instrument equipped with an Attenuated Total Reflectance (ATR)

accessory, a DTGS detector, a Glowbar source, a CsI beam splitter and Spectrum 10^{TM} software. The IR spectra were obtained in the range of 230-4000 cm^{-1} with a spectral resolution of 4 cm^{-1}, using 4 scans. The acquired spectra from both instruments were analyzed using the OMNIC Specta MCS Software. To identify the particles, an unknown spectrum matching approach was employed by comparing them with available databases such as HR Nicolet Sampler Library, Hummel Polymer Sample Library, Polymer Laminate Films, Wizard Library, Willey's Know It All as well as a custom library that was generated with more than 80 spectra. The standard criteria of ≥ 70% match between the sample and reference spectra were followed.

2.3.Quality assurance and quality control (QA/QC)

To ensure the integrity of the results and prevent cross-contamination, strict quality control measures were implemented in the present study. The laboratory environment was cleaned using a combination of 70% ethanol and MilliQ water. To minimize the risk of cross-contamination, laboratory personnel wore cotton laboratory coats exclusively. All equipment and tools used for sample processing were cleaned with 70% ethanol and MilliQ water. The study utilized analytical grade chemicals, and all reagents were prepared using MilliQ water. To verify accuracy of the analysis, multiple blank samples were included as control measures. The total number of blank samples analyzed can be found in *Supplementary material (Table S3)*. MPs were detected in all of the blank samples. It is highly likely that airborne fibers were the source of this contamination, as they were observed in the blank samples from both laboratories. The blank sample consisted of an open petri dish that was placed in the hoods during the sample pre-treatment process. The fiber deposition rates for the blank samples were determined to be 1.13 and 0.5 fibers/day/cm^2 at the two laboratories in the California and Spain, respectively. Subsequently, the appropriate corrections were applied to all the samples based on their exposure time.

Furthermore, a field blank was collected from the Lower South Bay, and despite taking precautions to prevent cross-contamination at the sampling site, it revealed the presence of 2 fibers, 3 films, and 1 fragment. Given the isolated nature of this finding, the decision was made not to blank-correct the results based on this field blank. However, it underscores the importance of maintaining rigorous sampling protocols and ongoing monitoring for potential sources of contamination in future studies.

Additionally, a laboratory duplicate was analyzed for the bottom layer of the Core 2 sediment, and the coefficient of variation, calculated as a percentage, was found to be 24.22%. Recovery rate experiments were conducted in a previous study (Dronjak et al., 2023) for the extraction of

MPs. The average recovery for triplicate samples was 75% for spiked MPs (*fluorescent polyethylene (PE) microspheres*, *Cospheric innovations*, *CA*, *USA*) with a diameter size of 53-63 µm, 74% for spiked MPs with a diameter size of 125-150 µm, 70% for particles with a diameter size of 250-300 µm, and 100% for particles with diameter sizes of 450-500 µm. These results indicate satisfactory laboratory performance in terms of recovery rates.

3. Results and discussion

3.1. Occurrence of microplastics in surface sediments of San Francisco Bay area

MPs were identified in all sediment samples; however, their concentration exhibited significant variability across different sampling locations (Table 1). The detected MPs concentrations in surface sediments from the Bay ranged from 2.1 to 11.9 MPs/g dry weight (dw), with an average value of 6.2 MPs/g dw. The highest concentration was observed in the Lower South Bay, while the lowest concentration was found in the North Bay, near two river sites (Sacramento River and San Joaquin). The higher concentrations of MPs observed in the Lower South Bay can be attributed to factors such as limited water flushing and the influence of wastewater and urban stormwater discharges (Smith and Hollibaugh, 2006). Previous studies in the area have also highlighted elevated levels of various contaminants in sediments from South and Lower South Bay including pharmaceuticals (Klosterhaus et al., 2013), triclosan (Kerrigan et al., 2015), perfluorooctane sulfonate (Sedlak et al., 2017a), and specific alternative flame retardants (Sutton et al., 2019).

Table 1 Microplastics concentration (MPs/g dw) and morphology (%) in different surface sediment samples

Sample ID	***Location***	***MPs concentration (MPs/g dw)***	***MPs morphology (%)***		
			fibers	***films***	***fragments***
LSB01	Lower South Bay	11.9	65.4	9.2	25.4
LSB02	Lower South Bay	9.7	65.3	14.2	20.5
SB01	South Bay	6.7	62.8	12.3	24.9
SB02	South Bay	9.2	68.7	7.7	23.6
CB01	Central Bay	4.3	67.7	8.1	24.2
CB02	Central Bay	3.7	70.2	8.5	21.3
NB01	North Bay (River site)	2.3	81.6	10.8	7.6
NB02	North Bay (River site)	2.1	84.7	13.5	1.8

Zhu et al. (2021) also conducted a study on MPs debris in sediment samples from the San Francisco Bay and their findings revealed similar MPs levels ranging from 0.03 to 42.10 particles/g dw, with a mean concentration of 5.18 particles/g. Notably, the highest concentration on average was consistently observed in the Lower South Bay. Bayo et al. (2022) reported the presence of MPs in coastal sediments near the harbor in Cartagena, Spain, with an average abundance of 0.02 items/g. The areas with the highest MPs abundance were close to car roads and areas with beachgoer activity. Another study in Spain, by Expósito et al. (2021), investigated marine sediments from the Western Mediterranean and found MPs mean abundance of 0.03 MPs/g. Bronzo et al. (2021) found the occurrence of MPs in coastal sediments from the inner Oslofjord, Norway, with MPs abundance ranging from 0.02 to 1.71 MPs/g. The highest abundance of MPs was observed near the input of sewage treatment plants, and areas with high human activities. Comparatively, our study demonstrates similar results as previous MPs study in the sediment of the San Francisco Bay (Zhu et al., 2021) and significantly higher MPs concentrations than those reported in the previous studies conducted worldwide (Bayo et al., 2022; Bronzo et al., 2021; Expósito et al., 2021). It is noteworthy, that comparison between the studies should be approached with caution since different studies are likely to employ diverse methodologies for MPs analysis, such as variations in sampling techniques, sample processing procedures, and analytical instrumentation. These methodological differences can lead to differences in the quantification and identification of MPs, making direct comparisons challenging. Furthermore, sampling locations, hydrodynamic conditions, sediment characteristics, and local ecosystem dynamics can also impact the accumulation and distribution of MPs.

In the present study, the analysis categorized MPs into three distinct shapes: fibers, fragments, and films. Although MPs pellets were also detected in the sediment samples, their concentration was minimal (less than 1%) compared to other morphology types such as fibers, fragments, and films, leading to their exclusion from separate categorization; nonetheless, acknowledging their presence remains important to highlight these specific MPs types. Fibers were found to be the most prevalent morphology, accounting for over 60% of the MPs in all sediment samples. The size of detected particles ranged from 25 µm to 5 mm, and the samples were further divided into size categories: those larger and smaller than 0.5 mm. Figure 3 showcases illustrative images of particles with various shapes captured using a Leica FLEXACAM C1, IC90 E, ICC50 W/E camera.

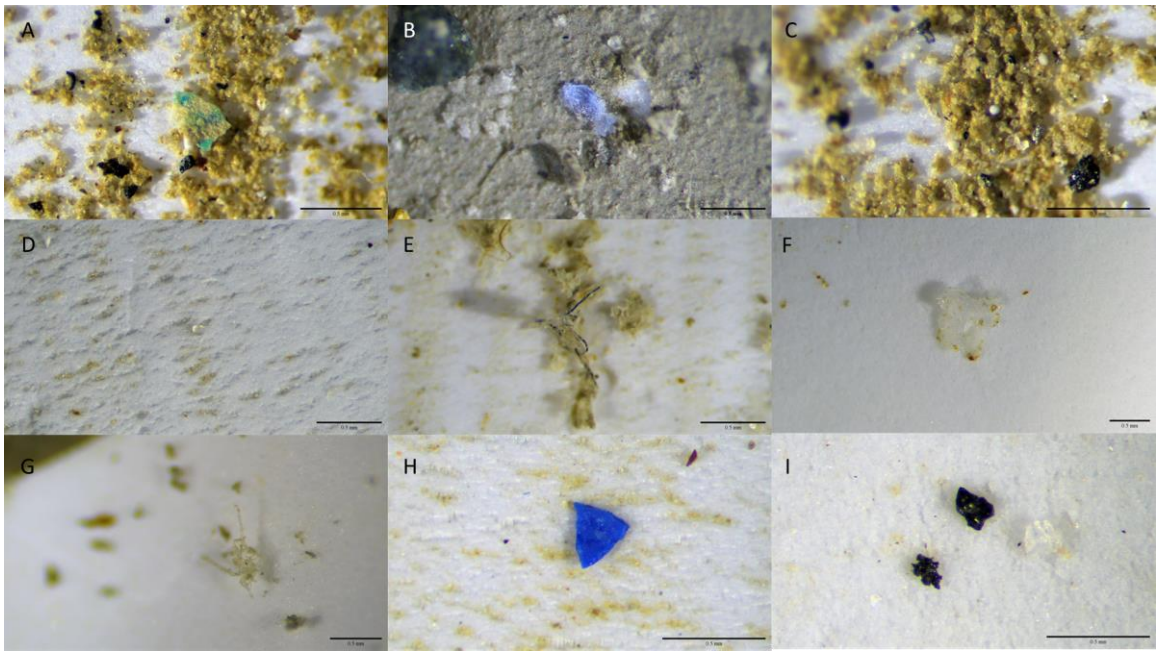


Figure 3 Microplastics found in different sediment samples: scale bar 0.5 mm (E: fibers; G: fiber ball; A, H, I: fragments; C, D: pellets; B, F: films)

Fibers derived from synthetic textiles have been identified as a significant source of MPs in the environment (Browne et al., 2011; Carney Almroth et al., 2018; Henry et al., 2019; Hernandez et al., 2017; McIlwraith et al., 2019; Melody M. Bomgardner, 2017; Pirc et al., 2016). These fibrous MPs can be released into environment through various pathways. Airborne fibers, originating from textile shedding (e.g., during use or drying) can be transported to the Bay via stormwater or direct settling onto the water surface. Furthermore, washing of synthetic textiles contribute to the presence of MPs in wastewater, as observed in studies conducted in different locations (Browne et al., 2011; Carney Almroth et al., 2018; Hernandez et al., 2017; Mintenig et al., 2017; Murphy et al., 2016; Wolff et al., 2019) and potentially can be released into the environment through final effluent discharge and the application of biosolids to the land (He et al., 2019; Nizzetto et al., 2016; Zubris & Richards, 2005). Additionally, fishing and other aquatic industries contribute to fiber pollution. The issue of fiber ball formation is also an important consideration, as it was observed in a sample from South Bay area and is commonly found in the marine environment. A previous case study conducted in Tarragona, Spain, also reported the presence of fiber balls on the sediment bottom, indicating that the primary mechanism for fiber ball formation is aggregation, and once they settle on the seafloor are expected to continue fragmenting until reaching the nanoscale (Expósito et al., 2021). These synthetic fiber balls can distort the actual values of fibers and total MPs abundance, as they were counted as a single fiber item due to challenges in separating them and quantifying each individual fiber.

MPs pellets were present in the sediment samples; however, their concentration was minimal compared to other morphology types like fibers, fragments, and films, accounting for less than 1%. Due to their low prevalence, they were not categorized separately, but their mention remains significant to draw attention to these specific MPs type. Previous research by Zhu et al. (2021) has indicated their utilization in various applications such as personal care products, biomedical research, and water treatment and purification (Ballent et al., 2016; Mani et al., 2019). Additionally, it is noteworthy that US federal law implemented the Microbead-Free Waters Act (MFWA) in 2015, aimed at prohibiting the use of plastic microbeads in rinse-off personal care products to prevent their pollution in water bodies (FDA, 2015; McDevitt et al., 2017).

In various sediment samples, another prevalent MPs fragment type was observed, characterized by its rubbery structure and black color. While spectroscopic analysis did not definitively determine the chemical composition of these particles, secondary characteristics such as compression, color, and texture strongly suggest that they originate from tire wear. Furthermore, another characteristic that support tire composition is that these particles were more prevalent in the denser fraction based on visual inspection. Other studies have established connection between tire wear and the presence of abundant rubber MPs in sediment samples (Gray et al., 2018). Modeling studies further indicate that tire wear may rank among the primary sources of MPs globally (Boucher and Friot, 2017; Jan Kole et al., 2017). Previous study of Bay area stormwater found that nearly half the MPs entering the Bay from this pathway were tire wear particles (Werbowski et al., 2021). Future studies should consider employing complementary techniques such as thermoextraction desorption gas chromatography mass spectrometry (TED-GC-MS) and pyrolysis-GC-MS methods, which have successfully identified similar particles in previous investigations (Eisentraut et al., 2018) to provide a more comprehensive understanding of the origins of these particles and its variations in the Bay. This will enable a more accurate assessment of the specific MPs sources and potential environmental impacts associated with the different polymer types.

3.2. Microplastics occurrence in sediment cores

MPs have been analyzed in sediment cores internationally. However, no previous sediment core MPs studies have been conducted in San Francisco Bay. The analysis of two sediment cores was conducted in the present study. Figure 4 shows the MPs concentrations in Core 1 and Core 2. Both cores were also analyzed for PCB concentrations as part of a separate study [data not published] to evaluate the PCB concentration profile in sediment. The PCB profiles offer a

valuable reference to benchmark the relative time period during which sediment layers were deposited. We expect the PCB sediment concentration peak to approximately correspond to sediment deposition from the 1960s, during which PCB uses in the US were the highest. Therefore, in Core 2, in which the PCB concentration peak was detected within the top 5 cm depth, we estimate the bottom of Core 2, at a depth of 28-33 cm, to likely predate the 1900s. This bottom layer can serve as a valuable reference to validate the methods employed in the present study, as MPs should not be present in significant quantities in the bottom layer unless there are mechanisms such as bioturbation or other mixing processes that can transport MPs to the bottom or potential contamination issues.

The analysis of Core 2 provides insights into both historical trends and method validation. The PCB profile observed in Core 2 indicates lower deposition rates, suggesting the possibility of averaging data from multiple decades as the samples were fractioned into 5 cm layers (data not published).

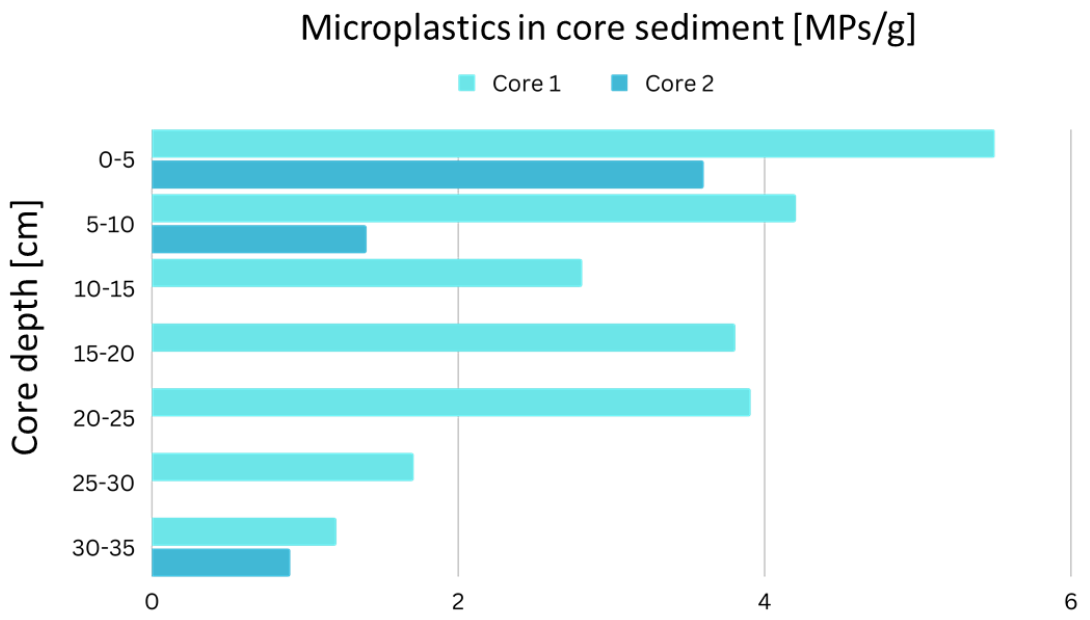


Figure 4 Concentrations of MPs in Core 1 (Pulgas Creek) and Core 2 (Redwood Marina) (Core 2 is 2 cm shorter than Core 1, placing its bottom layer within the depth range of 28-33 cm)

MPs were found in all core layers; however, their concentration varied greatly depending on the core depth. An increasing trend was observed from bottom to top layers with exception of two samples from Core 1 (15-20 cm and 20-25 cm) (Figure 4). This trend has also been observed in previous studies. In environments characterized by minimal disturbance (i.e., potential long-

term sinks), there seems to be a consistent global pattern of decreasing MPs concentrations as sediment depth increases (Martin et al., 2022).

Although the hypothesis was that there would be absence of MPs in the bottom layer from Core 2 based on temporal estimations, several MPs were found with an average concentration of 0.9 MPs/g dw calculated from laboratory duplicates. Similar findings have been observed in different studies. The study conducted by (Xue et al., 2020) hypothesized that MPs would be present only up to a depth of 22 cm (corresponding to a deposit from 1933 CE). However, the results revealed MPs extending down to 60 cm (representing a deposit from 1897 CE), suggesting sediment reworking by local invertebrates as the likely cause for this unexpected occurrence. In a separate study by Turner et al. (2019), MPs were detected as deep as 50 cm below sediment dating back to 1950 (45 cm versus 95 cm depth). These MPs fibers, which were chemically identical to fibers found in more recent layers, provided evidence for the reworking of MPs within the sediment column. Remarkably, the presence of these fibers dating back to the mid-nineteenth century, decades before plastic production began, further supports the notion of long-term reworking and accumulation of MPs in the sediments.

The surface layers of Core 1, located in the South Bay, exhibited higher MPs concentrations compared to Core 2 in the Lower South Bay. This difference in findings can be attributed to the significant influence of Pulgas Creek discharge in sediment Core 1, whereas sediment Core 2 is situated in a comparatively more ambient environment at Redwood Creek Marina.

In terms of MPs morphology, fibers once again emerged as the predominant shape, followed by fragments and films (Figure 5). This is consistent with multiple other studies that also report fibers consistently dominated the MPs compositions, constituting 100% of MPs in certain instances (Dong et al., 2020). Similarly, in another study, fibrous MPs were found to be highly prevalent, accounting for over 98% within different layers of each sediment core (Pervez and Wang, 2022). However, contrasting these findings, a relatively small fraction of MPs was identified as fibers (6.7%) in a study by (Kukkola et al., 2022), which deviates from the majority of existing literature where fibers have been recognized as the most common morphology of MPs in shelf environments. In fact, the overall median value reported across 16 studies suggests that fibers comprise approximately 64% of MPs (Harris, 2020). Fibers emerged as the predominant morphology not only in sediment samples but also within organisms. For instance, in San Francisco Bay, resident mussels and clams displayed fibers as the most dominant morphology, constituting over 96% of all particles observed (Klasios et al., 2021).

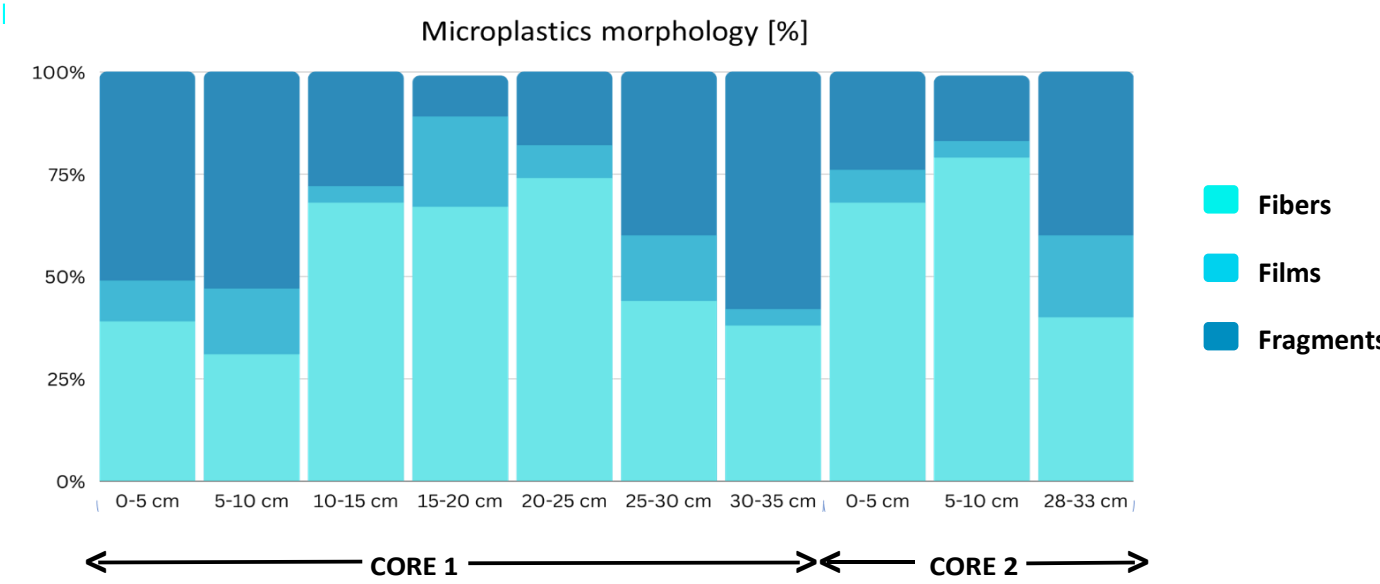


Figure 5 Morphology contribution in core samples

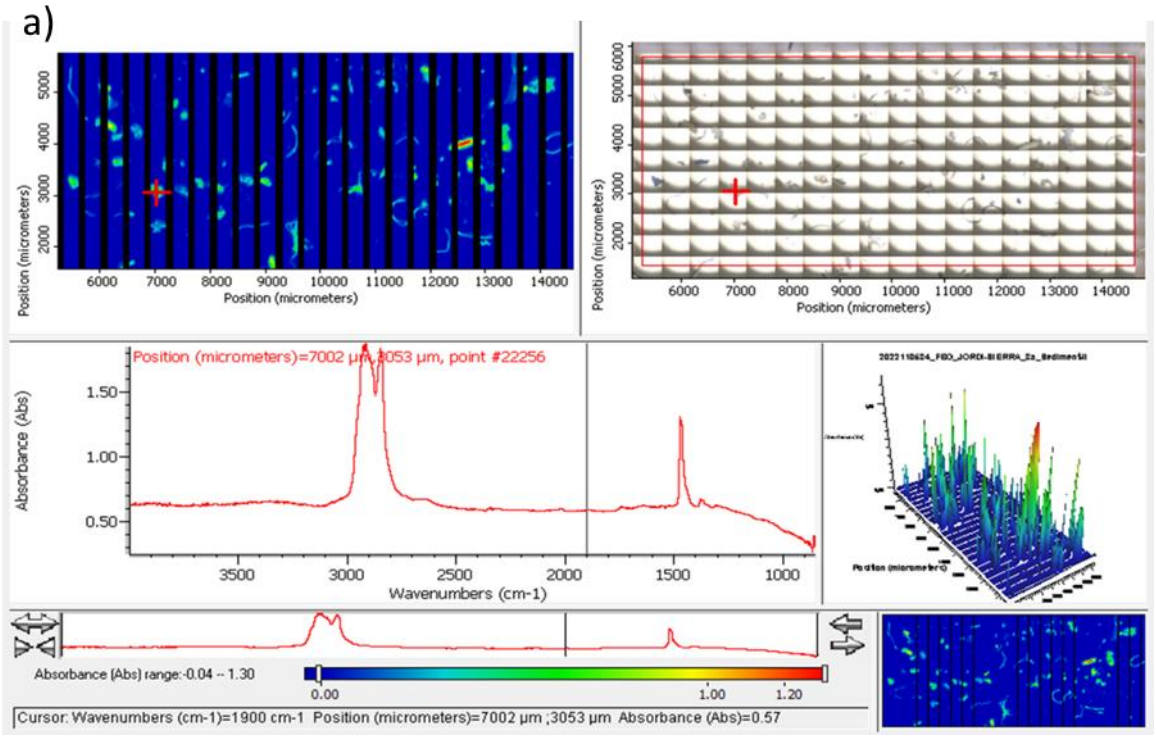
3.3.Polymer composition of microplastics particles

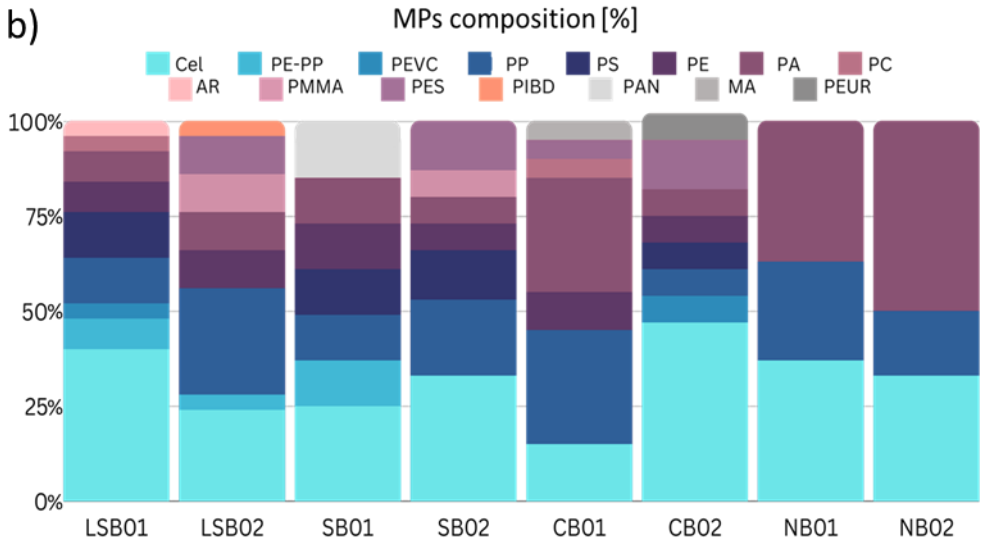
The identification of MPs polymer type is crucial for understanding the sources and potential environmental impacts of these particles. For this purpose, in the present study, spectroscopic techniques were employed to analyze the polymer composition of the MPs found in the surface sediments of the Bay. A representative subsample of (250 individual MPs), taking into account different morphologies, colors and sizes, was extracted for chemical composition analysis, comprising 119 MPs from surface grab samples and 131 MPs from the cores. The successful performance in distinguishing between MPs and other materials was evident as only 3 particles from surface grab samples and 2 particles from the cores were not identified as plastic. Particles were extracted on calcium fluoride slides for the FTIR mapping analysis and total of 19 different polymers were detected (Figure 6).

For the surface sediment samples, among the analyzed MPs, synthetic cellulose, polypropylene (PP), polystyrene (PS), polyethylene (PE), poly (propylene-ethylene) copolymer (PE-PP), polyamide (PA), and polyester (PES) were identified as the most prevalent polymers. The prevalence of these polymers varied among the samples, with synthetic cellulose ranging from 15 to 47%, PP from 7 to 28%, PS from 0 to 13%, PE from 0 to 12%, PE-PP from 0 to 12%, PA from 7 to 50%, and PES ranging from 0 to 13%.

The core sediment analysis revealed varying proportions of different polymer types, with synthetic cellulose (8-61%), polyethylene acrylate (PEA) (9-25%), PE (8-50%), PS (8-21%), PP (7-38%), and PE-PP (4-23%) emerging as the predominant polymers. As noted previously, tire wear

particles are not readily identifiable via spectroscopy, preventing confirmation of the black, rubbery fragments as derived from tires (Baensch-Baltruschat et al., 2020).





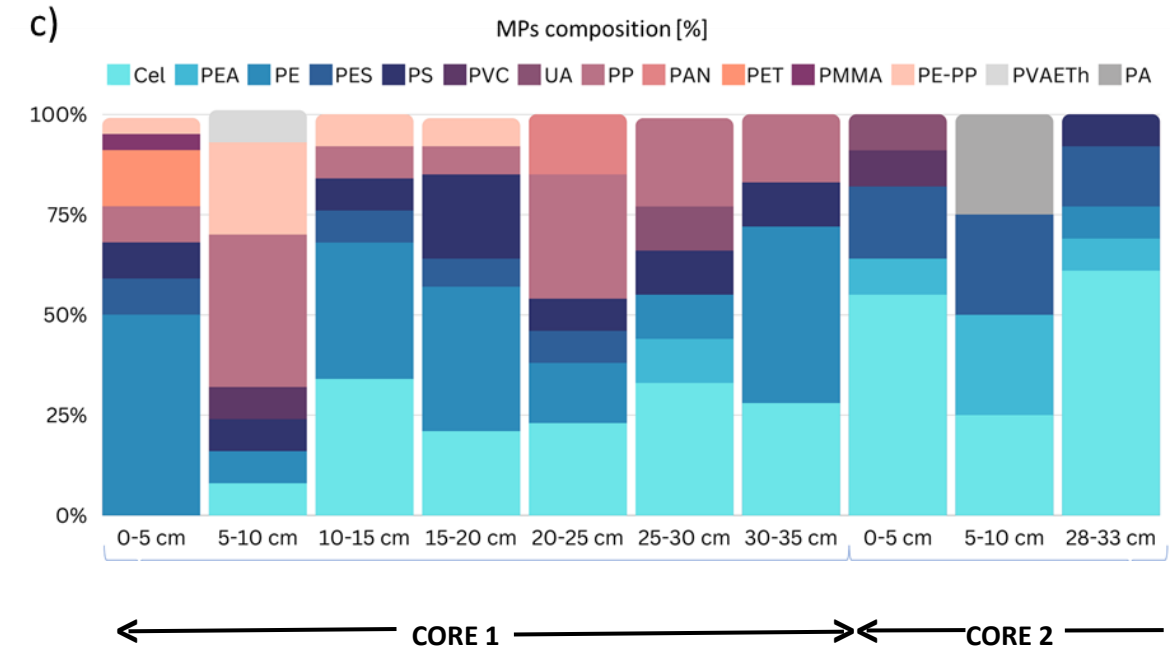


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Figure 6 Advanced software for microplastics identification (a) and polymer composition of surface grab samples (b) and core sediment samples (c):

synthetic cellulose (Cel); PE-PP (poly(ethylene-propylene) copolymer); PEVC (polyethylene-vinyl chloride); PP (polypropylene); PS (polystyrene); PE (polyethylene); PA (polyamide); PC (polycarbonate); AR (alkyd resin); PMMA (polymethyl methacrylate); PES (polyester); PIBD (polyisobutadiene); PAN (polyacrilonitrile); MA (methan alkyd); PEUR (polyether urethane); PEA (polyethyl acrylate); PVC (polyvinyl chloride); UA (urethane alkyd); PVAETH (Poly vinyl acetate:ethanol 3:1)

An effective strategy for mitigating MPs pollution in the environment is to control the release of plastic waste from potential sources of the pollution. Thereby, emphasizing the need to identify the sources of MPs in sediment (Sarkar et al., 2022; Yu and Ma, 2022). Several studies have contributed to the development of MPs pollution source analysis systems. These studies highlight the potential of using MPs characteristics such as color, shape, polymer type, and land-use patterns to trace the pollution source in sediments (Tampang and Viswanathan, 2022; Sambandam et al., 2022; C. Wang et al., 2021; Wang et al., 2019). A study conducted by Tampang and Viswanathan (2022) investigated the main pollution sources of MPs in sediments from the Miri coast in NW Borneo. The findings revealed that textiles, food packaging, and plastic containers were the primary sources of PET, PS, and PE MPs, respectively. Wang et al. (2019) proposed an analysis system based on the correspondence between MPs types and pollution sources reported in previous studies. Wang et al. (2021) assessed different polymer types of MPs based on their physical and chemical properties and daily applications, linking them to

potential pollution sources. Sambandam et al. (2022) analyzed the relationship between polymer types and MPs pollution sources in terrestrial and marine environments.

In the present study, we attempted to infer the potential sources of MPs pollution, based on previous research. Source identification was based on the consideration of various factors such as morphology, size, and polymer type. These considerations enabled us to identify a list of potential sources of MPs pollution, which is presented in the Table 2.

Table 2 Possible pollution sources

Source	Common polymer	Particle Characteristics	Reference
Textile-related sources	Acrylic; Nylon; Polyester	fiber; fiber ball	Browne et al., 2011; Carney Almroth et al., 2018; Dris et al., 2017; Hartline et al., 2016; Henry et al., 2019; Hernandez et al., 2017; Pirc et al., 2016
Fishing area ropes/lines/net	Polypropylene; Polyacrylonitrile; Polyvinylchloride; Polyamide	fiber	Wang et al., 2019
Cigarette filters	Cellulose acetate	fiber; fiber ball	Slaughter et al., 2011
Single-use bags and wraps	Polyethylene; Polypropylene	film	McKeen, 2013
Microbeads used in water softening and purification as well as other medical and industrial processes	Polystyrene	pellet	Ballent et al., 2016; Mani et al., 2019
Personal care products	Polyethylene; Polypropylene; Polyethylene terephthalate; Polystyrene	pellet	Wang et al., 2019
Tire particles and OTHER recycled tire material	Synthetic (styrene-butadiene) rubber; Natural (isoprene) rubber; Polyurethane	fragment	Edil, 2008; Eunomia, 2018; Jan Kole et al., 2017; Lassen et al., 2015; Sommer et al., 2018
Road markings (thermoplastic)	Styrene copolymer; Ethylene/vinyl acetate Copolymer (EVA); Nylon	fragment	Vogelsang et al., 2020

4. Conclusion

This study revealed the presence of MPs in sediment samples across the Bay area, with varying concentrations depending on the location and depth. The concentration of MPs detected ranged from 2.1 MPs/g dw to 11.9 MPs/g dw, with an average concentration of 6.2 MPs/g dw. The highest concentrations were observed in the Lower South Bay subembayment, while samples from the river confluence exhibited the lowest concentrations. Additionally, the analysis of sediment cores indicated an increasing trend in MPs concentrations from bottom to top layers, ranging from 1.2 MPs/g dw to 5.5 MPs/g dw. Fibers were found to be the most abundant morphology, followed by fragments and films. The most abundant polymers found among the samples were: PE, PP, PES, synthetic cellulose, PS, PEA, PA, and PE-PP. Tire particles were also tentatively identified in many samples. These findings emphasize the widespread distribution and varying levels of MPs contamination in the Bay area, highlighting the need for continued monitoring and mitigation strategies to address this environmental concern. The findings from the present study will contribute to the knowledge base on MPs pollution in urban coastal environments, providing valuable insights for environmental management and policy development.

References

Al Nahian, S., Rakib, M. R. J., Haider, S. M. B., Kumar, R., Mohsen, M., Sharma, P., & Khandaker, M. U. (2022). Occurrence, spatial distribution, and risk assessment of microplastics in surface water and sediments of Saint Martin Island in the Bay of Bengal. *Marine Pollution Bulletin*, *179*. https://doi.org/10.1016/j.marpolbul.2022.113720

anak Alexander Tampang, A. M., & Mohan Viswanathan, P. (2022). Occurrence, distribution and sources of microplastics in beach sediments of Miri coast, NW Borneo. *Chemosphere*, *305*. https://doi.org/10.1016/j.chemosphere.2022.135368

Baensch-Baltruschat, B., Kocher, B., Stock, F., & Reifferscheid, G. (2020). Tyre and road wear particles (TRWP) - A review of generation, properties, emissions, human health risk, ecotoxicity, and fate in the environment. In *Science of the Total Environment* (Vol. 733). https://doi.org/10.1016/j.scitotenv.2020.137823

Ballent, A., Corcoran, P. L., Madden, O., Helm, P. A., & Longstaffe, F. J. (2016). Sources and sinks of microplastics in Canadian Lake Ontario nearshore, tributary and beach sediments. *Marine Pollution Bulletin*, *110*(1). https://doi.org/10.1016/j.marpolbul.2016.06.037

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*. https://doi.org/10.1016/j.chemosphere.2019.124593

Bayo, J., Rojo, D., & Olmos, S. (2022). Weathering indices of microplastics along marine and coastal sediments from the harbor of Cartagena (Spain) and its adjoining urban beach. *Marine Pollution Bulletin*, *178*. https://doi.org/10.1016/j.marpolbul.2022.113647

Ben-Haddad, M., Abelouah, M. R., Hajji, S., Rangel-Buitrago, N., Hamadi, F., & Alla, A. A. (2022). Microplastics pollution in sediments of Moroccan urban beaches: The Taghazout coast as a case study. *Marine Pollution Bulletin*, *180*. https://doi.org/10.1016/j.marpolbul.2022.113765

Boucher, J., & Friot, D. (2017). Primary microplastics in the oceans: A global evaluation of sources. In *Primary microplastics in the oceans: A global evaluation of sources*. https://doi.org/10.2305/iucn.ch.2017.01.en

Bronzo, L., Lusher, A. L., Schøyen, M., & Morigi, C. (2021). Accumulation and distribution of microplastics in coastal sediments from the inner Oslofjord, Norway. *Marine Pollution Bulletin*, *173*. https://doi.org/10.1016/j.marpolbul.2021.113076

Browne, M. A., Crump, P., Niven, S. J., Teuten, E., Tonkin, A., Galloway, T., & Thompson, R. (2011). Accumulation of microplastic on shorelines woldwide: Sources and sinks. *Environmental Science and Technology*, *45*(21), 9175–9179. https://doi.org/10.1021/es201811s

Capillo, G., Savoca, S., Panarello, G., Mancuso, M., Branca, C., Romano, V., D'Angelo, G., Bottari, T., & Spanò, N. (2020). Quali-quantitative analysis of plastics and synthetic microfibers found in demersal species from Southern Tyrrhenian Sea (Central Mediterranean). *Marine Pollution Bulletin*, *150*. https://doi.org/10.1016/j.marpolbul.2019.110596

Carney Almroth, B. M., Åström, L., Roslund, S., Petersson, H., Johansson, M., & Persson, N. K. (2018). Quantifying shedding of synthetic fibers from textiles; a source of microplastics released into the environment. *Environmental Science and Pollution Research*, *25*(2). https://doi.org/10.1007/s11356-017-0528-7

Deme, G. G., Ewusi-Mensah, D., Olagbaju, O. A., Okeke, E. S., Okoye, C. O., Odii, E. C., Ejeromedoghene, O., Igun, E., Onyekwere, J. O., Oderinde, O. K., & Sanganyado, E. (2022). Macro problems from microplastics: Toward a sustainable policy framework for managing microplastic waste in Africa. In *Science of the Total Environment* (Vol. 804). https://doi.org/10.1016/j.scitotenv.2021.150170

Dong, M., Luo, Z., Jiang, Q., Xing, X., Zhang, Q., & Sun, Y. (2020). The rapid increases in microplastics in urban lake sediments. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-57933-8

Dris, R., Gasperi, J., Mirande, C., Mandin, C., Guerrouache, M., Langlois, V., & Tassin, B. (2017). A first overview of textile fibers, including microplastics, in indoor and outdoor environments. *Environmental Pollution*, *221*. https://doi.org/10.1016/j.envpol.2016.12.013

Dris, R., Gasperi, J., Saad, M., Mirande, C., & Tassin, B. (2016). Synthetic fibers in atmospheric fallout: A source of microplastics in the environment? *Marine Pollution Bulletin*, *104*(1–2). https://doi.org/10.1016/j.marpolbul.2016.01.006

Dronjak, L., Exposito, N., Sierra, J., Schuhmacher, M., Florencio, K., Corzo, B., & Rovira, J. (2023). Tracing the fate of microplastic in wastewater treatment plant: A multi-stage analysis of treatment units and sludge. *Environmental Pollution*, *333*. https://doi.org/10.1016/j.envpol.2023.122072

Edil, T. B. (2008). A review of environmental impacts and environmental applications of shredded scrap tires. *Proceedings of the International Workshop on Scrap Tire Derived Geomaterials - Opportunities and Challenges, IW-TDGM 2007*.

Eisentraut, P., Dümichen, E., Ruhl, A. S., Jekel, M., Albrecht, M., Gehde, M., & Braun, U. (2018). Two Birds with One Stone - Fast and Simultaneous Analysis of Microplastics: Microparticles Derived from Thermoplastics and Tire Wear. *Environmental Science and Technology Letters*, *5*(10). https://doi.org/10.1021/acs.estlett.8b00446

Ellen MacArthur Foundation. (2016). The New Plastics Economy: Rethinking the future of plastics. *Ellen MacArthur Foundation*, *January*.

Eo, S., Hong, S. H., Song, Y. K., Han, G. M., Seo, S., & Shim, W. J. (2021). Prevalence of small high-density microplastics in the continental shelf and deep sea waters of East Asia. *Water Research*, *200*. https://doi.org/10.1016/j.watres.2021.117238

Eunomia. (2018). Investigating options for reducing releases in the aquatic environment of microplastics emitted by (but not intentionally added in) products. In *Report for DG Env EC: Vol. Vol. 62, N* (Issue February).

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*. https://doi.org/10.1016/j.scitotenv.2021.147453

Expósito, N., Rovira, J., Sierra, J., Gimenez, G., Domingo, J. L., & Schuhmacher, M. (2022). Levels of microplastics and their characteristics in molluscs from North-West Mediterranean Sea: Human intake. *Marine Pollution Bulletin*, *181*. https://doi.org/10.1016/j.marpolbul.2022.113843

FDA. (2015). *The Microbead-Free Waters Act: FAQs | FDA*. U.S Food and Drug Administration.

Goswami, P., Vinithkumar, N. V., & Dharani, G. (2021). Microplastics particles in seafloor sediments along the Arabian Sea and the Andaman Sea continental shelves: First insight on the occurrence, identification, and characterization. *Marine Pollution Bulletin*, *167*. https://doi.org/10.1016/j.marpolbul.2021.112311

Gray, A. D., Wertz, H., Leads, R. R., & Weinstein, J. E. (2018). Microplastic in two South Carolina Estuaries: Occurrence, distribution, and composition. *Marine Pollution Bulletin*, *128*. https://doi.org/10.1016/j.marpolbul.2018.01.030

Harris, P. T. (2020). The fate of microplastic in marine sedimentary environments: A review and synthesis. In *Marine Pollution Bulletin* (Vol. 158). https://doi.org/10.1016/j.marpolbul.2020.111398

Hartline, N. L., Bruce, N. J., Karba, S. N., Ruff, E. O., Sonar, S. U., & Holden, P. A. (2016). Microfiber Masses Recovered from Conventional Machine Washing of New or Aged Garments. *Environmental Science and Technology*, *50*(21). https://doi.org/10.1021/acs.est.6b03045

Hartmann, N. B., Hüffer, T., Thompson, R. C., Hassellöv, M., Verschoor, A., Daugaard, A. E., Rist, S., Karlsson, T., Brennholt, N., Cole, M., Herrling, M. P., Hess, M. C., Ivleva, N. P., Lusher, A. L., & Wagner, M. (2019). Response to the Letter to the Editor Regarding Our Feature "are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris." In *Environmental Science and Technology* (Vol. 53, Issue 9). https://doi.org/10.1021/acs.est.9b02238

He, P., Chen, L., Shao, L., Zhang, H., & Lü, F. (2019). Municipal solid waste (MSW)landfill: A source of microplastics? -Evidence of microplastics in landfill leachate. *Water Research*, *159*. https://doi.org/10.1016/j.watres.2019.04.060

Henry, B., Laitala, K., & Klepp, I. G. (2019). Microfibres from apparel and home textiles: Prospects for including microplastics in environmental sustainability assessment. *Science of the Total Environment*, *652*. https://doi.org/10.1016/j.scitotenv.2018.10.166

Hernandez, E., Nowack, B., & Mitrano, D. M. (2017). Polyester Textiles as a Source of Microplastics from Households: A Mechanistic Study to Understand Microfiber Release during Washing. *Environmental Science and Technology*, *51*(12). https://doi.org/10.1021/acs.est.7b01750

Hidayaturrahman, H., & Lee, T. G. (2019). A study on characteristics of microplastic in wastewater of South Korea: Identification, quantification, and fate of microplastics during treatment process. *Marine Pollution Bulletin*, *146*. https://doi.org/10.1016/j.marpolbul.2019.06.071

Horton, A. A., & Dixon, S. J. (2018). Microplastics: An introduction to environmental transport processes. *Wiley Interdisciplinary Reviews: Water*, *5*(2). https://doi.org/10.1002/WAT2.1268

Jan Kole, P., 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. In *International Journal of Environmental Research and Public Health* (Vol. 14, Issue 10). https://doi.org/10.3390/ijerph14101265

Kerrigan, J. F., Engstrom, D. R., Yee, D., Sueper, C., Erickson, P. R., Grandbois, M., McNeill, K., & Arnold, W. A. (2015). Quantification of hydroxylated polybrominated diphenyl ethers (OH-BDEs), triclosan, and related compounds in freshwater and coastal systems. *PLoS ONE*, *10*(10). https://doi.org/10.1371/journal.pone.0138805

Klasios, N., De Frond, H., Miller, E., Sedlak, M., & Rochman, C. M. (2021). Microplastics and other anthropogenic particles are prevalent in mussels from San Francisco Bay, and show no

correlation with PAHs. *Environmental Pollution*, *271*. https://doi.org/10.1016/j.envpol.2020.116260

Klöckner, P., Reemtsma, T., Eisentraut, P., Braun, U., Ruhl, A. S., & Wagner, S. (2019). Tire and road wear particles in road environment – Quantification and assessment of particle dynamics by Zn determination after density separation. *Chemosphere*, *222*. https://doi.org/10.1016/j.chemosphere.2019.01.176

Klosterhaus, S. L., Grace, R., Hamilton, M. C., & Yee, D. (2013). Method validation and reconnaissance of pharmaceuticals, personal care products, and alkylphenols in surface waters, sediments, and mussels in an urban estuary. *Environment International*, *54*. https://doi.org/10.1016/j.envint.2013.01.009

Kühn, S., van Werven, B., van Oyen, A., Meijboom, A., Bravo Rebolledo, E. L., & van Franeker, J. A. (2017). The use of potassium hydroxide (KOH) solution as a suitable approach to isolate plastics ingested by marine organisms. *Marine Pollution Bulletin*, *115*(1–2), 86–90. https://doi.org/10.1016/j.marpolbul.2016.11.034

Kukkola, A. T., Senior, G., Maes, T., Silburn, B., Bakir, A., Kröger, S., & Mayes, A. G. (2022). A large-scale study of microplastic abundance in sediment cores from the UK continental shelf and slope. *Marine Pollution Bulletin*, *178*. https://doi.org/10.1016/j.marpolbul.2022.113554

Lares, M., Ncibi, M. C., Sillanpää, M., & Sillanpää, M. (2018). Occurrence, identification and removal of microplastic particles and fibers in conventional activated sludge process and advanced MBR technology. *Water Research*, *133*. https://doi.org/10.1016/j.watres.2018.01.049

Lassen, C., Hansen, S. F., Magnusson, K., Hartmann, N. B., Rehne Jensen, P., Nielsen, T. G., & Brinch, A. (2015). *Microplastics: Occurrence, effects and sources of releases to the environment in Denmark — Welcome to DTU Research Database*. Danish Environmental Protection Agency.

Löder, M. G. J., Imhof, H. K., Ladehoff, M., Löschel, L. A., Lorenz, C., Mintenig, S., Piehl, S., Primpke, S., Schrank, I., Laforsch, C., & Gerdts, G. (2017). Enzymatic Purification of Microplastics in Environmental Samples. *Environmental Science and Technology*, *51*(24). https://doi.org/10.1021/acs.est.7b03055

Mani, T., Blarer, P., Storck, F. R., Pittroff, M., Wernicke, T., & Burkhardt-Holm, P. (2019). Repeated detection of polystyrene microbeads in the lower Rhine River. *Environmental Pollution*, *245*. https://doi.org/10.1016/j.envpol.2018.11.036

Martin, J., Lusher, A. L., & Nixon, F. C. (2022). A review of the use of microplastics in reconstructing dated sedimentary archives. In *Science of the Total Environment* (Vol. 806). https://doi.org/10.1016/j.scitotenv.2021.150818

Masura, J., Baker, J., Foster, G., & Arthur, C. (2015). Laboratory methods for the analysis of microplastics in themarine environment: Recommendations for quantifying synthet- ic particles in waters and sediments. NOAA Technical Memorandum NOS-OR&R-48. *NOAA Technical Memorandum*, *July*.

McDevitt, J. P., Criddle, C. S., Morse, M., Hale, R. C., Bott, C. B., & Rochman, C. M. (2017). Addressing the Issue of Microplastics in the Wake of the Microbead-Free Waters Act - A New Standard Can Facilitate Improved Policy. *Environmental Science and Technology*, *51*(12). https://doi.org/10.1021/acs.est.6b05812

McIlwraith, H. K., Lin, J., Erdle, L. M., Mallos, N., Diamond, M. L., & Rochman, C. M. (2019). Capturing microfibers – marketed technologies reduce microfiber emissions from washing

machines. *Marine Pollution Bulletin*, *139*. https://doi.org/10.1016/j.marpolbul.2018.12.012

McKeen, L. W. (2013). Introduction to Use of Plastics in Food Packaging. In *Plastic Films in Food Packaging: Materials, Technology and Applications*. https://doi.org/10.1016/B978-1-4557-3112-1.00001-6

Melody M. Bomgardner. (2017). The great lint migration. *C&EN Global Enterprise*, *95*(2). https://doi.org/10.1021/cen-09502-bus1

Mintenig, S. M., Int-Veen, I., Löder, M. G. J., Primpke, S., & Gerdts, G. (2017). Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging. *Water Research*, *108*, 365–372. https://doi.org/10.1016/J.WATRES.2016.11.015

Morgado, V., Palma, C., & Bettencourt da Silva, R. J. N. (2023). Determination of microplastic contamination levels and trends in vast oceanic sediment areas with uncertainty. *Science of the Total Environment*, *884*. https://doi.org/10.1016/j.scitotenv.2023.163612

Murphy, F., Ewins, C., Carbonnier, F., & Quinn, B. (2016). Wastewater Treatment Works (WwTW) as a Source of Microplastics in the Aquatic Environment. *Environmental Science and Technology*, *50*(11), 5800–5808. https://doi.org/10.1021/acs.est.5b05416

Niu, L., Li, Y., Li, Y., Hu, Q., Wang, C., Hu, J., Zhang, W., Wang, L., Zhang, C., & Zhang, H. (2021). New insights into the vertical distribution and microbial degradation of microplastics in urban river sediments. *Water Research*, *188*. https://doi.org/10.1016/j.watres.2020.116449

Nizzetto, L., Futter, M., & Langaas, S. (2016). Are Agricultural Soils Dumps for Microplastics of Urban Origin? In *Environmental Science and Technology* (Vol. 50, Issue 20). https://doi.org/10.1021/acs.est.6b04140

Pervez, R., & Wang, Y. H. (2022). Microplastic distribution within core sediments of beach and its responses to anthropogenic activities. *Marine Pollution Bulletin*, *174*. https://doi.org/10.1016/j.marpolbul.2021.113256

Pirc, U., Vidmar, M., Mozer, A., & Kržan, A. (2016). Emissions of microplastic fibers from microfiber fleece during domestic washing. *Environmental Science and Pollution Research*, *23*(21). https://doi.org/10.1007/s11356-016-7703-0

Prokić, M. D., Radovanović, T. B., Gavrić, J. P., & Faggio, C. (2019). Ecotoxicological effects of microplastics: Examination of biomarkers, current state and future perspectives. In *TrAC - Trends in Analytical Chemistry* (Vol. 111). https://doi.org/10.1016/j.trac.2018.12.001

Rochman, C. M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Bucci, K., Athey, S., Huntington, A., McIlwraith, H., Munno, K., Frond, H. De, Kolomijeca, A., Erdle, L., Grbic, J., Bayoumi, M., Borrelle, S. B., Wu, T., Santoro, S., Werbowski, L. M., … Hung, C. (2019). Rethinking microplastics as a diverse contaminant suite. In *Environmental Toxicology and Chemistry* (Vol. 38, Issue 4). https://doi.org/10.1002/etc.4371

Sambandam, M., Dhineka, K., Sivadas, S. K., Kaviarasan, T., Begum, M., Hoehn, D., Sivyer, D., Mishra, P., & Murthy, M. V. R. (2022). Occurrence, characterization, and source delineation of microplastics in the coastal waters and shelf sediments of the central east coast of India, Bay of Bengal. *Chemosphere*, *303*. https://doi.org/10.1016/j.chemosphere.2022.135135

Sánchez-Soberón, F., Sutton, R., Sedlak, M., Yee, D., Schuhmacher, M., & Park, J. S. (2020). Multi-box mass balance model of PFOA and PFOS in different regions of San Francisco Bay. *Chemosphere*, *252*. https://doi.org/10.1016/j.chemosphere.2020.126454

Sarkar, B., Dissanayake, P. D., Bolan, N. S., Dar, J. Y., Kumar, M., Haque, M. N., Mukhopadhyay, R., Ramanayaka, S., Biswas, J. K., Tsang, D. C. W., Rinklebe, J., & Ok, Y. S. (2022). Challenges and opportunities in sustainable management of microplastics and nanoplastics in the environment. In *Environmental Research* (Vol. 207). https://doi.org/10.1016/j.envres.2021.112179

Sedlak, M. D., Benskin, J. P., Wong, A., Grace, R., & Greig, D. J. (2017). Per- and polyfluoroalkyl substances (PFASs) in San Francisco Bay wildlife: Temporal trends, exposure pathways, and notable presence of precursor compounds. *Chemosphere*, *185*. https://doi.org/10.1016/j.chemosphere.2017.04.096

Sedlak, M., Sutton, R., Box, C., Sun, J., & Lin, D. (2017). *Sampling and Analysis Plan for Microplastic Monitoring in San Francisco Bay and Adjacent National Marine Sanctuaries FINAL*.

Slaughter, E., Gersberg, R. M., Watanabe, K., Rudolph, J., Stransky, C., & Novotny, T. E. (2011). Toxicity of cigarette butts, and their chemical components, to marine and freshwater fish. *Tobacco Control*, *20*(1 SUPPL). https://doi.org/10.1136/tc.2010.040170

Smith, S. V., & Hollibaugh, J. T. (2006). Water, salt, and nutrient exchanges in San Francisco Bay. *Limnology and Oceanography*, *51*(1 II). https://doi.org/10.4319/lo.2006.51.1_part_2.0504

Sommer, F., Dietze, V., Baum, A., Sauer, J., Gilge, S., Maschowski, C., & Gieré, R. (2018). Tire abrasion as a major source of microplastics in the environment. *Aerosol and Air Quality Research*, *18*(8). https://doi.org/10.4209/aaqr.2018.03.0099

Sutton, R., Chen, D., Sun, J., Greig, D. J., & Wu, Y. (2019). Characterization of brominated, chlorinated, and phosphate flame retardants in San Francisco Bay, an urban estuary. *Science of the Total Environment*, *652*. https://doi.org/10.1016/j.scitotenv.2018.10.096

Sutton, R., Mason, S. A., Stanek, S. K., Willis-Norton, E., Wren, I. F., & Box, C. (2016). Microplastic contamination in the San Francisco Bay, California, USA. *Marine Pollution Bulletin*, *109*(1). https://doi.org/10.1016/j.marpolbul.2016.05.077

Thornton Hampton, L. M., Brander, S. M., Coffin, S., Cole, M., Hermabessiere, L., Koelmans, A. A., & Rochman, C. M. (2022). Characterizing microplastic hazards: which concentration metrics and particle characteristics are most informative for understanding toxicity in aquatic organisms? *Microplastics and Nanoplastics*, *2*(1). https://doi.org/10.1186/s43591-022-00040-4

Turner, S., Horton, A. A., Rose, N. L., & Hall, C. (2019). A temporal sediment record of microplastics in an urban lake, London, UK. *Journal of Paleolimnology*, *61*(4). https://doi.org/10.1007/s10933-019-00071-7

Vogelsang, C., Lusher, A., Dadkhah, M. E., Sundvor, I., Umar, M., Ranneklev, S. B., Eidsvoll, D., & Meland, S. (2020). Microplastics in road dust - characteristics, pathways and measures REPORT SNO. 7526-2020. In *Integrated environmental assessment and management* (Issues 7361–2019).

Wang, C., Zhao, J., & Xing, B. (2021). Environmental source, fate, and toxicity of microplastics. In *Journal of Hazardous Materials* (Vol. 407). https://doi.org/10.1016/j.jhazmat.2020.124357

Wang, T., Zou, X., Li, B., Yao, Y., Zang, Z., Li, Y., Yu, W., & Wang, W. (2019). Preliminary study of the source apportionment and diversity of microplastics: Taking floating microplastics in the South China Sea as an example. *Environmental Pollution*, *245*. https://doi.org/10.1016/j.envpol.2018.10.110

Werbowski, L. M., Gilbreath, A. N., Munno, K., Zhu, X., Grbic, J., Wu, T., Sutton, R., Sedlak, M. D., Deshpande, A. D., & Rochman, C. M. (2021). Urban Stormwater Runoff: A Major Pathway for Anthropogenic Particles, Black Rubbery Fragments, and Other Types of Microplastics to Urban Receiving Waters. *ACS ES&T Water*, *1*(6). https://doi.org/10.1021/acsestwater.1c00017

Wolff, S., Kerpen, J., Prediger, J., Barkmann, L., & Müller, L. (2019). Determination of the microplastics emission in the effluent of a municipal waste water treatment plant using Raman microspectroscopy. *Water Research X*, *2*. https://doi.org/10.1016/j.wroa.2018.100014

Xue, B., Zhang, L., Li, R., Wang, Y., Guo, J., Yu, K., & Wang, S. (2020). Underestimated Microplastic Pollution Derived from Fishery Activities and "Hidden" in Deep Sediment. *Environmental Science and Technology*, *54*(4). https://doi.org/10.1021/acs.est.9b04850

Yu, J., & Ma, X. (2022). Exploring the management policy of marine microplastic litter in China: Overview, challenges and prospects. *Sustainable Production and Consumption*, *32*. https://doi.org/10.1016/j.spc.2022.05.018

Zhu, X., Munno, K., Grbic, J., Werbowski, L. M., Bikker, J., Ho, A., Guo, E., Sedlak, M., Sutton, R., Box, C., Lin, D., Gilbreath, A., Holleman, R. C., Fortin, M.-J., & Rochman, C. (2021). Holistic Assessment of Microplastics and Other Anthropogenic Microdebris in an Urban Bay Sheds Light on Their Sources and Fate. *ACS ES&T Water*, *1*(6). https://doi.org/10.1021/acsestwater.0c00292

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 Research*, *112*. https://doi.org/10.1016/j.watres.2017.01.042

Zubris, K. A. V., & Richards, B. K. (2005). Synthetic fibers as an indicator of land application of sludge. *Environmental Pollution*, *138*(2). https://doi.org/10.1016/j.envpol.2005.04.013

Discussion

In this thesis, an extensive investigation into MPs characterization was carried out in two distinct regions: Catalonia, Spain, and San Francisco, California. The study encompassed diverse environments, including WWTPs, DWTPs, OAs, and marine sediments. The research followed a consistent methodology, which consisted of MPs sampling, sample pre-treatment, visual sorting, and conclusive spectroscopic confirmation using advanced techniques like FTIR and Raman spectroscopy.

Sample pre-treatment was tailored to the specific nature and demands of each sample type. This entailed established methods commonly employed in MPs research, such as advanced Fenton's oxidation, alkaline, and enzymatic digestion, as well as density separation, aimed at eliminating organic and inorganic matter. Notably, the density separation approach employed the floating method, enabling MPs to surface, with subsequent extraction of the supernatant containing microplastics, while denser inorganic components remained at the solution's bottom.

Following pre-treatment, a visual examination was conducted utilizing stereoscopic and optical microscopes. During this phase, suspected MPs were classified into prevalent morphologies: fibers, films, and fragments. In unique instances, additional morphologies like pellets or fiber balls were identified and recorded. Additionally, particle sizes were determined through sieving, employing stainless-steel sieves with varying mesh sizes. Morphology and size distribution analysis were imperative due to their significance in understanding MPs' behavior and impact.

The subsequent step involved a chemical analysis of subsample compositions. Particles exceeding 0.5 mm were manually identified using ATR spectroscopy, while those smaller were prepared on calcium fluoride slides for FTIR mapping. Spectral identification relied on comparisons with established data libraries.

The results of this thesis provide valuable data that advance our understanding of the sources and pathways of these contaminants, as well as their movement and fate within these compartments and their release into the natural environment.

Wastewater treatment plants

The study presents an investigation into the prevalence, fate, and removal of MPs within WWTPs, shedding light on their distribution and potential environmental implications. MPs were detected across all WWTP units, from influent to effluent, as well as in sludge. The analysis encompassed different morphology types and size ranges, revealing concentrations mainly below 2 mm. Interestingly, seasonal variations in MPs concentrations were observed, with

higher levels during winter, potentially attributed to precipitation and altered water consumption patterns.

The removal efficiency of MPs throughout the treatment process emerged in different percentages. Remarkably, removal rates exhibited substantial variability across treatment stages and morphology types. Secondary clarifiers, as the last treatment process, proved highly effective in MPs removal for both sampling techniques. Composite samples collected over a 24-hour period and filtered samples revealed slightly differing values, however, the study reported notably high removal rates exceeding 96% across all effluent samples, with some reaching up to 99%, corroborating the efficacy of WWTPs in reducing microplastic loads in treated water.

A significant concern arises from the accumulation of microplastics in sludge, particularly the implications of utilizing sludge as fertilizer. The substantial concentrations of MPs in thickened and dry sludge highlight the potential for reintroducing MPs into the environment through agricultural practices. The delicate balance between circular economy benefits and environmental risks necessitates a nuanced approach to sludge management and reuse.

The study focused also on categorizing MPs based on their predominant types: fibers, fragments, and films. Morphological diversity holds significance due to its influence on removal efficacy and interactions with other contaminants. Notably, the distribution of MPs morphology varied across different treatment units, with fibers emerging as the dominant type. Fibers, followed by fragments and films, constituted the primary morphologies observed within WWTP, consistent with previous studies. Pellets, although rare, were also detected, however at negligible concentrations. These findings resonate with the broader environmental context, where fibers released from washing processes and films from plastic packaging bags contribute significantly to MPs pollution. Moreover, fibers often aggregate into clusters, forming fiber balls, which can impact removal efficiency and necessitating specialized handling techniques.

The study investigated also the size distribution of MPs, revealing variations across treatment units and sampling periods. While no distinct size-dependent removal trends were evident, seasonal variations in particle size distribution emerged, underscoring the complexity of MPs behavior within WWTP.

Further exploration into MPs composition unveiled a diverse range of polymers, with polyethylene (PE), synthetic cellulose, and polypropylene (PP) being the most abundant. These polymers closely aligned with the most widely produced plastics globally, reflecting their prevalence in everyday materials. The study's findings offer insights into the polymer landscape

of WWTPs, potentially guiding future research and prioritizing mitigation efforts based on prevalent polymer types.

Drinking water treatment plants

This study examines the presence, removal, and composition of MPs in a DWTP located in Catalonia, Spain, providing insights into the abundance and behavior of MPs throughout the treatment process. The DWTP study is connected to the WWTP study in the same region, emphasizing the broader context of MPs pollution in the local water systems.

MPs were detected across all treatment stages of the DWTP, from influent to effluent, with their concentrations and removal efficiencies varying throughout the treatment process. The most prevalent morphological type in the DWTP influent was fibers, followed by fragments and films which were also found in the previous WWTP study. The concentration of MPs in the river water (influent) was 4.23 ± 1.26 MPs/L, primarily in the size range of 500-100 µm. Notably, the DWTP's overall MPs removal rate was 98.3%, demonstrating the effective removal of MPs from the water during the treatment process.

The study identifies the treatment units responsible for the highest MPs removal rates: clarifiers and sand filters. These stages notably reduced fiber concentration, likely due to the ability of fibers to form flocs and settle, aided by their weight and surface characteristics. However, the activated carbon filter and subsequent treatment units, such as microfiltration, electrodialysis, and remineralization, showed a slight increase in MPs content. This phenomenon may be attributed to the breakdown of MPs under shear forces or the possible release of MPs from DWTP materials. The study suggests that remineralization and post-chlorination stages may contribute to MPs pollution in the treated water.

In terms of composition, a wide range of polymers were identified in MPs, within the DWTP, including synthetic cellulose, PES, PP, PA, PE, and PET, among others. The prevalence of these polymers aligns with their common usage in various industries. Synthetic cellulose was a significant component, previously reported as a non-plastic particle in some studies. However, this study considered it a synthetic polymer due to its behavior after pre-treatment. The composition analysis highlights the potential sources of MPs, including textiles, packaging, and various consumer products.

The study's findings underscore the importance of proper management of DWTP sludge, as it accumulates MPs removed during the treatment process. Both dry sludge and water from sludge centrifugation were found to contain MPs, emphasizing the need to prevent their introduction

into terrestrial ecosystems through agricultural practices or discharge. The study recommends the development of technologies to effectively remove MPs from sludge to support a more sustainable approach to plastic waste management.

Despite the presence of MPs in the DWTP effluent, with a concentration of 0.075 ± 0.019 MPs/L, the overall intake of MPs through drinking tap water was estimated to be relatively low compared to other sources, such as food consumption.

Organic waste amendments

The findings from both previous studies of WWTP and DWTP revealed the accumulation of MPs in sludge, emphasizing the need for proper waste disposal practices. Specifically, the studies highlight that the agricultural usage of sludge should be avoided due to the potential for MPs to contaminate soil and impact the environment. Despite this clear recommendation, the practice of using sludge as fertilizers and organic waste amendments continues in Spain. This practice raises concerns about the potential reentry of MPs into the soil and subsequent ecological impact. To address this pressing issue, the third chapter of the doctoral thesis delves into the analysis of organic waste amendments within Catalonia, Spain, aiming to provide an understanding of the current practices and potential environmental implications. By investigating the use of organic waste amendments, this chapter seeks to contribute valuable insights for policymakers and stakeholders to make informed decisions regarding sludge management practices and mitigate the potential risks associated with MPs in terrestrial ecosystems.

The study conducts a comprehensive analysis of both micro and macro plastic particles present in various organic waste amendments. The analysis involves two replicates for each sample, incorporating statistical measures of variability for accuracy enhancement. The research focuses on samples expressed in terms of plastic item count per kilogram of dry sample, encompassing seven distinct types of organic waste amendments. MPs, ranging from 0.02 mm to 5 mm in size, and macro plastics, larger than 5 mm, were examined in the samples.

The results highlight varying levels of micro and macro plastic contamination across different organic waste amendment types. Sewage sludge displayed the highest plastic pollution concentration, followed by digestate from municipal waste and agri-food industry waste. Notably, fragments were the predominant morphology in most samples except the sewage sludge which was mainly composed of fibers.

Chemical composition analysis revealed prevalent polymers such as polyethylene (PE), polypropylene (PP), and polystyrene (PS) across the samples. These findings align with global plastic production statistics. Furthermore, fiber composition analysis identified polyester (PES) as the most abundant polymer, with variations observed in other types of fibers.

Marine sediments

The concluding chapter of this thesis constitutes a pivotal investigation focused on marine sediments within the San Francisco Bay area, an extensively urbanized and heavily polluted region with a pronounced presence of MPs. Recognizing marine sediments as the ultimate repository for MPs, this study rigorously examines spatial and temporal trends in MPs across diverse locations within the Bay area. Through the analysis of surface sediment grab samples and core sediments, this chapter unveils concentrations of MPs that surpass those documented in numerous global studies. This important discovery highlights the urgent and serious nature of the MPs issue, emphasizing the need for awareness and proactive steps to reduce their impact. By revealing the high levels of MPs in a heavily urbanized and polluted area, this study serves as a strong reminder of the necessity to address MPs concerns promptly. It urges both stakeholders and policy-makers to pay close attention to developing effective ways to tackle this problem. The findings in this chapter also emphasize the wider environmental impact and the urgent need for collective actions to fight MPs pollution at its root and protect marine ecosystems.

The research conducted focuses on the occurrence and characteristics MPs within the marine sediments of the San Francisco Bay area. The study encompasses various sampling locations, revealing significant variability in MPs concentrations. The concentration levels ranged from 2.1 to 11.9 MPs per gram of dry weight (dw) in surface sediments across the Bay. Notably, the Lower South Bay displayed the highest MPs concentration, attributed to factors such as limited water circulation and the input of wastewater and urban stormwater discharges. Similar elevated contaminant levels in South and Lower South Bay sediments have been reported in prior studies. Comparative analyses with other global studies emphasize the remarkable concentration of MPs in this urbanized ecosystem. Fibers constitute the dominant MPs morphology, with sizes ranging from 25 µm to 5 mm, indicating their prevalence. Synthetic textiles are identified as a significant source of these fibrous MPs. Various pathways contribute to their release into the environment, including textile shedding, washing, and other industrial activities. Additionally, tire wear is identified as a significant contributor, with rubbery particles likely originating from tire fragments. The study highlights the formation of fiber balls, particularly in the South Bay area, possibly originating from aggregation mechanisms.

Sediment core analysis presents a novel investigation, as previous sediment core MPs studies were absent in San Francisco Bay. The study examines two sediment cores, revealing variable MPs concentrations across core depths. Contrary to expectations, MPs were found even in the bottom sediment layers, suggesting long-term reworking and accumulation processes. The predominant morphology in core sediments, as in surface samples, is fibers, followed by fragments and films.

Polymer composition analysis of MPs is crucial for understanding sources and potential impacts. Synthetic cellulose, polypropylene (PP), polystyrene (PS), polyethylene (PE), poly (propylene-ethylene) copolymer (PE-PP), polyamide (PA), and polyester (PES) emerge as the most prevalent polymers in surface sediments. A similar analysis of core sediments reveals variations in polymer types and proportions. The study aims to identify potential pollution sources based on MPs characteristics, highlighting textiles, fishing-related materials, cigarette filters, single-use bags, microbeads, personal care products, tire particles, recycled tire material, and road markings as potential contributors to MPs pollution.

The culmination of diverse MPs studies across various environmental compartments underscores the complexity and urgency of addressing this pervasive pollution issue. From WWTP, DWTP, and organic waste amendments in Catalonia, Spain, to marine sediments in the San Francisco Bay area, these studies collectively shed light on the widespread presence, sources, distribution, and potential impacts of MPs. Examining the interplay between these studies offers crucial insights into the link between terrestrial and aquatic ecosystems and underscores the need for concerted efforts to mitigate MPs pollution.

The studies conducted in Spain's WWTP and DWTP emphasize the ubiquitous nature of MPs, as these particles persist even after conventional treatment processes. The contamination of both drinking water and wastewater effluents underscores the potential for human exposure to MPs, highlighting a critical concern for public health. The presence of MPs in treated drinking water also underscores the limitations of current treatment methods in removing these small and diverse particles. Furthermore, the incorporation of organic waste amendments into agricultural soils reveals a potential pathway for MPs to enter terrestrial ecosystems, emphasizing the intricate connections between land and water environments. This suggests that agricultural practices and organic waste management strategies require careful evaluation to prevent further pollution.

The investigation of MPs in marine sediments of the San Francisco Bay area offers a comprehensive understanding of MPs distribution, sources, and morphology in a highly

urbanized and polluted environment. The identification of various MPs shapes, including fibers, fragments, and films, suggests diverse sources contributing to the pollution. The prevalence of fibers, particularly from synthetic textiles, in both sediments and organisms underscores the need for stringent control measures throughout the product lifecycle. Additionally, the identification of tire wear particles as a significant contributor highlights the role of transportation-related activities in MPs pollution. The persistence of MPs even in deep sediment layers underscores the long-term impacts of pollution and the importance of comprehensive mitigation strategies.

The linkages between these studies lie in the intricate web of pollution sources, pathways, and impacts that extend from urban areas to aquatic ecosystems. The studies collectively highlight the potential for MPs to infiltrate water sources, traverse treatment systems, accumulate in soils, and impact marine environments. These findings underscore the need for holistic and interdisciplinary approaches to tackle MPs pollution effectively. Collaborative efforts between government agencies, industries, researchers, and communities are essential to develop and implement effective measures to mitigate MPs pollution.

Moving forward, a multifaceted approach is warranted. Firstly, stricter regulations and policies should be enforced to reduce the production, use, and disposal of single-use plastics and other potential sources of MPs. Enhanced treatment methods in both WWTPs and DWTPs are imperative to minimize the release of MPs into water bodies. Innovations in waste management and recycling practices can help curb the influx of MPs into agricultural soils. Additionally, public awareness campaigns and educational initiatives can encourage responsible consumer behavior and foster a culture of sustainability. Long-term monitoring and continued research are essential to assess the efficacy of mitigation measures, track emerging sources of MPs, and understand the potential ecological and human health risks.

These studies are of important as they present the extent of MPs pollution and its pervasive presence across ecosystems. They underscore the need for immediate action to prevent further contamination and protect the environment and human well-being. The collective findings emphasize the urgency of addressing MPs pollution at its source, implementing effective treatment strategies, and fostering a shift towards more sustainable consumption and waste management practices. The studies serve as a clarion call for global cooperation to combat one of the most pressing environmental challenges of our time.

Conclusion

Wastewater Treatment Plants

The investigation into MPs fate within WWTPs underscores their vital role in curtailing microplastic pollution. The study revealed remarkable reduction efficiencies, especially within pre-treatment and primary clarifier units, where MPs concentrations were significantly lowered by up to 99%. However, the impact of secondary treatment on microplastic reduction was found to be less pronounced. The morphologies of MPs, predominantly fibers, fragments, and films originating from diverse sources like washing processes, were elucidated. The presence of various polymers, such as PE, PP, PES, among others, underscores the complexity of microplastic compositions. While WWTPs act as crucial barriers to microplastic dispersion, an equally critical facet is the management of sludge, where microplastics tend to accumulate. A systematic approach to sludge handling is essential to prevent the inadvertent transmission of microplastics to terrestrial ecosystems.

Drinking Water Treatment Plants

The assessment of microplastic presence within Drinking Water Treatment Plants (DWTPs) highlighted the intricate interplay between treatment processes and microplastic retention. DWTPs exhibit the potential for substantial microplastic reduction, with clarifiers and sand filtration units emerging as effective microplastic removers, achieving impressive removal efficiencies exceeding 99%. However, it is noteworthy that residual microplastics persist in the effluent streams of DWTPs, emphasizing the necessity for vigilant monitoring and control. The absence of standardized analytical methods across European DWTPs underscores the complexity of microplastic assessment. In this context, effective management practices for both treated water and sludge assume paramount importance to prevent the unintended propagation of microplastics, prompting the exploration of innovative technologies for sludge microplastic removal.

Marine Sediments:

The comprehensive investigation into microplastics within marine sediments of the San Francisco Bay area illuminates the pervasive nature of microplastic contamination across this urban coastal environment. The varying concentrations observed across distinct locations and sediment depths underscore the dynamic nature of microplastic distribution. Notably, fibers, fragments, and films emerged as the prevalent microplastic morphologies, highlighting the diverse sources of these pollutants. The identification of polymers such as PE, PP, PES, among

others, elucidates the complex composition of microplastics. Importantly, the increasing concentrations from bottom to top sediment layers accentuate the cumulative nature of microplastic deposition over time. This study not only enriches our understanding of microplastic pollution in urban coastal areas but also emphasizes the indispensable need for sustained monitoring and strategic interventions to mitigate its ecological impact.

Organic Waste Amendments:

The in-depth exploration of microplastic introduction via organic waste amendments underscores their substantial role in terrestrial microplastic contamination. The meticulous examination of various organic waste amendment treatments revealed a spectrum of micro and macro plastic particle concentrations. While macroplastics are less prevalent, their larger mass and surface area render them a concern. In light of these findings, a multi-faceted strategy emerges. Implementing source separation, effective pre-treatment measures, enhanced sorting practices, and comprehensive awareness campaigns collectively hold the potential to diminish microplastic contamination. Furthermore, fostering research, rigorous monitoring, and the establishment of stringent regulations are imperative to comprehensively comprehend and control microplastic pollution. The pursuit of innovative technologies, coupled with a drive for sustainable plastic use and collaborative engagement across stakeholders, is pivotal in forging a path toward mitigating microplastic pollution within organic waste amendments. This comprehensive approach aligns with the broader goal of safeguarding ecosystems and fostering sustainable waste management practices.

Literature

Al Nahian, S., Rakib, M. R. J., Haider, S. M. B., Kumar, R., Mohsen, M., Sharma, P., & Khandaker, M. U. (2022). Occurrence, spatial distribution, and risk assessment of microplastics in surface water and sediments of Saint Martin Island in the Bay of Bengal. Marine Pollution Bulletin, 179. https://doi.org/10.1016/j.marpolbul.2022.113720

Alimi, O.S., Farner Budarz, J., Hernandez, L.M., Tufenkji, N. (2018). Microplastics and Nanoplastics in Aquatic Environments: Aggregation, Deposition, and Enhanced Contaminant Transport. Environmental Science and Technology, 52(4), 1704-1724. https://doi.org/10.1021/acs.est.7b05559.

Allen, S., Allen, D., Phoenix, V.R., Le, R.G., Jimenez, P.D., Simonneau, A., Binet, S., Galop, D. (2019). Atmospheric transport and deposition of microplastics in a remote mountain catchment. Nature Geoscience, 12, 339–344. https://doi.org/10.1038/s41561-019-0335-5.

Amrutha, K., & Warrier, A.K. (2020). The first report on the source to-sink characterization of microplastic pollution from a riverine environment in tropical India. Science of the Total Environment, 739, 140377.

anak Alexander Tampang, A. M., & Mohan Viswanathan, P. (2022). Occurrence, distribution and sources of microplastics in beach sediments of Miri coast, NW Borneo. Chemosphere, 305. https://doi.org/10.1016/j.chemosphere.2022.135368

Andrady, A. L. (2011). Microplastics in the marine environment. Marine pollution bulletin, 62(8), 1596-1605.

Andrady, A.L., & Neal, M.A. (2009). Applications and societal benefits of plastics. Philosophical Transactions of the Royal Society B: Biological Sciences, 364(1526), 1977-1984.

Arthur, C., Baker, J. E., & Bamford, H. A. (2009). Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris, September 9-11, 2008, University of Washington Tacoma, Tacoma, WA, USA.

Ashton, K., Holmes, L., & Turner, A. (2010). Association of metals with plastic production pellets in the marine environment. Marine Pollution Bulletin, 60(11), 2050–2055. https://doi.org/10.1016/J.MARPOLBUL.2010.07.014

Assured Biosolids Limitied. Assured Biosolids Limited (ABL) Biosolids Assurance Scheme (BAS) Position Statement on the potential impact of microplastics on biosolids recycling to agricultural land. Available at: https://assuredbiosolids.co.uk/wp-content/uploads/2018/07/M icroplastics-Position-Statement-27th-July-2018.pdf, 2018.

Baensch-Baltruschat, B., Kocher, B., Stock, F., & Reifferscheid, G. (2020). Tyre and road wear particles (TRWP) - A review of generation, properties, emissions, human health risk, ecotoxicity, and fate in the environment. In Science of the Total Environment (Vol. 733). https://doi.org/10.1016/j.scitotenv.2020.137823

Ballent, A., Corcoran, P. L., Madden, O., Helm, P. A., & Longstaffe, F. J. (2016). Sources and sinks of microplastics in Canadian Lake Ontario nearshore, tributary and beach sediments. Marine Pollution Bulletin, 110(1). https://doi.org/10.1016/j.marpolbul.2016.06.037

Barceló, D., Picó, Y., & Alfarhan, A. H. (2023). Microplastics: Detection in human samples, cell line studies, and health impacts. Environmental toxicology and pharmacology, 104204.

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. https://doi.org/10.1016/j.chemosphere.2019.124593

Bayo, J., Rojo, D., & Olmos, S. (2022). Weathering indices of microplastics along marine and coastal sediments from the harbor of Cartagena (Spain) and its adjoining urban beach. Marine Pollution Bulletin, 178. https://doi.org/10.1016/j.marpolbul.2022.113647

Ben-David, E.A., Habibi, M., Haddad, E., Hasanin, M., Angel, D.L., Booth, A.M., Sabbah, I. (2021). Microplastic distributions in a domestic wastewater treatment plant: Removal efficiency, seasonal variation and influence of sampling technique. Science of the Total Environment, 752, 141880. https://doi.org/10.1016/j.scitotenv.2020.141880.

Ben-Haddad, M., Abelouah, M. R., Hajji, S., Rangel-Buitrago, N., Hamadi, F., & Alla, A. A. (2022). Microplastics pollution in sediments of Moroccan urban beaches: The Taghazout coast as a case study. Marine Pollution Bulletin, 180. https://doi.org/10.1016/j.marpolbul.2022.113765

Blair, R. M., Waldron, S., & Gauchotte-Lindsay, C. (2019). Average daily flow of microplastics through a tertiary wastewater treatment plant over a ten-month period. Water Research, 163, 114909. https://doi.org/10.1016/J.WATRES.2019.114909

Blair, R.M., Waldron, S., Phoenix, V.R., Gauchotte-Lindsay, C. (2019). Microscopy and elemental analysis characterisation of microplastics in sediment of a freshwater urban river in Scotland, UK. Environmental Science and Pollution Research, 26(12), 12491–12504. https://doi.org/10.1007/s11356-019-04678-1.

Blasing, M., & Amelung, W. (2018). Plastics in soil: analytical methods and possible sources. Science of the Total Environment, 612, 422–435.

Bondelind, M., Sokolova1, E., Nguyen, A., Karlsson, D., Karlsson, A., Bjorklund, K. (2020). Hydrodynamic modelling of traffic-related microplastics discharged with stormwater into the gota river in Sweden. Environmental Science and Pollution Research, 27, 24218–24230.

Boucher, J., & Friot, D. (2017). Primary microplastics in the oceans: A global evaluation of sources. In Primary microplastics in the oceans: A global evaluation of sources. https://doi.org/10.2305/iucn.ch.2017.01.en

Braun, M., Mail, M., Heyse, R., & Amelung, W. (2021). Plastic in compost: prevalence and potential input into agricultural and horticultural soils. Science of The Total Environment, 760, 143335.

Bronzo, L., Lusher, A. L., Schøyen, M., & Morigi, C. (2021). Accumulation and distribution of microplastics in coastal sediments from the inner Oslofjord, Norway. Marine Pollution Bulletin, 173. https://doi.org/10.1016/j.marpolbul.2021.113076

Browne, M. A., Crump, P., Niven, S. J., Teuten, E., Tonkin, A., Galloway, T., & Thompson, R. (2011). Accumulation of microplastic on shorelines woldwide: Sources and sinks. Environmental Science and Technology, 45(21), 9175–9179. https://doi.org/10.1021/es201811s

Campbell, D., Pethrick, R.A., White, J.R. (2000). Polymer Characterization (Physical Techniques). Second Edition, Stanley Thornes LTD.

Capillo, G., Savoca, S., Panarello, G., Mancuso, M., Branca, C., Romano, V., D'Angelo, G., Bottari, T., & Spanò, N. (2020). Quali-quantitative analysis of plastics and synthetic microfibers found in demersal species from Southern Tyrrhenian Sea (Central Mediterranean). Marine Pollution Bulletin, 150. https://doi.org/10.1016/j.marpolbul.2019.110596

Carney Almroth, B. M., Åström, L., Roslund, S., Petersson, H., Johansson, M., & Persson, N. K. (2018). Quantifying shedding of synthetic fibers from textiles; a source of microplastics released into the environment. Environmental Science and Pollution Research, 25(2). https://doi.org/10.1007/s11356-017-0528-7

Carpenter, E. J., & Smith Jr, K. L. (1972). Plastics on the Sargasso Sea surface. Science, 175(4027), 1240-1241.

Carr, S. A., Liu, J., & Tesoro, A. G. (2016). Transport and fate of microplastic particles in wastewater treatment plants. Water Research, 91, 174–182. https://doi.org/10.1016/J.WATRES.2016.01.002

Carrasco Silva, G., Galleguillos Madrid, F. M., Hernández, D., Pincheira, G., Peralta, A. K., Urrestarazu Gavilán, M., ... & Fuentes Peñailillo, F. (2021). Microplastics and Their Effect in Horticultural Crops: Food Safety and Plant Stress.

Catarino, A.I., Macchia, V., Sanderson, W.G., Thompson, R.C., Henry, T.B. (2018). Low levels of microplastics (MP) in wild mussels indicate that MP ingestion by humans is minimal compared to exposure via household fibres fallout during a meal. Environmental Pollution, 237, 675-684. https://doi.org/10.1016/j.envpol.2018.02.069.

Chae, Y., & An, Y.J. (2018). Current research trends on plastic pollution and ecological impacts on the soil ecosystem: a review. Environmental Pollution, 240, 387–395.

Chen, G., Feng, Q., Wang, J. (2020). Mini-review of microplastics in the atmosphere and their risks to humans. Science of the Total Environment, 703, 135504. https://doi.org/10.1016/j.scitotenv.2019.135504.

Cherniak, S.L., Almuhtaram, H., McKie, M.J., Hermabessiere, L., Yuan, C., Rochman, C.M., Andrews, R.C. (2022). Conventional and biological treatment for the removal of microplastics from drinking water. Chemosphere, 288, 132587. https://doi.org/10.1016/j.chemosphere.2021.132587.

Coates, J. (2000). Interpretation of Infrared Spectra, A Practical Approach in Encyclopedia of Analytical Chemistry, R.A. Meyers (Ed.), John Wiley & Sons Ltd.

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

Conesa, J.A., Ortuño, N. (2022). Reuse of Water Contaminated by Microplastics, the Effectiveness of Filtration Processes: A Review. Energies, 15, 2432. https://doi.org/10.3390/en15072432.

Constant, M., Ludwig, W., Kerhervé, P., Sola, J., Charrière, B., Sanchez-Vidal, A., Canals, M., Heussner, S. (2020). Microplastic fluxes in a large and a small Mediterranean river catchment: The Têt and the Rhône, Northwestern Mediterranean Sea. Science of the Total Environment, 716, 136984. https://doi.org/10.1016/j.scitotenv.2020.136984.

Costa, M. F., Ivar do Sul, J. A., Silva-Cavalcanti, J. S., Araújo, M. C. B., Spengler, Â., & Tourinho, P. S. (2010). On the importance of size of plastic fragments and pellets on the strandline: a snapshot of a Brazilian beach. Environmental monitoring and assessment, 168, 299-304.

Cox, K.D., Covernton, G.A., Davies, H.L., Dower, J.F., Juanes, F., Dudas, S.E. (2019). Human Consumption of Microplastics. Environmental Science and Technology, 53(12), 7068-7074. https://doi.org/10.1021/acs.est.9b01517.

D'Angelo, S., & Meccariello, R. (2021). Microplastics: a threat for male fertility. International Journal of Environmental Research and Public Health, 18(5), 2392. https://doi.org/10.3390/IJERPH18052392.

da Silva Brito, W.A., Mutter, F., Wende, K., Cecchini, A.L., Schmidt, A., Bekeschus, S. (2022). Consequences of nano and microplastic exposure in rodent models: the known and unknown. Particle and Fibre Toxicology, 1–24. https://doi.org/10.1186/S12989-022-00473-Y.

Dadashi, S., Sepanlou, M. G., & Mirnia, S. K. (2019). Influence organic compost compounds on soil chemical and physical properties. International Journal of Human Capital in Urban Management, 4(1).

Dalmau-Soler, J., Ballesteros-Cano, R., Ma, R., Boleda, R., Paraira, M., Ferrer, N., Lacorte, S. (2021). Microplastics from headwaters to tap water: Occurrence and removal in a drinking water treatment plant in Barcelona Metropolitan area (Catalonia, NE Spain). Environmental Science and Pollution Research, 28, 59462-59472. https://doi.org/10.1007/s11356-021-13220-1.

de Souza Machado, A.A., Lau, C.W., Till, J., Kloas, W., Lehmann, A., Becker, R., & Rillig, M. C. (2018). Impacts of microplastics on the soil biophysical environment. Environmental Science & Technology, 52(17), 9656–9665. https://doi.org/10.1021/acs.est.8b01308.

Dehaut, A., Cassone, A. L., Frère, L., Hermabessiere, L., Himber, C., Rinnert, E., Rivière, G., Lambert, C., Soudant, P., Huvet, A., Duflos, G., & Paul-Pont, I. (2016). Microplastics in seafood: Benchmark protocol for their extraction and characterization. Environmental Pollution, 215, 223–233. https://doi.org/10.1016/J.ENVPOL.2016.05.018

Deme, G. G., Ewusi-Mensah, D., Olagbaju, O. A., Okeke, E. S., Okoye, C. O., Odii, E. C., Ejeromedoghene, O., Igun, E., Onyekwere, J. O., Oderinde, O. K., & Sanganyado, E. (2022). Macro problems from microplastics: Toward a sustainable policy framework for managing microplastic waste in Africa. In Science of the Total Environment (Vol. 804). https://doi.org/10.1016/j.scitotenv.2021.150170

Desforges, J.W., Galbraith, M., Dangerfield, N., & Ross, P.S. (2013). Widespread distribution of microplastics in surface water seawater in the North-East Pacific Ocean. Marine Pollution Bulletin, 79, 94–99.

Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption (recast). Official Journal of the European Union, L 435, 1-62.

Dong, M., Luo, Z., Jiang, Q., Xing, X., Zhang, Q., & Sun, Y. (2020). The rapid increases in microplastics in urban lake sediments. Scientific Reports, 10(1). https://doi.org/10.1038/s41598-020-57933-8

Dris, R., Gasperi, J., Mirande, C., Mandin, C., Guerrouache, M., Langlois, V., & Tassin, B. (2017). A first overview of textile fibers, including microplastics, in indoor and outdoor environments. Environmental Pollution, 221. https://doi.org/10.1016/j.envpol.2016.12.013

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. Environmental Chemistry, 12(5), 592–599. https://doi.org/10.1071/EN14167.

Dris, R., Gasperi, J., Saad, M., Mirande, C., & Tassin, B. (2016). Synthetic fibers in atmospheric fallout: a source of microplastics in the environment. Marine Pollution Bulletin, 104(1–2), 290–293. https://doi.org/10.1016/j.marpolbul.2016.01.006.

Dronjak, L., Exposito, N., Rovira, J., Florencio, K., Emiliano, P., Corzo, B., Schuhmacher, M., Valero, F., & Sierra, J. (2022). Screening of microplastics in water and sludge lines of a drinking water treatment plant in Catalonia, Spain. Water Research, 225, 119185. https://doi.org/10.1016/J.WATRES.2022.119185

Dronjak, L., Exposito, N., Sierra, J., Schuhmacher, M., Florencio, K., Corzo, B., & Rovira, J. (2023). Tracing the fate of microplastic in wastewater treatment plant: A multi-stage analysis of treatment units and sludge. Environmental Pollution, 333. https://doi.org/10.1016/j.envpol.2023.122072

Edil, T. B. (2008). A review of environmental impacts and environmental applications of shredded scrap tires. Proceedings of the International Workshop on Scrap Tire Derived Geomaterials - Opportunities and Challenges, IW-TDGM 2007.

Edo, C., Fernández-Piñas, F., & Rosal, R. (2022). Microplastics identification and quantification in the composted organic fraction of municipal solid waste. Science of The Total Environment, 813, 151902.

Eisentraut, P., Dümichen, E., Ruhl, A. S., Jekel, M., Albrecht, M., Gehde, M., & Braun, U. (2018). Two Birds with One Stone - Fast and Simultaneous Analysis of Microplastics: Microparticles Derived from Thermoplastics and Tire Wear. Environmental Science and Technology Letters, 5(10). https://doi.org/10.1021/acs.estlett.8b00446

Elkhatib, D., Oyanedel-Craver, V. (2020). A Critical Review of Extraction and Identification Methods of Microplastics in Wastewater and Drinking Water. Environmental Science and Technology, 54(12), 7037-7049. https://doi.org/10.1021/acs.est.9b06672.

Ellen MacArthur Foundation. (2016). The New Plastics Economy: Rethinking the future of plastics. Ellen MacArthur Foundation, January.

Eo, S., Hong, S. H., Song, Y. K., Han, G. M., Seo, S., & Shim, W. J. (2021). Prevalence of small high-density microplastics in the continental shelf and deep sea waters of East Asia. Water Research, 200. https://doi.org/10.1016/j.watres.2021.117238

Eriksen, M., Mason, S., Wilson, S., Box, C., Zellers, A., Edwards, W., & Amato, S. (2013). Microplastic pollution in the surface waters of the Laurentian Great Lakes. Marine Pollution Bulletin, 77(1–2), 177–182.

Erni-Cassola, G., Gibson, M.I., Thompson, R.C., Christie-Oleza, J.A. (2017). Lost, but Found with Nile Red: A Novel Method for Detecting and Quantifying Small Microplastics (1 mm to 20 µm) in Environmental Samples. Environmental Science and Technology, 51(23), 13641-13648. https://doi.org/10.1021/acs.est.7b04512.

Espinosa, C., Esteban, M.A., & Cuesta, A. (2016). Microplastics in aquatic environments and their toxicological implications for fish. In S. Soloneski & M.L. Larramendy (Eds.), Toxicology - New Aspects to This Scientific Conundrum (pp. 113–145). IntechOpen. https://doi.org/10.5772/64815.

Essel, R, Engel, L, Carus, M, and Ahrens, RH (2015) Sources of microplastics relevant to marine protection in Germany, Publication as pdf : www umweltbundesamt de/ publikationen/sources-of-microplastics-relevant-to-marine, 45pp

Eunomia. (2018). Investigating options for reducing releases in the aquatic environment of microplastics emitted by (but not intentionally added in) products. In Report for DG Env EC: Vol. Vol. 62, N (Issue February).

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. https://doi.org/10.1016/j.scitotenv.2021.147453

Expósito, N., Rovira, J., Sierra, J., Gimenez, G., Domingo, J. L., & Schuhmacher, M. (2022). Levels of microplastics and their characteristics in molluscs from North-West Mediterranean Sea: Human intake. Marine Pollution Bulletin, 181. https://doi.org/10.1016/j.marpolbul.2022.113843

FDA. (2015). The Microbead-Free Waters Act: FAQs | FDA. U.S Food and Drug Administration.

Fischer, E.K., Paglialonga, L., Czech, E., & Tamminga, M. (2016). Microplastic pollution in lakes and lake shoreline sediments–a case study on Lake Bolsena and Lake Chiusi (central Italy). Environmental Pollution, 213, 648–657.

Franco, A. A., Arellano, J. M., Albendín, G., Rodríguez-Barroso, R., Quiroga, J. M., & Coello, M. D. (2021). Microplastic pollution in wastewater treatment plants in the city of Cádiz: Abundance, removal efficiency and presence in receiving water body. Science of the Total Environment, 776. https://doi.org/10.1016/j.scitotenv.2021.145795

Franco, A. A., Arellano, J. M., Albendín, G., Rodríguez-Barroso, R., Zahedi, S., Quiroga, J. M., & Coello, M. D. (2020). Mapping microplastics in Cadiz (Spain): Occurrence of microplastics in municipal and industrial wastewaters. Journal of Water Process Engineering, 38, 101596. https://doi.org/10.1016/J.JWPE.2020.101596

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. Marine Pollution Bulletin, 85(1), 156–163.

Frias, J. P., & Nash, R. (2019). Microplastics: Finding a consensus on the definition. Marine pollution bulletin, 138, 145-147.

Frias, J., Pagter, E., Nash, R., O'Connor, I., Carretero, O., Filgueiras, A., ... & Gerdts, G. (2018). Standardised protocol for monitoring microplastics in sediments. Deliverable 4.2.

Fries, E., Dekiff, J. H., Willmeyer, J., Nuelle, M. T., Ebert, M., & Remy, D. (2013). Identification of polymer types and additives in marine microplastic particles using pyrolysis-GC/MS and scanning electron microscopy. Environmental Sciences: Processes and Impacts, 15(10), 1949–1956. https://doi.org/10.1039/c3em00214d

Gao, J., Pan, S., Li, P., Wang, L., Hou, R., Wu, W.M., & Hou, D. (2021). Vertical migration of microplastics in porous media: multiple controlling factors under wet-dry cycling. Journal of Hazardous Materials, 419, 126413.

Gatidou, G., Arvaniti, O. S., & Stasinakis, A. S. (2019a). Review on the occurrence and fate of microplastics in Sewage Treatment Plants. Journal of Hazardous Materials, 367, 504–512. https://doi.org/10.1016/J.JHAZMAT.2018.12.081

Gautam, R., Jo, J.H., Acharya, M., Maharjan, A., Lee, D.E., Pramod, P.B., Kim, C.Y., Kim, K.S., Kim, H.A., & Heo, Y. (2022). Evaluation of potential toxicity of polyethylene microplastics on human derived cell lines. Science of the Total Environment, 838, 156089. https://doi.org/10.1016/j.scitotenv.2022.156089.

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

Goswami, P., Vinithkumar, N. V., & Dharani, G. (2021). Microplastics particles in seafloor sediments along the Arabian Sea and the Andaman Sea continental shelves: First insight on the occurrence, identification, and characterization. Marine Pollution Bulletin, 167. https://doi.org/10.1016/j.marpolbul.2021.112311

Gray, A. D., Wertz, H., Leads, R. R., & Weinstein, J. E. (2018). Microplastic in two South Carolina Estuaries: Occurrence, distribution, and composition. Marine Pollution Bulletin, 128. https://doi.org/10.1016/j.marpolbul.2018.01.030

Gregory, M. R. (1996). Plastic 'scrubbers' in hand cleansers: a further (and minor) source for marine pollution identified. Marine pollution bulletin, 32(12), 867-871.

Guerranti, C., Perra, G., Martellini, T., Giari, L., Cincinelli, A. (2020). Knowledge about microplastic in Mediterranean tributary river ecosystems: Lack of data and research needs on such a crucial marine pollution source. Journal of Marine Science and Engineering, 8(3), 216. https://doi.org/10.3390/jmse8030216.

Guo, J.J., Huang, X.P., Xiang, L., Wang, Y.Z., Li, Y.W., Li, H., & Wong, M.H. (2020). Source, migration and toxicology of microplastics in soil. Environmental International, 137, 105263.

Harley-Nyang, D., Memon, F. A., Jones, N., & Galloway, T. (2022). Investigation and analysis of microplastics in sewage sludge and biosolids: A case study from one wastewater treatment works in the UK. Science of the Total Environment, 823, 153735.

Harris, P. T. (2020). The fate of microplastic in marine sedimentary environments: A review and synthesis. In Marine Pollution Bulletin (Vol. 158). https://doi.org/10.1016/j.marpolbul.2020.111398

Hartline, N. L., Bruce, N. J., Karba, S. N., Ruff, E. O., Sonar, S. U., & Holden, P. A. (2016). Microfiber Masses Recovered from Conventional Machine Washing of New or Aged Garments. Environmental Science and Technology, 50(21). https://doi.org/10.1021/acs.est.6b03045

Hartmann, N. B., Hüffer, T., Thompson, R. C., Hassellöv, M., Verschoor, A., Daugaard, A. E., Rist, S., Karlsson, T., Brennholt, N., Cole, M., Herrling, M. P., Hess, M. C., Ivleva, N. P., Lusher, A. L., & Wagner, M. (2019). Response to the Letter to the Editor Regarding Our Feature "are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris." In Environmental Science and Technology (Vol. 53, Issue 9). https://doi.org/10.1021/acs.est.9b02238

He, D., Luo, Y., Lu, S., Liu, M., Song, Y., & Lei, L. (2018). Microplastics in soils: analytical methods, pollution characteristics and ecological risks. Trends in Analytical Chemistry, 109, 163–172.

He, P., Chen, L., Shao, L., Zhang, H., & Lü, F. (2019). Municipal solid waste (MSW)landfill: A source of microplastics? -Evidence of microplastics in landfill leachate. Water Research, 159. https://doi.org/10.1016/j.watres.2019.04.060

Henry, B., Laitala, K., & Klepp, I. G. (2019). Microfibres from apparel and home textiles: Prospects for including microplastics in environmental sustainability assessment. Science of the Total Environment, 652. https://doi.org/10.1016/j.scitotenv.2018.10.166

Hernandez, E., Nowack, B., & Mitrano, D. M. (2017). Polyester Textiles as a Source of Microplastics from Households: A Mechanistic Study to Understand Microfiber Release during Washing. Environmental Science and Technology, 51(12). https://doi.org/10.1021/acs.est.7b01750

Hidayaturrahman, H., & Lee, T. G. (2019). A study on characteristics of microplastic in wastewater of South Korea: Identification, quantification, and fate of microplastics during treatment process. Marine Pollution Bulletin, 146. https://doi.org/10.1016/j.marpolbul.2019.06.071

Horton, A. A., & Dixon, S. J. (2018). Microplastics: An introduction to environmental transport processes. Wiley Interdisciplinary Reviews: Water, 5(2). https://doi.org/10.1002/WAT2.1268

Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C. (2017). Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. Science of the Total Environment, 586, 127-141. https://doi.org/10.1016/j.scitotenv.2017.01.190.

Huerta Lwanga, E., Gertsen, H., Gooren, H., Peters, P., Salánki, T., Van Der Ploeg, M., ... & Geissen, V. (2016). Microplastics in the terrestrial ecosystem: implications for Lumbricus terrestris (Oligochaeta, Lumbricidae). Environmental science & technology, 50(5), 2685-2691.

Huerta Lwanga, E., Mendoza Vega, J., Ku Quej, V., Chi, J. D. L. A., Sanchez del Cid, L., Chi, C., ... & Geissen, V. (2017). Field evidence for transfer of plastic debris along a terrestrial food chain. Scientific reports, 7(1), 14071.

Iñiguez, M.E., Conesa, J.A., Fullana, A. (2017). Microplastics in Spanish Table Salt. Scientific Reports, 7(1), 1-7. https://doi.org/10.1038/s41598-017-09128-x.

Isobe, A., Uchida, K., Tokai, T., & Iwasaki, S. (2015). East Asian Seas: a hot spot for pelagic microplastics. Marine Pollution Bulletin, 101, 618–623.

Jan Kole, P., 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. In International Journal of Environmental Research and Public Health (Vol. 14, Issue 10). https://doi.org/10.3390/ijerph14101265

Jenner, L. C., Rotchell, J. M., Bennett, R. T., Cowen, M., Tentzeris, V., & Sadofsky, L. R. (2022). Detection of microplastics in human lung tissue using μFTIR spectroscopy. Science of The Total Environment, 831, 154907. https://doi.org/10.1016/J.SCITOTENV.2022.154907

Jiang, X., Chang, Y., Zhang, T., Qiao, Y., Klobučar, G., & Li, M. (2020). Toxicological effects of polystyrene microplastics on earthworm (Eisenia fetida). Environmental Pollution, 259, 113896.

Johnson, A.C., Ball, H., Cross, R., Horton, A.A., Jürgens, M.D., Read, D.S., Vollertsen, J., Svendsen, C. (2020). Identification and Quantification of Microplastics in Potable Water and Their Sources within Water Treatment Works in England and Wales. Environmental Science and Technology, 54(19), 12326-12334. https://doi.org/10.1021/acs.est.0c03211.

Karami, A., Romano, N., Galloway, T., Hamzah, H. (2016). Virgin microplastics cause toxicity and modulate the impacts of phenanthrene on biomarker responses in African catfish (Clarias gariepinus). Environmental Research, 151, 58-70. https://doi.org/10.1016/j.envres.2016.07.024.

Karapanagioti, H. K., & Kalavrouziotis, I. K. (Eds.). (2019). Microplastics in water and wastewater. Iwa Publishing.

Katrivesis, F.K., Karela, A.D., Papadakis, V.G., Paraskeva, C.A. (2019). Revisiting of coagulation-flocculation processes in the production of potable water. Journal of Water Process Engineering, 27, 193-204. https://doi.org/10.1016/j.jwpe.2018.12.007.

Kazour, M., Terki, S., Rabhi, K., Jemaa, S., Khalaf, G., & Amara, R. (2019). Sources of microplastics pollution in the marine environment: Importance of wastewater treatment plant and coastal landfill. Marine Pollution Bulletin, 146, 608–618. https://doi.org/10.1016/J.MARPOLBUL.2019.06.066

Kerrigan, J. F., Engstrom, D. R., Yee, D., Sueper, C., Erickson, P. R., Grandbois, M., McNeill, K., & Arnold, W. A. (2015). Quantification of hydroxylated polybrominated diphenyl ethers (OH-BDEs), triclosan, and related compounds in freshwater and coastal systems. PLoS ONE, 10(10). https://doi.org/10.1371/journal.pone.0138805

Klasios, N., De Frond, H., Miller, E., Sedlak, M., & Rochman, C. M. (2021). Microplastics and other anthropogenic particles are prevalent in mussels from San Francisco Bay, and show no correlation with PAHs. Environmental Pollution, 271. https://doi.org/10.1016/j.envpol.2020.116260

Klein, M., & Fischer, E.K. (2019). Microplastic abundance in atmospheric deposition within the Metropolitan area of Hamburg, Germany. Science of the Total Environment, 685, 96–103. https://doi.org/10.1016/j.scitotenv.2019.05.405.

Klöckner, P., Reemtsma, T., Eisentraut, P., Braun, U., Ruhl, A. S., & Wagner, S. (2019). Tire and road wear particles in road environment – Quantification and assessment of particle dynamics by Zn determination after density separation. Chemosphere, 222. https://doi.org/10.1016/j.chemosphere.2019.01.176

Klosterhaus, S. L., Grace, R., Hamilton, M. C., & Yee, D. (2013). Method validation and reconnaissance of pharmaceuticals, personal care products, and alkylphenols in surface waters, sediments, and mussels in an urban estuary. Environment International, 54. https://doi.org/10.1016/j.envint.2013.01.009

Kosuth, M., Mason, S.A., Wattenberg, E. v. (2018). Anthropogenic contamination of tap water, beer, and sea salt. PLoS ONE, 13, 1-18. https://doi.org/10.1371/journal.pone.0194970.

Kühn, S., van Werven, B., van Oyen, A., Meijboom, A., Bravo Rebolledo, E. L., & van Franeker, J. A. (2017). The use of potassium hydroxide (KOH) solution as a suitable approach to isolate plastics ingested by marine organisms. Marine Pollution Bulletin, 115(1–2), 86–90. https://doi.org/10.1016/j.marpolbul.2016.11.034

Kukkola, A. T., Senior, G., Maes, T., Silburn, B., Bakir, A., Kröger, S., & Mayes, A. G. (2022). A large-scale study of microplastic abundance in sediment cores from the UK continental shelf and slope. Marine Pollution Bulletin, 178. https://doi.org/10.1016/j.marpolbul.2022.113554

Kurt, Z., Özdemir, I., & James R., A. M. (2022). Effectiveness of microplastics removal in wastewater treatment plants: A critical analysis of wastewater treatment processes. Journal of Environmental Chemical Engineering, 10(3). https://doi.org/10.1016/j.jece.2022.107831

Kwak, J. I., & An, Y. J. (2021). Microplastic digestion generates fragmented nanoplastics in soils and damages earthworm spermatogenesis and coelomocyte viability. Journal of Hazardous Materials, 402, 124034.

Lares, M., Ncibi, M. C., Sillanpää, M., & Sillanpää, M. (2018). Occurrence, identification and removal of microplastic particles and fibers in conventional activated sludge process and advanced MBR technology. Water Research, 133. https://doi.org/10.1016/j.watres.2018.01.049

Lares, M., Ncibi, M. C., Sillanpää, M., & Sillanpää, M. (2018). Occurrence, identification and removal of microplastic particles and fibers in conventional activated sludge process and advanced MBR technology. Water Research, 133. https://doi.org/10.1016/j.watres.2018.01.049

Lassen, C., Hansen, S. F., Magnusson, K., Hartmann, N. B., Rehne Jensen, P., Nielsen, T. G., & Brinch, A. (2015). Microplastics: Occurrence, effects and sources of releases to the environment in Denmark — Welcome to DTU Research Database. Danish Environmental Protection Agency.

Lebreton, L. C., Van Der Zwet, J., Damsteeg, J. W., Slat, B., Andrady, A., & Reisser, J. (2017). River plastic emissions to the world's oceans. Nature communications, 8(1), 15611.

Leslie, H. A., van Velzen, M. J. M., Brandsma, S. H., Vethaak, A. D., Garcia-Vallejo, J. J., & Lamoree, M. H. (2022). Discovery and quantification of plastic particle pollution in human blood. Environment International, 163, 107199. https://doi.org/10.1016/J.ENVINT.2022.107199

Li, Y., Li, W., Jarvis, P., Zhou, W., Zhang, J., Chen, J., Tan, Q., Tian, Y. (2020). Occurrence, removal and potential threats associated with microplastics in drinking water sources. Journal of Environmental Chemical Engineering, 8, 104527. https://doi.org/10.1016/j.jece.2020.104527.

Liebezeit, G., & Liebezeit, E. (2014). Synthetic particles as contaminants in German beers. Food Additives & Contaminants: Part A, 31(9), 1574-1578.

Liebezeit, G., Liebezeit, E. (2015). Origin of synthetic particles in honeys. Polish Journal of Food and Nutrition Sciences, 65, 1574-1578. https://doi.org/10.1515/pjfns-2015-0025.

Liu, W., Zhang, J., Liu, H., Guo, X., Zhang, X., Yao, X., Cao, Z., & Zhang, T. (2021). A review of the removal of microplastics in global wastewater treatment plants: Characteristics and mechanisms. Environment International, 146, 106277. https://doi.org/10.1016/J.ENVINT.2020.106277

Löder, M. G. J., Imhof, H. K., Ladehoff, M., Löschel, L. A., Lorenz, C., Mintenig, S., Piehl, S., Primpke, S., Schrank, I., Laforsch, C., & Gerdts, G. (2017). Enzymatic Purification of Microplastics in Environmental Samples. Environmental Science and Technology, 51(24). https://doi.org/10.1021/acs.est.7b03055

Lusher, A. (2015). Microplastics in the marine environment: distribution, interactions and effects. Marine anthropogenic litter, 245-307.

Lusher, A. L., Hurley, R., Vogelsang, C., Nizzetto, L., & Olsen, M. (2017). Mapping microplastics in sludge.

Lwanga, E. H., Gertsen, H., Gooren, H., Peters, P., Salánki, T., van der Ploeg, M., ... & Geissen, V. (2017). Incorporation of microplastics from litter into burrows of Lumbricus terrestris. Environmental Pollution, 220, 523-531.

Ma, B., Xue, W., Hu, C., Liu, H., Qu, J., Li, L. (2019). Characteristics of microplastic removal via coagulation and ultrafiltration during drinking water treatment. Chemical Engineering Journal, 359, 159-167. https://doi.org/10.1016/j.cej.2018.11.155.

MacArthur, D. E., Waughray, D., & Stuchtey, M. R. (2016). The new plastics economy, rethinking the future of plastics. In World Economic Forum. London, UK: Ellen MacArthur Foundation and McKinsey & Company.

Magnuson, K , Eliason, K , Frane, A , Haikonen, K , Hulten, J , Olshammar, M , Stadmark, J , and Voisin, A (2016) Swedish sources and pathways for microplastics to the marine environment wwwivlse/webdav/files/Rapporter/C183pdf

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. Environmental Science and Technology, 51(2), 810–818. https://doi.org/10.1021/acs.est.6b04048

Mani, T., Blarer, P., Storck, F. R., Pittroff, M., Wernicke, T., & Burkhardt-Holm, P. (2019). Repeated detection of polystyrene microbeads in the lower Rhine River. Environmental Pollution, 245. https://doi.org/10.1016/j.envpol.2018.11.036

Martin, J., Lusher, A. L., & Nixon, F. C. (2022). A review of the use of microplastics in reconstructing dated sedimentary archives. In Science of the Total Environment (Vol. 806). https://doi.org/10.1016/j.scitotenv.2021.150818

Martínez, M.A., Rovira, J., Sharma, R.P., Nadal, M., Schuhmacher, M., Kumar, V. (2017). Prenatal exposure estimation of BPA and DEHP using integrated external and internal dosimetry: A case study. Environmental Research, 158, 566-575. https://doi.org/10.1016/j.envres.2017.07.016.

Mason, S.A., Welch, V.G., Neratko, J. (2018). Synthetic Polymer Contamination in Bottled Water. Frontiers in Chemistry, 6, 1-11. https://doi.org/10.3389/fchem.2018.00407.

Masura, J., Baker, J., Foster, G., & Arthur, C. (2015). Laboratory methods for the analysis of microplastics in themarine environment: Recommendations for quantifying synthet- ic particles in waters and sediments. NOAA Technical Memorandum NOS-OR&R-48. NOAA Technical Memorandum, July.

Matsuguma, Y., Takada, H., Kumata, H., Kanke, H., Sakurai, S., Suzuki, T., & Newman, B. (2017). Microplastics in sediment cores from Asia and Africa as indicators of temporal trends in plastic pollution. Archives of Environmental Contamination and Toxicology, 73(2), 230–239.

McCormick, A., Hoellein, T. J., Mason, S. A., Schluep, J., & Kelly, J. J. (2014). Microplastic is an abundant and distinct microbial habitat in an urban river. Environmental Science and Technology, 48(20), 11863–11871. https://doi.org/10.1021/es503610r

McDevitt, J. P., Criddle, C. S., Morse, M., Hale, R. C., Bott, C. B., & Rochman, C. M. (2017). Addressing the Issue of Microplastics in the Wake of the Microbead-Free Waters Act - A New Standard Can Facilitate Improved Policy. Environmental Science and Technology, 51(12). https://doi.org/10.1021/acs.est.6b05812

McIlwraith, H. K., Lin, J., Erdle, L. M., Mallos, N., Diamond, M. L., & Rochman, C. M. (2019). Capturing microfibers – marketed technologies reduce microfiber emissions from washing machines. Marine Pollution Bulletin, 139. https://doi.org/10.1016/j.marpolbul.2018.12.012

McKeen, L. W. (2013). Introduction to Use of Plastics in Food Packaging. In Plastic Films in Food Packaging: Materials, Technology and Applications. https://doi.org/10.1016/B978-1-4557-3112-1.00001-6

Meixner, K., Kubiczek, M., & Fritz, I. (2020). Microplastic in soil–current status in Europe with special focus on method tests with Austrian samples. AIMS Environmental Science, 7(2).

Melody M. Bomgardner. (2017). The great lint migration. C&EN Global Enterprise, 95(2). https://doi.org/10.1021/cen-09502-bus1

Micha, R., Khatibzadeh, S., Shi, P., Andrews, K.G., Engell, R.E., Mozaffarian, D. (2015). Global, regional and national consumption of major food groups in 1990 and 2010: A systematic analysis including 266 country-specific nutrition surveys worldwide. BMJ Open, 5(9), e008705. https://doi.org/10.1136/bmjopen-2015-008705.

Mintenig, S. M., Int-Veen, I., Löder, M. G. J., Primpke, S., & Gerdts, G. (2017). Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging. Water Research, 108, 365–372. https://doi.org/10.1016/J.WATRES.2016.11.015

Mintenig, S.M., Löder, M.G.J., Primpke, S., Gerdts, G. (2019). Low numbers of microplastics detected in drinking water from ground water sources. Science of the Total Environment, 648, 631–635. https://doi.org/10.1016/j.scitotenv.2018.08.178.

Morgado, V., Palma, C., & Bettencourt da Silva, R. J. N. (2023). Determination of microplastic contamination levels and trends in vast oceanic sediment areas with uncertainty. Science of the Total Environment, 884. https://doi.org/10.1016/j.scitotenv.2023.163612

Mukotaka, A., Kataoka, T., Nihei, Y. (2021). Rapid analytical method for characterization and quantification of microplastics in tap water using a Fourier-transform infrared microscope. Science of the Total Environment, 790, 148231. https://doi.org/10.1016/j.scitotenv.2021.148231.

Murphy, F., Ewins, C., Carbonnier, F., & Quinn, B. (2016). Wastewater Treatment Works (WwTW) as a Source of Microplastics in the Aquatic Environment. Environmental Science and Technology, 50(11), 5800–5808. https://doi.org/10.1021/acs.est.5b05416

Napper, I. E., & Thompson, R. C. (2016). Release of synthetic microplastic plastic fibres from domestic washing machines: Effects of fabric type and washing conditions. Marine Pollution Bulletin, 112(1–2). https://doi.org/10.1016/j.marpolbul.2016.09.025

Ncube, L. K., Ude, A. U., Ogunmuyiwa, E. N., Zulkifli, R., & Beas, I. N. (2020). Environmental impact of food packaging materials: A review of contemporary development from conventional plastics to polylactic acid based materials. Materials, 13(21), 4994.

Negrete Velasco, A., Ramseier Gentile, S., Zimmermann, S., Stoll, S. (2022). Contamination and Removal Efficiency of Microplastics and Synthetic Fibres in a Conventional Drinking Water Treatment Plant. Frontiers in Water, 4, 835451. https://doi.org/10.3389/frwa.2022.835451.

Ngo, P. L., Pramanik, B. K., Shah, K., & Roychand, R. (2019). Pathway, classification and removal efficiency of microplastics in wastewater treatment plants. Environmental Pollution, 255, 113326. https://doi.org/10.1016/J.ENVPOL.2019.113326

Nicholson, F. A. (2018). Biosolids to Market: A Strategic Proposal to Explore the Threats to Biosolids to Land: Now and in the Future. UK Water Industry Research Limited..

Nikiema, J., Mateo-Sagasta, J., Asiedu, Z., Saad, D., & Lamizana, B. (2020). Water pollution by plastics and microplastics: a review of technical solutions from source to sea.

Niu, L., Li, Y., Li, Y., Hu, Q., Wang, C., Hu, J., Zhang, W., Wang, L., Zhang, C., & Zhang, H. (2021). New insights into the vertical distribution and microbial degradation of microplastics in urban river sediments. Water Research, 188. https://doi.org/10.1016/j.watres.2020.116449

Nizzetto, L., Bussi, G., Futter, M. N., Butterfield, D., & Whitehead, P. G. (2016). A theoretical assessment of microplastic transport in river catchments and their retention by soils and river sediments. Environmental Science: Processes and Impacts, 18(8), 1050–1059. https://doi.org/10.1039/c6em00206d

Nizzetto, L., Futter, M., & Langaas, S. (2016). Are Agricultural Soils Dumps for Microplastics of Urban Origin? In Environmental Science and Technology (Vol. 50, Issue 20). https://doi.org/10.1021/acs.est.6b04140

Novotna, K., Cermakova, L., Pivokonska, L., Cajthaml, T., Pivokonsky, M. (2019). Microplastics in drinking water treatment – Current knowledge and research needs. Science of the Total Environment, 667, 730-740. https://doi.org/10.1016/j.scitotenv.2019.02.431.

Nuelle, M. T., Dekiff, J. H., Remy, D., & Fries, E. (2014). A new analytical approach for monitoring microplastics in marine sediments. Environmental Pollution, 184. https://doi.org/10.1016/j.envpol.2013.07.027

O'Brien, B. J. (2019). Physicochemical properties of residuals from anaerobic digestion of dairy manure and food waste: Nutrient cycling implications and opportunities for edible mushroom cultivation. The University of Vermont and State Agricultural College.

O'Connor, D., Pan, S., Shen, Z., Song, Y., Jin, Y., Wu, W.M., & Hou, D. (2019). Microplastics undergo accelerated vertical migration in sand soil due to small size and wet-dry cycles. Environmental Pollution, 249, 527–534.

Oldfield, E. E., Wood, S. A., & Bradford, M. A. (2018). Direct effects of soil organic matter on productivity mirror those observed with organic amendments. Plant and soil, 423, 363-373.

Patel, M. M., Goyal, B. R., Bhadada, S. V., Bhatt, J. S., & Amin, A. F. (2009). Getting into the brain: approaches to enhance brain drug delivery. CNS drugs, 23, 35-58.

Pervez, R., & Wang, Y. H. (2022). Microplastic distribution within core sediments of beach and its responses to anthropogenic activities. Marine Pollution Bulletin, 174. https://doi.org/10.1016/j.marpolbul.2021.113256

Picariello, E., Pucci, L., Carotenuto, M., Libralato, G., Lofrano, G., & Baldantoni, D. (2020). Compost and sewage sludge for the improvement of soil chemical and biological quality of Mediterranean agroecosystems. Sustainability 13: 26.

Pirc, U., Vidmar, M., Mozer, A., & Kržan, A. (2016). Emissions of microplastic fibers from microfiber fleece during domestic washing. Environmental Science and Pollution Research, 23(21). https://doi.org/10.1007/s11356-016-7703-0

Pivokonsky, M., Cermakova, L., Novotna, K., Peer, P., Cajthaml, T., Janda, V. (2018). Occurrence of microplastics in raw and treated drinking water. Science of the Total Environment, 643, 1644–1651. https://doi.org/10.1016/j.scitotenv.2018.08.102.

Pivokonský, M., Pivokonská, L., Novotná, K., Čermáková, L., Klimtová, M. (2020). Occurrence and fate of microplastics at two different drinking water treatment plants within a river catchment. Science of the Total Environment, 741, 140236. https://doi.org/10.1016/j.scitotenv.2020.140236.

PlasticEurope. (2021). Plastics - the Facts 2021. An analysis of European plastics production, demand and waste data. Available from: https://plasticseurope.org/knowledge-hub/plastics-the-facts-2021/. Last accessed: 22/03/2022.

Plastics Europe. (2018). Plastics - the facts 2018. An analysis of European plastics production, demand and waste data 16. Retrieved from https://www.plasticseurope.org/application/files/6315/4510/9658/Plastics_the_facts_2018_AF_web.pdf.

Porterfield, K. K., Hobson, S. A., Neher, D. A., Niles, M. T., & Roy, E. D. (2023). Microplastics in composts, digestates, and food wastes: A review. Journal of Environmental Quality, 52(2), 225-240.

Prata, J. C. (2018). Airborne microplastics: Consequences to human health? In Environmental Pollution (Vol. 234). https://doi.org/10.1016/j.envpol.2017.11.043

Prata, J. C., da Costa, J. P., Duarte, A. C., & Rocha-Santos, T. (2019). Methods for sampling and detection of microplastics in water and sediment: A critical review. In TrAC - Trends in Analytical Chemistry (Vol. 110). https://doi.org/10.1016/j.trac.2018.10.029

Prata, J.C. (2018). Airborne microplastics: Consequences to human health? Environmental Pollution, 234, 115-126. https://doi.org/10.1016/j.envpol.2017.11.043.

Prata, J.C., da Costa, J.P., Duarte, A.C., Rocha-Santos, T. (2019a). Methods for sampling and detection of microplastics in water and sediment: A critical review. TrAC - Trends in Analytical Chemistry, 110, 150-159. https://doi.org/10.1016/j.trac.2018.10.029.

Prata, J.C., da Costa, J.P., Girão, A.V., Lopes, I., Duarte, A.C., Rocha-Santos, T. (2019b). Identifying a quick and efficient method of removing organic matter without damaging microplastic samples. Science of the Total Environment, 686, 131-139. https://doi.org/10.1016/j.scitotenv.2019.05.456.

Prata, J.C., da Costa, J.P., Lopes, I., Duarte, A.C., Rocha-Santos, T. (2020). Environmental exposure to microplastics: An overview on possible human health effects. Science of the Total Environment, 702, 134455. https://doi.org/10.1016/j.scitotenv.2019.134455.

Primpke, S., Christiansen, S. H., Cowger, W., De Frond, H., Deshpande, A., Fischer, M., ... & Wiggin, K. J. (2020). Critical assessment of analytical methods for the harmonized and cost-efficient analysis of microplastics. Applied Spectroscopy, 74(9), 1012-1047.

Prokić, M. D., Radovanović, T. B., Gavrić, J. P., & Faggio, C. (2019). Ecotoxicological effects of microplastics: Examination of biomarkers, current state and future perspectives. In TrAC - Trends in Analytical Chemistry (Vol. 111). https://doi.org/10.1016/j.trac.2018.12.001

Qiu, Q., Tan, Z., Wang, J., Peng, J., Li, M., & Zhan, Z. (2016). Extraction, enumeration and identification methods for monitoring microplastics in the environment. In Estuarine, Coastal and Shelf Science (Vol. 176). https://doi.org/10.1016/j.ecss.2016.04.012

Qiu, Y., Zhou, S., Zhang, C., Zhou, Y., & Qin, W. (2022). Soil microplastic characteristics and the effects on soil properties and biota: A systematic review and meta-analysis. Environmental Pollution, 120183.

Ragusa, A., Notarstefano, V., Svelato, A., Belloni, A., Gioacchini, G., Blondeel, C., Zucchelli, E., De Luca, C., D'avino, S., Gulotta, A., Carnevali, O., & Giorgini, E. (2022). Raman Microspectroscopy Detection and Characterisation of Microplastics in Human Breastmilk. Polymers, 14(13). https://doi.org/10.3390/polym14132700

Ragusa, A., Svelato, A., Santacroce, C., Catalano, P., Notarstefano, V., Carnevali, O., Papa, F., Rongioletti, M. C. A., Baiocco, F., Draghi, S., D'Amore, E., Rinaldo, D., Matta, M., & Giorgini, E. (2021). Plasticenta: First evidence of microplastics in human placenta. Environment International, 146, 106274. https://doi.org/10.1016/J.ENVINT.2020.106274

Ramsperger, A.F.R.M., Bergamaschi, E., Panizzolo, M., Fenoglio, I., Barbero, F., Peters, R., et al. (2023). Nano- and microplastics: a comprehensive review on their exposure routes, translocation, and fate in humans. NanoImpact, 29, 100441. https://doi.org/10.1016/j.impact.2022.100441.

Rashti, M. R., Hintz, J., Esfandbod, M., Bahadori, M., Lan, Z., & Chen, C. (2023). Detecting microplastics in organic-rich materials and their potential risks to earthworms in agroecosystems. Waste Management, 166, 96-103.

Rasmussen, L. A., Iordachescu, L., Tumlin, S., & Vollertsen, J. (2021). A complete mass balance for plastics in a wastewater treatment plant - Macroplastics contributes more than microplastics. Water Research, 201, 117307. https://doi.org/10.1016/J.WATRES.2021.117307

Revel, M., Châtel, A., Mouneyrac, C. (2018). Micro(nano)plastics: A threat to human health? Current Opinion in Environmental Science and Health, 1, 17-23. https://doi.org/10.1016/j.coesh.2017.10.003.

Rillig, M. C., Ingraffia, R., & de Souza Machado, A. A. (2017). Microplastic incorporation into soil in agroecosystems. Frontiers in plant science, 8, 1805.

Rillig, M. C., Ziersch, L., & Hempel, S. (2017). Microplastic transport in soil by earthworms. Scientific reports, 7(1), 1362.

Rillig, M.C. (2012). Microplastic in terrestrial ecosystems and the soil. Environmental Science & Technology, 46. River plastic emissions to the world's oceans. Nat. Commun. 8 (1), 1–10.

Rist, S., Carney Almroth, B., Hartmann, N. B., & Karlsson, T. M. (2018). A critical perspective on early communications concerning human health aspects of microplastics. In Science of the Total Environment (Vol. 626). https://doi.org/10.1016/j.scitotenv.2018.01.092

Rochman, C. M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Bucci, K., Athey, S., Huntington, A., McIlwraith, H., Munno, K., Frond, H. De, Kolomijeca, A., Erdle, L., Grbic, J., Bayoumi, M., Borrelle,

S. B., Wu, T., Santoro, S., Werbowski, L. M., … Hung, C. (2019). Rethinking microplastics as a diverse contaminant suite. In Environmental Toxicology and Chemistry (Vol. 38, Issue 4). https://doi.org/10.1002/etc.4371

Rodrigues, M. O., Abrantes, N., Gonçalves, F. J. M., Nogueira, H., Marques, J. C., & Gonçalves, A. M. M. (2019). Impacts of plastic products used in daily life on the environment and human health: What is known? In Environmental Toxicology and Pharmacology (Vol. 72). https://doi.org/10.1016/j.etap.2019.103239

Roig, N., Sierra, J., Martí, E., Nadal, M., Schuhmacher, M., & Domingo, J. L. (2012). Long-term amendment of Spanish soils with sewage sludge: Effects on soil functioning. Agriculture, ecosystems & environment, 158, 41-48.

Rose, P. K., Jain, M., Kataria, N., Sahoo, P. K., Garg, V. K., & Yadav, A. (2023). Microplastics in multimedia environment: A systematic review on its fate, transport, quantification, health risk, and remedial measures. Groundwater for Sustainable Development, 100889.

Ryan, P. G. (1988). The characteristics and distribution of plastic particles at the sea-surface off the southwestern Cape Province, South Africa. Marine Environmental Research, 25(4), 249-273.

Sajid, M., Ihsanullah, I., Tariq Khan, M., & Baig, N. (2023). Nanomaterials-based adsorbents for remediation of microplastics and nanoplastics in aqueous media: a review. Separation and Purification Technology, 305, 122453. https://doi.org/10.1016/J.SEPPUR.2022.122453.

Sakali, A., Coello, D., Brigui, J., Albendín, G., Arellano, J., Quiroga, J. M., & Rodríguez-Barroso, R. (2022). Annual estimates of microplastics in municipal sludge treatment plants in southern Spain. Journal of Water Process Engineering, 49, 102956.

Sambandam, M., Dhineka, K., Sivadas, S. K., Kaviarasan, T., Begum, M., Hoehn, D., Sivyer, D., Mishra, P., & Murthy, M. V. R. (2022). Occurrence, characterization, and source delineation of microplastics in the coastal waters and shelf sediments of the central east coast of India, Bay of Bengal. Chemosphere, 303. https://doi.org/10.1016/j.chemosphere.2022.135135

Sánchez-Soberón, F., Sutton, R., Sedlak, M., Yee, D., Schuhmacher, M., & Park, J. S. (2020). Multi-box mass balance model of PFOA and PFOS in different regions of San Francisco Bay. Chemosphere, 252. https://doi.org/10.1016/j.chemosphere.2020.126454

Sarkar, B., Dissanayake, P. D., Bolan, N. S., Dar, J. Y., Kumar, M., Haque, M. N., Mukhopadhyay, R., Ramanayaka, S., Biswas, J. K., Tsang, D. C. W., Rinklebe, J., & Ok, Y. S. (2022). Challenges and opportunities in sustainable management of microplastics and nanoplastics in the environment. In Environmental Research (Vol. 207). https://doi.org/10.1016/j.envres.2021.112179

Sarkar, D.J., das Sarkar, S., Das, B.K., Praharaj, J.K., Mahajan, D.K., Purokait, B., Mohanty, T.R., Mohanty, D., Gogoi, P., Kumar V, S., Behera, B.K., Manna, R.K., Samanta, S. (2021). Microplastics removal efficiency of drinking water treatment plant with pulse clarifier. Journal of Hazardous Materials, 413, 125347. https://doi.org/10.1016/j.jhazmat.2021.125347.

Sarkar, D.J., Sarkar, S.D., Das, B.K., Manna, R.K., Behera, B.K., & Samanta, S. (2019). Spatial distribution of meso and microplastics in the sediments of river Ganga at eastern India. Science of the Total Environment, 694, 133712.

Schell, T., Hurley, R., Nizzetto, L., Rico, A., & Vighi, M. (2021). Spatio-temporal distribution of microplastics in a Mediterranean river catchment: The importance of wastewater as an environmental pathway. Journal of Hazardous Materials, 420, 126481. https://doi.org/10.1016/J.JHAZMAT.2021.126481

Schymanski, D., Oßmann, B.E., Benismail, N., Boukerma, K., Dallmann, G., von der Esch, E., Fischer, D., Fischer, F., Gilliland, D., Glas, K., Hofmann, T., Käppler, A., Lacorte, S., Marco, J., Rakwe, M.E., Weisser, J., Witzig, C., Zumbülte, N., Ivleva, N.P. (2021). Analysis of microplastics in drinking water and other clean water samples with micro-Raman and micro-infrared spectroscopy: minimum requirements and best practice guidelines. Analytical and Bioanalytical Chemistry, 413, 5969–5994. https://doi.org/10.1007/s00216-021-03498-y.

Sedlak, M. D., Benskin, J. P., Wong, A., Grace, R., & Greig, D. J. (2017). Per- and polyfluoroalkyl substances (PFASs) in San Francisco Bay wildlife: Temporal trends, exposure pathways, and notable presence of precursor compounds. Chemosphere, 185. https://doi.org/10.1016/j.chemosphere.2017.04.096

Sedlak, M., Sutton, R., Box, C., Sun, J., & Lin, D. (2017). Sampling and Analysis Plan for Microplastic Monitoring in San Francisco Bay and Adjacent National Marine Sanctuaries FINAL.

Shahi, N.K., Maeng, M., Kim, D., Dockko, S. (2020). Removal behavior of microplastics using alum coagulant and its enhancement using polyamine-coated sand. Process Safety and Environmental Protection, 141, 9-17. https://doi.org/10.1016/j.psep.2020.05.020.

Sharma, S., & Chatterjee, S. (2017). Microplastic pollution, a threat to marine ecosystem and human health: a short review. Environmental Science and Pollution Research, 24(27), 21530–21547.

Shen, M., Zeng, Z., Wen, X., Ren, X., Zeng, G., Zhang, Y., & Xiao, R. (2021). Presence of microplastics in drinking water from freshwater sources: the investigation in Changsha, China. Environmental Science and Pollution Research, 28(31). https://doi.org/10.1007/s11356-021-13769-x

Shen, M., Zeng, Z., Wen, X., Ren, X., Zeng, G., Zhang, Y., Xiao, R. (2021). Presence of microplastics in drinking water from freshwater sources: the investigation in Changsha, China. Environmental Science and Pollution Research, 28(31), 42313-42324. https://doi.org/10.1007/s11356-021-13769-x.

Shim, W.J., & Thomposon, R.C. (2015). Microplastics in the ocean. Archives of Environmental Contamination and Toxicology, 69(3), 265–268.

Slaughter, E., Gersberg, R. M., Watanabe, K., Rudolph, J., Stransky, C., & Novotny, T. E. (2011). Toxicity of cigarette butts, and their chemical components, to marine and freshwater fish. Tobacco Control, 20(1 SUPPL). https://doi.org/10.1136/tc.2010.040170

Slootmaekers, B., Carteny, C.C., Belpaire, C., Saverwyns, S., Fremout, W., Blust, R., & Bervoets, L. (2019). Microplastic contamination in gudgeons (Gobio gobio) from Flemish rivers (Belgium). Environmental Pollution, 244, 675–684. https://doi.org/10.1016/j.envpol.2018.09.136.

Smith, S. V., & Hollibaugh, J. T. (2006). Water, salt, and nutrient exchanges in San Francisco Bay. Limnology and Oceanography, 51(1 II). https://doi.org/10.4319/lo.2006.51.1_part_2.0504

Sol, D., Laca, A., Laca, A., & Díaz, M. (2020). Approaching the environmental problem of microplastics: Importance of WWTP treatments. Science of The Total Environment, 740, 140016. https://doi.org/10.1016/J.SCITOTENV.2020.140016

Sommer, F., Dietze, V., Baum, A., Sauer, J., Gilge, S., Maschowski, C., & Gieré, R. (2018). Tire abrasion as a major source of microplastics in the environment. Aerosol and Air Quality Research, 18(8). https://doi.org/10.4209/aaqr.2018.03.0099

Steinmetz, Z., Wollmann, C., Schaefer, M., Buchmann, C., David, J., Tröger, J., ... & Schaumann, G. E. (2016). Plastic mulching in agriculture. Trading short-term agronomic benefits for long-term soil degradation?. Science of the total environment, 550, 690-705.

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 Research, 152, 21–37. https://doi.org/10.1016/J.WATRES.2018.12.050

Sundt, P , Schulze, P -E , and Syversen, F (2014) Sources of microplastic- pollution to the marine environment (MEPEX) www miljodirektoratet no/Documents/publikasjoner/M321/ M321 pdf

Sutton, R., Chen, D., Sun, J., Greig, D. J., & Wu, Y. (2019). Characterization of brominated, chlorinated, and phosphate flame retardants in San Francisco Bay, an urban estuary. Science of the Total Environment, 652. https://doi.org/10.1016/j.scitotenv.2018.10.096

Sutton, R., Mason, S. A., Stanek, S. K., Willis-Norton, E., Wren, I. F., & Box, C. (2016). Microplastic contamination in the San Francisco Bay, California, USA. Marine Pollution Bulletin, 109(1). https://doi.org/10.1016/j.marpolbul.2016.05.077

Sutton, R., & Sedlak, M. (2017). Microplastic monitoring and science strategy for San Francisco Bay. California: SFEI Contribution.

Tagg, A. S., Harrison, J. P., Ju-Nam, Y., Sapp, M., Bradley, E. L., Sinclair, C. J., & Ojeda, J. J. (2017). Fenton's reagent for the rapid and efficient isolation of microplastics from wastewater. Chemical Communications, 53(2), 372–375. https://doi.org/10.1039/c6cc08798a

Talvitie, J., Mikola, A., Koistinen, A., & Setala, O. (2017). Solutions to microplastic pollution–Removal of microplastics from wastewater effluent with advanced wastewater treatment technologies. Water Research, 123, 401–407. https://doi.org/10.1016/j.watres.2017.07.005.

Tamargo, A., Molinero, N., Reinosa, J.J., Alcolea-Rodriguez, V., Portela, R., Bañares, M.A., Fernández, J.F., Moreno-Arribas, M.V. (2022). PET microplastics affect human gut microbiota communities during simulated gastrointestinal digestion, first evidence of plausible polymer biodegradation during human digestion. Scientific Reports, 12(1), 1-15. https://doi.org/10.1038/s41598-021-04489-w.

Tanaka, K., Takada, H. (2016). Microplastic fragments and microbeads in digestive tracts of planktivorous fish from urban coastal waters. Scientific Reports, 6(1), 1-8. https://doi.org/10.1038/srep34351.

Thompson, R. C., Olsen, Y., Mitchell, R. P., Davis, A., Rowland, S. J., John, A. W., ... & Russell, A. E. (2004). Lost at sea: where is all the plastic?. Science, 304(5672), 838-838. Total Environ. 575, 1369–1374. https://doi.org/10.1016/j.scitotenv.2016.09.213.

Thornton Hampton, L. M., Brander, S. M., Coffin, S., Cole, M., Hermabessiere, L., Koelmans, A. A., & Rochman, C. M. (2022). Characterizing microplastic hazards: which concentration metrics and particle characteristics are most informative for understanding toxicity in aquatic organisms? Microplastics and Nanoplastics, 2(1). https://doi.org/10.1186/s43591-022-00040-4

Tong, H., Jiang, Q., Hu, X., Zhong, X. (2020). Occurrence and identification of microplastics in tap water from China. Chemosphere, 252, 126493. https://doi.org/10.1016/j.chemosphere.2020.126493.

Tunali, M., Adam, V., & Nowack, B. (2023). Probabilistic environmental risk assessment of microplastics in soils. Geoderma, 430, 116315

Turner, S., Horton, A. A., Rose, N. L., & Hall, C. (2019). A temporal sediment record of microplastics in an urban lake, London, UK. Journal of Paleolimnology, 61(4). https://doi.org/10.1007/s10933-019-00071-7

UK Water Industry Research, 2019. Sink to river - river to tap: review of potential risks from microplastics.[Online] Available at: https://ukwir.org/sink-to-river-river-to-tap-reviewof-potential-risks-from-microplastics.

Van den Berg, P., Huerta-Lwanga, E., Corradini, F., & Geissen, V. (2020). Sewage sludge application as a vehicle for microplastics in eastern Spanish agricultural soils. Environmental Pollution, 261, 114198.

Vogelsang, C., Lusher, A., Dadkhah, M. E., Sundvor, I., Umar, M., Ranneklev, S. B., Eidsvoll, D., & Meland, S. (2020). Microplastics in road dust - characteristics, pathways and measures REPORT SNO. 7526-2020. In Integrated environmental assessment and management (Issues 7361–2019).

Wagner, M., & Lambert, S. (2018). Freshwater microplastics: emerging environmental contaminants? (p. 303). Springer Nature.

Walkinshaw, C., Lindeque, P.K., Thompson, R., Tolhurst, T., & Cole, M. (2020). Microplastics and seafood: lower trophic organisms at highest risk of contamination. Ecotoxicology and Environmental Safety, 190, 110066. https://doi.org/10.1016/j.ecoenv.2019.110066.

Wang, C., Zhao, J., & Xing, B. (2021). Environmental source, fate, and toxicity of microplastics. In Journal of Hazardous Materials (Vol. 407). https://doi.org/10.1016/j.jhazmat.2020.124357

Wang, T., Zou, X., Li, B., Yao, Y., Zang, Z., Li, Y., Yu, W., & Wang, W. (2019). Preliminary study of the source apportionment and diversity of microplastics: Taking floating microplastics in the South China Sea as an example. Environmental Pollution, 245. https://doi.org/10.1016/j.envpol.2018.10.110

Wang, W., Ge, J., & Yu, X. (2020). Bioavailability and toxicity of microplastics to fish species: a review. Ecotoxicology and Environmental Safety, 189, 109913. https://doi.org/10.1016/J.ECOENV.2019.109913.

Wang, W., Ndungu, A.W., Li, Z., & Wang, J. (2017). Microplastics pollution in inland waters of China: a case study in urban surface waters of Wuhan, China. Science of the Total Environment, 575, 1369–1374. https://doi.org/10.1016/j.scitotenv.2016.09.213.

Wang, W., Yuan, W., Chen, Y., & Wang, J. (2018). Microplastics in surface waters of Dongting Lake and Hong Lake, China. Science of The Total Environment, 633, 539–545. https://doi.org/10.1016/J.SCITOTENV.2018.03.211

Wang, Z., Lin, T., Chen, W. (2020). Occurrence and removal of microplastics in an advanced drinking water treatment plant (ADWTP). Science of the Total Environment, 700, 134520. https://doi.org/10.1016/j.scitotenv.2019.134520.

Waring, R.H., Harris, R.M., Mitchell, S.C. (2018). Plastic contamination of the food chain: A threat to human health? Maturitas, 115, 64-68. https://doi.org/10.1016/j.maturitas.2018.06.010.

Weithmann, N., Möller, J. N., Löder, M. G., Piehl, S., Laforsch, C., & Freitag, R. (2018). Organic fertilizer as a vehicle for the entry of microplastic into the environment. Science advances, 4(4), eaap8060.

Werbowski, L. M., Gilbreath, A. N., Munno, K., Zhu, X., Grbic, J., Wu, T., Sutton, R., Sedlak, M. D., Deshpande, A. D., & Rochman, C. M. (2021). Urban Stormwater Runoff: A Major Pathway for Anthropogenic Particles, Black Rubbery Fragments, and Other Types of Microplastics to Urban Receiving Waters. ACS ES&T Water, 1(6). https://doi.org/10.1021/acsestwater.1c00017

WHO. (2019). Microplastics in Drinking-Water. Available from: https://www.who.int/water_sanitation_health/publications/information-sheet190822.pdf. Last Accessed: 22/03/2022.

Wolff, S., Kerpen, J., Prediger, J., Barkmann, L., & Müller, L. (2019). Determination of the microplastics emission in the effluent of a municipal waste water treatment plant using Raman microspectroscopy. Water Research X, 2. https://doi.org/10.1016/j.wroa.2018.100014

Woodward, J., Li, J., Rothwell, J., & Hurley, R. (2021). Acute riverine microplastic contamination due to avoidable releases of untreated wastewater. Nature Sustainability, 4(9), 793-802.

Wright, S.L., Kelly, F.J. (2017). Plastic and Human Health: A Micro Issue? Environmental Science and Technology, 51(12), 6634-6647. https://doi.org/10.1021/acs.est.7b00423.

Xu, E.G., Ren, Z.J. (2021). Preventing masks from becoming the next plastic problem. Frontiers of Environmental Science and Engineering, 15(6), 125. https://doi.org/10.1007/s11783-021-1413-7.

Xu, P., Ge, W., Chai, C., Zhang, Y., Jiang, T., & Xia, B. (2019). Sorption of polybrominated diphenyl ethers by microplastics. Marine Pollution Bulletin, 145. https://doi.org/10.1016/j.marpolbul.2019.05.050

Xue, B., Zhang, L., Li, R., Wang, Y., Guo, J., Yu, K., & Wang, S. (2020). Underestimated Microplastic Pollution Derived from Fishery Activities and "Hidden" in Deep Sediment. Environmental Science and Technology, 54(4). https://doi.org/10.1021/acs.est.9b04850

Xue, J., Samaei, S.H.-A., Chen, J., Doucet, A., Ng, K.T.W. (2022). What have we known so far about microplastics in drinking water treatment? A timely review. Frontiers of Environmental Science & Engineering, 16(5), 58. https://doi.org/10.1007/s11783-021-1492-5.

Yang, D., Shi, H., Li, L., Li, J., Jabeen, K., Kolandhasamy, P. (2015). Microplastic Pollution in Table Salts from China. Environmental Science and Technology, 49(22), 13622-13627. https://doi.org/10.1021/acs.est.5b03163.

Ye, Y., Yu, K., & Zhao, Y. (2022). The development and application of advanced analytical methods in microplastics contamination detection: A critical review. Science of The Total Environment, 818, 151851.

Yong, C.Q.Y., Valiyaveettil, S., & Tang, B.L. (2020). Toxicity of microplastics and nanoplastics in mammalian systems. International Journal of Environmental Research and Public Health, 17, 1509. https://doi.org/10.3390/IJERPH17051509.

Yu, J., & Ma, X. (2022). Exploring the management policy of marine microplastic litter in China: Overview, challenges and prospects. Sustainable Production and Consumption, 32. https://doi.org/10.1016/j.spc.2022.05.018

Yu, X., Peng, J., Wang, J., Wang, K., Bao, S. (2016). Occurrence of microplastics in the beach sand of the Chinese inner sea: The Bohai Sea. Environmental Pollution, 214, 722-730. https://doi.org/10.1016/j.envpol.2016.04.080.

Zhang, G.S., & Liu, Y.F. (2018). The distribution of microplastics in soil aggregate fractions in southwestern China. Science of the Total Environment, 642, 12–20.

Zhang, Q., Zhao, Y., Du, F., Cai, H., & Wang, G., & Shi, H. (2020). Microplastic fallout in different indoor environments. Environmental Science and Technology, 54(11), 6530–6539. https://doi.org/10.1021/acs.est.0c00087.

Zhao, S., Danley, M., Ward, J.E., Li, D., Mincer, T.J. (2017). An approach for extraction, characterization and quantitation of microplastic in natural marine snow using Raman microscopy. Analytical Methods, 9(9), 1470-1478. https://doi.org/10.1039/c6ay02302a.

Zheng, Y., Li, J., Cao, W., Liu, X., Jiang, F., Ding, J., Yin, X., & Sun, C. (2019). Distribution characteristics of microplastics in the seawater and sediment: A case study in Jiaozhou Bay, China. Science of The Total Environment, 674, 27–35. https://doi.org/10.1016/J.SCITOTENV.2019.04.008

Zhou, B., Wang, J., Zhang, H., Shi, H., Fei, Y., Huang, S., Tong, Y., Wen, D., Luo, Y., & Barcelo, D. (2020). Microplastics in agricultural soils on the coastal plain of Hangzhou Bay, east China: multiple sources other than plastic mulching film. Journal of Hazardous Materials, 388, 121814.

Zhu, X., Munno, K., Grbic, J., Werbowski, L. M., Bikker, J., Ho, A., Guo, E., Sedlak, M., Sutton, R., Box, C., Lin, D., Gilbreath, A., Holleman, R. C., Fortin, M.-J., & Rochman, C. (2021). Holistic Assessment of Microplastics and Other Anthropogenic Microdebris in an Urban Bay Sheds Light on Their Sources and Fate. ACS ES&T Water, 1(6). https://doi.org/10.1021/acsestwater.0c00292

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 Research, 112. https://doi.org/10.1016/j.watres.2017.01.042

Ziajahromi, S., Neale, P. A., Telles Silveira, I., Chua, A., & Leusch, F. D. L. (2021). An audit of microplastic abundance throughout three Australian wastewater treatment plants. Chemosphere, 263, 128294. https://doi.org/10.1016/J.CHEMOSPHERE.2020.128294

Zitko, V., & Hanlon, M. J. (1991). Another source of pollution by plastics: skin cleaners with plastic scrubbers. Marine Pollution Bulletin, 22(1), 41-42.

Zubris, K. A. V., & Richards, B. K. (2005). Synthetic fibers as an indicator of land application of sludge. Environmental Pollution, 138(2). https://doi.org/10.1016/j.envpol.2005.04.013

Annex I Chapter 1

Tracing the fate of microplastic in wastewater treatment: A multi-stage analysis of treatment units and sludge

1. Sampling of microplastics

Composite samples of 24 hours were collected in the water line (influent of WWTP, pretreatment units, biological reactor, secondary clarifier and effluent of WWTP) automatically and sequentially: 300 mL were sampled every 20 minutes in the influent; 250 mL every 15 min at the effluent of pretreatment unit and biological reactor; 300 mL every 15 min was sampled at the effluent of secondary clarifier and WWTP effluent). The retention times of each operation were not considered given that the treatment plant was operating stably without significant alterations and the organic matter removal efficiency was constant.

At the effluent of primary clarifier, the automatic sampling equipment was damaged, and for this reason 20 L of the sample was taken manually every 4 hours without taking night hours into account.

Additionally, another sample of the effluent of the WWTP (400 L) was also collected manually for 9 h without taking night hours into account and in order to compare to different sampling techniques.

In the sludge line, grab samples of 2 L of thickened sludge, 10 L of water from the centrifugation process, and 2 kg of dry sludge were sampled.

Samples were then homogenized and the subsamples were taken for further analysis considering the complexity and impurities of the matrix. Please check the table below.

Table S1 Sample volumes

Section	Unit	Sample volume		Analyzed volume	
		Summer	Winter	Summer	Winter
Water line	Influent	2 L	2 L	1 L	1 L
	Pretreatment	2 L	5 L	1 L	2 L
	Primary clarifier	20 L	20 L	5 L	5 L
	Biological reactor	15 L	25 L	10 L	10 L
	Effluent (composite 24 h)	25 L	25 L	25 L	25 L
	Effluent (filtered 9h)	100-400 L	24-400 L	100-400 L	24-400 L
Sludge line	Thickened sludge	2 L	2 L	0.5 L	0.5 L
	Centrifugation water	10 L	5 L	7 L	3 L
	Dry sludge	2 kg	2 kg	100 g	100 g

2. Analytical method recovery evaluation

Table S2 Recovery tests for thickened sludge

Thickened sludge	Particle size [µm]	Weight (g)	Initial number	Final number	Recovery [%]
	53-63	0.00161	15762	11250	71
	53-63	0.00156	15272	12100	79
	53-63	0.00176	17230	12943	75
	125-150	0.02300	1690	1175	70
	125-150	0.05000	3675	2850	78
	125-150	0.03430	2521	1873	74
	250-300	0.05500	504	370	73
	250-300	0.04200	385	258	67
	250-300	0.03980	365	256	70
	450-500	0.04800	93	92	99
	450-500	0.03200	62	62	100
	450-500	0.03460	67	68	101

3. Estimations of WWTP loads

- Water treat annually by Wastewater: **12.8×10^6 m^3/year**
- The average number of MPs removed per litter for both sampling periods:

  Summer: (369- 2.3) MPs/L= 366.7 MPs/L

  Winter: (1058- 5.6)MPs/L = 1052.4 MPs/L

  Average summer and winter: **709.55 MPs/L**

- How much MPs is avoided from entering the river annually

  12.8×10^6 m^3/year x 709,550 MPs/m^3 = **9.1×10^{12} MPs/year**

- How much MPs will reach the aquatic environment annually

Average MPs in the effluent 3.95 MPs/L = 3,950 MPs/m^3

3,950 MPs/m^3 x 12.8×10^6 m^3/year = **5.1×10^{10} MPs/year**

- How much MPs would reach terrestrial ecosystems if all sludge were recycled in agricultural soils:

  Average dry sludge: (51 450 + 133 200) MPs/kg: 2 = 92 325 MPs/kg

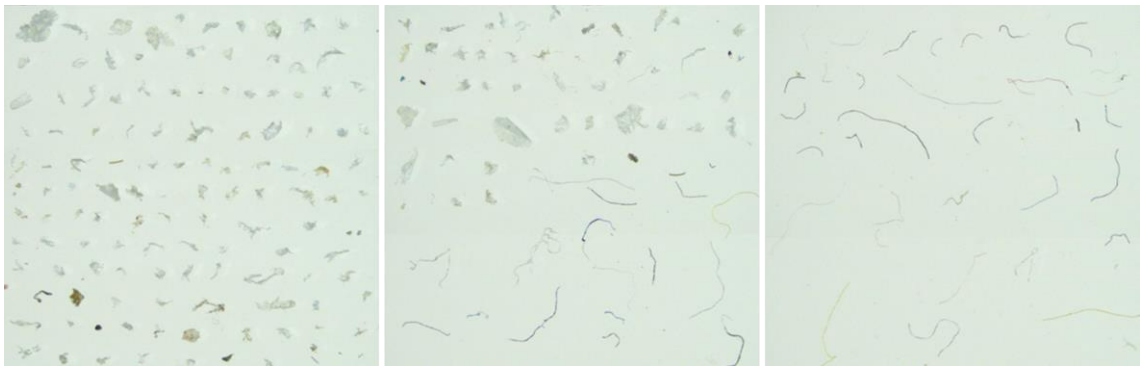
  944kg/h x 92 325 MP/kg x 24h/day x 365days/1year= **7.6×10^{11} MP/year**

4. Elaboration of the maps

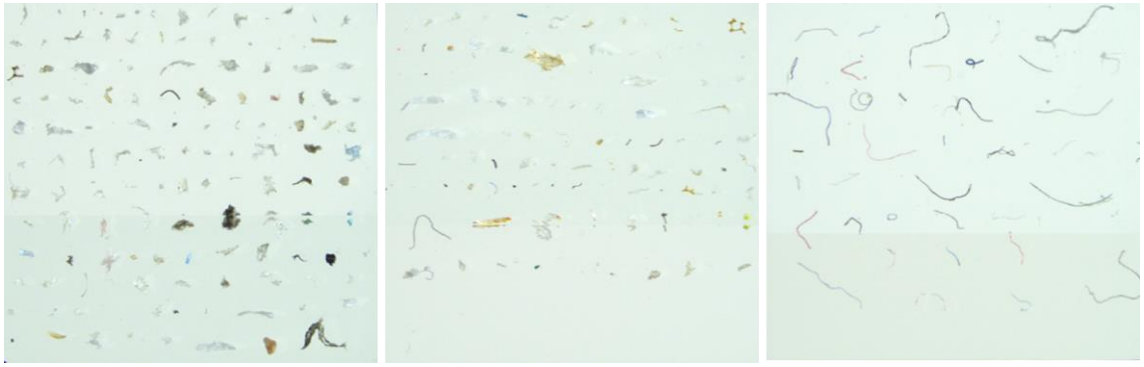
Subsamples of microplastic particles were prepared and extracted on calcium fluoride slides for mapping with micro-FTIR technique. Photos of the maps are presented below.

Influent of WWTP 0.02-2 mm (1 x 1 cm square)

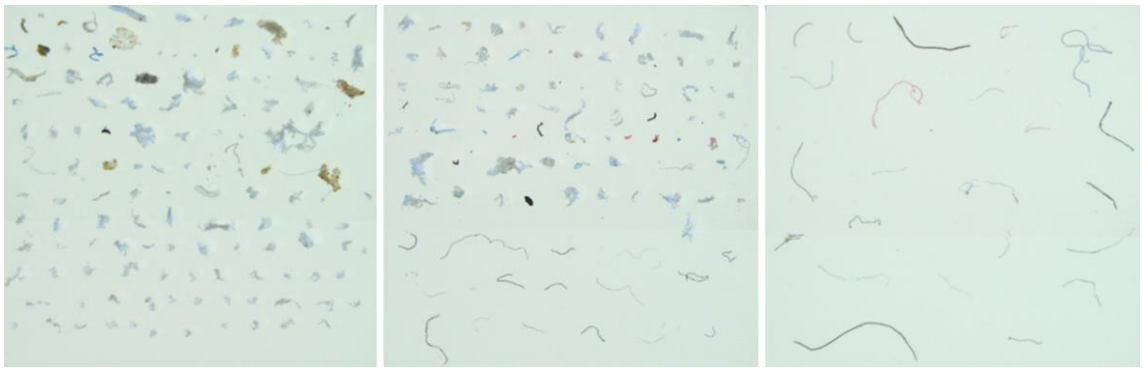
Pre-treatment of WWTP 0.02-2 mm (1 x 1 cm square)



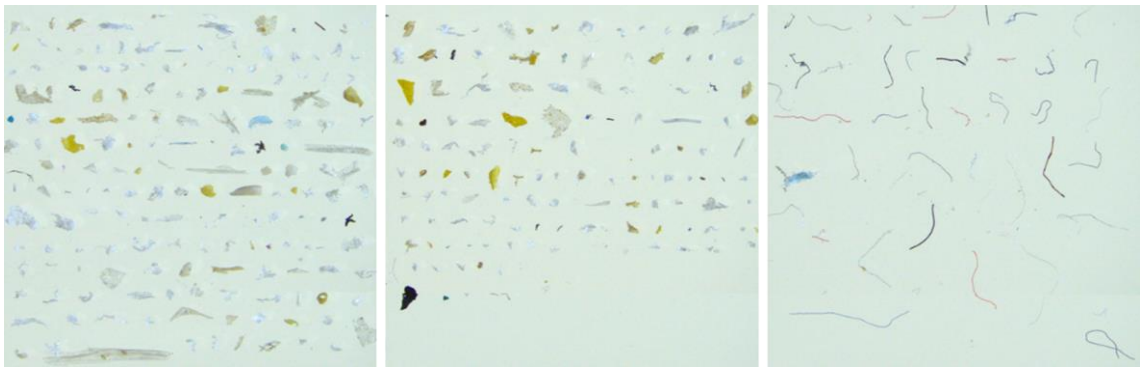
Primary clarifier 0.02-2 mm (1 x 1 cm square)



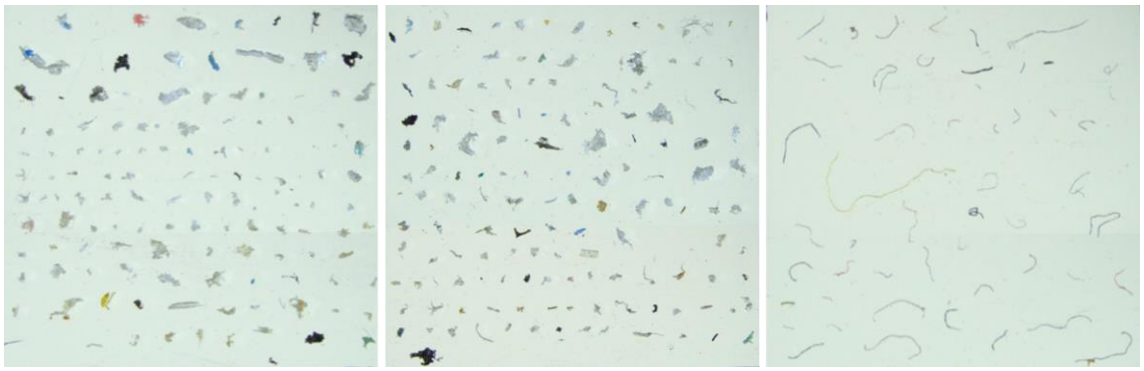
Biological reactor 0.02-2 mm (1 cm x 1 cm square)



Secondary clarifier 0.02-2 mm (1 cm x 1 cm square)



Dry sludge 0.02-2 mm (1 cm x 1 cm square)



Thickened sludge 0.02-2 mm (1 cm x 1 cm square)

5. *Composition of microplastics*

Table S2 Composition of microplastics for each sampling period

Influent of WWTP	[%] winter	[%] summer
PE	30	79
Synthetic cellulose	25	0
PP	9	7
PES	7	0
PE-PP	7	2
PA	6	0
PET	4	0
PVC	3	2
PEEA	2	5
PU	2	0
Other	5	0
PS	0	3
EVOH/EVA	0	2
Primary clarifier	[%] winter	[%] summer
PE	34	50
Synthetic cellulose	26	0
PP	12	33
PES	9	0
PA	4	0
PTFE	4	0
Other	11	0
PVC	0	17
Biological reactor	[%] winter	[%] summer
PE	47	33
PP	11	13
Synthetic cellulose	15	0
PES	12	0
PS	3	0
PU	4	0
PA	2	4
PET	2	0
Other	4	8
PE-PP	0	21
acrylamide	0	13
polyisobutadiene	0	8
Effluent of WWTP	[%] winter	[%] summer
PE	58	77
PP	4	4
PP-PE	6	2
Synthetic cellulose	20	0
PES	6	0
Other	6	9
PA	0	8

Dry sludge	[%] winter	[%] summer
PE	45	10
PE-PP	3	2
PP	4	0
PEEA	13	65
Synthetic cellulose	11	0
PES	6	0
PU	4	0
PET	3	0
PC	2	0
Alkyd resin	2	0
PS	2	0
Other	5	0
PA	0	21
PVC	0	2
Thickened sludge	[%] winter	[%] summer
PE	46	8
PP	12	0
Synthetic cellulose	11	0
PEEA	7	91
PE-PP	8	0
PES	5	0
PU	4	0
PET	2	0
Other	5	1
Centrifugation water	[%] winter	[%] summer
PE	44	45
PP	39	17
Synthetic cellulose	11	0
PES	4	0
PVC	1	0
PA	1	0
PEEA	0	3
PE-PP	0	16
Other	0	19

6. **Size distribution of microplastic particles**

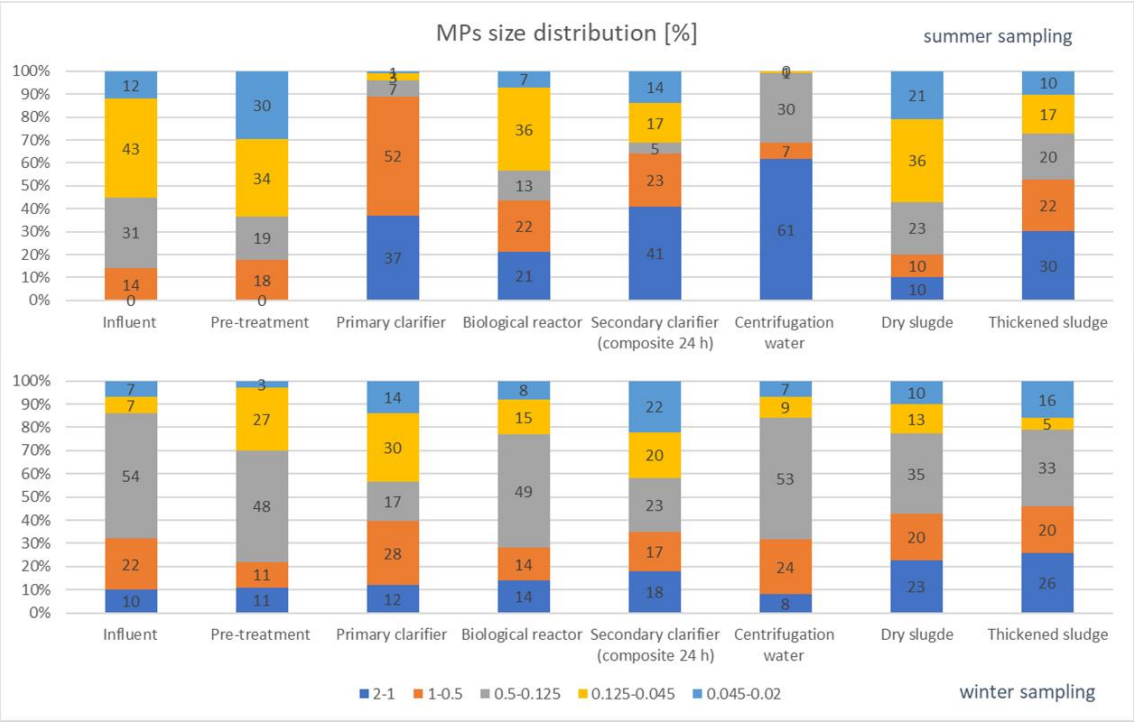


Figure S1 Microplastics size distribution within different treatment units of WWTP

Figure S1 presents the proportion of different size ranges of MPs across the WWTP. The size range of microplastic particles was from 5 mm to 0.02 mm divided into 5 sub-ranges: 2-1; 1-0.5; 0.5-0.125; 0.125-0.045; and 0.045-0.02 mm.

Annex II Chapter 2

Microplastics presence in water and sludge lines of a drinking water treatment plant in Catalonia, Spain.

1. Sampling of microplastics

Table S1: Water and sludge lines samples volumes per replicate

WATER LINE			
Clarifier line 1		**Clarifier line 2**	
PTFE Filter	**Volume (L)**	**PTFE Filter**	**Volume (L)**
1	4.8	1	3.1
2	4.0	2	3.6
3	4.6	3	2.8
4	5.2	4	2.5
5	2.7	5	5.3
6	4.6	6	5.6
7	3.1	7	5.1
Total	**29.0**	**Total**	**28.1**
Sand filter		**Activated carbon filter**	
PTFE Filter	**Volume (L)**	**PTFE Filter**	**Volume (L)**
1	78.6	1	211.8
2	89	2	107.8
3	99	3	280.2
Total	**266.6**	**Total**	**599.8**
Plant Influent (river water)		**Plant effluent**	
Subsamples	**Volume (L)**	**PTFE Filter**	**Volume (L)**
1	120	1	132.4
2	120	2	128.6
3	120	3	130.5
Total	**360**	4	143.3
		5	142.4
		6	148.3
		7	139.2
		Total	**964.6**
SLUDGE LINE			
Water from centrifugation		**Dry sludge**	
Subsamples	**Volume (L)**	**Subsamples**	**Weight (g)**
1	5	1	300
Total	**5**	**Total**	**300**

2. Size of fibers in water line

Size of fibers was determined with software *ImageJ* previously calibrated with reference material. Size is referring to the length of each fiber (Figure S1, Table S1).

Table S1. Size of fibers in the water line [%]

	Length of fibres [mm]				
	>5	5-2	2-1	1-0.5	0.5-0.1
Influent of DWTP	12.5%	30%	27.5%	22.5%	7.5%
Clarifier line 1	4.9%	34.2%	24.4%	14.6%	21.9%
Clarifier line 2	1.8%	14.0%	19.3%	28.1%	36.8%
Sand and carbon filter	<0.1%	5.7%	25.7%	42.9%	25.7%
Effluent of WWTP	3.2%	22.6%	38.7%	22.6%	12.9%

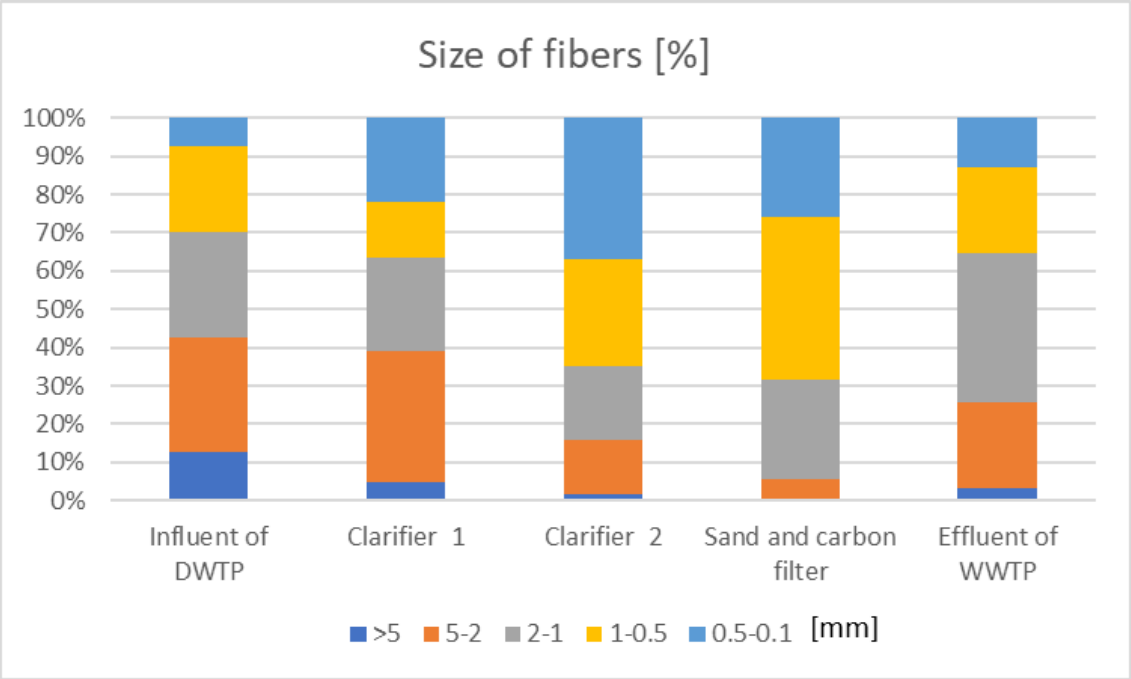


Figure S1. Lenght range of fibers in the water line

3. Composition of Microplastics.

Influent of DWTP	**%**
Polyester-PET	27
Synthetic cellulose	23
Polypropylene	23
Polyacrylonitrile	9
Polyamide	4
POLYETHYLENE	3
Others (less than 5%)	11%
Polyurethane	4
Polymethyl methacrylate	3
Polyvinyl stearate	2
PP-PE copolymer	2
Total	100
Primary clarifier	**%**
Synthetic cellulose	43
POLYPROPYLENE	26
Polyester-PET	11
Polyamide	11
Others (less than 5%)	9%
Viscose	5
Polycarbonate	2
EVOH/EVA	2
Total	100
Secondary clarifier	**%**
Synthetic cellulose	44
Polyamide	21
Polyester-PET	18
POLYETHYLENE	5
Others (less than 5%)	12%
Polyacrylonitrile	2.4
Polyvinyl acetate	2.4
Synthetic polymer	2.4
PVC	2.4
POLYPROPYLENE	2.4
Total	**100**
Sand and carbon filters	**%**
Synthetic cellulose	53
Polyester-PET	21
POLYETHYLENE	16
POLYPROPYLENE	5
Polyamide	5
Total	100
Effluent of DWTP	**%**
Synthetic cellulose	47
POLYETHYLENE	12

Polyamide	12
Polyester-PET	12
Polyurethane	6
Tygon B 44-4	6
Others (less than 5%)	5%
Fluoroelastomer	2.5
Polyisobutene	2.5
Total	**100**
Dry sludge	**%**
Synthetic cellulose	29
Polyamide	16
Polyester-PET	19
POLYPROPYLENE	10
Polyethylene	9
Alqui-resin	5
Others (less than 5%)	17%
Polyurethane	2
PP-PE copolymer	2
PVC	2
Polyacrylonitrile	1
Polyisobutadiene	1
Epoxy resin	0.8
Tygon B 44-4	0.8
EVOH/EVA	0.8
Polystyrene	0.8
Synthetic polymer	0.8
Total	**100**

Annex III Chapter 3

Evaluating Micro and Macro Plastic Environmental Hazards: An In-Depth Analysis of Organic Waste Amendments

1. Raw data regarding micro and macro plastic concentration in different organic waste amendments

MW-C		Sample 1							Sample 2								
	size(mm)	Fragments	Fibres	Films	Pellet	Foam		size(mm)	Fragments	Fibres	Films	Pelet	Foam				
	0.02-0.05 mm	2414	491	126	0	0		0.02-0.05 mr	12220	682	4476	0	0				
	0.05-0.1 mm	9492	2164	1382	4	0		0.05-0.1 mm	18424	2127	6638	0	0				
	0.1-0,5 mm	55701	37569	16175	0	0		0.1-0,5 mm	87111	24601	7179	0	0				
	0.5-1 mm	790	1423	891	0	0		0.5-1 mm	558	1327	292	0	0				
	1-2 mm	235	280	215	0	0		1-2 mm	270	159	80	0	0				
	2-5 mm	105	125	205	0	0	Total	2-5 mm	53	106	270	0	0	Total	mean	ds	%CV
	>5 mm	35	175	95	0	0	130093	>5 mm	5	80	27	0	0	166686	148389	25875	17,44
MW-D	0.02-0.05 mm	1802	211	87	0	0		0.02-0.05 mr	28264	3211	8732	0	0				
	0.05-0.1 mm	27942	10505	1157	0	0		0.05-0.1 mm	37533	973	11941	0	0				
	0.1-0,5 mm	262253	123269	33075	0	0		0.1-0,5 mm	145612	20973	8030	0	0				
	0.5-1 mm	230	549	4484	0	0		0.5-1 mm	195	390	1676	0	78				
	1-2 mm	240	779	3475	0	0		1-2 mm	78	1279	1131	0	0				
	2-5 mm	50	989	809	0	20		2-5 mm	0	491	117	0	0		mean	ds	%CV
	>5 mm	0	270	130	0	0	472326	>5 mm	0	819	390	0	0	271913	372119	141713	38,08
MW-B	0.02-0.05 mm	5048	566	307	0	0		0.02-0.05 mr	18442	3201	6162	0	0				
	0.05-0.1 mm	11667	2657	1273	0	0		0.05-0.1 mm	25702	1528	7973	0	0				
	0.1-0,5 mm	34619	22158	12068	0	0		0.1-0,5 mm	48251	16708	7730	0	0				
	0.5-1 mm	859	1099	2277	0	0		0.5-1 mm	1325	802	3355	0	0				
	1-2 mm	529	1019	1099	0	0		1-2 mm	475	1135	1261	0	0				
	2-5 mm	130	629	340	0	10	Total	2-5 mm	293	379	611	0	10		mean	ds	%CV
	>5 mm	40	509	170	0	0	99073	>5 mm	189	774	312	0	0	146617	122845	33619	27,37
AF-D	0.02-0.05 mm	4350	7862	1357	0	0		0.02-0.05 mr	89125	27779	34559	0	0				
	0.05-0.1 mm	28424	6939	7440	0	0		0.05-0.1 mm	49631	13082	15375	0	0				
	0.1-0,5 mm	76659	18182	2621	0	0		0.1-0,5 mm	76905	27300	9655	0	0				
	0.5-1 mm	440	806	2711	0	10		0.5-1 mm	264	716	1885	0	0				
	1-2 mm	160	150	460	0	10		1-2 mm	151	678	688	0	10				
	2-5 mm	40	480	470	0	0		2-5 mm	131	38	278	0	10		mean	ds	%CV
	>5 mm	30	130	80	0	10	159821	>5 mm	26	22	18	0	0	348325	254073	133293	52,46
HM																	
	0.02-0.05 mm	311	444	133	0	0		0.02-0.05 mr	79	296	43	0	0				
	0.05-0.1 mm	792	236	28	0	0		0.05-0.1 mm	217	166	29	0	0				
	0.1-0,5 mm	3148	1114	702	0	0		0.1-0,5 mm	2774	2246	330	0	0				
	0.5-1 mm	0	10	0	0	0		0.5-1 mm	10	25	0	0	0				
	1-2 mm	0	60	0	0	0		1-2 mm	0	100	0	0	0				
	2-5 mm	0	110	20	0	0		2-5 mm	0	84	0	0	0		mean	ds	%CV
	>5 mm	0	30	0	0	0	7137	>5 mm	0	10	0	0	0	6410	6774	514	7,59
AF-C	0.02-0.05 mm	17281	2659	390	0	0		0.02-0.05 mr	8192	1252	4534	0	0				
	0.05-0.1 mm	35847	5149	1742	0	0		0.05-0.1 mm	34300	3492	10929	0	0				
	0.1-0,5 mm	7455	22178	6651	0	0		0.1-0,5 mm	64019	27477	28255	0	0				
	0.5-1 mm	47	55	269	0	0		0.5-1 mm	158	32	63	0	0				
	1-2 mm	43	90	236	0	10		1-2 mm	32	63	222	0	0				
	2-5 mm	8	10	126	0	20		2-5 mm	32	32	32	0	0		mean	ds	%CV
	>5 mm	0	4	16	0	0	100286	>5 mm	0	0	0	0	0	183116	141701	58570	41,33
WW-SS		Fragments	Fibres	Films	Pellet	Foam			Fragments	Fibres	Films	Pellet	Foam				
	0.02-0.05 mm	109556	10860	665	0	0		0.02-0.05 mr	11818	988	6124	0	0				
	0.05-0.1 mm	109254	95131	12689	0	0		0.05-0.1 mm	30175	10228	3879	0	0				
	0.1-0,5 mm	30293	110732	1874	0	0		0.1-0,5 mm	159807	391596	2415	0	0				
	0.5-1 mm	171	679	141	0	0		0.5-1 mm	351	1579	378	0	0				
	1-2 mm	170	239	421	0	0		1-2 mm	96	476	254	0	0				
	2-5 mm	0	68	0	0	0		2-5 mm	0	284	0	0	0		mean	ds	%CV
	>5 mm	0	0	0	0	0	482943	>5 mm	0	0	0	0	0	620448	551695	97231	17,62

Annex IV Chapter 4

Spatial and Temporal Trends of Microplastics Accumulation in the Sediments of the San Francisco Bay Area

1. Sampling of microplastics

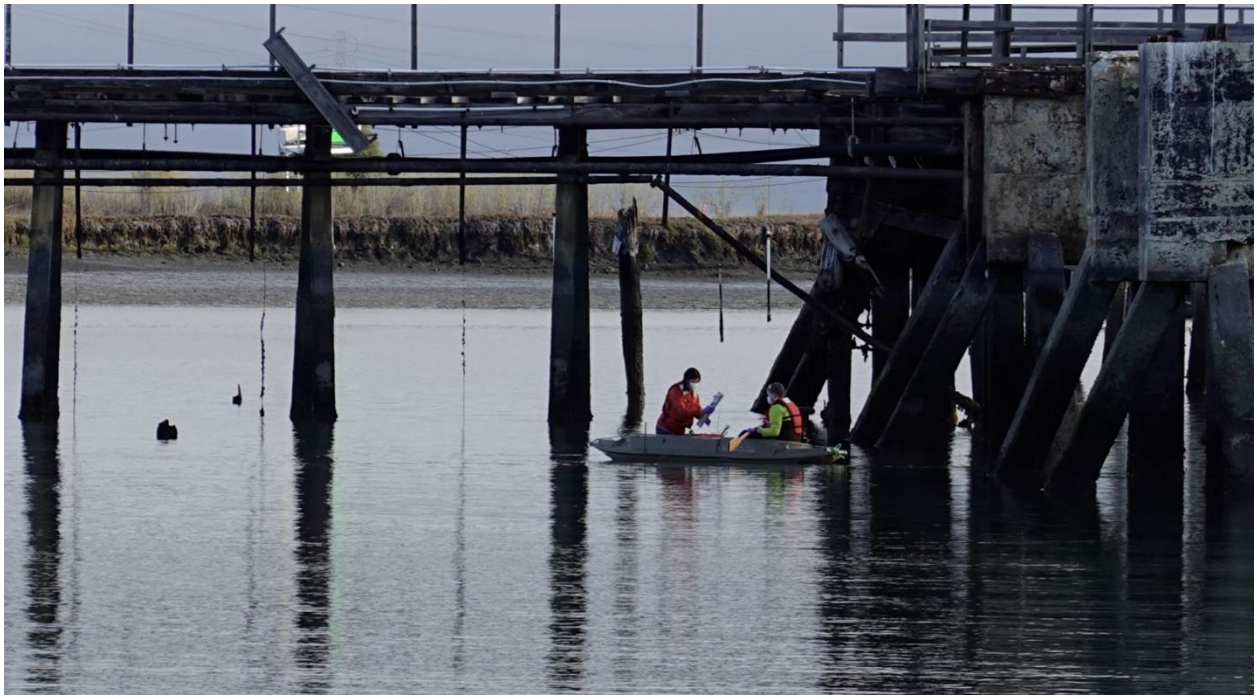


Figure S1 Sediment sampling and storage

Table S1 MPs sampling points

N	SAMPLE ID	TARGET LATITUDE	TARGET LONGITUDE	DEPTH [M]	LOCATION
1	LSB01	37.46823888	-122.0639734	3.7+	Lower South Bay
2	LSB02	37.49183613	-122.0985143	3.7+	Lower South Bay
3	SB01	37.55903527	-122.210577	3.7+	South Bay
4	SB02	37.61019366	-122.1673764	0.3 to 0.9	South Bay
5	CB01	37.82232768	-122.3492815	3.7+	Central Bay
6	CB02	37.87631112	-122.3615019	1.8 to 3.7	Central Bay
7	NB01	38.05895268	-121.8143678	3.7+	North Bay
8	NB02	38.02282086	-121.8083671	3.7+	North Bay
C1	Core 1	37.507007	-122.245120		South Bay
C2	Core 2	37.504393	-122.215279		Lower South Bay

2. Surface sediment characteristics

Sample characteristics are presented in Table S2.

Table S2. Total mass analyzed per sample

Sample ID	Mass of the sample-wet weight [$g_{w.w.}$]	Humidity %	Mass of the sample-dry weight [$g_{d.w.}$]
1024 BA41	100.50	61.14	39.05
1036 BC11	100.60	54.60	45.67
1011 BA10	100.00	44.11	55.89
1201 SB002S	100.30	63.01	37.10
1161 LSB001S	100.80	50.92	49.47
1107 CB001S	102.10	50.42	50.63
1070 BG20	100.70	20.61	79.95
1082 BG30	100.10	23.31	76.77

Table S3. Cross-contamination

BLANK SAMPLE	CROSS-CONTAMINATION OF MPS DETECTED [TOTAL NUMBER]		
	fibers	*Films*	*fragments*
1. LABORATORY 1	34	1	-
2. TRANSPORTATION	1	-	-
3. LABORATORY 2	15	-	
4. FIELD BLANK 1137	2	3	1

- Laboratory 1: 1.13 fibers/day/cm^3
- Laboratory 2: 0.5 fibers/day/cm^3

3. Size distribution of surface sediment samples

The analysis of MPs size distribution in the studied areas revealed distinct patterns. In Lower South Bay and North Bay, the dominant size range for MPs was found to be between 5 mm and 500 µm, with a lower abundance in the size fraction of 500-25 µm. On the contrary, South Bay exhibited a higher abundance of MPs in the size fraction 500-25 µm. In Central Bay, one sample showed a dominant presence of higher fraction, while the other sample had a higher abundance in the lower 500-25 µm fraction. These findings emphasize the variability in MPs size distribution among the different Bay areas.

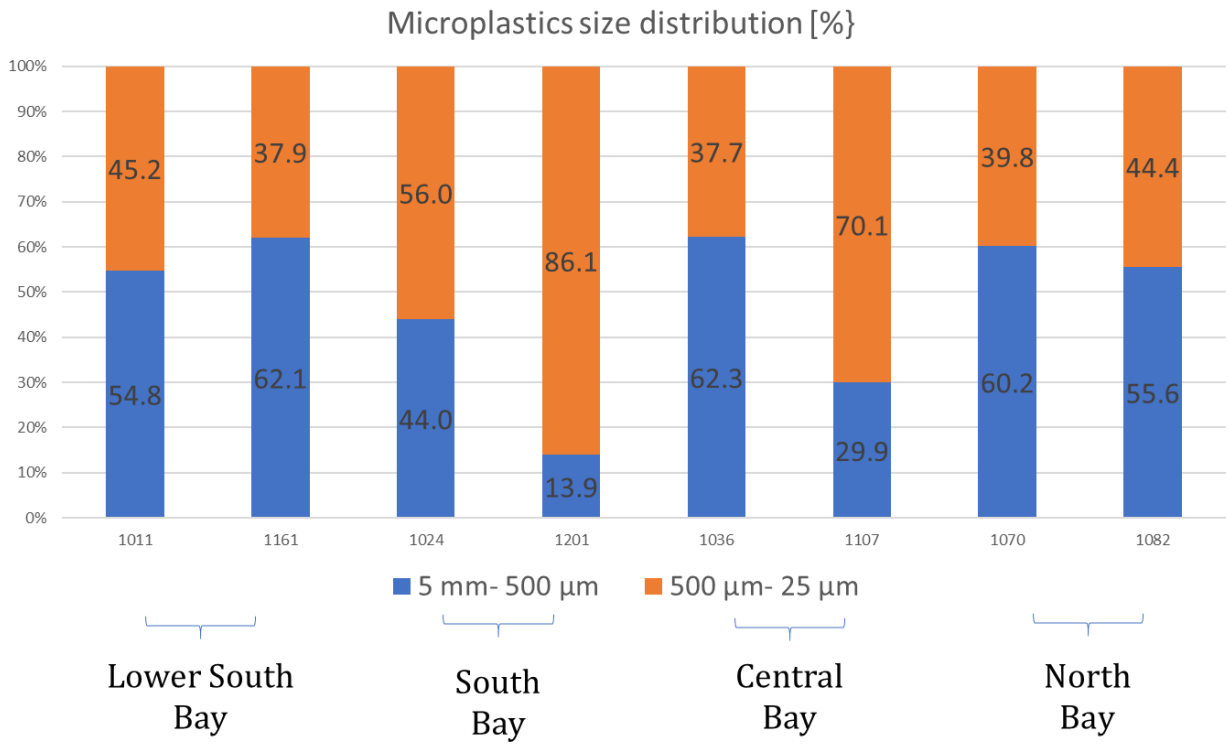


Figure S2 Microplastics size distribution in surface sediments

4. Elaboration of the maps on calcium fluoride slides

In order to identify chemical composition of microplastic particles, FTIR maps were prepared on calcium fluoride slides.

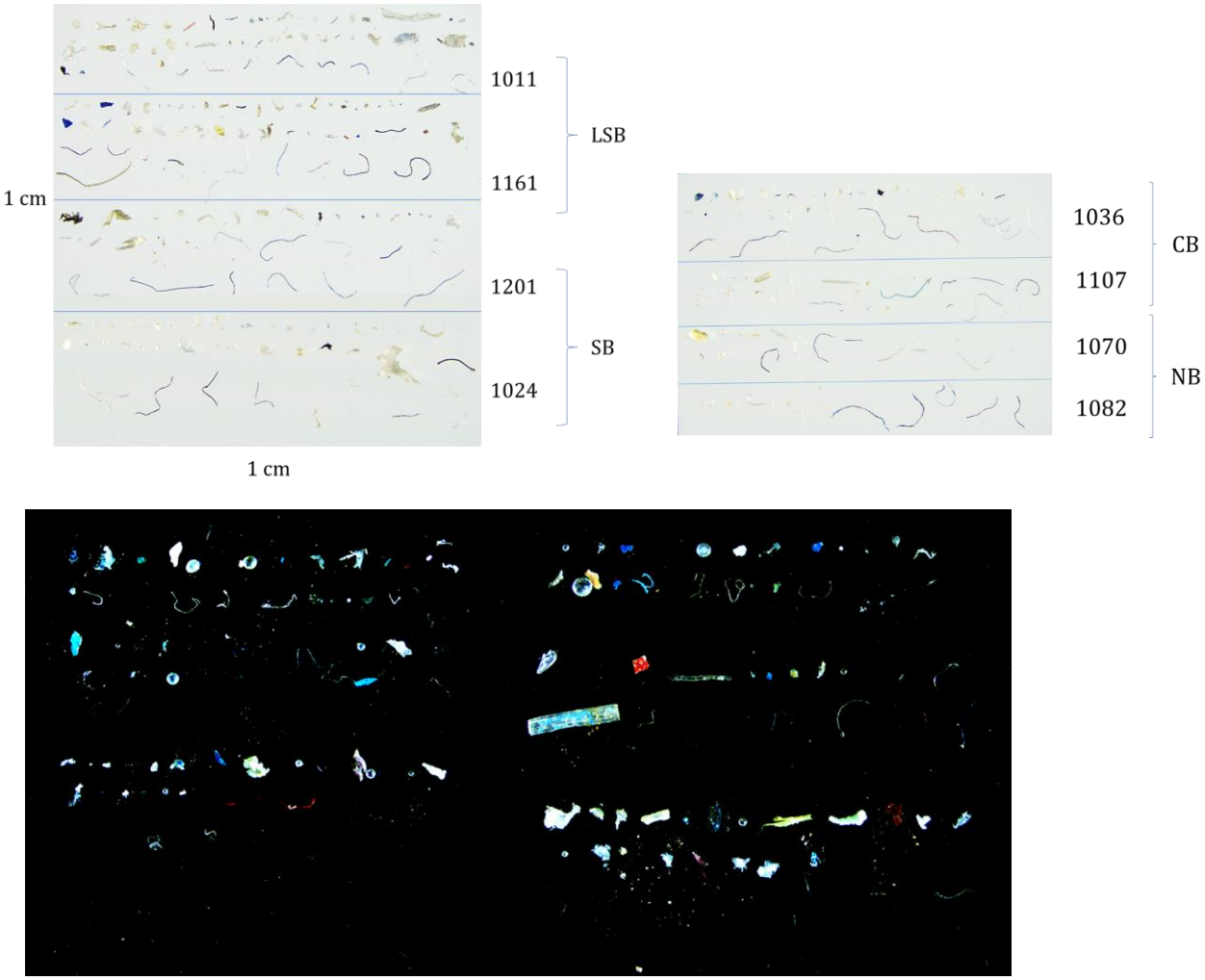


Figure S3 FTIR maps for microplastic identification

5. Software for advanced identification of microplasic particles

In order to analyze the spectra Omnic Picta software for advance microplastics identification was used. The acquired spectra were analyzed using the OMNIC Specta MCS Software. To identify the particles, an unknown spectrum matching approach was employed by comparing them with available databases such as HR Nicolet Sampler Library, Hummel Polymer Sample Library, Polymer Laminate Films, Wizard Library, Willey's Know It All as well as an own library that was generated with more than 80 spectra. The standard criteria of ≥ 70% between the sample and reference spectra were followed.

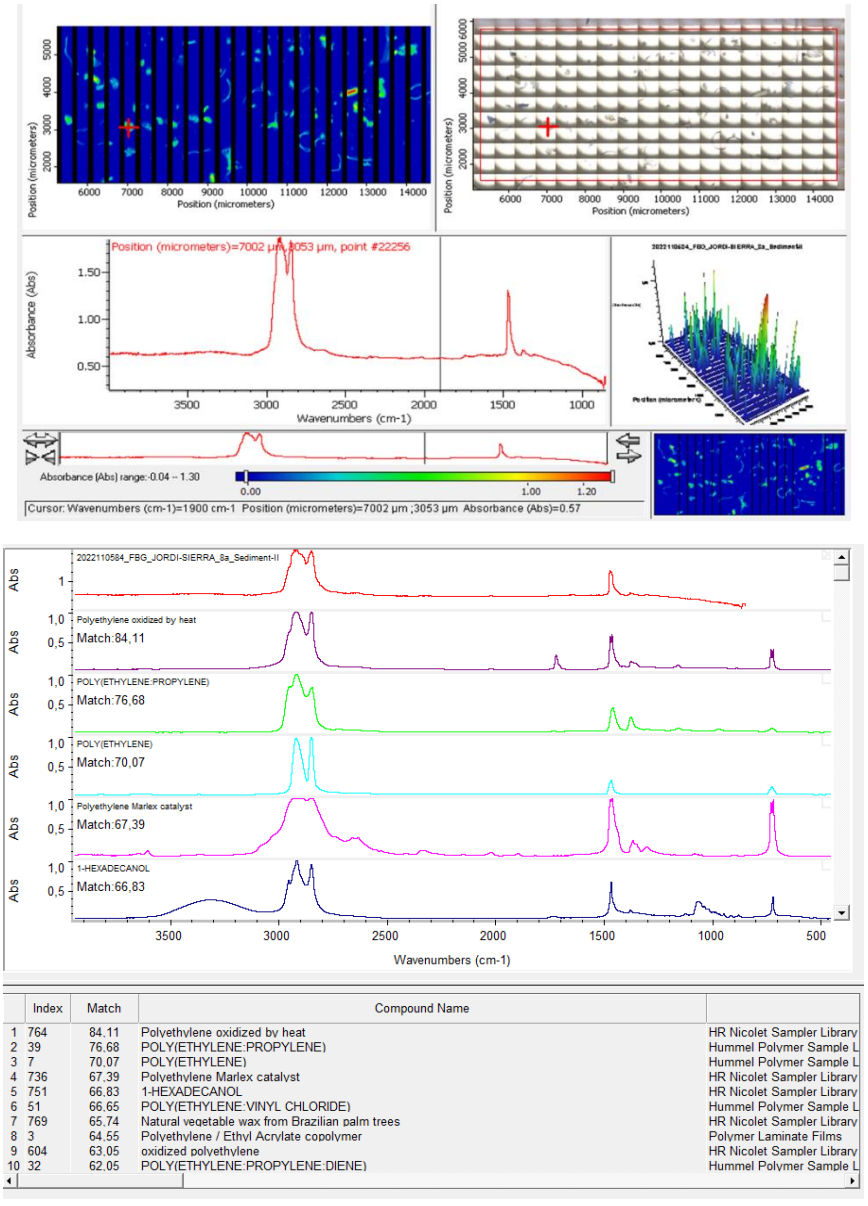


Figure S4 OMNIC Specta MCS Software for microplastics identification

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