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EFFECT OF TRADITIONAL COOKING METHOD ON THE HEAVY METAL CONTENT OF FOUR SELECTED FARMED CARP SPECIES AND ASSESSMENT OF POSSIBLE HUMAN HEALTH RISK

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ABSTRACT

The present study was conducted to evaluate the effect of traditional Bengal culinary practice on the heavy metal content of four carp species (*Labeo rohita, Catla catla, Cirrhinus cirrhosus,* and *Hypophthalmichthys molitrix)*. Fish samples were collected from seven different farms located in the Rajshahi district of Bangladesh. The collected samples were processed and cooked in a traditional currying method and concentrations of five heavy metals (Pb, Co, Cr, Cd, and Ni) were assessed in raw and cooked fish using a flame atomic spectrophotometer. Heavy metal concentrations were significantly reduced in the cooked samples with a few exceptions. The metal pollution index (MPI) indicated that the most amount of heavy metals was lost in *C. cirrhosus* followed by *H. molitrix, L rohita,* and *C. catla*. Pb was the most abundant and consumed of the tested heavy metals in the cooked fish followed by Co, Ni, Cd, and Cr respectively. Health risk index (HRI) and target hazard quotient (THQ) revealed that individual heavy metal intake from cooked fish does not pose any threat to human health. However, the hazard index (HI) of the accumulated heavy metals in the cooked fish suggested that the long-term effect of consuming heavy metal contaminated fish could collectively lead to possible non-carcinogenic health complexities. Additionally, HI also implied that the highest degree of health risk is associated with the consumption of *H. molitrix* and the least with *L. rohita.*

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INTRODUCTION

Fish serves as one of the major source of animal protein in Bangladesh. It also plays a crucial role as a supplier of essential micronutrients in our daily diet. Fish also has been an integral part of traditional Bengal cuisine from ancient times. Traditionally fish is mainly consumed as curry (Rahman and Islam, 2020) however, other forms of cooking methods as frying or grilling are also popular in Bangladesh. Currently, Bangladesh mostly relies on aquaculture production for its nationwide supply of freshwater fish. As a result of abundant natural resources and widespread farming activities, Bangladesh now ranks third in inland open-water capture fisheries and fifth in aquaculture production (FAO, 2022). Feed-based aquaculture operation has greatly increased domestic fish production over recent years. Farmers are now more oriented towards intensive culture practices to boost the existing production figures of Indian major carp within a relatively short period, following a process known as “Carp Fattening”. However, this novel approach has raised several concerning issues, among which heavy metal contamination is of particular importance.

The term heavy metal refers to any metallic chemical element that has a relatively high density ($<5g/cm^3$) and is toxic or poisonous even at low concentrations. Heavy metals enter aquatic ecosystems from different natural and anthropogenic sources such as mining, industrial and domestic effluents, urban stormwater, leaching of metals and solid wastes dump, textiles and cosmetics, atmospheric sources, and petroleum industrial activities (Mannan et al., 2018). However, in absence of such contaminant sources nearby, fish feed is considered to be the major contributor to heavy metal pollution in aquaculture systems (Ali and Khan, 2018; Sabbir et al., 2018). Heavy metal contamination in fish feed is largely attributed to the use of contaminated feed ingredients like unprocessed tannery waste (Sarker et al., 2017). These heavy metals ultimately find their way into the fish body and biomagnify in humans producing a wide array of health complexities. Therefore, it is important to assess the heavy metal content of edible fish and evaluate the possible effect of the cooking method over its quantity.

Several previous studies have shown a significant effect of cooking methods on the heavy metal content of food products (Inobeme et al., 2020). Numerous researchers have carried out their investigation of different cooking methods on the heavy metal content of different species of fish as well (Diaconescu et al., 2013; Huque et al., 2014; Lahiri et al., 2019). However, comparative studies on the effect of traditional cooking methods on the heavy metal content of different farmed carp species in Bangladesh are scarce. Therefore, the present investigation was carried out to determine the effect of traditional cooking methods on the heavy metal content of four selected farmed carp species and to evaluate the possible human health risk associated with its consumption.

MATERIAL AND METHODS

Study duration and location

The study was carried out from July, 2019 to December, 2019 for a period of six months in the Rajshahi district of Bangladesh. Fish samples were collected from seven distinct fish farms scattered in three different Upazila namely Paba, Mohonpur, and Godagari where carp fattening is widely practiced.

Species selection and sample collection

Based on consumer demand and culture practices, three Indian major carp (*Labeo rohita, Catla catla, Cirrhinus cirrhosus*) and one Chinese carp (*Hypophthalmichthys molitrix*) species were selected for this study. Fourteen fish samples were collected for each of the species generating a total sample size of fifty-six. The average length and weight of the collected specimen are presented in Table 1.

Table 1. Mean length and weight of the collected fish sample

Parameters	*L. rohita*	*C. catla*	*C. cirrhosus*	*H. molitrix*
Length (cm)	33.81±2.76	37.02±2.34	26.61±3.04	32.36±3.15
Weight (g)	445.56±58.94	816.13±60.08	232.06±28.28	346.75±37.43

Sample preparation, cooking, and digestion

After collecting the raw fish, they were cleaned with deionized water and muscle samples in the form of fillets were obtained after necessary processing (beheading, skinning, gutting). The samples were then washed again with deionized water and divided into two categories, one for the heavy metal analysis of raw muscle and the other to be analyzed after cooking. The cooking procedure was carried out following a traditional currying method. Each of the fish species was cooked separately using conventional spices with added water and soybean oil (Table 2) for thirty minutes in a preheated oven at 200°C.

Table 2. Ingredients used in cooking

Ingredients	Amount per kg fish
Onion	100g
Garlic	30g
Turmeric	10g
Ginger	25g
Peeper	20g
Cumin	20g
Cardamom seed pods	3 pcs
Salt	20g
Oil	100 ml
Water	200 ml

The raw and cooked fish samples were then fragmented, homogenized, and heated in an oven at 120°C for 48 hours until gaining constant weight. Then the samples were cooled in a desiccator and powdered using a grinder machine. For digestion, each of the samples was kept in a muffle furnace at 600°C in a separate crucible to obtain ash. After 6 hours, the samples were taken out from the muffle furnace and cooled and mixed with 20ml 1N HCl acid and also added distilled water until reaching 100 ml solution in a 500 ml beaker. Then this solution was sieved through filter paper.

Determination of heavy metal concentrations

The heavy metal concentrations of lead (Pb), cobalt (Co), chromium (Cr), cadmium (Cd), and nickel (Ni) from raw and cooked fish samples were determined in the central lab of the University of Rajshahi through atomic absorption spectrophotometer (AAS) technique. A flame atomic absorption spectrophotometer (Shimadzu AA-7000) was used to analyze the concentrations of heavy metals in the collected samples, where acetylene gas and air were used as fuel and oxidizer respectively. The absorption wavelengths and detections thresholds were 217.0 nm and 0.001 ppm for Pb, 247.7 nm and 0.02 ppm for Co, 357.9 nm and 0.01 ppm for Cr, 228.8 nm and 0.002 ppm for Cd, and 232.0 nm and 0.01 ppm for Ni.

Human health risk assessment

To evaluate the effect of heavy metals in cooked fish, the following parameters were estimated.

Metal pollution index (MPI)

The metal pollution index (MPI) expresses the cumulative heavy metal concentration in fish samples and was calculated as a geometric mean using the following equation (Usero et al., 1997) for both raw and cooked samples.

$$MPI\ \frac{mg}{kg} = \sqrt[n]{(Cf1 \times Cf2 \times Cf3 \times\ \dots\ Cfn)} \ldots\ldots\ldots\ldots\ldots(1)$$

Where, Cfn = amount of individual heavy metal in the sample “n” expressed in mg/kg.

Daily intake of metal (DIM)

The daily intake of each heavy metal (DIM) by a human individual was estimated using the following equation as suggested by Islam et al. (2017).

$$DIM = \frac{CM \times FIR \times CF}{WAB} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

where CM is the metal concentration in fishes (mg/kg), FIR is the average daily fish ingestion rate measuring 0.06258 kg/persons/day (DoF, 2020), WAB is the average body weight which is 70 kg for males and 50 kg for female (BBS, 2019). The conversion factor CF= 0.289 which was estimated from the mean moisture content of the collected fish samples to convert the dry weight of fish samples into fresh weight.

Health risk index (HRI)

The health risk index (HRI) was estimated as the ratio of DIM and oral reference dose (Rf_D). Rf_D is an approximation of daily oral exposure to the human populace that is expected to have no significant risk of deleterious effects over the course of their lives (USEPA-IRIS, 2006). The value of Rf_D for Pb, Co, Cr, Cd, and Ni is 0.0035, 0.003, 0.003, 0.001, 0.02 (USEPA, 2011). A food product with an HRI value of less than 1 is considered safe for human consumption.

The HRI for the heavy metals was calculated using the following equation (Cui et al., 2004).

$$HRI = \frac{DIM}{Rf_D} \ldots\ldots\ldots\ldots\ldots\ldots\ldots..(3)$$

Non-carcinogenic health hazard (THQ)

The target hazard quotient (THQ) was calculated to determine the non-carcinogenic health hazards related to the intake of heavy metal polluted fish using the following equation provided by USEPA (2011).

$$THQ = \frac{EF \times ED \times FIR \times CF \times CM}{WAB \times ATn \times Rf_D} \times 10^{-3} \ldots\ldots\ldots\ldots\ldots\ldots.(4)$$

where EF = the exposure frequency (365 days), ED = the exposure duration which is the estimated life expectancy of 71.2 years for males and 74.5 years for females in Bangladesh (BBS, 2020). ATn = the average exposure duration for non-carcinogens (EF×ED) as used in depicting noncancerous risk (USEPA, 2011). The rest of the parameters are explained before.

Hazard index (HI)

The hazard index (HI) is another indicator of human health risk due to heavy metal contamination which is the summation of THQ of all heavy metals (n) present in the fish samples (USEPA, 2011). The HI is calculated by using the following equation.

$$\sum_{i=1}^{n} THQ \ldots\ldots\ldots\ldots\ldots\ldots..(5)$$

According to USEPA (2011) the acceptable threshold for both THQ and HI is less than 1.

Statistical analysis

The statistical analysis and development of graphs were carried out using SPSS 25 and Microsoft Excel 2016. A paired sample T-test was conducted to determine the statistical difference between the mean of each heavy metal content of raw and cooked fish. The significance level was set at P<0.05.

RESULTS

Heavy metal content in fish samples

The concentration of Pb, Co, Cr, Cd, and Ni in raw and cooked fish samples is shown in Table 3. Pb was the most abundant of the tested heavy metals followed by Co and Ni in raw and cooked samples whereas Cr and Cd exhibited alternating levels of accumulation in the selected fish species. In most cases, the amount of different heavy metals in cooked fish fillets was significantly lower than in raw fish. However traditional cooking method appeared to have no significant effect on the Pb and Co content of *C. catla*, the Pb and Ni content of *H. molitrix*, and the Ni content in *C. cirrhosus.*

Table 3. Comparison of heavy metal content in raw and cooked fish (mg/kg)

Heavy Metals	Samples	*L. rohita*	*C. catla*	*C. cirrhosus*	*H. molitrix*
Pb	Raw	5.63±2.38	5.74±2.41	6.95±3.18	5.97±2.55
	Cooked	3.89±1.99	4.64±2.74	5.15±2.77	5.23±2.61
	P value	0.00*	0.15	0.01*	0.09
Co	Raw	3.67±1.75	2.38±1.3	3.72±1.02	3.66±1.85
	Cooked	2.56±1.02	2.44±2.03	2.50±0.75	2.19±1.86
	P value	0.00*	0.93	0.01*	0.01*
Cr	Raw	1.83±0.74	2.09±0.85	1.8±0.64	1.67±0.71
	Cooked	1.37±0.59	1.35±0.71	0.92±0.55	1.21±0.55
	P value	0.00*	0.00*	0.00*	0.00*
Cd	Raw	1.84±0.59	2.02±0.71	1.83±0.53	1.76±0.61
	Cooked	1.37±0.52	1.62±0.8	1.26±0.75	1.12±0.64
	P value	0.00*	0.01*	0.03*	0.00*
Ni	Raw	1.88±0.48	2.31±0.77	2.31±0.71	2.26±0.73
	Cooked	1.36±0.47	1.59±0.51	1.98±0.78	2.19±0.25
	P value	0.00*	0.00*	0.09	0.07

MPI of raw and cooked fish samples

The MPI of the experimental samples are illustrated in Figure 1. For raw samples, the highest heavy metal accumulation was measured in *C. cirrhosus* followed by *C. catla* while the MPI of *L. rohita* and *H. molitrix* were quite similar. The MPI of the raw fish sample of the experimental fish can be decennially arranged as *C. cirrhosus> C. catla> H. molitrix> L. rohita.* Nonetheless, this order of MPI was found to be substantially altered in cooked fish samples. Heavy metal content in cooked samples declined significantly in all the experimental fish, however, this reduction didn't correlate to their previous MPI values. Samples from different fish species exhibited different levels of heavy metal reduction upon cooking. The highest MPI for cooked muscle was estimated in *C. catla* followed by *L. rohita, H. molitrix,* and *C. cirrhosus.* As estimated from the difference between MPI values*, C. cirrhosus* showed the greatest amount of heavy metal reduction and *C. catla* the least due to cooking.

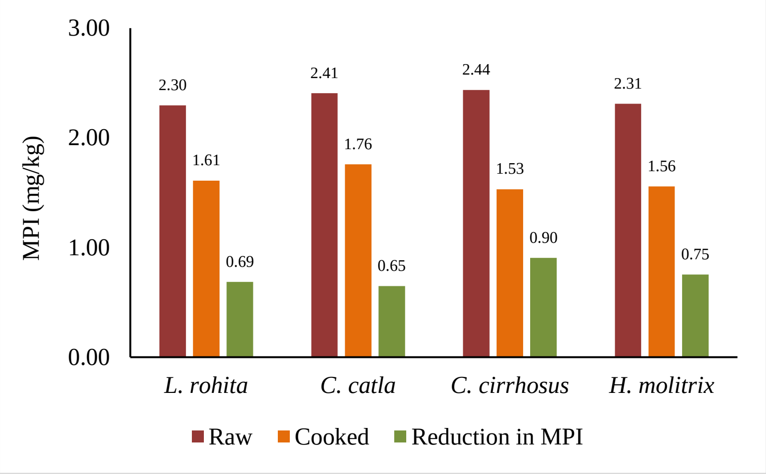

Figure 1. MPI of raw of cooked samples and their reduction due to cooking

DIM from the consumption of the experimental fish

The average daily intake of metals from the consumption of the selected fish species is given in Table 4. Presumably, Pb was the highest consumed heavy metal in all the experimental fish. Based on the average DIM value, *C. cirrhosus* was identified as the largest contributor to the tested heavy metals in the daily diets of men and women. The DIM for *L. rohita* was lowest among the experimental fish while *C. catla* and *H. molitrix* demonstrated an identical DIM value. In general, women showed a higher intake of each heavy metal per kg body weight compared to men due to their low body mass.

Table 4. Estimated daily heavy metal intake (mg/kg body weight/day)

Heavy Metals	*L. rohita*		*C. catla*		*C. cirrhosus*		*H. molitrix*		Average
	Men	Women	Men	Women	Men	Women	Men	Women	
Pb	9.98×10^{-04}	14.0×10^{-04}	11.9×10^{-04}	16.7×10^{-04}	13.2×10^{-04}	18.5×10^{-04}	11.9×10^{-04}	16.7×10^{-04}	14.1×10^{-04}
Co	6.58×10^{-04}	9.21×10^{-04}	6.27×10^{-04}	8.77×10^{-04}	6.42×10^{-04}	8.99×10^{-04}	6.27×10^{-04}	8.77×10^{-04}	7.66×10^{-04}
Cr	3.52×10^{-04}	4.93×10^{-04}	3.46×10^{-04}	4.85×10^{-04}	2.37×10^{-04}	3.31×10^{-04}	3.46×10^{-04}	4.85×10^{-04}	3.84×10^{-04}
Cd	3.51×10^{-04}	4.92×10^{-04}	4.16×10^{-04}	5.83×10^{-04}	3.25×10^{-04}	4.55×10^{-04}	4.16×10^{-04}	5.83×10^{-04}	4.53×10^{-04}
Ni	3.49×10^{-04}	4.88×10^{-04}	4.07×10^{-04}	5.70×10^{-04}	5.09×10^{-04}	7.13×10^{-04}	4.07×10^{-04}	5.70×10^{-04}	5.02×10^{-04}
Average	6.50×10^{-04}		7.17×10^{-04}		7.29×10^{-04}		7.17×10^{-04}		7.03×10^{-04}

Estimated HRI in men and women

HRI is an indicator of possible health complexities that may arise upon the consumption of toxic trace elements. A value greater than 1 indicates that the average consumption of that particular element is higher than the recommended limit and can possibly be detrimental to human health. In our investigation, Cd showed the highest HRI value among the heavy metals in all the species and was closely tailed by Pb (Table 5). HRI for Co was found to be higher compared to that of Cr and Ni. Species-wise, *C. catla,* and *H molitrix* exhibited the highest HRI on average while *L. rohita* the least. However, all the tested heavy metals across the four experimental fish demonstrated an HRI value of less than 1 indicating no probable health risk for daily individual heavy metal intake.

Table 5. HRI values of the tested metals across the selected fish species

Heavy Metals	*L. rohita*		*C. catla*		*C. cirrhosus*		*H. molitrix*	
	Men	Women	Men	Women	Men	Women	Men	Women
Pb	0.29	0.4	0.34	0.48	0.38	0.53	0.34	0.48
Co	0.22	0.31	0.21	0.29	0.21	0.3	0.21	0.29
Cr	0.12	0.16	0.12	0.16	0.08	0.11	0.12	0.16
Cd	0.35	0.49	0.42	0.58	0.32	0.45	0.42	0.58
Ni	0.02	0.02	0.02	0.03	0.03	0.04	0.02	0.03
Average	0.24		0.27		0.25		0.27	

THQ and HI in men and women

The THQ and HI values for five heavy metals calculated from the estimated consumption of the experimental fish are given in Table 6. THQ denotes the possibility of non-carcinogenic health hazards originating from the lifelong consumption of hazardous trace elements by humans and a THQ value of less than 1 is considered to be safe. In this study, the THQ value for each of the tested heavy metals across all the experimental fish was found to be less than 1 indicating no harmful impact of individual heavy metal intake (Table 6). However, food items can be contaminated by more than one heavy metal, and therefore a more holistic approach to risk estimation known as HI is used to determine the health risk associated with heavy metal consumption. Similar to the THQ, an HI value greater than 1 is regarded as unsafe for human consumption. The HI values found in this study show that the cumulative effect of the heavy metals present in all the experimental fish can be detrimental to human health in the long run (Table 6). The highest health risk is associated with the intake of *H. molitrix* followed by *C. catla, C. cirrhosus*, and lastly *L. rohita*. Women are at a greater health risk compared to men here as well.

Table 6. THQ and HI values of the tested metals across the selected fish species

Heavy Metals	*L. rohita*		*C. catla*		*C. cirrhosus*		*H. molitrix*	
	Men	Women	Men	Women	Men	Women	Men	Women
Pb	0.29	0.40	0.34	0.48	0.38	0.53	0.44	0.61
Co	0.22	0.31	0.21	0.29	0.21	0.30	0.31	0.44
Cr	0.12	0.16	0.12	0.16	0.08	0.11	0.14	0.20
Cd	0.35	0.49	0.42	0.58	0.32	0.45	0.45	0.63
Ni	0.02	0.02	0.02	0.03	0.03	0.04	0.03	0.04
Hazard Index	0.99	1.39	1.10	1.54	1.02	1.43	1.38	1.93

DISCUSSION

Cooking food items can significantly affect their heavy metal content as explored in several previous studies (Inobeme et al., 2020). However, these effects are not consistent across all cooking methods and can vary depending on the type of food as well. Heavy metals are soluble in water and therefore their content tends to reduce when foods are cooked in a watery medium (Lee et al., 2019). In traditional Bengal culinary practices, the fish fillets go through rigorous heating and boiling treatment. This thermal processing of fish fillets leads to their loss of heavy metal content as they are leached in the cooking medium as salts (Bryan and Hummerstone, 1971). Heating and boiling also denature the protein and amino acid content of fish muscle to some extent and facilitate the release of heavy metals that usually remains bonded to them (Ersoy et al., 2006; Ganbi, 2010).

Various species of fish respond differently to exposure to different cooking methods as loss of heavy metal is largely dependent on temperature, duration, and ingredients of the cooking method as well as on size, age, and weight of fish (Lahiri et al., 2019; Kalogeropoulos et al., 2012). Despite some exceptions, the result of this study indicates that the traditional cooking method can significantly reduce the heavy metal content in cooked fish in most cases (Table 3). Fillets of *L. rohita* and *H. molitrix* showed a decreased level of Pb content in cooked samples whereas the traditional cooking method imparted no significant effect on the Pb content of *C. catla* and *C. cirrhosus.* Co content in cooked muscle also declined in all the experimental fish except *C. catla.* The concentration of Cr and Cd was significantly reduced in all samples due to cooking while Ni content was unchanged *in C. cirrhosus* and *H. molitrix.* Evidence of decreased levels of heavy metal content in cooked fish has been previously reported in numerous studies. Ibrahim et al. (2018) reported a decreased level of Cd and Pb in *Oreochromis niloticus* cooked by grilling and frying. Atta et al. (1997) also reached a similar conclusion that heat treatment such as steaming and baking can significantly reduce certain heavy metals in *O. niloticus* including Pb and Cd. Significantly reduced levels of heavy metals (Pb, Cd, Cu, and Zn) in shellfish muscle under various cooking practice has also been reported (Lahiri et al., 2019). The findings of this study also coincide with Diaconescu et al. (2013) who argued that cooking has a significant effect on reducing the contamination of Pb, Cd, Cr, and Ni in several fin fish species. However, these findings are not always consistent and often tend to vary according to the difference in fish species and the cooking method followed. For instance, a comparative evaluation of heavy metal content in raw and boiled silver pomfret revealed that boiling had no significant effect on the manganese, lead, cadmium, and chromium content of the experimental fish (Huque et al., 2014). Antagonistic to the findings of this study, Zafar et al., (2019) concluded that cooking procedures especially currying could lead to an increase in certain heavy metals (Cd, Cr, Cu, Mn, and Zn) in some coastal fish species. Perellóet al. (2008) demonstrated that cooking elevated the concentrations of Cd, Pb, Hg, and As in sardine, hake, and tuna fish. In another investigation, Kalogeropoulos et al. (2012) reported that metal concentrations in cooked fish after pan frying and grilling were higher than in raw fish and explained that higher water loss and oil uptake can result in increased concentration of heavy metals in the muscle.

In the existing research works, little importance has been given to estimating the health hazards associated with the consumption of heavy metal contaminated cooked fish compared to raw fish. Our finding showed that the MPI values of the cooked fish ranged from 1.53 to 1.76 mg/kg which is fairly lower than the values determined by Ghosh et al. (2021) in raw freshwater fish (4.85 to 6.65 mg/kg) and Islam et al. (2017) in raw prawn (2.38 to 4.89 mg/kg) in Bangladesh. We also found that Pb was the most abundant of the five heavy metals (Pb, Co, Cr, Cd, and Ni) tested in this study and the most consumed as well. This result is in line with the previous findings of Ahmed et al. (2019) and Atique Ullah et al. (2017) where Pb was the predominant heavy metal present in both cultured and captured commercially important fish species in Bangladesh.

HRI values in this study exhibited that the daily intake of individual heavy metals is less than their oral reference doses and indicates no health risk for daily consumption of a particular metal (Table 5). All the THQ values obtained in this investigation were below 1 and implied that the possible non-carcinogenic health hazards due to prolonged fish consumption are negligible (Table 6). Similar assumptions have been proposed by Sarker et al. (2020) and Akter et al. (2021). However, the HI values demonstrated that the cumulative effect of the studied heavy metals is likely to impart hazardous effects on human health under long-term exposure. Ghosh et al. (2021) and Atique Ullah et al. (2017) also argued that the HI values of the accumulated heavy metals indicated that their collective influence could have a detrimental non-carcinogenic effect even when the individual THQ values are considered safe.

CONCLUSIONS

Heavy metal contamination in commercially produced fish is an alarming human health concern. The traditional cooking method followed in this study exhibited a variable degree of impact and significantly reduced most of the contaminating heavy metals present in the muscle of *L. rohita, C. catla, C. cirrhosus, and H. molitrix.* Health risk assessment revealed that although individual heavy metal intake might not pose a threat imminently, the cumulative effect of different heavy metals may lead to significant health hazards over long-term exposure.

COMPETING INTEREST

The authors stated that they have no known conflicting financial or non-financial interests or personal ties that may be seen as having influenced the work described in this manuscript.

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