Doctoral Program in Hydrology and Water Resource Management

MICROPLASTICS IN FRESHWATER ECOSYSTEMS: SOURCES, PATHWAYS, AND RISKS

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2022

Universidad de Alcalá

Doctoral Program in Hydrology and Water Resource Management

Microplastics in Freshwater Ecosystems: Sources, Pathways, and Risks

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Alcalá de Henares, 2022

To my parents

Numerous people and institutions contributed to and supported the research work carried out in this thesis, and also many people have supported me during this wonderful but challenging experience.

First of all, I would like to thank IMDEA as well as my supervisors, Andreu Rico and Marco Vighi, for allowing me to conduct this thesis. I am incredibly grateful to Andreu and Marco for everything they have thought me, their guidance and encouragement throughout this period. Thanks to them, this doctorate has allowed me to grow scientifically and personally. Thank you, Andreu for reading my numerous drafts and for your patience. Marco, your level of knowledge and ingenuity is something that will always keep aspiring me, and I will, of course, never forget the many Italian dinners.

I would also like to express my sincere gratitude to Luca Nizzetto and Rachel Hurley, for making my research experience at NIVA possible, teaching me so much about microplastic analysis, but also for their help, insightful comments and suggestions with paper writing. My special thanks also to Nina Buenaventura, for assistance in the laboratory work, especially with the soil sample analysis when the pandemic did not allow me to continue working in the lab.

Furthermore, I would like to thank the personnel of IMIDRA and especially Pedro V. Mauri, for their cooperation in the field experiment and the personnel of the Animal Experimental Centre of the University of Alcalá for their collaboration in the bioconcentration experiment.

Many thanks also to all the lab mates and colleagues from IMDEA who made this work possible. My special thanks to Sara and Raquel for their help with the toxicity studies despite the many struggles we encountered with the animal cultures. Thank you, Raquel, for bringing so much positivity and cheerfulness into the lab, and Sara, I’m glad that you still decided to pursue a doctorate in microplastics research. I would like to also thank Ivan and Ana María for their help in the laboratory studies. Thanks to Paula for her helpful comments on a draft of Chapter 5. My gratitude extends, of course, to the whole Ecotoxicology group of IMDEA. Their warm welcome, kindness, and support have made my start and life in Spain such a wonderful experience. A very special thanks to Alba, Ari, Francesco, Melina, and Amaia for the wonderful and fun

times at IMDEA but also for the moments we shared outside of the office. Many thanks to Jorge for the numerous times he helped me with R and to Alberto for his help with GIS.

I also want to thank my friends, who supported me along the way, especially Lilli for her many trips to Spain, Kym for always listening and providing advice, and Nadja for always bringing some optimism. Lastly, my family deserved endless gratitude. Without my parents' tremendous understanding, love, support, and encouragement, it would have been impossible for me to complete this doctorate. Thanks also to my brother, Opa and uncle Norbert for always believing in me. Danke.

Contents

Summary

Plastics and microplastics (MPs, plastics below 5 mm) are now ubiquitously found in the natural environment. They are a versatile group of materials that are made of different polymers and can have different shapes (e.g., bead, fragment, fiber). MPs are either directly emitted into the environment in this size or form due to the breakdown of larger plastic particles. However, the contribution of different MP sources and their pathways into the environment and between compartments are not well understood. Wastewater and sewage sludge have been suggested as major MP entry pathways into aquatic and terrestrial environments. Wastewater and stormwater converge a wide range of potential MP sources. These MPs can be released directly into the environment if wastewater treatment infrastructures are not in place or due to combined sewer overflows. In wastewater treatment plants, most MPs are captured and concentrated in the sewage sludge, with a smaller proportion not retained and released with treated effluents to aquatic receptors. Moreover, if sludge is used as fertilizer on agricultural lands, these wastewater-derived MPs can re-enter terrestrial ecosystems. Subsequently, they may be driven to aquatic environments through surface water runoff. For instance, MP fibers released from textiles and MP particles from the abrasion of vehicle tires during driving are expected to be emitted in large quantities into the environment along these pathways. Once in the environment, MPs may negatively affect organisms through various physical and chemical mechanisms such as entanglement, external damage, internal abrasive damage or blockage of the digestive tract after ingestion, food dilution, or leaching of harmful additives. Furthermore, MPs can interact with other environmental pollutants through sorption processes, thus acting as transport vectors of these pollutants for aquatic organisms.

This thesis aims: (1) To assess the current state of knowledge and identify data gaps regarding the sources, environmental pathways, loads and fate of plastic and MPs in aquatic and terrestrial ecosystems. (2) To determine MP pollution in a Mediterranean river catchment and elucidate the role of wastewater as a MP entry pathway. (3) To uncover the fate of MPs in agricultural soils following sewage sludge application and the importance of agricultural surface water runoff as a transport mechanism for MPs into aquatic ecosystems under semi-arid conditions. (4) To quantify the impacts of frequent MP types on freshwater invertebrates by considering

environmentally relevant shapes, sizes, and concentrations and to perform a risk assessment for these organisms. (5) To assess the relevance of MPs as vectors of other chemical contaminants in the aquatic environment and their contribution to ecological risks.

Following the general introduction in Chapter 1, Chapter 2 summarizes the current knowledge regarding the sources of plastics and MPs, their environmental pathways and load rates, and their occurrence and fate in different environmental compartments. It shows that knowledge of the sources of large-sized plastics, MPs, and nanoplastics to aquatic and terrestrial ecosystems is limited. Data on MPs occurrence in freshwaters is restricted to certain geographical regions, while data on soil ecosystems are generally limited. Furthermore, data limitations exist for pathways and fluxes into the environment and between different environmental compartments. This literature review also shows that the presence of MPs in wastewater is relatively well studied. However, the contribution of this pathway for MP contamination in aquatic ecosystems, compared to other pathways such as surface water runoff or the occurrence of large-sized plastics and their disintegration into MP and nanoplastic, are urgent research needs. Finally, this chapter highlights research areas that need to be advanced to improve the understanding of potential ecological risks of plastic pollution and provides recommendations to improve management and reduce plastic and MP inputs into the environment.

In the next chapter (Chapter 3), MP pollution in a Mediterranean river catchment (Henares River catchment, central Spain) is assessed, and the contribution of wastewater to the MP catchment discharge is identified. This chapter shows that although wastewater treatment plants retain on average 93% of the MPs in the influent, the treated effluents and small amounts of untreated wastewater inlets still contribute up to 50% of the total MP catchment discharge. Thus, the wastewater system represents a major environmental pathway for MPs into Mediterranean rivers. Moreover, it shows that MP concentrations in river water and sediment strongly depend on land use and significantly increase with increasing anthropogenic pressure. Finally, MP pollution patterns in the catchment are found to be influenced by season due to high flow periods in Spring, with higher water concentrations but lower sediment concentrations.

In Chapter 4, MP fate in agricultural soils receiving different sewage sludge treatments and the role of agricultural soil as contributors to MP pollution in aquatic ecosystems through surface water runoff is investigated. This chapter shows that the application of sewage sludge significantly increases MP concentrations in soils. These soil concentrations remain relatively constant over one year and little transport into deeper soil layers is observed. Additionally, the study indicates that MP concentrations in historically treated soils remain high five years after sludge application. Finally, this chapter highlights that surface water runoff has a negligible influence on the export of MPs from agricultural soils. Therefore, it concludes that agricultural lands can be considered long-term MP accumulators under the studied soil and weather conditions.

In Chapter 5, the ingestion and effects of polyester fibers and car tire particles are assessed for freshwater invertebrates with different feeding strategies and habitat preferences. It shows that ingestion capability depends on particle shape and size, while ingestion quantity also depends on the exposure pathway and the feeding strategy of the test organism. No adverse effects on survival or reproductive output for benthic macroinvertebrates are observed in this study. However, it concludes that chronic exposure of *Daphnia magna* (pelagic species) to both MP types could affect reproduction and survival at concentrations, that are for the most part not environmentally realistic yet. Finally, this chapter shows that MPs affect organisms through different effect mechanisms.

In Chapter 6, the relevance of MPs to act as vectors of organic pollutants to freshwater fish is investigated. This is done by assessing the bioconcentration and chemical stress caused by two hydrophobic organic chemicals in *Danio rerio* with and without MPs. This chapter shows that the presence of MPs does not enhance but rather decreases the bioconcentration of these chemicals. Moreover, the decrease in bioconcentration is greater for the more hydrophobic chemical. Chemical stress, determined through different biomarkers, is not enhanced in the presence of MPs. This confirms that MP contamination in freshwater ecosystems is not expected to aggravate the risks associated with the bioconcentration of organic contaminants in aquatic organisms. Other exposure pathways (i.e., uptake via respiration, skin permeability) may instead be of higher importance.

Finally, the overall conclusions of this thesis are presented in Chapter 7, together with some recommendations for future research and MP pollution management.

Resumen

Los plásticos y microplásticos (MPs, plásticos de menos de 5 mm) ahora se encuentran ubicuamente distribuidos en el entorno natural. Son un grupo versátil de materiales que se fabrican a partir de diferentes polímeros y pueden tener diferentes formas (p. ej., perla, fragmento, fibra). Los MPs pueden llegar al medio ambiente directamente o producirse por la partición de basura plástica más grande. Todavía se desconocen las principales fuentes y rutas de entrada de MPs en el medio ambiente, al mismo tiempo, que no se termina de comprender cómo éstos se transfieren entre los distintos compartimentos ambientales. Por un lado, la emisión de aguas residuales no tratadas y aguas de tormenta han sido señaladas como una de las principales fuentes de MPs en el medio ambiente. Por otro lado, las depuradoras, aunque consigue eliminar parte de éstos, también constituyen una fuente de emisión continua de MPs sobre ecosistemas acuáticos. Además, gran parte de los MPs se concentran en los lodos de las depuradoras. El uso de esos lodos como fertilizante agrícola, puede contribuir a su emisión sobre los ecosistemas agrícolas y contribuir a aumentar la carga de éstos sobre los ríos a través de la escorrentía superficial. Por ejemplo, las fibras textiles sintéticas y las partículas de neumáticos de vehículos procedentes de la abrasión producida durante la conducción se han encontrado en grandes cantidades en el medio ambiente. Una vez en el medio ambiente, los MPs pueden afectar a los organismos vivos negativamente por varios procesos, tanto físicos como químicos, incluyendo la reducción en la movilidad, daños externos, daños abrasivos internos, obstrucción del tracto digestivo por su ingestión, inanición o incluso por la lixiviación de aditivos perjudiciales para su salud. Además, los MPs pueden interaccionar con otros contaminantes que haya en el ambiente a través de procesos como la adsorción química, y por tanto pueden actuar como vectores de estos otros contaminantes en los organismos acuáticos.

Los principales objetivos de esta tesis doctoral son: (1) Evaluar el estado de conocimiento actual sobre la contaminación de plásticos y MPs en ambientes acuáticos y terrestres, así como su destino ambiental y sus principales vías de transporte. (2) Evaluar la contaminación por MPs en una cuenca Mediterránea y la influencia de la descarga de aguas residuales sobre esta. (3) Determinar el destino ambiental de los MPs en suelos agrícolas tratados con lodos de depuradora y evaluar la importancia de la escorrentía superficial como medio de transporte de MPs hacia

ecosistemas acuáticos bajo condiciones semi-áridas. (4) Cuantificar los impactos de los MPs típicamente encontrados en el medio ambiente de acuerdo a su forma, tamaño y concentración sobre los invertebrados de agua dulce para realizar una evaluación de riesgo ambiental. (4) Evaluar la importancia de los MPs como vector de otros contaminantes químicos en el medio acuático y sus potenciales riesgos ecotoxicológicos.

A continuación de la introducción general del Capítulo 1, en el Capítulo 2 se sintetiza el conocimiento actual relativo a las fuentes de plásticos y MPs, sus rutas de entrada al medio ambiente y concentraciones medias, así como su presencia y destino en cada compartimento ambiental. También se muestra como el conocimiento de las fuentes de macroplásticos, MPs y nanoplásticos en ecosistemas acuáticos y terrestres es limitado. Los datos acerca de la presencia de MPs en aguas dulces se restringe a determinadas localizaciones geográficas, mientras que la existencia de datos de ésta en suelos terrestres es aún más reducida. Además, también existe información limitada sobre las vías de entrada y transporte de MPs entre los distintos compartimentos ambientales. Esta revisión bibliográfica también revela que la presencia de MPs en aguas residuales está relativamente bien documentada. Sin embargo, la contribución de éstas como fuente de contaminación de MPs en ecosistemas acuáticos, comparada con otras como la escorrentía superficial o la presencia y posterior degradación de plásticos más grandes en MPs y nanoplásticos necesita ser urgentemente investigada. Finalmente, este capítulo subraya los potenciales riesgos ambientales de la contaminación plástica y proporciona algunas recomendaciones para mejorar la gestión de estos residuos y reducir la entrada de plásticos y MPs en el medio ambiente.

En el siguiente capítulo (Capítulo 3) se evalúa la contaminación por MPs en una cuenca hidrográfica de carácter mediterráneo (cuenca del río Henares, España) incluyendo el aporte procedente de las aguas residuales. Este capítulo muestra que, aunque las plantas de tratamiento de aguas llegan a retener una media del 93% de los MPs que llegan por el afluente, los efluentes resultantes y otras pequeñas masas de agua no tratada contribuyen hasta el 50% del vertido total de MPs en esta cuenca. Así pues, el sistema de aguas residuales representa una importante vía medioambiental de entrada de MPs en los ríos mediterráneos. Además, se muestra que las concentraciones de MPs en el agua y los sedimentos de los ríos dependen en

gran medida del uso del suelo y aumentan significativamente con el incremento de la presión antropogénica. Por último, se observa que los patrones de contaminación por MPs en la cuenca están influidos por la estación del año como los períodos de mayor caudal en primavera, con mayores concentraciones en el agua, pero menores en los sedimentos

En el capítulo 4 se investiga el destino de los MPs en los suelos agrícolas que reciben diferentes tratamientos de lodos de depuradora y el papel de los suelos agrícolas como contribuyentes a la contaminación por MPs en los ecosistemas acuáticos a través de la escorrentía de aguas superficiales. Este capítulo muestra que la aplicación de lodos de depuradora aumenta significativamente las concentraciones de MPs en los suelos. Además, estas concentraciones se mantienen relativamente constantes a lo largo de un año y se observa un escaso transporte hacia las capas más profundas del suelo. El estudio indica que las concentraciones de MPs en los suelos históricamente tratados siguen siendo elevadas cinco años después de la aplicación de los lodos. Por último, este capítulo destaca que la escorrentía de las aguas superficiales tiene una influencia insignificante en la exportación de MPs en suelos agrícolas. Por lo tanto, se concluye que los suelos agrícolas se pueden considerar grandes acumuladores de MPs a largo plazo en las condiciones edafológicas y meteorológicas estudiadas.

En el capítulo 5 se evalúan las tasas de ingestión y los efectos de las fibras de poliéster y las partículas de neumáticos de automóvil en invertebrados de agua dulce con diferentes estrategias de alimentación y preferencias de hábitat. Se demuestra que la capacidad de ingestión depende de la forma y el tamaño de las partículas, mientras que la cantidad de ingestión también depende de la vía de exposición y de la estrategia de alimentación del organismo de ensayo. En este estudio no se observan efectos adversos en la supervivencia o el rendimiento reproductivo de los macroinvertebrados bentónicos. Sin embargo, se concluye que la exposición crónica de *Daphnia magna* (especie pelágica) a ambos tipos de MP podría afectar a su reproducción y la supervivencia a unas concentraciones que no son ambientalmente realistas del todo. Por último, este capítulo muestra que los MPs afectan a los organismos a través de diferentes mecanismos.

En el capítulo 6 se investiga la importancia de los MPs como vectores de contaminantes orgánicos en peces de agua dulce. Para ello, se evalúa la bioconcentración y el estrés químico causado por dos sustancias químicas orgánicas hidrofóbicas en el pez cebra *Danio rerio* con y sin MPs. Este capítulo muestra que la presencia de MPs no aumenta, sino que disminuye la bioconcentración de estas sustancias químicas. Además, la disminución de la bioconcentración es mayor para el compuesto más hidrofóbico. El estrés químico, determinado a través de diferentes biomarcadores, no aumenta en presencia de MP. Este capítulo confirma que no se espera que la contaminación por MP en los ecosistemas de agua dulce agrave los riesgos asociados a la bioconcentración de sustancias químicas orgánicas en los organismos acuáticos. En cambio, otras vías de exposición (por ejemplo, la absorción a través de la respiración o la permeabilidad de la piel) pueden tener mayor importancia.

Por último, en el capítulo 7 se presentan las conclusiones generales de esta tesis, junto con algunas recomendaciones para la investigación futura y la gestión de la contaminación con MPs.

Zusammenfassung

Kunststoffe und Mikroplastik (MP, Kunststoffe kleiner als 5 mm) sind heute allgegenwärtig in der Umwelt zu finden. Sie stellen eine vielseitige Werkstoffgruppe dar, die aus verschiedenen Polymeren besteht und in unterschiedlichen Formen vorkommen kann (z. B. Kugel, Fragment, Faser). MP werden entweder direkt in dieser Größe in die Umwelt abgegeben oder entstehen durch die Fragmentierung größerer Kunststoffpartikel. Der Beitrag verschiedener MP-Quellen und -Eintragspfade in die Umwelt und zwischen verschiedenen Kompartimenten ist jedoch weitgehend ungeklärt. Es wird vermutet, dass Abwasser und Klärschlamm als Haupteintrittspfade für MP in aquatische und terrestrische Ökosysteme fungieren. Abwasser und Niederschlagswasser führen eine Vielzahl potenzieller MP-Quellen zusammen die direkt in die Umwelt freigesetzt werden können, wenn keine Kläranlagen vorhanden sind oder aufgrund von Mischwasserüberläufen. In Kläranlagen werden die meisten MP aus dem Abwasser entfernt und im Klärschlamm aufkonzentriert, wobei ein kleinerer Anteil der MP nicht zurückgehalten und mit dem geklärten Abwasser in aquatische Systeme eingeleitet wird. Durch das Ausbringen von Klärschlamm als Düngemittel auf landwirtschaftlichen Flächen können die aus dem Abwasser stammenden MP außerdem terrestrische Ökosysteme erreichen. Von dort könnten sie wiederum durch Oberflächenwasserabfluss in aquatische Systeme transportiert werden. Es wird vermutet, dass vor allem MP-Fasern von Textilien und Reifenabrieb über diese Pfade in großen Mengen in die Umwelt gelangen. Einmal in der Umwelt können MP Organismen durch verschiedene physikalische und chemische Mechanismen negativ beeinflussen. Dazu gehören das Verfangen in MP, äußere und innere Verletzungen oder die Blockierung des Verdauungstrakts, Verringerung des Nahrungsgehalts durch den Verzehr von Plastik anstatt Nahrung, oder das Auslaugen schädlicher Zusatzstoffe. Darüber hinaus können MP durch Sorptionsprozesse mit anderen Umweltschadstoffen in Wechselwirkung treten und somit als Transportvektoren dieser Schadstoffe für aquatische Organismen fungieren.

Die Hauptziele dieser Arbeit sind: (1) Den aktuellen Wissensstand bezüglich der Quellen, Umweltpfade, Konzentrationen und dem Verbleib von MP in aquatischen und terrestrischen Ökosystemen zu bewerten und Datenlücken zu identifizieren. (2) Die MP-Kontamination in einem Flusseinzugsgebiet in einer Mediterranen Klimazone zu bestimmen und die Rolle von Abwasser als Eintragspfad für MP aufzuklären. (3) Das

Schicksal von MP in landwirtschaftlichen Böden nach der Klärschlammausbringung zu bestimmen und die Bedeutung des Oberflächenwasserabflusses von landwirtschaftlich genutzten Flächen als Transportmechanismus für MP in aquatische Ökosysteme unter semi-ariden Klimabedingungen zu identifizieren. (4) Die Auswirkungen häufiger MP-Typen auf Süßwasserinvertebraten unter Berücksichtigung umweltrelevanter Formen, Größen und Konzentrationen zu analysieren und eine Risikobewertung für diese Organismen durchzuführen. (5) Die Relevanz von MP als Vektoren für andere chemische Schadstoffe in der aquatischen Umwelt und ihren Beitrag zu ökologischen Risiken zu bestimmen.

Nach der allgemeinen Einführung in Kapitel 1 fasst Kapitel 2 den aktuellen Wissensstand zu den Quellen von Kunststoffen und MP, ihren Pfaden in die Umwelt und Belastungsraten sowie ihrem Vorkommen und Verbleib in verschiedenen Umweltkompartimenten zusammen. Es zeigt, dass das Wissen über die Herkunft von Makroplastikteilen, MP und Nanoplastik in aquatischen und terrestrischen Ökosystemen begrenzt ist. Daten zum Vorkommen von MP in Süßgewässern sind auf bestimmte geografische Regionen beschränkt, während Daten zu Bodenökosystemen im Allgemeinen unzureichend sind. Darüber hinaus bestehen Wissenslücken bezüglich der Eintragspfade in die Umwelt und dem Transport und Verbleib zwischen verschiedenen Umweltkompartimenten. Diese Literaturrecherche zeigt auch, dass das Vorkommen von MP im Abwasser relativ gut untersucht ist. Es besteht jedoch dringender Forschungsbedarf bezüglich des Beitrags von Abwasser zur MP-Kontamination in aquatischen Ökosystemen im Vergleich zu anderen Eintragspfaden wie dem Oberflächenwasserabfluss oder dem Auftreten von größeren Kunststoffteilen und deren Fragmentierung in MP und Nanoplastik. Schließlich hebt dieses Kapitel Forschungsbereiche hervor die vorangetrieben werden müssen um das Verständnis potenzieller ökologischer Risiken der Plastikverschmutzung zu erweitern, und gibt Empfehlungen zum Management und der Reduzierung von Plastik- und MP-Einträgen in die Umwelt.

Im nächsten Kapitel (Kapitel 3) wird die MP-Kontamination in einem Flusseinzugsgebiet in der Mediterranen Klimazone (Einzugsgebiet des Flusses Henares, Zentralspanien) bewertet und der Beitrag von Abwasser zum MP-Gesamtabfluss im Einzugsgebiet ermittelt. Dieses Kapitel zeigt, dass obwohl Kläranlagen im Durchschnitt 93 % der MP zurückhalten, die behandelten Abwässer

und kleine Mengen an unbehandeltem Abwasser, immer noch bis zu 50 % der gesamten MP-Fördermenge des Einzugsgebietes beitragen können. Somit stellt das Abwassersystem einen wichtigen Eintragspfad für MP in Fließgewässer der Mediterranen Klimazone dar. Darüber hinaus wird gezeigt, dass MP-Konzentrationen in der Wassersäule und in Sedimenten stark von der Landnutzung abhängen und mit zunehmendem anthropogenem Druck signifikant zunehmen. Schließlich wird festgestellt, dass die MP-Kontamination im Wassereinzugsgebiet aufgrund von Hochwasserperioden im Frühjahr mit höheren Wasserkonzentrationen, aber niedrigeren Sedimentkonzentrationen von der Jahreszeit beeinflusst werden.

In Kapitel 4 wird der Verbleib von MP in landwirtschaftlichen Böden mit unterschiedlichen Klärschlammbehandlungen und der Beitrag von Oberflächenwasserabfluss von landwirtschaftlichen Böden zur MP-Verschmutzung in aquatischen Ökosystemen untersucht. Dieses Kapitel zeigt, dass die Ausbringung von Klärschlamm die MP-Konzentrationen in Böden signifikant erhöht. Die Bodenkonzentrationen bleiben im Verlauf eines Jahres relativ konstant und der Transport in tiefere Bodenschichten ist gering. Darüber hinaus weist die Studie darauf hin, dass MP-Konzentrationen in Böden auch noch fünf Jahre nach der Klärschlammausbringung hoch sind. Schließlich hebt dieses Kapitel hervor, dass der Einfluss von Oberflächenwasserabfluss für den MP-Export aus landwirtschaftlichen Böden vernachlässigbar ist. Daher kommt es zu dem Schluss, dass landwirtschaftliche Flächen unter den untersuchten Boden- und Wetterbedingungen als langfristige MP-Akkumulatoren betrachtet werden können.

In Kapitel 5 werden die Aufnahme und die Wirkung von Polyesterfasern und Autoreifenpartikeln für Süßwasserinvertebraten mit unterschiedlichen Strategien zur Nahrungsaufnahme und unterschiedlichen Lebensraumpräferenzen evaluiert. Das Kapitel zeigt, dass die Fähigkeit zum Verzehr von der Form und Größe der MP abhängt, während die Aufnahmemenge auch vom Expositionsweg und der Strategie zur Nahrungsaufnahme des Testorganismus beeinflusst wird. In dieser Studie werden keine negativen Auswirkungen auf das Überleben oder die Fortpflanzungsleistung benthischer Makroinvertebraten beobachtet. Sie kommt jedoch zu dem Schluss, dass eine chronische Exposition von *Daphnia magna* (pelagische Art) gegenüber beiden MP-Typen deren Fortpflanzung und Überleben beeinträchtigen könnte. Dies tritt jedoch bei Konzentrationen auf, die derzeit zum größten Teil noch nicht ökologisch

realistisch sind. Schließlich zeigt dieses Kapitel, dass MP über unterschiedliche Mechanismen auf Organismen einwirken.

In Kapitel 6 wird die Relevanz von MP als Vektoren für organische Schadstoffe in Süßwasserfischen betrachtet. Dazu wird die Biokonzentration von zwei hydrophoben organischen Chemikalien in An- und Abwesenheit von MP in *Danio rerio* und deren Einfluss auf chemischen Stress untersucht. Dieses Kapitel zeigt, dass MP die Biokonzentration dieser Chemikalien nicht erhöhen, sondern eher verringern. Die Reduktion der Biokonzentration ist darüber hinaus größer für die hydrophobere Chemikalie. Auch chemischer Stress, gemessen an Biomarkerantworten, wird in Gegenwart von MP nicht verstärkt. Dies bestätigt, dass die MP-Kontamination in Süßwasserökosystemen die Risiken, die mit der Biokonzentration organischer Chemikalien in aquatischen Organismen einhergehen, voraussichtlich nicht erhöht. Andere Expositionswege (z. B. die Aufnahme über die Atmung oder Haut) erscheinen von größerer Bedeutung zu sein.

Abschließend werden in Kapitel 7 die allgemeinen Schlussfolgerungen dieser Arbeit präsentiert, zusammen mit einigen Empfehlungen für die zukünftige Forschung und das Management für MP-Verschmutzung.

Publication List

Scientific paper 1 (published), as Chapter 2:

Schell T, Rico A, Vighi M. 2020. Occurrence, fate and fluxes of plastics and microplastics in terrestrial and freshwater ecosystems. Reviews of Environmental Contamination and Toxicology (Continuation of Residue Reviews), 250. Springer, New York, NY. https://doi.org/10.1007/398_2019_40

Scientific paper 2 (published), as Chapter 3:

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 Material 420: 126481. https://doi.org/10.1016/j.jhazmat.2021.126481

Scientific paper 3 (published), as Chapter 4:

Schell T, Hurley R, Buenaventura NT, Mauri PV, Nizzetto L, Rico A, Vighi M. 2022. Fate of microplastics in agricultural soils amended with sewage sludge: Is surface water runoff a relevant environmental pathway? Environmental Pollution 293: 118520. https://doi.org/10.1016/j.envpol.2021.118520

Scientific paper 4 (published), as Chapter 5:

Schell T, Martinez S, Dafouz R, Hurley R, Vighi M., Rico A., 2022. Effects of polyester fibers and car tire particles on freshwater invertebrates. Environmental Toxicology and Chemistry 41:1555-1567. https://doi.org/10.1002/etc.5337

Scientific paper 5 (published), as Chapter 6:

Schell T, Rico A, Cherta L, Nozal L, Dafouz R, Giacchini R, Vighi M. 2022. Influence of microplastics on the bioconcentration of organic contaminants in fish: Is the "Trojan Horse" effect a matter of concern? Environmental Pollution 306:119473. https://doi.org/10.1016/j.envpol.2022.119473

CHAPTER 1

General Introduction

1.1. Plastics and microplastics in the environment

The rise in global plastic production and consumption over the past decades is no surprise, given plastics' many advantages and unique properties (e.g., durability, malleability, lightness, and low production costs). Large-scale plastic production began in the 1950s and has been steadily growing since. However, recycling rates are still low, and 79 % of plastic waste accumulates in landfills or is released into the natural environment (Geyer *et al.* 2017). Moreover, because plastics are manufactured to be very durable, they persist over long timescales after leaking into the environment (Chamas *et al.* 2020; Krause *et al.* 2020). This has led to a global plastic pollution problem that is expected to grow further, particularly in countries with less developed economies (Geyer *et al.* 2017; Lebreton & Andrady 2019).

Plastic products encompass a diversity of materials which can be differentiated into various groups depending on their chemical composition, function, size, and shape. A broad distinction is made between thermoplastics, thermosetting plastics, and elastomers. Within these broad categories, plastics are further distinguished based on their polymer type. Depending on the polymer type and added additives, properties such as density, stability, durability, and resistance vary (Hahladakis *et al.* 2018). As environmental contaminants, plastics are classified based on their size into macroplastics (> 1 cm), mesoplastics (5 - 10 mm), microplastics (1 µm - 5 mm), and nanoplastics (< 1 µm). These size classifications slightly vary depending on the literature (Hartmann *et al.* 2019). The microplastics (MPs) class can be split up further into primary MPs, produced in this size range (e.g., microbeads added in cosmetics or cleansers), and secondary MPs formed due to the fragmentation of larger plastic products or debris (Cole *et al.* 2011). Finally, MPs can be grouped by shape or morphology, such as beads (or spheres), pellets, fibers, fragments, films, and foams (Hartmann et al. 2019; Figure 1.1). Therefore, MPs can be considered versatile contaminants that stem from various sources and reach ecosystems following diverse exposure pathways.

Research into the environmental side effects of plastic originated in the marine environment. Reports on the presence of plastic particles in marine waters go back to the 1970s (Carpenter *et al.* 1972; Colton *et al.* 1974). Also the term MP was first used to describe small plastic particles in marine environments (Thompson *et al.* 2004). As

environmental research expands, it has become more and more apparent that MP contamination is ubiquitous and not confined to the marine environment but spreads out to freshwater and terrestrial ecosystems and the atmosphere (Horton *et al.* 2017b; Allen *et al.* 2019; Wong *et al.* 2020). In fact, MPs have been found in very remote areas, including the deep sea, glaciers, and Antarctica (Wagner *et al.* 2014; González-Pleiter *et al.* 2020).

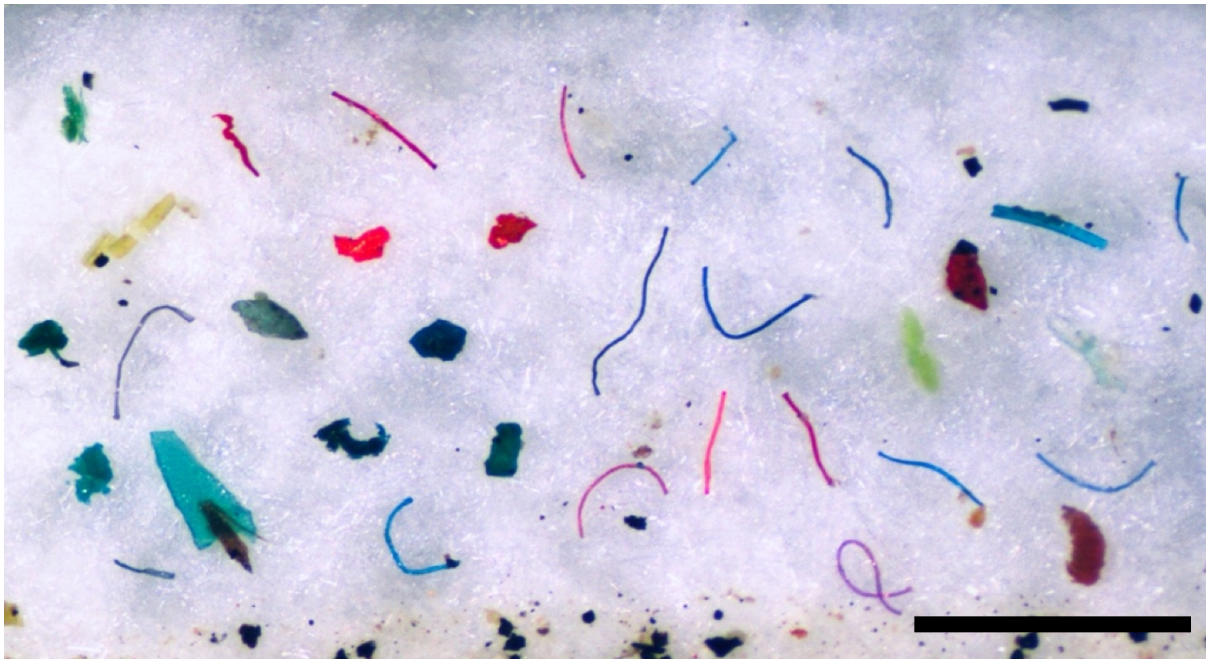


Figure 1.1. Example of MPs with different shapes from an environmental water sample. The scale bar represents 1000 µm. Picture taken by Theresa Schell.

In recent years, MP pollution has received a great deal of scientific and public attention, with scientific publications on MPs increasing exponentially (Zhou *et al.* 2021). Yet, important knowledge gaps remain regarding their sources, environmental fate and exposure to organisms. While it is well known that most plastics are produced and emitted into the environment on land and are then transported along rivers to the marine environment (Jambeck *et al.* 2015; Schmidt *et al.* 2017), the importance of different primary sources, as well as their pathways and fluxes between land and freshwater ecosystems are not well understood (Horton *et al.* 2017b). Deeper knowledge, especially on the relevance of the numerous different sources that contribute to MP contamination, is needed to fully understand and tackle MP pollution. Entry pathways for MPs into freshwater and terrestrial ecosystems include wastewater and sewage sludge, atmospheric transport or drifting, surface runoff and industrial spills (Waldschläger *et al.* 2020). However, once in the environment, MP transport

processes among environmental compartments are yet poorly quantified (Petersen & Hubbart 2021). Holistic approaches such as the assessment of MP pollution at the catchment level are needed to better understand their fate and transport processes, and to derive environmental management measures that limit their mobilization and exposure to living organisms (Windsor *et al.* 2019a; Hoellein & Rochman 2021).

Current literature points towards wastewater and sewage sludge being among the most important entry pathways for MPs into terrestrial and freshwater ecosystem (Nizzetto *et al.* 2016c; Ziajahromi *et al.* 2016; Corradini *et al.* 2019). In wastewater, MPs from different sources such as textiles, paints, cleaning products, and personal care products, and in combined sewer systems, traffic-related MPs are converged (Ziajahromi *et al.* 2016; Kole *et al.* 2017; Fältström *et al.* 2021). MPs not retained during wastewater treatment are emitted into the environment. Most retained MPs are accumulated in the sewage sludge (Liu *et al.* 2019b). The application of this solid by-product as fertilizer to agricultural lands is a widespread practice. In Europe the use of sludge for this purpose varies greatly between countries. For example, while sludge application to agricultural lands is prohibited in the Netherlands, in Spain about 80% of the produced sludge is reused as agricultural fertilizer (Eurostat 2020). It has been predicted that between 63,000 to 430,000 tons of MP may be added to European farmlands along this pathway annually (Nizzetto *et al.* 2016c). Thus plastic inputs into terrestrial environments through sludge application may exceed those released into marine environments (Nizzetto *et al.* 2016c). Thus far, not only the impact of MPs on soil ecosystem processes and soil organisms are largely unknown, but also their persistence and fate within this compartment (Horton *et al.* 2017b; Boots *et al.* 2019). For instance, whether MPs are retained in soil, transported into deeper soil layers, taken up by plants or transported by surface water runoff into aquatic ecosystems is still unclear (McCormick *et al.* 2016; Nizzetto *et al.* 2016c; Ng *et al.* 2018; Windsor *et al.* 2019a). As pointed out by some authors (Wagner *et al.* 2014; Horton *et al.* 2017b), water runoff is expected to be an important pathway for MPs from terrestrial into surface water ecosystems, but this has never been quantified experimentally.

Elucidating contamination patterns and assessing exposure concentrations is fundamental to determining the environmental risks associated with MP pollution. As mentioned above, MPs are a diverse suite of different polymer types, sizes, and shapes (Hartmann *et al.* 2019) and may affect different organisms in different ways.

Therefore, it is important to determine which MP types and sizes are prevalent in the environment as this may not only affect their behavior and fate (Nizzetto *et al.* 2016a) but also their capacity to be ingested and affect different living organisms (Koelmans *et al.* 2020). Plastic pollution is usually assessed based on MP particle abundance (e.g., number/volume or number/area), however it is important to additionally quantify MPs based on mass to compare total plastic loads between different sampling areas and studies.

1.2. Impacts of MPs in freshwater ecosystems

A variety of physical and chemical mechanisms are related to the risks of MPs for freshwater organisms. MPs may act as physical or chemical stressors. Negative physical impacts encompass entanglement, external damage, internal abrasive damage, or blockage of the digestive track after ingestion, as well as food dilution (Cole *et al.* 2011; De Ruijter *et al.* 2020; Amariei *et al.* 2022). In addition, plastics may contain potentially harmful additives (e.g., plasticizers, flame retardants), which can leach out into the water phase or be released into organisms after ingestion (Beiras *et al.* 2021). Furthermore, MPs may serve as vectors for other environmental contaminants such as hydrophobic organic chemicals, metals, or antibiotics, which may sorb to MPs (Yu *et al.* 2019; Syberg *et al.* 2020; Amelia *et al.* 2021). While several studies showed that MPs can transport other environmental contaminants into animals (Rochman *et al.* 2013; Batel *et al.* 2016, 2018), there is currently a disagreement in the scientific community about the relevance of MPs as vectors for these chemical contaminants, in comparison to other uptake routes (e.g., food or surrounding medium) (Hartmann *et al.* 2017; Lohmann 2017; Koelmans *et al.* 2022; Sun *et al.* 2022). Thus, this yet needs to be proven experimentally.

Moreover, there is currently a mismatch between MPs found in environmental samples and those used for toxicity testing (Rochman *et al.* 2019; De Ruijter *et al.* 2020). For example, while fibers represent only about 8% of MPs used in effect studies, they are the most abundant MP types in water and sediment (De Ruijter *et al.* 2020). To fully understand the risk associated with MPs it is imperative to determine their effects and ingestion capacity in a variety of living organisms, taking into account the quantity of different sizes, shapes and polymer types to which they are exposed to and their specific morphological and ecological traits (Scherer *et al.* 2017; Redondo-

Hasselerharm *et al.* 2018a; Koelmans *et al.* 2020). In this regard, environmentally realistic exposure studies assessing both physical and chemical effects of frequently encountered MPs are urgently needed.

1.3. Research objectives

This thesis aims to expand our understanding on the fluxes and pathways of MPs into terrestrial and freshwater ecosystems, and to quantify their risks for freshwater organisms.

The specific research objectives of this thesis are:

1. To assess the current state of knowledge and identify data gaps regarding the sources, environmental pathways, loads and fate of plastics and MPs in aquatic and terrestrial ecosystems.
2. To determine MP pollution in a Mediterranean river catchment and elucidate the role of wastewater as a MP entry pathway.
3. To uncover the fate of MPs in agricultural soils following sewage sludge application and the importance of agricultural surface water runoff as a transport mechanism for MPs into aquatic ecosystems under semi-arid conditions.
4. To evaluate the impacts of frequent MP types on freshwater invertebrates by considering environmentally relevant shapes, sizes, and concentrations and to perform a risk assessment for these organisms.
5. To assess the relevance of MPs as vectors of other chemical contaminants in the aquatic environment and their contribution to ecological risks.

This thesis was developed as part of the water-JPI funded IMPASSE project (Impacts of MicroPlastics in AgroSystems and Stream Environment), which was supported by the Spanish Ministry of Science and Innovation (PCIN- 2017-016). This project was coordinated by the Norwegian Institute for Water Research and was carried out in cooperation with the Department of Biology of the Biotechnical Faculty at University of Ljubljana, the department of Aquatic Sciences and Assessment at the Swedish University of Agriculture, the Department of Ecological Science of the Faculty of Science at Vrije Universiteit Amsterdam, the Department of Earth and Environmental Sciences at Windsor University, and the IMDEA Water Institute. The project aimed to contribute to improve our understanding on MP pollution and their potential ecological

impacts in agricultural soils and freshwaters focusing on MP entering these environments through wastewater or sludge. The current thesis has contributed to this by investigating the pathways and loads of MPs from wastewater and sludge in case study on the river catchment level and a field experiment under Mediterranean conditions in central Spain, and by assessing the effects of MPs on freshwater organisms.

CHAPTER 2

Occurrence, Fate and Fluxes of Plastics and Microplastics in Terrestrial and Freshwater Ecosystems

This Chapter has been published as:

Schell T, Rico A, Vighi M. 2020. Occurrence, fate and fluxes of plastics and microplastics in terrestrial and freshwater ecosystems. Reviews of Environmental Contamination and Toxicology (Continuation of Residue Reviews), 250. Springer, New York, NY. https://doi.org/10.1007/398_2019_40

Abstract

Plastics and microplastics are nowadays ubiquitously found in the environment. This has raised concerns on possible adverse effects for human health and the environment. To date, extensive information exists on their occurrence in the marine environment. However, information on their different sources and their transport within and across different freshwater and terrestrial ecosystems is still limited. Therefore, we assessed the current knowledge regarding the industrial sources of plastics and microplastics, their environmental pathways and load rates, and their occurrence and fate in different environmental compartments; thereby highlighting important data gaps which are needed to better describe their global environmental cycle and exposure. This study shows that the quantitative assessment of the contribution of the different major sources of plastics, microplastics and nanoplastics to aquatic and terrestrial ecosystems is challenged by some data limitations. While the presence of microplastics in wastewater and freshwater is relatively well studied, data on sediments and especially soil ecosystems are too limited. Moreover, the overall occurrence of large-size plastics, the patterns of microplastic and nanoplastic formation from them, the presence and deposition of plastic particles from the atmosphere, and the fluxes of all kinds of plastics from soils towards aquatic environments (e.g., by surface water runoff, soil infiltration) are still poorly understood. Finally, this study discusses several research areas that need urgent development in order to better understand the potential ecological risks of plastic pollution, and provide some recommendations to better manage and control plastic and microplastic inputs into the environment.

2.1. Introduction

Over the past century, plastic has made the journey from being virtually non-existent to a ubiquitous and integral part of modern life. While plastic has numerous advantages compared to alternative materials, we are facing severe environmental, economic and ethical issues due to the vast plastic waste production and rapid disposal. Up until 2015, the total amount of plastic produced was 8300 million tons, 6300 million tons of which were discarded as waste (Geyer *et al.* 2017). Much of this waste (79%) is accumulated in landfills or the natural environment, and this amount is expected to increase significantly in the future (up to 12,000 million tons by 2050) if management actions are not immediately taken (Geyer *et al.* 2017).

Most macroplastics break down due to mechanical and chemical fragmentation into smaller pieces, which are commonly termed microplastics (particles < 5 mm; MPs) or nanoplastics (particles < 1µm) (Gigault *et al.* 2018). The breakdown process may take between 50 - 600 years and usually depends on several factors such as the polymer composition and the environmental condition. MPs that are formed due to the breakdown of macroplastics are commonly referred to as secondary MPs, while MPs intentionally produced in this size range are referred to as primary MPs. Nowadays, macroplastics, MPs and nanoplastics can be found floating or in suspension in many water bodies, accumulated in sediments or terrestrial ecosystems, and even transported and deposited in pristine environments due to wind and currents (Dris *et al.* 2015, 2016; Ballent *et al.* 2016; Fischer *et al.* 2016; Hurley & Nizzetto 2018).

The widespread distribution of plastic and its variability in size and shape allow the ingestion by organisms across many trophic levels and habitats (Wright *et al.* 2013; Kühn *et al.* 2015). Large plastic debris (macroplastics) can cause adverse effects on coastal and marine animals (marine mammals, fish, and seabirds) due to ingestion as well as entanglement, which impedes their mobility (Van Franeker *et al.* 2011; Knowlton *et al.* 2012; Schuyler *et al.* 2012; Kühn *et al.* 2015). Fishing gear, balloons, plastic bags and bottle caps have been identified to be the most harmful type of macroplastics to marine organisms (Hardesty *et al.* 2015). Although most research has focused on the marine environment, freshwater and terrestrial organisms are expected to suffer from the same sort of effects. For example, cattle have been reported to

suffocate and die due to the ingestion of plastic bags, which can block airways and stomachs (Ramaswamy & Sharma 2011).

Similar to macroplastics, environmental exposure to MPs has raised concerns about their potentially adverse effects in smaller organisms. Ecotoxicological studies with MPs have been primarily conducted using marine organisms (77%), while freshwater organisms have been less researched (23%) (de Sá *et al.* 2018), and research involving terrestrial organisms is still in its beginnings (Chae & An 2018). MPs may cause physical effects such as internal and external abrasion or blockages of the digestive tract in small invertebrates and fish (Wright *et al.* 2013; Karami *et al.* 2016; Jovanović 2017). Research also shows that MPs ingested by freshwater organisms, either on purpose when they are confounded for prey, or accidentally due to non-selective feeding strategies (i.e., water filtration or deposit feeding) (de Sá *et al.* 2015; Windsor *et al.* 2019b), may reduce their feeding efficiency and lower the energy uptake, which often results in reduced growth, reproduction and survival (Foley *et al.* 2018). In addition, MPs may affect the growth, chlorophyll content, photosynthesis activity and reactive oxygen species of microalgae at high, currently not realistic, concentrations (Prata *et al.* 2019).

Although several cases evidence deleterious impacts of macroplastics on aquatic and terrestrial organisms under laboratory conditions, the capacity of MPs or even nanoplastics to pose a real threat for ecosystems and human health is disputable. This is because the majority of studies showing some impacts of MPs on terrestrial or freshwater organisms have been performed with very high exposure concentrations, while risk at environmentally relevant concentrations has yet to be disclosed (Lenz *et al.* 2016).

Despite physical effects, the release of additives such as phthalates, chlorinated paraffins and bisphenols present in some macroplastics and MPs (Stenmarck *et al.* 2017) have been reported to induce endocrine-disrupting effects (Rochman *et al.* 2014). Furthermore, hydrophobic pollutants (e.g., some pesticides, PCBs, PAHs) can be adsorbed to plastics and may be released into the body of the organisms after ingestion, leading to the so-called "Trojan Horse" effect (Teuten *et al.* 2009; Koelmans *et al.* 2016; Crawford & Quinn 2017; Bouhroum *et al.* 2019). In contrast, it has been suggested that ingested clean MPs may reduce the concentration of bioaccumulated

chemicals in the body of contaminated organisms (Lohmann 2017). Furthermore, MPs could not only act as carriers for chemicals but can also transport bacteria or pathogens (Keswani *et al.* 2016; Kirstein *et al.* 2016) across different environmental compartments and regions. In order to provide some responses to the concern produced by the perception of the potential risks for human health and the environment of MPs and of chemicals associated to them, Koelmans et al. (2017a) proposed adverse outcome pathways for assessing and comparing the risk of macroplastics, MPs and nanoplastics and highlighted the uncertainties that still exist in both, exposure and effect assessment.

The continuous emission patterns and the breakdown of plastic litter into smaller pieces in the environment may contribute to future concentrations that are orders of magnitude higher than the ones currently monitored (Everaert *et al.* 2018), thus contributing to a yet uncertain risk scenario. Policies dedicated to control emissions and manage risks of macroplastics, MPs and nanoplastics in the environment require a proper understanding of the main emission routes, the current exposure levels and the fluxes among environmental compartments. The available literature describing the exposure and impacts of plastics in the environment, providing a comparative assessment of the global occurrence, transport and fate, has so far mainly focused on the marine environment (see for example; GESAMP 2015; Auta *et al.* 2017). Although the freshwater environment is considered in some recent reviews (see, for example: (Eerkes-Medrano *et al.* 2015; SAPEA 2019), most studies consider specific emission routes and local monitoring campaigns.

Therefore, this study aimed to assess the state of the knowledge regarding the overall sources of plastic and its occurrence, fate, fluxes and loads into and within different environmental compartments of terrestrial and freshwater ecosystems. This study identifies data gaps that need to be addressed in order to understand the life cycle of the different plastic types in the environment, particularly in the soil-water interface, and provides relevant information to support research into the accumulation and ecotoxicological characterization of plastics to living organisms. Ultimately, this study provides guidance information to derive effective management measures aimed at reducing plastic discharges into the environment and attaining a more sustainable use and consumption of plastics in the nearby future.

2.2. Environmental sources of plastics

Nowadays, Asia is producing 50% of the world's plastic, followed by Europe and North America, producing 19% and 18%, respectively (PlasticsEurope 2018). The majority of plastics can be classified into the two main categories: thermoplastics (pellets that are re-melted to manufacture the final product), and thermoset plastics (thermally produced into the commercial shape). Thermoplastics constitute 80% of the total plastic and are the main source of primary MPs. Thermoplastics are mainly formed by polyethylene, polypropylene or polyvinylchloride, while thermoset plastics are formed, among others, by polyester, polyurethane, silicone and polyamide.

Sources of plastics can be classified in terms of the life expectancy of the produced plastics before disposal. Here we classify plastic sources into those with a short-term (single-use or very limited number of times with a useful lifespan up to 1 year), mid-term (up to 10 years), or long-term (more than 10 years) use expectancy.

Plastics with short-term use expectancy

Single-use items are mainly formed by packaging material, which is the biggest plastic sector worldwide (almost 36% in 2015; Figure 2.1) and accounts for almost 50% of the generated plastic waste (Geyer *et al.* 2017). The vast majority of packaging plastics are polyethylene, polypropylene and polyethylene terephthalate (PET) (Geyer *et al.* 2017). Except for refillable PET bottles used in some countries, packaging is single-use with a life span of less than six months. Most foods are wrapped in plastic and single-use plastic bags have been widely used all over the world due to their convenience, availability and low price. Plastic bags are known to cause severe environmental problems, especially in countries without proper waste management (Adane & Muleta 2011). Thus, many countries have put bans or levies in force to reduce their use or to encourage voluntary reductions (Xanthos & Walker 2017). Many African countries, for instance, have banned single-use plastic bags, while the EU Directive 2015/720 encourages member states to reduce the number of ''lightweight'' carrier bags by 2025. Those bans and restrictions have already reduced the plastic bag use drastically in some countries (e.g., Ireland, England, Italy). Moreover, other single-use items like cutlery, plates, cups, and straws are planned to be banned in Europe by 2021 (EC 2019).

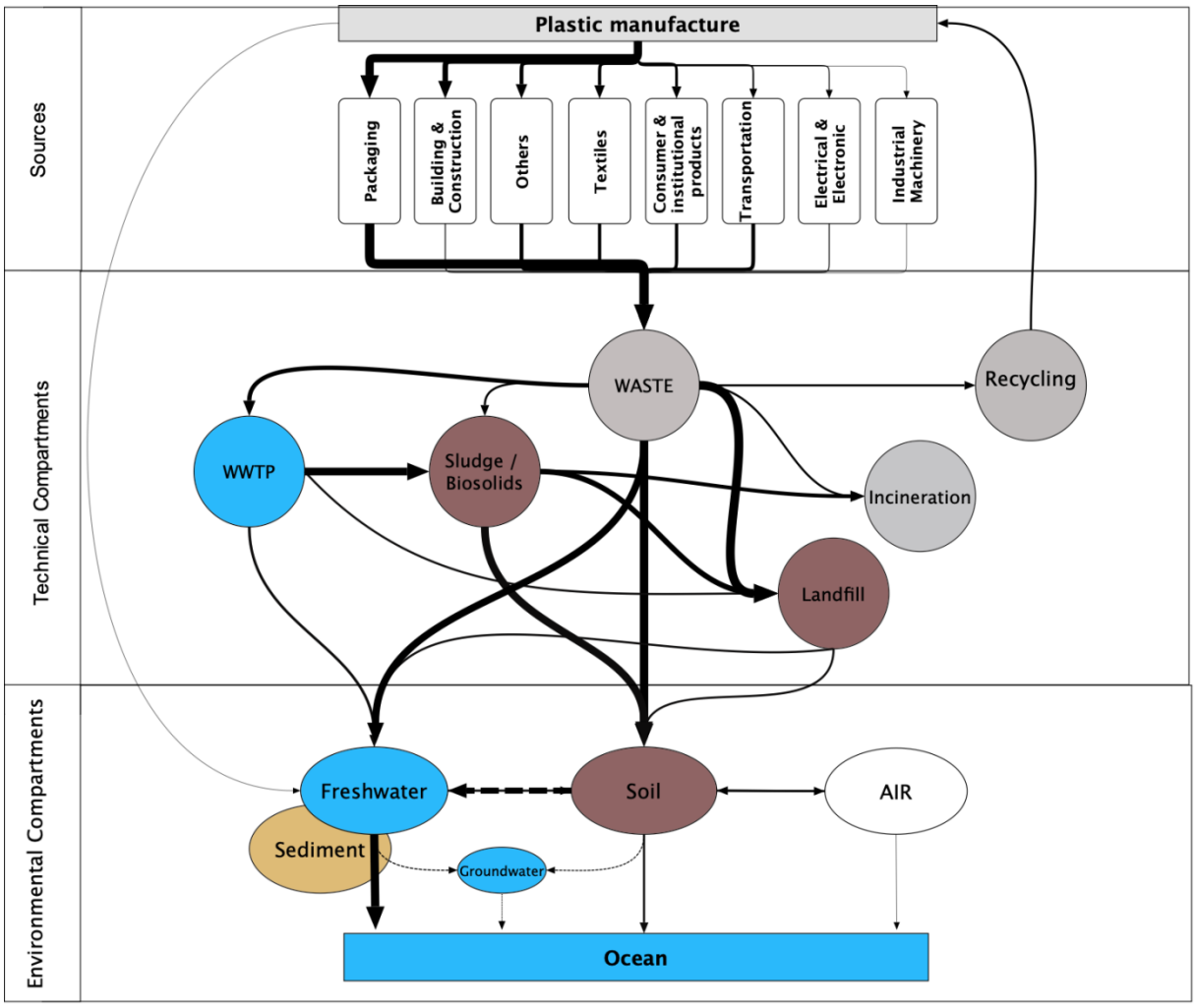


Figure 2.1. Production and pathways of plastics into the different environmental compartments. Thickness of the different arrows is related to the quantitative relevance of the different mass flows. The relevance of the different plastic sources mass flows is based on Geyer et al. (2017), while the relevance of the technical compartments environmental flows is based on the reviewed literature or assumptions. Dashed lines indicate yet completely unexplored pathways with unknown relevance.

Another important sector using single-use plastic is agriculture. Plastic films are used for plastic mulching, for the construction of greenhouses and tunnels, or to wrap silage to store animal fodder. The global plastic consumption in agricultural production is estimated to be about 2.5 million tons per year (Hussain & Hamid 2003). A variety of different plastic types are used in agriculture, including polyethylene, polypropylene, Ethylene-Vinyl Acetate Copolymer, polyvinyl chloride and poly-methyl-methacrylate (Scarascia-Mugnozza *et al.* 2012).

MPs added to consumer products (e.g., as a component of personal care, cosmetic and cleaning products) are specially manufactured to be used once and then washed down the drain. They are often referred to as microbeads, even though they

are mostly irregular in shape in order to obtain an abrasive effect (Fendall & Sewell 2009; Napper *et al.* 2015; Kalčíková *et al.* 2017). The majority of microbeads in facial and body scrubs are made of polyethylene, with average concentrations of 4.82 g/100 mL body scrub and 0.74g/100 mL facial scrub (Gouin *et al.* 2015; Kalčíková *et al.* 2017). Other plastic polymers used in cosmetic products include polylactic acid, PET, polyethylene isoterephthalate, nylon-12, nylon-6, poly-methyl-methacrylate, polytetrafluoroethylene, and polyurethane (Leslie 2014; Rochman *et al.* 2015). Additionally, microbeads are used in industry as abrasives/scrubbers and sand-blasting media as well as in anti-slip, anti-blocking applications and for medical applications. It has been calculated that more than 4000 tons of polyethylene microbeads were used in cosmetic products all over the EU (including Norway and Switzerland) in 2012 (Gouin *et al.* 2015), and the US is emitting 263 tons of polyethylene microbeads per year (2.4 mg per person per day) (Gouin *et al.* 2011). A ban of MPs intentionally added to products (i.e., microbeads) has been proposed in the EU (EC 2019), while the US Microbead free waters act of 2015 (US Congress 2015) prohibits the manufacturing, packaging, and distribution of rinse-off cosmetics containing plastic microbeads already. This only applies to rinse-off products, while MPs are still permitted as a component in 'leave on' products (e.g., lotions, sunscreens, make-up and deodorants).

Plastics with mid-term use expectancy

Plastics with a mid-term lifespan are mainly found in the sectors of electronics, household, tires and textiles. The production of electrical and electronic products counts to the fastest growing manufacturing and waste generation sectors (Geyer *et al.* 2017; Kumar *et al.* 2017) and as many textiles are made, entirely or to a certain extent, of synthetic plastic fibers (e.g. polyamide, polyester, acrylic) also production rates of synthetic plastic fibers have increased over the last decade. Nowadays, two-thirds of the total fiber production is synthetic plastic fibers and worldwide 59 tons of plastic textiles were produced in 2015 (Geyer *et al.* 2017; Gasperi *et al.* 2018).

Synthetic polymers with rubber-like characteristics are the principal component of vehicle tires. They are composed of a mixture of natural and synthetic rubbers (styrene-butadiene rubber). While driving, tire and road wear particles are formed which contain styrene-butadiene rubber in a mix with natural rubber, pavement parts

and many other additives (Unice *et al.* 2013; Sundt *et al.* 2014). While tires contain almost 50% of polymers, tire wear particles, which are a mix of pavement part and polymers contain only 16 - 23% of polymers (Kreider *et al.* 2010).

Plastics with long-term use expectancy

Plastics designed for long-term use belong to the following categories: parts of transportation (i.e., vehicle, plane and train parts), building and construction, industrial machinery, consumer and institutional products. While plastics for the building and construction sector account for the second highest plastic consumption, only a small portion enters the waste stream directly (Figure 2.1; Geyer *et al.* 2017). As these categories do not belong to the items that are usually littered, they are not expected to contribute significantly to the plastic load in the environment. However, their breakdown rate into MPs and nanoplastics (due to exposure to light and weathering), also during their useful lifetime, is not clear.

2.3. Pathways of plastic to the environment

Hereafter plastic waste will refer to all plastic material that is discarded, while litter will include only those items that are not properly discarded. Packaging material is accounting for almost 50% of the generated plastic waste, followed by textiles (almost 14%) (Geyer *et al.* 2017). Most plastic waste is generated in Asia, while America, Japan and the European Union are the world's largest producers of plastic packaging waste per capita.

2.3.1. Collected solid waste

Collected plastic waste is either landfilled, incinerated or recycled. In Europe, 27.3% are landfilled, 31.1% is recycled, and 41.6% is incinerated for energy recovery (PlasticsEurope 2018). The percentage of collected plastic waste varies strongly between different countries, depending on the applied waste management plans and policies. While worldwide, the plastic recycling rate is still low, it has increased by almost 79% within the last ten years in the EU, including Norway and Switzerland (PlasticsEurope 2018).

Large scale industrial plastic production began in the 50s, but plastic recycling was not established until the 80s. It is estimated that only 9% of the total produced

plastic waste up to 2015 has been recycled (Geyer *et al.* 2017). From this again only a small portion is submitted to primary recycling in which the recycled plastic is used to replace all or a least a proportion of the virgin polymer resins (Hopewell *et al.* 2009). While high-income countries have sorting and processing facilities, in low-income countries, plastic recycling is not well established. Moreover, certain types of plastic are difficult to recycle. For example, thermoset plastics, including textiles, are usually not recycled.

Plastic that is not recycled but still collected is landfilled or incinerated. In eight EU countries, Norway and Switzerland, a landfill ban for plastic is in force, leading to a very small percentage of plastic being used for landfill applications (PlasticsEurope 2018). On average, 27.3% of the generated plastic waste is landfilled in Europe. In contrast, in low-income countries, waste is mainly stored in open, poorly managed dumps, from where plastic can be transported by wind force. In middle-income countries, some controlled landfills are in place, but open dumping is still common practice. The advantages of combustion of plastic waste are that it can be used for energy recovery, and the incinerated plastic cannot enter the environment anymore. At the same time, incineration results in the generation of air pollutants (Verma *et al.* 2016).

2.3.2. Wastewater

Both MPs, as well as macroplastics, enter wastewater either directly if products containing plastic are flushed down the drain (e.g., fibers detached during laundry of textiles, microbeads in consumer products, cotton buds or sanitary products), or in combined sewer systems from street dust and litter. Macroplastics escape wastewater treatment only on rare occasions and mainly enter the environment with untreated wastewater due to combined sewer overflows for example after heavy rainfall events or snowmelts (Williams & Simmons 1999), or if untreated wastewater enters the environment because wastewater treatment plants (WWTPs) are not in place. Although high-income countries treat on average 70% of the wastewater, yet globally only 20% of the generated wastewater is treated (Sato *et al.* 2013). For MPs, the situation is different, due to their small size, they can escape the treatment and are also released with treated effluents (Ziajahromi *et al.* 2016). This pathway for MPs has been increasingly investigated. To date, 24 studies have measured MPs in wastewater

(Table S1), from which three have not exclusively assessed MPs but included other litter items in the micro range (microlitter; HELCOM 2014; Michielssen *et al.* 2016; Talvitie *et al.* 2017b). Such studies were mainly carried out in northern and western Europe (14 studies), followed by North America (5 studies).

The number of MPs in raw wastewater varies greatly between WWTPs, from a few MPs L^{-1} to exceptional maximum values of more than 10,000 MPs L^{-1} (Figure 2.2). The data shown in Figure 2.2 are described in more detail in Table S1 where the removal rate, identification method, particle shape and polymer composition are reported. Especially high concentrations have been observed in raw wastewaters in Denmark (Vollertsen & Hansen 2017; Simon *et al.* 2018). The Danish studies assessed MPs in the smaller size range (i.e., between 10 or 20 and 500 µm), while other studies assessing MPs down to 20 µm found much lower MP concentrations (Talvitie *et al.* 2015; Leslie *et al.* 2017).

WWTPs have, in general, a large retention potential for MPs, often higher than 95% (Table S1). However, in treated wastewater the number of MPs varies greatly too, from less than 1 MP L^{-1} (Browne *et al.* 2011; Carr *et al.* 2016; Murphy *et al.* 2016; Ziajahromi *et al.* 2017b) to several hundred (Simon *et al.* 2018), and up to several thousand MPs L^{-1} (Vollertsen & Hansen 2017) (Figure 2.2). Larger MPs are usually better retained during the treatment, so the most frequently observed MPs in treated wastewater are smaller than 300 µm (Dris *et al.* 2015; Mintenig *et al.* 2017; Talvitie *et al.* 2017a; Gündoğdu *et al.* 2018; Lee & Kim 2018; Wolff *et al.* 2018; Liu *et al.* 2019b; Magni *et al.* 2019). For example, Magni et al. (2019) found that 94% of the MPs between 5–1 mm were retained by an Italian WWTP, while only 65% of the MPs between 0.1–0.01 mm were retained. Moreover, the number of MPs seems to be increasing with decreasing particle size. Wolff et al. (2018) reported the results of small-size MPs measured in treated wastewater and indicated that 44% of the measured MPs are between 10 and 30 µm, while 51% are between 30 and 100 µm. Furthermore, current research indicates that the amount of MPs retained by WWTPs is not only influenced by the size, but also by the particle shape. Usually, fibers are better retained in WWTPs as compared to microbeads or other irregular particles (Magnusson & Norén 2014; Talvitie *et al.* 2017b; Gündoğdu *et al.* 2018). Fibers and fragments are the most frequently occurring MP types in WWTP effluents (Table S1). Regarding polymer composition, polyethylene particles or polyester fibers are the most

common plastic types (Table S1). Although a huge amount of tire debris is suspected to enter WWTPs (Kole *et al.* 2017), they have not been frequently reported in treated effluents (Table S1). Only Lee and Kim (2018) have reported the presence of black particles possibly being tire fragments.

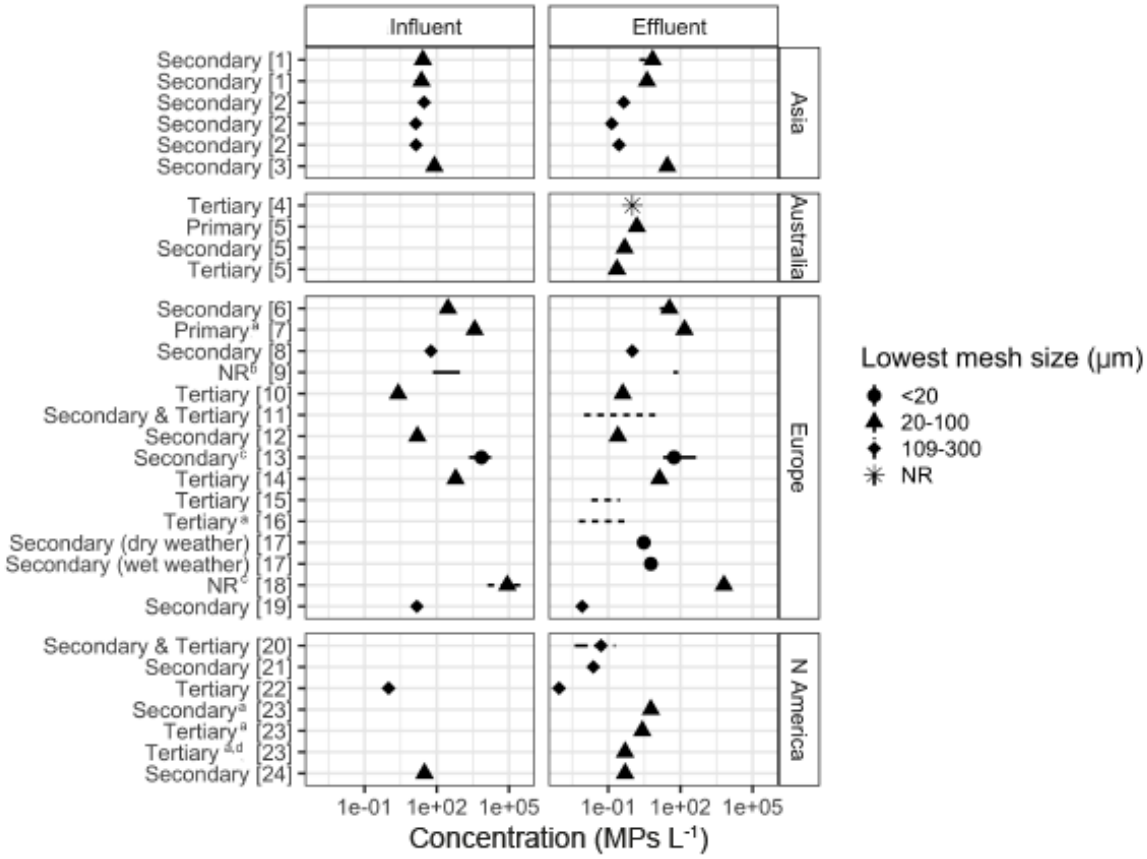


Figure 2.2. Mean and/or minimum-maximum MP concentrations (MPs L^{-1}) in influent and effluent of municipal WWTPs with different treatment types. NR= not reported. N America = North America. Notes: [a]All anthropogenic litter in the micro range and not only MPs considered. [b]Range of mean concentrations between seven different WWTPs. [c]Upper size limit of 500 µm. [d]Pilot scale anaerobic membrane bioreactor.

References: [1] Gündoğdu et al. (2018) [2] Lee and Kim (2018) [3] Liu et al. (2019b) [4] Browne et al. (2011) [5] Ziajahromi et al. (2017b) [6] Dris et al. (2015) [7] HELCOM (2014) [8] Lares et al. (2018) [9] Leslie et al. (2017) [10] Magni et al. (2019) [11] Mintenig et al (2017) [12] Murphy et al. (2016) [13] Simon et al. (2018) [14] Talvitie et al. (2015) [15] Talvitie et al. (2017a) [16] Talvitie et al. (2017b) [17] Wolff et al. (2018) [18] Vollertsen and Hansen (2017) [19] Magnusson and Noren (2014) [20] Mason et al. (2016) [21] Dyachenko et al. (2017) [22] Carr et al. (2016) [23] Michielssen et al. (2016) [24] Gies et al. (2018).

Concentrations of MPs in wastewaters show some seasonal and diurnal variations related to water consumption rates and human activity (Mintenig *et al.* 2017; Talvitie *et al.* 2017b; Lares *et al.* 2018). For instance, Talvitie et al. (2017b) reported

that night time concentrations were slightly lower (average concentrations 476.7 and 0.8 microlitter L^{-1} in influent and effluent respectively) compared to day time concentrations (584 and 1.7 microlitter L^{-1} in influent and effluent, respectively). Therefore, MPs occurrence seems to be highly variable and depending on a variety of different environmental (weather, season) and behavioral variables but also methodological procedures (i.e., sampling method, including mesh sizes and sample volume), extraction method, and determination method. Despite the high retention of MPs by WWTPs, considering the large volumes treated daily, it is considered that more than one million particles can enter the aquatic environment via this pathway per WWTP (Ziajahromi *et al.* 2017b; Gündoğdu *et al.* 2018), which constitutes one of the main sources of MPs into the environment.

2.3.3. Sludge and other agricultural amendments

WWTPs retain the majority of MPs during pre- and primary treatment (mechanical treatment and sludge settling processes) and MPs are therefore concentrated in the grease or sludge phase (Murphy *et al.* 2016; Leslie *et al.* 2017; Talvitie *et al.* 2017b). While solids intercepted by grids and grease removal steps are disposed of in landfills, sludge is often reused as fertilizers in agriculture. The amount trapped in the sludge roughly constitutes 50 - 90% of the MPs present in raw wastewater (Table S2); (Magnusson & Norén 2014; Carr *et al.* 2016; Lee & Kim 2018). MP concentrations measured in sludge range between 650 MPs kg^{-1} dw to more than 240,300 MPs kg^{-1} dw (Figure S1, Table S2). Murphy et al. (2016) found significantly bigger sized MPs in the sludge phase compared to MPs in treated wastewater, confirming the differential retention potential of WWTPs regarding MPs size. Furthermore, the sludge treatment process (thickening, digestion, drying, stabilization, dewatering) may affect the MP size (Mahon *et al.* 2017). Similar to wastewater, sludge samples usually show high numbers of fibers, followed by fragments (Table S2), and the main detected polymer is usually PES (particularly when there are many fibers present), followed by polyethylene and PP.

Plastics can end up in compost used as agricultural amendment due to wrong recycling or separation of waste, e.g., if plastic food packaging is disposed of in the organic waste (Mercier *et al.* 2017; Weithmann *et al.* 2018). Weithmann et al. (2018)

reported that organic fertilizers may contain up to 895 MPs kg^{-1}, and Fuller and Gautam (2016) found on average 23,000 mg MPs kg^{-1} in composted waste materials.

2.4. Occurrence and fluxes of plastics in environmental compartments

It is reasonable to hypothesize that in different countries, relevant differences in the emissions of plastic waste and the presence of macroplastics and MPs in environmental compartments exist. This may, in particular, originate from differences in waste management between high-income and low-income countries. In the following sections, some comparisons at the continental level are made. However, as the availability of information is not adequately balanced among countries and continents (for example, data from Africa and South America are very scarce), the comparison is only partial.

2.4.1. Air

Studies assessing the occurrence of airborne plastic particles have identified mainly fibers (Dris *et al.* 2015; Abbasi *et al.* 2019). Atmospheric fallout of fibers in the area of Paris (France) showed a high variability, with values ranging between 2 and 355 fibers m^{-2} day^{-1}; however, half of those were natural (50%; cotton or wool), and only 17% were purely synthetic (mainly PET) (Dris *et al.* 2016). Based on these samples, the same authors estimated that the fiber deposition rate in highly populated urban environments can roughly range between 1.2 and 4 kg km^{-2} $year^{-1}$, and concluded that atmospheric fallout might constitute a relevant pathway of MPs. The limited data on atmospheric MPs deposition rates makes it is difficult to draw conclusions on the relevance of this pathway for the environmental distribution of MPs. In the study by Dris et al. (2016) suburban fallout was found to be only about 50% of that observed in urban areas (53 particles m^{-2} day^{-1} compared to 110 particles m^{-2} day^{-1}), and thus it may be assumed that fiber fallout is even lower in natural and agricultural environments.

In addition to fibers, MPs in street dust are also likely to become airborne (Dall'Osto *et al.* 2014; Gasperi *et al.* 2018). According to Kole et al. (2017), 12% of the generated tire dust (1040 tones $year^{-1}$) in the Netherlands ends up in the air. The particles are generated by the interaction of tires with the road while driving and are generally found along roadside areas (Kreider *et al.* 2010). Wind and rainfall might

influence the atmospheric transport and fallout of MPs, while deposited fibers and street dust in urban environments may be transported via water runoff into sewer systems or directly to terrestrial or aquatic ecosystems. However, studies properly describing such processes are lacking.

2.4.2. Soil

It has been suggested that agricultural soils could constitute larger MP sinks than marine ecosystems (Hurley & Nizzetto 2018). However, research on the quantification of plastics in soils (for both macroplastics and MPs) is still very limited and mostly contracted to the last four years. We identified twelve studies reporting plastics in soil, from which three considered only a limited number of plastic types (Table S3). The available studies provide first indications of the scale of the pollution and suggest the ubiquitous presence of MPs in terrestrial ecosystems, also beyond agricultural areas. Most studies report plastic quantities in terms of particles, while some others provide concentrations based on mass measurements, which hampers to some extent direct comparisons among them. The highest MP concentration based on mass has been measured in soils from an industrial area in Australia, which was historically used to produce chlorinated plastic, containing 6700 mg MP Kg^{-1} dw (Fuller & Gautam 2016). The highest concentration based on the number of MP particles was provided by Vollertsen and Hansen (2017), who described Danish agricultural soils containing about 145,000 MPs kg^{-1}, in the size range of 20 to 500 µm which was based on weight however only 12 mg kg^{-1}. Also Chinese farmland soils were found to contain a high MP content, ranging between 70 and 18,760 MPs kg^{-1} dw (Figure S2; Liu *et al.* 2018; Zhang & Liu 2018; Zhang *et al.* 2018). In contrast, farmlands in Germany showed a much lower MP occurrence (0.34 MPs kg^{-1} dw) (Piehl *et al.* 2018). This might be partly related to differences in the considered MP sizes during the study and due to differences in agricultural practices. While Piehl et al. (2018) assessed MPs of a size between 1 and 5 mm, the study by Vollertsen and Hansen (2017) considered MPs between 20 µm and 500 µm. However, the different ranges in concentrations may also be attributed to the presence of different input sources.

The application of sewage sludge as agricultural fertilizer (biosolids) is considered to be a major source of MPs to soils. Nizzetto et al. (2016c) estimated that between 63,000-430,000 and 44,000-300,000 tons of MPs could be yearly added to

agricultural land in Europe and North America, respectively. Corradini et al. (2019) found that an increasing number of sludge applications were positively correlated to increasing MP concentrations in soils. Zubris and Richards (2005), report up to 1210 fibers kg^{-1} in soils five years after sewage sludge application and detected fibers still 15 years after application, which is another indication for MP accumulation in soil due to sludge application. On the other hand, almost twice the concentration of MPs was found in Danish fields not treated with sludge compared to treated fields (Vollertsen & Hansen 2017). Additional studies investigating the presence of MPs in soil after application of wastewater sludge are fundamental to estimate the importance of this pathway better.

Irrigation with reclaimed wastewater and the usage of plastic material in agriculture constitute additional sources of plastics in soil ecosystems. Based on studies from China, the latter one seems to be one of the most important plastic sources for elevated MPs concentrations in soil in addition to sewage sludge application (Zhang & Liu 2018; Zhang *et al.* 2018). In contrast to those concentration hot spots, agricultural areas in Germany without plastic mulching or use of sewage sludge as fertilizer the MP concentration seems much lower (i.e., on average 0.34 MPs kg^{-1} dw soil) (Piehl *et al.* 2018). As the frequency of the observed macroplastic polymer types was reflected by the types of MPs, MP particles in this study most likely come from the degradation of (littered) macroplastic (Piehl *et al.* 2018). The breakdown of macroplastic into MPs in terrestrial ecosystems may be dependent on their whereabouts in the soil and on soil cultivation. Williams and Simmons (1996) assessed low-density polyethylene degradation over a period of four months in different environments (river beach, in trees at the river bench and buried by soil). They found that macroplastics on the soil surface degrade faster as compared to buried plastics and assumed light to be the main influencing driver (although rainfall and other weathering processes may have affected degradation).

Littering, drift from landfills or spills from industry can also become important sources of plastics into soils. As described above, deposition of MPs from the air can additionally add MPs to soils. This seems, however, more relevant close to urban areas and streets with heavy traffic. Finally, during flood events, plastics from the aquatic environment can be deposited on the shores of rivers (Scheurer & Bigalke 2018). Therefore, based on the data that is available up to now, the primary inputs of MPs

into soil seem to come from agricultural practices (sewage sludge, plastic mulching) and the fragmentation of plastic litter.

The most common polymer types reported in soils are polyethylene and polypropylene (Table S3). Macroplastics reported in terrestrial systems are polyethylene films and bottles (Ramos *et al.* 2015; Huerta Lwanga *et al.* 2017b; Piehl *et al.* 2018). In a more remote place (desert in southern Arizona) plastic that is more mobile due to transportation by wind like plastic bags and balloons have been reported (Zylstra 2013).

The fate of MPs within the soil is not completely clear yet. MPs in soils may be transported along with water runoff and soil erosion into adjacent streams and rivers. So far, there is no knowledge of the importance of this pathway as it has not been experimentally proven. Translocation into deeper soil layers can occur through soil cultivation (Hurley & Nizzetto 2018) or transport by soil organisms. Earthworms and collembolans have been shown to ingest and transport MPs from the soil surface into deeper soil layers (Huerta Lwanga *et al.* 2017a; Maaß *et al.* 2017; Rillig *et al.* 2017b). Also other animals, e.g., birds or domestic animals, which have been shown to take up MPs (Zhao *et al.* 2016; Huerta Lwanga *et al.* 2017b), can transport MPs over longer distances. To date, it is yet unclear whether low sized MPs can be transported through soil pores into groundwater, but low concentrations of MPs (0 to 7 MPs m^{-3}) have been reported in raw drinking waters from groundwater wells (Mintenig *et al.* 2019). Uptake of plastics by plants is another potential source of mobilization of plastics from soil ecosystems, particularly for nanoplastics, however no studies have investigated this using whole plants (Ng *et al.* 2018). The only study available in this respect is the one provided by Bandmann et al. (2012), who demonstrated uptake of 20 and 40 nm polystyrene beads by tobacco BY-2 cells in cell culture via endocytosis, while 100 nm beads were excluded.

2.4.3. Freshwaters

Plastic pollution along rivers has been already observed and assessed in the 1990s (Williams & Simmons 1996, 1999). Nevertheless, few studies have reported plastic pollution in freshwaters until the whole environmental movement was initiated a few years ago. Some studies assessing litter in rivers have not exclusively focused on plastic, but also included other litter items like glass, paper and wood. Those studies

show that about 80% of the litter items are plastics, but do not provide concentrations or mass estimates (Crosti *et al.* 2018; González-Fernández *et al.* 2018; Castro-Jiménez *et al.* 2019).

Studies focusing on providing concentrations of macroplastics in the environment are very limited (Tab S4). Macroplastics concentrations have been reported for example for the Los Angeles River, in California (819 macroplastics m^{-3}) (Moore *et al.* 2011), the Yangtze River in China (8.74 x 10^3 macroplastics km^{-2}) (Xiong *et al.* 2019), and in Lakes (1800 macroplastics km^{-2}) and Rivers (0.012 macroplastics m^{-3}) in Switzerland (Faure *et al.* 2015). It has been estimated that in the Seine River in France, 28,000 kg of floating plastic are trapped annually by floating debris retention booms (Gasperi *et al.* 2014) and floating macroplastics in the Saigon River in Vietnam were estimated to range between 7500 - 13,700 tons $year^{-1}$ (van Emmerik *et al.* 2018). As only buoyant plastics were considered in those studies, the total loads may be underestimated as plastic is also transported by sub-surface transport (Morritt *et al.* 2014). The most common macroplastics reported in freshwater environments are plastic bottles, food packaging items, plastic bags and sewage-related plastic, like handles from buds of cotton wool and sanitary towels (Table S4). Regarding polymer composition, polypropylene and polyethylene are the plastic types that were omnipresent, and to a lesser extent, polystyrene and PET have been reported (Table S4).

MPs in water have been assessed using different sampling methods and are reported in different units (i.e., particles per water volume or particles per area) (Figure 2.3). To be able to compare the results of the different studies, we choose 38 studies which either reported the number of MPs per water volume or gave sufficient information to transform the reported unit. Like in other environmental compartments, the concentrations varied greatly among studies (Figure 2.4). As for Figure 2.2, the data shown in Figure 2.4 are described in more detail in Table S5 where, besides the identification method, the particle shape, the polymer composition, and the sampling methods are reported. Moreover, two additional studies are listed in Table S5, which are not included in the figure because they reported the MP concentration in weight per water volume instead of number per water volume, which hampers a direct comparison. Most studies in Europe found average concentrations of less than 1 to less than 100 MPs m^{-3}, while the highest average concentration of 100,000 MPs m^{-3}

(with a maximum concentration of 187,000 MPs m^{-3}) was measured in the Amsterdam Canals (Leslie *et al.* 2017). Furthermore, Liu et al. (2019a) reported up to 22,849 MPs m^{-3} (average: 1,409 MPs m^{-3}) in stormwater ponds receiving urban runoff in Denmark. The highest peak concentration from all studies was found in the Snake River in North America and was as high as 5,405,000 MPs m^{-3} (average: 91 MPs m^{-2}) (Kapp & Yeatman 2018). The second highest peak concentration was reported by Lahens et al. (2018), and corresponds to 519,223 MPs m^{-3} (minimum 17,210 MPs m^{-3}) monitored in the Saigon River (Vietnam). Overall, reported concentrations of MPs appear to be higher in Asia, as compared to Europe and North America (Figure 2.4). However, most of the studies carried out in Asia were performed in China and focused on assessing lower size classes than those studied in Europe. The only two studies conducted in Europe that considered a very small size (MPs below 20 µm) were the ones by Leslie et al. (2017) and Liu et al. (2019a), who observed by far the highest concentrations. Current research shows that smaller particles (< 0.5 mm) are usually the most frequent ones (e.g., Leslie *et al.* 2017; Yan *et al.* 2019). Therefore, the higher concentrations found in Asia may be not exclusively related to higher pollution but also to the sampling methods used. The results of this overview are comparable with those reported by Li et al. (2018) on the occurrence of MPs in freshwater.

Studies assessing the concentration of MPs using different net sizes at the same sampling sites found substantial differences in the number of particles intercepted by plankton nets vs trawling nets (Dris *et al.* 2015; Xiong *et al.* 2019). Kapp and Yeatman (2018) used different sampling methods to assess the occurrence of particles larger than 100 µm and found that on average there were higher concentrations in grab samples (glass containers were filled with water from the surface) as compared to net samples (Table S5). Also, other differences in study design, such as sample volume, sample depth, or sample location in the river could influence the measured MP concentration. For example, Vermaire et al. (2017) found higher concentrations in grab samples close to the river shore, which were subsequently filtered through a 100 µm net compared to open water samples taken using a 100 µm manta trawl.

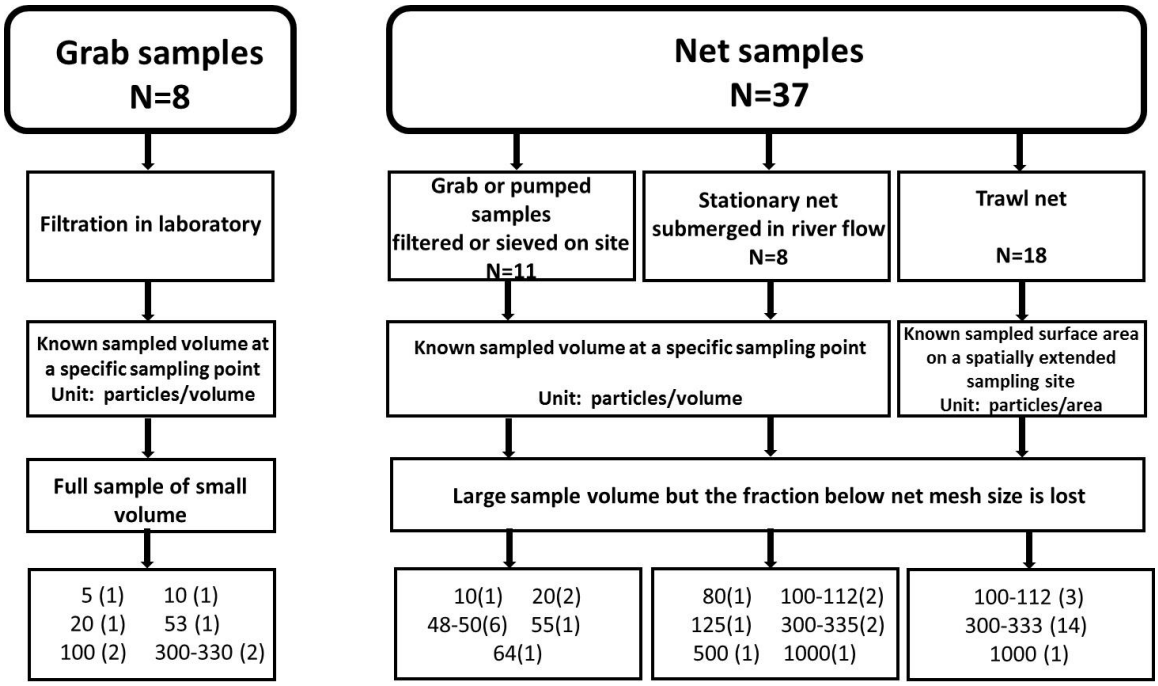


Figure 2.3. Overview of the most common sampling methods used for freshwater MPs sampling. N is the number of studies that applied the respective sampling method. This does not correspond to the number of studies listed in table S5 because some studies used more than one sampling method. In the last row of boxes, the minimum mesh size (µm) used in the different studies is reported (in brackets the number of studies).

Although MPs have been found in remote locations and rural areas, there is evidence that MP concentration increases with proximity to cities (Wang *et al.* 2017b; Di & Wang 2018; Tibbetts *et al.* 2018). A modelling study identified the Yangtze River catchment as the catchment transporting the highest plastic loads into the ocean (Schmidt *et al.* 2017). The four case studies looking at MP concentrations in the Yangtze river found highly variable concentrations but were also amongst the highest observed (Zhang *et al.* 2015; Wang *et al.* 2017b; Di & Wang 2018; Xiong *et al.* 2019). However, concentrations in the same order of magnitude were also monitored in other rivers in China such as the Pearl River, which was also ranked under the top ten catchments transporting plastic into the ocean (Schmidt *et al.* 2017).

Not only spatial hot spots but temporal hot spots based on weather condition may exist in freshwater ecosystems. Storms and rainfall can increase plastic concentration in waters from both lateral (land-based) and sewage effluent discharge points (Fischer *et al.* 2016), and MPs that had been deposited on river beds can re-enter the water phase after flood events (Hurley *et al.* 2018a).

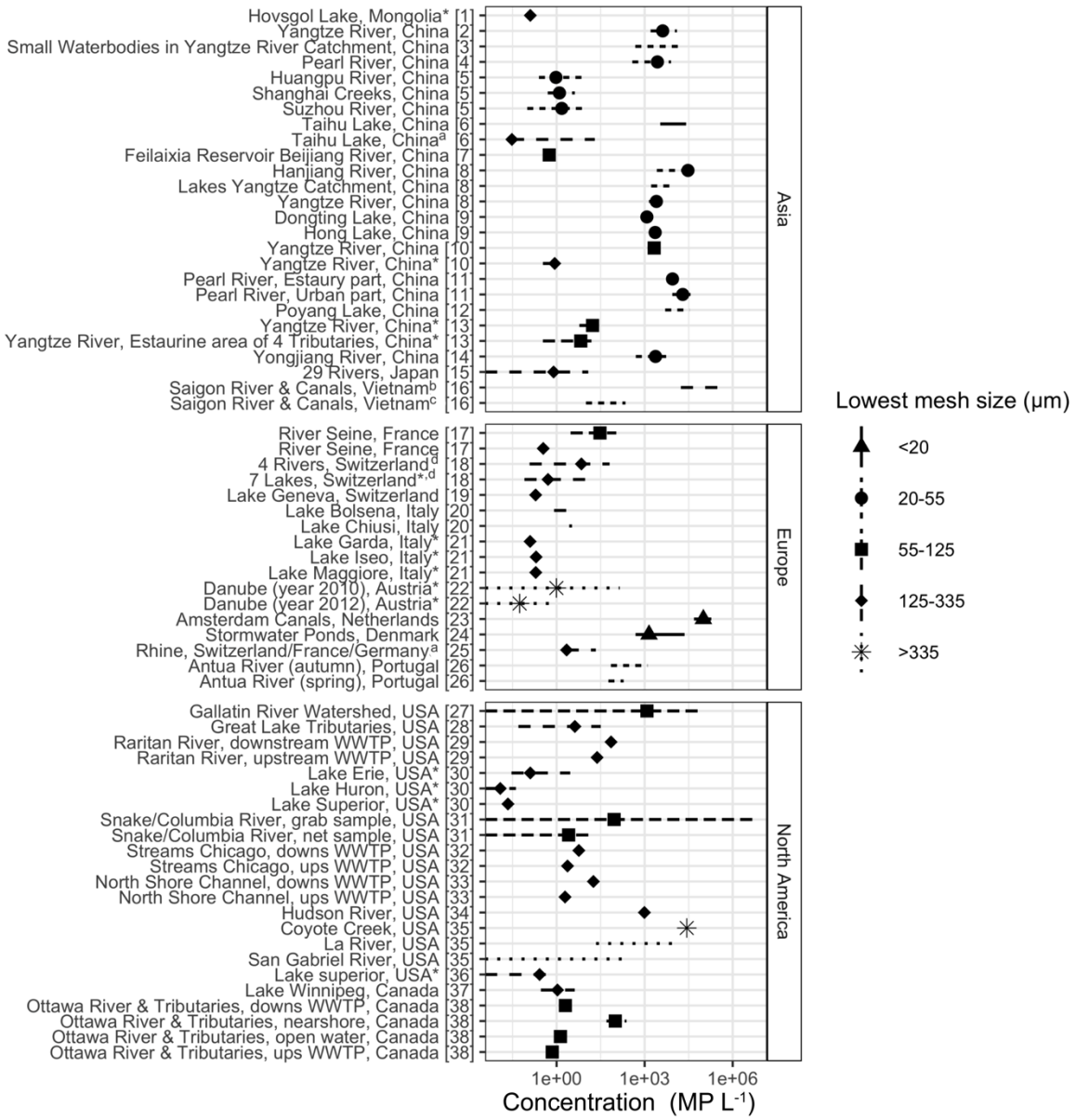


Figure 2.4. Mean and/or minimum-maximum MP concentrations (MPs m^{-3}) in freshwater samples. downs = downstream. ups= upstream. Notes: * Concentration in MPs m^{-3} was estimated by dividing the reported concentration in particles per area by the height of the net used for sampling. [a]Minimum concentration no specified. [b]Only fibers assessed. [c]Only fragments assessed. [d]Mean across all sample sites with minimum and maximum mean concentrations across sample sites.

References: [1] Free et al. (2014) [2] Di and Wang (2018) [3] Hu et al. (2018) Lin et al. (2018) [5] Luo et al. (2018) [6] Su et al. (2016) [7] Tan et al. (2019) [8] Wang et al.(2017b) [9] Wang et al. (2018) [10] Xiong et al. (2019) [11] Yan et al. (2019) [12] Yuan et al. (2019) [13] Zhang et al. (2015) [14] Zhang et al. (2019) [15] Kataoka et al. (2019) [16] Lahens et al. (2018) [17] Dris et al. (2015) [18] Faure et al. (2015) [19] Faure et al. (2012) [20] Fischer et al. (2016) [21] Sighicelli et al. (2018) [22] Lechner et al.(2014) [23] Leslie et al. (2017) [24] Liu et al. (2019a) [25] Mani et al. (2016) [26] Rodrigues et al. (2018) [27] Barrows et al. (2018) [28] Baldwin et al. (2016) [29] Estahbanati and Fahrenfeld (2016) [30] Eriksen et al. (2013) [31] Kapp and Yeatman (2018) [32] McCormick et al. (2016) [33] McCormick et al. (2014) [34] Miller et al. (2017) [35] Moore et al. (2011) [36] Hendrickson et al. (2018) [37] Anderson et al. (2017) [38] Vermaire et al. (2017).

Fragments and fibers formed by polyethylene and polypropylene are the most frequently observed particles across all studies evaluating MP pollution in freshwater ecosystems; whereas pellets or beads are only rarely reported as the main occurring plastic types (Table S5). The latter are mainly found in studies along the rivers Rhine and Danube, in the proximity to plastic processing plants and are thus assumed to be pre-production pellets (Lechner *et al.* 2014; Lechner & Ramler 2015; Mani *et al.* 2016). The prevalence of secondary MPs (fragments and fibers) suggests wastewater and runoff as sources for plastic pollution in freshwater ecosystems (Table S5). Several studies confirmed that by demonstrating that MP concentrations are higher downstream of WWTP as compared to sampling sites in upstream areas (McCormick *et al.* 2014; Estahbanati & Fahrenfeld 2016; Vermaire *et al.* 2017; Kay *et al.* 2018). For example, in the Ottawa River (Canada), 0.71 particles m^{-3} were found upstream of a WWTPs compared to 1.99 MPs m^{-3} downstream. In the Raritan River and the North Shore Channel (USA) 24 MPs m^{-3} and 1.94 MPs m^{-3} were found upstream the WWTP, and 71.7 particles m^{-3} and 17.93 MPs m^{-3} were detected downstream, respectively (McCormick *et al.* 2014; Estahbanati & Fahrenfeld 2016; Vermaire *et al.* 2017). As mentioned above, the majority of MPs in wastewater is smaller than 300 µm. Thus, it may be presumed that larger MPs enter via different pathway like surface runoff or stem from the breakdown of macroplastics directly in the aquatic environment. However, with untreated wastewater, for instance, during sewage overflows, large MPs and macroplastics can enter river ecosystems. For example, Morritt et al. (2014) identified pollution hotspots in the vicinity of WWTPs that were mainly constituted of sanitary products. MPs hotspots were also detected in areas with low population density but high agricultural use, also pointing to agricultural runoff as an important source (Kapp & Yeatman 2018). Finally, poor waste management likely increases plastic input into aquatic ecosystems (Lahens *et al.* 2018), where they can break down into smaller particles. Xiong et al. (2019), for example, found that the abundance of MP is positively related to the presence of macroplastics.

2.4.4. Sediments

Similar to macroplastics in surface waters also macroplastics in sediments are only rarely assessed and the way macroplastic occurrence is reported is highly variable and difficult to compare (Table S6). Macroplastics along river banks have been observed while assessing buoyant litter in general (Williams & Simmons 1999; Rech

et al. 2014), and river beach sediments in Switzerland contained on average 90 macroplastics m^{-2} (Faure *et al.* 2015). Across different lakeshores, macroplastics concentrations have been shown to vary notably (Imhof *et al.* 2013; Fischer *et al.* 2016). While high macroplastic concentrations have been observed at the south shore of Lake Garda (Italy; with an average concentration of 483 macroplastics m^{-2}), the occurrence at the north shore was significantly lower (i.e., 0 - 8.3 macroplastics m^{-2}) (Imhof *et al.* 2013). Food packaging is among the most frequently observed macroplastics but also bottles, bags and ropes are described by several studies. Regarding the polymer composition, polyethylene and polypropylene, as well as Styrofoam, are reported (Tab S6).

As for macroplastics and the other compartments, the concentration of MPs in freshwater sediments has not been reported in consistent units across all studies. Therefore, we focused on studies that have reported the concentration in MPs kg^{-1} sediment. However, studies reporting MPs per sediment area, which gave sufficient information to estimate the concentration in MPs kg^{-1}, were also included. Therefore, from the 34 studies that were found during the literature search (Table S7), 30 were chosen for comparisons (Figure 2.5). The data shown in Figure 2.5 are described in more detail in Table S7 where the type of analysis, particle shapes and polymer composition are reported. The highest sediment concentration of 2071 MPs kg^{-1} dw has been found in the urban canals of Amsterdam, where also the highest water concentrations were observed (Leslie *et al.* 2017). This value is the average of six urban canals with high variability in MP concentration, particularly fibers, where the presence of a hotspot is evident. MP concentrations in riverbed sediments seem, in general, higher than in river beach and shore sediments (Figure 2.5; Table S7). Most studies on MPs in riverbed sediments report concentrations between 100 MPs kg^{-1} and a few thousand MPs kg^{-1}. Studies from Asia were exclusively carried out in China and reported similar concentration ranges as those described in Europe. Interestingly, the study on the Yangtze River (China), which has been estimated to be the highest contributor of plastic to the sea (Schmidt *et al.* 2017) and is amongst the highest MP concentrations reported in water (Figure 2.4, Table S5), had a comparably low sediment concentration 7-66 MP kg^{-1}. The only study carried out in Africa (in a semi-arid South African basin) assessing the concentration of MPs in river sediment reports notable differences between concentrations in summer (1 - 14.6 MP kg^{-1} dw) and winter (13.3 - 563.8 MP kg^{-1} dw), which were related to a reduced flow condition in

winter in the studied region (Nel *et al.* 2018). Subsequently, the hydrological variation shown by many rivers seems to be one of the main factors contributing to MP deposition and re-mobilization from riverbeds. This was also demonstrated by Hurley et al. (2018a), who report that about 70% of the MPs in the sediments of the upper Mersey and Irwell catchments (UK) were exported after a flooding event. Several studies show that, after transportation with the river flows, MPs tend to (re-)deposit in low energy environments, such as meanders, deltas, dams, harbors and coastal lagoons (Claessens *et al.* 2011; Vianello *et al.* 2013; Shruti *et al.* 2019). The deposition of low-density polymers in sediment environments is also related to a density increase by biofouling (e.g., Ye & Andrady 1991; Andrady 2011; Zettler *et al.* 2013; McCormick *et al.* 2014).

For lakes, mainly beach and shore sediment concentrations have been reported. In Europe, average concentrations for beach and shore sediments ranged between 0.94 and 44 MPs kg^{-1}, while beach and shore sediments from Lake Ontario (Canada) contained much higher concentrations (20 - 27,830 MPs kg^{-1}; Figure 2.5, Table S7). Several studies have noted that plastic concentrations differ strongly between different areas of the same lake (Zbyszewski & Corcoran 2011; Imhof *et al.* 2013; Zbyszewski *et al.* 2014; Zhang *et al.* 2016), suggesting that accumulation is patchy and the formation of contamination hotspots is influenced by winds, waves and/or beach morphology (Imhof *et al.* 2016, 2018). Similar observations were made at Lake Huron (Canada), in which 94% of all monitored pellets were found to accumulate in one single beach (Zbyszewski & Corcoran 2011). In the Taihu Lake (China), MPs concentrations ranged from 11 to 235 MP kg^{-1} in different bed areas, and the average MPs abundance in sediments in the northwest area was approximately six times higher than the abundance of the southeast area (Su *et al.* 2016). Fibers followed by fragments were usually the most common particle types monitored (Table S7). Spheres, beads or pellets were, in rare occasions, reported to be dominant, and mostly in the vicinity to plastic industries (Zbyszewski & Corcoran 2011; Zbyszewski *et al.* 2014; Hurley *et al.* 2018a; Peng *et al.* 2018). Based on polymer type, polyethyleneand polypropylene were the most common, despite their buoyant properties, as well as polystyrene (Table S7).

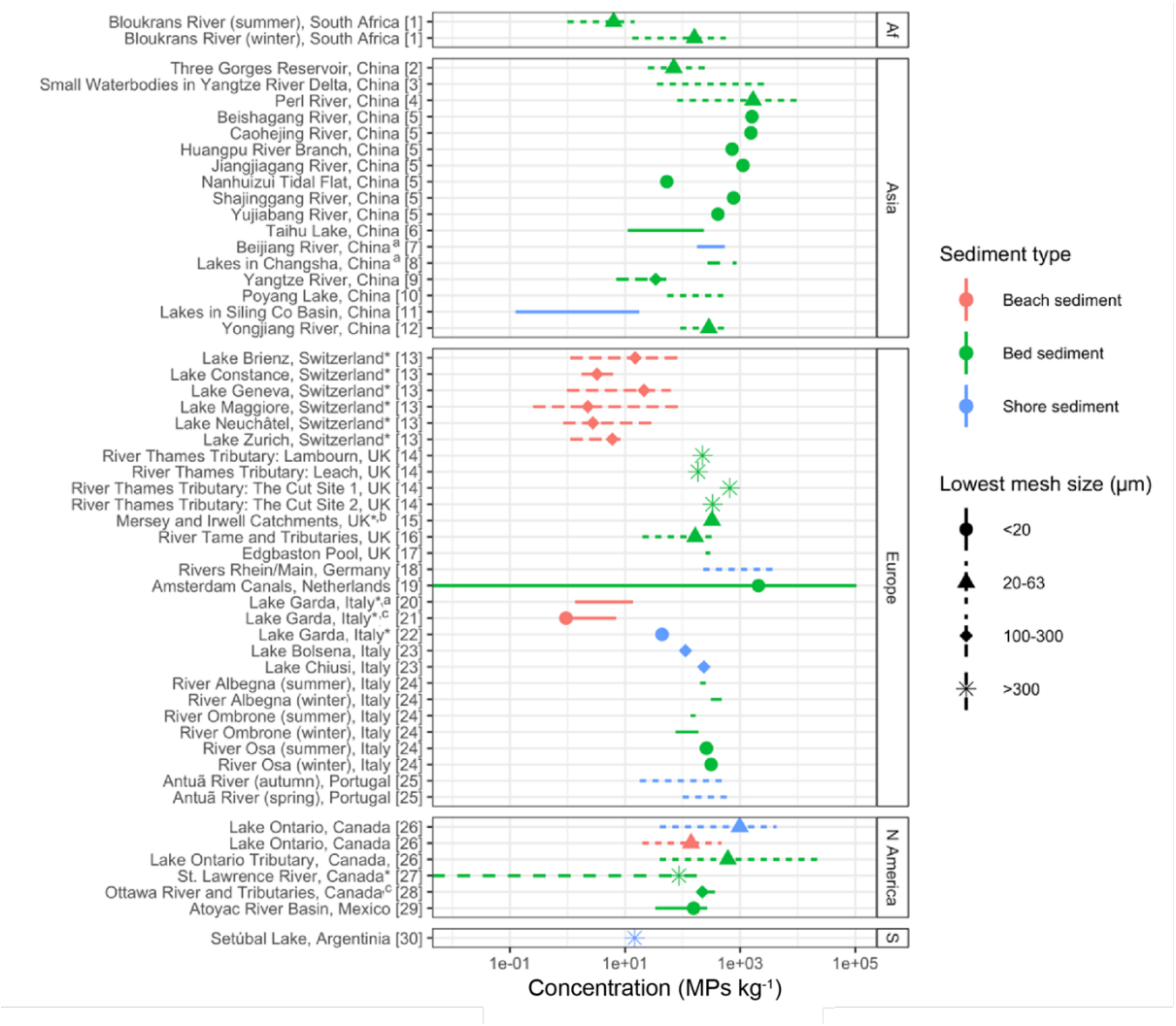


Figure 2.5. MP concentrations in different types of sediment samples (MPs kg^{-1}). Notes: The sediment type for river sediment was categorized as bed sediment if the type was not clearly stated. *Concentration in MPs kg^{-1} was estimated by using the sample depth and assuming a density of 1.6 g cm^{-3} for the sediment. [c]Range of mean concentrations across different sampling sites. [b] maximum value is shown; [c]no lower value reported. Af=Africa. N America = North America. S = South America.

References: [1] Nel et al. (2018) [2] Di and Wang (2018) [3] Hu et al. (2018) [4] Lin et al. (2018) [5] Peng et al. (2018) [6] Su et al. (2016) [7] Wang et al.(2017a) [8] Wen et al. (2018b) [9] Xiong et al. (2019) [10] Yuan et al. (2019) [11] Zhang et al.(2016) [12] Zhang et al. (2019) [13] Faure et al. (2015) [14] Horton et al. (2017a) [15] Hurley et al. (2018a) [16] Tibbetts et al. (2018) [17] Vaughan et al. (2017) [18] Klein et al. (2015) [19] Leslie et al. (2017) [20] Imhof et al. (2013) [21] Imhof et al. (2016) [22] Imhof et al. (2018) [23] Fischer et al. (2016) [24] Guerranti et al. (2017) [25] Rodrigues et al. (2018) [26] Ballent et al. (2016) [27] Castañeda et al. (2014) [28] Vermaire et al. (2017) [29] Shruti et al. (2019) [30] Blettler et al. (2017).

2.4.5. Marine

Rivers are estimated to be the main pathways for plastics entering the oceans. Estimations on the amount of plastic waste entering the ocean through this pathway range between 0.41 and 4 x 10^6 tons $year^{-1}$ (Lebreton *et al.* 2017; Schmidt *et al.* 2017). From the top ten river catchment that transport 88 - 95% of the global plastic load into the oceans, eight are located in Asia (Schmidt *et al.* 2017). Oceans have been assumed to be the final sink for macroplastics and MPs. As this review is focused on terrestrial and freshwater ecosystems, this compartment will not be discussed in detail. A number of articles and reviews have been published on the topic within the last few years which describe plastic occurrence in the oceans and its effects on marine life (see, e.g. Barboza & Gimenez 2015; Jambeck *et al.* 2015; Auta *et al.* 2017).

2.5. Discussion

We fully agree with the statement provided by the SAPEA (2019) report: "The number of papers is growing exponentially in this field, but knowledge is not growing at the same rate — there is some redundancy and marginality in the papers". Furthermore, many papers on plastic pollution do not assess and describe important plastic sources and flows. This review paper made an attempt to describe the available information regarding global environmental loads and the plastic life cycle, and to show which further research studies are needed to fully understand specific plastic sources and pathways. This section describes the areas that need further research commitment and development to improve exposure assessments and to evaluate the long-term risks of plastics to terrestrial and freshwater ecosystems.

2.5.1. The need for advancing and standardizing sampling and analysis techniques

As indicated in several parts of this review, the sampling methods reported in the literature are extremely variable and, in many cases, difficult to compare. In marine monitoring studies, the most commonly used method for sampling is the so-called manta trawl, a device similar to a large plankton net with a mesh size usually larger than 300 µm (GESAMP 2015). Using a manta trawl allows to sample a thin layer of surface water and, therefore, the results are generally reported as MPs (number or weight) per surface area (m^2 or km^2). The same device is frequently used also in

freshwater, together with other sampling methods (Figure 2.3), that produce results expressed as MPs per volume unit (e.g., L or m^3) and that may consider different size fractions, sometimes down to 20 µm. The results from studies considering the different sampling methods are hardly comparable. Data for surface units may be converted into data for unit volume, by calculating the mouth surface area of the manta trawl. However, this is a rough approximation because the trawl is not always fully immersed. Moreover, with the manta trawl, all particles below 300 µm are lost. This is shown by studies using both sampling methods (Kapp & Yeatman 2018; Lahens *et al.* 2018; Xiong *et al.* 2019). Small particles generally represent the largest share of the total amount of particles present in natural waters. Therefore, the manta trawl method largely underestimates the actual MP concentrations, at least in terms of particle numbers.

A recent report (GESAMP 2019) describes and compares methods for sampling macroplastics and MPs, with particular focus on the marine environment. The report highlights advantages and disadvantages of the different sampling methods. However, particularly for MPs, precise indications or suggestions of the methods to be used for a better exposure and risk characterization are not provided. It should be noted that the impact of different size fractions may be extremely different on the various components of the aquatic ecosystem (e.g., small fishes, macro-invertebrates, micro-invertebrates, bacteria). Therefore, methods capable to provide quantitative samples of different fractions, including relatively small MPs (e.g., down to 20 µm, achievable with fine-meshed phytoplankton nets), should be used whenever possible.

The available data on soil and sediments is relatively scarce. This may be partly related to the complex and time-consuming procedure required to extract MPs from these matrices (Hurley *et al.* 2018b). Some studies report MP concentrations as the number of particles per kg, while others provide the weight of MPs per kg. In other cases, data is reported as MP number or weight per surface unit (e.g., mg m^{-2}). Therefore, the comparison of literature data is not straightforward.

Besides this, existing methods to identify and count MPs are variable. Until recently, it was common practice to solely rely on visual detection (using a microscope), which may lead to false positives or false negatives. In more recent studies, visual examination is usually combined with Fourier Transform Infrared (FTIR)

or Raman Spectroscopy, which allows polymer Identification. This is, however, time-consuming and thus frequently only a sub-sample is subjected to spectroscopic methods. Other studies use different methods like SEM (Scanning Electron Microscopy), XRF (X-Ray Fluorescence), Pyr-GC/MS (Pyrolysis interfaced with gas chromatography/mass spectrometry) (Klein *et al.* 2018). It has been observed that MP abundance often varies with the methods used (Song *et al.* 2015; Mai *et al.* 2018; Picó & Barceló 2019), therefore analytical results may be difficult to compare across studies. The previously mentioned GESAMP report (GESAMP 2019) also compares methods for processing and analyzing MPs but does not provide clear suggestions for standardization.

There is an urgent need for standardizing methodologies to be applied to the exposure assessment, which include those related to sample processing, MP extraction, identification and counting, as well as the units to be used for reporting data. The major gap refers to very small-sized MPs (below 20 µm) and nanoplastics. Particularly the latter can currently not be included in monitoring programs because suitable sampling methods are lacking and analytical methods, such as pyrolysis-GC/MS (Fischer & Scholz-Böttcher 2017), are exploratory (GESAMP 2019).

Most procedures commonly applied to date allow sampling, processing and measuring particles down to a minimum size of 20 µm. Only very few studies measured smaller particles, down to 10 µm (e.g.,Leslie *et al.* 2017; Simon *et al.* 2018). In theory, very small particles and, especially, nanoplastics should be more abundant in the environment, and their concentrations are expected to increase. The development of methods for the evaluation and quantification of small-sized MPs and nanoplastics is one of the major research needs to assess the potential risks for human and environmental health. In particular, detection technologies to identify nano-sized plastic particles are still lacking (Mai *et al.* 2018). A promising approach, at least to quantify the mass and the composition (if not the number of particles), could be the use of Pyr-GC/MS (Hendrickson *et al.* 2018; Mintenig *et al.* 2018) coupled with methods of small-sized particle separation based on ultrafiltration membrane technologies (Mulder 1996; Judd & Jefferson 2003).

2.5.2. Towards a microplastic mass balance and suitable evaluation of environmental fluxes

The difficulties in getting reliable and comparable results for the concentrations of MPs in the different environmental compartments, and the limited information regarding some fluxes among compartments make the evaluation of a regional and global mass balance of plastics challenging. However, some first estimates can be made based on the available data, to at least give an approximate order of magnitude of the contribution of different sources to surface waters.

From the data reported in Figure 2.2 and Table S1, it can be concluded that the range of particles in effluents from WWTPs that include secondary and tertiary treatments spans from 1 to 5800 MPs L^{-1}, with a geometric mean around 29 MPs L^{-1}. In non-treated wastewaters, the concentrations range from a few particles L^{-1} up to more than 100,000 particles L^{-1}, with a geometric mean of about 242 MPs L^{-1}. These data are in reasonable agreement with the percentage of retention by WWTPs reported by several authors, which ranges from 80% to 99% of the number of inflowing particles (see Section 4.3).

The approximated per capita consumption of water in Europe is 140 L per day (EEA 2018). Although with some regional differences, it may be estimated that about 85% of the EU population (525 million in the EU plus Norway and Switzerland) is connected to WWTPs with secondary or tertiary treatment, while the remaining population (15%) is connected to WWTPs with only primary treatment or not connected at all (Table 2.1).

From these data, it can be estimated that the daily input of MPs (in the range of 20 to 5000 µm) via wastewater into European surface waters is:

- from treated wastewater: an average value of 1,800 x 10^9 particles per day (possible range from 9 x 10^9 to 130 x 10^{12} particles day^{-1})
- from untreated wastewater: an average value of 2,700 x 10^9 particles per day (possible range from 27 x 10^9 to 1,400 x 10^{12} particles day^{-1}).

Transforming these data on a weight basis is challenging because, in general, only numbers of MPs are reported, while size/weight conversion factors are not readily

available. Combined data on numbers and weight are reported in a Danish report (Vollertsen & Hansen 2017) assessing MPs occurrence in ten different WWTPs and in the study by Simon et al. (Simon *et al.* 2018). However, both studies took only MPs between 10 or 20 and 500 µm into account. Therefore, estimating the load on a weight basis from the particle numbers is not possible.

Table 2.1. Percentage of EU population connected to WWTPs in 2015 (EEA 2019).

	No treatment or no connection with sewerage	**Primary treatment**	**Secondary treatment**	**Tertiary treatment**
Northern	15.1	5.6	2.3	77
Central	3.4	0	16.5	80.1
Southern	23	2.2	21.3	53.4
Eastern	26	0.2	13.6	60.6
South-Eastern	40	16.7	22.8	20.6
Weighted average respect to population	13	2	18	67

Despite their wide range of variability, these estimates give a first approximation of the load of MPs in surface waters from urban wastewater and allow the following observations. First, the load that may be attributed to the relatively small percentage of European untreated wastewaters is much higher than the load deriving from treated wastewater, which points towards a definite need of implementing secondary and tertiary WWTPs in areas that are still not connected to reduce total MPs emission. Taking into account that untreated wastewater is concentrated in south-eastern Europe, it may be hypothesized that some watersheds (e.g., lower Danube) are subjected to higher contamination than those located in other European regions (Lechner *et al.* 2014). Unfortunately, data on MP concentrations in surface waters of south-eastern Europe are not available. Siegfried et al. (2017) implemented a modelling approach based on estimations of mass-based per capita consumption rates of personal care products, plastic fibers from textiles, plastic fibers in household dust

and tire debris, and concluded that the largest emitted mass from rivers to the sea occurs for tire debris, followed by textile fibers. Furthermore, they estimated that the majority of plastic particles emitted in Europe flow into the Mediterranean and the Black seas as a result of different socio-economic development and technological status of sewage treatment facilities.

Due to the scarcity of data of water consumption and WWTP implementation, a comparable evaluation cannot be done for other continents. However, it may be hypothesized that the percentage of treated wastewater in Asia and Africa is much lower than in Europe or North America.

The problem is also complicated by the fact that only a relatively small part of the population is connected to sewerage systems. Data from the WHO/UNICEF Joint Monitoring Programme (JMP), referred to 2015, indicate that in Eastern, South-eastern and Central Asia, with a population of more than four billion inhabitants, only 25% of the population is connected with sewerage systems; and in Sub-Saharan Africa the percentage is lower than 6% (WHO/UNICEF 2019). The high concentrations of MPs in surface waters of Asia (mostly assessed in China), as compared to those measured in Europe (Figure 2.4), supports the hypothesis regarding the large influence of WWTP on surface water emissions. The dominant shape in WWTP effluents are fibers, followed by fragments. Only in one case a minor amount (< 10%) of pellets that may be assimilated to primary microbeads was observed (Dyachenko *et al.* 2017).

Wastewater represents only one of the possible pathways of MPs into surface waters, and as discussed in this study, surface runoff from agricultural and urban soils may also represent a major source. Unfortunately, a comparable estimate of MPs emissions from soils due to water runoff is not possible due to field data limitations. On the other hand, this review shows that MP concentrations in WWTP sludge (mainly from Europe) range between 10 x 10^3 and 10 x 10^5 particles kg^{-1} dw. Nizzetto et al. (2016c) estimated that the total yearly input of MPs from sewage sludge to farmland is about 63,000 - 430,000 tons in Europe, and 44,000 - 300,000 tons in North America. Data on MP concentrations in soil are scarce and scattered (Figure S2 and Table S3). The majority of data on agricultural soils refer to China and indicate a reduced range of variability (from about 60 to 200 particles kg^{-1} dw), except for a couple of higher values (more than 10,000 particles kg^{-1} dw) from soils sampled in a greenhouse.

Overall, this study shows that soil could be considered as a sink as well as a source of MPs to surface water. Therefore, further research is urgently required to assess fluxes of MPs from soils into surface water ecosystems and to assess the fate of MPs in the soil ecosystems, investigating its retention potential and the capacity of MPs to reach groundwater ecosystems. An additional source of MPs to soil and surface water may be atmospheric fall-out (Dris *et al.* 2016). However, the information available to date does not yet allow a quantitative estimate (Wetherbee *et al.* 2019). An attempt to perform a quantitative evaluation of emissions to all environmental compartments (air, soil, WWTP, surface waters) has been made for tire debris indicating that urban and road runoff, as well as atmospheric deposition, may represent relevant contributions (Kole *et al.* 2017).

The formation or disappearance of MPs within the compartments also has to be considered in an overall mass balance. Macroplastic fragmentation in the different compartments is reasonably one of the major sources of MPs in the environment. However, the patterns of macroplastic fragmentation, their characterization and quantification in terms of amount produced and time to produce them are still largely unknown. The only fragmentation pattern that is sufficiently documented and quantified is the production of fibers during laundry of synthetic fabrics (Browne *et al.* 2011; Eerkes-Medrano *et al.* 2015). Although the amount of fibers may vary depending on the type of clothes (e.g. polymer composition, weave type, age), the type of washing machine, and the washing condition, it has been estimated that several thousand fibers are generated per washing cycle (Hartline *et al.* 2016; Napper & Thompson 2016; Pirc *et al.* 2016; Carney Almroth *et al.* 2018).

For any other type of plastic breakdown process, reliable experimental quantitative information is not yet available, although a modelling approach to predict the contribution of macroplastic breakdown to the MPs bulk in the ocean has been proposed (Koelmans *et al.* 2017b). Plastic fragmentation in the environment may be extremely variable in function of factors like light intensity, temperature, erosion and other physical impacts. The number and weight of MPs and nanoplastics that may be produced by a macroplastic item (e.g., a bag or a bottle) in a given time under environmental conditions is still largely unknown. This is an important knowledge gap that must be investigated in depth, and may be somewhat inferred based on the

amount and type of polymers of macroplastic litter in the environment and their documented half-lives.

Although plastic polymers are persistent compounds, some polymers can undergo biodegradation (Albertsson *et al.* 1987). Scientific evidence of biodegradation through bacterial activity and invertebrate digestion mechanisms has increased recently (Briassoulis *et al.* 2015; Yoshida *et al.* 2016; Yang *et al.* 2018). Compared to macroplastics, MPs and nanoplastics may be more prone to form complex structures with organic matter particles and be readily attacked by bacteria and invertebrates. Therefore, a real possibility of their complete disappearance exists. Nevertheless, to date, the extent of these degradation processes in environmental compartments, their time scale as well as the patterns and the end-products are relatively unknown (SAPEA 2019). Although plastic polymers are practically inert molecules, with low biological and toxicological activity, many monomers, that can be formed during the degradation of plastic, are not. Monovinylchloride (the monomer of polyvinyl chloride), for instance, is a recognized carcinogenic compound (Brandt-Rauf *et al.* 2012).

2.5.3. Microplastics in environmental compartments: what does it mean in terms of risks for living organisms?

As discussed above, information on the presence of MPs in environmental compartments is often biased by the inconsistency of units (e.g., n L^{-1}, n m^{-2}, mg L^{-1}, n kg^{-1}, mg kg^{-1}), by the variability in size classes sampled and measured, and by the complexity in shape and composition that are often not clearly reported. These inconsistencies make the assessment of their possible impact on living organisms rather complex, so the actual environmental risks of different plastics and their associated chemicals remain largely unknown (Koelmans *et al.* 2017a). It is important to highlight that quantifying the effects of MPs on living organisms by a simple concentration-response relationship of the whole mass of MPs of a certain type found in environmental samples is more complicated than for most chemical contaminants. Their impacts on aquatic organisms depend on a number of factors such as:

- the shape: the physical effect determined by long and thin fibers may be completely different from those determined by microspheres or by irregular fragments (Au *et al.* 2015; Lambert *et al.* 2017);

- the size range: the definition of MPs in term of size is extremely wide (from 5 mm to 1 µm) and the living organisms that may be affected by MPs are also extremely variable in size. For example, in the aquatic environment, from fish to zooplankton; for any type and size of organism, different MP size classes may be ingested, including small sizes (below 20 µm) and nanoplastics, that are practically never measured;
- the composition: for most MP polymers, being the effects mainly physical, it may be hypothesized that the response is not related to the polymer composition; however, for some particular MP particles, such as for tire debris, the composition is much more complex and the effects may also be determined by the leaching of non-polymeric chemicals.

Some recent effect studies took these parameters into account, used exposure conditions in relation to the traits of the organisms (i.e., feeding type; substrate preference) or provided dose-response relationships (e.g., Au *et al.* 2015; Scherer *et al.* 2017; Ziajahromi *et al.* 2017a; Redondo Hasselerharm *et al.* 2018). This allows to conduct an ecological risk assessment with preliminary data for a range of species based on a comparison between an environmental exposure (e.g., a PEC: predicted environmental concentration) and an effect level (e.g., a PNEC: predicted no effect concentration). So far five studies carried out a provisional ecological risk assessment for MPs (Burns & Boxall 2018; Everaert *et al.* 2018; Adam *et al.* 2019; Besseling *et al.* 2019; Zhang *et al.* 2019). Burns and Boxall (2018) did not identify any ecological risk for measured concentrations in water or sediment. Adam et al. (2019) conducted risk assessment for different continents and found that although no risk is expected for Europe and North America, "a risk cannot completely be excluded in Asia", where the highest MP pollution occurred. These findings are supported by a case study in the Yongjiang River in south China (Zhang *et al.* 2019). The authors calculated a risk quotient for each of their sample sites by dividing the measured MP concentrations by PNEC values derived from species sensitivity distribution based on literature toxicity data. They found that for most sites, a risk threshold was not exceeded, except for the two most contaminated sites, which were close to the urban center of Nanning City (Zhang *et al.* 2019). Besseling et al. (2019) concluded that hazardous MPs concentrations do not occur for freshwaters, while hotspot locations of near-shore marine surface waters, may exceed safe concentrations. Similarly, Everaert et al.

(2018) derived for the marine environment that at current average concentrations no risk is expected but that a risk cannot be excluded for heavily polluted sites. As MP concentrations in the environment are usually reported in particles number while effect data is based on mass, a transformation was necessary in these studies to be able to compare effect and exposure data. To perform a refined ecological risk assessment of MPs will require much more detailed information on MP exposure with a precise assessment of number (or weight) of particles per size classes, shape and composition. Considering that current methods for the analysis of MPs are complex, expensive and time-consuming, this level of detail is, to date, difficult to achieve. Further research should be devoted to both areas, to refine exposure assessments including areas that are expected to be heavily polluted but on which information is still completely missing such as the Ganges in India or the Amazon in South America (Adam *et al.* 2019). Moreover, effect assessments should be performed taking into account ecologically relevant combinations of organisms and MPs sizes, shapes and types. It is most likely that future risk assessments need to consider MP particle mixtures taking into account different polymer types, shapes and sizes, and that exposure and risk indicators are derived taking all these variables into account.

For nanoplastics, risk assessment is currently not feasible as they cannot be detected in environmental samples thus far. Also, regarding the effect assessment, the major unknown issues are related to small and very small particles (Koelmans 2019). nanoplastics may cross cellular membranes and enter into cells if they are below a given size. Within the cells, nanoplastics can possibly interact with the cellular content, structure and function. This represents a substantial difference in comparison to macroplastics or MPs. Indeed, MPs cannot be accumulated in biological organs and tissues and may produce mainly physical stress on living organisms, although the consequences of that may result in physiological and metabolic alterations. The size threshold below which small plastic particles may enter into cells is still unknown. Recent studies on nanoplastics performed with reference materials labeled with fluorescent dye demonstrate their capacity to be taken up, enter tissues, and accumulate in small organisms (Cui *et al.* 2017; Lee *et al.* 2019). However, some authors discuss that this may be an artefact either created by the leaching of those dye paints, which can be taken up into cells or due to the autofluorescence of the evaluated biological tissues (Catarino *et al.* 2019; Schür *et al.* 2019).

2.5.4 How can MP inputs into the environment be controlled?

From all the considerations mentioned above, it is evident that the precautionary principles strongly push towards the control of MPs and nanoplastics. From the available literature on MP presence in the environment, it appears that primary MPs represent a relatively small amount of the total bulk of MPs detected, being secondary MPs the largest majority. It is difficult to quantify the percentage of primary MPs in the environment precisely. However, in general, it seems to be never higher than 10%, and in most cases, the percentage is much lower, sometimes almost negligible. For example, in urban wastewater, the majority of MPs is represented by textile fibers (see for example Dris *et al.* 2015; Vollertsen & Hansen 2017; Wang *et al.* 2017b) while in runoff water the most abundant particles are fragments from macroplastic breakdown (see for example Liu *et al.* 2019a). Therefore, the recent proposal of ECHA (ECHA 2019) for a ban or restriction of primary MPs may have a limited relevance and effectiveness for the reduction of the presence of MPs in the environment.

Based on the information available to date, the most plausible solution to reduce the environmental emission and exposure to MPs seems to be the control of macroplastics. The restrictions on single-use plastic items that will be active in Europe starting from 2021 (EC 2019) seem to be an excellent starting point. Comparable restrictions should be applied in the short-term on food and other kinds of packaging, which represent the largest amount of plastic wastes. In addition to restrictions, a more efficient recycling strategy and improvement of the circular economy related to plastic products would be beneficial (Barra & Leonard 2018). However, in some cases, different types of measures should be developed. As shown above, fibers represent the most abundant type of MPs present in wastewater. Since it is almost impossible to ban synthetic fabrics, that today makes up the majority of our clothing, the solution should be sought in another direction (e.g., by means of retaining fibers in washing machines, water treatment procedures, etc.).

Finally, the substitution of traditional plastic polymers, based on the petrochemical industry, with new generation polymers, based on biological resources (e.g., PLA: polylactic acid; PHA: polyhydroxyalkanoates) is often proposed as a suitable solution. However, present knowledge on the toxicological properties of these new compounds and of their degradation products must be improved (Lambert &

Wagner 2017; Picó & Barceló 2019). Understanding possible biodegradation patterns of traditional and emerging plastic polymers is important for future management and remediation of plastics in the environment.

2.6. Conclusions

In this study we have described the state of the knowledge regarding the occurrence of macroplastics and MPs in different environmental compartments. It has been highlighted that some data gaps still exist in order to better understand their life cycle, to develop a precise mass balance and to quantitatively assess the contribution of the different main sources of macroplastics, MPs and nanoplastics in the environment. The emission of MPs from WWTPs into aquatic ecosystems is the environmental pathway that has been most researched. However, there are other pathways that may have similar or even larger contributions, and that require further investigation. Those pathways are, for example, the fluxes of plastics from landfills and agricultural soils towards surface and groundwater ecosystems by water runoff or deep-horizon infiltration, or the transport and deposition of plastic particles from the atmosphere. Moreover, quantitative evaluations of the occurrence of large-size plastics in natural environments need to be performed, and their breakdown rates into MPs and nanoplastics still need to be assessed under different environmental conditions (i.e., temperature and light intensities, water currents).

There is enough experimental evidence demonstrating that the presence of macroplastics in aquatic ecosystems represent an environmental risk, particularly for large animals. Regarding MPs, a risk for human and environmental health has not been demonstrated. Available toxicological evidence indicates that some effects on aquatic and terrestrial organisms, vertebrates and invertebrates, have been observed only at concentrations that are orders of magnitude higher than the maximum levels measured in the environment. Other possible effects, such as a potential increase in the bioaccumulation of chemicals due to their transport into the organisms adsorbed on MPs (the "Trojan Horse" effect) seems to be context dependent, and negligible in comparison to direct accumulation from the surrounding environment (e.g., from water) or from food (Koelmans *et al.* 2013, 2014; Lohmann 2017; Mohamed Nor & Koelmans 2019). However, research still needs to demonstrate this experimentally. An additional gap are the toxicological risks of nanoplastics that has to be investigated further, taking

their bioaccumulative and reactive potential in biological tissues, organs and cells into account.

Current knowledge gaps regarding environmental fluxes and breakdown of MPs and nanoplastics are still too large in order to assess future risks for man and the environment. Furthermore, the bias on sampling and analysis makes a precise quantification challenging. This is particularly difficult for small MPs and nanoplastics, which are probably the more concerning particles from a toxicological point of view. Moreover, although present exposure seems to be far away from levels of concern, it is difficult to predict future emission patterns since they will be closely related to plastic use and management policies. This review shows that the construction of waste-water treatment facilities and the proper management of sludge applications in agriculture are efficient means to reduce MPs emissions. Moreover, the ban of single-use plastics, the substitution of some plastic polymers with biodegradable compounds, and the reduction of MPs emission at a source are key to control plastic pollution. From now onwards, we expect technological solutions to be developed and implemented in this direction. There is no doubt that plastics changed our life in the middle of the last century, and the control of plastics will again change our life in the near future.

Acknowledgements

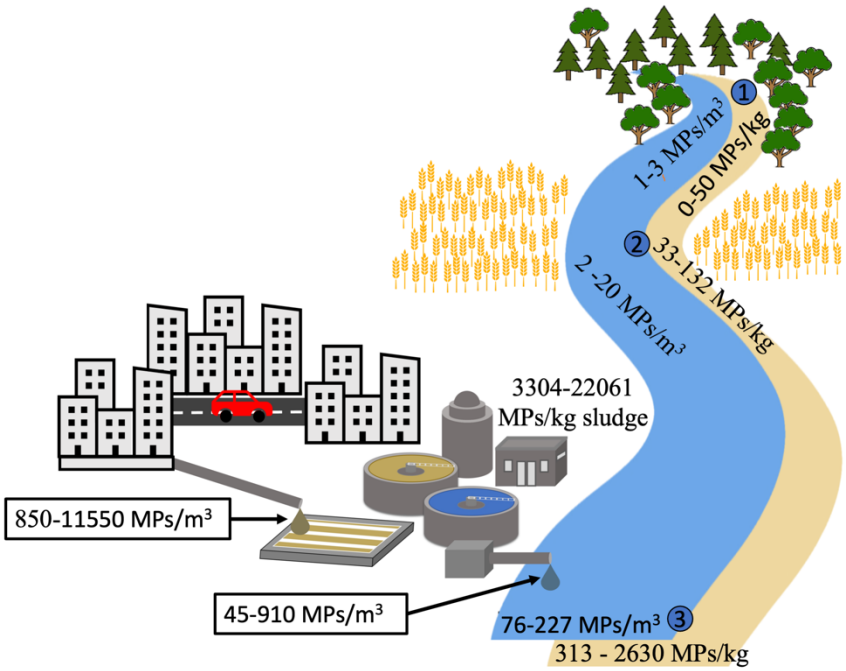
The study has been conducted as part of the EU JPI-Water initiative IMPASSE project (Impacts of MicroPlastics in AgroSystems and Stream Environments, PCIN-2017-016). A. Rico was supported by a postdoctoral grant provided by the Spanish Ministry of 1083 Science, Innovation and University (IJCI-2017-33465)

Supporting Information

The Supporting Information for this Chapter can be downloaded at: https://doi.org/10.1007/398_2019_40

CHAPTER 3

Spatio-Temporal Distribution of Microplastics in a Mediterranean River Catchment: The Importance of Wastewater as an Environmental Pathway



This Chapter has been published as:

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 Material 420: 126481. https://doi.org/10.1016/j.jhazmat.2021.126481

Abstract

Microplastics (MPs) are considered to be ubiquitous contaminants in freshwater ecosystems, yet their sources and pathways at the river catchment scale need to be better determined. This study assessed MP (55 - 5000 µm) pollution in a Mediterranean river catchment (central Spain) and aimed to identify the importance of wastewater as an environmental pathway. We sampled treated and untreated wastewaters, and raw and digested sludge from five WWTPs during two seasons. River water and sediments were sampled at three locations with different anthropogenic influences during three seasons. On average, 93% (47 - 99%) of MPs were retained by WWTPs. Concentrations in river water and sediment ranged between 1 - 227 MPs m^{-3} and 0 - 2630 MPs kg^{-1} dw, respectively. Concentrations strongly depended upon land-use, with pollution levels increasing significantly downstream of urban and industrial areas. Seasonality influenced the observed MP concentrations strongly. During high flow periods, higher water but lower sediment concentrations were observed compared to low flow periods. We estimate that 1×10^{10} MPs are discharged into the catchment via treated and untreated wastewater annually, which constitutes up to 50% of the total MP catchment discharge. Thus, we conclude that the wastewater system represents a major environmental pathway for MPs into Mediterranean rivers with low dilution capacity.

3.1. Introduction

Microplastics (MPs; defined as plastic particles < 5 mm) have been identified in numerous environmental compartments globally (Auta *et al.* 2017; Cera *et al.* 2020; Schell *et al.* 2020). Pollution by MPs raises environmental concerns as they can be ingested by and potentially affect a wide range of organisms, from primary producers to fish and mammals (de Sá *et al.* 2018). Yet, quantitative information about MP sources, pathways, and exposure is still limited, especially in freshwater systems. Wastewater has been suggested as one of the main pathways for MPs release to the aquatic environment and represents the convergence of a wide range of potential MP sources (e.g., fibers from clothing, various types of fragments, tire and road wear particles; Ziajahromi *et al.* 2016; Wagner *et al.* 2018). Although studies show that wastewater treatment plants (WWTPs) are efficient at capturing MPs (about 60 - 99.9% are retained; Ngo *et al.* 2019; Barchiesi *et al.* 2021), they still constitute a potentially significant source due to the very large volumes of effluents discharged (Ziajahromi *et al.* 2017b; Edo *et al.* 2020). Thus, despite low concentrations typically reported for treated effluents, the number of particles released to recipient waters can still be very high over relevant temporal scales (Mason *et al.* 2016; Liu *et al.* 2021). Furthermore, in some cases, untreated wastewater may enter the environment directly i.e., due to the lack of wastewater treatment infrastructure or the role of combined sewer overflows, which may discharge untreated water during heavy rainfall events.

During wastewater treatment the bulk of MPs in wastewater influents is believed to be sequestered and concentrated into the sewage sludge (Mintenig *et al.* 2017; Liu *et al.* 2019b). This solid by-product is frequently reused as fertilizer on agricultural land and these wastewater-derived MPs can enter the terrestrial environment along this pathway (Corradini *et al.* 2019; Van Den Berg *et al.* 2020). Thus, surface water runoff from agricultural soils, which receive sewage-sludge or plastic input through other agricultural processes (e.g., plastic mulching), has been suggested as another possible MP pathway to rivers (Horton & Dixon 2018; Qi *et al.* 2020).

Industrial spillages, emissions from road traffic, atmospheric deposition, wind-blown debris from littering or loss during waste disposal and the degradation of larger plastic debris directly in the aquatic environment may further contribute to MP contamination in freshwater ecosystems (Lechner & Ramler 2015; Cai *et al.* 2017; Dris

et al. 2018; Knight *et al.* 2020; Piñon-Colin *et al.* 2020). Quantitatively assessing the relative contributions of each of these pathways is challenging, and further research is needed to understand and characterize them in relation to different anthropogenic pressures and geographical settings. Catchment characteristics such as topography, hydrology, land use and soil characteristics are likely to influence MPs sources, transport and sinks further, but the underlying processes are poorly understood and quantified (Windsor *et al.* 2019a).

In arid or semi-arid regions, MP inputs through surface water runoff from urban or agricultural lands is expected to be lower than in wetter regions. WWTP effluents, however, contribute, during most parts of the year, a large volume of the rivers' total water flow and may therefore be of higher relevance compared to other MPs sources and pathways. Yet, the number of studies assessing MP contamination in freshwater ecosystems in semi-arid areas − and particularly in the Mediterranean region − is very low (Guerranti *et al.* 2020). Rivers in semi-arid areas are subject to high temporal flow fluctuations, which may affect MP transport and sedimentation. Further work to clarify the sources, environmental pathways, fate, and storage of MPs on the catchment scale in semi-arid systems is required to establish the relative importance of environmental variables and processes, and to design efficient measures to reduce MP pollution.

Hence, the main objective of this study was to elucidate the role of wastewater as MP source to rivers in a semi-arid Mediterranean catchment by (1) assessing the concentration of MPs in influent, effluent and sludge of WWTPs with different treatment types, as well as their removal efficiency, (2) establishing the spatial (land use based) and temporal (seasonal) distribution of MPs in river water and sediment in the same catchment, and (3) determining the contribution of wastewater to the total MP catchment discharge.

3.2. Material and methods

3.2.1. Study area

This study was conducted in the Henares River catchment (4,144 km^2), which is located in the upper Tagus River Basin, central Spain (Figure 3.1). The Tagus River is the longest river in the Iberian Peninsula, emptying into the Atlantic Ocean near Lisbon, Portugal. The Henares River catchment has an average discharge of 2.66 x 10^8 m^3 $year^{-1}$ (based on the latest available 20-year average; CEDEX 2021).

The area is characterized by a continental Mediterranean climate, with hot and dry summers and mild-to-cold winters. The flow regime of the Henares comprises high flow during the winter and spring, and low flow during the summer and autumn (Camargo 2006). While the upper part of this catchment is mostly characterized by forest areas or extensive agriculture, the lower part is influenced by industrial and urban areas and wastewater discharges (Figure 3.1). The main industry sectors relate to chemical and metal products, machinery and electrical equipment, transport equipment, as well as paper and printing materials. The two main cities in the catchment, Alcalá de Henares and Guadalajara have a population of around 197,500 and 87,500 inhabitants, respectively.

We sampled five out of the 19 WWTPs in the catchment. The sampled WWTPs contribute to approximately 75% of the wastewater discharge. About 1.5% of the wastewater is released untreated into the catchment, mainly corresponding to small villages. Additional untreated wastewater may also be discharged into the rivers during heavy storm events, when the influent volume exceeds WWTP capacities. The five monitored WWTPs differ in regard to treated water volume, treatment steps and influent water characteristics (urban wastewater or a combination of urban and industrial wastewater; Table A1).

3.2.2. MP sampling

At each WWTP, the untreated influent, treated effluent, and raw and processed sludge (after anaerobic or aerobic digestion and dehydration) were sampled. Samples were taken during the summer (25. - 27. July) and autumn (6. - 8. November) of 2017. Influent (20 L) and effluent (200 L) were sampled at each WWTP by filtering the water through a battery of nylon nets with different mesh sizes (55, 150, 300 µm). Recommended sampling volumes differ depending on the water type: the sample volume should be sufficient to be representative but small enough to prevent clogging of the nets (Koelmans *et al.* 2019). After filtering, the concentrated samples were stored in glass flasks, along with the milli-Q water used for rinsing the nets. Raw sludge was sampled prior to sludge treatment steps – like digestion and dehydration − while the processed sludge was sampled after these steps, directly from the sludge hopper. Both sludge types were dried (50°C for 48 - 72 hours) and stored in glass flasks until further analysis.

Three sampling sites were chosen to assess the MP concentration in water and sediments of the Henares River catchment (Figure 3.1). Site 1 (Sorbe River; UTM 30T 484735 4526717) was located downstream of forested and vegetated areas; Site 2 (Badiel River; UTM 30T 493150 4516208) was located close to the outlet of a sub-catchment mainly influenced by agriculture, but was also subjected to the discharges from untreated wastewaters from very small villages; and Site 3 (Henares River; UTM 30T 464261 4478887) was located at the outlet of the catchment. Water and sediment samples were taken during summer (27. July to 1. August), and autumn (6. - 9. November) of 2017, and spring (23. - 24. April) of 2018.

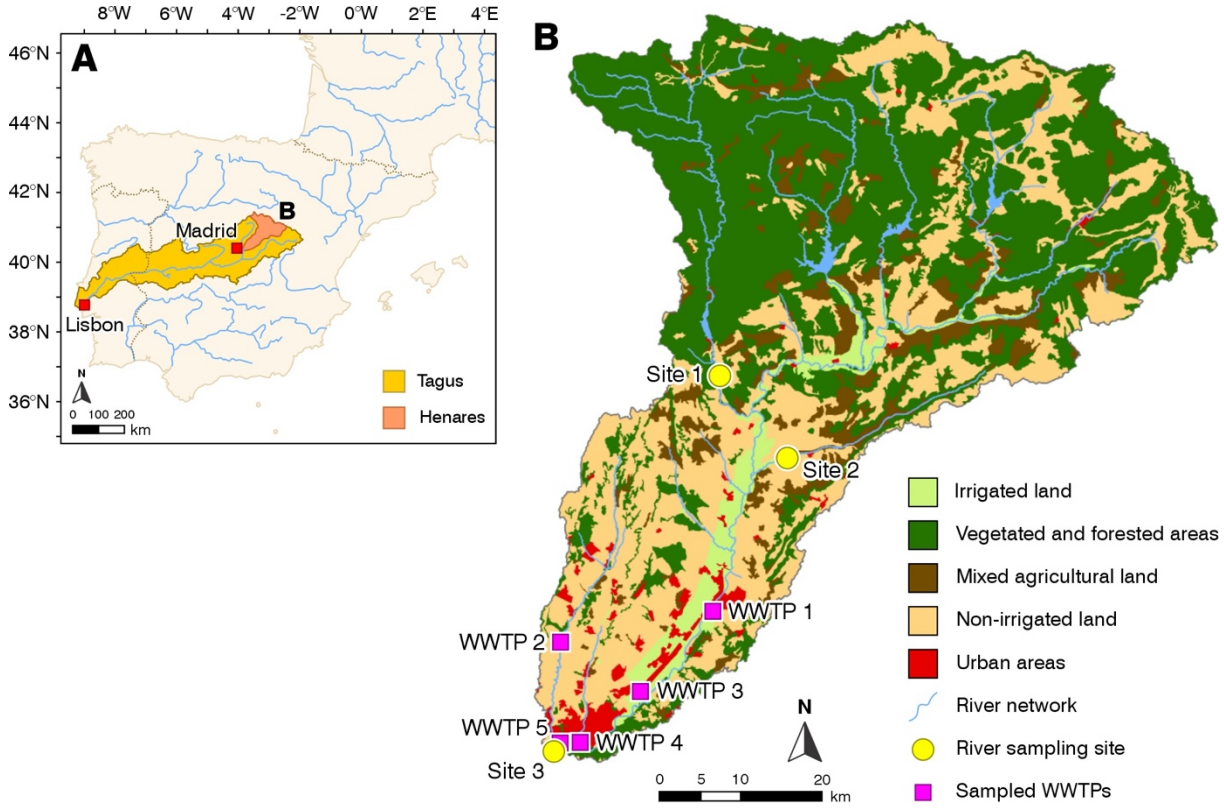


Figure 3.1. Location of the Henares river catchment within the Tagus River Basin in the Iberian Peninsula (A). The major river networks of the region are also shown. Location of river sampling sites and the sampled WWTPs within the Henares catchment (B), with a description of land use retrieved from the Corine land cover database (Corine Land Cover 2006).

River water was filtered through the same battery of nets as were used for wastewater. During each sampling event, 10,000 L of river water were pumped into the nets using a submersible pump (Jardín y Natura Outdoor & Garden Products S.L, WP30/3, with a flow of 5000 L h^{-1}). This is except for two samples where only 5000 L were filtered: sampling site 2 in summer, where rapid clogging of the net with organic

material prevented the sampling of a larger volume, and at sampling site 3 in spring, in response to very high MP concentrations recorded in the earlier samples. At site 1 and 2, the pump was placed directly onto the riverbed with no or very little overlying water. At site 3, the water level was higher, and the pump was submerged directly below the water surface. The filtrate of each size fraction was collected in glass flasks along with Milli-Q water that was used to rinse the nets. These flasks were stored at 4°C until analysis. Sediment samples (approximately 0.5 kg wet weight) were taken from riverbed areas composed of sand and slick with a core sampler (sediment depth: 10 cm). The samples were dried (50°C for 72 hours) and stored in glass flasks until analysis.

3.2.3. MP extraction and analysis

Water samples (wastewater and river water) were − depending on their organic matter content − either directly vacuum filtered onto filter papers (Whatman GF/A; Ø 47 mm; 1.6 µm pore size) and retained for analysis or first treated to remove organic material. Some river water and influent samples were largely clear or only contained large pieces of organic material that could be removed and inspected for MPs. These samples were vacuum filtered directly onto filter papers and retained for analysis. Samples with high organic matter content were first left to stand until the overlying water was clear. This was then decanted off and vacuum filtered on a filter paper. The remaining slurry was transferred to clean, pre-rinsed Erlenmeyer flasks and treated using Fenton's reagent, following the procedure outlined in Hurley et al. (2018b). After the digestion was complete, the flasks were filled with filtered RO water and permitted to settle out overnight. The overlying water was decanted and vacuum filtered onto a filter paper, representing a freshwater density extraction (ρ = 1.0 g cm^{-3}). The residual material in the flasks was transferred to clean pre-rinsed polyethylene tubes and subjected to a further freshwater density extraction, followed by two high density extractions using saturated NaI (ρ => 1.75 g cm^{-3}) to isolate MPs from the solid matrix. All filter papers were stored in petri dishes and retained for analysis.

Solid samples (sludge and sediment samples) were subjected to both density separation and organic matter removal. For the sludge, subsamples of between approximately 2.5 and 10 g were weighed into clean, pre-rinsed Erlenmeyer flasks. Initial subsamples of 10 g were found to be too large for some samples, which

contained high MP counts. These samples were repeated with smaller sub-sample sizes, which explains the final range that was used. The samples were then treated with Fenton's reagent to reduce the organic content, following Hurley et al. (2018b). Following digestion, the flasks were filled with filtered RO water to loosen any particles adhering to the sides of the flask and to wash any residual hydrogen peroxide in the sample. The samples were left to settle out overnight and the overlying water was decanted and vacuum filtered. The remaining material in the flasks was then transferred to clean, pre-rinsed polyethylene tubes for a second freshwater density extraction, followed by two high density extractions, as was performed for the high organic content water samples. For the sediment samples, 20 - 30 g replicates were weighed into clean, pre-rinsed polyethylene tubes. They were first subjected to density separation using two high saturated NaI extractions. The first extraction was typically characterized by a large amount of organic material, which was collected using a 38 µm stainless steel sieve and transferred to an Erlenmeyer flask and treated with Fenton's reagent. Both the digested material and the second extractions from density separation (which had significantly low organic content) were filtered onto filter papers (Whatman GF/A Ø 47 mm) and retained for analysis.

All filter papers were visually analyzed for MP particles using a Nikon SMZ 745T stereomicroscope at 20 - 50 x magnification. Each suspected MP particle was photographed using an Infinity 1 camera and classified into beads (spherical particles), fragments, fibers, films, foams, granules, glitter, or pellets (pre-production pellets). On rare occasions fiber clumps (fiber bundles) occurred, that could not be separated. These were therefore counted as such. The long and short dimensions of each particle were measured using the Infinity Analyze (v.6.5.4) software package, following calibration using a measurement standard. Particle depth was estimated to the nearest of 25 µm for particles extracted from sludge and sediment and the river water samples taken in spring 2018. The ratio of the minor axis to the depth axis was calculated for those samples, which had a median ratio of 0.5. Following the approach by Simon et al. (2018), this ratio was then used to estimate the depth for MP fragments, granules, foams, and glitter in wastewater and river samples from previous samplings (summer and autumn 2017). For films a depth of 10 µm was assumed.

The polymer composition of suspected MPs was characterized using Fourier transform infrared spectroscopy (FTIR). Large MPs (> 300 µm; excluding fibers) were

analyzed using an Agilent Cary 630 ATR-FTIR equipped with a diamond crystal accessory. Each measurement comprised four co-scans, taken at a spectral resolution of 4 cm^{-1}. A new background measurement was taken before each individual particle was analyzed. Small MPs and all fibers (55 - 300 µm) were analyzed using a Perkin Elmer Spotlight 400 µFTIR in transmission mode. The particles were first compressed using a diamond compression cell (DC-3, Perkin Elmer) before being loaded onto the machine, to improve spectral quality. Four co-scans, taken at a spectral resolution of 4 cm^{-1}, were taken for each particle measurement. A new background scan was made each time the diamond compression cell was loaded onto the machine (approximately every 1 - 10 particles). Each spectrum was compared to a series of commercial (PerkinElmer Polymer library, Agilent Polymer library), open source (Primpke *et al.* 2018), and in-house libraries and manually verified to confirm the polymer type. In addition to fibers that were suspected to be plastic after visual assessment using the criteria of Lusher et al. (2020), a small number of likely cellulosic fibers were analyzed using FTIR to confirm their composition. Fibers that were cellulose (e.g., cotton) or other natural materials (e.g., wool, silk) were not included in the dataset as they are not made of plastic polymers. Semi-synthetic fibers were included in the analysis due to the ongoing debate regarding their inclusion in the definition for MP. For MPs in wastewater (influent, effluent) the polymer composition of 86% of the MPs were determined. The remaining 14% were fibers that visually resembled plastic fibers (Lusher *et al.* 2020). The polymer composition of > 99% of particles in river water was analyzed and 100% for all other sample types.

3.2.4. Quality assurance/quality control (QA/QC)

All sample processing and analysis was performed in the NIVA Microplastic Laboratory, in a positive pressure room with HEPA-filtered (class H13) air input. Several contamination reduction procedures are implemented in the laboratory, including: the use of natural fiber clothing and lab coats, removal of loose fibers using a lint roller upon entry to the laboratory, and regular removal of dust from all areas of the laboratory. To further reduce the potential for contamination, all processing steps in which samples were exposed to the laboratory environment (e.g., during subsampling) were undertaken in a laminar flow cabinet present inside the Microplastic Laboratory. All laboratory water or solutions used in the sample processing were pre-filtered (0.22 µm for RO water, 1.2 µm for NaI and H_2O_2) immediately prior to use. All

containers were rinsed with filtered RO water three times before use, to remove any potential contamination.

A total of three blanks were included for each batch of sludge and sediment samples as well as for river water samples from April 2018. Blanks comprised combined procedural, container, and solution blanks, that were treated to an identical sample processing procedure within each sample batch. All suspected MPs found in the blanks were visually and chemically characterized in the same way as the environmental samples.

3.2.5. MP mass estimation and concentrations

The mass of each MP particle was estimated using the three analyzed size axes to provide an estimate of particle volume. According to the different morphologies the volume was calculated using the following approaches: fibers were treated as cylinders; fragments, films, glitter and granules were treated as cuboids; beads were treated as spherules; and fiber bundles were treated as cuboids and then divided to reflect the estimated percentage of the cuboid that was taken up by fiber versus empty space. Polymer density was established from a literature search, where the most commonly reported densities for each polymer were used (Table A2). To calculate the mass of the remaining MPs for which the polymer composition was not available, a density of 0.9 g cm^{-3} was assumed for fragments and of 1.3 g cm^{-3} for fibers, based on the median density of the current dataset. Finally, MPs were grouped into four size classes according to the longest measured axes (55 - 150 µm; 150 - 300 µm; 300 - 1000 µm and 1000 - 5000 µm). A few particles > 5000 µm and lower than 55 µm were observed, which were excluded from the analysis.

3.2.6. Calculation of MP discharge by wastewater

To determine the contribution of wastewater to the total catchment MP discharge, the amount of wastewater annually produced in the Henares catchment was first obtained from the Tagus River Basin Authority (Confederación Hidrográfica del Tajo 2019). Effluents were grouped according to wastewater type (i.e., urban or urban combined with industrial effluents; untreated urban effluents; treated industrial effluents). MP loads emitted with wastewater were estimated based on the observed concentrations in influent and effluent in this study. First, the flow based weighted

average was calculated for the influent and effluent across all sampling WWTPs. Furthermore, the flow based weighted average of WWTPs 1, 3 and 5 was used as an estimation for entirely industrial effluents, although MP composition in industrial wastewater is expected to vary greatly based on the industrial sector. The flow based weighted average for influents was used as an approximation to account for wastewater from small villages that are discharged into the rivers without treatment. Finally, the respective average MP concentration was calculated by multiplying the respective volume of wastewater discharge by the obtained weighted average for the wastewater type.

3.2.7. Statistical analysis

A Redundancy Analysis (RDA) was performed to assess the influence of different independent variables on the size, particle type and polymer type distribution of MPs in wastewater, sludge, river water and river sediments. For the wastewater, we tested the differences between the untreated and treated water, and subsequently the influence of the sampled WWTP, date, and type of water in the influent water dataset. The same variables, plus WWTP treatment type, were tested for the effluent water samples. Statistical differences were assessed between raw and processed sludge, and in each dataset, we evaluated the possible influence of WWTP, date, treatment type and type of water on the MP distribution. Finally, we tested the influence of the sampling site and the sampling date on the MP dataset obtained from the river water and sediment samples. RDAs were performed with 499 Monte Carlo permutations using the statistical software Canoco v5.1 (Ter Braak & Šmilauer 2018). Statistical influence of the tested parameters was determined when the calculated p-value was < 0.05.

3.3. Results and discussion

3.3.1 MPs in untreated and treated wastewaters

Concentrations based on particle counts in untreated wastewaters ranged between 850 and 11,550 MPs m^{-3}, while the estimated concentration based on mass ranged between 4.51 and 194 mg m^{-3} (Table 3.1). The lowest and highest estimated mass concentrations did not necessarily correspond with the lowest and highest particle count concentrations. For example, the highest particle count concentration was observed during the autumn sampling at WWTP 1, while the highest mass

concentration was measured at the same WWTP but during summer. Fibers (11 - 86%; average: 41%) and fragments (12 - 69%; average: 42%) were the main MP types observed in untreated wastewaters (Figure A1; Table B1). Granules, foams, beads, films, and glitter were also recorded, and together accounted on average for 12% (2 - 38%) of the observed MPs. Most fibers had lengths between 1000 - 5000 µm (74% of all observed fibers), and no fibers smaller than 300 µm were identified (Figure A2). The majority of the other particle shapes – mainly fragments − were between 300 - 1000 µm (59%) in size (based on the long axis). In total, 24 different polymer types and suspected tire particles were identified by the FTIR analysis (Table B2). The most frequent polymer types based on particle counts were polyester, polyethylene, and polypropylene (Figures 3.2 and A3, Table B2).

The sampling month and type of influent (urban or urban combined with industrial) did not significantly influence the particle type, size, or polymer distribution of MPs in untreated wastewater (Table A3).

MP concentrations in effluent (excluding paint particles originating from the WWTP tanks) ranged between 45 and 535 MPs m^{-3}, or 0.28 to 48.5 mg m^{-3} (Table 3.1). The highest particle-based concentration was measured during the autumn sampling at WWTP 2, while the highest mass-based concentration was measured in the same WWTP but during the summer sampling. On average, the majority of MPs emitted with the effluent were fragments (29 - 96%; average: 69%) followed by fibers (4 - 64%; average: 19%). Beads, films, foams, and granules were also observed (0 - 43%; average: 11%; Figure A1; Table B1). An exception to this trend was the autumn sampling at WWTP 5, where more fibers were emitted, and the summer sampling at WWTP 3, where almost equal parts of granules and fragments were emitted (Figure A1; Table B1). The majority of fibers in treated wastewater were between 1000 - 5000 µm (80% of all observed fibers) while the majority of the other particle types were 300 - 1000 µm based on the long axis (49%; Figure A2). Fourteen different polymer types were identified, as well as suspected paint fragments and tire particles (Figure 3.2). Paint fragments were the most frequent MP type observed in treated wastewater. They seem to originate from the settling tanks which are coated to protect the tanks against corrosion and bacterial action. Paint fragments were included in the MP analysis because paints are often polymer based or contain polymers as binders (Gaylarde *et al.* 2021). All paint particles identified from FTIR

analysis were matched to a polymeric component of paint. This shows that WWTPs can not only act as a pathway for MPs but also as a source. Apart from paint fragments, most of the MPs in the effluent were composed of polyethylene or polypropylene (Figures 3.2 and A3; Table B2). Only a single suspected tire particle was observed across all effluents. There was no statistically significant influence of the treatment type, sampling month, or type of influent on the size, particle type, and polymer composition, suggesting that MP outputs from WWTPs are rather homogeneous (Table A3). As expected, we identified statistically significant differences in terms of size, particle type, and polymer composition ($p < 0.01$ in all cases; Table A3) between the MPs in the influent and effluent water. Almost all polymer types were found in higher abundance in untreated wastewater compared to treated wastewater, except for paint particles which were more prevalent in the effluent.

The MP concentrations in untreated and treated wastewater varied substantially not only across different WWTPs, but also within the same WWTP across the different sampling events (particularly for WWTP 1). A wide concentration range has also been reported in literature with concentrations ranging between 1.5 and 10,044 MPs L^{-1} in the influent and between 0 and 447 MPs L^{-1} in the effluent (Sun *et al.* 2019; Barchiesi *et al.* 2021). It has previously been shown that MP concentrations in wastewater are subject to strong temporal fluctuations and depend on factors such as weather conditions or even the time of the day (Wolff *et al.* 2018; Cao *et al.* 2020; Xia *et al.* 2020). The observed variability between different WWTPs may be further related to other external factors such as the served area, type of sewage collection system, plant treatment capacity (Barchiesi *et al.* 2021). The MP removal efficiency of the WWTPs, calculated based on the particle concentrations in untreated and treated wastewater excluding paint fragments, ranged between 47% and 99%, with a flow-based weighted average of 93%. This is in line with literature reports where efficiencies between 60 and 99.9% have been reported, with lower efficiencies (< 90%) being less frequently observed (e.g., Talvitie *et al.* 2017b; Gündoğdu *et al.* 2018; Conley *et al.* 2019; Akarsu *et al.* 2020; Bayo *et al.* 2020b). The highest removal efficiency was obtained by WWTP 1, which relies on primary and secondary treatments. Although WWTP 2 showed a high removal rate during the summer sampling, the poorest MPs removal was observed at this WWTP during the autumn sampling. As the effluent measured corresponds to an influent that entered the WWTPs many hours before the sampling

time, it could be also assumed that a peak in MP inflow may have occurred during the early morning hours, leading to a higher concentration in the effluent sampled.

We did not observe a significant increase in the removal efficiency by WWTPs applying additional tertiary treatment compared to those that relied solely on primary and secondary treatments (Table A3). Previous studies have shown that secondary treatments can achieve already up to 99% removal (Magnusson & Norén 2014; Simon *et al.* 2018). The evaluation of tertiary treatments such as rapid sand filters, disc filters and membrane bioreactors show a potential to increase removal efficiencies (Michielssen *et al.* 2016; Talvitie *et al.* 2017a, b; Bayo *et al.* 2020a).

In the current study, the overall retention capacity was higher for fibers than for other MP types (Table A4). This has also previously been observed by Lares et al. (2018) and Gündoğdu et al. (2018) and may be related to the increased tendency to form fiber bundles or greater retention in wastewater treatment filtration steps due to their elongated and irregular shape. Yet, this trend is not always consistent. For example, Michielssen et al. (2016) and Conley et al. (2019) found that fragments were more effectively removed during wastewater treatment. The increase in fiber concentrations observed in our study during autumn may be due to increased laundry frequencies or loads during this time period (e.g., whereby use of synthetic fabrics compared to natural fibers increased in this season). The breakdown of textiles during laundry is an important source for fibers: depending on the fabric up to 700,000 fibers can be released from a 6 kg wash load (Napper & Thompson 2016). This may represent an important temporal aspect affecting the nature of MP release by WWTPs in the study area.

The relatively high number of fragments compared to fibers in wastewater in some samples (e.g., during summer sampling of WWTPs 1, 2, and 3) may be partially related to the fact that the WWTPs collected wastewater from combined sewer systems, which aggregate rainwater runoff and wastewater in the same system (Sun *et al.* 2019). More fragments from street cleaning and urban runoff may therefore have entered the WWTPs. Stormwater runoff collected in combined sewer systems – which may reach WWTPs or be discharged directly into water bodies – has been suggested as one of the main pathways for tire and road wear particles into the environment (Kole *et al.* 2017). In this study, however, the percentage of particles that were suspected to

be tire particles (rubbery, elastic, black particles that presented a result indicative of high carbon black content in FTIR analysis) and particles found to be composed of major tire rubbers (e.g., styrene-butadiene copolymer) was relatively low (1.23% and 0.14% in untreated and treated water, respectively). Occurrence of tire fragments in combined WWTP influents is possible during rain periods, hence a continuous supply of these particles is not expected. Another explanation for the limited reports of tire particles in wastewater relates to the inherent analytical challenges associated with the determination of these materials in environmental samples. Tire material is typically characterized by high carbon black content, which is used as a filler ingredient. This leads to a near-complete absorption of the IR beam during FTIR analysis and results in low quality spectra that cannot be accurately interpreted. In addition, tire particles derived from road environments may contain minerogenic material, that can significantly increase the particle density and may, therefore, not be isolated using saturated NaI solutions during sample processing. Finally, tire particle sizes below 100 µm are more frequently expected in the environment (Kreider *et al.* 2010; Järlskog *et al.* 2020), and their size is therefore close to or below the lower size limit used in the current study. This may explain the low occurrence of tire particles observed.

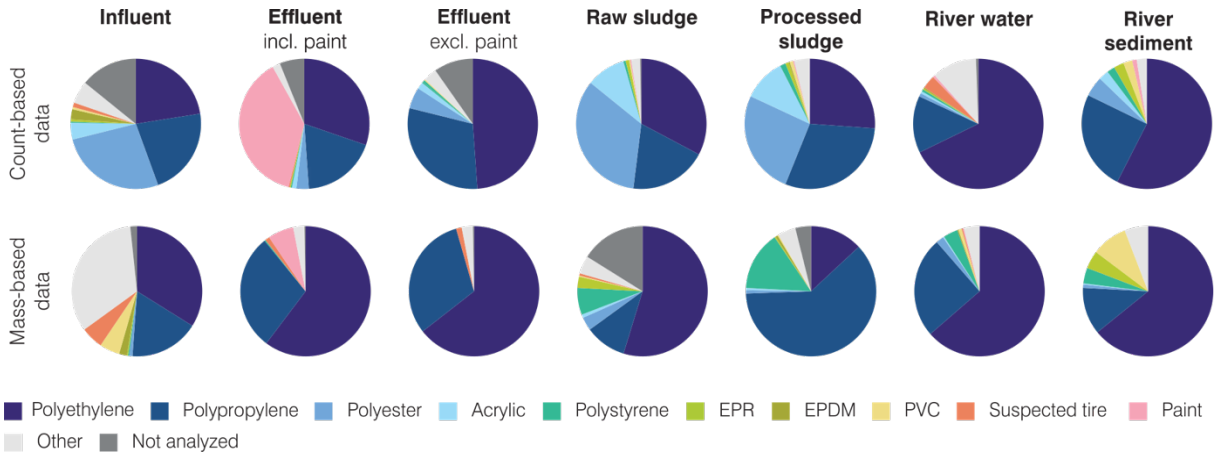


Figure 3.2. Mean count-based and mass-based polymer distribution across all sampling sites and sampling events in different sample types. Acrylic refers only to fibers and does not include acrylic paint. Paint includes paint acrylate and other types of paint fragments matched to a polymeric component of paint. EPDM = Ethylene propylene diene monomer; EPR = Ethylene propylene rubber; PVC = Polyvinyl chloride. Polyethylene terephthalate fragments (included in 'other') and polyester fibers were kept separated to help identify these different particles. Details on the concentration of different polymer types included in the category 'other' can be found in Table B2 and B3.

Table 3.1. Characteristics of the different WWTPs, concentration of MPs in untreated and treated wastewaters and raw and processed sludge and calculated MP emissions. Numbers marked with an asterixis (*) indicate paint fragments observed in the effluent which seem to originate from the painting of the outlet tank and were thereby emitted by the WWTPs themselves and did not enter with the influent. The retention capacity of the WWTPs has been calculated without considering paint fragments. IT = influent type; TS = treatment steps; Se = season; R = retention; D = discharge; U = urban wastewater; I = industrial wastewater; S = secondary; T = tertiary; Su = Summer; Au = Autumn.

WWTP	IT	TS	Se	Untreated Wastewater		Treated wastewater		Raw sludge mean (min-max)		Processed sludge mean (min-max)		R	D	Emitted on sampling day
				MPs m^{-3}	mg m^{-3}	MPs m^{-3}	mg m^{-3}	MPs kg^{-1} dw	mg kg^{-1} dw	MPs kg^{-1} dw	mg kg^{-1} dw	%	m^3 day^{-1}	MPs day^{-1}
1	U/I	S	Su	5050	194	110+10*	8.00*	8213 (7161-10,167)	32.8 (25.1-44.5)	6276 (3878-7876)	92.1 (37.8-196)	97.8	24,675	202,070
			Au	11,550	146	70	1.41	11,954 (9517-14,545)	82.0 (54.8-129)	5640 (5222-6256)	20.6 (18.1-22.9)	99.4	19,989	148,470
2	U	S	Su	2650	179	165	48.5	37,163 (31,086-43,320)	131 (118-153)	18,852 (11,283-23,767)	31.0 (8.20-48.1)	93.8	520	327,030
			Au	1000	23.5	535	6.47	45,043 (37,716-50,167)	349 (209-553)	22,061 (19,949-24,828)	37.6 (24.1-58.1)	46.5	370	1,075,885
3	U/I	T	Su	850	69.2	255	35.2	10,569 (9633-11,123)	54.6 (37.3-73.5)	3304 (2432-4749)	49.2 (5.12-136)	70.0	1837	8,179,635
			Au	3550	95.1	135+10*	0.58*	60,277 (54,941-66,260)	305 (70.9-716)	9038 (8388-9743)	141 (23.1-364)	96.2	2121	5,071,545
4	U	S	Su	1000	18.6	195+65*	3.65+0.28*	30,486 (25,000-40,945)	19.2 (12.7-31.4)	16,600 (12,500-21,348)	46.0 (7.75-99.6)	80.5	1982	101,400
			Au	1400	1.86	450+460*	16.4+4.0*	13,737 (12,550-15,949)	65.7 (52.2-85.1)	3978 (3352-4972)	516 (5.05-1525)	67.9	2011	166,500
5	U/I	T	Su	3350	16.5	45+240*	0.28+0.11*	36,730 (35,185-39,431)	129 (60.0-215)	9487 (7648-11,257)	142 (38.0-312)	98.7	32,077	1,110,375
			Au	2000	4.51	280+600*	5.3+4.4*	37,373 (31,809-43,035)	127 (93.6-159)	7340 (4677-11,300)	59.1 (8.91-129)	86.0	37,567	5,596,920

3.3.2 MPs in sludge

Blanks from the sludge analysis contained mainly viscose fibers and expanded polystyrene fragments. While expanded polystyrene fragments were not present in any of the sludge samples, viscose fibers were present in low numbers. To eliminate the potential contribution from airborne contamination, all viscose fibers were excluded from the data (Table A5).

MPs in the raw sludge ranged from about 8000 to 60,000 MPs kg^{-1} dw, and from about 33 to 350 mg kg^{-1} dw (Table 3.1). In the processed sludge, concentrations ranged between about 3300 and 22,000 MPs kg^{-1} dw, and, in terms of mass, between 21 and 516 mg kg^{-1} dw (Table 3.1). As can be seen from the range of measurements in Table 3.1, MP contamination in sludge is highly heterogeneous. Recent literature shows similarly heterogenous contamination patterns, with concentrations reported based on dry weight ranging from approximately 1500 to 170,000 MPs kg^{-1} dw (Sun *et al.* 2019). High variation occurred not only between different studies but also between different WWTPs within the same study. For example, Mintening et al. (2017) observed between 10,000 and 240,000 MPs kg^{-1} dw sludge across different WWTPs in Germany. Mass-based concentrations are, however, not commonly reported (Koyuncuoğlu & Erden 2021).

While we found that the MP concentrations based on mass are generally comparable between the raw and processed sludge, based on particle counts, the concentration is much higher in raw sludge (Table 3.1). Raw sludge contained a significantly higher number of particles of all sizes but particularly the larger size fractions (> 150 µm; $p = 0.004$; Table A3). Degradation and fragmentation of MPs during sludge digestion and stabilization processes within wastewater treatment (Mahon *et al.* 2017) may explain why the raw sludge contained a higher number of large particles. In both sludge types, the majority of fibers were between 1000 and 5000 µm (55% for raw sludge and 60% for processed sludge), while the majority of all other MPs (based on longest axis) were between 300 and 1000 µm (43 and 40%), and between 150 and 300 µm (40 and 35%) (Figure A2). In both raw and processed sludge, most MPs were fragments (6 - 82% with an average of 52% and 17 - 79% with an average of 56%, respectively) and fibers (16 - 93% with an average of 47% and 19 - 83% with an average of 44%, respectively; Figure A1; Table B1). In general, the

concentration of fibers and fragments was higher in raw sludge ($p = 0.018$). A small number of glitters, granules and beads were found in the processed sludge (1.6%), and glitter, granules and pellets in the raw sludge (> 1%). Polyester and polyethylene were the most frequently recorded polymer types in the raw sludge, while polypropylene, polyester and polyethylene occurred most frequently in the processed sludge (Figure A3; Table B2). In raw sludge, 30 different polymer types, suspected paint fragments and tire particles were identified, while in processed sludge only 20 polymer types were identified. Furthermore, based on abundance, polyethylene and polystyrene tended to be more abundant in raw sludge, while ethylene propylene diene monomer and silicone were more abundant in the processed one ($p = 0.06$). Due to the large heterogeneity of MPs in the samples, no clear pattern was observed whether any polymer types were preferentially emitted with the effluent or retained in sludge.

MPs in sludge can enter terrestrial ecosystems if used as agricultural fertilizer, which is the case for 98% of the sludge produced at the investigated WWTPs. With repeated sewage sludge applications MPs may accumulate over time in soils (Corradini *et al.* 2019; Van Den Berg *et al.* 2020). Edo et al. (2020) estimated that the use of sludge on agricultural soils in the Madrid area could add up to 10×10^{13} MP particles per year. However, they found much higher MPs concentrations in sludge and soil amendments ($133{,}000 \pm 59$ MPs kg^{-1} ww and $101{,}000 \pm 10$ MPs kg^{-1} dw, respectively) compared to the current study, which may be related to the lower size limit of 25 µm that was used. Soil amendments corresponded, in this case, to dry sludge pellets, which undergo thermal drying at 300°C as an additional step after digestion. In the summer months, the treated wastewaters from WWTP 3 are used for the irrigation of a golf camp, which may represent an additional source of MPs for the terrestrial environment.

3.3.3. MPs in river water

Blanks from April 2018 samples showed no presence of MPs, with only cellulose fibers observed (Table A6). Therefore, a negligible background contamination was assumed for all other water samples.

MPs were found in all river water samples. At site 1, the MP concentration in river water was relatively low: up to 3.1 MPs m^{-3} or 0.89 mg m^{-3}. Concentrations were around an order of magnitude higher at site 2 (up to 19.7 MPs m^{-3} or 4.0 mg m^{-3}), and

about two orders of magnitude higher at site 3 (up to 227.0 MPs m^{-3} or 26.9 mg m^{-3}). The highest concentrations were recorded during the spring sampling (Table 3.2). The most frequently observed particle morphologies were fragments (62 - 91%, with an average of 81%), followed by fibers and beads (Figure A1). Granules, films, glitter and foams were also detected (Figure A1; Table B1). According to the existing literature, fragments and fibers are the most frequently observed MPs types in river water, except for a few studies reporting significant contamination from beads or pellets (e.g. Lechner *et al.* 2014; Mani *et al.* 2016; Sarijan *et al.* 2021). Most fibers were 300 - 1000 µm in length (58% of all observed fibers; Figure A2). Smaller fibers below 300 µm were only observed at sampling site 2. The fiber size distribution was slightly smaller than that emitted by WWTPs in the effluent. Other MP types were largely between 55 and 150 µm (41% of all particles) or 150 and 300 µm (41%) in size. Particles > 300 µm were less frequent.

In total, 30 different polymer types, suspected paint fragments and tire particles were identified. Polyethylene was the most common polymer type followed by polypropylene (Figure 3.2; Table B3). Statistically significant differences between sites in terms of MP size ($p = 0.004$), particle type ($p = 0.004$), and polymer composition ($p = 0.012$) were observed (Table A3). Particles of all types and size categories dominated at site 3 over sites 1 and 2. The observed polymer distribution is comparable to other studies assessing MP contaminated river water, which also reported mainly polyethylene and polypropylene (Cera *et al.* 2020; Sarijan *et al.* 2021). Suspected tire particles, as well as styrene-butadiene rubber, were found at sites 2 and 3, at very low concentrations (0.3 and 3%, respectively).

Our study shows that MP pollution varies strongly depending on land-use and increases significantly in areas with anthropogenic activity and wastewater discharges, as observed by other researchers (Estahbanati & Fahrenfeld 2016; Vermaire *et al.* 2017; Wang *et al.* 2017b; Tibbetts *et al.* 2018; Wu *et al.* 2020b). The concentration at site 2 was relatively low, despite the influence of untreated wastewater discharge and agriculture as potential sources.

In addition to spatial variability in MP concentration, we also observed temporal fluctuations. This has been recently described by other studies looking at the spatio-temporal distribution of MPs in rivers (Fan *et al.* 2019; Mintenig *et al.* 2020; Stanton *et*

al. 2020). Temporal variations seem to be strongly related to rain and storm events. For example, the considerable increase in MP concentration at site 3 during the spring sampling period (Table 3.2), may be related to the heavy rain events that occurred prior to the sampling day. High flow velocities have been linked to higher MP concentrations (Watkins *et al.* 2019; Mintenig *et al.* 2020), possibly due to MP re-suspension from the sediment phase (Hurley *et al.* 2018a). Heavy rain and flood events may also result in an increased MP input from non-point sources, along with stormwater (Kataoka *et al.* 2019; Mak *et al.* 2020). In contrast, Fan et al. (2019) and Han et al. (2020) observed lower MP concentrations during the wet season probably due to a dilution effect caused by the precipitation. Therefore, MP river concentration may show low temporal variations under similar weather conditions but strong fluctuations after rainfall events (Cheung *et al.* 2019; Mintenig *et al.* 2020; Xia *et al.* 2020).

Table 3.2. Concentration of MPs in river water and sediment at different sampling sites and sampling events.

		Water		Sediment	
		MPs m^{-3}	**mg m^{-3}**	**MPs kg^{-1} dw mean (min - max)**	**mg kg^{-1} dw mean (min - max)**
Site 1	Summer	1.30	0.54	49.7 (0 - 99.4)	0.32 (0 - 0.91)
	Autumn	1.70	0.89	0	0
	Spring	3.10	0.84	11.1 (0 - 33.3)	0.01 (0 - 0.04)
Site 2	Summer	7.03	4.00	32.9 (0 - 98.7)	0.073 (0 - 2.19)
	Autumn	2.10	1.73	49.6 (0 - 99.9)	0.04 (0 - 0.07)
	Spring	19.7	1.13	132 (0 - 246)	1.91 (0 - 5.53)
Site 3	Summer	147	14.6	2630 (2466 - 2910)	33.6 (22.7 - 44.3)
	Autumn	75.8	9.63	1143 (896 - 1594)	11.4 (4.57 - 22.4)
	Spring	227	26.9	313 (195 - 347)	4.36 (0.60 - 9.16)

3.3.4. MPs in river sediment

MPs observed in the blanks were entirely composed of viscose fibers (Table A7), which were not observed in any of the sediment samples.

The pattern of MP contamination in river sediments corresponded well to that described for the water samples. The lowest MP concentration was found at site 1 (up to 49.7 MPs kg^{-1} dw or 0.32 mg kg^{-1} dw), followed by site 2 (up to 132 MPs kg^{-1} dw or 1.91 mg kg^{-1} dw), with the highest concentration observed at site 3 (up to 2630 MPs

kg^{-1} dw or 33.6 mg kg^{-1} dw sediment; Table 3.2). MPs in sediment consisted only of fragments (33 - 100% with an average of 87%) and fibers (0 - 67%; average: 13%) except for a small number of glitter particles recorded at sampling site 1 in summer (Figure A1; Table B1). In spring, only fragments were present at all sampling sites, suggesting that fibers may be preferentially exported during the high flow events characteristic of this season. Many studies report fragments or fibers as the dominant MPs in freshwater sediments and only in rare occasions other MP types (i.e., pellets, films) have been reported as dominant (Yang *et al.* 2021). In total, 19 different polymer types and paint fragments were identified. The polymer distribution in the sediment compartment was less diverse than in the water phase. Similar to river water, polyethylene was found to dominate in the sediments, followed by polypropylene. These polymer types were observed across all sediment samples (Figure 3.2, Figure A3), despite their low density compared to water. Alterations to particle density due to aging, biofilm formation, and heteroaggregation (Lagarde *et al.* 2016; Nguyen *et al.* 2020; Wu *et al.* 2020a) may have contributed to their sedimentation. The majority of fibers were between 1000 and 5000 µm (63%), and most fragments, including glitter particles, were between 300 and 1000 µm (50%). The fraction of larger MPs was higher compared to the MPs in the water column, where most MP particles were smaller than 300 µm (Figure A2).

In addition to inter-site variability, seasonality also influenced sediment MP concentrations. Yet, in contrast to the water concentrations which were highest in spring, the sediment concentrations at site 3 were instead at their lowest in spring. This supports the assumption that MPs are resuspended from channel bed sediments during high flow and that sediments represent dynamic compartments, serving as temporary sinks or sources of MPs to the water column (Nizzetto *et al.* 2016a; Hurley *et al.* 2018a; Ockelford *et al.* 2020). It should be taken into account that sediment samples were taken from sandy and clayey areas from the sides of the riverbed, where lower flow conditions occurred compared to the center of the riverbed, which was typically made up of gravel and rocks. This may influence MP settling and accumulation, as both flow rate and grain size have been demonstrated to influence MPs concentration in riverbeds (Enders *et al.* 2019; Ockelford *et al.* 2020). Further research should investigate the spatial and temporal aspects of this trend and elucidate the role of river sediments throughout the annual regime of a river.

3.3.5. Wastewater contribution to river contamination

We estimate that about 1.0 x 10^{10} MPs (or 389 kg), from which 1.5 x 10^{9} MPs (or 6 kg) are fibers, enter the Henares River and its tributaries via wastewater annually (Table 3.3). This estimation is based upon the amount of treated and untreated wastewater being discharged to surface waters in the catchment, and the measured MP concentrations in the influent and effluent of the monitored WWTPs. Furthermore, based on the measurements taken at the mouth of the catchment (site 3), we estimate an annual MP export of approximately 4.1 x 10^{10} MPs (2.1 x 10^{10} - 6.2 x 10^{10} MPs) or about 4000 kg (2646 - 5331 kg), which is discharged into the Tagus River system. Fibers represent 3.3 x 10^{8} - 1.4 x 10^{9} MPs (0.23 - 175 kg), while the remaining 1.9 x 10^{10} - 6.2 x 10^{10} (2472 - 7379 kg) are other MP types, mainly fragments. This was calculated based on the discharge for the Henares catchment for the period from May 2017 to April 2018 (2.75 x 10^{8} m^3; Table A8) (CEDEX 2021). These calculations indicate that wastewater releases contribute approximately 15 to 50% of the total river MP contamination based on particle counts, but only about 7 to 15% based on particle mass. The sediment concentrations were not included in this calculation, which would reduce the estimated contribution from wastewater as a source of MP to the Henares River. Yet, potential sewage overflow due to heavy rain events was also not captured in this estimate, which may represent significant pulses of MP to the river.

The total amount of wastewater entering the catchment was estimated to be 5.2 x 10^{7} m^3 per year, of which 7.6 x 10^{5} m^3 was untreated. Wastewater, therefore, contributed approximately 1/5 of the total streamflow. The river discharge in our sampling period (May 2017 - April 2018) was comparable to the annual average of the past 20 years (2.66 x 10^{8} m^3). However, the annual discharge in 2017 was only half of this (1.36 x 10^{8} m^3) due to a dry Spring (Table A8). Wastewater may have therefore contributed a greater proportion in 2017 than in other years. Whilst this does not affect the total MP export, decreased streamflow may result in a lower dilution of MPs and therefore higher risk of exposure for aquatic organisms. Discharging untreated wastewater during low flow conditions may also influence the partitioning of MPs between the sediment and water phase, potentially creating hotspots of MP contamination (Woodward *et al.* 2021). Modeling of fluvial response and impacts related to climate change suggest a significant reduction in inputs (i.e., from precipitation and runoff) and greater interannual variability characterized by increased

drought risk for arid and semi-arid regions in Spain (Estrela *et al.* 2012). This could lead to the conditions of 2017 becoming the norm for the Henares River catchment, which would translate to an increased potential risk from MP exposure for aquatic organisms, which will already be under pressure from the other factors related to this environmental shift. This highlights the importance of local climate conditions on the potential significance of MP releases.

Table 3.3. Estimated amount of MPs (in particle counts and mass) entering the Henares River catchment by wastewater.

	Amount of wastewater produced in the catchment in 2017 in m^3	**Concentration of MPs in wastewater**		**Emitted into the river per year**	
		MPs m^{-3}	mg m^{-3}	Number	kg
Treated urban and industrial wastewater	49,225,286	161[a]	6.47[a]	7.9×10^9	318.7
Untreated urban wastewater	765,752	2439[b]	76[b]	1.9×10^9	58.2
Treated industrial wastewater	2,276,415	123[c]	5.5[c]	2.8×10^8	12.5
Total amount	52,267,453			1.0×10^{10}	389.2

[a] *based on a flow based weighted average in the sampled effluent*

[b] *based on a flow based weighted average in the sampled influent*

[c] *flow based weighted average of sampling WWTPs 1, 3 and 5 were used as an estimation as they were receiving in addition to urban also industrial wastewater, although the MP composition in industrial wastewater is expected to vary greatly based on the industrial sector.*

Wastewater largely represents a pathway for MPs from other sources, which converge and are concentrated within wastewater systems and as a result of wastewater treatment processes. Thus, the majority of MPs do not originate from the wastewater system itself. Improving treatment steps to retain more MP and assessing the processing and reuse of sludge represent potential mechanisms to reduce the environmental release of MP, but the original sources of MP to wastewater should also be addressed to make meaningful and long-term reductions in environmental contamination. The MP contamination recorded here was composed of a diverse suite of different particle morphologies and polymer compositions. Yet, insights from the contribution of potential sources emerged from the dataset. For example, a proportion of the polypropylene particles observed in this study shared a visual resemblance.

These particles were all green, approximately the same thickness, and are present as rectangular pieces that appear to fragment along their length. Figure 3.3 presents examples of these particles. One possible source could be artificial turf, which is often composed of green polypropylene shards (e.g., Morehouse 1992; Ekstrand *et al.* 2006; Watterson 2017). These particles were observed in all sample types investigated in this study, demonstrating that this source is an input to wastewater treatment systems. Some are transferred to the sludge, but a proportion is not captured by wastewater treatment processes and are discharged with WWTP effluents. The occurrence of these particles in river water and sediment may relate to wastewater releases, or other release pathways connecting the original source to the river channel, such as surface water runoff. Further work should refine such source apportionment exercise and unfold different transport pathways of MPs to river systems to help inform policy and mitigation strategies.

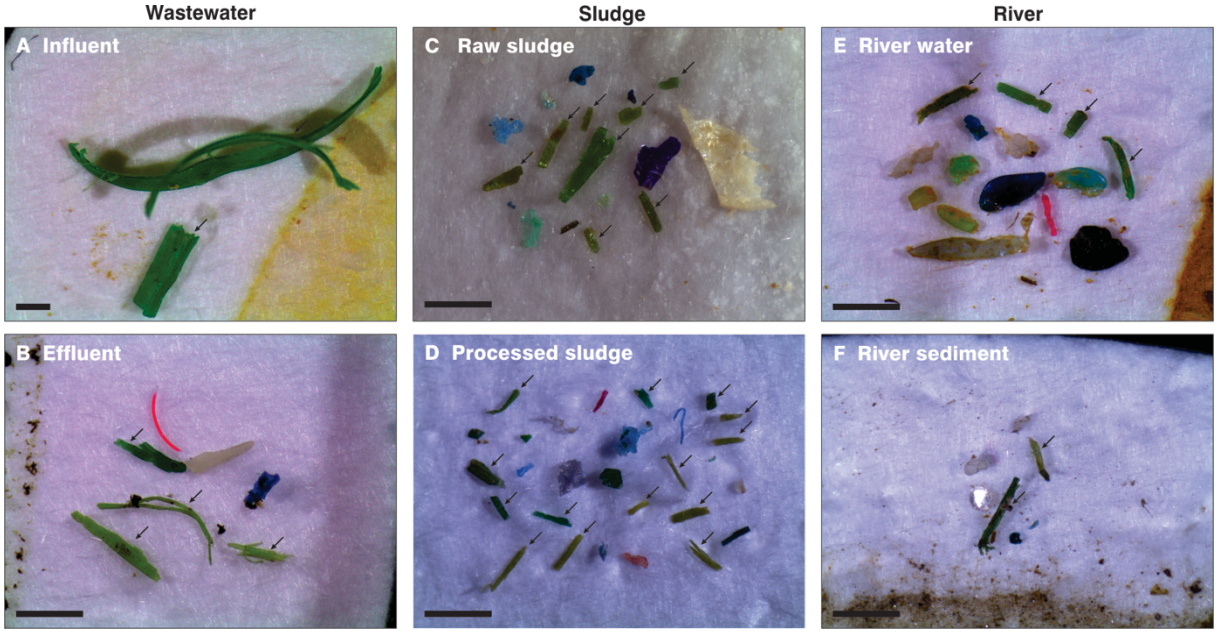


Figure 3.3. Examples of green polypropylene fragments that share a visual resemblance, observed in all different sample types. The relevant particles are indicated with an arrow. The black scale bar represents 1000 µm in each image.

The results of this study indicate that although wastewater is an important pathway of MPs to surface water contamination (explaining up to 50% of the total riverine load of MPs), other sources to river water and sediment are also relevant. Rivers are highly dynamic environments with a multitude of potential inputs, thus these sources are difficult to detect and quantify. For example, although we found a significantly higher MP concentration downstream of the WWTPs, this sampling point

was also influenced by urban and industrial areas. These environments are also known to represent sources of MP contamination to rivers (Kataoka *et al.* 2019). This explains the discrepancies in the assemblage of MP observed in the river samples versus the wastewater samples (Figures A1 - A3). Urban and agricultural runoff may constitute additional sources, although rain events, strong enough to produce water runoff, occur only rarely in the study area. Atmospheric fallout may be of lower importance as it has been shown to be made up mainly of fibers (Cai *et al.* 2017; Stanton *et al.* 2020), and therefore it is less likely to be the source of MP fragments. During the sampling events, macroplastics were observed at the river shores and directly in the water close to sampling site 3 (Figure A4). Those plastics have possibly served as a source for MP fragments, which were the main MP type observed in river water and sediment. The contribution of each of these different sources to the total MP loads in the Henares River catchment will be evaluated in a follow-up study using the INCA model described by Nizzetto et al. (2016b).

3.3.6. Analytical challenges and sample harmonization

The results reported for wastewater and sludge are characterized by a discrepancy between count and mass-based concentrations. This was largely governed by the high proportion of fibers, which typically contribute less to the total mass based on their low particle volume compared to other particle types. MP counts and mass concentrations in river water and sediment − which mostly contained fragments − were in better agreement. Discrepancies between count and mass-based concentrations have been observed previously (Kataoka *et al.* 2019; Constant *et al.* 2020). This disparity has the potential to result in different trends being described for MP contamination in the environment. Mass represents a more robust metric for the comparison of total plastic loads amongst studies, due to the fact that MPs may fragment during MP sample processing and analysis (Simon *et al.* 2018). On the other hand, the possibility to report particle counts and describe individual particle characteristics such as size, shape, and polymer composition, is more important for environmental risk assessment. These factors determine the ability of organisms to ingest MPs and are likely to influence the associated risk (e.g., Carbery *et al.* 2018). Currently available methods for assessing these variables are typically costly and time intensive. Additional work is required to produce mass-based estimates for these particles. This is in contrast to methods that provide outputs as mass-based

quantification, such as pyrolysis gas- chromatography mass-spectrometry, which may be more time and cost efficient but does not assess factors such as particle size or shape (Hendrickson *et al.* 2018; Mintenig *et al.* 2018). In addition, pyrolysis-based mass-spectrometry methods require that problems of sample homogenization and representativeness are first solved, as a single particle may already saturate the detector. Thus, the analytical method used here represents a valuable synthesis to produce datasets useful for different scopes. Aligning datasets that report count or mass-based concentrations across differing size ranges is important. Kooi and Koelmans (2019) suggested using continuous probability distributions to allow a comparison between studies using mass and count-based data and to fill in the gaps on small-sized MPs assessment, which are below the sampled threshold. Further work to harmonize this process and test its application should be undertaken.

3.4. Conclusions

This study represents one of the first investigations assessing the role of wastewater as a release pathway for MP contamination at the river catchment scale under semi-arid conditions. Moreover, it describes the spatio-temporal variability of MP exposure over a gradient of anthropogenic pressures. We show that WWTPs, despite retaining a large number of MPs, provide (together with minor untreated wastewater discharges) about 15 - 50% of the MPs river catchment discharge. We did not observe any influence of the different wastewater treatment types on the MP removal efficiency. MP river concentrations varied strongly based on land-use, increasing significantly downstream of urban and industrial areas. Seasonality influenced the observed MP concentrations, with increasing water concentrations during spring. To identify and quantify different sources and better understand resulting contamination patterns in environmental settings, high resolution temporal and spatial sampling may be necessary. This may also help to detect and quantify as yet less well-constrained MP sources to river catchments, including sources that are both spatially and temporally variable. Potential additional sources that warrant further attention include the occurrence of macroplastic contamination in the catchment and their potential to form MPs, and the fate of MPs after applied with sewage sludge to agricultural fields, which may reach aquatic systems via surface water runoff.

Acknowledgements

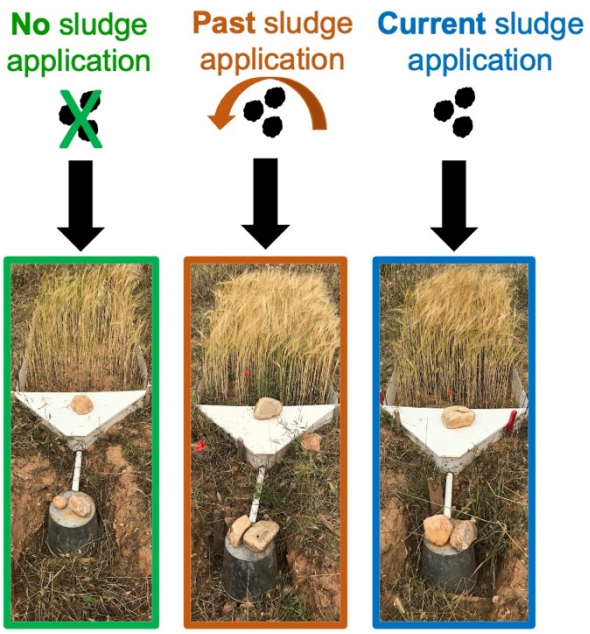
The study has been conducted as part of the EU JPI-Water initiative IMPASSE project (Impacts of MicroPlastics in AgroSystems and Stream Environments) supported by the spanish Ministry of Economy, Industry and Competitiveness (grant number: PCIN- 2017-016) and the Norwegian Research Council (grant number: 271825/E50). A. Rico was supported by a Ramon y Cajal grant provided by the Spanish Ministry of Science and Innovation (RYC2019-028132-I). The authors thank Nina Buenaventura for assistance in the laboratory work.

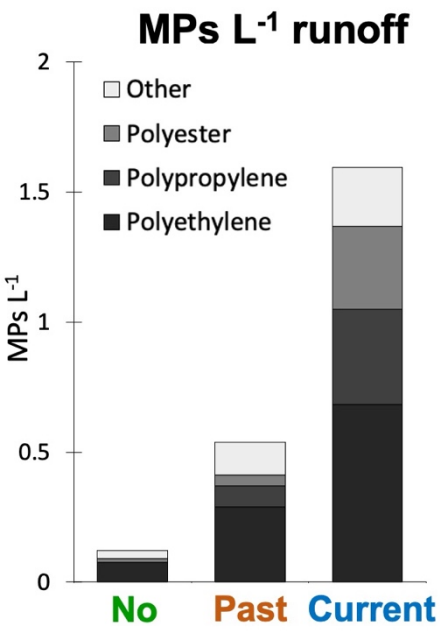
Supporting Information

The Supporting Information for this Chapter can be downloaded at: https://doi.org/10.1016/j.jhazmat.2021.126481

CHAPTER 4

Fate of Microplastics in Agricultural Soils Amended with Sewage Sludge: Is Surface Water Runoff a Relevant Environmental Pathway?





This Chapter has been published as:

Schell T, Hurley R, Buenaventura NT, Mauri PV, Nizzetto L, Rico A, Vighi M. 2022. Fate of microplastics in agricultural soils amended with sewage sludge: Is surface water runoff a relevant environmental pathway? Environmental Pollution 293: 118520. https://doi.org/10.1016/j.envpol.2021.118520

Abstract

Sewage sludge used as agricultural fertilizer has been identified as an important source of microplastics (MPs) to the environment. However, the fate of MPs added to agricultural soils is largely unknown. This study investigated the fate of MPs in agricultural soils amended with sewage sludge and the role of surface water runoff as a mechanism driving their transfer to aquatic ecosystems. This was assessed using three experimental plots located in a semi-arid area of Central Spain, which were planted with barley. The experimental plots received the following treatments: (1) control or no sludge application; (2) historical sludge application, five years prior to the experiment; and (3) sludge application at the beginning of the experiment. MPs were analyzed in surface water runoff and in different soil layers to investigate transport and infiltration for one year. The sewage sludge used in our experiment contained 5972 - 7771 MPs kg^{-1} dw. Based on this, we estimated that about 16,000 MPs were added to the agricultural plot amended with sludge. As expected, the sludge application significantly increased the MP concentration in soils. The control plot contained low MP concentrations (31 - 120 MPs kg^{-1} dw), potentially originating from atmospheric deposition. The plot treated five years prior to the experiment contained 226 - 412 and 177 - 235 MPs kg^{-1} dw at the start and end of the experiment, respectively; while the recently treated plot contained 182 - 231 and 138 - 288 MPs kg^{-1} dw. Our study shows that MP concentrations remain relatively constant in agricultural soils and that the MP infiltration capacity is very low. Surface water runoff had a negligible influence on the export of MPs from agricultural soils, mobilizing only 0.2 - 0.4% of the MPs added with sludge. We conclude that, in semi-arid regions, agricultural soils can be considered as long-term accumulators of MPs.

4.1. Introduction

Wastewater treatment plants (WWTPs) are important pathways for microplastics (MPs) into the environment (Nizzetto *et al.* 2016c; Ziajahromi *et al.* 2016). MPs are efficiently retained during wastewater treatment, mainly during mechanical treatment and sludge settling processes (Murphy *et al.* 2016; Leslie *et al.* 2017). It has been estimated that about 60 to 99% of MPs in WWTP inflow waters are ultimately transferred into sewage sludge, in which MP concentrations from around 1000 to 186,700 MPs kg^{-1} dw have been reported (Gao *et al.* 2020). As sludge is rich in organic matter and nutrients, it is commonly used as an agricultural soil conditioner or fertilizer and has been shown to enhance soil properties by increasing the microbial activity and improving carbon and nitrogen mineralization processes, as well as some enzymatic functions (Roig *et al.* 2012). Around 35% of the sludge produced in Europe is applied on land (Table S1). In some countries, such as Spain, about 80% of the sludge produced by WWTPs is applied to agricultural soils, thus constituting a major environmental pathway for MPs (Rolsky *et al.* 2020). The relevance of sludge as a MP input source into soil ecosystems has been investigated recently (Corradini *et al.* 2019; Crossman *et al.* 2020; Van Den Berg *et al.* 2020; Zhang *et al.* 2020). These studies show that sludge applications increase the MP content in soils and that successive sludge applications can lead to considerable MP accumulation. Other sources of plastic pollution into agricultural soils include compost, plastic mulching, wastewater irrigation, road runoff, atmospheric deposition, and littering (Bläsing & Amelung 2018; Piehl *et al.* 2018; Corradini *et al.* 2019; Huang *et al.* 2020; Zhang *et al.* 2020). Hence, also soils never treated with sludge can become contaminated by MPs.

To date, there is a paucity of information on the fate of MPs in agricultural soils. MP fate may depend on the polymer type, size, and morphology of the MPs. Smaller MPs, for instance, may be more readily transported into deeper soil layers (Liu *et al.* 2018; O'Connor *et al.* 2019). The fate of MPs in agricultural soils may also depend on the environmental conditions, soil characteristics and agricultural management (Zhang *et al.* 2020; Kim *et al.* 2021b). Due to soil tillage and bioturbation, MPs may be translocated into deeper soil layers (Huerta Lwanga *et al.* 2017a; Maaß *et al.* 2017; Rillig *et al.* 2017a; Zhang *et al.* 2020; Kim *et al.* 2021b), which can hinder photoinduced degradation and disintegration processes (De Souza Machado *et al.* 2018), thus increasing their persistence. For example, synthetic fibers have been shown to remain

for more than 15 years in sludge-amended soils (Zubris & Richards 2005). While there are several studies indicating that MPs are relatively persistent in soils, other studies point at environmental processes that contribute to their transport and dissipation, such as water runoff (Crossman *et al.* 2020; Kim *et al.* 2021b). However, the efficiency of vertical and lateral water flows in mobilizing MPs from soil to freshwater ecosystems has not been studied empirically thus far (Schell *et al.* 2020). Obtaining this information is especially relevant to assess the environmental risks of MPs and to support appropriate sludge management.

Therefore, this study aimed to assess the fate of MPs applied with sewage sludge to agricultural soils and their transport to surface water ecosystems using experimental plots. We assessed to what degree MPs are retained by agricultural soils, their infiltration capacity into deeper soil horizons, and their capacity to be mobilized from agricultural soils due to transport via surface water runoff. Furthermore, this study provides first estimates of MP loads emitted from agricultural soils into freshwater ecosystems and discusses potential implications for sustainable sludge applications in European agriculture.

4.2. Material and Methods

4.2.1. Experimental set-up

This study was carried out at the Madrid Institute for Rural, Agricultural and Food Research and Development (IMIDRA), which is located near Madrid (Central Spain). The area is characterized by a semi-arid climate, with an average yearly rainfall of 430 mm (data 1957 - 2000), mainly concentrated during spring and autumn. The experiment was set up on an agricultural field without historical sewage sludge application, which was assured by records of the experimental farm. Three agricultural plots ($2m^2$; Figure S1) with comparable soil characteristics, composition (Table S2), and a slope of 5% (facing south-east) were prepared. Each plot received a different treatment. Plot 1 served as a control (no sludge application). Plot 2 was prepared with soil from an adjacent field (located approximately 150 m north-west of the test plots) to which sludge (5 kg m^{-2} ww) was applied only in 2013 (i.e., five years prior to the start of the experiment). To prepare this plot, the upper 15 to 20 cm of sludge treated soil were removed using an excavator. During this process, the original soil structure was impaired; however, the transportation of soil to the experimental plot was required to

allow planting the same crop and to ensure the same environmental conditions. Plot 3 was treated with sewage sludge immediately prior to the start of the experiment. The sludge was applied following standard agricultural practices in the region, using a dose of 5 kg m^{-2} ww (1.1 kg m^{-2} dw). After spreading it over the agricultural plot surface on October 30th, 2017, it was left to dry for two days. The sludge was then manually incorporated into the soil using a hoe to a depth of approximately 10 cm. All plots were sown with barley (Hordeum vulgare, 350 seeds m^{-2}) on November 24, 2017. The barley was harvested on July 4, 2018. Subsequently, the soil was manually plowed, mimicking regular agricultural procedures for soil aeration and preparation for the next crop. The experiment was terminated on October 14, 2018. Details on the source and handling of the sludge used in Plot 3 are outlined in the Supporting Information (SI).

4.2.2. Soil sampling

The migration of MPs into deeper soil horizons was assessed by taking samples in all plots at the start and the end of the experimental period, producing soil samples for three different soil horizons: 0 - 5, 5 - 10, and 10 - 15 cm. The soil below 15 cm was very compacted, which hampered the sampling of deeper soil layers. At the start of the experiment, 5 samples of each soil horizon were taken with a stainless-steel core sampler (Ø 4 cm) with minimal disturbance of the experimental plots. Each sample contained about 50 - 100 g of soil. Since the soil samples taken at the start of the experiment showed a very heterogeneous MP distribution, the sample volume was increased for the sampling at the end of the experiment. Five samples of each soil horizon were taken with a metal shovel, which contained approximately 500 g each. Soil samples were transferred into glass jars and stored at room temperature until further analysis.

4.2.3. Runoff sampling

Surface water runoff was collected from the experimental plots during the 12 months of the experiment using runoff collectors modified after Pinson et al. (2004). The collectors were made entirely of metal. The runoff was collected through a metal pipe into a metal bucket (Figure S1), which was emptied following each rainfall event that generated runoff. The buckets used for runoff water collection were kept covered to avoid air contamination. Runoff volume from the plots was measured, preconcentrated by filtering it through a 20 µm plankton net and transferred into glass

bottles. The buckets and nets were carefully rinsed with filtered (0.22 µm) Milli-Q water, which was also transferred into the bottles.

Runoff samples collected from the three plots after the first rain event were unfortunately lost during shipment to the NIVA MP laboratory (Norway). This unfortunate event was considered to be of critical importance for the results of the study since, in Plot 3, sludge was added recently, and MPs were most likely present on the soil surface where erosion would be maximal. For this reason, this part of the runoff experiment was repeated (for further details, see SI). During two rain events in March 2018 and one in October 2018, runoff unexpectedly exceeded the capacity of some of the collecting buckets. The overflow volume was monitored, and data were corrected accordingly.

4.2.4. MP analysis

4.2.4.1. MP extraction

Surface water runoff samples contained both water and some soil material. The overlying water was carefully decanted, vacuum filtered onto Whatman GF/A (Ø 47 mm) filter papers and retained for analysis. The soil material was subjected to two freshwater density extractions (filtered (0.22 µm) RO water; $\rho = 1.0$ g cm^{-3}) followed by two saturated NaI solution (ρ => 1.75 g cm^{-3}) extractions to isolate higher density plastic particles. The overlying liquid of each extraction step was filtered onto a new filter paper and retained for visual analysis and chemical characterization (see below).

Soil and sludge samples were dried at 50°C for 48 hours and homogenized using a stainless-steel sample splitter (Haver RT; Haver & Boecker, Germany). Three 75 g subsamples were taken from each soil sample, with the exception of the control sample at 10 - 15 cm and the historical application at 10 - 15 cm for which a lower sub-sample amount was used, as the collected sample volume was not sufficient (Table S3). MPs were isolated from the soil using two density separation extractions using saturated NaI solution (ρ => 1.75 g cm^{-3}). Soil samples had a high organic content and required treatment with 30% (v/v) H_2O_2. After organic matter removal, the samples were filtered onto Whatman GF/A (Ø 47 mm) filters and retained for analysis.

For the sewage sludge, three 10 g subsamples were processed. The organic matter content was reduced by treating the samples with Fenton's reagent, followed by two freshwater density extractions (ρ = 1.0 g cm^{-3}). Following this, two high density extractions were performed using a saturated NaI solution (ρ => 1.75 g cm^{-3}). Each density extract was filtered onto a separate Whatman GF/A filter paper. Full details of the MP extraction procedure for all sample types are outlined in the SI.

4.2.4.2. Quality assurance/quality control (QA/QC)

All sample processing was performed in the NIVA MP Laboratory, which is a positive pressure room with HEPA-filtered (class H13) air input. Several contamination reduction procedures are in place, including the use of natural fiber clothing and lab coats, removal of loose fibers using a lint roller upon entry to the laboratory, and regular removal of dust from all areas of the laboratory. All steps during which samples were exposed to the laboratory environment (e.g., during weighing or sample homogenization) were undertaken in a laminar flow cabinet inside the MP Laboratory, to further reduce the potential for contamination. Samples were kept covered at all other stages of processing. All laboratory water or solutions used in the sample processing were pre-filtered (0.22 µm for RO water, 1.2 µm for NaI and H_2O_2) prior to use. All containers were rinsed with filtered RO water three times before use, to remove any potential contamination.

A total of three blanks were included for each batch of samples that were processed. These were combined procedural, container, and solution blanks, that were treated to an identical sample processing procedure within each sample batch. All suspected MPs found in the blanks were treated to the same visual and chemical characterization as in the environmental samples. The method used in this study has previously been validated using spiked samples for a range of MP particle types in Hurley et al. (2018b) and Crossman et al. (2020).

4.2.4.3. Visual analysis

All filter papers were first visually analyzed for MP particles following Lusher et al. (2020). Based on the inclusion of this visual analysis step, a lower size limit of detection of 50 µm was set for all the samples. Each filter was traversed at 20 - 50 x magnification using a Nikon SMZ 745T stereomicroscope. All suspected MPs

were photographed using an Infinity 1 camera and their long and short dimension were measured using the Infinity Analyze (v.6.5.4) software package, following calibration using a measurement standard. In addition, the depth axis of each particle was estimated to the nearest 25 µm. The particles were classified by size and shape (bead, fiber, film, fragment, glitter, granule). Beads were defined as spherical particles; fibers as textile fibers/threads with an elongated shape and cylindrical form indicating extrusion; films as particles with a thickness of 10 µm or below; fragments as irregularly shaped particles that derive from the breakdown of larger plastic items; glitter as particles that have a reflective layer embedded within plastic with a shape that is hexagonal or formerly hexagonal (in the case of broken glitter pieces); and granules as semi-rounded particles with similar depth and width dimensions. Additionally, a small number of fiber bundles that could not be untangled were recorded as such.

4.2.4.4. Chemical characterization

All suspected MP particles were further chemically characterized to confirm their plastic composition. Large MPs (> 300 µm; excluding fibers) were analyzed using an Agilent Cary 630 ATR-FTIR equipped with a diamond crystal. Small MPs and all fibers (50 - 300 µm) were analyzed using a Perkin Elmer Spotlight 400 µFTIR in transmission mode. Each spectrum was compared to a series of commercial (PerkinElmer Polymer library, Agilent Polymer library), open source (Primpke *et al.* 2018), and in-house libraries and were manually verified to confirm the polymer type. In the soil samples, 6% of the particles were lost after visual inspection prior to chemical characterization, while transferring them to the diamond compression cell. Across the remaining particles, 85% of all suspected MP particles were confirmed by FTIR to be plastic and 15% as non-plastic particles. Therefore, 15% of the lost particles were randomly excluded as non-plastic particles. The datasets presented here represent the MP component only. Full details of the chemical characterization are outlined in the SI.

4.2.5. Particle mass estimation

Following physical and chemical characterization of MP particles, the mass of each particle was estimated. This utilized the three analyzed axes related to the size of the particle to provide an estimate of particle volume. The calculation used to assess volume differed for different morphologies: fibers were treated as cylinders; fragments, films, glitter and granules were treated as cuboids; beads were treated as spheres;

and fiber bundles were treated as cuboids and then divided to reflect the estimated percentage of the cuboid that was taken up by fiber versus empty space. Particle volume was converted to mass using the density of the polymer of each particle, as identified by FTIR analysis. Polymer density was established from a literature search, where the most commonly reported densities for each polymer were used (Table S4). For particles lost prior to FTIR analysis a density of 1 g cm^{-3} was assumed.

4.2.6. Statistical analyses

To test significant differences between soil MP concentrations at the start and end of the experiment, t-tests were applied. All soil data were checked for normality using Shapiro-Wilk's tests. To assess statistical differences between soil treatments, analysis of variance (ANOVA) was performed, followed by Tukey tests. Data not meeting requirements for parametric testing were tested using Kruskal-Wallis tests. All analyses were done using R version 4.0.3 (R Core Team, 2020). Statistically significant differences were assumed when the calculated p-value was < 0.05.

4.3. Results and Discussion

4.3.1. MPs in sludge

Blanks from the sludge analysis (n = 3) contained only non-plastic particles (Table S5). The MP analysis of the sludge samples shows that the count-based concentration of MPs > 50 µm in sludge was around 7000 MPs kg^{-1} dw (Table 4.1). Only a small numerical fraction (< 3%) of plastics was > 5000 µm in their longest dimension, which comprised almost 25% of the mass (Table S6). MPs in the sludge consisted of fragments (48 - 56%), of which most were < 500 µm, and fibers (44 - 52%), of which most were > 1000 µm (Figure S2; Table S7). No other shapes were observed.

Table 4.1. MPs in sludge applied to the runoff plot treated with sludge at the beginning of the experiment (n = 3). Plastic particles > 5000 µm that were observed were between 5000 and 6000 µm with one exception that was 11,750 µm.

	Mean particle count in kg^{-1} dw (min - max)	**Mean estimated particle mass in mg kg^{-1} dw (min - max)**
Including plastics > 5000 µm	7266 (6351 - 7956)	74.4 (53.6 - 115.4)
MPs 50 - 5000 µm	7078 (5972 - 7771)	56.1 (52.1 - 60.6)

Fifteen different polymer types were identified in addition to a type of paint for which the specific polymer type could not be further determined. Fragments consisted mainly of polyethylene, followed by polypropylene, whilst fibers were mostly composed of polyester, followed by polypropylene and acrylic (Figure S3; Table S8).

4.3.2. MPs in soil

Blanks from the soil analysis (n = 18) contained mainly non-plastic particles and three MP particles (Table S9), hence the background contamination was judged to be negligible, and no data corrections were made. The control soil (Plot 1) contained on average 57 (31 - 84) MPs kg^{-1} at the start and 99 (79 - 120) MPs kg^{-1} at the end of the experiment. The historically treated plot (Plot 2) contained 330 (226 - 412) and 204 (177 - 235) MPs kg^{-1} and the recently treated plot (Plot 3) contained 215 (182 - 231) and 211 (138 - 288) MPs kg^{-1}, at the start and the end of the experiment, respectively (Figure 4.1A). These concentrations refer to MPs < 5000 µm. Concentrations including plastic particles up to 12800 µm (maximum length measured) are shown in Table S10. At the start of the experiment, the concentrations were significantly higher in the plot that received the historical application ($p = 0.003$) and the plot that received the recent application ($p = 0.04$), compared to the control. No statistically significant differences were observed between the historical and recent application ($p = 0.12$). At the start of the experiment, the total MP concentrations were not significantly different from the concentrations at the end of the experiment in any of the investigated plots, indicating losses resulting from runoff or other processes were likely small and that MP concentrations in soil are relatively stable over time. This is further supported by the similar concentrations measured in the historical and recently treated plot. In the control plot, the concentration slightly increased at the end of the experiment, but this could be an effect of the larger soil volume taken in the last sampling.

In general, fibers were by far the most commonly observed MP type (44 - 91%) in soil, followed by fragments (4 - 44%; Figure S4; Table S11 and S12). Most fibers were > 1000 µm in size, with exception of fibers in the upper layers (0 - 5 and 5 - 10 cm) of the historically treated plot at the start of the experiment, where a higher proportion of fibers < 1000 µm was observed. Most other MP types were < 500 µm, with almost no particles > 1000 µm (Figure S2). A small fraction of particles > 5000 µm were observed (Table S13 and S14). The presence of fibers in soil has previously been

related to sewage sludge application (Habib *et al.* 1998; Zubris & Richards 2005; Corradini *et al.* 2019). However, the current study shows that soil without historical sludge application can also contain MP fibers, which may originate from atmospheric deposition (Cai *et al.* 2017; Allen *et al.* 2019). The most common polymer types in the soil samples were polyester (22 - 53%) and acrylic (22 - 37%; Figure 4.1B; Table S15 and S16). A higher polymer diversity was observed in the soils amended with sludge compared to the control. At the start of the experiment 12 different polymer types were observed in total, and at the end of the experiment 20 were detected. Higher sample volumes taken at the end of the experiment may be more representative of the polymer diversity and less affected by the heterogeneous distribution of MPs observed in the soil.

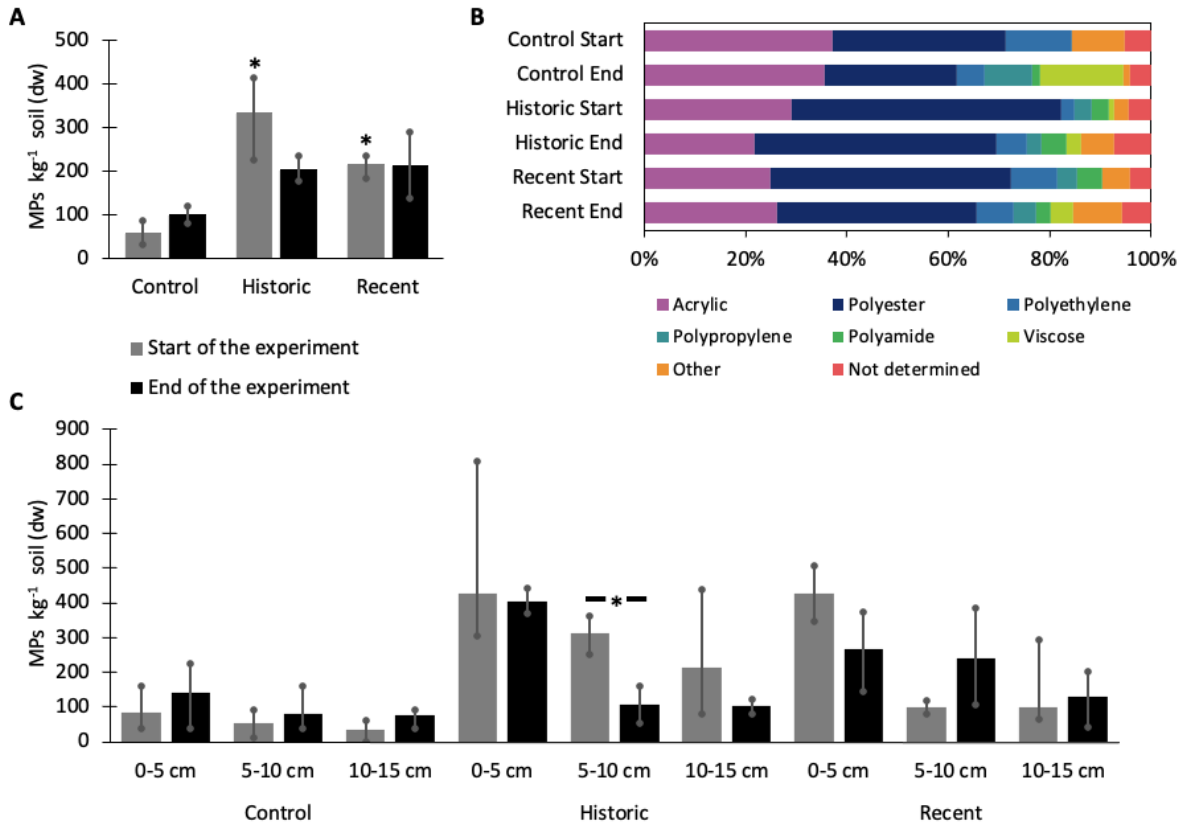


Figure 4.1. MPs detected in soils, subjected to different sludge treatments, at the start and the end of the experiment reported by A) mean, min and max (error bars) concentration (MP kg^{-1}) per experimental plot; B) polymer type observed at the start of the experiment (Start) and the end of the experiment (End); and C) mean, min and max (error bars) concentration (MP kg^{-1}) according to different soil sample depth (0 - 5 cm; 5 - 10 cm and 10 - 15 cm). Statistically significant differences compared to the control are indicated by asterisks (*), while significant differences between the start and the end of the experiment are indicated by asterisks in between dashes (-*-).

Regarding the vertical distribution in soil, MPs were found to be more abundant in the uppermost layer in all plots, both at the start and end of the experiment (Figure 4.1; Table S10). In the plot receiving the recent application, a trend of MP infiltration towards lower soil layers was observed, with a slight increase in the number of MP particles at the end of the experiment in the intermediate and bottom layers (Figure 4.1C). No clear influence of MP properties (i.e., size, shape, and polymer composition) on their vertical distribution in soil was observed. Soil properties likely impact the integration of MPs into soils and their lateral and vertical transport through them. The analyzed soils were fine textured with a high silt content, and low organic matter content (Table S2). Coarse textured soil with larger pore spaces may allow for a higher infiltration compared to the soils analyzed here. Crossman et al. (2020) − who monitored MP concentrations in soils with and without biosolid application in Ontario, Canada − observed that in a field with lower soil density, MP abundance increased over time at deeper layers (10 - 15 cm) and MPs were better retained in this soil during heavy rainfall events, compared to MPs in soils with higher densities. Additionally, the MP size may affect the vertical movement. It has been shown using soil column experiments that smaller MPs have a greater movement potential, and that the migration depth significantly increases with an increasing number of wet-dry cycles (O'Connor *et al.* 2019). However, the smallest particle size (21 µm) investigated by these authors was below the size threshold considered in the current study.

Results for mass estimates show different patterns compared to those derived from the analysis of MP counts (Figure S5). Different replicates showed a higher variation based on mass, caused by a few large and therefore heavy fragments with an estimated mass of > 0.1 mg. Following the estimation frame, the mass of a particle is fundamentally linked to the particle size (e.g., measured by the length along three main direction) through a cubic power law. This explains why a few rare, large particles can have a large influence on total mass estimates. This behavior obviously also amplifies the uncertainty associated to the relatively low resolution of the sampling to consistently detect such rarer particles. For example, a very high mass-based concentration was observed at the end of the experiment in the recently treated plot, which was caused by one large polyethylene particle of 1 mg in the 10 - 15 cm soil layer.

Comparing MP contamination between studies reporting MP in estimated mass data and particle count is a useful exercise. Obtaining robust mass estimates is possible if sampling effort is properly sized, enabling consistent observations of rare large particles. Increased resolution can be achieved by increasing the size of individual soil samples or increasing the frequency and total number of observations. While the first case seems a more cost-efficient approach, processing large samples through multiple extraction, clean-up, particle detection and counting steps imposes serious analytical hindrances. A consolidated theoretical frame for optimizing sampling effort that simultaneously considers expectation of MP contamination characteristics and analytical limitations is not yet available. The results presented here provide some useful insights into the challenges and current limitations for MP assessment in soils, especially concerning the large heterogeneity of their spatial distribution (even when operating at relatively small scales) and their physical characteristics. Taking this into consideration, assessing the particle shape, size, and counts should be considered as a priority in soil MP assessment as impacts on both the soil properties and organisms are likely to depend more on these particle characteristics (De Souza Machado *et al.* 2018, 2019; Boots *et al.* 2019).

4.3.3. MPs in surface water runoff

Blanks from the runoff analysis did not contain MPs (Table S17). The observed annual precipitation during the study period (490 mm) was comparable with the long-term average annual precipitation for the study area (430 mm), and individual rainfall events within the study period were considered as representative for the study area too (Table S18).

From a visual inspection of the soils, it appeared that there was a slight difference in texture, which may have determined the observed difference in runoff generation between soil plots (Figure S6A; Table S18). Furthermore, the recent addition of sludge as a soil amendment expectedly affects water infiltration rates and hydraulic properties of soil, contributing to a reduction in the volume of macropores that favor runoff (Mamedov *et al.* 2016). The repeat of the first runoff events of the study confirmed that these events did not yield a significantly higher MP mobilization compared to subsequent events and was therefore excluded from the data analysis (see SI section S3 for further details). During the runoff events, in which some runoff-

collector buckets were overflowing due to unexpectedly high runoff formation (Table S18), the water loss appeared to be minimal for the control and the historically treated plot. Although the collector buckets were embedded in the soil and weighted down (Figure S1), the bucket from the recently treated plot had tipped to the side slightly during these three runoff events, causing additional water loss. During the last of these runoff events, runoff from the recently treated plot was completely lost. This is not expected to have substantially affected the results, as measured MP levels in runoff samples from events where overflowing occurred were comparable to those observed during runoff events capturing the total runoff volume (Table S18). For the two runoff events from the recently treated plot during which the total runoff was lost (first and last runoff event), data was corrected for the mass balance estimations.

MPs were mobilized by runoff from all plots, yet in very small numbers, as shown in Figure 4.2. The highest average MP concentration in runoff water was obtained from the recently treated plot and was 1.6 MPs L^{-1} (total MP number observed in runoff during the study period divided by total runoff volume collected). In this plot, the MP concentration in individual runoff events ranged between 0 and 14 MPs L^{-1}. Runoff water from the historically treated plot contained 0.5 MPs L^{-1} (0 - 0.7 MPs L^{-1}), and from the control plot 0.12 MPs L^{-1} (0 - 2.5 MPs L^{-1}; Table S18). The same pattern was observed based on mass concentration (Table S19).

In terms of MP mobilization from surface area, about 4 MPs m^{-2} were mobilized from the control plot over the period of one year, while 6.5 MPs m^{-2} were mobilized from the plot subject to historic sludge application. From the recently treated plot, 17.5 MPs m^{-2} were mobilized. Considering corrections applied to account for lost runoff, this value may be up to 31.5 MPs m^{-2} (Table S20). Despite similar soil MP concentrations in the recently and historically treated plots, the concentration and total number of MPs in runoff water was much lower in the historically treated plot than in the recently treated plot. Possible reasons for this may be that: i) MPs in recently treated soils are more mobile as they are less well incorporated into the soil matrix, and/or ii) particles with physical/chemical characteristics more prone to be mobilized by runoff (e.g., low density polymers, more regular morphologies) were lost from the soil in the historically treated plot well before the beginning of the experiment.

No direct relationship was found between the total MP count in the runoff and the rainfall volume (Figure S6B). However, there was a trend towards a positive correlation between the mass of soil exported via runoff and the number of MPs detected in the total runoff material (Figures 4.3 and S7). This suggests that there may be some similarities in the thresholds for the mobilization of both soil and MP particles in surface water runoff.

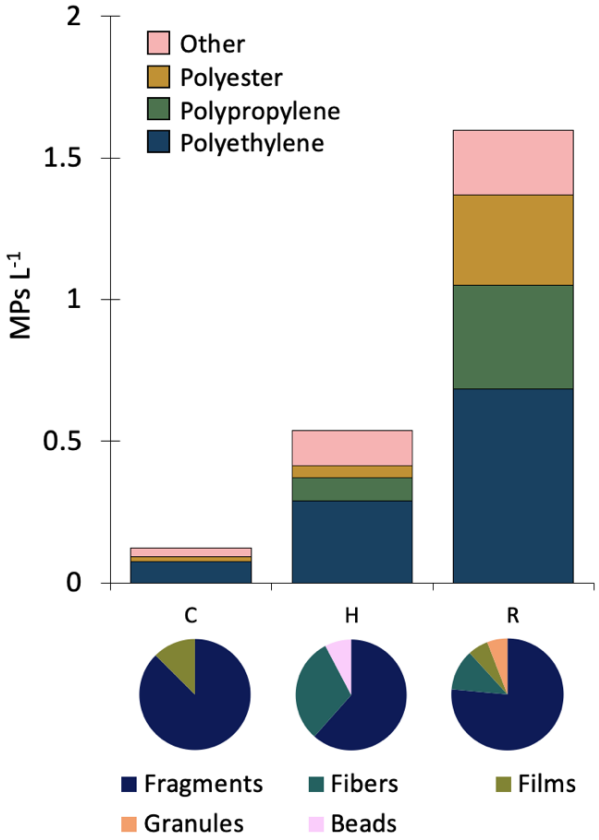


Figure 4.2. MP concentration (MPs L^{-1}) in the runoff water from the different sludge application treatments (C= control; H = historic application; R = recent application) aggregated for the whole experimental period (one year). The pie charts show the distribution of particle shapes.

Fragments were the dominant particle type (62 - 88%) in runoff from all three plots. Fibers represented only 0 - 31% and were absent in the runoff from the control plot (Figure 4.2; Table S21). This indicates that fragments are more easily mobilized, while fibers are preferentially retained in the soil compartment. The increased fiber retention could be explained by their morphology, which may promote a more efficient entangling in soil aggregates compared to fragments. MPs in runoff were ≤ 3420 µm in size (Figure S3; Table S22). In total, 12 different polymer types were observed. Polyethylene (48%), polypropylene (18%) and polyester (16%) were the most common polymers in the runoff (Figure 4.2; Table S23). The prevalence of polyethylene and

polypropylene, which are both less dense than water, may suggest that low density polymers are also preferentially mobilized.

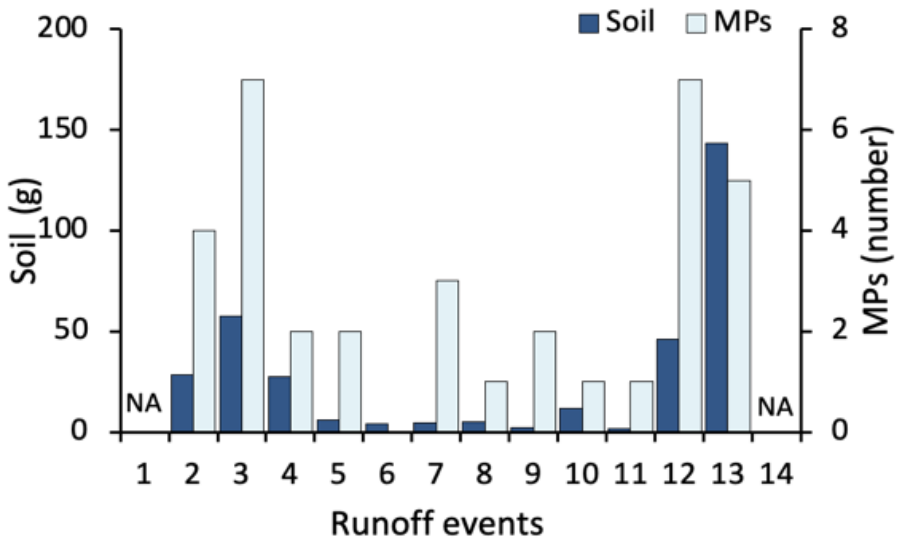


Figure 4.3. Total mass of soil material transported by runoff into the collector bucket (grams) and number of MPs detected in the runoff from the recently treated plot. NA in indicates that runoff occurred but the measurement was lost.

4.3.4. Mass balance and upscaling

Based on the MP concentration in sludge and the mass of sludge added to the plot, about 16,000 particles or 164 mg were added to the recently treated experimental plot (Table 4.2). This includes plastic particles up to 12,800 µm (maximum particle length observed). According to the soil analysis results, approximately 80,000 MPs or 364 mg of MP were calculated to be present in the recently treated soil plot (2 m^2 with a depth of 15 cm) at the start of the experiment. Thus, the calculated MP count in the soil plot was twice as expected, considering the background contamination based on the MP presence in the control soil (22,200 MPs) and the MPs added by sludge (16,000 MPs). The calculated mass-based estimate, however, was found to be congruent with the MP mass added at the start of the experiment and the background contamination (Table 4.2).

In the current experiment, the MP concentration in soil did not significantly change over the period of one year. The persistence of MP in soil is reinforced by the very low losses measured through runoff. Based on count-based data, we estimate

that about 0.2 - 0.4% (measured value - value corrected for lost runoff events) of the MPs added by sewage sludge, and about 0.04 - 0.08% the MPs measured in the soil, were exported via surface water runoff in one year. Regarding mass-based data, we estimate that 0.3 - 0.6% (2.3 - 4.1%; considering the mass of one silicone particle that influence the data greatly) of the MPs added by sludge to the soil, and 0.1 - 0.2% (1.0 - 1.8%) of the MPs present in the soil, were exported by runoff. The mass-based export is higher as mainly fragments were transported by runoff, which tended to have a higher mass compared to fibers.

Based on the MP concentration observed in sewage sludge in the current study and the estimated sludge volume applied to agricultural soils in Spain (941.6 thousand tons; Eurostat, 2020), it can be estimated that 5.9×10^{12} - 7.5×10^{12} MPs or 5.0×10^{4} - 1.1×10^{5} kg MPs are added to soils annually in this country. This is a rough estimate, as observed MP concentrations in sludge vary greatly, not only between different WWTPs but also between different sampling events at the same WWTP (Schell *et al.* 2021).

Our results show that most of the MPs applied by sewage sludge are retained in the soil (> 99% based on count-based data or > 95% based on mass-based data). Thus, it can be assumed that soils act as important long-term stores of MPs, with repeated sludge applications leading to MP accumulation over time. The estimated annual runoff emissions from agricultural settings in Spain are in the range of 1.2×10^{10} - 3.0×10^{10} MPs (assuming the observed 0.2 - 0.4% export for count-based data) or 150 - 4455 kg MPs (considering the observed 0.3% - 4.1% export for mass-based data). It is important to note that not all MPs removed from soil by runoff will directly reach surface water bodies, as some may be transported to forested areas or to other agricultural fields instead. However, these estimations provide a first assessment of MP sources from agricultural soils treated with sewage sludge. Such estimations may vary notably depending on soil type, crop type, agricultural practices or geographic location, but are useful for future comparisons and MP source apportionment exercises at regional scales.

Table 4.2. Calculated and measured input (by sludge) and output (runoff) of MPs (including small plastic particles up to 12,800 µm, which was the maximum particle length observed) for count based (#) and mass based (mg) data per soil plot and per kg soil or L runoff from different soil treatments. Calculations are based on the concentration measured in soil and the approximated amount of soil in the runoff plot based on soil density and plot dimensions. The MP mass transported by runoff from the recently treated plot is shown with and without the mass of one silicone particle which determined most of the observed mass (3.18 mg) and thus strongly influenced the data.

	MPs per soil plot or average concentration per kg soil or L runoff	**MPs added by sludge to soil plot**		**MPs at the start of the experiment**		**MPs at the end of the experiment**		**MPs transported by runoff water in one year**	
		#	mg	#	mg	#	mg	#	mg
Control	plot	-	-	22,200	215	37,100	44	8	0.071
	kg or L	-	-	62	0.57	103	0.12	0.12	0.001
Historical application	plot	unknown	unknown	121,600	277	73,350	174	13	0.074
	kg or L	unknown	unknown	338	0.76	208	0.46	0.54	0.003
Recent application	plot	16,000	164	80,000	365	79,100	935	35[a]-63[b]	0.52/3.7[a] - 0.94/6.67[b]
	kg or L	44	0.44	226	0.97	219	2.48	1.6	0.17/0.02

[a] *measured value*

[b] *corrected value considering lost runoff events (see Table S20 for further details)*

4.3.5. Critical remarks

We observed highly heterogeneous MP distributions in the experimental soils and a discrepancy between the measured count-based and estimated mass-based concentrations. Follow-up studies should increase the number of replicates and/or the sample volume to improve the representativeness of the results. Furthermore, the analysis of smaller MPs in environmental matrices is still challenging and time-consuming, while it is possible that in a relatively long-term study (e.g., one year or more), MPs undergo further fragmentation due to various biotic and abiotic agents (e.g., UV exposure, erosion, soil invertebrate ingestion; Ng et al., 2018). Therefore, small-sized MPs and nanoplastics may represent a currently non-quantifiable but important fraction, potentially hampering the mass balance. This highlights the need for more efficient analytical methods for small-sized MP determination in soil and other environmental matrices (Vighi *et al.* 2021).

Different MP types have been shown to negatively affect soil properties. They can lead to changes in soil hydrology (i.e., increase the water holding capacity) and microbial activity, which in turn can affect the crop performance (De Souza Machado *et al.* 2018, 2019; Boots *et al.* 2019). Furthermore, although research on the effects of MPs on soil organisms is still very limited, studies have indicated that inhibition in growth and reproduction, mortality, and internal injuries after ingestion may occur at high concentrations (Huerta Lwanga *et al.* 2016; Cao *et al.* 2017; Song *et al.* 2019; Selonen *et al.* 2020). Test concentrations in those studies were several orders of magnitude higher than maximum concentrations (500 MP kg^{-1} dw or 16 mg kg^{-1} dw) observed in the current study. However, as MPs accumulate in the soil over time (Corradini *et al.* 2019; Van Den Berg *et al.* 2020), repeated sludge applications as well as other input sources such as atmospheric deposition (Allen *et al.* 2019), wind erosion (Rezaei *et al.* 2019) and plastic mulching (Huang *et al.* 2020), may cause soil MP contamination to reach these levels in the future.

The sustainability of sludge application in agriculture has been debated due to its potential human and environmental health consequences. To date, sewage sludge must fulfill the quality requirements regulated under the European Sewage Sludge Directive (86/278/EEC) to be used as agricultural fertilizer. Thus far, MPs have not been included in this directive, but there is a currently undergoing initiative to evaluate

whether certain contaminants of emerging concern, including MPs, should be regulated. To understand at what contamination level the presence of MPs outweighs the benefits of sludge application, further research is needed to assess the long-term persistence and infiltration of MPs in soils under different conditions, their potential mobilization into other environmental compartments (i.e., surface and groundwaters), and their ecological effects.

4.4. Conclusions

To our knowledge, this paper represents one of the first attempts to assess MP fluxes in agricultural soils empirically. The study shows that sludge application significantly increases MP content in soils and that those concentrations remain relatively stable over time. Mobilization of MPs, both into deeper soil layers and along surface water runoff, was very low during the period of one year under the environmental conditions tested in this study. Thus, we conclude that in semi-arid regions, surface water runoff has a negligible influence on the export of MPs from agricultural soils and that agricultural soils can be considered long-term MP accumulators.

Acknowledgments

The study has been conducted as part of the EU JPI-Water initiative IMPASSE project (Impacts of MicroPlastics in AgroSystems and Stream Environments) supported by the Spanish Ministry of Economy, Industry and Competitiveness (grant number: PCIN- 2017-016) and the Norwegian Research Council (grant number: 271825/E50). A. Rico was supported by the Talented Researcher Support Programme - Plan GenT (CIDEGENT/2020/043) of the Generalitat Valenciana.

Supporting Information

The Supporting Information for this Chapter can be downloaded at: https://doi.org/10.1016/j.envpol.2021.118520

CHAPTER 5

Effects of Polyester Fibers and Car Tire Particles on Freshwater Invertebrates

This Chapter has been published as:

Schell T, Martinez S, Dafouz R, Hurley R, Vighi M, Rico A. 2022. Effects of polyester fibers and car tire particles on freshwater invertebrates. Environmental Toxicology and Chemistry 41:1555-1567. https://doi.org/10.1002/etc.5337

Abstract

Microplastic (MP) ingestion has been shown for various organisms; however, knowledge of their potential adverse effects on freshwater invertebrates remains limited. Here we assessed the ingestion capacity and the associated effects of polyester fibers (26 - 5761 µm) and car tire particles (25 - 75 µm) on freshwater invertebrates under acute and chronic exposure conditions. A range of MP concentrations were tested on *Daphnia magna*, *Hyalella azteca, Asellus aquaticus* and *Lumbriculus variegatus* using water only (up to 0.15 g L^{-1}) or spiked sediment (up to 2 g kg^{-1} dw), depending on the habitat of the species. While *D. magna* did not ingest any fibers, low levels of fibers were ingested by all tested benthic invertebrate species. Car tire particle ingestion rose with increasing exposure concentration for all tested invertebrates and was highest in *D. magna* and *L. variegatus*. No statistically significant effects on mobility, survival or reproductive output were observed after acute and chronic exposure at the tested concentrations in most cases. However, fibers affected the reproduction and survival of *D. magna* (NOEC: 0.15 mg L^{-1}) due to entanglement and limited mobility under chronic conditions. Car tire particles affected the reproduction (NOEC: 1.5 mg L^{-1}) and survival (NOEC: 0.15 mg L^{-1}) of *D. magna* after chronic exposure at concentrations that are in the same order of magnitude as modeled river water concentrations, suggesting that refined exposure and effect studies should be performed with these MPs. This study confirms that MP ingestion by freshwater invertebrates depends on particle shape and size and that ingestion quantity depends on the exposure pathway and the feeding strategy of the test organism.

5.1. Introduction

Microplastics (MPs) are ubiquitous contaminants in freshwater ecosystems (Li *et al.* 2020; Schell *et al.* 2020; Yang *et al.* 2021) and can be ingested by a wide range of organisms (Scherer *et al.* 2018). Ingestion of MPs has been shown to cause adverse effects such as intestinal damage, reduced growth, decreased reproduction, and decreased survival of aquatic organisms (Foley *et al.* 2018; Kögel *et al.* 2020). Most studies assessing the effects and ingestion of MPs under laboratory conditions have been performed with polyethylene and polystyrene fragments or beads, which are not necessarily the most abundant MP types in the environment (De Ruijter *et al.* 2020; Kögel *et al.* 2020; Kutralam-Muniasamy *et al.* 2020; Miloloža *et al.* 2021). Furthermore, data for freshwater benthic organisms remain very limited (Bellasi *et al.* 2020; Kögel *et al.* 2020), with most toxicity tests performed without sediment, thus limiting the applicability of these data for conducting sediment risk assessments.

Fibers are often reported as the dominant MPs in freshwater ecosystems (Li *et al.* 2020; Rebelein *et al.* 2021; Sarijan *et al.* 2021; Yang *et al.* 2021). The fragmentation of textiles during laundry and subsequent environmental discharge of treated and untreated wastewaters constitute one of the most important pathways for these MPs into the aquatic environment (Ziajahromi *et al.* 2016; De Falco *et al.* 2019; Schell *et al.* 2020). Another important pathway for fibers is atmospheric transport and deposition, which is also responsible for the contamination of remote areas (González-Pleiter *et al.* 2020; Stanton *et al.* 2020). Most field monitoring studies report the dominance of specific MP shapes and polymer types by showing their total percentage instead of providing their actual concentrations (e.g., Rebelein et al., 2021; Yang et al., 2021). Therefore, only a limited number of field studies reported specific MP fiber concentrations in freshwater ecosystems. Measured fiber concentrations in freshwater have been found to range from less than 0.1 fibers L^{-1} to 519 fibers L^{-1} (Lahens *et al.* 2018; Martínez Silva & Nanny 2020). Several studies show that MP fibers are retained in freshwater sediments (Tibbetts *et al.* 2018; Deng *et al.* 2020; Martínez Silva & Nanny 2020). Concentrations as high as 1,323 MPs kg^{-1} dw sediment were measured in a textile industrial area in Shaoxing city, China, with up to 79% being fibers (Deng *et al.* 2020). Mass fractions of fibers are less frequently reported, but the mass concentration in the water of the Saigon River (Vietnam) has been estimated to vary between 0.05 mg L^{-1} and 0.22 mg L^{-1} (Lahens *et al.* 2018). In river sediments of central Spain,

concentrations up to 1.58 mg fibers kg^{-1} dw were measured (Schell *et al.* 2021); while MP concentrations (including all shapes) reached 1 g kg^{-1} dw (equivalent to 4000 MPs kg^{-1} dw) in the river shore sediments of the Rhine-Main area in Germany (Klein *et al.* 2015).

Recently, tire related particles have been included under the MP contaminant group (Hartmann et al., 2019; Kole et al. 2017; Wagner et al. 2018). The terminology used for those particles differs between studies; thus, hereafter the term tire particles is used for MPs consisting only of tire material. Tire wear particles, if not specified otherwise, will refer to particles that are formed by the friction of tires on the road surface and are therefore emitted into the environment as particles consisting of polymer tread with pavement encrustations (Kreider *et al.* 2010; Unice *et al.* 2013). Global annual tire wear emissions have been estimated to be up to 3.4 million tons (Baensch-Baltruschat *et al.* 2020). These particles are prone to reach aquatic environments via stormwater runoff and wastewater effluents of combined sewer systems (Kole *et al.* 2017; Wagner *et al.* 2018; Unice *et al.* 2019). To date, assessing aquatic exposure concentrations for tire wear particles is challenging, mainly because of their high black carbon content, which produces a low-quality spectrum that cannot accurately be interpreted by the spectroscopic techniques often used for MP identification (Wagner *et al.* 2018). Nonetheless, 15% - 38% of the MPs (80 - 260 MPs kg^{-1} dw) measured in the sediment of a stormwater treatment wetland were most likely derived from tires (Ziajahromi *et al.* 2019) and up to 1833 suspected tire wear particles kg^{-1} ww sediment (corresponding to 0.0023 g kg^{-1} ww) were observed in the Ashley River (South Carolina, USA; Leads and Weinstein, 2019). Some studies have relied on chemical markers to assess tire particle exposure (Wik & Dave 2009; Baensch-Baltruschat *et al.* 2020). For example, using markers for rubber polymers, Unice et al. (2013) reported a maximum concentration of 5.8 g kg^{-1} dw of tire particle material in sediments (which amounts to 11.6 g kg^{-1} dw of tire wear particles, assuming a 50:50 ratio of polymer tread and mineral road encrustations) of the Seine River catchment (France), and in a follow up modeling study, these authors reported that water concentrations may be up to 0.12 mg L^{-1} (Unice *et al.* 2019).

The ingestion capacity of fibers and tire particles by freshwater organisms may significantly differ from that described for other MPs, as not only the polymer type but also the size and morphology are expected to play an important role (Ogonowski *et al.*

2016; Ziajahromi *et al.* 2017a). For example, longer fibrous materials may have different ingestion rates and gut retention times than spherical particles (Wright *et al.* 2013; Qiao *et al.* 2019a). Previous studies also show that plastic fibers can exert greater adverse effects on benthic and planktonic invertebrates than other MP types, for example due their longer gut residence time or entanglement capacity (Au *et al.* 2015; Ziajahromi *et al.* 2017a).

While fibers are expected to mainly cause effects of a physical/mechanical nature, tire particles contain a complex mixture of elastomers and chemical additives (Kreider *et al.* 2010), which may be taken up by living organisms. Therefore, they may pose a combination of both physical and chemical hazards. Thus far, research on the environmental effects of tire particles has been mainly related to the toxicity of their chemical leachates obtained with different chemical and physical methods that force their desorption (e.g., Hartwell *et al.* 1998; Wik & Dave 2005, 2006; Wik 2007; Turner & Rice 2010; Marwood *et al.* 2011). The effects of the leachates may, however, not be the only cause for the tire-related toxicity as the tire particles themselves may influence the bioavailability of these chemicals and pose additional effects (Khan *et al.* 2019). There is likely to be a complex interplay between physical particle characteristics and the potential chemical hazards which govern the risk posed by this MP type (Selonen *et al.* 2021). Therefore, to reliably assess the impacts of tire particles on living organisms, long-term toxicity studies with entire particles and environmentally relevant exposure and desorption conditions are needed.

The aim of this study was to determine the ingestion of MP fibers and car tire particles by a selection of freshwater invertebrates with different habitat preferences and feeding strategies and to assess their short- and long-term effects. Laboratory experiments were performed with *Daphnia magna* (pelagic; filter feeder), *Hyalella azteca* (epibenthic; shredder), *Asellus aquaticus* (epibenthic; deposit feeder) and *Lumbriculus variegatus* (endobenthic; deposit feeder) using concentrations that range from present-day environmentally relevant conditions to projected future MP pollution scenarios, using water only and water-sediment exposure. The ultimate goal of this paper was to generate toxicity data that can be used to assess the risks of these MPs for freshwater ecosystems, and to discuss the mechanisms that explain the differences in uptake and toxicity among freshwater organisms.

5.2. Materials and Methods

5.2.1. Test materials

The MPs used in this study were obtained from the Norwegian Institute for Water Research (NIVA), Oslo Norway. Fibers were generated by washing polyethylene terephthalate (PET) fleece blankets ('Skogsklocka', IKEA, Norway) in a clean washing machine (Candy Smart, model no. CS 1692D3-S) on a 15 min cycle at 40°C and 1200 rpm. During the washing process no detergents or softener were added. To obtain the fibers from the effluent, the effluent was collected in a stainless-steel pressure vessel (Pope Scientific, Wisconsisn, USA) and vacuum-filtered through a 10 µm nylon membrane. The fibers were of a cylindrical shape (Figure S1), had a density of 1.38 g cm^{-3}, a mean length of 600 µm (min - max: 26 - 5761 µm; SD: 554 µm, n = 618), and a width of 20 µm. The fiber sizes used in this study are in the range of those monitored in freshwater ecosystems (Schell *et al.* 2021; Yang *et al.* 2021). Fiber stability in water and sediment was not assessed as they were expected to be stable given the relatively short duration of the experiments.

Tire particles were obtained from the Danish tire granulate manufacturer Genan. The particles were a by-product of granulate production. The granulate was milled from end-of-life passenger tires, which represents the primary source of tire debris found in the environment. Prior to granulate production, the material was separated from metallic tire scaffolding and textile components. Finally, the material was purified at NIVA (i.e., separating residual metal particles, plastic, fiber contamination) and sieved into a size range of 25 - 75 µm. This size range was chosen because most tire particles in the environment are expected to be smaller than 100 µm (Kreider *et al.* 2010; Järlskog *et al.* 2020). The lower size limit of 25 µm was selected to facilitate the visual assessment step included in the analysis of particle ingestion by organisms. However, during the sieving process some particles stuck together and particles below 25 µm were included in the experiments. Furthermore, particles agglomerated following the sieving process, and as a consequence, few particles larger than 75 µm were measured. The measured mean size of the long axis of the tire particles (n = 896) was 38.7 (min - max: 3 - 200 µm; SD: 27.7 µm). Tire particles had a density of 1.16 g cm^{-3} and were irregularly shaped (Figure S1). The chemical composition of the tire material, including the content of trace metals and polycyclic aromatic hydrocarbons, is described in Selonen et al. (2021).

Stock solutions were prepared to allow a more precise dosage based on MP mass of low concentrations of the test materials in the experiments. The stock solutions of fibers were prepared in ethanol, as it was not possible to achieve a homogeneous solution in water. Despite this, few fiber clumps were observed in the stock solutions and the two highest exposure concentrations based on visual inspection. However, fiber clumps have also been observed in environmental samples (e.g., Schell et al., 2021) and should therefore be included as part of environmentally relevant exposure scenarios for freshwater organisms. The stock solutions of tire particles were prepared in Milli-Q water. The latter were placed in an ultrasonic bath for 15 mins to break up agglomerates prior to the addition to the test beakers. All stock solutions were prepared just before adding to the test units (i.e., prior to the first spike). The stock solutions used in the chronic *Daphnia magna* tests were prepared directly before the test start and used for the test medium renewal throughout the entire experiment. In order to estimate the nominal dose of particles added per test system, subsamples of the stock solutions (20 - 1000 µL depending on the concentration of the stock solution; n = 6) were taken, and the MPs were counted using a stereo microscope (Olympus SZX7 coupled to an Olympus DP21 camera system). The count-based concentration was estimated based on the particle count and the dilution factor in the test medium. Additionally, count-based concentrations were calculated based on the nominal mass following Leusch and Ziajahromi (2021), using the particle density and the average measured fiber length or tire particle diameter. Tire particles were assumed to be spherical for the calculation.

5.2.2. Test species

All test organisms came from the in-house cultures of the IMDEA Water Institute, which were held at 20 ± 1 °C with a 16:8 h (light:dark) photoperiod. *D. magna* were kept in beakers containing synthetic hard water (ASTM E729 – 96; ASTM, 2007) or mineral water (AquaBona; FuenMajor Spring), which was renewed two times a week, and were fed with the green algae *Chlorella vulgaris*. *H. azteca* and *A. aquaticus* were cultured in aquaria with water from an artificial pond (filtered through a 20 µm plankton net and autoclaved prior to use) and fed with previously inoculated *Populus s*p. leaves (to obtain microbial and fungal communities to increase their palatability) and a solution of fish food (Tetramin, Tetra GmbH, Germany). *L. variegatus* were kept

in aquaria with quartz-sand sediment. The overlying water and the fish food used for their maintenance were the same as described above.

5.2.3. Experimental design

The characteristics of the different tests performed, such as the exposure route, exposure duration, and endpoints, are summarized in Table 5.1. For the pelagic organisms (*D. magna*), water-only tests were performed, while for the epibenthic organisms (*H. azteca* and *A. aquaticus*) water-only and water-sediment tests were performed. For the endobenthic organisms (*L. variegatus*), water-sediment experiments were performed. The test organisms were exposed to five fiber or tire particle concentrations. In the water-only experiments, the exposure concentrations ranged between 0 and 0.15 g L^{-1}, while in the water-sediment tests, the exposure concentrations ranged between 0 and 2 g kg^{-1} of sediment dry weight (Table 5.1). The number of replicates per concentration varied depending on the test (Table 5.1). In the tests performed with fibers, a solvent control was included in addition to the control, to which the same amount of ethanol was added as to the test beakers.

Acute and chronic effects were assessed in the different test organisms. The endpoints evaluated at the end of the experiments were ingestion, immobility, reproduction, or survival, depending on the exposure duration and the test organism (Table 5.1). In the chronic tests with macroinvertebrates, ingestion was also assessed on day 14 after the start of the experiment as an intermediate endpoint evaluation. All tests were performed under controlled climate conditions with a photoperiod of 16:8h (light:dark) and a temperature of 20 ± 1°C. Water temperature, pH, conductivity, and dissolved oxygen were measured at the start and the end of the tests with a multiparameter meter (Hanna HI91894), as well as after the renewal of the exposure medium to assure that they were within the acceptable range of the guidelines.

Table 5.1. Summary of the different toxicity tests, including test species, exposure pathway, test duration, exposure concentrations for fibers and tire particles, evaluated endpoints, number of replicates, and the test protocol used as reference.

Species	Exposure pathway	Test duration	Exposure concentrations	Endpoints	Replicates	Test protocol
D. magna	Water	48 hours	0; 0.00015; 0.0015; 0.015; 0.15 g L^{-1}	Ingestion; Immobility	4	OECD 202
D. magna	Water	21 days	0; 0.00015; 0.0015; 0.015; 0.15 g L^{-1}	Ingestion; Survival; Reproduction	10	OECD 211
H. azteca	Water	4; 14; 28 days	0; 0.00015; 0.0015; 0.015; 0.15 g L^{-1}	Ingestion; Survival;	4	ASTM E1706-05
H. azteca	Water-Sediment	4; 14; 28 days	0; 0.002; 0.02; 0.2; 2 g kg^{-1} dw	Ingestion; Survival;	4	ASTM E1706-05
A. aquaticus	Water	4; 14; 28 days	0; 0.00015; 0.0015; 0.015; 0.15 g L^{-1}	Ingestion; Survival;	4	ASTM E1706-05
A. aquaticus	Water-Sediment	4; 14; 28 days	0; 0.002; 0.02; 0.2; 2[a] g kg^{-1} dw	Ingestion; Survival;	3	ASTM E1706-05
L. variegatus	Water-Sediment	4; 14; 28 days	0; 0.002; 0.02; 0.2; 2 g kg^{-1} dw	Ingestion; Survival; Reproduction	4	OECD 225

[a]Tire particles only: the highest concentration tested for fibers was 0.2 g kg^{-1} dw

5.2.3.1. Tests with *D. magna*

Standard acute tests were carried out for each MP type following the OECD guideline 202 (OECD 2004). The fiber stock solution was added to the empty beakers at the respective concentrations, and the ethanol was allowed to evaporate until completely dry in a fume hood. This step was unnecessary for tire particles as the stock solutions were prepared in water. Then, 50 mL of test medium (AquaBona; FuenMajor Spring) and five organisms were added per beaker (n = 4). Mobility was checked after 24 and 48 hours of exposure. Organisms were considered to be immobile if no movement was recognized after gently moving the beaker for 10 seconds.

Chronic toxicity tests were carried out under semi-static conditions according to the OECD guideline 211 (OECD 2012). Test units were prepared in the same way as for the acute tests, but the medium was replaced three times per week, and organisms were kept individually in 50 mL of test medium (n = 10). Offspring were counted and removed three times per week, prior to medium renewal, to assess reproduction. *D. magna* reproduction was calculated as the total number of living offspring per parent animal that did not accidentally or inadvertently die during the test. Adult survival was recorded at the same time as offspring was counted. After each medium renewal, *D. magna* were fed with *C. vulgaris* corresponding to 0.15 mg organic Carbon/individual when they were adults, and one third and half this amount when they were neonates and juveniles, respectively.

While the chronic fiber experiment was performed once, the chronic tire particle experiment was carried out twice: one with synthetic hard water (ASTM 2007) and *D. magna* strain A and one with mineral water (AquaBona; FuenMajor Spring) and a new strain of *D. magna* (strain B). Strain B was also used in the acute tests and the fiber experiment. The two different strains were used because a new culture was established at the laboratory.

5.2.3.2. Tests with *H. azteca* and *A. aquaticus*

The tests with *H. azteca* and *A. aquaticus* were performed based on ASTM E1706-05 (ASTM 2005) with filtered and autoclaved natural water, as described above for the culturing of organisms. Only adults between 0.5 - 1 mm were used for the experiments. In the water-only tests, 200 mL were added per test beaker. In the water-

sediment tests, sediment was prepared following the OECD guideline 225 (OECD 2007) except for the addition of clay. The final sediment mixture contained 94.5% sand (< 2mm grain size), 5% peat (heated at 100°C and ground with a ball mill; Retsch MM400), and 0.5 % *Urtica* powder. Two days before the start of the experiment, the peat was mixed with milli-Q water (50% of the sediment dry weight), and the pH was corrected (5.5 ± 0.5 pH) using $CaCO_3$. The mixture was kept under constant stirring for 48 hours to allow the pH to adjust to 5.5 - 6.5 and was then mixed with the remaining ingredients. Finally, an amount corresponding to 20 g of dry sediment was added to each test beaker.

For fibers, the stock solutions were added to 250 mL beakers containing 5 g dry sand (previously subtracted and not added to sediment mixture). The ethanol added with the stock solutions was evaporated in a fume hood. This process took approximately 2 hours. Then, the sand-fiber mixture was added to the sediment mixture and mixed properly. For tire particles, the respective amount of the stock solution was directly added into the final sediment mixture. The overlying water used was the same water as for the water-only test and the culturing of organisms. In each test system, 150 mL water and 10 organisms were added. Each test beaker was continuously aerated and covered to avoid contamination from the air and evaporation of the test medium.

Test organisms were fed with pre-conditioned *Populus sp*. leaf disks (diameter: 2 cm). Each test unit consisted of 10 organisms with three leaf disks. In the water-only tests with *H. azteca*, 300 µL Tetramin solution (6.66 g ground Tetramin per L Milli-Q water, Tetra GmbH, Germany) was also added weekly as an additional food source to achieve optimal maintenance of the test organisms (e.g., Besser et al., 2005; Soucek et al., 2016). Each test beaker was continuously aerated and covered to avoid contamination and evaporation of the test medium. Aeration in the water-only test ensured the distribution of MPs throughout the water phase. Mortality was assessed as the difference between the initial number of organisms added and the number of living organisms after each test period.

5.2.3.3. Tests with *L. variegatus*

Each test unit consisted of a 250 mL beaker with 10 individuals. The test organisms used in the experiment were of a similar size (18.4 ± 3.3 cm; mean ± SD).

According to OECD 225 (OECD 2007), they were cut in half 14 days before the start of the test to synchronize reproduction. Mortality was assessed after 4 and 14 days. Reproduction was assessed in the chronic test (28 days) as the difference between the initial number of organisms and the number of living organisms after the test period. The preparation of the test units followed the same steps as described above for the *H. azteca* and *A. aquaticus* water-sediment tests. The *Urtica* powder in the sediment served as a food source.

5.2.4. MP extraction and visual assessment

At the end of each test, all living organisms were transferred into Milli-Q water and were subjected to five rinsing steps with Milli-Q water to remove MPs potentially attached to their body surface. *D. magna* exposed to fibers were first visually analyzed under a stereo microscope (Olympus SZX7) to assess the external entanglement with fibers. To isolate the ingested particles, the tissue was digested using a potassium hydroxide solution (KOH; 10%) for *L. variegatus*, and a hydrogen peroxide solution (H_2O_2; 15%) for the remaining test organisms for 48 hours at 50°C. Recovery tests with the here tested MPs from biota have been carried out in previously published studies showing satisfactory results (Bråte *et al.* 2020; Kallenbach *et al.* 2021, 2022). However, in the current study H_2O_2 was used without the addition of chitinase, as pre-tests showed sufficient digestion of the test organisms' exoskeleton. These solutions were then vacuum filtered onto filter papers (glass microfiber filters; 0.7 µm; Scharlau) and the number of ingested particles and their size was assessed using a stereo microscope (Olympus SZX7) with a camera attachment (Olympus DP21). Fibers long and tire particles long and short dimensions were measured with the Olympus DP2-Twain software. In total, 618 fibers and 896 tire particles from the stock solutions were measured. The size of all ingested fibers was analyzed. For tire particles, the size of all particles ingested by benthic invertebrates after chronic exposure (28 days) was analyzed except for those experiments in which organisms ingested a high number of particles (i.e., 70% of particles ingested by *H. azteca* in the water only test and 8.5% of particles ingested by *A. aquaticus* in the water only test). For *D. magna* all ingested tire particles were measured in the acute experiment, and 3.7% of particles ingested in the chronic experiment using strain A. For each batch of samples processed, 3 blanks were included, which were used to assess for potential procedural, container, solution, and air contamination. Blanks were treated with an identical sample

processing and microscope analysis procedure as the experiment samples. Blanks contained no fibers and a total of three tire particles. Background contamination was therefore considered negligible.

5.2.5. Data analyses

The No Observed Effect Concentration (NOEC) and, if possible, the Lowest Observed Effect Concentration (LOEC) were determined for all evaluated endpoints in the toxicity tests. For this, the normal distribution and homogeneity of variances of the response data were tested using Shapiro test and Levene's test, respectively. As parametric assumptions were not met for all data, non-parametric Kruskal-Wallis tests followed by Bonferroni-adjusted Wilcoxon rank-sum tests were carried out. This was done for all assessed endpoints except for *D. magna* survival in chronic toxicity tests, which was analyzed using Fisher's Exact tests to identify the NOEC and LOEC values. Wilcoxon rank-sum tests were used to check for differences between the controls and solvent controls. Statistically significant differences between the observed effects in the different treatments and the controls were assumed when the p-value was < 0.05. All statistical analyses were carried out using the software R Version 4.1.1 (R Core Team, 2021) in RStudio (RStudio Team 2021) and the required extension packages (Wickham 2016; Kassambara 2017; Fox & Weisberg 2019).

5.3. Results and Discussion

5.3.1. MP ingestion and effects on *D. magna*

The results of the experiments show that *D. magna* did not ingest fibers, while tire particle ingestion increased with increasing exposure concentrations (Figure 5.1). After 21 days of exposure (assessed only during the first test with strain A), a higher number of tire particles (about 20 to 60 times higher) was observed inside the gut compared to after 48 hours. The higher ingestion was probably caused by the longer exposure time and the increase in body size, allowing adults to ingest particles of larger sizes as well as agglomerates. This confirms the importance of life stage and body size for MP ingestion, as indicated previously (Scherer *et al.* 2017). Furthermore, after 21 days of exposure, adults' ingestion was on average higher at the second-highest concentration (0.015 g L^{-1}) compared to the highest concentration (0.15 g L^{-1}). The lower ingestion rate at the highest exposure concentration may have been caused by the increased agglomeration of tire particles in the test beakers, which has also been

observed elsewhere (Milološa *et al.* 2021). *D. magna* commonly feeds on small, suspended particles in the water (planktonic algae) and their particle selectivity mainly depends on the particle size (Gophen & Geller 1984; Ebert 2005). They usually ingest particles between 1 and 50 µm, but larger particles with a diameter of up to 70 µm may also be taken up (Ebert 2005). Thus, the tire particles were within this ingestible size range (Figure S2), while the length of most fibers used in the current study (mean ± SD: 600 ± 554 µm) exceeded this size range, which could have prevented their uptake.

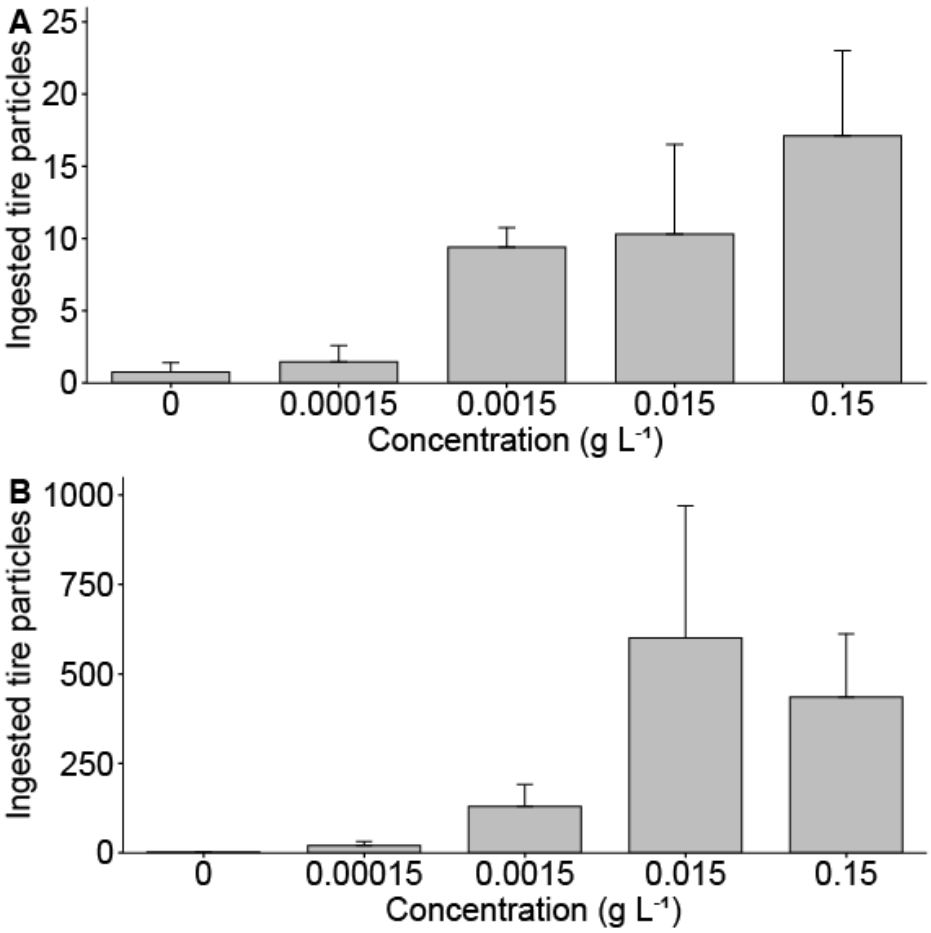


Figure 5.1. Mean number and corresponding standard deviation of ingested tire particles per *D. magna* at (A) 48 hours (juveniles) and (B) 21 days (adults) after the start of the exposure period.

No effects on *D. magna* mobility were observed after acute exposure (48 hours) to fibers or tire particles at the tested concentrations. However, chronic exposure to fibers and tire particles negatively affected survival and reproduction. For fibers, the observed NOEC for reproduction and survival was 0.00015 g L^{-1} (Figure 5.2). There was no significant difference between the solvent control and the control. At concentrations above ≥ 0.0015 g L^{-1}, fibers were observed to form agglomerates with the green algae provided as food. Adult *D. magna* got entangled in those

agglomerates, which impeded their movement (Figure 5.3), and probably caused the observed reduction in reproduction (up to 85%) and survival (up to 90%) compared to the control (Figure 5.2). Furthermore, algae-fiber agglomerates may have reduced food availability and food quality. Similarly, Ziajahromi et al. (2017a) showed that *Ceriodaphnia dubia* did not ingest PET fibers of a length between 100 and 400 µm but was negatively affected due to external physical damage (i.e., carapace and antenna deformities) caused by the entanglement with fibers.

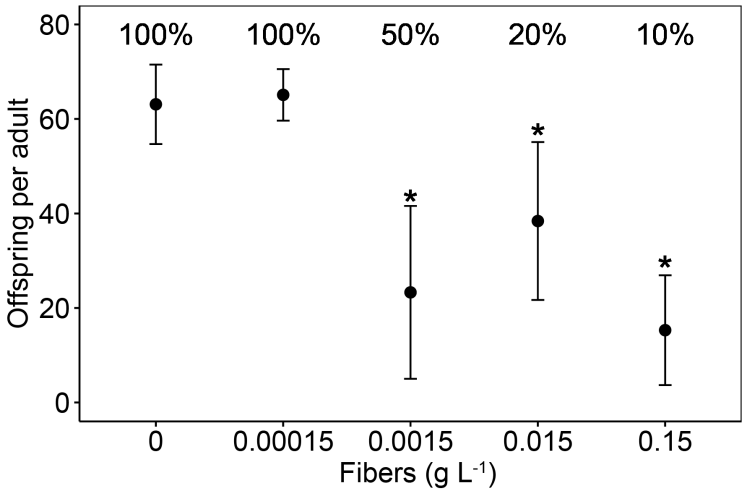


Figure 5.2. Reproduction displayed as mean number of offspring (± 95% CI, n = 10) per adult *D. magna* after 21 days of exposure to increasing fiber concentrations. Statistically significant differences in reproduction between the control and the different fiber concentrations are displayed by asterisks. The percentage of surviving adults is shown above the respective treatment.

In contrast to the results of the current study, fiber ingestion by juvenile *D. magna* has been observed in previous studies (Jemec *et al.* 2016; Kim *et al.* 2021a). For example, Kim et al. (2021a) reported that the ingested fiber sizes ranged between 10 and 70 µm and that longer fibers were not ingested. Jemec et al. (2016), however, reported the size of ingested fibers was mainly around 300 µm, but also longer ones (up to 1400 µm) were found inside *Daphnias*' guts. The authors explained that this was probably possible because fibers were twisted prior to ingestion. Furthermore, the procedure used to generate the fibers may not only influence the length but also the shape of fibers, which in turn may affect ingestion and entanglement capacity. Similar to this study, Jemec et al. (2016) used PET fleece textile to obtain fibers, but the procedure to generate them differed. These researchers used a ball mill to grind the textile, while in the current study, fibers were obtained by washing the PET fleece blankets. Based on the reported fiber dimension by Jemec et al. (2016), the fibers used

in their experiment were more flattened out compared to the fibers used in the current study, which were more cylindrical. Furthermore, the mortality rate observed in *D. magna* that were not pre-fed prior to the exposure did not increase following a dose-response pattern (i.e., it was between 20 - 40% at concentrations from 12.5 to 100 mg L^{-1}); while the *D. magna* that were fed with algae before the experiment showed no increased mortality at the same test concentrations (Jemec *et al.* 2016). This is in contrast to our results and the ones by Ziajahromi et al. (2017a), where concentration-dependent effects were clearly observed. Ziajahromi et al. (2017a) observed a significant reduction in the reproductive output with increasing concentration and 40% mortality at the highest test concentration (0.001 g L^{-1}) during chronic exposure of *C. dubia*. Moreover, the authors reported an LC_{50} of 0.0015 g L^{-1} (1.3 x 10^4 fibers L^{-1}) for acute exposure (Ziajahromi *et al.* 2017a), while we did not find any influence on *D. magna* mobility at this concentration.

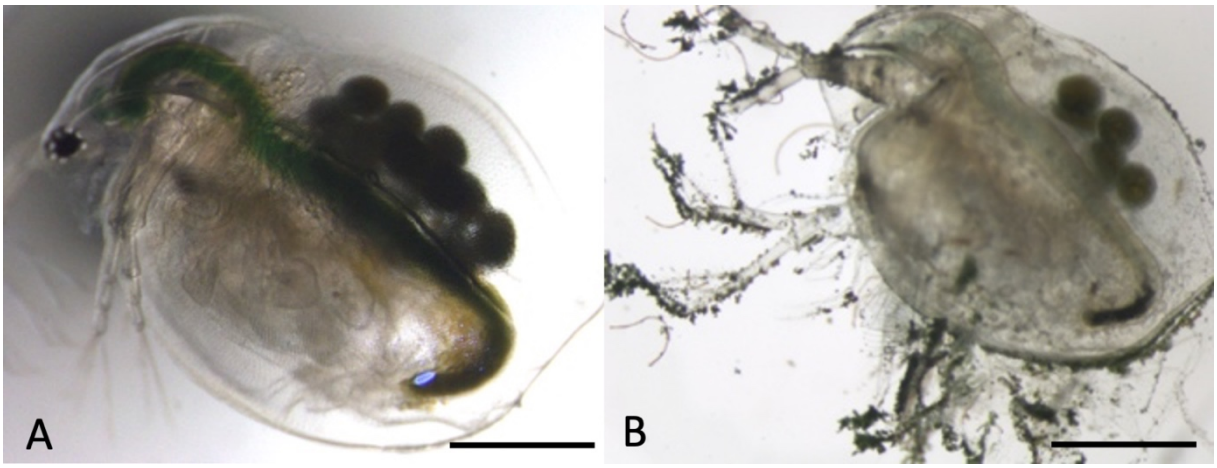


Figure 5.3. *D. magna* after exposure for 21 days to (A) the control and (B) 0.015 g fibers L^{-1}, which resulted in the entanglement of the *D. magna* in algae-fiber agglomerates. The black scale represents 1000 µm.

For tire particles, the 21-day NOECs for reproduction in the two experiments conducted were 0.015 and 0.0015 g L^{-1} (Figure 5.4). While adult survival was not affected during the first experiment (using synthetic hard water and strain A), the NOEC for survival was 0.00015 g L^{-1} in the second experiment (using mineral water and strain B). This might be due to different sensitives of the two *D. magna* strains used (e.g., see Toumi et al. 2015). The reduced reproductive output and survival may be caused by a physical effect of the particles themselves and/or due to incorporated compounds (e.g., metals; organic compounds) that leached out into the test medium or into the organisms' body after ingestion of the particles. Tire particle leachates have previously

been shown to be toxic to aquatic organisms; however, leachates from different tire material can vary considerably in toxicity (Wik & Dave 2005, 2006; Wik *et al.* 2009; Lu *et al.* 2021a). Differing compositions depending on the tire type and manufacturer, different wear of tires, and the method used to generate the test material may affect toxicity (Baensch-Baltruschat *et al.* 2020). Halle et al. (2021) compared the toxicity of tire particles ground from pristine and road-worn tires and observed a greater toxicity of pristine particles for *H. azteca*, which was attributed to a higher abundance of chemical compounds in these particles.

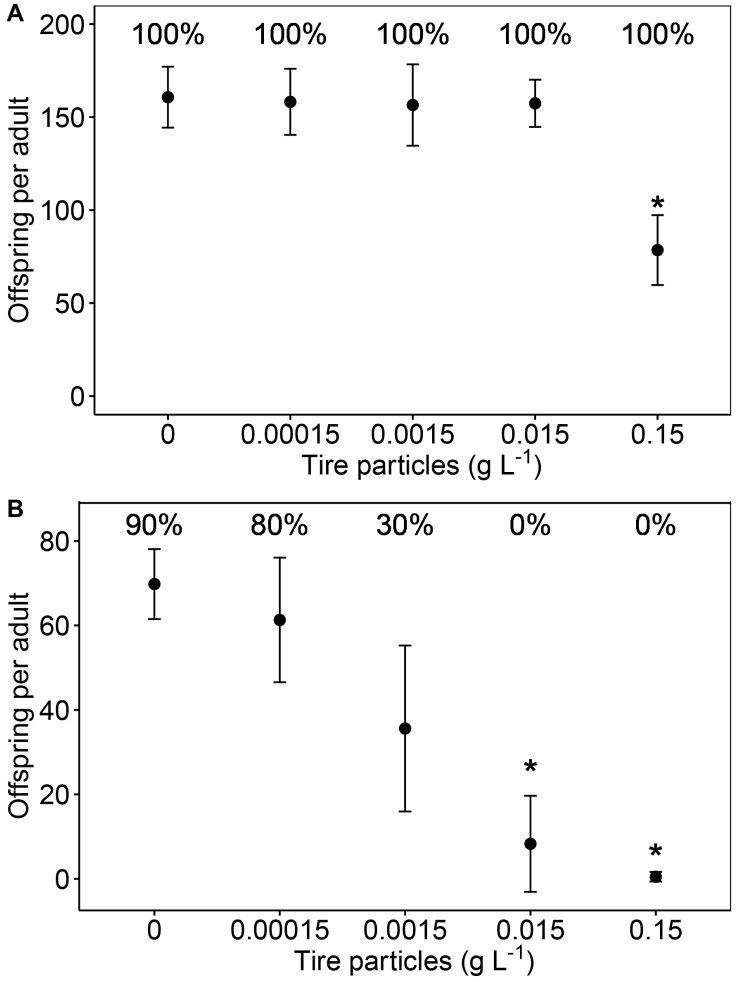


Figure 5.4. Reproduction displayed as mean number of offspring (± 95% CI, n = 10) per adult *D. magna* after 21 days of exposure to increasing tire particle concentrations in (A) the first experiment using synthetic hard water and strain A, and (B) the second experiment using mineral water and strain B. Statistically significant differences in reproduction between the control and the different tire particle concentrations are displayed by asterisks. The percentage of surviving adults is shown above the respective treatment.

The toxicity of tire leachates to *D. magna* has been previously related to metals, primarily Zn, and different organic compounds, including benzothiazoles and

phthalates (Wik & Dave 2006; Wik *et al.* 2009; Marwood *et al.* 2011; Capolupo *et al.* 2020; Lu *et al.* 2021a). The tire material used in this study contained a mixture of different metals (with Zn concentrations being by far the highest with 21.9 g kg^{-1}) and polycyclic aromatic hydrocarbons (see Selonen et al., 2021 for further details). Roadway particles (collected during outdoor driving) and tire wear particles (collected on a simulated laboratory driving course) have been shown to have lower Zn concentrations than tire particles cryogenically ground from unused tires but contained other metals at higher concentrations, probably originating from asphalt (Kreider *et al.* 2010). Moreover, particles ground from tires, such as those used in this study, usually have a higher polymer content but lower mineral content (Kreider *et al.* 2010). Therefore, future studies should be performed with tire wear MPs recovered from environmental samples to increase the realism of the ecological risk assessment. Also, it remains unclear if the effects observed here are caused by chemical leaching, the physical effects of the tire particles, or a combination of both, which remains to be investigated in follow-up studies.

5.3.2. MP ingestion and effects on *H. azteca*, *A. aquaticus* and *L. variegatus*

After 4, 14 and 28 days of exposure all tested macroinvertebrate species showed very low fiber ingestion, suggesting that no accumulation occurred within the organisms' bodies (Figures 5.5 and S3). This is supported by previous MP accumulation experiments, which showed that *Gammarus fossarum* egests polyamide fibers within similar time frames as food items (Blarer & Burkhardt-Holm 2016). In our study, fiber ingestion was higher at the highest test concentration for all organisms and varied slightly depending on the exposure type (i.e., water vs sediment). For example, ingestion by *H. azteca* was higher when exposed to fibers in the water phase compared to fibers mixed into the sediment (Figures 5.5 and S3). *A. aquaticus* ingested, on average, more fibers than *H. azteca* when exposed to fibers in the water phase at the highest concentration; however, the difference was not statistically significant. In the test systems with sediment exposure, *L. variegatus* ingested more fibers compared to *H. azteca* at the highest test concentration ($p = 0.01$, after 28 days of exposure). The highest fiber concentration tested for *A. aquaticus* was 0.2 g kg^{-1}, where hardly any ingestion was observed for all species. While the average size of fibers in the stock solution was 600 µm, ingested fibers were on average slightly smaller, especially for *A. aquaticus* and *H. azteca* (Figures 5.6 and S4). Furthermore, the average size of

ingested fibers differed slightly depending on the species. *L. variegatus* ingested on average the longest and *A. aquaticus* the shortest fibers (Figure 5.6).

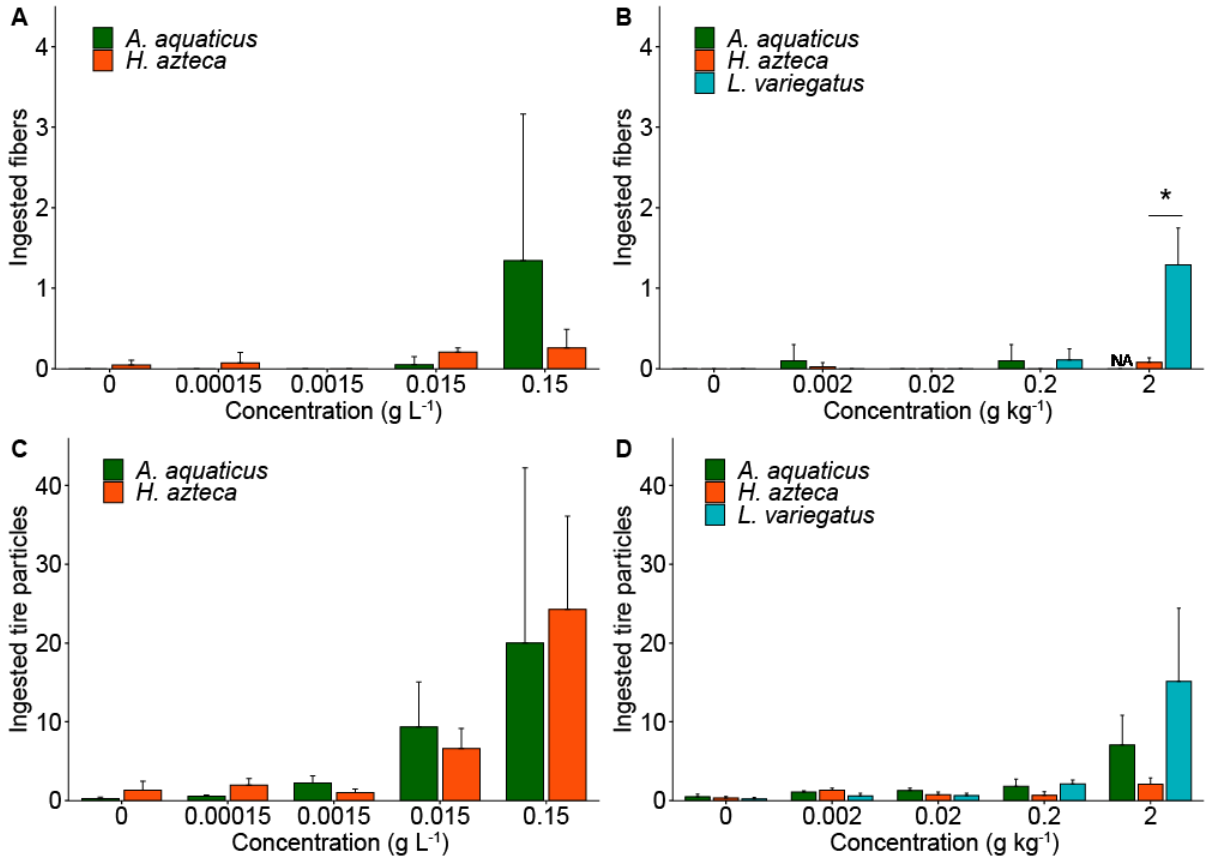


Figure 5.5. Mean number and corresponding standard deviation of ingested MPs per organisms after 28 days of exposure to increasing concentrations of (A) fibers in water, (B) fibers in sediment, (C) tire particles in water, and (D) tire particles in sediment. NA indicated that this concentration was not tested for *A. aquaticus*. Statistically significant differences in ingestion between species are indicated by an asterisk.

Tire particles were ingested by all three macroinvertebrate species. However, no apparent differences in the number of ingested particles were found from day 4 to 28, indicating that tire particles did not accumulate within the organisms (Figures 5.5 and S3). As has been previously observed, some MPs can pass through the digestive tract without accumulation, causing little or no observed adverse effects (Gouin 2020). For instance, Khan et al. (2019) reported a gut clearance time of 24-48h for *H. azteca* exposed to tire particles. The whole size range of particles present in the tire stock solutions was ingested by the tested macroinvertebrates (Figure S2).

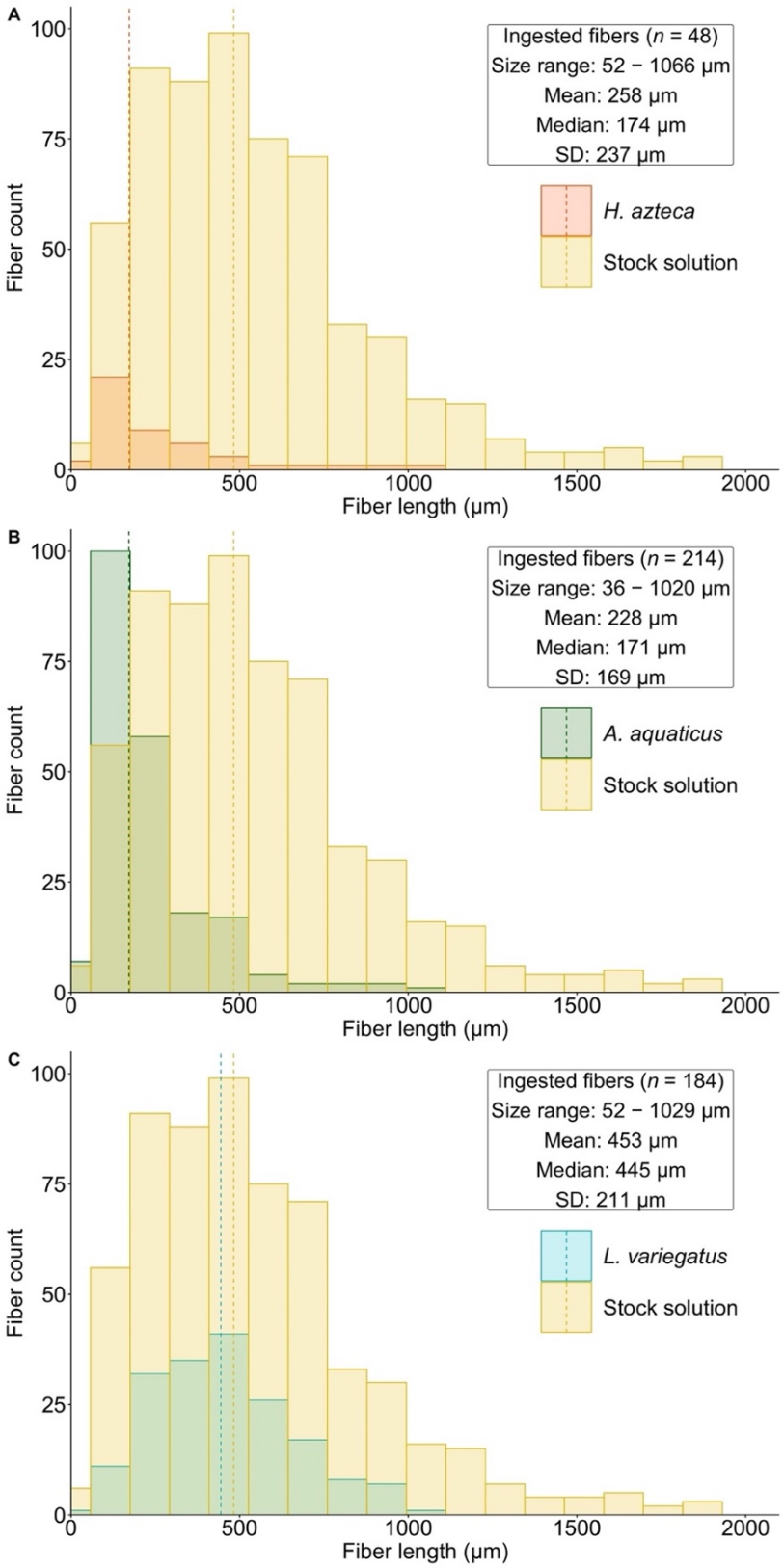


Figure 5.6. Fiber size distribution in the stock solutions and ingested by (A) *H. azteca* and (B) *A. aquaticus* after exposure to fibers dispersed in water, and (C) *L. variegatus* after exposure to fibers mixed into the sediment. The dashed lines display the respective median of the fiber size distribution. The exposure distribution of the stock solutions was: 26 - 5761 µm; mean: 600 µm; median: 482 µm; SD: 559 µm (n = 618). For the stock solutions the size distribution is only shown up to 2000 µm because only 12 out of the 618 measured fibers were outside of this range.

Tire particle uptake was much higher than fiber uptake by all species (Figure 5.5), probably due to the difference in particle size and shape. Lower fiber ingestion compared to other particle types has been documented previously (e.g. fragments; Gray and Weinstein 2017). Furthermore, the exposure pathway influenced MP uptake, i.e., ingestion by *A. aquaticus* and *H. azteca* was higher when exposed to particles in the water phase compared to particles mixed into the sediment (Figure 5.5). As for fibers, *L. variegatus* showed the highest ingestion for tire particles from all three benthic species during sediment exposure. *A. aquaticus* and *H. azteca* – both epibenthic species – were probably not in direct contact with the MPs and thus ingested fewer particles than *L. variegatus*, which is an endobenthic species and feeds directly on sediment particles. MPs partly bury and accumulate in the sediment (Yao *et al.* 2019; Scherer *et al.* 2020), thus endobenthic species may encounter MPs in their natural environment more frequently than epibenthic or pelagic species. Moreover, a higher tire particle ingestion by species following a non-selective feeding strategy (i.e., *L. variegatus* and *D. magna*) was observed. These findings are in agreement with previous studies showing that MP ingestion depends not only on the MPs' size and shape but also on species characteristics like feeding strategy, habitat, or the developmental stage (Scherer *et al.* 2017; Redondo-Hasselerharm *et al.* 2018a; Fueser *et al.* 2020).

No significant effects on survival were observed for *H. azteca*, *A. aquaticus* and *L. variegatus* after acute or chronic exposure to fibers or tire particles (Figures S5 - S7). The solvent controls of the fiber tests showed that the ethanol used in the stock solutions did not influence the survival of *H. azteca* and *A. aquaticus*, nor the reproduction of *L. variegatus*. A slight but not significant decrease in reproduction of *L. variegatus* was observed at the highest tire particle concentration after 28 days (Figure S8). In line with these results, several studies have reported no effects of MPs (including fibers and tire particles) on freshwater organisms (Redondo-Hasselerharm et al. 2018; Setyorini et al. 2021). For instance, Setyorini et al. (2021) assessed the effects of 50,000 PET fibers kg^{-1} with a length of 50 µm on *Chironomus riparius*, showing ingestion but no significant effects. Similarly, Au et al. (2015) observed fiber ingestion by *H. azteca* (polypropylene marine rope; length: 20-75 µm; diameter: 20 µm) but also observed a 10-day LC_{50} of 71,000 MP L^{-1}. The current study shows no effects at such concentration, which may be related to the larger fiber size used in our study and the lower ingestion. In a chronic experiment, polyamide fibers with a length of

500 µm were ingested and decreased food assimilation efficiency of *G. fossarum* at a concentration of 2680 fibers cm^{-2}, which was possibly caused by physical damage inside the digestive tract (Blarer & Burkhardt-Holm 2016).

The NOEC values for tire particles to benthic invertebrates were > 0.15 g L^{-1} for water exposure and > 2 g kg^{-1} dw for sediment exposure. This is in line with previous studies showing no effects on benthic freshwater invertebrates exposed to sediments spiked with tire wear particles up to 10 g kg^{-1} (Panko *et al.* 2013) or with tire particles ground from used tires up to a concentration of 100 g kg^{-1} (Redondo-Hasselerharm *et al.* 2018b). However, tire particles ground from worn and pristine tires have been shown to negatively affect *H. azteca* survival, when dispersed in water only, at concentrations slightly higher than the ones tested in the current study (i.e., 0.2 - 1 g L^{-1} for acute exposure, and 0.6 g L^{-1} for chronic exposure; Halle et al., 2021; Khan et al., 2019).

5.3.3. Risk assessment

To compare our test concentrations with measured environmental concentrations, the mass-based concentrations and the estimated count-based concentrations are shown in Table S1. For water exposure to fibers, the lowest NOEC value observed (0.00015 g L^{-1}) corresponds to approximately 700 fibers L^{-1}. Highest measured fiber concentrations in freshwater ecosystems were in the same order of magnitude (Table 5.2). For tire exposure in the water column, the lowest NOEC observed (0.00015 g L^{-1}) corresponds to about 30,000 particles L^{-1}. Based on mass, the observed NOEC is in the same order of magnitude as modeled maximum tire particle concentrations water column concentrations for the Seine River catchment (France; Table 5.2).

For sediment exposure to fibers, no effects were observed at the highest test concentration (2 g kg^{-1} dw), therefore, it can be concluded that the NOEC for all tested species is above this concentration. This corresponds to approximately 3.20 x 10^7 fibers kg^{-1}, which is much higher than the highest reported environmental concentration (about 1000 fibers kg^{-1}; Deng et al., 2020). Based on mass, the highest concentration of MPs in general (not only fibers) was used as a proxy, which is 1 g kg^{-1} dw sediment (Klein *et al.* 2015), and thus half of the NOEC concentration observed in our study.

Table 5.2. Maximum measured environmental concentrations (MEC) in different environmental compartments and acute EC_{50}-values and chronic NOECs derived from the current study. NA: Endpoint not assessed or concentration not available. Sediment concentrations are reported per kg dw sediment if not indicated otherwise.

		Maximum MEC	**48-h EC_{50}**	**NOEC reproduction**			**NOEC mortality**	
		Fibers/Tire particles	*D. magna*	*D. magna*	*L. variegatus*	*D. magna*	*H. azteca*	*A. aquaticus*
Fibers in water	g L^{-1}	0.00022[a]	> 0.15	0.00015	NA	0.00015	> 0.15	> 0.15
	# L^{-1}	519[a]	NA	6,920	NA	6,920	194,550	194,550
Fibers in sediment	g kg^{-1}	0.00158[b]	NA	NA	> 2	NA	> 2	> 0.2
	# kg^{-1}	1045[c]	NA	NA	> 3.20 x 10^7	NA	> 3.20 x 10^7	> 4.62 x 10^6
Tire particles in water	g L^{-1}	0.0008[d] 0.00012[e]	> 0.15	0.0015	NA	0.00015	> 0.15	> 0.15
	# L^{-1}	NA	NA	120,000	NA	29,300	> 1.25 x 10^7	> 1.25 x 10^7
Tires particles in sediment	g kg^{-1}	0.0023[f] 5.8[g]	NA	NA	> 2	NA	> 2	> 2
	# kg^{-1}	1833[f]	NA	NA	> 7.33 x 10^{10}	NA	> 7.33 x 10^{10}	> 7.33 x 10^{10}

[a]Lahens et al. (2018)

[b]Schell et al. (2021)

[c] Deng et al. (2020); measured 1323 MPs of which 79% were fibers

[d] orignial study by Ni et al. 2008; tire wear concentraition estimated by Baensch-Baltruschat et al. (Baensch-Baltruschat *et al.* 2020)

[e] Unice et al. (2019); concentration modled

[f] Leads and Weinstein (2019); concentration shown in particles kg^{-1} ww

[g] Unice et al. (2013); concentration measured based on polymer maker

Also, for tire particles, the NOEC values for sediments are above 2 g kg^{-1} or 7.33 x 10^{10} particles kg^{-1} dw sediment, which is significantly larger than the highest monitored concentration of tire particles in freshwater sediments (1,833 particles kg^{-1} ww; Leads and Weinstein, 2019). On the other hand, the maximum sediment concentration estimated by Unice et al. (2013) based on polymer markers (5.8 g kg^{-1}) is almost three times as high as the highest tested concentration in this study. However, a previous study observed no adverse effects on benthic freshwater invertebrates exposed to up to 100 g kg^{-1} (Redondo-Hasselerharm *et al.* 2018b), suggesting that no risks are expected for this environmental compartment.

5.4. Conclusions

This study shows that MP type, size, and exposure pathway determine the ingestion capacity of freshwater invertebrates, as well as the observed effect mechanism. Although adverse effects were only observed for the pelagic species tested (*D. magna*), sediment often contains much higher concentrations than the overlying water. Therefore, epibenthic and endobenthic species are more likely to encounter and ingest MPs. Based on the comparison between measured environmental concentrations and the NOEC values determined here, it can be concluded that the current risks for benthic and epibenthic macroinvertebrates are generally low or insignificant, while for pelagic organisms such as *D. magna*, refined exposure and effect studies with fibers and tire particles are recommended.

Acknowledgements

The authors would like to thank Ana María Martín Quesada and Ariadna García-Astillero Honrado for their support in the laboratory. Furthermore, we would like to thank Paula Redondo-Hasselerharm for comments on an earlier version of the manuscript. This study has received funding from the EU JPI-Water initiative IMPASSE project (PCIN- 2017-016), and the μNanoCare (RTC2019-007261-5) and the nanoCLEAN (PID2019-111519RA-I00/AEI/ 10.13039/501100011033) projects, all supported by the Spanish Ministry of Science and Innovation. A. Rico was supported by the Talented Researcher Support Programme - Plan GenT (CIDEGENT/2020/043) of the Generalitat Valenciana.

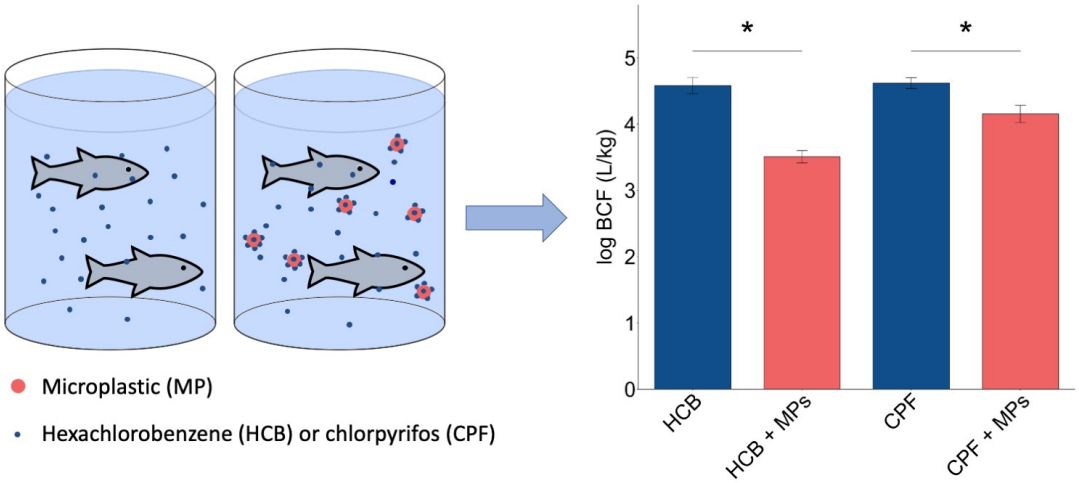
Supporting Information

The Supporting Information for this Chapter can be downloaded at:

https://doi.org/10.1002/etc.5337

CHAPTER 6

Influence of Microplastics on the Bioconcentration of Organic Contaminants in Fish: Is the "Trojan Horse" Effect a Matter of Concern?



This Chapter has been published as:

Schell T, Rico A, Cherta L, Nozal L, Dafouz R, Giacchini R, Vighi M. 2022. Influence of microplastics on the bioconcentration of organic contaminants in fish: Is the "Trojan Horse" effect a matter of concern? Environmental Pollution 306:119473 https://doi.org/10.1016/j.envpol.2022.119473

Abstract

Microplastics (MPs) have been shown to act as sorbent phases and thus carriers of organic chemicals in the aquatic environment. Therefore, concerns exist that MP ingestion increases the uptake and accumulation of organic chemicals by aquatic organisms. However, it is unclear if this pathway is relevant compared to other exposure pathways. Here we compared the bioconcentration capacity of two hydrophobic organic chemicals (i.e., chlorpyrifos and hexachlorobenzene) in a freshwater fish (*Danio rerio*) when exposed to chemicals through water only and in combination with contaminated polyethylene MPs. Additionally, a suite of biomarker analyses (acetylcholine esterase, glutathione S-transferase, alkaline phosphatase, catalase) was carried out to test whether MPs can enhance the toxic stress caused by chemicals. Two 14-day semi-static experiments (one for each chemical) were carried out with adult fish. Each experiment consisted of (1) a control treatment (no chemicals, no MPs); (2) a treatment in which fish were exposed to chlorpyrifos or hexachlorobenzene only through water; (3) a treatment in which fish were exposed to the chemicals through water and contaminated polyethylene MPs (100 mg MPs L^{-1}). Two additional treatments were included for the biomarker analysis. These contained MPs at two different concentrations (5 and 100 mg MPs L^{-1}) but no chemicals. The presence of contaminated MPs in contaminated water did not enhance but rather decreased the bioconcentration of both chemicals in fish compared to the treatment that contained contaminated water in absence of MPs. This was more pronounced for hexachlorobenzene, which is more hydrophobic than chlorpyrifos. Enzyme activity levels in fish were only significantly altered in the presence of MPs for alkaline phosphatase. This study indicates that MP presence in freshwater ecosystems is not expected to increase the risks associated with chemical bioconcentration in aquatic organisms and that other exposure pathways (i.e., uptake via respiration, skin permeability) may be of higher importance.

6.1. Introduction

Microplastic (MP) ingestion has been reported for different freshwater organisms (Galafassi *et al.* 2021; Kumar *et al.* 2021), which may have detrimental physical consequences (Gray & Weinstein 2017; Lei *et al.* 2018; Qiao *et al.* 2019b, a). For instance, histopathological damage in intestines following MP ingestion has been reported in zebrafish (Lei *et al.* 2018; Qiao *et al.* 2019b) and European sea bass (Pedà *et al.* 2016). Furthermore, MPs may pose a risk to aquatic organisms due to the additional effects caused by associated chemicals. Plastics can contain several types of additives (e.g., plasticizers, flame retardants, and stabilizers), which are incorporated during plastic production to receive specific product properties (Hahladakis *et al.* 2018). The leaching of these additives may exert harmful effects in aquatic organisms (Ke *et al.* 2019; Oliviero *et al.* 2019). Moreover, due to MPs' big surface-to-volume ratio and their sorption affinity for hydrophobic organic contaminants, MPs can sorb other environmental contaminants (Teuten *et al.* 2007; Velzeboer *et al.* 2014). The sorption of chemicals to MPs has been confirmed in laboratory studies and for MPs recovered from environmental samples, which contained hydrophobic organic pollutants as well as different drugs and metals (Yu *et al.* 2019; Syberg *et al.* 2020; Amelia *et al.* 2021). Several studies have provided evidence for the transfer of organic contaminants from MPs into animals (Rochman *et al.* 2013; Batel *et al.* 2016, 2018). Consequently, MPs have been proposed as possible contaminant vectors, influencing the uptake of hydrophobic organic chemicals, a phenomenon also termed "Trojan Horse" effect. It has been suggested that the bioaccumulation of these contaminants in environmental media can be enhanced in the presence of MPs as high quantities of sorbed chemicals may be released into the organisms' gut following ingestion, thus favoring chemical uptake (Bakir *et al.* 2014; Coffin *et al.* 2019). Outcomes of a meta- analysis of existing data demonstrated that the presence of MPs significantly enhanced the bioaccumulation of other contaminants by 31% in aquatic organisms under laboratory conditions (Sun *et al.* 2022). However, in most laboratory studies, contaminated MPs are fed to organisms in a clean environment, while in the natural environment, other media also contribute to the organisms' contamination (such as water, sediment, and food) (Koelmans *et al.* 2016; Gerdes *et al.* 2019). Furthermore, modeling studies suggest that the role of MPs as contaminant vectors may have limited relevance compared to other uptake routes (Gouin *et al.* 2011; Lohmann 2017; Mohamed Nor & Koelmans 2019). Thus, current

literature is rather inconclusive on the relevance of contaminated MP ingestion as a novel pathway that enhances the ecological risk of chemicals, and experimental proof using environmentally relevant exposure conditions is rare, especially for freshwater ecosystems (Hartmann *et al.* 2017; Sun *et al.* 2022).

This study aimed to assess the capacity of MPs to act as contaminant vectors for freshwater fish and to compare the bioconcentration capacity of two organic chemicals: chlorpyrifos (CPF) and hexachlorobenzene (HCB). CPF and HCB are both listed as priority substances under the European Water Framework Directive (European Commission 2013), and were chosen in this study due to their environmental relevance and their different hydrophobicity (log K_{OW} 4.7 and 5.8, respectively). In our study, zebrafish (*Danio rerio*) were exposed to CPF and HCB through water only and in combination with contaminated MPs for 14 days. Our experiment was designed to understand to what extent MP contamination may enhance bioconcentration and exacerbate the impact of other contaminants on freshwater organisms. Additionally, the capacity of MPs to enhance or decrease the toxic stress caused by chemicals (i.e., neurotoxicity, general stress, and antioxidant defense) in zebrafish was assessed using a multi-biomarker approach.

6.2. Material and methods

6.2.1. Fish maintenance

Adult zebrafish with an average length of 5 cm and 663 ± 26 mg weight (mean ± SD; n = 6) were purchased from Pisciculture Superior S.L. and were maintained at 26 °C, with a photoperiod of 12/12 hours (light/dark) during three weeks before the start of the experiment. They were fed daily with fish food (Tetramin, Tetra GmbH, Germany) and checked visually to assess their active behavior and ensure proper health conditions.

6.2.2. Test materials

Polyethylene MPs (irregularly shaped with a mean particle size of 150 µm, Sigma-Aldrich) were chosen due to their widespread occurrence in the environment (Schell *et al.* 2020) and their higher sorption capacity for organic chemicals compared to other plastic types observed in the laboratory as well as in field studies (Alimi *et al.* 2018; Syberg *et al.* 2020).

To obtain MPs with sorbed chemicals, 2 g L^{-1} polyethylene particles were added to milli-Q water containing CPF or HCB (Analytical standards, Merck) at concentrations of 200 µg L^{-1} and 36 µg L^{-1}, respectively. Prior to spiking, each chemical was dissolved in methanol. MPs were previously coated with 0.5 ml of the non-ionic detergent Tween® 20 (Scharlau) per g of MP to allow an even dispersion in water. This was performed in glass bottles and the suspension was kept under constant stirring. The same concentration of chemicals and Tween® 20 was added to a water control containing only Milli-Q water and no MPs. The procedure was done twice for each chemical. Sorption of chemicals to MPs and equilibrium state were confirmed by chemical analysis of water at different time points (up to 168 h) before the addition of the contaminated MPs to the experiment. Further details on the chemical-MP partitioning process are provided in the Supplemental Information (SI; Section S1, Tables S1 - S4).

6.2.3. Experimental design

This study consisted of two parts, a bioconcentration analysis and a biomarker analysis. For the bioconcentration analysis two 14-day semi-static experiments (one for each chemical) were carried out with adult zebrafish. Each experiment consisted of: (1) a control without addition of chemicals nor MPs (combined for both experiments; n = 6); (2) a treatment in which fish were exposed to CPF or HCB only through water (n = 6); (3) a treatment in which fish were exposed to the respective chemical through water and contaminated MPs (in this case, 100 mg MPs L^{-1} were added (n = 6)). For the biomarker analysis, the same chemical treatments were used, and in addition, two MP controls containing clean MPs at two different concentrations (5 and 100 mg MPs L^{-1}, n = 3, Table 6.1). The high MP concentration tested (100 mg L^{-1} or 57000 MPs L^{-1}) is around two orders of magnitude higher than maximum measured concentrations in freshwaters based on particle counts (Lu *et al.* 2021b). However, the study was designed as proof-of-principle to test whether MPs could enhance bioconcentration compared to the uptake through water.

Two different scenarios were tested in our study: (1) the presence of contaminated MPs in contaminated water (with CPF as an example) and (2) the addition of highly contaminated MPs to water with a very low chemical background concentration (with HCB as an example). The nominal concentrations are shown in

Table 6.1. Each test unit consisted of a 3 L beaker with dechlorinated tap water containing six fish. MPs were kept distributed throughout the water phase by aerating the beakers. The test medium was renewed on days 3, 7, and 10 after the start of the experiment. Fish were fed before the start of the experiment and before each medium renewal. Fish, water, and MP samples were taken at the start of the experiment and after each exposure period (i.e., at each medium renewal and the end of the experiment after 14 days). In each sampling event, one fish sample was taken per replicate and euthanized by rapid cooling (Wallace *et al.* 2018). The digestive tract was separated from the remaining fish tissue and samples were stored frozen until analysis. Water samples were taken from the test beakers before and after medium renewal using glass pipettes (4 mL for CPF and 10 ml for HCB approximately 30 min after spiking, and 150 ml for both chemicals at the end of each exposure period). To obtain the MP samples, 50 mL of stock suspensions (to measure initial chemical load) and 500 mL of test medium before medium renewal were taken using glass pipettes. These samples were filtered onto glass filter papers (Borosilicate filter GMFF, Scharlau) to separate the MPs. All samples were stored frozen until analysis. Water temperature, pH, conductivity, and dissolved oxygen were measured before and after each medium renewal with a multiparameter meter (Hanna HI91894).

Table 6.1. Nominal test concentrations of the different treatments used in the experiments.

Treatment	Chemical water concentration (µg L^{-1})	Chemical concentration sorbed to MPs (µg g^{-1})	MP concentration (mg L^{-1})
Control	0	-	0
CPF	15.5	-	0
CPF + MPs	15.5	45	100
HCB	4.2	-	0
HCB + MPs	0.6	16	100
MP Low	0	0	5
MP	0	0	100

6.2.4. Analytical method

A gas chromatograph (GC) system (Agilent 7890A) coupled to a mass spectrometer (MS) with a triple quadrupole analyzer (Agilent 7000 GC/MS Triple Quad) was used to determine the concentration of CPF and HCB in water, MPs, and fish samples. Details on the GC-MS conditions are provided in the SI (Section 2.1, Table

S5). Prior to the instrumental analysis, different extraction methods of CPF and HCB from the different matrices were applied. Water sample treatments were based on Stir Bar Sorptive Extraction (SBSE, also called Twister extraction). For MP and fish tissue, sample treatments were based on a solid-liquid extraction and an additional cleanup step for fish. Details on all sample treatments are outlined in the SI (Section S2.2). Isotopically labeled internal standards (ILIS), hexachlorobenzene-13C6 and chlorpyrifos-(diethyl-d10), were used to correct possible losses during sample treatment and/or instrumental deviations.

Validation of the three analytical methodologies (including sample treatment and instrumental analysis) was performed in order to provide quality assurance of the results (conditions of the validation study are shown in the SI, Section 2.3). The method was validated for linearity, accuracy, precision, and limits of detection (LOD) and quantification (LOQ), with satisfactory results. Mean recovery (n = 3) was between 90 and 103% for chemicals extracted from water and fish samples, and between 83 and 104% from MPs, with good precision expressed as relative standard deviation. Detailed results can be seen in Table S6.

During sample analysis, daily quality controls (spiked matrices as done in the validation study) were injected to ensure the reliability of the results.

6.2.5. MPs detection and counting

To assess MP ingestion by fish, the digestive tract of the organisms exposed to MPs was digested using 15% H_2O_2 for 48 hours at 50 °C. The solution was then vacuum filtered onto filter papers (Borosilicate filter GMFF, Scharlau) and retained until visual assessment. MPs were counted using a stereo microscope (Olympus SZX7) with 40x magnification. Recovery tests (n = 3) were carried out by adding 25 polyethylene MPs to the digestive tract of two clean fish from the fish stock and 10 ml H_2O_2. This was kept for 48 hours at 50°C, followed by the same filtration and visual analysis procedure as described above. Recovery of MPs based on MP count showed satisfactory results (between 88 and 92%), with no visible changes of the MP particles observed.

6.2.6. Biomarker determination

The activity of four enzymes was measured in the current study: acetylcholinesterase (AChE), catalase (CAT), glutathione S-transferase (GST), and alkaline phosphatase (ALP). Seven treatments were considered for the biomarker analysis (Table 6.1). Fish samples were taken at the end of the experiment (day 14) from each treatment and immediately frozen in liquid nitrogen. Samples were stored at -80°C until analysis. Control fish from the fish stock were also sampled at the start (day 0) of the experiment. Fish specimens were divided into head (for AchE analysis) and body (for the remaining enzymes). For each treatment, six specimens were analyzed, and two replicates of the spectrophotometric measurement were performed. The detailed analytical procedures are outlined in the SI (Section S3).

6.2.7. Data analyses

The CPF and HCB bioconcentration factors (BCFs) were calculated as the ratio of measured chemical concentrations in fish and water at the end of each exposure period (i.e., 3, 7, 10 and 14 days), for the treatments containing chemicals alone and for the treatments containing chemicals in the presence of MPs. Normal distribution and equality of variances of the calculated BCF values were tested using Shapiro–Wilk test and Bartlett's test, respectively. Significant differences between the calculated BCF in the water only treatment and the water and MPs treatment were assessed for each sampling day by preforming student t-tests using a significance level of 0.05.

For the analysis of biomarker responses, the measured enzymatic activities were log transformed when data did not meet the requirements for parametric testing. Student t-tests were used to compare the enzymatic activities of the control treatments on day 0 and day 14. One-way analysis of variance (ANOVA) followed by a Tukey post hoc multiple comparison tests was used to test differences in enzymatic activities levels induced by different MP concentrations compared to the control. To assess differences in enzymatic activities between the treatments containing only MP at the higher concentration, only chemicals, and chemicals with MP, a two-way ANOVA followed by a Tukey post hoc multiple comparison tests was performed. Statistical analyses, including the construction of graphs, were performed using the software R Version 4.1.1 (R Core Team, 2021) in RStudio (RStudio Team, 2021) or the R language-based software Jamovi (The jamovi project 2021) and the required extension

packages (Wickham 2016; Kassambara 2017; Fox & Weisberg 2019). Statistically significant differences were assumed when the calculated p-value was < 0.05.

6.3. Results

6.3.1. Fish survival and MP ingestion

No mortality was observed due to exposure to chemicals, MPs, or the combination of both during the experiment. One fish died in the MPs only treatment containing the lower MP concentration (5 mg L^{-1}), possibly due to damage during medium renewal and manipulation. Ingestion of MPs by fish was confirmed but varied between fish and sampling times (Table 6.2). The number of ingested MPs in the CPF with MP treatment was relatively stable over time with an outlier on day 7. In the HCB with MP treatment, the concentration of MPs extracted from the digestive tract of the fish seemed to increase over time. Small amounts of contamination in control samples have been observed. However, this contamination likely occurred during sample processing and not due ingestion of MPs by fish.

Table 6.2. Mean MP particle ingestion (min-max) displayed in number of MPs for different treatments at different time points (day 0 to day 14). Calculated by dividing the total number of MPs extracted from the digestive tracts of the fish of one sample by the number of fish. NA: time point was not assessed.

Treatment	Day 0	Day 3	Day 7	Day 10	Day 14
Control	0 (0 - 0)	0.2 (0 - 0.5)	0.3 (0 - 1)	0 (0 - 0)	2.5 (0.5 - 4)
CPF	NA	0.2 (0 - 0.5)	0 (0 - 0)	0 (0 - 0)	0.2 (0 - 0.5)
HCB	NA	0.2 (0 - 0.5)	0.2 (0 - 0.5)	0 (0 - 0)	0 (0 - 0)
CPF + MP	NA	163 (15 - 353)	962.5 (203 - 1720)[a]	167 (29 - 436)	80.3 (53 - 150)
HCB + MP	NA	10.8 (3.5 - 21.5)	18 (5 - 42.5)	66.7 (14 - 112)	417 (256 - 632)

[a] one sample was lost, the mean ingestion was calculated from two samples only.

6.3.2. Chemical analysis

The results of the measured chemical water concentrations in the stock suspension are provided in the SI (Table S8). The chemical concentrations on MPs before their addition to the test medium were relatively stable over time (Tables S9 and S10). The CPF concentration on MPs decreased strongly after the three to four days in the test medium (measured prior to medium renewal), probably caused by the establishment of a new equilibrium as a result of the lower CPF concentrations and the

different physical chemical conditions in the test medium compared to the stock suspension. However, an increase in concentration was observed along with a temporal trend. The calculated MP-water partitioning coefficients (K_{PW}; Table S11) were higher than those calculated for the stock suspension, suggesting that CPF was strongly sorbed to the MPs. The HCB concentrations measured on MPs in the test medium after the three to four days were also lower, indicating a new partitioning equilibrium was established. Again, the K_{PW} were higher than those calculated for the stock suspension, suggesting that also HCB is strongly sorbed to the MPs (Table S11).

The measured CPF concentrations in the test medium are shown in Tables S12 and S13. Most of the chemical dissipated (on average 96% for the CPF only treatment and 93% for the CPF with MPs treatment) within the three to four days until the next medium renewal. Dissipation was probably a cause of microbial degradation, uptake by fish, sorption to MPs and the wall of the test vessels or organic suspended material. The measured HCB concentrations are shown in Tables S14 and S15. In both treatments (HCB only and HCB with MPs), the HCB water concentrations were lower than the nominal values. As for CPF, most of the chemical dissipated within three to four days until the next medium renewal (i.e., 93% for the HCB only treatment, and 73% for the HCB with MPs treatment).

The CPF concentration in fish tissue increased over time in the CPF only and the CPF with MP treatment (Figure 6.1A). The CPF concentrations were almost identical in the two treatments (i.e., CPF only and the CPF with MP) after three and seven days relative to the start of the experiment. At days 10 and 14, relative to the start of the experiment, a lower CPF concentration was observed in fish in the CPF with MP treatment compared to the CPF only treatment. Although a steady state had not been reached within the 14 days of the experiment, the CPF concentration increase rate in fish tissue notably slowed down over time (Figure 6.1A).

For HCB, the steady state in the body concentration was reached within the time of the experiment (Figure 6.1B). The concentration in the HCB only treatment was higher compared to the HCB with MP treatment. This was expected based on the experimental design, as less HCB was added to the water containing MPs.

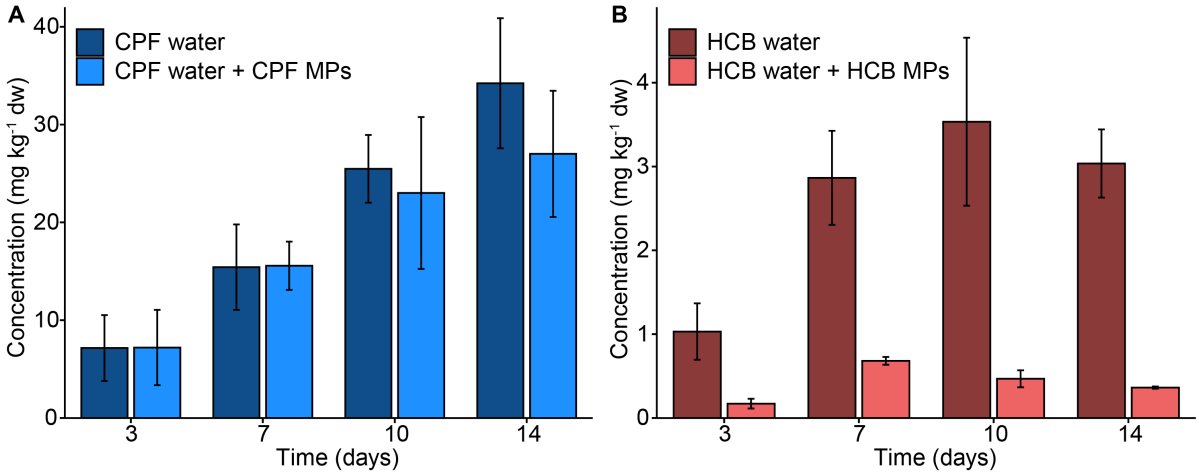


Figure 6.1. Concentration of (A) chlorpyrifos and (B) hexachlorobenzene measured in fish tissue exposed only through water or through the combination of water and MPs over a period of 14 days. The values show the mean of the three measurements per treatment on sampling days 3 - 10 and the six measurements on sampling day 14 with their corresponding Standard Deviation.

6.3.3. Bioconcentration factors

In general, the BCF of CPF was higher in the CPF only treatment compared to the CPF with MP treatment, showing statistically significant differences on day 14 relative to the start of the experiment (Figure 6.2A). The BCF for the HCB only treatment was also higher (about one order of magnitude) as compared to the HCB with MP treatment, showing statistically significant differences for the entire exposure period (Figure 6.2B).

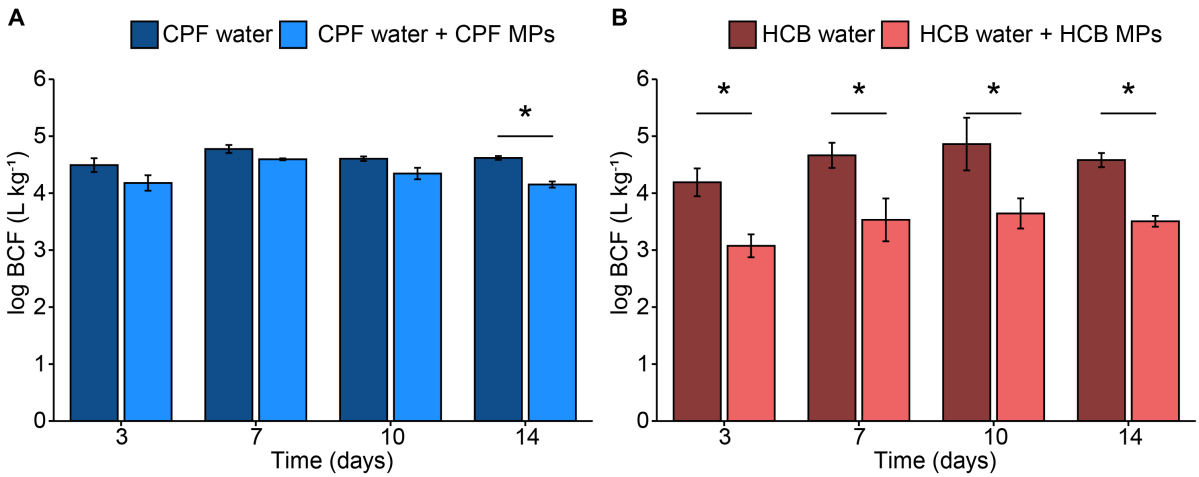


Figure 6.2. Bioconcentration factors of (A) chlorpyrifos and (B) hexachlorobenzene for fish exposed only through water or through the combination of water and MPs over a period of 14 days. The values show the mean of the three measurements per treatment on sampling days 3 - 10 and the six measurements on sampling day 14 and the corresponding Standard Deviation. Asterisks indicate statistically significant differences ($p < 0.05$) between treatments in each sampling time.

6.3.4. Biomarkers

The measurements of the enzymatic activities are shown in Figure 6.3 No statistically significant differences between the control fish samples taken at the start of the experiment (day 0) and the control fish samples taken at the end of the experiment (day 14) were observed. The different chemical and MP treatments were only sampled at the end of the experiment; thus, the following enzymatic activity comparisons refer to statistical tests performed between the different chemical and MP treatments and the control samples taken at the end of the experiment.

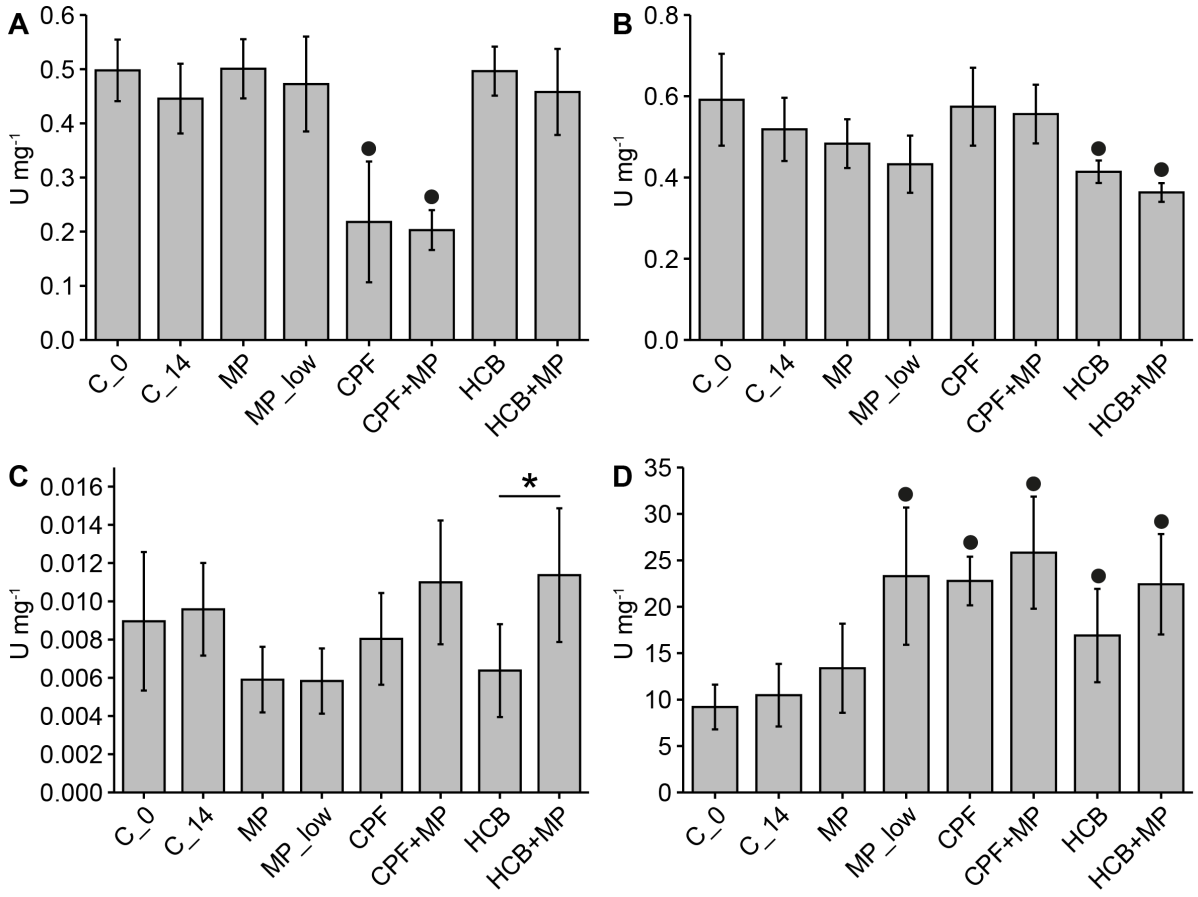


Figure 6.3. Enzymatic activities of (A) acetylcholinesterase, (B) glutathione S-transferase, (C) alkaline phosphatase, and (D) catalase to the different treatments. The graphs show mean enzymatic units "U" per mg protein with the corresponding standard deviation. Dots indicate statistically significant ($p < 0.05$) differences between C_14 and all other treatments. The asterisk indicates statistically significant ($p < 0.05$; Tukey post hoc multiple comparison test) interaction between the treatments containing HCB and the treatment containing HCB and MP. C_0: control at the start of the experiment; C_14: control at the end of the experiment; MP_low: MP only treatment containing 5 mg MP L^{-1}; MP: MP only treatment containing 100 mg MP L^{-1}.

The results of the ANOVA show that AChE was significantly inhibited by CPF, while there was no significant interaction between the AChE activity measured in the CPF only treatment and the CPF with MP treatment. No statistically significant differences were observed between the AChE activities of HCB only and the control, neither with those in the HCB with MP treatment (Figure 6.3A).

The GST activity was not influenced by CPF nor by the treatment containing CPF and MPs. The GST activity was significantly decreased by the HCB treatment, and the treatment containing HCB and MPs, however there were no significant differences between these two treatments (Figure 6.3B).

The exposure to HCB and CPF alone, and to MPs alone, resulted in a slight (not significant) reduction of the ALP production, however the combination of the test chemicals with MPs increased the ALP production, becoming statistically significant for the HCB with MP treatment (Figure 6.3C).

All treatments, except MP alone at the higher concentration, produced a statistically significant increase of CAT. For both chemicals, the presence of MPs induced a slight increase in the enzyme's activity, however the differences were not statistically different compared to the chemical only treatments (Figure 6.3D).

6.4. Discussion

The presence of MPs in contaminated water did not enhance but rather decreased the bioconcentration of both tested chemicals in fish compared to the absence of MPs. Therefore, our results show that direct water uptake (via skin and gills) is a more relevant uptake route for fish compared to the ingestion of contaminated MPs, even though MPs were present at higher concentrations than those reported in the environment. Therefore, under environmentally relevant conditions, the MP portion taken up by fish would be lower, which further reduces the importance of MPs for the chemical uptake.

These findings are in agreement with other experimental studies which observed that the surrounding medium is more important for the uptake of chemicals compared to the uptake along with MPs. In these studies, MPs either did not affect the bioaccumulation of polychlorinated biphenyls (Besseling *et al.* 2017) or the

bioconcentration 4-n-Nonylphenol and 4- Methylbenzylidene-camphor (Beiras *et al.* 2019), or decreased the bioconcentration of the triazole fungicide difenoconazole (Li et al. 2021) compared to chemical exposure only through the surrounding medium. Chemical uptake via gills and skin goes directly into the bloodstream, while ingested chemicals may be partly metabolized in the intestinal tract prior to uptake into blood (e.g., Pickford et al. 2003). Nonetheless, also food is likely a more relevant uptake route compared to MPs, particularly for chemicals with high biomagnification potential (i.e., persistent, not eliminated or reduced through metabolism by the organism). Higher transfer of contaminants from dried fish food compared to MPs has been shown in marine amphipods (Scopetani *et al.* 2018). During digestion, the substances that are accumulated in the food are released into the digestive tract while MPs are non-digestible and thus may maintain their sorption capacity (although possibly influenced by the digestive fluids, e.g., Bakir et al. 2014; Coffin et al. 2019) and therefore their chemical load.

Previously a "cleaning effect" following plastic ingestion has been proposed by experimental and modeling studies (Gouin *et al.* 2011; Gerdes *et al.* 2019; Mohamed Nor & Koelmans 2019). Gouin et al. (Gouin *et al.* 2011) predicted that for chemicals with a log K_{OW} between 6.5 and 7.5, the chemical body burden can be reduced by > 20%, if the diet is composed of 10% MP. The underlying principle is that the ingestion of clean MPs and their high sorption affinity for organic chemicals favor the transfer to plastic within the gastrointestinal tract and decreases the body burden (Gouin *et al.* 2011). This was also shown by the modelling study of Mohamed Nor and Koelmans (2019), where polychlorinated biphenyls were estimated to be transferred from contaminated food to clean polyethylene MPs in a simulated gut condition. However, these authors also observed that, in an environmentally relevant chemical and MP exposure scenario, the presence of plastic might not influence the chemical uptake (Mohamed Nor & Koelmans 2019). This is supported by an experimental study, in which no higher elimination rates of polychlorinated biphenyls by rainbow trout were observed if clean polyethylene microspheres were ingested with contaminated food compared to the fish that did not ingest plastic (Rummel *et al.* 2016). Both sorption and desorption of chemicals to and from MPs in the organism's gut depend on the gut retention time; therefore, a short gut retention time may not allow sufficient time to transfer chemicals from or to biota (Chua *et al.* 2014; Mohamed Nor & Koelmans 2019). In our study, we did not observe a temporal increase of the number of MP particles

inside the fish gut in the treatment containing CPF with MPs, indicating that MPs were not accumulated and that the gut retention time of CPF was not prolonged. A slightly different pattern in ingested MPs was observed for the HCB with MP treatment, which was very low during the first two sampling days and increased slightly on day 10 and 14.

Besides the potential "cleaning effect" described above, it has been suggested that MPs can lower the bioconcentration/bioaccumulation potential of chemicals because they can alter the equilibrium between biota and the exposure media, reducing chemical bioavailability (Teuten *et al.* 2007; Chua *et al.* 2014; Rehse *et al.* 2018). From the available bioconcentration results, it was difficult to decide whether MPs facilitated a higher depuration from the organisms by sorbing chemicals in the digestive tract or if MPs sorbed a higher proportion of chemicals already from the water and made them therefore less available, or a combination of both. However, based on the measured concentration of chemicals on MPs and in the test medium, the K_{PW} calculated for both CPF and HCB was much higher in the test medium compared to the ones calculated in the stock suspensions (Table S11), suggesting reduced availability of the chemicals. Such an increase in the K_{PW}, may have been influenced by the different physicochemical conditions (i.e., pH, temperature, concentration of dissolved carbon) of the test medium as discussed in several studies (Velzeboer *et al.* 2014; Wang & Wang 2018; Seidensticker *et al.* 2019; Wang *et al.* 2020). Moreover, the decrease in bioconcentration in presence of MPs was higher for HCB, which is the most hydrophobic chemical of the two tested. This supports the already established relationship between hydrophobicity and the sorption affinity of chemicals to MPs (Razanajatovo *et al.* 2018). HCB was less available to fish because it was bound strongly to MPs, indicated by the higher log K_{PW} (Table S11).

In our study, we evaluated two different environmental scenarios: the presence of contaminated MPs in contaminated water (with CPF as example), where a theoretical equilibrium between MPs and water was reached, and the addition of contaminated MPs into water with a low exposure concentration (with HCB as example). The later represents a scenario in which MP are transported from contaminated areas to remote environments. Our experiment showed that even in a scenario of low water exposure concentration (i.e., the HCB one), MPs did not increase the bioconcentration of chemicals in fish. Furthermore, as contaminated MPs were

added to water with a very low background exposure concentration, desorption of HCB from MPs occurred and a new equilibrium between the MPs and water was reached, which would also happen under natural conditions.

Our study showed that the exposure to MPs alone, at two different concentrations, had no significant effects on the tested biomarkers, with exception for the CAT response to the lower MPs concentration. Most studies assessing effects of MPs on biomarker response were carried out using small MPs (≤ 20 µm). These showed that MPs can alter biomarker responses including oxidative stress, neurotoxicity, and inflammatory effects depending on the MP size, dose and the exposure time (Karami *et al.* 2017; Prokić *et al.* 2019). The here observed influence of CPF on AChE was expected as AChE is an indicator for neurotoxicity and its activity is usually decreased in the presence of organophosphate and carbamate insecticides (Amiard-Triquet *et al.* 2013). However, MPs (despite slightly reducing CPF internal concentrations) did not alter the fish response to CPF. CAT and GST are both indicators for oxidative stress. GST is involved in detoxification processes of xenobiotics (Nimmo 1987) and although a decreased activity of this enzyme was observed in fish exposed to HCB alone, the presence of MP did not significantly affect the inhibitory effect of HCB. Similar to our results, no effect of MPs on the AChE and GST activity were found in juveniles of the common goby *Pomatoschistus microps* when exposed to pyrene (Oliveira *et al.* 2013). CAT belongs to the first line defense enzymes and protects cells from oxidative damage. Its activity was increased by both chemical treatments, indicating a stress condition. However, the presence of MPs did not induce significantly higher levels of CAT, suggesting that MPs had no influence on chemical stress. Finally, ALP is a plasma membrane-bound enzyme which is involved in phosphate hydrolysis. There was a trend of a slight reduction in ALP activity compared to the control by exposure to any of the tested chemicals, and by MPs alone at both tested concentrations. Fish exposed to HCB with MPs showed a significantly higher ALP activity compared to the fish exposed to HCB only. This trend was also observed for CPF. Food intake and food quality have previously been reported to cause changes in intestinal ALP (Lallès 2020). MP ingestion may have led to food dilution (Amariei *et al.* 2022) and thus affected the ALP activity. However, in this study, the ALP activity was measured in the whole-body extract of the fish, therefore, the alteration may also be attributed to ALP changes in other body parts (e.g., in blood or liver). Similar to our results, the ALP activity in the body tissue of juvenile Amazonian

cichlids (*Symphysodon aequifasciatus*) was significantly reduced at 200 µg L^{-1} of MPs, which the authors attributed to deficits in digestive capabilities (Wen *et al.* 2018a). A significant increase in ALP activity was measured in the blood of the common carp (*Cyprinus carpio*) after exposure to MPs alone (2 mg L^{-1}), the herbicide paraquat alone, and a combination of both (Nematdoost Haghi & Banaee 2017). These authors attributed the increase of ALP in plasma to the damage of cell membranes in the liver, bile ducts, and damage to the mucous lining of the intestine and the renal tract.

6.5. Conclusions

Our study shows that the presence of MPs in water exposure media does not increase the bioconcentration of hydrophobic organic chemicals in fish. This indicates that MP ingestion could be considered a negligible chemical uptake route as compared to other exposure routes, supporting the findings shown by former experimental and modeling studies. No consistent trends on the influence of MPs on the fish enzymatic activities could be elucidated from this study. Therefore, we conclude that MP concentrations above current exposure levels are not likely to increase fish stress levels, neither increase hydrophobic organic chemical exposure and risks to fish in freshwater ecosystems.

Acknowledgements

This study has been conducted as part of the EU JPI-Water initiative IMPASSE project (PCIN- 2017-016) supported by the Spanish Ministry of Science and Innovation. A. Rico acknowledges the Talented Researcher Support Programme - Plan GenT (CIDEGENT/2020/043) of the Generalitat Valenciana. The fish experiment was approved by the Spanish Competent Authority (Reference: PROEX 222/18). We thank the personnel of the Animal Experimental Centre of the University of Alcalá for their collaboration in the experiment and Ana María Martín Quesada for her support in the laboratory.

Supporting Information

The Supporting Information for this Chapter can be downloaded at: https://doi.org/10.1016/j.envpol.2022.119473

CHAPTER 7

Conclusions and Recommendations

7.1. Conclusions

Plastic pollution is an increasingly recognized problem of global scale. Research into microplastic (MP) contamination and its consequences has been rapidly expanding in recent years. Yet important data gaps hinder reliable source apportionment and environmental risk assessment. This thesis was undertaken to determine the current state of knowledge on freshwater and terrestrial plastic and MP pollution, their sources, environmental fate and pathways, and to identify data gaps to support research into environmental plastic accumulation and their ecotoxicological characterization. Furthermore, this thesis assessed MP pollution in rivers and agricultural soils of the Mediterranean climate zone focusing on wastewater and sewage sludge as key entry pathways, and the role of surface water runoff as a transport mechanism for MPs into aquatic ecosystems. Finally, this thesis contributed to determining the impacts of two specific MP types, polyester fibers and car tire particles, on freshwater invertebrates and has provided a deeper insight into the relevance of MP as vectors for other chemical pollutants into aquatic organisms ("Trojan Horse" effect).

The following main conclusions were derived from this thesis:

- Quantitative information on MP sources, their pathways into and between freshwater and terrestrial ecosystems, and exposure concentrations (especially of small-sized MPs, nanoplastics and macroplastics) in these ecosystems are still limited and, if available, mostly restricted to Europe, North America, and China (at the time the literature review was completed in Spring 2019) (Chapter 2).
- MP exposure in river water and sediment of the Henares River and its tributaries increases considerably with anthropogenic pressures (downstream wastewater treatments plant inlets and industrial and urban areas) and varies temporally related to season and weather events (Chapter 3).
- Wastewater represents a significant environmental pathway for MPs into the Henares River catchment, contributing up to 50% of the MP discharge, despite high MP retention by wastewater treatment plants (WWTPs) and minor untreated wastewater discharge (Chapter 3).

- Sludge application significantly increases MP soil contamination, and these MPs are found to remain in the soil over time, with low infiltration into deeper soil layers, suggesting that agricultural soils can be considered long-term MP accumulators (Chapter 4).
- Agricultural surface runoff from sewage sludge amended fields appears to be of limited relevance as a pathway for MPs into aquatic ecosystems under semi-arid conditions (mobilizing only 0.2 - 0.4% of the MPs added with sludge to soil) (Chapter 4).
- Based on the comparison between measured or modeled environmental concentrations and experimentally determined No Observed Effect Concentrations (NOECs), risk of polyester fibers and car tire particles, is currently low for benthic and epibenthic macroinvertebrates, while some pelagic organisms (shown for *D. magna*) may be negatively affected (Chapter 5).
- Ingestion capacity and quantity of MPs by freshwater invertebrates and their effect mechanisms depend on MPs' size and shape. Ingestion quantity further depends on species-specific traits such as their habitat, their MP encounter frequency, body size, and feeding strategy (Chapter 5).
- MPs have a negligible contribution to the uptake of hydrophobic organic contaminants by fish and do not enhance the biological stress caused by them (measured as biomarker response) (Chapter 6).

7.2. Recommendations for further research and MP pollution management

In this thesis, adverse effects of MPs for freshwater organisms were only observed for pelagic organisms at MP water concentrations that, for the most part, are not yet environmentally relevant. However, the persistence of plastics and their continued release suggest increased pollution levels in the future, possibly reaching those concentrations and further elevating sediment contamination levels. Therefore, future research efforts should focus on courses of action that limit environmental MP release.

Based on the results of this thesis, effective measures to reduce environmental MP emissions include limiting MP release from urban wastewater by implementing WWTPs in areas that are not yet connected to the sewerage system (particularly in less developed regions). Furthermore, it is important to increase MP retention

efficiencies in existing WWTPs, especially for smaller MPs, by optimizing wastewater and stormwater treatment where treatment facilities are already in place. This is also expected to help minimize the environmental release of the MP types (textile fibers and car tire particles) that were studied in this thesis and have been shown to pose adverse effects (Chapter 5). Intercepting fibers already before they enter wastewater by adding filters to washing machines and dryers could be another possibility to increase their retention. However, not all of these particle types can be captured in this way. For instance, airborne fibers and tire wear particles not collected in sewer systems would still be emitted. These diffuse pollution sources are hard to avoid and more challenging to capture. Therefore, improved road runoff management solutions, such as vegetated buffer strips or artificial wetlands, may be proposed to retain MPs before reaching natural water bodies.

Moreover, MPs retained by WWTPs in the sewage sludge (Chapters 2, 3, and 4) enter the environment if sludge is re-purposed as agricultural fertilizer. Although this may limit their release to aquatic ecosystems, MPs added with sludge are maintained in the soil over extended periods, possibly building up concentrations after repeated sludge applications (Chapter 4). In this context, there is an urgent need to determine at which concentrations MPs may harm different parameters of soil ecosystems, including soil physicochemical properties, the structure, and function of microbial and invertebrate communities, as well as crop yields and development. Concentrations of MPs in sludge are currently not regulated, and no restrictions to MP quantities in sludge applied to agricultural soils exist. The importance of this MP pathway compared to MPs formation from other sources, such as plastic mulch and mismanaged plastic waste, needs to be determined to ponder the risks and benefits of sludge application and to provide recommendations for adequate mitigation decisions for policy frameworks.

Furthermore, the here derived estimations on the relevance and contribution of different MP pathways (wastewater vs. agricultural runoff; Chapters 2 and 3) may vary notably depending on soil type, crop type, agricultural practices (e.g., sludge vs. plastic mulch application), or geographic settings (i.e., drier vs. wetter areas, frequency of heavy rain and storm events, land use, efficiency of plastic waste collection and recycling rates). Agricultural runoff may be a relevant MP release pathway into freshwater ecosystems in locations with higher annual precipitation or heavy events, such as northern Europe or the tropics, whereas the relative contribution of treated

wastewater may be reduced in these settings due to a higher dilution of the discharged wastewater by the receiving water bodies. While this does not affect the total MP export, dilution directly influences the exposure risk for aquatic organisms, which may not only vary spatially but as shown in this thesis, temporally according to season and weather events (Chapter 3). Therefore, additional efforts are required to reliably determine exposure concentrations, including high-resolution temporal and spatial samplings in different geographical settings, to fully understand MP sources and spatial and temporal pollution patterns at the river catchment scale.

While this thesis provided some new insight into the role of two environmental pathways, it is imperative to determine the origin of the remaining MP loads at a catchment scale, to which no source could be assigned based on the research conducted within this thesis. Fragmentation of macroplastics is expected to be a major source for the river catchment. This assumption is based on macroplastic waste accumulated at the river shores based on the visual inspection of the area around the sample site located at the mouth of the river catchment and fragments being the primary MP type observed in river water and sediment (Chapter 3). During rain events, urban surface runoff and combined sewer overflow may be another important MP contribution to the catchment that has to be quantified. The quantification of these sources with experimental and modeling studies will aid in the identification of specific risk mitigation measures.

Finally, MPs are found as complex mixtures in the environment (Chapters 2, 3, and 4). Therefore, not only the effect assessment of frequent MP types but of their mixtures, taking into account the ratio of different sizes, shapes, and polymer types, are recommended to predict the vulnerability of aquatic organisms to MP pollution. Based on the results of the current thesis (Chapter 5), refined water exposure assessments with polyester fibers and car tire particles are required; and follow-up studies are needed with a wider array of aquatic organisms to determine risks considering different effect mechanisms (i.e., entanglement, ingestion, leaching). Furthermore, the specific properties of the particles responsible for the tire particles' toxicity, which are the particle itself, incorporated metals and additives, or a combination of both, could not be elucidated within this thesis and should be determined further.

REFERENCES

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

Adam, V., Yang, T. & Nowack, B. (2019). Toward an ecotoxicological risk assessment of microplastics: Comparison of available hazard and exposure data in freshwaters. *Environ. Toxicol. Chem.*, 38, 436–447.

Adane, L. & Muleta, D. (2011). Survey on the usage of plastic bags, their disposal and adverse impacts on environment: A case study in Jimma City, Southwestern Ethiopia. *J. Toxicol. Environ. Heal. Sci.*, 3, 234–248.

Akarsu, C., Kumbur, H., Gökdağ, K., Kıdeyş, A.E. & Sanchez-Vidal, A. (2020). Microplastics composition and load from three wastewater treatment plants discharging into Mersin Bay, north eastern Mediterranean Sea. *Mar. Pollut. Bull.*, 150, 110776.

Albertsson, A.-C., Andersson, S.O. & Karlsson, S. (1987). The mechanism of biodegradation of polyethylene. *Polym. Degrad. Stab.*, 18, 73–87.

Alimi, O.S., Farner Budarz, J., Hernandez, L.M. & Tufenkji, N. (2018). Microplastics and Nanoplastics in Aquatic Environments: Aggregation, Deposition, and Enhanced Contaminant Transport. *Environ. Sci. Technol.*, 52, 1704–1724.

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

Amariei, G., Rosal, R., Fernández-Piñas, F. & Koelmans, A.A. (2022). Negative food dilution and positive biofilm carrier effects of microplastic ingestion by D. magna cause tipping points at the population level. *Environ. Pollut.*, 294, 118622.

Amelia, T.S.M., Khalik, W.M.A.W.M., Ong, M.C., Shao, Y.T., Pan, H.J. & Bhubalan, K. (2021). Marine microplastics as vectors of major ocean pollutants and its hazards to the marine ecosystem and humans. *Prog. Earth Planet. Sci.*, 8,12.

Amiard-Triquet, C., Amiard, J. & Rainbow, P. (2013). *Ecological Biomarkers. Indicators of Ecotoxicological Effects*. CRC Press, Boca Raton.

Anderson, P.J., Warrack, S., Langen, V., Challis, J.K., Hanson, M.L. & Rennie, M.D. (2017). Microplastic contamination in Lake Winnipeg, Canada. *Environ. Pollut.*, 225, 223–231.

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

ASTM. (2005). Standard test methods for measuring the toxicity of sediment-associated contaminants with freshwater invertebrates. ASTM E1706-05. In: *ASTM Annual Book of Standards*. ASTM International, pp. 1138–1220.

ASTM. (2007). Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians. ASTM Standard E729 - 96. In: *ASTM annual book of*

standards. ASTM International.

Au, S.Y., Bruce, T.F., Bridges, W.C. & Klaine, S.J. (2015). Responses of Hyalella azteca to acute and chronic microplastic exposures. *Environ. Toxicol. Chem.*, 34, 2564–2572.

Auta, H.S., Emenike, C.U. & Fauziah, S.H. (2017). Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions. *Environ. Int.*, 102, 165–176.

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. *Sci. Total Environ.*, 733, 137823.

Bakir, A., Rowland, S.J. & Thompson, R.C. (2014). Enhanced desorption of persistent organic pollutants from microplastics under simulated physiological conditions. *Environ. Pollut.*, 185, 16–23.

Baldwin, A.K., Corsi, S.R. & Mason, S.A. (2016). Plastic Debris in 29 Great Lakes Tributaries: Relations to Watershed Attributes and Hydrology. *Environ. Sci. Technol.*, 50, 10377–10385.

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. *Mar. Pollut. Bull.*, 110, 383–395.

Bandmann, V., Müller, J.D., Köhler, T. & Homann, U. (2012). Uptake of fluorescent nano beads into BY2-cells involves clathrin-dependent and clathrin-independent endocytosis. *FEBS Lett.*, 586, 3626–3632.

Barboza, L.G.A. & Gimenez, B.C.G. (2015). Microplastics in the marine environment: Current trends and future perspectives. *Mar. Pollut. Bull.*, 97, 5–12.

Barchiesi, M., Chiavola, A., Di Marcantonio, C. & Boni, M.R. (2021). Presence and fate of microplastics in the water sources: focus on the role of wastewater and drinking water treatment plants. *J. Water Process Eng.*, 40, 101787.

Barra, R. & Leonard, S.A. (2018). *Plastics and the circular economy*. Scientific and Technical Advisory Panel to the Global Environment Facility, Washington, DC.

Barrows, A.P.W., Christiansen, K.S., Bode, E.T. & Hoellein, T.J. (2018). A watershed-scale, citizen science approach to quantifying microplastic concentration in a mixed land-use river. *Water Res.*, 147, 382–392.

Batel, A., Borchert, F., Reinwald, H., Erdinger, L. & Braunbeck, T. (2018). Microplastic accumulation patterns and transfer of benzo[a]pyrene to adult zebrafish (Danio rerio) gills and zebrafish embryos. *Environ. Pollut.*, 235, 918–930.

Batel, A., Linti, F., Scherer, M., Erdinger, L. & Braunbeck, T. (2016). Transfer of benzo[a]pyrene from microplastics to Artemia nauplii and further to zebrafish via a trophic food web experiment: CYP1A induction and visual tracking of persistent organic pollutants. *Environ.*

Toxicol. Chem., 35, 1656–1666.

Bayo, J., López-Castellanos, J. & Olmos, S. (2020a). Membrane bioreactor and rapid sand filtration for the removal of microplastics in an urban wastewater treatment plant. *Mar. Pollut. Bull.*, 156, 111211.

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

Beiras, R., Muniategui-Lorenzo, S., Rodil, R., Tato, T., Montes, R., López-Ibáñez, S., *et al.* (2019). Polyethylene microplastics do not increase bioaccumulation or toxicity of nonylphenol and 4-MBC to marine zooplankton. *Sci. Total Environ.*, 692, 1–9.

Beiras, R., Verdejo, E., Campoy-López, P. & Vidal-Liñán, L. (2021). Aquatic toxicity of chemically defined microplastics can be explained by functional additives. *J. Hazard. Mater.*, 406, 124338.

Bellasi, A., Binda, G., Pozzi, A., Galafassi, S., Volta, P. & Bettinetti, R. (2020). Microplastic contamination in freshwater environments: A review, focusing on interactions with sediments and benthic organisms. *Environ. - MDPI*, 7,30.

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. *Environ. Pollut.*, 261, 114–198.

Besseling, E., Foekema, E.M., Van Den Heuvel-Greve, M.J. & Koelmans, A.A. (2017). The Effect of Microplastic on the Uptake of Chemicals by the Lugworm Arenicola marina (L.) under Environmentally Relevant Exposure Conditions. *Environ. Sci. Technol.*, 51, 8795–8804.

Besseling, E., Redondo-Hasselerharm, P., Foekema, E.M. & Koelmans, A.A. (2019). Quantifying ecological risks of aquatic micro- and nanoplastic. *Crit. Rev. Environ. Sci. Technol.*, 49, 32–80.

Besser, J.M., Brumbaugh, W.G., Brunson, E.L. & Ingersoll, C.G. (2005). Acute and chronic toxicity of lead in water and diet to the amphipod Hyalella azteca. *Environ. Toxicol. Chem.*, 24, 1807–1815.

Blarer, P. & Burkhardt-Holm, P. (2016). Microplastics affect assimilation efficiency in the freshwater amphipod Gammarus fossarum. *Environ. Sci. Pollut. Res.*, 23, 23522–23532.

Bläsing, M. & Amelung, W. (2018). Plastics in soil: Analytical methods and possible sources. *Sci. Total Environ.*, 612, 422–435.

Blettler, M.C.M., Ulla, M.A., Rabuffetti, A.P. & Garello, N. (2017). Plastic pollution in freshwater ecosystems: macro-, meso-, and microplastic debris in a floodplain lake. *Environ. Monit. Assess.*, 189.

Boots, B., Russell, C.W. & Green, D.S. (2019). Effects of Microplastics in Soil Ecosystems: Above

and below Ground. *Environ. Sci. Technol.*, 53, 11496–11506.

Bouhroum, R., Boulkamh, A., Asia, L., Lebarillier, S., Halle, A. Ter, Syakti, A.D., *et al.* (2019). Concentrations and fingerprints of PAHs and PCBs adsorbed onto marine plastic debris from the Indonesian Cilacap coast and the North Atlantic gyre. *Reg. Stud. Mar. Sci.*, 29, 100611.

Ter Braak, C.J.F. & Šmilauer, P. (2018). *Canoco reference manual and user's guide: software for ordination, version 5.1.* Microcomputer Power, Ithaca, USA.

Brandt-Rauf, P., Long, C., Kovvali, G., Li, Y., Monaco, R. & Marion, M.-J. (2012). Plastics and carcinogenesis: The example of vinyl chloride. *J. Carcinog.*, 11, 5.

Bråte, I.L.N., Hurley, R., Lusher, A., Buenaventura, N., Hultman, M., Halsband, C., *et al.* (2020). *Microplastics in marine bivalves from the Nordic environment*. *Nor. Environ. Agency Publ. Ser. M-1629|2020*. Norwegian Environment Agency publication series M-1629|2020. Nordic Council of Ministers, TemaNord report TN2020:504, ISSN 0908-6692.

Briassoulis, D., Babou, E., Hiskakis, M. & Kyrikou, I. (2015). Analysis of long-term degradation behaviour of polyethylene mulching films with pro-oxidants under real cultivation and soil burial conditions. *Environ. Sci. Pollut. Res.*, 22, 2584–2598.

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., *et al.* (2011). Accumulation of microplastic on shorelines woldwide: Sources and sinks. *Environ. Sci. Technol.*, 45, 9175–9179.

Burns, E.E. & Boxall, A.B.A. (2018). Microplastics in the aquatic environment: Evidence for or against adverse impacts and major knowledge gaps. *Environ. Toxicol. Chem.*, 37, 2776–2796.

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

Camargo, J.A. (2006). *Ecología y Conservación del Río Henares y sus Tributarios.*

Cao, D., Wang, X., Luo, X., Liu, G. & Zheng, H. (2017). Effects of polystyrene microplastics on the fitness of earthworms in an agricultural soil. *IOP Conf. Ser. Earth Environ. Sci.*, 61, 012148.

Cao, Y., Wang, Q., Ruan, Y., Wu, R., Chen, L., Zhang, K., *et al.* (2020). Intra-day microplastic variations in wastewater: A case study of a sewage treatment plant in Hong Kong. *Mar. Pollut. Bull.*, 160, 111535.

Capolupo, M., Sørensen, L., Jayasena, K.D.R., Booth, A.M. & Fabbri, E. (2020). Chemical composition and ecotoxicity of plastic and car tire rubber leachates to aquatic organisms. *Water Res.*, 169, 115270.

Carbery, M., O'Connor, W. & Palanisami, T. (2018). Trophic transfer of microplastics and mixed contaminants in the marine food web and implications for human health. *Environ. Int.*, 115, 400–409.

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. *Environ. Sci. Pollut. Res.*, 25, 1191–1199.

Carpenter, E.J., J, A.S., Harvey, G.R., Miklas, H.P. & Peck, B.B. (1972). Polystyrene Spherules in Coastal Waters. *Science*, 178, 749–750.

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

Castañeda, R.A., Avlijas, S., Simard, M.A., Ricciardi, A. & Smith, R. (2014). Microplastic pollution in St. Lawrence River sediments. *Can. J. Fish. Aquat. Sci.*, 71, 1767–1771.

Castro-Jiménez, J., González-Fernández, D., Fornier, M., Schmidt, N. & Sempéré, R. (2019). Macro-litter in surface waters from the Rhone River: Plastic pollution and flows to the NW Mediterranean Sea. *Mar. Pollut. Bull.*, 146, 60–66.

Catarino, A.I., Frutos, A. & Henry, T.B. (2019). Use of fluorescent-labelled nanoplastics (NPs) to demonstrate NP absorption is inconclusive without adequate controls. *Sci. Total Environ.*, 670, 915–920.

CEDEX. (2021). *Centro de Estudios y Experimentación de Obras Públicas*. Available at: https://ceh.cedex.es/anuarioaforos/afo/estaf-datos.asp?indroea=3062. Last accessed 19 May 2021.

Cera, A., Cesarini, G. & Scalici, M. (2020). Microplastics in freshwater: What is the news from the world? *Diversity*, 12, 276.

Chae, Y. & An, Y.-J. (2018). Current research trends on plastic pollution and ecological impacts on the soil ecosystem: A review. *Environ. Pollut.*, 240, 387–395.

Chamas, A., Moon, H., Zheng, J., Qiu, Y., Tabassum, T., Jang, J.H., *et al.* (2020). Degradation Rates of Plastics in the Environment. *ACS Sustain. Chem. Eng.*, 8, 3494–3511.

Cheung, P.K., Hung, P.L. & Fok, L. (2019). River Microplastic Contamination and Dynamics upon a Rainfall Event in Hong Kong, China. *Environ. Process.*, 6, 253–264.

Chua, E.M., Shimeta, J., Nugegoda, D., Morrison, P.D. & Clarke, B.O. (2014). Assimilation of polybrominated diphenyl ethers from microplastics by the marine amphipod, allorchestes compressa. *Environ. Sci. Technol.*, 48, 8127–8134.

Claessens, M., Meester, S. De, Landuyt, L. Van, Clerck, K. De & Janssen, C.R. (2011). Occurrence and distribution of microplastics in marine sediments along the Belgian coast. *Mar. Pollut. Bull.*, 62, 2199–2204.

Coffin, S., Lee, I., Gan, J. & Schlenk, D. (2019). Simulated digestion of polystyrene foam enhances desorption of diethylhexyl phthalate (DEHP) and In vitro estrogenic activity in a size-dependent manner. *Environ. Pollut.*, 246, 452–462.

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

Colton, J.B., Knapp, F.D. & Bums, B.R. (1974). Plastic Particles in Surface Waters of the Northwestern Atlantic: The abundance, distribution, source, and significance of various types of plastics are discussed. *Science*, 185, 491–497.

Confederación Hidrogáfica del Tajo. (2019). *Censo de vertidos*. Available at: http://www.chtajo.es/LaCuenca/Calidad Agua/ControlVertidos/Paginas/default.aspx#Censo de Vertidos. Last accessed 4 December 2020.

Conley, K., Clum, A., Deepe, J., Lane, H. & Beckingham, B. (2019). Wastewater treatment plants as a source of microplastics to an urban estuary: Removal efficiencies and loading per capita over one year. *Water Res. X*, 3, 100030.

Constant, M., Ludwig, W., Kerhervé, P., Sola, J., Charrière, B., Sanchez-Vidal, A., *et al.* (2020). Microplastic fluxes in a large and a small Mediterranean river catchments: The Têt and the Rhône, Northwestern Mediterranean Sea. *Sci. Total Environ.*, 716, 136984.

Corine Land Cover. (2006). *https://www.eea.europa.eu/themes/landuse/interactive/cic-download*.

Corradini, F., Meza, P., Eguiluz, R., Casado, F., Huerta-Lwanga, E. & Geissen, V. (2019). Evidence of microplastic accumulation in agricultural soils from sewage sludge disposal. *Sci. Total Environ.*, 671, 411–420.

Crawford, C.B. & Quinn, B. (2017). The interactions of microplastics and chemical pollutants. In: *Microplastic Pollutants*. pp. 131–157.

Crossman, J., Hurley, R.R., Futter, M. & Nizzetto, L. (2020). Transfer and transport of microplastics from biosolids to agricultural soils and the wider environment. *Sci. Total Environ.*, 724, 138334.

Crosti, R., Arcangeli, A., Campana, I., Paraboschi, M. & González-Fernández, D. (2018). 'Down to the river': amount, composition, and economic sector of litter entering the marine compartment, through the Tiber river in the Western Mediterranean Sea. *Rend. Lincei. Sci. Fis. e Nat.*, 29, 859–866.

Cui, R., Kim, S.W. & An, Y.J. (2017). Polystyrene nanoplastics inhibit reproduction and induce abnormal embryonic development in the freshwater crustacean Daphnia galeata. *Sci. Rep.*, 7, 1–10.

Dall'Osto, M., Beddows, D.C.S., Gietl, J.K., Olatunbosun, O.A., Yang, X. & Harrison, R.M. (2014). Characteristics of tyre dust in polluted air: Studies by single particle mass spectrometry (ATOFMS). *Atmos. Environ.*, 94, 224–230.

Deng, H., Wei, R., Luo, W., Hu, L., Li, B., Di, Y., *et al.* (2020). Microplastic pollution in water and sediment in a textile industrial area. *Environ. Pollut.*, 258, 113658.

Di, M. & Wang, J. (2018). Microplastics in surface waters and sediments of the Three Gorges

Reservoir, China. *Sci. Total Environ.*, 616–617, 1620–1627.

Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N. & Tassin, B. (2015). Microplastic contamination in an urban area: A case study in Greater Paris. *Environ. Chem.*, 12, 592–599.

Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N. & Tassin, B. (2016). Microplastic in urban sources and receiving water within an urban area : a case study in Greater Paris. *Environ. Chem.*, 12, 592–599.

Dris, R., Gasperi, J., Rocher, V. & Tassin, B. (2018). Synthetic and non-synthetic anthropogenic fibers in a river under the impact of Paris Megacity: Sampling methodological aspects and flux estimations. *Sci. Total Environ.*, 618, 157–164.

Dyachenko, A., Mitchell, J. & Arsem, N. (2017). Extraction and identification of microplastic particles from secondary wastewater treatment plant (WWTP) effluent. *Anal. Methods*, 9, 1412–1418.

Ebert, D. (2005). *Ecology, Epidemiology, and Evolution of Parasitism in Daphnia [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Books*.

EC. (2019). *Press Release: Parliament seals ban on throwaway plastics by 2021*. *Press Release Database*. Available at: http://europa.eu//rapid/press-release_STATEMENT-19-1873_en.html. Last accessed 28 April 2019.

ECHA. (2019). *Proposal for a restriction on intentionally added microplastics Version n. 1.1. 20 March 2019*. Helsinki, Finland.

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

EEA. (2018). *Water is life. EEA Signals*. Copenhagen. Available at https://www.eea.europa.eu//publications/eea-signals-2018-water-is-life.

EEA. (2019). *Urban waste water treatment*. Available at: https://www.eea.europa.eu/data-and-maps/indicators/urban-waste-water-treatment. Last accessed 10 June 2019.

Eerkes-Medrano, D., Thompson, R.C. & Aldridge, D.C. (2015). Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. *Water Res.*, 75, 63–82.

Ekstrand, J., Timpka, T. & Hägglund, M. (2006). Risk of injury in elite football played on artificial turf versus natural grass: A prospective two-cohort study. *Br. J. Sports Med.*, 40, 975–980.

van Emmerik, T., Kieu-Le, T.-C., Loozen, M., van Oeveren, K., Strady, E., Bui, X.-T., *et al.* (2018). A Methodology to Characterize Riverine Macroplastic Emission Into the Ocean. *Front. Mar. Sci.*, 5, 1–11.

Enders, K., Käppler, A., Biniasch, O., Feldens, P., Stollberg, N., Lange, X., *et al.* (2019). Tracing

microplastics in aquatic environments based on sediment analogies. *Sci. Rep.*, 9, 1–15.

Eriksen, M., Mason, S., Wilson, S., Box, C., Zellers, A., Edwards, W., *et al.* (2013). Microplastic pollution in the surface waters of the Laurentian Great Lakes. *Mar. Pollut. Bull.*, 77, 177–182.

Estahbanati, S. & Fahrenfeld, N.L. (2016). Influence of wastewater treatment plant discharges on microplastic concentrations in surface water. *Chemosphere*, 162, 277–284.

Estrela, T., Pérez-Martin, M.A. & Vargas, E. (2012). Impacts of climate change on water resources in Spain. *Hydrol. Sci. J.*, 57, 1154–1167.

European Commission. (2013). *Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy Text with EEA relevance*.

Eurostat. (2020). *Sewage sludge production and disposal from urban wastewater*. Available at: http://appsso.eurostat.ec.europa.eu/nui/show.do?dataset=env_ww_spd&lang=en#. Last accessed 20 November 2020.

Everaert, G., Van Cauwenberghe, L., De Rijcke, M., Koelmans, A.A., Mees, J., Vandegehuchte, M., *et al.* (2018). Risk assessment of microplastics in the ocean: Modelling approach and first conclusions. *Environ. Pollut.*, 242, 1930–1938.

De Falco, F., Di Pace, E., Cocca, M. & Avella, M. (2019). The contribution of washing processes of synthetic clothes to microplastic pollution. *Sci. Rep.*, 9, 1–11.

Fältström, E., Olesen, K.B. & Anderberg, S. (2021). Microplastic types in the wastewater system—a comparison of material flow-based source estimates and the measurement-based load to a wastewater treatment plant. *Sustainability*, 13, 5404.

Fan, Y., Zheng, K., Zhu, Z., Chen, G. & Peng, X. (2019). Distribution, sedimentary record, and persistence of microplastics in the Pearl River catchment, China. *Environ. Pollut.*, 251, 862–870.

Faure, F., Corbaz, M., Baecher, H. & De Alencastro, L.F. (2012). Pollution due to plastics and microplastics in lake Geneva and in the Mediterranean Sea. *Arch. des Sci.*, 65, 157–164.

Faure, F., Demars, C., Wieser, O., Kunz, M. & De Alencastro, L.F. (2015). Plastic pollution in Swiss surface waters: Nature and concentrations, interaction with pollutants. *Environ. Chem.*, 12, 582–591.

Fendall, L.S. & Sewell, M.A. (2009). Contributing to marine pollution by washing your face: Microplastics in facial cleansers. *Mar. Pollut. Bull.*, 58, 1225–1228.

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.

Fischer, M. & Scholz-Böttcher, B.M. (2017). Simultaneous Trace Identification and Quantification

of Common Types of Microplastics in Environmental Samples by Pyrolysis-Gas Chromatography–Mass Spectrometry. *Environ. Sci. Technol.*, 51, 5052–5060.

Foley, C.J., Feiner, Z.S., Malinich, T.D. & Höök, T.O. (2018). A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. *Sci. Total Environ.*, 631–632, 550–559.

Fox, J. & Weisberg, S. (2019). *Car: An R Companion to Applied Regression*. Third. Sage, Thousand Oaks CA.

Van Franeker, J.A., Blaize, C., Danielsen, J., Fairclough, K., Gollan, J., Guse, N., *et al.* (2011). Monitoring plastic ingestion by the northern fulmar Fulmarus glacialis in the North Sea. *Environ. Pollut.*, 159, 2609–2615.

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

Fueser, H., Mueller, M.T. & Traunspurger, W. (2020). Ingestion of microplastics by meiobenthic communities in small-scale microcosm experiments. *Sci. Total Environ.*, 746, 141276.

Fuller, S. & Gautam, A. (2016). A Procedure for Measuring Microplastics using Pressurized Fluid Extraction. *Environ. Sci. Technol.*, 50, 5774–5780.

Galafassi, S., Campanale, C., Massarelli, C., Uricchio, V.F. & Volta, P. (2021). Do freshwater fish eat microplastics? A review with a focus on effects on fish health and predictive traits of MPs ingestion. *Water*, 13, 2214.

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

Gasperi, J., Dris, R., Bonin, T., Rocher, V. & Tassin, B. (2014). Assessment of floating plastic debris in surface water along the Seine River. *Environ. Pollut.*, 195, 163–166.

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

Gaylarde, C.C., Neto, J.A.B. & da Fonseca, E.M. (2021). Paint fragments as polluting microplastics: A brief review. *Mar. Pollut. Bull.*, 162, 111847.

Gerdes, Z., Ogonowski, M., Nybom, I., Ek, C., Adolfsson-Erici, M., Barth, A., *et al.* (2019). Microplastic-mediated transport of PCBs? A depuration study with Daphnia magna. *PLoS One*, 14, 1–15.

GESAMP. (2015). Sources, fate and effects of microplastics in the marine environment: a global assessment (Kershaw, P. J., ed.). (IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection). *Rep. Stud. GESAMP*, No. 90, 96 p.

GESAMP. (2019). Guidelines or the monitoring and assessment of plastic litter and microplastics in the ocean (Kershaw P.J., Turra A. and Galgani F. editors), (IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP/ISA Joint Group of Experts on the Scientific Aspects of Marine En. *Rep. Stud. GESAMP*, No. 99, 130 p.

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

Gies, E.A., Lenoble, J.L., Noël, M., Etemadifar, A., Bishay, F., Hall, E.R., *et al.* (2018). Retention of microplastics in a major secondary wastewater treatment plant in Vancouver, Canada. *Mar. Pollut. Bull.*, 133, 553–561.

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

González-Fernández, D., Hanke, G. & Network, and the R. (2018). *Floating Macro Litter in European Rivers - Top Items*. *EUR 29383 EN, Publ. Off. Eur. Union*. Luxembourg.

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

Gophen, M. & Geller, W. (1984). Filter mesh size and food particle uptake by Daphnia. *Oecologia*, 64, 408–412.

Gouin, T. (2020). Toward an Improved Understanding of the Ingestion and Trophic Transfer of Microplastic Particles: Critical Review and Implications for Future Research. *Environ. Toxicol. Chem.*, 39, 1119–1137.

Gouin, T., Avalos, J., Brunning, I., Brzuska, K., Graaf, J. De, Kaumanns, J., *et al.* (2015). Use of Micro-Plastic Beads in Cosmetic Products in Europe and Their Estimated Emissions to the North Sea Environment. *SOFW J.*, 141, 40–46.

Gouin, T., Roche, N., Lohmann, R. & Hodges, G. (2011). A Thermodynamic Approach for Assessing the Environmental Exposure of Chemicals Absorbed to Microplastic. *Environ. Sci. Technol*, 45, 1466–1472.

Gray, A.D. & Weinstein, J.E. (2017). Size- and shape-dependent effects of microplastic particles on adult daggerblade grass shrimp (Palaemonetes pugio). *Environ. Toxicol. Chem.*, 36, 3074–3080.

Guerranti, C., Cannas, S., Scopetani, C., Fastelli, P., Cincinelli, A. & Renzi, M. (2017). Plastic litter in aquatic environments of Maremma Regional Park (Tyrrhenian Sea, Italy): Contribution by the Ombrone river and levels in marine sediments. *Mar. Pollut. Bull.*, 117, 366–370.

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. *J. Mar. Sci. Eng.*, 8, 216.

Gündoğdu, S., Çevik, C., Güzel, E. & Kilercioğlu, S. (2018). Microplastics in municipal wastewater treatment plants in Turkey: a comparison of the influent and secondary effluent concentrations. *Environ. Monit. Assess.*, 190, 626.

Habib, D., Locke, D.C. & Cannone, L.J. (1998). Synthetic Fibers As Indicators of Municipal Sewage Sludge, Sludge Products, and Sewage Treatment Plant Effluents. *Water. Air. Soil Pollut.*, 103, 1–8.

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

Halle, L.L., Palmqvist, A., Kampmann, K., Jensen, A., Hansen, T. & Khan, F.R. (2021). Tire wear particle and leachate exposures from a pristine and road-worn tire to Hyalella azteca: Comparison of chemical content and biological effects. *Aquat. Toxicol.*, 232, 105769.

Han, M., Niu, X., Tang, M., Zhang, B.T., Wang, G., Yue, W., *et al.* (2020). Distribution of microplastics in surface water of the lower Yellow River near estuary. *Sci. Total Environ.*, 707, 135601.

Hardesty, B.D., Good, T.P. & Wilcox, C. (2015). Novel methods, new results and science-based solutions to tackle marine debris impacts on wildlife. *Ocean Coast. Manag.*, 115, 4–9.

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. *Environ. Sci. Technol.*, 50, 11532–11538.

Hartmann, N.B., Hüffer, T., Thompson, R.C., Hassellöv, M., Verschoor, A., Daugaard, A.E., *et al.* (2019). Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris. *Environ. Sci. Technol.*, 53, 1039–1047.

Hartmann, N.B., Rist, S., Bodin, J., Jensen, L.H.S., Schmidt, S.N., Mayer, P., *et al.* (2017). Microplastics as vectors for environmental contaminants: Exploring sorption, desorption, and transfer to biota. *Integr. Environ. Assess. Manag.*, 13, 488–493.

Hartwell, S., Jordahl, D., Dawson, C. & Ives, S. (1998). Toxicity of scrap tire leachates in estuarine salinities: Are tires acceptable for artificial reefs? *Trans. Am. Fish. Soc.*, 127, 796–806.

HELCOM. (2014). BASE project 2012-2014: Preliminary study on synthetic microfibers and particles at a municipal waste water treatment plant. *Balt. Mar. Environ. Prot. Comm. HELCOM*.

Hendrickson, E., Minor, E.C. & Schreiner, K. (2018). Microplastic Abundance and Composition in Western Lake Superior As Determined via Microscopy, Pyr-GC/MS, and FTIR. *Environ. Sci. Technol.*, 52, 1787–1796.

Hoellein, T.J. & Rochman, C.M. (2021). The “plastic cycle”: a watershed scale model of plastic pools and fluxes. *Front. Ecol. Environ.*, 19, 176–183.

Hopewell, J., Dvorak, R. & Kosior, E. (2009). Plastics recycling: challenges and opportunities. *Philos. Trans. R. Soc. B Biol. Sci.*, 364, 2115–2126.

Horton, A.A. & Dixon, S.J. (2018). Microplastics: An introduction to environmental transport processes. *Wiley Interdiscip. Rev. Water*, 5, e1268.

Horton, A.A., Svendsen, C., Williams, R.J., Spurgeon, D.J. & Lahive, E. (2017a). Large microplastic particles in sediments of tributaries of the River Thames, UK – Abundance, sources and methods for effective quantification. *Mar. Pollut. Bull.*, 114, 218–226.

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

Hu, L., Chernick, M., Hinton, D.E. & Shi, H. (2018). Microplastics in Small Waterbodies and Tadpoles from Yangtze River Delta, China. *Environ. Sci. Technol.*, 52, 8885–8893.

Huang, Y., Liu, Q., Jia, W., Yan, C. & Wang, J. (2020). Agricultural plastic mulching as a source of microplastics in the terrestrial environment. *Environ. Pollut.*, 260, 114096.

Huerta Lwanga, E., Gertsen, H., Gooren, H., Peters, P., Salánki, T., van der Ploeg, M., *et al.* (2017a). Incorporation of microplastics from litter into burrows of Lumbricus terrestris. *Environ. Pollut.*, 220, 523–531.

Huerta Lwanga, E., Gertsen, H., Gooren, H., Peters, P., Salánki, T., Van Der Ploeg, M., *et al.* (2016). Microplastics in the Terrestrial Ecosystem: Implications for Lumbricus terrestris (Oligochaeta, Lumbricidae). *Environ. Sci. Technol.*, 50, 2685–2691.

Huerta Lwanga, E., Vega, J.M., Quej, V.K., Angeles, J.D.L., Sanchez, L., Chi, C., *et al.* (2017b). Field evidence for transfer of plastic debris along a terrestrial food chain. *Sci. Rep.*, 7, 14071.

Hurley, R., Woodward, J. & Rothwell, J.J. (2018a). Microplastic contamination of river beds significantly reduced by catchment-wide flooding. *Nat. Geosci.*, 11, 251–257.

Hurley, R.R., Lusher, A.L., Olsen, M. & Nizzetto, L. (2018b). Validation of a method for extracting microplastics from complex, organic-rich, environmental matrices. *Environ. Sci. Technol*, 52(13), 7409–7417.

Hurley, R.R. & Nizzetto, L. (2018). Fate and occurrence of micro(nano)plastics in soils: Knowledge gaps and possible risks. *Curr. Opin. Environ. Sci. Heal.*, 1, 6–11.

Hussain, I. & Hamid, H. (2003). Plastics in agriculture. In: *Plastics and the Environement* (ed. Andray, A.L.). John Wiley & Sons, Inc., pp. 185–209.

Imhof, A.H.K., Ivleva, N.P., Schmid, J., Niessner, R., Imhof, H.K., Ivleva, N.P., *et al.* (2013). Contamination of beach sediments of a subalpine lake with microplastic particles. *Curr. Biol.*, 23, 867–868.

Imhof, H.K., Laforsch, C., Wiesheu, A.C., Schmid, J., Anger, P.M., Niessner, R., *et al.* (2016).

Pigments and plastic in limnetic ecosystems: A qualitative and quantitative study on microparticles of different size classes. *Water Res.*, 98, 64–74.

Imhof, H.K., Wiesheu, A.C., Anger, P.M., Niessner, R., Ivleva, N.P. & Laforsch, C. (2018). Variation in plastic abundance at different lake beach zones - A case study. *Sci. Total Environ.*, 613–614, 530–537.

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

Järlskog, I., Strömvall, A., Magnusson, K., Gustafsson, M., Polukarova, M., Gal, H., *et al.* (2020). Occurrence of tire and bitumen wear microplastics on urban streets and in sweepsand and washwater. *Sci. Total Environ.*, 729, 138950.

Jemec, A., Horvat, P., Kunej, U., Bele, M. & Kržan, A. (2016). Uptake and effects of microplastic textile fibers on freshwater crustacean Daphnia magna. *Environ. Pollut.*, 219, 201–209.

Jovanović, B. (2017). Ingestion of microplastics by fish and its potential consequences from a physical perspective. *Integr. Environ. Assess. Manag.*, 13, 510–515.

Judd, S. & Jefferson, B. (2003). *Membrane for Industrial Wastewater Recovery and Re-Use*. Elsevier Science.

Kalčíková, G., Alič, B., Skalar, T., Bundschuh, M. & Gotvajn, A.Ž. (2017). Wastewater treatment plant effluents as source of cosmetic polyethylene microbeads to freshwater. *Chemosphere*, 188, 25–31.

Kallenbach, E.M.F., Friberg, N., Lusher, A., Jacobsen, D. & Hurley, R.R. (2022). Anthropogenically impacted lake catchments in Denmark reveal low microplastic pollution. *Environ. Sci. Pollut. Res.*

Kallenbach, E.M.F., Hurley, R.R., Lusher, A. & Friberg, N. (2021). Chitinase digestion for the analysis of microplastics in chitinaceous organisms using the terrestrial isopod Oniscus asellus L. as a model organism. *Sci. Total Environ.*, 786, 147455.

Kapp, K.J. & Yeatman, E. (2018). Microplastic hotspots in the Snake and Lower Columbia rivers: A journey from the Greater Yellowstone Ecosystem to the Pacific Ocean. *Environ. Pollut.*, 241, 1082–1090.

Karami, A., Groman, D.B., Wilson, S.P., Ismail, P. & Neela, V.K. (2017). Biomarker responses in zebrafish (Danio rerio) larvae exposed to pristine low-density polyethylene fragments. *Environ. Pollut.*, 223, 466–475.

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). *Environ. Res.*, 151, 58–70.

Kassambara, A. (2017). *Ggplot2' Based Publication Ready Plots Version 0.4.0*. https://cran.r-project.org/package=ggpubr.

Kataoka, T., Nihei, Y., Kudou, K. & Hinata, H. (2019). Assessment of the sources and inflow processes of microplastics in the river environments of Japan. *Environ. Pollut.*, 244, 958–965.

Kay, P., Hiscoe, R., Moberley, I., Bajic, L. & McKenna, N. (2018). Wastewater treatment plants as a source of microplastics in river catchments. *Environ. Sci. Pollut. Res.*, 25, 20264–20267.

Ke, A.Y., Chen, J., Zhu, J., Wang, Y.H., Hu, Y., Fan, Z.L., *et al.* (2019). Impacts of leachates from single-use polyethylene plastic bags on the early development of clam Meretrix meretrix (Bivalvia: Veneridae). *Mar. Pollut. Bull.*, 142, 54–57.

Keswani, A., Oliver, D.M., Gutierrez, T. & Quilliam, R.S. (2016). Microbial hitchhikers on marine plastic debris: Human exposure risks at bathing waters and beach environments. *Mar. Environ. Res.*, 118, 10–9.

Khan, F.R., Halle, L.L. & Palmqvist, A. (2019). Acute and long-term toxicity of micronized car tire wear particles to Hyalella azteca. *Aquat. Toxicol.*, 213, 105216.

Kim, D., Kim, H. & An, Y.J. (2021a). Effects of synthetic and natural microfibers on Daphnia magna–Are they dependent on microfiber type? *Aquat. Toxicol.*, 240, 105968.

Kim, S.K., Kim, J.S., Lee, H. & Lee, H.J. (2021b). Abundance and characteristics of microplastics in soils with different agricultural practices: Importance of sources with internal origin and environmental fate. *J. Hazard. Mater.*, 403, 123997.

Kirstein, I. V., Kirmizi, S., Wichels, A., Garin-Fernandez, A., Erler, R., Löder, M., *et al.* (2016). Dangerous hitchhikers? Evidence for potentially pathogenic Vibrio spp. on microplastic particles. *Mar. Environ. Res.*, 120, 1–8.

Klein, S., Dimzon, I.K., Eubeler, J. & Knepper, T.P. (2018). Analysis, Occurrence, and Degradation of Microplastics in the Aqueous Environment BT - Freshwater Microplastics: Emerging Environmental Contaminants? In: *Freshwater Microplastics. The Handbook of Environmental Chemistry* (eds. Wagner, M. & Lambert, S.). Springer International Publishing, Cham, pp. 51–67.

Klein, S., Worch, E. & Knepper, T.P. (2015). Occurrence and spatial distribution of microplastics in river shore sediments of the rhine-main area in Germany. *Environ. Sci. Technol.*, 49, 6070–6076.

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

Knowlton, A.R., Hamilton, P.K., Marx, M.K., Pettis, H.M. & Kraus, S.D. (2012). Monitoring North Atlantic right whale Eubalaena glacialis entanglement rates: A 30 yr retrospective. *Mar. Ecol. Prog. Ser.*, 466, 293–302.

Koelmans, A.A. (2019). Proxies for nanoplastic. *Nat. Nanotechnol.*, 14, 307–308.

Koelmans, A.A., Bakir, A., Burton, G.A. & Janssen, C.R. (2016). Microplastic as a Vector for Chemicals in the Aquatic Environment: Critical Review and Model-Supported Reinterpretation

of Empirical Studies. *Environ. Sci. Technol.*, 50, 3315–3326.

Koelmans, A.A., Besseling, E., Foekema, E., Kooi, M., Mintenig, S., Ossendorp, B.C., *et al.* (2017a). Risks of Plastic Debris: Unravelling Fact, Opinion, Perception, and Belief. *Environ. Sci. Technol.*, 51, 11513–11519.

Koelmans, A.A., Besseling, E. & Foekema, E.M. (2014). Leaching of plastic additives to marine organisms. *Environ. Pollut.*, 187, 49–54.

Koelmans, A.A., Besseling, E., Wegner, A. & Foekema, E.M. (2013). Plastic as a carrier of POPs to aquatic organisms: A model analysis. *Environ. Sci. Technol.*, 47, 7812–7820.

Koelmans, A.A., Diepens, N.J. & Mohamed Nor, N.H. (2022). Weight of Evidence for the Microplastic Vector Effect in the Context of Chemical Risk Assessment. In: *Microplastic in the Environment: Pattern and Process* (ed. Bank, M.S.). Springer International Publishing, Cham, pp. 155–197.

Koelmans, A.A., Kooi, M., Law, K.L. & Van Sebille, E. (2017b). All is not lost: Deriving a top-down mass budget of plastic at sea. *Environ. Res. Lett.*, 12.

Koelmans, A.A., Mohamed Nor, N.H., Hermsen, E., Kooi, M., Mintenig, S.M. & De France, J. (2019). Microplastics in freshwaters and drinking water: Critical review and assessment of data quality. *Water Res.*, 155, 410–422.

Koelmans, A.A., Redondo-Hasselerharm, P.E., Mohamed Nor, N.H. & Kooi, M. (2020). Solving the non-alignment of methods and approaches used in microplastic research in order to consistently characterize risk. *Environ. Sci. Technol.*, 54, 12307–12315.

Kögel, T., Bjorøy, Ø., Toto, B., Bienfait, A.M. & Sanden, M. (2020). Micro- and nanoplastic toxicity on aquatic life: Determining factors. *Sci. Total Environ.*, 709, 136050.

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

Kooi, M. & Koelmans, A.A. (2019). Simplifying Microplastic via Continuous Probability Distributions for Size, Shape,and Density. *Environ. Sci. Technol. Lett.*, 6, 551–557.

Koyuncuoğlu, P. & Erden, G. (2021). Sampling, pre-treatment, and identification methods of microplastics in sewage sludge and their effects in agricultural soils: a review. *Environ. Monit. Assess.*, 193, 175.

Krause, S., Molari, M., Gorb, E. V., Gorb, S.N., Kossel, E. & Haeckel, M. (2020). Persistence of plastic debris and its colonization by bacterial communities after two decades on the abyssal seafloor. *Sci. Rep.*, 10, 1–15.

Kreider, M.L., Panko, J.M., McAtee, B.L., Sweet, L.I. & Finley, B.L. (2010). Physical and chemical characterization of tire-related particles: Comparison of particles generated using different methodologies. *Sci. Total Environ.*, 408, 652–659.

Kühn, S., Bravo Rebolledo, E.L. & van Franeker, J.A. (2015). Deleterious Effects of Litter on Marine Life. In: *Marine Anthropogenic Litter* (eds. Bergmann, M., Gutow, L. & Klages, M.). Springer International Publishing, Cham, pp. 75–116.

Kumar, A., Holuszko, M. & Espinosa, D.C.R. (2017). E-waste: An overview on generation, collection, legislation and recycling practices. *Resour. Conserv. Recycl.*, 122, 32–42.

Kumar, R., Sharma, P., Manna, C. & Jain, M. (2021). Abundance, interaction, ingestion, ecological concerns, and mitigation policies of microplastic pollution in riverine ecosystem: A review. *Sci. Total Environ.*, 782, 146695.

Kutralam-Muniasamy, G., Pérez-Guevara, F., Elizalde-Martínez, I. & Shruti, V.C. (2020). An overview of recent advances in micro/nano beads and microfibers research: Critical assessment and promoting the less known. *Sci. Total Environ.*, 740, 139991.

Lagarde, F., Olivier, O., Zanella, M., Daniel, P., Hiard, S. & Caruso, A. (2016). Microplastic interactions with freshwater microalgae: Hetero-aggregation and changes in plastic density appear strongly dependent on polymer type. *Environ. Pollut.*, 215, 331–339.

Lahens, L., Strady, E., Kieu-Le, T.C., Dris, R., Boukerma, K., Rinnert, E., *et al.* (2018). Macroplastic and microplastic contamination assessment of a tropical river (Saigon River, Vietnam) transversed by a developing megacity. *Environ. Pollut.*, 236, 661–671.

Lallès, J.P. (2020). Intestinal alkaline phosphatase in the gastrointestinal tract of fish: biology, ontogeny, and environmental and nutritional modulation. *Rev. Aquac.*, 12, 555–581.

Lambert, S., Scherer, C. & Wagner, M. (2017). Ecotoxicity testing of microplastics: Considering the heterogeneity of physicochemical properties. *Integr. Environ. Assess. Manag.*, 13, 470–475.

Lambert, S. & Wagner, M. (2017). Environmental performance of bio-based and biodegradable plastics: the road ahead. *Chem. Soc. Rev.*, 46, 6855–6871.

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 Res.*, 133, 236–246.

Leads, R.R. & Weinstein, J.E. (2019). Occurrence of tire wear particles and other microplastics within the tributaries of the Charleston Harbor Estuary, South Carolina, USA. *Mar. Pollut. Bull.*, 145, 569–582.

Lebreton, L. & Andrady, A. (2019). Future scenarios of global plastic waste generation and disposal. *Palgrave Commun.*, 5, 1–11.

Lebreton, L.C.M., Van Der Zwet, J., Damsteeg, J.W., Slat, B., Andrady, A. & Reisser, J. (2017). River plastic emissions to the world's oceans. *Nat. Commun.*, 8, 1–10.

Lechner, A., Keckeis, H., Lumesberger-Loisl, F., Zens, B., Krusch, R., Tritthart, M., *et al.* (2014). The Danube so colourful: A potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. *Environ. Pollut.*, 188, 177–181.

Lechner, A. & Ramler, D. (2015). The discharge of certain amounts of industrial microplastic from a production plant into the River Danube is permitted by the Austrian legislation. *Environ. Pollut.*, 200, 159–160.

Lee, H. & Kim, Y. (2018). Treatment characteristics of microplastics at biological sewage treatment facilities in Korea. *Mar. Pollut. Bull.*, 137, 1–8.

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

Lei, L., Wu, S., Lu, S., Liu, M., Song, Y., Fu, Z., *et al.* (2018). Microplastic particles cause intestinal damage and other adverse effects in zebrafish Danio rerio and nematode Caenorhabditis elegans. *Sci. Total Environ.*, 619–620, 1–8.

Lenz, R., Enders, K. & Gissel, T. (2016). Microplastic exposure studies should be environmentally realistic. *Proc. Natl. Acad. Sci. U. S. A.*, 113, 2–3.

Leslie, H.A. (2014). *Review of Microplastics in Cosmetics: Scientific background on a potential source of plastic particulate marine litter to support decision-making.* IVM Institute for Environmental Studies.

Leslie, H.A., Brandsma, S.H., van Velzen, M.J.M. & Vethaak, A.D. (2017). Microplastics en route: Field measurements in the Dutch river delta and Amsterdam canals, wastewater treatment plants, North Sea sediments and biota. *Environ. Int.*, 101, 133–142.

Leusch, F.D.L. & Ziajahromi, S. (2021). Converting mg/L to Particles/L: Reconciling the Occurrence and Toxicity Literature on Microplastics. *Environ. Sci. Technol.*, 55, 11470–11472.

Li, C., Busquets, R. & Campos, L.C. (2020). Assessment of microplastics in freshwater systems: A review. *Sci. Total Environ.*, 707, 135578.

Li, C., Yuan, S., Zhou, Y., Li, X., Duan, L., Huang, L., *et al.* (2021). Microplastics reduce the bioaccumulation and oxidative stress damage of triazole fungicides in fish. *Sci. Total Environ.*, 806, 151475.

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

Lin, L., Zuo, L.Z., Peng, J.P., Cai, L.Q., Fok, L., Yan, Y., *et al.* (2018). Occurrence and distribution of microplastics in an urban river: A case study in the Pearl River along Guangzhou City, China. *Sci. Total Environ.*, 644, 375–381.

Liu, F., Olesen, K.B., Borregaard, A.R. & Vollertsen, J. (2019a). Microplastics in urban and highway stormwater retention ponds. *Sci. Total Environ.*, 671, 992–1000.

Liu, M., Lu, S., Song, Y., Lei, L., Hu, J., Lv, W., *et al.* (2018). Microplastic and mesoplastic pollution in farmland soils in suburbs of Shanghai, China. *Environ. Pollut.*, 242, 855–862.

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

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

Lohmann, R. (2017). Microplastics are not important for the cycling and bioaccumulation of organic pollutants in the oceans—but should microplastics be considered POPs themselves? *Integr. Environ. Assess. Manag.*, 13, 460–465.

Lu, F., Su, Y., Ji, Y. & Ji, R. (2021a). Release of Zinc and Polycyclic Aromatic Hydrocarbons From Tire Crumb Rubber and Toxicity of Leachate to Daphnia magna: Effects of Tire Source and Photoaging. *Bull. Environ. Contam. Toxicol.*, 107, 651–656.

Lu, H.C., Ziajahromi, S., Neale, P.A. & Leusch, F.D.L. (2021b). A systematic review of freshwater microplastics in water and sediments: Recommendations for harmonisation to enhance future study comparisons. *Sci. Total Environ.*, 781, 146693.

Luo, W., Su, L., Craig, N.J., Du, F., Wu, C. & Shi, H. (2018). Comparison of microplastic pollution in different water bodies from urban creeks to coastal waters. *Environ. Pollut.*, 246, 174–182.

Lusher, A., Bråte, I.L.N., Munno, K., Hurley, R. & Welden, N. (2020). Is It or Isn't It: The Importance of Visual Classification in Microplastic Characterization. *Appl. Spectrosc.*, 74, 1139–1153.

Maaß, S., Daphi, D., Lehmann, A. & Rillig, M.C. (2017). Transport of microplastics by two collembolan species. *Environ. Pollut.*, 225, 456–459.

Magni, S., Binelli, A., Pittura, L., Avio, C.G., Della Torre, C., Parenti, C.C., *et al.* (2019). The fate of microplastics in an Italian Wastewater Treatment Plant. *Sci. Total Environ.*, 652, 602–610.

Magnusson, K. & Norén, F. (2014). Screening of microplastic particles in and down-stream a wastewater treatment plant. *IVL Swedish Environ. Res. Inst.*, C 55, 22.

Mahon, A.M., O'Connell, B., Healy, M.G., O'Connor, I., Officer, R., Nash, R., *et al.* (2017). Microplastics in sewage sludge: Effects of treatment. *Environ. Sci. Technol.*, 51, 810–818.

Mai, L., Bao, L.J., Shi, L., Wong, C.S. & Zeng, E.Y. (2018). A review of methods for measuring microplastics in aquatic environments. *Environ. Sci. Pollut. Res.*, 25, 11319–11332.

Mak, C.W., Tsang, Y.Y., Leung, M.M.-L., Fang, J.K.-H. & Chan, K.M. (2020). Microplastics from effluents of sewage treatment works and stormwater discharging into the Victoria Harbor , Hong Kong. *Mar. Pollut. Bull.*, 157, 111181.

Mamedov, A.I., Bar-Yosef, B., Levkovich, I., Rosenberg, R., Silber, A., Fine, P., *et al.* (2016). Amending Soil with Sludge, Manure, Humic Acid, Orthophosphate and Phytic Acid: Effects on Infiltration, Runoff and Sediment Loss. *L. Degrad. Dev.*, 27, 1629–1639.

Mani, T., Hauk, A., Walter, U. & Burkhardt-Holm, P. (2016). Microplastics profile along the Rhine River. *Sci. Rep.*, 5, 17988.

Martínez Silva, P. & Nanny, M.A. (2020). Impact of microplastic fibers from the degradation of nonwoven synthetic textiles to the magdalena river water column and river sediments by the city of Neiva, Huila (Colombia). *Water*, 12, 1210.

Marwood, C., McAtee, B., Kreider, M., Ogle, R.S., Finley, B., Sweet, L., *et al.* (2011). Acute aquatic toxicity of tire and road wear particles to alga, daphnid, and fish. *Ecotoxicology*, 20, 2079–2089.

Mason, S.A., Garneau, D., Sutton, R., Chu, Y., Ehmann, K., Barnes, J., *et al.* (2016). Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent. *Environ. Pollut.*, 218, 1045–1054.

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. *Environ. Sci. Technol.*, 48, 11863–11871.

McCormick, A.R., Hoellein, T.J., London, M.G., Hittie, J., Scott, J.W. & Kelly, J.J. (2016). Microplastic in surface waters of urban rivers: Concentration, sources, and associated bacterial assemblages. *Ecosphere*, 7.

Mercier, A., Gravouil, K., Aucher, W., Brosset-Vincent, S., Kadri, L., Colas, J., *et al.* (2017). Fate of Eight Different Polymers under Uncontrolled Composting Conditions: Relationships between Deterioration, Biofilm Formation, and the Material Surface Properties. *Environ. Sci. Technol.*, 51, 1988–1997.

Michielssen, M.R., Michielssen, E.R., Ni, J. & Duhaime, M.B. (2016). Fate of microplastics and other small anthropogenic litter (SAL) in wastewater treatment plants depends on unit processes employed. *Environ. Sci. Water Res. Technol.*, 2, 1064–1073.

Miller, R.Z., Watts, A.J.R., Winslow, B.O., Galloway, T.S. & Barrows, A.P.W. (2017). Mountains to the sea: River study of plastic and non-plastic microfiber pollution in the northeast USA. *Mar. Pollut. Bull.*, 124, 245–251.

Miloloža, M., Grgić, D.K., Bolanča, T., Ukić, Š., Cvetnić, M., Bulatović, V.O., *et al.* (2021). Ecotoxicological assessment of microplastics in freshwater sources—a review. *Water*, 13, 56.

Mintenig, S.M., Bäuerlein, P.S., Koelmans, A.A., Dekker, S.C. & Wezel, A.P. Van. (2018). Closing the gap between small and smaller: Towards a framework to analyse nano- and microplastic in aqueous environmental samples. *Environ. Sci. Nano*, 5, 1640–1649.

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 Res.*, 108, 365–372.

Mintenig, S.M., Kooi, M., Erich, M.W., Primpke, S., Hasselerharm, P.E.R.-, Dekker, S.C., *et al.*

(2020). A systems approach to understand microplastic occurrence and variability in Dutch riverine surface waters. *Water Res.*, 176, 115723.

Mintenig, S.M., Löder, M.G.J., Primpke, S. & Gerdts, G. (2019). Low numbers of microplastics detected in drinking water from ground water sources. *Sci. Total Environ.*, 648, 631–635.

Mohamed Nor, N.H. & Koelmans, A.A. (2019). Transfer of PCBs from Microplastics under Simulated Gut Fluid Conditions Is Biphasic and Reversible. *Environ. Sci. Technol.*, 53, 1874–1883.

Moore, C.J., Lattin, G.L. & Zellers, A.F. (2011). Quantity and type of plastic debris flowing from two urban rivers to coastal waters and beaches of Southern California. *Rev. Gestão Costeira Integr.*, 11, 65–73.

Morehouse, C.A. (1992). Artificial Turf. In: *Turfgrass*, Agronomy Monographs (eds. Waddington, D., Carrow, R. & Shearman, R.). pp. 89–127.

Morritt, D., Stefanoudis, P. V., Pearce, D., Crimmen, O.A. & Clark, P.F. (2014). Plastic in the Thames: A river runs through it. *Mar. Pollut. Bull.*, 78, 196–200.

Mulder, M. (1996). Basic Principles of Membrane Technology. Springer Dordrecht.

Murphy, F., Ewins, C., Carbonnier, F. & Quinn, B. (2016). Wastewater Treatment Works (WwTW) as a Source of Microplastics in the Aquatic Environment. *Environ. Sci. Technol.*, 50, 5800–5808.

Napper, I.E., Bakir, A., Rowland, S.J. & Thompson, R.C. (2015). Characterisation, quantity and sorptive properties of microplastics extracted from cosmetics. *Mar. Pollut. Bull.*, 99, 178–185.

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

Nel, H.A., Dalu, T. & Wasserman, R.J. (2018). Sinks and sources: Assessing microplastic abundance in river sediment and deposit feeders in an Austral temperate urban river system. *Sci. Total Environ.*, 612, 950–956.

Nematdoost Haghi, B. & Banaee, M. (2017). Effects of micro-plastic particles on paraquat toxicity to common carp (Cyprinus carpio): biochemical changes. *Int. J. Environ. Sci. Technol.*, 14, 521–530.

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

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

Nguyen, T.H., Tang, F.H.M. & Maggi, F. (2020). Sinking of microbial-associated microplastics in

natural waters. *PLoS One*, 15, 1–20.

Nimmo, I.A. (1987). The glutathione S-transferases of fish. *Fish Physiol. Biochem.*, 3, 163–172.

Nizzetto, L., Bussi, G., Futter, M.N., Butterfield, D. & Whitehead, P.G. (2016a). A theoretical assessment of microplastic transport in river catchments and their retention by soils and river sediments. *Environ. Sci. Process. Impacts*, 18, 1050–1059.

Nizzetto, L., Butterfield, D., Futter, M., Lin, Y., Allan, I. & Larssen, T. (2016b). Assessment of contaminant fate in catchments using a novel integrated hydrobiogeochemical-multimedia fate model. *Sci. Total Environ.*, 544, 553–563.

Nizzetto, L., Futter, M. & Langaas, S. (2016c). Are Agricultural Soils Dumps for Microplastics of Urban Origin? *Environ. Sci. Technol.*, 50, 10777–10779.

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

Ockelford, A., Cundy, A. & Ebdon, J.E. (2020). Storm Response of Fluvial Sedimentary Microplastics. *Sci. Rep.*, 10, 1–10.

OECD. (2004). *Test No. 202: Daphnia sp. Acute Immobilisation Test*. OECD Guidelines for the Testing of Chemicals, Section 2.

OECD. (2007). *Test No. 225: Sediment-Water Lumbriculus Toxicity Test Using Spiked Sediment*. OECD Guidelines for the Testing of Chemicals, Section 2.

OECD. (2012). *Test No. 211: Daphnia magna Reproduction Test*. OECD Guidelines for the Testing of Chemicals, Section 2.

Ogonowski, M., Schür, C., Jarsén, Å. & Gorokhova, E. (2016). The effects of natural and anthropogenic microparticles on individual fitness in Daphnia magna. *PLoS One*, 11(5).

Oliveira, M., Ribeiro, A., Hylland, K. & Guilhermino, L. (2013). Single and combined effects of microplastics and pyrene on juveniles (0 + group) of the common goby Pomatoschistus microps (Teleostei , Gobiidae). *Ecol. Indic.*, 34, 641–647.

Oliviero, M., Tato, T., Schiavo, S., Fernández, V., Manzo, S. & Beiras, R. (2019). Leachates of micronized plastic toys provoke embryotoxic effects upon sea urchin Paracentrotus lividus. *Environ. Pollut.*, 247, 706–715.

Panko, J.M., Kreider, M.L., McAtee, B.L. & Marwood, C. (2013). Chronic toxicity of tire and road wear particles to water- and sediment-dwelling organisms. *Ecotoxicology*, 22, 13–21.

Pedà, C., Caccamo, L., Fossi, M.C., Gai, F., Andaloro, F., Genovese, L., *et al.* (2016). Intestinal alterations in European sea bass Dicentrarchus labrax (Linnaeus, 1758) exposed to microplastics: Preliminary results. *Environ. Pollut.*, 212, 251–256.

Peng, G., Xu, P., Zhu, B., Bai, M. & Li, D. (2018). Microplastics in freshwater river sediments in

Shanghai, China: A case study of risk assessment in mega-cities. *Environ. Pollut.*, 234, 448–456.

Petersen, F. & Hubbart, J.A. (2021). The occurrence and transport of microplastics: The state of the science. *Sci. Total Environ.*, 758, 143936.

Pickford, K.A., Thomas-Jones, R.E., Wheals, B., Tyler, C.R. & Sumpter, J.P. (2003). Route of exposure affects the oestrogenic response of fish to 4-tert-nonylphenol. *Aquat. Toxicol.*, 65, 267–279.

Picó, Y. & Barceló, D. (2019). Analysis and Prevention of Microplastics Pollution in Water: Current Perspectives and Future Directions. *ACS Omega*, 4, 6709–6719.

Piehl, S., Leibner, A., Löder, M.G.J., Dris, R., Bogner, C. & Laforsch, C. (2018). Identification and quantification of macro- and microplastics on an agricultural farmland. *Sci. Rep.*, 8, 17950.

Piñon-Colin, T. de J., Rodriguez-Jimenez, R., Rogel-Hernandez, E., Alvarez-Andrade, A. & Wakida, F.T. (2020). Microplastics in stormwater runoff in a semiarid region, Tijuana, Mexico. *Sci. Total Environ.*, 704.

Pinson, W.T., Yoder, D.C., Buchanan, J.R., Wright, W.C. & Wilkerson, J.B. (2004). Design and Evaluation of an Improved Flow Divider for Sampling Runoff Plots. *Appl. Eng. Agric.*, 20, 433–438.

Pirc, U., Vidmar, M., Mozer, A. & Kržan, A. (2016). Emissions of microplastic fibers from microfiber fleece during domestic washing. *Environ. Sci. Pollut. Res.*, 23, 22206–22211.

PlasticsEurope. (2018). *Plastics – the Facts*. PlasticsEurope Associaion of Plastic Manufacturers.

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

Primpke, S., Wirth, M., Lorenz, C. & Gerdts, G. (2018). Reference database design for the automated analysis of microplastic samples based on Fourier transform infrared (FTIR) spectroscopy. *Anal. Bioanal. Chem.*, 410, 5131–5141.

Prokić, M.D., Radovanović, T.B., Gavrić, J.P. & Faggio, C. (2019). Ecotoxicological effects of microplastics: Examination of biomarkers, current state and future perspectives. *TrAC - Trends Anal. Chem.*, 111, 37–46.

Qi, R., Jones, D.L., Li, Z., Liu, Q. & Yan, C. (2020). Behavior of microplastics and plastic film residues in the soil environment: A critical review. *Sci. Total Environ.*, 703, 134722.

Qiao, R., Deng, Y., Zhang, S., Wolosker, M.B., Zhu, Q., Ren, H., *et al.* (2019a). Accumulation of different shapes of microplastics initiates intestinal injury and gut microbiota dysbiosis in the gut of zebrafish. *Chemosphere*, 236, 124334.

Qiao, R., Sheng, C., Lu, Y., Zhang, Y., Ren, H. & Lemos, B. (2019b). Microplastics induce intestinal inflammation, oxidative stress, and disorders of metabolome and microbiome in zebrafish.

Sci. Total Environ., 662, 246–253.

R Core Team. (2020). R: A Language and Environment for Statistical Computing. *R Found. Stat. Comput.*

Ramaswamy, V. & Sharma, H.R. (2011). Plastic bags - threat to environment and cattle health: A retrospective study from Gondar City of Ethiopia. *IIOAB J.*, 2, 7–12.

Ramos, L., Berenstein, G., Hughes, E.A., Zalts, A. & Montserrat, J.M. (2015). Polyethylene film incorporation into the horticultural soil of small periurban production units in Argentina. *Sci. Total Environ.*, 523, 74–81.

Razanajatovo, R.M., Ding, J., Zhang, S., Jiang, H. & Zou, H. (2018). Sorption and desorption of selected pharmaceuticals by polyethylene microplastics. *Mar. Pollut. Bull.*, 136, 516–523.

Rebelein, A., Int-Veen, I., Kammann, U. & Scharsack, J.P. (2021). Microplastic fibers — Underestimated threat to aquatic organisms? *Sci. Total Environ.*, 777, 146045.

Rech, S., Macaya-Caquilpán, V., Pantoja, J.F., Rivadeneira, M.M., Jofre Madariaga, D. & Thiel, M. (2014). Rivers as a source of marine litter - A study from the SE Pacific. *Mar. Pollut. Bull.*, 82, 66–75.

Redondo-Hasselerharm, P.E., Falahudin, D., Peeters, E. & Koelmans, A.A. (2018a). Microplastic effect thresholds for freshwater benthic macroinvertebrates. *Environ. Sci. Technol.*, 52, 4, 2278–2286.

Redondo-Hasselerharm, P.E., De Ruijter, V.N., Mintenig, S.M., Verschoor, A. & Koelmans, A.A. (2018b). Ingestion and Chronic Effects of Car Tire Tread Particles on Freshwater Benthic Macroinvertebrates. *Environ. Sci. Technol.*, 52, 13986–13994.

Rehse, S., Kloas, W. & Zarfl, C. (2018). Microplastics reduce short-term effects of environmental contaminants. Part I: Effects of bisphenol a on freshwater zooplankton are lower in presence of polyamide particles. *Int. J. Environ. Res. Public Health*, 15, 280.

Rezaei, M., Riksen, M.J.P.M., Sirjani, E., Sameni, A. & Geissen, V. (2019). Wind erosion as a driver for transport of light density microplastics. *Sci. Total Environ.*, 669, 273–281.

Rillig, M.C., Ingraffia, R. & de Souza Machado, A.A. (2017a). Microplastic Incorporation into Soil in Agroecosystems. *Front. Plant Sci.*, 8, 8–11.

Rillig, M.C., Ziersch, L. & Hempel, S. (2017b). Microplastic transport in soil by earthworms. *Sci. Rep.*, 7, 1362.

Rochman, C.M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Bucci, K., *et al.* (2019). Rethinking microplastics as a diverse contaminant suite. *Environ. Toxicol. Chem.*, 38, 703–711.

Rochman, C.M., Hoh, E., Kurobe, T. & Teh, S.J. (2013). Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. *Sci. Rep.*, 3, 3263.

Rochman, C.M., Kross, S.M., Armstrong, J.B., Bogan, M.T., Darling, E.S., Green, S.J., *et al.* (2015).

Scientific Evidence Supports a Ban on Microbeads. *Environ. Sci. Technol.*, 49, 10759–10761.

Rochman, C.M., Kurobe, T., Flores, I. & Teh, S.J. (2014). Early warning signs of endocrine disruption in adult fish from the ingestion of polyethylene with and without sorbed chemical pollutants from the marine environment. *Sci. Total Environ.*, 493, 656–661.

Rodrigues, M.O., Abrantes, N., Gonçalves, F.J.M., Nogueira, H., Marques, J.C. & Gonçalves, A.M.M. (2018). Spatial and temporal distribution of microplastics in water and sediments of a freshwater system (Antuã River, Portugal). *Sci. Total Environ.*, 633, 1549–1559.

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. *Agric. Ecosyst. Environ.*, 158, 41–48.

Rolsky, C., Kelkar, V., Driver, E. & Halden, R.U. (2020). Municipal sewage sludge as a source of microplastics in the environment. *Curr. Opin. Environ. Sci. Heal.*, 14, 16–22.

RStudio Team. (2021). RStudio: Integrated development environment for R.

De Ruijter, V.N., Redondo-Hasselerharm, P.E., Gouin, T. & Koelmans, A.A. (2020). Quality Criteria for Microplastic Effect Studies in the Context of Risk Assessment: A Critical Review. *Environ. Sci. Technol.*, 54, 11692–11705.

Rummel, C.D., Adolfsson-Erici, M., Jahnke, A. & MacLeod, M. (2016). No measurable “cleaning” of polychlorinated biphenyls from Rainbow Trout in a 9 week depuration study with dietary exposure to 40% polyethylene microspheres. *Environ. Sci. Process. Impacts*, 18, 788–795.

de Sá, L.C., Luís, L.G. & Guilhermino, L. (2015). Effects of microplastics on juveniles of the common goby (Pomatoschistus microps): Confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. *Environ. Pollut.*, 196, 359–362.

de Sá, L.C., Oliveira, M., Ribeiro, F., Rocha, T.L. & Futter, M.N. (2018). Studies of the effects of microplastics on aquatic organisms: What do we know and where should we focus our efforts in the future? *Sci. Total Environ.*, 645, 1029–1039.

SAPEA. (2019). *A Scientific Perspective on Microplastics in Nature and Society*. Science Advice for Policy by European Academies, Evidence Review Report No. 4, Berlin:SEPEA.

Sarijan, S., Azman, S., Said, M.I.M. & Jamal, M.H. (2021). Microplastics in freshwater ecosystems: a recent review of occurrence, analysis, potential impacts, and research needs. *Environ. Sci. Pollut. Res.*, 28, 1341–1356.

Sato, T., Qadir, M., Yamamoto, S., Endo, T. & Zahoor, A. (2013). Global, regional, and country level need for data on wastewater generation, treatment, and use. *Agric. Water Manag.*, 130, 1–13.

Scarascia-Mugnozza, G., Sica, C. & Russo, G. (2012). Plastic Materials in European Agriculture: Actual Use and Perspectives. *J. Agric. Eng.*, 42, 15.

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. *J. Hazard. Mater.*, 420, 126481.

Schell, T., Rico, A. & Vighi, M. (2020). Occurrence, fate and fluxes of plastics and microplastics in terrestrial and freshwater ecosystems. In: *Reviews of Environmental Contamination and Toxicology (Continuation of Residue Reviews), vol 250*. Springer, New York, NY, pp. 1–43.

Scherer, C., Brennholt, N., Reifferscheid, G. & Wagner, M. (2017). Feeding type and development drive the ingestion of microplastics by freshwater invertebrates. *Sci. Rep.*, 7, 1–9.

Scherer, C., Weber, A., Lambert, S. & Wagner, M. (2018). Interactions of Microplastics with Freshwater Biota. In: *Freshwater Microplastics: Emerging Environmental Contaminants? The Handbook of Environmental Chemistry.* (eds. Wagner, M. & Lambert, S.). Springer International Publishing, pp. 153–180.

Scherer, C., Weber, A., Stock, F., Vurusic, S., Egerci, H., Kochleus, C., *et al.* (2020). Comparative assessment of microplastics in water and sediment of a large European river. *Sci. Total Environ.*, 738, 139866.

Scheurer, M. & Bigalke, M. (2018). Microplastics in Swiss Floodplain Soils. *Environ. Sci. Technol.*, 52, 3591–3598.

Schmidt, C., Krauth, T. & Wagner, S. (2017). Export of Plastic Debris by Rivers into the Sea. *Environ. Sci. Technol.*, 51, 12246–12253.

Schür, C., Rist, S., Baun, A., Mayer, P., Hartmann, N.B. & Wagner, M. (2019). When Fluorescence is not a particle: The tissue translocation of microplastics in Daphnia magna seems an artifact. *Environ. Toxicol. Chem.*, 38(7), 1495–1503.

Schuyler, Q., Hardesty, B.D., Wilcox, C. & Townsend, K. (2012). To eat or not to eat? debris selectivity by marine turtles. *PLoS One*, 7, e40884.

Scopetani, C., Cincinelli, A., Martellini, T., Lombardini, E., Ciofini, A., Fortunati, A., *et al.* (2018). Ingested microplastic as a two-way transporter for PBDEs in Talitrus saltator. *Environ. Res.*, 167, 411–417.

Seidensticker, S., Zarfl, C., Cirpka, O.A. & Grathwohl, P. (2019). Microplastic–Contaminant Interactions: Influence of Nonlinearity and Coupled Mass Transfer. *Environ. Toxicol. Chem.*, 38, 1635–1644.

Selonen, S., Dolar, A., Jemec Kokalj, A., Sackey, L.N.A., Skalar, T., Cruz Fernandes, V., *et al.* (2021). Exploring the impacts of microplastics and associated chemicals in the terrestrial environment – Exposure of soil invertebrates to tire particles. *Environ. Res.*, 201, 111495.

Selonen, S., Dolar, A., Jemec Kokalj, A., Skalar, T., Parramon Dolcet, L., Hurley, R., *et al.* (2020). Exploring the impacts of plastics in soil – The effects of polyester textile fibers on soil invertebrates. *Sci. Total Environ.*, 700, 134451.

Setyorini, L., Michler-Kozma, D., Sures, B. & Gabel, F. (2021). Transfer and effects of PET microfibers in Chironomus riparius. *Sci. Total Environ.*, 757, 143735.

Shruti, V.C., Jonathan, M.P., Rodriguez-Espinosa, P.F. & Rodríguez-González, F. (2019). Microplastics in freshwater sediments of Atoyac River basin, Puebla City, Mexico. *Sci. Total Environ.*, 654, 154–163.

Siegfried, M., Koelmans, A.A., Besseling, E. & Kroeze, C. (2017). Export of microplastics from land to sea. A modelling approach. *Water Res.*, 127, 249–257.

Sighicelli, M., Pietrelli, L., Lecce, F., Iannilli, V., Falconieri, M., Coscia, L., *et al.* (2018). Microplastic pollution in the surface waters of Italian Subalpine Lakes. *Environ. Pollut.*, 236, 645–651.

Simon, M., van Alst, N. & Vollertsen, J. (2018). Quantification of microplastic mass and removal rates at wastewater treatment plants applying Focal Plane Array (FPA)-based Fourier Transform Infrared (FT-IR) imaging. *Water Res.*, 142, 1–9.

Song, Y., Cao, C., Qiu, R., Hu, J., Liu, M., Lu, S., *et al.* (2019). Uptake and adverse effects of polyethylene terephthalate microplastics fibers on terrestrial snails (Achatina fulica) after soil exposure. *Environ. Pollut.*, 250, 447–455.

Song, Y.K., Hong, S.H., Jang, M., Han, G.M., Rani, M., Lee, J., *et al.* (2015). A comparison of microscopic and spectroscopic identification methods for analysis of microplastics in environmental samples. *Mar. Pollut. Bull.*, 93, 202–209.

Soucek, D.J., Dickinson, A. & Major, K.M. (2016). Selection of food combinations to optimize survival, growth, and reproduction of the amphipod Hyalella azteca in static-renewal, water-only laboratory exposures. *Environ. Toxicol. Chem.*, 35, 2407–2415.

De Souza Machado, A.A., Lau, C.W., Kloas, W., Bergmann, J., Bachelier, J.B., Faltin, E., *et al.* (2019). Microplastics Can Change Soil Properties and Affect Plant Performance. *Environ. Sci. Technol.*, 53, 6044–6052.

De Souza Machado, A.A.S., Lau, C.W., Till, J., Kloas, W., Lehmann, A., Becker, R., *et al.* (2018). Impacts of microplastics on the soil biophysical environment. *Environ. Sci. Technol.*, 52, 9656–9665.

Stanton, T., Johnson, M., Nathanail, P., MacNaughtan, W. & Gomes, R.L. (2020). Freshwater microplastic concentrations vary through both space and time. *Environ. Pollut.*, 263, 114481.

Stenmarck, Å., Belleza, E.L., Fråne, A., Busch, N., Larsen, Å. & Wahlström, M. (2017). *Hazardous substances in plastics - ways to increase reycling*. Nordic Council of Ministers.

Su, L., Xue, Y., Li, L., Yang, D., Kolandhasamy, P., Li, D., *et al.* (2016). Microplastics in Taihu Lake, China. *Environ. Pollut.*, 216, 711–719.

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

Sun, T., Wang, S., Ji, C., Li, F. & Wu, H. (2022). Microplastics aggravate the bioaccumulation and toxicity of coexisting contaminants in aquatic organisms: A synergistic health hazard. *J. Hazard. Mater.*, 424, 127533.

Sundt, P., Schulze, P.-E. & Syversen, F. (2014). Sources of microplastic- pollution to the marine environment Project report. *Nor. Environ. Agency*.

Syberg, K., Knudsen, C.M.H., Tairova, Z., Khan, F.R., Shashoua, Y., Geertz, T., *et al.* (2020). Sorption of PCBs to environmental plastic pollution in the North Atlantic Ocean: Importance of size and polymer type. *Case Stud. Chem. Environ. Eng.*, 2, 100062.

Talvitie, J., Heinonen, M., Pääkkönen, J.P., Vahtera, E., Mikola, A., Setälä, O., *et al.* (2015). Do wastewater treatment plants act as a potential point source of microplastics? Preliminary study in the coastal Gulf of Finland, Baltic Sea. *Water Sci. Technol.*, 72, 1495–1504.

Talvitie, J., Mikola, A., Koistinen, A. & Setälä, O. (2017a). Solutions to microplastic pollution – Removal of microplastics from wastewater effluent with advanced wastewater treatment technologies. *Water Res.*, 123, 401–407.

Talvitie, J., Mikola, A., Setälä, O., Heinonen, M. & Koistinen, A. (2017b). How well is microlitter purified from wastewater? – A detailed study on the stepwise removal of microlitter in a tertiary level wastewater treatment plant. *Water Res.*, 109, 164–172.

Tan, X., XubiaoYu, Cai, L., Wang, J. & Peng, J. (2019). Microplastics and associated PAHs in surface water from the Feilaixia Reservoir in the Beijiang River, China. *Chemosphere*, 221, 834–840.

Teuten, E.L., Rowland, S.J., Galloway, T.S. & Thompson, R.C. (2007). Potential for plastics to transport hydrophobic contaminants. *Environ. Sci. Technol.*, 41, 7759–7764.

Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Bjorn, A., *et al.* (2009). Transport and release of chemicals from plastics to the environment and to wildlife. *Philos. Trans. R. Soc. B Biol. Sci.*, 364, 2027–2045.

The jamovi project. (2021). jamovi. (Version 1.6). Retrieved from https://www.jamovi.org.

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., *et al.* (2004). Lost at Sea : Where Is All the Plastic ? *Science*, 304, 2004.

Tibbetts, J., Krause, S., Lynch, I. & Smith, G.H.S. (2018). Abundance, distribution, and drivers of microplastic contamination in urban river environments. *Water*, 10(11), 1597.

Toumi, H., Boumaiza, M., Millet, M., Radetski, C.M., Camara, B.I., Felten, V., *et al.* (2015). Investigation of differences in sensitivity between 3 strains of Daphnia magna (crustacean Cladocera) exposed to malathion (organophosphorous pesticide). *J. Environ. Sci. Heal. B*, 50(1), 34–44.

Turner, A. & Rice, L. (2010). Toxicity of tire wear particle leachate to the marine macroalga, Ulva lactuca.

Unice, K.M., Kreider, M.L. & Panko, J.M. (2013). Comparison of tire and road wear particle concentrations in sediment for watersheds in France, Japan, and the United States by quantitative pyrolysis GC/MS analysis. *Environ. Sci. Technol.*, 47, 8138–8147.

Unice, K.M., Weeber, M.P., Abramson, M.M., Reid, R.C.D., van Gils, J.A.G., Markus, A.A., *et al.* (2019). Characterizing export of land-based microplastics to the estuary - Part I: Application of integrated geospatial microplastic transport models to assess tire and road wear particles in the Seine watershed. *Sci. Total Environ.*, 646, 1639–1649.

US Congress. (2015). *Microbead-Free Waters Act of 2015. Public Law 114 – 190 114th Congress An Act*. Available at: https://www.faa.gov/air%7B%5C_%7Dtraffic/publications/controller%7B%5C_%7Dstaffing/%0Amedia/2018-ABA-200-CWP%7B%5C_%7D2019%7B%5C_%7DReport%7B%5C_%0A%7D508.pdf. Last accessed 26 May 2019.

Vaughan, R., Turner, S.D. & Rose, N.L. (2017). Microplastics in the sediments of a UK urban lake. *Environ. Pollut.*, 229, 10–18.

Velzeboer, I., Kwadijk, C.J.A.F. & Koelmans, A.A. (2014). Strong sorption of PCBs to nanoplastics, microplastics, carbon nanotubes, and fullerenes. *Environ. Sci. Technol.*, 48, 4869–4876.

Verma, R., Vinoda, K.S., Papireddy, M. & Gowda, A.N.S. (2016). Toxic Pollutants from Plastic Waste- A Review. *Procedia Environ. Sci.*, 35, 701–708.

Vermaire, J.C., Pomeroy, C., Herczegh, S.M., Haggart, O. & Murphy, M. (2017). Microplastic abundance and distribution in the open water and sediment of the Ottawa River, Canada, and its tributaries. *Facets*, 2, 301–314.

Vianello, A., Boldrin, A., Guerriero, P., Moschino, V., Rella, R., Sturaro, A., *et al.* (2013). Microplastic particles in sediments of Lagoon of Venice, Italy: First observations on occurrence, spatial patterns and identification. *Estuar. Coast. Shelf Sci.*, 130, 54–61.

Vighi, M., Rosal, R., Bayo, J., Fernández-Piñas, F., Gago, J., Gómez, M., *et al.* (2021). Micro and nano-plastics in the environment: Research priorities for the near future. *Rev. Env. Contam. Toxicol.*, 257.

Vollertsen, J. (ed) & Hansen, A.A. (ed). (2017). *Microplastic in Danish wastewater: Sources, occurrences and fate*. Danish Environmental Protection Agency.

Wagner, M., Scherer, C., Alvarez-muñoz, D., Brennholt, N., Bourrain, X., Buchinger, S., *et al.* (2014). Microplastics in freshwater ecosystems: what we know and what we need to know. *Environ. Sci. Eur.*, 26:12, 1–9.

Wagner, S., Hüffer, T., Klöckner, P., Wehrhahn, M., Hofmann, T. & Reemtsma, T. (2018). Tire wear particles in the aquatic environment - A review on generation, analysis, occurrence, fate and effects. *Water Res.*, 139, 83–100.

Waldschläger, K., Lechthaler, S., Stauch, G. & Schüttrumpf, H. (2020). The way of microplastic through the environment – Application of the source-pathway-receptor model (review). *Sci. Total Environ.*, 713, 136584.

Wallace, C.K., Bright, L.A., Marx, J.O., Andersen, R.P., Mullins, M.C. & Carty, A.J. (2018). Effectiveness of Rapid Cooling as a Method of Euthanasia for Young Zebrafish (Danio rerio). *J. Am. Assoc. Lab. Anim. Sci.*, 57, 58–63.

Wang, F., Zhang, M., Sha, W., Wang, Y., Hao, H., Dou, Y., *et al.* (2020). Sorption behavior and mechanisms of organic contaminants to nano and microplastics. *Molecules*, 25, 1827.

Wang, J., Peng, J., Tan, Z., Gao, Y., Zhan, Z., Chen, Q., *et al.* (2017a). Microplastics in the surface sediments from the Beijiang River littoral zone: Composition, abundance, surface textures and interaction with heavy metals. *Chemosphere*, 171, 248–258.

Wang, W., Ndungu, A.W., Li, Z. & Wang, J. (2017b). Microplastics pollution in inland freshwaters of China: A case study in urban surface waters of Wuhan, China. *Sci. Total Environ.*, 575, 1369–1374.

Wang, W. & Wang, J. (2018). Comparative evaluation of sorption kinetics and isotherms of pyrene onto microplastics. *Chemosphere*, 193, 567–573.

Wang, W., Yuan, W., Chen, Y. & Wang, J. (2018). Microplastics in surface waters of Dongting Lake and Hong Lake, China. *Sci. Total Environ.*, 633, 539–545.

Watkins, L., Sullivan, P.J. & Walter, M.T. (2019). A case study investigating temporal factors that influence microplastic concentration in streams under different treatment regimes. *Environ. Sci. Pollut. Res.*, 26, 21797–21807.

Watterson, A. (2017). Artificial turf: Contested terrains for precautionary public health with particular reference to Europe? *Int. J. Environ. Res. Public Health*, 14.

Weithmann, N., Möller, J.N., Löder, M.G.J., Piehl, S., Laforsch, C. & Freitag, R. (2018). Organic fertilizer as a vehicle for the entry of microplastic into the environment. *Sci. Adv.*, 4, 1–8.

Wen, B., Zhang, N., Jin, S.R., Chen, Z.Z., Gao, J.Z., Liu, Y., *et al.* (2018a). Microplastics have a more profound impact than elevated temperatures on the predatory performance, digestion and energy metabolism of an Amazonian cichlid. *Aquat. Toxicol.*, 195, 67–76.

Wen, X., Du, C., Xu, P., Zeng, G., Huang, D., Yin, L., *et al.* (2018b). Microplastic pollution in surface sediments of urban water areas in Changsha, China: Abundance, composition, surface textures. *Mar. Pollut. Bull.*, 136, 414–423.

Wetherbee, G.A., Baldwin, A.K. & Ranville., J.F. (2019). *It is Raining Plastic. U.S. Geological Survey. Open-File Report 2019–1048. Open-File Rep.* available at https://doi.org/10.3133/ofr20191048.

WHO/UNICEF. (2019). Joint Monitoring Programme for water supply, sanitation and hygiene (JMP). Available at: https://ec.europa.eu/knowledge4policy/online-resource/whounicef-joint-

monitoringprogramme-water-supply-sanitation-hygiene-jmp_en?pages=1. Last accessed 10 June 2019.

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.

Wik, A. (2007). Toxic components leaching from tire rubber. *Bull. Environ. Contam. Toxicol.*, 79, 114–119.

Wik, A. & Dave, G. (2005). Environmental labeling of car tires-toxicity to Daphnia magna can be used as a screening method. *Chemosphere*, 58, 645–651.

Wik, A. & Dave, G. (2006). Acute toxicity of leachates of tire wear material to Daphnia magna-Variability and toxic components. *Chemosphere*, 64, 1777–1784.

Wik, A. & Dave, G. (2009). Occurrence and effects of tire wear particles in the environment - A critical review and an initial risk assessment. *Environ. Pollut.*, 157, 1–11.

Wik, A., Nilsson, E., Källqvist, T., Tobiesen, A. & Dave, G. (2009). Toxicity assessment of sequential leachates of tire powder using a battery of toxicity tests and toxicity identification evaluations. *Chemosphere*, 77, 922–927.

Williams, A.T. & Simmons, S.L. (1996). The degradation of plastic litter in rivers: Implications for beaches. *J. Coast. Conserv.*, 2, 63–72.

Williams, A.T. & Simmons, S.L. (1999). Sources of riverine litter: The river Taff, South Wales, UK. *Water. Air. Soil Pollut.*, 112, 197–216.

Windsor, F.M., Durance, I., Horton, A.A., Thompson, R.C., Tyler, C.R. & Ormerod, S.J. (2019a). A catchment-scale perspective of plastic pollution. *Glob. Chang. Biol.*, 25, 1207–1221.

Windsor, F.M., Tilley, R.M., Tyler, C.R. & Ormerod, S.J. (2019b). Microplastic ingestion by riverine macroinvertebrates. *Sci. Total Environ.*, 646, 68–74.

Wolff, S., Kerpen, J., Prediger, J., Barkmann, L. & Müller, L. (2018). Determination of the Microplastics Emission in the Effluent of a Municipal Waste Water Treatment Plant using Raman Microspectroscopy. *Water Res. X*, 2, 100014.

Wong, J.K.H., Lee, K.K., Tang, K.H.D. & Yap, P.S. (2020). Microplastics in the freshwater and terrestrial environments: Prevalence, fates, impacts and sustainable solutions. *Sci. Total Environ.*, 719, 137512.

Woodward, J., Li, J., Rothwell, J. & Hurley, R. (2021). Acute riverine microplastic contamination due to avoidable releases of untreated wastewater. *Nat. Sustain.*, 2019.

Wright, S.L., Thompson, R.C. & Galloway, T.S. (2013). The physical impacts of microplastics on marine organisms: A review. *Environ. Pollut.*, 178, 483–492.

Wu, N., Zhang, Y., Li, W., Wang, J., Zhang, X., He, J., *et al.* (2020a). Co-effects of biofouling and inorganic matters increased the density of environmental microplastics in the sediments of Bohai Bay coast. *Sci. Total Environ.*, 717, 134431.

Wu, P., Tang, Y., Dang, M., Wang, S., Jin, H., Liu, Y., *et al.* (2020b). Spatial-temporal distribution of microplastics in surface water and sediments of Maozhou River within Guangdong-Hong Kong-Macao Greater Bay Area. *Sci. Total Environ.*, 717, 135187.

Xanthos, D. & Walker, T.R. (2017). International policies to reduce plastic marine pollution from single-use plastics (plastic bags and microbeads): A review. *Mar. Pollut. Bull.*, 118, 17–26.

Xia, W., Rao, Q., Deng, X., Chen, J. & Xie, P. (2020). Rainfall is a significant environmental factor of microplastic pollution in inland waters. *Sci. Total Environ.*, 732, 139065.

Xiong, X., Wu, C., Elser, J.J., Mei, Z. & Hao, Y. (2019). Occurrence and fate of microplastic debris in middle and lower reaches of the Yangtze River – From inland to the sea. *Sci. Total Environ.*, 659, 66–73.

Yan, M., Nie, H., Xu, K., He, Y., Hu, Y., Huang, Y., *et al.* (2019). Microplastic abundance, distribution and composition in the Pearl River along Guangzhou city and Pearl River estuary, China. *Chemosphere*, 217, 879–886.

Yang, L., Zhang, Y., Kang, S., Wang, Z. & Wu, C. (2021). Microplastics in freshwater sediment: A review on methods, occurrence, and sources. *Sci. Total Environ.*, 754, 141948.

Yang, S.-S., Brandon, A.M., Andrew Flanagan, J.C., Yang, J., Ning, D., Cai, S.-Y., *et al.* (2018). Biodegradation of polystyrene wastes in yellow mealworms (larvae of Tenebrio molitor Linnaeus): Factors affecting biodegradation rates and the ability of polystyrene-fed larvae to complete their life cycle. *Chemosphere*, 191, 979–989.

Yao, P., Zhou, B., Lu, Y.H., Yin, Y., Zong, Y.Q., Chen, M. Te, *et al.* (2019). A review of microplastics in sediments: Spatial and temporal occurrences, biological effects, and analytic methods. *Quat. Int.*, 519, 274–281.

Ye, S. & Andrady, A.L. (1991). Fouling of Floating Plastic Debris Under Biscayne Bay Exposure Conditions. *Mar. Pollut. Bull.*, 22, 608–613.

Yoshida, S., Hiraga, K., Takanaha, T., Taniguchi, I., Yamaji, H., Maeda, Y., *et al.* (2016). A bacterium that degrades and assimilates poly(ethyleneterephthalate). *Science*, 351, 1196–1199.

Yu, F., Yang, C., Zhu, Z., Bai, X. & Ma, J. (2019). Adsorption behavior of organic pollutants and metals on micro/nanoplastics in the aquatic environment. *Sci. Total Environ.*, 694, 133643.

Yuan, W., Liu, X., Wang, W., Di, M. & Wang, J. (2019). Microplastic abundance, distribution and composition in water, sediments, and wild fish from Poyang Lake, China. *Ecotoxicol. Environ. Saf.*, 170, 180–187.

Zbyszewski, M. & Corcoran, P.L. (2011). Distribution and degradation of fresh water plastic particles along the beaches of Lake Huron, Canada. *Water. Air. Soil Pollut.*, 220, 365–372.

Zbyszewski, M., Corcoran, P.L. & Hockin, A. (2014). Comparison of the distribution and degradation of plastic debris along shorelines of the Great Lakes, North America. *J. Great*

Lakes Res., 40, 288–299.

Zettler, E.R., Mincer, T.J. & Amaral-Zettler, L.A. (2013). Life in the “Plastisphere”: Microbial Communities on Plastic Marine Debris. *Environ. Sci. Technol.*, 47, 7137–7146.

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

Zhang, K., Gong, W., Lv, J., Xiong, X. & Wu, C. (2015). Accumulation of floating microplastics behind the Three Gorges Dam. *Environ. Pollut.*, 204, 117–123.

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

Zhang, L., Xie, Y., Liu, J., Zhong, S., Qian, Y. & Gao, P. (2020). An Overlooked Entry Pathway of Microplastics into Agricultural Soils from Application of Sludge-Based Fertilizers. *Environ. Sci. Technol.*, 54, 4248–4255.

Zhang, S., Yang, X., Gertsen, H., Peters, P., Salánki, T. & Geissen, V. (2018). A simple method for the extraction and identification of light density microplastics from soil. *Sci. Total Environ.*, 616–617, 1056–1065.

Zhang, X., Leng, Y., Liu, X., Huang, K. & Wang, J. (2019). Microplastics ’ Pollution and Risk Assessment in an Urban River: A Case Study in the Yongjiang River, Nanning City South China. *Expo. Heal.*, 12, 141–151.

Zhao, S., Zhu, L. & Li, D. (2016). Microscopic anthropogenic litter in terrestrial birds from Shanghai, China: Not only plastics but also natural fibers. *Sci. Total Environ.*, 550, 1110–1115.

Zhou, M., Wang, R., Cheng, S., Xu, Y., Luo, S., Zhang, Y., *et al.* (2021). Bibliometrics and visualization analysis regarding research on the development of microplastics. *Environ. Sci. Pollut. Res.*, 28, 8953–8967.

Ziajahromi, S., Drapper, D., Hornbuckle, A., Rintoul, L. & Leusch, F.D.L. (2019). Microplastic pollution in a stormwater floating treatment wetland: Detection of tyre particles in sediment. *Sci. Total Environ.*, 713, 136356.

Ziajahromi, S., Kumar, A., Neale, P.A. & Leusch, F.D.L. (2017a). Impact of Microplastic Beads and Fibers on Waterflea (Ceriodaphnia dubia) Survival, Growth, and Reproduction: Implications of Single and Mixture Exposures. *Environ. Sci. Technol.*, 51, 13397–13406.

Ziajahromi, S., Neale, P.A. & Leusch, F.D.L.L. (2016). Wastewater treatment plant effluent as a source of microplastics: review of the fate, chemical interactions and potential risks to aquatic organisms. *Water Sci. Technol.*, 74, 2253–2269.

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

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

Zylstra, E.R. (2013). Accumulation of wind-dispersed trash in desert environments. *J. Arid Environ.*, 89, 13–15.

Microplastics are ubiquitously detected in the natural environment. However, major knowledge gaps exist regarding their sources and pathways into the environment and the risks of microplastic pollution for freshwater ecosystems. This thesis assesses microplastic contamination in freshwaters and agricultural soils and the importance of wastewater and agricultural surface runoff as microplastic pathways into aquatic environments under Mediterranean conditions. Furthermore, the impacts of microplastic fibers and tire particles on freshwater invertebrates and the relevance of microplastics as vectors of organic contaminants in the aquatic environment are investigated.