

Microplastic Ingestion by Fishes from Jamuna River, Bangladesh

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ABSTRACT

Microplastics (MP) have been an evolving global concern by dint of the escalation of plastic pollution in the aquatic environment. However, few data document MP ingestion and accumulation in freshwater fauna as compared to marine organisms. This study investigates the prevalence of MPs in the gastrointestinal tracts (GIT) of 45 individuals belonging to seven commonly found Bangladeshi freshwater fish species with different feeding types (herbivore, carnivore, and omnivore). A total of 81 MP items of varying shapes were detected in 76% of individuals investigated, with an average abundance of 1.80 ± 1.65 items/individual. Of these, fiber was identified as the most prevalent ingested MP type (70%) followed by film (14%), line (10%), fragment (4%), and foam (2%). Black-colored MPs were the most dominant (27%) followed by white (26%), blue (24%), red (17%), and green (6%). The results demonstrated a higher number of MPs in the carnivore (1.95 items/individual) and omnivore (1.85 items/individual) fish species as compared to herbivore fish species. Among carnivores, *Wallago attu* registered the highest amount of ingested MP items (3.5 items/individual), while *Anguilla bengalensis* registered the highest amount of ingested MP items (2.14 items/individual) among the omnivores. The amount of ingested MPs was significantly correlated ($P < 0.05$) with body size, body weight, and gut weight, while an insignificant correlation ($P > 0.05$) was found between the number of consumed MPs and trophic fractions. The results provide valuable insights into the prevalence of MPs in freshwater fish in Bangladesh and associated bioaccumulation through trophic transfer.

1. INTRODUCTION

Plastics are recognized as artificial substances composed of synthetic or semi-synthetic natural polymers manufactured from petro-based chemicals that are cost-effective, lightweight, durable, and corrosive resistant (Boucher and Friot, 2017; Denuncio et al., 2011). Plastic production has been increased about 43% over the last decade. According to data from the Association of Plastic Europe, worldwide plastic production hit 322 million tons during 2015 (Plastics Europe and EPRO, 2016), 335 million tons in 2017 (Lahens et al., 2018), and demand is presumed to increase to 1,000 million tons by 2050 (Lusher et al., 2017). Freshwater ecosystems are the primary destination of many pollutants delivered in the watershed since aquatic environments are generally situated in valleys and low-height

landscapes. Plastic discarded inaccurately (e.g., roads, streets, and open landfills) are conveyed by pluvial flows to water bodies (Faure et al., 2015). Upon reaching freshwaters, plastics may get entangled by streambed structures (e.g., riverbanks, shrubs, trees, and cliffs), carry with the current to floodplains, or become entrained in adjacent sediments (Azevedo-Santos et al., 2021). In Brazil's Amazonian ecosphere, plastic comprises 15.7% of total solid waste and it is precisely estimated that 182,085 metric tons of plastic are dumped yearly, which is potentially transported by the Amazon River to the Atlantic Ocean, presently the world's second most plastic-polluted river, trailing only China's Yangtze River (Giarrizzo et al., 2019). Rivers currently dump 1.15 to 2.41 million tonnes of plastic waste each year into the ocean. The world's 20 most polluting waterways, primarily in Asia, represent

67% of overall pollution (Lebreton et al., 2017). Admittedly, rivers have gotten very little consideration concerning the issue of microplastic (MP) pollution (Costa and Barletta, 2015). Plastic particles less than 5 mm are commonly known as microplastics (Hartmann et al., 2019). In the early 1970s, microplastics were documented in seawater. (Guzzetti et al., 2018). Microplastics have been marked as deriving environmental pollutants that have also procured research concern at present. The micropollutants are present in both terrestrial and aquatic environments having deleterious effects on existing ecosystems (Karim et al., 2020).

The largest densities of microplastic debris have been reported from plankton (Yu et al., 2020), water bodies (Deng et al., 2020), and sediment (Castañeda et al., 2014). Predominantly found plastic particles within the aquatic environment are: fragments, fibers, and pellets (Veiga et al., 2016). Prior studies have used various methods to detach, identify, and validate plastic pollution in fish (Boerger et al., 2010; Vendel et al., 2017). For example, plastic objects were examined in the whole gastrointestinal tract (GIT) (Liboiron, 2019). Their origin classifies microplastics. Primary microplastics are cosmetic (i.e., shower gel, lipstick, and shaving cream), cleaning products, exfoliating scrubs, and medicines. Secondary microplastics result from micro-and macro debris fragmentation, subjected to mechanical forces, oxidation, and photochemical processes (Mathalon and Hill, 2014). Plastic enters an aquatic ecosystem by river drainage, storm water, and wastewater treatment plant (WWTP) discharges (Dris et al., 2015). A range of 19 to 447 particles/L of microplastics was found in the effluent of ten of Denmark's largest tertiary WWTPs and emitted up to 1 to 30 particles/L from three secondary WWTPs (Conley et al., 2019). The most polymer confronted; specifically, microplastics are polyethylene terephthalate (PET), polypropylene (PP), polyvinylchloride (PVC), polyethylene (PE), polystyrene (PS), and polytetrafluoroethylene (PTFE) (Rocha-Santos and Duarte, 2015; Xu et al., 2020).

A broad range of marine organism ingested microplastics have been reported in fishes (Bellas et al., 2016; Jovanović, 2017), turtles (Duncan et al., 2019), sea birds (Lavers et al., 2019), and mammals (Zantis et al., 2021). Obvious consequences of microplastic ingestion by aquatic organisms are blockage of GIT, growth retardation, reproduction failure, and alteration of feeding (Cole et al., 2015;

Nelms et al., 2018; Sussarellu et al., 2016). In addition, contaminants accumulating on the surface of plastic materials may have detrimental health effects on fish (Critchell and Hoogenboom, 2018; Pannetier et al., 2020). Plastic has been shown to influence DNA damage, oxidative pressure, cirrhosis, embryo-toxicity, aberrant riposte, and lipid peroxidation (Brandts et al., 2018) in fishes. The prime goal of the following study was to identify the presence of MPs in the gastrointestinal tract (GIT) of freshwater fish. Particularly, the research objectives were to 1) assess the abundance, morphotype, and color of MPs in fish gut contents, 2) compare the MPs concentration among diverse species of fishes from different feeding habitats and tropic fractions, and 3) determine the association between MPs intake rate and fish length, body weight, and gut-weight. These findings provided the early sign of microplastic contamination of Jamuna River's fishes. The findings demonstrated that fishes of Jamuna River incorporate varying amounts of microplastics in the gastrointestinal tract.

2. METHODOLOGY

2.1 Study area

This study was carried out on Jamuna River, between 24°22'41.17"N, 89°47'49.08"E (S1; close to Bangabandhu Bridge, Tangail District) and 24°11'42.17"N, 89°46'36.74"E (S2; near Chauhali Upazila, Sirajganj District) (Figure 1). A delta plain with tributaries of the Ganges (Padma), Brahmaputra (Jamuna), and Meghna Rivers occupying 79% of the nation. Jamuna-Brahmaputra Rivers are roughly victims of industrial (pulp, paper, textiles, fertilizers, and detergent) toxic discharges, lubricants, and heavy metals. Plastic particles scattered over the branches by stream gliding or weaving and tourist spots near Jamuna Bridge could be one of the basic explanations of plastic contamination in the normal natural way of life (Hossain et al., 2019; Uddin and Jeong, 2021).

2.2 Sample collection

In total, 45 individuals representing seven species with 20.74±8.65 cm average length were collected by 5 mm mesh nets during the time period of 20th April to 5th July 2019. During sampling, divergent feeding zones (demersal, benthopelagic, and pelagic) with feeding habits were considered (herbivore, carnivore, and omnivore) (Table 1). Sample collection also relied on the conventional fish consumption nature and accessibility of fishes for that certain

fishing period. Therefore, resulting in different numbers of individuals for each species. Then, samples were put into an icebox to preserve and transport, and taken to the laboratory for storage in a refrigerator at -4°C for analysis (Peters and Bratton, 2016).

2.3 Sample preparation

In the laboratory, fish were allowed to thaw for about 30 minutes at room temperature before examining total length (TL) and body weight (W). Consequently, the GIT of each fish was dissected. After weighing, samples were transferred into individual clean beakers (Figure 2).

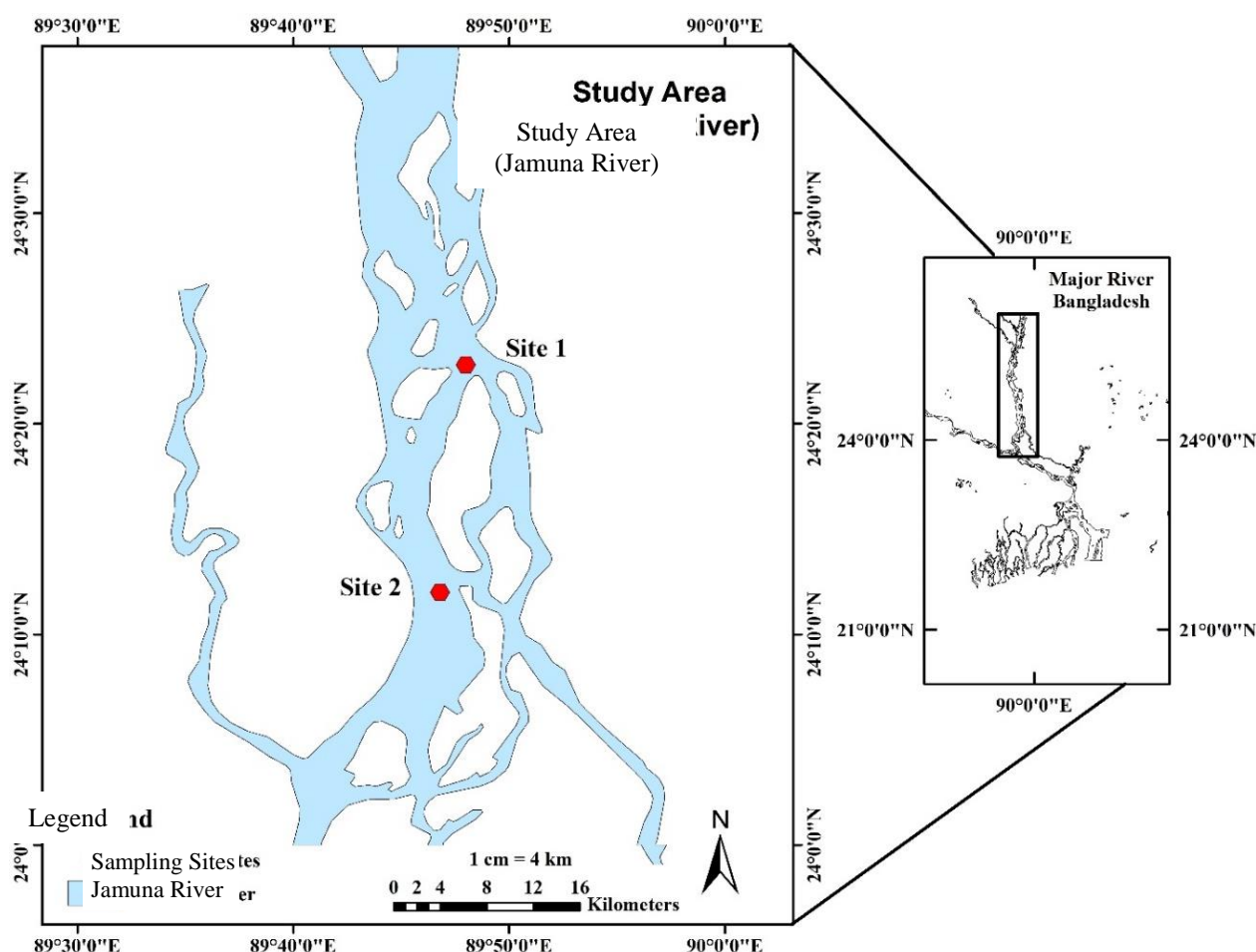


Figure 1. Map showing the study area (Jamuna River)

Table 1. Generic information about sample fishes, i.e., scientific name, local name, trophic fraction, feeding zone, and feeding group

Scientific name	Local name	Number (n)	Trophic fraction	Feeding zone	Feeding group	Total length (cm)	Total weight (g)	Gut weight (g)
<i>Wallago attu</i>	Boal	8	3.70 ± 0.56	Demersal	Carnivore	31.46 ± 3.73	142.50 ± 50.90	3.62 ± 0.70
<i>Anguilla bengalensis</i>	Biam	7	3.80 ± 0.70	Benthopelagic	Omnivore	26.27 ± 13.70	94.28 ± 143.82	2.58 ± 2.25
<i>Labeo Calbasu</i>	Karlbaous	8	2.00 ± 0.00	Demersal	Omnivore	19.61 ± 1.52	95.37 ± 19.96	3.84 ± 1.09
<i>Ailia coila</i>	Kajali	5	3.60 ± 0.60	Pelagic	Carnivore	11.16 ± 1.05	5.20 ± 1.30	0.22 ± 0.02
<i>Cirrhinus reba</i>	Tatkini	5	2.50 ± 0.20	Benthopelagic	Herbivore	12.56 ± 0.61	18.80 ± 1.64	0.79 ± 0.15
<i>Ompok pabda</i>	Pabda	7	3.80 ± 0.60	Demersal	Carnivore	17.05 ± 0.59	26.71 ± 3.25	0.57 ± 0.13
<i>Clupisoma garua</i>	Gaira	5	3.70 ± 0.59	Demersal	Omnivore	20.52 ± 1.77	50.40 ± 12.62	2.80 ± 1.17

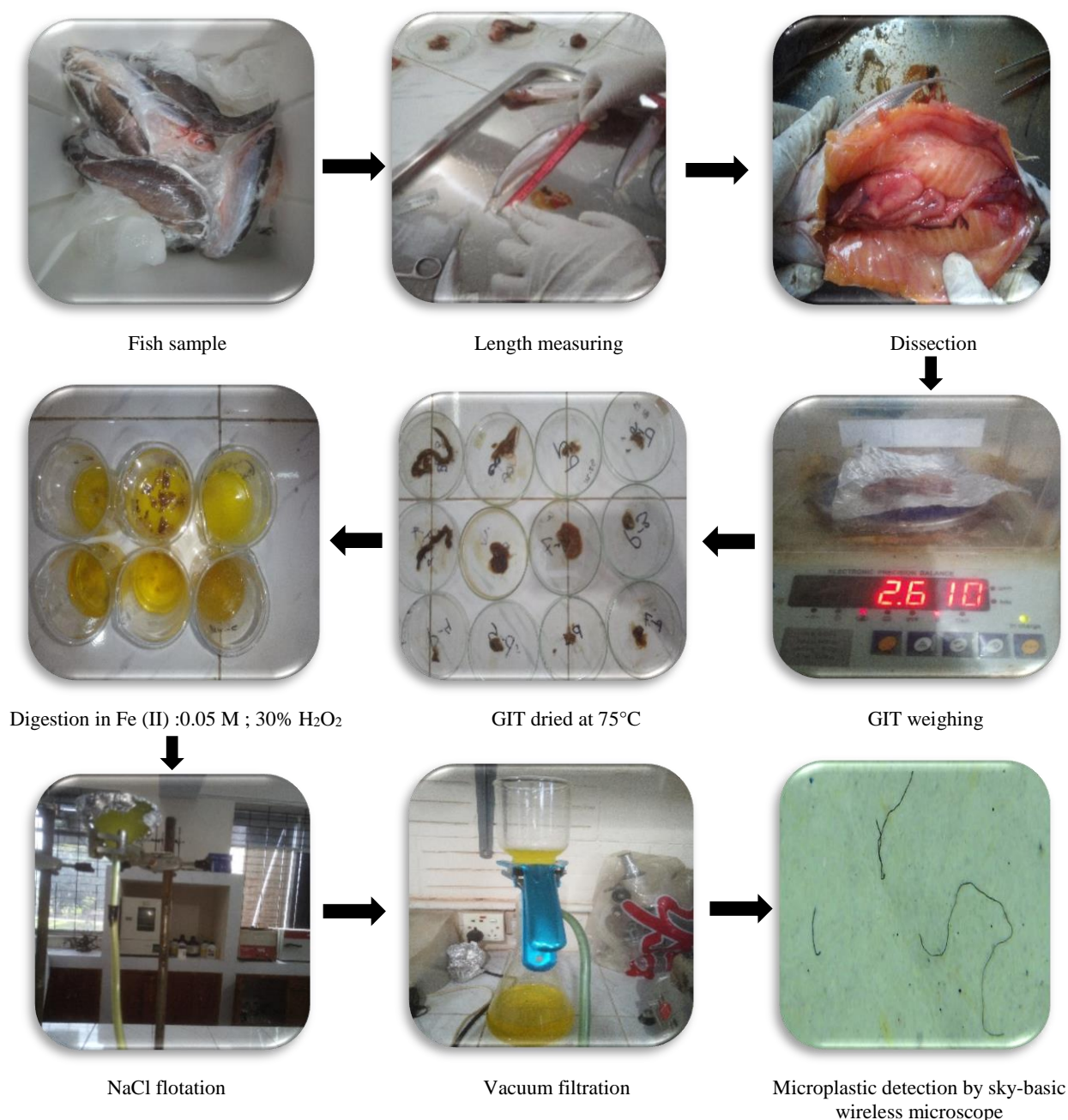


Figure 2. Preparation of samples, digestion, and analytical processes to identify microplastics in fish samples

2.4 Digestion and hydrogen peroxide (H₂O₂) treatment

The removed digestive tissue was dried a minimum of 24 h at 75°C in a hot air oven and added to 20 mL 0.05 M Fe(II) (7.5 g FeSO₄·7H₂O) (278.02 g/mole) in 500 mL water with 3 mL concentrated H₂SO₄ and 20 mL (H₂O₂ 30%) at 75°C. The mixture was left to stand on a lab bench at room temperature for five minutes, then heated to 75°C on a hotplate. As gas bubbles affirmed, the beaker was removed from

the hotplate and approximately 6 g of salt (NaCl) was added to increase the aqueous density. The mixture was heated to 75°C until the salt dissolved (McNeish et al., 2018). The developed analytical techniques have both advantages and disadvantages (Strungaru et al., 2019) for MPs detection in aquatic organisms. NaCl solution was used in this study because of its efficacy, low cost, and non-hazardous features. Instead of H₂O₂, HNO₃ (Chan et al., 2019), and NaOH (Yuan et al., 2019) was used to digest organic matter.

2.5 Density separation, floating, and vacuum filtration

The solution was transferred to the density separator (glass funnel fitted with a 50 mm segment of latex tube), putting a pinch clamp on top to control liquid flow. The beaker was rinsed with distilled water and lined overnight by aluminum foiling to transfer all the remaining organs to the density separator. Visually inspected floating microplastics and remaining settled organs were vacuum filtered through 1.2 μm Whitman GF/Microfiber filter papers (Masura et al., 2015).

2.6 Detection of microplastics

Filters were observed under a sky-basic wireless digital microscope, and images of plastic items were taken by installing Max-See software in the android phone at 50X-100X magnifications at different resolutions (1,920×1,080, 1280×720, and 640×480). Then, visually assessed the plastic images and categorized them by color and shape (fiber, fragment, thread line, film, or foam) (Figure 3).

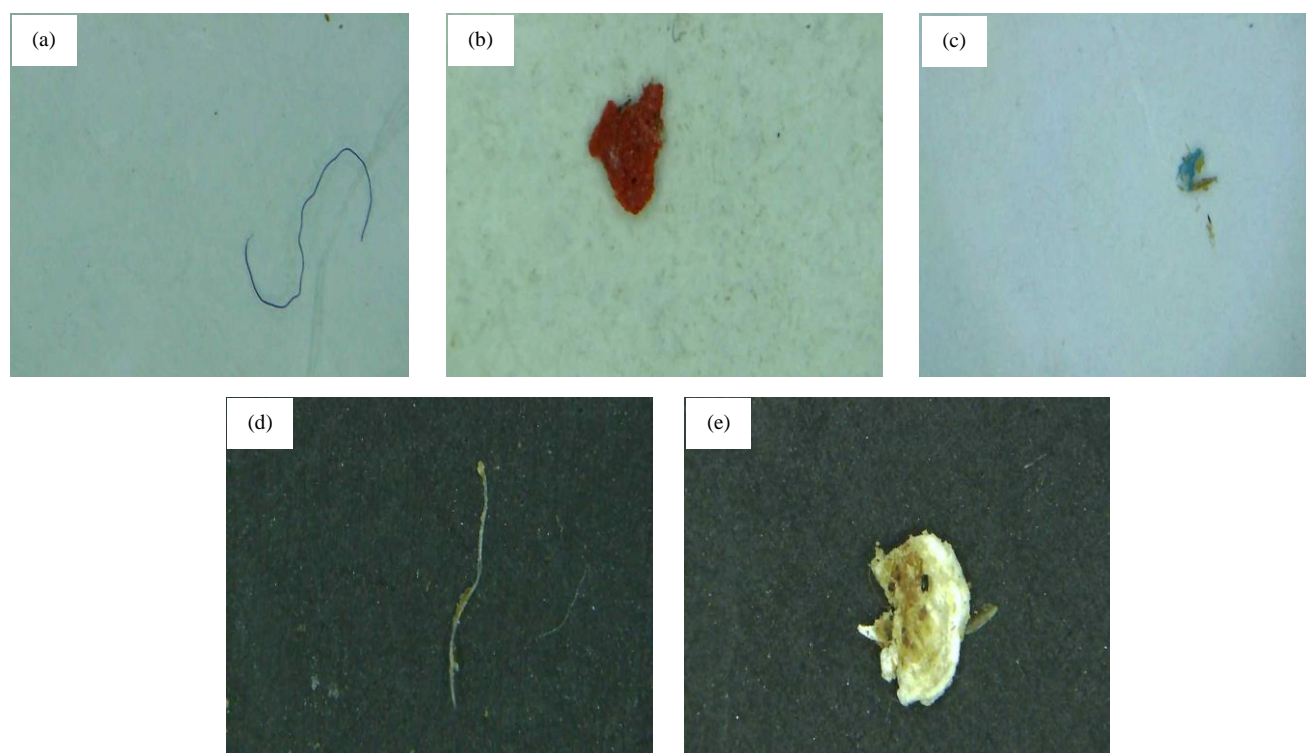


Figure 3. Example of microplastic found in fish from Jamuna River. The shapes included fiber (a), fragment (b), film (c), thread line (d), and foam (e).

3. RESULTS

3.1 Abundance of microplastic in fish

Out of seven species collected from Jamuna River, 34 of 45 individuals (76%) contained an average of 1.80 ± 1.65 particles (SD) per total fish. Fish ranged in length from 54.3 to 10.2 cm and weight between 400 to 4 g. On average, the highest (3.50 ± 1.93 , 35%) microplastic particles per species are extracted from *Wallago attu* and the lowest MPs exhibited from *Ompok pabda* (1.00 ± 0.58 , 9%) followed by *Labeo calbasu* (2.12 ± 1.55 , 21%), *Anguilla bengalensis* (2.14 ± 1.21 , 18%), *Clupisoma garua* (1.00 ± 1.12 , 6%), *Cirrhinus reba* (1.00 ± 1.41 , 6%) and *Ailia coila* (0.80 ± 1.30 , 5%) (Figure 4(a) and 4(b)).

3.2 Morphotype and color distribution of microplastic

From the 81 particles, 57 were fiber (70%), 11 were film (14%), 8 line (10%), 3 fragment (4%), and 2 foam (2%) (Figure 4(c)). Color distribution of ingested microplastic was not homogenous. Five different colors of microplastic were found among the species. Black particles were most commonly found (27%), subsequently, white (26%), blue (24%), red (17%), and green (6%) (Figure 5(a)), which differs from Bessa et al. (2018) observed blue as most specific color (47%) led by transparent (30%) and black (11%). This study shows, the predominant fiber colors were black (39%) and blue (28%), followed by red (21%), green (9%), and white (3%) (Figure 5(b)).

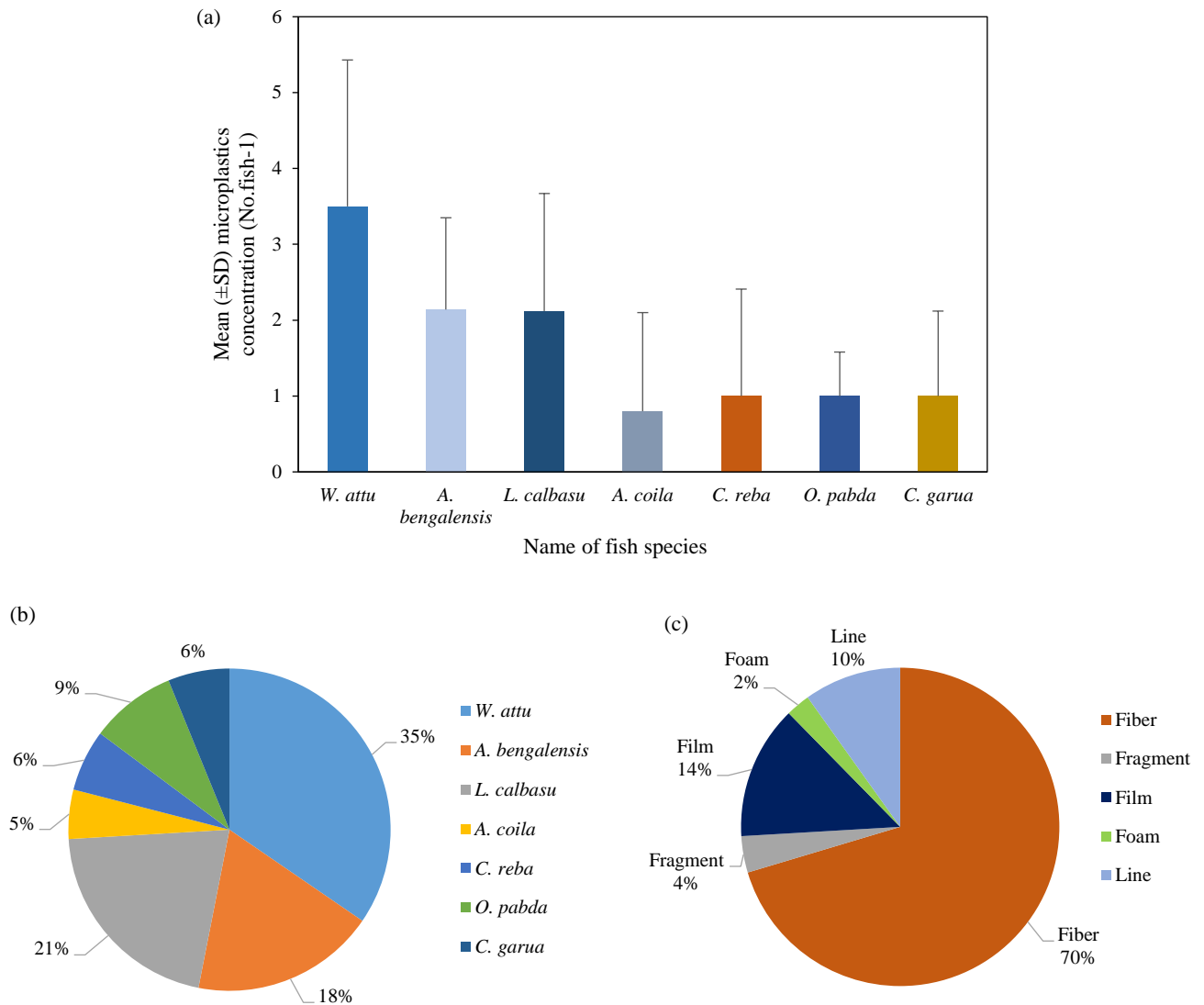


Figure 4. Average: (a) percentage (%), (b) morhotype, and (c) of microplastic in identified fishes

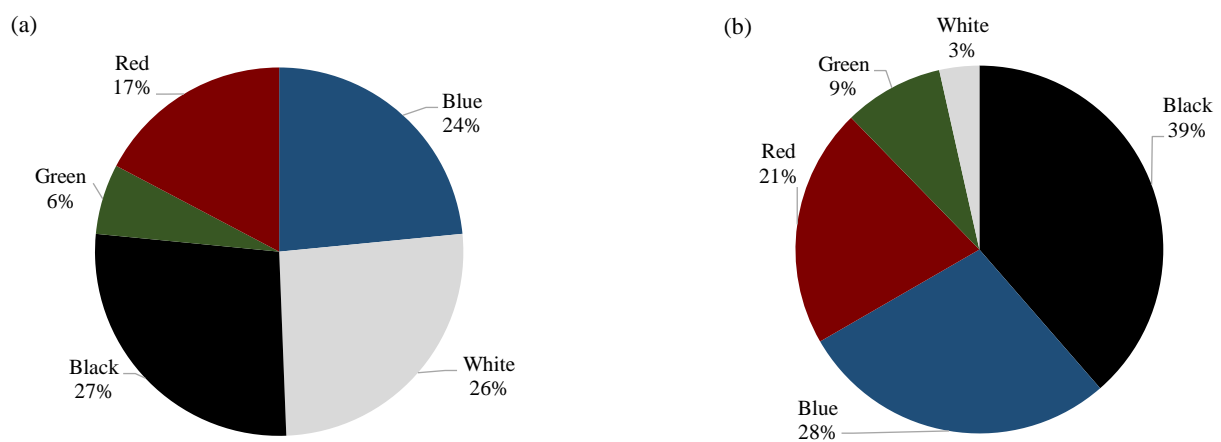


Figure 5. Percentage of MPs by color (a), frequency of fibers color (b), average of microplastic in different fish feeding zone of seven fish species (c)

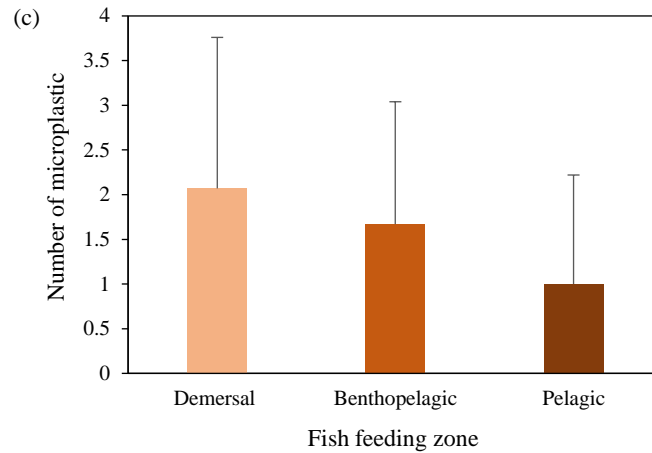


Figure 5. Percentage of MPs by color (a), frequency of fibers color (b), average of microplastic in different fish feeding zone of seven fish species (c) (cont.)

3.3 MPs abundance with fishing habitat and tropic level

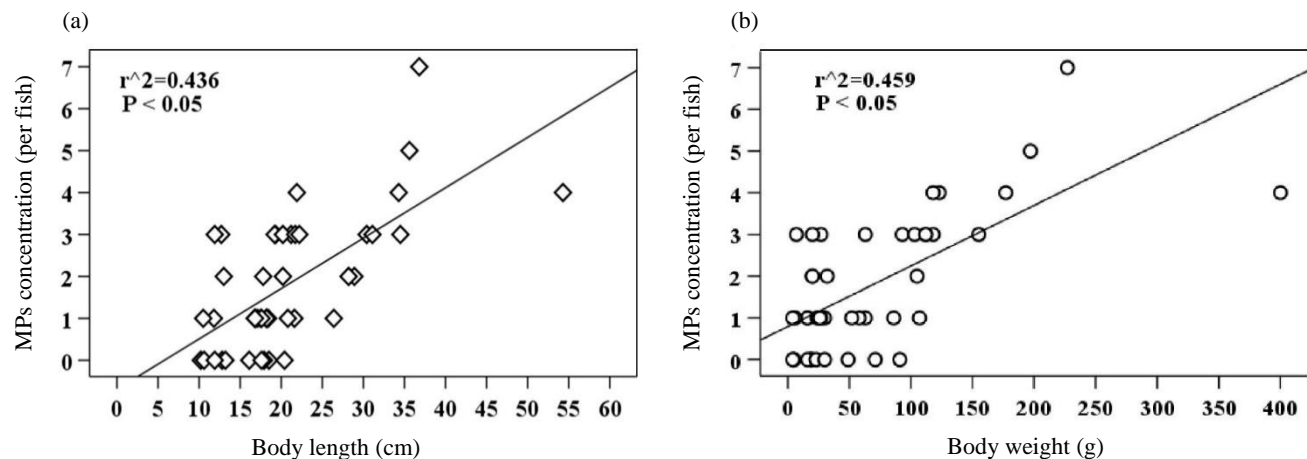
A one-way ANOVA indicated that the mean microplastic particles were significantly different among the seven taxa ($F=3.062$, $df=6$, and $P=0.015$), but an insignificant effect of fish FFG on microplastic concentration was found ($F=0.736$, $df=2$, and $P=0.485$) (Table 2).

3.4 Influence of MPs absorption among fish body length, body weight, and gut weight

Fish body size ($R^2=0.436$, $P<0.05$), body weight ($R^2=0.459$, $P<0.05$) were positively correlated with the microplastic abundance in fish specimens. The relationship between the rate of microplastic intake and gut-weight was statistically significant ($R^2=0.439$, $P<0.05$) (Figure 6(a), 6(b), and 6(c)). Whereas, number of microplastics and tropic fraction showed an indistinct correlation. (Spearman's correlation, $\rho=0.119$, $P>0.05$).

Table 2. Comparison of mean microplastic concentration among taxa and fishing groups, using one-way ANOVA and Kruskal-Wallis statistical analyses

Sample type	df	ANOVA		Kruskal-Wallis		
		F Value	P Value	H	X ²	P Value
Fish Taxa	6	3.062	0.015	13.331	12.591	0.038
Fish FFG	2	0.736	0.485	1.896	5.991	0.395



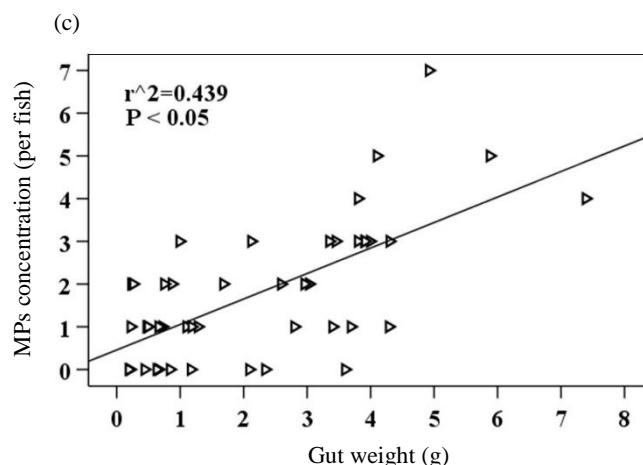


Figure 6. Linear Regression analysis for number of microplastic with fish size (a), body weight (b), and gut weight (c) (cont.)

4. DISCUSSION

In spite of the nutrient value of fish and its crucial role in the aquatic ecosystem, few studies particularly look at plastic burden in freshwater species. Our study is reputedly the first to portray ingestion of plastic by Jamuna River fishes. Bangladesh produced approximately 400 to 4,500 tons of solid debris daily which usually carries mismanaged plastic, more than half of this waste is disposed of in low-lying soil or freshwater (Arefin and Mallik, 2017). Thus, Bangladesh placed 10th over 20 improperly managed plastic generator countries around the globe. ESDO (2016) asserted Bangladesh has 7,928 billion microbeads entering its rivers, canals, and alternative water resources.

More than 250 particles were sorted from the gut contents and examined under a microscope. By characterizing the morphotype, we determined 81 particles as plastic those found in several studies (e.g., Vendel et al., 2017; Herrera et al., 2019; Arias et al., 2019). Almost all synthetic fibers are released from textile and domestic washing (Cesa et al., 2017). The line probably derives from fishing gear, nets, and sewing thread. Even so, the fragments and films come from an array of indefinite sources, from land and aquatic features. In this research, the size and nature of plastic could not be confirmed by applying FTIR spectroscopy due to the small width of plastic.

In the current study, 78% of sample fish contained microplastic with fiber (70%) most dominated. This result can be closely analogous to the findings of other research conducted in various regions. The ingestion of MPs reported in 68% in *Boops boops* from Balearic Island (Nadal et al., 2016), 95.7% in freshwater fishes from china (Jabeen et al., 2017), 85% in lake Michigan (USA) where 97-100%

of all particles were fiber (McNeish et al., 2018), 8% in fishes from Mexican Gulf (Phillips and Bonner, 2015).

Sanchez et al. (2014) exposed the first proof on MPs ingestion by freshwater fish in wild gudgeons (*Gobio gobio*), about 12%. In particular, MPs were retrieved from *Wallago attu* (35% and *Labeo calbasu* (21%) in the present study. *Lepomis macrochirus* and *L. megalotis* had 45% particles that were collected from the Central River Basin of Brazos, Texas (Peters and Bratton, 2016) and ESDO (2016) found 35% microplastic in rui (*Labeo rohita*) and 2.2% in sharputi (*Puntius sarana*). MPs reported about 33%, 49%, and 18% in *H. translucens*, *H. nehereus*, and *S. gibbosa* fishes, respectively, from the coastline of northern Bay of Bengal, carried fiber mostly ascendant, while MPs differed significantly among fish species and relevant with body and GIT weight (Hossain et al., 2019). Pazos et al. (2017) reported 96% of the fiber in fish from Río de la Plata estuary, merging no correlation between MPs quantity and fish length, weight, and feeding habit. In constant, particles constituted fragments at 54% in South-western Germany (Roch et al., 2019). Pegado et al. (2018) showed the number of MPs was not correlated with weight and tropic level.

Based on feeding habits, Carnivore and omnivore guild contained 20 fishes, and the Herbivore guild had five fish. (Table 1). As a result, determining which feeding habit incorporates more MPs ingestion was difficult. According to Ismail et al. (2018), microplastic density in omnivore and carnivore fishes was lower comparing herbivore fishes from Biawak Island. Nevertheless, Garnier et al. (2019) reported herbivore fish *Siganus* spp. had the lowest MPs (0.15 ± 0.10) and carnivore fish *Epinephelus merra* contained the highest number of MPs (0.39 ± 0.14) per

fish. Andrade et al. (2019) depicted herbivores had observed lowest percentage of plastics (13.3% for *Myloplus rubripinnis* and rose to 27.3% for *Metynnis guaporensis*). Omnivores had the greatest degree of frequency, with *Acnodon normani* accounted for 25.0% and *Myloplus rhom boidalis* making up 100%. In Carnivore stomachs, microplastic was found at a sufficient amount ranging from 14.3% in *Semasalmus manueli* to 75.0% in *Pygocentrus nattereri*. The current study found that the feeding zones have an influence on MPs assimilation by fish. The presence of MPs in demersal (2.07 ± 1.69) fishes were higher than benthopelagic (1.67 ± 1.37) and pelagic (1.00 ± 1.22) fishes (Figure 5(c)) as demersal species are both carnivorous and omnivorous, eating a wide range of plant and animal-based foods (e.g., larvae, insects, crustaceans, mollusks, and algae).

5. CONCLUSION

Based on the study, it can be concluded that fishes from the Jamuna River are at risk of being contaminated with microplastics. Approximately 76% of processed microplastics 70% were fibers and 14% were films. We observed a significant difference in the frequency of microplastic occurrence between the different fish species. Microplastics were most abundant in carnivore species, *Wallago attu* and the lowest recorded in herbivore: *Ailia coila*. This study also showed that the number of the microplastics ingested are independent of the feeding habits but had reliant on feeding zone. Our findings include a benchmark estimate that should be considered not just in future studies aimed at describing the possible effects of microplastic in fish assemblages and potential ecological threats, as well as in studies aimed at assessing the impacts of microplastic contamination on native communities that rely on fish as food supply. Furthermore, research should be carried out within a wide variety of fish and organisms to understand the dormant effects and to clarify the MPs movement across the terrestrial-aquatic boundary on the freshwater ecosystem in Bangladesh.

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