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Release of Microplastics to the Environment Through Wastewater Treatment Plants: Study on Four Types of Wastewater Treatment Processes

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Abstract Wastewater treatment plants (WWTPs) are one of the most important ways of releasing microplastics (MPs) into the environment. In this study, the size, number, color, and shape of MPs during the wastewater treatment process were investigated in six WWTPs with different processes, which include WWTPs A and B (activated sludge process, ASP), WWTP C and D (aerated lagoon, AL), WWTP E (sequencing batch reactor process, SBR), and WWTP F (stabilization pond, SP). The MP particles were detected by the polarized light microscopy. In all six WWTPs, the clear color was observed as the dominant color in the effluent. Among the forms of MPs, fibers had the highest removal efficiency in WWTPs A (97.3%), B (99.2%), C (95.5%), and D (94.3%). In both WWTPs E and F, the highest removal rate of MP shapes was related to films (96.1%) and granules (86.1%), respectively. MPs with size 25–125 μm had the highest amount (0.39 to 4.08 MP/L) in the effluent of WWTPs compared to larger sizes. With respect to the type of the wastewater treatment process, the number of MPs during the treatment process decreased from 3.75–25.31 to 0.51–6.28MP/L. Based on the results of this study, ASP with a removal rate of 91.87% had the highest efficiency compared to other processes. However, daily 4.95×10^4 to 1.49×10^8 MP enter the environment via the effluent of these WWTPs. The study recommends reducing the use of MPs and plasticizers in widely used products as much as possible and replacing them with nature-friendly materials.

Highlights

- MPs with size 25-125μm had the highest amount in the effluent of WWTPs.
- The highest MPs removal efficiency was obtained by ASP (89.4% - 91.87%).
- Daily 4.95×104 to 1.49×108 MP enter the environment through the effluent of WWTPs.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11270-023-06594-0.

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Keywords Microplastic · Wastewater · Wastewater treatment process

1 Introduction

MPs are small plastic particles the size of their particles is less than 5 mm (Abeynayaka et al., 2022; Nguyen et al., 2023; Takdastan et al., 2021; Zhao et al., 2022). These particles as environmental pollutants are of concern due to their adverse effects (Dong et al., 2022; Hajji et al., 2023; Kutralam-Muniasamy et al., 2023; Lofty et al., 2022). MPs are an anthropogenic contamination detected in oceans, rivers, and WWTP outlet. These particles accumulate in organisms and they cause chronic toxicity (Takdastan et al., 2021). The recent studies confirmed the presence of MPs in human blood, strongly supporting its high human toxicological hazard (Leslie et al., 2022).

MPs have different sources including industrial activities (microbeads, nurdles), construction activities (construction dust), road runoff, and household activities (domestic waste, laundry and cosmetics, and personal care products). A large number of MPs from the above sources enter WWTPs via the urban wastewater collection network (Chand et al., 2022; Harley-Nyang et al., 2022; Shan et al., 2022; Tadsuwan & Babel, 2022a; Wu et al., 2022; Zhang et al., 2023). WWTPs decrease the large number of MPs, but it acts as an entry point for MP particles to the environment through sewage effluent (Al-Azzawi et al., 2022; Hajji et al., 2023; Luo et al., 2023; Sadia et al., 2022).

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Estahbanati and Fahrenfeld (2016) showed that the number of MPs in the Raritan River (the USA), downstream of WWTPs, was significantly increased, indicating that WWTP effluent is an important entry point for MPs to enter the environment. WWTPs cause the transfer of MPs to the environment; despite the high efficiency of MP removal by WWTPs, they are not completely removed (Gao et al., 2023a; Khan et al., 2022; Krishnan et al., 2023; LaRue et al., 2022; Martín-García et al., 2023; Reddy & Nair, 2022).

Takdastan et al. (2021) studied the characteristics of MPs during the wastewater treatment process (ASP) in Ahvaz City (Iran). They reported that the removal efficiency of MPs in this WWTP arrived at 90.87% and the number of MPs released into the environment was about 2.419×10^7 MP/day. Naji et al. (2021) also found that MP concentration in wastewater effluent of Bandar Abbas City in Iran was 70.66 MP.35 L^{-1}.

Simon et al. (2018) found that in 10 cases from Denmark's WWTPs, the number of MPs removed ranged from 79.9 to 98.7%. However, due to the high volume of WWTPs effluent, the rate of MPs entering the environment is high (Simon et al., 2018). Tang et al. (2020) reported that MPs released from WWTPs in China is about 9.1×10^{10} MP particles per day (Tang et al., 2020), while Mason et al. (2016) estimated that about $3–23 \times 10^{23}$ MP particles per day discharged into the aquatic environment from the municipal wastewater in the USA (Mason et al., 2016).

Despite the many studies that have been done on the high efficiency of MPs removal by WWTPs, due to the different research conditions, it is difficult to compare the studied WWTPs with each other.

Liu et al. (2021) reported that the abundance of MP particles was $0.28–3.14 \times 10^4$ items/L in the influent and $0–2.97 \times 10^2$ items/L in the effluent. In addition, the removal rate of MP particles was reported as 50.00 to 99.57% (Liu et al., 2021). The studies conducted on MPs of wastewater mostly focus on the characteristics of MPs. Various factors such as population density, and wastewater treatment processes cause changes in the abundance and number of MPs (Sönmez et al., 2023; Vuori & Ollikainen, 2022). Jiang et al. (2020) compared the ASP process with the SBR process. They found that the efficiency of MP removal by ASP (96.7%) was higher than the SBR process (95.7%). In another study, Lares et al. (2018) reported that the removal rate of MP particles by the membrane bioreactor (MBR) process was higher than the ASP process. Few studies investigated the operational characteristics and the processes of wastewater treatment systems for the removal of MPs. It is essential to study the performance and removal of MPs by different wastewater treatment processes. In addition, to obtain a better treatment process to remove MPs, it is necessary to use the same analytical techniques to study the wastewater treatment processes in a region.

In this study, all WWTPs in Ardabil province were investigated for the first time to identify MPs.

Ardabil province is an economically developing city located in the northwest of Iran. This province has six WWTPs, and the effluents of these WWTPs enter either into surface water or into agricultural lands. Therefore, investigating the fate of MPs in WWTPs of Ardabil province is of great importance. To this aim, WWTPs of Ardabil province with different treatment processes were investigated to (1) determine the size, number, color, and shape of MPs as well as the daily emission of MPs from WWTPs and (2) to compare the six wastewater treatment processes to remove MPs. This study can help introduce a new perspective for the efficient control of the MPs in wastewater.

2 Material and Methods

2.1 Sampling Site

The sampling was done in August 2022 from different stages of the wastewater treatment in 6 WWTPs of Ardabil province, Iran. These WWTPs are treated by different treatment processes, which include WWTPs A and B (ASP), WWTP C and D (AL), WWTP E (SBR), and WWTP F (SP). The information on each of WWTPs is shown in Table S1.

The influent of wastewater in five WWTPs A, B, C, E, and F originates from domestic wastewater, while in WWTP D a part of industrial wastewater also enters this treatment plant. MPs in the influent of five WWTPs with domestic wastewater are mainly caused by washing textile clothes, cosmetics, and personal care products. In WWTP D, MPs of industrial sewage effluent from raw plastic materials or plastic production enter this network. In each of the WWTPs, sampling was done during the wastewater treatment process, and the sampling points are shown in Fig. 1. In total, 78 samples were collected with three replications.

2.2 Sample Collection

In each of the WWTPs, the samples were collected from different points of wastewater treatment. The samples were collected by a technician in a 20-L stainless steel bucket. Then, the samples collected from each point of WWTPs were separately passed through the steel mesh screens (Damavand Company, Iran). Four mesh screens of different sizes were used, which were placed on top of each other and fixed (Fig. S1). The sizes of these mesh screens were from top to bottom 840, 420, 125, and 25 µm, respectively (diameter: 20 cm). To avoid contamination of samples with plastics, each of the samples was covered with aluminum foil and then transported to the laboratory for analysis.

2.3 Sample Processing

The sample processing was carried out to determine the characteristics of MPs. For better detection of MPs, the organic matter of the collected samples was removed through the wet peroxidation (WPO) method (Gao et al., 2023b; Sadia et al., 2022; Wang et al., 2022; F. Yang et al., 2022). The sediment samples containing MPs were washed from the mesh screen with deionized water with a volume of about 300 mL and transferred into the glass beakers. Then, the contents of the beakers were slowly concentrated in the oven at 60 °C until their volume reaches 100 mL. To digest the organic material, 20 mL of 30% hydrogen peroxide (H_2O_2) solution was added to each of the beakers. Then, the solution containing the samples in each of the beakers was stirred at a temperature of 60 °C until all the H_2O_2 evaporated. To separate and float MPs from other materials, 9 mL of sodium iodide (≥99.5%, molar mass: 149.89 g/mol, Sigma Aldrich) solution was added to the sample content of the beakers. The samples were centrifuged at 3500×*g* for 10 min. Then, the floating particles from the surface of these tubes were transferred to glass plates (Alvim et al., 2022; De-la-Torre et al., 2022; Eibes & Gabel, 2022; Koyuncuoğlu & Erden, 2023; Ridall et al., 2023; Yang et al., 2023; Ziajahromi et al., 2021).

The use of Rose-Bengal solution (4,5,6,7-tetrachloro-20,40,50,70-tetraiodofluorescein) is a staining method that separates the plastic and the non-plastic particles from each other. The non-plastic particles were colored pink by this method. Therefore, the non-plastic particles were easily separated. Ten milliliters of 0.2 mg/mL Rose-Bengal solution was added to each of the samples,

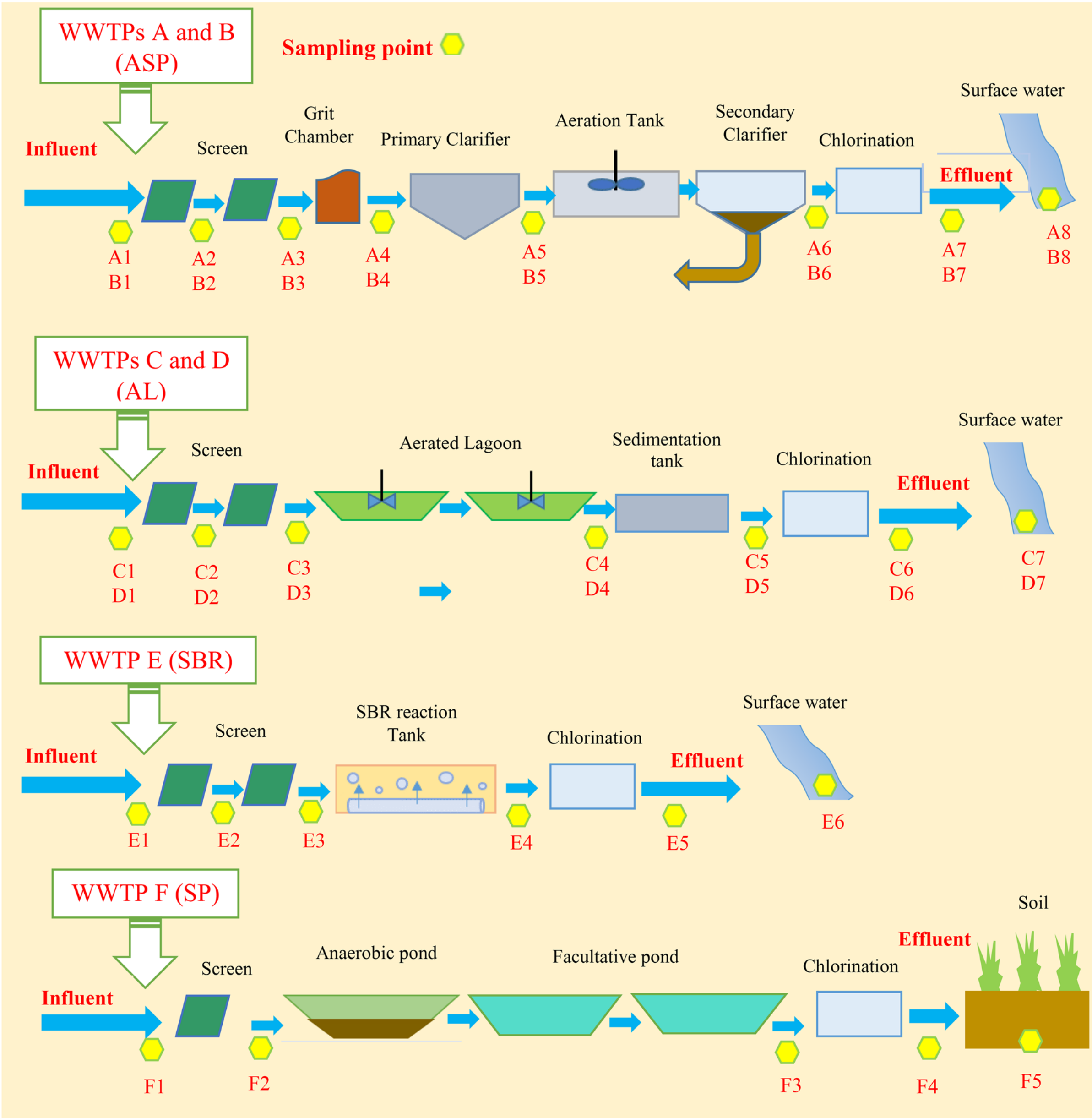

Fig. 1 The schematic of six WWTPs with locations of sampling points

and then, they were kept at a temperature of 24 °C for 10 min (Becucci et al., 2022; Kılıç et al., 2023; Mishra et al., 2022; Monteiro & Costa, 2022). The characteristics of MPs were studied by polarized light microscopy (PLM) (Olympus BX60, Japan) with applied visual sorting methods (Fig. 2). This microscope creates a clearer image of smaller MPs with the available magnifications in eyepieces (100×, 200×, 1000×).

3 Result and Discussion

3.1 Color Distribution of MPs

The color distribution of MPs collected from six different WWTPs is presented in Fig. 3, which are classified into clear, black, green, yellow, red, white, and blue colors. In all six WWTPs, the clear color (46.2%,

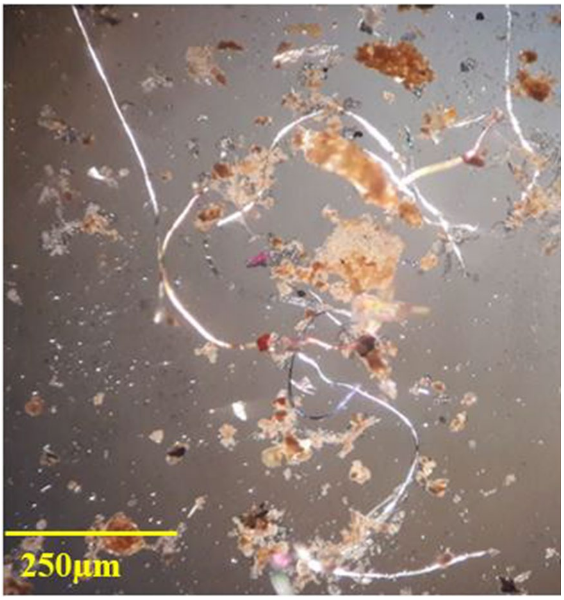

Fig. 2 MP particles found using PLM in WWTP

30.2%, 29%, 80.4%, 25%, and 47.3% for WWTPs A, B, C, D, E, and F respectively) was observed as the dominant color in the influent. After that, green was the dominant color in five WWTPs (30.3%, 26.3%, 24.1%, 22.4%, and 29.3% for WWTP A, B, C, E, and F respectively) (Fig. 3A, B, C, E, and F) and black for WWTP D with a value of 60.9% (Fig. 3D). At the sampling points of WWTPs, this proportion of the color of MPs changed a little. Nevertheless, in the effluent of all WWTPs, the highest amount belonged to the clear color. As in the reports of Takdastan et al. (2021), the clear was found as the dominant color of MPs in sewage effluent. Kinds of plastic packaging and bags can be a source of MPs of clear color (Haque et al., 2022; Sakali et al., 2022). The Wilcoxon signed-rank test demonstrated no significant differences between the distribution of colors at different stages of wastewater treatment in WWTPs. These results showed that the distribution of MP color may not be influenced by different stages of wastewater treatment.

3.2 Shape of MPs

The shapes of MPs were classified into three groups which include fiber, film, and granule (Fig. 4). The dominant shape in the influent of all six WWTPs was fiber with 63.2 to 28.3% followed by film with 36.1 to 21.1% and granule with 22.8 to 10.3%. These findings are consistent with other reports that indicated fibers were the highest rate in the influent of WWTP compared to other shapes (Kwon et al., 2022; Wu et al., 2022). The high amount of MPs in the form of fiber in the sewage can be due to the washing of clothes, so that 700,000 fibers can be released from washing 6 kg of polyacrylic clothes (Tadsuwan & Babel, 2022b). The type of wastewater treatment process can play a role in removing forms of MPs (Zöhre & James, 2022). The results of this study showed that WWTPs A and B, which are treated by ASP, had the highest removal efficiency of MP shapes. Among the forms of MPs, fibers had the highest removal efficiency in these WWTPs. In WWTPs A and B, the removal efficiency of fiber was 97.3% and 99.2% respectively, followed by film 94.2% and 96.1% respectively as well as granule 91.8% and 95.3% respectively (Fig. 4A and B). According to the reports of Takdastan et al. (2021), the highest removal rate was obtained for fibers in WWTP treated by the ASP process.

As seen in Fig. 4C and D, the highest removal efficiency was found for fiber, film, and granule respectively in WWTPs C (95.5%, 94.2%, and 93.1% respectively) and D (94.3%, 92.1%, and 91.3% respectively), which are treated by AL process. In WWTP E (SBR), films (96.1%) had the highest removal efficiency compared to other shapes, followed by fibers (92.3%) and granule (90.4%) (Fig. 4E). These results are consistent with Jiang et al. (2020) who indicated that the removal efficiency of film and foam was high in WWTP treated by the SBR process (Jiang et al., 2020). In WWTP F, the removal efficiency of fiber, film, and granule was obtained as 82.6%, 77.9%, and 86.1%, respectively. The highest removal rate of MP shapes in this WWTP, which was treated by the SP process, was related to granules and is presented in Fig. 4F.

The Wilcoxon signed-rank test demonstrated significant differences between the distribution of Fiber at sampling points one and two as well as at points two and three in WWTPs A (with p-values of 0.039 and 0.026, respectively) and B (with p-values of 0.041 and 0.035, respectively). In addition, for both film and granule distributions, significant differences were observed between sampling points 2 and 3 in WWTP A with p-values of 0.028 and 0.016, respectively. In WWTP B, there were statistically significant differences between the distribution of granule at all

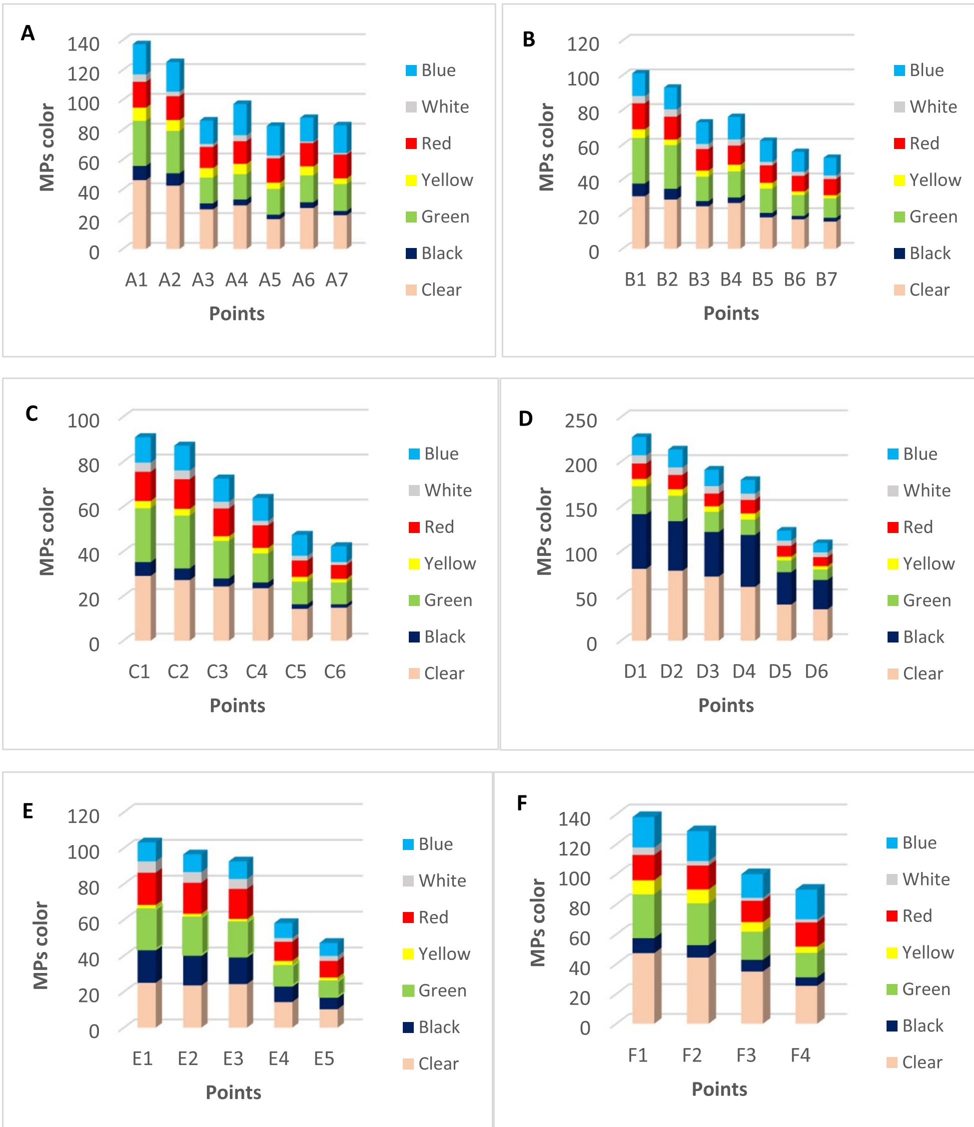

Fig. 3 Distribution of color of MPs per liter (%) during treatment processes of **A**, **B**, **C**, **D**, **E**, **F** WWTPs

sampling points. Wilcoxon signed-rank analysis also demonstrated significant differences between the distribution of all shapes of MPs at sampling points in WWTPs C, D, E, and F (Table S2).

3.3 Size of MPs

The sizes of MPs collected from sampling points in six WWTPs were divided into four groups with sizes 25–125 µm, 125–420 µm, 420–840 µm, and > 840 µm, as shown in Fig. 5. At the influent of five WWTPs of A, B, D, E, and F, the highest amount of MPs was placed in size 125–420 µm, which were 1.07 MP/L, 2.36 MP/L, 13.01 MP/L, 2.01 MP/L, and 1.81 MP/L, respectively (Fig. 5A, B, D, E, and F). In WWTP C, the majority of MPs was detected in size 420–840 µm (1.3 MP/L) (Fig. 5C). According to recent reports, the largest amount of MPs in the influent of WWTP had a size of less than 190 µm (Takdastan et al., 2021; Ziajahromi et al., 2017). In the stage after the screening, MP particles with a size of less than 420 µm increased. So that MPs with size 25–125 µm increased

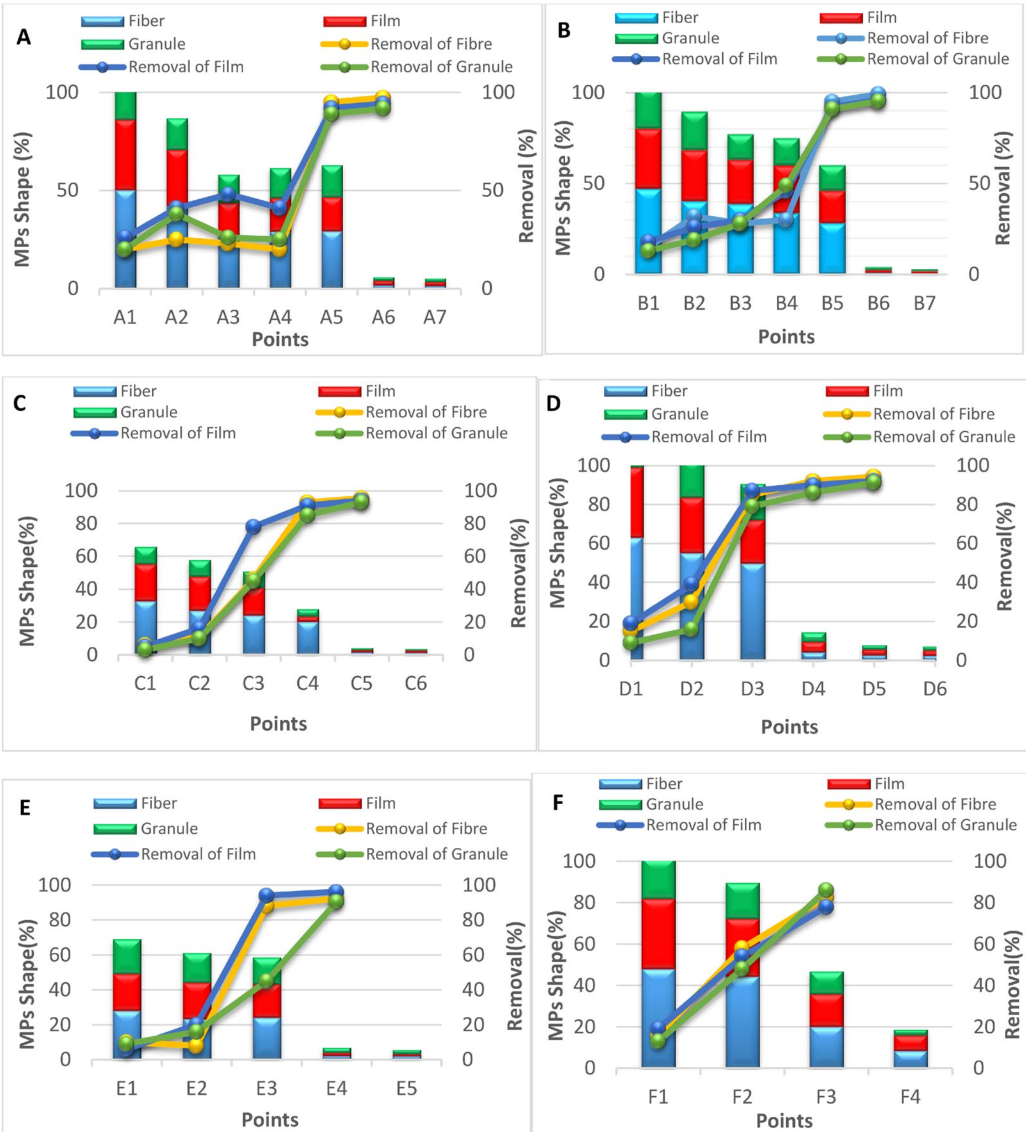

Fig. 4 The shape of MPs per liter (%) during treatment processes of **A**, **B**, **C**, **D**, **E**, **F** WWTPs

from 0.93 to 1.28 MP/L, 1.01 to 2.36 MP/L, 0.81 to 1.7, 3.6 to 8.6 MP/L, 0.91 to 1.98 MP/L, and 0.71 to 1.94 MP/L in the points of influent to screening of WWTPs of A, B, C, D, E, and F, respectively. Also, the amount of MPs with size 125–420 μm increased at the screening points relative to the influent. This can be due to the breaking of MPs in the stage after the screening, which causes an increase in the rate of small-sized plastics. In all six WWTPs, the amount of MPs with a size > 840 μm at points 4 was zero. These results are consistent with the findings of Ziajahromi et al. (2017) who showed that during the wastewater treatment process, MPs larger than 500 μm were completely removed in 3 WWTPs. In this study, in the effluent of WWTPs, the rate of MPs with size 25–125 μm for WWTPs A, B, C, D, E, and F was 0.39 MP/L, 0.46 MP/L, 0.83 MP/L, 4.08 MP/L, 0.91 MP/L, and 0.31 MP/L, respectively, and the rate of size 125–420 μm for these WWTPs was 0.12 MP/L, 0.09 MP/L, 0.4 MP/L, 2.2 MP/L, 0.06 MP/L, and 0.99 MP/L, respectively. The amount of MPs larger than 420 μm in the effluent of all six WWTPs was found

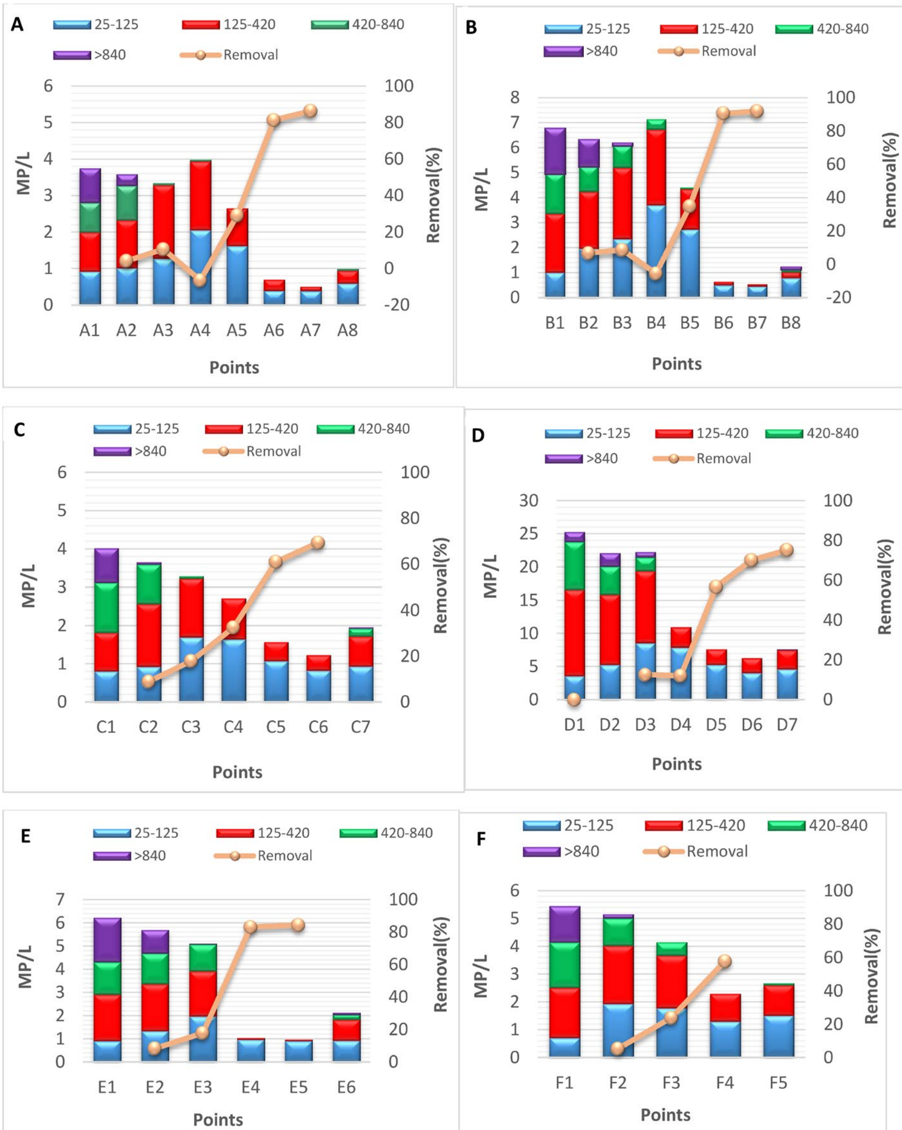

Fig. 5 Size, number, and removal of MPs during treatment processes of **A**, **B**, **C**, **D**, **E**, **F** WWTPs

to be zero and they were completely removed during the treatment process. In the final sampling points, the size of MPs increased again, so that particles larger than 420 µm were found at the points A8 and F5 for WWTPs A and F, respectively (Fig. 5A and F), as well as MPs > 840 µm were observed at the points B8, C7, D7, and E6 in WWTPs B, C, D, and E, respectively (Fig. 5B, C, D, and E). This increase in the size of the particles can be due to the re-entry of MPs from the outside environment to the final sampling points.

3.4 Comparison of MPs Number and Removal Efficiency in Six WWTPs

The number of MP particles and their removal efficiency during different processes of wastewater treatment is exhibited in Fig. 5. The number of MPs in the influent of six WWTPs of A, B, C, D, E, and F was 3.75 MP/L, 6.77 MP/L, 4.02 MP/L, 25.31 MP/L, 6.22 MP/L, and 5.45 MP/L, respectively. The rate of MPs in the influent of WWTP D was higher than

Table 1 Estimates on the daily discharge of MPs to the environment through WWTPs

WWTPs	Treatment processes	Capacity (L/d)	Number of MPs in effluents (MP/L)	Discharge (MP/day)
A	ASP	1×10^7	0.51	5.1×10^6
B	ASP	9×10^6	0.55	4.95×10^4
C	AL	8×10^6	1.23	9.84×10^4
D	AL	2.38×10^7	6.28	1.49×10^8
E	SBR	1×10^7	0.97	9.7×10^5
F	SP	1.3×10^7	2.3	2.99×10^7

the others, which could be due to the high population covered by this WWTP as well as the entry of effluent of industrial WWTPs to WWTP D. The rate of removal of the number of MPs after screening was different in six WWTPs, so that the removal efficiency was obtained 4.27%, 6.8%, 8.95%, 12.6%, 8.36%, and 5.32% for points A2, B2, C2, D2, E2, and F2, respectively. After the grit chambers unit at points A4 and B4, the number of MPs increased from 3.35 to 3.99 MP/L and from 6.18 to 7.14 MP/L, respectively. Rotary grit chamber was used in WWTPs A and B, in which the mechanical stirring in these unit can break MPs to smaller particles and thus increase their number. These results are corresponding with the findings of Jiang et al. (2020), who reported that after the grit chamber, the abundance of MPs increased from 78.5 items/L to 85.2 items/L compared to the previous unit. In wastewater treatment with the AL process (WWTPs C and D) at points C5 and D5 after the aeration and sedimentation ponds, the MP removal efficiency was found to be 61% and 70.1%, respectively. In WWTP E, the removal efficiency of the number of MPs after the SBR process (E4) was 83.1%, which is close to the reported findings (88.1%) (Jiang et al., 2020). In WWTPs A and B, which are treated with ASP process, the number of MPs at A6 and B6 was 0.7 MP/L (with a removal efficiency of 86.33%) and 0.64 MP/L (with a removal efficiency of 90.54%). The results of the study showed that the greatest amount of MP removal was obtained after secondary treatment by the ASP process. Takdastan et al. (2021) also showed that the number of MPs decreased from 9.8 to 1.3 MP/L after secondary treatment (ASP). In this study, the overall efficiency of MP removal in WWTPs is arranged as follows: B (ASP, 91.87%) > A (ASP, 89.4%) > E (SBR, 84.4%) > D (AL, 75.19%) > C (AL, 69.4%) > F (SP, 57.8%). Despite the high removal efficiency of MPs during wastewater treatment processes, due to the high volume of effluent, between 4.95×10^4 and 1.49×10^8 MP enter to the environment daily through these WWTPs (Table 1).

The Wilcoxon signed-rank test demonstrated a statistically significant difference between sampling points two and three in WWTPs C, D, E, and F with *p*-values of 0.039, 0.041, 0.386, and 0.023, respectively. In addition, a significant difference was observed between sampling points 3 and 4 in WWTPs E (p-value = 0.006) and F (p-value = 0.002). There was also a significant difference between sampling points four and five as well as five and six in WWTPs A, B, C, and D with $p \leq 0.05$ (Table S3).

3.5 MPs in the Receiving Sources of the Effluent

The number of MPs at the final points of sampling (points of entry of the effluent into the environment) was enhanced compared to the effluent of WWTPs, so that it increased in surface water from 0.51 to 1 MP/L, 0.55 to 1.22 MP/L, 1.23 to 1.96 MP/L, 6.28 to 7.63 MP/L, and 0.97 to 2.13 MP/L in points of A8, B8, C7, D7, and E6, respectively (Fig. 5A, B, C, D, and E). Also, the number of MPs in F5 (agricultural land) increased to 2.68 MP/L (Fig. 5F). Estahbanati and Fahrenfeld (2016) detected the concentration of MPs in the river that was located on the way to WWTP, and they observed that the amount of MPs in the upstream and downstream of the WWTP was 24 No/m^3 and 71.7 No/m^3, respectively. In this study, the Wilcoxon signed-rank analysis showed that there is a statistically significant difference between sampling points six and seven in WWTPs A, B, and C with *p*-values of 0.046, 0.038, and 0.039, respectively (Table S3). The USA focuses on eliminating MP particles used in personal care products, and Belgium, Austria, Sweden, and the

Netherlands are also supporting the ban on the use of MPs in these products (Stein, 2015; Graney, 2015). This action can cause the reduction of MPs in the effluent of WWTPs and as a result reduce it in the receiving waters or soil (Estahbanati & Fahrenfeld, 2016).

4 Conclusions

We investigated the size, number, color, and shape of MPs during six WWTPs with treatment processes ASP, AL, SBR, and SP, as well as determined the number of MPs in the receiving sources of this effluent. The clear color was the dominant color at the influent as well as at the effluent of WWTPs. MPs with size of larger were completely removed during the treatment process. A high amount of MPs was removed by these WWTPs. Generally, different processes of wastewater treatment and the type of operating units have a major impact on the removal of MPs. Best removal efficiency of MPs was obtained by the ASP process. However, a large volume of MPs enters the environment via the effluent of these WWTPs. Therefore, the effluent is a source of the entry of MP particles into the environment. The number of MP downstream of WWTPs increased compared to the final effluent. More research is needed to determine the rate of MPs in the upstream points of these WWTPs as well as in more points of these receiving sources of effluent.

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Data Availability All data generated or analyzed during this study are included in this published article.

Declarations

Conflict of Interest The authors declare no competing interests.

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