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Microplastics composition and load from three wastewater treatment plants discharging into Mersin Bay, north eastern Mediterranean Sea

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ABSTRACT

Copious quantities of microplastics enter the sewage system on a daily basis, and hence wastewater treatment plants (WWTPs) could be an important source of microplastic pollution in coastal waters. Influent and effluent discharges from three WWTPs in Mersin Bay, Turkey were sampled at monthly intervals over a one-year period during 2017. When data from all WWTPs were combined, fibers constituted the dominant particle form, accounting for 69.7% of total microplastics. Although notable oscillations in microplastic particle concentrations were observed throughout the year influent waters on average contained about 2.5-fold greater concentrations of microplastics compared to the effluent waters. An average of 0.9 microplastic particles were found per liter of effluent from the three WWTPs amounting to around 180 × 10^6 particles per day to Mersin Bay. This shows that despite their ability to remove 55–97% of microplastics, WWTPs are one of the main sources of microplastics to the northeast Mediterranean Sea.

1. Introduction

Annual global plastic production exceeds 336 million metric tons in year and is expected to increase at an annual rate of 4% (Plastic Europe, 2018). With growing plastic production, plastic litter in the environment is increasing and accumulating in various environments, including marine habitats (Andrady, 2011). Similar to other regions, coastal marine litter in the northeastern Mediterranean consists of 60–80% of plastics (Aydın et al., 2016). Over the past decade, there has been increased awareness of micro-size plastic litter found in the marine environment. Particles which are very small (< 5 mm) termed as microplastics (Thompson et al., 2004; Barnes et al., 2009).

Experimental studies have shown that many marine invertebrates such as bivalves, echinoderms, amphipods and zooplankton ingest microplastics (Browne et al., 2008; Cole et al., 2013) which can be transferred through the food chain from prey to predator (Setälä et al., 2013). In different regions of the world, microplastics (MPs) have also been found in the digestive systems of many species of fish (Foekema et al., 2013; Zhu et al., 2019; Garnier et al., 2019). In Mersin Bay, Turkey, of 1337 fish specimens (belonging to 28 species), 58% had microplastics in their digestive system (Güven et al., 2017). Besides direct harm to many marine organisms, one other main concern is that the plastics entering the food chain can ultimately pass to humans through the consumption of edible fish and shellfish.

Accumulation of microplastics in surface waters (of lakes, ponds, estuaries and seas) is reported to originate from a variety of sources such as domestic and urban activities, industrial sources and roads (mainly through tyre degradation) which could often end up in wastewater treatment plants (WWTPs) in many densely populated coastal cities (Faure et al., 2012; Sadri and Thompson, 2014; Lechner et al., 2014; Estahbanati and Fahrenfeld, 2016). Processed municipal wastewaters contain, for example, synthetic textile fibers from household laundry and abrasive plastic particles from cleaning agents (Browne et al., 2011). In addition, almost all microplastics break down into even smaller pieces further contributing to the microplastic pool that may reach the WWTPs through city liquid-waste collection systems.

Microplastics concentrations in the influent of wastewater treatment plants could vary substantially ranging from 10^3 to as high as 10^8 microplastic particles/m^3 (Carr et al., 2016; Murphy et al., 2016; Hidayaturrahman and Lee, 2019). It has been reported that the microplastic removal efficiencies of wastewater treatment plants are rather high with values of 80–95% (Magnusson and Wahlberg, 2014; Carr et al., 2016; Leslie et al., 2017). However, overall, the number of discharged microplastics in effluent could still be very significant despite such high removal efficiencies.

The Mediterranean Sea which is the largest sea in the world and

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surrounded by 22 countries is susceptible to high levels of pollution due to its huge coastal population. Marine debris, including microplastics, is a particularly important problem for the unique biodiversity of the Mediterranean (Deudero and Alomar, 2015; Pedrotti et al., 2016) which is further aggravated by a low level of environmental awareness in particular among populations living in the eastern and southern coasts. Although other kinds of pollution studies have been investigated for decades in the Mediterranean (e.g. at least 50 years for the metal pollution) (Fernex and Migon, 1994; Kütting, 1994; Marmer and Langmann, 2005; Ochoa-Hueso et al., 2017), microplastic pollution studies are relatively new (Pedrotti et al., 2016; Abidli et al., 2018; Giani et al., 2019; De Haan et al., 2019), particularly in the eastern Mediterranean (relevant references cited in the next paragraph). Therefore, any studies on microplastics from wastewater treatment facilities should be very important for the determination of sources of microplastics in the Mediterranean Sea.

Despite several published studies reporting microplastic levels at the sea surface, in sediment and the digestive systems of fish from Turkish coasts (Gündoğdu et al., 2017; Gündoğdu et al., 2018; Güven et al., 2017), so far there is only one published study on wastewater contribution to the microplastic load in the eastern Mediterranean Sea based on only one-week sampling at two WWTPs in Adana, Turkey (Gündoğdu et al., 2018). The amount of unretained microplastics from WWTPs is expected to differ depending on many conditions, such as their level, capacity, location, region as well as season, rainfall, urban waste etc. (Conley et al., 2019). For example, due to large increases in seasonal populations, WWTPs must operate at their highest capacities in the summer which may decrease their efficiency to retain the microplastic load. Moreover, calculation of total load from WWTPs to a specific marine region requires sampling from different areas and seasons. Therefore, it is also important to analyze the spatial-temporal changes in microplastic composition.

The aim of this study is to evaluate abundances and forms/types of microplastics in influent and effluent waters from three main WWTPs of Mersin, in Turkey, in order to understand their role in microplastic pollution in the northeastern Mediterranean.

2. Materials and methods

2.1. Sampling area and wastewater treatment plants (WWTP)

Mersin Bay, Turkey is located on the northeastern Mediterranean Sea, and together with neighboring Iskenderun Bay to the east is one of the most populated regions both in Turkey and the eastern Mediterranean. In this study, wastewater samples were collected monthly from three municipal treatment plants namely Karaduvar, Tarsus and Silifke located around Mersin Bay during 2017. Collectively the above-mentioned WWTPs treat approximately 90% of Mersin Greater Municipality's wastewater (Fig. 1 and Table 1).

The first treatment plant (i.e. Karaduvar) applies tertiary treatment processes, which include screening (mesh size of 6 mm), ventilated sand and an oil chamber, preliminary sediment tank biological and chemical phosphorus removal units, aeration tanks, final sediment tank followed by deep sea discharge (2 km offshore, 10 m contour depth at sea). The other two treatment plants (Tarsus and Silifke) apply secondary treatment processes, which includes screening (mesh size of 6 mm), preliminary sediment, aeration tanks and final sediment tanks. While effluent from the Karaduvar WWTP is discharged directly at sea, effluent at the Silifke and Tarsus WWTPs is first discharged to the Göksu and Berdan rivers, respectively, at river points about 12 km before reaching to the sea. Scheme of WWTPs were given in Fig. 2.

2.2. Sampling method

Wastewater samples were collected from the influent and effluent of WWTP's during one year at monthly intervals from January to December. Samples (each 10 L volume) were collected in triplicate (with the exception of Karaduvar sampling in January, which was only one replicate of 10 L).

2.3. Particle filtration and analyses

At the laboratory, collected samples were filtered with a standard zooplankton sampling net (mesh diameter 26 μm). In general, the standard EC guideline for processing of microplastic samples was considered (Euoropean Commission, 2013). Hydrogen peroxide (35%) was used to remove organics on the filter prior to microscopic observation and counting.

The detected particles under microscope were categorized as one of the following 4 major forms: fiber, soft plastic, hard plastic and others (styrofoam or polystyrene, rubber, etc.). A small force is applied with the help of forceps to separate the soft plastics from the hard ones and the classification is made according to the reaction of the particle. It is thought that it will be easier to identify the source of the plastic by classifying in this way. For example, while hard plastics are often part of a bottle cap or similar hard macroplastic product, soft plastic often comes out of shopping bags or local greenhouse material. Microplastic particles in each form were further sub-divided into categories based on colour. All filters were visually examined under a digital optical microscope (Olympus SZX16 Stereomicroscope) with maximum magnification of 30×. All microplastics and non-plastic particles (including pieces of wood, paper and metal) were collected and transferred to a clean grid filter in a petri dish. Photos were taken using a DP26 - Olympus 5.0 MP High Color Fidelity Microscope Digital Camera and all particles were further examined for secondary visual classification. After discarding non-plastic natural particles, microplastic particles were processed using Olympus cellSens (Image Analysis software) in order to determine the diameter/length of each individual particle. Only pieces of plastic litter with a diameter < 5 mm were considered as microplastic while pieces with a diameter > 5 mm were excluded from any further analysis.

The amounts of microplastics (MPs) discharged per day by each WWTP were calculated as the microplastic concentration determined (particles/L) multiplied by the WWTP capacity (L/day).

Some of the particles were also analyzed with a Spectra-Tech IR-Plan microscope coupled to a Bomem ABB FTLA Fourier transform infrared spectrometer at the Scientific and Technological Centers of the University of Barcelona in order to confirm the particles as plastic and identify their polymer type. In this way, error originating from the presence of non-microplastic particles was removed (Lenz et al., 2015). On average 15–20 microplastics (from a total of 50 MPs) were selected from each WWTP for FTIR analyses according to Lares et al. (2018). Samples were taken to produce the spectra with wavelengths between 600 and 4000 cm^{-1} with a spectral resolution of 4 cm^{-1}. Spectra were compared to the spectra libraries supplied by Perkin Elmer including polyethylene (PE), polypropylene (PP), polyamide (PA), polyvinyl chloride (PVC), polystyrene (PS), polyester (PES), polyethylene terephthalate (PET), polyurethane (PUT), and acrylic (AC).

2.4. Statistical analyses used

For testing possible differences in the particle sizes of microplastics from influent and effluent waters, a t-test analysis was used. Pearson correlation analysis was used for evaluating relationship between rainfall and microplastic quantities in influent waters of the three WWTPs. Any possible trend in monthly microplastic data was investigated using Mann-Kendall statistical test.

2.5. Contamination control

In the present study, great care at all times was taken to prevent contamination during sample collection and laboratory work. Cotton

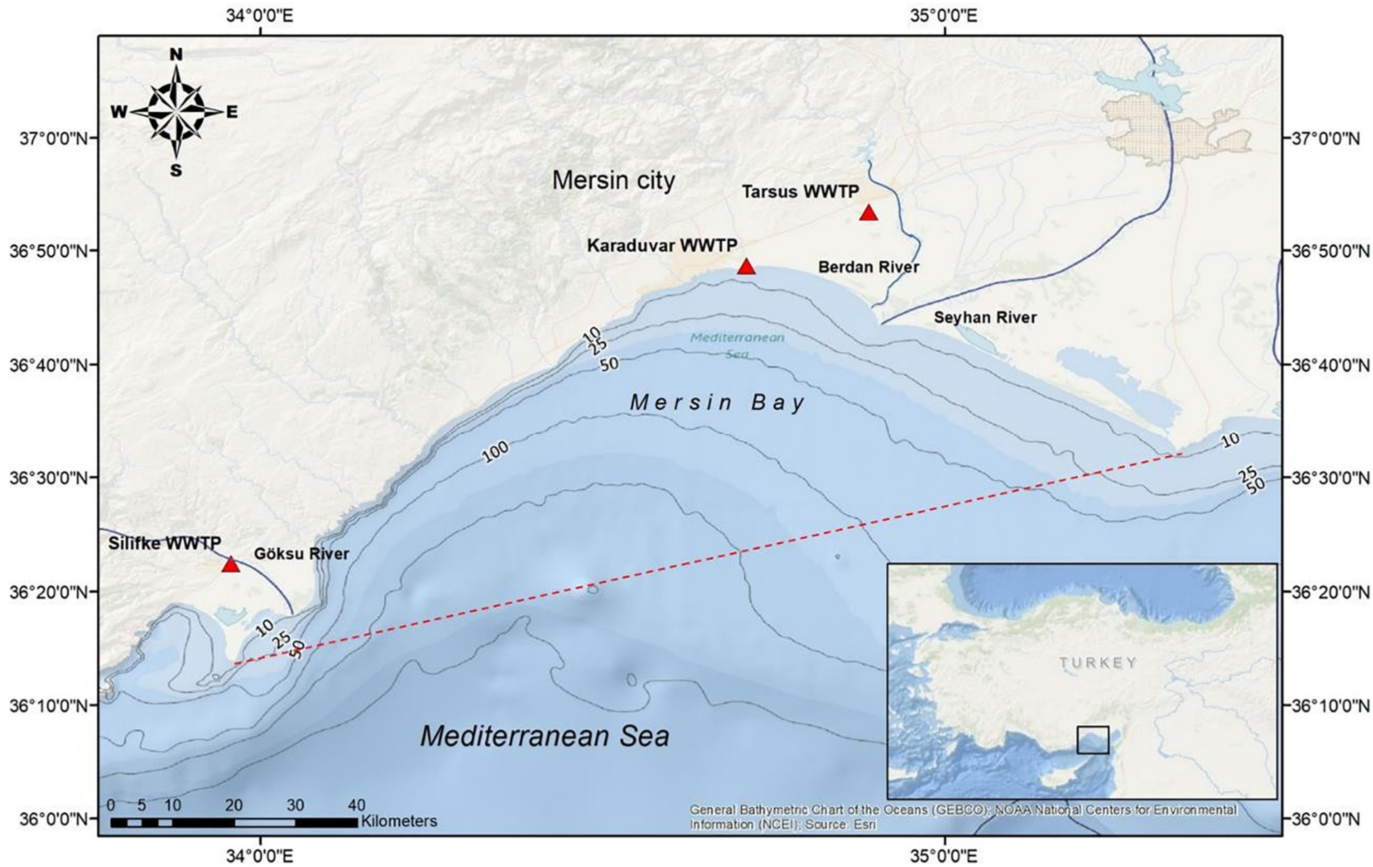

Fig. 1. Locations of the three wastewater treatment plants (WWTPs) in Mersin province, Turkey, on the northeastern Mediterranean. The dotted line shows the assumed outer border of Mersin Bay.

lab coats were worn to prevent fiber contamination (Hidalgo-Ruz et al., 2012; Güven et al., 2017; Lares et al., 2018). The filtration unit was rinsed with distilled water at all times prior to filtration. Two control samples (one in the filtration unit and one in the lab) were filtered using the same procedure as detailed for all other samples to determine the level of microplastic contamination.

Despite the precautions, contamination could not be completely avoided. As expected, only fibers were detected in the contamination control samples. These microplastics were cross-examined to separate contaminants from the detected microplastics. The number of microplastics due to contamination detected numbered between 0 and 4 for each filtering period. Contaminating particles were removed from the analysis data.

3. Results

Microplastics were detected for all months in both the influent and effluent of the three WWTPs, with equal sample volumes every month (in triplicate, 3 × 10 L), with the exception of influent sampling for Karaduvar (1 × 10 L water, no replicate). A total of 2584 microplastic particles (MPs) from influent samples and 1041 MPs from effluent samples were obtained from the three WWTPs during the entire study period. This corresponds to approximately an overall 57% removal efficiency for microplastics for all WWTPs combined. However, combined removal efficiencies fluctuated between 20 and 87% for different months (Fig. 3A). There was no clear pattern throughout the year for the removal rate, however, generally in summer months higher values were observed for the combined value from all three WWTPs. Microplastic numbers varied between 1.1 and 3.6 particles/L (in November and January, respectively) in influent waters and 0.4 and 2.2 particles/L (in May and July) in effluent waters (Fig. 3A).

When data for each WWTP evaluated separately (Fig. 3B), an average of 2.8 particles/L at Karaduvar, 3.1 particles/L at Tarsus and 1.5 particles/L at Silifke were detected in influent samples. Mean microplastic counts in effluent samples after treatment decreased to average values of 1.6, 0.7 and 0.6 particles/L in Karaduvar, Tarsus and Silifke, respectively. The highest concentrations of detected MPs in effluent waters were 5.1, 1 and 1 particles/L for Karaduvar, Tarsus and Silifke, respectively.

While microplastic concentrations in the influent of Karaduvar and Tarsus WWTPs were determined to be very similar to each other, the number of microplastics detected at the Silifke WWTP was considerably lower. It is also important to note that the volume of wastewater discharged from Silifke is 3.5 fold less than Tarsus and 12 fold less than Karaduvar. The highest microplastic concentrations were detected in Tarsus (6 particles/L in March) followed by Karaduvar (5.8 particles/L

Table 1
Daily water volume capacities and process used at the waste water treatment plants (WWTPs) sampled in Mersin province, Turkey.

WWTP name	Process level used	Capacity (m^3/day)	Approx. population served (excluding tourist numbers in summer season)
Karaduvar	Tertiary	150,000	1,010,000
Tarsus	Secondary	43,000	340,000
Silifke	Secondary	12,000	120,000

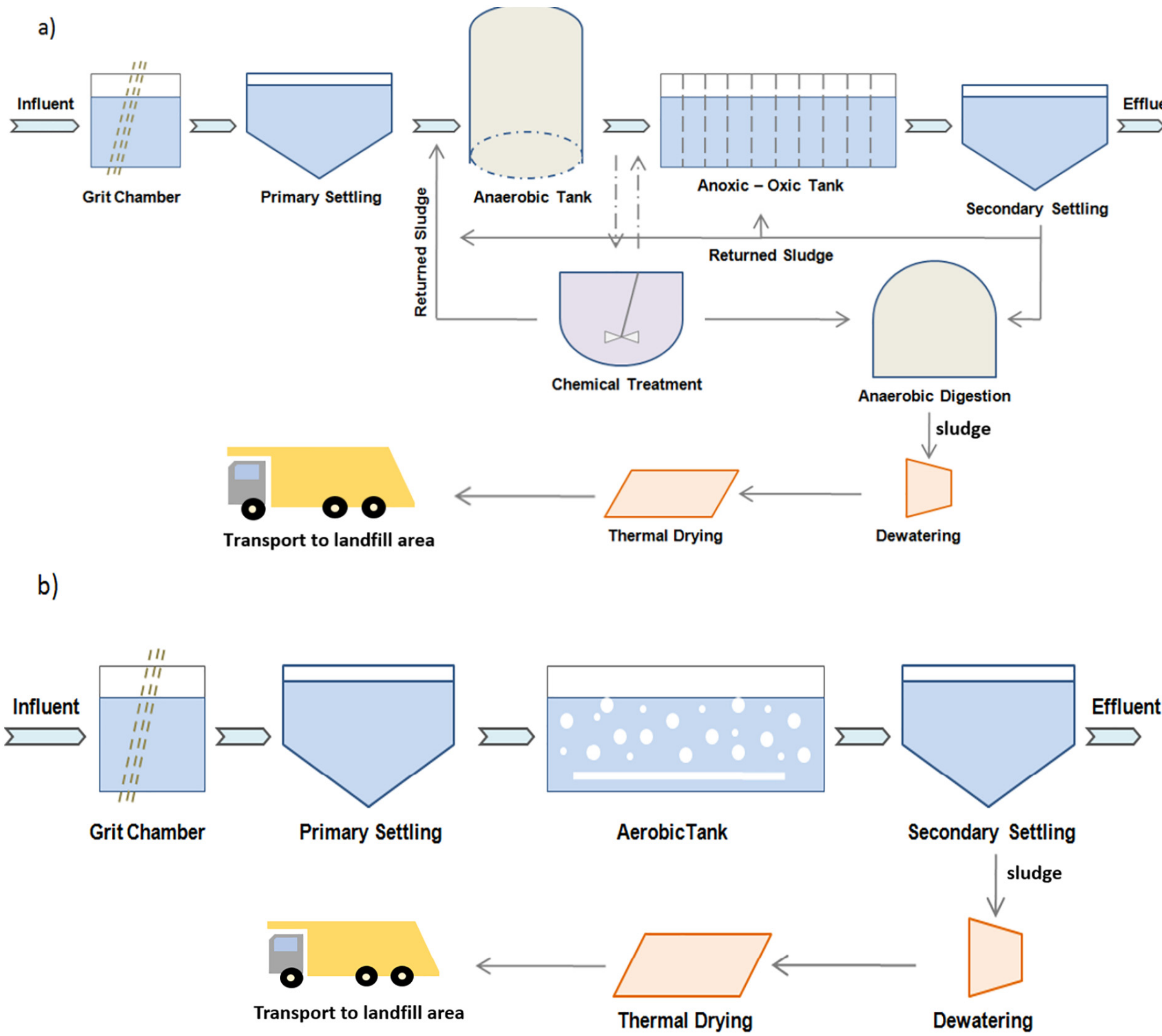

Fig. 2. Schematic diagram of Karaduvar WWTP (a, tertiary treatment), and Silifke & Tarsus WWTPs (b, secondary treatment).

in August) (Fig. 3). Similar to influent data, Silifke also displayed the lowest values for microplastics in its effluent waters (Fig. 3).

Mann-Kendall statistical analyses showed no apparent trend in monthly microplastics data for influent (Kendall's tau = −0.183, p = 0.435) or effluent samples (Kendall's tau = +0.183, p = 0.435).

When data for the individual WWTPs was evaluated separately, for certain months (in 4 out of 36 cases) influent waters were observed to display somewhat lower values than corresponding effluent waters (February and November for Karaduvar, October for Tarsus and November for Silifke WWTP; see Fig. 3B) indicating negative removal efficiencies.

In this study, visual classification disclosed mainly 4 forms of microplastics: soft, hard, and fibers; only about 0.6% of particles were categorized as "other" microplastics (rubber, styrofoam, miscellaneous etc.). Examples of microplastics detected at the WWTPs are shown in Fig. 4.

When data from all WWTPs was combined, fibers were the dominant particle form found constituting 69.7% of total, more notably in influent waters (79%), for the entire year (Fig. 5A). However, surprisingly, the share of hard plastics increased substantially (from an annual average of 7.5% to 32.8%) in effluent waters (Fig. 5A). So, while the bulk of fibers (81%, from a total of 2104 particles in the influent to 478 particles in the effluent) were retained by the WWTPs, the number of hard plastics increased in effluent waters (from a total of 200 particles to 341 particles for all samples).

The percentage share of fibers (66% of total for the year) at Karaduvar WWTP was lower compared to the other two WWTPs (95% for Tarsus and 94% for Silifke) in influent waters (Fig. 5B). The proportions of different plastic forms were also more similar between Tarsus and Silifke compared to those at Karaduvar.

Main colours of the majority of particles observed were crystal transparent, brown or black (for all data combined 71%, for all particles from the effluent only 59% or for fibers in effluent 86.6%). For hard plastics, besides crystal transparent particles, orange and purple colours were also most common (57.5% for all data combined).

Particle sizes of microplastics are shown separately for influent and effluent waters in Fig. 6A for all microplastics groups and WWTPs combined, and Fig. 6B for all microplastics groups combined for each WWTP. It was found that two- thirds of detected microplastics sizes were > 500 μm. Overall average microplastic length was surprisingly higher in effluent waters (1309 μm) compared to influent (1135 μm). A *t*-test confirmed difference in mean sizes between influent and effluent values ($p < 0.001$). Excepting the February, July and May values, average microplastic sizes for effluent waters were also higher than those for influent waters throughout the year (Fig. 6A).

Average microplastic sizes for the three WWTPs are shown separately in Fig. 6B. Microplastics identified from the influent and effluent waters of Tarsus and Silifke WWTPs were found to be at similar size range with annual average lengths between 1057 and 1095 μm. Karaduvar WWTP displayed higher values for both influent (annual

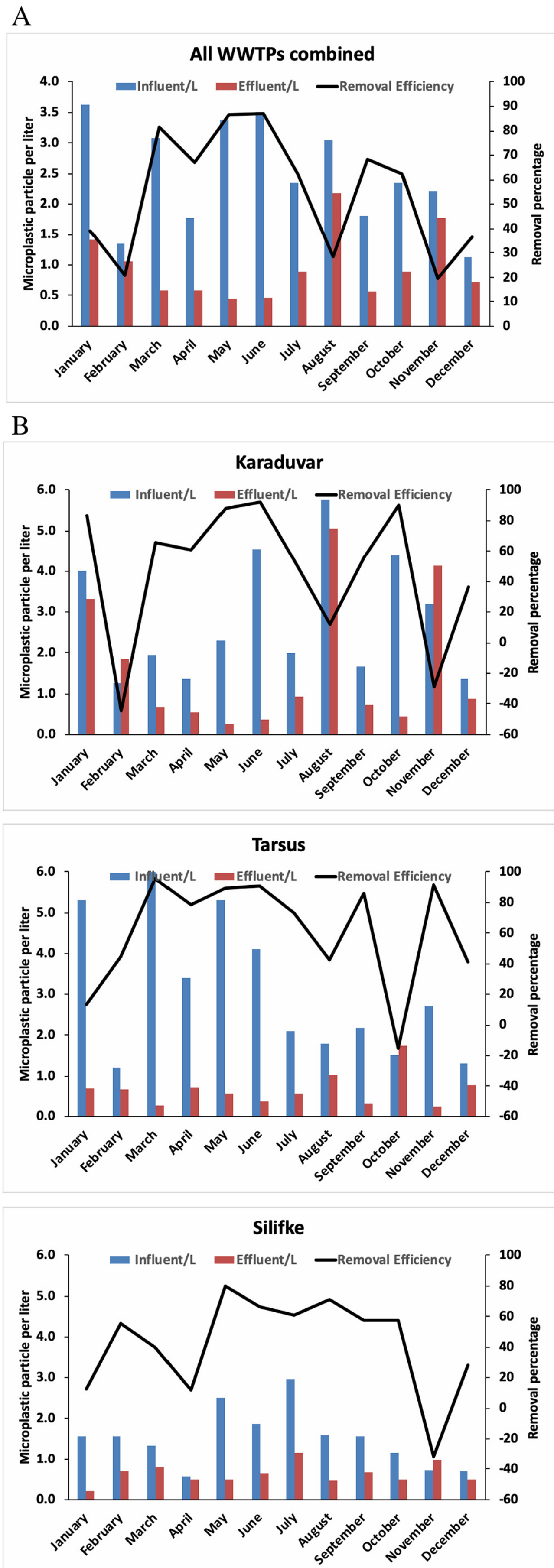

Fig. 3. A. Average monthly microplastic levels (particles/L) in influent and effluent waters sampled from all three WWTPs, for Mersin Bay, Turkey, during 2017.
B. Average monthly microplastic levels (particles/L) in influent and effluent waters from each WWTP, in Mersin Bay, Turkey, during 2017.

average length 1242 μm) and effluent waters (annual average length 1499 μm).

Of the 49 particles (obtained from effluent) examined by FTIR analysis, all were confirmed as being plastic. Among the plastic polymers identified; 51% were polyethylene (PE), 35% were polypropylene (PP), 6% were acrylic fiber, 4% were polystyrene (PS), and 4% were cellulose acetate. Twelve of the fiber particles analyzed by FTIR were composed of the 6 PP, 2 PS, 3 acrylic fiber and 2 cellulose acetate but none were found to belong to the PE group. Polyethylene (PE) and polypropylene (PP) particles had a wide range of colours, such as orange, white, yellow, transparent, blue, red etc. Only acrylic and cellulose type polymers had black colour in the FTIR subsample (Fig. 7).

4. Discussion

4.1. Microplastic form, polymer type, colour and size

For both influent and effluent samples, fibers were the dominant microplastics form in WWTPs investigated in our study. This is in parallel to findings of many other studies from the literature (Martin and Eizhvertina, 2014; Mason et al., 2016). The source of the high levels of fibers is suggested to be laundry and/or textile industry connected to the WWTPs (Lares et al., 2018).

Confirmation of polymer type in effluent was of additional significance for the potential impact at the final destination, the sea. Due to the high cost of FTIR analysis, a subsample of microplastics from effluent samples only was chosen for polymer confirmation. However, subsampling may or may not necessarily reflect the actual polymer concentrations. The percentage of microplastics confirmed by FTIR corresponds to approximately 5% in our study. This value is higher than or similar to some other studies (i.e. 1.4% in Lares et al., 2018 and 4.8% in Gies et al., 2018). Murphy et al. (2016) also undertook limited FTIR identification relying upon mainly visual analysis.

In the subsample used for FTIR analyses, the most common polymer type was polyethylene (PE), followed by polypropylene (PP), acrylic fiber, polystyrene (PS) and cellulose acetate. In contrast to our results, polymers of Mersin Bay microplastics were composed of mainly copolymers (e.g.; polystyrene: isoprene), while polymer type PE was less frequently encountered (Güven et al., 2017). However, from a total of 431 particles sampled from seawater and sediment of Mersin Bay during 2017 and analyzed by FTIR, 186 (43%) were polyethylene (unpublished data of A. E. Kideys). Polyethylene is one of the cheapest and most widely used plastic polymer types (e.g. food packaging, toys, etc.). Literature studies indicate that the probability of detected microplastics being polypropylene may be up to 27%, while the probability of being polyethylene varies between 4% and 51% (Sun et al., 2019). Twelve of the fibers analyzed by FTIR were composed of the PP, PS, acrylic and cellulose acetate groups but none were found to belong to the PE group. Similar to our findings, Lares et al. (2018) found that no fiber was in PE type. The overall conclusion from these evaluations is that the major source of PE in the marine environment is not from fibers via WWTPs but other forms of plastics, either directly from treatment plants or though riverine input or non-point sources.

Transparent, brown and black were the dominant colours observed in our study (for all samples combined as well as for effluent only or for fibers in effluent only). However, 53% of all hard plastics were black, blue or transparent. These results are somewhat similar with the results of previous studies. Fortin et al. (2019) found that the most common colour of detected MPs was black. Conversely, Murphy et al. (2016)

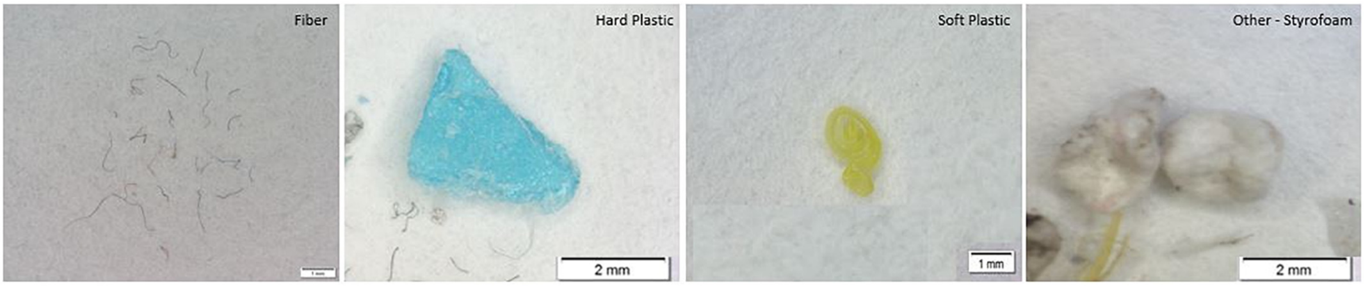

Fig. 4. Examples of microplastics detected from WWTPs of Mersin, Turkey.

reported that half of the detected MPs in sewage were red and blue. Güven et al. (2017) reported that the dominant colour was blue in the digestive systems of fishes from Mersin Bay.

In our study, two-thirds of detected microplastics sizes were > 500 μm. Similarly, in many other studies, > 70% of the detected microplastics were found to be > 500 μm (Sun et al., 2019; Dris et al., 2015; Lares et al., 2018). Overall average microplastic length was surprisingly higher in effluent waters (1309 μm) compared to influent (1135 μm). A *t*-test confirmed difference in mean sizes between influent and effluent values ($p < 0.001$). With the exception of February and May values, average microplastic sizes for effluent waters were also higher than those for influent waters throughout the year (Fig. 6A).

4.2. Microplastics in influent waters

Besides domestic waste, WWTPs receive waste water loaded with microplastics from diverse sources such as industry, agriculture, urban and highway storm water runoff. Dry and wet atmospheric fallout may

A

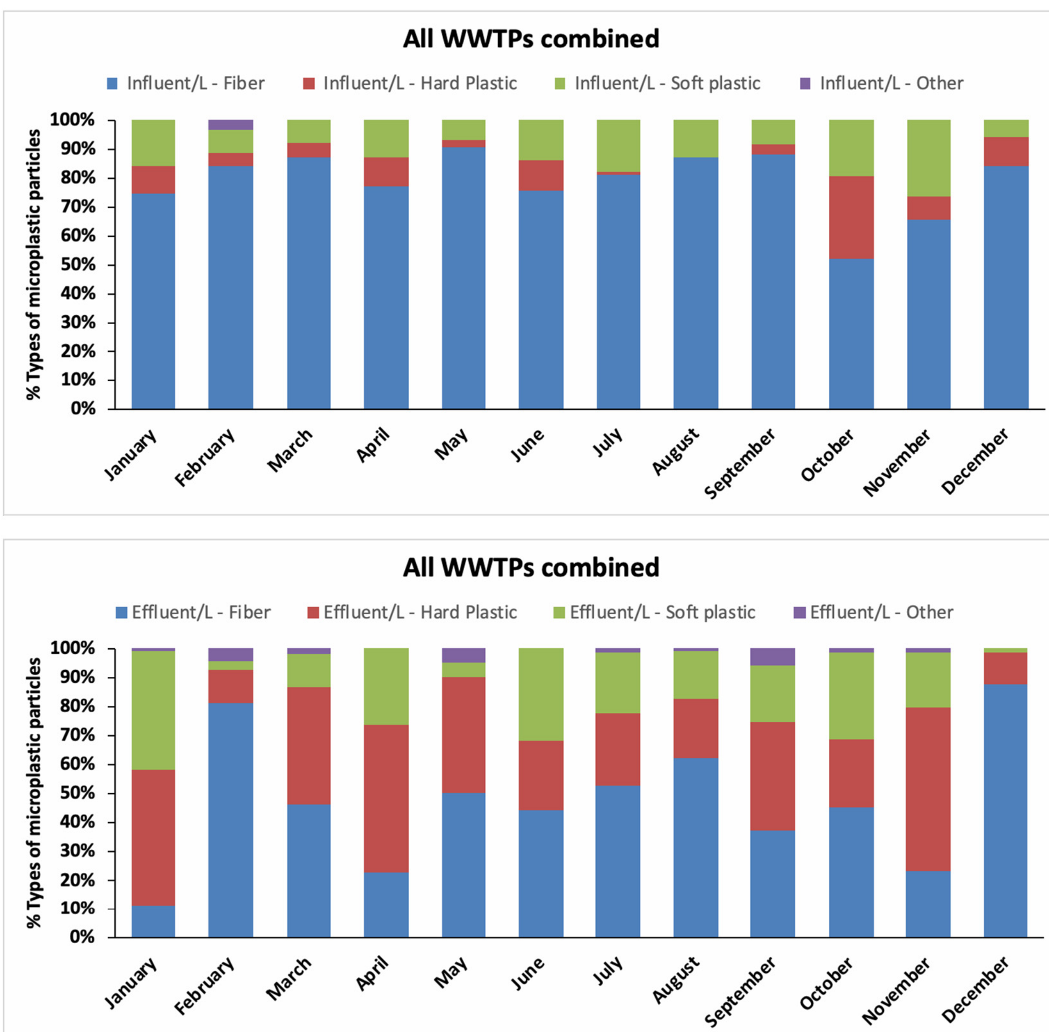

Fig. 5. A. Percentage shares of different microplastic forms in influent (top) and effluent waters (bottom) of WWTPs (all three combined), for Mersin Bay, Turkey, during 2017.

B. Percentage shares of different microplastic forms in influent (top) and effluent (bottom) waters at each of the WWTPs, for Mersin Bay, Turkey, during 2017.

B

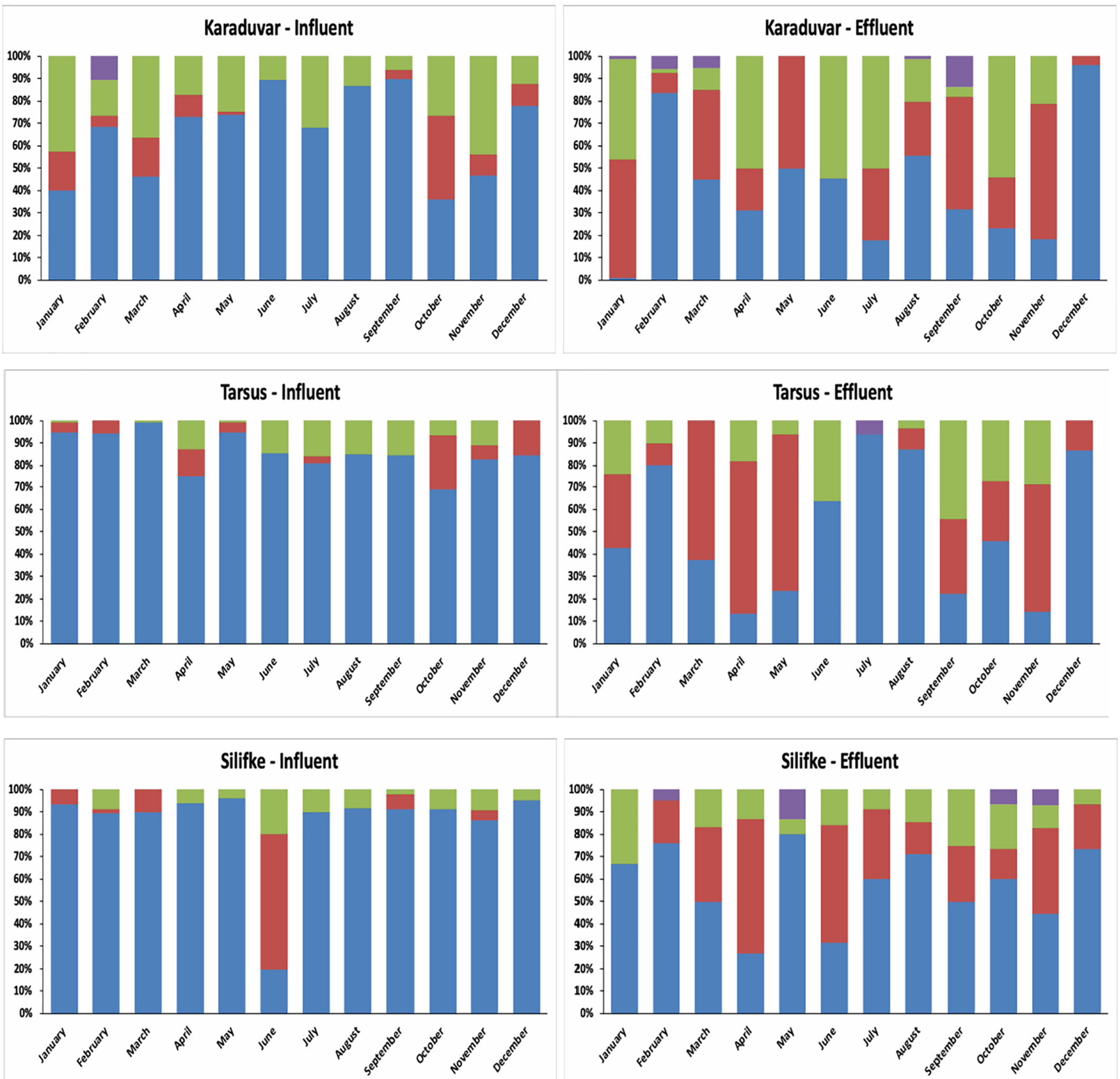

Fig. 5. (*continued*)

also contribute microplastics to waters entering the WWTPs.

Microplastic loads for the influent waters of the Karaduvar and Tarsus WWTPs were found as 3.1 and 2.6 microplastics/L, respectively, whereas waters entering the Silifke WWTP displayed lower values (1.5 microplastics/L). One of the reasons for the observed differences could be the acceptance of pre-treated wastewater from industrial facilities at the Tarsus and Karaduvar wastewater treatment plants, but absent at the Silifke WWTP. The positive effects of sewer system networks receiving pre-treated waste were also indicated in other studies for higher microplastic concentrations (Michielssen et al., 2016; Sun et al., 2019). On the other hand, the capacity of the Karaduvar WWTP and population size in the catchment area is significantly higher, which may suggest a direct link between population density and microplastics load in influent waters. However, when calculated per capita, Karaduvar WWTP generated the lowest values for microplastics emitted per day in the influent based on the approximated population served (223 microplastics/person/day), while Tarsus and Silifke showed similar values of 329 and 310 microplastics/person/day, respectively (Table 2). This may be caused by the different origins of the influent waters.

Karaduvar is located near the densely populated Mersin city center, receiving principally waters from domestic waste. In contrast, the other two WWTPs are located in rural areas, collecting waste from lower populations but with significant contributions from agriculture and runoff in particular during rainy seasons. Higher inflow rates and increased microplastic leakage per capita in the Silifke area from the sewage system due to summer tourism (because of the proximity to popular beach resorts) may have resulted in highest values observed

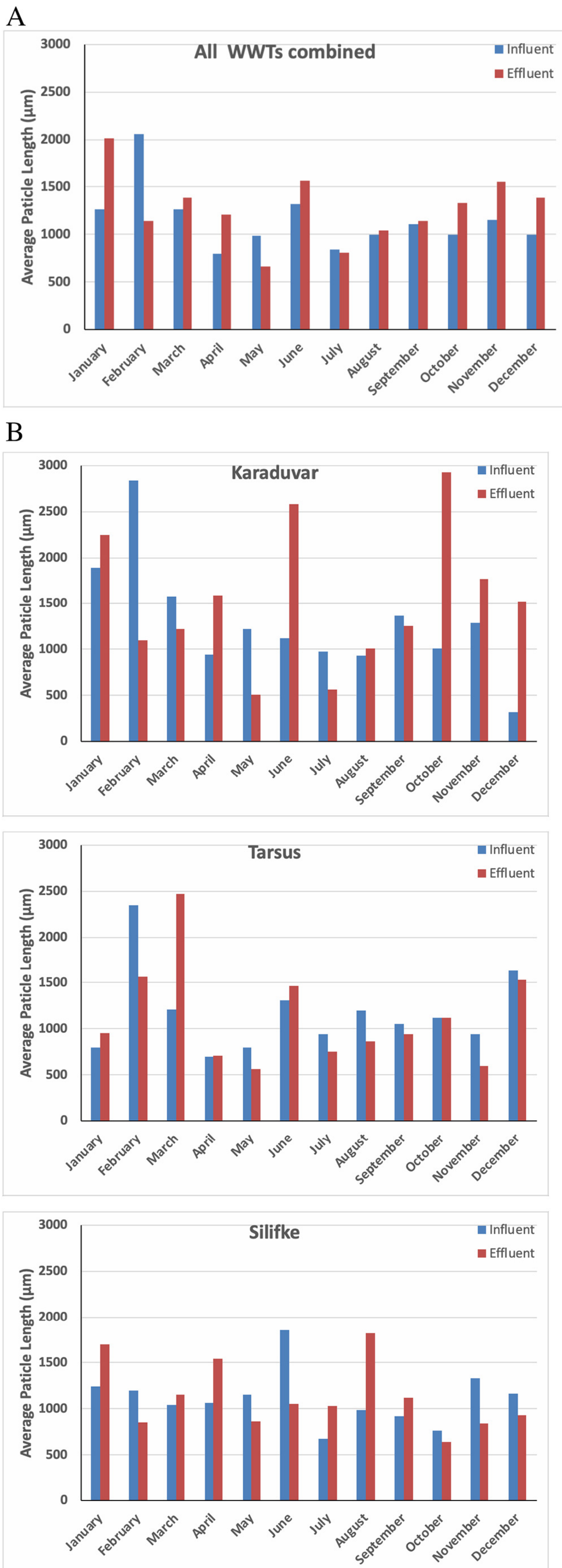

Fig. 6. A. Average monthly lengths of microplastic particles in influent and effluent waters at the three WWTPs (all data combined) for Mersin Bay, Turkey, during 2017.
B. Average monthly lengths of microplastic particles in influent and effluent waters at each WWTP in Mersin Bay, Turkey, during 2017.

here during May, June and July (2.5, 1.9 and 3.0 microplastics/L, respectively, see Fig. 3B).

A possible relationship between rainfall and microplastic quantities in influent waters was evaluated for the three WWTPs studied here. While no significant statistical relationship was determined between these two parameters for the Karaduvar and Tarsus plants, microplastic particle concentrations in the influent water of Silifke WWTP correlated significantly with rainfall values for the same region (Pearson correlation 0.83, $p < 0.001$; Fig. 8). Again, this supports the conclusion that storm runoff may be a significant contributor to the microplastics load in influent waters outside densely populated areas (Magnusson et al., 2016).

4.3. Microplastics in effluent waters and removal efficiencies

The average microplastic concentrations in effluent waters ranged between 0.6 and 1.6 microplastics/L which are within the range of findings in other studies (Table 3). While microplastic concentrations were significantly higher than those reported for many countries (Magnusson and Norén, 2014 for Sweden; Carr et al., 2016 for the USA; Ziajahromi et al., 2017 for Australia), they were determined as being lower than those found for example in the Netherlands (Leslie et al., 2017), Germany (Mintenig et al., 2017) or Slovenia (Kalčíkováa et al., 2017).

A short-term study (carried out during a single week in August) conducted in a neighboring region (Iskenderun Bay) also reported higher MP values than those found in our study (Table 3). Interestingly, this value is very similar to the three values recorded in August at the three WWTPs of our study (Fig. 3A) which suggests that microplastic sampling during short-time scales may not be sufficient to present valid annual loads from WWTPs.

The microplastic removal efficiencies of WWTPs determined range from 40% to 99% (see Table 3). In this 12-month study, removal efficiencies of the WWTPs varied throughout the year. Despite having a tertiary treatment system, Karaduvar WWTP demonstrated a lower removal efficiency (38%) compared to Silifke (58%) and Tarsus (78%) which both use a secondary treatment process. In general, WWTPs with tertiary treatment processes displayed somewhat lower microplastic concentrations in the effluent waters than those with only primary or secondary treatment processes (Sun et al., 2019). However, studies have also shown that tertiary treatment in some WWTPs did not further decrease microplastics concentrations in effluent waters (Mason et al., 2016; Mintenig et al., 2017). In our study microplastics removal rates were not determined at the end of each process of the WWTPs, hence removal efficiencies may be overestimated. However, it should be noted that recent studies have reported that up to 45% of microplastics from 100 µm to 5000 µm size range were removed by primary treatments in various WWTPs (Dris et al., 2018; Murphy et al., 2016; Sutton et al., 2016), probably by sinking flocculating particles or floating grease and oil. Secondary treatments were found to further remove 50% of microplastics from wastewater, by chemical flocculants and bacteria settling in the clarification tanks (Mintenig et al., 2017; Talvitie et al., 2017; Ziajahromi et al., 2017). Microplastics smaller than 500 µm were also shown to be completely removed from the secondary treatment effluent (Mintenig et al., 2017; Talvitie et al., 2017; Ziajahromi et al., 2017). Consequently, only about 2% of total microplastics entering via influent waters were detected in the effluent of tertiary/advanced level treatments. These studies show that microplastic removal efficiencies of WWTPs could be more related with other external factors rather than

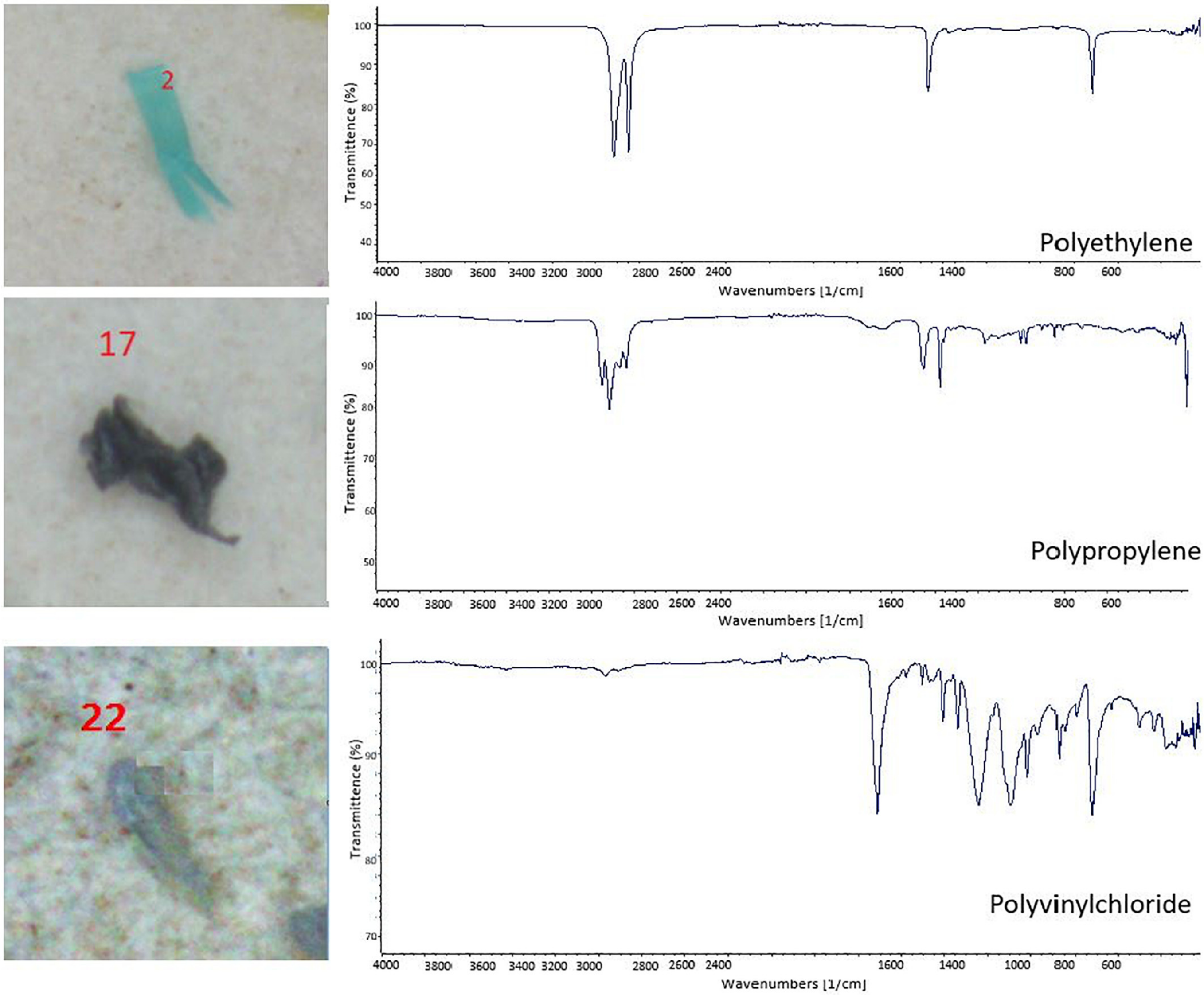

Fig. 7. Fourier transform infrared (FT-IR) spectra of dominant microplastics including polyethylene (top), polypropylene (middle) and polyvinylchloride (bottom) from Mersin WWTPs in 2017.

Table 2
Daily water volume, approximate population served, microplastic abundance in inflow waters and calculated inflow rate per capita at the waste water treatment plants (WWTPs) in Mersin province, Turkey.

WWTP	Capacity (m^3/day)	Population (excl. summer tourists)	Abundance inflow (microplastics/L)	Inflow rate (microplastics/person/day)
Karaduvar	150,000	1,010,000	3.1	223
Tarsus	43,000	340,000	2.6	329
Silifke	12,000	120,000	1.5	310

levels of treatment processes between secondary and tertiary systems.

For certain months (4 out of 36 cases) negative values were observed for removal efficiencies (i.e. February and November for Karaduvar, October for Tarsus and November for Silifke WWTP; see Fig. 3B). The fact that these are mainly rainy months (autumn season) suggest that high runoff during sporadic events may displace some of the microplastics retained for longer times in the WWTP, unbalancing monthly entrance-exit values. In addition, changes in the composition of microplastics (i.e. forms and sizes) could be other reasons for the observed differences in removal efficiencies among the WWTPs throughout the year. While the length of fibers and hard plastics in effluent waters was not smaller than that in influent, it was indeed slightly shorter for the soft plastic particles (1560 μm in effluent waters vs. 1636 μm in influent waters) (Fig. 9). The duration of wastewater treatment (from entry to plant and exit) yielded up to maximum values of 12 h for the three studied WWTPs indicating that the sampled influent and effluent waters could not be compared directly. Therefore, some inherent differences between the samples of influent and effluent waters should be expected causing disparity in results.

4.4. Microplastic discharge to the Eastern Mediterranean Sea

Effluent from the Karaduvar WWTP is directly discharged to the sea (2 km distance to shore at a 10 m depth contour) while effluent waters

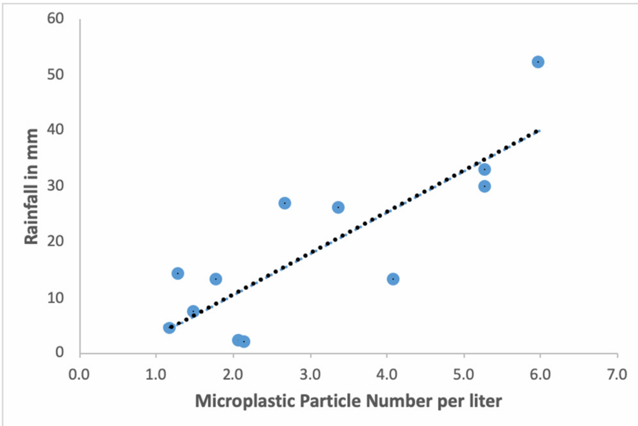

Fig. 8. Relationship between rainfall and monthly microplastic levels in influent waters of Silifke WWTP during 2017.

of Silifke and Tarsus plants are first discharged to Göksu and Berdan rivers respectively, then to the sea. These two rivers have a high flow rate and hence there is possibility that most, if not all, microplastics could be transported 12 km to the sea. Whether they arrive via rivers or via a sea bottom pipe at 10 m, some microplastics may be sedimented (especially fibers; Gökdağ et al. 2017) while some circulate in the water column or sea surface under the influence of currents occurring in the Bay.

The enormous quantities of water discharge from the three WWTPs, coupled with their relatively low microplastic removal efficiencies result in a significant input of microplastics to the Mersin Bay in the northeastern Mediterranean. Considering the daily capacity values given in Table 1, the microplastic input from the Karaduvar WWTP amounts 240 million microplastics/day (Table 4). Corresponding values for Tarsus and Silifke WWTPs are 30.1 and 7.2 million microplastics/day, respectively. Therefore, the total number of microplastic particles entering Mersin Bay from the three WWTPs investigated in this study amounts to 277.3 million microplastics/day or > 100,000 million microplastics/year, mostly of polyethylene and polypropylene types.

With an average concentration of 172,723 microplastic particles/km^2 at the sea surface, 3.4 microplastic particles/m^3 in the water column, and 274 microplastic particles/L in the sediment (Güven et al., 2017; Gökdağ, 2017), Mersin Bay has already been classified among one of the most polluted regions in the Mediterranean Sea.

Mersin Bay covers an area of approximately 3500 km^2 (see Fig. 1), therefore assuming an average depth of 50 m; the seawater volume of Mersin Bay can be approximated as 175 km^3. Using these figures, we can deduce the total amount annual of microplastics in Mersin Bay to be 595,000 million particles. Hence, the yearly microplastic load from the three WWTPs to Mersin Bay equals about 17% of total MP particles already in the bay. This indicates that the Mersin WWTPs are a very significant source of microplastics pollution in the northeastern Mediterranean. It is thereby alarming to consider that such an enormous number of microplastics particles are discharged from a mere 100 km stretch of this shoreline.

5. Conclusions

Our study establishes that the three WWTPs studied here receive significant amounts of microplastics from their surrounding environments. While MP concentrations obtained from influent waters of Karaduvar and Tarsus WWTPs were determined as being very similar, the number of microplastics detected at the Silifke WWTP was considerably lower. Among the three WWTPs, microplastic levels at only the Silifke plant influent showed close correlation with rainfall. Despite most microplastic particles being retained by the WWTPs, huge numbers were observed to reach the aquatic environment. The overall microplastic removal efficiency percentage for the three WWTPs was calculated as 57%.

Turkey is surrounded by seas on three sides with a total coastline length of > 8 thousand km. Therefore, the high number of WWTPs along the Turkish coastline would appear to be an important pollutant source for microplastics, not only for Mersin Bay but for the entire Mediterranean Sea, due to the counterclockwise circulation of the Atlantic Ocean from Mersin Bay to the Levantine Sea and ultimately to the entire Mediterranean Sea. Our conclusion is similar to the findings of Ziajahromi et al. (2017) who suggested that although low concentrations of microplastics are detected in wastewater effluent, WWTPs still have the potential to act as a pathway to release microplastics given the large volumes of effluent discharged to the aquatic environment.

Our 12-month sampling study shows that WWTPs discharge substantial amounts of microplastics to the marine environment and hence, underlines the necessity for innovative actions to decrease this pollution source. Indeed, the most common forms of microplastics we found in effluent waters were fibers and hard plastics (79%), similar to those found by Güven et al. (2017) in sediments and digestive systems of fishes from Mersin Bay. Overall, this suggests that microplastics which enter the marine environment from effluent waters of WWTPs may represent an important threat to marine biota.

Declaration of competing interest

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

Table 3
Reported microplastic concentrations in effluent waters and removal efficiencies for waste water treatment plants (WWTPs) in the literature and for this study.

Country	Influent conc. (MP/L)	Effluent conc. (MP/L)	Removal efficiency (%)	Treatment system	Reference
Turkey (Karaduvar)	3.1	1.6 (0.3–5.1)	48	Tertiary	This study
Turkey (Tarsus)	2.6	0.7 (0.2–1.0)	73	Secondary	This study
Turkey (Silifke)	1.5	0.6 (0.2–1.2)	60	Secondary	This study
Turkey	18.75	4.5	76	Secondary	Gündoğdu et al., 2018
Australia	0.76	0.28	66	Primary-secondary-tertiary	Ziajahromi et al., 2017
Canada	31.1	0.5	98	Primary-secondary	Gies et al., 2018
Finland	6.9	(0.005–0.3)	40–99.9	Tertiary	Talvitie et al., 2017
Netherlands	5–220	20–225	72	Primary-secondary-tertiary	Leslie et al., 2017
Scotland	15,6	0.25	98.4	Secondary	Murphy et al., 2016
Slovenia	–	21.7	–	Secondary	Kalčíkováa et al., 2017
South Korea	13,813	132	99	Primary-secondary-tertiary	Hidayaturrahman and Lee, 2019
Sweden	0.8	0.00825	99	Tertiary	Magnusson and Norén, 2014
USA	0.08	0.00088	99	Tertiary	Carr et al., 2016

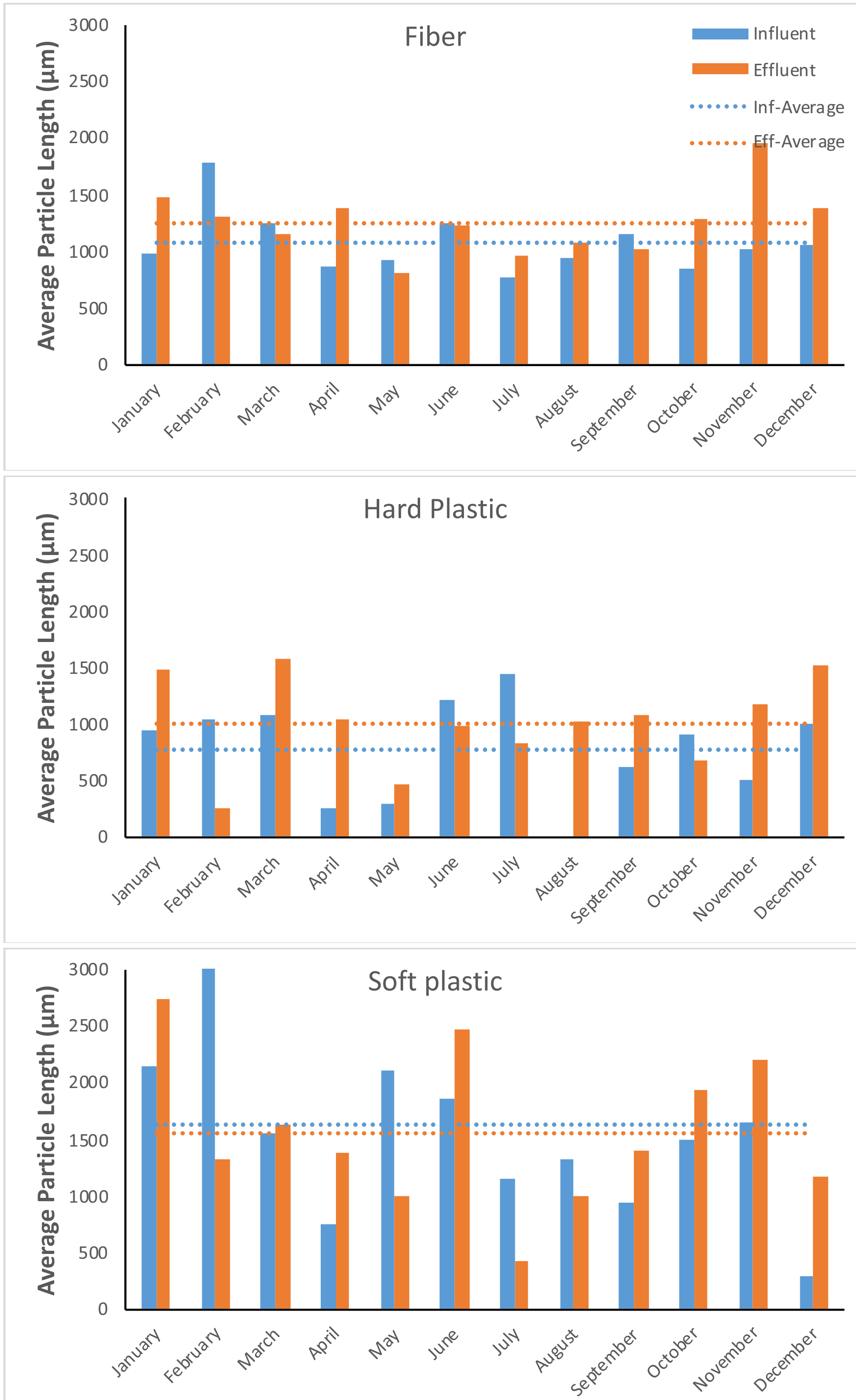

Fig. 9. Average monthly lengths of different microplastic forms in influent and effluent waters (all WWTPs combined).

Table 4
Daily water volume, microplastic abundance in effluent waters and calculated outflow rates and microplastic discharge into the Mersin Bay.

WWTP	Location of discharge points	Capacity (m^3/day)	Abundance in effluent (MP/L)	Outflow rate (MP/day) ($\times 10^6$)	Microplastic discharge in a year ($\times 10^9$)
Karaduvar	Deep sea discharge (2 km offshore, 10 m contour depth at sea)	150,000	1.6	240	87.6
Tarsus	Berdan River, at 12 km distance to the sea	43,000	0.7	30.1	11
Silifke	Goksu River, at 12 km distance to the sea	12,000	0.6	7.2	2.6
Total				277.3	101.2

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