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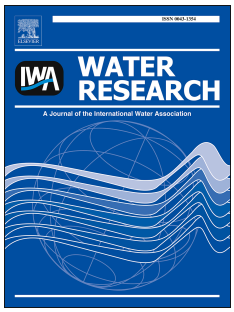

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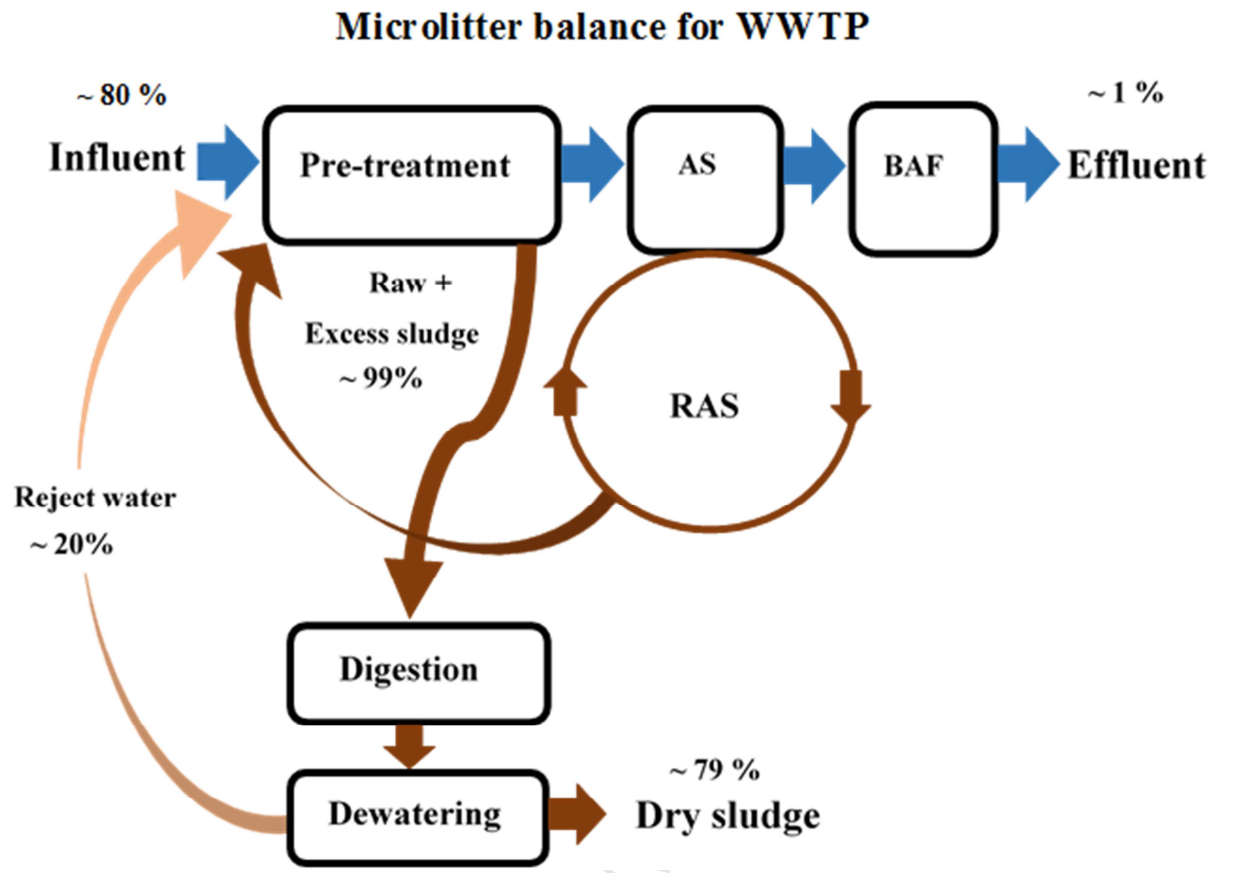


How well is microlitter purified from wastewater? – A detailed study on the stepwise removal of microlitter in a tertiary level wastewater treatment plant

Julia Talvitie [a], Anna Mikola [a], Outi Setälä [b] Mari Heinonen [c] and Arto Koistinen [d]

[a] Department of Built Environment, Aalto University, PO Box 15200, FI-00076, AALTO, Finland; [b] Finnish Environment Institute, Marine Research Center, PO Box 140, FI-00251 Helsinki, Finland; [c] Helsinki Region Environmental Services Authority HSY, PO Box 100, FI-00066 HSY, Finland; [d] University of Eastern Finland Sib Labs, PO Box 1627, FI-70211, Kuopio, Finland

Abstract

Wastewater treatment plants (WWTPs) can offer a solution to reduce the point source input of microlitter and microplastics into the environment. To evaluate the contributing processes for microlitter removal, the removal of microlitter from wastewater during different treatment steps of mechanical, chemical and biological treatment (activated sludge) and biologically active filter (BAF) in a large (population equivalent 800 000) advanced WWTP was examined. Most of the microlitter was removed already during the pre-treatment and activated sludge treatment further decreased the microlitter concentration. The overall retention capacity of studied WWTP was over 99% and was achieved after secondary treatment. However, despite of the high removal performance, even an advanced WWTP may constitute a considerable source of microlitter and microplastics into the aquatic environment given the large volumes of effluent discharged constantly. The microlitter content of excess sludge, dried sludge and reject water were also examined. According to the balance analyses, approximately 20% of the microlitter removed from the process is recycled back with the reject water, whereas 80% of the microlitter is contained in the dried sludge. The study also looked at easy microlitter sampling protocol with automated composite samplers for possible future monitoring purposes.

Key words: WWTP, microlitter, microplastics, wastewater, sludge, reject water, microlitter budget

1. Introduction

Litter has become a serious problem in aquatic environments worldwide. Litter includes both organic and inorganic materials like glass, metals, rubber, wood, paper, textiles and, for the most, plastics (OSPAR 2014). Microlitter comprises litter particles smaller than 5 millimetres. Microlitter, and particularly its plastic subtype, microplastics, has received considerable attention over the past decade (Thompson *et al*. 2004, Barnes *et al*. 2009, Ladewig *et al*. 2015). Microplastics are of concern because of their durability and potential to be transferred within food webs (Cole *et al*. 2013, Setälä *et al*. 2014). Microplastics may cause mechanical stress when ingested, but also expose marine organisms to various hazardous substances, such as plasticizers (Fries *et al*. 2013), toxic metals (Rochman *et al*. 2014) and persistent organic pollutants (POPs) (Rios *et al*. 2010, Chua *et al*. 2014). These micropollutants are either added to the plastics during production or adsorbed from the surrounding water (Teuten *et al*. 2009). In aquatic environments, microplastics can also function as artificial "microbial reefs" and transport non-indigenous and possibly harmful species (Zettler *et al*. 2013). In addition to microplastics, also non-synthetic textile fibers has been proposed to have potential to transport chemical pollutants throughout the aquatic environment (Ladewig *et al*. 2015).

Microlitter consists of primary and secondary particles. Primary particles are intentionally microscopic in, e.g microbeads in peeling lotions and textile fibers, while secondary microlitter is fragmented from larger particles (Barnes *et al*. 2009). Both aquatic and land-based sources have been identified contribute to the amount of litter in marine environments (Law *et al*. 2010). Land base sources include public littering, poorly managed landfills, riverine transport, stormwater and untreated municipal sewage.

Recently, wastewater treatment plants (WWTPs) have been suggested to act as one of the land base sources or entrance routes for microlitter to the aquatic environment (Magnusson & Norén 2014, Talvitie *et al*. 2015, Murphy *et al*. 2016). First studies have shown that microlitter can be efficiently (> 98 %) removed from the wastewater during the wastewater treatment (Magnusson & Norén

2014, Carr *et al*. 2016, Murphy *et al*. 2016). However, treated effluents still contain microlitter particles like plastic microbeads from toothpaste and textile fibers (Browne *et al*. 2011, Talvitie *et al*. 2015, Carr *et al*. 2016).

As vast volumes of effluent waters are discharged continuously into aquatic environments globally and the amounts are expected to grow due the population growth and urbanization (UN Water 2015), the role of WWTPs as an entrance route of microlitter to aquatic environments may be significant. At the same time, WWTPs can offer solutions to reduce the input of microlitter into the environment. Despite of this potential, very little attention has yet been drawn to the actual removal of microlitter during different type of wastewater treatment processes. Here we report detailed data on the removal of microlitter during different treatment steps in a large (population equivalent 800 000) advanced WWTP. The balance of microlitter in WWTP were estimated to further evaluate the removal and distribution of microlitter during the treatment processes. Also, the effect of microlitter size and shape on their removal in different treatment steps were determined. The further objective of this study was to establish an easy-to-use protocol for monitoring of WWTPs. In the end, we report the evaluation of microlitter and microplastic load discharged into the marine environment with effluents.

2. Materials and Methods

2.1 Description of the selected WWTP

Selected WWTP (Viikinmäki, Helsinki Region Environmental Services Authority, HSY) is the largest wastewater treatment plant in Finland and the Nordic Countries, treating the wastewaters of ca. 800,000 inhabitants in the Helsinki metropolitan area. An average of 270 000 cubic meters of treated wastewaters are discharged from the WWTP into a Gulf of Finland, Baltic Sea every day. The treatment process in Viikinmäki WWTP is based on activated sludge method and has multiple treatment steps based on pre-, chemical- and biological treatment. The nitrogen removal has been

enhanced with a tertiary denitrifying biological filter. In 2015, 95% of organic material (BOD_7), 98% of suspended solids (SS), 95% of total phosphorus (P-tot) and 90% of total nitrogen (N-tot) were removed during the treatment process of the selected WWTP.

Pre-treatment includes coarse screening (10 mm), grit removal, chemical treatment and primary sedimentation. In order to remove phosphorus, ferrous sulphate is dosed in the sand removal prior to secondary clarifier. In biological treatment biodegradable matter and nitrogen are removed from the wastewater with activated sludge method. Activated sludge process includes aeration tanks and secondary clarifiers. Hydraulic retention time in the process is approximately 25 hours and sludge retention time varies between 6 – 12 days. Most of the activated sludge is recycled from secondary clarifiers into the aeration tanks as return activated sludge but part of it is also continuously removed from the process. This excess sludge is returned to primary sedimentation and sent to sludge treatment together with raw sludge. The nitrogen removal is further improved in tertiary treatment process with biologically active filter (BAF). During the BAF process, wastewater flows through tightly packed polystyrene beads. The beads provide a surface for micro-organisms to attach and grow. While growing, they consume organic material as well as phosphorus and convert nitrates to nitrogen gas.

Viikinmäki WWTP has also a solids handling treatment. Organic matter in the sludge is anaerobically digested to produce biogas, i.e. methane and consecutively used for the plant's own energy consumption. After the digestion, sludge is dewatered with centrifuges. For dewatering, the sludge is conditioned with flocculation chemical polyacrylamide (PAM). PAM induces a release of the water during dewatering by enhancing the aggregation of sludge particles into larger particle groups called flocs. Dewatering generates reject water, which is conducted via a settling tank into the beginning of the wastewater treatment process. The dried sludge is processed further in composting fields and used in green construction. The plant produces annually around 60,000 tonnes of dried sludge which has a dry solids (TS) content of 29 %.

2.2 Sampling methods

The samples were collected from the plant influent, after pre-treatment, after the activated sludge (AS) process, plant effluent, excess sludge, reject water and dried sludge (SD fig. S1). Sampling was carried out during a seven-day period 14.9 – 20.9.2015 with three different sampling methods; grab sampling (here meaning one sampling occasion at a certain time), 24-hour composite sampling and 24- hour sequential sampling (Table 1.).

Table 1. Sampling methods (grab sampling, 24h composite sampling, 24h sequential sampling), sampling locations (wastewater, sludge) and sampling days.

	Monday 14.9.2015	**Tuesday 15.9.2015**	**Wednesday 16.9.2015**	**Thursday 17.9.2015**	**Friday 18.9.2015**	**Saturday 19.9.2015**	**Sunday 20.9.2015**
Grab sampling (wastewater)	**X**		**X**			**X**	
Grab sampling (sludge)			**X**				
Grab sampling (reject water)			**X**				
24h composite sampling (wastewater)		**X**		**X**			**X**
24h sequential sampling (wastewater)				**X**			

2.2.1 Grab sampling of the wastewater and sludge

Grab samples from wastewater were collected from each sampling site in water process. Three replicates (n = 3) were taken from each sample types consecutively. Sampling was done by pumping water from the wastewater stream was (at depth ~ 1 m) onto the designated filter with an electric pump (Biltema art.17-953). Filtering set up previously designed for microplastic sampling in wastewaters was used (Talvitie *et al*. 2015). The respective filter mesh sizes were 300, 100 and 20 µm, giving size fractions of > 300 µm, 100 – 300 µm and 20 – 100 µm (SD fig S2). The volume of each sample (Table 2.) was measured with a flow meter (Gardena Water Smart Flow Meter) attached to the pump. This volume of filtered water depended on the water quality and filter size. The volumes of replicate samples differed, since the water quality varied.

This sampling method is not applicable for influent water due to its high amount of organic material which rapidly clogs the filters allowing only small water volumes to pass. For the influent sampling a metallic beaker to collect water from the wastewater stream surface was used and the samples were later filtered in laboratory with the same filter set up.

Samples from the excess sludge and reject water were collected with the same method as influent samples, while dried sludge was collected by hands from the conveyor belt after dewatering process. All sludge and reject water samples were placed into pre-cleaned plastic containers and transported into the laboratory for filtering. The sludge and reject water samples were diluted before filtering by mixing subsamples of wet sludge (1 gram), reject water (10 gram) and dry sludge (0,2 gram) with 1 liter of tap water. Diluted sludge and reject water samples were then filtered with the filtering device as the wastewater samples. Details of different samples are presented in table 2. To prevent contamination during the sampling, all equipment was rinsed carefully with tap water prior to sampling.

135 Table 2. The sample volumes/weights with sampling locations and mesh sizes of the filters

Sampling sites	sample volume/weight 300 µm filter	sample volume/weight 100 µm filter	sample volume/weight 20 µm filter
Influent	0.1 l	0.1 l	0.1 l
After pre-treatment	50 – 333 l	7 – 10 l	0.5 l
after AS	200 – 333 l	20 – 30 l	1 l
Effluent	1000 l	100 l	2 l
Excess sludge	1.2 ml = 1 g	1.2 ml = 1 g	1.2 ml = 1 g
Dry sludge	0.2 g	0.2 g	0.2 g
Reject water	10 ml = 10 g	10 ml = 10 g	10 ml = 10 g

136

137 **2.2.2 Composite sampling**

138 24-hour composite samples were collected from all sites in water process (Table 3.). Composite
139 samplers (ISCO 3700) in each sampling location took flow proportional, discrete samples at regular
140 15 min. intervals over 24-hour period of time. The samples were collected into plastic containers
141 placed in refrigerators. Both, the containers and refrigerators were carefully cleaned prior to
142 sampling to avoid contamination from room dust. From the containers, samples were poured into
143 the filter device using a measuring glass. Sampling was performed three times during the week;
144 Tuesday, Thursday and Sunday (Table 1.). To assess possible contamination, blank samples of tap
145 water were used as controls following the same methodological procedure as the actual samples.

146

147

148

149 Table 3. The 24-hour composite sample volumes with sampling locations and mesh sizes of the
150 filter.

Sampling site	sample volume with 300 µm filter	sample volume with 100 µm filter	sample volume with 20 µm filter
Influent	0.1 l	0.1 l	0.1 l
After pre-treatment	13.2 – 14.5 l	8.5 – 10.5 l	0.5 l
After AS	11 – 14.5 l	11 – 14.5 l	1 l
Effluent	10.5 – 13.5 l	10.5 – 13.5 l	2 l
Control	14.5 l	14.5 l	2 l

151

152 **2.2.3 Sequential sampling**

153 Influent and effluent sampling were performed simultaneously with automated samplers (ISCO
154 3700). The samplers took 24 samples at 1-hour interval. After sampling, 3 samples (3 hours) were
155 pooled together, resulting 8 samples per sampling. To avoid contamination, the bottles and the
156 samplers were cleaned carefully before the sampling. The sampler device formed a closed system
157 for entire sampling period. After the sampling, bottles were immediately closed until filtering.
158 Control samples prepared of tap water were preserved in pre-cleaned bottles in the sampler for 24-
159 hours and treated the same way as the actual sequential samples.

160 **2.3 Analyses of microlitter**

161 **2.3.1 Analyses with stereo microscope**

162 All the filtered samples were stored in clean Petri dishes. Samples were visually examined using a
163 stereomicroscope (Fiberoptic-Heim LQ 1100, magnification ×50), light projected from above to get
164 good image of surface structure of microlitter particles. The particles were counted, divided into

fibers, fragments, flakes, films and spheres and their coloration documented. The morphological properties of the particles were inspected with micro tweezers to exclude soft, easily disintegrating organic materials. Organic litter like food scraps and paper (cellulose), were excluded from the examination. To detect possible contamination during transport and microscopic analyses, three control samples were carried along during the sampling in the WWTP.

2.3.2 Material identification of microlitter

The chemical composition of microlitter particles from effluent samples were analysed with imaging Fourier Transform infrared spectrometer (FTIRi) (Spectrum Spotlight 300, PerkinElmer, Waltham, MA, USA). All together three effluent samples, one replica from each day (Monday, Wednesday and Saturday) were chosen for analyses and all together 752 particles were included into the analyses. Particles were hand-sorted from the filters with fine tip tweezers under the stereomicroscope and carefully rinsed with distilled water to remove organic matter that was attached to them. Rinsed particles were placed onto ZnSe windows designed for FTIRi microscopy and let to dry for few hours. Each sample window was photographed with the FTIRi and particles identified from the picture. The particles were exposed one by one to infrared radiation in wave length region of 700 - 4000 cm^{-1} using single point transmission mode. To analyse the spectra obtained with FTIRi, the Thermo Scientific™ Hummel Polymer and Additives FT-IR Spectral Library was used. The materials of textile fibers were analyzed with textile fiber library made ourselves from pure model fibers (purchased from Lahti University of Applied Sciences, Faculty of Technology) (SD fig. S3 – S10). The model fibers included cotton, wool, linen, silk, viscose, polyester, polyacryl, polyamide, and polypropene fibers.

188 ### 2.3.3 The data analyses

189 The removal of microlitter during different treatment steps were statistically analyzed using analysis
190 of variance (ANOVA).

191 For the microlitter balance analyses, the average microlitter flow was calculated by multiplying the
192 average microlitter concentrations with the corresponding average flow along each wastewater
193 treatment step. The equation can be expressed as:

194 $ML = Q\ C$

195 ML (microlitter d^{-1}) is the flux of microlitter and Q (L d^{-1}) is wastewater, reject water or sludge
196 flow. C (ML L^{-1}) is the average microlitter concentration measured in the wastewater, reject water
197 or sludge.

198 The retention capacity of microlitter in WWTP was calculated as:

199 ([Influent] – [Effluent] / [Influent]) x 100

200

201 ## 3. Results and Discussion

202 ### 3.1 Grab sampling of wastewater and sludge

203 Most of the microlitter particles (97.4% - 98.4%) were removed already during mechanical and
204 chemical pre-treatment phase (table 4). During pre-treatment, microlitter concentrations decreased
205 significantly in all three sampling occasions (Monday: $F = 190.668 > 7.709$; $P < 0.05$, Wednesday:
206 $F = 14.246 > 7.709$; $P < 0.05$ and Saturday: $F = 37.689 > 7.709$; $P < 0.05$). This finding is consistent
207 with previous studies examining the removal of microlitter during wastewater treatment processes
208 (Talvitie *et al.* 2015, Carr *et al.* 2016, Murphy *et al.* 2016). Pre-treatment in Viikinmäki WWTP
209 includes screening, grit removal and chemically enhanced primary sedimentation. Purpose of the
210 treatment is to remove grit, particulate materials as well as phosphates and colloidal matter from the

211 wastewater. According to our results, these techniques remove efficiently also microlitter. In
212 influent, majority of microlitter particles are probably attached to grit and larger particles and
213 removed from the wastewater along with them.

214 Concentration continued to decrease significantly also during AS process, except on Wednesday
215 when the variation between the replicates were so high that although microlitter was clearly
216 removed to a lower level, the result was insignificant with P-value 0.05 (Monday: $F = 7.966 >$
217 7.709; $P < 0.05$, Wednesday: $F = 7.472 > 7.709$; $P < 0.05$, Saturday: $F = 42.097 > 7.709$; $P < 0.05$).
218 Together AS process further decreased the microlitter concentration in range [7% - 20%] (table 4).
219 During the treatment, microlitter particles are mixed with flocs and settled into the sludge during
220 secondary sedimentation. Small part of the microlitter, however, escapes the treatment.

221 According to our results, the tertiary treatment, biologically active filter (BAF), didn't decrease
222 microlitter concentration (table 4). The main purpose of BAF in Viikinmäki WWTP is to remove
223 nitrogen from the wastewater but according to the preliminary study (Talvitie *et al*. 2015) the
224 filtering effect was expected to remove a proportion of the microlitter particles left in the secondary
225 effluent. After the BAF, the discharged effluent contained 0.7 – 3.5 microlitter particle per liter of
226 wastewater. This result differs from the study of Carr *et al.* (2016), where no microlitter after the
227 tertiary treatment was detected. Differences in the sampling methods, especially volumes make this
228 comparison difficult, but both works clearly underline the efficiency of the first steps of the
229 processes in the purification efficiency. The two WTTPs also differ in the techniques used for
230 tertiary treatments, which may have caused differences in the removal result. Where BAF appears
231 to be inefficient to remove microlitter, other techniques, like gravity filters, may have a better
232 removal capacity.

233

234

Table 4. The average microlitter concentration, including all the fractions (> 300 μm, 100 – 300 μm and 20 – 100 μm), in influent, after pre-treatment, after AS and in effluent. Data is given in number of microlitter particles per L^{-1} of wastewater. Figures represent mean values ±SE, n =3.

Sampling site	Monday	Wednesday	Saturday
Influent	636.7 (±38.8)	686.7 (±155.0)	380 (±52.2)
After pre-treatment	14.2 (±4.0)	10.9 (±2.9)	9.9 (±1.0)
After AS	1.0 (±0.6)	1.3 (±0.9)	2.0 (±0.2)
Effluent	3.2 (±0.7)	0.7 (±0.6)	3.5 (±1.3)

To evaluate the microlitter balance of the WWTP, excess sludge, dry sludge and reject water were also analyzed for one sampling day. The results are collected to table 5. The average wastewater flow during the sampling day (Wednesday) was 2.82×10^8 L d^{-1}.

Table 5. The average microlitter concentrations and flow rates in excess + raw sludge mixture, dry sludge and reject water. Data is given in number of microliter particles per g^{-1} or L^{-1} of sludge. Concentrations are mean values ±SE, n=3 and flows day average.

Sampling point	Recess + raw sludge	Dry sludge	Reject water
Microlitter per gram	76.3 (±4.3)	186.7 (±26.0)	12.9 (±0.3)
Microlitter per liter	63611.1 (±3543.7)	NA	12866.7 (±275.4)
Average flow (L d^{-1})	2.92×10^6	1.84×10^5	2.74×10^6

A microlitter balance over the treatment process is presented in Fig. 3. The overall balance and the balance of the pre-treatment are summing up to an error of approximately 20% of the influent load. This indicates that the microlitter sampling and analysing have been successful in different parts of the water and solid flows. When not removed from the WWTP with effluent or dried sludge,

microlitter particles are recycled inside the WWTP with the activated sludge and reject water. During the digestion organic matter is degraded, but non-biodegradable or slowly degradable particles like plastics are not affected by this sludge handling. In dewatering of raw and excess sludge, the reject water is separated and conducted into the beginning of the wastewater treatment process. Balance analysis indicates that reject water acts as considerable inner source of microlitter particles back into the process (fig 1.). Approximately 20% of the microlitter removed from the process is recycled back in the reject water whereas 80% of the microlitter is contained in the dried sludge.

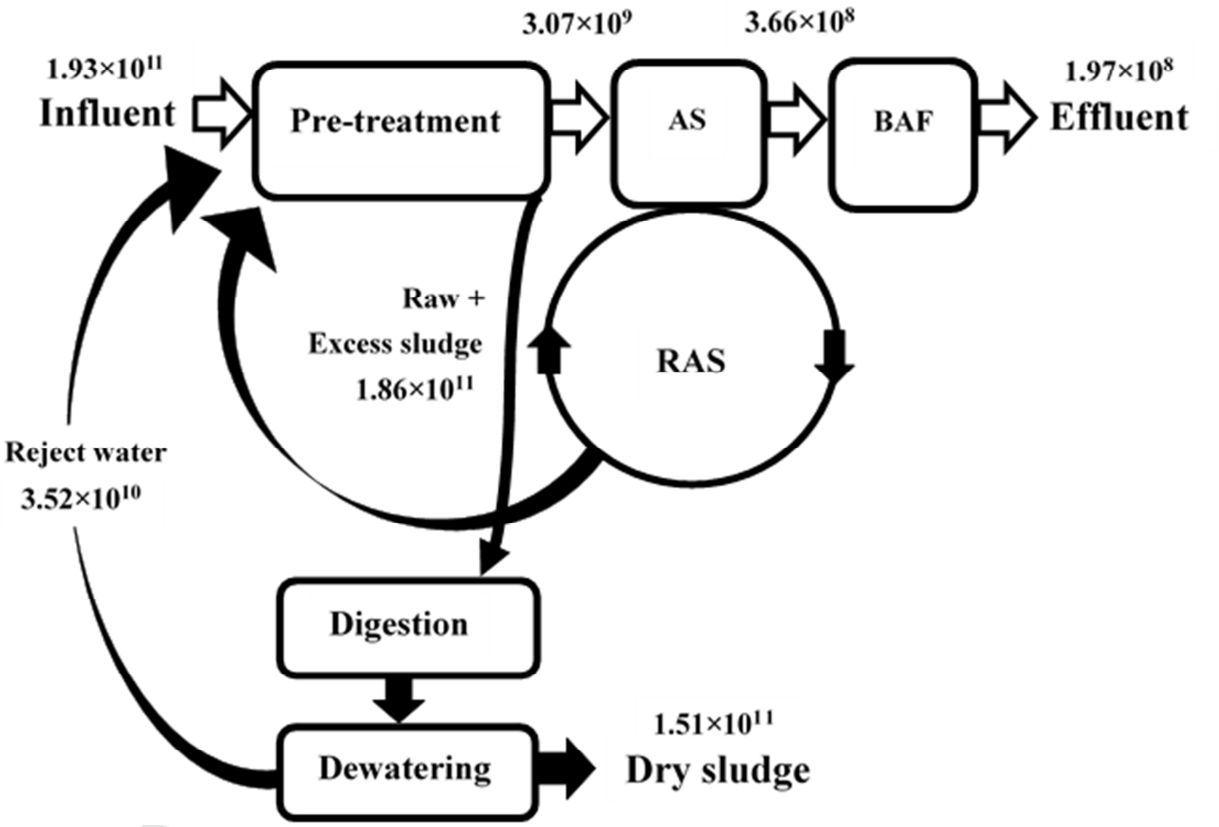


Fig 1. Microlitter balance and distribution in the WWTP. White arrows represents wastewater and black-coloured represents sludge. Figures are microlitter flow per day (ML/d).

The dried sludge is transported to composting fields. After composting, the product is used in green construction. As Viikinmäki WWTP produces annually around 60,000 tonnes of dried sludge,

enormous amounts of microlitter are ending up to the environment with sludge.

3.2 24-hour composite samples

24-hour composite sampling was performed to study the average concentration and removal of microlitter during the wastewater treatment over one day period. Sampling was performed three times during the sampling week; Monday to Tuesday, Wednesday to Thursday and Saturday to Sunday (Table 6.). The method was also evaluated for possible microlitter monitoring purposes.

Table 6. 24-hour composite samples. The microlitter concentrations in influent, after pre-treatment, after AS and in effluent. Data is given in number of microlitter particles per L^{-1} of wastewater.

Sampling point	Tuesday	Thursday	Sunday
Influent	900.0	390.0	630.0
After pre-treatment	23.8	10.1	4.1
After AS	2.8	3.1	1.5
Effluent	2.8	1.4	1.4
Blank	0.4	0.8	0,6

The trend of microlitter removal during different treatment steps determined with 24 – hour composite sampling was very well in line with grab sampling results. Also the microlitter content per liter was in the same range. Surprisingly the variation between sample days especially in influent wastewater was larger with 24h composite samples (390.0 – 900.0 particles per L^{-1}) than with grab samples (380±52.2 – 686.7±155.0).

Automated samplers provide an easy microlitter sampling protocol. This can be utilized in monitoring. However, if the microlitter concentration is very low, composite samples might give false zero results, as the sample volumes are so small (in our study, only 10-20 liters). In this case,

grab sampling with larger sample volumes might be required. Another clear disadvantage of composite sampling is the microlitter contamination. Microlitter type that easily contaminates the samples is textile fiber. As it is also one of the most common microlitter types in wastewater (Habib *et al*. 1996, Zubris *et al*. 2005, Talvitie *et al*. 2015), the contamination distorts the results and easily leads to overestimation of microlitter and microplastics in wastewater. During this study, a great care was taken to minimize contamination during the composite sampling. However, the efforts, contamination of microlitter particles was continuously detected, being between 0.4 – 0.8 microlitter per L^{-1} of water, which accounts for over 30% of the estimated microlitter content in the effluent water. No contamination was detected due to the transportation and analyses.

24-hour sequential sampling

In-day variation in the microlitter concentration was detected in both influent and effluent samples (Fig 2). The night time (10 pm – 7 am) concentrations were generally lower, average concentrations in influent 476.7 and effluent 0.8 microlitter L^{-1}, compared to day time (7 am – 10 pm) concentrations of 584 in influent and 1.7 microlitter per L^{-1} in effluent. No contamination was detected from blank sequential samples. The lowest microlitter concentration during day time was detected during 1 – 4 pm and microlitter concentration increases towards the early evening hours. This might have had an impact on our results from grab sampling, as the sampling was performed always in day time during low concentration hours.

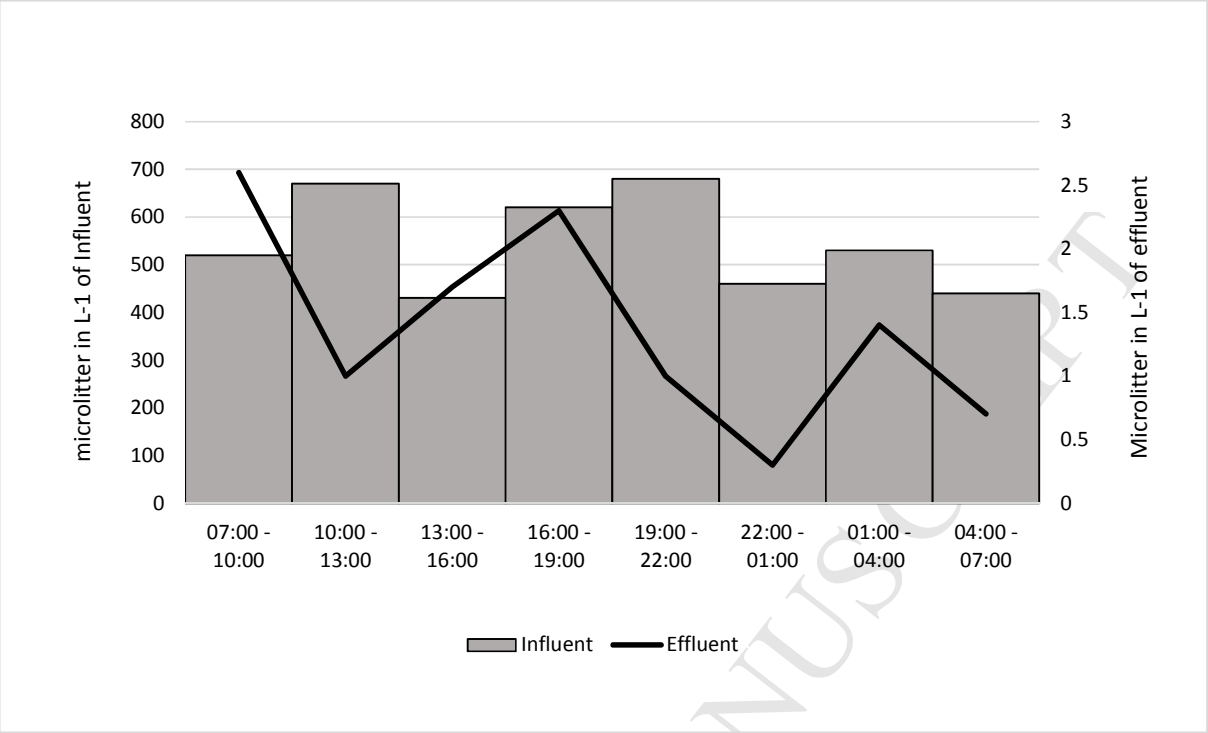


Fig 2. 24-hour sequential samples. Microlitter concentrations in influent and effluent. Data is given in number of microlitter particles per L^{-1} of wastewater. One sample consist of three-hour period pooled together.

The variation trend in microlitter concentration is in line with day-time activities in households which influence the amount of microlitter coming to the WWTPs. The in-day variation in microlitter concentration has to be taken into account when evaluating the total amounts of microlitter entering and leaving the WWTP with discharged effluents. These results supports the use of composite sampling or e.g long-term large-scale sampling described by Carr *et al.* (2016) when evaluating the role of WWTPs as source of microlitter pollution.

3.3 Analyses of microlitter

3.3.1 Analyses with stereomicroscope

The proportion of the different microlitter size fractions differed between the wastewater treatment steps (Fig. 3). Pre-treatment had the greatest impact on microlitter size distribution, efficiently removing larger size fractions (≥ 300 and 100 – 300 µm), and particles in the smallest fraction (20-100µm) became most abundant. In effluent samples, the slight increase of particles of 100-300 µm in size was caused by the emergence of clear crystal fragments in this fraction. Origin of these fragments remained unknown.

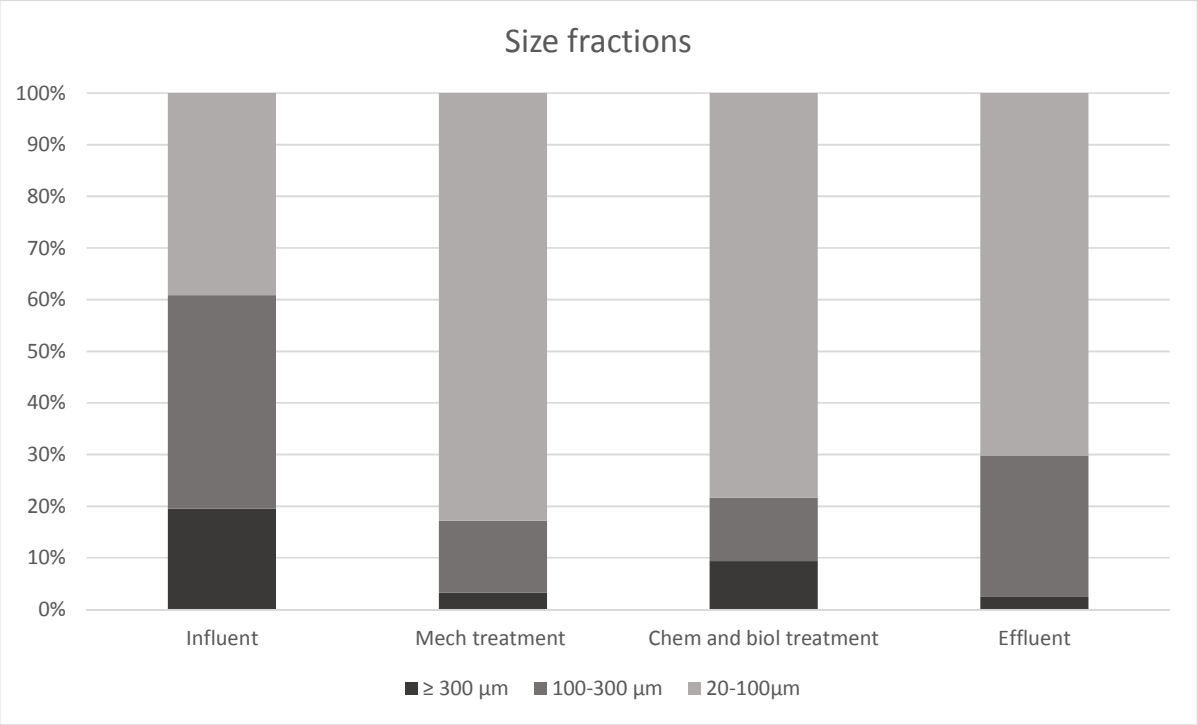


Figure 3. The proportion of the different size classes of microlitter during the wastewater treatment steps. The distribution is based on number of particles.

Also the proportion of particle types/shapes changed during the purification process (Fig. 4). In the influent the fibers made up around 70 % of the total microlitter particles, and in effluent only 30 %. Most of the fibers were removed already during pre-treatment. In influent, textile fibers probably

attach easily to grit and to other larger organic waste material and settle down to sludge efficiently during primary sedimentation. The proportion of fragments increased during the treatment. Majority of the fragments detected in effluent consisted of clear fragments. Relative proportion of flakes was approximately the same in all treatment phases.

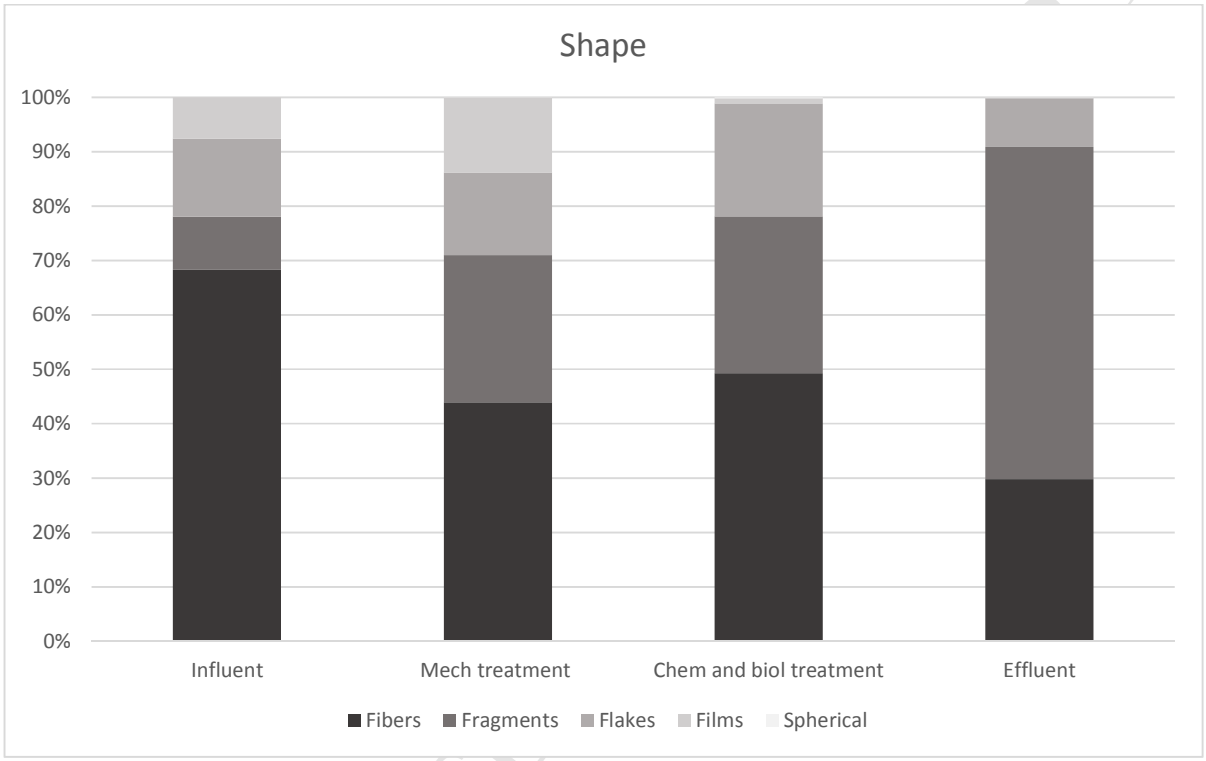


Figure 4. The proportion of microlitter shape categories during the wastewater treatment phases. The distribution is based on number of particles.

3.3.2 Material identification

All together 752 microlitter particle were included in the material identification. However, the overall success rate for material identification was relatively low, only 18 % (table 8). Especially clear fragments remained unrecognized. Reasons for overall weak success rate were unrecognizable particles (e.g. inorganic minerals and metals), limits of the FTIR spectroscopy and weak quality infrared spectra. The cause for weak quality spectra can be e.g the biofilm contaminating the particles. The biofilm in our wastewater samples generally gave spectrum peaks at wavenumber of

1000 – 2500 cm^{-1}. In those wavenumbers the biofilm was blocking the possible peaks from the actual material of the particle and hindered the analyses. Scanning electron microscopy with energy dispersive spectrometer (SEM-EDS) could probably increase the percentages for successful analyses by e.g. confirming inorganic composition of the particles. Some chemical treatments have been tested for removal of biological matter from samples when analysing the microplastics from wastewater (Tagg *et al*. 2015). However, if natural materials e.g cotton textile fibers are included into the examination, the chemical treatments can harm the materials and complicate the analyses.

Table 8. Microlitter types from effluent samples included into the material analyses, the success rate of the analyses and detected materials (PE=polyethylene, PS=polystyrene, PP= polypropylene PES=polyester, NA = not available).

Particle types	Included	Successfully analyzed	%	Materials
Fibers	108	108	100	See fig.7
Fragments				
Blue	26	3	12	PE x 2, PS
Black	18	3	17	PE x 2, PP
Red	4	1	25	PP
Yellow	3	3	100	PES
Green	6	0	0	NA
Brown	1	0	0	NA
White	4	1	25	PP
Clear	507	14	2,8	PE (minerals?)
Silver	2	0	0	NA (metals?)
All	571	25	4	
Flakes				

Blue	3	0	0	NA
Black	4	0	0	NA
Red	2	0	0	NA
Clear	1	0	0	NA
Silver	55	0	0	NA (metals?)
All	65	0	0	
Films				
Black	1	0	0	NA
Yellow	1	0	0	NA
Clear	3	1	33	PP
All	5	1	20	
Spherical				
Black	1	0	0	NA
Yellow	1	0	0	NA
White	1	0	0	NA
All	3	0	0	

All of the 108 fibers from effluent samples were successfully analysed with FTIRi. Textile fiber is easily identifiable litter type and this helps further material analyses. However, majority of the natural textile fibers gave relatively weak spectrums and it was hard to found a match for them in commercial polymer libraries. To solve this problem, the spectrums were analysed with textile fiber library made from model fibers. With the help of the library, materials of all textile fibers were ascertained.

The top two textile fibers were cotton (44%) and polyester (33%) (Fig 5.). In overall, 66% of the all textile fibers were natural fibers of cotton, linen or wool. Natural textile fibers have been more or

less excluded from the scientific microlitter studies and the focus has been more on synthetic fibers. This is probably due the general perception that because natural fibers degrade faster and are "organic" by nature, they do not pose harm to the environment. Opposite opinions state that natural fibers may actually act as carriers to harmful substances because textiles from organic fibres have often been treated with harmful chemicals, such as flame retardants, Also, like microplastics, natural fibers may also absorb pollutants from surrounding water and faster degradation compared to synthetic ones can lead to quicker release of chemicals into the surrounding environment (Ladewig *et al*. 2015). As the textile fiber, from washing of clothes (Browne *et al*. 2011), is one of the most common litter types in wastewater and both the natural and synthetic fibers can have negative impacts in environment, we recommend that when evaluating the role of WWTPs as point source of microlitter, natural textile fibers would be included into the examination.

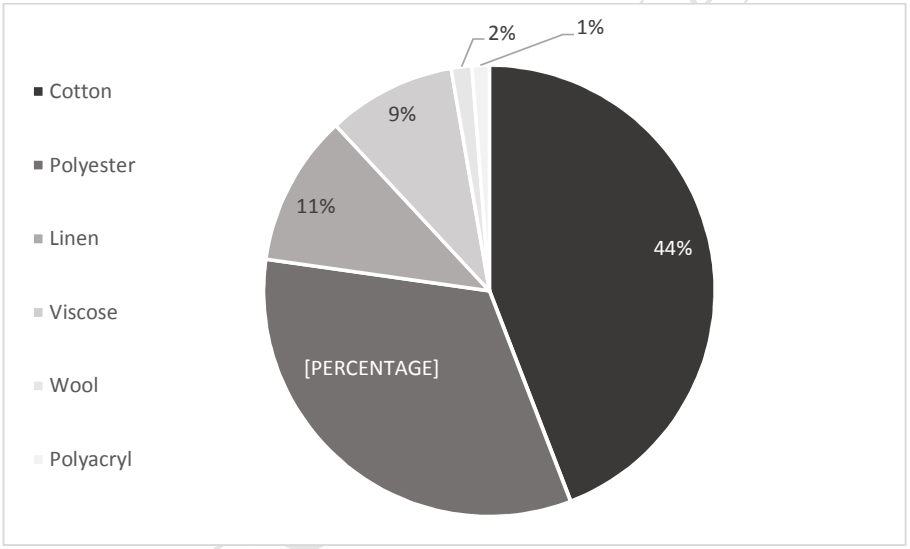
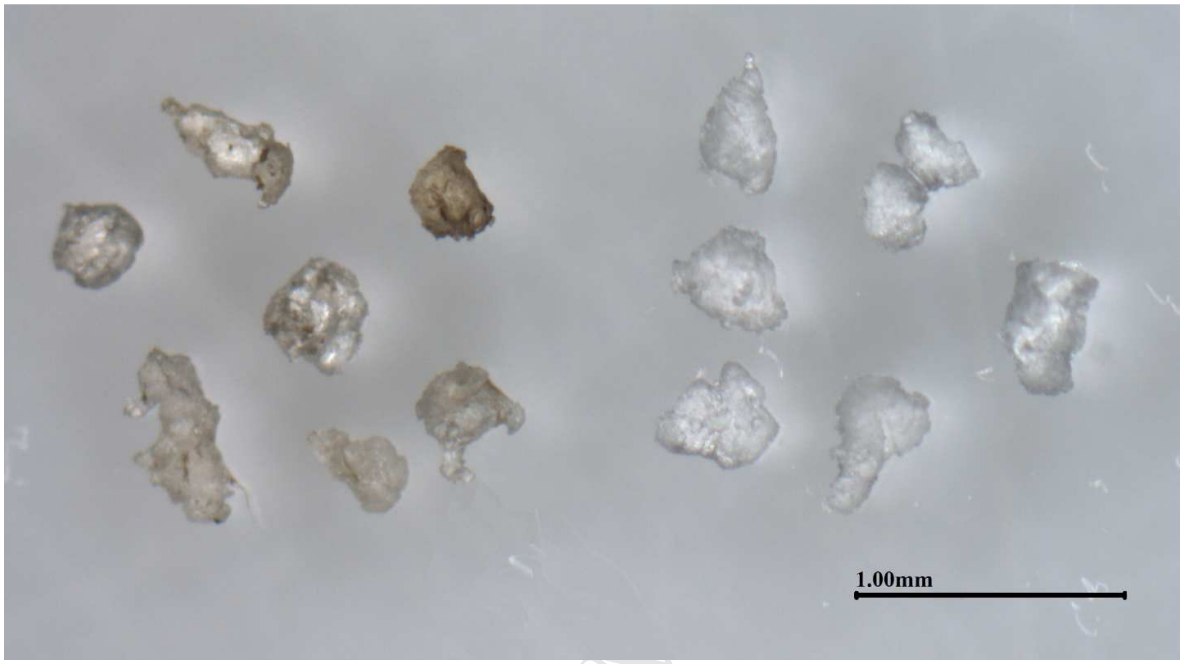


Figure 5. Materials of fibers and their percentage in effluent samples.

Microlitter type observed frequently in our effluent samples was clear polyethylene fragment, the type found in some widely used cleansing scrubs (fig 6, SD fig S11). When a particle has a very distinct and recognizable profile (size, shape and color), it is possible to find its origin. For example

374 Carr *et al.* (2016) discovered that the most common particle found in their effluent samples, blue
375 polyethylene fragments, resembled the ones found in toothpaste.
376



377

378 Figure 6. On the left: polyethylene fragments, covered with brownish biofilm, from effluent
379 samples. On the right: polyethylene fragments from cleansing scrubs.

380 However, clear PE fragments consisted only a small part from all clear particles found from our
381 effluent samples (Table 8). Most of the clear particles were hard crystals which remained
382 uncharacterized. Wastewater contains a lot of microlitter and microplastic particles that are
383 unrecognizable and it is difficult to estimate their possible material before material analyses.
384 Particles are either secondary microparticles deriving from fragmentation of larger litter items or
385 primary microparticles from unknown source. Analyzing these particles is particularly challenging
386 when material is not suitable for FTIR spectroscopy and hence the spectra gained from the analyses
387 are unclear.
388
389

3.4.3 The role of WWTPs as an entrance route for microlitter

The amount of microlitter entering the marine environment with the effluent varied between 2.0×10^8 to 7.9×10^8 particles per day and the ascertained average MP load varied between 1.7×10^6 to 1.4×10^8 particles per day (Table 9).

Table 9. Flow rates (m^3/d), microlitter concentrations (MLs/m^3) and microlitter load (ML/d), microplastic concentrations (MPs/m^3) and microplastic load (MPs/h) from WWTP with effluent.

Date	Monday 14.9	Wednesday 16.9	Saturday 19.9
Flow rate (m^3/d)	219024	281750	224294
ML concentration (ML/m^3)	3200	700	3500
ML outflow (ML/d)	~ 7.0×10^8	~ 2.0×10^8	~ 7.9×10^8
MP concentration (MP/m^3)	651	6	161
MP outflow (MP/d)	~ 1.4×10^8	~ 1.7×10^6	~ 2.0×10^7

In their study Murphy *et al*. 2016 estimated the daily discharge of 6.5×10^7 MPs in large secondary WWTP (PE 650 000, treating 260 954 m^3 of wastewater per day) and Carr *et al*. (2016) calculated a daily discharge of 0.93×10^6 MPs also in large (treating 1.06 million m^3 of wastewater per day) secondary WWTP. Our results, together with these studies, indicate that secondary and even tertiary WWTPs may constitute a considerable source of microlitter and microplastics given the large volumes of effluent discharged to the aquatic environment constantly.

4. Conclusions

Our work provides a detailed information on the stepwise removal of microlitter in a tertiary level WWTP. Most of (97%) the microlitter in wastewater was removed during the pre-treatment and AS

treatment further decreased (7% - 20%) the microlitter concentration. Tertiary treatment BAF didn't have any significant impact on microlitter concentration. Pre-treatment had the greatest effect also on microlitter size distribution, efficiently removing larger size classes of ≥ 300 and 100 – 300 µm. Of the different types of microlitter, fibers and films were most efficiently removed from the wastewater. The microlitter balance confirmed the applicability of our sampling and analysing methods and showed that 0.1% of the microlitter entering the plant ended up in the effluent and the rest was removed with the sludge. 20% of the microlitter in the removed sludge returned to the process with reject water.

Automated composite samplers can provide relatively easy microlitter sampling protocol. With grab sampling, larger sample volumes and hence statistically more representative results are gained. The method is neither so sensitive to contamination. However, the grab sampling method requires much more time and effort compared to composite sampling with automated equipment. The grab sampling also ignores the in-day variations in microlitter concentration. If microlitter is examined over longer period or regularly e.g. for monitoring purposes composite sampling with automated samplers can offer a practical sampling tool.

According to our results 2.0×10^{8} to 7.9×10^{8} microlitter per day and 1.7×10^{6} to 1.4×10^{8} microplastics per day was discharged into the Baltic Sea with effluent. Given the large volumes of effluent discharged to the aquatic environment constantly, even tertiary level WWTPs may constitute a considerable source of microlitter and microplastics.

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Highlights

- The microlitter retention capacity in investigated WWTP was over 99%.
- The size and shape of microlitter particles has impact on their removal in WWTP.
- Microlitter balance analysis for the wastewater treatment process for performed.
- Microlitter sampling protocol for monitoring purposes was investigated.
- WWTPs can constitute entrance route for microlitter into the aquatic environment.