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Determining an optimized laboratory procedure to extract microplastics from wastewater treatment plant effluents using the acid-washing process

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ABSTRACT

Nowadays, due to population growth and changing consumption patterns, environmental damage has considerably increased. One of these problems is the microplastics present in aqueous media. The important point is that wastewater treatment plants cannot completely remove microplastics and they end up entering aquatic and terrestrial receiving environments, which in turn can endanger living organisms. In the present study, an optimized method is introduced to efficiently detect and extract microplastics from the input effluent and output waste streams of the wastewater treatment plants. First, the seasonal sampling was performed in three seasons: spring, summer, and autumn, to determine the season with the most microplastic production. Then, the acid washing using 30% hydrogen peroxide (H_2O_2) and 0.05 M divalent iron was performed on the prepared samples. Also, since this method is based on creating density differences, sodium chloride (NaCl) salt was used. As a result of this procedure, the number of microparticles and microfibers along with their size (up to lower than 10 μm) and morphology were detected in the domestic effluents with an efficiency of more than 98%. Notably, the performance of the studied wastewater treatment plant in terms of microplastic removal efficiency was also evaluated using this method.

Keywords: Microplastics; Extraction; Municipal wastewater; Acid leaching; Oxidation

1. Introduction

Among various contaminating sources, plastics and their derivatives play an important role in environmental issues. A considerable portion of these plastics is composed of micro and Nano plastics. One of the major pathways for these particles to enter nature is wastewater treatment plants that are unable to remove these particles and drain them into the accepting environments [1,2].

One of these receptive environments is the seas and oceans. By entering marine environments, plastics can enter the marine food chain, and thousands of species of aquatic animals such as whales, dolphins, turtles, and seabirds die due to suffocation every year [1,3,4].

Despite the mentioned issues originating from plastic contamination, the studies related to the abundance of microplastics in aquatic environments are limited [5,6]. Because these particles are invisible due to their ultra-small size, negligible attention has been focused on this kind of

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contamination. The emission of these particles (microplastics) in different countries varies by the amount and pattern of consumption and lifestyle and is also debatable. According to these explanations, the studies were conducted on marine environments, in which the accumulation of microplastics in lakes and rivers was also reported. Also, studies were conducted in the field of identification and separation of particles in wastewater treatment plants [7–9].

In a research, the presence of microplastics was detected through differential scanning calorimetry (DSC) analysis of three wastewater treatment plants. The results showed the presence of polyethylene (PE), polystyrene (PS), polypropylene (PP), and polyethylene terephthalate (PET), which were classified as fragments, fibers, or granules. During the evaluation of the wastewater treatment plants, it was determined that the preliminary treatment did not remove more than 58% of the microplastics, while the wastewater treatment plants applying a secondary treatment with activated sludge achieved microplastic removal effectiveness between 90% and 96.9% [10].

Also, a study was conducted by Ziajahromi et al. [11] in Australia. The main purpose of this study was to establish a validation method for sampling microplastics existing in effluent waste and using a method for determining and measuring microplastics in waste streams. The acid-washing method using hydrogen peroxide and sodium iodide salt was utilized in this study, which is based on creating a density difference between particles to separate them. With the method and materials used in their study, the extraction of particles was done through an optimized procedure.

Considering the importance of the subject, identification methods have an advantage when they can determine the shape and size of microplastic particles down to very small sizes. For example, in a research, an improved method was determined. This method has been able to determine the size, shape, polymer type, and particle composition using a combination of oxidation, fluorescent dye, and attenuated total reflectance FTIR (ATR-FTIR) analysis in wastewater. In this method, particles as small as 20 microns were identified [12].

In a research, a new method was reported for analyzing microplastic fibers' characteristics using Fourier-transform infrared spectroscopy (FTIR). Based on this method, a new quantifying procedure and simultaneous detection of fiber polymers using the FTIR analysis were developed. Simulating the washing process was done using commercially available domestic products and wastes were filtered using the GF/F (0.7 μm) or 0.2 μm filters to collect the smallest fibers. In addition, a new method for wastewater treatment was also proposed. After that, filters were investigated using the scanning electron microscope (SEM) to confirm the length and width of fibers. This method allows better release of fibers and identification of fiber polymers [13].

Based on the studies that were conducted, in a research, the existing methods for removing microplastics were evaluated in a comparative manner. In this study, the existing techniques using hydrogen peroxide, proteinase, trypsin, and potassium hydroxide were compared. Also, the refining ability, the digestion effect, the ability to recover microplastics, and polymer detection were investigated using Raman spectroscopy and matching software [14].

In a study on a wastewater treatment plant in China, which was about the release of microplastic particles through the effluent, this issue was examined. In this research, it was determined how much microplastic particles are removed through sludge and how much is removed through the effluent [15].

It was determined in this study that microplastics exist in the input and output streams of the wastewater treatment plant in combination with various polymers, which subsequently enter the aquatic environments. The level of microplastic removal in different stages of the wastewater treatment plants was also determined. Because the final destination of most of these microplastics is the marine environments, a study was performed by Abiola et al. in 2017 considering this problem, entitled analyzing microplastics and their removal from water. In this study, polystyrene as one of the microplastics existing in water was considered and the results have shown that different filtration methods can show acceptable performance in removing it [16].

However, the source of transporting particles to seas is the more important problem [17]. Therefore, numerous studies have been oriented to investigate microplastic's source of production and entrance to aquatic and marine environments. Most of these studies have concluded that the output waste of wastewater treatment plants is one of the major sources of this environmental problem. For example, as a result of various studies performed in the USA, it was revealed that the waste stream from wastewater treatment plants has increased the concentration of microplastics in the Chicago River [18–20].

A study was performed by Magnusson et al. [21] in Sweden, entitled the pathways of entering microplastics to marine environments. They have demonstrated the ways in which microplastics enter marine environments. Accordingly, it was determined that the waste stream from wastewater treatment plants is one of the major entering pathways.

Further investigations, it was revealed that microplastics are not completely removable through the existing processes in the primary and secondary treatments. However, before removing microplastics from effluents, there is a more important stage, which is detecting and extracting them from the streams of effluents [22–24].

Because there are other organic and inorganic compounds in effluents along with microplastics and microfibers, their separation is of great importance [25,26]. The presence of other particles along with microplastics makes it difficult to observe and detect them and it cannot be claimed with complete certainty that the observed or removed particles were microplastics or not. Based on that, multiple types of research have been performed to determine a high-yield method to detect and separate microplastics from effluents. Some of the methods to detect microplastic particles were performed on seawater and some others were performed on municipal sewage [1,11,27,28].

According to this, the present study has been devoted to determining an optimal methodology to detect and extract the particles from wastewater and effluent streams. The method used in this study is different in several ways from all the methods used before. This difference is related to parameters such as the type of materials used, temperature, mixing time, and optimization in the number of materials used.

Contrary to the previous methods performed on effluent samples, a different and optimized acid-washing method and a different salt for creating density differences were used in the present study. These changes are not limited to these cases and new materials, techniques, and parameters were also applied during our investigations. Also, the methods of sampling and preparation of wastewater samples were done in such a way that can be cited to be a reliable criterion to a large extent in terms of the statistical population. As a result, it was demonstrated how differences between performing methods can affect the efficiency of particle detection.

2. Materials and methods

According to the aforementioned content in section (2) and the previous studies conducted in this field on various samples, such as seawater, the present study is focused on municipal wastewater with its specific characteristics, which will be discussed later in detail. First of all, the conditions of the sampling location related to the samples used in this study as the human wastewater were presented. The studying wastewater treatment plant (WWTP) is located in Iran, which is important to be studied due to its vicinity with aquatic environments that can result in disposing contaminated waste streams to the agricultural lands. This wastewater treatment plant has a capacity of 20,000 m^3/d that supplies drinking water to about 120,000 people. This wastewater treatment plant is one of the most advanced and equipped centers in the country. The present study not only performed the separation of microplastic particles but also determined what percentage of particles can be removed by a typical treatment system in existing effluent and what percentage needs a more advanced treatment system to be removed.

2.1. Sampling methodology

The sampling method is of great importance in any research. Sampling must be done in such a way that provides an acceptable and universal statistical population. Therefore, sampling in this study was conducted in such a way that microplastic particles can be estimated to a reasonable level. Sampling from the waste streams of the Wastewater treatment plant was performed on both the input and output streams. The sampling was performed in three seasons' spring, summer, and autumn, 10 consecutive days in each season, and 100 L in each day, which resulted in 1 m^3 for each season. The reason for sampling in different seasons was to investigate the relationship between seasons and the production of microplastic particles. The sampling was also performed on the input effluent and output waste to evaluate the performance of the Wastewater treatment plant in removing these particles. Finally, the samples were passed through sieves to separate other components existing in the waste. Then, the materials and particles were washed with distilled water and sent to the laboratory for the next stages.

2.2. Microplastic particles identification

Due to the presence of microparticles other than microplastics in municipal sewage, their identification and separation are challenging and of great importance. The presence of other particles along with microplastics makes it difficult to observe and detect them and it cannot be claimed with complete certainty that the observed or removed particles were microplastics or not. Therefore, before performing any experiment to separate and remove microplastic particles, these particles must be segregated to determine the amount of them in the input effluent and output waste, which can also get the removal percentage. Based on that, determining any method for wastewater treatment and separation of microplastic particles from the wastewater stream needs precise detection. Therefore, the process and steps for this research are considered as shown in figure number one. These steps include 1- Passing the prototype over the sieves, 2- Placing the sample obtained from washing the surface of the sieves into the oven, and 3- Pouring H_2O_2, Fe, and NaCl into the sample container (It is explained in detail below), 4- Sedimentation and collection of the liquid surface using a pipette 5- Viewing the final sample containing microplastic particles with a Stereo microscope.

At first, the wastewater was first passed through the prepared sieves to separate the waste ingredients and then thoroughly washed for further laboratory processes. It should be mentioned that because the consumption pattern varies by season, this stage was performed on the samples in three seasons' spring, summer, and autumn. According to the established procedure, 5 sieves with grades of 100, 200, 270, 400, and 500 with mesh opening diameters of 150, 75, 53, 38, and 25 μm, respectively, were used in this study to classify existing particles in term of size, by passing the wastewater stream through these specified sieves.

After passing the wastewater and effluent, the samples on the mesh of the sieve were washed with 400 mL distilled water (the amount of washing water is optional) and then oven dried at 90°C for 24 h, to reduce its amount to about 50 mL.

From this point on, the acid-washing process was performed. First, 25 mg of 0.05 M divalent iron was poured into a beaker containing the distilled water resulting from washing the input effluent and output waste. Afterward, the beakers were placed on magnetic stirrers to obtain a complete mixture. Then, the 30% hydrogen peroxide solution was added in the amount of 50 and 30 mL to the beakers containing the input effluent (due to the high concentration of organic compounds) and output waste, respectively.

At this point, due to the presence of an acid, interactions occur that lead to the production of foam and its possible overflow from the beaker. However, this problem can be managed by distilled water [27]. After the solution has calmed down, the beaker is placed on the magnetic stirrer again. The temperature of the magnetic stirrer should be fixed at 70°C and the mixing process should be done for 45 min. All the processes performed up until now were aimed to remove organic compounds to inhibit the formation of precipitates. As the separation mechanism is based on density difference, 7–8 g NaCl was added to the beaker solution for every 20 mL of samples to increase the density.

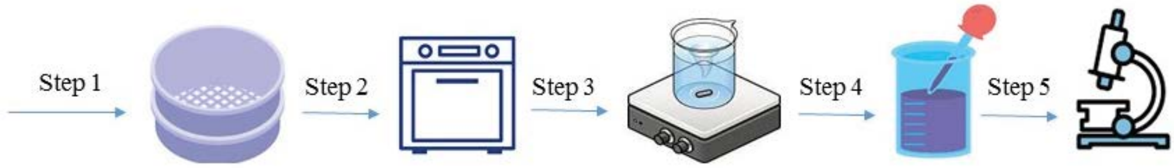


Fig. 1. General steps of conducting the experiment.

Then, the solution was allowed to further mix at 70°C to form a complete mixture with NaCl.

After the NaCl particles were completely dissolved in the mixture, the magnetic stirrer was turned off and the beaker remained immobilized for 24 h to complete the separation process by particle precipitation. Then, the heavier particles were precipitated after 24 h, and microplastic and microfiber particles remained suspended on the surface.

The liquid in the beaker was collected from the surface and intermediate depth of the solution using a laboratory pipette and was washed with water by passing through a sieve. Then, the size of microplastic particles was determined using a stereo microscope. After the collection and extraction, the distilled water (containing microplastic particles) was evaporated at the ambient temperature and the dried sample was prepared for observation, detection, and counting of particles.

2.3. Detection of suspicious particles

Given the possibility of existing particles in the effluent, the detection and separation of microplastics and microfibers from other materials are of great importance to avoid any mistakes in their detection. Based on that, to detect suspicious microplastics, the Rose Bengal coloring method was applied [11]. In this method, natural particles such as fibers, which are similar in appearance to plastic fibers, are colored in a way that can be observable with the naked eye and separable. To do so, the strainer containing suspicious microplastics was colored with 5 mL of the 0.2 mg/L Rose Bengal solution for 5 min at ambient temperature. After that, the strainer was further washed with distilled water under the vacuum condition to remove the residual color. Then, the strainer was oven dried at 60°C for 15 min to get prepared for further investigations by stereo microscope and spectroscopy.

2.4. Observation and measurement steps

The method of measuring microplastics and determining their types consists of two parts. The stereo microscope is a kind of optical microscope that usually works based on the reflected light from an object and is applied to observe samples in more magnification. This device shines a light on the object from two separate paths and magnifies objects up to about 160 times through optical lenses. Also, the separated particles were later detected using the FTIR analysis to obtain the types and spectra of microplastics present in the sample. The results of this spectroscopy are given in the next chapter.

3. Results and discussion

After performing the separation and extraction of particles, the samples were prepared for the observation and counting. Then, the polymeric structure of microplastic particles was determined using the spectroscopy method. Based on that, the primary version of effluent was observed using stereo microscope at the beginning and before performing any extraction, to demonstrate the difference before and after the extraction steps. This approach has clearly revealed that the separation and performing a suitable method can substantially reduce the error of detecting particles and destroying materials that can be considered as microplastic by mistake.

3.1. Results of the observing samples and spectroscopy of particles

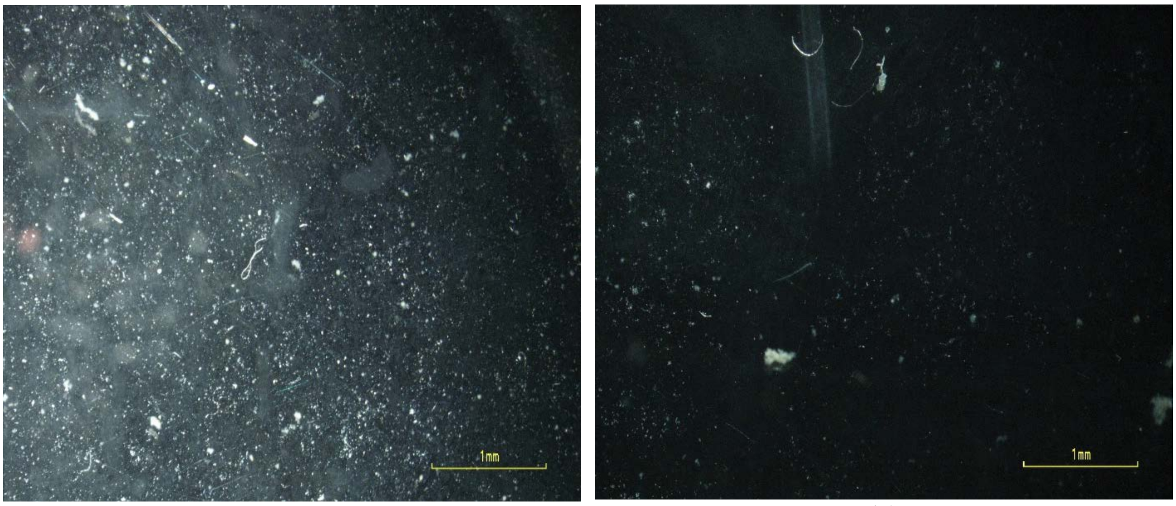
According to the previously mentioned points and observations, the results are illustrated in this subsection. In the figures depicted in Fig. 2, which is related to the primary version of the effluent, it is evident that some various particles and materials make it challenging to detect microplastic particles from others.

Afterward, the acid-washed samples were observed by the stereo microscope, which is depicted in Fig. 3. Accordingly, microplastic particles were successfully segregated from sludge carcasses by acid washing and microplastic particles are visible.

Therefore, by comparing Fig. 2 with Fig. 3, it can be concluded that the error possibility in observing and detecting microplastics mitigates by determining a suitable separation/extraction method. Another noteworthy point is that the primary and secondary treatments in the wastewater treatment plant can not completely separate particles from the effluent. In addition, it was proved by the FTIR analysis that the extracted particles are microplastics, and based on that, types of polymers were determined and illustrated in Table 1.

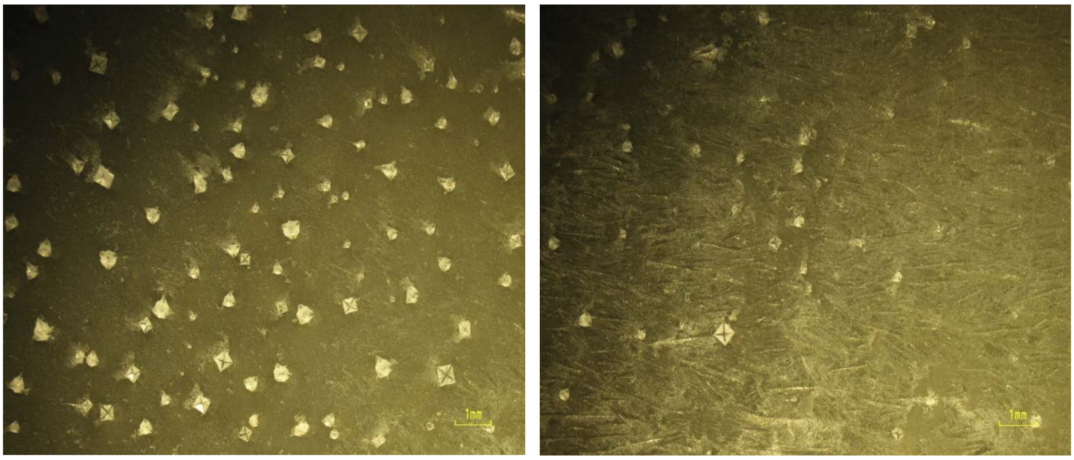
3.2. Counting particles

After spectroscopic results were determined, the liquid sample was poured into the container. Then, by dividing the surface of the container (glass plate) into four equal parts, the number of microplastics was counted using a stereo microscope. Particle counting was performed by considering their appearance and the results for the input effluent and output waste of the treated stream for all three seasons are presented in Table 2.



(1) (2)

Fig. 2. Photo of the microplastics present in the wastewater sample entering the treatment plant (No. 1) and the effluent sample leaving the treatment plant (No. 2).



(1) (2)

Fig. 3. Photo of microplastics existing in the sample of primary effluent after performing the acid washing process (No. 1) and Figures of microplastics existing in the sample of output waste of the treatment plant after performing the acid washing process (No. 2).

Table 1
Spectroscopy results for microplastic particles existing in the effluent (Approximate)

Type of polymer	Frequency in the effluent (%)	Application
Polyethylene	30	Bags, bottle, kitchen appliances, pipes
Polystyrene	25	Disposable tableware, electronics
Polyester	15	Garment industry, some plastic accessories
Polypropylene	10	Food packaging industries, bottle, industrial applications
Polyamide	20	In the production of carpets, rugs, and sportswear

According to the results obtained from the input effluent and output waste, the efficiency of the treatment process in removing microplastic particles from the effluent can be evaluated. On average, 95% of these particles were separated in the treatment process and disposed of with sludge. Based on the results depicted in Tables 2 as well as Fig. 4, the number of particles in different seasons is clear.

Accordingly, in terms of the amount of microplastic particles, summer has the highest frequency compared to spring and autumn. Also, in terms of geometric shape, the number

Table 2
Results of counting particles by appearance and type in spring, summer and autumn

Particle form	Number of particles in the sample of inlet effluent per m^3	Number of particles in the sample of outlet waste per m^3
	Spring	
Sharp corner	9,132 ± 557.05	510 ± 31.11
Rounded	15,156 ± 1,030.60	490 ± 33.32
Microfiber	3,110 ± 161.72	190 ± 9.88
Total	27,398 ± 1,562.48	1,190 ± 67.03
	Summer	
Sharp corner	11,297 ± 689.117	610 ± 41.48
Rounded	16,730 ± 1,137.64	456 ± 31.08
Microfiber	4,720 ± 245.44	275 ± 14.3
Total	32,747 ± 1,876.57	1,341 ± 78.43
	Autumn	
Sharp corner	6,230 ± 380.03	475 ± 28.97
Rounded	11,340 ± 771.12	396 ± 26.92
Microfiber	2,850 ± 148.2	292 ± 15.18
Total	20,420 ± 1,143.94	1,163 ± 64.29

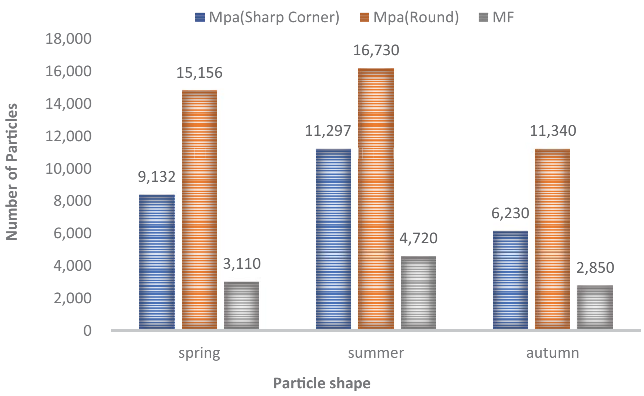


Fig. 4. Comparison of the number of microplastic particles in the incoming wastewater for different seasons.

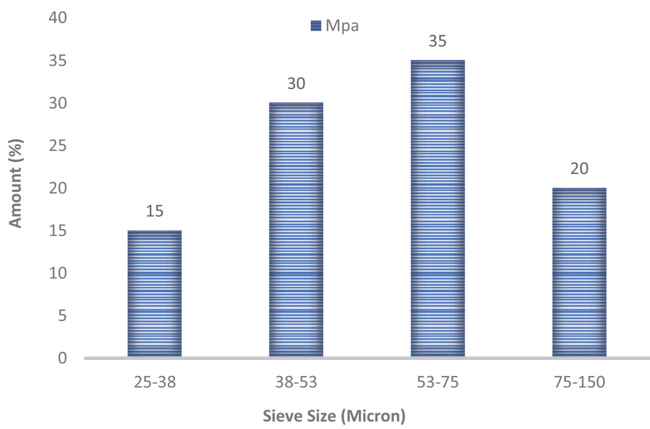


Fig. 5. Determination of particle size based on the order of sieving.

of rounded particles was more than sharp-corner particles and microfibers. Also, particle measurements were performed for each of the sieves. Based on this, the surface of each sieve was washed and the acid was washed separately. The result of this share is shown in Fig. 5.

Based on this, the percentage of the abundance of microplastics in the inlet and outlet of the wastewater treatment plant was calculated separately based on the shape of the particles, which is shown in Fig. 6. Also, using the numbers in this figure, the percentage of removal of microparticles (sharp-corners and round-corners) and microfibers in the wastewater treatment process was calculated, which are 96.2 and 94.17, respectively. This result shows that during the usual treatment process in the wastewater treatment plant, the removal percentage of microparticles is higher than microfibers.

According to the obtained results and determined number and form of particles, per capita microplastic particles

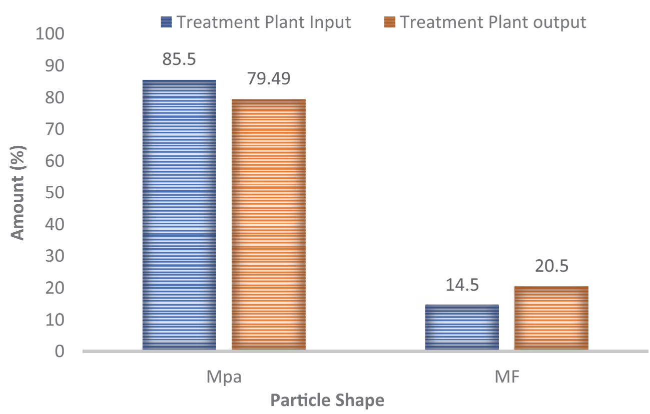


Fig. 6. The amount of particles in the incoming and outgoing wastewater.

production can be estimated by considering the population and the volume of produced effluents per day in the city or country that the wastewater treatment plant is located in. The covering population is about 120,000 and the volume of produced effluent is nearly 20,000 m^3/d. It should be noted that the per capita determination was based on both incoming effluent and outgoing waste stream. Also, calculations were conducted based on the summer data as the time of maximum particle production. Initially, the per capita production was determined based on the sample of inlet effluent of the wastewater treatment plant and the results are illustrated in Table 3.

After that, the daily per capita of each person was calculated based on the particles obtained from the output waste as shown in Table 4, which indicates how many microplastic particles pass through the wastewater treatment plant output waste and enter the aquatic and terrestrial accepting environments.

3.3. Evaluating the separation of particles smaller than 25 μm using the existing method

As mentioned in the sampling section, the largest sieve used for washing the waste to obtain samples was a grade of 500 with a mesh opening diameter of 25 μm. In this step of the research, for validating and evaluating the efficiency of the proposed procedure, the waste stream passed through the aforementioned sieve was washed by acid to determine the size range of particles that can be extracted. As discussed in the introduction section, previous methods have been able to extract particle sizes with a minimum range of 25 μm. According to the method proposed in this study, by use of the divalent iron, different salt to create density difference, altering the sampling method, optimizing the use of hydrogen peroxide, and the performed trial and error, it is possible to detect particles with smaller than 25 μm in size. According to the obtained results using this optimized method, smaller particles were detected in the output waste stream and the removal efficiency was also enhanced, which can be seen in Fig. 7. Also, the number of extracted microplastics smaller than 25 μm in size was calculated to be 117 ± 9.52 particles/m^3, which the majority portion of them was microfibers. Overall, the size of these particles was in the range of 8–23 μm.

It should be noted that the difference in salts had a significant effect on the separation of particles and formed precipitates. According to the conducted experiments, using sodium chloride salt has resulted in more acceptable results in making density differences compared with other salts. Notably, the type of salt should be selected based on the density of polymers existing in the wastewater, which is determined using spectroscopy tests. Based on that, sodium chloride was selected for this study according to the aforementioned considerations. Up now, various methods have been used to extract particles from seawater and municipal sewage, each of which has included different parameters and instructions. In the cases reviewed in the introduction section, the acid washing process was performed on the dried sample of effluent or seawater, and in some other cases, the chemical extraction process was performed on the liquid sample up to 100 mL. With the trial and error

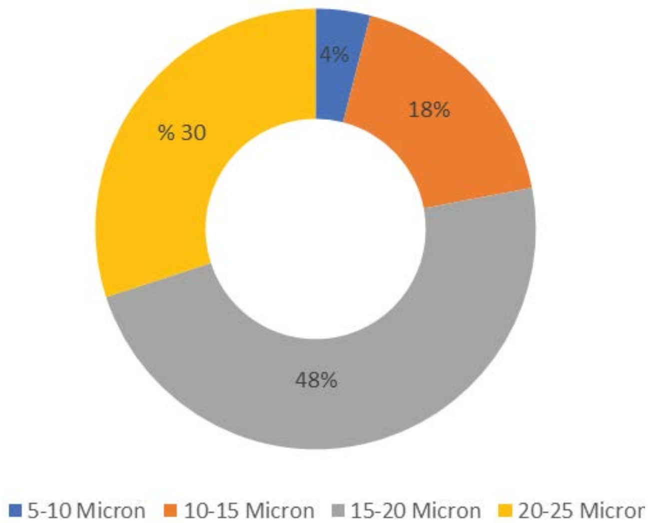


Fig. 7. The size of particles smaller than 25 μm and their percentage.

Table 3
Per capita production of microplastic particles according to the input effluent

Type	Amount of production (m^3)	Amount of production (d)	Per capita production (person/d)
Microparticles	28,027 ± 1,765.70	560,540,000 ± 35,311,012	4,671.16 ± 294.26
Microfiber	4,720 ± 245.41	94,400,000 ± 4,908,778	786.67 ± 40.90
Total	32,747 ± 1,866.57	654,940,000 ± 37,331,574	5,457.83 ± 311.09

Table 4
Per capita production of microplastic particles according to the ouput waste

Type	Amount of production (m^3)	Amount of production (d)	Per capita production (person/d)
Microparticles	1,066 ± 67.14	21,320,000 ± 1,343,160	177.60 ± 11.14
Microfiber	275 ± 17.31	5,500,000 ± 345,460	45.83 ± 2.86
Total	1,341 ± 76.42	26,820,000 ± 1,528,738	223.43 ± 12.73

conducted in this study, it was concluded that the acid-washing method on dry samples is suitable only if microplastic particles are existing in large sizes. It was also found that in liquid samples, the lower the amount of liquid volume, the better and the acid-washing operation and consequently better the detection of microplastics from other organic matter because the precipitates will be formed better.

4. Conclusions

Given the importance of the subject and the threats that microplastics pose to aquatic and terrestrial environments, using appropriate methods to detect and extract particles is essential. In the current study, an attempt has been made to provide an optimal and appropriate method compared with other methods, through which microplastic particles can be well detected and extracted. Up now, various methods have been used to extract particles from seawater and municipal sewage, each of which has included different parameters and instructions. Also, by determining an appropriate amount of hydrogen peroxide injection in the input effluent, which contains a lot of organic matter, the sedimentation efficiency was substantially increased and particle extraction was achieved to a desirable level, which shows better performance compared with previous research. Considering the mentioned cases, it was found that the results of the optimized method in this research are extremely suitable and it has been able to separate the microplastic particles in the wastewater from its organic materials. Also, optimizing the consumption, time, and temperature parameters and innovation in using the 0.05 M iron solution have resulted in higher efficiency in detecting smaller particles (smaller than 10 μm). It should be mentioned that the conventional process of the wastewater treatment plant was found to dispose of 94%–96% of microplastic particles with sludge. The noteworthy point is that a fraction of particles, which their number was determined, was not separated by the conventional treatment process and entered the aquatic and terrestrial environments. To separate these particles, advanced approaches should be evaluated. Based on the seasonal sampling performed in this study, the frequency of microplastics was related to summer. It was also found that differences in salts, the use of divalent iron, and changing the volume of effluent to perform acid washing, have a significant effect on oxidation, sediment formation, and finally particle detection.

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