

CHAPTER 13

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THE SYNERGISTIC EFFECTS OF HEAVY METALS AND MICROPLASTICS ON FRESH WATER FISH HEALTH

In developing countries, the combination of plastic & heavy metals is increasing high population density, rapid industrialization, poor waste management and urbanization which have placed serious stress on human and fishes health and the environment. (Microscopic plastic particles <5 mm to <8 mm) Microplastics are having a severe and devastating impact on the health of freshwater fish. The synergistic effect of heavy metals and (MPs) microplastic on freshwater fish mainly creates a “TROJAN HORSE” effect, where microplastic absorbs toxic metals (e.g., Pb, Cd, Cu) and transport them into the fishes, mainly causing greater, combined toxicity than either pollutant alone. Studies in major INDIA rivers like the GANGAS and YAMUNA and other waterbodies have shown that microplastics are not only making fish sick and but also increasing their mortality rates.

1. Major effects on freshwater fish health :

- **Digestive system and internal injuries :** Fish mistake microplastics for food and eat them, causing

blockages in their stomach and intestines (gastrointestinal tract). This can result in internal, inflammation , and stomach ulcers.

- **Damage to liver and gills :** Microplastics particles accumulate in the liver , causing liver damage (lipid accumulation, fibrosis). Additionally, these particles stick to the gills , making breathing difficult .
- **Nutritional deficiency and stunted growth :** Due to plastic filling their stomachs, fish feel less hungry , leading to malnutrition and inhibited growth .
- **Oxidative stress :** The leakage of harmful chemicals (additives) from microplastics causes oxidative stress , DNA damage , and a weakened immune system in fish bodies. Co- exposure increases reactive oxygen species (ROS), causing oxidative harm, especially in the liver and gills .
- **Behavioral changes :** Research has shown that fish consuming microplastics exhibit changes in their swimming and hunting behavior, reducing their chances of survival .
- **Physical and Chemical hazards :** Physical injury from ingested plastics occurs alongside the chemical toxicity of released additives and absorbed heavy metals .

2. Key synergistic mechanisms and effects :

- **“Trojan Horse” Mechanism :** Microplastics act as vectors for heavy metals due to their high surface area and hydrophobic nature, witch attract metal ions in aquatic environments.
- **Increased Bioavailability :** The digestive system of fish, with its low pH and high enzyme concentration, promotes the desorption of metals from MPs, allowing them to be absorbed more readily into fish’s bloodstream and tissues.

- **Enhanced Toxicity** : Combined exposure leads to higher mortality, severe oxidative stress, liver/kidney damage, and behavioral changes compared to individual exposure.
- **Physiological Disruptions** : Studies indicate a significant reduction in red blood cells (RBCs), lymphocytes, and thrombocytes (e.g. in African catfish), indicating immune suppression.
- **Reproductive and Growth Impairment** : The combined pollution harms reproductive systems by disrupting steroidogenesis, reducing egg and sperm quality, and hindering growth.

Table - 1

Information about fish species.

Serial no	Fish species common name	Scientific name	Sampling area	No. of individuals (n)	Feeding zone	Length range cm	Average length cm±SD	Weight range (g)	Mean weight (g) ± S D
1	Taki	<i>Channa punctata</i>	Jashore	10	Benthopelagic	14.3–17.0	15.33 ± 0.77	34.66–53.35	42.51 ± 5.52
2	Taposi	<i>Polynemus paradiseus</i>	Khulna	10	Demersal	12.9–18.5	15.74 ± 1.96	15.17–27.2	21.56 ± 4.52
3	Khoira	<i>Gudusia chapra</i>	Jashore	10	Pelagic	7.4–9.3	8.47 ± 0.52	3.93–7.26	5.57 ± 0.89
4	Chela	<i>Salmosto</i>	Jashore	10	Benthopelagic	5.1–6.1	5.66 ± 0.34	0.99–	1.16 ±

Serial no	Fish species common name	Scientific name	Sampling area	No. of individuals (n)	Feeding zone	Length range cm	Average length cm±SD	Weight range (g)	Mean weight (g) ± SD
		<i>ma bacaila</i>			elagic			1.38	0.15
5	Bata	<i>Labeo bata</i>	Jashore	10	Benthopelagic	12.3–13.5	13.04 ± 0.58	18.74–28.42	23.31 ± 3.69
6	Kachki	<i>Corica soborna</i>	Narail	10	Pelagic-neritic	3.6–4.2	3.96 ± 0.22	0.49–0.77	0.58 ± 0.09
7	Chanda	<i>Parambassis ranga</i>	Narail	10	Demersal	4.5–6.7	5.33 ± 0.63	1.03–2.97	1.66 ± 0.51
8	Kakila	<i>Xenentodon cancila</i>	Narail	10	Pelagic-neritic	9.2–10.8	10.12 ± 0.59	1.82–3.06	2.49 ± 0.47
9	Parshe	<i>Mugil cephalus</i>	Sathkhira	10	Benthopelagic	11.3–13	11.9 ± 0.47	12.78–20.72	17.11 ± 2.25
10	Khailsha	<i>Trichogaster fasciata</i>	Jashore	10	Benthopelagic	3.9–6.4	4.42 ± 0.72	1.3–4.71	1.86 ± 1.05
11	Puti	<i>Puntius sophore</i>	Jashore	10	Benthopelagic	7–7.7	7.33 ± 0.25	4.73–6.86	5.96 ± 0.78
12	Darkina	<i>Esomus danrica</i>	Jashore	10	Benthopelagic	5–5.6	5.13 ± 0.25	0.83–1.22	1.04 ± 0.12

Serial no	Fish species common name	Scientific name	Sampling area	No. of individuals (n)	Feeding zone	Length range cm	Average length cm±SD	Weight range (g)	Mean weight (g) ± SD
13	Bele	<i>Glossogobius giuris</i>	Jashore	10	Benthoplaegic	11.0–13.0	11.66 ± 0.62	8.58–14.85	12.05 ± 1.69
14	Tengra	<i>Mystus tengara</i>	Bagherhat	10	Demersal	9.0–10.3	9.43 ± 0.46	8.75–13.87	10.82 ± 1.92
15	Shing	<i>Heteropneustes fossilis</i>	Narail	10	Demersal	10.5–14.9	12.95 ± 1.5	6.88–20.22	14.03 ± 4.46
16	Baim	<i>Mastacembelus armatus</i>	Jashore	10	Demersal	9.1–13	10.31 ± 1.2	2.26–7.48	3.95 ± 1.61
17	Mola	<i>Amblypharyngodon mola</i>	Jashore	10	Benthopelagic	4.6–5.1	4.87 ± 0.18	0.93–1.24	1.01 ± 0.09

3. Microplastic extraction from fish species

For the extraction of microplastics, each sample's body length (cm) and weight (gm) were measured (Table 1). In the laboratory, 7 individuals from each species (whole-body) were examined for the extraction of microplastics. Each fish was placed into a 100 mL beaker and digested by using an H₂O₂ digestion protocol with slight modifications adopted from Ref. In brief, fish samples were digested by adding 10 mL H₂O₂ and 10 mL of 0.05 M Fe (II) solution and heated at 40 °C for 72 h. Additionally, if any organic matter remains visible, add more than 5 mL of H₂O₂ solution and wait until complete digestion process. After digestion, samples were

passed through GF/B: 1 mm, 47mmWhatman, UK filter papers using the vacuum filtering process. In a separate glassware Petri dish, all air-drying filter papers were put in, sealed, and wrapped using aluminum foil paper for further analysis.



1.2. Identification and characterization of microplastics

All the filter papers were visually observed under a HumaScope Advanced LED, Human, Germany binocular microscope by adjusting the magnification from 10_ to 100X, beside ImageJ software was applied for further analysis of the captured image (OPTIKA, Italy) (Ghosh *et al.*, 2020;). Each of the microplastic particles was observed visually and categorized as the total number, color (transparent, white, black, blue, and red), shape (film, fragment, and fiber), and size (1e5 mm and <1 mm). In particular cases, the hot-point test was performed for item verification. Identified microplastic origin and chemical composition were determined by Fourier Transform Infrared Spectroscopy (FTIR) (FTIR- 4600, JASCO Inc., Tokyo, Japan) equipped with an Attenuated Total Reflection (ATR) unit . Each measurement was performed by sixteen co-scans within the 500-4000 cm₋₁ spectral range with a spectrum resolution of 4 cm.

3.3. Potential risk assessment from microplastics

The risks that chemicals associated with the wide variety of plastic items pose to the environment, humans, and other life forms are only poorly understood . Chemical toxicity was considered to determine the ecological impact caused by various types of microplastic polymers . The polymer hazard index (PHI) is a method utilized recently to calculate the potential risk posed by microplastics. Additionally, plastic polymers' monomers and chemical toxicities can be used to evaluate the potential damage to the health of people and the environment . The following formula was used to determine the polymer hazard assessment for microplastics:

$$\text{Polymer Hazard Index (PHI)} = \sum_n^p x S_n$$

Here, the polymer hazard index of microplastics is referred to as 'PHI', P_n represents the polymer types percentage of identified microplastics, and S_n represents the average hazard of the polymer . The microplastic hazard categories and risk levels were evaluated using the PHI score, reported in several studies . Therefore, the polymer risk index, which is based on hazard grades ranging from 1 to 10,000, is divided into five degrees of risk and used to evaluate the microplastic's health risk level .

3.4. Fish heavy metals analysis

For heavy metal extraction; three samples of each species (whole-body) were dried, crushed, and weighted at 0.5 g using an electric balance before being placed in a beaker with a volume of 100 mL. The tri-acid mixture of HNO₃ (69%), H₂SO₄ (98%), and HClO₄ (70%) at a 5:1:1 ratio detailed explained by Ref. During the experimental procedures, all conical flask containing fish samples was covered by a watch glass to control the possible contamination and loss of volatile elements. After cooling to around room temperature, the digested solution was filtered using a 0.1 M HNO₃ pre-washed Whatman 42 filter paper and then diluted to a volume of 50 mL with double-distilled water; (Kawser *et al.*, 2016 for the metal detection in Atomic Absorption Spectrometry (AAS) (Model: AA-7000, SHIMADZU, Japan). All the experiments were conducted with laboratory-grade chemicals (Merck, Germany) and double-distilled water.

3.5 Health risk assessment from heavy metals

The estimated consumption of microplastics per week by considering the recommended dietary intake of 50 g of fish muscle per week for children and 300 g of fish muscle per week for adults. Additionally, microplastics and associated toxic metals have been linked to endocrine problems such as carcinogenesis and mutagenesis. Therefore, the human health risk from metals in the edible fish of the studied species was analyzed with Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), Hazard Index (HI), and Carcinogenic risk (CR). The exposure to hazardous metals was calculated by considering the average concentration of a metal found in fish muscles and accounting for an intake of 50 g per day for children and 100 g per day for adult.

4. Microplastics and heavy metals relationship

Indicating varying degrees of pollution from light to severe. A scatter plot diagram between microplastics and the metal pollution index (MPI) was used to explore the relationship between microplastics and the heavy metals (Cu, Cr, Pb, and Cd) found in the muscles of 17 freshwater fish species. As demonstrated in , there were fewer or no correlations ($R^2 \leq 0.0013$) observed because of the minimal interaction between microplastics and heavy metals in the freshwater fish species. A paired t-test revealed no statistically significant differences between the abundance of microplastics and the MPI value in various fish species ($t \leq 0.14$; $p \leq 0.89$). Similar results were also reported for fish species *Atropus* and *Leiognathus brevis* in the Gulf of Manner and *S. jello* from the northeast of Persian Gulf. Therefore, the current study findings also support the notion that the accumulation of microplastics and heavy metals in various fish species may have differed depending on factors such as age, gender, food references, habitat, seasonal changes, as well as pollutant size, shape, color, types, and forms.

Conclusions

Microplastics are not only destroying the freshwater ecosystem, but they can also enter the human body through contaminated fish, which is a serious health risk for humans and fishes both. The study results revealed that the highest abundance of microplastics was found in *C. punctata* and lowest in *A. mola* fish species and black fiber in < 1 mm size made up the majority of the microplastics. Additionally, microplastics contained polyethylene, polypropylene, and polystyrene types of polymers identified by ATR-FTIR analysis. Heavy metals concentration was extensively varied in different species and the level of Cu, Cr, Pb, and Cd are

lower than the maximum allowable limit.

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