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Microplastic distributions in a domestic wastewater treatment plant: removal efficiency, seasonal variation and influence of sampling technique

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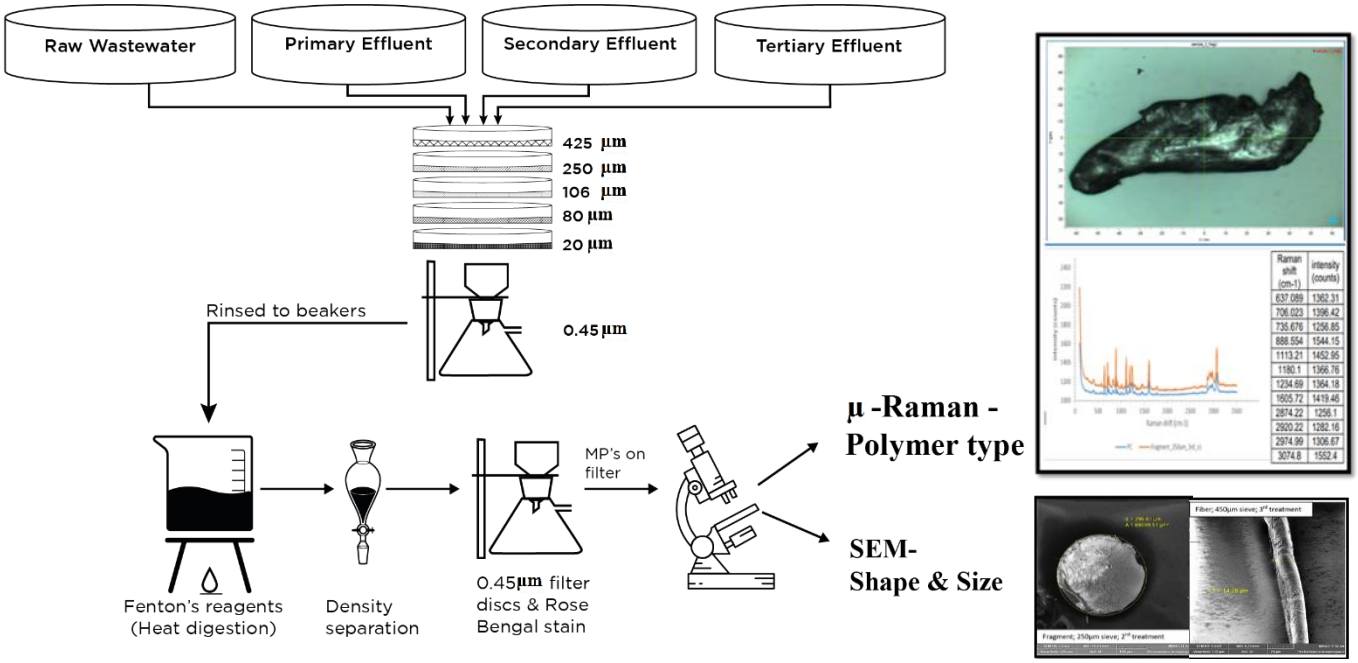
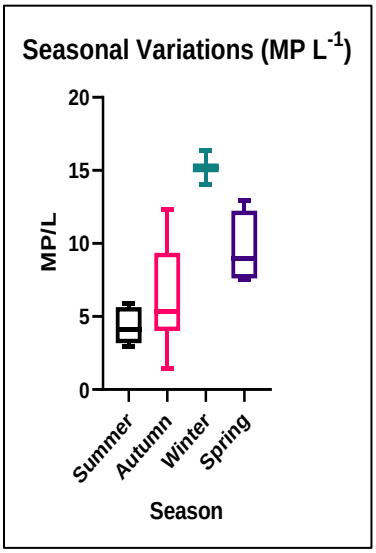
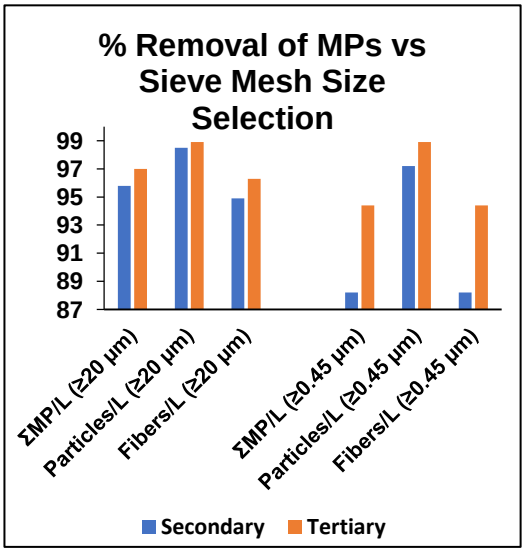
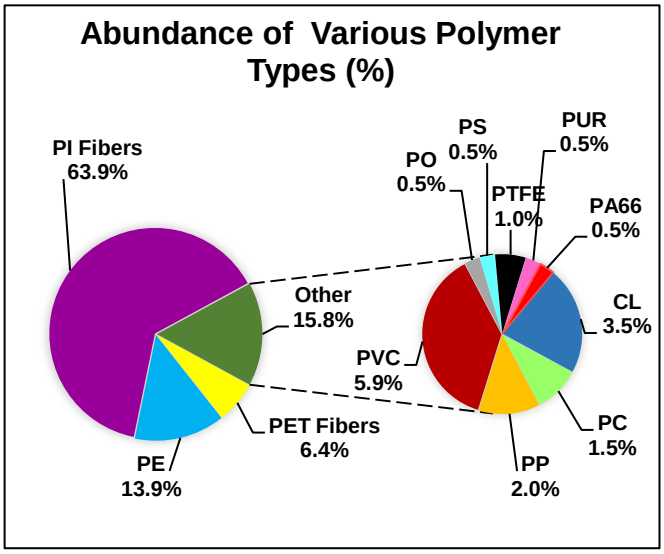
*Graphical Abstract









***Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)**

1. Effective microplastics sampling methodologies are needed for better control
2. MPs removal in WWTP was evaluated during a year campaign using different methods
3. MPs concentrations was higher in winter and spring compared with summer and autumn
4. Large or 24h composite samples contributed to minimize variability between samples

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Abstract

Wastewater treatment plants (WWTPs) serve as an important route of microplastics (MP) to the environment. Therefore, more effective MP sampling and detection methodologies, as well as a better understanding of their influence on MP occurrence and distributions in WWTP effluents, are needed for better removal and control. In this work, the efficiency of a municipal WWTP to remove MP was assessed by collecting samples from raw to tertiary effluent during a 12-month sampling campaign (season-based) using different sampling methods (containers, 24-h composite and large grab samples). MP retrieved from different treatment units within the WWTP were identified and quantified using plastic/non-plastic staining followed by optical microscopy, SEM and μ–Raman microscopy. Overall, the mean removal efficiency of MP in the WWTP was 97%, with most MP removed by the secondary stage and a mean effluent concentration of 1.97 MP L^{-1} after sand filtration. The relative abundance of particles was lower than fibers in treated effluent compared with the raw wastewater, with MP fibers constituting 74% of the total MP in raw wastewater and 91% in treated effluent. Taking seasonal variations into account is important as total MP in the effluent was notably higher in winter and spring compared with summer and autumn. Increasing the sampled volume using large samples or 24-h composite samples significantly reduced the variability between samples. However, total MP L^{-1} post tertiary stage was significantly lower using morning sampling (9 am) by large grab sampling method (1.2 MP L^{-1}) compared to 24-h composite sampling (3.2 MP L^{-1}). These differences may be attributed to intra-daily changes. Using a finer mesh size (0.45μm) to capture MP beyond the size range typically studied (≥20 μm) effectively doubled the number of MP detected in the tertiary effluent and highlights the importance of standardizing sampling procedures.

Keywords:

microplastic capture efficiency; composite sampling; grab sampling; wastewater effluent; seasonal variation; rapid sand filter (RSF)

1. Introduction

Microplastic (MP; <1 mm) can easily be transported by air and water over long distances resulting in ubiquitous environmental distribution and the potential to accumulate in diverse natural habitats; from urban beaches to pristine seafloor sediments to polar regions (Bergmann et al. 2017, Imhof et al. 2013, Thompson et al. 2009), as well as in biota (Mathalon and Hill 2014, Ribeiro et al. 2019). Mechanical effects from MP and nanoplastics (NP; <1 µm) may involve physical damage during ingestion, hindering mobility and clogging of the digestive tract, while chemical effects include inflammation, decreased predatory performance, endocrine disruption, hepatic stress, intestinal alterations, oxidative stress and decreased growth (Barboza et al. 2019, Bergmann et al. 2017, Browne et al. 2011, Hollman et al. 2013). Additive chemicals and adsorbed pollutants associated with MP may also pose environmental and health risks (Capolupo et al. 2020, Hermabessiere et al. 2017, Turner 2016, Ziccardi et al. 2016, Zimmermann et al. 2019). Furthermore, MP may serve as substrates that enable pathogens to 'hitchhike' to new habitats via direct discharge of wastewater or urban surface run-off (Dris et al. 2015, Zettler et al. 2013).

Wastewater treatment plants (WWTPs) concentrate large amounts of MP from urban areas (Kay et al. 2018, McCormick et al. 2014). The WWT process consists of physical, chemical and biological treatment barriers broadly organized in three main stages: primary, secondary and tertiary. The tertiary treatment aims to produce effluents appropriate for unrestricted irrigation or safe disposal to land and natural water bodies. Although WWT does not target MP specifically, there is a consensus that 97-99% of MP are removed to the sewage sludge, mainly during the primary and secondary stages (Carr et al. 2016, Freeman et al. 2020, Murphy et al. 2016, Talvitie et al. 2017a) (Table S1). Despite the efficient removal rates, WWTPs are considered an entrance route for MP into the environment due to the large volumes discharged (Talvitie et al. 2017a). A recent study reported emissions of up to 1.83×10^{10} MP day^{-1} (~6.7×10^{12} MP annually) from a single, medium-sized (30-50,000 m^3/day) WWTP (Leslie et al. 2017). It is noteworthy that emission figures vary

considerably depending on specific WWTP capacity, raw wastewater MP content and MP removal efficiency (Freeman et al. 2020).

Polyester (PET) microfibers and polyethylene (PE) microparticles are typically the most abundant MP in secondary and tertiary effluents (Browne et al. 2011, Lares et al. 2018, Murphy et al. 2016, Ziajahromi et al. 2017), reflecting the fact that PET and PE are the most commonly used synthetic polymers in textiles and plastic manufacturing, respectively (Lares et al. 2018). The prevalence of polyamide (nylon) microfibers in WWTPs effluents has been also reported and attributed to washing of synthetic textiles (Vollertsen and Hansen 2017). A contributing factor to the dominance of fibers in effluents is their long thin shape, which allows them to pass through even fine-grained filters. A high abundance of PE fragments with uneven shapes in wastewater effluents has also been reported and is attributed to MP in personal care and household cleaning products (Talvitie et al. 2017b).

A comparison of studies investigating MP distribution in WWTPs suggests that the capture methods employed primarily used mesh sizes ≥20 μm (Sun et al. 2019), meaning MP <20 μm will pass through and lead to an underestimation of total MP abundance. In addition, uncertainties concerning the types and quantities of MP present in raw wastewater and effluent are compounded by recent findings that point to high capture rates for larger MP in WWTPs (Michielssen et al. 2016, Ziajahromi et al. 2017) and enrichment of smaller MP (low micron to nanometer in size), as well as fibers in effluents (Kay et al. 2018, McCormick et al. 2014). However, a lack of standard protocols for MP sampling (e.g. use of both small and large grab samples, and 24-h composite samples), sample preparation, analysis (i.e. visual sorting vs spectroscopy) of MP in WWTP effluents and onsite sampling limitations means that existing studies are not directly comparable (Freeman et al. 2020, Gies et al. 2018, Kay et al. 2018, Lares et al. 2019, Pedersen and Winther-Nielsen 2015).

The aims of the current study were to: (i) investigate the occurrence and distribution of MP in a domestic WWTP in different stages and (ii) assess the effect of different sampling techniques on the abundance and characteristics (polymer type, size, shape) of MP collected from the same effluent samples (mainly secondary and tertiary). The sampling strategy was based on three different sampling methods that were applied at regular intervals (weekly) over a period of 12 months (different seasons): a) small grab samples (30 L), b) composite sampling of 6-24 L to integrate diel variations, and c) large grab samples (100 L). MP were grouped into a "typical" size range (≥20 µm) to facilitate comparison with other studies and an "extended" size range (≥0.45 µm) to allow data for MP <20 µm to be included. To the best of our knowledge, combining assessment of different sampling techniques, together with using a 0.45 µm mesh cutoff in tertiary effluents, the length of assessment (12 months) and investigation of seasonal variability has not been previously attempted.

2. Materials and Methods

2.1 Description of Karmiel WWTP

Raw wastewater, primary, secondary and tertiary effluents were sampled at the Karmiel domestic WWTP, situated in northern Israel between July 2018 and July 2019. Operational details of the WWTP are provided in Table S2 (SI). Briefly, this system has an average flow rate of 30,000 m^3/day, treating domestic sewage of 13 municipalities with a population of roughly 210,000 people and providing tertiary effluents for unrestricted irrigation. Karmiel WWTP is a typical activated sludge-based wastewater treatment system that consists of bar screens, grit removal, primary clarifiers, biological nutrient removal, activated sludge reactors with low-speed surface aerators for the first 2 modules and a diffused air system for the third module, final clarifiers, effluent filtration and disinfection as the last stage of the treatment. As is common in Israel and many other countries, both the final (tertiary) effluent (mainstream) and digested sludge (side stream) are used in

agricultural applications (irrigation and soil amendment, respectively). This means that most of the MP removed in the processing of the wastewater will subsequently be dispersed in the open environment (mainly agricultural fields). Many countries (i.e. western European countries, USA, Canada) dispose of the final treated effluent into rivers/lakes or directly into the sea as there is no need in these countries for water re-use (Loos et al. 2012). Figure S1 illustrates the different treatment units of the Karmiel WWTP and the four sampling points used in the current study.

2.2 Wastewater sampling

At each sampling point, volume and method of sampling were dependent on the ability to filter samples with high organic matter that rapidly clogs the sieves and cause overflow. Thus, only post-secondary and -tertiary effluents were sampled using the large grab sample method (100 L). Accordingly, only small volumes of raw (3 L) and primary treated wastewater (6 L) were sampled (small grab and 24-h composite sampling, respectively). Sampling was conducted on a weekly basis for a year (July 2018 until July 2019) at the locations described in Figure S1. In campaign 1, small grab samples were collected into acid washed containers (Murphy et al. 2016) on a weekly basis (mornings) from July 2018 to March 2019, while in campaign 2 sampling was conducted from April to July 2019 using an ISCO model 5800 autosampler (24-h composite sampling) or by direct filtration in the WWTP (large grab samples) to assess the variability between samples (Table 1). Due to regulatory constrictions, primary effluents replaced influent sampling in campaign 2.

The 24-h composite sampling (Michielssen et al. 2016, Talvitie et al. 2017a) and large grab samples (Mason et al. 2016, Mintenig et al. 2017, Talvitie et al. 2015, Ziajahromi et al. 2017) were collected onsite on a weekly basis using sample volumes of 6-24 L and 100 L, respectively (as described above and in Table 1). The wastewater filtration method was adapted from a previous study (Dyachenko et al. 2017). MP were isolated from all sampling points using a stack of six 8”-diameter

144 stainless steel sieves (Gilson Best, USA) with mesh sizes of 425 µm, 300 µm, 212 µm, 106 µm, 80
145 µm and 20 µm. Approximately 10 L of filtrate from the 20 µm sieve of each sample was further
146 passed through a 0.45 µm filter (GN-6 Metricel® Grid, Pall Corporation) using a Buchner funnel.
147
148 For the large grab sampling method, the wastewater flow was directed to the series of stacked
149 sieves and samples filtered onsite at the WWTP. Each large grab sampling event took 2-3 h to
150 process, with a maximum flow rate of 200 mL min^{-1} through the sieves. The sieves used for onsite
151 sampling were removed after sampling and stored at room temperature wrapped with aluminum foil
152 prior to their transfer to the laboratory. To avoid cross contamination, the sieves were washed with
153 tap water between sampling events.
154
155 2.3 Sample preparation
156 The results are presented as two sets of data corresponding to (i) a "typical" size range (≥20 µm)
157 comparable to other studies and (ii) an "extended" size range (≥0.45 µm) to allow data for MP <20
158 µm to be included. In addition, MP were separated into fibers and particles, where particles
159 collectively refer to the total amount of fragments, beads, films and pellets, whereas fibers are
160 defined as MP objects with an aspect ratio of >3 (WHO 1997). A schematic illustrating sample
161 processing and analysis is presented in Figure 1.
162
163 Recovery of MP from the sieves was accomplished by repeatedly washing each sieve with a total of
164 100 mL filtered (0.45µm) distilled deionized water (DDW). The material recovered (water +
165 particles) from each sieve was transferred to a 600 mL acid washed glass beaker (pre-weighed)
166 covered with aluminum foil and stored at room temperature until further processing, using a
167 protocol adapted from (Masura et al. 2015). Briefly, 40 mL of 0.05 M $FeSO_4$ and 80 mL 30% H_2O_2
168 were added to each beaker and the slurry was heated to 70 ºC while stirring for 30 min. Once cooled

to room temperature, the extract was filtered through a 0.45 µm, 47 mm diameter membrane filter (GN-6 Metricel® Grid, Pall Corporation) or evaporated to dryness for a subsequent optional density separation step (usually applied to samples from raw, primary and secondary treated wastewater due to their high level of organic matter). The material from this digestion step was transferred using distilled water into a separation funnel containing a 5M solution of NaCl (20 mL). Undigested particulate material was allowed to settle overnight, and the residual suspended solids were collected on a 0.45 µm, 47 mm diameter filter (GN-6 Metricel® Grid, Pall Corporation). The filter was allowed to dry at room temperature while loosely covered with aluminum foil for 24-h (Figure 1).

2.4 QA/QC

To prevent contamination, samples and equipment were covered with aluminum foil to minimize exposure to airborne MP and personal protective equipment (e.g. white cotton lab coats) were worn at all times. All glassware and filtration equipment were thoroughly washed with filtered (0.45 µm) DDW water and procedural blanks (absent of MP) consisting of 0.45 µm filtered DDW water (3 x 30 L) were processed in parallel with each batch of WWTP samples to quantify and ultimately subtract background MP contamination. For positive controls, 3 x 30 L of filtered DDW (0.45 µm) samples were spiked with known numbers of MP (30-50) including PVC microspheres (250 µm, Goodfellow, Cat. No. CV316010), polyethylene microspheres (40-48 µm, Sigma, Cat. No. 434272) and acrylic and polyester fibers (1-2 mm in length, 10-20 µm in width, produced internally from commercially available yarns) and processed as above.

2.5 MP quantification and characterization

Staining methods were used to determine which particles in the samples were MP (Ziajahromi et al. 2017) (Figure 1). Rose-Bengal solution (Sigma-Aldrich) was first used to differentiate between

non-plastic particles and MP in the samples (Liebezeit and Liebezeit 2014). The surface of the membrane filter (0.45 µm, 47 mm diameter) was covered with Rose-Bengal solution (0.2 mg/mL) and allowed to react for 5 min at room temperature (Liebezeit and Liebezeit 2014). The dye was then washed off with filtered (0.45 µm) ultrapure water and the filter was dried at 60 ºC for 15 min. The dried filters were inspected using a dissecting microscope and the pink-stained particles suspected to be natural particles were further agitated with a stainless-steel micro spatula to test each particle's durability. Each piece that was able to endure this test without breaking apart was suspected as MP (Liebezeit and Liebezeit 2014). Conversely, microlitter that exhibited cellular structures, uneven color distribution, or metallic sheen were rejected. Questionable MP were set aside for further analysis using µ-Raman spectroscopy (see below).

The total number of MP counted in each sample was expressed as MP abundance (MP L^{-1}). The retention capacity of MP within the "typical" range (≥20 µm) and the "extended" range (≥0.45 µm) in Karmiel was calculated as ([Incoming water] - [Effluent water] / [Incoming water]) x 100. MP particles were split into five categories according to their visual appearance (Jayasiri et al. 2013, Lee et al. 2014): (a) fragments, (b) films, (c) pellets, (d) beads, and (e) foams. MP categorized by appearance were stored in a sealed and labelled 24-well plate for further processing.

Individual MP (~450) isolated from secondary and tertiary effluent samples were mounted on carbon adhesive discs and photographed using a MIRA3 FEG-SEM scanning electron microscope. Examination was performed at accelerating voltages of 10−20 kV; an emission current (Ic) of 10 µA; working distance of 12−24 mm. A representative subset (in terms of shapes, color and sizes) of approximately 10% of the suspected MP from the same effluent samples were mounted on glass slides and left to dry at room temperature in a dust-free box. The dried samples were analyzed using a Horiba Jobin Yvon (LabRAM HR Evolution®) µ-Raman instrument equipped with a 532 nm

laser using the following setting: grating – 1800 gr/mm, acquisition time – 5 sec., accumulation – 3, range – 100-3500 cm. The *Know It All* (Bio-Rad, Israel) software was employed for identification of the polymers examined by µ-Raman. MP were considered tentatively identified when the match was 90% or above. Statistical analyses were performed using GraphPad Prism version 8.00 for Windows (GraphPad Software, La Jolla California USA).

The efficiency of the tertiary sand filtration step was tested in a laboratory setting using a sand packed column with similar sand and flow rate specifications as in Karmiel WWTP. Glass column with a height of 20 cm and a diameter of 1.6 cm was purchased from GE-Healthcare. Acid washed quartz sand particles with an average size of 0.8 mm were used to pack the column. Particles (PC and PP) and red colored PET fibers of various sizes (Figure S2) were prepared in house by grinding and cutting commercially available pellets and fibers. PVC particles (250 µm) were purchased from Goodfellow, UK. In each test, 40 fibers and 30 particles of each type were mixed with 3 L DDW in a clean Erlenmeyer flask and were transferred to the top of the sand column and eluted using a peristaltic pump (model BT100M-YZ2515x, Baoding Chuang Rui Precision Pump Co., Ltd) while maintaining a column flow rate of 18 mL min^{-1}. These tests were repeated 13 times. The outflow was filtered using a 0.45 µm filter and MP that eluted from the sand column were counted manually.

3. Results and Discussion

3.1 MP distributions in raw wastewater and treated effluents

Table 2 shows the concentrations of the separate particle and fiber fractions, together with the combined particle and fiber MP concentrations in the raw wastewater and the secondary and tertiary treated wastewater (effluent) collected using small grab samples (July 2018-March 2019). The total MP concentration in Karmiel WWTP raw wastewater was ~65 MP L^{-1} in the "typical" range but doubled to ~130 MP L^{-1} when the "extended" range was applied. Although reported MP raw

wastewater concentrations vary greatly from 1 to 10044 MP L^{-1} (Sun et al. 2019), the most comparable values were found in studies by Dris et al. (2015) and Lares et al. (2019), with 293 and 58 MP L^{-1}, respectively.

Following the secondary treatment step, the mean MP concentration in the raw wastewater was reduced by nearly 24-fold to 2.7 MP L^{-1} (95.8% reduction) using the "typical" mesh size range and by approximately 8-fold to 15.6 MP L^{-1} (88.2% reduction) using the extended range. The mean MP concentration in the secondary effluent was reduced only slightly further during the tertiary treatment step to 1.9 MP L^{-1} (from 95.8% to 97.0%) using the "typical" range. In contrast, the tertiary treatment step was not as efficient when using the "extended" range, with a total MP concentration of 7.3 MP L^{-1} in the tertiary effluent (from 88.2 to 94.4%). Irrespective of the MP type or size range measured, the most significant reduction in MP concentration occurred prior to the tertiary treatment, with the tertiary sand filtration treatment generally proving ineffective in reducing MP levels further. These findings are consistent with other studies that observed effective removal during preliminary (35-59%) and post primary (50-98%) stages and a further 0.2-14% post the secondary treatment (Sun et al. 2019). The efficiency of the tertiary sand filtration step to remove MP fibers and particles of various sizes was simulated in laboratory sand column tests using Karmiel WWTP sand and flow parameters (Figure S2). The results were comparable with the field test, showing a significantly reduced ($p<0.0001$) removal rate for the fibers (89% capture) compared to the particles of various sizes and shapes (97-100% capture).

Table 2 also shows that the total MP content of the raw wastewater determined using the "typical" range is dominated by fibers (73.8%) rather than particles (26.2%). This pattern is similar in the "extended" range, where the distribution of fibers and particles is 69.5% and 30.5%, respectively. When looking at the influence of the different treatment steps on removing particles and fibers (Table 2), the secondary treatment step removed particles from the raw wastewater more efficiently (from 17 to 0.26 particles L^{-1}; 98.5% decrease) than fibers (from 47.8 to 2.44 fibers L^{-1}; 94.9%

decrease) in the "typical" range. For the "extended" range, secondary treatment was also more efficient at removing particles (from 39.56 to 1.11 particles L^{-1}; 97.2% decrease relative to raw wastewater) than at removing fibers (from 90.11 to 15.17 fibers L^{-1}; 83.2% decrease). However, the results suggest that fibers with a diameter <20 µm are the MP fraction that is least effectively removed during secondary treatment. The mean particle concentration in the secondary effluent was reduced only slightly further during the tertiary treatment step to 0.19 particles L^{-1} (from 98.5% to 98.9%) using the "typical" range and 0.44 particles L^{-1} (from 97.2 to 98.9%) using the "extended" range. Similarly, the mean fiber concentration was reduced only slightly further during the tertiary treatment step to 1.78 fibers L^{-1} (from 94.9% to 96.3%) using the "typical" range. However, the tertiary treatment step appeared to have a greater influence on reducing fiber concentration in the secondary effluent to 6.86 fibers L^{-1} (from 83.2 to 92.4%) when applying the "extended" range, possibly reflecting the higher load of fibers in the secondary effluent compared to particles. At the end of the tertiary process, fibers accounted for the vast majority of MP in the final effluents, with values of 90.4% and 94.0% in the "typical" and "extended" ranges, respectively. This relative increase in fibers in final effluents compared to the raw wastewater suggests fibers are less efficiently retained in WWT processes than particles. In the current study where the Karmiel WWTP has an average flow rate of 30,000 m^3 day^{-1}, the final effluent concentrations of 1.97 MP L^{-1} ("typical" range) and 7.30 MP L^{-1} ("extended" range) correspond to a discharge of 59 million and 219 million MP day^{-1}, respectively, or 21.5×10^9 and 79.9×10^9 MP $year^{-1}$, respectively.

Although reported MP concentrations in the final effluents from different WWTPs around the world are highly variable (0.005 to 447 MP L^{-1}) (Lares et al. 2018, Sun et al. 2019), the results of the current study are consistent with other recent studies of tertiary WWTPs, where an anticipated trend of decreased MP concentrations in final effluents is not observed for WWTPs equipped with advanced treatment units (*i.e.* membrane-based system). For example, some of the highest reported MP concentrations, an average of 4.9 fibers L^{-1} and 8.6 particles L^{-1} of treated wastewater, were

296 reported by Talvitie et al. (2015) for effluents from tertiary biological filtration treatment. However,
297 it is important to consider that high values in specific studies may also reflect the use of more robust
298 sampling, sample preparation and analysis as compared to other studies. As a result, the observed
299 variability between studies can be attributed to several factors, including the composition of raw
300 wastewaters, the procedures used in the studied WWTPs (Mahon et al. 2017), different sampling
301 strategies and sample processing, and different characterization methods in particular (Lares et al.
302 2018). For example, Lares et al. (2018) used 250 µm mesh sieves and found that the majority of MP
303 in the final effluent ranged between 0.5 to 1 mm. In contrast, Talvitie et al. (2017a) used a 20 µm
304 sieve and reported that only 20% of the MP they retrieved were larger than 300 µm. Importantly,
305 this variability in approaches makes direct comparison of data sets challenging, an issue which is
306 compounded by a lack of a standard units for reporting MP concentrations (Freeman et al. 2020,
307 Frias and Nash 2019).
308
309 3.2 Partitioning between different shapes of MP
310 The relative distribution of different MP types (fibers, fragments, films, pellets, beads and foam)
311 varied between the raw wastewater and secondary and tertiary effluents. In the "typical" range, the
312 relative abundance of particles was lower than for fibers in both the raw wastewater (26%) and
313 lower still in the secondary (9.5%) and tertiary (9.4%) effluents (Figure 2a). This indicates that the
314 secondary and tertiary treatment processes remove particles more efficiently than the fibers, leading
315 to a relative increase in the abundance of fibers in final tertiary effluent (91%) relative to the raw
316 wastewater (74%). The predominance of fibers over particles has been reported previously for
317 wastewater effluents (Lares et al. 2018, Leslie et al. 2017, Mason et al. 2016, Talvitie et al. 2015,
318 Ziajahromi et al. 2017) and illustrates the large impact of household laundry and textile handling on
319 MP occurrence in wastewater and in effluents (Lares et al. 2018). When looking in more detail at
320 the behavior of the different sub-types of MP within the particle fraction of the "typical" range,
321 fragments constituted 25% of MP in the raw wastewater (Figure 2b). This increased to 68% and

73% after secondary and tertiary treatment processes, indicating poor removal of fragments. Films were the most abundant sub-type in raw wastewater (72%) but exhibited the highest removal efficiency following secondary and tertiary treatment (22 and 18%, respectively). Beads and foam particles in the raw wastewater (2 and 1%, respectively) remained minor fractions following the tertiary process.

In the "extended" range (Figure S2a), the relative abundance of fibers displayed a similar trend to the "typical" range (Figure 3a). However, the proportion of fibers in secondary and tertiary effluents was higher (93% and 96%, respectively), suggesting fibers <20 µm are less efficiently removed than larger fibers and particles. As in the "typical" range, fragments became the most abundant MP type in the particle fraction after the secondary and tertiary treatment steps (Figure S2b), increasing from 21% (raw wastewater) to 53% and 78%, respectively. Lares et al. (2018) attributed the low abundance of fragments in raw wastewater to a combination of diurnal variations and hydraulic retention time. Film and foam particles were effectively removed with each treatment step. Films were reduced from 72% in raw wastewater to 25% and 13% secondary and tertiary effluents, while foam particles were reduced from 5% in the raw wastewater to 3% and 1%. The efficient removal of films from wastewater was also reported by Talvitie et al. (2017b). Irrespective of the MP type, the overall removal efficiency of MP was increased with each additional treatment step.

3.3 MP color distribution

Irrespective of treatment stage or mesh size, the retrieved MP fibers were predominantly black (50-85%), with blue being the second most common (10-20%) (Figure S3a,c). However, black fibers decreased (from ~85% in the raw wastewater to ~70% and 50% subsequent to the secondary and tertiary treatments, respectively) while blue fibers increased from 10% in the influent to 20% in the tertiary wastewater. The colors of MP particles were predominantly brown (35%) followed by white (30%), black (20%) and transparent (15%) in the raw wastewater but primarily transparent (45%)

and white (20%) in the tertiary wastewater (Figure S3b,d). The percentage of pink and green colored particles was also elevated subsequent to the secondary and tertiary treatments. The color of MP particles and fibers play an important role in quantification by Raman spectroscopy, with black MP difficult to identify.

3.4 Fiber dimensions

The length and width of MP fibers extracted from the raw wastewater and secondary and tertiary effluent samples were measured from images produced using scanning electron microscopy (Figures 1, 3a-b and S5). In the "typical" range, fibers in raw wastewater and treated effluent (secondary and tertiary) were on average 1687-1879 µm in length and 16-19 µm in width (Figure 3a-b), with most fibers being 1000-2000 µm in length and 10-20 µm in width, irrespective of treatment level (Figure S5a-b). It is interesting to observe fibers with a relatively high range of lengths after the sand filtration system. However, differences between treatment stages or sieve sizes were not statistically significant due to the high standard deviation (Figures 3a-b, and S5c-d). Furthermore, there was no obvious difference between average fiber lengths and widths between the "typical" and "extended" ranges (Figure S5c-d), most likely reflecting the fact that all fibers had widths within range 10-20 µm and could therefore pass through all of the sieves with the exception of the 0.45 µm filter. The occurrence and distribution of MP fibers therefore appear to be predominantly governed by the width rather than the length of the fibers. The current study found that MP fibers with a median width of 10-20 µm dominate WWTP effluent samples, which is in line with the observations of Raju et al. (2020) and raises questions about the ability of current membrane technologies to remove them.

3.5 Particle dimensions

The dimensions of MP particles (fragments, films, beads, pellets and foam) extracted from the samples are shown in Figure 3c-d. The average normalized values (root*[width*Length]) are shown

374 in Table S3, where average particle dimensions ranged from 568 µm (raw wastewater) to 308 µm
375 (tertiary effluent). Most particles from the treated wastewater ranged from 200 to 450 µm, with a
376 peak abundance at 350 µm, while most of the particles from the raw wastewater were larger (350-
377 850 µm). The average dimensions of particles extracted from raw wastewater were significantly
378 larger (36-46%) than those extracted from the treated effluents. Conversely, smaller particles were
379 found after the tertiary treatment, although these were not statistically significant. The smaller
380 average size of MP in the tertiary treatment compared with MP from primary and secondary
381 treatments is consistent with previous studies (Dris et al. 2018, Talvitie et al. 2017a, Vollertsen and
382 Hansen 2017).
383
384 3.6 Seasonal variations in WWTP MP
385 The mean abundance of MP in the tertiary effluent was significantly higher ($p = 0.0002$) in winter
386 and spring samples (15 and 9 MP L^{-1} respectively) compared with the summer season (~4 MP L^{-1})
387 (Figure 4a). The higher MP abundance in the winter season may reflect a higher contribution of
388 terrestrial runoff during this period, where ~45% increase in flow rate was observed as result of
389 high rainfall during the winter (Table S4). This increase in flow rate corresponded to a comparable
390 increase in the MP concentration in the winter as compared to the summer and autumn (Table S4).
391 The high winter flow rate, typical for many middle eastern countries, appears to significantly
392 influence the number of MP in effluents, as well as corresponding emissions to agricultural land,
393 water bodies and the sea. This pattern of elevated concentrations of MP during the rainy season has
394 been also observed in marine and riverine environments adjacent to urban centers. For example, the
395 abundances of MP in Nakdong River, South Korea, increased from 260 to 1410 items/m^3 (dry
396 season) to 210 to 15,560 items/m^3 (rainy season) (Kang et al. 2015). Similarly, a greater abundance
397 of MP was observed in Hong Kong during the rainy season (Fok and Cheung 2015). In this study,
398 fibers were the dominant type of MP in each season, representing over 80% in all cases (Figure 4b).
399 The winter season was characterized by the highest relative abundances of particles (~16%)

compared with the summer and autumn seasons (3-4%), while almost no particles were found during the spring season. The variation in MP particle and fiber relative abundance between different seasons may also reflect the higher contribution of terrestrial runoff during the winter season, while the lower rainfall in other seasons means domestic MP sources dominate the raw wastewater.

3.7 Micro Raman analysis

Identifying the polymer composition of MP that are smaller than 500 µm requires the application of diagnostic characterization methods such as FTIR or Raman spectroscopy (Löder and Gerdts 2015). In this study, µ-Raman spectroscopy was used to obtain interpretable spectra from a sub-set of approximately 10% sub-set of the suspected MP particles and fibers. A breakdown of the polymer composition of the MP is shown in Figure 5. Particles were predominantly composed of polyethylene (PE; 13.9%), polyvinyl chloride (PVC; 5.9%), polypropylene (PP; 2.0%), polycarbonate (PC; 1.5%), polytetrafluoroethylene (PFTE; 1.0%), polyolefin elastomer (PO; 0.5%), polystyrene (PS; 0.5%), polyurethane (PUR; 0.5%) and nylon 66 (PA66; 0.5%). The remaining 3.5% of suspect MP were identified as cellulose-based. Approximately 71% of the total suspected MP were fibers, of which 6.4% were tentatively identified as polyethylene terephthalate (PET) and 0.5% as nylon 66 (PA66). The identification of the remaining fibers was hindered by their mostly black colored pigment. Black samples strongly absorb laser light, warm up and emit intense background fluorescence that masks the Raman signal. PET is the most common thermoplastic polymer resin of the polyester family and the PET MP originated exclusively from fibers, suggesting synthetic textiles as the main source. Interfering fluorescence due to polymer additive compounds (i.e. organic colorants, pigments and dyes) and residues of biological origin from bacterial biofilm were the main limitation encountered. Consistent with other studies, clean-up of samples by hydrogen peroxide (or enzymatic digestion) was not always sufficient to overcome the problem of fluorescence from biological residues (Löder and Gerdts 2015). Another issue that hindered obtaining interpretable spectra was the partial degradation of samples due to UV exposure,

426 which leads to chemical changes at the MP surface (Silva et al. 2018). A breakdown of MP
427 according to their corresponding density values suggests that the tentatively identified MP (those
428 with % match >90) are roughly divided between floating lighter density PE particles (13.9%, 0.88-
429 0.96 g cm^{-3}) and heavier sinking PET fibers (6.4%, 1.37 g cm^{-3}) and PVC particles (5.9%, 1.31-1.45
430 g cm^{-3}). As density separation is key to the wastewater treatment process (primary and secondary
431 clarifiers in the sampled plant), the density of particle polymers, in addition to their shape, should
432 be the focus of future studies.
433
434 3.8 Sampling methodology
435 Small grab sampling containers have been frequently applied in related studies due to their ease of
436 use and the ability to collect samples with high organic matter (Sun et al. 2019). Due to low
437 concentrations and uneven distributions of MP in treated wastewater, recent efforts have employed
438 a more representative sampling approach by increasing the sampling volume (Mason et al. 2016,
439 Talvitie et al. 2017b) and/or by taking 24-h composite samples (Dris et al. 2015, Michielssen et al.
440 2016, Simon et al. 2018, Talvitie et al. 2017b). To assess whether the latter two methods minimize
441 errors and contamination sources associated with the small grab sampling procedure, large grab
442 samples (100 L) and 24-h composite (6-24 L) were collected in the period April-July 2019. To
443 facilitate comparison with reported sampling methods, only the "typical" range (≥20 μm) was
444 examined. As sampling of large volumes of raw and primary treated wastewaters was not feasible
445 due to onsite regulations, only small volumes of primary treated wastewater (6 L) were sampled
446 using an autosampler. Based on the 24-h composite results, the secondary treatment process reduced
447 the total MP abundances 4-fold as compared with the primary treatment process (from 29 to 7.2 MP
448 L^{-1}). Again, the contribution of the tertiary process to the further removal of MP from the final
449 effluent was more moderate as compared with the secondary process (~2-fold reduction from 7.2 to
450 3.2 MP L^{-1}) (Figure 6a). The total MP concentrations determined for the secondary effluent using
451 the two different sampling methods were comparable, with values of 7.2 ± 1.2 and 6.7 ± 0.7 MP L^{-1}

452 for the 24-h composite and large grab methods, respectively (Figure 6a). These values were
453 markedly higher compared to those obtained for secondary effluent using the small grab method
454 (2.7 ± 0.48) (Table 2), possibly reflecting high variation inherent in the latter sampling method.
455 However, there was a significant variation ($p = 0.0013$) in the total MP concentrations determined
456 in the tertiary effluent, with a higher value of 3.2 ± 0.7 MP L^{-1} for the 24-h composite samples
457 compared to 1.2 ± 0.2 MP L^{-1} for large grab samples and to 1.6 ± 0.6 MP L^{-1} for small grab
458 samples. Significantly higher levels of MP detected by the 24-h composite sampling may reflect
459 diurnal fluctuations in the WWTP flow rate, the types and concentrations of MP (i.e. discharge from
460 washing machines vs industry). Overall, the greater variability of the small grab samples was
461 indicated by the higher average coefficient of variation (CV) (54.3%) compared with that of large
462 grab samples (15.6%) and the 24-h composite samples (15.4%). Irrespective of the sampling
463 method employed, an efficient removal of MP particles was observed but this was less efficient for
464 MP fibers (Figures 6b-c). The low standard deviation that characterized the 24-h composite and the
465 large grab samples implies that sampling over a 24-h period or increasing grab sample volume
466 significantly lowers the error relative to the small grab sampling.
467
468 The results of the second campaign agree with the small grab samples from first campaign, where
469 fibers are the dominant form of MP in terms of absolute and relative abundance. Furthermore,
470 consecutive stages of the WWT process proved to be efficient in removal of MP particles, but less
471 efficient in reducing the number of MP fibers. The number of MP particles was effectively reduced
472 from 6.4 in the primary stage to 1.0 in the secondary stage and finally to 0.04 MP L^{-1} in the tertiary
473 stage (Figure 6b). The largest reduction in the number of MP fibers occurred during secondary
474 treatment (from 22.5 to 6.1 MP L^{-1}), while further removal during the tertiary treatment step was
475 more moderate (from 6.1 to 3.2 MP L^{-1}) (Figure 6b).
476
477 3.9 Recommendations

Recent studies of MP in WWTPs have primarily used mesh sizes ≥20 μm (Sun et al. 2019), although the specific lower cut-off varies between studies. This has the potential to significantly impact the resulting quantification of MP in WWTP samples and makes it difficult to draw meaningful comparisons between data from different studies. We suggest that an internationally recognized standard operating procedure for assessment and monitoring of MP in wastewater effluents should be established, where a minimum mesh size and specific size sub-classes are defined. It is recommended that the lower-cut off should extend to a minimum of 20 μm, since collection and analysis of the smaller MP has a crucial impact on the overall number of recovered MP and provides a more accurate picture of MP size distributions, especially as smaller and potentially more toxic MP (due to the relative ease in which MP cross biological barriers increases with decreasing size) are more abundant in final effluents (Blair et al. 2017). This highlights the need for assessing the occurrence, behavior and fate of smaller MP and NPs in WWTPs. In addition, to fully grasp the magnitude of MP emissions, it is suggested that MP concentrations determined in final effluents should be used to estimate the daily/annual discharge of the WWTP studied.

It is further recommended to employ the 24-h composite sampling method since it accounts for the intraday variations of source loading during the day-basis (morning, midday and evening) and peak flow rates, therefore providing a more representative snapshot of MP distribution in each treatment stage compared with morning sampling (Conley et al. 2019, Talvitie et al. 2017a, Wolf et al. 2019). These daily snapshots can provide a basis for more accurate calculations of weekly, seasonal and diurnal MP emissions. Using the large grab sampling method (100 L) is also a useful way to reduce the variability between samples. Given the high proportion of microfibers in the total MP load entering WWTPs, extraction, identification and analytical techniques require further development and optimization for this sub-class of MP in order to provide a more robust characterization and

quantification. Finally, seasonal variations in concentrations and types of MP have to be taken into account in the experimental design when assessing MP emissions from WWTPs.

4. ;Conclusions

WWTPs are a focal point for the removal of MP before they are released into aquatic environments or used for agriculture irrigation. Although WWTPs are not specifically designed to remove MP pollution, the current and previous studies demonstrate they can achieve very high retention rates. The removal capacity of MP in the Karmiel WWTP was ~97%, with most of the MP fraction removed prior to the tertiary treatment stage. Expanding the mesh size used for filtering effluent from 20 µm to 0.45 µm significantly increased the abundance of MP in final effluents, suggesting many studies underestimate the real amount of MP in WWTPs effluents. However, the large volumes of wastewater processed mean that WWTP effluents still represent sizable annual emissions to the environment and do not adequately prevent MP pollution. Furthermore, fibers are both one of the main MP sub-types entering WWTPs and the sub-type least effectively retained. Large grab sampling provides more representative samples than conventional small grab sampling, while 24-h composite sampling allows intra daily fluctuations to be measured. Furthermore, seasonal variations suggest that snap-shot monitoring does not provide adequate overviews of MP concentrations in effluents.

Acknowledgement

This article was supported by the GoJelly (A Gelatinous Solution to Microplastic Pollution) project which receives funding from the European Union's Horizon 2020 research and innovation program under Grant agreement No. 774499. A. Booth also received support through the Norwegian Research Council project 'MICROFIBRE' (grant no. 268404).

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Table

Table 1. Sampling regimes (small grab sampling, large grab sampling, 24-hr composite sampling), sampling locations (wastewater, sludge) and sampling days.

	Volume (L)	Sampling mode	Sampling dates	Process sampled	Sampling location
Small grab sampling	3 - 30	Plastic containers	July 2018 - March 2019	- Raw wastewater (3L) - Secondary (30L) - Tertiary (30L)	• Between bar & grit chamber • Post-secondary settling • Post disinfection
Large grab sampling	100	Direct (onsite) filtration	April 2019 – July 2019	- Secondary (100L) - Tertiary (100L)	• Post-secondary settling • Post disinfection
24-hr composite sampling	6 - 24	Automated sampler	April 2019 – July 2019	- Primary (6L) - Secondary (17-20L) - Tertiary (24L)	• Post primary settling • Post-secondary settling • Post disinfection

Table 2. Total MP concentrations in raw, secondary and tertiary treated wastewater. Data is given as number of MP (MP L^{-1}) for the "typical" range (≥20 µm) and the "extended" range (≥ 0.45 µm). Values in parentheses represent the standard deviation (n=3, 19 and 22 for raw, secondary and tertiary wastewater, respectively).

		Particles L^{-1}	% Decrease	Fibers L^{-1}	% Decrease	ΣMP L^{-1}	% Decrease
≥20 µm ("Typical")	**Raw**	17.00 (*7.49*)	-	47.78 (*18.52*)	-	64.78 (*18.88*)	-
	Secondary	0.26 (*0.06*)	98.5	2.44 (*0.48*)	94.9	2.72 (*0.48*)	95.8
	Tertiary	0.19 (*0.05*)	98.9	1.78 (*0.20*)	96.3	1.97 (*0.21*)	97.0
≥0.45 µm ("Extended")	**Raw**	39.56 (*12.49*)	-	90.11 (*24.64*)	-	129.67 (*27.23*)	-
	Secondary	1.11 (*0.25*)	97.2	15.17 (*2.75*)	83.2	15.62 (*2.52*)	88.2
	Tertiary	0.44 (*0.17*)	98.9	6.86 (*1.00*)	92.4	7.30 (*1.08*)	94.4

Figure
Click here to download Figure: Ben-David et al._STOTEN_Figures-final.docx

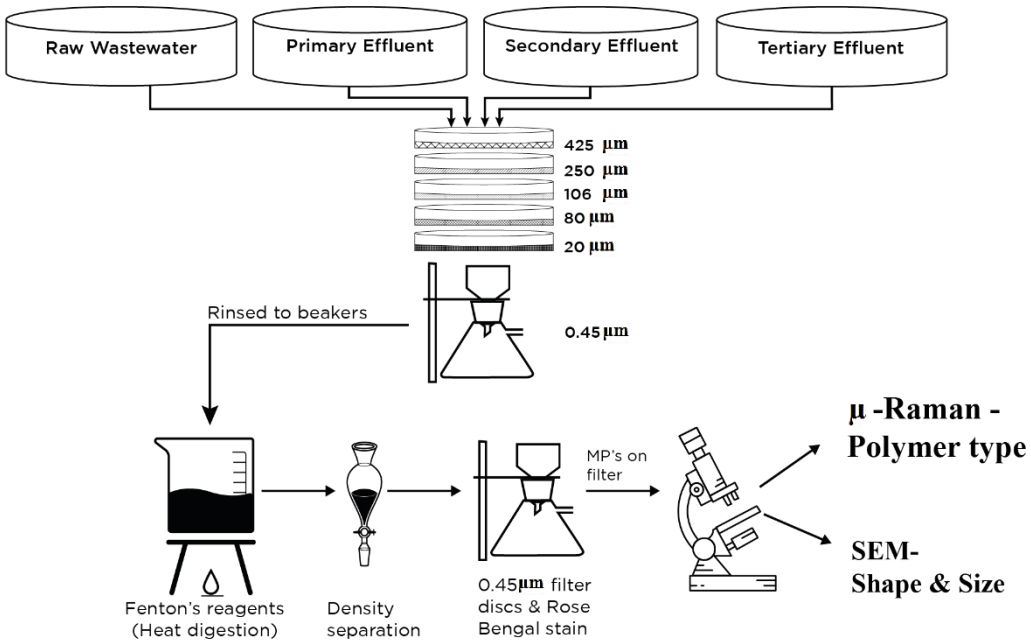

Figure 1. *Flow diagram for the analysis of microplastics in wastewater samples. Water from the various treatment stages was passed through a series of sieves, and the material captured on the 20 µm sieve was loaded onto a 0.45 µm filter, digested, collected on another 0.45 µm filter and inspected by microscope after staining with Rose Bengal.*

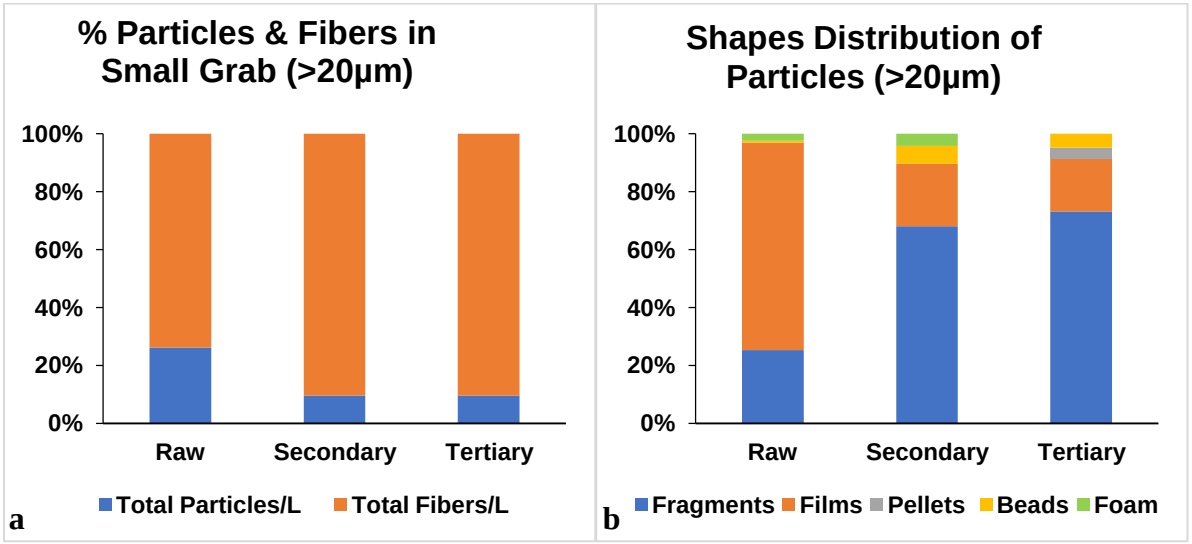

Figure 2. *Relative abundance of MP fibers and particles in raw wastewater and secondary and tertiary effluents in (a) the "typical" range (≥20 µm). A breakdown of the different shapes (i.e. fragments/films/pellets/beads/foam) within the particle fraction is shown for (b) the "typical" range (≥20 µm).*

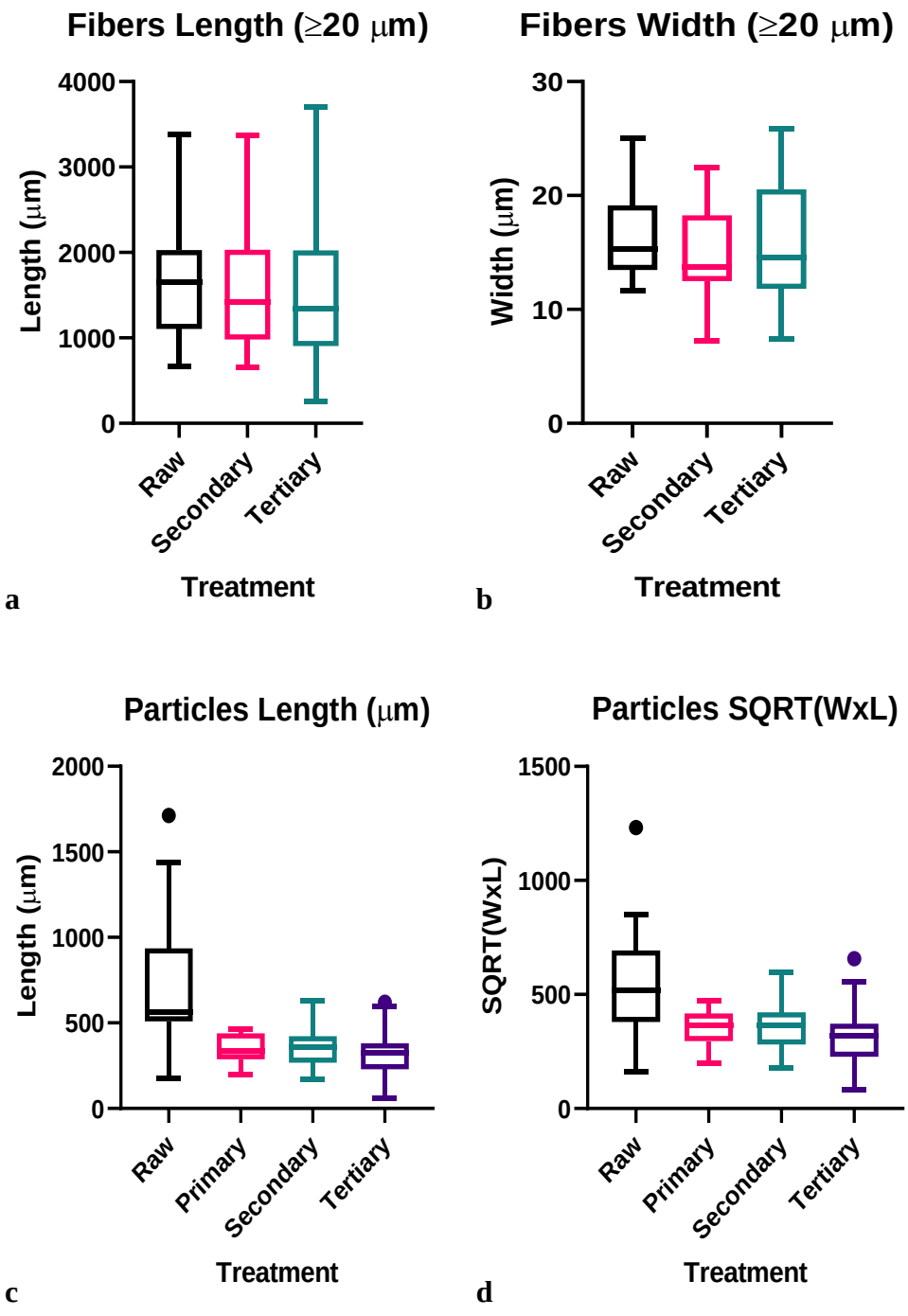

Figure 3. *(a-b) Box and whiskers plots of the width and length of fibers extracted from raw, secondary and tertiary treated wastewater (≥20 μm). (c-d) Box and whiskers plots of the length and the normalized size (root*[width*Length]) of MP particles extracted from raw, primary, secondary and tertiary treated wastewater.*

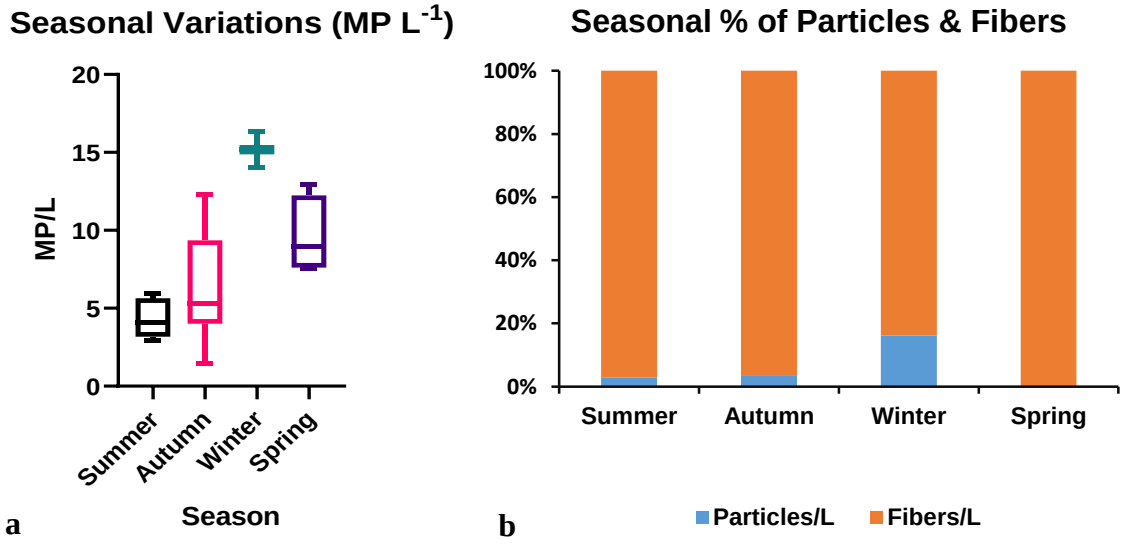

Figure 4. *(a) Box and whiskers plot of the seasonal fluctuations in MP concentration (MP L^{-1}). (b) Seasonal fluctuations in the relative abundance (%) of MP particles and fibers.*

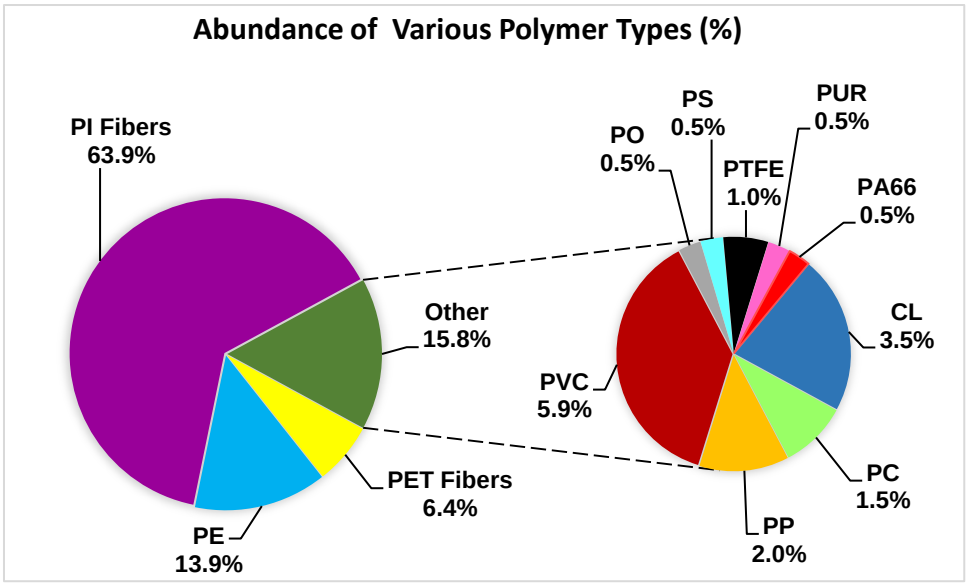

Figure 5. *Polymer type of MP particles and fibers that were extracted from primary, secondary and tertiary treated wastewater. PI Fibers=fibers with low % match or masking plastic pigment/colorant, PE=polyethylene (0.88-0.96 g cm^{-3}), PET Fibers= polyethylene terephthalate (1.37 g cm^{-3}), PVC = polyvinyl chloride (1.31-1.45 g cm^{-3}), CL= cellulose (1.5 g cm^{-3}), PP=polypropylene (0.90-0.92 g cm^{-3}), PC = polycarbonate (1.20-1.22 g cm^{-3}), PFTE = polytetrafluoroethylene (2.2 g cm^{-3}), PO = polyolefin (0.94-0.97 g cm^{-3}), PS=polystyrene (0.96-1.05 g cm^{-3}), PUR= polyurethane (1.05 g cm^{-3}) and PA66=nylon 66 (1.14 g cm^{-3}). Density is indicated in parentheses. Polymers were identified by means of micro-Raman.*

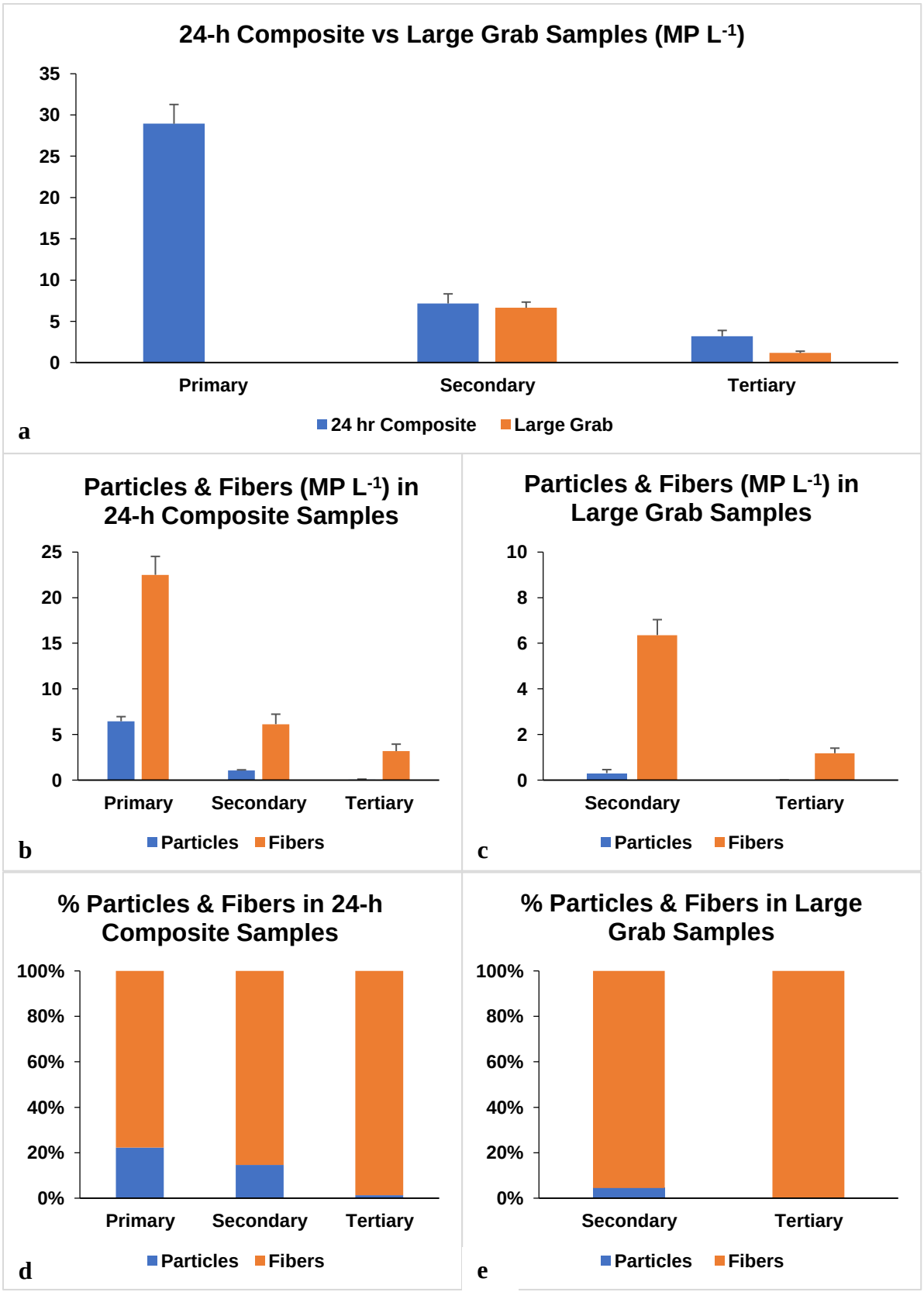

Figure 6. (a) *Comparison of total MP concentrations (MP L^{-1}) in primary, secondary and tertiary treated wastewater using 24-h composite and large grab sampling methods. (b) Absolute abundance of particles and fibers using the 24-h composite sampling method. (c)*

Relative abundance of particles and fibers using 24-h composite sampling method. Error bars represent the standard deviation of the mean (n=3).