Pollution 2021, 7(2): 405-416
DOI: 10.22059/poll.2021.317403.996

RESEARCH PAPER

No Seasonal Differences in the Emission of Microplastics from an Urban Wastewater Treatment Plant on the Southern Coast of the Caspian Sea

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Received: 16 January 2021, Revised: 03 March 2021, Accepted: 15 March 2021

ABSTRACT
This paper is the first to report on the role of a wastewater treatment plant (WWTP) in Sari, as a source of microplastics (MPs) in the Caspian Sea. Composite 270-liter/24-hour samples were taken the treated effluent of the WWTP in winter and spring, two seasons with different levels of human activity. The effluent contained 380±52.5 and 423±44.9 MPs/m^3 in winter and spring, respectively, with the total numbers of MPs/m^3 not differing between the two seasons. The dominant type of MPs in the effluent was microfibers with 237±68.7 and 328±33.4 per m^3 in winter and spring, respectively. In both seasons, fiber sizes of <300 µm were the most abundant in comparison to larger sizes. For the microparticles, sizes of <300 and ≥500 µm were the most abundant in spring and winter, respectively. The predominant types of fibers and particles were polyester and polyethylene, respectively, likely originating from the washing of synthetic clothing and from microbeads in toothpaste and cosmetics. The results of this study show that the Sari wastewater treatment plant is an important source of MP release to the aquatic environment and the difference in sampling time has no significant effect on the amount of microplastics released.

KEYWORDS: microplastic, wastewater, treatment plant, Caspian Sea.

INTRODUCTION

Annual demand for plastics worldwide is steadily increasing. It already amounted 322 million tons in 2015, and is projected to further increase in the near future (Lusher et al., 2017). It is estimated that currently between 4.8 and 12.7 million metric tons of plastic enter the oceans every year (Jambeck et al., 2015). Microplastics (MPs) are plastic pieces smaller than 5 mm in size (Raju et al., 2018), and can be distinguished into two different types, primary and secondary MPs. Primary MPs are produced in microscopic sizes (Cole et al., 2011) and Secondary MPs are generated from the decomposition of larger plastic debris released into the environment through chemical, physical or biological processes (Andrady, 2011; Hidalgo-Ruz et al., 2012). Based on their physical features, MPs are classified as particles (fragments, films, pellets, granules, lines, foams and beads) and fibers (Magni et al., 2019).

MPs have spread all over the world and are of increasing environmental concern (Mintenig et al., 2017; Wu et al., 2017). These small plastic debris have been detected in different

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environments such as soils, seas, rivers, lakes, runoff waters and effluents of wastewater treatment plants (WWTP), and also in food, water and air (Michielssen et al., 2016; Ziajahromi et al., 2017a; Smith et al., 2018; Eerkes-Medrano et al., 2019).

Due to their small size, MPs are ingested by marine and terrestrial organisms, potentially causing health problems (Michielssen et al., 2016; Miranda and De Carvalho-Souza, 2016). Another concern about MPs is related to their high potential to adsorb persistent organic pollutants (POPs) and heavy metals, facilitating their transfer into the food chain (Avio et al., 2015). Consumed MPs may enhance the transmission of harmful pollutants to organisms and ultimately affect nutrient availability. Hence, the presence of MPs in seafood is considered a potential threat to human health (Rochman et al., 2015; Carr et al., 2016; Miranda and De Carvalho-Souza, 2016).

There is a variety of entry sources to the environment, but one of the most important inputs of MPs into aquatic environments are WWTPs, which may emit large amounts of synthetic fibers and particles in the treated effluent (Murphy et al., 2016; Talvitie et al., 2017; Magni et al., 2019). In most previous studies, the sampling of the WWTPs was incidentally or momentarily (grab sample), which may not be representative of the number of microplastics released into the environment due to diurnal changes in sewage discharge. To cope with this, large volume composite sampling has been suggested to cover the intra-day fluctuations of MPs loading (Talvitie et al., 2017; Cloni et al., 2019).

Despite the great importance of studies on microplastics in the world, few studies have been carried out in this field in Iran. The available studies mainly focused on the presence of MPs in coastal sediments of the Persian Gulf in southern Iran (Akhbarizadeh et al., 2017; Naji et al., 2017a,b; Esmaili and Naji, 2018; Dobaradaran et al., 2018), wind-eroded sediments of desert areas (Rezaei et al. 2019), urban and industrial areas (Abbasi et al., 2017; Dehghani et al., 2017; Abbasi et al., 2019), and marine mussels (Naji et al., 2018; Abbasi et al., 2018; Akhbarizadeh et al., 2018, Akhbarizadeh et al., 2019; Hanachi et al., 2019). Mehdinia et al. (2020), for the first time, reported on the occurrence of microplastics in sediments of the Caspian Sea, the largest closed lake in the world, and found an average of 107.6 microplastics per kilogram sediment in the coastal area. They emphasized the need to investigate the possible sources of microplastic release into the Caspian Sea and mentioned WWTPs as one of the possible sources of synthetic fibers and plastic particles that need to be investigated.

This study therefore reports the presence of MPs in the treated effluent of the wastewater treatment plant of Sari, which discharges into the Tajan River that leads into the Caspian Sea. Considering the large number of tourists in the region, differences in the amount of water consumed and the type of consumption of tourists compared to local people, and the fact that human activity (including water use) is highly dependent on ambient temperature, sampling took place in winter and spring, as the low and high tourist seasons, respectively.

MATERIALS AND METHODS

The study was carried out at the WWTP of Sari in northern Iran, located at 36°36′18″N latitude and 53°04′38″E longitude, which is responsible for treating the wastewater of approximately 105,800 inhabitants (Fig. 1). This WWTP uses a conventional activated sludge process and discharges the treated effluent with an average flow of 22,000 m^3/day into the Tajan Rive which leads into the Caspian Sea. Since both aquatic environments have extremely ecologically important and valuable aquatic ecosystems, contaminants entering cannot only have a negative impact on the ecosystem but may also affect economic activities such as fishing and tourism.

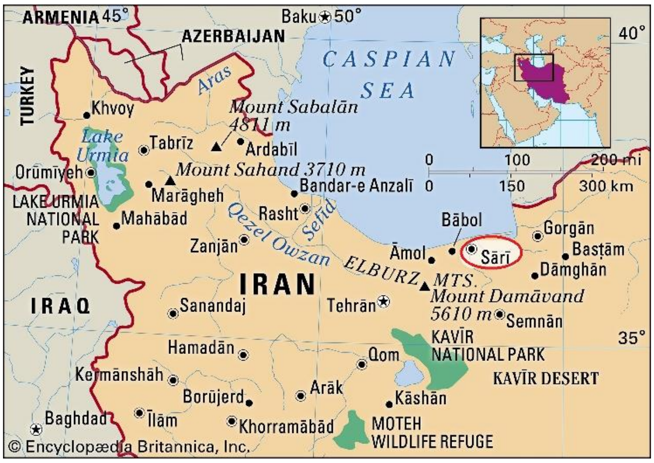

Figure 1. Sari city on the southern coast of the Caspian Sea (Britannica, 2018)

Sampling was performed in 2018, in February (winter) and April (spring) as the low and high tourist seasons, respectively. Sampling was done by taking a 24-hour composite sample of treated effluent (Sun et al., 2019). After 24 hours, a total of 270 liters (for each replicate) of effluent had passed through the sieve collection (37, 300 and 500 μm). The mesh sizes of the sieves were selected to separate the MP size classes and examine their numbers. In order to increase the accuracy of the estimate of the number of particles in the treated effluent, three replicate samples were taken on three consecutive days with the same weather conditions. The final results are based on the average of three repetitive samplings.

Each sieve was washed with 1 liter of ultrapure water and the collected matters was transferred into clean glass bottles and stored at 4°C in the dark (Mintenig et al., 2017), and transferred to the laboratory for further processing. .

In the laboratory, the glass sampling bottles were emptied into clean beakers, and the volume was concentrated to 100 mL by drying at 70°C. Then the beakers were placed on magnetic heater stirrers at 60°C and a few droplets of hydrogen peroxide (H_2O_2) solution (30%) were added to digest the small amount of organic matter (Ziajahromi et al., 2017a). After digestion of the organic matter and full hydrogen peroxide evaporation, 15 mL of sodium iodide (NaI) solution with a density of 1.70-1.75 g/cm^3 were added to the sample for density separation of the MPs from the sand particles. MPs floating in the NaI solution were collected by centrifugation and filtering the supernatant over a 37 μm screen (Ziajahromi et al., 2017a).

To avoid overestimation of MP numbers, natural particles and fibers were stained by adding 5 mL of a 0.2 mg/mL Bengal Rose solution to each screen After 5 min at room temperature, the Bengal Rose solution was washed off with ultrapure water. The samples were dried at 60°C for 15 min and visually analyzed for morphological characteristics using a stereomicroscope. Pink-stained fibers and particles, assumed to be natural organic matter, were removed from the samples after confirming their natural origin by Micro-Raman spectrometry (Ziajahromi et al., 2017a).

Blank samples were collected at the sampling site before and after sampling, and in the laboratory during sample processing to investigate any potential MP contamination. No MPs were found in the blanks which indicated that our samples were not contaminated during the transportation and analyses, including the filtration step.

After preparing the samples, the fibers and particles on each screen (except for the pink fibers and particles) were examined and counted using a stereomicroscope (KERN, OZL-45). All pieces identified as MPs were separated and divided into two groups: fibers and particles.

They were examined using a Micro-Raman spectrometer (Confocal Raman microscope, LabRAM HR Evolution - HORIBA) operating at laser wavelengths of 785 nm and 633 nm (diode). High resolution spectra were recorded in the range 400-3000 cm^{-1} to identify the type of particles and fibers, respectively, using the LabSpec6 software.

The Independent T-test and the Mann-Whitney test were applied to determine differences in the total numbers of released MPs (microparticles and microfibers) and the numbers of MPs in different size classes in the winter and spring samples. All statistical analyses were performed using the SPSS16 software.

RESULTS AND DISCUSSION

This study presents, for the first time, reliable data on the presence of microparticles and synthetic microfibers in the effluent of an urban WWTP in Iran. MPs were found in all samples taken from the Sari WWTP.

On average 380 and 423 MPs/m^3 of treated effluent were found in winter and spring, respectively (Table 1). The total number of MPs/m^3 did not significantly differ between the two seasons (Independent T-test: T=628, P>0.05).

Microscopic examination showed that fibers were the dominant type of MPs in the treated effluent, with on average (±SE) 237±68.7 and 328±33.4 fibers and 143 ±16.6 and 95.1±12.2 synthetic particles per m^3 in the treated effluent in winter and spring, respectively (Table 1). The Independent T-test showed no significant difference (T=2.1-1.2, P>0.05) between the two seasons for the total numbers of both particles and fibers. The lack of a significant difference in the total number of microplastics between the spring (high tourist) and winter (low tourist) seasons therefore is due to the absence of a difference in the numbers of microfibers and microparticles between the two seasons. The somewhat higher number of released microplastics in the April samples may be attributed to differences in people's lifestyles in winter and spring.

Table 1. The total numbers of microplastics (MP), synthetic microfibers (MFi) and plastic microparticls (MPa) per m3 (±SE, n=3) in the treated effluent of the Sari wastewater treatment plant in Iran, sampled in the spring and winter of 2018.

Season	MFi/m^3	MPa/m^3	MP/m^3
Spring	328±33.4[a]	95.1±12.2[a]	423±44.9[a]
Winter	237±68.7[a]	143±16.6[a]	380±52.5[a]

a: there is no difference in the numbers of MP, MFi and MPa between the two seasons.

The reason for the lack of difference in the number of microplastics released at different sampling times may be attributed to the operation of this treatment plant. Alavian and Hashemi (2020), in a study on the number of microplastics in the influent of the Sari wastewater treatment plant, stated that there is a significant difference in the number of microplastics in the wastewater in the spring and winter that can be due to seasonal variation in the activities of people and the activities of tourists. Although there is a significant difference in the number of microplastics in the influent for two seasons, the high efficiency of microplastics removal of the treatment plant (~97%) (Alavian et al., 2020) leads to insignificant differences in the number of microplastics in the treated wastewater in spring and winter. However, the role of tourists in contaminating the Caspian Sea through microplastic release by WWTP effluents needs further investigation.

Sari WWTP effluents showed higher amounts of microplastics compared to municipal wastewater treatment plants effluent in Scotland (250±40, 65 μm) and Sweden (8.25 ± 0.85, 300 μm) (Magnusson and Norén, 2014; Murphy et al., 2016), but much lower than the values found in urban wastewater treatment plants in Finland (1050±400, 250 μm) and China (28400±7000, 47 μm) (Lares et al., 2018; Liu et al., 2019). Also, the numbers of fibers and particles in the treated effluent of the Sari WWTP were much lower than the amounts reported for a Finnish WWTP (≥20 μm), which also used a tertiary filtration system (Talvitie et al., 2015). This may be due to a difference in the amount of MPs entering the WWTPs or a difference in the mesh size of the sieves used for sampling.

The differences in the number of MPs reported in different studies may be related to the use of different sampling (simple or composite, sampling time, mesh size, etc.), extraction (digestion and density separation method), and identification methods, but also to social, economic and climatic differences that affect the number of particles released into the sewage. At present, the comparison of the results of different studies is hampered by the lack of standardization of sampling and analytical methods (Magni et al., 2019). This illustrates the importance of developing standard protocols for the sampling and measurement of MPs, as well as developing an identical unit to report their frequency to facilitate data comparisons between different sampling sites.

In our study, fibers were the dominant MP type in the Sari WWTP effluent in spring (77.5%) and winter (62.3%). This dominance of fibers agrees with Dris et al. (2015) and Magnusson and Norén (2014). Dris et al. (2015) reported that most of the MPs in the secondary treated effluent of a WWTP in Paris were fibers. Magnusson and Norén (2014) found that synthetic fibers comprised approximately 50% of the detected MPs in a secondary WWTP in Sweden.

Although the total number of MPs per m^3 was relatively low, given the amount of treated effluent discharged daily, it does make the Sari WWTP in northern Iran an important source of MP emission to the Tajan River and potentially also to the Caspian Sea. Considering that the Sari WWTP discharges with an average flow of 22,000 m^3/d to the Tajan River, on average approximately 8.4 and 9.3 million MPs are emitted to the river per day in winter and spring, respectively. The Tajan River is one of the most important rivers in the Mazandaran province and plays a major role in providing water to a large area of agricultural land. It also is one of the habitats for native and migratory fish. The volume of MPs discharged by the Sari WWTP effluent therefore may threaten the aquatic ecosystems of the Tajan River and the Caspian Sea, and also lead to MP pollution of agricultural fields.

The release of the high volume of microfibers in the treated effluent to the Tajan River is of environmental importance as it is likely that fibers can cause problems for aquatic organisms in the river as well as the Caspian Sea. Ziajahromi et al. (2017b), for instance, found that fibers were more toxic to the cladoceran *Ceriodaphnia dubia* compared to particles. Further research is needed to assess the potential risk of the MPs released by the Sari WWTP to the aquatic ecosystems of the Tajan River and the Caspian Sea.

The numbers of MPs in the sizes 37-300 and 300-500 μm did not significantly differ (T=1.4-1.8; P>0.05), but the numbers of MPs ≥500 μm did significantly differ (T=-2.9, P<0.05) between the two seasons (Fig. 2). There was no significant difference between the number of microfibers in the different size classes between the two seasons (T=0.2-1.5; P>0.05). The number of microparticles differed only between the two seasons for the size class ≥500 μm (U=0.0, P<0.05), for the two other sizes there was no difference between the two seasons ($U^{(37\text{-}300)}$: 2.0; $T^{(300\text{-}500)}$:-0.4; P>0.05) (Fig. 2). The greater number of particles ≥500 μm in winter compared to spring may be due to fact that the sampling of treated effluent in winter coincided with the cleaning of the primary and secondary sedimentation tanks of the Sari WWTP.

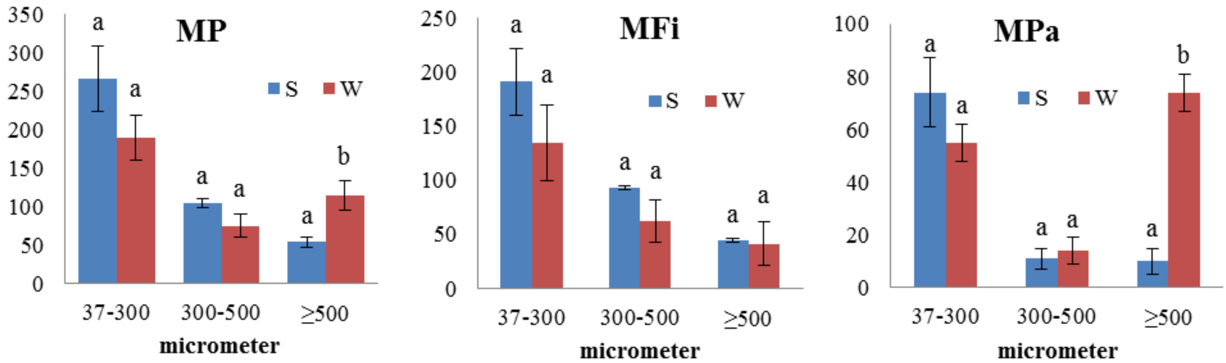

Figure 2. The numbers of microplastics (MP), synthetic microfibers (MFi) and plastic microparticles (MPa) in different size classes in treated effluent from the Sari wastewater treatment plant in Iran in Spring (S) and Winter (W). (±SE, n=3). Bars with the same letter do not differ significantly between spring and winter.

Independent T-tests showed that in spring the numbers of MPs in the three size classes (37-300, 300-500 and ≥500 µm) were significantly different (T=3.7-5.7, P<0.05). The average number of MPs in the size of 37-300 µm was significantly higher than in the other two groups, followed by the sizes of 300-500 and ≥500 µm. In winter, the number of MPs in the size range of 37-300 µm was significantly different from the size range of 300-500 µm (T=3.5, P<0.05), but the number of MPs in the size of ≥500 µm was not significantly different from the other two groups (T=-1.6-2.2, P>0.05). There was no significant difference between the numbers of MPs in the sizes of 37-300 µm and ≥500 µm, but the higher number of MPs in size 37-300 µm compared with ≥500 µm in both seasons suggests that the smaller MPs are contributing most to the emission of MPs from the Sari WWTP into the surface water (Fig. 3).

In spring, the numbers of fibers in the three sizes were significantly different (T=3.2-16.8, P<0.05) with the size range of 37-300 µm being significantly more abundant than the larger size classes. In winter, the smaller size range was also most abundant, but the differences in concentrations of the different fiber size classes were not significant (T=0.7-2.3; P>0.05) (Fig. 3). These results indicate the importance of small fibers in the treated effluent.

Among the microparticles, in spring the size class 37-300 µm was significantly more abundant compared to the larger sizes (T=4.3-4.4; P<0.05) while there was no difference in abundance of the two larger size classes (T=0.2, P>0.05). In winter, the numbers of particles in all three sizes were significantly different, with the particles with sizes ≥500 µm being significantly more abundant than the 37-300 and 300-500 µm size classes (U=0.0; P<0.05) (Fig. 3).

According to our results, most fibers and particles discharged by the Sari WWTP in both spring and winter were less than 300 µm in size, except for the particles emitted in winter. This probably can be attributed to the lower capability of processes in the wastewater treatment plant to remove fibers and particles smaller than 300 µm (Ziajahromi et al., 2017a; Talvitie et al., 2017, Alavian et al., 2020). According to Talvitie et al. (2017), the smallest sized MPs (20-100 µm) were the most abundant in primary or secondary effluents, because the larger size fractions can be removed efficiently by pre-treatment. In fact, primary treatment plays a significant role in the removal of MPs, and counts for up to 98% removal of MPs from wastewater (Talvitie et al., 2017; Yang et al., 2019). It therefore seems likely that large-sized MPs and MPs with a higher density than water are separated from the sewage during the primary treatment processes like Screen, grit, primary clarification and flotation (Zhang et al., 2020). Zhang et al. (2020) found that high-density microplastics such as PET are removed from the wastewater during primary settling. They also found large amounts of medium-density microplastics in wastewater, most of which were removed during primary treatment. Most small microplastics (less than 300 microns) are separated from the wastewater by secondary treatment (Liu et al., 2019). According to previous research on treated effluents, it however, can be stated that despite the ability of WWTPs to remove most of the microplastics, the amount of microplastics present in the treated effluent is still significant (Alavian et al., 2020).

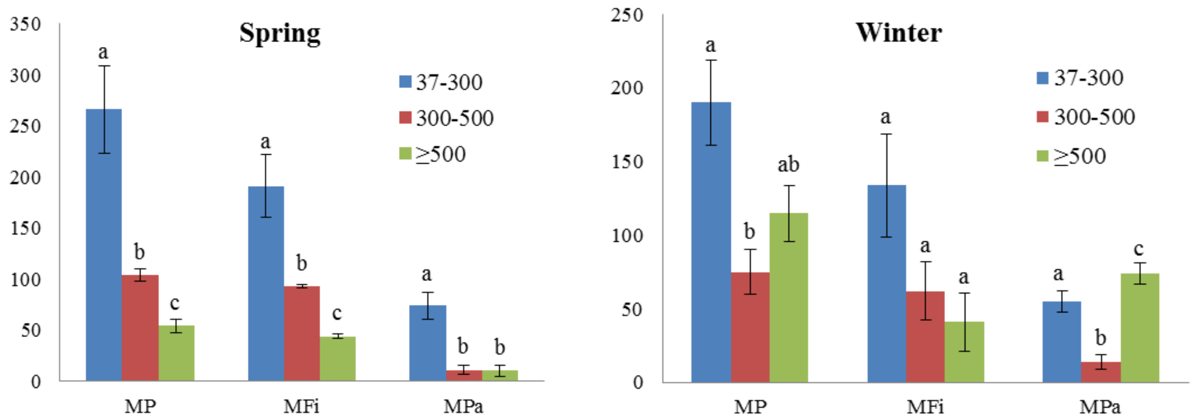

Figure 3. The numbers of microplastics (MP), synthetic microfibers (MFi) and plastic microparticles (MPa) in different size classes in the treated effluent from the Sari wastewater treatment plant in Iran, sampled in spring (left) and winter (right) (±SE, n=3). Bars with the same letter do not differ significantly between size classes.

In our study, MPs smaller than 300 µm were most abundant, followed by MPs smaller than 500 µm. This may be due to the less efficiency of the Sari wastewater treatment plant in removing particles smaller than 500 µm which leads to their large numbers in the treated effluent (Alavian et al., 2020). Mehdinia et al. (2020) found that most microplastics had a size of <500 µm and that microfibers represented the most common MP shape and had the higher MP concentrations in sediments near the mouth of permanent rivers. This is consistent with the results of the present study. The presence of a large number of microplastics with a size <500 µm in the treated effluent discharged into the Tajan River, one of the most important permanent rivers leading to the Caspian Sea, indicates the important role of the Sari wastewater treatment plant as a main source of releasing microplastics.

Different types of polymers were found in the Sari WWTP effluents sampled in winter and spring. In total, four types of polymers were identified among the collected fibers: polyester (PES) was the dominant type, followed by polyamide (PA), acrylic and polypropylene (PP) (Table 2).

Table 2. The type of microfibers in the treated effluent of Sari wastewater treatment plant in Iran

Season	**Microfibers**			
	PES (%)	**Acrilic (%)**	**PA (%)**	**PP (%)**
Winter	72	14	14	-
Spring	40	27	27	6

These fibers are likely to originate from the washing of synthetic clothing or from the carpet washing industry. Brown et al. (2011) stated that wastewater from domestic washing machines can produce >1900 fibers per garment during washing. Five kg of polyester textile (Falco et al., 2018) and one fleece clothing (Almroth et al., 2018) can produce about

6,000,000 and 110,000 microfibers, respectively. According to Hartline et al. (2016) a population of 100,000 people would produce about 1.02 kg of fibers daily through washing, which would eventually enter wastewater treatment plants. Aalipour et al. (2020) in his study on carpet washing industry wastewater in Iran showed that washing one square meter of machine-woven carpet can release between 1825 and 3098 microfibers. Since acrylic is one of the main fiber types used in Iranian carpets, the presence of this type of fibers in the Sari WWTP effluent may be due to the washing of carpets.

Morphological analysis by stereomicroscope showed that most of the microparticles extracted from the Sari effluent were spherical or irregular blue particles and mostly in the range of 37-300 μm. Micro-Raman analysis showed that the blue particles in the treated effluent were made of polyethylene and had a morphology and structure similar to fragments and microbeads from personal care products, both in winter and spring. Therefore, these particles likely are related to the use of personal care products, such as toothpaste and facial scrubs (Carr et al., 2016; Ziajahromi et al., 2017a). Except for polyethylene, which was the most abundant type of polymer for the particles, polypropylene and polyethylene terephthalate were the other particle types identified in the Sari effluent (Table 3). The abrasion of products made of these polymers may also result in the production of large numbers of such microplastics.

Table 3. The type of microparticles in the treated effluent of Sari wastewater treatment plant in Iran

Season	**Microparticles**		
	PE (%)	**PET (%)**	**PP (%)**
Winter	58	-	42
Spring	73	19	8

According to our results, a large number of particles ≥500 μm were observed in the winter effluent. Examination by Micro-Raman spectrometry revealed that all these particles were polypropylene and polyethylene. As indicated above, the abundance of particles ≥500 μm in winter may be due to the cleaning of the primary and secondary sedimentation tanks of the Sari WWTP, which released particles from equipment such as tank brushes or from the walls of the tanks. However, the origin of these particles requires further investigation.

CONCLUSION

This study showed no difference between the total numbers of MPs released by the Sari wastewater treatment plant in spring and winter. There were differences in the size and shape of the MPs in the treated effluent between the two seasons, with microfibers being present in higher numbers than microparticles. This suggests that synthetic fibers are a more important source of MPs than particles. Although the total number of MPs per m^3 was low, given the amount of effluent discharged daily, it does make the Sari WWTP in northern Iran an important source of MP emission to the Tajan River and potentially also to the Caspian Sea. Further research is needed to determine the possible consequences of this MP discharge for the aquatic and terrestrial ecosystems as well as for human health in this region. Also seasonal variation in MP emission from the Sari WWTP needs further investigation to allow more accurately assessing the amount of discharged microplastics into the environment and the possible effects of mitigation measures.

ACKNOWLEDGMENT

We would like to express our great appreciation to the Shahid Beheshti University, to Dr. Freek Ariese of the Vrije Universiteit Amsterdam, as well as the Mazandaran Water and Wastewater Company for their valuable and constructive support of this research project.

GRANT SUPPORT DETAILS

The present research has been financially supported by Shahid Beheshti University and Mazandaran Provincial Company (grant No. 3154.150).

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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