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Original Article

Properties and abundance of microplastics found in fish feed, tissues, and culture water of catfish (*Heteropneustes fossilis*)

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Abstract: Microplastics are threatening to public and environmental health for their potential toxicity. Thus, understanding the ecological occurrence of MPs is crucial, but research on MPs in Bangladesh has yet in its infancy. To understand the existence of MPs in aquaculture, we investigated fish flesh, commercial fish feed, and rearing water of *Heteropneustes fossilis* farms (n = 10). The density separation method to extract and light microscopic technique to analyze the MPs were employed. A total of 191 MPs particles were recorded, whereas 134 in rearing water ranging from 8-53 μm, 45 in fish feed ranging from 10-88 μm, and 12 in fish flesh ranging from 7-15 μm. Fiber type MPs were the most frequent, contributing 77% in water, 62% in fish feed, and 5% in fish flesh, following fragments and films. Identified MPs were obtained with various colors such as black, blue, brown, translucent, and mixed. MPs were found with the highest abundance in the fish feed (6 particles gm-1), subsequent in water and fish flesh. Identifying the sizes, shapes, colors, and types of MPs is crucial as it affects the interaction and uptake in fish. This study provides an important baseline for future research on the transfer of MPs via the food chain.

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Introduction

Approximately 6300 million tons of plastic wastes have been generated worldwide from 1950 to 2015 due to improper management, a fraction of which ultimately entered the aquatic environment (Geyer et al., 2017). Once entering the environment, plastic may fragment into microplastics (MPs) through weathering processes (Peng et al., 2020). The term 'microplastic' refers to any synthetic polymer with a size of below 5 mm (Kershaw, 2015) and varies in color, size, and shape (Wright et al., 2013). Among the most common shapes, fiber, film, fragment, and granule (Rocha-Santos and Duarte, 2017) are included. Different sizes and shapes result in varying densities of plastic particles (Wright et al., 2013). Due to their small size, MPs are easily ingested by organisms and transferred to high-trophic-level organisms through the food chain (Chagnon et al., 2018). Such pollution poses a serious threat to organisms, ecosystems, and even human health (Ma et al., 2020). Although it is unknown how much damage microplastics could have on humans nor how many particles would have to be ingested to cause any significant damage, it is suggested that the health hazards MPs cause on aquatic biota may also affect the human body (Smith et al., 2018). Marine plastic pollution has been of concern since the late 1960s (Kenyon and Kridler, 1969) and early '70s (Carpenter et al., 1972), but plastic waste in freshwater aquatic systems has become a relatively recent focus of research (Moore et al., 2011; Zbyszewski and Corcoran, 2011; Wagner et al., 2014; Eerkes-Medrano et al., 2015), where the abundance of microplastics is comparable or even higher compared to that in a marine environment (Lebreton et al., 2017). Studies showed that MPs enter freshwater primarily through surface runoffs,

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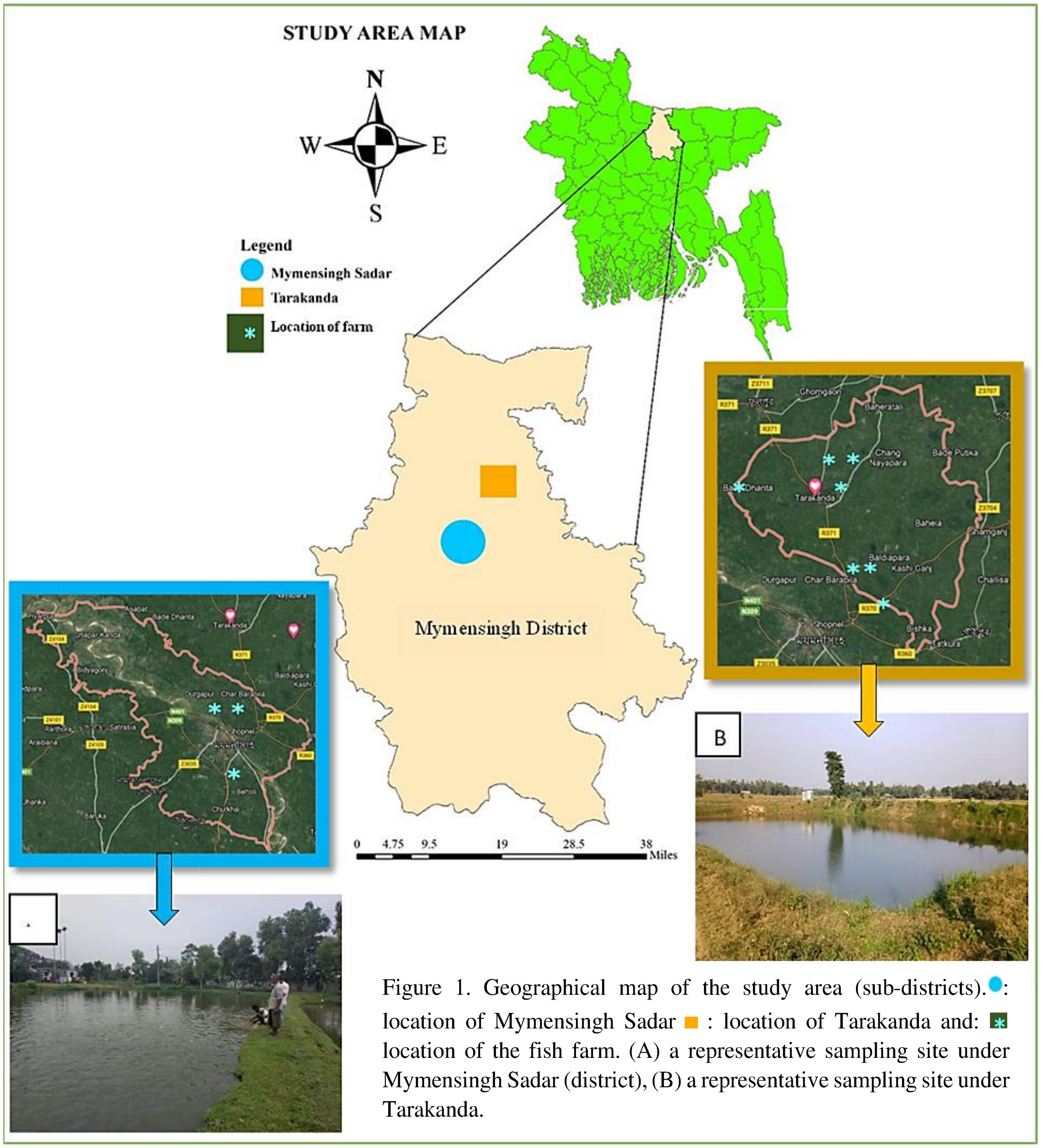

Figure 1. Geographical map of the study area (sub-districts). ●: location of Mymensingh Sadar ■ : location of Tarakanda and: ✱ location of the fish farm. (A) a representative sampling site under Mymensingh Sadar (district), (B) a representative sampling site under Tarakanda.

domestic wastewater (Ziajahromi et al., 2017; Long et al., 2019), or other human activities (Ng et al., 2018; Deng et al., 2020). However, knowledge of the presence and abundance of various MPs in a freshwater aquaculture system, *viz.,* rearing water, fish feed, fish flesh, is scarce, particularly in Bangladesh.

Bangladesh is ranked 3rd in inland open water capture production and 5th in world aquaculture production (excluding aquatic plants) (FAO, 2018). Inland aquaculture of indigenous and commercially important exotic species has been expanded massively and drew particular attention among the farmers (DoF, 2020). Thus, aquaculture plays a vital role in the economy of Bangladesh, providing food, nutrition, incomes, livelihoods, and export earnings (Dey et al., 2010; E-Jahan et al., 2010). Different sub-districts (Upazila) of the Greater Mymensingh area is one of the most important places for freshwater aquaculture in Bangladesh from where around 40% of the country's aquaculture production comes and has also become known as the freshwater aquaculture hub (Ahmed and Toufique, 2015), because of the availability of hatchery-produced fry, favorable resources, and climatic conditions (Ahmed, 2009). However, rapid intensification in commercial aquaculture enhances overall production and invites water pollution and fish disease problems.

The newly emerging MPs pollution and dietary

Figure 2. Collected (A) fish feed and (B) catfish (*Heteropneustes fossilis*).

exposure to MPs through the consumption of contaminated fish will be recognized as a recent threat in aquaculture. However, no in-depth research based on the regional aquaculture environment is available in Bangladesh. Therefore, the present study aims to explore the existence of MPs in the edible catfish, *Heteropneustes fossilis*, particularly in rearing water, fish feed, and fish flesh in the Mymensingh aquaculture zone, Bangladesh. The findings of this study could be helpful to understand the source and occurrence along with ingestion of MPs by edible freshwater fish in aquatic environment contaminants with plastic.

Materials and Methods

Sampling: The experiment was carried out from July to November 2019 at Fish Disease Laboratory, Department of Aquaculture and Food Safety and Environmental Toxicology Laboratory, Department of Agricultural Chemistry, Bangladesh Agricultural University (BAU), Mymensingh. To detect microplastics in farm water and commercial fish feed, a total of 10 fish were collected from farms in Tarakanda (24°51′9.91″N, 90°18′47.83″E) and Mymensingh Sadar (24°45′14″N 90°24′11″E) sub-district (Upazila) under Mymensingh district (Fig. 1).

Three juvenile fish rearing in the same pond was collected (with a weight of 15.18±6.39 g and a total length of 11.69±3.82 cm). Simultaneously, 1 litre of surface water from 3 different points of farm and 250 g floating pelleted fish feed used for *H. fossilis* feeding in that farm were collected (Fig. 2A) and transported to Fish Disease Laboratory, BAU, Mymensingh. To ensure the sample quality, fish (Fig. 2B) were placed in an icebox filled with ice, and water and fish feed were placed in sterile plastic bottles for transportation. The collected feeds were oven drying at 80°C for 3 h, then powdered using an automated grinder. Sampled fish were dissected in the laboratory, and 1 g flesh was taken from each fish for MPs analysis.

Extraction of microplastics from water: Saturated NaCl was used as a density separator to extract microplastics from pond water because it is nonhazardous, cheap, and readily available. A fully saturated solution was prepared using a conical flask by dissolving 26 g NaCl /100 mL of pond water. The mixture was swirled with a magnetic stirrer for 1 h. The exact process was repeated for the remaining 9 water samples.

Extraction of microplastics from fish feed and flesh: Saturated NaBr solution was used as a density separator to extract MPs from fish samples and feed samples. Two hundred grams of NaBr was mixed in 500 ml of distilled water to make a saturated 40% NaBr solution. Fifteen mL of 40% NaBr solution was poured into the conical flask containing 1 g of the feed sample. The whole mixture was swirled using a magnetic stirrer. When the sample and saturated NaBr solution get adequately mixed, it was allowed to settle down for a few hours, allowing higher densities of particles to settle down on the base. The supernatant was pipetted to the conical flask. The same process was repeated for the remaining feed and fish flesh samples.

Table 1. Microplastics (MPs) type and abundance in rearing water, fish feed, and fish flesh at Mymensingh Sadar and Tarakanda, Bangladesh.

	Particles				**Abundance**
	Fiber	Fragments	Film	Total particles	
Mymensingh Sadar (No. investigated of farm: 3)					
Water	41 (75.92%)	13 (24.07%)	0 (0%)	54 MPs	1.80 particles mL^{-1}
Feed	4 (66.67%)	1 (16.67%)	1 (16.67%)	6 MPs	2 particles gm^{-1}
Fish flesh	1 (100%)	0 (0%)	0 (0%)	1 MPs	0.33 particles gm^{-1}
Tarakanda (No. investigated of farm: 7)					
Water	62 (77.5%)	18 (22.5%)	0 (0%)	80 MPs water	1.14 particles mL^{-1}
Feed	22 (56.41%)	11 (28.21%)	6 (15.38%)	39 MPs	5.57 particles gm^{-1}
Fish flesh	8 (72.72%)	3 (27.27%)	0 (0%)	11 MPs	1.57 particles gm^{-1}

Figure 3. Extraction of microplastics from (A) pond water, (B) fish feed, and (C) *Heteropneustes fossilis* flesh samples.

Peroxide oxidation and filtration: About 30% hydrogen peroxide was added to the conical flask, which contained a sample at a ratio of 1:1. The mixtures were kept at room temperature overnight and 2 h hot bath to destroy the organic matter presents in the samples. Mixtures were poured through a cellulose nitrate filter paper (pore size: 0.22 µm) in a Buchner funnel for filtration. MPs particles got trapped in the cellulose nitrate filter paper, and the liquid was drawn through the funnel into the flask below by vacuum filtration. The filter papers were dried at room temperature overnight, and Petri dishes, marked according to the sampling sites, were used to store filter papers for further microscopic examinations.

Microscopic observation: The observation was done using a light microscope (ZEISS Primo star, Germany) under different magnifications (10× and 40× magnifications). The total count of microplastic particles was calculated and noted down as well as shapes such as fiber, film, fragment, and granule were also determined. Counting was done twice for each sample.

Results

Microplastics in farm water: MPs were found in all the water samples of fish farms at Mymensingh Sadar and Tarakanda with the abundances of 1.80 particles mL^{-1} and 1.14 particles mL^{-1}, respectively (Table 1). Types of MPs, including colors and sizes, in catfish rearing water of experimental farms, are depicted in Figure 4A-F and Table 1. Using a density separation method with saturated NaCl, 134 microplastics belonging to two different types were identified in water samples. Different colored fibers such as black, orange, mixed colored, magenta, etc., were dominant in addition to the colored polyurethane foams such as brownish orange, blue, etc., in pond water. Samples collected from Mymensingh Sadar showed 75.92% fibers and 24.07% polyurethane foams, whereas Tarakanda showed 77.5% fibers and 22.5% foams. No films were found from the water. MPs' sizes in water ranged from 8.32-52.90 µm, measured under 40× magnification.

Microplastics in fish feed and fish flesh: All the feed samples collected from the *H. fossilis*showed the presence of MPs with the abundances of 2 particles gm^{-1} (Mymensingh Sadar) and 5.57 particles g^{-1} (Tarakanda) were observed (Table 1). Fibers were more abundant in all the fish feed, followed by the fragments. In addition, fewer films were revealed. 45

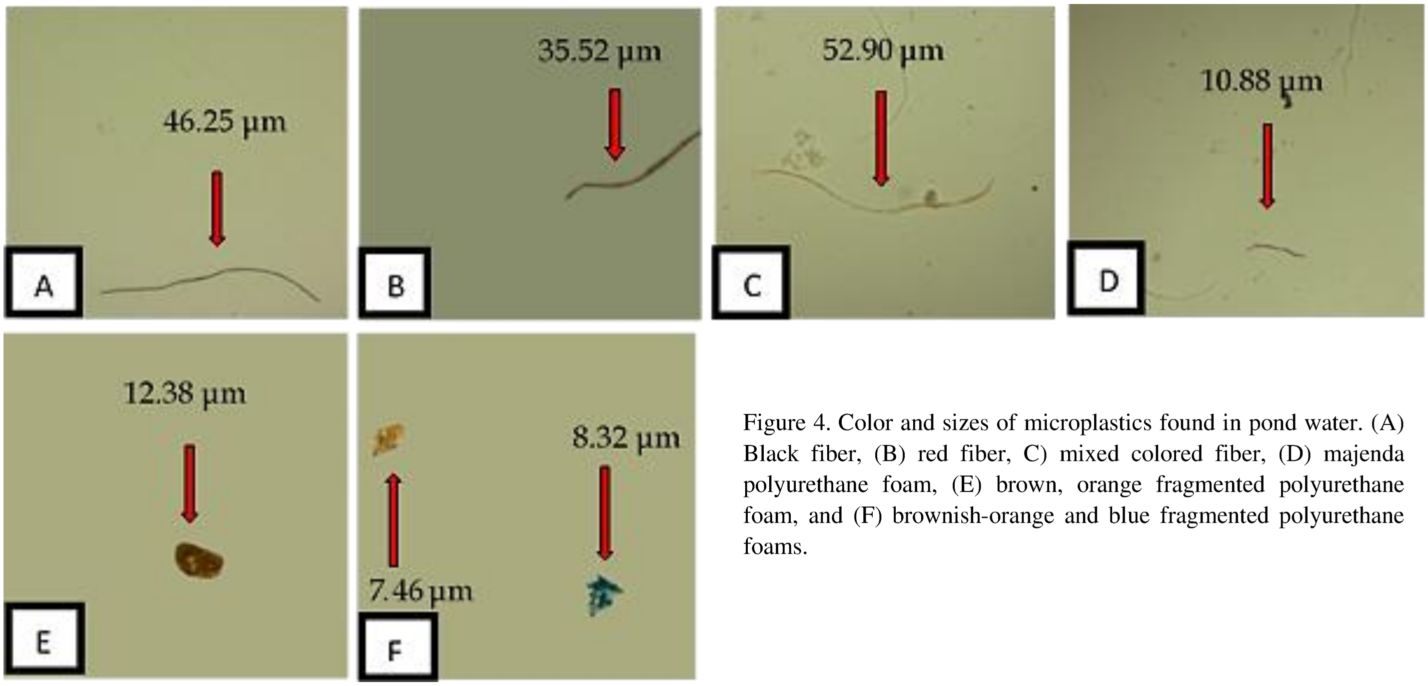

Figure 4. Color and sizes of microplastics found in pond water. (A) Black fiber, (B) red fiber, C) mixed colored fiber, (D) majenda polyurethane foam, (E) brown, orange fragmented polyurethane foam, and (F) brownish-orange and blue fragmented polyurethane foams.

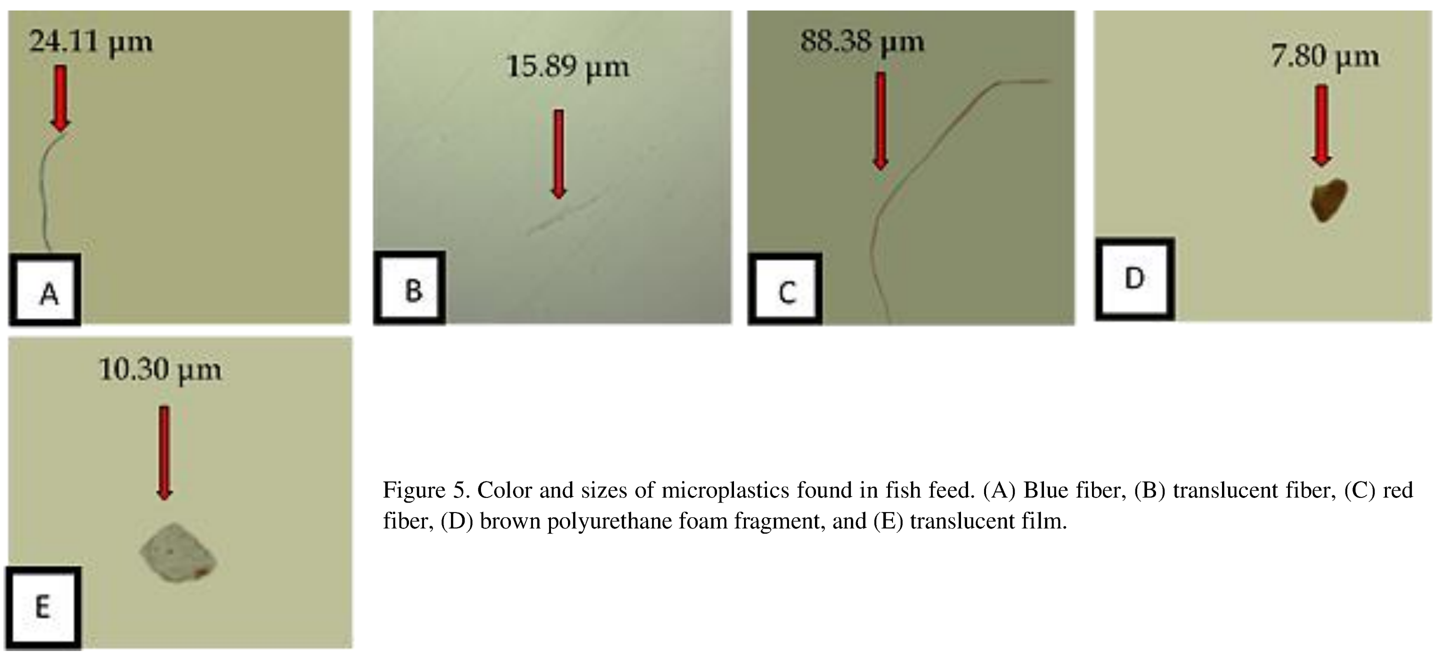

Figure 5. Color and sizes of microplastics found in fish feed. (A) Blue fiber, (B) translucent fiber, (C) red fiber, (D) brown polyurethane foam fragment, and (E) translucent film.

MPs were found from the fish feed by density separation method using saturated NaBr solution. The sizes of these MPs ranged from 10.08-88.38 µm (Fig. 5A-E). On the other hand, 12 MPs were detected from fish flesh with an abundance of 0.33 particles g^{-1} in Mymensingh Sadar but 1.57 particles g^{-1} (Table 1) in Tarakanda, which indicated very few MPs were available in fish flesh. Different colored fibers such as black, blue, translucent, and brownish colored polyurethane foam were found in the fish flesh, and their sizes were in a range of 7.44-15.20 µm (Fig. 6A-D).

Discussions

MPs in consumable species are becoming a global concern for public health. It is now well-known that MPs are highly persistent and accumulate in different ecosystems at increasing rates (Andrady, 2017). For this reason, MPs are considered an emerging global public health issue, and the long-term effects of continuous MP accumulation followed by transfer along the food chain are of great concern (Smith et al.,

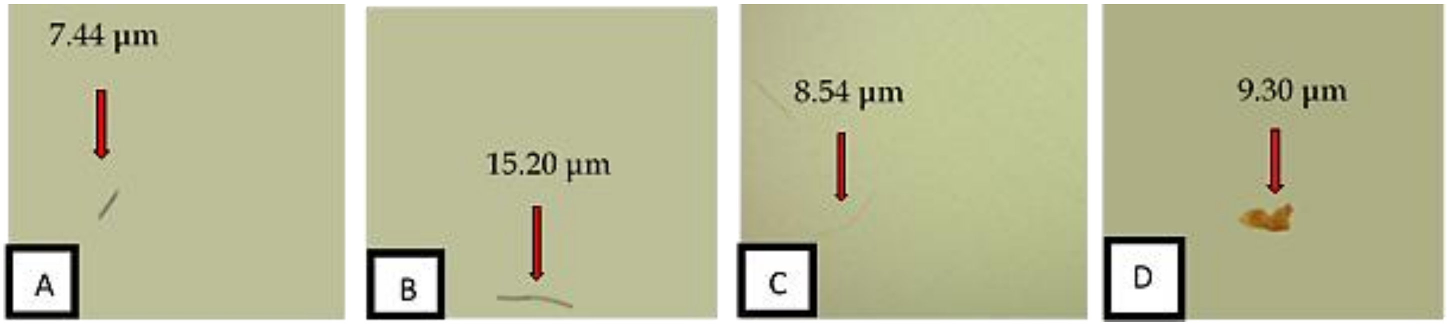

Figure 6. Color and sizes of microplastics found in fish flesh. (A) Blue fiber, (B) black fiber, (C) translucent fiber, and (D) brownish polyurethane foam fragment.

2018). Fewer studies addressed the presence of MPs in freshwater environments, but several recent publications enriched the knowledge of MPs in freshwater (Wagner et al., 2014; Driedger et al., 2015; Dris et al., 2015; Eerkes-Medrano et al., 2015; Horton et al., 2017; Besseling et al., 2017). MPs availability in freshwater bodies results from the mismanagement of plastic waste. The plastic-goods production sector holds the twelve position of export earning sectors of Bangladesh (Hossain et al., 2020) and shares a larger portion of mismanaged plastic waste each year, about 1.89 million tons (Ritchie and Roser, 2018). Therefore, the presence of MPs in freshwater aquaculture may become a threat to sustainability.

Several digestion techniques have been reported in the previous studies, including acid (Cole et al., 2014), alkali (Karami et al., 2017), oxidizers (Prata et al., 2019), enzyme (Löder et al., 2017), and the improved separation method by the combination of PAC and ethanol (Ma et al., 2020). However, the present study successfully detected 191 colored MPs belong to various sizes ranging from 7.44-52.90 µm from the collected samples of *H. fossilis* culture system where 26% saturated NaCl solution was used as density separator for a water sample, and 40% saturated NaBr solution for fish flesh and fish feed samples. The density separation is the most reliable and standard method for separating MPs from aquatic systems, including sediment or sand (Quinn et al., 2017). Thus, the present technique was proved to be a more accessible and efficient method for investigating various MPs in the fish farming system in Bangladesh. A high level of MP pollution in aquaculture water of fish ponds at two experimental stations in China was observed by Ma et al. (2020), with the abundances of 10.3-87.5 particles L^{-1} compared to that reported in freshwater fish ponds in the Carpathian basin of Europe (3.52-32.05×10^{-3} particles L^{-1}) (Bordós et al., 2019). The present investigation revealed abundant MPs from pond water, *i.e.,* 1.80 particles mL^{-1} and 1.14 particles mL^{-1} in Mymensingh Sadar and Tarakanda sub-districts, respectively. Until recently, no standardized sampling and separation methods of MPs have been established worldwide (Stock et al., 2019). The discrepancies in MP abundance observed might have been attributed to the differences in the applied methods for sampling and separation across the studies (Ma et al., 2020), geographical locations, level of microplastic pollutions, and overall aquaculture management techniques.

There may be several routes for the MPs contamination in aquaculture farms of the investigated areas. One possible route is the overflowing water of the Old-Brahmaputra River, which passes beside Mymensingh District. This river receives massive runoff water from the surroundings during the rainy season, carrying sewage disposal, plastic waste, and industrial effluents (Muyen et al., 2016). Besides, due to heavy rainfall, the aquaculture ponds have been subjected to devastating floods each year, which may act as a route of MPs contamination (Muyen et al., 2016; Hossain et al., 2020). Some of the *H. fossilis* ponds of the study areas were either generally affected by the devastating floods or runoff water due to heavy rainfall. Again, the Old-Brahmaputra river receives a vast amount of industrial effluents, including textiles, garments, jute, cotton, and dying industries, which leads to fiber type MP abundance in the river water (Katzenberger and Thorpe, 2015; Bhuyan et al., 2019). Our study represents a higher availability of fiber type MPs (*viz.,* 75.92 and 77.5% of total study) in pond waters of 2 sub-districts due to partial fill-up of the ponds floodwater or runoff water contaminated highly with fiber type MP. Priscilla and Patria (2020) also represented similar findings in milkfish farms, where they found a higher abundance of fiber type MPs (55.00±11.7 particles L^{-1} and 55.60±13.8 particles L^{-1}) on the surface water of two different locations in Indonesia.

The present study revealed 45 MPs in different commercial catfish feeds. It can be pronounced that the MPs were contaminated with the ingredients used in the manufacturing process of feed. Fishmeal, the key ingredient of fish feed, becomes a concerning matter due to the presence of MP in fish meal, which may also play the primary role of MP introduction in the freshwater aquaculture system. In a finding of Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) (Kershaw and Rochman, 2015), it was observed that aquaculture systems where fish, shrimps, or other farmed species are fed with feeding materials produced from fish and other animals (*viz.,* fishmeal) might be contaminated with MPs present in these products. Large numbers of small pelagic fishes, at risk of direct uptake of MPs mistaken for food items, are converted into fishmeal and fish oil each year (FAO, 2016), a proportion generally used in the aquaculture feed industry. In addition to using contaminated feeds in aquaculture, fisheries production may also influence environmental plastic availability (Lusher et al., 2017).

Generally, most fish species are gutted before consumption by humans, and hence, direct human exposure to MPs from fish becomes negligible. However, it is stated that the presence of MPs in the eviscerated flesh (whole fish excluding the viscera and gills) of two commonly consumed dried fish species (*Chelon subviridis* and *Johnius belangerii*) was significantly higher than excised organs (viscera and gills), evidencing that the evisceration does not necessarily eliminate the risk of MPs intake by human consumers (Karami et al., 2017). In addition, small marine pelagic fish species, such as sardines, anchovies, and many other small-sized freshwater fish, are sometimes eaten whole, including the digestive system. Small indigenous freshwater species such as *Amblypharyngodon mola* and *Esomus danricus* are also eaten whole and are commonly consumed in rural diets in Bangladesh (Lusher et al., 2017). Abbasi et al. (2018) investigated commercially important fish species and a crustacean from four sites in the Musa Estuary and a site in the Persian Gulf to confirm the presence and location of MPs. A total of 828 MPs were detected in the guts (gastrointestinal tracts), skin, muscle, gills, and liver of demersal and pelagic fish (*Platycephalus indicus*, *Saurida tumbil*, *Sillago sihama* and *Cynoglossus abbreviatus*) from all five sites and in the exoskeleton and muscle of the tiger prawn, *Penaeus semisulcatus*, from three sites. In the present study, no viscera of *H. fossilis* were investigated but detected 12 MPs in the flesh, ranging from 7.44-15.20 μm. The presence of MPs in fish feed and rearing water might be the important sources of MPs in the flesh as it is recognized that MP particle size of less than 10 μm can easily pass the epithelial wall of the gill and intestine of fish (Abbasi et al., 2018). Thus, MP particle size has a vital role in determining the effects of MP and its translocation in cellular parts (Katzenberger and Thorpe, 2015).

Conclusion

The present study provides the first evidence of the existence of MPs in an aquaculture farm of catfish (*H. fossilis*) in Bangladesh. Various MPs were observed in rearing water, commercial fish feed, and fish flesh. In total, 191 MP particles were identified where various fibers and colors such as black, blue, red, brownish, and translucent were more abundant than fragments and translucent films. The highest concentration of MPs particles was observed in the fish feed, followed by rearing water and fish flesh, indicating that the fish feed could be an MPs source. In-depth studies are required to elucidate the existence of MPs in the aquaculture system, e.g., fish farms, and the factors influencing their biological impact on overall aquatic biota. Future investigations are necessary to determine the ingestion and egestion rates of MPs in commercially important fish species in Bangladesh and their transfer rates to different tissues from the gut. However, there is a lack of management processes to control and prevent MPs pollution in aquaculture feed in Bangladesh. Further in-depth studies of MPs in feeds are crucial to providing data support for feed safety evaluations.

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