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 treatment plants (WWTPs) 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. River water and sediment concentrations 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 MP concentrations strongly, with higher water but lower sediment concentrations observed during high flow events. We estimate that $1x10^{10}$ MPs are discharged into the catchment via wastewater annually, which constitute about 50% of the total MP catchment discharge. Thus, WWTPs represent a major environmental pathway for MPs into Mediterranean freshwater ecosystems. However, other MP sources should be quantified (i.e., plastic fragmentation, urban and agricultural runoff).

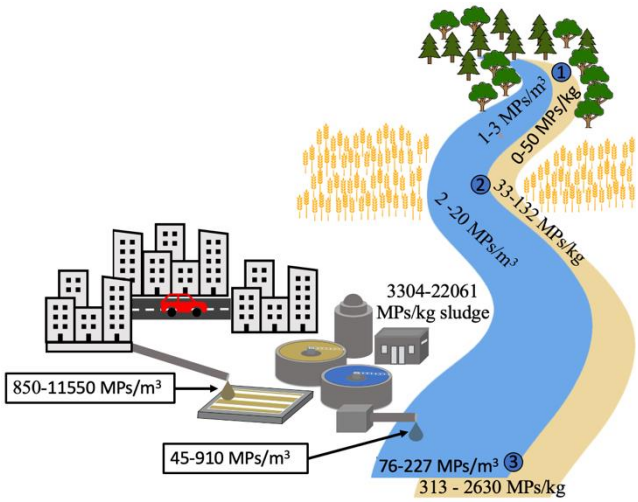
Keywords: microplastics, wastewater treatment plants, environmental monitoring, freshwater ecosystems, Mediterranean region

Highlights

- We assessed wastewater as a pathway for MP contamination in a Mediterranean catchment
- WWTPs remove 93% of MPs but still contribute to 50% of catchment discharge

- MP river concentrations increase strongly downstream of urban and industrial areas
- High temporal variations are observed in relation to rain events
- Additional sources are likely to contribute to the observed MP concentrations

Graphical abstract



1. Introduction

Microplastics (MPs; defined as plastic particles < 5 mm), have now 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. It represents the convergence of a wide range of potential MP sources (e.g., fibers from clothing, fragments, tire and road wear particles; Wagner et al., 2018; Ziajahromi et al., 2016). Although wastewater treatment plants (WWTPs) are efficient at capturing MPs (on average between 88 and 94%; Iyare et al., 2020), 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 rain 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 runoff from agricultural soils, which receive sewage-sludge or plastic input through other agricultural processes such as plastic mulching, has been suggested as another possible important release 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 largely quantified (Windsor et al., 2019).

In arid or semi-arid regions, MP inputs from 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 river's total water flow and may therefore be of higher relevance compared to other MPs sources and pathways (Schell et al, 2020). Yet, the number of studies assessing MP contamination in freshwater ecosystems in the 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 elucidate 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 the design efficient measures to reduce MP pollution.

Hence, the main objective of this study was to elucidate the role of WWTP as MP sources to rivers in a semiarid 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) and temporal (season) distribution of MPs in river water and sediment in the same catchment, and (3) determining the contribution of wastewater to the total MPs 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. 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 $272x10^6$ m^3 $year^{-1}$ (based on the last 20 years average; CEDEX 2017). The area is characterized by a continental Mediterranean climate, with hot and dry summers and mild-to-cold dry 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 and paper and printing materials. The two main cities, Alcalá de Henares and Guadalajara, have a population of around 197,500 und 87,500, respectively.

We sampled five out of the 19 WWTPs in the catchment, which 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 anerobic or aerobic digestion and dehydration) were sampled (Tab A1). 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). 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 is 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 with the exception of two samples where only 5000 L were sampled: 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. 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 flask until analysis.

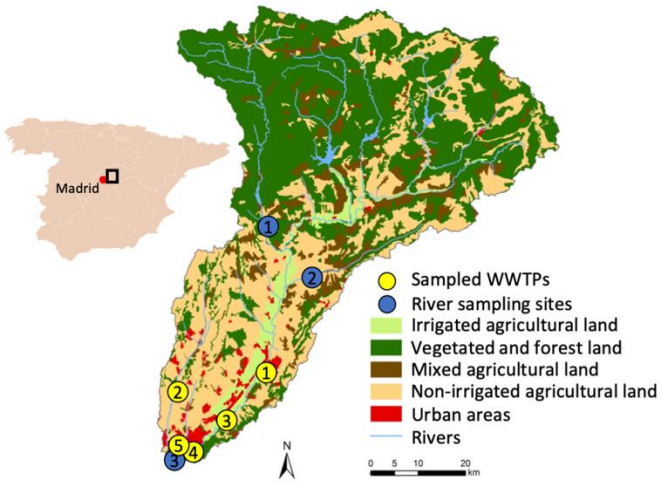


Fig. 1 Location of the sampled WWTPs and river sampling sites in the Henares River catchment, together with a description of the land uses. Land use information was retrieved from the Corine land cover database (Corine land cover, 2006).

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) 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 second extractions (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 first 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 MP shapes were classified into beads (spherical particles), fragments, fibers, films, foams, granules, glitter and pellets (pre-production pellets). On rare occasions fiber clumps occurred, that could not be separated, and where therefore counted as such. The long and short dimension 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. For MPs in wastewater (influent, effluent) the polymer composition of 86% of the MPs were determined. 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 reductions 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. For the other water samples (wastewater and river water from July and November 2017) sampling blanks were taken, which formed the processing blanks. However, the content of these blanks revealed a large number of particles (clear/yellowish fibres) that were not observed in any of the environmental samples. Thus these blanks were disregarded. 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. The results of the particle analysis in blanks are described for each sample type and presented in the SI.

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. Finallym, 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 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

3.6 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

193.62 mg m^{-3} (Tab. 1). The lowest and highest estimated mass concentrations do 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%; mean: 41%,) and fragments (12-69%; mean: 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 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 48.52 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%; mean: 69%) followed by fibers (4-64%; mean: 19%). Beads, films, foams and, granules were also observed (0-43%; mean: 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. 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 WWTP1). It has previously been shown that MP concentrations in wastewater are subject to strong daily fluctuations and depend on factors such as weather conditions (Cao et al. 2020; Wolff et al., 2018; Xia et al., 2020). 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 70 and 99% have been reported,

with lower efficiencies (<90%) being less frequently observed (e.g., Bayo et al., 2020b; Conley et al., 2019; Gündoğdu et al., 2018; Talvitie et al., 2017b). 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 already 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., 2020a; Michielssen et al., 2016; Talvitie et al., 2017a, 2017b). Although the WWTPs sampled here apply sand filtration as part of the tertiary treatment, no enhanced MP retention was observed in our study.

In the current study, the overall retention capacity was higher for fibers than for other MP types (Tab. A6). 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 a greater retention in wastewater treatment filtration steps due to their elongated and irregular shape. Yet, this trend is not always consistent: Michielssen et al. (2016) and Conley et al. (2019) found that fragments were in fact removed slightly more during wastewater treatment. The increase in fiber concentrations during autumn, observed in this study, may be due to increased laundry frequencies or loads during this time, (e.g., whereby use of synthetic fabrics compared to natural fibers increased in this season) compared to the summer. 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 this region.

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 low 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 sampling 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.

Table. 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 outlet tank and where 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; I = industrial; 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**	**MPs 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}	
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 (3878-7876)	92.1 (37.8-196)	97.8	24675	202070
			Au	11,550	146	70	1.41	11,954 (9,517-14,545)	82.0 (54.8-128.8)	5,640 (5222-6256)	20.6 (18.1-22.9)	99.4	19989	148470
2	U	S	Su	2,650	179	165	48.5	37,163 (31,086-43,320)	131 (118-153)	18,852 (11,283-23767)	31.0 (8.18-48.1)	93.8	520	327030
			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 (2432-4749)	49.2 (5.12-135.9)	70.0	1837	8179635
			Au	3,550	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	5071545
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)	16600 (12500-21348)	46.0 (7.75-99.5)	80.5	1982	101400
			Au	1,400	1.9	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	166500
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)	9487 (7648-11257)	142 (38.0-312)	98.7	32077	1110375
			Au	2,000	4.51	280+600*	5.3+4.4*	37,373 (31,809-42,035)	127 (93.6-159)	7340(4677-11300)	59.1 (8.91-129)	86.0	37567	5596920

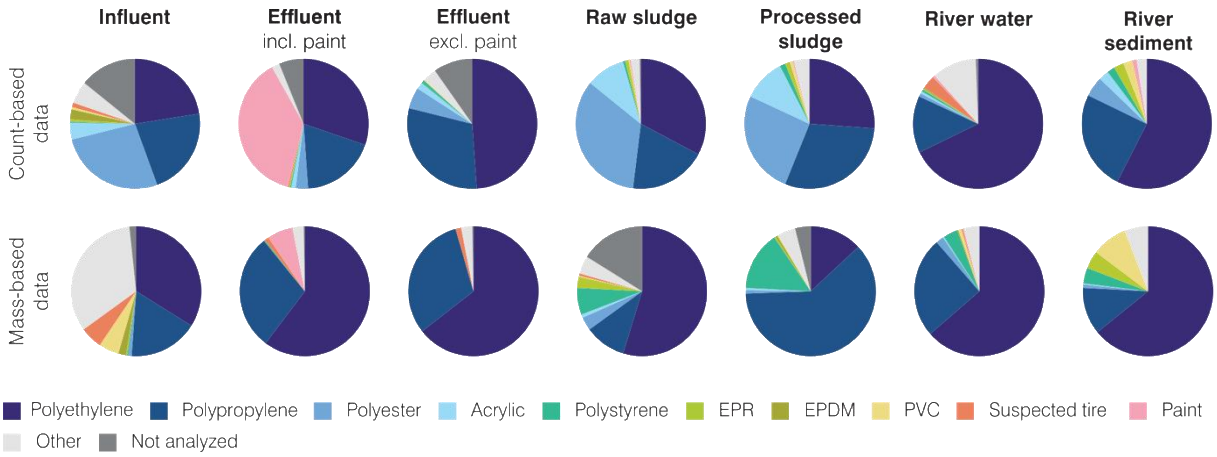


Figure 2. Mean count-based and massed-based polymer distribution across all sampling sites and sampling events in different sample types. Acrylic refers only to fibers and does not include acrylic paint; acrylic paint is included in paint. EPDM = Ethylene propylene diene monomer; EPR = Ethylene propylene rubber; PVC = Polyvinyl chloride Polymer type included in others can be found in Table 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. A3).
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 (Tab. 1). In the processed sludge, concentrations ranged between 3300 and 22,061 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. While 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. S4). 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 1000-5000 µm (55% for raw sludge and 60% for processed sludge) while the majority of all other MPs (based on longest axis) were between 300-1000 µm (43 and 40%) and 150-300 µm (40 and 35%) (Fig. S1). In both raw and processed sludge, most MPs were fragments (6-82% with a mean of 52 % and 17-79% with a mean of 56%, respectively) and fibers (16-93% with a mean 47% and 19-83% with a mean 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 treated 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). Furthermore, based on abundance, polyethylene and polystyrene tend to be more abundant in raw sludge, while ethylene propylene diene monomer and silicone are 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 preferently 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 amendment (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 now presence of MPs, with only Cellulose fibers observed (Tab. A5). Therefore, a negligible background contamination was also 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 a mean of 81%), followed by beads and fibers (Fig. A1). Granules, films, glitter and foams were also detected (Fig A1; Tab. B2). According to the existing literature, fragments and fibers are the most frequently observed MPs types in river water, with exception of a few studies reporting significant contamination from beads or pellets (e.g. Lechner et al., 2014; Mani et al., 2016). 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 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, 34 different polymer types were identified. Polyethylene was the most common polymer type followed by polypropylene (Fig. 2; Tab. B2). 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). Suspected tire particles, as well as styrene-butadiene rubber, were found at site 2 and 3, in 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 dumping and agriculture as potential sources and despite the high MP concentrations observed in untreated wastewater and sludge in this study (see section 3.1).

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 (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. Higher 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). Therefore, MP river concentration may show low temporal variations under similar weather conditions but strong fluctuations after rain events (Cheung et al., 2019; Mintenig et al., 2020; Xia et al., 2020).

Table 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	1.72	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	19.4	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. A4), 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 246 MPs kg^{-1} dw or 5.53 mg kg^{-1} dw), with the highest concentration observed at site 3 (up to 2630 MPs kg^{-1} dw or 34 mg kg^{-1} dw sediment; Fig. 2, Tab. S7). MPs in sediment consisted only of fragments (33-100% with a mean of 87%) and fibers (0-67%; mean: 13%) with the exception of a small number of glitter particles recorded at sample 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 higher flow events during this season. In total, 19 different polymer types 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 below 300 µm (Fig. A2).

In addition to intersite variability, seasonality also had an influence on 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., 2018; 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 have an influence on MPs 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 393 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 $2.1x10^{10}$ MPs (between $1.1x10^{10}$ and $3.2x10^{10}$ MPs) or 2400 kg (between 1,373 to 3,829), that is discharged into the Tagus river system. Fibers represent $1.7x10^{8}$-$7.3\ x10^{8}$ particles (0.12-137 kg) where the remaining $1.0x10^{10}$-$3.2x10^{10}$ (1,282-3,829 kg) are MP particles, mainly fragments. This was calculated based on the annual discharge for the Henares catchment in 2017 ($1.43x10^{8}$ m^3; CEDEX 2017). These calculations indicate that wastewater releases contribute approximately half of the total river MP contamination based on particle counts, but only 1/6 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 were 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 $5.2\ x10^{7}$ m^3 per year (based on data from 2017), of which $7.9x10^{5}$ m^3 was untreated. Wastewater therefore contributed approximately 1/3 of the total streamflow. The annual river discharge in 2017 was much lower than the average of the past 20 years ($272x10^{6}$ m^3), and wastewater may have therefore contributed a greater proportion than for other years. Yet, this does not affect the total MP export, decreased streamflow may result in a lower dilution of particles and therefore higher risk of exposure for aquatic organisms. Modelling 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 in the future (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.

Wastewater largely represents a pathway for MP from other sources, which converge and are concentrated within wastewater systems and as a result of wastewater treatment processes. Thus, the majority of MP 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 are not captured by wastewater treatment processes and are discharged in the effluent. 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 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 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 site 3. Those plastics have possibly served as a source for MP fragments, which were the main MP type observed in river water and sediment (Fig. A4). 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).

Table. 3. Estimated amount of MPs (in particle counts and mass) entering the river by wastewater.

	Amount of wastewater produced in the catchment in 2017 in m^3	**Concentration of MPs in wastewater**		**Emitted into the river in kg/per year**	
		mg m^{-3}	MPs m^{-3}	kg m^{-3}	MPs m^{-3}
Treated urban and industrial wastewater	49,225,286	6.47[a]	161[a]	318.7	$7.94x10^9$
Untreated urban wastewater	789,574	76[b]	2439[b]	60.6	$1.93x10^9$
Industrial effluents unknown treatment	2,276,415	5.5[c]	123[c]	12.5	$2.8x10^8$
Total amount	52,161,976			391.2	$1.01x10^{10}$

[a] *based on a flow based weighted average in sampled treated wastewater*
[b] *based on a flow based weighted average in sampled untreated wastewater*

[c] *flow based weighted average of sampling WWTPs 1, 3 and 6 were used as an estimation as they were receiving in addition to urban also industrial wastewater, although industrial wastewater is expected to vary greatly based on MPs composition based on the industrial sector.*

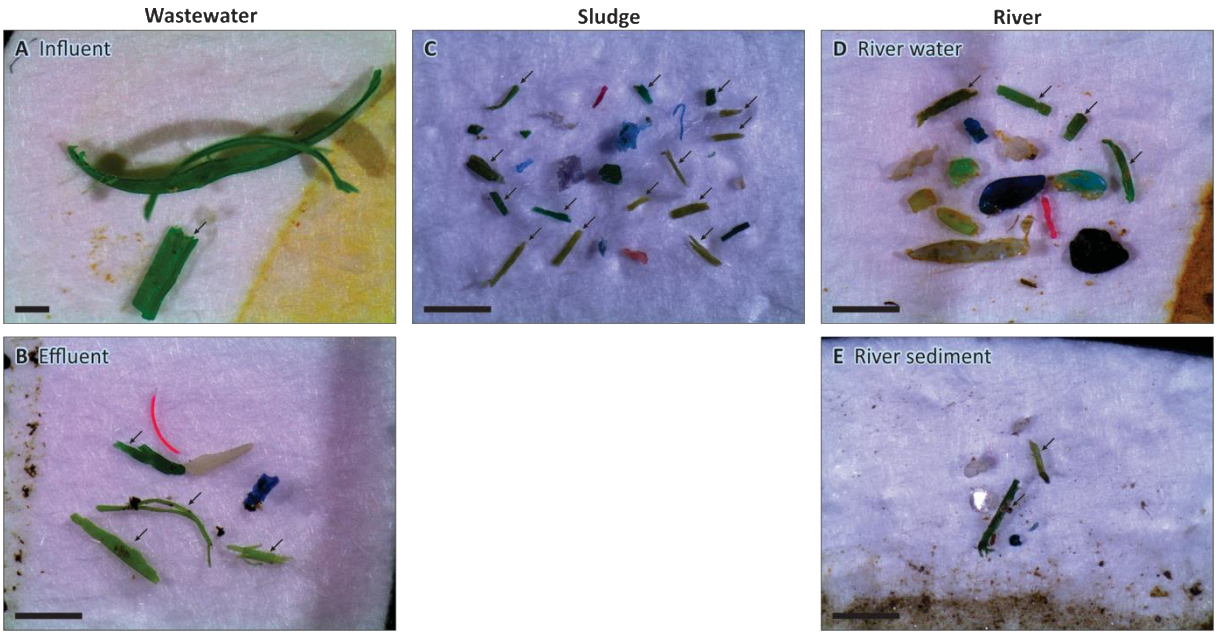


Figure 3: Examples of green polypropylene fragments that share a visual resemblance, observed in all different sample types. The relevant particles are indicated with an arrow. The black scale bar represents 1000 µm in each image.

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 (Constant et al., 2020; Kataoka et al., 2019). 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 assessments. 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 particles may already saturate the detector. Thus, the analytical method used here represents a valuable synthesis to produce datasets useful for different scopes. Aligning datasets which report count or mass-based concentrations across differencing 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 assesing the role of wastewater as a release pathway for MP contamination at the river catchment scale and with respect to spatiotemporal variability. We show that WWTPs, despite retaining a large number of MPs, provide about 50% of the MP 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 both land-use and season. To identify and fully 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 MPs 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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