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Research Article

Microplastic and Heavy Metal Accumulation in Cultured Fish: Concerns for Food Safety

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Abstract

Microplastic (MP) and heavy metal contamination in aquatic ecosystems pose serious risks to environmental and human health due to their toxic effects. This study investigates the presence of microplastics (MPs) and heavy metals in five cultured freshwater fish species collected from aquaculture ponds in Rajshahi, Bangladesh, and evaluates related environmental and human health risks. MPs were found in the gastrointestinal tracts and edible tissues of 96% and 88% of the sampled fish, respectively, with an average of 1.52 particles/g GIT and 0.54 particles/g flesh. *Pangasius pangasius* exhibited the highest MP levels. The most common types of MPs were blue fibers less than 1 mm in size. FTIR analysis identified that the MPs consisted of polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET). The pollution load index (*PLI*) indicated significant MP contamination (*PLI* > 1). Heavy metal analysis showed elevated levels of Cr, Mn, Cd, and Pb in fish tissues, exceeding FAO/WHO guidelines. Human health risk assessments revealed non-carcinogenic risks (*HI* > 1) and carcinogenic risks (*TCR* > 1.0×10^{-4}) from consuming fish. The Metal Pollution Index (*MPI*) ranked *P. pangasius* as the most contaminated species. Positive correlations between contaminant levels and fish size suggest that larger fish are more vulnerable. The findings emphasize the importance of improved waste management, pollution control, and ongoing monitoring to protect food safety and the environment.

Keywords: Microplastics, cultured fish; polymer type; heavy metals; contamination; health risk

1. Introduction

Environmental contaminants in aquaculture have raised concerns in recent years, especially microplastics and heavy metals in freshwater fish. Aquaculture is a vital food source, driven by the growing global demand for fish [1]. The global expansion of aquaculture has been crucial in maintaining livelihoods and ensuring food security, particularly in densely populated regions such as South Asia. Pond-based fish culture dominates the inland freshwater system in Bangladesh, where aquaculture accounts for more than 50% of the nation's total fish production [2]. However, farmed fish are collecting dangerous compounds as a result of pollution and intensive aquaculture [3] Fish consume microplastics through their food or water [4]. Aquatic ecosystems have been exposed to several pollutants, such as heavy metals and microplastics (MPs), as a result of aquacultural intensification, urbanization, industrial discharge, and agricultural runoff [5-6], posing significant bioaccumulation and biomagnification risks along the food chain [7]. Public health issues are exacerbated by the confluence of environmental pollution and food safety concerns, underscoring the need for enhanced knowledge and control of pollutants in aquaculture. In 2019, microplastics accounted for 88% of the 20 million metric tons of plastic entering the environment, mainly

from single-use items. Plastic particles smaller than 5 mm are known as microplastics (MPs). They are either produced at microscopic sizes (primary MPs) or result from the breakdown of bigger plastic waste (secondary MPs) [8]. MPs have been extensively documented in freshwater environments, where they are consumed by fish and other aquatic species [9]. In aquatic food webs, their hydrophobic surfaces can concentrate and adsorb heavy metals and other persistent organic pollutants, which increase their toxicity and bioavailability [10].

Bangladesh is currently facing significant environmental and public health challenges stemming from the escalating contamination of plastics and heavy metals. These problems are intensified by high population density, rapid urbanization, industrial expansion, and inefficient waste management systems [11, 12]. Plastics are widely used in various industries because they are inexpensive, strong, lightweight, and resistant to deterioration [13]. Global plastic production has increased dramatically, reaching over 413 million tons in 2023, compared to just 1.5 million tons in the 1950s [14]. A growing global concern is the contamination of ecosystems with microplastics, which have been detected in terrestrial, freshwater, and marine environments. These particles can enter the human body through ingestion or inhalation and pose serious threats to environmental and human health [15]. Microplastics act as vectors for various environmental pollutants, including heavy metals, pesticides, and pathogenic microorganisms, thus intensifying their toxicological impacts [16, 17]. Fish are exceptional bioindicators of water pollution because they are more susceptible to ingesting microplastics, either accidentally or through selective feeding [18]. Microplastics have been identified in edible fish species in several studies, which raises concern about public health and food safety as they can carry harmful toxins such as heavy metals and persistent organic pollutants [19, 20]. Microplastics are made even more toxic by their physicochemical characteristics, which allow them to absorb poisonous compounds used in the production of plastic, including plasticizers, flame retardants, and biocides [21]. In addition to pollutants originating from plastic, heavy metals can reach aquatic habitats through a variety of man-made channels, such as runoff from agriculture, industrial effluents, and municipal wastewater discharge [22]. It has been demonstrated that microplastics help these pollutants become bioavailable and transfer to aquatic life. They frequently build up in fish gastrointestinal tracts and have sublethal consequences like decreased feeding efficiency, stunted growth, and physiological stress [21]. These impacts could eventually spread across the food chain and endanger human health. The ecological and toxicological risks posed by microplastic pollution in freshwater ecosystems are now recognized as a global issue [23]. Investigations have documented the presence of microplastics and associated heavy metals in fish from diverse marine environments, including the South China Sea [7], Persian Gulf, Caspian Sea, and the Gulf of Mannar [24-26]. In the context of Bangladesh, although a few studies have reported the occurrence of microplastics and heavy metals in surface water, sediments, fish feed [27-30], and fish tissues [20], there remains a substantial gap in the scientific understanding of their sources, distribution, bioaccumulation, and ecological consequences in freshwater systems. Comprehensive assessments are urgently needed to evaluate the extent of contamination and its implications for aquatic life and public health.

Therefore, the study investigates the abundance, types, and polymer composition of microplastics, alongside the concentration of heavy metals, in five commonly cultured freshwater fish species collected from aquaculture ponds in the Rajshahi district of Bangladesh, and assesses heavy metal-based human health risks (carcinogenic and non-carcinogenic) associated with consuming these fish species. The findings provide critical insight into food safety concerns and inform strategies for pollution control in freshwater aquaculture systems.

2. Materials and methods

2.1. Sample collection and preparation

In the current investigation, samples of freshwater cultured fish (5 species) were collected from five distinct fish culture ponds, 2, 3, 4, 6, and 10 (Figure 1), in the Rajshahi District of Bangladesh during November and December 2023. During sampling, different zones were considered, including residential,

market, and village regions. For this study, five species were chosen based on the availability of commercial fish, public demand, and the specific fishing season. The collected fish species were *Pangasius Pangasius* (Pangas catfish), *Hypophthalmichthys Nobilis* (Bighead carp), *Ctenopharyngodon idella* (Grass carp), *Labeo rohita* (Rui), and *Oreochromis niloticus*_(Tilapia), which were commercially significant. The collected fish samples were wrapped in aluminum foil, placed in an icebox, and then promptly transported to the Institute of Environmental Science (IES) Laboratory at the University of Rajshahi, Bangladesh. The samples were cleaned in the lab using distilled water to remove any impurities that might have adhered externally, then stored at -20°C until defrosted. In this investigation, 5 samples per individual of the same species of fish were taken into consideration for the extraction of MPs and heavy metals. The frozen fish samples were defrosted and washed twice with distilled water to extract microplastics. The length and weight of each specimen were measured and documented (Supplementary file-table S1). Using a knife, scissors, and forceps, each specimen was dissected in a metal tray. The gastrointestinal tract (GT) was then removed, and 20 g of the flesh was moved to a Petri dish, weighed, and put in a 1000 mL beaker. Each pooled specimen was placed in a 1000 mL beaker, and three replicates of each species were prepared [19].

2.2. Microplastic isolation from fish species

To decompose the organic matter, each beaker was filled with a 4 M NaOH solution at a 1:10 (gastrointestinal tract) weight-to-volume ratio of NaOH, and the mixture was stirred at 360 rpm and 50°C for 1 to 1.5 h on a magnetic plate. After cooling to room temperature, H_2O_2 was added to the solution at a 6:1 NaOH ratio, and the entire solution was agitated for 30 min. at the same speed to complete the decomposition of the organic matter. The solution was then left to settle for 2 to 16 h. The solution was filtered through a 53 µm stainless steel sieve and returned to the beaker following the rest interval. Each beaker was filled with 50 ml of 30% H_2O_2 and 50 ml of 0.05% $FeSO_4$, and the mixture was swirled for 30 min. at 50°C and 360 rpm.

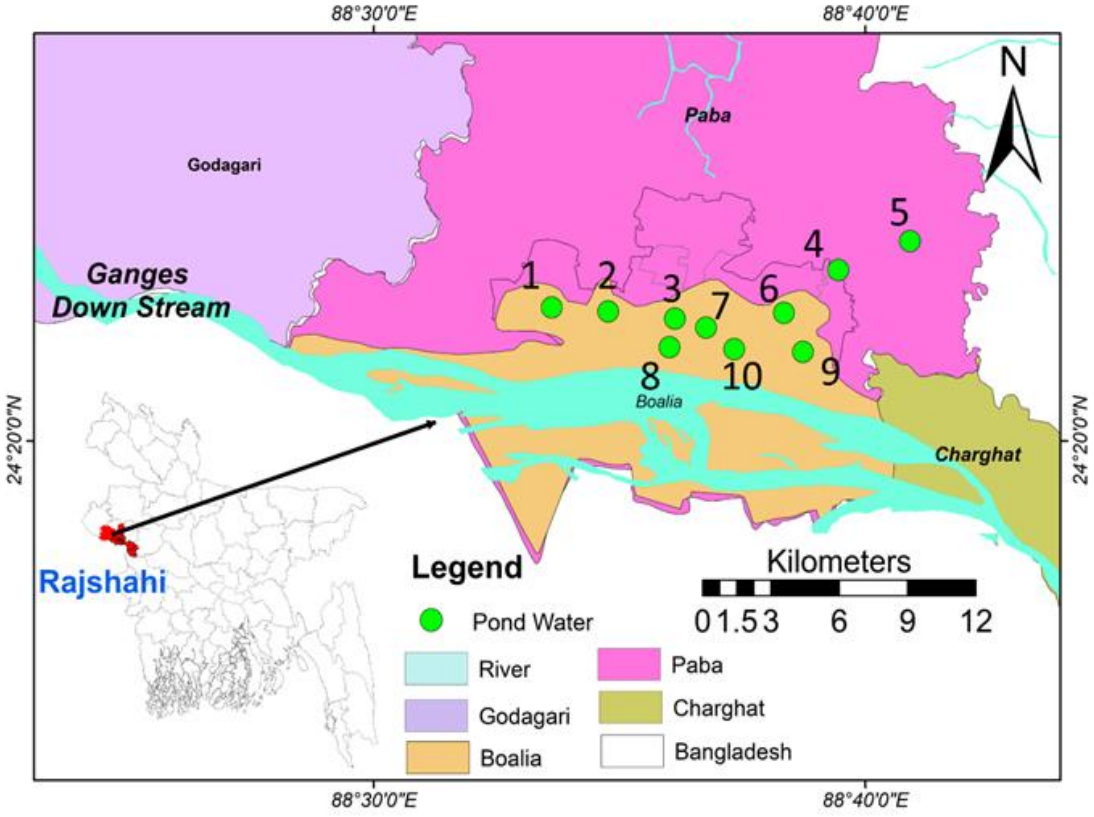

Figure 1. Location map of the sampling sites in Rajshahi district, Bangladesh.

According to [31], microplastics were isolated from the dissolved solution of soft tissues using a saturated saline solution (1.2 g/mL NaCl) by flotation. To each beaker, 600 mL of filtered saturated NaCl solution was added, and the samples were allowed to settle at room temperature for 24 h. Then, the digested fluids were filtered through a 20 µm glass microfiber filter paper with a 47 mm diameter (GF/C, UK) using the vacuum filtering method. All of the air-drying filter papers were placed in different glassware Petri dishes, sealed, and covered with aluminum foil for further examination.

2.3. Identification and characterization of microplastics

The filter papers were observed visually using a 100x Optika digital binocular microscope (model B-190TB, Italy) equipped with a tablet and a 3.2 MP camera (magnification 10x to 100x). Each microplastic particle was visually inspected and classified according to its size (<0.5 mm, 0.5–0.9 mm, 1–1.9 mm, and 2–5 mm), shape (film, fragment, fiber, and granule), color (transparent, blue, red violet, and green), and total quantity. The SEM-EDS (FESEM model JSM-IT 800, JEOL, Japan, with coater model Dll-29030SCTR, JEOL, Japan) analyses provided information on the morphology, surface roughness, and elemental composition of microplastic particles. The composition of the polymers was determined by a Fourier Transform Infrared (FTIR) spectrophotometer (PerkinElmer 100 FTIR, PerkinElmer, Inc., USA). Sixteen co-scans with a spectral resolution of 4 cm^{-1} were used for each measurement, which was conducted in the 500 cm^{-1} - 4000 cm^{-1} spectral regions.

2.4. Contamination and health hazard assessment indices for MPs in fish

The pollution load index (*PLI*) and contamination factor (*CF*) are commonly used to assess ecological risk in terrestrial and aquatic habitats [32]. This study evaluated the risk to humans and the environment by counting the number of MPs in samples of five farmed fish species that were taken from five different ponds in the Rajshahi District. Equations 1, 2, and 3 were applied to compute the Pollution Load Index (*PLI*), with categorization based on the values recommended by [32]. It is noteworthy that no previous scientific research was conducted to determine baseline levels of microplastic contamination in cultured fish. Therefore, the minimal concentration of MPs in the GIT and flesh was utilized as the background value for different fish species.

$$CF = C_i / C_0 \quad (1)$$

where C_i is the concentration of MPs, and C_0 is the background concentration of MPs.

$$CD = \sum CF \quad (2)$$

where *CD* is the degree of contamination and *CF* is the contamination factor, determined by Eq. 1.

$$PLI = (CF_1 \times CF_2 \times CF_3 \times \ldots\ldots CF_n)^{1/n} \quad (3)$$

where n is the total number of samples, and *CF* is calculated as described in the earlier equation (Equation. 1).

The *CF* values were classified into four groups: $CF < 1$ for low contamination, 1-3 for moderate contamination, 3-6 for considerable contamination, and > 6 for extremely high contamination. The *CD* values were also classified into four groups: $CD < 5$ for low contamination, 5-10 for moderate contamination, 10-20 for considerable contamination, and > 20 for extremely high contamination. Conversely, when $PLI < 1$, no pollution, and $PLI > 1$, the sample is considered to be polluted.

2.5. Fish heavy metals analysis

Fish samples were defrosted, cleaned with distilled water, and then dissected with non-metallic, sterile instruments to prevent contamination. Muscle tissues were separated, chopped, and dried for 24 h at 103–105°C in a microwave oven until they reached a consistent weight. Using a sterile mortar and pestle, the dry samples were ground into a fine powder. All equipment was cleaned overnight using 2% HNO_3 and then rinsed with distilled water. To analyze heavy metals, 1 g of powdered sample was placed in a 250 mL beaker, treated with 4 mL of aqua regia (HCl: HNO_3 = 3:1), and heated to 103 to 105 °C for 1 h. Afterward, 6 mL of 30% H_2O_2 was added, and the mixture was then digested at 90°C for 2 h, until a clear solution formed. After that, the samples were allowed to cool to ambient temperature. 10 milliliters of distilled water were added and left overnight. The samples were then filtered through Whatman No. 40 filter paper to ensure a clean and clear solution for analyzing

heavy metals using an atomic absorption spectrophotometer (AAS) (AAS-220FS, Shimadzu Corporation, Japan) [33]. The metal concentrations in fish samples are shown on a dry weight basis. On the other hand, the Food and Agriculture Organization's (FAO) recommended maximum acceptable concentration (MAC) of several metals in foods has been expressed on a wet weight basis. The following equation (4), adopted from [34], has been used to convert the FAO data (wet-to-dry) weight to obtain an accurate result of the measured metal concentrations. An average moisture content of 79% was used to convert the weights to dry weight.

$$MCWW = \frac{(100-PM)}{100} \times dw \quad (4)$$

Where, *MCWW* is the metal concentration in wet weight, PM is the percentage of moisture in samples and *dw* is the dry weight, and their unit is mg/kg.

2.6. Health risk assessment of heavy metals

Microplastics and harmful metals have been linked with endocrine issues like mutagenesis and carcinogenesis. To evaluate the health risk posed to humans from consuming contaminated fish, various techniques have been developed. Therefore, the Estimated Daily Intake (*EDI*), Target Hazard Quotient (*THQ*), Hazard Index (*HI*), and Carcinogenic Risk (*CR*) were used to analyze the human health risk associated with metals in edible fish of the species. The *EDI* and Target Hazard Quotient (*THQ*) have a direct relationship with the levels of potentially dangerous heavy metals in food and daily food consumption.

2.6.1. Estimated Daily Intake (EDI)

The estimated daily intake was measured by the following equation (5) in mg/kg body weight/day [35].

$$EDI = \frac{E_F \times E_{ED} \times F_{IR} \times C_f \times C_M}{W_{AB} \times AT_n} \times 10^{-3} \quad (5)$$

where E_F is the frequency of exposure (365 days a year), E_D is the duration of exposure (72 years), F_{IR} is the ingestion rate (67.8 g per person per day) [36], C_f is the conversion factor (C_f = 0.208) to convert fresh weight to dry weight considering 79 % of the fish fillet's moisture content, C_M is the heavy metal concentration in fish fillet (mg/kg dry weight basis), W_{AB} is the average adult Bangladeshi person's body weight (60 kg), and AT_n is the average exposure period for non-carcinogens [35].

2.6.2. Target Hazard Quotient (THQ)

THQ is a dimensionless, non-carcinogenic danger. The target hazard quotients (*THQs*) were used in this study to assess the non-carcinogenic health hazards related to the consumption of fish and crustacean species. The *THQs* were calculated using the standard assumption for an integrated USEPA risk analysis, as follows: equation (6) [37].

$$THQ = \frac{E_F \times E_{ED} \times F_{IR} \times C_f \times C_M}{RfD \times W_{AB} \times AT_n} \times 10^{-3} \quad (6)$$

E_F, E_D, F_{IR}, C_f, C_M, W_{AB}, and AT_n are explained in the earlier section. *RfD* is the reference dose of an individual metal (mg/kg/day). *RfD* for Cu, Cr, Ni, Pb, and Cd were 0.04, 1.5, 0.02, 0.004, and 0.0005 [20], and for Fe, Zn, and Mn are 0.7, 0.3, and 0.14 mg/kg/day, respectively [38]. The *RfD* is an estimate of the daily exposure to which the general population is susceptible throughout life without posing a serious risk of negative consequences. The exposed population is unlikely to show overt negative values if the *THQ* is less than 1. There may be a health risk if the *THQ* is equal to or more than 1, in which case relevant actions and preventative measures are to be implemented [38].

2.6.3. Hazard Index (HI)

The hazard index (*HI*), which is used to evaluate the entire potential health risk associated with numerous metals, is calculated by adding the target hazard quotients (*THQs*) of each metal. If the *HI* is less than 1, exposed populations are unlikely to show obvious adverse effects. The hazard index (*HI*) was calculated by using equation (7) [38].

$$HI = THQ_{Fe} + THQ_{Cr} + THQ_{Mn} + THQ_{Ni} \ldots (7)$$

HI values of less than 1 indicate non-carcinogenic risk, whereas values greater than indicate significant risk for exposed consumers [39].

Among the analyzed heavy metals, Cd, Cr, Ni, and Pb were considered potent carcinogens. The following equations (8 and 9) define the carcinogenic risk (*CR*) [35].

$$CR = CSF \times EDI \quad (8)$$

$$TCR = \sum 1nCR \quad (9)$$

Where *EDI* stands for estimated daily intake and *CSF* for the cancer slope factor in (mg/kg/day). The values of cancer slope factors (*CSF*), concentrations of Cr, Pb, Cd, and Ni were 0.5, 0.38, 0.01 (mg/kg/day) [40], and 0.91 (mg/kg/day) [41], respectively. According to USEPA (2011) [37], carcinogenic risk *CR* values below 1.0×10-6 are generally regarded as negligible, cancer risk *CR* values above 1.0×10^{-4} are seen as undesirable, and risk *CR* values between 1.0×10^{-4} and 1.0×10^{-6} are typically regarded as an acceptable range [42].

2.7. Metal Pollution Index (MPI)

The Metal Pollution Index (*MPI*) was calculated to analyze the total heavy metal concentration levels in various fish species. The geometrical mean of the concentrations of each metal found in the fish samples was computed to yield this index.

$$MPI\,(\text{mg/kg}) = (C_1 \times C_2 \times C_3 \times \cdots \times C_n)^{1/n} \quad (10)$$

Where, C_n = Concentration of metal *n* in the sample [43].

2.8 QA/QC and matrix effects in heavy metal analysis using AAS

To accurately measure heavy metals in complex biological or environmental samples (such as fish tissue, water, or sediment) using Atomic Absorption Spectroscopy (AAS), strict quality assurance and control (QA/QC) procedures are essential. These procedures ensure the traceability, reproducibility, and reliability of analytical results. Common QA/QC practices include using certified reference materials (CRM), method blanks, spike-and-recovery tests, duplicate analyses, and instrument calibration. Without these controls, it becomes difficult to assess matrix effects or the influence of co-extracted substances from the sample matrix, which can either increase or decrease the target metal ion absorption signal. If not properly managed, these matrix interferences, a known limitation of AAS, can cause biased results [44]. In spike-and-recovery studies, a known amount of the target metal is added to the sample, and the recovery rate is measured. Ideally, recoveries should be within the acceptable range of 80–120% in the presence of the matrix, indicating the method's accuracy in quantifying the analyte [45]. Without this step, it remains uncertain whether any analyte loss, interference, or complete metal extraction resulted from the digestion process. The use of CRMs, which are standardized samples with verified metal concentrations, provides a reference for method validation. Including CRM data helps verify the precision and traceability of the measurements. In this study, blanks were used to detect contamination, and the instrument was calibrated with standard solutions.

3. Results

3.1. MPs abundance in freshwater cultured fish species

The present investigation was conducted on five freshwater fish species (5 individuals of each species) from the fish culture ponds of Rajshahi, Bangladesh. Supplementary file Table S1 and Figure 2 provide detailed information on the fish species, and Table 1 provides detailed information about the gastrointestinal tract (GIT) and flesh sample taken for analysis. Fish can swallow plastic either directly through primary ingestion, accidentally as food, or indirectly through secondary digestion from eating prey that has already been contaminated with plastic [46]. In this investigation, microplastics were found in the GIT of 96% of fish and the flesh of 88% of fish. The identified microplastics were fibers, fragments, film, and granules (Figure 3).

A total of 1022 microplastic particles were identified in the GIT of all fishes, with an average of 40.88 ±26.95 particles/GIT, with a range of 05 to 106 particles/ individual fish. (Table 2). The average number of MPs in GIT per species was 66 ± 35.75 (*P. Pangasius*), 49.4± 24.17 (*C. Idella*), 43.4 ± 21.33 (*H. Nobilis*), 21.8 ± 13.16 (*L. Rohita*), 23.8 ± 12.61 (*O. niloticus*), and average number of MPs /g GIT per species was 1.06 ± 0.37 (*P. Pangasius*), 2.15± 0.89 (*C. Idella*), 2.12± 0.92 (*H. Nobilis*), 0.99± 0.57 (*L. Rohita*), 1.29± 0.49 (*O. niloticus*) (Table 2). In the flesh of all fish samples, 270 microplastic particles were identified, with an average of 0.54 ±0.109 particles /g flesh, with the range of 0.25 to 0.95 particles /g flesh. The average number of MPs in flesh per species was 0.69±0.23 (*P. Pangasius*), 0.62±0.22 (*C. Idella*), 0.49±0. 29 (*H. Nobilis*), 0.46 ± 0.29 (*L. Rohita*), 0.44 ±0.28 (*O. niloticus*) (Table 2). The scatter plot indicates a strong positive relationship between the number of MPs in the GIT and fish's body length and weight shown in supplementary file figure S1(a, b).

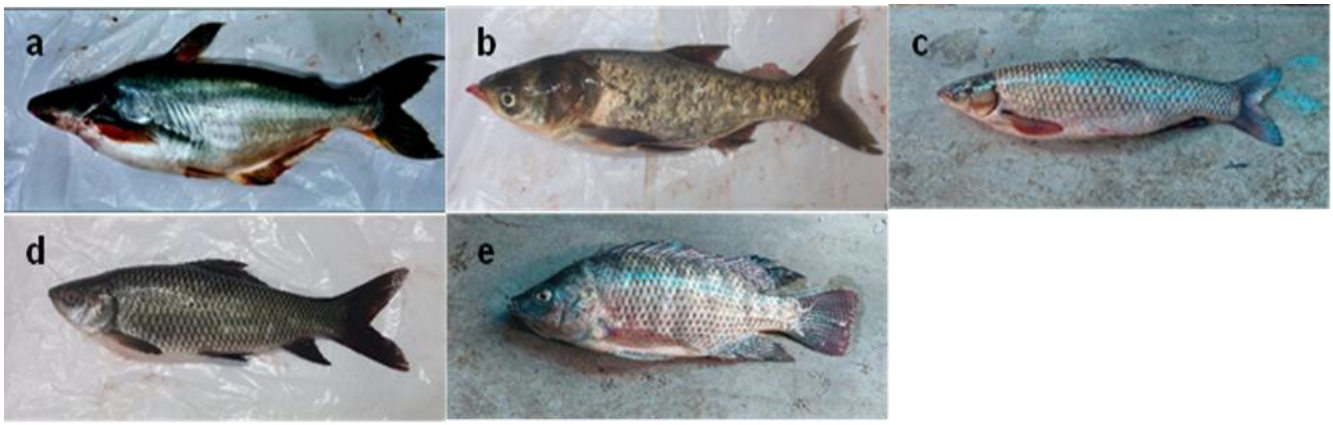

Figure 2. Collected freshwater cultured fish species: (a) Pangus catfish (*Pangasius Pangasius*), (b) Bighead carp (*Hypophthalmichthys Nobilis*), (c) Grass carp (*Ctenopharyngodon idella*), (d) Rui (*Labeo rohita*), (e) Tilapia (*Oreochromis niloticus*).

Table 1. Gastrointestinal tract (GIT) and flesh sample of collected fish species.

Fish species common name	Scientific name	No. of individuals	Wet weight range (g) of GIT	Mean wet weight (g) ± SD of GIT	Wet weight (g) of flesh
Pangus catfish	P. pangasius	5	51.4 - 75.5	61.99±10.21	20
Bighead carp	H. nobilis	5	15.5- 33.85	22.83±7.14	20
Grass carp	C. idella	5	20.25-28.15	23.85±3.39	20
Rui	L. rohita	5	15.75-30.5	20.96±6	20
Tilapia	O. niloticus	5	14.2-25.1	19.51±4.67	20

This study shows a strong positive correlation ($R^2 = 0.4886$) between fish length and fish body weight with microplastic concentration. Additionally, the number of microplastics (MPs) in the flesh showed a moderate positive correlation with the fish's body length and weight shown in supplementary file figure S1(c, d). The number of microplastics tends to increase with the increase in fish length and body weight.

3.2. Morphology and polymer type of microplastics

This study revealed that, in fish GIT, blue (60.37%) was the most dominant color of isolated microplastics, followed by red (20.98%), transparent (6.98%), violet (6.41%), and green (4.27%). In fish flesh, blue (68.61%) was also the most dominant color, followed by red (17.7%), transparent (6.31%), violet (5.05%), and green (3.89%) displayed in Figure 4(a, b). The largest percentage of microplastic particles in the GIT was fiber (57.09%), which was followed by fragment (29.74%), film (7.84%), and granule (5.32%). In the case of fish flesh fiber (66.70%), it was also the dominant shape, which was followed by fragments (26.97%) and film (6.34%) shown in Figure 4 (c, d). In addition, a total of 90.73% of extracted microplastics were in the less than 1 mm size range in GIT, where the remaining 9.27% of microplastics were within the 1–5 mm size range, and in flesh, 96.8% of microplastics were in the less than 1 mm size range, and the remaining 3.2% of microplastics were within the 1–5 mm size range, as shown in Figure 4 (e, f).

Details on the shape and surface roughness, elemental composition, and types of minerals and metals that have accumulated on the surface of microplastic particles were disclosed by the SEM-EDX research. Due to degradation, SEM images revealed irregular and rough areas on the smooth surfaces of microplastics (Figure 5). The FT-IR spectra of microplastic polymers are shown in Figure 6. FT-IR spectra identify microplastics by comparing their distinct

"fingerprint" of infrared absorbance, which is based on the vibrational energy of the molecular bonds, to known polymer spectra in a reference database [47-48].

The distinctive absorption bands in the mid-infrared range (4000–500 cm^{-1}) of the FTIR spectra easily identify microplastics and distinguish them from one another. In this study, Spectrum (a) shows significant CH stretching (2920 and 2851 cm^{-1}), CH_2 bending (1464–1384 cm^{-1}), CH_2 rocking and bending (1097, 719 cm^{-1}), and a small oxidative carbonyl (1746 cm^{-1}). Spectrum (b) shows strong C–H stretching (2959, 2927, and 2850 cm^{-1}), CH bending (1377 cm^{-1}), methyl rocking (1167 cm^{-1}), crystalline isotactic peaks (974, 668 cm^{-1}), and a weak C=O stretching (1739 cm^{-1}) band. In addition, spectrum (c) shows O-H stretching (3431 cm^{-1}), aliphatic C-H stretching (2922, 2852 cm^{-1}), a prominent ester carbonyl peak (1720 cm^{-1}), aromatic C=C (1670 cm^{-1}), and C–O stretching and C–H bending (1384, 1095, 718 cm^{-1}).

3.3. Assessment of contamination level by CF and PLI values

The contamination factor (*CF*) is significant for assessing microplastic pollution because it indicates how much an organism or environment is affected. Contamination factors provide important insights for well-informed decision-making and successful mitigation measures by taking into account parameters such as concentration and dispersion to address the growing concern of microplastic pollution. The *CF*, *CD,* and *PLI* values of the digestive tract were 0.99 to 5.42, 4.95 to 27.11, and 1.49 to 1.88, and the values of fish muscle were 1.09 to 2.48, 5.44 to 12.4, and 1.50 to 1.65, respectively (Supplementary file figure S2). *P. pangasius, H. nobilis,* C. *idella, L. rohita,* and *O. niloticus* were shown in Figure S2.

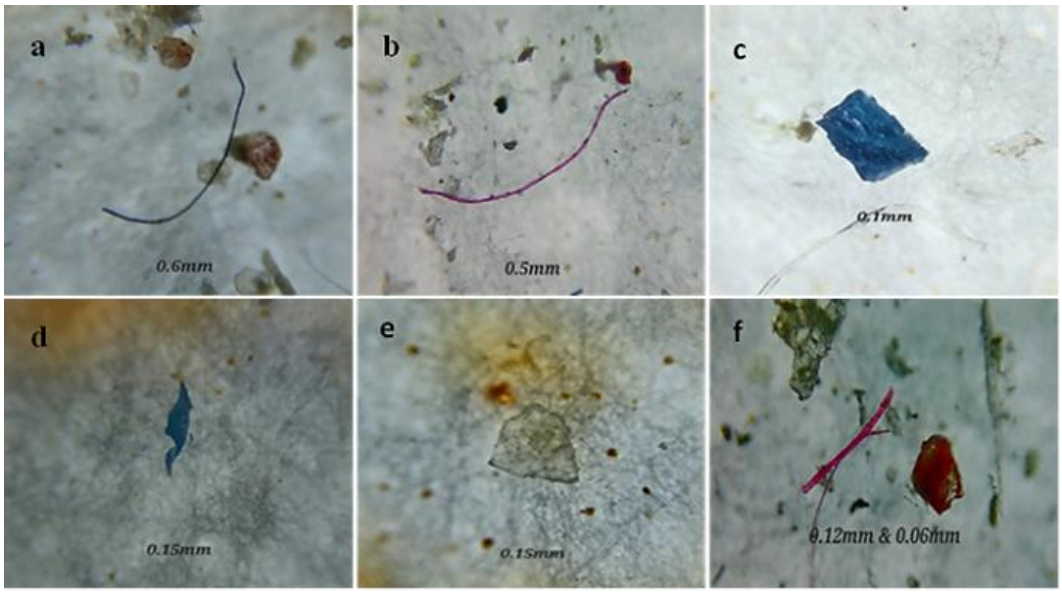

Figure 3. Isolated microplastics (a) blue fiber, (b) red fiber, (c, d) blue fragment, (e) transparent film, and (f) red fiber and red granule from freshwater cultured fish species at 40x magnification.

Table 2. Microplastics in the GIT and Flesh of each fish species.

Fish species	No. of MPs particles / individual GIT			No. of MPs particles/g GIT			No. of MPs particles/g Flesh		
	Min.	Max.	Avg.	Min.	Max.	Avg.	Min.	Max.	Avg.
P. Pangasius	14	106	66±35.75	0.46	1.41	1.06±0.37	7	19	0.69±0.23
H. Nobilis	8	63	43.4±21.35	0.83	2.91	2.12±0.92	0	15	0.49±0.29
C. Idella	11	70	49.4±24.17	0.83	3.23	2.15±0.89	5	16	0.62±0.22
L. Rohita	0	35	21.8±13.16	0	1.49	0.99±0.57	0	16	0.46±0.29
O. niloticus	5	38	23.8±12.61	0.487	1.69	1.29±0.49	0	14	0.44±0.28

In this study, *CF values* of the GIT *of P. pangasius, H. nobilis,* C. *idella,* and *O. niloticus* showed (3 < *CF* < 6) and *L. rohita* (*CF* < 1). *CF values* of the flesh of all fish species were within the range of (3 < *CF* < 6), indicating considerable contamination The *CD* values of GIT of *P. pangasius, H. nobilis,* C. *idella,* and *O. niloticus* showed extremely high contamination; however, the *CD* values of GIT of *L. rohita* showed low contamination. The *CD* values of the flesh of fish species were within 5 < *CD* < 10 except *C. idella* (10 < *CD* < 20). The Pollution Load Index (*PLI*), which combines several factors and offers a detailed picture of pollution levels, is crucial for evaluating microplastic pollution. This approach helps us understand how different sources of microplastics add up, making it easier to take specific actions and manage the environment in a long-lasting way. The *PLI* values of the fish samples were >1, implying that the fish were contaminated. *PLI* scores were assigned in the following descending order: *P. pangasius* (1.88), > *O. niloticus* (1.87), > *C idella* (1.86), > *H. nobilis* (1.83), > *L. rohita* (1.49) (Supplementary file figure S2).. Based on the ratio between the occurrence of MPs and the background value, it appears that the polymer type of MPs has a minimal impact on the *PLI* [49].

3.4. Heavy metal concentration in freshwater fish species

In this study, the concentrations of heavy metals in five fish species were analyzed. The results, shown in Table 3, compare the heavy metal concentrations in the fish species with the FAO/WHO recommended limits for foodstuffs on a dry weight basis. This study investigated the concentrations of eight metals, including Cr, Mn, Fe, Ni, Cu, Zn, Cd, and Pb, in five native freshwater cultured fish species from five selected fish cultured ponds in Rajshahi District, Bangladesh. Elevated levels of heavy metal accumulation are found in fish species (Table 3). The trends in heavy metal concentrations (mg/kg) were: Fe (31.64±4.2) > Ni (13.77±3.7) > Cu (12.14±3.2) > Zn (9.79±1.0) > Mn (7.99±3.5) > Cr (7.31±2.3) > Pb (3.15±0.6) > Cd (1.71±0.7), respectively. Among them, several metals exceeded the maximum allowable concentration (MACs) set by [50]. The highest concentration of Cr, Mn, Fe, and Cd was found in *P. pangasius*. In all species, Cd and Pb exceeded MACs, especially Pb, for *H. nobilis* and *C. idella*.

Human health risks from heavy metals were assessed through both non-carcinogenic and carcinogenic evaluations. In this study, the estimated daily intake (*EDI*) values of the heavy metals were compared with the maximum tolerable daily intake (*MTDI*) limits established by organizations such as WHO, FAO, JECFA, and EFSA. Table 4 compares the estimated daily intake (*EDI*) of eight heavy metals of five freshwater fish species with maximum tolerable daily intake (*MTDI*) limits. The highest *EDI*s were found in *P. pangasius* for Cr, Mn, Fe, and Cd, with Cr (2.27×10^{-3}) being close to the *MTDI* (3.0×10^{-3}), suggesting a probable health risk from regular consumption. Significant concerns were raised by Cd, as the *EDI* in *C. idella* (5.69×10^{-4}) and *P. pangasius* (5.99×10^{-4}) were higher than the *MTDI* (8.3×10^{-4}). Across species, *EDI*s of Pb varied from 5.52×10^{-4} to 8.98×10^{-4}, with sensitive groups at higher risk of exposure. Although cumulative exposure is possible, Fe, Cu, Ni, and Zn all stayed within acceptable consumption levels. The lowest *EDI*s were found in *O. niloticus*, indicating that it is a safer food option [50-51].

The Target Hazard Quotient (*THQ*) and the Hazard Index (*HI*) for eight heavy metals of five fish species are shown in Table 5. *THQ* evaluates non-carcinogenic risk from specific metals, whereas *HI* represents total risk. *HI* > 1 indicates possible health risks associated with everyday fish consumption. The *P. pangasius* showed the highest *HI* (2.317) due to the *THQ* value of Cd (1.199), with Pb and Ni posing supplementary hazards. Additionally, due to Cd, the *HI* value of *C. idella* (1.622) and *L. rohita* (1.04) exceeded the safe limit. Cd, the predominant contributor, and the *HI* values of *H. nobilis* (0.999) and *O. niloticus* (0.885) were below 1, indicating reduced but significant hazards. Across all species, Cd consistently presented the highest non-carcinogenic risk, with Pb and Ni playing a role. This raises concerns about prolonged exposure and its harmful effects [50, 52].

3.5. Metal Pollution Index (MPI) assessment

The total metal accumulation in different species and sizes of cultured fish from the Rajshahi region was assessed using the Metal Pollution Index (*MPI*). While traditional chemical, biochemical, and biological methods remain essential, *MPI* can

serve as a valuable addition to complex freshwater monitoring programs by providing further insights into metal input, bioavailability, and bioconcentration in the environment [43]. The *MPI* is a precise and dependable technique for tracking metal contamination in food samples. The *MPI*s of individual fish samples are presented in Figure S3.

3.6. Relation between microplastics and heavy metals in freshwater culture fish species

The scatter plot revealed a strong positive correlation between the metal pollution index (*MPI*) and the number of microplastics (*MPs*) in fish flesh (R^2 = 0.888) (Figure 7). This suggests that elevated levels of heavy metals in fish flesh are closely related to an increase in MP concentration.

3.7. Correlation of heavy metals with fish body length and body weight

Fish length showed a significant positive correlation with Cr (r = 0.74) and Cd (r = 0.64), supporting the concept of length-dependent accumulation of certain metals. The significant correlations between fish length and heavy metal concentrations such as Cr, Cd, Ni, and Fe are shown in Table S2.

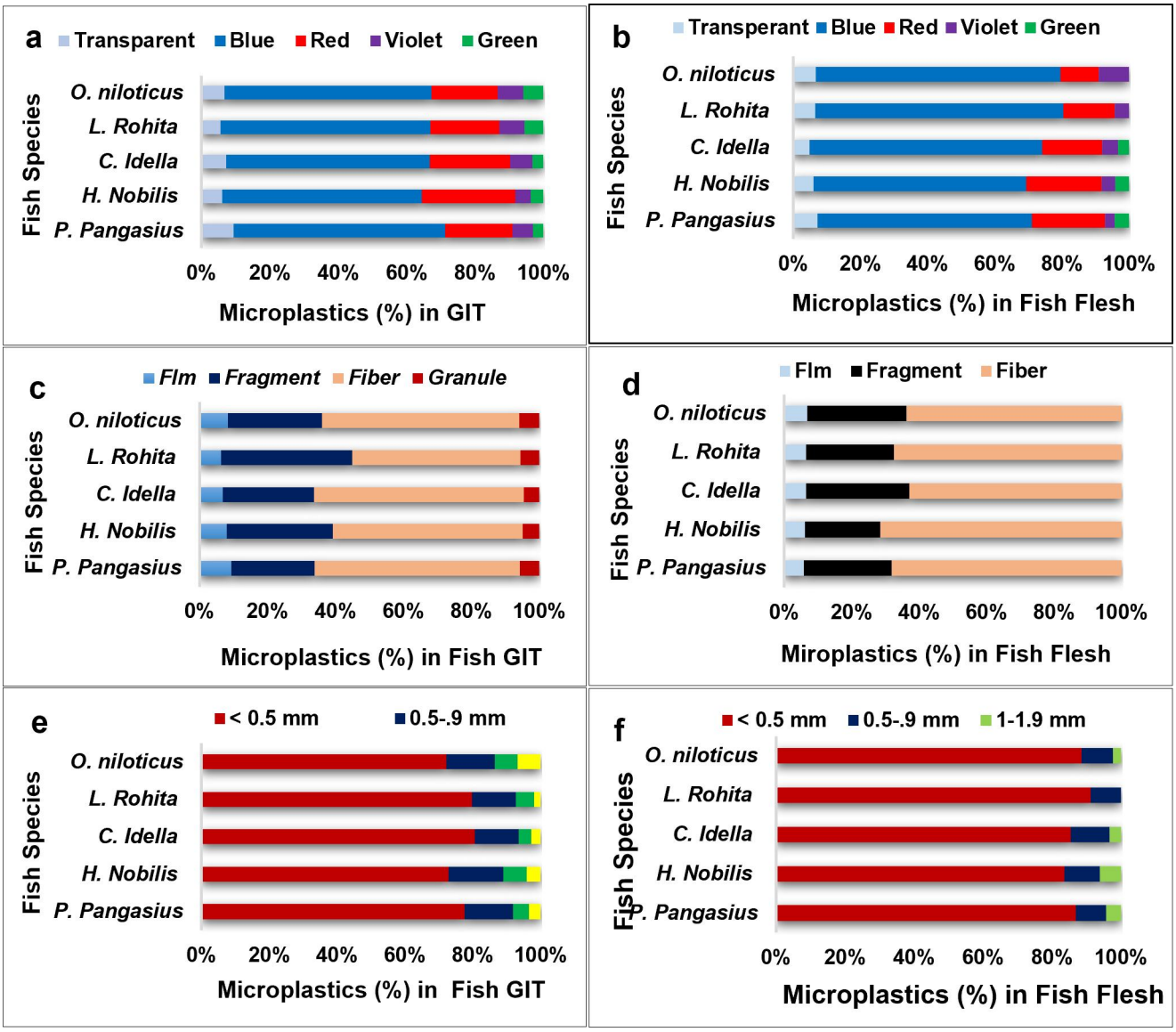

Figure 4. Color (a, b), shape (c, d), and size (e, f) distribution of microplastics in the GIT and in the flesh of fish species.

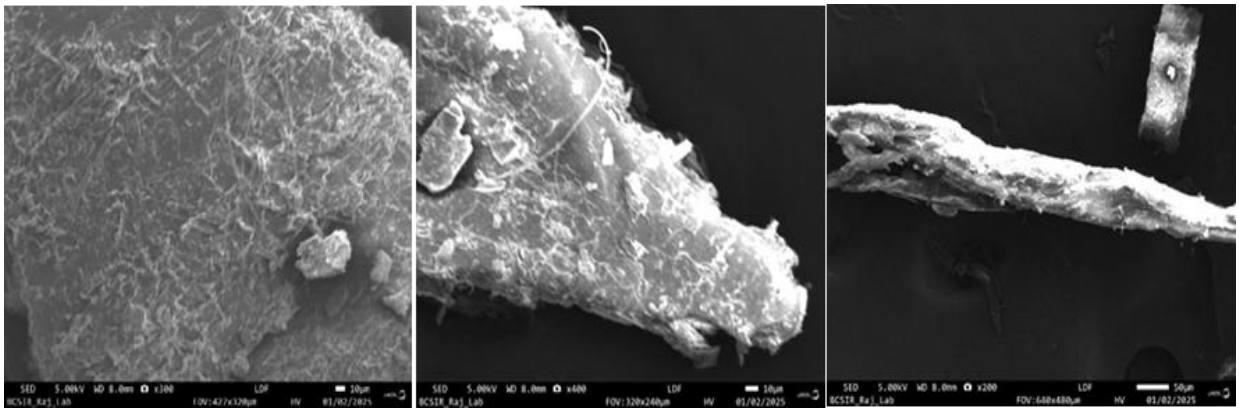

Figure 5. SEM image of microplastic isolated from cultured fish species.

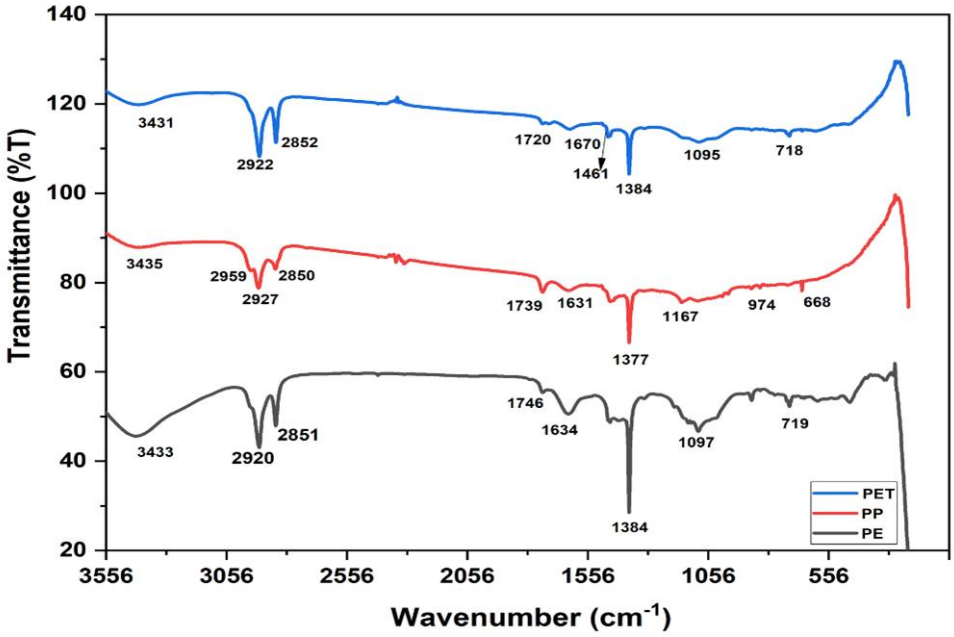

Figure 6. FTIR spectra of isolated microplastic polymer found in fish species.

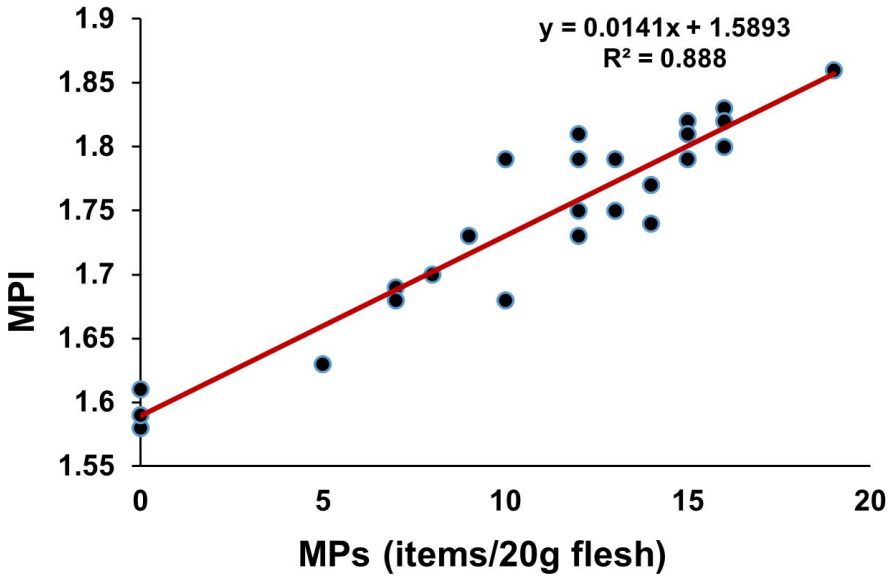

Figure 7. Relationship between microplastics and heavy metals in freshwater fish species.

Table 3. Heavy metals in fish muscles mg/kg (dry weight)

Fish species	Cr	Mn	Fe	Cd	Ni	Cu	Zn	Pb
P. pangasius	9.65	12.80	36.91	2.55	18.18	10.52	10.55	3.32
H. nobilis	7.13	5.69	31.74	1.23	9.46	9.79	10.85	3.82
C. idella	8.85	9.50	34.40	2.42	14.59	13.42	9.89	3.37
L. rohita	7.17	3.89	28.89	1.19	16.02	17.12	9.52	2.88
O. niloticus	3.78	8.1	26.27	1.14	10.59	9.82	8.18	2.35
Mean ± SD	7.31±2.3	7.99±3.5	31.64±4.2	1.71±0.7	13.77±3.7	12.14±3.2	9.79±1.0	3.15±0.6
*MAC (FAO/WHO) Dry weight	4.35	4.35	434	0.43	347	120	120	2.17

* MAC = Maximum Allowable Concentration

Table 4. Estimated daily intake (*EDI*) (mg/kg-body weight/day) of heavy metals from freshwater fish consumption.

Fish species	Cr	Mn	Fe	Cd	Ni	Cu	Zn	Pb
P. pangasius	2.27×10^{-3}	3.01×10^{-3}	8.68×10^{-3}	5.99×10^{-4}	4.27×10^{-3}	2.47×10^{-3}	2.48×10^{-3}	7.8×10^{-4}
H. nobilis	1.68×10^{-3}	1.34×10^{-3}	7.46×10^{-3}	2.89×10^{-4}	2.22×10^{-3}	2.30×10^{-3}	2.55×10^{-3}	8.98×10^{-4}
C. idella	2.08×10^{-3}	2.23×10^{-3}	8.09×10^{-3}	5.69×10^{-4}	3.43×10^{-3}	3.15×10^{-3}	2.32×10^{-3}	7.92×10^{-4}
L. rohita	1.69×10^{-3}	9.09×10^{-4}	6.79×10^{-3}	2.79×10^{-4}	3.77×10^{-3}	4.02×10^{-3}	2.24×10^{-3}	6.77×10^{-4}
O. niloticus	8.88×10^{-4}	1.90×10^{-3}	6.17×10^{-3}	2.68×10^{-4}	2.49×10^{-3}	2.31×10^{-3}	1.92×10^{-3}	5.52×10^{-4}
Maximum Tolerable Daily Intake. (MTDI)	3×10^{-3} [a]	0.14 [a]	0.8 [b]	8.3×10^{-4} [c]	2.8×10^{-3} [d]	0.1 [b]	1.0 [e]	3.6×10^{-3} [c]

a = USEPA, 2011 b = WHO, 1996 c = JECFA, 2011 d = EFSA, 2020 e = WHO, FAO/2011

Table 5. Target hazard quotient (*THQ*) and hazard index (*HI*) of heavy metals from freshwater fish consumption.

Species	THQ								HI (TTHQ)
	Cr	Mn	Fe	Cd	Ni	Cu	Zn	Pb	
P. pangasius	1.51×10^{-3}	2.15×10^{-2}	1.19×10^{-2}	1.199	2.14×10^{-1}	6.18×10^{-2}	8.26×10^{-3}	1.95×10^{-1}	2.317
H. nobilis	1.12×10^{-3}	9.55×10^{-3}	1.02×10^{-2}	5.78×10^{-1}	1.11×10^{-1}	5.75×10^{-2}	8.5×10^{-3}	2.24×10^{-1}	0.999
C. idella	1.39×10^{-3}	1.59×10^{-2}	1.11×10^{-2}	1.138	1.71×10^{-1}	7.88×10^{-2}	7.75×10^{-3}	1.98×10^{-1}	1.622
L. rohita	1.12×10^{-3}	6.49×10^{-3}	9.3×10^{-3}	5.59×10^{-1}	1.88×10^{-1}	1.0×10^{-1}	7.45×10^{-3}	1.69×10^{-1}	1.04
O. niloticus	5.92×10^{-4}	1.36×10^{-2}	8.46×10^{-3}	5.36×10^{-1}	1.24×10^{-1}	5.77×10^{-2}	6.41×10^{-3}	1.38×10^{-1}	0.885

Table 6. Carcinogenic risk (*CR*) and total carcinogenic risk (*TCR*) of heavy metals from freshwater fish consumption.

Fish species	*CR*				*TCR*
	Cr	Cd	Ni	Pb	
P. pangasius	1.135×10^{-4}	5.99×10^{-6}	3.88×10^{-3}	2.96×10^{-4}	4.29×10^{-3}
H. nobilis	8.4×10^{-5}	2.89×10^{-6}	2.02×10^{-3}	3.41×10^{-4}	2.45×10^{-3}
C. idella	1.04×10^{-4}	5.69×10^{-6}	3.12×10^{-3}	3.01×10^{-4}	3.53×10^{-3}
L. rohita	8.45×10^{-5}	2.79×10^{-6}	3.43×10^{-3}	2.57×10^{-4}	4.24×10^{-3}
O. niloticus	4.44×10^{-5}	2.68×10^{-6}	2.26×10^{-3}	2.09×10^{-4}	2.91×10^{-3}

The correlation analysis reveals strong positive relationships among Cr, Fe, Zn, Cd, Ni, and Pb ($r > 0.70$). The Pearson correlation analysis indicates that fish weight was positively correlated with several heavy metals, notably Cr ($r = 0.67$), Mn ($r = 0.65$), and Cd ($r = 0.63$) (Table S3). Moderate correlations were also found with Fe ($r = 0.52$) and Ni ($r = 0.49$). Conversely Cu showed no significant relationship with weight.

Table 6 shows carcinogenic risk (*CR*) values of Cr, Cd, Ni, and Pb, along with total carcinogenic risk (*TCR*) values for five fish species. According to [52], CR values above the acceptable range (1.0×10^{-6} to 1.0×10^{-4}) indicate possible public health risks. In this study, the *TCR* values of all species were above the threshold, with *P. pangasius* having the highest value (4.29×10^{-3}), due to elevated Pb (2.96×10^{-4}) and Ni (3.88×10^{-3}) levels Significant cancer risks were also posed by *C. idella* (3.53×10^{-3}) and *L. rohita* (4.24×10^{-3}). Although the *CR* values of Cr (1.135×10^{-4} in *P. pangasius*, for example) approached or exceeded safe limits, Cd and Cr made up a smaller portion of *CR*. The lowest *TCR* of *H. nobilis* (2.45×10^{-3}) exceeded the acceptable levels.

4. Discussion

4.1. Abundance and characterization of MPs

These results of the study showed that microplastics are present in all the investigated fish species, which is comparable with some prior studies that reported microplastic abundance in freshwater fish species from Bangladesh as: 1.80 ± 1.65 particles/g [53], 0.65–3.82 particles/g [27], and 1.21±1.13 particles/g [54]. Additionally, the current study result is higher than 1.80 ± 1.65, 0.147 particles/g, 0.13–0.50 particles /g, and 1.21±1.13 particles/g [53,55,26,54], and lower than 0.65-3.82 particles/g [27] (Table S4). The positive correlation between fish body weight and length and microplastic accumulation may be explained by increased food consumption, swimming habits, preferred habitats, and prolonged exposure to contaminated environments [56]. According to Seetapan & Prommi (2023) [57], MP ingestion is probably to occur in large fish due to their high energy requirements and capacity for food intake. In contrast, MP ingestion by fish increases as fish body weight increases [58]. However, a positive correlation was found between the microplastic accumulation rate and fish body size, but it did not show a significant correlation. Therefore, the microplastics ingestion rate not only depends on the fish body weight and size but also on the level of plastic pollution in the water body. As plastic pollution varies globally, there is a relationship between trophic level, feeding strategy, biogeography, habitat, and ecological niche, and how much and what kind of plastic is ingested by fish [59]. In this investigation, various types of microplastics were identified, and the most dominant type was blue fibers less than 1mm in size. Microplastics of various colors have been exposed to originate from commonplace plastic-based objects, including clothes, packaging, fishing nets, and more [60].

Due to inadequate waste management, these tiny pieces of plastic fragments and garbage may be discharged into water bodies, creating colored microplastics that could deceive aquatic

life and raise the risk of plastic ingestion [61]. According to Islam et al. [62], fish may also inadvertently consume the vibrant microplastics as part of their regular feeding habits. The expanding aquaculture and agriculture industries increase the use of fishing nets and other outdated fishing gear, which increases the amount of microplastic fibers [63]. Additionally, Browne et al. [64] claim that the increased fiber content is instigated by treated laundry effluent from a nearby wastewater system. The biodegradation of larger plastic items through various environmental processes over time produces smaller microplastic particles, which may be more hazardous than the larger items [65]. Additionally, tiny microplastics have a considerable capacity to absorb water from hydrophobic organic pollutants, which can be extremely hazardous to freshwater organisms [63]. According to the distinctive functional groups, the spectra a, b, and c indicate that the polymers were polyethylene (PE), polypropylene (PP), and polypropylene terephthalate (PET), respectively, which are shown in Figure 6 [47-48]. The results of polymer identification can be used to determine the origin of plastic particles. PE and PP are widely utilized in everyday life because they are affordable and simple to process; for example, PP is regularly used in fishing gear [60]. Furthermore, Xu et al. [63] suggested that fishing activities and pondside household laundry effluent may be possible sources of these microplastics. Higher *CF, CD,* and *PLI* values indicate that the fish species were moderately to considerably contaminated. The occurrence of microplastics (MPs) in pond water is significantly influenced by human activities, including industrialization, disposal of plastic waste, household wastewater, agricultural runoff, fishing activities, recreational activities, and so on, in ponds [4,65]. MP abundance is also closely associated with socioeconomic features such as population density, industrialization level, economic development status, human lifestyle, and fishing intensity. As a result, these extensive sources of MPs in ponds cause pollution and influence the value of *CF*, *CD*, and *PLI*.

4.2. Heavy metal-based health risk

The mean values of Cr, Mn, Cd, and Pb concentrations exceeded tolerable limits throughout the species, indicating widespread contamination. The long-term accumulation of Fe, Ni, Cu, and Zn is concerning, even though their levels remained below acceptable limits. There may be health concerns for the people who consume these fish. The study results are similar to those obtained from an earlier investigation [34,43], but the concentration is higher than that reported by [20]. These elevated levels of heavy metal pollution in freshwater ecosystems in Bangladesh have been driven by rapid urbanization, high population density, industrial discharge, agricultural runoff, and wastewater discharge. Research has also shown that the uptake and accumulation of heavy metals in fish are strongly affected by environmental factors such as water pH and temperature, as well as by biological variables including fish species, gender, and dietary habits [66]. The values of *THQ*, *HI*, and *TCR* exceeded the acceptable levels, suggesting that regular consumption of these fish may have long-term carcinogenic risks. Most likely as a result of contaminated feed, water, or sediment, Ni and Pb were the main contributors due to contaminated sediment, water, or feed [51,67]. The significant correlations between fish length and heavy metal concentrations (Table S3) suggest bioaccumulation with growth, a pattern consistent with previous studies. Conversely, Cu displayed a negligible correlation with length and weak relations with most other metals, suggesting an independent uptake mechanism. These findings highlight the importance of fish size in understanding metal exposure and accumulation outlines. The correlation analysis reveals strong positive relationships among the heavy metals, indicating possible common sources or synergistic bioaccumulation in fish tissues [68]. Strong inter-metal correlations, such as between Fe and Zn, indicate common sources or synergistic accumulation mechanisms. These findings are consistent with past research, signifying that older or larger fish typically have higher concentrations of specific heavy metals due to their varying metabolic rates and longer exposure times [68]. *MPI* values of the cultured fish species (Fig.7), indicating that the heavy metal accumulation pattern was directly related to the size and body weight of the fish. These differences in heavy metal accumulation could be explained by ecological behaviors, trophic levels, and dietary patterns.

According to Ali et al. [69], *P. pangasius* and other omnivorous or benthic feeders are more likely to ingest contaminated sediments or debris, which increases their absorption of heavy metals. However, because it interacts less with sediments, *O. niloticus*, a known filter feeder, tends to collect lower amounts of heavy metals [70]. Considering the potential of heavy metal exposure from fish intake, these findings are important and could aid in the regulation of aquaculture operations in contaminated areas. Heavy metals in fish are closely related to an increase in MP concentration, and microplastics may act as a path for heavy metals. According to these findings, consuming more microplastics may cause metals to bioaccumulate unusually, raising the ecotoxicological danger to consumers and fish. [71] validated the transfer of ingested microplastics and related metals into crab tissues, while [72] demonstrated that plastic trash can adsorb trace metals in marine habitats. In terms of ecological health and food safety, this link emphasizes the possible risk of MP contamination in the aquaculture environment.

4. Conclusion

This study presents a comprehensive assessment of microplastic (MP) and heavy metal contamination in five cultured freshwater fish species from aquaculture ponds in Rajshahi, Bangladesh, providing critical insight into environmental and human health risks. Microplastics were detected in the gastrointestinal tract (GIT) of 96% and the flesh of 88% of the sampled fish, with an average of 1.52 particles/g in the GIT and 0.54 particles/g in the flesh. *Pangasius pangasius* showed the highest MP levels. The study revealed that the majority of the identified microplastics were blue-colored fibers, and <1 mm in size. The SEM-EDX and FTIR investigations revealed that the identified polymers were primarily composed of polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET), mostly originating from household garbage, packaging products, and fishing gear. The abundance of MPs correlated positively with fish length and weight, suggesting bioaccumulation potential associated with feeding behavior, trophic level, and environmental exposure. The contamination factor (*CF*), degree of contamination (*CD*), and pollution load index (*PLI* > 1) results indicated moderate to high levels of contamination, with *P. pangasius* having the highest level of pollution. Heavy metal concentrations, particularly Cr, Mn, Cd, and Pb, exceeded maximum allowable concentrations in several species, posing substantial non-carcinogenic (*THQ, HI* > 1) and carcinogenic ($TCR > 10^{-4}$) risks upon regular consumption. Among the species, *Pangasius pangasius* exhibited the highest levels of both MPs and heavy metals, while *Oreochromis niloticus* showed comparatively lower contamination, likely due to differences in feeding behavior and habitat interaction. The strong positive correlation ($R^2 = 0.888$) between the Metal Pollution Index (*MPI*) and MP abundance in fish flesh highlights the potential role of microplastics as vectors for heavy metal transport and bioaccumulation. Overall, the findings underscore the urgent need for improved waste management, monitoring, and regulation of plastic and metal pollutants in aquaculture systems. These results can inform risk assessments, consumer safety guidelines, and policy measures aimed at minimizing pollutant exposure from freshwater fish consumption in Bangladesh and similar contexts globally.

Authors Contribution

Md Ohidur rahman: Conceptualization; methodology; investigation; sample collection; sample analysis, data curation; data analysis; software computation; writing-original draft. Md Golam Mostafa: Conceptualization; methodology; investigation; validation; software; supervision; writing-review & editing. All authors have read and agreed with the published version of the manuscript.

Supplementary Material

The Supplementary Material for this article can be found online at:

https://www.jspae.com/index.php/jce/article/view/742/319

Conflicts of Interest

There are no conflicts of interest reported by the writers.

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Data Availability statement

The data presented in this study are available on request from the corresponding author.

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