Source tracking microplastics pollution from Qarasu basin rivers to Gorgan Bay, Caspian Sea

ebrahim masoudi

Gorgan University of Agricultural Sciences and Natural Resources

Aliakbar Hedayati (✉ hedayati@gau.ac.ir)

Gorgan University of Agricultural Sciences and Natural Resources <https://orcid.org/0000-0001-7658-5314>

tahere bagheri

Iranian Fisheries Research Organization

AmirParviz salati

Khorramshar Marine Science and Technology University: Khorramshahr Marine Science and Technology University

Roghayeh safari

Gorgan University of Agricultural Sciences and Natural Resources

mohammad Gholizadeh

Gonbad Kavous University

mohammad zakeri

Hormozgan University

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Abstract

Concerns about the negative effects of MPs on human health have led to increasing attention to the occurrence of MPs in the aquatic environment. Recent studies have focus from the marine environment to inland waters, particularly the spatio-temporal distribution of MPs in rivers for residential and agricultural area. Qarasu river basin, watershed to Gorgan Bay, is the site of many permanent rivers and the levels of MP pollution in those rivers are unknown. This research was conducted in three different type of land use: forest, residential and agricultural along 8 different rivers. A total of 9 fish species were identified and the presence of MPs in 87% of fish was confirmed. The prevalence of MP among species was *Liza* sp.> *C.gibelio*> *G.holbrooki*> *R.rutilus*> *N.melanostomus*> *C.carpio* = *V.vimba*> *R.frisii*> *Barbus* sp. The highest frequency of MPs was observed in Qarasu river (1880 ± 251 n/kg) and Mohammadabad river (184 ± 1340 n/kg) in agricultural land use, the lowest frequency was related to Baghu and Ziarat rivers with 660 ± 77 and 600 ± 91 n / kg was found in forest land use respectively. The highest type, color and size of MP sediments were fragment, black and 1-2 mm, respectively. The highest type, color and size of MP in fishes were fiber, black and 0.5-0.1mm respectively. Overallly according to the source tracking result, agricultural land use was the main source of MP pollution of Gorgan Bay. The Bay is strongly affected by the incoming rivers, the Qarasu River Basin as the most important river supplying water.

Introduction

Annual plastic production increased from 1.5 million tons in the 1950s to 368 million tons in 2019 (Plastics Europe, 2019). Plastics are made of a wide variety of materials. MP particles (MP) are plastics smaller than 5 mm in size that result from primary and secondary MPs (Ryan et al., 2009). MP pollution is a global environmental concern due to its ubiquity in the environment and the dangers they pose to ecosystems (Rachman et al., 2018). In general, MPs cause the most damage to the natural environment, because they are readily available to higher species and are concentrated as they move upward in the biological food chain (Canesi et al., 2015). Since the beginning of present century, contamination from large disposable items such as plastic bags has attracted much attention, while smaller and more harmful MPs have only been studied (Browne et al., 2007).

The effects of MPs are present not only in surface waters, but also in deep water, sediments, soils and organisms (Zhang et al., 2018). MPs, with their specific size and stable properties, are breeding grounds for microorganisms and carriers of contaminants (Zhang et al., 2018). Plastics, flame retardants and other chemicals are added to plastic products to improve their performance. These chemicals can be transported to the environment by MPs and pose complex environmental hazards (Abarghouei et al., 2021). MPs can be ingested by aquatic animals, seabirds or mammals; After accumulating in the food chain, they eventually enter the bodies of top predators, including humans (Liu et al., 2019). Given the presence of MPs in water, the importance of marine MP contamination research is obvious. However, freshwater areas are very important, most ocean MPs come from land, freshwater ecosystems play an important role in MP transport (Bagheri et al., 2020). It is useful to study the locations and characteristics of MP contamination in freshwater areas. There are few studies here that focus on MP consumption by freshwater fish, Wagner (2014) shows that only five studies have examined its occurrence in freshwater fish. Among these, we can refer to a study in China,

where it was found that most of the particles eaten by freshwater fish are MP fragments (Jabeen, 2017). In Brazil, a study by Silva-Silva-Cavalcanti (2017) on the Pajeú River in the city of Serra Talhada, which there were 75% contained MPs in gastric contents. Few reports are available to describe research on MPs in freshwater environments, but limited data indicate widespread MP contamination in rivers, lakes, and reservoirs (Zhang et al., 2015; Peng et al., 2018; Wang et al., 2018). In this study according to the land use, MP samples from upstream, middle and downstream rivers of Qarasu Basin in forest, residential and agricultural land uses were measured to estimate source tracking of MP distribution. The aim of this study was to compare the frequency of MPs in different land use and also detection of their shape, size and color of biotic and abiotic samples in the rivers of Qarasu Basin river.

Materials And Methods

Qarasu River with a catchment area of 1762 Km² and a discharge of 54.9 million cubic meters, originates from the heights of Qaleh Maran Mountain and after a 160-kilometer route, flows into Gorgan Bay, Caspian Sea. These rivers flows along the east-west in the south of Golestan province, but the source of most of its branches, before joining the Qarasu river, is from the Alborz mountain range and in the south-north direction (Caspian Water Consulting Engineers, 1998). The average height of the basin is 624 meters above sea level, the minimum of which is -26 meters on the shores of the Caspian Sea and the maximum is about 3200 meters in the southern heights of the basin. Up to a height of about 1000 m, the slopes are covered with forest and gradually decreases in height with decreasing forest density and increasing agricultural activities (MasoomPour, 2005). Qarasu River, which flows into Gorgan Bay (southeast of the Caspian Sea) near Qarasu village (54° 02' 23" east and 36° 50' 52" north) (Fig. 1).

In the present study, 24 stations were sampled in three types of land use (forest, residential and agricultural) (Figure 1). Criteria for sampling sites selection at different land use was according to the initial filed study, monitoring of point and non point pollution sources and literature review of related reports. Source tracking of MPs was measured according to the comparison of MPS entrance to three land use of river basin including forest, residential and agricultural. Agricultural land use was the main source of MP pollution of Gorgan Bay Sediment samples were taken from all stations, fish were caught only from Mohammadabad rivers for residential and agricultural land use, Baqo river for agricultural land use and the end of Qarasu river for residential and agricultural land uses near the estuary. To study the MP contamination of sediments in Qarasu Basin Rivers, samples were taken from 8 rivers (Mohammad Abad, Shast Kola, Shamuskh, Mohammadabad Fazelabad, Baqo, Ziarat, Tuskestan and Qarasu) each river with agricultural, forest and residential uses. Sampling was often done from the top 5 cm of sediment according to the European Marine Waste Monitoring Guide (TSG_ML). To separate MP particles from sediments, the density difference between plastic particles and sediments were taken into account.

Sediment MPs were extracted in the laboratory according to the method of Claessens et al. (2013). In general, 200 g of precipitate was dried overnight at 60 ° C and then added by adding 400 ml of NaI solution at a density of 1.8 g per cubic centimeter. The materials were stirred again and the samples were centrifuged at 5000 rpm for 5 minutes, after which the samples were immobilized for one and a half hours. Finally, the solution was centrifuged for 10 minutes and the separated solution was filtered through a strainer. After the

filters dried, the particles on the filter paper were transferred to a petri dish for microscopic examination. The fish were frozen immediately after catching and be defrosted at room temperature for testing.

The digestive organs of each fish were removed separately from the top of the esophagus and at the end of anus and immediately placed in plastic zippered bags. All used equipment were examined under a microscope to check for polymer contamination before the test. The wet samples were weighed before drying, then the samples were placed in pre-cleaned glass containers. Place the dishes in an oven at 60 ° C for 72 to 24 hours, after which the dry weight was measured again.

10% (w / v) KOH (Merck, Germany) was used for digestion and added to all organs and tissues. The product were placed in glass containers and in an oven at 40 ° C for 72 to 48 hours to complete the digestion process. Once the viscera are completely dissolved, it will be filtered through filter paper (Whatman PLC 122 United Kingdom). The filter paper was transferred to sodium iodide solution (4/4 M) Nal (Merck, Germany) and then subjected to sonication and severe shock. The Nal solution was then centrifuged and filtered again, visual observation and recording were also performed under a stereomicroscope.

The extraction method used for fish was used by Karimi et al., (2017). All particles were analyzed by stereomicroscope (NOVEL, Ningbo Yongxin Optics Co., Ltd., China) with IC80 HD camcorder and software (LAS). Totally 8800 certain plastic particle were detected from 72 sites, the maximum length and width of the MPs were identified by image analysis in all certain particles. MP-like particles were examined for further detection by a hot needle, so the hot needle causes deformation and adhesion of the plastic (Devriese et al., 2015). 72 MP particle were investigated using FTIR Vertex70 (Germany) to identify the polymer composition of MPs. The FTIR absorption spectrum recorded an average of 64 scans in the infrared range of 4000 to 400 cm with a resolution of 4.4 cm. The type of polymer was determined based on the frequency of adsorption of specific chemical bonds in the specified polymer samples. Statistical analysis was with SPSS software version 24. Principal component analysis (PCA) was performed using a data matrix of polymer types found at each sampling site to assess the relationship between MP compounds and sampling sites.

Results

1-4 MP contamination in different river sediments

According to ANOVA analysis, a significant difference in MP abundance was observed between the sample areas ($p < 0.05$). The highest frequency of MPs was observed in agricultural use site of Qarasu river (1880 ± 251 n / kg) and Mohammadabad river ($720 (184 \pm 1340$ n / kg)), the lowest frequencies was also in forest use related to Baqo and Ziarat rivers with 660 ± 77 and 600 ± 91 n/kg respectively (Table 1).

Table 1. Mean content of MPs in the sediments of sampling rivers

Site	MPs abundance (n/kg)	Sizes of MPs (mm)	Number of colors (min–max)
Mohammad-Abad	1040 ± 193 ^a	1.28 ± 0.3 ^a	2 – 6
Shastkola	1200 ± 231.4 ^b	1.41 ± 0.1+2 ^b	3 – 5
Shamoshak	1200 ± 175 ^b	1.62 ± 0.19 ^c	2 – 5
Mohamadabad	1340 ± 293 ^c	1.75 ± 0.37 ^d	2 – 5
Baqo	660 ± 191 ^d	0.98 ± 0.14 ^e	1 – 5
Ziarat	600 ± 66 ^e	1.78 ± 0.26 ^f	2 – 6
Tuskestan	880 ± 81 ^f	1.58 ± 0.19 ^g	1 – 5
Qarasu	1880 ± 255 ^g	1.8 ± 0.25 ^h	2 – 6

MP concentrations in sediments showed an increase from forest area to agriculture. The MP content in the sediments of agricultural stations with 38% had the highest and the forest margin with 25% had the lowest amount of MPs. Among the sampled rivers, the highest frequency of MPs was related to Qarasu river (21.36%) and Mohammadabad-Kordkoy river (15.23%). Baqo and Ziarat rivers had the lowest frequency of MPs in sampling stations with 7.5 and 6.82 percent, respectively.

Different abundances of MPs were observed in the sediments of sampling stations in Qarasu basin (Figure 2). The stations were classified into 3 groups: forest, residential and agricultural, according to different uses that affect the MP abundance. The average MP abundance in each group were 2180 ± 288, 3280 ± 409 and 3340 ± 377, respectively.

A number of MPs found in sediments are shown in the Figure 3. In the forest area, Fiber was the most MP (mean 55.45%), while bead was the lowest 1%. In residential and agricultural areas, the most abundance were belonged to Fragment. No bead was observed in the agricultural area. The highest diversity of MPs were observed in Ziarat and Tuskestan rivers. In general, the size ratios of MPs in the range of 3-5 mm, 2-3 mm, 1-2 mm, 0.5-1 mm and 0.1-0.5 mm, were 8.79, 75.12, 35.16, 29.45 and 13.85%, respectively (Figure 4).

The highest size of 1-2 mm were observed in stations with residential use (214. 2180 n / kg) and the lowest size was in 3-5 mm in forest land use (160 ± 31 n / kg). In all sampling stations, most MPs were black (64.22%) and gray 16.89% (Figure 4). The lowest color variation were observed in Baqo and Mohammadabad rivers.

2-4 MP contamination in fishes of different rivers

Morphometric measurements including the number and size of MPs in the species are presented in Table 1. The results show that *C.gibelio* had the highest total length (10.23± 1.56 cm), standard length (7.15± 1.1 cm) and body weight (8.33±0.4 g) (Table 2). The highest number of MPs in gastrointestinal tract of *C.gibelio* and

Liza sp were recorded with a range of 1-7 MPs. The lowest number of MPs was reported in *C. carpio*. The smallest MP size between 130 and 1500 mm was recorded only in *C. carpio*.

Table 2. Fish biometric data, number, and sizes of MPs (MPs) recorded

	SL (cm)	TL (cm)	BW (gr)	GW (gr)	No. of MPs	Size of MPs (mm)
<i>Liza</i> sp. (n=22)	5.65	7.47	3.66	0.092	7	100-3960
<i>Neogobius melanostomus</i> (n=15)	5.7	7.98	6.57	0.014	3	105-3300
<i>Carassius gibelio</i> (n=21)	7.15	10.23	8.33	0.197	7	250-5000
<i>Gambusia holbrooki</i> (n=18)	4.05	5.47	1.75	0.031	5	160-5000
<i>Cyprinus carpio</i> (n=13)	5.4	7	3.34	0.063	2	130-1500
<i>Vimba vimba</i> (n=13)	5.3	6.65	2.5	0.003	4	120-4900
<i>Rutilus frisii</i> (n=12)	5.1	6.35	1.99	0.037	4	115-3150
<i>Rutilus rutilus</i> (n=17)	4.75	5.1	1.26	0.021	6	100-4600
<i>Barbus</i> sp. (n=9)	4.4	5.05	1.33	0.015	4	190-5000

SL= standard length, TL= total length, BW= body weight, GW= gutted weight (g), No. of MPs= number of MPs, Size of MPs= Size of MPs (µm)

A total of 140 fish belonging to 9 species were analyzed in different habitats (forest, agriculture and residential), which shows 87% of the prevalence of MPs, that means only 13% of the fish samples were found without MPs (Fig.2a).

Highest MP with 15.71% and 15% were in *Liza* sp. and *C. gibelio*, while the lowest was recorded in *Barbus* sp. with 43.6%. Also, the prevalence of MP in different fishes were *Liza* sp.> *C. gibelio*> *G. holbrooki*> *R. rutilus*> *N. melanostomus*> *C. carpio* = *V. vimba*> *R. frisii*> *Barbus* sp (Fig. 5b). It is important to note that the occurrence and prevalence of MPs directly reflects the number of fish samples (n) for each species. The most MPs observed in fishes were related to Qarasu river with agricultural application (1340± 241). The lowest observed rate was also in MahammadAbad river (40±10) with residential use.

The frequency and size distribution of MPs in the sampling areas in the Qarasu Basin is shown in Figure 6. Fiber was the most significant MP (55%) followed by fragment (40.71%), while bead was below 5% (Figure 6b). The highest frequency of MPs were in agricultural and residential stations in fiber type. The highest percentage of observed colors were belonged to black (56.93%) and gray (18.25%) and the lowest to blue White and red colors were observed only in the agricultural area. The highest amount of blue MP (2.92%) was observed in the estuary of Qarasu river (Figure 6c). The highest size range of MPs were observed in the agricultural area. Size 2-3 mm didnot found in residential area, however the largest size was 1-2 mm in the (residential area (Figure 6d

PCA results (Figure 7) of the sediments showed that Gh.U and M.A stations are located in the second quarter and are associated with MPs with size <1 mm and fragment. Agricultural stations (Gh.A, Shk.A and Sh.A) and residential (TU, AU, ZU, Sh.U and Shk.U) were located in the third quarter with a strong correlation with color, size (> 1mm), and type of MP (fiber and willow). Similarly, the results of fish gastrointestinal tract analysis, Gh.E and Gh.A stations were located in the fourth ridge, which belongs to the downstream area of Qarasu River and its estuary. These stations are located in the downstream area of Qarasu and Misab rivers, which are in black and gray colors with a size of 1-2 mm.

Discussion And Conclusion

MP is a pervasive worldwide contamination, but research into its abundance, movement, and biological interactions in freshwater ecosystems is rare. In this study, we present evidence that MPs are present in the tributaries of the Qarasu Basin, including fish digestion tract and sediments. Understanding the factors that create MP patterns in freshwater food networks is crucial for management policies. The aim of this study was to investigate the frequency, distribution and diversity of MPs in the sediments of study stations from the rivers of Qarasu Basin. The MP contents of some of the fish caught were also examined to show how MPs can be effective in different habitats. The development of residential, agricultural and recreational areas around rivers has increased environmental degradation in recent years. The entry of large amounts of untreated residential and rural wastewater, agricultural effluents and waste from recreational areas in forested areas along rivers has caused the current degradation of the environment in the rivers of Qarasu Basin. Plastics and other human wastes are commonly found in marine fish, but the frequency and route of introduction and the effects of different land uses on freshwater fish are largely unknown.

In this study, 87% of fish had MPs in their digestive tissue, which was approximately with an average of 6 particles per individual in which fiber was predominant MP. Pazos et al, 2017 showed MP abundance was approximately 8–55 particles per fish, and the fibers were predominant in all categories of fishes from the estuary of Rio de la Plata, Argentina, which was comparable to intestinal MPs in our study. In contrast, Lusher et al, 2016 found 11% (84 out of 761) of MPs of mesoplastic fish from the northeastern Atlantic with an average of 1.2 particles per fish. In the study of MP extraction of sediments, the thickness of the collected sediments and their extraction methods were different, however, there was no uniform standard for sediment collection and its thickness varied from 2 to 50 cm (Cannas et al., 2017). The MP content (335µm- 5 mm) in Indian rivers was 64.1% fiber (Lechthaler et al., 2021).

In the present study, 5 cm above the surface of MP sediments was collected so that the abundance of MPs in the agricultural area was significantly higher than forest sediments (upstream). The frequency of MPs was in the range of 600-1880 n/kg, which is comparable to other coastlines and seas. The most observed MPs was Fragment (51.76%), which is consistent with the study of Mateos-Cárdenas et al. (2020). Shastkolah, Shamushk, Ziarat and Tuskestan rivers were upstream and forested with the highest percentage of fiber. MPs of 1-2 mm (34.98%) and 0.5-1 mm (28.77%) were predominant, which is comparable to the results of Park et al. (2020) with a MP dominance of <1 mm. Also, the smallest amount of MPs with large size (3-5 mm) was 8.17%. Small MPs may have more harmful effects on aquatic organisms. Lusher et al. (2013) showed that the small size of MPs compared to MPs facilitates their absorption by organisms. Also, most of the samples

were black (64.22%), which is the same as the results of Govender et al. (2020). Based on the results of this study, it can be recommended that more research could be on small plastics of 100-500 µm and on their collection at different sediment depths in the future.

Overall, the results showed that the MP pollution of Gorgan Bay is strongly affected by the incoming rivers, including the Qarasu river basin as the most important river supplying water of the Gorgan bay. In the study of 8 different rivers, Qarasu river was introduced as the most polluted river and agricultural use as the most polluting type of use. In the study of 9 different fish species, *Liza* sp. introduced as the most polluted species. The most type, color and size of MP sediments were 1-2 mm, fragment and black respectively. The highest type, color and MP size of fish were fiber, black, 0.5-0.1 mm, respectively. MPs have become widespread in the environment, leading to potential concerns about their impact on environmental health. Due to the importance of the seas and its role in human food needs, and considering that Gorgan Bay is a semi-enclosed environment, they are very vulnerable to pollution. As well as the outlet of residential and industrial wastewater that enters directly into the Gorgan bay, it can be used as an indicator of changes in the amount of plastic waste in certain areas and can also be presented as a suggested topic for future studies.

Declarations

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Figures

Figure 1

Sampling site of rivers in Qarasu basin, Gorgan bay. Sampling sites for sediment and fish were at same sites.

Figure 2

MP abundance observed in sediment from three different land use in the studied rivers.

Figure 3

MPs in sediments of the studied stations (left to right: Fiber, Fragment, Bead)

Figure 4

Percent of MP particles by abundance (a), shape (b) color (c) and size (d) in the sediment collected from studied rivers.

Figure 5

MP abundance observed in fish from the studied rivers. Gh= Qarasu, M= Mohammad Abad, Agri= Agriculture, Estu= Esturay

Figure 6

MP particles by abundance (a), shape (b) color (c) and size (d) in the digestive tract of fishes collected from three different sites

Figure 7

PCA results of MP contamination in sediment (A) and fishes (B) of Qarasu basin rivers

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- [graphicabstractmicroplastic.pptx](#)