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Microplastic pollution in fish from Halda river, a natural carp breeding habitat in Bangladesh

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Abstract

Microplastics (MPs) are among the emergent contaminants that have attracted increasing global concern in recent years. These particles are now pervasive across nearly all ecological compartments, posing substantial risks to both environmental integrity and human health. This study investigated the MPs contamination in commonly consumed fish species from an ecologically critical river in Bangladesh, the Halda. The MPs particles in muscle (including skin) and gastrointestinal tract (GIT) of fish were extracted using H_2O_2 digestion followed by density separation. Across the species, per individual abundance of MPs was higher in GIT (6.5 ± 3.6 particles/individual) than that of muscle (6.1 ± 3.3 particles/individual). Apparent higher concentration of MPs in GIT and muscle of herbivores (9.0 ± 2.9 and 5.8 ± 2.6 particles/individual, respectively) coupled with lower MPs abundance in carnivores (6.8 ± 2.7 and 4.2 ± 1.8 particles/individual in GIT and muscle, respectively) suggested that the pelagic food source is likely to be the primary pathway of MPs accumulation in the Halda river fishes. Contrastingly, lower MPs accumulation in carnivores and insignificant correlation between fish size and MPs abundance suggested biomagnification of MPs was not substantial. Therefore, it was evident that MPs contamination in fish of this river was spatially dispersed due to heterogenous sources along the river course. Among the identified MPs, fiber-shaped and small-sized ones (<500μm) were the most prevalent. The ATR-FTIR spectroscopy revealed that Polypropylene constituted the major proportion indicating potential high MPs discharge by domestic and textile industries. This study suggests that Halda river is polluted with MPs which could ultimately pose health risks to humans through fish consumption.

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Introduction

Plastic has occupied a large proportion of our daily life. Globally, the output of plastics has grown tremendously from 1.5 million tons in 1950 to over 415 million tons in 2021 and is predicted to exceed 33 billion tons by 2050[1]. With the increase in plastic use, the generation of Microplastics (MPs) has also increased. MPs are defined as small plastic pieces less than 5 mm long. Unfortunately, MPs have indeed been reported in various types of media and ecosystems including both aquatic and terrestrial and have become an emerging pollutant across the world[2–4]. These pollutants are of serious concern due to their potential to bioaccumulate and biomagnify in humans through the food chain, their ability to act as carriers of heavy metals and their toxicity[5]. Studies have reported that MPs from water bodies and soil surfaces enter the food chain through the interaction of biological and non-biological factors, affecting ecological security[6]. MPs can have an effect on a fish's biological system when they are swallowed and accumulated in fish tissues[7–9]. These polymeric materials suppress the functioning of acetylcholinesterase (AChE) in the fish brain[9]. Since fish are ubiquitous, the identification of MPs in fish species can be employed to evaluate the quality of aquatic ecosystems[10]. The accumulation of MPs in fish depends on several factors including but not limited to habitat type and compartment, body size, feeding habit and nature[11–15].

The Halda river, a highly dynamic river in the southeast Bangladesh, runs a wide range of geographic setups along its 98 Km long path. It originates from a pristine mountainous area and flows through hilly region, floodplain, farmlands and dense human settlements increasing towards downstream[16]. This river is the only tidal river located close to the coastline where Indian major carp have been naturally spawning and considered as one of the richest and oldest natural spawning grounds of Indian major carps in the world. Therefore, fish health in this river is of extreme importance form conservation and commercial point of view. This river also delivers drinking water to residents in Chattogram city and provides a fishing area for local fishermen. However, the Halda river is exposed to indiscriminate waste discharges from diverse sources. Studies have already reported various forms of pollution in the Halda such as heavy metals[17–19]. These pollutants are known for their environmental and toxicological impacts which also has human health implications. In Bangladesh, other rivers with similar to that pollutant profiles of Halda have also reported the emergent pollution MPs in some cases[20–24]. Considering the anthropogenic stress on the Halda river, it is expected that it could also experience MPs pollution. On the other hand, MPs pollution of a waterbody is reflected in organisms inhabiting there including zooplankton to predatory fish[25]. The presence of MPs in water not only have direct impact as pollutant but also it facilitates the accumulation of other pollutants such as heavy metals and other organic pollutants on the surface of the MPs[26,27]. On the other hand, MPs can affect the fish food web through lower trophic level organisms[28]. Therefore, MPs pollution has multidimensional ecological consequences. In this context, monitoring of MPs pollution in fish from the Halda river will indicate the overall ecosystem health as well as provide valuable information regarding the potential risks to human health. However, no study has yet assessed MPs in the Halda river. Therefore, this study aims to determine the MPs abundance in frequently consumed fish species from the Halda river and characterize the MPs based on their shape, size, color, polymeric types and surface morphology. The findings of this study are expected to contribute in establishing a baseline data on the MPs contamination status of Halda river fishes which will help in the identification of potential risks arising from the consumption of contaminated fish and finally in designing a proper management regime for the conservation of the Halda river.

Materials and Methods

Study area

The Halda river, lying within the border of Bangladesh, is the third largest river in Chattogram and is resourceful with different added values[(29)]. The river originates from Halda chora in Ramgarh upazila under Khagrachari district, Bangladesh. It flows through several upazillas and a thana of Chattogram metropolitan city before falling into the Karnaphuli river[(30)]. Halda is an exceptional natural treasure of Bangladesh serving as a natural breeding habitat for several important Indian carps, including Rui *(Labeo rohita*), Catla (*Gibelion catla*), Mrigel (*Cirrhinus cirrhosus*), Kalibaush (*Labeo calbasu*) and others[(30,31)]. This river receives effluents from Anannya Housing Estate, Abul Khair Consumer Goods, KDS Textile Mills Ltd, Madina Tannery, Asian paper mills and Hathazari 100 MW Peaking Power Plant[(32)]. These are responsible for the pollution of this important river. Industrial waste is the major source of pollution in the Halda river contributing 53% of total wastes followed by sewage effluent, tobacco farming, unplanned rubber dams and illegal sand extraction[(33)].

Sample collection

A total of 48 individual fish belonging to 8 different species (4–10 individuals of each species) were directly collected from fishermen at three stations (Nazirhat, Sattarghat and Modunaghat) in the study area (Fig. 1). The stations were selected to cover the areas with maximum human settlement and fishing activity.

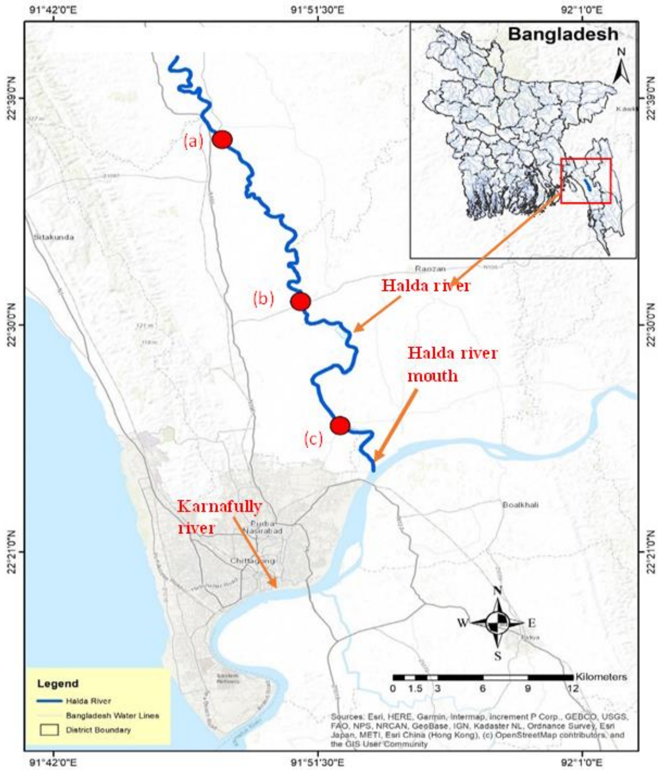

Fig. 1. Study Area (Halda River) with three sampling sites (a) Nazirhat, (b) Sattarghat, (c) Modunaghat.

Samples were collected to ensure the presence of fish from diverse habitat layers (demersal, benthopelagic and benthic) and feeding guilds (omnivore, carnivore and herbivore) (Table 1). Immediately after collection, samples were stored in ice and brought to the laboratory for further analysis. The individuals, feeding guilds and habitat layers were identified following various literatures[34,35].

Laboratory Analysis

Sample Preparation

In the laboratory, the samples were thawed on clean stainless-steel trays and the total length (cm) and weight (g) of fish were recorded upto first decimal. After that, each individual was dissected to extract the full gastrointestinal tract (GIT). The muscle of the whole fish was separated from the bones. The GIT and muscle weight were also recorded up to first decimal (Table 1). During dissection, all equipment were properly washed before starting a new sample to avoid cross contamination.

Table 1. Description of the collected fish samples. Values are reported as (Mean ± SD). (n = sample size)

Species Name (n)	Habitat layer	Feeding guild	Length (cm)	Body Weight (g)
Panna microdon (4)	Demersal	Carnivore	12.0 ± 2.8	18.9 ± 9.2
Silonia silondia (6)	Demersal	Carnivore	18.8 ± 2.5	44.5 ± 13.8
Macrobrachium rosenbergiii (10)	Benthic	Omnivore	14.2 ± 3.6	23.9 ± 22.8
Glossogobius giuris (8)	Demersal	Omnivore	14.8 ± 3.8	26.4 ± 20.5
Cirrhinus cirrhosus (4)	Benthopelagic	Herbivore	26.8 ± 5.5	266.6 ± 26.7
Gibelion catla (4)	Benthopelagic	Herbivore	24.9 ± 4.8	285.4 ± 93.1
Pseudapocryptes elongatus (6)	Benthic	Carnivore	10.8 ± 1.4	5.9 ± 1.2
Periophthalmodon schlosseri (6)	Benthic	Carnivore	8.1 ± 1.2	5.8 ± 2.5

Digestion

Digestion plays the most crucial part in the methodology of extracting MPs from GIT and muscle. Although several chemicals like Hydrogen peroxide (H_2O_2), Hydrochloric acid (HCl), Potassium Hydroxide (KOH) and Sodium Hydroxide (NaOH) are used for the digestion of organic matter of the gut and muscle. Among them, Hydrogen peroxide (H_2O_2) was used in this study as it had been found to be the most suitable considering the cost, usefulness, environmental impact and most importantly effectiveness(36). Both GIT and muscle were digested using 30% H_2O_2 by placing them at a stirring hot plate at a temperature of 80°C at 300 rpm for 24 hours and 72 hours respectively.

Density Separation

The digested solutions were first screened using a 20 μm sieve to separate them from the digestion solution following which they were subjected to density separation using saturated (1.2 g/mL) NaCl solution following Lusher(37). After that, the samples were filtered through a cellulose nitrate filter paper (0.45 μm, Sartorious Stedim Biotech GmbH, Germany) under gentle pressure by a vacuum pump (VP-1-C2, Aruki, China). The filter paper with the residues on them were kept in a petri dish, dried in the oven at 80°C for short period and covered with aluminum foil until further analysis.

Quantitative and qualitative analysis

The MPs were identified through microscopic observation and the abundance of MPs particles was recorded according to the shapes, sizes and colors(38). The identified MPs were also classified based on their morphological types(36). Suitable magnifications (from 20x to 40x) were used with a digital microscope (Humascope, Advanced LED, Human, Germany) for this purpose. Images of picked up MPs were taken using a digital microscope (Novel Biological Binocular Microscope XSZ-107T) with Scope image bioimage software.

To determine the polymer type of the identified MPs, Fourier Transform Infrared (FTIR) spectrophotometer (PerkinElmer FT-IR/NIR Spectrometer Frontier, Germany) equipped with an Attenuated Total Reflectance (ATR) adapter was used. The spectrum range of the device for MPs identification was 650-4000 cm^{-1} with 4 scans and with a resolution of 4 cm^{-1}.

MPs also experience substantial environmental weathering reactions(39) and go through changes in physiochemical properties. Weathered plastics have a greater adsorption capacity than virgin plastics(40) which can be detected by Scanning Electron Microscope (SEM). Therefore, SEM was used to analyze the surface morphology of randomly selected MPs particles to comprehend the MPs consumed by the fish.

Quality control and blank test

All possible measures were taken to avoid possible contamination during laboratory treatment. All equipment, dissection tray, dissecting tools, laboratory desk and samples were thoroughly cleaned before and after to avoid cross contamination. A laboratory blank sample test without fish gastrointestinal tract and muscle was conducted following the same procedure to correct the potential contamination.

Data analysis

MPs abundance was subjected to Kolmogorov-Smirnov test for normality testing. The homogeneity of variance was determined by Levene's test. The Analysis of Variance (One Way ANOVA) followed by Tukey HSD test were also performed to compare the variability in MPs among the species, habitat layers or feeding guilds. In all cases, $p < 0.05$ was considered statistically significant.

Results

Microplastic Abundance

All fish individuals under investigation contained MPs in their GIT and muscles. The overall MPs abundance was higher in GIT than that of muscles with 6.5 ± 3.6 and 6.1 ± 3.3 particles/individual, respectively. However, the abundance of MPs in GIT differed significantly among species (One-way ANOVA, $F = 3.669$, $p = 0.004$). Among the investigated species, the silond catfish (*S. silondia)* had the highest MPs abundance in both GIT and muscle with 10.8 ± 4.0 and 8.2 ± 3.1 particles/individual, respectively. On the other hand, the lowest MPs in GIT was recorded in giant mudskipper (*P. schlosseri)* with 3.2 ± 0.9 particles/individual while in case of muscle, panna croaker (*P. microdon)* had the lowest abundance with 1.5 ± 1.3 particles/individual (Table 2). MPs abundance also differed significantly among the fish from different habitat layers (One-Way ANOVA, $p < 0.05$). The order of MPs abundance in GIT was Benthopelagic > Demersal > Benthic species, whereas it was Demersal > Benthopelagic > Benthic species for MPs from muscle (Fig. 2).

Table 2. Abundance of microplastics in gastrointestinal tract (GIT) and muscle of different fish collected from the Halda river. Values are reported as Mean ± SD

Habitat	Common name	Species Name	Abundance/individual	
			GIT	Muscle
Benthic	Elongate Mudskipper	*Pseudapocryptes elongatus*	5.2 ± 1.8	4.3 ± 1.8
	Giant Mudskipper	*Periophthalmodon schlosseri*	3.2 ± 0.9	3.0 ± 1.1
	Giant freshwater prawn	*Macrobrachium rosenbergii*	6.8 ± 2.6	4.9 ± 1.4
		Mean ± SD of Benthic	5.4 ± 2.5	4.2 ± 1.6
Demersal	Silond Catfish	*Silonia silondia*	10.8 ± 4.0	8.2 ± 3.1
	Panna Croaker	*Panna microdon*	8.3 ± 4.2	1.5 ± 1.3
	Tank Goby	*Glossogobius giuris*	8.9 ± 4.2	6.5 ± 2.3
		Mean ± SD of Demersal	6.8 ± 4.4	8.5 ± 3.7
Benthopelagic	Mrigal	*Cirrhinus cirrhosus*	9.0 ± 1.9	5.3 ± 2.2
	Catla	*Gibelion catla*	9.0 ± 3.9	6.3 ± 2.9
		Mean± SD of Benthopelagic	9.0 ± 2.9	5.9 ± 2.6

MPs accumulation in GIT and muscle also varied among the four feeding guilds. Interestingly, both in GIT and muscle, herbivores demonstrated the highest abundance followed by omnivores and carnivores (Fig. 2). However, the differences were not statistically significant (One Way ANOVA, $p > 0.05$). A linear regression analysis also suggested that there was no significant association of body weight with either the abundance of MPs in GIT ($R^2 = 0.132$, $p = 0.18$) or in muscle ($R^2 = 0.023$, $p = 0.18$).

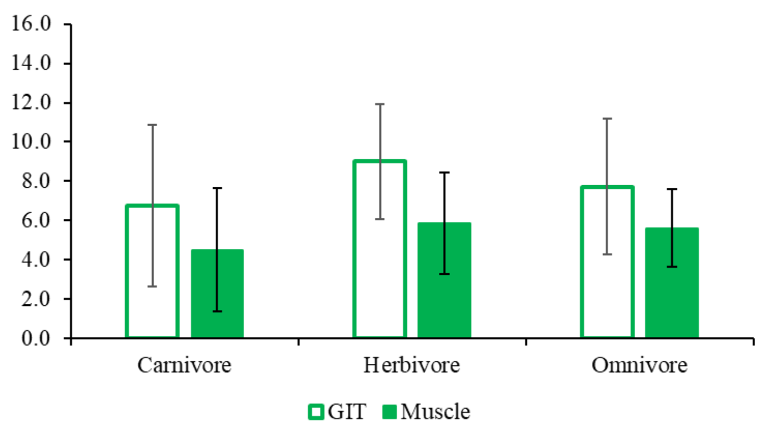

Fig. 2. Abundance (per individual) of microplastics in fish collected from the Halda river grouped by their feeding guilds.

Shape and size types of MPs

MPs of five different morphological shapes namely fragment, film, fiber, foam and pellet and three different sizes (<500 µm, 500-1000 µm, and >1000 µm) were observed during this study (Fig. 3). Among the shapes, fiber was the most abundant in both GIT and muscle followed by fragment. Pellet was the least abundant shape. The results of the One-way ANOVA revealed contrasting results. While the relative abundance of fiber shaped MPs differed significantly in the muscle of different species, such differences were not observed in the abundance of other shapes (One-way ANOVA, $p < 0.05$ and $p > 0.05$ for fiber shape and other shapes, respectively). Representative pictures of MPs of different shapes are presented in Fig. 5.

Particles of all 3 size classes were found in GIT and muscle of the studied individuals throughout all 8 species collected, except the muscle of Panna Croaker, Elongate and Giant Mudskipper (*P. microdon*, *P. elongatus* and *P. schlosseri*, respectively) where large (>1000 µm) size MPs (Fig. 4) were absent. Nearly in all cases, the smallest MPs were the most dominant. However, such dominance was particularly clear in muscle samples where the smallest MPs constituted more than 80% of the total MPs extracted.

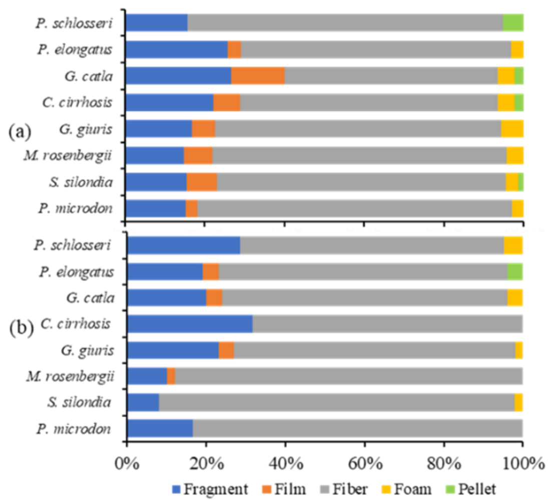

Fig. 3. Relative abundance (%) of microplastic shapes recorded in Gastro intestinal tract (GIT) (a) and muscle (b) of different fish species collected from the Halda river, Bangladesh.

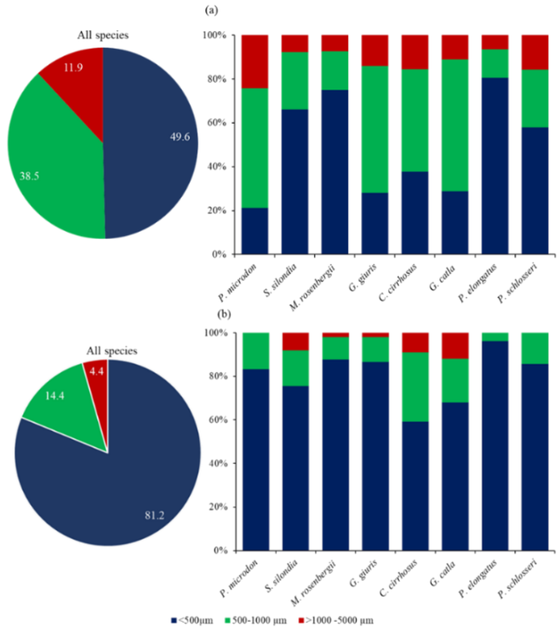

Fig. 4. Overall and species-wise relative abundance (%) of microplastic sizes recorded in Gastro intestinal tract (GIT) (a) and muscle (b) of different fish species collected from the Halda river, Bangladesh.

Color types of MPs

Visual identification by microscope confirmed the presence of MPs of seven colors (Transparent, Green, Pink, Red, Blue, Black, Brown) in the sample. Across the samples (both GIT and muscle), relatively lighter colors were common with green color MPs being the most prevalent (Fig. 6). Interestingly, the benthic omnivore tank goby (*G. giuris)* had the maximum absolute number of green particles among the species. The other color MPs were in the order of Red > Blue > Pink > Transparent > Black > Brown. Representative images of different MPs of different colors and shapes are presented in Fig. 5.

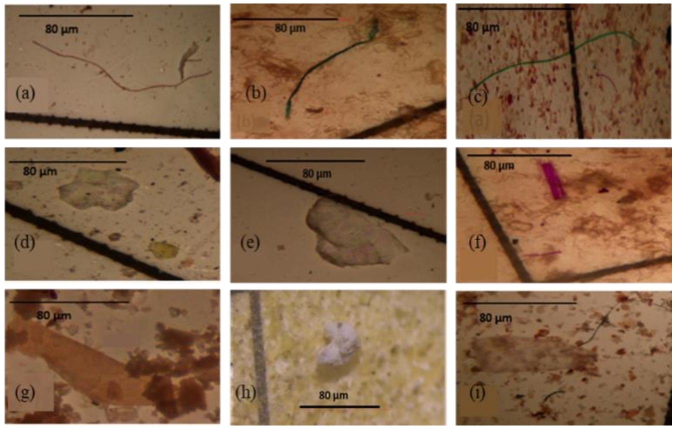

Fig. 5. Stereo microscopic images of different microplastic in fish from the Halda river (a) red fiber, (b) green fiber, (c) green fiber, (d) transparent film, (e) transparent fragment, (f) pink fragment, (g) pink film, (h) transparent foam and (i) transparent film.

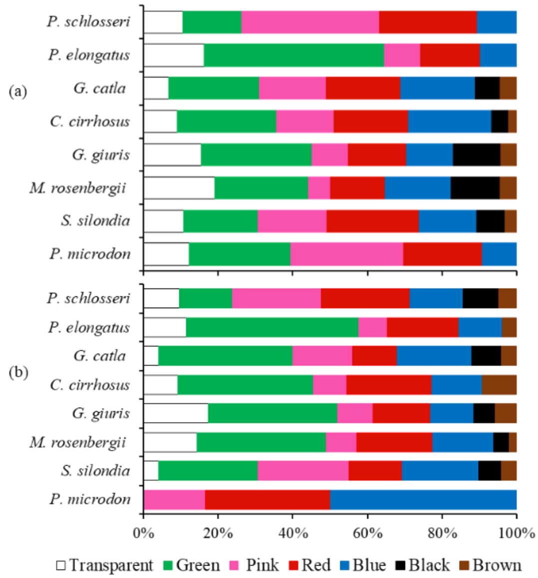

Fig. 6. Relative abundance (%) of microplastic colors recorded in Gastro intestinal tract (GIT) (a) and muscle (b) of different fish species collected from the Halda river, Bangladesh.

Polymer types MPs

72 particles (36 particles from GIT and 36 particles from Muscle) of MPs of different shapes were picked randomly and tested for polymer type using FTIR. 4 types of polymers were identified, namely Polyethylene (PE), Polypropylene (PP), Polyester (PES) and Polyethylene terephthalate (PETE) (Fig. 7). Among those PP was the most prevalent,

constituting half of the total sample followed by PE, PES and PETE. Major portion of the fiber and film shaped particles were PP, whereas PE was the component material of a major portion of the fragments (Fig. 7).

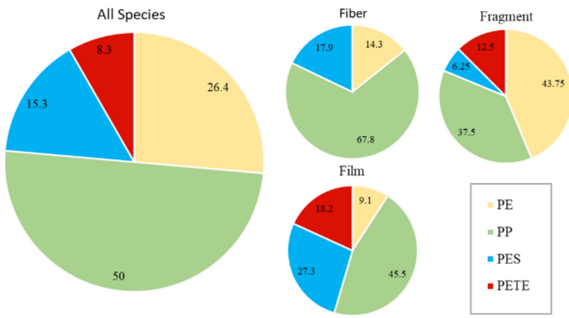

Fig. 7. Overall and shape-wise relative abundance (%) of different polymer types of microplastics in fish collected from the Halda river, Bangladesh, as revealed by ATR-FTIR spectroscopy of randomly selected particles.

Discussion

This study revealed MPs contamination in different species of fish, frequently consumed by people in Bangladesh. Both edible (Muscle, 6.1 ± 3.2 per individual) and inedible (GIT, 6.5 ± 3.6 per individual) tissues were found to contain MPs. It is certain from the observed results that the Halda river is already affected by MPs pollution. The observed abundances of MPs are comparable to those reported from other studies including the northern Bay of Bengal(41), Egypt and China(42,43). However, the abundances of GIT MPs were lower in this study than that of other studies on fish(44-46). Polluting industries are comparatively less prevalent in the study area compared to others which might be a reason for this result.

Although there is a lack of general consensus, several studies have reported that the degree of MPs accumulation in fish is related to their feeding habits or their habitat compartment(47–50). In this study, benthopelagic fish were found to be the greatest MPs accumulators in their GIT. It could be because of their tendency to frequently move in both pelagic and benthic zones of waterbody(51). Both of these regions are reported to be contaminated with MPs. The pelagic region, on the one hand, often contains a very large number of low-density MPs particles due to their tendency to float for a long time(52). The bottom region is also found to be contaminated as particles floating on the surface eventually sink to the bottom due to biofouling which increases their weight(53). This could make the MPs more available to the benthopelagic fish as they feed on the busy traffic between pelagic and benthic region. It is important to note that this study covered only few representative species. Therefore, the representation of pelagic fish might be underrepresented which is a limitation of this study. The highest MPs accumulation in *S. silondia,* in spite of not being a benthopelagic species, could be well explained by its feeding habit. They have the ability to feed in different zones as well as they feed on zoobenthos living on detritus(54). Bour *et al.*(55) reported that feeding mode is more important than habitat layer in MPs accumulation.

In muscle samples, the highest abundance was observed in demersal fishes which might have resulted because of the tendency of these fish to feed on organisms that accumulate settled MPs in large numbers(56). It is important to note that we included fish skin in the edible part since many small fish are frequently consumed in Bangladesh without the skin being removed and previous studies have reported that washing did not effectively remove MPs stuck to the fish skin(57,58). Besides, previous studies also reported adhesion to be another potential pathway for MPs presence in edible tissues(59). Therefore, muscle MPs in this study could be partially overestimated. Although not statistically significant, the relatively higher MPs in herbivore indicates a lose connection between trophic level and MPs accumulation. The results are comparable with the findings of previous studies(47,48,55). The results of polymeric analysis revealed that PP was the most abundant constituting about 50% of the total MPs which is similar to the study conducted by Sultan *et al*.(4) from the beach sediments of the coastal region of Bangladesh. PP is widely used in packaging industries, plastic parts in machinery and even in fibers and textile industries which serve as possible sources of this polymer. Shape morphology also shows that fiber is the most abundant shape observed in both GIT and muscle. Similar results are also observed in several fish and shellfish species from the mangrove estuary of Bangladesh(4). Fibers, among all the shapes, pose the greatest risk to organisms as they tend to stay in the gastrointestinal tract for an extended period. Moreover, the morphology of fiber mimics that of the prey of the fish. So, the possibilities of consuming mistakenly are higher which may cause several health issues. High proportion of PP and dominance of fiber shape materials in this study combinedly suggest that packaging and textile industries can be the major source of MPs in that water body. Apart from accumulation in fish, MPs have other environmental consequences. Bakir(60) for example showed that PP, PE and PES have high sorption capacity for different heavy metals and hydrocarbons. So, the presence of these plastic materials in the waterbody might facilitate heavy metal accumulation in fish.

Besides, MPs particles of the smallest size was the most abundant in both muscle and GIT samples. It has been reported that freshwater fish species(61) and fish species from the northern Bay of Bengal(44,46) also accumulated large number of small size MPs. Smaller MPs particles have higher chances of entering the muscle through endocytosis compared to the larger ones. So, they are often more abundant. Also, there is a huge controversy about the preferential ingestion of different colors of MPs by organisms(62,63). According to previous studies(64–66), fishes seem to be more likely to consume MPs which have colors that resemble their natural food[67]. The analyzed samples also showed the prevalence of green MPs in both GIT and muscle.

Exposure of fishes to MPs may also cause alterations in the transcription of important genes engaged in several signaling processes[68,69]. According to Rochman *et al.*[70] the contaminated polyethylene plastic debris caused endocrine disruption in Japanese medaka by suppressing the gene expression for vitellogenin, choriogenin and estrogen receptor. Additionally, Law *et al.*[71] observed that Zebra fish larvae's locomotor activity was affected by the up-regulation of the visual gene expression brought on by MPs. Moreover, results of SEM analysis showed the presence of fractures, cracks, pits and foreign particles on the assessed particles. The flake, scratches and grooves on the surface of the MPs particles enhance the surface area and resultantly facilitate the adsorption of foreign particles. It thus results in enhanced concentration of these particles in the environment[72]. Chemical toxicants that usually become adsorbed include Persistent Organic Pollutants (POPs), Persistent bio-accumulative toxic chemicals and heavy metals. In brief, weathered MPs particles may result in synergistic risk and hazards to the riverine organisms[73]. Bangladesh has a high population density and on an average 62.58g of fish are consumed by each person every day[74]. As MPs were present in every species of Bangladeshi urban river fish that was looked into, eating this fish may be a direct source of human exposure to MPs. Moreover, the deteriorated MPs items are more likely to go through cellular internalization[75]. So, there could be major public health issues because of the MPs that are found in subtropical fish. However, a study incorporating a larger sample size covering several other regularly consumed species would have enabled a more robust assessment of the MPs contamination status in Halda river fish but it was not possible due to the fishing ban in Halda river. To ensure sample integrity, individuals collected directly from the main river channel under the supervision of the relevant authority were included.

Conclusion

This study aimed to investigate the abundance and characteristics of MPs in fish from the Halda river. MPs were found in all studied fish species and the mean abundance was 6.5 ± 3.6 items/individual and 6.1 ± 3.3 items/individual in gastrointestinal tract and muscle samples, respectively. MPs contamination in fish pose ecological risks besides the consumption of fish from this river is also likely to pose human health risks. There was no significant association of the MP abundance with their body weight rather it possibly depends on the habitat and feeding guilds. However, lack of clear relationships with consumer's feeding habit or size together with low MPs abundance suggested that the whole ecosystem of the Halda river has not been affected yet. Therefore, immediate interventions to stop domestic and industrial discharges may effectively conserve this ecologically significant riverine fish breeding ground. Failing to do so could eventually result in drastic reduction in the natural breeding of Indian major carps.

To the best of knowledge, this is the first investigation of the distribution of MPs in the fish species of Bangladesh's Halda river. However, intraspecific variations, sample size, seasons, shortage of available fund etc. are responsible for the lack of detailed study. Future research on MPs in fisheries could perhaps concentrate on identifying and quantifying the various types of MPs particularly in-situ detection of them; identifying sources; zonal distributions and so on in various compartments along the river channel. Moreover, spatial and seasonal variations in the abundance of MPs should also be checked to help design future management strategies for this ecologically important river.

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