Investigation of Microplastic Pollution in *Sargassum* sp. Macroalgae on Rocky Shores of Bushehr Province

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Abstract

Nowadays, the increase in microplastic pollution has become a global problem. The food chain can be polluted by the presence of microplastics in macroalgae. To measure the presence and amount of microplastics in macroalgae and water, sampling was performed from the rocky shores of Bushehr province during the winter season of 2021 and summer season of 2022 from four stations of Jofreh, Rishehr, Bojikdan, and Halileh. The result indicates the presence of microplastics in the macroalgae and the water, which were separated using a stereomicroscope. The microplastics were analyzed by FTIR-ATR device to determine the type of microplastic. The dominant species of the sampling stations were macroalgae *Sargassum* sp.

Although, according to the results, the microplastic pollution in macroalgae samples was higher in the summer than in winter, the microplastic pollution in the water was more in the winter season. The average abundance of microplastics was calculated as 173 ± 96.96 and 116.75 ± 63.36 microplastics/kg in the summer and winter seasons, respectively. Jofreh and Halileh stations, with a mean frequency of 225 ± 50.48 and 23 ± 5.69 microplastics/kg, were the most polluted and clean stations, respectively. The sphere form of the microplastic fragments was more frequent after the fiber type. The results of the FTIR-ATR analysis showed that the microplastic polymers in *Sargassum* sp. and water were polyamide (nylon)> polystyrene> polyvinyl chloride, respectively. Since the main activities that pollute the beaches of Bushehr province are fishing, shipping, tourism, etc., and they are more in the summer. So it can be seen as evidence for the results of this study.

Keywords: Microplastic, Marine Pollution, Macroalgae, Bushehr Province, *Sargassum* sp.

Introduction

Due to the expansion of plastic production techniques with high volume and low price following the invention of the first modern plastic in 1907, the production speed of plastic products has increased, and they are mass-produced (Cole et al., 2011). Today, it

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is impossible to imagine life without plastics and often used in various applications such as food and beverage packaging, drugs, cosmetics, detergents, chemicals, medicine, electronics, construction, automobiles, and aircraft parts (Kumar et al. al, 2020; Galgani et al., 2010). Plastic pieces smaller than 5 mm are called microplastics. Plastics that are produced in microscopic size are known as primary microplastics. These plastics are commonly used in facial cleansers and cosmetics (Zitko and Hanlon, 1991). Secondary microplastics describe small plastic fragments obtained from the breakdown of larger plastic residues, in the sea, and on land (Ryan et al., 2009). Coastal zones, around industrial units and harbors, have the highest abundance of microplastic fragments (Claessens et al., 2011; Desforges et al., 2014). Sources that directly introduce plastic into the marine environment include coastal tourism, marine fleets, recreational and commercial fishing, and marine industries e.g. oil and gas platforms, fish farming can pose a risk to both plants and animals (Thompson et al., 2009).

Plastics harm communities and their economies, as well as aquatic ecosystems. Also, there are concerns about human health and the impact of microplastics on organisms and their risks and side effects. Microplastic accumulation in marine macrophytes, such as macroalgae and seagrasses, is a potentially critical pathway that has been neglected. One of the ways how microplastics enter the marine food web is through macroalgae (Saeb Mehr et al., 2016). Previous research shows that microplastics are ingested by various marine organisms, from zooplankton to fish (Saeb Mehr et al., 2016). This phenomenon causes inhibition of growth, shorter life span, and reproductive capacity in these organisms. Fishes and crustaceans are the most studied groups, but studies on other organisms are much fewer. Microplastics also hurt the growth and psummerosynthesis of algae (Zhang et al., 2017). it has been stated that sometimes marine organisms mistake plastic waste for bait, and this causes plastics to enter the food chain (Cole et al., 2011).

Bushehr province, despite having more than 625 km of the coastal strip and proximity to sensitive coastal and marine areas and habitats such as coral reefs, mangrove forests, and estuaries, due to the establishment of economic and industrial centers of marine origin in its coasts and marine areas, it has always faced serious environmental risks. Oil and gas facilities located onshore and offshore, sources and facilities of thermal power plants located on land that cause severe thermal changes, agricultural and aquaculture activities, shipping, marine accidents, and urban sewage are one of the major causes of pollution and threats to the biodiversity of these beaches (Heidari et al., 2013).

Kurd and Naji (2018), intended to investigate the contamination of microplastics in 5 species of dominant fish in Chabahar Bay (Sistan and Baluchistan Province). They concluded that all fish sampled from Chabahar Bay contained microplastics. Naji and Esmaili

(2015) investigate the microplastic pollution in the coastal sediments of the tidal areas of Hormozgan province. They concluded that the highest frequency of microplastics was in the stations near the industrial zones, and the lowest microplastics were in the stations less influenced by human factors. Feng et al. (2020) have suggested that microplastics absorbed by edible macroalgae may be transferred to humans leading to a high potential risk to human health. Jiang et al. (2020) revealed the characteristics of microplastics in the surface waters of the South Yellow Sea under the influence of season. They concluded that the abundance of microplastics, especially in small sizes, was positively correlated with seawater salinity. These results show that microplastic pollution in the surface waters of the South Yellow Sea varies with different seasons due to differences in land resources and marine hydrological dynamics.

Considering the high biological capacities of Bushehr province as well as its many pollution sources, it is necessary to research their relationship. This study aims to investigate the relationship between microplastics in macroalgae and water as well as identify the polymers that make up microplastics trapped by *Sargassum* sp.

Materials and methods

Study Area

Bushehr province is located in the south of Iran and on the edge of the Persian Gulf. Algae have always existed on the rocky shores of this province as a stable ecosystem for many years (Saeb Mehr et al., 2016). After the investigations carried out along the rocky coasts of Bushehr province, four stations named Jofreh, Rishehr, Halileh village, and Bojikdan were selected as sampling stations, and their location was recorded by GPS (CX120) . The geographical location of these stations can be seen in Figure 1 and Table 1. The selection of stations was based on the number of human activities, incoming effluents, and the presence of algal species.

Table 1. Coordinates of research stations

Station No.	Station	Longitude	Latitude
1	Jofreh	50° 82' 52.4" E	28° 97' 45.57" N
2	Rishehr	50° 82' 60.48" E	28° 90' 00.41" N
3	Halileh	50° 87' 40.5" E	28° 83' 57.2" N
4	Bojikdan	51° 07' 54.19" E	28° 64' 51.17" N

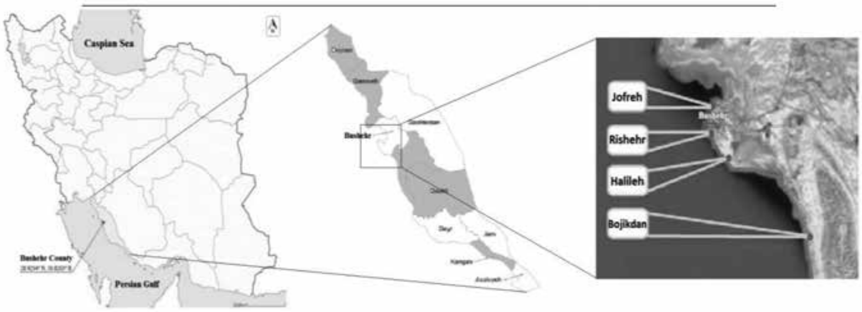

Fig. 1. Bushehr province map

Physicochemical factors

Environmental factors such as temperature, salinity, pH, and electrical conductivity of water were measured by a Hach multimeter. The measurement results in the two winter and summer seasons are shown in Tables 2 and 3. These factors are measured to understand the changes and relationship between environmental factors and microplastics in both seasons.

macroalgae and water sampling

Sampling was done during the winter and warm seasons from November 2021 to June 2022 from four stations in the studied area. Among the algae observed, the macroalgae *Sargassum* sp. of different sizes and healthy appear in tidal areas, and during the full tide, were manually sampled. Then samples were carefully placed in aluminum foil, and the station specifications and sample code were specified. Samples were transferred to the laboratory in a flask filled with ice (Feng et al., 2020). Water samples were taken at a distance of 10 meters from the coast of each station in the amount of two numbers of 5 L barrels from the coastal waters and transferred to the marine biology laboratory of Khorramshahr University of Marine Sciences and Technologies for filtration inside the glacier containing ice.

Identification of macroalgae and microplastics in the laboratory

In the laboratory, macroalgae samples were identified using a stereomicroscope (Olympus SZ40) and using an Olympus TL2 microscope, as well as using an algae identification key)Baldock et al., 2009; Bast et al., 2014 Shams et al., 2013). Then, the microplastics in the macroalgae were transferred to the Petri dishes of each sample using forceps. After checking the appearance of microplastics, they were psummerographed (figure 2-b,c,d). Also, 10 liters of water were collected from each station and transported to the laboratory. In the laboratory, using Gff (glass fiber filter) filter paper, the microplastics in the water sample were separated from the water (Prata et al., 2018). The microplastic samples in filter paper were observed and psummerographed by stereomicroscope and microscope. Both types of microplastics found in macroalgae and water were transferred to the laboratory of Amirkabir University (Tehran, Iran) to measure organic and polymeric compounds and determine functional groups in microplastics and were measured by FTIR infrared spectrometer.

Statistical methods

To investigate the presence or absence of significant differences between physicochemical factors and the abundance of microplastics in macroalgae samples, ANOVA statistical method and Pearson coefficient were used in SPSS ver. 22, and Excel 2010 was used to draw the graphs.

Results

Physicochemical factors

The results related to the physicochemical factors of the water of each of the studied stations in the two winter and summer seasons are given in tables 2 and 3, respectively. The water temperature ranges from 21.9 degrees to 23.7 degrees Celsius in the winter season and 29.1 degrees to 30.5 degrees Celsius in the summer season. In winter and summer seasons, the range of water salinity changes between 38.6 to 39.7 and 39.1 to 41.2 ppt, the water acidity from 8.06 to 8.11 and 8.06 to 8.22 ppt, respectively. Moreover, the electrical conductivity of water is between 51.6 to 53.3 and 52.1 to 54.6 mS/cm.

Microplastics in macroalgae

The results showed that the dominant species of the study area is *Sargassum* sp. (Figure 2a). It also showed that microplastics were found in this species in all stations (Figure 2b, c, d).

The abundance of microplastics found in macroalgae *Sargassum* sp. is shown for 4 stations in the two seasons (Figure 3). Jofreh station has the highest amount of microplastic pollution with an average frequency of 180 ± 10.14 microplastics/kg in the winter season and 270 ± 9.84 microplastics/kg in the summer season (Figure 3). Also, Halileh station, which had the lowest amount of microplastic contamination, 19 ± 3.46 and 27 ± 4.58 microplastics/kg in winter and summer seasons, respectively, was considered as a control station. According to the graph of abundance in summer and winter, the average of microplastic trapped in the summer is 173 ± 96.96 microplastics/kg, which is more compared to the winter with an average of 116.75 ± 63 microplastics/kg of macroalgae (Figure 3).

The examined microplastics have different shapes including the film, piece, bullet, and fiber shape, which can be seen in Figure 4 in terms of abundance percentage. Fiber-shaped microplastics with 71% and film-shaped with 5% had the highest and lowest form of microplastics, respectively.

Also, the abundance percentage of each

Table 2. Physicochemical factors of water in the winter season

Station	Temp (°C)	pH	Salinity (ppt)	Electrical Conductivity (mS/cm)
Jofreh	21.9^{a}	8.06^{a}	38.8^{a}	52.6^{a}
Rishehr	22.1^{a}	8.10^{b}	38.6^{a}	51.6^{b}
Halileh	22.4^{b}	8.11^{b}	39.7^{b}	53.3^{c}
Bojikdan	23.7^{c}	8.11^{b}	39.5^{b}	53.1^{c}

Dissimilar letters in each column indicate a significant difference ($P < 0.05$)

Table 3. Physicochemical factors of water in the summer season

Station	Electrical Conductivity (mS/cm)	Salinity (ppt)	pH	Temp (°C)
Jofreh	29.5^{a}	8.06^{a}	40^{a}	53.7^{a}
Rishehr	30.1^{b}	8.07^{b}	39.1^{b}	52.1^{b}
Halileh	29.1^{c}	8.22^{c}	40.1^{a}	54.1^{c}
Bojikdan	30.5^{d}	8.11^{d}	41.2^{c}	54.6^{d}

Dissimilar letters in each column indicate a significant difference ($P < 0.05$)

Fig. 2. Sample of *Sargassum* sp. macroalgae (a). The forms of microplastics in the sample of *Sargassum* sp. macroalgae (b,c,d)

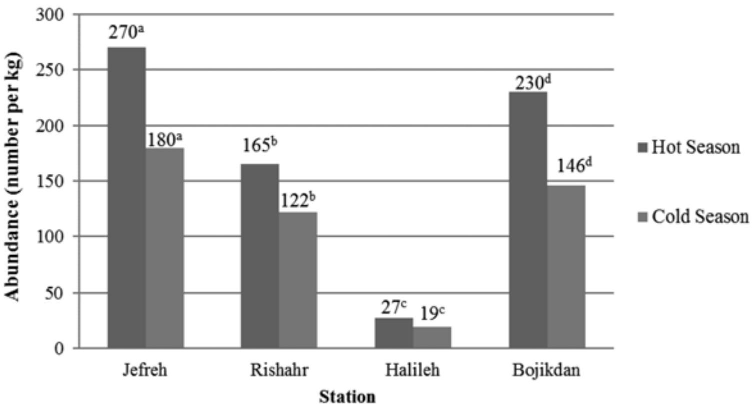

Fig. 3. Abundance of microplastic/Kg of *Sargassum* sp. in winter and summer seasons

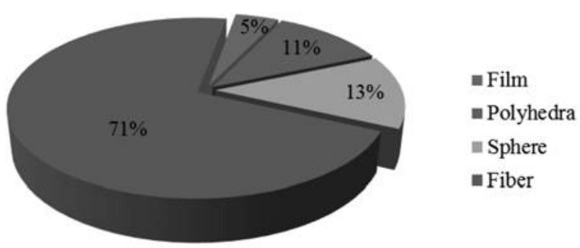

Fig. 4. The percentage of abundance of microplastic found in *Sargassum* sp. based on appearance

microplastic form in the macroalgae Sargassum sp. can be seen at different stations in Figure 5.

Forms and abundance of microplastics in water

Microplastics were also observed in filtered water (Figure 6). The abundance of microplastics in water is also listed in Table 4. The highest amount of microplastics in water was determined in both seasons at Jofreh station. Also, the frequency of microplastics in the winter was higher than in the summer (Table 4).

Identification of types of polymers

The results of FTIR-ATR analysis showed that the most abundant polymers found in microplastics are polyamide (PA) or nylon, polystyrene (PS), and polyvinyl chloride (PVC) (Tables 5 and 6). According to Table 5, polyamide polymer (nylon) was found in the macroalgae samples of all stations, followed by polystyrene polymer, which was the predominant percentage of macroalgae contamination. Also, according to Table 6, most polymers are polystyrene type, but the information in this Table cannot be expanded due to the low abundance of microplastics found in water samples.

The spectrum diagram can be seen in Figures 7, 8, and 9. The output spectrum of the FTIR

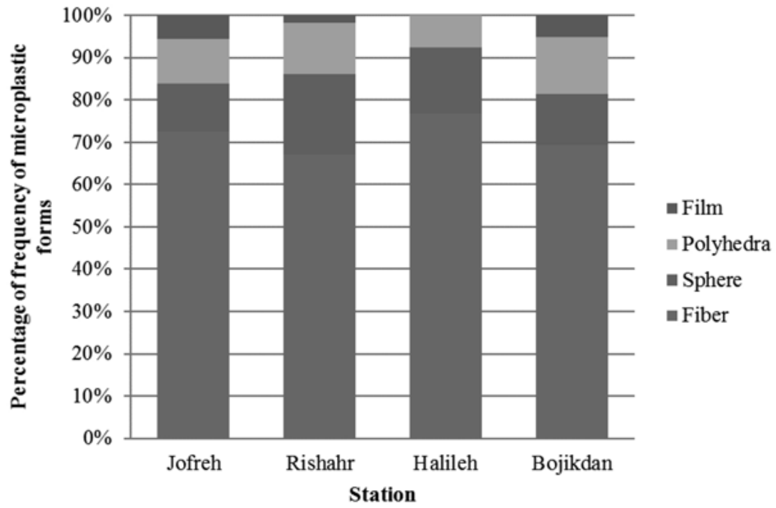

Fig. 5. The abundance percentage of different forms of microplastics found in samples of *Sargassum* sp.

Table 4. The abundance of microplastics in one cubic meter of water

Station	Summer Season	Winter Season
Jofreh	100	100
Rishehr	0	100
Halileh	0	0
Bojikdan	0	100

Fig. 6. Microplastic found in water filter sample

Table 5. The number of microplastics found in samples of *Sargassum* sp. at each station according to the type of polymer

Station	The most abundant polymers found	Polyvinyl chloride (PVC)	Polystyrene (PS)	Nylon
Jofreh	Nylon – PS – PVC	15	24	103
Rishehr	Nylon – PS	7	12	39
Halileh	Nylon	1	2	10
Bojikdan	Nylon	8	10	41

Table 6. The number of microplastics found in water samples at each station according to the type of polymer

Station	Polymers found in water	Polystyrene (PS)	Nylon
Jofreh	Nylon – PS	1	1
Rishehr	PS	1	0
Halileh	-	-	-
Bojikdan	PS	1	0

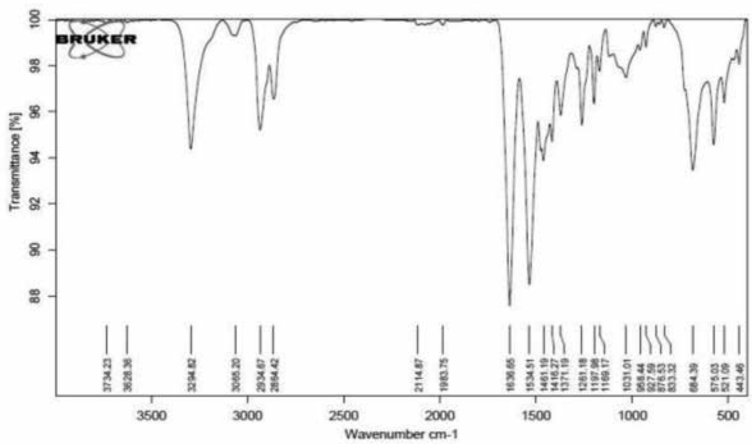

Fig. 7. The result of FTIR-ATR analysis of the microplastic sample in the *Sargassum* sp. which is similar to the standard spectrum of PA (Nylone) polymer

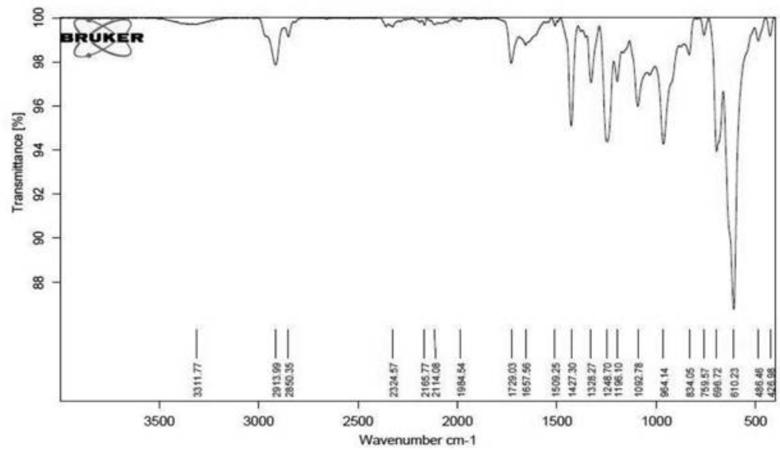

Fig. 8. The result of FTIR-ATR analysis of the microplastic sample in the *Sargassum* sp. which is similar to the standard spectrum of PVC

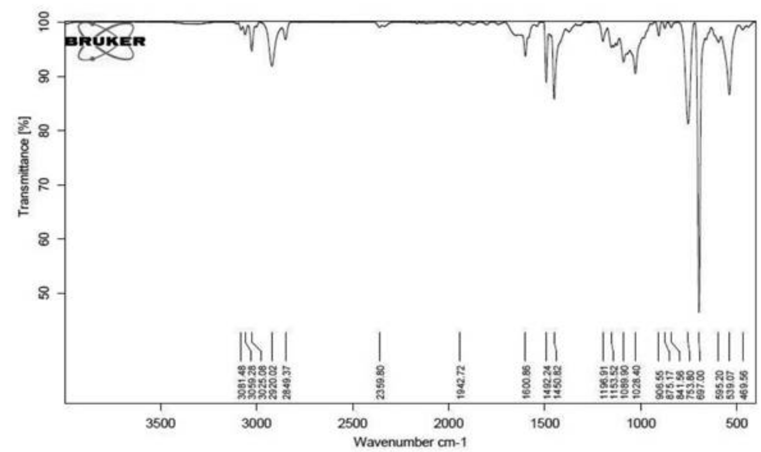

Fig. 9. FTIR-ATR analysis of the microplastic sample in the *Sargassum* sp. macroalgae which is similar to the standard spectrum of PS

device, which belongs to the microplastics in the Sargassum sp. sample, is shown in Figure 7. The peaks can be seen at the wavelengths of 3294.82, 2934.67, 1636.65, and 1534.51 cm-1, and the resulting spectrum is similar to the standard polyamide (nylon). Figure 8 also shows a similar spectrum of another type of microplastic found in macroalgae Sargassum sp. and the polyvinyl chloride polymer.

Correlation between physicochemical and microplastic factors

According to Pearson's statistical test results, the correlation between salinity and temperature factors with microplastics was not significan. The one-way ANOVA statistical test showed a significant difference in the abundance of microplastics in the stations in both seasons.

Discussion

The area investigated in this research is the shores of Bushehr province, which includes industrial and fishing wharves. By their nature, they can be a source of pollution in the environment. The presence of microplastics, plastic particles smaller than 5 mm in marine environments, has caused a growing global concern due to their low weight, long shelf life, and risk to related marine communities. Considering the importance of microplastic pollution, monitoring the release of plastics by the tourism and fishing beaches of Chabahar Bay can be proposed as a topic for future studies. Pollution of the marine environment is a global phenomenon, and plastics are one type of pollutant introduced by humans into aquatic ecosystems, especially in the sea environment (Naji et al., 2017). Sea-based resources such as shipping, transportation activities, fishing, and land-based resources such as factories and industries adjacent to the sea and tourism, introduce plastics into the sea and ocean.

According to the results of measuring the abundance of microplastics in macroalgae Sargassum sp. in both seasons (Figure 3), Jofreh station, which is near urban residential areas, surface water inlets, urban sewage, fishing, and recreational piers, as well as coastal parks, has the highest abundance of microplastics in two seasons, on average 225 ± 50.48 microplastics/kg of macroalgae. After Jofreh, Bojikdan station has the highest level of microplastic pollution, 188 ± 48.01 microplastics/kg of macroalgae. Bojikdan station is in the vicinity of the coastal village, which seems today development in the tourism industry, the creation of eco-tours and residences, as well as the lack of proper waste management and proper culture, has caused the disposal of plastic waste and the transfer of untreated sewage to the sea. Rishehr station is near urban residential areas with a lower population concentration than Jofreh, the swimming area, and the surface water inlet, hand as an average of 143.5 ± 25.82 microplastics/kg of macroalgae. Finally, Halileh station is located in the vicinity of rural residential areas, a local fishing pier, and a coastal park outside the city, and due to the small scale of the fishing industry in this place compared to other fishing piers, with an average of 23 ± 5.69 microplastics/kg of macroalgae. It is the cleanest station and witness station.

According to the results of the one-way ANOVA statistical test (Table 7), there is a significant difference in the abundance of microplastics in the stations in both seasons. The reason for this can be seen from what was said about the type and amount of pollution sources at each station. ($P< 0.05$).

By comparing the abundance of microplastics in macroalgae in both seasons, it can be seen that the entrapment of microplastics in macroalgae was more in the summer. The reason for this can be seen as the decrease in the viscosity of seawater due to heat and as a result, the reduction in the buoyancy of microplastics in water. Also, the onset of heat and fishing activity does not affect this difference. Other reasons include the high tourism capacity of Bushehr province in April and May and the increase in the amount of plastic waste entering the sea.

The results showed that about 71% of the found microplastics are fibers, 13% are spherical, 11% are multifaceted, and 5%

are in the form of films (Figure 4). The origin of fibrous and stringy microplastics can be from nets, ropes, and threads used in fishing (Jang et al., 2014) or clothing made from plastic fibers (Frias et al., 2016). Based on the studies, it has been shown that stringy microplastics are often produced in urban areas, and spherical and polyhedral microplastics are produced more often in industrial areas (Abbasi et al., 2019). Also, considering that one of the critical industries of the shores of the study area of Bushehr province is fishing, it seems that fishing nets containing plastic fibers are one of the sources of increasing microplastics in the region.

The amounts of microplastics found in the water of the study area in both seasons were insignificant. The results showed that more microplastics were found in surface water in the winter than in the summer, with 75 ± 50 microplastics/m^3 of water in the winter season and 25 ± 50 microplastics/m^3 of water in the summer season (Table 4). The main reason is that viscosity has an inverse relationship with microplastic sedimentation. Due to the low temperature in the winter season, the viscosity is high which increases microplastic sedimentation, and more microplastics can float on the water. But in the summer season, this situation is reversed, the increase in temperature reduces the intermolecular force of water particles, and the viscosity reduction may result in microplastic sedimentation increases, and this is the reason why fewer microplastic particles are seen in surface water in the summer season (Kooi et al., 2017).

The results of the polymer analysis of microplastics showed that the polyamide polymer (nylon) was found in macroalgae Sargassum sp. Polystyrene and polyvinyl chloride were the most abundant in macroalgae and water samples. Nylon is used as raw material in manufacturing fishing nets and ropes, clothing, plastic fibers, electrical insulation, consumer or industrial goods, electronic industries, etc. (Deopura et al., 2008). Polystyrene is mainly used in disposable containers, sports equipment, toys, winter insulation (Styrofoam), and the packaging of goods. (Marsh and Bugusu, 2007). Polyvinyl chloride is also used in making all kinds of pipes, construction works, etc. (Titow, 2012).

Based on the results (Tables 5 and 6), polyamide polymer (nylon) is the most abundant in macroalgae and water in the Jofreh station. Residents of the areas around Jofreh station have many fishing activities. Therefore, amounts of fishing nets and ropes can be placed on macroalgae and water surfaces in the form of microplastics.

Considering the fishing activity and the entry of rural and urban residential sewage sampling stations, the presence of nylon polymer is probable.

Also, the results of Table 5 showed that polyvinyl chloride is more in the macroalgae of the Jofreh station than in other stations. One of the justifications for the increase of this polymer can be mentioned in the increasing urban constructions and residential areas in the Jofreh region. Also, according to Table

6, this type of polymer was not found in water samples. According to Tables 5 and 6, polystyrene polymer was the most abundant in macroalgae and water samples in Jofreh station due to the increase in plastic wastes such as disposable containers, toys, tourism, and water activities.

Few studies have been done regarding the investigation of microplastics in macroalgae (Table 8). In the present study, the amount of microplastic pollution in the macroalgae Sargassum sp., on average, is 145 pieces per kilogram. Feng et al. conducted research on several species of macroalgae in 2020, in the Yellow Sea region, Haizhou Bay. Their results showed that *Ulva prolifera* had the highest microplastic contamination of 190 pieces/kilogram. *U. pertusa* also had the lowest amount of contamination with microplastic contamination of 60 pieces/kg. Also, Feng and his colleagues conducted another study in 2020 in the Yellow Sea, as a result of which microplastic contamination of 660 pieces/kilogram of *U. prolifera* macroalgae was recorded. According to the research conducted in this study, the amount of microplastic found shows a lower level of pollution in Bushehr province. The reason for this can be seen as less connection with open waters, fewer industries and sources of pollution (tourism, fishing, factories, etc.), less population, and therefore less plastic consumption.

Relatively extensive research has been done on the microplastic pollution in water bodies. According to the present study, this type of pollution in the Persian Gulf, the shores of Bushehr Province, is 50 microplastics/m3 of water. According to Table 8, it was found that there is a significant difference in the amount of this pollution all over the world. Microplastic pollution in the water is as high as 5300 microplastics/m3 in the southern Yellow Sea (Jiang et al., 2020). Besides, Aytan et al. (2016) recorded a pollution level of 1100 microplastics/m3. On the other hand, Doyle et al. (2011) concluded the contamination at the level of 0.004-0.19 microplastics/m3 in the North East Pacific Ocean. These results show that the pollution

Table 8. Abundance of microplastics in macroalgae in other studies in the world

Region	Species	Abundance (number per kg)	Reference
Haizhou bay	*Ulva. prolifera*	190	Feng et al., 2020
Haizhou bay	*Pyropia yezoensis*	170	Feng et al., 2020
Haizhou bay	*Sargassum horneri*	140	Feng et al., 2020
Haizhou bay	*Ulva Pertusa*	60	Feng et al., 2020
Yellow sea	*Ulva. prolifera*	660	Feng et al., 2020
Bushehr province	*Sargassum* sp.	145	Recent study

of Bushehr beaches is in the moderate range compared to other regions of the world (Table 9).

It is expected that in the waters of Iran, this amount of pollution will be lower than the global level due to the less population, fewer factories and plastic production industries, etc. However, due to the advancement of technology and proper management in other countries of the world to eliminate these pollutions, including advanced purification systems, the use of suitable alternatives instead of plastic, extensive training, etc., one should not rely only on the current data and take action to eliminate this. The problem did not become global.

As stated by the results of the present study, it can be seen that macroalgae play a significant role in trapping microplastics. Also, the beginning of the summer and the increase in fishing, and the tourism industry cause an increase in microplastic contamination in the current study areas. Jofreh station had the highest microplastic pollution, which can be attributed to the higher input volume of surface water, urban sewage, fishing, recreational activities, etc. Also, the most microplastics found in all the samples were polyamide (nylon) and string-shaped (fiber), which can make the main tools of fishing activity in these areas.

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Table 9. Abundance of microplastics in water in other studies in the world

Region	Abundance (number per m^3)	Reference
Black sea	1100	Aytan et al. 2016
Mediterranean sea	0.15	De Lucia et al. 2014
Hong Kong beach	3.973	Cheung et al. 2018
North east Pacific Ocean	0.004-0.19	Doyle et al. 2011
Seto inland sea	0.39	Isobe et al. 2014
California waters	3.92	Lattin et al. 2004
Xiangshan bay	8.91	Chen et al. 2018
Southern Yellow sea	5300	Jiang et al. 2020
Haizhou bay	1450	Feng et al. 2020
Chabahar bay	0.49	Khamarzadeh et al. 2019
Bushehr province	50	Recent study

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