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Characteristics of Microplastics in the Sludge of Wastewater Treatment Plants

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ABSTRACT

In recent decades, one of the environmental concerns is contamination with emerging pollutants of microplastics. Microplastics enter the environment through wastewater treatment plants and can absorb harmful pollutants. This study investigated microplastic pollution in the sludge of Zahedan wastewater treatment plants. To investigate microplastic pollution in the sludge of Zahedan wastewater treatment plants, 5 kg samples of sludge were passed through stainless-steel sieves and digested using H2O2 solution. NaCl was used based on density to separate microplastics. FESEM and FTIR analyses were used to investigate the surface morphology and polymer type of microplastics. The abundance of microplastic particles in the sludge ranged from 71-95 $N/Kg_{dry.sludge}$, and their size varied from 25-500 micrometers. The most common color observed was transparent, and the fiber shape was the most prevalent. The study highlights the importance of addressing microplastic pollution in wastewater treatment plants to prevent harmful effects on the environment. Also, the data obtained from this study can be used to improve the treatment process and understand the removal of microplastics in urban wastewater treatment plants.

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INTRODUCTION

The production of plastic in the world between 1950 (1.7 × 10^9 kilograms) and 2022 (4.3 × 10^{11} kilograms) has increased by 218 times (Schütze et al. 2022). Nowadays, environmental pollution from various types of plastics is a threat to ecosystems worldwide .This threat is increasing day by day with the increasing production and consumption of plastics (Al-Oufi et al. 2004; Castañeda et al. 2014; Tagg et al. 2022; Jahantiq et al., 2020) .Plastics in the environment are divided into megaplastics (more than 100 mm), macroplastics (20–100 mm), mesoplastics (5–20 mm), microplastics (0.1–5 mm), and nanoplastics (less than 100 nm) (Kamani et al. 2024; X. Sun et al. 2020). Among these groups, microplastics are of particular importance due to their small size (Boakes et al. 2023; Kishipour 2020). Nowadays, environmental pollution by microplastics is one of the new concerns of environmental researchers and scientists, as microplastics can be found in water resources, sediments, soil, and many high-altitude areas, even in the Arctic and Antarctic ice (Hosseinipour Dizgah et al. 2018). The destruction of microplastics leads to the leakage of the raw materials that make up the plastic structure into the environment (Al-Oufi et al. 2004; Miller et al. 2019; Rios et al. 2010; Norabadi et al., 2020). Microplastics can

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also absorb pollutants such as Polycyclic Aromatic Hydrocarbon (PAH), chlorinated organic pesticides, Poly Chlorinated Biphenyl (PCB), and heavy metals. Microplastics are easily absorbed by microorganisms due to their small size, or they are ingested by various aquatic organisms in aquatic environments. Microplastic particles in humans can cause endocrine disorders, hormonal disorders, carcinogenesis, disease (liver, kidney, lung, and heart), immune system disorders (T cell suppression, cytokine production, proliferation of white blood cells, inflammatory response, etc.), and so on. Due to the small size of these particles, they can easily pass through the respiratory system (entering deep into the lung and causing inflammation, alveolus and lower airway fibrosis and carcinogenesis, etc.), cell membrane, blood-brain barrier, and placenta, and they may also penetrate other organs and cause serious health damage (Al-Oufi et al. 2004; Boakes et al. 2023; Castañeda et al. 2014; Eriksen et al. 2013; Ghayebzadeh, Taghipour, and Aslani 2020; Gupta 2017; Jambeck et al. 2015; Kishipour 2020; Mehrabian et al., 2018). Microplastics are generally classified into two categories: primary microplastics, which are used as raw materials in the plastic industry, artificial textiles, electronic and electrical equipment, cosmetics and hygiene products, and toothpaste. Secondary microplastics are mainly due to the decomposition of larger plastic parts following processes such as weathering, photooxidation by UV rays, hydrolysis, mechanical breakage due to sand abrasion or water turbulence, and the presence of waste in the environment (Al-Oufi et al. 2004; Boakes et al. 2023; Gupta 2017; Jambeck et al. 2015; Kishipour 2020; Mansoorian 2016; Balarak et al., 2020). The number of microplastics in the environment depends on the amount of plastic consumption in society, the recycling of plastic in waste, and waste management (Gupta 2017; Jambeck et al. 2015; Kishipour 2020). Microplastics easily find their way into wastewater and ultimately into wastewater treatment plants, where some of them settle in the sludge of the treatment plants and some enter the environment through the effluent of the treatment plants. It should be kept in mind that the use of sewage sludge from sewage treatment plants varies greatly around the world, but in general the final sludge of the treatment plants and the effluent from the wastewater treatment plants are important sources of microplastics entry into the environment. This includes water sources, agricultural soil, and fertilizer produced from the sludge of the treatment plants, which ultimately humans will face (Boakes et al. 2023; Castañeda et al. 2014; Eriksen et al. 2013; Ghayebzadeh, Taghipour, and Aslani 2020; Gupta 2017; Jambeck et al. 2015; Kishipour 2020; Bazgir et al., 2019). The health and ecological risks of microplastics to humans and the environment depend on the degree of risk and exposure to them, which come in three forms: biological, physical, and chemical risks. The toxicity of these particles depends on their surface properties, size, shape, polymer type, etc (Samiha et al. 2023). Therefore, understanding microplastics and providing solutions to control their entry into the environment is one of the goals of environmental researchers. On the other hand, limited data and information are available on the number, type, and shape of microplastics in wastewater and sludge settled in treatment plants. Therefore, the aim of this study is to investigate the abundance, shape, size, color, and type of polymer of microplastics hazards in the sludge of Zahedan wastewater treatment plants.

MATERIALS AND METHODS

For this purpose, a sample was taken from the settled sludge at the bottom of the secondary settling tank using a rake (Fig. S1). The 5 kg sludge samples were poured into glass containers washed with filtered distilled water. The samples were quickly transferred to the laboratory located at the Zahedan School of Health, and to prevent bacterial activity, the samples were kept in a refrigerator at a temperature of 4 °C in the laboratory (Ganji et al. 2024; Glöckler, Foschum, and Kienle 2023; Yli-Rantala et al. 2022).

The screening method was used to separate microplastic particles from sludge. Samples were passed through stainless steel sieves in a 2000 µm (mesh 10), 500 µm (mesh 35), 250 µm (mesh 60), and 25 µm (mesh 500) manner. After passing the samples, large microplastic particles were visible to the naked eye on the sieves. The digestion process (removal of organic matter without affecting the structure of microplastics) was performed using hydrogen peroxide solution (H_2O_2 Manufactured by Merck, Germany with 30% purity) under optimal conditions (24 h, 75 °C, and H_2O_2 = 20ml) in the oven. Optimal conditions were obtained after performing full analysis steps under the title of the initial test. After the digestion process, in order to extract and separate the microplastic particles from the construction, the "separate by density" method (NaCl=1.2 mg/L) was used. Then the digested solutions were filtered using a membrane filter (diameter 47 mm, pore size 45 µm) and a vacuum pump (Jiang et al. 2020; Mahon et al. 2017; Yli-Rantala et al. 2022). Finally, the microplastic particles were classified based on their size, shape, and color. Visual analysis of the particles was performed using an Olympus microscope equipped with a controller and camera DP12.

The Fourier Transform Infrared Spectrometer (FTIR, model TENSOR II, made by BRUKER factory, Germany) was used to determine the type of polymer that makes up microplastics. Each substance has a specific spectrum and bond that can be identified by comparing its spectrum with the spectrum of known substances. Also, the FTIR can distinguish and separate microplastics from organic and mineral materials. Furthermore, in this study, the field Emission Scanning Electron Microscope (FESEM, model EM620 Eco, made by KYKY factory, China) was used to identify the surface morphology of microplastics. The images taken from the surface of particles have high resolution, facilitating the differentiation of microplastics from organic particles. The EDX analysis is used for further analysis (Böke, Popp, and Krafft 2022; Taghipour et al. 2023; Kamani et al., 2023).

To prevent contamination of tools, equipment, and samples during the testing process, all laboratory equipment was first washed three times with tap water and then with filtered distilled water. Then, all the equipment was covered with aluminum foil (Binda et al. 2023; Sbrana et al. 2023). Hands and forearms were also washed to prevent potential contamination. During the test, to reduce possible contamination (dust from traffic, fiber from clothing, and chemicals), non-polymer gloves, non-plastic containers, and aluminum covers were used. All stages (except for the sieving stage) were performed under the hood. Plastic tools were used to minimize errors. The work surfaces and equipment were regularly washed with a 2% (Decon 90%; Fisher Scientific) cleaning solution. To identify the level of error and contamination during sample preparation and testing, 4 filtered distilled water samples were kept in the testing environment for 7 days. In addition, nitrate cellulose (NC) filters (4 filters on the Petri dish) were placed near the microscope, hood, and the testing environment for 7 days (Jiang et al. 2020; Meng et al. 2023; Sharifi, Attar, and Bina 2023).

RESULTS AND DISCUSSION

On average, less than 2 microplastic particles were detected in each of the water-blank samples. In blank filter samples placed to control and investigate contamination and possible errors, an average of less than 1 microplastic particle was detected (Table S1). The results of the control and quality analyses were very small compared to the volume and number of microplastics in this analysis, which showed a very low error rate for this experiment and analysis.

Abundance, color, and shape detection, as well as photography of microplastics were done by an Olympus microscope (Fig. 1). The results showed that microplastics were present in all samples taken from the sludge of the treatment plants. Table 1 shows the highest number of microplastic particles in the No. 1 wastewater treatment plant sludge and the lowest number of microplastic particles in the No. 2 wastewater treatment plant sludge. The highest elimination

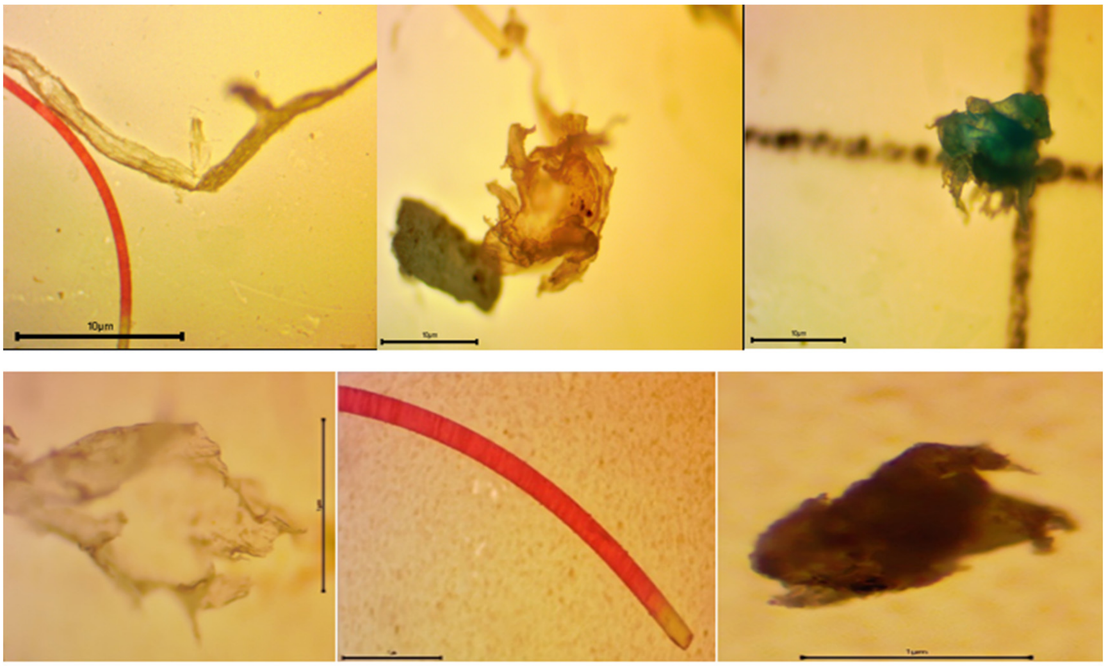

Fig. 1. Pictures of MPs were magnified 10 time and the scale bars represent 0.1 mm

Table 1. Abundance of MPs in influent, effluent and secondary sedimentation sludge, distribution of microplastics, and removal

WWTPs	Number of microplastics in influent (Number of MPs/Liter (N/L))	Number of microplastics in effluent (N/L)	Removal (%)	Number of microplastics in sludge (Number of MPs/kilogram dry.sludge (N/Kg dry.sludge)	Removal (%)	Secondary sedimentation removal (%)
WWTP 1	635	57	91	95	14.96	85
WWTP 2	540	59	89	92	17.03	83
WWTP 3	589	41	93	71	12.05	88
Mean	588 ± 38.79	52.33 ± 8.05	91 ± 1.63	86 ± 10.67	14.68 ± 2.04	85.33 ± 2.05

efficiency belongs to the NO. 3 wastewater treatment plant and the lowest elimination efficiency belongs to the NO. 2 wastewater treatment plant. According to the study of the distribution of microplastics in sludge samples, a large reduction of microplastic particles relative to the input unit can be concluded, which indicates the high efficiency of the processes of wastewater treatment units.

The reasons affecting the number of the large number of microplastics observed in the results of this study include the lower size of the sieves used. The smaller the size of the sieves, the higher number of microplastics detected (Alavian Petroody, Hashemi, and van Gestel 2020; Sol et al. 2023). Other factors impacting the number and efficiency of microplastics removal include the treatment process, the amount of influent to the wastewater treatment plant, and the population covered by each of the wastewater treatment plants (Harley-Nyang et al. 2023). In the secondary settling tank, suspended impurities form clots and settle in the sludge on the floor (Wu et al. 2021). Features such as shape, size, material density, Hydraulic Retention Time

Table 2. Type of shapes of MPs

WWTPs	Type of shapes Percentages of shapes (%)			
	Fiber	Granule	Film	Fragment
WWTP 1	36 37.89	19 20	15 15.78	25 26.31
WWTP 2	32 34.78	17 18.47	20 21.73	23 25
WWTP 3	30 42.25	14 19.71	6 8.45	21 29.57
Mean (N/Kg)	32.66 ± 2.49 38.3 ± 3.06	16.66 ± 2.05 19.39 ± 0.66	13.66 ± 5.79 15.32 ± 5.43	23 ± 1.63 26.96 ± 1.92

(HRT), and flow conditions (laminar or turbulent) affect the removal efficiency and deposition of microplastic particles during the filtration process (J. Sun et al. 2019). Jiahui Jian et al.'s (2020) study on wastewater treatment plant sludge showed that the abundance of microplastics in the sludge was 6 $N/Kg_{dry.sludge}$, which is less than the number of microplastics found in the study, but Alavian et al.'s (2020) study showed that the sludge of the primary sedimentation tank contained 214 $N/Kg_{dry.sludge}$ (Alavian Petroody, Hashemi, and van Gestel 2020; Jiang et al. 2020). A study by A.M. Mahon et al. (2017) found that microplastics in wastewater treatment plant sludge varies from 4196–15385 $N/Kg_{dry.sludge}$[1] (Mahon et al. 2017).

The particle size distribution of microplastics indicates a high abundance of these particles. The distribution of the size of the sludge microplastics in this study was determined based on the mesh of the sieves used (sizes 25 µm, 250 µm, 500 µm, and 2000 µm), which was detectable by mesh 10 (2000 µm) due to the size of large pores if microplastics were present with the naked eye. No microplastic particle was detected on the 2000 µm sieve in the study. So the microplastic particles found were 25–500 µm sized based on the sizeing of the sieves used. According to the results obtained from other studies, during wastewater treatment processes, large microplastic particles are converted into smaller particles, so smaller particles are present in larger numbers in the wastewater (Sharifi, Attar, and Bina 2023; J. Sun et al. 2019). Many of the microplastics observed are about the size of microplastics caused by cosmetics, carpets, and clothing (Guerranti et al. 2019; Jessieleena et al. 2023; Leslie 2014; Ustabasi and Baysal 2019). Alavian and Hashemi's (2020) study on the sludge and effluent of the Sari city wastewater treatment plant showed that a large number of microplastics of 300 µm size enter the environment daily through sludge and effluent (Alavian Petroody, Hashemi, and van Gestel 2020). The study by Jie Yang et al. (2021) in China indicated that most microplastic particles in sludge and effluent are particles of 1-3 mm in size (Yang et al. 2021).

Although, the results of Table 2 indicate that the observed microplastics in this study are in the form of fragments, films, fibers, and granules. The most and least observed forms in sludge No. 1 and 3, respectively, fiber and film, while in the sludge of wastewater treatment plant NO. 2, the most and least observed forms are, respectively, fiber and granule.

The results of Table 2 and Fig. 2 indicate that in this study, the most observed form of microplastic particles is fiber particles. The abundant number of fibers is due to the entry of wastewater from laundry, textiles, carpets, etc., which constitute the largest volume of household wastewater entering the wastewater collection system. Studies show that washing 1 m^2 of carpet can introduce 1825–3098 fiber microplastics into the wastewater, or washing one clothing each time can introduce 1900 fiber-shaped microplastics into the wastewater (Ou and Zeng 2018; Turan, Erkan, and Engin 2021; Zarfl and Matthies 2010). In this study, the abundance of fiber

1 Number of MPs/kilogram $_{dry.sludge}$

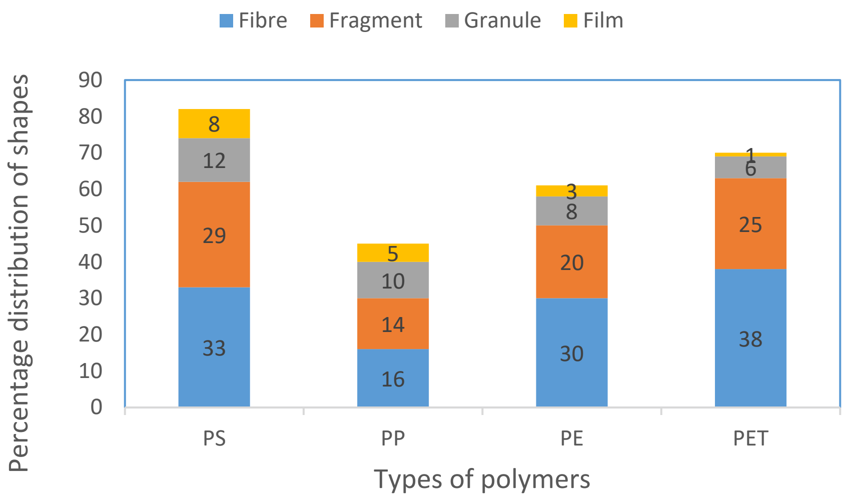

Fig. 2. Percentages of polymers by type

Table 3. Type of polymers of MPs

WWTPs	Type of polymers Percentages of polymers (%)			
	PET	PP	PE	PS
WWTP 1	33 34.73	14 14.73	20 21.05	28 29.47
WWTP 2	29 31.52	16 17.39	22 23.91	25 27.17
WWTP 3	20 28.16	15 21.12	19 26.76	17 23.94
Mean (N/Kg)	27.33 ± 5.43 31.47 ± 2.68	15 ± 0.81 17.74 ± 2.64	20.33 ± 1.5 23.09 ± 2.33	23.22 ± 4.64 26.86 ± 2.26

shapes in sludge samples (71–91 $N/Kg_{dry.sludge}$) can be justified by their high density compared to sludge density, which causes sedimentation and consequently increases removal efficiency in the secondary sedimentation unit. Additionally, the high surface-to-volume ratio of fibers compared to other shapes (fragment, film, and granule) does not allow them to settle and be removed by upstream units such as bar screen, grit chamber, and primary sedimentation. As a result, more fiber-shaped microplastics are found in the secondary sedimentation tank in settled sludge. Suspended particles such as microplastics in the aeration reactor are influenced by the mixed liquor suspended solids (MLSS) and consequently settle in the secondary sedimentation tank along with other flocs (Z. Zhang and Chen 2020).

A study by Elina Yli-Rantala et al. (2022) showed that the majority of microplastic forms in wastewater treatment plant sludge are fiber and granule. However, another study by Yanfei Zhou et al. (2019) and Xuemin Lv et al. (2019) demonstrated that in wastewater and wastewater treatment plant sludge, the majority of microplastics exist in fiber forms. According to the published articles, the sources of fiber production are mainly synthetic textile materials that enter the wastewater network through the effluent discharge of washing machines and settle in the sedimentation tank (Lv et al. 2019; Yli-Rantala et al. 2022; Zhou, Liu, and Wang 2019).

The FTIR method was used to identify the type of polymer that makes microplastics (Fig. S2). The spectrum obtained from the FTIR analysis belongs to the PET polymer. The results of Table 3 shows that the type of polymer that constitutes microplastics in this study is mainly Poly

Table 4. Type of colors of MPs

WWTPs	Type of colors Percentages of colors (%)						
	Green	Transparent	White	Blue	Yellow	Red	Black
WWTP 1	9 9.47	26 27.36	18 18.94	7 7.36	11 11.57	8 8.42	16 16.84
WWTP 2	6 6.25	24 26.08	21 22.82	11 11.95	10 10.86	7 7.6	13 14.13
WWTP 3	8 11.26	22 30.98	13 18.3	3 4.22	6 8.45	4 5.63	15 21.12
(N/Kg)	7.66 ± 1.24	24 ± 1.63	17.33 ± 3.29	7 ± 3.26	9 ± 2.16	6.33 ± 1.69	14.66 ± 1.24
Mean	9.08 ± 1.95	28.14 ± 2.07	20.02 ± 1.99	7.84 ± 3.17	10.29 ± 1.33	7.21 ± 1.17	17.36 ± 2.87

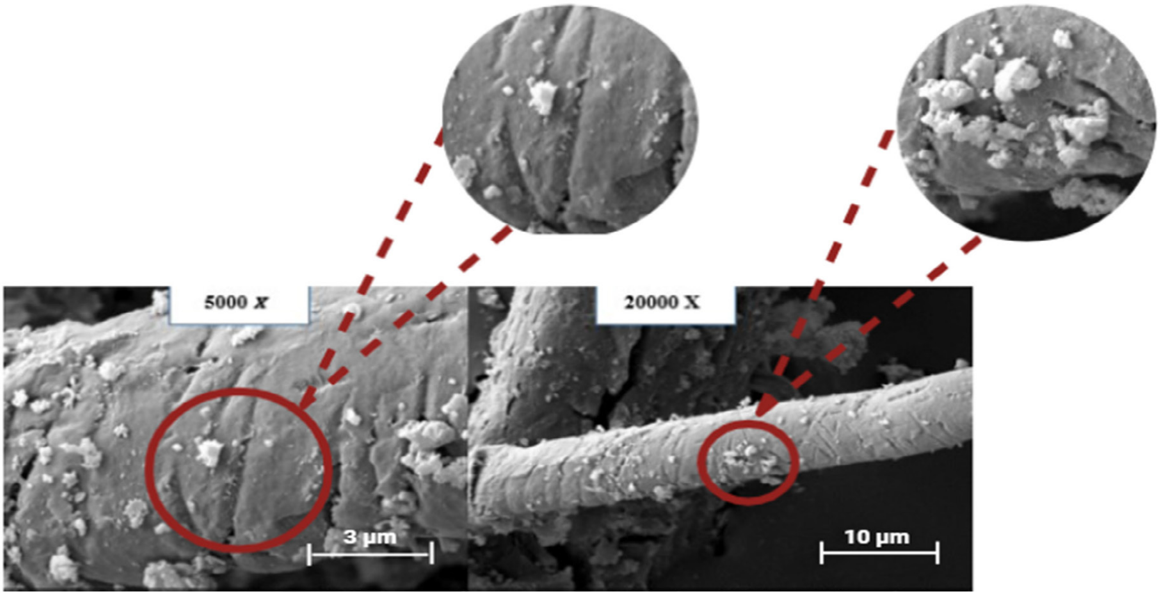

Fig. 3. SEM images of selected microplastics magnified 5000 X and 20000 X

ethylene terephthalate (PET), poly styrene (PS), Poly ethylene (PE), and Poly propylene (PP) polymers, which are among the most widely used and common polymers. After consumption in human life, these polymers eventually enter wastewater and wastewater treatment plants sludge. The results of the study by Xiaolei Zhang et al. (2020) showed that in the process of conventional wastewater treatment and digestion of sludge by anaerobic method, most of the polymers in wastewater and sludge are PET, PE, PP, and PS polymers (X. Zhang, Chen, and Li 2020). The results of all three studied treatment plants indicate that the frequency of PET is higher than other polymers (220–298 $N/Kg_{dry.sludge}$), whereas the frequency of polymer PP is lower than other polymers. The high frequency of PET in the sludge of wastewater treatment plants can be attributed to the widespread use and high consumption of this type of polymer in human daily life. On the other hand, the density of PET particles is higher than the density of wastewater. Therefore, such polymers with high density tend to settle to a greater extent in the sludge.

Table S2 presents the characteristics of common and widely used polymers. Nowadays, polymer PET is widely used in the packaging of all kinds of drinks and mineral water. Due to the lack of a comprehensive waste management program in Zahedan, such polymers easily enter the environment and eventually find their way into wastewater. Another factor contributing to the abundance of polymer PET compared to other polymers is the high resistance that polymer

PET exhibits against weathering processes and chemical oxidation, and as a result, it delays their destruction and decomposition (Benosman et al. 2011; J. Sun et al. 2019).

The examination of the color of microplastics in this study showed that microplastic particles are predominantly black, red, yellow, blue, white, transparent, and green, which is consistent with other previous studies. The most frequent microplastic color observed in the investigated samples is transparent (164–202 $N/Kg_{dry.sludge}$), and the least frequent observed color is red (20–50 $N/Kg_{dry.sludge}$) (Table 4). Generally, the predominance of the transparent color can be attributed to the large amount of microplastic with the transparent color used in personal care and cosmetic products such as toothpaste, detergents, equipment, soap, and gel. Most of these microplastics settle in the secondary sedimentation unit (Juliano and Magrini 2017; Khalid and Abdollahi 2021; Lei et al. 2017). Xiaowei Li et al.'s (2018) study showed that most of the microplastics observed in the sludge of treatment plants are white, while Alavian and Hashemi's (2020) study on wastewater treatment plant sludge showed that most of the microplastics in the sludge are black (Alavian Petroody, Hashemi, and van Gestel 2020; Li et al. 2018; Ren et al. 2020).

FESEM analysis was employed to investigate both the surfaces and surface morphology of microplastics. Fig. 3, image FESEM illustrates the microplastic particles studied in this research. By investigating the surface of the microplastic particles in the samples, it can be inferred that the microplastic particles are breaking and being crushed. These particles are under the influence of destructive factors such as photooxidation, impacts, some microorganisms, etc. The micro white particles observed on the microplastic surface are the remnants of larger particles that have fractured and disintegrated. The findings of FESEM further reveal that the particles' surfaces exhibit roughness and cracks. If these microplastics are introduced into the environment, they can facilitate the transportation of other pollutants to different ecosystems (Amrutha et al. 2023).

CONCLUSIONS

In this study, the abundance, color, size, shape, and constructive polymer of microplastics present in the sludge of wastewater treatment plants in Zahedan city were investigated. The results of this study showed that despite the different processes of wastewater treatment plants, there are many microplastics in the sludge of the treatment plants. Most of the shapes of microplastics in the sludge samples are fiber and fragment shapes, which remain in the wastewater until the end of the treatment process due to their low weight, and the treatment units have a lower removal efficiency for these shapes. However, in the secondary sedimentation unit, due to the flocculation process and the longer retention time, they settle to some extent.

Fiber forms, due to the inflow of effluent from washing machines and dry cleaners, are abundant in the effluent entering wastewater treatment plants. Fiber forms have a high surface-to-volume ratio, which increases their potential to create environmental hazards due to the surface adsorption of heavy metals and other organic pollutants on their surfaces. The most common polymer found in the sludge of treatment plants is the polymer PET, which is due to its wide application as food packaging materials, fabric fibers, insulation, building materials, etc. Therefore, measures should be taken to reduce and control the amount of these polymers, such as comprehensive management methods to reduce the consumption of microplastics and remove them from the exit effluent of wastewater treatment plants. As a result, preventing the entry of such microplastics into the environment can significantly reduce the effects of microplastics on the environment.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publica.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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