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# A complete mass balance for plastics in a wastewater treatment plant - Macroplastics contributes more than microplastics

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## ABSTRACT

A complete plastic particle mass balance was established at Sweden's second-largest wastewater treatment plant. It comprised material collected at its two bar screens, a 20 mm and a 2 mm one, in the influent water after the 20 mm screen, the effluent water, and the digested sludge. Macro- and microplastics above 500 µm were analysed individually applying ATR-FTIR, while microplastics of 10–500 µm were analysed by µFTIR imaging with automated particle recognition. Masses of plastics >500 µm were determined by weighting, while the mass of the smaller microplastics was estimated from the imaging. The total plastic load on the plant was 202.2 kg d<sup>-1</sup>, of which the two screens retained 73%. The remaining plastic mass was found in the sludge (13.6%) and the effluent (0.4%). The missing 12.7% could be caused by sampling and measuring uncertainties and potentially also fragmentation below the size detection limit of the analytical approach, or by degradation. The bar screens furthermore retained plastics smaller than the screen size, indicating that this material should be taken into account also when solely looking at smaller particles. The overall treatment efficiency of the plant was high: 99.6% considering both macro- and microplastics, and 98.8% considering only microplastics <500 µm.

## 1. Introduction

Plastics have become an integrate part of human life due to their versatile applicability and excellent longevity. The downside is that improper waste management has resulted in the accumulation of plastic pollution in the environment (Barnes et al., 2009; Derraik, 2002). Once present in the environment, plastic debris is subject to environmental factors such as sunlight and mechanical stress, which cause fragmentation into smaller pieces termed microplastics (MP), commonly defined as items smaller than 5 mm in their longest dimension (Arthur et al., 2009). From there, MP can get ingested by aquatic organisms (Cole et al., 2013; Derraik, 2002; Windsor et al., 2019), upon which the MP itself or additives and absorbed pollutants within it might cause adverse effects on the organisms (Andrady, 2011; Browne et al., 2013; Zhang and Chen, 2020).

In developed countries, liquid waste from households, institutions, and industries is usually collected by sewer systems. MP is present in the dry weather sewage, e.g. due to primary MP added to cosmetics and personal care products (Carr et al., 2016), and secondary MP released from breakdown of synthetic clothing during washing (Browne et al., 2011). Urban stormwater runoff is also conveyed in sewers, either

combined with the dry weather sewage or separately. This water contains MP from atmospheric deposition, traffic, and weathering of plastic materials, and enters the sewers during wet weather (Liu et al., 2019a, 2019c; Olesen et al., 2019). The ubiquitous presence of MP in urban dry and wet weather runoff entails further research into possible sources and sinks. In this context, the fluxes of MP from wastewater treatment plants (WWTPs) have received considerable attention (Liu et al., 2020; Mintenig et al., 2017; Simon et al., 2018). Most studies have investigated the presence of MP in inlet and effluent wastewater to assess the potential release of MP to the receiving environment. Indeed, several studies have reported elevated concentrations of MP downstream of WWTPs (Estabhanati and Fahrenfeld, 2016; Habib et al., 1998; Magnusson and Norén, 2014; McCormick et al., 2014). Once MP have been through the WWTP there is a risk of them having been colonized by pathogens residing in the wastewater and so MP might become a vector for their spreading (McCormick et al., 2014). Additionally, some studies have investigated MP in sewage sludge (Carr et al., 2016; Ljung et al., 2018) and found it to be a significant sink for MP in WWTPs. Although studies show that the vast majority of MP is removed from the wastewater during treatment, the sheer volume of treated wastewater has been argued to result in a significant discharge of MP to the aquatic

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environment (Ziajahromi et al., 2016).

Early studies have primarily focused on enumeration and description by employing visual identification by stereo microscopy based on physical characterization of type, morphology, and colour (Andrady, 2011; Derraik, 2002). This method, however, can be prone to bias. Relatively low magnification, poor contrast, e.g. when a particle has a colour similar to the background, operator dependency and difficulty in distinguishing between natural fibres such as cotton and synthetic fibres make MP identification difficult (Sun et al. 2019). It has been suggested to use a set of standardized criteria to improve characterization and avoid misidentification. However, even when these are used during visual sorting of suspected plastic particles (plastics), the risk for misidentification might still be as high as 70% (Hidalgo-Ruz et al., 2012) and no chemical information necessary for identifying the type of polymers is obtained (Xu et al. 2019).

Hence, identification of visually sorted particles should always be accompanied by chemical analysis (Hidalgo-Ruz et al., 2012). Fourier Transform Infrared spectroscopy (FTIR) and Raman spectroscopy have been highlighted as the most prominent technologies for acquiring information on the chemical structure of particles. The chemical fingerprint of unidentified particles obtained by these vibrational spectroscopic methods is commonly referenced to a library of spectra of known materials to identify them (Vianello et al., 2013a). A common practise is to perform visual inspection and separation of suspected MP followed by confirmation by ATR-FTIR or Raman spectroscopy (Bayo et al., 2020; Conley et al., 2019; Magni et al., 2019; Ziajahromi et al., 2021). While particles  $>500\ \mu\text{m}$  can readily be handled manually, and thus analysed as single particles using these techniques, this method becomes increasingly less feasible when particle size decreases. Methods for analysing MP  $<500\ \mu\text{m}$  using focal plane array (FPA)-FTIR have been developed (Löder et al., 2015) and improved upon to include mass estimation (Simon et al., 2018). While the size and abundance of MP are of importance for the ingestion and biological impacts of MP (Cole et al., 2013; Prata et al., 2020), these parameters are inadequate to describe the MP load from various sources. This owes partly to the continuous fragmentation of MP, decreasing their size while increasing abundance. By using mass as a conserved base quantity, measurements become independent of the change in apparent abundance. Several studies have hence suggested to report MP in terms of particle number, size, mass, and polymer types in order to ensure comparability of results and their versatile application (Simon et al., 2018).

This study seeks to expand the knowledge on the dynamics of plastics inside WWTPs by expanding the upper size limit of analysis and applying a mass balance approach for investigating their occurrence and fate. To this end, samples of bar screen material, inlet wastewater, digested sludge, and effluent from a Swedish WWTP were analysed for their content of plastics by FPA- $\mu$ FTIR imaging for MP of 10-500  $\mu\text{m}$  and ATR-FTIR for particles  $>500\ \mu\text{m}$ .

## 2. Materials and Methods

### 2.1. Sampling and description of WWTP

Ryaverket is one of the largest WWTPs in Sweden handling wastewater from approximately 790,000 inhabitants as well as industries in the greater Gothenburg area. Around 369,000  $\text{m}^3$  of treated wastewater is discharged daily, through Göta älv into the Kattegat Sea. The WWTP utilizes bar screens, sand and grease traps, primary settling, activated sludge, secondary settling, nitrifying trickling filters and nitrifying moving bed biofilm reactors, followed by post-denitrification, and, finally, tertiary mechanical filtration through disc filters (Hydrotech HSF2220-2FN) with a mesh size of 15  $\mu\text{m}$ . In this WWTP, fat, oil, and grease from the grease traps is skimmed and fed to the anaerobic digester together with fatty slurries from external sources (Tumlin and Bertholds, 2020). A full overview of the internal waste streams is found in Figure S1. To investigate the fluxes of plastics to and from the WWTP,

five different samples were taken: material from coarse (20 mm) and fine (2 mm) bar screens, influent wastewater after the 20 mm screen, digested sludge, and effluent. Fatty slurries from external sources were not sampled. Sampling took place through April-September 2018 (exact dates are in supplementary material Table S1). Influent dry weather wastewater was sampled flow-proportionally over 24 h using an ISCO 6700 Portable Sampler. Each day 5 L was sampled and stored in 1 L aluminium bottles. On the same days, bulk samples of material from bar screens and digested sludge were sampled and stored in glass jars. Two effluent samples were collected on the third day of the sampling campaign by continuously filtering from the effluent over the course of a work day, using a custom-made filtration device filtering onto 10  $\mu\text{m}$  stainless steel (Figure S2). The pump used contained no plastic or painted parts. Further details on the setup are found in (Liu et al., 2019b). In total, three filters were used for each sample. A flowmeter on the discharge line quantified the amount of filtered water, which in both cases were  $>550\ \text{L}$ .

For practical reasons it was not possible to completely avoid the use of plastic during sampling. The autosampler used for sampling inlet wastewater contained Teflon® (polytetrafluoroethylene, PTFE) tubes. A polyvinylchloride (PVC) hose was needed to connect the custom filtration device to the effluent sampling point at the wastewater treatment plant. Equipment used for sampling bar screen material and sludge were metal, while all sample containers were either glass or metal. Based on previous experience, any potential contamination from these sources is expected to be minimal and were not further assessed (Liu et al., 2020, 2019c).

### 2.2. Sample preparation

Two litres of each influent wastewater sample were used for the identification of MP down to 10  $\mu\text{m}$ . To reduce the volume, samples were filtered on a  $\varnothing 47\ \text{mm}$  stainless steel filter with a 10  $\mu\text{m}$  mesh and transferred into a 50  $\text{g L}^{-1}$  sodium dodecyl sulphate (SDS) solution by ultrasonication (Poornejad et al., 2016). A glass-covered magnetic stir bar was added and the samples were moderately stirred while heated to 50°C in a water bath for 48 h. Next, a two-step enzymatic digestion was applied to degrade proteinaceous and cellulosic material (Cole et al., 2014; Löder et al., 2017). First, proteinaceous material was degraded using 1 mL of Alcalase® 2.4 L FG (Novozymes, Bagsværd, Denmark) in a 0.2 M tris(hydroxymethyl) aminomethane buffer solution at pH 8.2 while being stirred and incubated at 50°C for 48 h. Afterwards, the buffer solution was replaced with an acetate buffer at pH 4.8 while 0.5 mL Cellulase, enzyme blend® and 0.5 mL Viscozyme® L (Merck KGaA, Darmstadt, Germany) were added, and incubated at 50°C for another 48 h. Subsequently, the samples were subjected to iron catalysed oxidation with hydrogen peroxide (Fenton reaction) to further remove organic matter. First, the samples were transferred into 200 mL Milli-Q water followed by 145 mL 50% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) while the source of iron(II) was 62 mL 0.1 M iron sulphate ( $\text{FeSO}_4$ ). The efficiency of the Fenton reaction is highly dependent on temperature and pH (Bautista et al., 2007; Tagg et al., 2017). Therefore, 0.1 M sodium hydroxide (NaOH) was used to adjust the pH to 3 while the temperature was kept around 20°C by cooling the beaker with ice. Finally, dense inorganic particles (e.g. sand) were removed through density separation using a  $\text{ZnCl}_2$  solution ( $\rho=1.7\ \text{g cm}^{-3}$ ) in 100 mL separator funnels (Imhof et al., 2012).

Hereafter, the samples were split into two size fractions by wet sieving with 15  $\text{g L}^{-1}$  SDS solution through a sieve with mesh sizes of 500  $\mu\text{m}$ . The particles in the filtrate were collected on a 10  $\mu\text{m}$  steel filter and transferred into 50% v/v ethanol by ultrasonication. Finally, the ethanol-water was completely evaporated into headspace vials using  $\text{N}_2$  before the volume was adjusted with 50% v/v ethanol to 5 mL of particle concentrate. To have enough particles for a representative analysis of microplastics above 500  $\mu\text{m}$  the remaining 3 L of influent wastewater were wet sieved over a 500  $\mu\text{m}$  sieve. Then the particles were oxidized

with 10% H<sub>2</sub>O<sub>2</sub> for 24 hours and dried at 55°C.

The effluent samples were transferred from the filters into 50 g L<sup>-1</sup> SDS solution by ultrasonication and subsequently followed the protocol described for wastewater samples. Likewise, a 20 g subsample of digested sludge was extracted from a larger sample of approx. 1 L upon mixing it thoroughly, pre-oxidized in 10% H<sub>2</sub>O<sub>2</sub> for 24 hours upon which it followed the same protocol as the wastewater samples. Beforehand, the water content of the digested sludge was determined with a Mettler Toledo Moisture Analyzer HE73.

### 2.2.1. Bar screen material

A subsample of approximately 100 g from each sample was dried to examine the water content. Then the coarse particles were sterilized and cleaned by oxidation with 10% H<sub>2</sub>O<sub>2</sub>. Finally, the samples were again dried, and all particles which potentially might be of plastic were manually sorted out under a stereo microscope. The sorted particles were further cleaned with ethanol prior to analysis. The size cut-off for the bar screen materials was 2 mm.

As coarse particles are retained on the bar screens, a filter cake builds up which could result in a reduction in the effective size of the screening. To investigate whether a significant amount of smaller particles were caught on the 2 mm bar screens, the amount of particles <2 mm in these samples was quantified. A subsample of 100 g bar screen material was dried and weighed. Afterwards, the particles were dispersed in 10 g L<sup>-1</sup> SDS solution and mixed on a shaking table for 1 h. Finally, the samples were wet sieved over a cascade of sieves ranging from 2 mm down to 20 µm. The sieves were dried and weighed before and after sieving to quantify the mass of smaller particles. The total mass of particles smaller than 2 mm on the bar screens was a few percent, and it was hence deemed sufficient to estimate the amount of microplastics below 2 mm in the bar screen material assuming a similar fraction of plastics in the small particles as was found for the fraction above 2 mm.

### 2.3. Spectroscopic analysis

In preparation for the spectroscopic analysis of the smaller size fraction (10-500 µm), a subsample of particle concentrate was deposited onto an infrared transparent Ø13 × 2 mm zinc selenide window held in a compression cell (Pike Technologies) which left an effective opening of Ø10 mm. The particle concentrate was homogenized by vortexing, and a subsample extracted with a glass capillary micropipette, added to the window, and dried on a heating plate at 50°C. Subsamples were added repeatedly in small steps of 50 µL until the window was sufficiently covered by particles, hereby avoiding particle agglomeration and overloading of the window. This process was repeated until the window was sufficiently covered by particles. In this way, 4-12% of the sample concentrates were deposited per window. Three separate windows with subsamples were prepared per analysed sample to account for heterogeneity during subsampling. The windows were analysed by µFTIR imaging using a Cary 670 FTIR spectrometer (Agilent Technologies, Santa Clara, CA, USA) combined with a Cary 620 FTIR microscope equipped with a 15x Cassegrain objective and a mercury cadmium telluride (MCT) detector with a 128 × 128 focal plane array (FPA), yielding a 5.5 µm pixel resolution. The samples were analysed in transmission mode applying a spectral range of 3750-850 cm<sup>-1</sup> at 8 cm<sup>-1</sup> spectral resolution with 30 co-added scans. The background was created by 120 co-added scans. After depositing the sub-samples, the whole effective area of the window (10 × 10 mm) was scanned, creating approximately 3.2 million spectra per scan, which then were processed using the siMPle software (formerly MPhunter) developed at Aalborg University, Aalborg, Denmark in association with Alfred Wegner Institute, Helgoland, Germany (Primpke et al., 2020). Each spectrum from the scan was correlated to more than 100 spectra of polymers and natural materials from a reference library. The 1<sup>st</sup> thresholds (the ones identifying if there is a microplastic particle, see Liu et al., 2019a) were varied to minimize the number of false positive and false negative identification based on an

expert evaluation of the goodness of the spectra comparison. The 2<sup>nd</sup> thresholds (the ones deciding the size of a microplastic particle) were varied and the particle size compared to a visual image of the scanned surface to optimize the size estimate. The evaluation was done on a subset of the automatically detected particles (Liu et al., 2019a; Primpke et al., 2017). Masses were estimated from the 2-dimensional images based on material density and an estimated thickness as described in Simon et al. (2018).

All sorted-out particles from the bar screen samples and particles >500 µm from wastewater and sludge samples were first photographed and then analysed using a Cary 630 ATR-FTIR spectrometer. Interpretation of the infrared spectra was done using software OMNIC 8.3 (Thermo Fisher Scientific Inc, MA, USA) in combination with commercial spectra libraries containing a wide variety of synthetic and natural materials (Vianello et al., 2013b). The masses of these particles were measured on an analytical balance (Mettler Toledo XSE205, d=0.01 mg).

### 2.4. Mitigation and assessment of contamination

Several precautions were taken to avoid contamination of the sample prior to analysis. During sampling, cotton work-suits were worn to reduce contamination with fibres from synthetic materials. Cotton lab coats were likewise worn during sample preparation in the laboratory. Glassware and other equipment were flushed thrice with filtered (0.7 µm glass fibre) water (Nuelle et al., 2014) while the steel filters were muffled before use (Simon et al., 2018). Additionally, the lab space was cleaned prior to sample preparation and the use of plastic equipment limited. Finally, air-born contamination was minimized by keeping beakers with samples covered with aluminium foil or glass watches during transfer in the lab and by conducting the sample preparation inside fume hoods. All reagents were filtered through 0.7 µm glass fibre filters before use.

Despite thorough care, complete avoidance of contamination is seldom possible. Consequently, triplicate laboratory blanks, following the preparation protocol for wastewater and sludge (six in total), were prepared and analysed along with the respective samples. Filtered (0.7 µm GF filter) Milli-Q water was used as a medium for blank samples. A similar volume of sub-sample from the blanks were analysed as were for their respective samples. The procedural blanks assess the contamination during sample preparation and analysis and the determined contamination hence refers to one sample preparation, and not to the volume of filtered water processed. Possible contamination during sampling was not assessed.

### 2.5. Statistical analysis

The normality of the size distribution of MP particles was assessed using Shapiro-Wilk normality test. A non-parametric Kruskal-Wallis test was performed to investigate whether there was significant difference in the size distribution between the influent wastewater and the other samples. Wilcoxon rank sum test was performed to assess differences in the mean of these samples. A significance level of p=0.05 was applied for all statistical analysis using the software R (v3.5.3).

## 3. Results and Discussion

### 3.1. Contamination

Analysis of 300 µL aliquots of the triplicate laboratory blanks for the wastewater protocol revealed a contamination by 13 MP particles. This corresponded to a contamination of 83.9 ng or 4.33 items per processed sample. Four polymer types were identified: PE, PP, polyester, and PS with polyester accounting for 69% of the contamination by number and 77% by mass. In the blanks following the sludge protocol, the contamination was even lower. Seven MP particles were identified in triplicate

**Table 1**

Concentration of plastics found in three independent samples of influent wastewater, sludge, effluent, and bar screen material (two for effluent) from Ryaverket in Sweden and the average flow of each process during sampling. The flow for digested sludge is calculated as dry weight (DW) based on measurements of the water content in the sludge samples. SD=standard deviation.

Sample	Concentration [average ( $\pm$ SD)]			Flow [average ( $\pm$ SD)]	
	10-500 $\mu$ m	>500 $\mu$ m			
Influent wastewater	63.41 ( $\pm$ 28.60)	93.33 ( $\pm$ 161.66)	$\mu$ g L <sup>-1</sup>	3.60 ( $\pm$ 0.51)	m <sup>3</sup> s <sup>-1</sup>
Effluent	0.88 ( $\pm$ 0.55)	1.51 ( $\pm$ 0.35)	$\mu$ g L <sup>-1</sup>	3.49 ( $\pm$ 0.08)	m <sup>3</sup> s <sup>-1</sup>
Digested sludge	305.34 ( $\pm$ 166.85)	264.32 ( $\pm$ 304.72)	$\mu$ g g <sup>-1</sup> DW	7.97 ( $\pm$ 1.11)*	ton DW h <sup>-1</sup>
20 mm bar screen	>2 mm 25.26 ( $\pm$ 34.39)	<2 mm n/a	mg g <sup>-1</sup>	90 ( $\pm$ 70)	kg h <sup>-1</sup>
2 mm bar screen	26.47 ( $\pm$ 26.60)	0.96 ( $\pm$ 1.29)	mg g <sup>-1</sup>	81 ( $\pm$ 54)	kg h <sup>-1</sup>

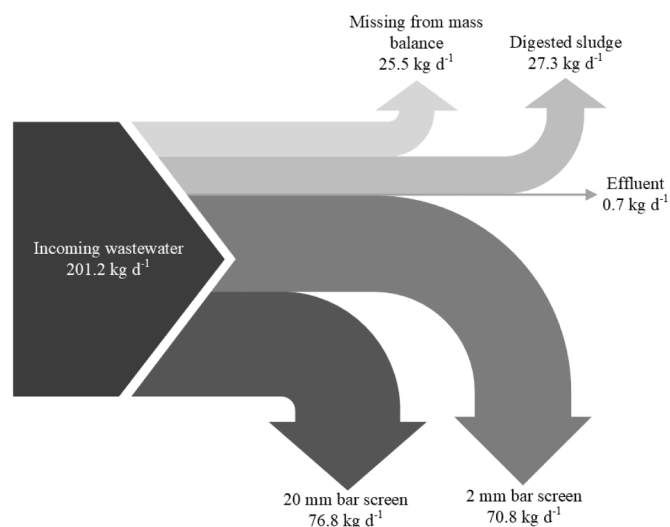
deposits of 200  $\mu$ L corresponding to 20.3 ng or 2.33 items per blank. Of the seven particles identified, six were of polyester and one was of PA. The contamination is expected to arise from occasional exposure to open air during sample preparation and not from the filtered Milli-Q used as matrix for the blanks. Considering the mass of MP identified in the blanks for the wastewater protocol in relation to the real wastewater samples, the possible contamination corresponds to just 1.20% of the average mass of MP found in similar sample aliquots. The blanks from the sludge protocol contained just 0.04% of the average MP mass from the sludge samples due to a much greater amount of MP identified in these samples.

A study on another WWTP by Mintenig et al. (2017) reported contamination levels of 21 particles and 130 fibres in procedural blanks consisting of 150 L filtered (3  $\mu$ m stainless steel filter) tap water treated subsequent to samples of treated wastewater. Simon et al. (2018) reported finding 2110 MP, corresponding to 84  $\mu$ g, in triplicates of 1 L filtered demineralized water processed in parallel with samples of raw wastewater. More recently, Liu et al. (2019) found MP corresponding to 22.2 items, with an estimated mass of 942 ng, in triplicates of 100 L of filtered (1.2  $\mu$ m glass fibre filter) Milli-Q water alongside stormwater samples following an protocol similar to the present one. In the case of Mintenig et al. (2017) and Simon et al. (2018), reported MP concentrations were corrected by subtracting possible contamination or used as detection limit, while Liu et al. (2019) did not correct the results due to the low levels of contamination detected. The levels of contamination found in the present study were low compared to the amount of particles found in the samples, on average corresponding to 1.2% and 0.04% of the plastics found in raw wastewater and sludge, respectively. Therefore, the results were not corrected for background contamination arising from the sample preparation.

### 3.2. Mass balance for plastics at Ryaverket wastewater treatment plant

In total, 14 samples of six different kinds were analysed for their plastic content, revealing a total of 3,909 plastic particles (plastics). Ranked in order of abundance: 2,455 were found in digested sludge, 719 in effluent, 576 in raw influent wastewater, and 159 in bar screen material. To establish the mass balance for plastics at the WWTP, the concentration of plastics in each sample was multiplied by the respective flow of the liquid or mass streams during the period of sampling. The range of the concentration in each kind of sample and corresponding flows is presented in Table 1. Details on individual values of plastic concentration and flow for each sample can be found in Table S1.

The overall mass balance was calculated using the average of the three independent samples (two in the case of effluent samples) and the



**Fig. 1.** Mass balance for plastics at Ryaverket wastewater treatment plant in Sweden.

average flow on the day of sampling. Fig. 1 shows the mass balance for plastics at the wastewater treatment plant. It shows that 201.2 kg of plastics entered the treatment plant daily while 0.7 kg was released through the effluent. This corresponds to a removal efficiency of 99.6%. The primary removal of plastics from the wastewater happened via the 20 mm and 2 mm bar screens with 38.2 and 35.2%, respectively. Finally, 13.6% of the plastics were recovered in the sludge fraction. These measurements account for 87.3% of the load to the wastewater treatment plant. The missing 12.7% of the plastic mass balance are expected to arise from sampling and measuring uncertainties and potentially by plastics loss in the digester and the activated sludge process tanks. The latter can either be chemical-biological degradation or simply a fragmentation below the size detection limit of the applied analytical technique. There is a great variability of the daily wastewater flow where factors such as time of sampling and the weather can have a significant influence. Therefore, it is important to report specifics about the time of sampling and antecedent weather patterns. Furthermore, the digested sludge represents an average over several weeks due to the retention time in the digester. These issues become of increasing importance when MP concentrations identified in small bulk samples are scaled to yearly discharges. Some of these uncertainties are evident when looking at variation in the plastic concentration in samples presented in Table 1.

No other studies have dealt specifically with the retention of MP in material from bar screens, likely because the size extends beyond what is considered MP. This study found that due to the build-up of a filter cake, retention of small particles can be expected and indeed 2.4 to 4.5% of the dry mass of 2 mm bar screen material consisted of particles below the actual size of the screens. Assuming a similar fraction of plastics in the small particles as was found for the fraction above 2 mm, the removal of MP with 2 mm bar screen material was 2.6 kg d<sup>-1</sup>, corresponding to 4.6% of MP <2 mm of what was identified in the influent wastewater samples. These findings highlight that bar screen material should be considered in future research dealing with the mass balance at wastewater treatment plants utilizing this technology even when targeting the MP <5 mm. However, as this study investigates the total mass balance of plastics in the WWTP, this fraction only accounted for 1.3% of all the incoming plastics. This indicates that the potential error from not directly measuring the content of small MP in the filter cake was small compared to the overall uncertainties.

Considering only the small fraction of MP <500  $\mu$ m, the removal through sludge was 65.8% of the inlet mass, highlighting this as a key process in the removal of small MP. Previous studies have reported

comparable MP removal of 72-98% through sludge (Leslie et al., 2017; Murphy et al., 2016; Talvitie et al., 2017b). It is estimated that 11.6% of the small MP were retained in the filter cake on the 2 mm bar screens. Finally, the effluent samples contained MP corresponding to 1.2%, giving a removal efficiency for MP <500  $\mu\text{m}$  of 98.8%, which falls in line with other studies reporting overall MP removal efficiencies of WWTPs above 98% (Carr et al., 2016; Magnusson and Norén, 2014; Murphy et al., 2016; Simon et al., 2018; Talvitie et al., 2017a). The 21.4% unaccounted for are most likely related to the previously explained analytical uncertainties.

In terms of numbers, the average concentration of plastics in raw wastewater was 533 items  $\text{L}^{-1}$  in influent wastewater, while the final effluent contained 4 items  $\text{L}^{-1}$ . The highest concentration in terms of numbers were found in the sludge with 1,401 items  $\text{g}^{-1}$  dry weight (DW). In material from the 20 mm and 2 mm bar screens, the concentrations were 0.20 and 0.28 items  $\text{g}^{-1}$ , respectively. The issues of reporting a concentration in terms of number are especially apparent when comparing the concentration of plastics found in bar screen materials. Despite a low number of plastics identified in these samples, the contribution in terms of mass was much greater than in, e.g. digested sludge.

Due to the novelty of this research topic, direct comparison with other studies can be difficult. Substantial differences in sampling methods, sampled volumes, sample preparation, higher or lower size cut-off and analytical techniques for MP identification have been applied in previous studies. To better compare studies, there is a need for standardization in the field, as highlighted by e.g. Hidalgo-Ruz et al. (2012) and Ziajahromi et al. (2016). These issues are apparent when comparing reports on the concentration of MP in WWTPs. In a recent review on the occurrence and fate of MP in wastewater treatment plants, Gatidou et al. (2019) reported influent concentrations varying from 1-7, 216 particles  $\text{L}^{-1}$ . Likewise to the present study, Talvitie et al. (2017) applied 24-hour flow proportional composite sampling and identified a similar concentration of MP of 630-900 items  $\text{L}^{-1}$  in influent samples, although using a different sample preparation and analysis approach. Simon et al. (2018) used a similar sample preparation and nearly identical analysis methods but reported much higher concentrations of MP, between 2,223-18,285 particles  $\text{L}^{-1}$  in influent samples from ten Danish WWTPs. Similar variation is apparent when considering the MP concentration reported in effluent and sludge. In this study the concentration of MP in the sludge samples was up to three orders of magnitude higher than those previously reported (Gatidou et al., 2019). It seems unlikely that this difference is due to true variability in plastic contents of different WWTP sludge, and more likely due to differences in analytical approaches.

The potential for the release of plastics to the environment from Ryaverket WWTP can be estimated based on the mass balance. Considering the annual effluent flow, an estimated 263 ( $\pm 42$ ) kg of MP  $\text{y}^{-1}$  is released into the environment through the outlet, corresponding to a per capita release of 0.33 ( $\pm 0.03$ ) g  $\text{capita}^{-1} \text{y}^{-1}$ . Another potential pathway for the spreading of MP to the environment is the fertilization of agricultural fields with sewage sludge (Browne et al., 2013; Zubris and Richards, 2005). In Sweden, 34% of the sewage sludge was spread on agricultural fields in 2016 (Naturvårdsverket, 2018). Based on the mass balance established in this study, a total of 3,372 ( $\pm 1,530$ ) kg of MP could hence annually be released to the terrestrial environment. However, in a study on the impact of using different amounts of sewage sludge as fertilization of a series of test-fields in Petersborg, Malmö (Ljung et al., 2018) found the concentration to be 3 to 10 times lower than what it theoretically should be assuming that plastics could not break down in nature. Therefore, more research is needed to understand the true impact of using sewage sludge for fertilization. Furthermore, other potential sources for MP in agricultural fields such as littering and mulching should also be accounted for.

Finally, the flow of plastics with the bar screen material was 50 tons  $\text{y}^{-1}$ . However, as this material is incinerated, the potential for release

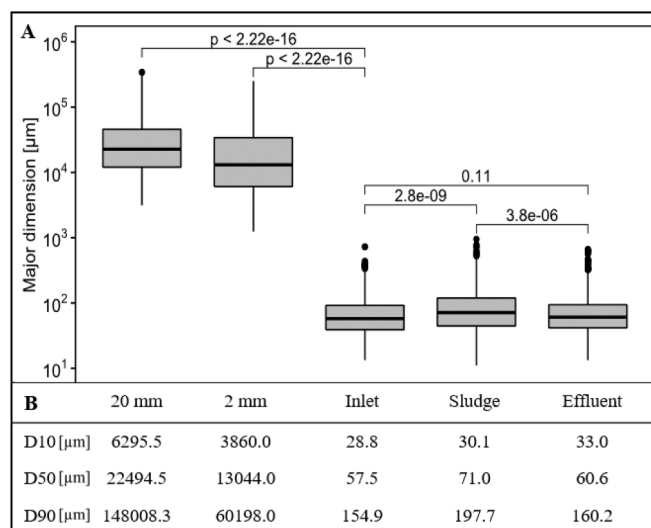


Fig. 2. Boxplot of the particle size of plastics identified in bar screen materials, influent wastewater, digested sludge, and effluent determined by their major dimension and represented on a logarithmic scale (A). The solid black line shows the median. The p-value for each comparison is given above comparison lines. A table with the D10, D50, and D90 values for each sample type is shown in (B).

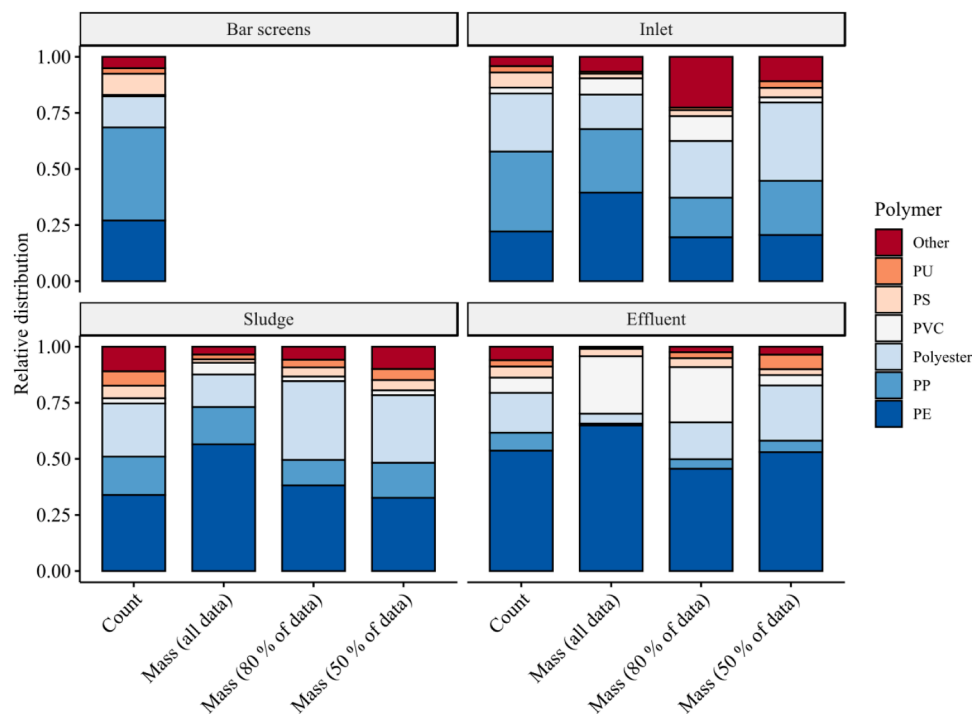
into the environment would only be through spills during transport to the incineration plant, which is unlikely to happen at a meaningful scale.

### 3.3. Size distribution of plastics

A boxplot of the size of identified plastics is presented in Fig. 2A together with the D10, D50 and D90 for each sample type in Fig. 2B. Particle size distributions can be found in supplementary material Figure S3. The size of the MP particles was defined by their major Feret-diameter determined by *siMple* for MP <500  $\mu\text{m}$  and manual measurement of the major dimension in ImageJ for plastics >500  $\mu\text{m}$ . A Shapiro-Wilk normality test revealed that the size of the plastics was non-normal distributed in all samples. Consequently, a non-parametric Kruskal-Wallis test was used to compare the size distribution of plastics in the inlet with other sample types, results of which are also shown in Fig. 2A. The test revealed a significant ( $p < 0.05$ ) difference in the size distribution of influent, bar screen, and sludge samples but an insignificant ( $p > 0.05$ ) difference between influent and effluent samples.

A pairwise Wilcoxon rank sum test showed that the mean size of plastics in the sludge was significantly larger than the ones in the influent and effluent, while plastics in the bar screen material were significantly larger than the plastics in other matrices. The large plastic particles on the screens originate from the wastewater. The fact that large particles were not also found in the inlet wastewater is likely an artefact of the sampling, as the concentration of large particles is low and the probability of finding one in the small volumes of inlet wastewater collected hence also low. No significant difference between plastics in coarse and fine bar screen material was found. It is furthermore worth noticing that plastics fitting the definition of MP (<5 mm) were identified in the 20 mm bar screen material, again highlighting that these screening processes should not be overlooked in studies of MP at WWTPs (an example of identified plastics is shown in Figure S4).

One likely reason for the difference between the size of plastics in influent wastewater and sludge could be that the large MP are rare in wastewater compared to smaller ones (Talvitie et al., 2017b). Although 24-hour flow-proportional sampling ensures a degree of representability for the day of sampling, the position of the intake of the autosampler could influence the size distribution as larger particles have a greater tendency to either sink or float. These particles would however, still be



**Fig. 3.** Distribution of polymers for influent wastewater, digested sludge, bar screen material, and effluent in terms of particle count and mass. Furthermore, the figure show plots of the mass distribution were 10% (80% of data) and 25% (50% of data) least and most massive particles are excluded.

present in the wastewater reaching the treatment plant and end up in the sludge fraction during treatment. Furthermore, while a wastewater sample represents a temporal span of one day, the solid retention time during anaerobic digestion is several weeks, meaning that the presence of MP particles with an extreme size is more likely to occur. This is evident when looking at Fig. 2B, where the sludge samples show not only a higher D90 value but also a greater spread than influent and effluent samples.

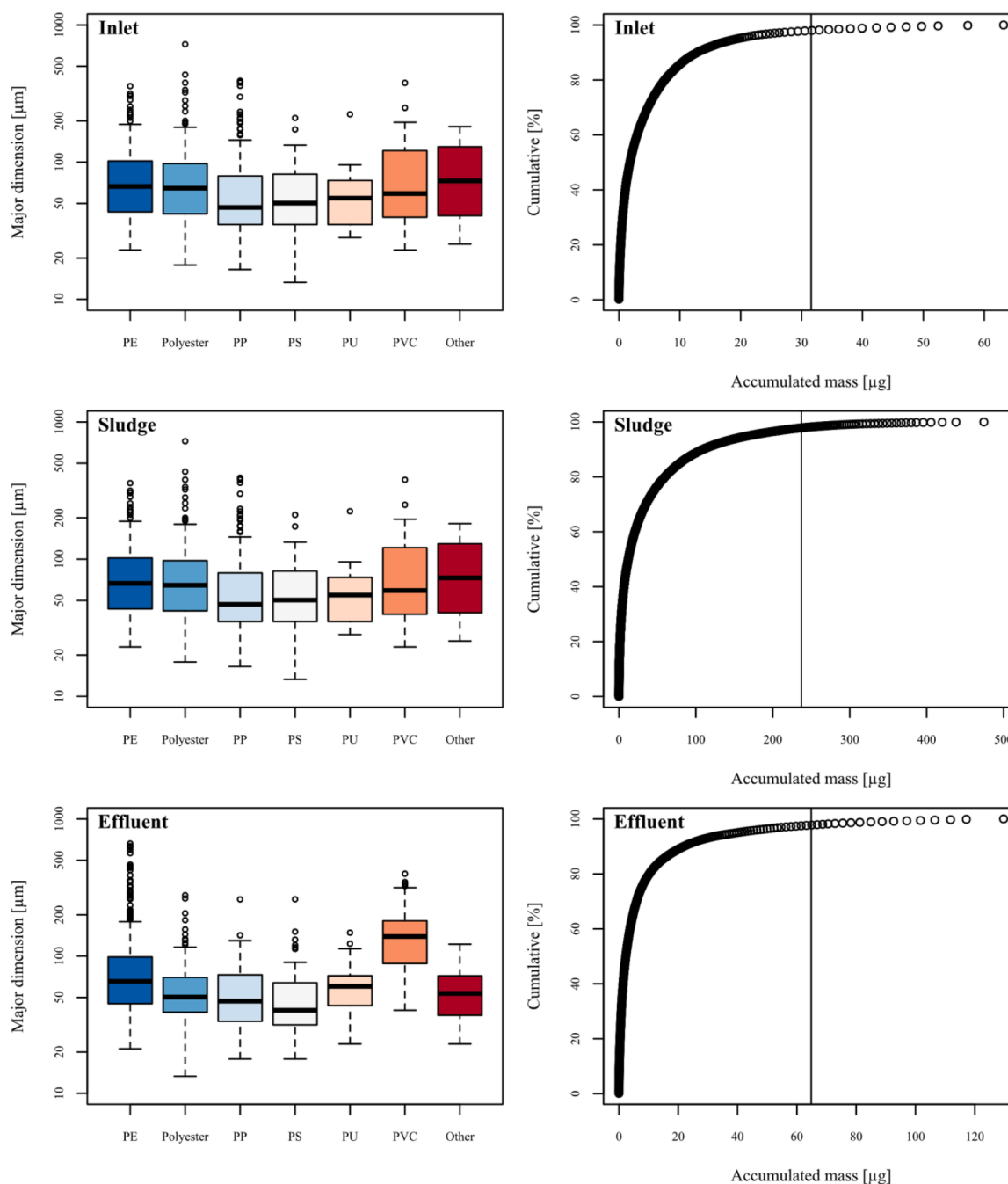
Although the wastewater treatment plant applied disc filtration with a mesh size of 15  $\mu\text{m}$ , larger MP were identified in the effluent. In a study of the disc filters at Ryaverket, Wilén et al. (2012) reported similar findings and attributed this to particles having non-spherical shapes. The characteristic was confirmed for MP by Liu et al. (2019b) who reported the shape of MP identified in stormwater ponds as plump ellipsoids having a major-to-minor dimension ratio of 1.9. Simon et al. (2019) studied the removal of MP by a disc filter and likewise reported some particles in the effluent having larger dimensions than the filter mesh. They contributed these findings to possible defects in the filter cloth due to mechanical stresses or unintended bypassing of wastewater.

In a study applying a similar methodology, Simon et al. (2018) reported the median size of MP to be 48  $\mu\text{m}$  with a D90 of 100  $\mu\text{m}$  for raw wastewater and a median of 45  $\mu\text{m}$  with a D90 of 91  $\mu\text{m}$  for treated wastewater. In a study on the retention of MP by disc filters with a 18  $\mu\text{m}$  mesh, Simon et al. (2019) found a median of 33.5  $\mu\text{m}$  before and 47.9  $\mu\text{m}$  after disc filtration. These figures are slightly below what was found for raw and treated wastewater in this study. Other studies reported MP size as abundance in certain size ranges. Mintenig et al. (2017) found 59.2% of MP identified in treated wastewater in the size range 50–100  $\mu\text{m}$ , making this the most abundant size fraction with only 8.5% of identified MP particles being above 200  $\mu\text{m}$ . Applying similar ranges, the abundance of MP within the size range 50–100  $\mu\text{m}$  were 36.0, 36.3 and 38.8% in wastewater, sludge, and effluent while the amount >200  $\mu\text{m}$  in those samples were 5.9, 9.3 and 6.7%. Similarly, Talvitie et al. (2017b) found that 70% of MP in treated wastewater was in the size range below 100  $\mu\text{m}$ . In the present study, 78.4, 70.0, and 76.6% of MP identified in wastewater, sludge, and effluent, respectively, were below 100  $\mu\text{m}$ . In

studies applying manual sorting prior to spectroscopic analysis, the size of the identified MP tended to be larger (Bayo et al., 2020; Magni et al., 2019). In the size range 10–100  $\mu\text{m}$ , Magni et al. (2019) found 12%, 24%, and 14% in raw wastewater, sludge, and treated wastewater, respectively, while reporting the highest abundance of 36–54% in the size range 100–500  $\mu\text{m}$ . Bayo et al. (2020) found the highest abundance of particles in the size range 400–600  $\mu\text{m}$ , accounting for 35.2% of all MP. The explanation for this discrepancy is most likely differences in applied quantification methodologies. As describe by Hidalgo-Ruz et al. (2012) and Sun et al. (2019), manual sorting can be prone to bias toward the larger size fraction due to the difficulties in identifying and handling small particles. This shows that the abundance of identified MP is highly dependent on the applied methodology, in many cases making direct comparison impossible.

### 3.4. Polymer distribution of plastics

The identified plastics could be categorized into 22 polymer groups. The most diverse type of sample was digested sludge covering 18 polymer groups. The least diverse sample type was 20 mm bar screen material, covering only 7 polymer groups. Throughout all matrices, the most common polymer types in terms of mass, ranked by relative contribution, were: PE, PP, polyester, PVC, PS, PU, acrylic, alkyd, PA, acrylic paints, EVA, ABS, SAN, epoxy, PVA, PVAC, vinyl copolymer, polycarbonate, PAN, PU paints, phenoxy resin, and PLA. Of these, 97% of the total plastic mass were identified within the first six groups. Previous studies likewise reported PE, PP, polyester, PVC, PS and PU as polymer commonly found in WWTPS (Mintenig et al., 2017; Murphy et al., 2016; Simon et al., 2018; Talvitie et al., 2017a; Ziajahromi et al., 2017) which, coincidentally, are also the six most common polymers in the European plastic demand (Plastics Europe, 2019). For bar screen material, only the total polymer mass was quantified and not the mass per polymer group. Fig. 3 shows the relative distribution of polymers identified in each type of sample in terms of both particle count and mass. As the range of identified plastics spans several orders of magnitude (Fig. 2A), some of the largest particles might dominate the polymer



**Fig. 4.** Left) Boxplots of polymer specific size distribution based on the major dimension of the particles plotted on a logarithmic scale for influent wastewater, digested sludge, and effluent samples. Right) Accumulated distribution of identified plastics ranked by mass. The solid black line shows the division for 50% of total plastic mass.

distribution in terms of mass. This issue is further illustrated in Fig. 4, which shows the accumulated distribution of plastics in each sample ranked by mass. The solid black line indicates half of the total plastic mass and shows that just 2.1, 2.1 and 2.4% of the plastics accounted for 50% of the total plastic mass for influent wastewater, sludge, and effluent, respectively. Consequently, Fig. 3 also contains plots where the 10 and 25% least and most massive particles were removed, an approach suggested by Olesen et al. (2018). In samples of influent wastewater, the removal of 20% of the extreme values put a larger emphasis on less common polymer types, as the few large particles tended to be of common polymers. The polymer distribution in sludge samples becomes increasingly similar in terms of particle count and mass when removing extreme values, indicating the presence of large particles dominating

certain polymer types.

The effluent samples, for example, showed a large difference in polymeric distribution between particle count and mass, especially for PVC. The explanation for the difference is likely the size of the particles. Fig. 4 shows a boxplot of the polymer specific size distribution for each sample type. The figure indicates a clear difference in the size distribution of the PVC particles in the effluent compared to other polymer types. A pairwise Wilcoxon rank sum test revealed that the mean size of the PVC particles in the effluent samples was significantly higher ( $p < 0.05$ ) than all other polymer types. The same was true for the mean size of the PE particles when compared to polyester, PP, PS, and the grouped polymers. The reason why the difference in particles size for PE is less pronounced when considering the mass distribution shown in



Fig. 4 is likely the difference in density of PE at  $0.95 \text{ g cm}^{-3}$  and PVC at  $1.4 \text{ g cm}^{-3}$ .

#### 4. Conclusions

The total plastic mass removal efficiency, taking all sizes entering the plant into account, was 99.6%. Considering only the small MP  $<500 \mu\text{m}$ , the removal efficiency became 98.8%. Behind the latter numbers was that the sludge retained 65.8%, while 11.6% were retained at the 2 mm bar screens, and 1.2% was found in the effluent. 21.4% were unaccounted for in this mass balance, a discrepancy believed caused by sampling and measuring uncertainties or fragmentation and degradation in the digester and process tanks.

The bar screen material retained much larger plastics than what was sampled in the influent wastewater, sludge, and effluent. In terms of plastic particle numbers, the bar screen material contributed little to the overall plastic load on Ryaverket WWTP. However, in terms of plastic mass, things were quite different. Here the material retained on the screen accounted for 73% of the total load. The material on the 2 mm bar screen furthermore contained particles below 2 mm, causing this screen to retain 4.6% of the microplastics mass load ( $<5 \text{ mm}$ ). Ryaverket applies two screens, a coarse 20 mm followed by a fine 2 mm one. The latter caught about half of all screened plastic mass, showing that WWTPs benefit from being equipped with fine screens as this reduces the plastic load on the plant quite significantly. Having this additional 2 mm bar screen meant that Ryaverket received just 46% of what it would have received, had it only had a 20 mm bar screen. This study shows, that in order to establish a complete mass balance of plastics at wastewater treatment plants, it is crucial to consider plastics larger than what is considered microplastics and to take plastics of all sizes into account at all treatment steps.

#### Declaration of competing interest

None.

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#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2021.117307.

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