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Detection and characterization of MPs in the human stool: an observational study in Bushehr, Iran

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Plastic particles smaller than 5 mm, known as microplastics (MPs), are considered emerging contaminants that may pose health risks to humans. The present observational-descriptive study aimed to detect and characterize MPs in stool samples of 30 adult individuals with endocrine disorders in Bushehr, Iran. The presence, morphology, polymer types, and elemental composition of MPs in human stool samples were analyzed using Micro-Raman spectroscopy, scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDX). All stool samples contained MPs, with a mean concentration of 6.22 items/g. The most frequently identified MPs were within the size range of 500–1000 µm, and fibers accounted for 97.9% of the observed shapes. White/transparent and black/gray were the most common colors detected. Raman analysis identified six types of polymers, with polypropylene (PP) being the most prevalent (36.36%). EDX results revealed major elements such as carbon and oxygen, followed by trace elements like Na, Mg, Si, Cl, K, Ca, Fe, and I. Overall, the findings indicate widespread exposure to MPs, likely via ingestion, and highlight their roles as vectors for environmental pollutants due to their ability to adsorb heavy metals and undergo surface modification through environmental weathering.

Keywords Microplastic, Human stool, Endocrine disorders, SEM-EDX

Microplastics (MPs), plastic particles measuring smaller than 5 mm[1,2], are categorized as either primary or secondary MPs based on their source of environmental release[3,4]. Primary MPs are produced for specific applications, including use in scrubs/facial cleansers, washing gels, and cleansing pads[5–7]. In contrast, secondary MPs are generated from bulk plastic materials through processes like erosion, corrosion, abrasion, photo-oxidation, and biological transformation[8–10]. Moreover based on their physicochemical properties, MPs can be classified into fiber, film, and fragment[11].

MPs can pose a major threat to the global ecosystem for several reasons. First they can be ingested by a wide range of marine organisms such as worms[12], shellfish[13], fish[14], and birds[15]. Second, they have the ability to absorb chemicals and transfer them through the food chain[16]. Finally, they contribute to environmental pollution through the release of their additives, including flame retardants, antioxidants, plasticizers, and pigments, into the environment[17].

Several studies have reported the presence of MPs in various environmental matrices, including surface water[18], sediments[19], runoff[20], wastewater[21], air[22–24], and dust[25]. Although MPs pollution in the aquatic environment, particularly oceans, has been studied for over a decade, the detection of these particles in honey[26], beer[27], milk[28], and drinking water[29], has recently increased concerns regarding their potential impacts on human health.

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Human exposure to MPs is now clearly recognized. The body may be exposed to MPs via different routes, including ingestion[30], inhalation[31], or through skin contact[32,33]. Following human exposure to MPs through diet, it is possible to uptake these particles by humans, because particles less than 150 μm can pass through the epithelial cells of the gastrointestinal tract of the body of mammals[34].

It is estimated that only about 0.3% of ingested MPs are absorbed by the human body, while an even smaller fraction, almost 0.1% including particles larger than 10 μm, may be capable of crossing organ and cell membranes, as well as the blood-brain barrier and placenta[30].

The first evidence of the plastic fiber accumulation in the lung was proven in 1998 for the first time, which suggests that MPs can accumulate in the lung[31]. Additionally, Amato-Lourenço et al. (2021)[35,36] have confirmed the occurrence of MPs in human lung samples.

Skin contact with MPs may occur through the use of cosmetics containing micro and nanoplastics (NPs) or through water during washing[32]. However, because only particles smaller than 100 nm can pass through the corneous layer of the skin, so the absorption of MPs through the skin is unlikely, while the absorption of NPs is more plausible[32].

Although human exposure to MPs has been established, evidence regarding their presence and effects within the human body remains limited. Previous studies have suggested possible association between MPs or their derivatives and endocrine disorders, such as diabetes and thyroid dysfunction. However, no research to date has investigated the presence of MPs in stool samples from individuals with endocrine disorders. The objectives of this study are to[1] quantify the abundance of MPs in stool samples from adults with endocrine disorders[2]; characterize the physicochemical properties of detected MPs, including size distribution, morphology, color, and polymer type; and[3] provide foundational data to inform future investigations into the potential health implications of MP exposure in vulnerable populations.

Materials and methods

Participant recruitment

This research is an observational-descriptive study focusing on 30 individuals with endocrine disorders, including both thyroid conditions (hypothyroidism and hyperthyroidism) and diabetes. The participants, all adults aged 20 and over, were patients referred to a specialized clinic in Bushehr, Iran. The study protocol was approved by the Ethics Committee of Shiraz University of Medical Sciences (Ethical cod IR.SUMS.REC.1402.469). Written informed consent was obtained from all participants. All methods were performed in accordance with the relevant guidelines and regulations.

Sampling

All study participants voluntarily contributed stool for analysis. Participants were instructed to collect stool samples in open glass containers covered with foil. The stool samples were obtained in a medical diagnostic laboratory setting. The samples were promptly transferred to the laboratory and maintained at −20 °C awaiting subsequent digestion and preparation steps.

MPs extraction

To digest the samples, 10 g of stool was weighed and transferred to a glass container, then 50 ml of 30% H_2O_2 (Merck, Germany) solution was added. Then the containers were subsequently incubated at 40 °C for 2 weeks. After digestion, particles larger than 5 mm were separated using a sieve. The liquid passing through the sieve was then filtered using 5 μ filter paper and finally rinsed with distilled water to eliminate the remaining H_2O_2. In the following step, 20 ml of saturated NaI solution (density 1.6 g/cm^3)[37,38] was added to the undigested sample residue, which could contain MPs. After 4 h of setting, the mixture was passed through a polytetrafluorethylene (PTFE) filter paper with a pore size of 0.45 μm. Finally, the filter papers were transferred to glass petri dishes and dried at room temperature.

MPs detection

An optical microscope (A. KRÜSS Optronic, Germany) with up to 400 × magnification was used to count the number of MP particles extracted from the samples. Particle analysis involved counting and classifying the particles based on their size, shape, and color. In some cases, the hot needle test was used for confirmation. The size of each identified MP particle was measured using a probe diameter. The shape of the MPs was categorized into three main types: fibers, fragments, and films. Similarly, the color of the MPs was categorized as black/gray, white/transparent, blue, red, brown, pink, and green.

Polymer identification

In addition to optical microscopy, micro-Raman analysis was used to precisely determine the chemical composition of the MPs. The polymer types were identified using a micro-Raman instrument (Lab Ram HR, Horiba, Japan), which determined polymer type by analyzing spectra in the range of 400–1800 cm^{-1} obtained through 785 nm laser irradiation. Finally, the results were matched with the reference spectra to identify the specific polymer types.

Morphological and elemental characteristics of MPs

The morphology of the MPs particles was examined using a scanning electron microscope (SEM) (TESCAN, Czech Republic). To assess the elemental composition, the SEM was equipped with an energy-dispersive X-ray spectrometry (EDS) system. Before analysis, MP particles were securely mounted on conductive carbon tape to facilitate effective electron conduction. Furthermore, a thin layer of gold was applied to the surface of the MPs to reduce charging effects and improve image clarity.

Quality assurance (QA)/Quality control (QC)

All sample handling and digestion steps were performed inside a clean bench with reduced airflow to minimize the deposition of airborne particle. A 100% cotton lab coat and nitrile gloves were used. Plastic tools and containers were avoided whenever possible, with glass or metal materials used instead. Prior to use all solutions were filtered through 0.22 µm membranes before use, and all working surfaces were thoroughly rinsed with filtered distilled water prior to analysis. A procedural blank consisting of distilled water was included in each batch and processed using the same digestion, filtration, and microscopic examination steps as the stool samples.

Statistical analysis

Microsoft Excel 2018 and SPSS 20 software were used for statistical analysis. The MPs results are reported as the mean ± standard deviation. The relationship among the levels of MPs and age, sex, and type of disorder were calculated by the Spearman test. The p-value < 0.05 was considered statistically significant. Additionally, all graphs were illustrated using Microsoft Excel 2018 and Origin 2022.

Results and discussions

MPs abundance

A total of sixteen men and thirteen women, aged 29–62 years, participated in this study. MPs were detected in all thirty stool samples, with a total of 904 MPs identified across all stool samples. Trace levels of background MPs were also observed in the blank controls, with an average of 2 items/filter, all of which were fibers. These background values were subtracted from the corresponding sample counts. As shown in Fig. 1 and Table S1, the average number of MPs was 6.22 ± 3.3 items/g, ranging from 1.37 to 15.75 items/g. According to Fig. S1, the average number of MPs was 6.7 ± 3.1 and 5.74 ± 3.5 in the Diabetes and Thyroid subgroups, respectively. Additionally, Table S2, indicates no significant association (p-value > 0.05) between MPs abundance and age, sex, and type of disorder.

Only a limited number of studies have so far reported MPs in human samples. In the one of the earliest investigation, MPs were detected in human stool across eight countries in Europe and Asia. MPs were detected in all eight samples, with a median concentration of 20 pieces/10 g MPs within the range of 18–172 (equivalent to 2 pieces/g), which is lower than the finding of the present study[39]. Another study conducted in Beijing, China, 26 young males participated in the study, and finally, 24 of the participants completed the study. Approximately all of the samples were positive for MPs (23 of 24 samples). Detected MPs were in the range of 1–36 particle/g, which is almost similar to our range (1.37–15.75 items/g)[40]. Also, a study was conducted for the first time was conducted on inflammatory bowel disease (IBD)[41] and healthy persons[42] in Nanjing, China. MPs were found in all 102 stool samples. The total number of MPs detected in healthy and IBD participants was 3070 and 5459 respectively, with average concentrations of 28 and 41.8 items/g, which is significantly higher than the present study[43]. A study in Hong Kong, China, investigated MPs in 8 adult samples. The result revealed a total of 129 MPs, with an average concentration of 50 particles/g, ranging from 20.4 to 138.9 particles/g, which was about 8 times higher than reported in the presented study[43].

A number of studies have also reported MPs by weight rather than by number. In a study conducted across three cities in China, 78 young students participated. MPs were detected in 77 out of 78 samples, with a total mass concentration of 54.7 µg/g (range: 10.1–102.7 µg/g)[44].

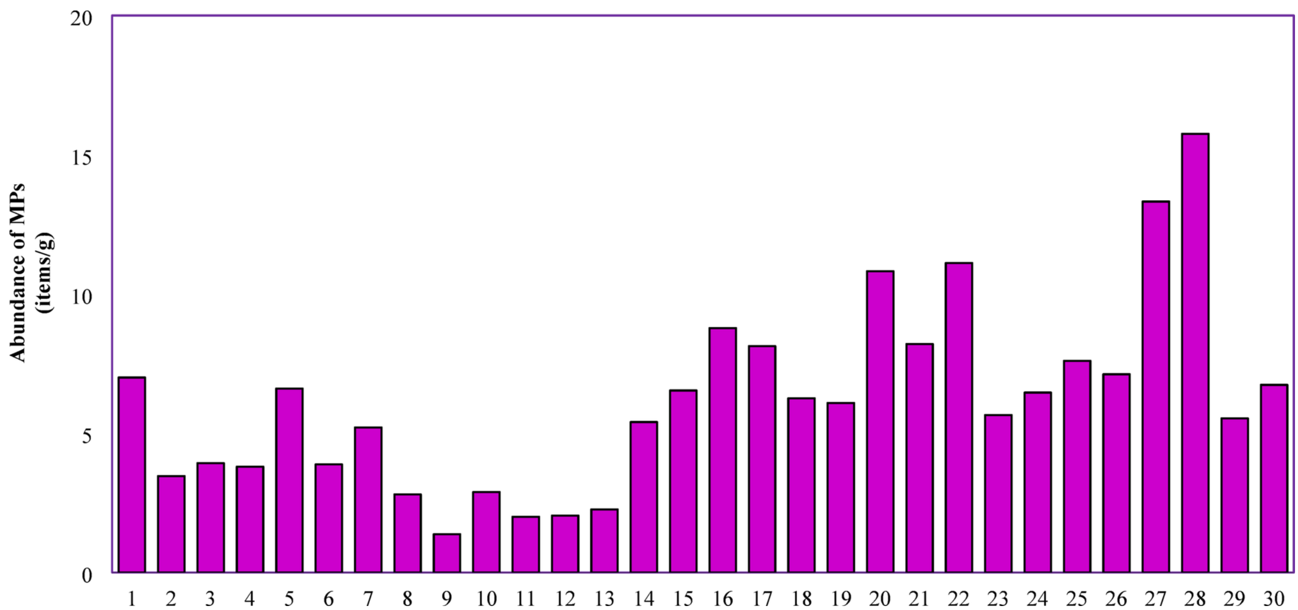

Fig. 1. Abundance of Microplastic (MPs) in the human stool samples.

Another study was conducted in Surabaya, Indonesia. Eleven healthy people from the coastal fishing community participated in the study. MPs were detected in more than half of the participants (6 out of 11 participants). The MPs were reported as 3.33 to 13.99 µg/g[45]. Also, Wibowo et al.[46] detected MPs in 7 out of 11 stool samples collected from eleven healthy adults from a rural farming village in Indonesia. MPs concentration was in the range of 6.94 to 16.55 µg/g.

These findings suggest that exposure to MPs is widespread and persistent, and has been detected in human samples almost worldwide. The differences in the amounts reported in different studies can be attributed to factors such as regional differences[47], diet[48,49], sampling, digestion, and detection methods[50,51]. Therefore, establishing a standardized method for sampling, digestion, and identification of MPs is essential in order to facilitate comparisons of results on a global scale.

Characteristics of MPs

Based on the Fig. 2 and Table S3, the detected MPs were classified into six size categories. Among the detected MPs, 16.04% were in the size range of 1000–5000 µm, 34.07% were between 500 and 1000 µm, 28.32% were between 250 and 500 µm, 17.92% were between 100 and 250 µm, 2.65% were between 50 and 100 µm, and 1% were < 50 µm. MPs in the size range of 500–1000 µm and < 50 µm had the highest and lowest numbers, respectively among all MPs. Based on the results, larger MPs were more abundant in this study, and the lower number of MPs under 50 µm could be due to limitations in the methods of separation and identification of these particles[51].

Among the few studies that have identified MPs in human samples, four them were conducted in China and reported the size of detected MPs. A study reported MPs within the size range of 50–500 µ[39], another study reported MPs in the size of 20–800 µ[40], another study reported MPs in the range of 40.2–481.9 µ[43], and a study in reported MPs in size range of 4.4–333.2 and 1.7–393.8 µ in healthy and IBD participants respectively[41]. Compared to the mentioned studies, the reported MPs in the presented study are in the broader size range.

Particle size is a key factor influencing the toxicity of particulate matter within the body[52,53]. It has been established that the most important route of entry of MPs into the body is ingestion[42,54]. The detection of MPs in human stool samples confirms that these particles are ingested and subsequently excreted from the body[39]. To date, no research has explored the fate of MPs after they enter the gastrointestinal tract[41]. MPs can likely be absorbed by the body through lymphatic tissue, particularly through phagocytosis or endocytosis[41,55].

According to Fig. 3 and Table S4, the identified MPs morphologies were categorized into fibers, films, and fragments. The majority of MPs in all samples were fibers, accounting for 97.9%. Fragments and films were other detected shapes of MPs with percentages of 1.55% and 0.6% respectively. Just three studies reported MPs shape, and all of them are in contrast with the present study. Detected MPs by Yan et al.[43] were in the order of sheet, fiber, fragment, and pellet. Schwabl et al.[39] reported MPs shaped as fragments, films, spheres, and fibers. Ho et al.[38] detected two MP shapes, with fragments being more abundant than fibers.

The higher levels of fiber compared to other forms of MPs could be due to several reasons. These include the presence of fibers in the air and dust, fiber deposition on food[56], cultural differences in cooking methods, packaging containers, and types of textiles used[40]. Previous studies on dust[25,57,58] reported that fibers were the dominant form of MPs, likely because of their distribution in the environment and subsequent entry into the body.

Figure 4 and Table S5 show the color of detected MPs. Detected colors were black/gray, red, brown, white/transparent, blue, pink, and green. White/transparent and black/gray were the dominant colors, with

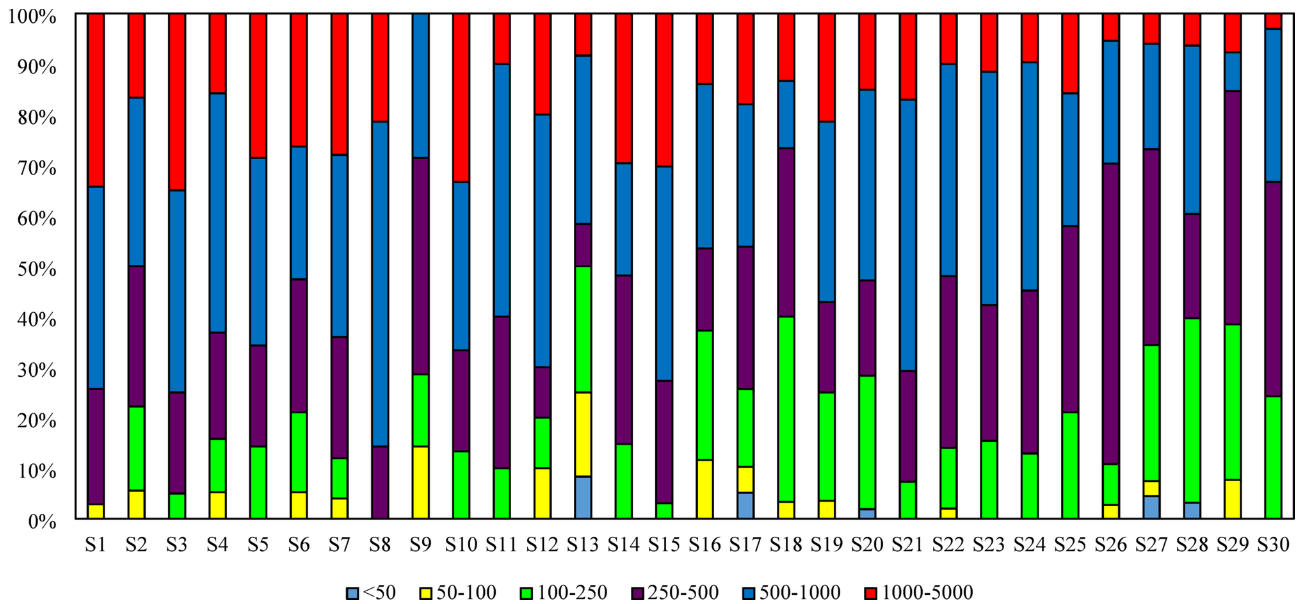

Fig. 2. Microplastic (MPs) size distribution in the human stool samples (µm).

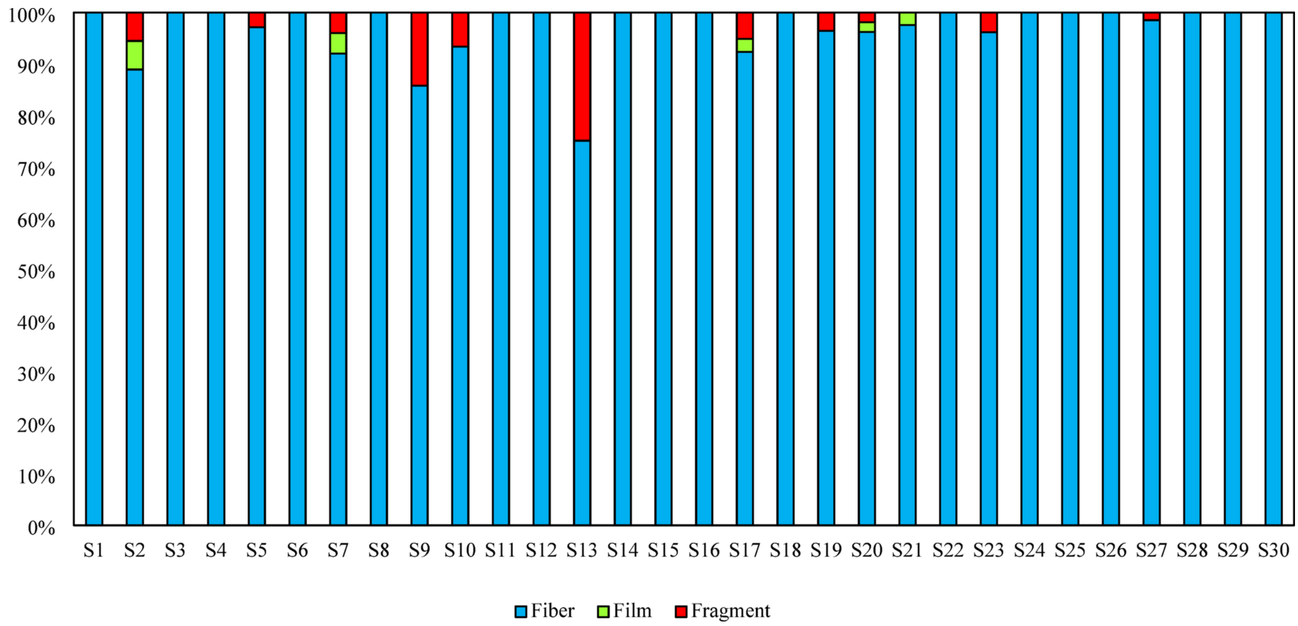

Fig. 3. Microplastic (MPs) shape distribution in the stool samples.

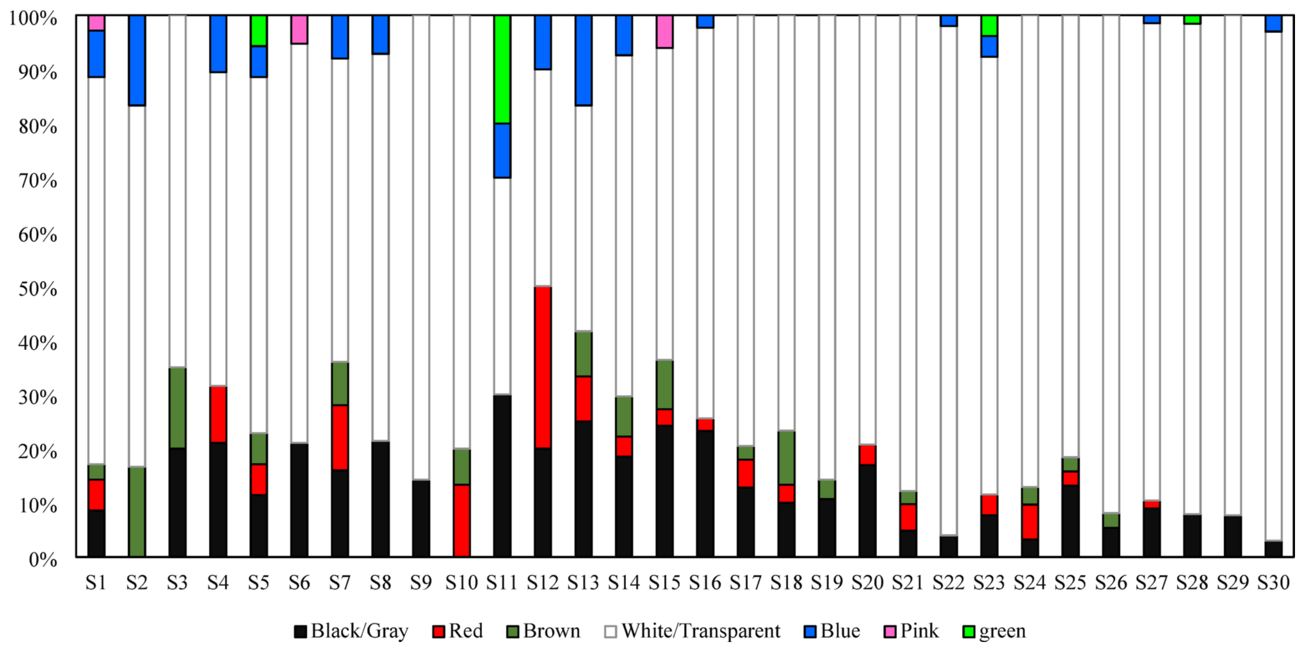

Fig. 4. Microplastic (MPs) color distribution in the stool samples.

percentages of about 78% and 12%. Up to now, there has not been any study on the stool that reported color for detected MPs, so in this study, for the first time, the color of MPs was reported. Some studies conducted on other parts of the body have reported the color of MPs. In a study conducted in Italy on placentas, 12 MPs were identified, with colors of blue, orange, dark, red, violet, and pink; blue was the dominant color[59]. Two studies on other human tissues are in agreement with the present studies. In a study that investigated MPs in human colectomy samples in Malaysia, the observed MPs exhibited a range of colors, including transparent, black, red, green, blue, brown, purple, and yellow, with the dominant color being transparent[60]. In this study, the high occurrences of transparent color of MPs were linked to the effect of digestive enzymes on these compounds during intestinal transit and their decolorization[60]. Another study in lung tissue in Brazil detected MPs were white/gray, brown, beige, transparent, black, blue, yellow, green, and orange, and bright colors were dominant[34]. Finally, the abundance of transparent color in this study is likely linked to the widespread presence of fibers, which attributed to human exposure to materials that release these compounds, including packaging containers.

Another reason could be interactions within the digestive system, although the effect of the digestive system and digestive enzymes on MPs has not been proven so far.

Polymer composition of MPs

Six polymers, including polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), medium-density polyethylene (MDPE), polyvinyl chloride (PCV), and polycarbonate (PC), were detected using a Raman spectrometer. The percentage of MPs polymers, and Raman spectra was presented in Figs. 5 and S2, the dominant polymer was PP (36.36%). In agreement with our study, three previous studies, Schwabl et al.[39], Wibowo et al.[46], and Zhang et al.[40] reported PP as the dominant polymer. In contrast with the present study, two studies conducted in China, Yan et al.[43] and Ho et al.[38] reported PET and PS as the dominant polymer.

PP is widely used in food packaging because of its heat resistance and chemical stability, which can leach from these containers into food and lead to ingestion of this polymer[61,62].

Chemical composition analysis of MPs

Figure 6 presents the SEM-EDX results, showing that various chemical elements including C, O, Na, Mg, Si, Cl, K, Ca, Fe, and I were detected in the samples. The highest abundance among all elements was related to C and O, which are the main elements of plastic. The prominent peaks of C and O in the EDX analysis may indicate the presence of MPs particles[63], although polymer identification is recommended for confirmation. In addition to C and O, trace amounts of other elements like Na, Mg, Si, Cl, K, Ca, Fe, and I were identified. These elements may be used in plastic manufacturing, or their presence could indicate that the MPs have been exposed to environmental weathering, which alters their surface and their capacity to absorb other components[64–66].

Study limitation

This study have several limitations, including the absence of a control group, the lack of subdivision of patients into smaller subgroups, and the unavailability of occupational data. Additionally, the analytical method used, with a resolution of ≥ 50 µ, may have led to an underestimation of smaller MPs. Classical detection limits and recovery efficiencies were not determined, and particle identification relied primarily on filter size ranges, supplemented by optical microscopy, Raman spectroscopy, and SEM.

Recommendation

Future studies should involve larger sample sizes and appropriate control groups. They should also include the acquisition and presentation of blank EDX spectra from unused filters to ensure that detected trace metals are not analytical artifacts. More sensitive techniques capable of detecting nanoplastics should be employed, and further research into the association between MPs and dietary habits is needed to better clarify exposure pathways.

Conclusion

This research confirms the occurrence of MPs in the stool samples of individuals with endocrine disorders, suggesting an association rather than a causal relationship. The results showed a predominance of fiber-shaped particles, mostly within the 500–1000 µm size range, and primarily composed of polypropylene. The detection of various inorganic and metallic elements suggests that MPs are not only synthetic in origin but also capable of interacting with environmental contaminants through surface sorption and weathering. These findings highlight the need for standardized methods in MPs analysis and for evaluating the potential health implications of chronic exposure, especially in vulnerable populations.

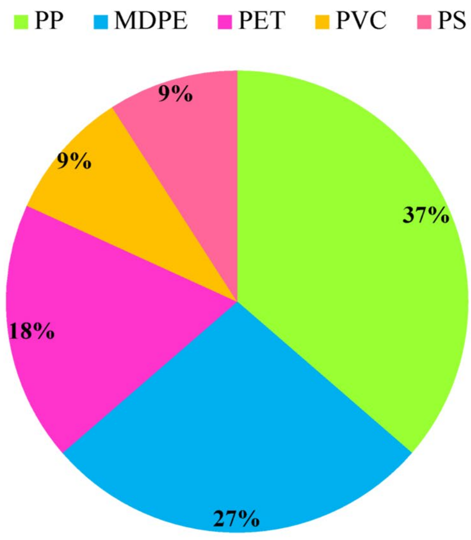

Fig. 5. Polymer type of detected Microplastic (MPs) in the human stool samples.

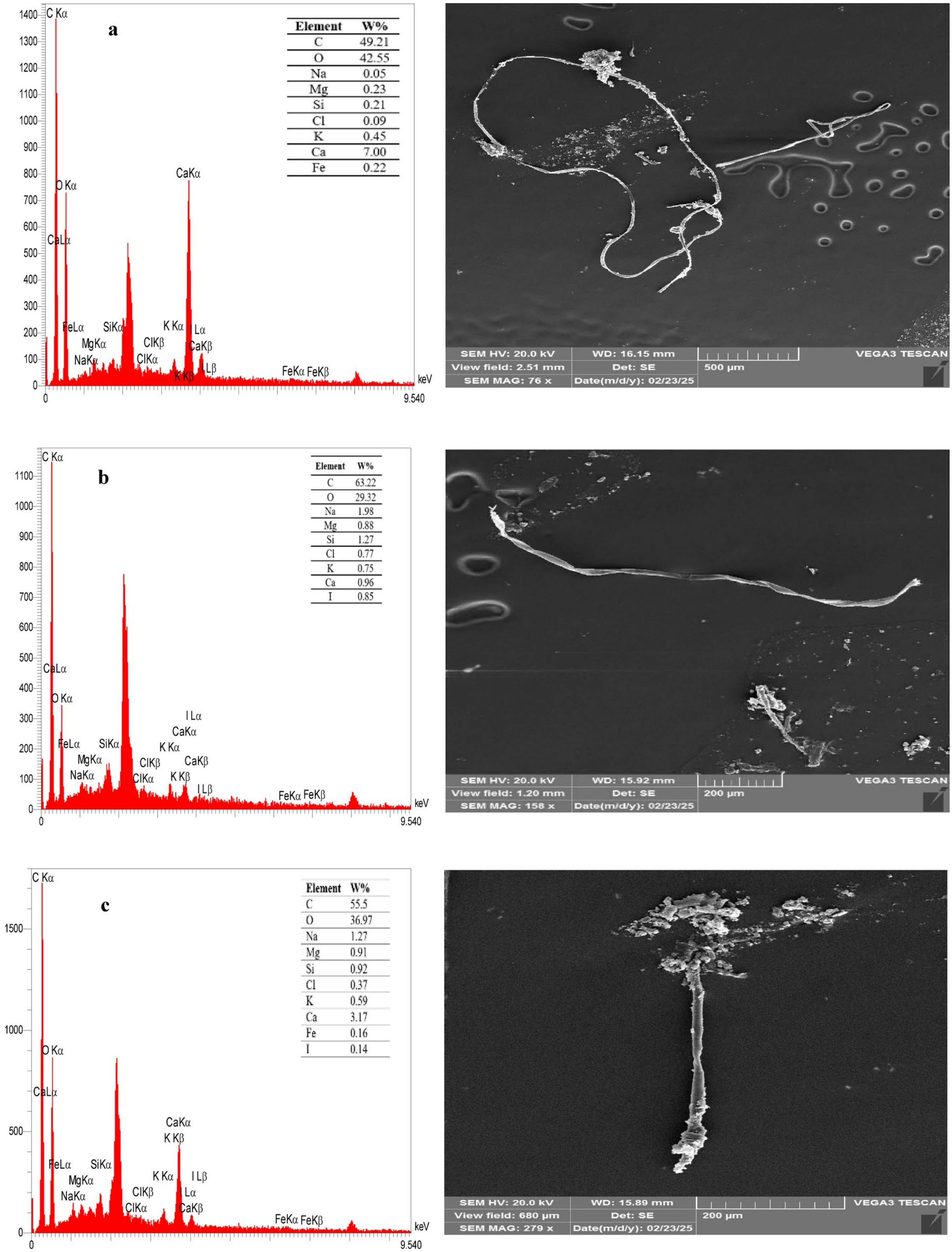

Fig. 6. Scanning electron microscopy (SEM) images and energy-dispersive X-ray spectroscopy (EDX) analysis (**a**–**f**) of some identified MPs in the human stool samples.

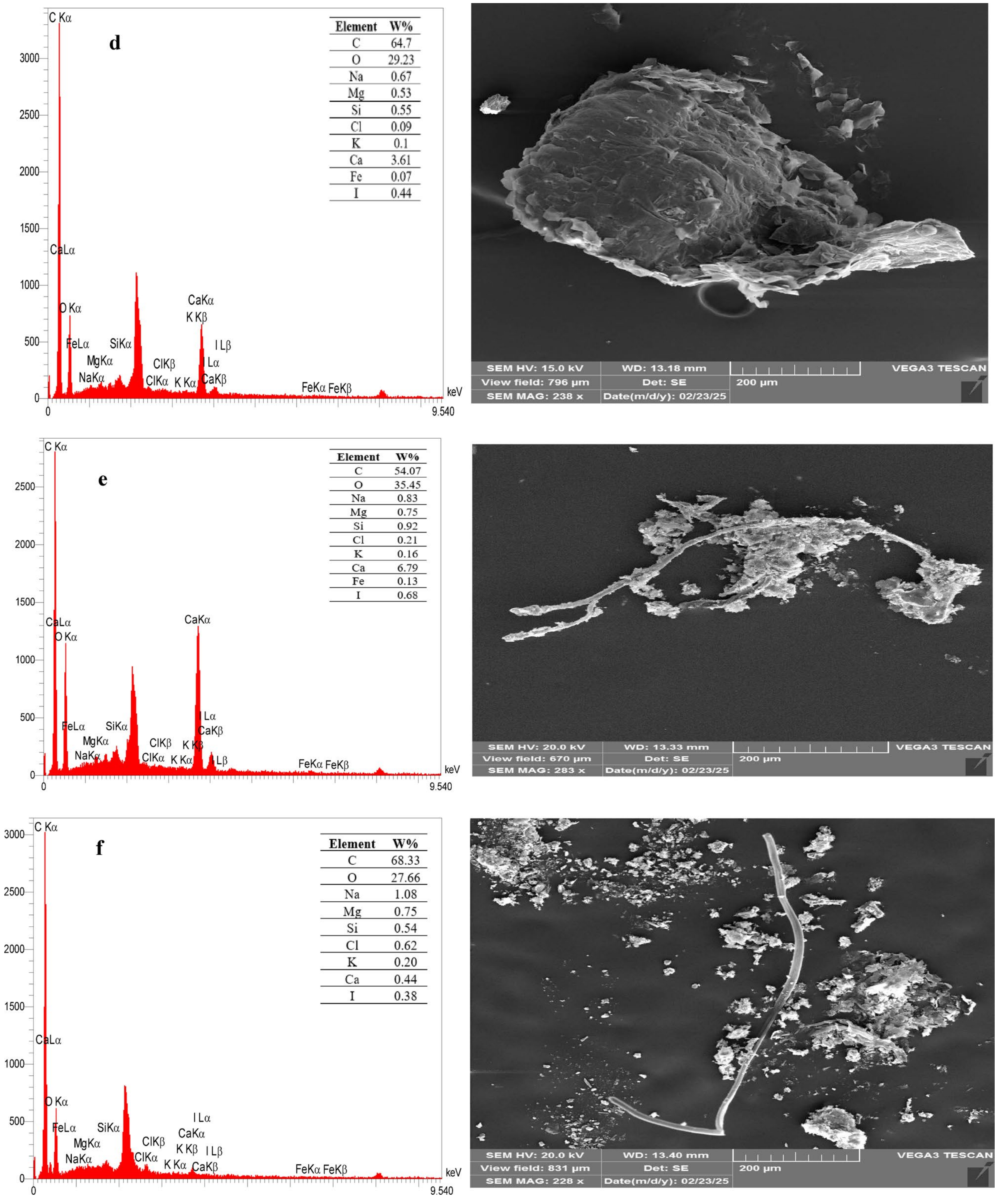

Fig. 6. (continued)

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

**Fatemeh Faraji Ghasemi: ** Sampling, Samples preparation, Software, Writing original draft, Writing-review & editing; **Mansooreh Dehghani: ** Supervision, Conceptualization, Methodology, Project administration, Writing-review & editing; **Sina Dobaradaran: ** Writing-review & editing; **Mohammad Hoseini: ** Writing-review & editing; **Mohammad Reza KalantarHormozi: ** Writing-review & editing; **Mohammad Reza Samaei: ** Writing-review & editing.

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Declarations

Competing interests

The authors declare no competing interests.

Ethical approval and consent to participate

The ethics committee of Shiraz University of Medical Sciences has approved this study (Ethical cod IR.SUMS.REC.1402.469). Informed consent was obtained from all individual participants included in the study.

Consent for publication

Not applicable.

Additional information

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