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Characterization of Microplastics in Gastointestinal Tracts of Some Selected Fish Species in Akhalia River, Fulbaria, Mymensingh

Nasrin Akter[1*], Effat Tyeba[2], Murad Ahmed Farukh[1] and Md Abdul Baten[1]

[1]Department of Environmental Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

[2]Department of Agroforestry, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

Correspondence: nasrin.23221315@bau.edu.bd , Phone: +8801793071056

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Abstract: Rivers have been identified as significant paths for the movement of microplastics into lake and marine environments. This study aimed to assess the abundance, distribution, and characteristics of microplastics in three freshwater fish species (*Channa punctatus*, *Macrognathus armatus*, and *Anabas testudineus*) from the Akhalia River, Fulbaria. During August through October of 2024, nine fish samples were collected at seven sampling stations. To identify the particles, microplastic concentrations were measured using the Daffodil MCX100 microscope. From the study it is found that the number of microplastics in the gut of freshwater fishes varied from 19.67±2.49 to 22.67±1.69 particles per 100L. Taki (*Channa punctatus*), a carnivorous fish species, consumed the most abundance microplastics (36%) and Baim (*Macrognathus armatus*), an omnivorous fish species, consumed the least amount (31%) of microplastics. The most commonly ingested types of microplastic were 34% filament, 28% fragment, 20% foam, 11% film, and 7% others. Black Microplastics were the least common (6%), while white microplastics were the most common (45%). Other colors include transparent (8%), blue (15%), green (18%), and red (8%). Around 46% of the microplastics in fish samples were greater than 4 mm and 23% microplastics were <0.5 mm in size. Fish body weight and length have a little positive association with microplastic abundance, according to linear regression analysis. From this study, it is revealed that microplastics pollute water bodies and hence, fishes. These findings highlight the need for rigorous environmental monitoring, public education, and governmental actions to prevent plastic pollution in river water.

Keywords: Microplastics; Akhalia river; Fish, Freshwater; Daffodil MCX100 microscope; Carnivore; Omnivore.

INTRODUCTION

Plastic pollution has become a critical global environmental issue, with microplastics emerging as a particularly persistent and hazardous form. Microplastics are defined as plastic particles smaller than 5 mm in size, either manufactured at microscopic sizes (primary microplastics) or formed by the degradation of larger plastic debris (secondary microplastics) (Cole, et al. 2011).

Aquatic creatures are harmed by the rapid growth of microplastics (MPs), which have become a global environmental problem. Fish constitutes a significant amount of protein supply (60%) to the citizens of Bangladesh; the country is considered the most prominent fish-producing nation in the world, with 4.62 million metric tons (MMT) of production in 2021–2022 (DOF 2023). Plastic pollution has become ubiquitous in the natural environment as waste management of plastics is insufficient in many countries of the world (Jambeck, et al. 2015). Although plastic waste can take many different forms, the smallest particles are referred to as "microplastics." There is no globally accepted definition of what size particle qualifies as a "microplastic (MP)," however the literature generally agrees on an upper limit of 5 mm (with a size range of 0.1–5000 µm) (Andrady and Zhu 2021). Global plastic production increased from 359 million to 368 million from 2018 to 2019. Construction materials (20.4%), automotive industries (9.6%), electronic and electrical appliances (6.2%), home, leisure, and sports items (3.4%), packaging (39.6%), and items in diverse industries (e.g., medical) account for 16.7% of plastic output. (Plastics 2020). The European Chemicals Agency defines microplastics as any piece of plastic smaller than 5 mm, rather than a particular kind of plastics (Collignon, et al. 2012). Microplastics, which are becoming hazardous pollutants, are created when large plastic particles break down into tiny fragments smaller than 5 mm. According to their origin, microplastics are divided into primary and secondary categories (Frias and Nash 2019).

Various studies conducted on microplastics in the Meghna, Turag, Jamuna, Old Brahmaputra, and other urban rivers revealed the presence of microplastics in fish species (Khan, et al. 2024); (Shaikh Tareq Arafat, et al. 2023); (Khan, et al. 2023); (Khan and Setu 2022); (Syed Rubaiyat Ferdous, et al. 2023). This research was conducted in rural rivers that were not considered before. This study is justified as it addresses a key knowledge gap by providing empirical evidence of microplastic presence in local freshwater fish species. This research aims to (1) investigate the abundance and distribution of microplastics in the gastrointestinal tracts of selected fish species, (2) assess the types and variety of microplastics (shape, size, color) ingested by fish species.

MATERIALS AND METHODS

Study Area

This research was directed at the Akhalia River, which is the main and only river in Fulbaria Upazilla. The Akhalia River originates from the Shirkhali River, which is a branch of the Old Brahmaputra River, and finishes at the Khirak/Khiru River. Fulbaria Upazila (Mymensingh district) with an area of 398.70 sq km, located in between 24°23' and 24°44' north latitudes and in between 90°08' and 90°28' east longitudes (Figure 1).

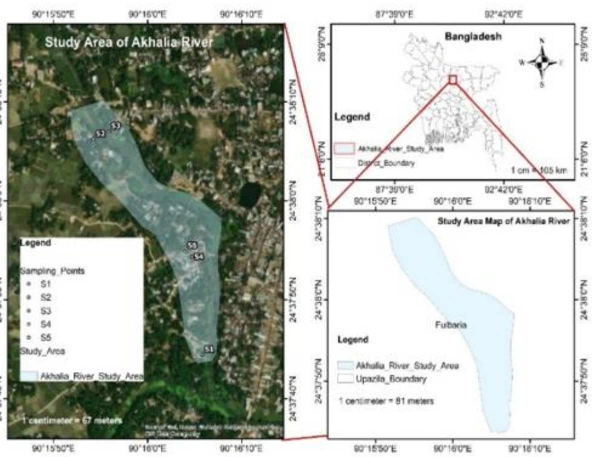

Figure 1. Map of the Study Area

Collection of Fish Samples

From August 2024 to October 2024, fish samples were gathered from various sites in the Akhalia River. A total of three species namely Koi (Anabas testudineus), Baim (Macrognathus armatus), Taki (Channa punctatus) were collected based on various feeding zones (demersal, benthopelagic) and feeding habits (omnivore and carnivore), especially two layers of fishes (middle and lower layer). Nine fishes of three species (3 individuals of each species) were collected from Akhalia River. Following fish collection, fish samples were kept in an icebox, brought to the Bangladesh Agricultural University's Department of Environmental Science lab, and kept in a freezer at -200° C for analysis using the procedures outlined by (Hossain, et al. 2020) and (Lusher, et al. 2017)

Fish Samples Processing

Fish samples were thawed at room temperature in the lab before analysis, and the species was then determined. Each fish's total length (TL), total width and total body weight (Table 1).

Sample Processing for Microplastic Identification

The fish samples were meticulously dissected with sterile equipment (forceps, anti-cutter) after being cleansed with ultrapure water. For the purpose of detecting microplastic, the gastrointestinal tracts (GITs, which include the stomach and intestine) and gills were taken out and moved to a Petri dish (Koongolla, et al. 2022). To extract potential plastic debris from the fish samples, the GITs were first inspected visually, followed by chemical digestion. Following the visual inspection, the GIT and all its contents from each sample were transferred to the glass beakers for chemical digestion.

Digestion, Hydrogen peroxide Treatment and Vacuum Filtration

To isolate MPs from the fish samples, we slightly modified the potassium hydroxide (KOH) and hydrogen peroxide (H_2O_2) combined digestion methods (Hurt, O'Reilly and Perry 2020); (Hasan, et al. 2022); (Modupe, Akorede and Alex 2024). The gastrointestinal tract from each sample was transferred to a 150-ml beaker containing 60 ml 1 M KOH and 30 ml 5% sodium lauryl sulfate ($C_{12}H_{25}NaSO_4$) and the beaker was covered with aluminum foil. The covered beaker was incubated at 60 °C for 72 h. Throughout the incubation period, the beaker was shaken gently at regular intervals. For wet peroxide oxidation, a thorough mixture of 6 g NaCl and 20 ml of 30% H_2O_2 was added to the beaker. The residual organic materials were then allowed to slowly degrade for 24 hours at room temperature in the beaker. Microplastics are resistant to wet peroxide oxidation (Masura, et al. 2015)The sample was then centrifuged (Laboratory Centrifuge 800, China) at 2000 rpm at room temperature for 10 min to amalgamate the dilution properly. Next, the sample was filtered using a 0.45-µm cellulose nitrate filter (Whatman), using glass vacuum filtration unit (Duran, Germany), and a vacuum pump (Rocker 300, Taiwan). The filter membrane was instantly covered up with a petri dish to avoid contamination.

Detection of Microplastics

Each of the filter membranes was placed under a Daffodil MCX100 microscope (Micros Austria) ranging between 40×and 100×for visual detection and counting the number of microplastics. Filter membranes were taken from petri dishes using clean tweezers and placed carefully on the slides under the microscope. Additionally, two to three little drops of adhesive were added to the filters' bottom edges, which curled. Filter membranes were found to be faint white. All microplastic particles were recognized and described based on their morphological features, including size, shape, and color. Based on their morphologies, microplastics were divided into six groups, including filaments, fragments, films, foams, and others. Each of the particles was photographed using Daffodil MCX100 microscope and IS capture software (Figure 2).

Table 1. Details information about collected fish samples, i.e., local name, scientific name, feeding zone, and feeding group, (Mean± SD) length, width, weight

Local name	Scientific name	Total No. of Samples	Feeding Zone	Feeding group	Mean± SD Length (cm)	Mean± SD Width (cm)	Mean± SD Weight (gm)
Baim	*Macrognathus armatus*	3	Benthopelagic	Omnivore	16.5±1.235	1.74±0.18	11.94±3.36
Koi	*Anabas testudineus*	3	Demersal	Omnivore	12.07±2.07	3.93±0.7	37.21±20.7
Taki	*Channa punctatus*	3	Demersal	Carnivore	15.1±1.27	2.77±0.5	35.16±14.12

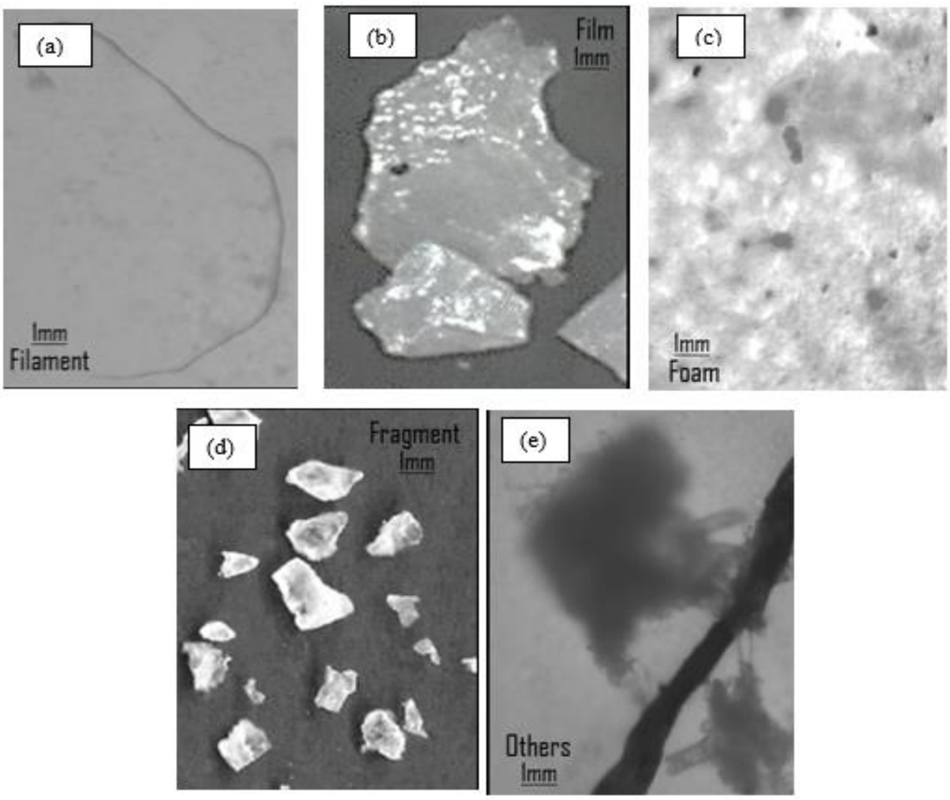

Figure 2. Typical images of microplastic isolated from fish by its shape (a) filament, (b) film, (c) foam, (d) fragment and (e) others

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RESULTS

Abundance of Microplastics According to Fish Species

A total number of 190 microplastics (particles per 100 L) were found across all fish samples. The abundance of microplastics ingested varied among the three fish species collected from the Akhalia River is shown in Figure 3. *Channa punctatus* exhibited the highest mean number of microplastics (22.67 particles per 100L) compared to *Macrognathus armatus* (21.00 particles per 100L) and *Anabas testudineus* (19.67 particles per 100L). Although there were slight differences in mean microplastic abundance among species, the variation was relatively small. Error bars indicate standard deviations, suggesting some degree of variability within species.

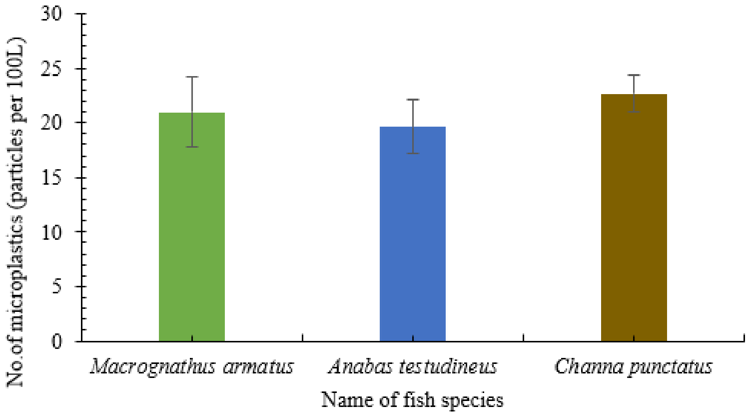

Figure 3. Abundance of microplastics per individual fish species

Prevalence of Microplastics According to Fish Feeding Group and Fish Feeding Zone

Based on the feeding habitat group, fish species were categorized into carnivore and omnivore group. carnivorous fish exhibited a higher mean microplastic load (approximately 22.7 particles per 100L) compared to omnivorous fish (approximately 20.2 particles per 100 L) (Figure 4a). Higher microplastics were found in the feeding zone of demersal fishes than benthopelagic fishes (Figure 4b). Baim, a benthopelagic fish, had the least quantity of microplastics, whereas Koi and Taki, which are demersal fish, had the most.

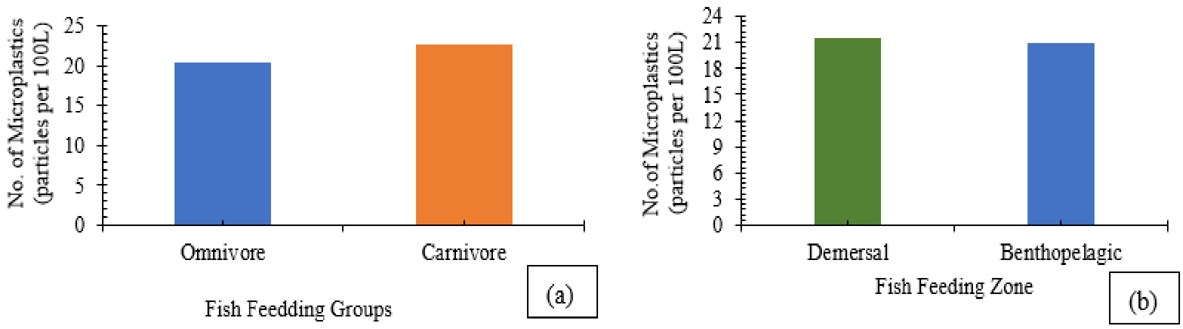

Figure 4. Prevalence of microplastics according to (a) feeding groups and (b) feeding zone

Shape and Color Distribution of Microplastics

Filaments were the most numerous shape category, while the "others" category was the least common. In total counts, there were approximately 64 filaments and 14 particles classified as others. By percentage filaments comprised 34% of all particles, fragments 28%, foam 20%, film 11%, and others 7% (Figure 5a).

Figure 5b shows the color-based percentage distribution of microplastics. White microplastics were the most dominant, making up 45% of the total particles identified. Green microplastics accounted for 18%, followed by blue microplastics and it was 15%. Red and transparent microplastics each contributed 8%, while black microplastics were the least common, comprising only 6% of the total.

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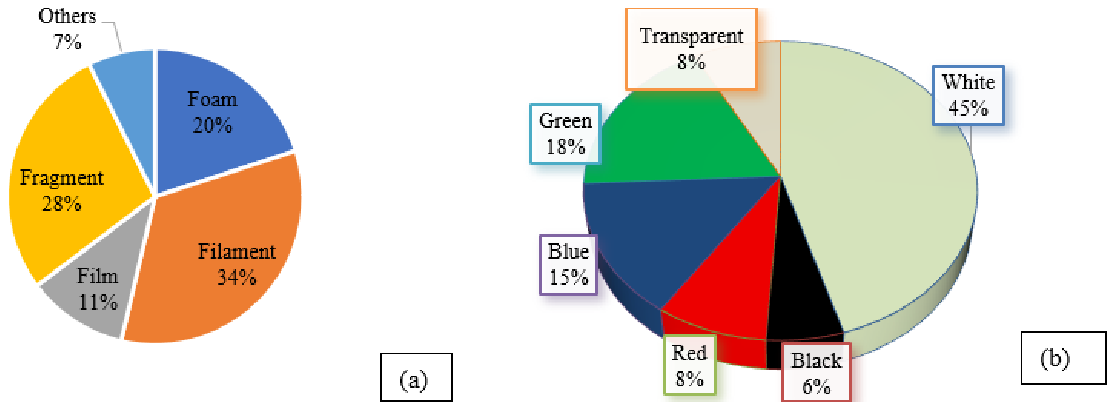

Figure 5. Percentage distribution of microplastic identified by (a) shape and (b) color

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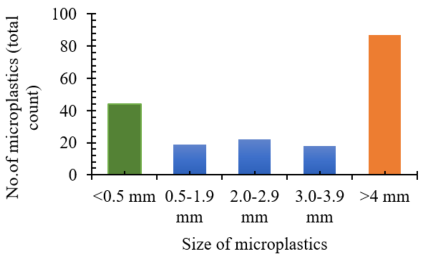

Figure 6. Number of microplastic particles by size category

DISCUSSION

The detection of 190 microplastics particles per 100L in the sampled fish from the Akhalia River clearly indicates widespread microplastic contamination in the aquatic environment. The variation in microplastic abundance among species reflects differences in ecological behavior, habitat preference, and feeding strategies. Taki (*Channa punctatus*), a carnivorous and demersal fish, exhibited the highest mean microplastic load (22.67 ± 1.69 particles per 100L). Carnivorous fish may ingest microplastics both directly from water and indirectly through their prey, leading to bioaccumulation across the food web. This aligns with findings from other studies where carnivorous species often display higher levels of microplastic ingestion compared to omnivorous or herbivorous species (Biginagwa, et al. 2016). Baim (*Macrognathus armatus*), an omnivorous benthopelagic species, exhibited intermediate levels (21.00 ± 3.26 particles per 100L). As bottom feeders, they are exposed to sediment bound microplastics, supporting the idea that both the water column and sediment act as major reservoirs of microplastic pollution. Koi (*Anabas testudineus*), an omnivorous demersal species, showed the lowest microplastic abundance (19.67 ± 2.49 particles per 100L). Although living close to the bottom, their feeding habits might involve less direct ingestion of microplastic contaminated sediment compared to species like Baim or Taki. The differences observed also suggest that species specific behaviors such as feeding selectivity, habitat zone usage, and water column interaction play significant roles in microplastic exposure and ingestion rates. Moreover, the standard deviations reported, especially for Baim, indicate individual variability in microplastic intake, possibly influenced by local microhabitat conditions or feeding intensity. Khan *et al.* (2024) found microplastics per sampled fish was recorded as 2.8 ± 1.30 and 1.8 ± 0.83 for *Puntius sophore*, 2.4 ± 0.54 and 3.4 ± 1.14 for *Anabas testudineus*, 3.8 ± 1.30 and 2.8 ± 0.83 respectively. The highest amount 3.0 ± 1.9 items fish^{-1} of microplastics was found in Ashulia Bridge fishes than the Mirpur Road. Arafat *et al.* (2023) found 90% fishes were contaminated, total of 179 microplastic particles were detected with an average of 5.96 ± 1.32 microplastic (particles fish^{-1}) in Meghna River. Ferdous *et al.* (2023) found the highest number of microplastics in freshwater eels, specifically *Mastacembelus armatus*, with a detection rate of 10.31 ±0.75 per fish, out of 58.93% of fish due to their feeding zone in old Brahmaputra River, Bangladesh. That is quite similar to this study.

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The analysis of microplastic shapes revealed that filaments were the most dominant type, accounting for 34% of all particles identified in the fish gut samples. This was followed by fragments (28%), foam (20%), film (11%), and others (7%). The dominance of filaments could be attributed to the prevalence of synthetic fibers in the aquatic environment, likely originating from domestic wastewater (e.g., washing machine discharges containing textile fibers), fishing gear, and industrial activities. Filamentous microplastics are easily transported by water currents and can be ingested unintentionally by aquatic organisms due to their resemblance to natural food sources such as algae or detritus.

Fragments are often irregularly shaped and are readily available for ingestion by various size classes of fish. Foam and film types, though present in smaller percentages (20% and 11% respectively), also highlight the diversity of plastic sources. The "others" category (7%) likely includes particles that were heavily weathered or irregular, making them difficult to classify under the main categories. Khan *et al.* (2024) found fiber (89-93%), fragments (21-35%), films (0-26%) and foams (0-5%). This is dissimilar as there is no fiber found in this study. The predominance of fibers in all fish samples is closely similar with other studies that have found fibers to be the most common shape of microplastics in fish (Compa, et al. 2018) and (Jabeen, et al. 2017)

The color analysis of microplastics retrieved from the fish samples revealed a clear dominance of white-colored particles, which accounted for 45% of the total microplastics observed. Green (18%), blue (15%), red (8%), and transparent (8%) microplastics followed, while black particles were the least abundant, representing only 6%. The predominance of white microplastics may reflect the common use of white or colorless plastics in packaging, disposable goods, and fishing equipment. White plastics are often used in a wide variety of commercial products and are more likely to fragment and accumulate in aquatic environments due to their extensive usage and widespread disposal. The notable presence of green and blue microplastics suggests that materials such as fishing nets, ropes, and food packaging typically manufactured in these colors are significant contributors to the river's plastic pollution. These colors are also visually attractive to aquatic organisms, possibly leading to preferential ingestion due to confusion with natural prey items such as algae or small invertebrates. Red and transparent microplastics, though less abundant (each 8%), also reflect diverse sources, including beverage containers, cosmetic products, and various household materials. The lower proportion of black microplastics (6%) might be attributed to the difficulty of detecting very small dark particles against the organic background during microscopic analysis, or to their lower buoyancy, which can cause black-colored plastics to settle in sediments rather than remain in the water column where fish feed. The dominant color white (45%) also points to packaging waste or synthetic materials, a trend similarly observed in other studies (Zitouni, et al. 2021)Arafat *et al.* (2023) found the dominant color among the seven color groups was white (30%). This result is nearly similar to the current study result. Khan *et al.* (2024) found the dominant color of microplastics was blue (69-83%), then red (20 - 33%), black (11-14%), green (8-20%), and purple (6-8%). This result is dissimilar as for the colored microplastics, it is inferred that fish ingest these microplastics by confusing them with their preferential prey.

The size distribution of microplastics recovered from fish samples indicated that the particles varied widely, ranging from less than 0.5 mm to greater than 4 mm. The largest proportion (46%) consisted of microplastics greater than 4 mm in size, while 23% were less than 0.5 mm. Particles measuring 2.0–2.9 mm, 0.5–1.9 mm, and 3.0–3.9 mm constituted 12%, 10%, and 9% of the total, respectively. The dominance of large-sized particles (>4 mm) suggests that fish are more likely to ingest bigger microplastics, possibly due to visual recognition or the accidental ingestion of larger debris present in their feeding zones. Larger particles are often more visible and may resemble natural food items such as aquatic invertebrates or plant matter, particularly for species that rely on sight for feeding. The substantial presence of smaller particles (<0.5 mm) (23%) highlights another concern: although less abundant than larger particles, small microplastics may pose a greater physiological risk due to their higher potential to penetrate biological tissues, cross cellular barriers, and carry toxic pollutants adsorbed on their surfaces. Khan *et al.* (2024) found the majority (56-71%) of the microplastics assessed were <0.5 mm in size, followed by 0.5-1.0 mm (29-33%) and 1-5 mm (38 -53%). Arafat *et al*. (2023) found the highest proportion of MPs (54%) occurred in the 45-100 µm. The most frequent size of microplastics observed in red sea as >5000µm (26%) (Hamed, et al. 2023). The difference in findings is attributed to variations in their feeding zones and pollution levels.

CONCLUSIONS

The study's findings suggest that fish from the Akhalia River may be at risk of microplastic contamination. The selection of three fish species in different habitats (benthopelagic and demersal) was the main focus of this study for the detection of microplastics. Taki (*Channa punctatus*) (36%) was found to have the greatest concentration of microplastics among the fish species that were collected. From 9 fish samples, the average microplastic abundance was 21.11 ± 2.85 pieces per individual. Filaments (34%) from the processed microplastics were predicted to be the most common in the fish GITs. The majority of microplastics (45%) were white, with transparent, red, black, green and blue MPs. Compared to the other sizes, the bulk of microplastics (87 particles, 46%) were large-sized (>4mm). Microplastic consumption and fish body weight and length were shown to be slightly correlated. This study has only assessed the microplastic contamination of fish that are significant to freshwater. However, further research on microplastic contamination in

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water and prey may indicate a feasible pathway for microplastic transfer. To ascertain these possible impacts on fish species and people, more investigation should be required. Identifying the sources would enable us to prioritize repair efforts. Building infrastructure and facilities will be necessary to establish a waste collection and management system that is more effective and efficient, as well as to formulate and implement pertinent policies.

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