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Vercauteren et al.

Contribution of wastewater on microplastic pollution

Towards a Better Understanding of the Contribution of Wastewater Treatment Plants to the Microplastic Pollution in Receiving Waterways

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Abstract: Microplastics (MPs; 1 µm - 5 mm), are ubiquitous in daily-use products and regularly end up in the wastewater. The main part of the wastewater is treated in wastewater treatment plants (WWTPs) which allow for at least partial removal of MP. The research aimed to understand the contribution of domestic wastewater on MP pollution in Flanders (Belgium) via two main discharge routes of MPs: (1) the effluent and (2) removed fractions. Furthermore the effect of effluent discharge on the microplastic contamination in the waterway was studied in both surface water and sediment samples of upstream and downstream locations of a discharge of three WWTPs. On average, 12.64 ± 20.20 MP per L enter a WWTP (10 µm – 5 mm). The effluent contained on average 0.41 ± 0.91 MP per L, resulting in an average removal efficiency of 97.46 ± 2.33 %, which is comparable with various (non-)European countries. Removal

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efficiencies are both polymer- and size-specific and data suggest that smaller particles are less efficiently removed from the wastewater, which also causes an increased input of smaller particles in the environment. The sludge is the most efficient treatment process to remove MPs. Despite the high removal efficiencies, still $1.11 \times 10^7 \pm 3.07 \times 10^7$ MPs end up in the nearby waterway daily. Nonetheless, based on the results gathered in this study, this does not seem to impact the MP concentration in the waterway significantly. In summary, this research offers a holistic approach in the research on the impact of wastewater on the microplastic pollution in the ecosystem, integrating different discharge routes and measuring the impact on environmental microplastic pollution.

Keywords: Microplastics; Emerging pollutants; Water quality; Fourier-transform infrared spectroscopy; Wastewater discharge

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Abbreviations

WWTP	Wastewater treatment plant
MP	Microplastic
FTIR	Fourier-transform infrared spectroscopy
PTFE	Polytetrafluoroethylene
LOD	Limit of detection

LOQ Limit of quantification

PET Polyethylene terephthalate

PE Polyethylene

PP Polypropylene

PVC Polyvinylchloride

PS Polystyrene

1. INTRODUCTION

Microplastics (MPs), defined as plastic particles between 1 µm and 5 mm in size (Arthur et al., 2009; GESAMP, 2015), are ubiquitous in daily-use products. Through the use of these products (e.g., in agriculture, transport, households, health care,…) or products containing (micro-)plastics (e.g., paints, personal care products,…), microplastics can end up in (domestic) wastewater. Depending on the route of this wastewater, they can end up in our aquatic ecosystem potentially causing adverse effects in aquatic organisms, such as decreased growth, reproduction and inflammation (reviewed in Besseling et al., 2019; Stienbarger et al., 2021). Alternatively, they can be ingested by humans through the consumption of drinking water originating from treated sewage water. For example, Semmouri et al. (2022) reported an average of 0.05 ± 0.02 MPs/L in purified drinking water from wastewater effluent in Flanders.

Currently in Flanders, the domestic wastewater of 83 % of the households is connected to a working wastewater treatment plant (WWTP) (Vlaamse milieumaatschappij, 2021). Influents of WWTPs, containing various sources of pollution, undergo a suite of mechanical (e.g., using filters and sand) and biological treatment (using activated sludge) processes that are aimed to remove pollutants from the water

before its discharge in the natural environment (Talvitie, Mikola, Koistinen, et al., 2017). These treatments, although originally intended to reduce other sources of contamination such as particles and dissolved nitrogen and phosphorus, have been proven to be (at least partially) successful in the elimination of MPs from the wastewater (Ben-David et al., 2021; Carr et al., 2016; Magnusson & Norén, 2014; Murphy et al., 2016; Tadsuwan & Babel, 2021; Ziajahromi et al., 2021).

Based on existing literature, WWTPs are considered to be either sinks or sources of MPs in the environment. An incomplete removal of MP from the wastewater can result in an extensive MP pollution through large volumes of effluent that are discharged in the receiving waterway (Magnusson & Norén, 2014; Tadsuwan & Babel, 2021; Vivekanand et al., 2021). This was for example reported in the Chicago river (USA) where the discharge of WWTP effluents caused increases in the MP concentrations in the river (McCormick et al., 2014). Due to the discharge of wastewater containing MPs, WWTPs have been suggested to be one of the important land base sources of microplastics in the aquatic environment (Magnusson & Norén, 2014; Murphy et al., 2016; Talvitie et al., 2015; Vivekanand et al., 2021). Despite an increasing number of studies concerning MPs and WWTPs, no data, besides from two non-peer reviewed, explorative studies from our lab (Lecompte and Janssen, 2015; Van Echelpoel, 2014), is available on the removal efficiencies of WWTPs in Flanders, a densely populated region in Belgium, Europe.

The possible impact on the environment, as observed in the example of the Chicago river (McCormick et al., 2014), urges us to improve our understanding of the contribution of domestic wastewater on MP pollution in Flanders. To support a more holistic study of the role of WWTP on microplastic pollution in the environment, two

main exit routes of MPs out of WWTP are being studied: (1) via the effluent and (2) via the removed fractions (e.g., sludge, sand, floating layer). We collected influent and effluent samples in six different WWTPs in Flanders. Additionally, in two of these WWTPs, samples from the removed fractions were investigated in order to assess the MP removal efficiency of each treatment process and linked risks for MP pollution in the environment. To further estimate the role of WWTPs as point sources of MP pollution in rivers, the effect on MP concentration in nearby rivers was investigated up- and downstream from the point of discharge of WWTP effluents. Based on the data gathered in this study, the impact of MP pollution (in the size range of 10 µm – 5 mm) via (domestic) wastewater is holistically estimated in Flanders, which will help to guide future research efforts and facilitate policy decision making.

2. MATERIAL & METHODS

2.1. Sample locations

Six Flemish WWTPs were sampled in triplicates (three replicates per WWTP collected on three consecutive days) between July and September 2019. Detailed information about sampling location, dates and number of samples is provided in Supplementary file 1. The WWTPs are distributed across Flanders and were selected based on the applied treatment processes (i.e. a treatment of wastewater using activated sludge). Information regarding the average influent flow, maximum influent capacity, as well as the unit processes of each WWTP and the hydraulic and sludge retention times can be found in Supplementary file 2. The selected WWTPs received wastewater of various sources (Supplementary file 2, 3), however, the main source was domestic wastewater with a maximum of 10% of industrial wastewater supply.

In two WWTPs (WWTP-Aartselaar and WWTP-Grimbergen), sampling was performed twice, once in a rainy period and once in a dry period to explore whether temporal differences in rainfall affect removal efficiencies of WWTPs. Other WWTPs were only sampled in dry periods. Dry periods are defined as starting on the 3rd day, after at least two days with less than 0.2 mm precipitation per day, of which the precipitation on the day itself is also less than 0.2 mm precipitation per day. A rainy period is defined as the day after a day with at least 5 mm precipitation per day.

2.2 Sampling strategy in WWTP

2.2.1 Influent and effluent samples. An integrated sample was gathered over a 24 hour period. Every 30 minutes, 210 ml of the influent or effluent was collected using an automated sampler (Bühler 4010, Hach-Lange, Ireland). Influent samples were taken immediately after the bar screens (with mesh of 6 mm, almost coinciding with the upper limit of the MP size range). All subsamples were integrated into a 10 L sample, transported in pre-rinsed bottles and closed of using aluminum foil. Samples were stored at 4 °C upon further processing. The sampling method did not affect the detected MP concentrations, in terms of contamination or loss.

2.2.2 Removed fractions. In two selected WWTPs (Aartselaar and Grimbergen) samples of the removed fractions (sand, sludge and floating fraction) were collected in triplicates (in three consecutive days).

At the end of each sampling day (after 24 hours), the collected sand and thickened sludge was sampled. Several subsamples of sludge were collected with a metal spoon, homogenized and transferred to 2 L pre-rinsed glass jars. The total removed sludge fraction of the WWTP for the 24h period was estimated based on the total weight. The

sand samples (this treatment is only used in WWTP-Aartselaar) were collected in a similar manner. The density of the sand was measured by weighing 10 L of sand. The glass jars were closed off using aluminum foil and transported to the lab where they were stored at 4° C until further processing.

The floating layer, that was gathered by one round of the scraper (approximately 30 min) in the final sedimentation tank, was collected by a plankton net (Hydro-Bios, pore size 10 μm) and transferred entirely to 4 L glass jars. Data was extrapolated for the entire sampling period. All glass jars were closed off using aluminum foil and transported to the lab where they were stored at 4° C until further processing.

2.3 Sampling strategy in the surrounding waterways

To study the role of WWTP effluent discharge in terms of MP pollution, water and sediment samples both upstream and downstream of an WWTP were collected at three out of the six locations (Heule, Aartselaar, Grimbergen; Supplementary file 4). At each sampling location, approximately 10 sub-samples of ~ 1 L of surface water were collected in a pre-rinsed 10 L bottle using a stainless steel sampling cup, mounted on a telescopic arm (Acuradmin, 1 L, up to 3 m) and a stainless steel funnel. Samples were taken maximally two meters from the bank and in the top 0.5 m of the water surface. For each location, three replicates (each 10 L) were collected over a period of 15 minutes, each 1 to 2 m further upstream of each other. The used material was rinsed with filtered water between consecutive samplings. The bottles were sealed with aluminum foil and transported to the lab. Samples were stored at 4°C until further processing.

A Van Veen grab (Van Eijkelkamp, 2 L) was used to sample the sediment, for which five to ten separate Van Veen samples were integrated into one sample. The

samples were collected in a stainless steel bucket and transferred to pre-rinsed 4 L glass jars. The jars were sealed with aluminum foil and transported to the lab where they were stored at 4°C until further processing. The sediment, in contrast to the surface water (both in dry and wet weather periods), was sampled only once per location because microplastic concentration in sediment is considered to be less variable over time.

2.4 Extraction and analysis of microplastics from the matrices

2.4.1 Microplastic extraction. For the aqueous samples, first, 50 g potassium hydroxide (KOH, AnalaR NORMAPUR) was added to each sample ~10 L) for digestion of the organic matter present (60°C, 48 hours) (based on Thiele et al., 2019). After digestion, the supernatant water was filtered through a cellulose nitrate filter (8.0 µm pore size, Whatman AE99). The sample sediment and rinse water were transferred to a pre-rinsed 200 mL polypropylene conical centrifuge tube (Thermo Scientific Nunc) and then centrifuged (megafuge 40R, Thermo Scientific) at 3500 rpm, equal to 2,675 g (RCF), for 5 minutes. The supernatant water from the centrifuge tube was filtered again over the cellulose nitrate filter. The pellet was then diluted with 150 mL of sodium iodide (density 1.6 g/cm^3; NaI, GPR RECTAPUR, VWR), solubilized and centrifuged again at the same settings (2,675 g (RCF), 5 min) to separate the microplastics present in the sample from the denser, residual inorganic fraction, as done by Claessens et al., (2013) and Van Cauwenberghe et al., (2013). The supernatant was filtered again over a cellulose nitrate filter. The density separation with NaI was repeated two more times, but in the final centrifugation step the rotational speed was increased to 3,853 g (RCF).. The cellulose filter(s) were mixed with KOH (50 mL per filter) in a glass beaker and then placed in a warm water bath (Memmert WTB) at 60°C for 24 hours to digest the cellulose filter(s).

The resulting solution was filtered over a polytetrafluoroethylene (PTFE) filter (pore size: 10.0 µm, Omnipore Membrane filter, Merck) through a glass filter system. Both the measuring cup and the filtration system were rinsed three times with filtered deionized water and once with a 0.1% Tween® 80 solution. Subsequently, the filter was dried at room temperature for 24 hours in a dust-free environment.

For the sediment and sludge samples, a subsample ~ 20 g wet weight) was collected after homogenization. First, the glass jar containing the entire sample was shaken thoroughly. Subsequently, the sample was mixed manually using a metal spoon to ensure homogeneity as much as possible. Approximately, 20 grams of the homogeneous sample was transferred to a measuring cup and 200 mL hydrogen peroxide H_2O_2 (30%, tested according to Ph.Eur, VWR) was added stepwise (per 25 mL) for digestion of the organic material. The sample was first kept at room temperature for 24 hours and then placed in a warm water bath (60°C, Memmert WTB) for 24 hours to stimulate digestion (based on Claessens et al., 2011; Thiele et al., 2019; Van Cauwenberghe et al., 2013, 2015a,b). After digestion, the supernatant water was filtered through a cellulose nitrate filter (pore size: 8.0 µm, Whatman AE99). An additional 25 mL of 69% nitric acid HNO_3 (Supelco®, Merck) was added to the remaining sediment for one hour at room temperature (based on Claessens et al., 2013; Nel et al., 2018). The acid sludge and rinse water of the sample were then transferred with a glass funnel into a pre-rinsed 200 ml polypropylene conical centrifuge tube (Thermo Scientific Nunc) to perform density separation using NaI. The obtained solution was then sieved (pore size: 15 µm) using a PVC sieve (based on in-house protocol described by Van Echelpoel et al., 2014). The fraction larger than 15 µm was diluted with 40 mL NaI in a 50 mL conical centrifuge

tube (Nerbeplus) and centrifuged again (2,675 g (RCF),, 5 min). Finally, the supernatant was filtered over a PTFE filter as described above. The filters were dried at room temperature for 24 hours in a dust-free environment.

2.4.2 Microplastic characterization. The dried PTFE filters were fully analyzed by FTIR spectroscopy (Nicolet iN10 FT-IR Microscope; Thermo Fisher Scientific, Madison, Wi, USA), with a theoretical lower particle size limit of 10 µm. The entire surface of the filter was scanned and the spectrum of each pinpointed particle was determined (100 µm step-size scanning, 150 x 150 µm aperture, spectral resolution 16 cm^{-1}, reflection mode, spectral range 1,300-4,000 cm^{-1}). The obtained spectra were identified based on their correlation (Pearson correlation, threshold match 75%) with known spectra in the reference library, both in-house and commercial library. Furthermore, information was collected on the length and width of the particle. The theoretical size range of the particles varied between 10 µm and 5 mm. All unidentified particles (non-plastics or match to reference plastics lower than 75 %) were removed from the data set.

2.4.3 Global comparison of reported removal efficiencies for WWTPs. Based on a literature search, results on the obtained microplastic removal efficiencies were compared to peer reviewed published results. A strict selection was applied, based on comparable sampling and analytical methods, to ensure reliable comparisons. Of the available peer reviewed literature reporting clearance rates in WWTP, 16 publications were deemed suitable to compare our results based on comparable sampling and analytical methods.

2.5 Quality control and quality assessment

2.5.1 Contamination mitigation. Precautions were applied to avoid contamination as part of the so-called good field and laboratory practices (GLP). These practices include wearing a cotton lab coat, using pre-rinsed (using deionized water) materials, working in a closed laboratory environment and under a clean laminar flow (Potteau, Heule). Airborne contamination was reduced as much as possible by storing all lab materials in a dust-free environment and by covering all cups, beakers and bottles with aluminum foil or with pre-rinsed watch glasses. Glass, metal or stainless steel laboratory equipment was used if possible. The use of plastic equipment was avoided as much as possible, but if it could not be replaced, possible contamination was tested *a priori* by the analysis of blank samples (e.g., the 50 mL conical polypropylene centrifuge tubes used for density separation) and methods were adjusted as needed.

2.5.2 Adequate control samples. Negative control samples (sample blanks) were produced to determine the extent of contamination in the samples during the sampling or extraction process. Negative controls for both effluent and influent samples were produced in the field by sampling 10 L of filtered deionized tap water by the autonomous sampler and, subsequently processed in the lab, using the same protocols as the samples. For the samples from the floating layer, negative control samples were produced by mimicking the passage of 1L of filtered water through the net. Negative control samples for the surface water were produced in the field (using 10 L of filtered deionized tap water) and were processed in the lab, like the other samples. For the removed fractions in the WWTP and the sediment in the waterways, no suitable negative control samples could be sampled.

Additionally, six positive controls for water samples (spiked blanks) were also prepared (47.33 ± 10.5 polyethylene particles L^{-1}, 90 - 106 μm diameter, Cospheric) and processed like the other samples, to obtain an indication of the recovery rate of microplastics in our samples. This preliminary work found a recovery rate of 82 ± 4% for the reference microplastics following the described extraction procedure.

To date, no standardized methods are available to account for these controls in analyses (Brander et al., 2020). We corrected our data for possible contamination during sampling and processing using the limit of detection (LOD) and the limit of Quantitation (LOQ) based on the negative control samples (Armbruster and Pry, 2008; Uhl et al., 2018):

$$LOD = Average_{Blanc} + 1{,}645 \times Standard\ deviation_{Blanc} \quad \text{(Eq. 1)}$$

$$LOQ = Average_{Blanc} + 3 \times Standard\ deviation_{Blanc} \quad \text{(Eq. 2)}$$

Calculation of LOD and LOQ values was done per matrix and per polymer type. We consider all concentrations found in samples below the LOQ for a particular polymer type to be unreliable to quantify due to possible contamination, and these values are thus reported as "<LOQ". The obtained LOD and LOQ values per polymer type are shown in Supplementary file 5.

2.6 Data analysis

All statistical tests were executed in R Studio. Graphs were produced using the ggplot2 package (version 4.0.3) available in R. Statistical differences were considered significant if the p-value was less than 0.05.

2.6.1 Microplastic concentrations The Shapiro-Wilk test of normality and Levene's test of homogeneity of variances were applied as pre-tests to all metric data.

Differences between locations and environmental factors in terms of microplastic concentrations were statistically analyzed using ANOVA or the non-parametric alternative, Kruskal-Wallis. Both analyses were followed by an appropriate post-hoc analysis to compare the groups in pairs.

Correlations with environmental factors or other metadata (continuous variables) were studied by calculating the Spearman Rank correlation.

2.6.2 Removal efficiency. The MP removal efficiency (*Z*) was calculated for each of the WWTP based on average MP concentration in influent and effluent from three consecutive samplings. The removal efficiency for a given WWTP (Z_c) was calculated based on following formula:

$$Z_c = 100\% * \left(1 - w_{c,eff}/w_{c,in}\right) \quad \text{(Eq. 3)}$$

With $w_{c,eff}$ representing the mean MP concentration per liter in the effluent for a given WWTP c and $w_{c,in}$ being the mean MP concentration per liter in the influent for the same WWTP. A mean removal efficiency (Z_{mean}) is calculated based on all the Z_c of all WWTPs.

To investigate the flow of microplastics, the particle flow in two WWTPs, namely WWTP-Aartselaar and WWTP-Grimbergen, was studied. The measured concentrations for influent and effluent were converted to a total number of microplastics per matrix, based on the flow rate measured during the sampling itself (three days). The concentration of microplastics per gram of wet weight of sludge and sand samples was recalculated to the total number of kg of sludge or sand that was removed from the WWTP per day or three days, taking into account the density of the matrix (determined at 100 ml for the sludge and 10 L for the sand). The concentration of microplastics in the

floating layer was recalculated to the total volume removed per skimmer pass, which was reconverted to the total volume removed per day (based on the average duration of one pass). The removed fraction of MPs of each step in the purification process was expressed as a percentage of the total number of particles recovered in the influent.

3. RESULTS AND DISCUSSION

All identified particles below the respective LOQ values were removed from the dataset. Hence, only those particles (in the size range of 10 µm – 5 mm) of which the quantification is considered reliable are reported below.

3.1 Microplastic concentrations in wastewater entering WWTP

Microplastics contamination was present in all influents of the six sampled WWTPs with an average microplastic concentration of 12.64 ± 20.20 MP per L, ranging between 0.97 and 86.45 MP per L (Table 1). When taking into account the total volume of water that is entering the WWTP ($1.70 \times 10^7 \pm 1.38 \times 10^7$ L per day), an average of 2.70×10^8 microplastics enter one WWTP daily. With 323 WWTPs distributed across Flanders, this results in an estimated total load of 8.72×10^{10} microplastics that enter all WWTPs daily in Flanders (or 3.18×10^{13} MPs per year). The integrated sampling method used to collect the influent samples allowed us to include the in-day variation of microplastic contamination entering the WWTP, resulting in a more reliable extrapolation to daily influent MP concentration compared to one time sampling (Ben-David et al., 2021; Talvitie, Mikola, Koistinen, et al., 2017). This method is however less recommended in case of expected low MP concentrations since the low volume of the samples might cause false zero results, as pointed out by Talvitie et al. (2017).

The six sampled WWTPs show no significantly different input of microplastics (Kruskal-Wallis, $p = 0.2027$). Microplastic concentrations in influent are similar in dry or rainy periods (Kruskal-Wallis, $p = 0.5591$). Microplastics found entering the WWTP were mainly polystyrene (58%), polypropylene (19%) or polyethylene terephthalate (12%). The polymer composition differed based on the location of the WWTP (Figure 1). Since most of microplastics are formed due to fragmentation (Lassen et al., 2015), we expected a higher abundance of smaller particles. This was indeed observed in the influent samples, where smaller particles (50-75 µm) were most represented based on their relative frequency in the samples (Figure 2). The relatively low number of particles with a size < 25 µm can probably be explained by size limitations of the spectrometer which likely situates around 20 µm in practice (rather than the official reported size limit of 10 µm).

3.2 Microplastic removal efficiency of WWTP

Effluent samples contained on average 0.41 ± 0.91 MP per L with a maximal measured concentration of 3.42 MP per L. Effluent concentrations for each individual sample are reported in Table 1. Based on influent and effluent concentrations, an average microplastic removal efficiency of 97.46 ± 2.33 % (min. 92.57 – max. 100.00 %) for the six Flemish WWTPs, was calculated using equation 3 (Table 1). The removal efficiencies do not seem to be impacted by rainfall ($p=0.505$), but for rainy periods only two removal efficiencies are available. Therefore, more research is necessary to corroborate these results. The removal efficiencies as calculated in this study are higher in comparison to the previously reported efficiencies in WWTP-Destelbergen in Flanders (range between 34 % and 65 %; Lecompte and Janssen, 2015; Van Echelpoel, 2014). The use of different

sampling and analytical methods (size range of 5 µm – 5 mm; sampling volume of 1 L used, sieved and treated with 15 % H_2O_2 prior to density separation; identification through light microscopy) most likely influenced these results and make a comparison less straightforward.

As illustrated in Figure 2, the size distribution observed in the effluent samples is different from that of the influent. The most dominant size range (50-75 µm) represents 22.5 % in the influent and up to 32% in the effluent. A similar pattern was found comparing the 25-50 µm range (Figure 2). Similar results, for size range between 20 and 100 µm, were also found by Talvitie et al. (2017b). This suggests that smaller particles are less efficiently removed from the wastewater, which highlights the need for further development of advanced final-stage treatment technologies to remove the smaller sized particles.

The measured removal efficiencies of the WWTPs in Flanders are comparable with various European and non-European countries, such as Finland (Lares et al., 2018; Talvitie et al., 2015; Talvitie, Mikola, Koistinen, et al., 2017), Scotland (Murphy et al., 2016), Germany (Mintenig et al., 2017), United States of America (Conley et al., 2019; Michielssen et al., 2016) and South Korea (Lee & Kim, 2018) (Figure 3). In Denmark, a higher removal efficiency (similar methods and size ranges) of 99.3 % was reported (Simon et al., 2018). The latter study, however, recalculated the particle numbers into mass concentrations and reported removal efficiencies based on mass concentrations. Since particle numbers are less conserved due to possible fragmentation, using particle concentrations would thus lead to lower removal efficiencies. Other countries such as China (Lv et al., 2019), France (Dris et al., 2018) and the Netherlands (Leslie et al., 2017)

reported lower microplastic removal efficiencies (< 90 %). The removal efficiency calculated by Lv et al. (2019) focused on a Chinese WWTP using other removal techniques, namely the oxidation ditch, which is a modified activated sludge biological treatment process (EPA, 2000), and a membrane bioreactor. The latter shows promising results in increasing the removal efficiencies even further (Lv et al., 2019), even though this was contradicted by Leslie et al. (2017).

The success of microplastic removal in the WWTP is known to be dependent on the applied treatment processes (Talvitie, Mikola, Koistinen, et al., 2017). Therefore, the treatment process of two of the six sampled WWTPs (WWTP-Aartselaar and WWTP-Grimbergen) was studied in more detail by analyzing the MP concentration in influent, effluent and in the removed fractions (sand, sludge and floating layer on sedimentation tanks; supplementary file 6). A particle balance was set up allowing for an exploration of the efficiency of the various treatment steps used in WWTPs (Figure 4). This particle balance was set up using total removed fractions measured on three consecutive sampling days to account for longer transit times in the WWTPs. The highest fraction of MP is captured in the sludge fraction where between 12.53% and 93.79% of the MP that enter the WWTP are removed, as also seen in previous studies (Leslie et al., 2017; Magnusson & Norén, 2014; Wei et al., 2022). Sand (only available in WWTP-Aartselaar) and the floating layer are, according to our measurements, capturing a lower amount of MPs of the total influx (< 3 % and < 1 %, respectively). In the activated sludge treatment, the microplastics might form aggregates with organic materials, causing a change in density of the plastics and thus causing sedimentation, as described before by Talvitie and colleagues (2017). The sludge was found to be the main sink of MP was as found in

previous studies as well, with reported retention rates of > 99 % (Magnusson & Norén, 2014) and 72 % (Leslie et al., 2017).

The physicochemical characteristics of different polymer types might result in a different behavior of the plastics in the WWTP, and thus could result in a different capture efficiency. Therefore we calculated a polymer-specific particle balance, as illustrated in supplementary file 7. The following figures should be interpreted carefully: due to the investigated size range (25 µm – 5 mm) and the limited particle counts in the effluent samples, zero counts are likely non-detects and the reported removal rates are, by consequences, only estimates. Polypropylene was efficiently caught in the sludge (between 43.66 % and >100 %). PS, a denser polymer type, also tends to end up in the sludge (max. 66.62 %), while other polymer types such as PET and PE also end up here in lower amounts (34.57 % and maximum 4.98 %, respectively). PVC, even though found in the effluent, was only measured in small amounts in the removed fractions, and therefore, it is unclear where exactly the PVC particles end up and whether this polymer type is efficiently removed from the wastewater. Particles with sizes smaller than 1,000 µm were mostly found in the sludge, while particles larger than 1,000 µm were predominantly found in the sand fraction in WWTP in Aartselaar, indicating size-specific removal efficiencies of different techniques.

The particle-balance was mostly incomplete, i.e. a fraction of the incoming MPs were not found in the removed fractions or effluent. It is possible that (a fraction of) the MPs have a higher retention time and due to the shorter measuring period (three days), this fraction had not yet passed the entire WWTP. It is also possible that, in the WWTPs, MPs

are fragmenting into smaller particles that cannot be detected by the FTIR. More research is necessary to study this observation in more detail.

3.3 Role of effluent discharge on microplastic contamination in waterways

Sampled effluent contained on average 0.41 ± 0.91 MP per L. Although the main fraction of microplastics is removed from the wastewater, the non-removed fraction is responsible for a discharge of $1.11 \times 10^7 \pm 3.07 \times 10^7$ microplastics (25-1,000 µm) per day in the nearby waterway (on average per WWTP), which evidently could influence the MP contamination levels in these waterways. To estimate the effect of this discharge on the MP concentrations, samples of surface waters and sediment were analyzed from up- and downstream locations from a WWTP discharge. The MP concentrations in the surface waters downstream of a WWTP were lower compared to the concentration upstream (Table 2), although no significant differences could be observed (Tangebeek p=0.1573; Grote struisbeek p=0.1797; Heulebeek p=1.000). Also, for the sediment all downstream locations contained less microplastics compared to the upstream locations except for the Tangebeek (Grimbergen) where higher MP concentrations were found downstream of the WWTP compared to upstream (Table 2). As no replicates for sediment samples were collected, no information on the variation in contamination levels is available and thus no statistical analysis can be performed. Due to the discharge of MP in the waterway, we would expected a higher MP concentration downstream of the WWTP), which was for example observed in both the Raritan and Chicago river (Estahbanati & Fahrenfeld, 2016; McCormick et al., 2014). However, this was not the case in the current study. A dilution effect, linked to the high volumes of water added, might take place downstream of the effluent discharge, resulting in a dilution of the MP

concentration in the total waterway. Magnusson and Norén (2014) showed that the increased MP concentration due to effluent discharge was mainly located in or near the effluent plume. At a further distance (200 m) the effect of this discharge was not noticeable anymore (Magnusson & Norén, 2014). The WWTP effluent discharge of Heule was closest to the downstream location (~ 47 m), but also here we did not observe an increased MP concentration. Furthermore, in previous studies, no correlation was found between distance from discharge location and MP concentration, indicating the action of various processes such as uptake by biota, dilution, settling of MP during transport (Estahbanati & Fahrenfeld, 2016). Furthermore, daily and seasonal variations have been reported by Magnusson and Norén (2014) and Ben-David et al. (2021), which could also offer a possible explanation for the data presented here given the observed large variation in MP concentrations in the surface waters.

Although the MP concentrations in the receiving waterway did not seem to be significantly affected by the effluent discharge, the particle composition might be influenced by the input of effluent discharge. In the surface waters, changes in polymer composition were observed in the Grote struisbeek (WWTP-Aartselaar) where an input from PS via the effluent could have possibly increased the PS fraction in the downstream location compared to the upstream location (Figure 5a). In the Heulebeek and Tangebeek however, no influence of effluent discharge could be observed (Figure 5a). In the sediment, an increase in PS was also observed downstream from the effluent discharge of WWTP-Aartselaar, as compared to upstream of the WWTP (from 7.69 % to 33.33 %). In other locations, no effect in polymer composition of the effluent discharge was obvious (Figure 5b).

When comparing the size distribution of MPs in the surface waters of upstream and downstream locations, it appeared that the fraction of smaller particles (25-50 µm) was higher in the upstream locations in comparison with the downstream locations (Figure 6a). Nonetheless, some particles with sizes between 20 and 25 µm were found in downstream locations and not in upstream locations (Figure 6a). This could be a result of the discharge of predominantly smaller particles via the effluent (see Figure 2). In the sediment, this effect was not present (Figure 6b). These smaller particles in the surface waters could exert more effects compared to larger particles when taken up by aquatic organisms (e.g., An et al., 2021). More research is necessary to further study the effect of effluent discharge on polymer composition and size distribution in the waterways.

3.4 Impact of wastewater on the environmental microplastic contamination

The general aim of this research was to adopt a more holistic approach to understand the contribution of domestic wastewater on MP pollution in Flanders. Microplastics may be emitted into the aquatic environment via various wastewater routes including via effluent discharge, removed fractions, storm water overflows or direct discharge (without treatment in WWTP). In this study two important routes, effluent discharge and removed fractions, were studied.

Based on the high removal efficiencies of the MPs (with size of 10 µm – 5 mm) measured in the WWTP, we conclude that WWTPs using a sludge treatment are efficient in retaining microplastics. Thus WWTP, and more specifically the sludge, functions as a sink of MP. However, due to the large volumes of water that are discharged on a daily basis, this incomplete removal results in significant amounts of MP that enter the environment via wastewater discharges, causing WWTPs to be referred to as sources of

MP pollution (Ben-David et al., 2021; Talvitie et al., 2015). Based on the results of this study, effluent discharge via the sewage system doesn't necessarily cause an increased concentration of MP in the nearby waterway in the sampled downstream locations, although a local increase in the effluent plume is possible. Nonetheless, based on an analysis of the size distribution, the effluent introduces more smaller particles in the surface water.

As shown by this study and previously published research, the sludge is the main removal route of MP. Hence, if these fractions, such as sludge, are not removed in an adequate way, WWTPs can be a source of MP pollution to the environment. For example, approximately 53 % of the sludge produced in the European Union is used as biosolids in agriculture (ECHA, 2019; Zubris & Richards, 2005). Those biosolids are known to introduce MPs in the terrestrial environment of which > 99% are estimated to be transported to the aquatic environment during rain events or by erosion (Crossman et al., 2020). Legislation regarding the application of biosolids needs to be revised to take into account this important route of MP pollution. In Flanders, none of the removed fractions (i.e., thickened sludge, sand and floating fraction) are land filled. The floating material is scraped off and added to the thickened sludge fraction upon removal. Approximately 2/3 of the sludge (based on mass, dry weight based) is incinerated, while the remaining fraction is dried to pellets and sold as fuel to the cement industry. The sand fraction undergoes a physicochemical cleaning treatment in soil remediation centers. Hence, in Flanders, thickened sludge is mainly incinerated and, thus, the captured MPs are destroyed; this is also the case in the Netherlands (Leslie et al., 2017). Other pathways of wastewater to the environment, such as stormwater overflow, were not studied in this

research. Moreover, in some cases the flow rate in the WWTPs can be too high and part of the incoming influent will be discharged without any treatment in the nearby water stream to avoid an overload of the WWTP (Sundt et al., 2014). However, only few studies (e.g., Liu et al., 2019; Tian et al., 2020; Vollertsen et al., 2007) have conducted research on the contribution of both stormwater overflow and run-off on MP pollution levels and their effects in the receiving environment. More research is definitely necessary on the impact of storm weather events on microplastic pollution in the environment.

It is important to mention that WWTPs are merely a pathway of wastewater and no direct source. The true source of microplastics is located more upstream the wastewater chain, more specifically the households, industry, … that are (sub)consciously discharging MPs in the wastewater. Policy and/or scientific efforts could focus on the improvement of the removal efficiencies of WWTPs. However, the cost-benefit is deemed to be limited due to the already high removal efficiencies. Focus on the limitation of discharge of MP in the wastewater at the actual source (households, industry,…) should be the main focus to lower MP emission in the environment. Furthermore, in Europe, a fraction of the wastewater (ranging between 0.027 % and 48.33 %) of 21 countries (e.g., Norway, Croatia, Poland Serbia and Belgium) is collected and discharged without treatment (EEA, 2020). In Flanders, this is the case for the wastewater of 14.5% of the households (Vlaamse Milieumaatschappij, 2021). This untreated wastewater will thus be discharged in the nearby water stream either directly, or after sinking processes in a small settling basin or sedimentation pond. As far as we know, no studies have quantified the MP removal efficiency of a settling basin. However,

a lower efficiency for microplastic removal as compared to the complex treatment processes of a WWTP can be expected.

4. CONCLUSION

The domestic wastewater that enters the WWTP contains up to 86.45 MP per L. Flemish WWTPs, using activated sludge as a treatment process, efficiently removed on average 97.46 % of the microplastic particles that enter the WWTP. Results suggest that smaller particles are less efficiently removed from the wastewater, which is also affecting the size distribution of the MPs found in the nearby waterway. Sludge appeared to contain the most microplastics (up to 94 %), compared to other removal processes, which warrants careful reuse of the sludge in agricultural applications. Nonetheless, on average $1.11 \times 10^7 \pm 3.07 \times 10^7$ microplastics, predominantly smaller particles (25-75 µm), are still discharged in the nearby waterway per WWTP on a daily basis. This, however, did not result in an increased concentration of microplastics in the receiving surface waters and sediments. While these results show a "snapshot" of the microplastic presence in the specific WWTPs, more accurate or reliable values could be obtained by the inclusion of longer and continuous studies, and additional visits at different times of the year. In summary, this research offers a holistic approach in the research on the impact of wastewater on the microplastic pollution in the ecosystem, integrating different discharge routes and measuring the impact on environmental microplastic pollution.

Supporting Information—The Supporting Information are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

Author Contributions

Maaike Vercauteren: Conceptualization; Formal analysis; Writing–Original draft. **Ilias Semmouri:** Conceptualization; Investigation; Methodology; Writing–Review & Editing. **Emmanuel Van Acker:** Conceptualization; Methodology; Investigation; Writing–Review & Editing. **Emmy Pequeur:** Conceptualization; Methodology; Writing – Review & Editing. **Colin Janssen:** Conceptualization; Writing – Review & Editing; Supervision. **Jana Asselman:** Conceptualization; Writing – Review & Editing; Supervision.

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Disclaimer

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

This research is part of a large research project of which not all aspects are published yet. Therefore, the data is not published (yet) in a data repository but are available upon request by contacting the corresponding author (maaike.vercauteren@ugent.be).

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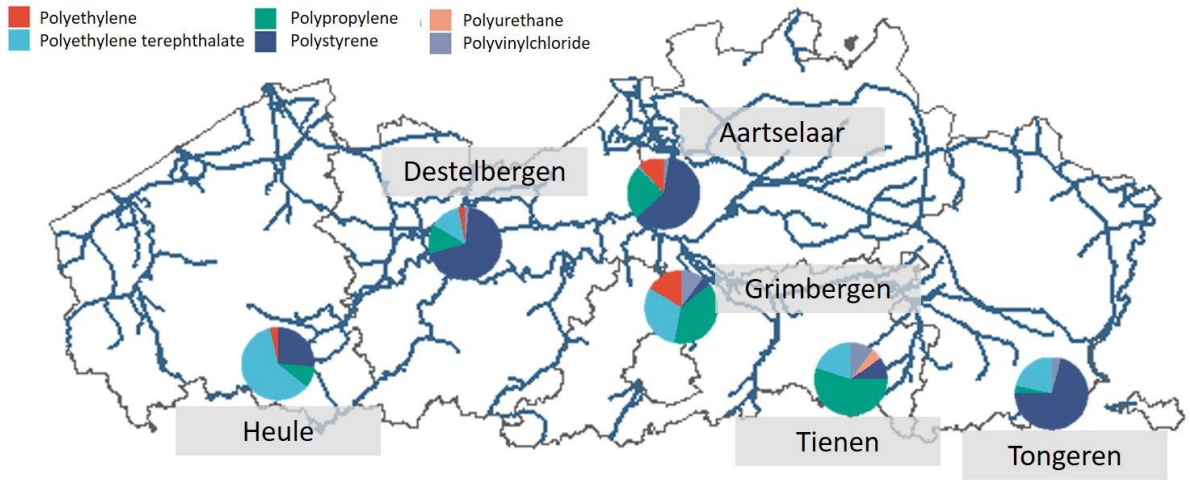

Figure 1: Polymer composition of the influent in the six sampled wastewater treatment plants.

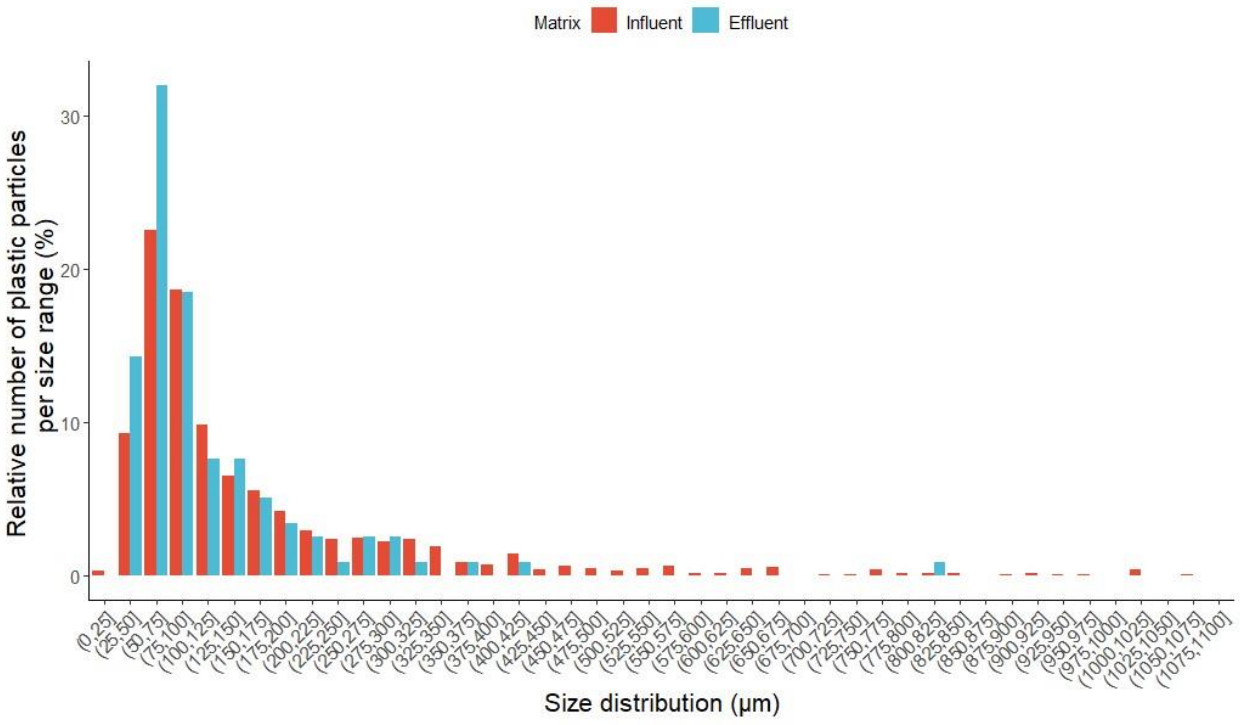

Figure 2: Relative size distribution of plastic particles found in influent and effluent samples of all wastewater treatment plants. The size distribution of particles from influent

samples are more widespread, containing particles of up to 1100 µm. In contrast, effluent samples contained mainly the smallest particles.

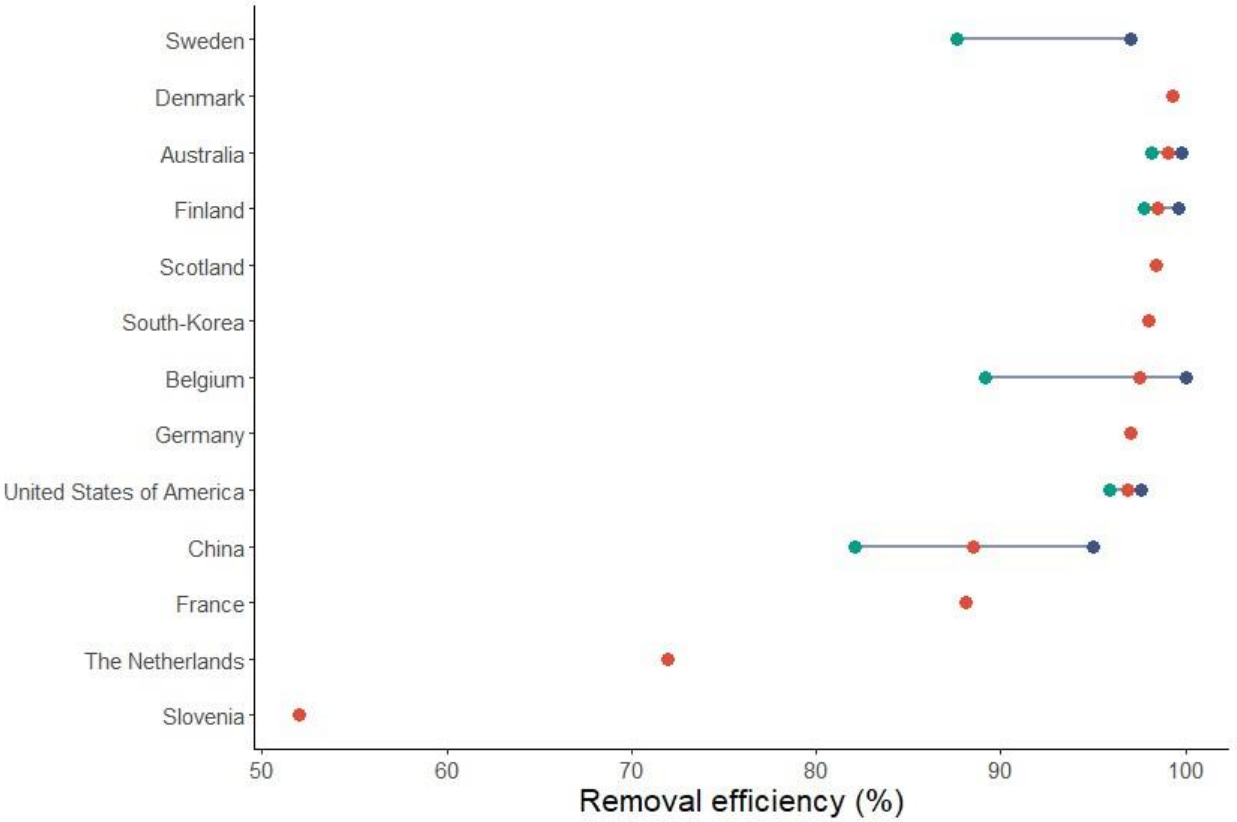

Figure 3: Comparison of the MP removal efficiency in different WWTP (minimum(green), mean (red), maximum (blue)) of different countries. Sources: Sweden (Magnusson & Norén, 2014); Denmark (Simon et al., 2018); Australia (Ziajahromi et al., 2021); Finland (Lares et al., 2018; Talvitie et al., 2015; Talvitie, Mikola, Koistinen, et al., 2017), Scotland (Murphy et al., 2016); South Korea (Lee & Kim, 2018); Belgium-Flanders: this study; Germany (Mintenig et al., 2017); United States of America (Conley et al., 2019; Michielssen et al., 2016); China (Lv et al., 2019; Yang et al., 2019); France (Dris et al., 2018); The Netherlands (Leslie et al., 2017); Slovenia (Kalčíková et al., 2017)

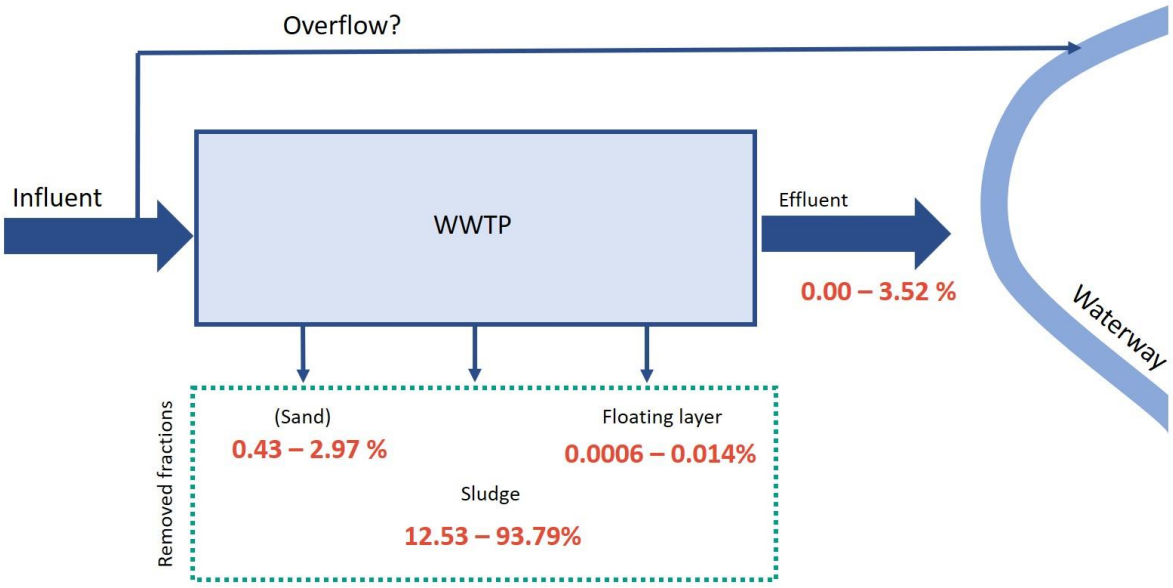

Figure 4: Schematic overview of the particle-balance analysis of the MP particles that enter a WWTP showing a range of the capture efficiency of all polymer types (in both wet and dry conditions) for each of the used techniques. We did not quantify the number of microplastics that leave the WWTP through overflow.

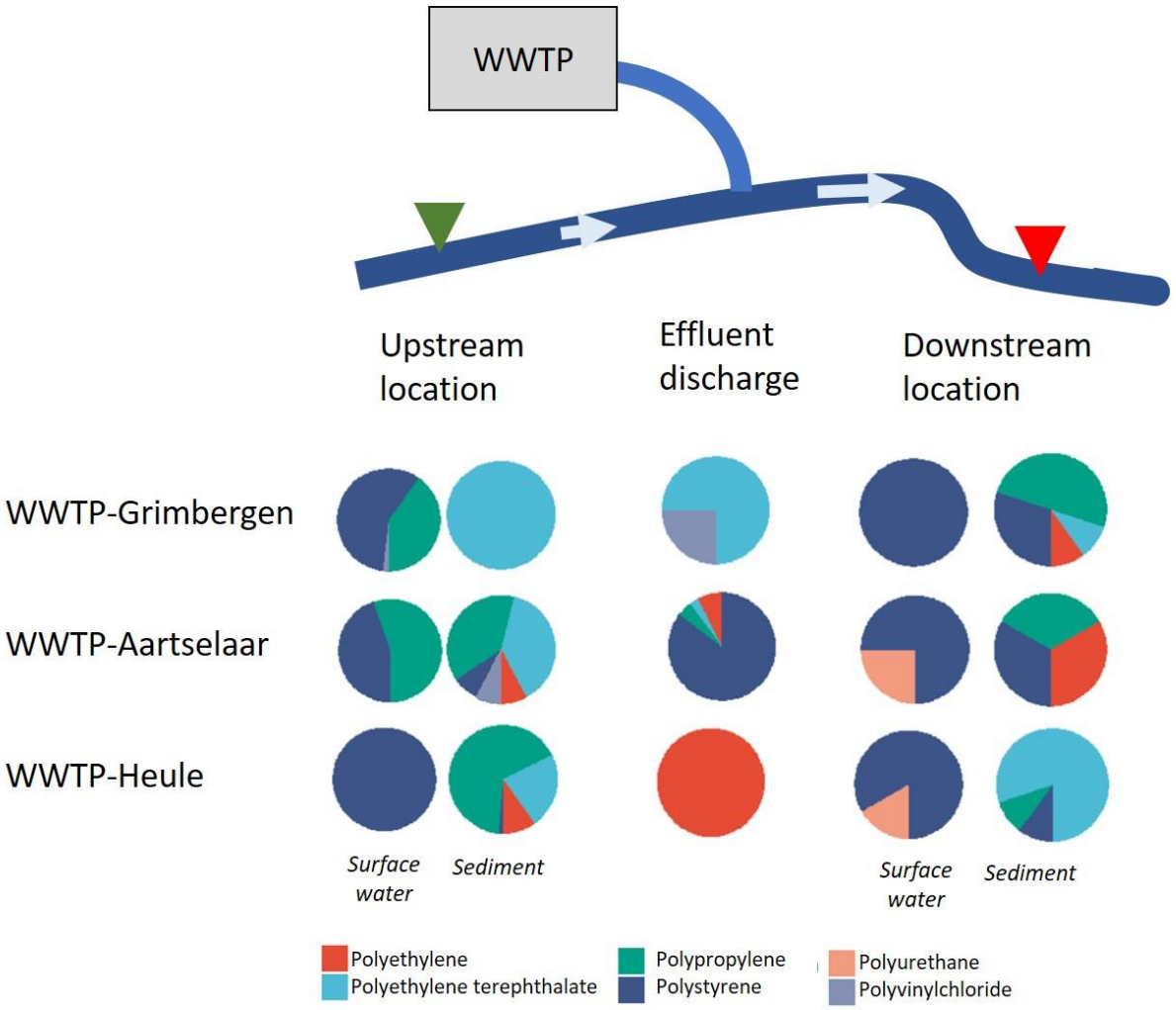

Figure 5: Comparison of the polymer composition in upstream and downstream location from an effluent discharge point in both water (A) and sediment (B)

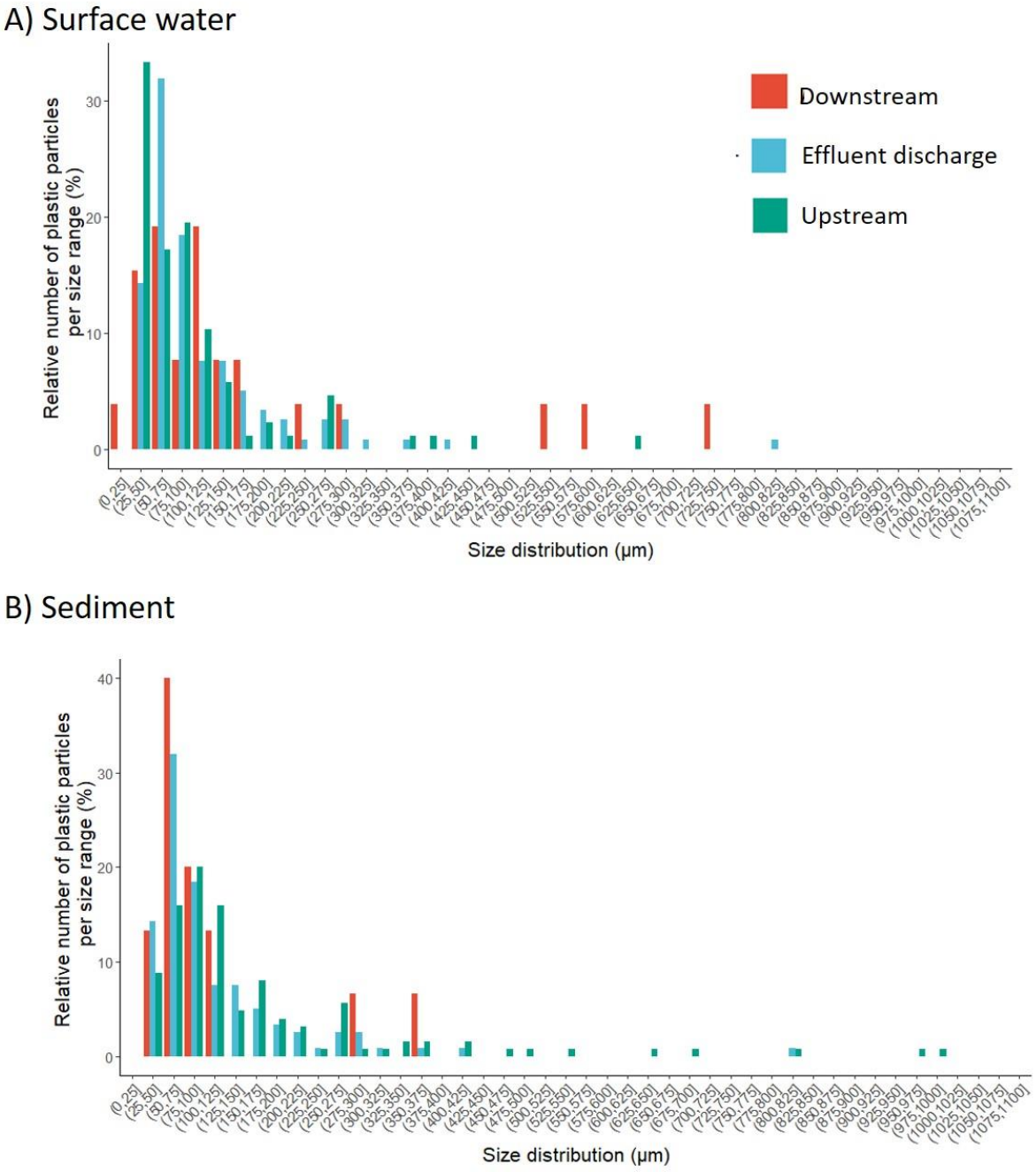

Figure 6: Comparison of relative size distribution in upstream and downstream location from an effluent discharge point in both water (A) and sediment (B).

Table 1: Overview of results of microplastic concentrations in influent and effluent samples with calculations of the removal efficiencies of the wastewater treatment plants.

WWTP	Weather conditions	Day	Influent			Effluent			Removal efficiency (%)
			Volume (L)	Particle numbers	Particle concentration (#/L)	Volume (L)	Particle numbers	Particle concentration (#/L)	
Aartselaar	Dry	1	2.18	14	6.42	8.83	<LOQ	0.00	
		2	2.06	6	2.91	8.49	0	0.00	
		3	2.29	8	3.50	8.13	3	0.37	
					4.28			**0.12**	**97.13 %**
	Rain	1	5.24	453	86.45	9.12	1	0.11	
		2	1.95	7	3.59	18.42	63	3.42	
		3	2.10	70	33.41	9.44	1	0.11	
					41.15			**1.21**	**97.05 %**
Destelbergen	Dry	1	5.39	274	50.88	8.49	26	3.06	
		2	2.06	17	8.25	8.63	10	1.16	
		3	2.14	8	3.74	8.94	4	0.45	
					20.96			**1.56**	**92.57 %**
Grimbergen	Dry	1	2.13	9	4.23	8.92	<LOQ	0.00	
		2	2.15	14	6.53	9.01	1	0.11	
		3	2.08	4	1.93	9.67	3	0.31	
					4.23			**0.14**	**96.68 %**
	Rain (sampling was not Performed on consecutive days)	1	2.20	16	7.27	9.03	0	0.00	
		2	2.06	2	0.97	9.40	0	0.00	
		3	2.22	4	1.81	7.96	0	0.00	
					3.35			**0.00**	**100.00 %**
Heule	Dry	1	4.27	14	3.28	9.28	1	0.11	
		2	4.22	55	13.03	8.76	<LOQ	0.00	
		3	4.49	15	3.34	9.20	<LOQ	0.00	
					6.55			**0.04**	**99.45 %**

Tienen	Dry	1	1.96	4	2.04	8.67	0	0.00	
		2	2.28	5	2.19	8.83	<LOQ	0.00	
		3	2.24	11	4.91	9.27	2	0.22	
					3.05			**0.07**	**97.64 %**
Tongeren	Dry	1	2.16	8	3.71	8.64	2	0.23	
		2	2.22	26	11.71	8.54	2	0.23	
		3	2.31	86	37.23	8.63	0	0.00	
					17.55			**0.16**	**99.12 %**
								Average	**97.46 %**
								Standard deviation	2.33 %

Table 2: Comparison of the microplastic concentrations in the waterways upstream and downstream of an effluent discharge. The distance between the discharge location and the downstream sampling location is also indicated.

	Distance between discharge and downstream location	**Surface water (MP particles per L)**		**Sediment (MP particles per kg DW)**	
		Upstream	**Downstream**	**Upstream**	**Downstream**
WWTP - Grimbergen (Tangebeek)	± 329 m	1.42 ± 1.38	0.16 ± 0.06	0.76	1.67
WWTP - Aartselaar (Grote struisbeek)	± 1.6 km	2.22 ± 2.26	0.39	1.56	0.61
WWTP - Heule (Heulebeek)	± 47 m	0.25 ± 0.20	0.19 ± 0.10	9.56	1.09