Spatio-temporal distribution of microplastics in a Mediterranean river catchment: the importance of wastewater as an environmental pathway

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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 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 $1x10^{10}$ 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.

Keywords: microplastics, wastewater treatment plants, environmental monitoring, freshwater ecosystems, Mediterranean region

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; Wagner et al., 2018; Ziajahromi et al., 2016). Although studies show that wastewater treatment plants (WWTPs) are efficient at capturing MPs (about 60-99.9% are retained; Barchiesi et al. 2021; Ngo et al., 2019), they still constitute a potentially significant source due to the very large volumes of effluents discharged (Edo et al., 2020; Ziajahromi et al., 2017). 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 (Liu et al., 2021; Mason et al., 2016). 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 (Liu et al., 2019; Mintenig et al., 2017). 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 and 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 (Cai et al., 2017; Dris et al., 2018; Lechner and Ramler, 2015; Piñon-Colin et al., 2020; Knight 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., 2019).

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.

2. Material and methods

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 (Fig. 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 2.66×10^8 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 (Fig. 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; Tab. A1).

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 (Fig. 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 of November) of 2017, and spring (23-24 April) of 2018.

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.

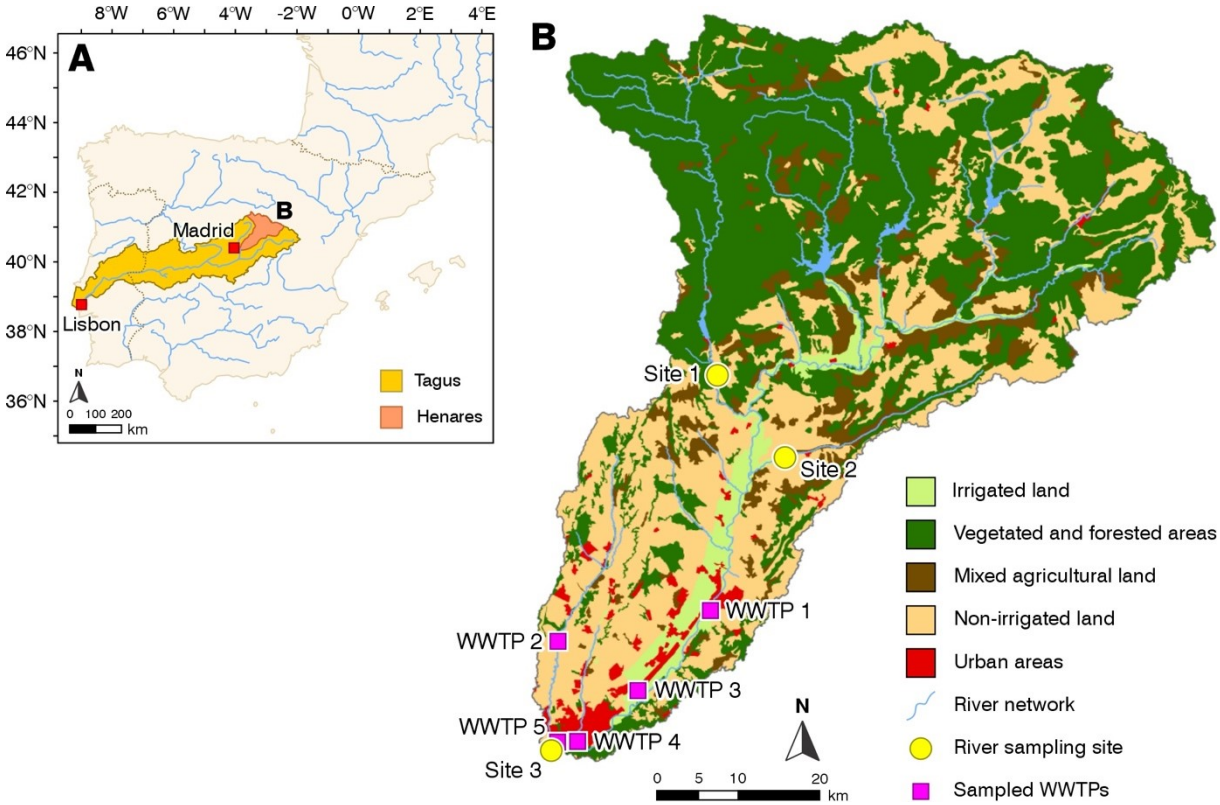


Fig. 1 Location of the Henares river catchment within the Tagus River Basin in the Iberian Peninsula (A). The major river networks of the region are also shown. Location of river sampling sites and the sampled WWTPs within the Henares catchment (B), with a description of land use retrieved from the Corine land cover database (Corine land cover, 2006).

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. (2018a). 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. (2018a). 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 20-50x 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 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.

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.

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 (Tab. 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.

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.

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 and Šmilauer, 2018). Statistical influence of the tested parameters was determined when the calculated p-value was < 0.05.

3. Results and discussion

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} (Tab. 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 (Fig. A1; Tab. 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 (Fig 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 (Tab. B2). The most frequent polymer types based on particle counts were polyester, polyethylene, and polypropylene (Fig. 2 and A3, Tab. 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 (Tab. 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} (Tab. 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%; Fig. A1; Tab. 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 (Fig. A1; Tab. 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%; Fig. A2). Fourteen different polymer types were identified, as well as suspected paint fragments and tire particles (Fig. 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 as 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 (Fig. 2 and A3; Tab. 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 (Tab. A3). As expected, we identified statistically significant differences in terms of size, particle type, and polymer composition ($p < 0.01$ in all cases; Tab. 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 (Barchiesi et al. 2021; Sun et al. 2019). 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 (Cao et al. 2020; Wolff et al., 2018; 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., Akarsu et al., 2020; Bayo et al., 2020a; Conley et al., 2019; Gündoğdu et al., 2018; Talvitie et al., 2017a). 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 (Tab. A3). Previous studies have shown that secondary treatments can achieve already up to 99% removal (Magnusson and 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 (Bayo et al., 2020b; Michielssen et al., 2016; Talvitie et al., 2017a, 2017b).

In the current study, the overall retention capacity was higher for fibers than for other MP types (Tab. 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 and 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.

Tab. 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	5,050	194	110+10*	8.00*	8,213 (7,161-10,167)	32.8 (25.1-44.5)	6,276 (3,878-7,876)	92.1 (37.8-196)	97.8	24,675	202,070
			Au	11,550	146	70	1.41	11,954 (9,517-14,545)	82.0 (54.8-129)	5,640 (5,222-6,256)	20.6 (18.1-22.9)	99.4	19,989	148,470
2	U	S	Su	2,650	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	1,000	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	1075885
3	U/I	T	Su	850	69.2	255	35.2	10,569 (9,633-11,123)	54.6 (37.3-73.5)	3,304 (2,432-4,749)	49.2 (5.12-136)	70.0	1,837	8,179,635
			Au	3,550	95.1	135+10*	0.58*	60,277 (54,941-66,260)	305 (70.9-716)	9,038 (8,388-9,743)	141 (23.1-364)	96.2	2,121	5,071,545
4	U	S	Su	1,000	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	1,982	101400
			Au	1,400	1.86	450+460*	16.4+4.0*	13,737 (12,550-15,949)	65.7 (52.2-85.1)	3,978 (3,352-4,972)	516 (5.05-1525)	67.9	2,011	166,500
5	U/I	T	Su	3,350	16.5	45+240*	0.28+0.11*	36,730 (35,185-39,431)	129 (60.0-215)	9,487 (7,648-11,257)	142 (38.0-312)	98.7	32,077	1,110,375
			Au	2,000	4.51	280+600*	5.3+4.4*	37,373 (31,809-43,035)	127 (93.6-159)	7,340 (4,677-11,300)	59.1 (8.91-129)	86.0	37,567	5,596,920

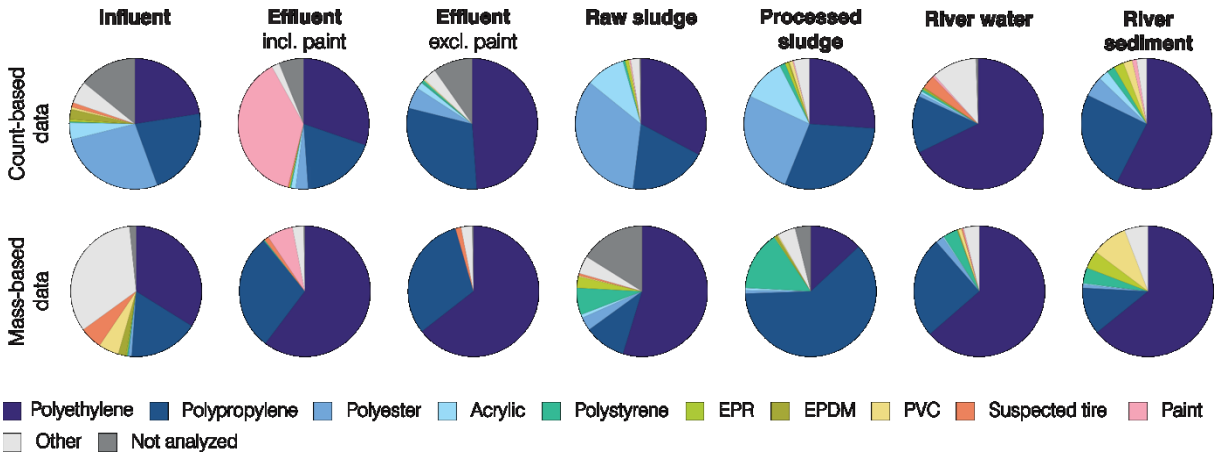


Fig. 2. Mean count-based and mass-based polymer distribution across all sampling sites and sampling events in different sample types. Acrylic refers only to fibers and does not include acrylic paint. Paint includes paint acrylate and other types of paint fragments matched to a polymeric component of 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.

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 (Tab. A5).

MPs in the raw sludge ranged from about 8,000 to 60,000 MPs kg^{-1} dw, and from about 33 to 350 mg kg^{-1} dw (Tab. 1). In the processed sludge, concentrations ranged between about 3,300 and 22,000 MPs kg^{-1} dw, and, in terms of mass, between 21 and 516 mg kg^{-1} dw (Tab. 1). As can be seen from the range of measurements in Table 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 1,500 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 and 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 (Tab. 1). Raw sludge contained a significantly higher number of particles of all sizes but particularly the larger size fractions (>150 µm; p=0.004; Tab. 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%) (Fig. 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; Fig. A1; Tab. 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 (Fig. A3; Tab. 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 $10x10^{13}$ MP particles per year. However, they found much higher MPs concentrations in sludge and soil amendments (133,000 ± 59 MPs kg^{-1} ww and 101,000 ± 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 MPs in river water

Blanks from April 2018 samples showed no presence of MPs, with only cellulose fibers observed (Tab. 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 (Tab. 2). The most frequently observed particle morphologies were fragments (62-91%, with an average of 81%), followed by fibers and beads (Fig. A1). Granules, films, glitter and foams were also detected (Fig A1; Tab. 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; Fig. 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 55-150 µm (41% of all particles) or 150-300 µm (41%; Fig. A1) 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 (Fig. 2; Tab. 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 (Tab. 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 and Fahrenfeld, 2016; Tibbetts et al., 2018; Vermaire et al., 2017; Wang et al., 2017; Wu et al., 2020a). 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 spatiotemporal 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 (Tab. 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 (Mintenig et al., 2020; Watkins et al., 2019), possibly due to MP re-suspension from the sediment phase (Hurley et al., 2018b). 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).

Tab. 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)

3.4 MPs in river sediment

MPs observed in the blanks were entirely composed of viscose fibers (Tab. 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; Tab. 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 (Fig. A1; Tab. 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 (Fig. 2, Fig. 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., 2020b) 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 (Fig. 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 (Hurley et al., 2018b; Nizzetto et al., 2016a; 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.

3.5 Wastewater contribution to river contamination

We estimate that about $1.0x10^{10}$ MPs (or 389 kg), from which $1.5x10^{9}$ MPs (or 6 kg) are fibers, enter the Henares River and its tributaries via wastewater annually (Tab. 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 $4.1x10^{10}$ MPs ($2.1x10^{10}$ -$6.2x10^{10}$ MPs) or about 4,000 kg (2,646-5,331 kg), which is discharged into the Tagus River system. Fibers represent $3.3.x10^{8}$-$1.4x10^{9}$ MPs (0.23-175 kg), while the remaining $1.9x10^{10}$-$6.2x10^{10}$ (2,472-7,379 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 ($2.75x10^{8}$ m^3; Tab. 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 $5.2x10^{7}$ m^3 per year, of which $7.6x10^{5}$ 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 ($2.66x10^{8}$ m^3). However, the annual discharge in 2017 was only half of this ($1.36x10^{8}$ m^3) due to a dry Spring (Tab. 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.

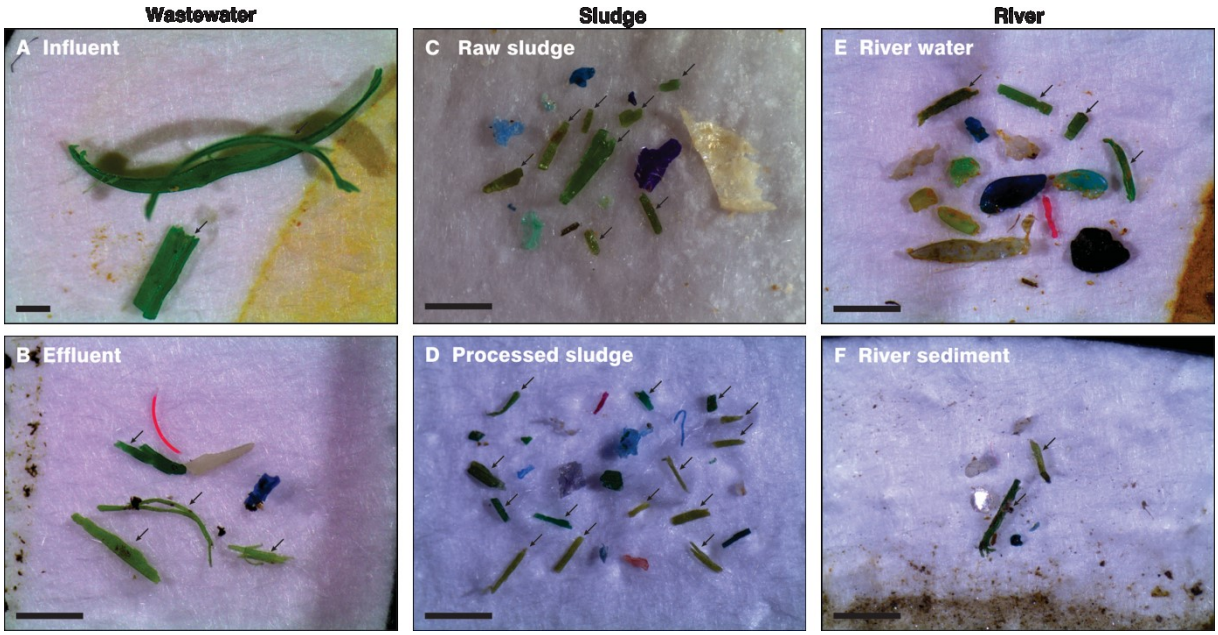
Wastewater largely represents a pathway for MPs from other sources, which converge and are concentrated within wastewater systems and as a result of wastewater treatment processes. Thus, the majority of MPs do not originate from the wastewater system itself. Improving treatment steps to retain more MP 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 presents examples of these particles. One possible source could be artificial turf, which is often composed of green polypropylene shards (e.g., Ekstrand et al., 2006; Morehouse, 1992; 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.

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 (Fig. 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 (Fig. 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).

Tab. 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]	$7.9x10^9$	318.7
Untreated urban wastewater	765,752	2439[b]	76[b]	$1.9x10^9$	58.2
Treated industrial effluents	2,276,415	123[c]	5.5[c]	$2.8x10^8$	12.5
Total amount	52,267,453			$1.0x10^{10}$	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.*



682
683 *Fig. 3. Examples of green polypropylene fragments that share a visual resemblance, observed in all different sample types.*
684 *The relevant particles are indicated with an arrow. The black scale bar represents 1000 µm in each image.*

685 ### 3.6 Analytical challenges and sample harmonization

686 The results reported for wastewater and sludge are characterized by a discrepancy between
687 count and mass-based concentrations. This was largely governed by the high proportion of
688 fibers, which typically contribute less to the total mass based on their low particle volume
689 compared to other particle types. MP counts and mass concentrations in river water and
690 sediment − which mostly contained fragments − were in better agreement. Discrepancies
691 between count and mass-based concentrations have been observed previously (Constant et al.,
692 2020; Kataoka et al., 2019). This disparity has the potential to result in different trends being
693 described for MP contamination in the environment. Mass represents a more robust metric for
694 the comparison of total plastic loads amongst studies, due to the fact that MPs may fragment
695 during MP sample processing and analysis (Simon et al. 2018). On the other hand, the
696 possibility to report particle counts and describe individual particle characteristics such as size,
697 shape, and polymer composition, is more important for environmental risk assessment. These
698 factors determine the ability of organisms to ingest MPs and are likely to influence the
699 associated risk (e.g., Carbery et al. 2018). Currently available methods for assessing these
700 variables are typically costly and time intensive. Additional work is required to produce mass-
701 based estimates for these particles. This is in contrast to methods that provide outputs as mass-
702 based quantification, such as pyrolysis gas- chromatography mass-spectrometry, which may be
703 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.

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 spatiotemporal 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.

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6. References

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. https://doi.org/10.1016/j.marpolbul.2019.110778

Auta, H.S., Emenike, C.U., Fauziah, S.H., 2017. Distribution and importance of microplastics in the marine environmentA review of the sources, fate, effects, and potential solutions. Environ. Int. 102, 165–176. https://doi.org/10.1016/j.envint.2017.02.013

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. https://doi.org/10.1016/j.jwpe.2020.101787

Bayo, J., Olmos, S., López-Castellanos, J., 2020a. Microplastics in an urban wastewater treatment plant: The influence of physicochemical parameters and environmental factors. Chemosphere 238. https://doi.org/10.1016/j.chemosphere.2019.124593

Bayo, J., López-Castellanos, J., Olmos, S., 2020b. Membrane bioreactor and rapid sand filtration for the removal of microplastics in an urban wastewater treatment plant. Mar. Pollut. Bull. 156, 111211. https://doi.org/10.1016/j.marpolbul.2020.111211

Cai, L., Wang, J., Peng, J., Tan, Z., Zhan, Z., Tan, X., Chen, Q., 2017. Characteristic of microplastics in the atmospheric fallout from Dongguan city, China: preliminary research and first evidence. Environ. Sci. Pollut. Res. 24, 24928–24935. https://doi.org/10.1007/s11356-017-0116-x

Camargo, J. A. (ed.), 2006. Ecología y Conservación del Río Henares y sus Tributarios. Cersa Ediciones, Madrid.

Cao, Y., Wang, Q., Ruan, Y., Wu, R., Chen, L., Zhang, K., Lam, P.K.S., 2020. Intra-day microplastic variations in wastewater: A case study of a sewage treatment plant in Hong Kong. Mar. Pollut. Bull. 160, 111535. https://doi.org/10.1016/j.marpolbul.2020.111535

Carbery, M., O'Connor, W., Palanisami, T., 2018. Trophic transfer of microplastics and

771 mixed contaminants in the marine food web and implications for human health. Environ.
772 Int. 115, 400-409. https://doi.org/10.1016/j.envint.2018.03.007.
773 CEDEX (Centro de Estudios y Experimentación de Obras Públicas) 2021.
774 https://ceh.cedex.es/anuarioaforos/afo/estaf-datos.asp?indroea=3062 (accessed 19 May
775 2021).
776 Cera, A., Cesarini, G., Scalici, M., 2020. Microplastics in freshwater: What is the news from
777 the world? Diversity 12. https://doi.org/10.3390/d12070276
778 Cheung, P.K., Hung, P.L., Fok, L., 2019. River Microplastic Contamination and Dynamics
779 upon a Rainfall Event in Hong Kong, China. Environ. Process. 6, 253–264.
780 https://doi.org/10.1007/s40710-018-0345-0
781 Confederación Hidrográfica del Tajo 2019.
782 http://www.chtajo.es/LaCuenca/CalidadAgua/ControlVertidos/Paginas/default.aspx#Cen
783 so%20de%20Vertidos (accessed 4 December 2020).
784 Conley, K., Clum, A., Deepe, J., Lane, H., Beckingham, B., 2019. Wastewater treatment
785 plants as a source of microplastics to an urban estuary: Removal efficiencies and loading
786 per capita over one year. Water Res. X 3, 100030.
787 https://doi.org/10.1016/j.wroa.2019.100030
788 Constant, M., Ludwig, W., Kerhervé, P., Sola, J., Charrière, B., Sanchez-Vidal, A., Canals,
789 M., Heussner, S., 2020. Microplastic fluxes in a large and a small Mediterranean river
790 catchments: The Têt and the Rhône, Northwestern Mediterranean Sea. Sci. Total
791 Environ. 716. https://doi.org/10.1016/j.scitotenv.2020.136984
792 Corine land cover, 2006.
793 https://www.eea.europa.eu/themes/landuse/interactive/clc-download.
794 Corradini, F., Meza, P., Eguiluz, R., Casado, F., Huerta-Lwanga, E., Geissen, V., 2019.
795 Evidence of microplastic accumulation in agricultural soils from sewage sludge disposal.
796 Sci. Total Environ. 671, 411–420. https://doi.org/10.1016/j.scitotenv.2019.03.368
797 de Sá, L.C., Oliveira, M., Ribeiro, F., Rocha, T.L., Futter, M.N., 2018. Studies of the effects
798 of microplastics on aquatic organisms: What do we know and where should we focus our
799 efforts in the future? Sci. Total Environ. 645, 1029–1039.
800 https://doi.org/10.1016/j.scitotenv.2018.07.207
801 Dris, R., Gasperi, J., Rocher, V., Tassin, B., 2018. Synthetic and non-synthetic anthropogenic
802 fibers in a river under the impact of Paris Megacity: Sampling methodological aspects
803 and flux estimations. Sci. Total Environ. 618, 157–164.

https://doi.org/10.1016/j.scitotenv.2017.11.009
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. https://doi.org/10.1016/j.envpol.2019.113837
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. https://doi.org/10.1136/bjsm.2006.027623
Enders, K., Käppler, A., Biniasch, O., Feldens, P., Stollberg, N., Lange, X., Fischer, D., Eichhorn, K.J., Pollehne, F., Oberbeckmann, S., Labrenz, M., 2019. Tracing microplastics in aquatic environments based on sediment analogies. Sci. Rep. 9, 1–15. https://doi.org/10.1038/s41598-019-50508-2
Estahbanati, S., Fahrenfeld, N.L., 2016. Influence of wastewater treatment plant discharges on microplastic concentrations in surface water. Chemosphere 162, 277–284. https://doi.org/10.1016/j.chemosphere.2016.07.083
Estrela, T., Pérez-Martin, M.A., Vargas, E., 2012. Impacts of climate change on water resources in Spain. Hydrol. Sci. J. 57, 1154–1167. https://doi.org/10.1080/02626667.2012.702213
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. https://doi.org/10.1016/j.envpol.2019.05.056
Gaylarde, C.C., Neto, J.A.B., da Fonseca, E.M., 2021. Paint fragments as polluting microplastics: A brief review. Mar. Pollut. Bull. 162. https://doi.org/10.1016/j.marpolbul.2020.111847
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. https://doi.org/10.3390/jmse8030216
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. https://doi.org/10.1007/s10661-018-7010-y
Han, M., Niu, X., Tang, M., Zhang, B.T., Wang, G., Yue, W., Kong, X., Zhu, J., 2020. Distribution of microplastics in surface water of the lower Yellow River near estuary.

Sci. Total Environ. 707, 135601. https://doi.org/10.1016/j.scitotenv.2019.135601
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. https://doi.org/10.1021/acs.est.7b05829
Horton, A.A., Dixon, S.J., 2018. Microplastics: An introduction to environmental transport processes. Wiley Interdiscip. Rev. Water 5, e1268. https://doi.org/10.1002/wat2.1268
Hurley, R. R., Lusher, A. L., Olsen, M., Nizzetto, L., 2018a. Validation of a Method for Extracting Microplastics from Complex, Organic-Rich, Environmental Matrices. Environ. Sci. Technol. 52(13), 7409–7417. https://doi.org/10.1021/acs.est.8b01517.
Hurley, R., Woodward, J., Rothwell, J.J., 2018b. Microplastic contamination of river beds significantly reduced by catchment-wide flooding. Nat. Geosci. 11, 251–257. https://doi.org/10.1038/s41561-018-0080-1
Järlskog, I., Strömvall, A., Magnusson, K., Gustafsson, M., Polukarova, M., Gal, H., Aronsson, M., Andersson-sköld, Y., 2020. Occurrence of tire and bitumen wear microplastics on urban streets and in sweepsand and washwater. Sci. Total Environ. 729, 138950. https://doi.org/10.1016/j.scitotenv.2020.138950
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. https://doi.org/10.1016/j.envpol.2018.10.111
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. https://doi.org/10.1007/s11356-020-08187-4
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. https://doi.org/10.1016/j.watres.2019.02.054
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. https://doi.org/10.3390/ijerph14101265
Kooi, M., Koelmans, A.A., 2019. Simplifying Microplastic via Continuous Probability Distributions for Size, Shape,and Density. Environ. Sci. Technol. Lett. 6, 551–557. https://doi.org/10.1021/acs.estlett.9b00379
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.

870 Monit. Assess. 193. https://doi.org/10.1007/s10661-021-08943-0
871 Kreider, M.L., Panko, J.M., McAtee, B.L., Sweet, L.I., Finley, B.L., 2010. Physical and
872 chemical characterization of tire-related particles: Comparison of particles generated
873 using different methodologies. Sci. Total Environ. 408, 652–659.
874 https://doi.org/https://doi.org/10.1016/j.scitotenv.2009.10.016
875 Lagarde, F., Olivier, O., Zanella, M., Daniel, P., Hiard, S., Caruso, A., 2016. Microplastic
876 interactions with freshwater microalgae: Hetero-aggregation and changes in plastic
877 density appear strongly dependent on polymer type. Environ. Pollut. 215, 331–339.
878 https://doi.org/10.1016/j.envpol.2016.05.006
879 Lares, M., Ncibi, M.C., Sillanpää, Markus, Sillanpää, Mika, 2018. Occurrence, identification
880 and removal of microplastic particles and fibers in conventional activated sludge process
881 and advanced MBR technology. Water Res. 133, 236–246.
882 https://doi.org/10.1016/j.watres.2018.01.049
883 Lechner, A., Keckeis, H., Lumesberger-Loisl, F., Zens, B., Krusch, R., Tritthart, M., Glas, M.,
884 Schludermann, E., 2014. The Danube so colourful: A potpourri of plastic litter
885 outnumbers fish larvae in Europe's second largest river. Environ. Pollut. 188, 177–181.
886 https://doi.org/10.1016/j.envpol.2014.02.006
887 Lechner, A., Ramler, D., 2015. The discharge of certain amounts of industrial microplastic
888 from a production plant into the River Danube is permitted by the Austrian legislation.
889 Environ. Pollut. 200, 159–160. https://doi.org/10.1016/j.envpol.2015.02.019
890 Lee, H., Kim, Y., 2018. Treatment characteristics of microplastics at biological sewage
891 treatment facilities in Korea. Mar. Pollut. Bull. 137, 1–8.
892 https://doi.org/10.1016/j.marpolbul.2018.09.050
893 Liu, X., Yuan, W., Di, M., Li, Z., Wang, J., 2019. Transfer and fate of microplastics during
894 the conventional activated sludge process in one wastewater treatment plant of China.
895 Chem. Eng. J. 362, 176–182. https://doi.org/10.1016/j.cej.2019.01.033
896 Liu, W., Zhang, J., Liu, H., Guo, X., Zhang, X., Yao, X., Cao, Z., Zhang, T., 2021. A review
897 of the removal of microplastics in global wastewater treatment plants: Characteristics
898 and mechanisms. Environ. Int. 146, 106277.
899 https://doi.org/10.1016/j.envint.2020.106277
900 Lusher, A., Bråte, I.L.N., Munno, K., Hurley, R., Welden, N., 2020. Is It or Isn't It: The
901 Importance of Visual Classification in Microplastic Characterization. Appl. Spectrosc.
902 74, 1139–1153. https://doi.org/10.1177/0003702820930733

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. https://doi.org/naturvardsverket-2226

Mahon, A.M., O'Connell, B., Healy, M.G., O'Connor, I., Officer, R., Nash, R., Morrison, L., 2017. Microplastics in sewage sludge: Effects of treatment. Environ. Sci. Technol. 51, 810–818. https://doi.org/10.1021/acs.est.6b04048

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. https://doi.org/10.1016/j.marpolbul.2020.111181

Mani, T., Hauk, A., Walter, U., Burkhardt-Holm, P., 2016. Microplastics profile along the Rhine River. Sci. Rep. 5, 17988. https://doi.org/10.1038/srep17988

Mason, S.A., Garneau, D., Sutton, R., Chu, Y., Ehmann, K., Barnes, J., Fink, P., Papazissimos, D., Rogers, D.L., 2016. Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent. Environ. Pollut. 218, 1045–1054. https://doi.org/10.1016/j.envpol.2016.08.056

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. https://doi.org/10.1039/C6EW00207B

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. https://doi.org/10.1016/j.watres.2016.11.015

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. https://doi.org/10.1039/C8EN00186C

Mintenig, S.M., Kooi, M., Erich, M.W., Primpke, S., Hasselerharm, P.E.R.-, Dekker, S.C., Koelmans, A.A., Wezel, A.P. Van, 2020. A systems approach to understand microplastic occurrence and variability in Dutch riverine surface waters. Water Res. 176, 115723. https://doi.org/10.1016/j.watres.2020.115723

Morehouse, C.A., 1992. Artificial Turf. Turfgrass, Agronomy Monographs.

936 https://doi.org/https://doi.org/10.2134/agronmonogr32.c3
937 Napper, I.E., Thompson, R.C., 2016. Release of synthetic microplastic plastic fibres from
938 domestic washing machines: Effects of fabric type and washing conditions. Mar. Pollut.
939 Bull. 112, 39–45. https://doi.org/10.1016/j.marpolbul.2016.09.025
940 Ngo, P.L., Pramanik, B.K., Shah, K., Roychand, R., 2019. Pathway, classification and
941 removal efficiency of microplastics in wastewater treatment plants. Environ. Pollut. 255.
942 https://doi.org/10.1016/j.envpol.2019.113326
943 Nguyen, T.H., Tang, F.H.M., Maggi, F., 2020. Sinking of microbial-associated microplastics
944 in natural waters. PLoS One 15, 1–20. https://doi.org/10.1371/journal.pone.0228209
945 Nizzetto, L., Bussi, G., Futter, M.N., Butterfield, D., Whitehead, P.G., 2016a. A theoretical
946 assessment of microplastic transport in river catchments and their retention by soils and
947 river sediments. Environ. Sci. Process. Impacts 18, 1050–1059.
948 https://doi.org/10.1039/C6EM00206D
949 Nizzetto, L., Butterfield, D., Futter, M., Lin, Y., Allan, I., Larssen, T., 2016b. Assessment of
950 contaminant fate in catchments using a novel integrated hydrobiogeochemical-
951 multimedia fate model. Sci. Total Environ. 544, 553–563.
952 https://doi.org/10.1016/j.scitotenv.2015.11.087
953 Ockelford, A., Cundy, A., Ebdon, J.E., 2020. Storm Response of Fluvial Sedimentary
954 Microplastics. Sci. Rep. 10, 1–10. https://doi.org/10.1038/s41598-020-58765-2
955 Piñon-Colin, T. de J., Rodriguez-Jimenez, R., Rogel-Hernandez, E., Alvarez-Andrade, A.,
956 Wakida, F.T., 2020. Microplastics in stormwater runoff in a semiarid region, Tijuana,
957 Mexico. Sci. Total Environ. 704. https://doi.org/10.1016/j.scitotenv.2019.135411
958 Primpke, S., Wirth, M., Lorenz, C., Gerdts, G., 2018. Reference database design for the
959 automated analysis of microplastic samples based on Fourier transform infrared (FTIR)
960 spectroscopy. Anal. Bioanal. Chem. 410, 5131–5141. https://doi.org/10.1007/s00216-
961 018-1156-x
962 Qi, R., Jones, D.L., Li, Z., Liu, Q., Yan, C., 2020. Behavior of microplastics and plastic film
963 residues in the soil environment: A critical review. Sci. Total Environ. 703, 134722.
964 https://doi.org/10.1016/j.scitotenv.2019.134722
965 Sarijan, S., Azman, S., Said, M.I.M., Jamal, M.H., 2021. Microplastics in freshwater
966 ecosystems: a recent review of occurrence, analysis, potential impacts, and research
967 needs. Environ. Sci. Pollut. Res. 28, 1341–1356. https://doi.org/10.1007/s11356-020-
968 11171-7

969 Schell, T., Rico, A., Vighi, M., 2020. Occurrence, fate and fluxes of plastics and microplastics
970 in terrestrial and freshwater ecosystems, in: Reviews of Environmental Contamination
971 and Toxicology (Continuation of Residue Reviews). Springer, New York, NY.
972 Simon, M., van Alst, N., Vollertsen, J., 2018. Quantification of microplastic mass and
973 removal rates at wastewater treatment plants applying Focal Plane Array (FPA)-based
974 Fourier Transform Infrared (FT-IR) imaging. Water Res. 142, 1–9.
975 https://doi.org/10.1016/j.watres.2018.05.019
976 Stanton, T., Johnson, M., Nathanail, P., MacNaughtan, W., Gomes, R.L., 2020. Freshwater
977 microplastic concentrations vary through both space and time. Environ. Pollut. 263,
978 114481. https://doi.org/10.1016/j.envpol.2020.114481
979 Sun, J., Dai, X., Wang, Q., van Loosdrecht, M.C.M., Ni, B.-J., 2019. Microplastics in
980 wastewater treatment plants: Detection, occurrence and removal. Water Res. 152, 21–37.
981 https://doi.org/10.1016/j.watres.2018.12.050
982 Talvitie, J., Mikola, A., Setälä, O., Heinonen, M., Koistinen, A., 2017a. How well is
983 microlitter purified from wastewater? – A detailed study on the stepwise removal of
984 microlitter in a tertiary level wastewater treatment plant. Water Res. 109, 164–172.
985 https://doi.org/10.1016/j.watres.2016.11.046
986 Talvitie, J., Mikola, A., Koistinen, A., Setälä, O., 2017b. Solutions to microplastic pollution –
987 Removal of microplastics from wastewater effluent with advanced wastewater treatment
988 technologies. Water Res. 123, 401–407. https://doi.org/10.1016/j.watres.2017.07.005
989 Ter Braak, C.J.F., Šmilauer, P., 2018. Canoco reference manual and user's guide: software for
990 ordination, version 5.1. Microcomputer Power, Ithaca, USA.
991 Tibbetts, J., Krause, S., Lynch, I., Smith, G.H.S., 2018. Abundance, distribution, and drivers
992 of microplastic contamination in urban river environments. Water (Switzerland) 10.
993 https://doi.org/10.3390/w10111597
994 Van Den Berg, P., Huerta-lwanga, E., Corradini, F., Geissen, V., 2020. Sewage sludge
995 application as a vehicle for microplastics in eastern Spanish agricultural soils. Environ.
996 Pollut. 261, 114198. https://doi.org/10.1016/j.envpol.2020.114198
997 Vermaire, J.C., Pomeroy, C., Herczegh, S.M., Haggart, O., Murphy, M., 2017. Microplastic
998 abundance and distribution in the open water and sediment of the Ottawa River, Canada,
999 and its tributaries. Facets 2, 301–314. https://doi.org/10.1139/facets-2016-0070
1000 Wagner, S., Hüffer, T., Klöckner, P., Wehrhahn, M., Hofmann, T., Reemtsma, T., 2018. Tire
1001 wear particles in the aquatic environment - A review on generation, analysis, occurrence,

fate and effects. Water Res. 139, 83–100. https://doi.org/10.1016/j.watres.2018.03.051
Wang, W., Ndungu, A.W., Li, Z., Wang, J., 2017. Microplastics pollution in inland freshwaters of China: A case study in urban surface waters of Wuhan, China. Sci. Total Environ. 575, 1369–1374. https://doi.org/10.1016/j.scitotenv.2016.09.213
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. https://doi.org/10.1007/s11356-019-04663-8
Watterson, A., 2017. Artificial turf: Contested terrains for precautionary public health with particular reference to Europe? Int. J. Environ. Res. Public Health 14. https://doi.org/10.3390/ijerph14091050
Windsor, F.M., Durance, I., Horton, A.A., Thompson, R.C., Tyler, C.R., Ormerod, S.J., 2019. A catchment-scale perspective of plastic pollution. Glob. Chang. Biol. 25, 1207–1221. https://doi.org/10.1111/gcb.14572
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. https://doi.org/10.1016/J.WROA.2018.100014
Woodward, J., Li, J., Rothwell, J., Hurley, R., 2021. Acute riverine microplastic contamination due to avoidable releases of untreated wastewater. Nat. Sustain. https://doi.org/10.1038/s41893-021-00718-2
Wu, P., Tang, Y., Dang, M., Wang, S., Jin, H., Liu, Y., Jing, H., Zheng, C., Yi, S., Cai, Z., 2020a. 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. https://doi.org/10.1016/j.scitotenv.2019.135187
Wu, N., Zhang, Y., Li, W., Wang, J., Zhang, X., He, J., Li, J., Ma, Y., Niu, Z., 2020b. 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. https://doi.org/10.1016/j.scitotenv.2019.134431
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. https://doi.org/10.1016/j.scitotenv.2020.139065
Yang L, Zhang Y, Kang S, et al (2021) Microplastics in freshwater sediment: A review on methods, occurrence, and sources. Sci Total Environ 754:141948. doi:

10.1016/j.scitotenv.2020.141948
Zhang, S., Han, B., Sun, Y., Wang, F., 2020. Microplastics influence the adsorption and desorption characteristics of Cd in an agricultural soil. J. Hazard. Mater. 388, 121775. https://doi.org/10.1016/j.jhazmat.2019.121775
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. https://doi.org/10.2166/wst.2016.414
Ziajahromi, S., Neale, P.A., Rintoul, L., Leusch, F.D.L., 2017. Wastewater treatment plants as a pathway for microplastics: Development of a new approach to sample wastewater-based microplastics. Water Res. 112, 93–99. https://doi.org/10.1016/j.watres.2017.01.042