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Occurrence of Microplastics in Influent, Sewage Sludge and Effluent of Municipal Wastewater Treatment Plant, A Case Study Center of Iran, Qom city

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ABSTRACT

The presence of Microplastics (MPs) in the environment poses a significant threat to both humans and ecosystems health. One common source of environmental MPs pollution is the sludge and effluent discharged by wastewater treatment facilities if no specific measures are implemented for post process MP removal. The purpose of this study is to investigate the MPs removal capacity under different conditions by analysis of MPs in the inlet wastewater, outlet effluent, and sludge of one of the wastewater treatment plants (WWTPs) in Qom city, Iran. Monthly sampling was conducted in spring and summer of 2022, resulting in analysis of a total of 18 samples from wastewater, effluent, and sludge. MPs were identified and separated according to established guidelines followed by further analysis using scanning electron microscopy (SEM) and µ-Raman spectroscopy. The average MPs concentration in wastewater and effluent were 710± 34.67 MP/L and 51± 4.42 MP/L, respectively, while it was 30.76±7.19MP/g in sludge. The treatment plant demonstrated an average MPs removal efficiency of 92.81%. Overall, polyethylene terephthalate (PET) and fibers were the most frequent type and shape of MPs identified across all samples. The dominant sizes of MPs in wastewater and sludge ranged between 250-500 µm, while in the effluent, the MPs were primarily within the 250-100 µm. Based on the findings, it is estimated that 2652×106 MPs enter the environment daily through effluent and contributing to the pollution in air, soil, and surface water. The results of this study showed that sludge and effluent from WWTP are rich in MPs, and if used as fertilizer or to irrigate fields and crops, they can cause high levels of MPs to accumulate in the soil, polluting ecosystems and posing serious risks to organisms.

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INTRODUCTION

Plastics are integral to modern life, used in industries like agriculture, packaging, clothing, medicine, transportation, and cosmetics (Fan et al., 2021; Reddy & Nair, 2022). However, improper waste management, unsafe disposal, industrial production of small plastic particles, and wastewater contribute to macroplastics and microplastics (MPs) entering the environment (Zhang & Chen, 2020). MPs, defined as particles smaller than 5 mm, originate directly from sources or result from macroplastic degradation through chemical, physical, or biological processes (He et al., 2019). The size, shape, and polymer type of MPs determine their ecological harm (He et al., 2019). MPs adsorb toxic chemicals, such as heavy metals and persistent organic pollutants, via hydrophobic interactions and electrostatic forces, forming micelle-like structures that concentrate pollutants (Wang et al., 2021). Their hydrophobic, low-polarity surfaces enhance the accumulation of harmful chemicals from water. In marine environments, MPs disrupt carbon fixation by affecting algae, contributing to climate change and global warming (Shen et al., 2020). They are found globally, from ocean depths to snow-covered mountains, impacting glacier melting and sea levels (Parolini et al., 2021; Stefánsson et al., 2021; Wu et al., 2019).

MPs are present in marine organisms and human breast milk, posing health risks by releasing toxic monomers and carcinogenic additives upon ingestion or inhalation (Ragusa et al., 2022; Sequeira et al., 2020). They also carry hydrophobic pollutants like polycyclic aromatic hydrocarbons, pesticides, and phthalates, causing endocrine disruption, lung damage, liver dysfunction, intestinal obstruction, brain oxidative stress, and metabolic disorders (Amato-Lourenço et al., 2021; Collard et al., 2017; Revel et al., 2018; Wang et al., 2021).

Urban influents, including surface runoff, domestic sewage, and precipitation, contain high MP levels, contaminating soil through irrigation with domestic and industrial wastewater (Bayo et al., 2020). Compost fertilizers and environmental waste further pollute soil, reducing fertility and affecting biota, with MPs entering the food chain via plants (Bläsing & Amelung, 2018). Sewage sludge, rich in microorganisms and organic matter, is a significant MP reservoir, widely used to enhance soils (Weithmann et al., 2018).

Annually, thousands of tons of MPs enter agricultural soils in Europe and North America through sewage sludge (Buta et al., 2021). Studies in Iran report high MP concentrations in sludge (6070±807.25 MP/kg) and effluent (5.3±0.31 to 11.13±3.14 MP/L), with daily releases of 2.3×10^9 MPs through effluent and 1.61×10^{10} through dried sludge (Buta et al., 2021; Naji et al., 2021; Oveisy et al., 2022; Sharifi et al., 2023). In China, improper sludge disposal transfers significant MPs to soil and freshwater (Jiang et al., 2020). In England, sludge used as fertilizer introduces 1.02×10^{10} to 1.61×10^{10} MPs into soil monthly (Harley-Nyang et al., 2022). These findings highlight the pervasive environmental and health challenges posed by MPs.

Globally, the concentrations of MPs in WWTP influents vary from 0.28 to 31,400 MPs/l, whereas the effluents exhibit a range from 0.01 to 297 MPs/l, demonstrating considerable removal efficiency, with some instances achieving up to 99%. Nevertheless, owing to the substantial volumes of wastewater generated, significant quantities of MPs continue to be discharged daily, with estimates suggesting a range from 500,000 to 1.39×10^{10} MPs/l entering aquatic ecosystems (Liu et al., 2021). In recent years, some studies have examined the effectiveness of advanced wastewater treatment methods in removing particulate pollutants. These methods can also effectively remove MPs from wastewater (Al-Amshawee et al., 2022; Alalwan et al., 2023; Habl et al., 2024; Mishra et al., 2022).

Investigating the characteristics of MPs in the sludge and effluent of treatment plants used for irrigation and fertilization of farms and pastures is necessary because of their harmful effects on ecosystems and people's health. In addition, Iran lacks comprehensive studies on MP occurrence in WWTPs, despite high plastic waste production. In Iran, there are limited studies on the

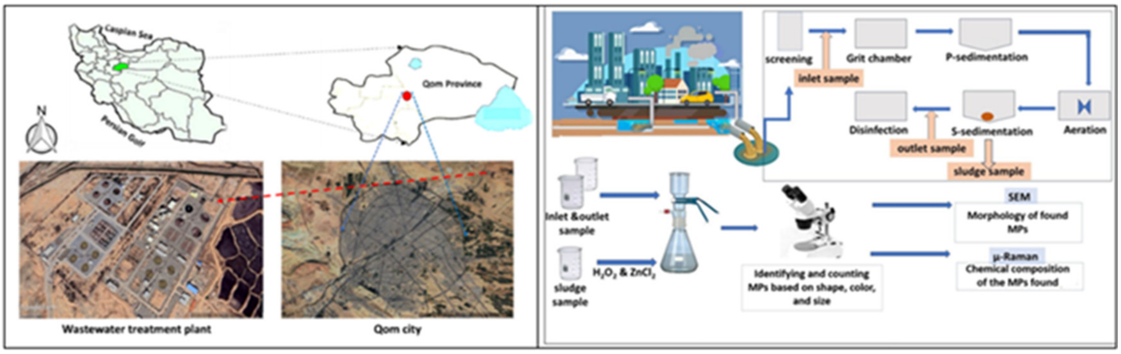

Fig. 1. Geographic location of wastewater treatment plant in Qom city and Schematic of sampling points and methodology of the present study.

characteristics and properties of effluent and sludge from treatment plants. Therefore, this study set out to shed light into the frequency and characteristics of MPs in the influent, effluent and sludge from the wastewater treatment plant of Qom city in Iran. Also, sampling was conducted during two seasons and the MPs removal efficiency was calculated for the units in the treatment plant. On the other hand, considering that Qom is a tourist city and sampling was conducted simultaneously with cultural events, new data was obtained on plastic consumption in events and the plastic load entering the wastewater collection network. For this purpose, samples were taken from the influent, effluent, and return activated sludge of the Qom wastewater treatment plant in spring and summer. After digestion and density separation, the samples were examined microscopically to count and identify MPs, and were subjected to µ-Raman analysis to identify the type of polymer.

MATERIALS AND METHODOLOGY

Study area

The city of Qom is the capital of Qom province, located 130 km southwest of the capital, Tehran in the central part of Iran. It has an arid and semi-arid climate with an annual rainfall of 161 mm (Ranjdoost et al., 2024). Qom is located at 34° 44' 37" north latitude, to 55° 33' 27" east longitude. This city has an area of approximately 730 (km^2) and a population of over one million (Fouladi Fard et al., 2018). Figure 1 shows the location of the study area as well as the location of wastewater treatment plant in the current study.

Sampling method

The introduction and characteristics of the investigated treatment plant are included in the supplementary file. Monthly sampling of influent, effluent, and sludge was conducted at a wastewater treatment plant (WWTP) from April to September 2022 to assess seasonal variations in microplastic (MP) concentrations and removal efficiency, following a modified methodology from Petroody et al. (2020). For detailed sampling procedures, refer to the supplementary file.

Extraction and identification of microplastics

Influent and effluent samples were allowed to settle in their transport containers for a few hours, after which the supernatant was filtered through a 0.45 µm filter. For MPs extraction from sludge, dried samples were treated with 15% hydrogen peroxide (H_2O_2) and zinc chloride ($ZnCl_2$), followed by centrifugation and vacuum filtering. To prevent contamination, strict protocols were followed during sample preparation, including using aluminum foil and cleaning surfaces with alcohol. MPs were identified and classified based on visual properties under a

Table 1. Distribution MPs in 6 samples of sludge, wastewater and effluent in wastewater treatment plant of Qom city.

	Sludge											
Sort	A	B	C	D	E	F	A	B	C	D	E	F
	Particles in 20 gr of dried sludge						Abundance per 1g					
Fiber	376	480	514	544	612	784	18.8	24	25.7	27.5	30.6	39.2
Sheet	46	32	30	31	43	56	2.3	1.6	1.5	1.65	2.15	2.8
Fragment	39	18	23	17	13	34	1.95	0.9	1.15	0.85	0.65	1.7
Total	461	530	567	592	668	874	23.05	26.5	28.35	29.6	33.4	43.7
	Influent											
	Particles in 3 liters						Abundance per 1 Liter					
Fiber	1836	1902	1890	1971	2081	2130	612	634	630	657	694	710
Sheet	123	128	106	112	58	135	41	43	35	37	19	45
Fragment	57	43	84	53	44	43	19	14	28	18	15	14
Total	2016	2073	2070	2136	2183	2308	672	691	690	712	728	769
	Effluent											
	Particles in 3 liters						Abundance per 1 Liter					
Fiber	112	130	140	138	142	150	37	43	47	46	47	50
Sheet	19	12	7	12	15	15	6	4	2	4	5	5
Fragment	5	3	5	3	3	9	2	1	2	1	1	3
Total	136	145	152	153	160	174	45	48	51	51	53	58
Sample	A	B	C	D	E	F						
Removal efficiency (%)	97.49	97.77	97.69	97.61	97.57	97.48						

Samples taken on the following dates. A: 4th April; B:5th May; C: 6th June; D: 5th July; E: 6th August; F: 6th September.

stereomicroscope, with morphology assessed via SEM and chemical composition analyzed using μ-Raman spectroscopy. The resulting data were analyzed using SPSS version 26 software. For more details on the method, see the supplementary file. Figure 1 shows a schematic of the working method.

RESULTS AND DISCUSSION

MPs in the shape of fibers, sheets, and fragments were found in all 18 samples of influent, effluent, and sludge. According to the μ-Raman results, 90%-75% of the particles that were visually considered MPs were plastic, which indicated the reliability of the identification method for the quantification of MPs (Li et al., 2018). See the supplementary file to view the QA and QC results (Table S1 and Table S2)

Frequency of Microplastics

Sludge samples

According to table 1, the frequency of MPs in sludge varied from 23.05 to 43.7 (MP/g) during spring and summer, which is consistent with the results of studies conducted in other countries such as Turkey, Australia, Finland, China, Spain, Italy, and Mauritius (Jiang et al., 2020; Magni et al., 2019; van den Berg et al., 2020; Vardar et al., 2021; Zhang & Chen, 2020). In the UK, the concentration of MPs varied from 37.7 - 286.5 (MP/g) of sludge (Harley-Nyang et al., 2022), which exceeds the results obtained in the present study. However, in the Netherlands, the frequency of MPs was reported to be between 0.2 - 0.45 (MP/g) (Brandsma, 2013). Of course, the type of WWTP units, the cover population, the type of influent and identification methods of MPs should not be ignored in these studies. For example, the sludge of a treatment plant in Turkey with a population of 2 million people, primary and secondary treatment of activated sludge, contained 32MP/g (Vardar et al., 2021).

MPs in influent are transferred to sludge cake due to filtration and sedimentation units, because these units are most effective in removing MPs from influent (Jiang et al., 2020). The use of sludge with this level of pollution for fertilizing fields causes large amounts of MPs to enter the soil. Soil erosion, plowing and wind flow cause MPs to release into the atmosphere (Pérez-Reverón et al., 2022). The presence of earthworms in agricultural soils causes the transmission of MPs. Likewise, MPs in soils with a higher degree of porosity can penetrate the groundwater due to infiltrating water flows (Yu et al., 2019).

Fibers were dominant in all samples. The frequency of sheets was more than that of fragments, which was consistent with the reports of studies conducted in Bandar Abbas (Iran), Bangkok and Australia (Naji et al., 2021; Tadsuwan & Babel, 2021; Ziajahromi et al., 2021). However, in some studies, more fragments than other forms of MPs were observed in sludge (Pittura et al., 2021; Yang et al., 2021; Zhang & Chen, 2020).

The average frequency of MPs in summer and spring was 35.56 (MP/g) and 26 (MP/g), respectively, indicating higher amounts of MPs in sludge in summer than in spring. However, this difference was not significant ($p > 0.05$). In the present study, the samples in the last two months of summer were taken at a time large-scale religious ceremonies were being held in the city, during which disposable plastic containers and cups were used to distribute food and drinks among people. This might be, considered as a possible reason for the difference in frequency of MPs in spring and summer.

influent and effluent samples

The average concentration MPs detected in influent and effluent samples were 710± 34.67 and 51± 4.42 (MP/L), respectively, which was similar to the reports in South Korea, America, and China (Conley et al., 2019; Hidayaturrahman & Lee, 2019; Wang et al., 2020). Other studies in other countries reported lower values (Simon et al., 2019). According to a study in Italy, the average levels of MPs in samples taken from the influent and effluent of a wastewater treatment plant serving a population of 1.2 million people were 2.5 ± 0.3 (MP/L) and 0.1 ± 0.4 (MP/L), respectively (Magni et al., 2019). In the domestic wastewater of the rural area of Hangzhou, China, MPs values of the influent were reported to be (430-2154) $\times 10^3$ (MP/L) (Wei et al., 2020). MPs from synthetic clothing are released into wastewater during laundering. MPs from various personal care products are discharged into wastewater. Tire wear and plastic road components contribute to runoff that enters water systems. Manufacturing granules and resin pellets may inadvertently contaminate wastewater. Disposable items like wipes and sponges release plastic fibres upon use and disposal. Degradation of plastic building materials leads to fragments entering wastewater. Food packaging can be washed into drains from both residential and commercial sources. Certain pharmaceuticals and medical products incorporate MPs that reach sewers. MPs in sludge from treatment plants can re-enter soils and water bodies when used as fertilizer. Rain can transport microplastics from various surfaces into wastewater systems (Osman et al., 2023; Talukdar et al., 2024). So, the difference in the values of MPs in different studies can be attributed to the type of wastewater treatment system and its efficiency in removing particles (Harley-Nyang et al., 2022). Sampling season, amount of rainfall, factors related to the studied area, such as population, culture, development and industries also cause fluctuations of MPs in wastewater and consequently in the concentration of MPs in sludge, influent, and effluent (Li et al., 2018). In Italy, it was reported that municipal wastewater is diluted with underground water up to 30%, which influenced the concentration of detected MPs. In some regions of the world, like the city of Qom in Iran, household waste and surface runoff are collected along with the wastewater network. The samples examined in the present study were taken from domestic sewage, while there was no industrial sewage. As a result, most identified MPs had a secondary origin (from street washing and domestic discharges). These discharges can include washing synthetic clothes and larger plastic materials like plastic bags, packaging, bottles, and pipes that degrade and produce MPs in various ways (Yang et al., 2021).

In the city of Qom, 48000 (m^3/day) of effluent enters the urban green space, and it is estimated to enter 2652×10^6 (MP/day) into the environment daily by effluent, which can introduce the air as a result of soil erosion or affect other parts of the ecosystem (Reddy & Nair, 2022). The observations in the current study were similar to those detected in the US, in which the release of 500 to 1000 million (MP/m^3) from three treatment plants were released (Conley et al., 2019). However, based on the removal efficiencies reported in the research, treating all influent sufficiently can reduce the global MPs load by 90% in water environments (Reddy & Nair, 2022).

In the present study, the concentration of MPs had an ascending trend from the first month of sampling to the last month, with the maximum concentration of MPs in influent being 679 (MP/L), and 58 (MP/L) in effluent. It should be noted that samples were for the last month of summer (September). One possible reason for this difference was explained in the sludge section. The values of the MPs were different between the months, but the difference was not significant ($p> 0.05$). Table 1 shows the trend of frequency changes of MPs during the sampling period.

There was a significant difference between influent and effluent ($p <0.001$). Fig. S1 shows the average concentration comparison of influent and effluent MPs during sampling.

The average MPs removal efficiency was 97.6%. In other studies, the impact of the various unit of treatment on the final MPs content in the effluent was significant. The treatment efficiency in the removal of MPs from the influent was stated to be 97% by Magni et al. (2019) and 90% by Bayo et al. (2020) (Bayo et al., 2020; Magni et al., 2019). Table 1 provides more details on the frequency of MPs in all 6 samples of influent and effluent. Table S3 shows the MPs removal efficiency in different studies.

The shape, color, size and type of polymer of microplastics

Tables S4-S6 present, the data for MPs identified in the samples. The fibers were the most frequent ones in all samples. In sludge, influent, and effluent, fibers had the frequency of 88%, 92%, and 88%, respectively. The frequency of fragments in all three samples was 3%. Figure 3a indicates the lower frequency of fragments than sheets and fibers in all three samples. In most studies on MPs content in sludge and influent, fibers were introduced as dominant MPs which is in agreement with to the results of the present study (Jiang et al., 2020; Li et al., 2018; Magni et al., 2019). A study in Bangladesh also found that microfibers had a frequency of more than 75% in the sludge samples taken from the textile industry (Hossain et al., 2023). Microfibers can be attributed to the influent caused by the washing of synthetic clothing and fabrics in homes and industries (Rochman, 2018). A synthetic cloth can produce more than 1900 fibers per wash with a washing machine (Browne et al., 2011). The reason for the presence of more fibers in the sludge was attributed to more contact surface compared to the othlier MPs because it is easily eliminated by the activated sludge system (Jiang et al., 2020). Fragments and sheets are directly produced from the MPs in the manufacture of cosmetics and other similar products or are produced by the breaking and fragmentation of macroplastics (Ben-David et al., 2021).

The reports related to the shape of MPs are very important because they can specify the origin of MPs and influent characteristics. Inhaled synthetic fibers can cause chronic lung inflammation. This may elevate the risk for diseases like cancer and diabetes. Also, fibers may disseminate to organs, causing systemic inflammation and immune responses (Lee et al., 2023). However, the treatment processes may also affect the shape of MPs, which requires more studies

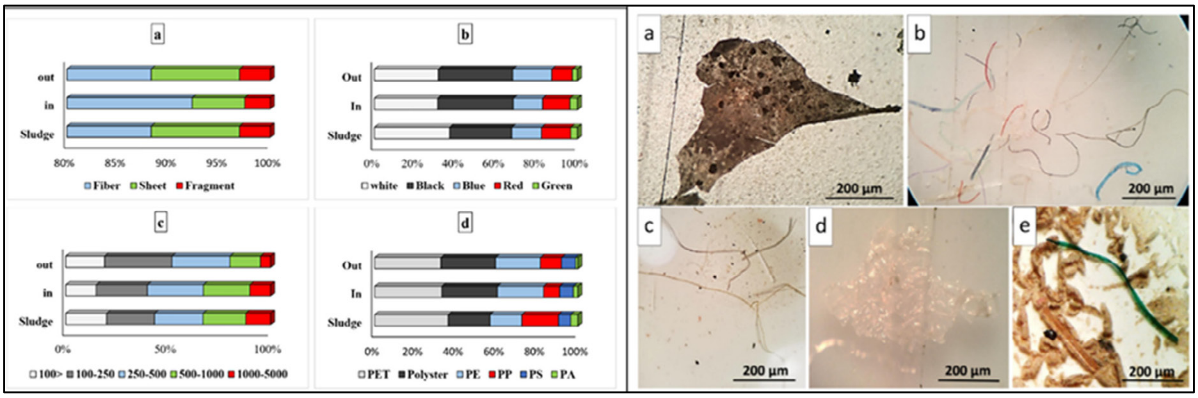

Fig. 3. Frequency percentage of: (a) shapes, (b) colors, (c) size and (d) polymer of MPs detected in this study; Photographs of MPs observed in samples. a, d: Sheets, b, c, e: Fibers.

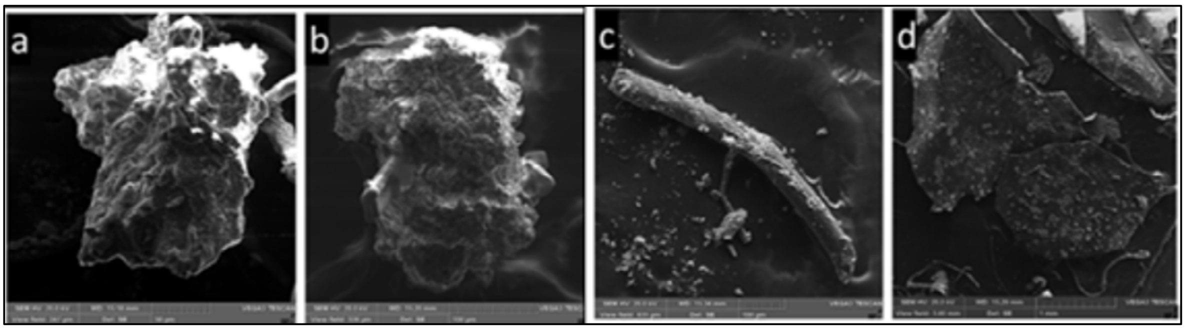

Fig. 4. SEM images of selected MPs. (a) Fragment, (b) Fragment, (c) Fragment, (d) Sheet.

(Collivignarelli et al., 2021). The color, size, and shape of MPs are changed under the influence of weathering and physico-chemical changes, and even due to chemical changes, objects with more dangerous chemical characteristics can be derived from them (Liu et al., 2020). In this study, fibers were also dominant in samples of influent and effluent, which supports the results of previous studies.

The difference in the size of MPs in the present study can be attributed to the mechanical forces caused by mixing or pumping influent In treatment plants, which can convert MPs into smaller pieces, and increase their number in the influent as well (Enfrin et al., 2020). On the other hand, according to the results of previous studies, MPs with a size larger than 500 µm are usually removed by treatment plants more easily than MPs with a size less than 100 µm (Freeman et al., 2020; Menéndez-Manjón et al., 2022). Studies have shown that some treatment units are capable of removing smaller MPs. For example, MF and UF membranes with pores of 0.1 to 10 µm had 81.5 to 100% removal efficiency for MPs smaller than 100 µm, which can be used to effectively remove MPs from the effluent (Marsono et al., 2022). Fig. 3c shows that MPs were found in all size groups, and in all three samples, the size group $1000 < L < 5000$ µm was the least abundant. Fig. 3 shows some MPs identified the present study.

Figure 3d shows the type of polymers and their frequency. PET was dominant in sludge, influent, and effluent samples. In addition, PP was more frequent in effluent and sludge than influent, while the lowest frequency was related to polyamide (PA). However other polymers such as poly Ethylene (PE), polystyrene (PS), and polyester (PES) were also observed in this study. Other studies also obtained similar results (Lv et al., 2019; Yang et al., 2019). In some studies, PE was predominant (Carr et al., 2016). This evidence suggests different sources of MPs in the urban influent network. PET and PES mainly originate from textiles and clothing washing operations, while PE and PP are released from personal and household care products (Lv et al., 2019). Table S7 provides a useful comparison between some past studies and the present study.

SEM results

SEM was used to obtain clear images of the surface properties of different MPs (Fig. 4). The images showed signs of fragmentation and flaking and physical and chemical decomposition. Fig. 4a shows the irregular surface of a MP in the sludge. Numerous, deep and relatively sharp pits that increase its surface area enhance its ability to carry microbial and chemical objects (Klein & Fischer, 2019). Fig. 4b shows the surface of a MP in the influent sample, which has deep pits with rounded edges, and erosion and corrosion can be clearly seen in it. Fig. 4c shows a young fiber with a relatively smooth surface and no signs of weathering. Along the same lines, Fig. 4d shows a sheet in the influent sample, which has a relatively smooth surface and many objects are attached to it. Overall, SEM images showed evidence of erosion and deep and shallow pits on the surface of MPs, which were similar to the results in other studies (Li et al., 2018; Mahon et al., 2017). Pits on the surface of MPs in influent may be attributed to impact and friction caused by its dynamics, while fractures may be caused by treatment processes (Klein & Fischer, 2019). These surface features cause the transport of other environmental particles by MPs, which increases their toxicity and environmental hazards (Cai et al., 2017).

CONCLUSIONS

This study investigated the occurrence and characteristics of microplastics (MPs) in the influent, effluent, and sludge of a wastewater treatment plant (WWTP) in Qom city, Iran, during spring and summer 2022. The results revealed significant MP contamination, with average concentrations of 710 ± 34.67 MP/L in influent, 51 ± 4.42 MP/L in effluent, and 30.76 MP/g in sludge. The WWTP demonstrated a high MP removal efficiency of 97.6%, primarily through filtration and sedimentation processes, which transferred most MPs from influent to sludge. Fibers, predominantly polyethylene terephthalate (PET), were the most common MP type across all samples, with black and white colors dominating and sizes primarily ranging from 100–500 μm. The estimated daily release of 2652 × 10^6 MPs into the environment via effluent underscores the potential ecological impact, particularly on agricultural soils and urban green spaces where effluent is used for irrigation.

These findings highlight the role of WWTPs as both a sink and a source of MPs, emphasizing the need for improved treatment technologies to enhance MP removal, especially for smaller particles (<100 μm). The dominance of fibers suggests that domestic sources, such as textile washing, are significant contributors to MP pollution in Qom's wastewater. Seasonal variations, potentially influenced by cultural events increasing plastic use, warrant further investigation to understand temporal MP dynamics. The study underscores the urgent need for strategies to mitigate MP release into the environment, such as advanced filtration systems or restrictions on sludge application in agriculture, to reduce ecological and health risks associated with MP contamination. Future research should focus on optimizing WWTP processes and exploring the long-term impacts of MPs in receiving environments, particularly in arid regions like Qom where water reuse is critical.

Limitations and recommendations

Enhancing detection limits for smaller MPs: In this study face difficulties in detecting microplastics under 50 μm size threshold, leading to potential underrepresentation. Future research should focus on advanced analytical methods, such as μ-FTIR or Raman spectroscopy, to improve sensitivity for small microplastics. Additionally, developing standardized protocols for measuring nanoplastics could enhance understanding of their concentrations in wastewater.

Ensuring representativeness of sampling: The choice of wastewater treatment facilities and sampling timing significantly influences results. To improve representativeness, studies should include a variety of plants with different treatment technologies and environmental contexts. Furthermore, sampling protocols should consider temporal variability by integrating seasonal changes, flow dynamics, and operational cycles. A stratified sampling approach combined with statistical variability analyses can better reflect actual conditions.

Given the extensive and potential sources of MPs in the environment, and the harmful effects of MPs on ecosystems and human and animal health, it is recommended that research be focused on determining the permissible limit of this pollutant in effluent discharged to wastewater treatment plants

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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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