



Bioaccumulation and Heavy Metal Contamination in Fish Species of the Dhaleswari River of Bangladesh and Related Human Health Implications

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Abstract

The release of a large quantity of heavy metals into the Dhaleswari River from the tannery, dyeing, and other industrial setups and their subsequent transfer to food chains through fish consumption have been an alarming issue in Bangladesh. To study the pollution level, a total of seven fish species, namely *Heteropneustes fossilis*, *Channa punctata*, *Nandus nandus*, *Chanda nama*, *Anabas testudineus*, *Mystus gulio*, and *Colisa fasciata*, were collected in winter from the Dhaleswari River and the total concentrations of Cr, Pb, Ni, and Zn in head and body tissues were analyzed separately. The concentrations of Cr, Pb, and Zn were found 300, 20, and 10 times higher, respectively, than the guideline value of the Food and Agriculture Organization (FAO)/World Health Organization (WHO), indicating possible health risks to humans. In most cases, bioaccumulation factors (BAFs) exceeded the highest limit, expressing that most of the species, especially *C. nama*, *A. testudineus*, and *C. fasciata*, were in the highly bioaccumulative state. The health risks associated with fish consumption were determined in terms of estimated daily intake (EDI), non-carcinogenic risks (THQ), and carcinogenic risk (TR) factors. The THQs for Cr and Pb crossed the maximum value of 1 in all the fish species except Pb in *Mystus gulio*, which might cause different non-carcinogenic diseases upon consumption of these fishes. In all the fish species, the carcinogenic risk factor for Cr exceeded the standard value (10^{-4}), indicating chronic cancer risk to humans. Although the estimated daily intake (EDI) values did not cross the permissible limit, continuous consumption of contaminated fish from the target area may cause serious health complications. This study revealed that consumption of these fishes exposed people to a higher risk of non-carcinogenic and carcinogenic consequences in terms of human health.

Keywords Bioaccumulation · Heavy metals · Estimated daily intake · Carcinogenic and non-carcinogenic risks · Permissible limit

Introduction

Heavy metal contamination of the aquatic ecosystem is a primary and worldwide environmental concern in recent times due to associated ecological and human health risks [1–3]. Heavy metals with high persistence time, non-bio-degradable, and bioaccumulative nature can be concentrated along with the food chain, delivering a poisonous impact at points that are far eliminated from the source of contamination [4–7]. Since Bangladesh is a developing country, most industrial activities are constructed along the riverbanks for cooling, cleaning, and disposal purposes. Consequently, thousands of tons of waste materials have been discharging into this river water daily from these anthropogenic sources [8]. Moreover, to achieve a high-profit goal, the industrial authorities do not follow the

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guidelines and legislations formulated by the government and many industries do not run effluent treatment plants (ETPs) despite having it to reduce pollution [9].

The Dhaleswari River of Bangladesh is a 160-km-long tributary of the Jamuna River in the country's central part. It starts off the Jamuna close to the north-western tip of Tangail District and meets the Shitalakshya River close to Narayanganj District. Along the path of its flow, it receives large quantities of pollutants including heavy metals from industrial wastes largely adjacent Hemayetpur tannery industries, steel mills, navigation activities, thousands of upstream dyeing, shipbreaking, and other anthropogenic activities [10]. Besides, toxic heavy metals released from indiscriminate application of pesticides ultimately drain into the adjoining water body and are carried downstream through the river flow. As an inherent product of the tanning process, chromium has been discharged in a substantial amount. By and large, just 60% of the chromium is taken up by the cured pelt, and the other 40% of chromium stays in the solid and fluid squanders, for the most part as spent chrome liquor [11]. Other metals such as Zn, Cu, Mn, and Pb are also released from tannery industries as salt impurities as well as dyeing industries [12].

Heavy metals, entering the water environment, are finally incorporated into the pelagic deposits, and organisms of these deposits accumulate heavy metals to variable degrees [1, 13]. Environmental stability and the diversity of aquatic organisms are greatly affected by high heavy metal concentrations. The major inhabitants of water are fishes, and they cannot escape the detrimental consequence of these pollutants. Besides, fishes can concentrate heavy metals to a great degree in their muscles. Through natural absorption, heavy metals can infiltrate fish tissues and as a consequence humans get exposed to these pollutants through the food web [14]. Despite human nutritional contribution from fish consumption, they must be cautiously screened to ensure that poisonous metals are not being transferred to humans through fish consumption because of potential health risks [15–17].

Consumption of toxic metal-contaminated fish can cause tumor, cancer, and numerous pulmonary diseases in the human body such as nephritis, fibrosis, anuria, renal failure, poor reproductive capacity, kidney dysfunction, lung inflammation, hypertension, and hepatic dysfunction [17–20]. Due to the Dhaleswari River's pollution, the situation has also led to strong demand for comprehensive health risk assessment and implementation in the ecosystem [21]. Considering the realities referenced previously [1, 5, 9, 10, 12, 14, 15], the fundamental purpose of this research was to find out the level of pollution of heavy metals in fish as well as the bioaccumulation factor and assess the risk in fish consumption of the Dhaleswari River.

Materials and Methods

Description of the Study Area

This study is conducted on the area adjacent to the tannery industrial setup located in the Hemayetpur area of Savar (Fig. 1). The area is 15 km west of the capital city of Bangladesh, Dhaka. The sampling area lies within the geographic coordinates of 23.79783333 N, 90.24527778 E to 23.76666667 N, 90.23666667 E. The tannery industries occupy an area of 200 acres. The land type of the study area is predominantly low-land. The topographic elevation of the site is 1.5 m above the mean sea level.

The water and fish samples were collected during October 2018 from the Dhaleswari River, exposed to different levels of environmental pollution.

Water Sample Collection

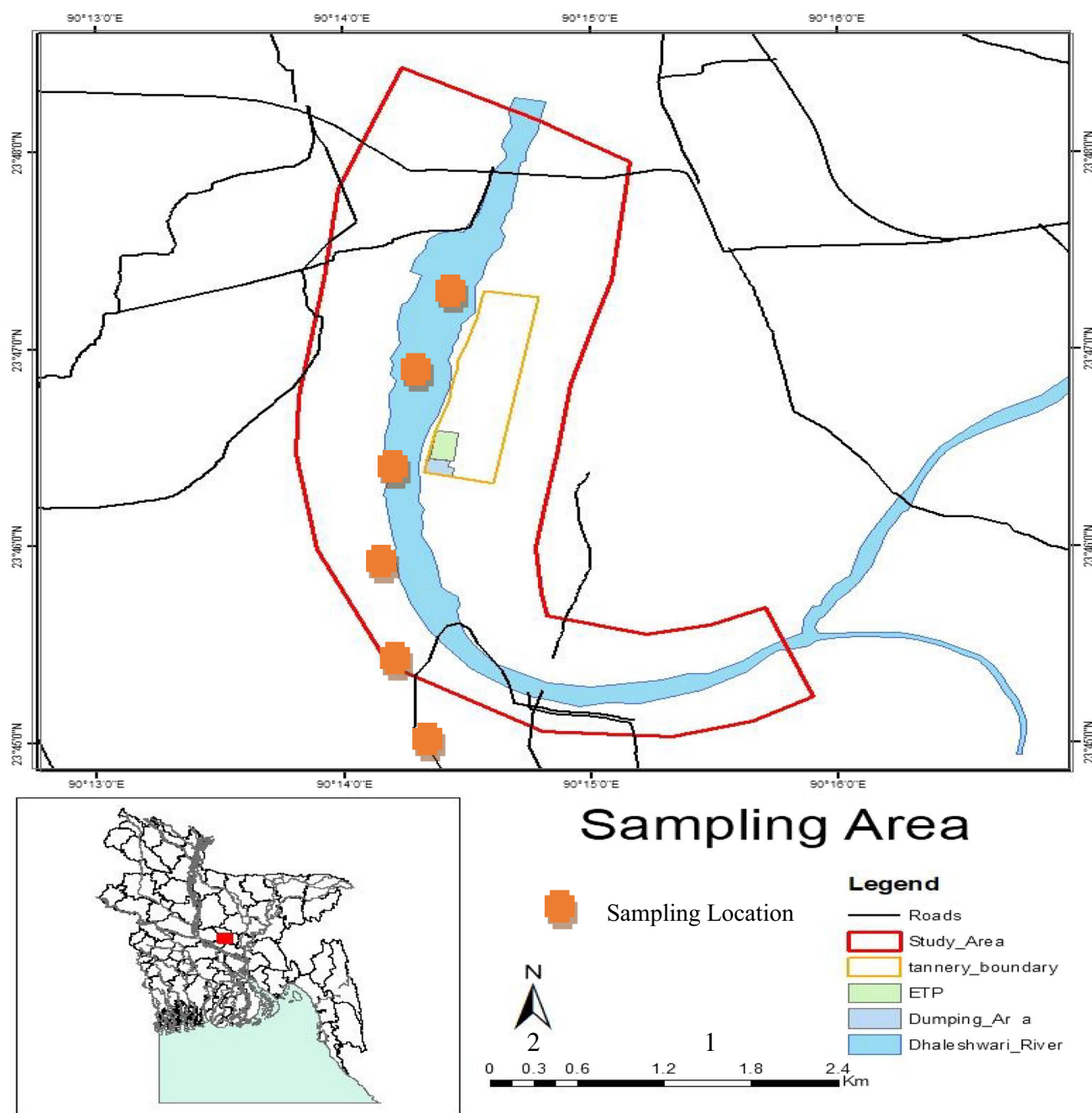
Water samples were collected from each fish sampling location. The water samples were sampled in clean plastic bottles (washed with 69% ultrapure nitric acid by Fisher Scientific, distilled water, and finally with sampled water), bottle caps were tightened carefully to prevent any contamination, and were taken to the lab for subsequent analyses. The water samples were preserved by acidification ($\text{pH} \leq 2$) using ultrapure nitric acid and stored at 4 °C.

Fish Sample Collection

A total of 84 fishes (12 for each species) were collected from six points of the Dhaleswari River that fishers had caught near the tannery industry. The collected seven variants of fish samples were immediately transferred into the icebox on the spot and brought into the laboratory for further processing. The edible and non-edible parts of the fishes were separated with steam-cleaned stainless steel knife and washed with deionized water to remove any adherents. The edible portions were dissected into two parts: body (skin, scales, and muscle) and head (no gills); each part was further cut separately into small pieces and homogenized in a food processor. Finally, the homogenized samples were preserved with proper labels in a freezer at -20 °C for laboratory analysis [22]. The local name, scientific name, and description of the collected fish species are depicted in Table 1.

Digestion of Water Samples

To determine heavy metal in water samples, 100 ml of acid-preserved and homogenized sample was taken in a Pyrex beaker after being acid-washed. After that, 5 ml of concentrated



1. The sampling points are out of the river.
2. You can easily move the sampling point to the river by cursor.

Fig. 1 The sampling site along the Dhaleswari River, Savar, Bangladesh

nitric acid (HNO_3) was added to the beaker. The volume of the mixture was lowered at almost 10–20 ml by digesting at 80°C on a hot plate. Until it gave a light-colored clear solution, the heating and adding of concentrated HNO_3 were continued. After digestion, the samples were cooled and then filtered using Whatman no. 42 filter paper; the filtrate was volumed to 50 ml with deionized water and stored at a cool place [23].

Digestion of Fish Samples

Homogenized samples of 2 g were taken in a 100-ml Pyrex beaker, charring at 150°C for 1 h and ash at 450°C for about 10 h using a muffle furnace. Then, the beaker was cooled down, 5 ml of 6 M HCl added, placed on a hot plate, and continued heating until dryness. Finally, 10 ml of 0.1 M

Table 1 Seven species of the studied fish samples including their feeding and living habit

Local name	Scientific name	Description of the species
Shingi fish	<i>Heteropneustes fossilis</i>	The Asian stinging catfish or fossil cat is a species of <i>Airsac</i> catfish. It can tolerate slightly brackish water, and it is omnivorous. The juveniles feed on crustaceans, plant matter, and insects while the adults feed on insects, detritus, and plant matter
Taki fish	<i>Channa punctata</i>	The spotted snakehead is a fish species of snakehead found in the Indian subcontinent and nearby areas. The species is mainly a carnivore. A favorite food of this species is other small fishes
Nurpur fish	<i>Nandus nandus</i>	Gangetic leafish is a species of Asian leafish native to South Asia and Indochina. They are common in slow-moving or stagnant water bodies, including ponds, lakes, ditches, and flooded fields of Bangladesh. <i>Nandus nandus</i> is a carnivorous fish that feeds primarily on shellfish, finfish, and other aquatic insects
Lomba Chanda fish	<i>Chanda nama</i>	The elongate glassy perchlet is a species of freshwater fish in the Asian glassfish family. The species are carnivores and feed on mosquito larvae and worms and eat the scales of other fishes (lepidophagy); the species may have potential use in controlling malaria and parasites
Koi fish	<i>Anabas testudineus</i>	The climbing perch is a fish species from the family of Anabantidae, the climbing gouramis. It is native to Asia, found in Bangladesh, India, and China. This predatory species consumes food from different feeding labels of waterbody, primarily protozoans, rotifers, crustaceans, insects, algae, diatoms, etc
Nuna-tengra fish	<i>Mystus gulio</i>	<i>Mystus gulio</i> , the long whiskers catfish, is a species of catfish of the family Bagridae, distributed around India to the Malay archipelago. It is found in Bangladesh, India, Sri Lanka, Pakistan, Nepal, and Vietnam. It is predominately a carnivore, feeding mainly on crustaceans, molluscs, and insect larvae. Primarily a low salinity brackish water fish that enters and lives in freshwater
Kholshe fish	<i>Colisa fasciata</i>	Kholshe fish is a tropical labyrinth perch found in some Asian countries like Bangladesh, Eastern India, Northeastern India, Nepal, Upper Myanmar, China, and Pakistan. The fish is omnivorous and can feed on live, frozen, and flake feeds

HNO₃ was added and heated for about 30 min. Filtering was done by using Whatman no. 42 filter paper in a 50-ml Pyrex volumetric flask and volumed up to the marks with 0.1 M HNO₃ [22]. The samples were kept in a pre-cleaned polyethylene bottle and finally ready to analyze Cr, Ni, Zn, and Pb with AAS (Varian AA-240, NJ, USA).

Quality Assurance and Quality Control

All chemicals used in this study were received from different companies. Acids (HNO₃, HCl) were supplied by Merck Germany; ultrapure nitric acid by Fisher Scientific, USA; and certified reference materials (CRM) DORM-2-dogfish mussels by the National Research Council, Canada. Quality assurance and quality control were ensured and assessed using reagent blank, method blank, and certified reference

materials as suggested by the APHA [24]. All samples were analyzed in total four batches, each with a method blank. Calibration curves with an R^2 value of a minimum of 0.9986 were considered for concentration calculation. Three replicate blank samples were digested (following fish sample digestion) to determine the limit of detection (LOD) for each metal. The accuracy of the analytical procedure of AAS was checked by the analysis of certified reference materials (CRM) DORM-2-dogfish mussels from the National Research Council, Canada. The recovery values are mentioned in Table 2. Before using DORM-2 in our study, we analyzed it in the Bangladesh Centre for Scientific and Industrial Research (BCSIR), Dhaka, and Centre for Advanced Research in Science (CARS), University of Dhaka, to check its integrity. We digested the DORM-2 fish tissues in six replicates and sent three samples to each

Table 2 Comparison between measured and certified values of heavy metal concentration as mg/kg dry weight, including the limit of detection

Metal name	Wavelength (nm)	Limit of detection (μM)	DORM-2 reference material		
			Certified value (mg kg ⁻¹)	Measured value (mg kg ⁻¹)	% recovery
Cr	357.9	0.0087	34.7 ± 5.5	33.1 ± 1.8	95.4
Ni	232.0	0.0035	19.4 ± 3.1	19.1 ± 0.9	98.5
Pb	217.0	0.0050	0.065 ± 0.007	0.063 ± 0.003	96.9
Zn	213.9	0.0086	25.6 ± 2.3	24.7 ± 1.6	96.5

laboratory. We found very accurate results in all the analyses and the values were 33.08 ± 38 and 34.16 ± 0.36 mg kg⁻¹ for Cr, 19.71 ± 0.51 and 19.64 ± 0.66 mg kg⁻¹ for Ni, 0.062 ± 0.007 and 0.06 ± 0.002 mg kg⁻¹ for Pb, and 25.95 ± 0.19 and 25.83 ± 0.14 mg kg⁻¹ for Zn.

We also used SRM 1643f reference material for the validation of water analysis results and the recovery percentage ranged from 91.5 to 96.8% for these four metals. The certified values for Cr, Pb, Ni, and Zn were 18.50 ± 0.1 , 18.488 ± 0.084 , 59.8 ± 1.4 , and 74.4 ± 1.7 µg L⁻¹, respectively, while measured values were 17.45 ± 0.07 (94.3%), 16.91 ± 0.02 (91.5%), 56.6 ± 0.41 (94.6%), and 72.05 ± 0.82 (96.8%) µg L⁻¹, respectively.

Statistical Analysis

The collected data were analyzed statistically using Microsoft Excel, Minitab-18, and SPSS-25 software following correlation matrix and presented in a graph, chart, and text form. The correlation coefficient matrix measures how well each constituent's variance can explain its relationship with others [25]. Limit of detection (LOD) was determined by using $\text{LOD} = \text{Reagent blank} + 3s$ formula, where s is the standard deviation. Reagent blank was calculated from seven blank replicates and standard deviation (s) was calculated from seven replicates of DORM-2 fish samples.

Assessment of Health Risk Due to Heavy Metal Pollution

Bioaccumulation Factor

The bioaccumulation factor reflects the amplitude of the metal concentration level within the accumulative fish organs over time [26, 27]. BAF is to assess by the ratio between metal concentration in the fish muscles and those in the water environment [28, 29].

$$\text{BAF} = \frac{\text{CM}_{\text{fish}}}{\text{CM}_{\text{water}}} \quad (1)$$

where CM_{Fish} is the metal concentration in fish tissue (mg/kg), and CM_{Water} is the metal concentration in a water medium (mg/l). Besides, BAF is categorized according to the following ranges: $\text{BAF} < 1000$: less probability of accumulation; $1000 < \text{BAF} < 5000$: bioaccumulative; $\text{BAF} > 5000$: highly bioaccumulative [30].

Estimated Daily Intakes (EDIs)

Estimated daily intakes (EDIs) determine the risks associated with the consumption of metal in food [31]. EDI was

calculated using the individual average concentration in food samples by the weight of food item consumed by a person (body weight 60 kg for adult in Bangladesh), which was obtained from the household income and expenditure survey [32], and EDI provided by the USEPA can be determined by using the following equation [33–35]:

$$\text{EDI} = (\text{FIR} \times C)/\text{BW} \quad (2)$$

where FIR is the fish ingestion rate (g/person/day), C is the heavy metal concentration in fish samples (mg/kg), and BW is the body weight assuming 60 kg for adult residents [36]. The daily consumption rate of fish by that city's population was 59.91 g on a wet weight basis [37].

Non-carcinogenic Risks

The non-carcinogenic risk was estimated using the method of the U.S. Environmental Protection Agency [38]. The non-carcinogenic risk for each specific metal through fish consumption was determined by the target hazard quotient (THQ) [39], which can be calculated using the following equation:

$$\text{THQ} = \left(\frac{\text{Efr} \times \text{ED} \times \text{FIR} \times C}{\text{RfD} \times \text{BW} \times \text{AT}} \right) \times 10^{-3} \quad (3)$$

$$\text{Total THQ (TTHQ)} = \text{THQ}_{\text{toxicant 1}} + \text{THQ}_{\text{toxicant 2}} + \dots + \text{THQ}_{\text{toxicant n}} \quad (4)$$

where THQ is the target hazard quotient, EFr is the exposure frequency (365 days/year), ED is the exposure duration (70 years), FIR is the fish ingestion rate (g/day), C is the heavy metal concentration in fish (mg/kg), and AT is the averaging time for non-carcinogens (365 days/year \times number of exposure years). The oral reference doses for Cr, Ni, Pb, and Zn were 0.003, 0.02, 0.0035, and 0.3, respectively [38]. In case the THQ values exceed the limit of 1, a potential health risk might occur, and relative protective measures should be taken [40].

In determining the non-carcinogenic risk from multiple heavy metals, a hazard index (HI) has been widely used as provided by the guideline of the USEPA [41]. Using the sum of the hazard quotients, the hazard index was calculated [38]. The formula of the hazard index is as follows:

$$\text{HI} = \sum \text{Total THQ} = \text{TTHQ}_{\text{fish 1}} + \text{TTHQ}_{\text{fish 2}} + \dots + \text{TTHQ}_{\text{fish n}} \quad (5)$$

$$\text{TTHQ (individual fish)} = \text{THQ}_{\text{toxicant 1}} + \text{THQ}_{\text{toxicant 2}} + \dots + \text{THQ}_{\text{toxicant n}} \quad (6)$$

Carcinogenic Risks

The carcinogenic risks related to Cr, Pb, and Ni were determined using the following equation provided by the USEPA:

$$TR = \left(\frac{EFr \times ED \times FIR \times C \times CSF}{BW \times AT} \right) \times 10^{-3} \quad (7)$$

where CSF is the oral carcinogenic slope factor provided by the USEPA [38]. The CSF values for Cr, Ni, and Pb were 0.041, 0.0017, and 0.0085 mg/kg/day, respectively.

Results and Discussions

The heavy metal concentrations (Cr, Ni, Zn, and Pb) of the eighteen water samples collected from 6 fish sampling area are shown in Table 3. Among the four heavy metals, it was evident that the Cr, Pb, and Zn concentration of the water sample crossed the permissible limit of ECR [42] and the USEPA [43]. The sequence of heavy metal concentrations in the river water is $Zn > Cr > Ni > Pb$. These metals are carcinogenic and have many other health-related issues [44]. Chromium is the major heavy metal that tannery industries had released since a large number of chromium salts were used for tanning processes. The high concentration of Pb and Zn in the Dhaleswari River might be due to tannery effluents, domestic construction and car-associated sources, untreated dyeing wastes, and so on [22]. Among the harmful metals, Cr, Pb, and As are liable for some intense and constant illnesses, for example, skin and mucous layer aggravation, sharpening, consumption, dermatitis, and persistent ulceration [45].

Heavy Metal Concentrations in Fish Samples

The metal accumulation in foodstuffs signifies environmental contamination that could lead to human health risk [46]. Metal contamination in fish samples is concerning because it directly affects human health. The metal concentration in fish samples varies with species, body parts, and trophic level.

Table 3 Studied heavy metal concentrations and their permissible limit of water sample

Heavy metals	Mean concentration (mg/l)	Permissible limit (mg/l)
Chromium (Cr)	0.653 ± 0.05	0.5 mg/l; ECR [42]
Nickel (Ni)	0.395 ± 0.148	0.47 mg/l; USEPA [43]
Lead (Pb)	0.225 ± 0.035	0.10 mg/l; USEPA [43]
Zinc (Zn)	1.341 ± 0.049	0.12 mg/l; USEPA [43]

Chromium Concentration in Fish Samples

The Cr concentration in fish samples ranged between 1.60–116.70 and 3.85–14.45 mg/kg in the body and head, respectively, that exceeded the recommended value (0.15 mg/kg) of the FAO [47]. The measured mean concentration of Cr in all the fish species is organized in the following order: *Anabas testudineus* (62.93 mg/kg) > *Colisa fasciata* (56.00 mg/kg) > *Heteropneustes fossilis* (16.03 mg/kg) > *Nandus nandus* (7.93 mg/kg) > *Mystus gulio* (5.18 mg/kg) > *Chanda nama* (4.65 mg/kg) > *Chanda nama* (4.03 mg/kg). The highest concentration was observed in the body sample of *Anabas testudineus*. The lowest concentration was recorded in the body sample of *Chanda nama*. Figure 2 indicates that the body part of fish samples showed a higher level of accumulation than the head part, which also varied with the species. The higher level of chromium in Dhaleswari River fishes could be exposed to a large amount of untreated tannery effluent every day. Bashar et al. [48] found a similar chromium level in the Buriganga River fishes, ranging from 49.36 to 164.73 mg/kg. On the other hand, Brishti et al. [49] found a lower chromium level (0.322–0.407 mg/kg) in the Shitalakkhya River fishes.

Lead Status in Fish Samples

The Pb concentration in the body and head of fish samples ranged from 1.5–8.5 to 3–11.5 mg/kg, respectively (Fig. 3). A higher concentration of Pb was found in the head of five fish species, namely *Channa punctata*, *Nandus nandus*, *Chanda nama*, *Mystus gulio*, and *Colisa fasciata*, whereas *Heteropneustes fossilis* and *Anabas testudineus* species contained higher Pb in their body parts. The recommended MPL value of Pb impurities provided by the FAO is 0.50 mg/kg [47] which suggests a highly polluted condition of the Dhaleswari River. Bashar et al. [48] observed a similar kind of Pb accumulation in fish samples of the Buriganga River, ranging from 11.05 to 18.16 mg/kg, whereas a study in the Shitalakkhya River by Brishti et al. [49] showed a lower concentration (0.01–0.3159 mg/kg) than this study. The elevated concentrations of Pb in fishes might come from dyeing industries, Pb battery industries, and impurities of salts used in tannery industries.

Nickel Status in Fish Samples

The highest and lowest concentrations of Ni were determined in the head sample of *Colisa fasciata* and *Nandus nandus*, respectively (Fig. 4). In this experiment, body parts showed higher Ni concentrations in four species and head in three species. Results showed that Ni concentrations in fish samples are low and ranged from 1.7 to 8.1 and nearly not detectable in *Nandus nandus* to 8.5 mg/kg in the body

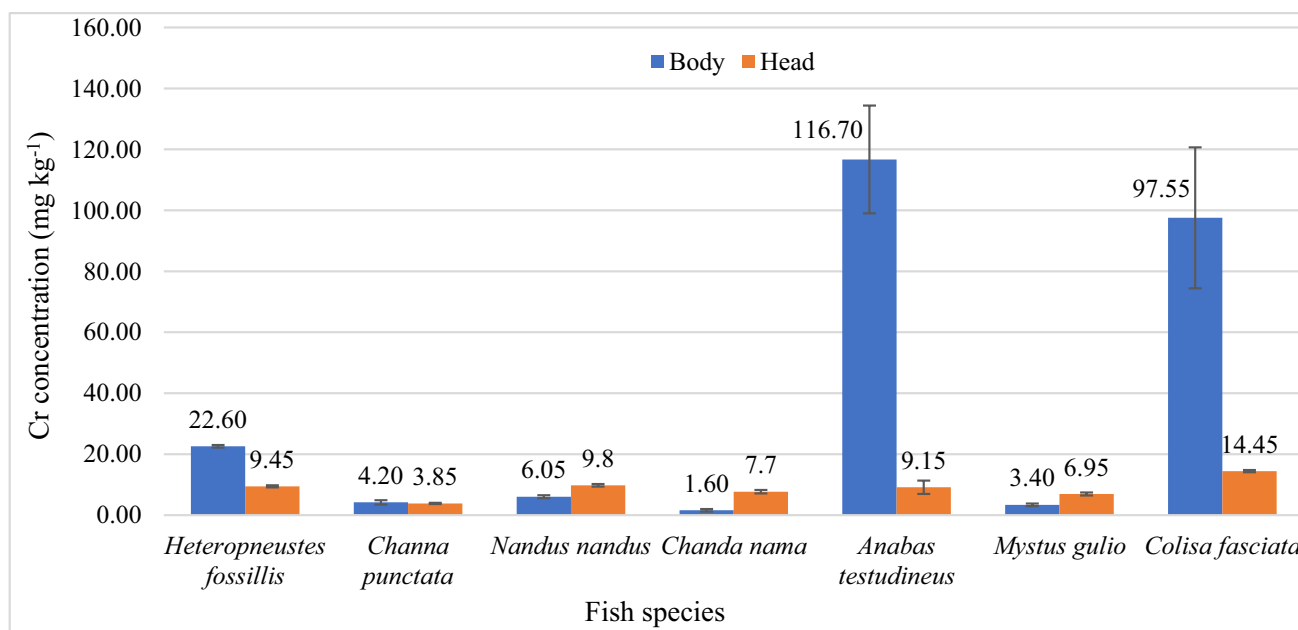


Fig. 2 Variation of Cr concentration in the body and head of seven fish species ($n=6$)

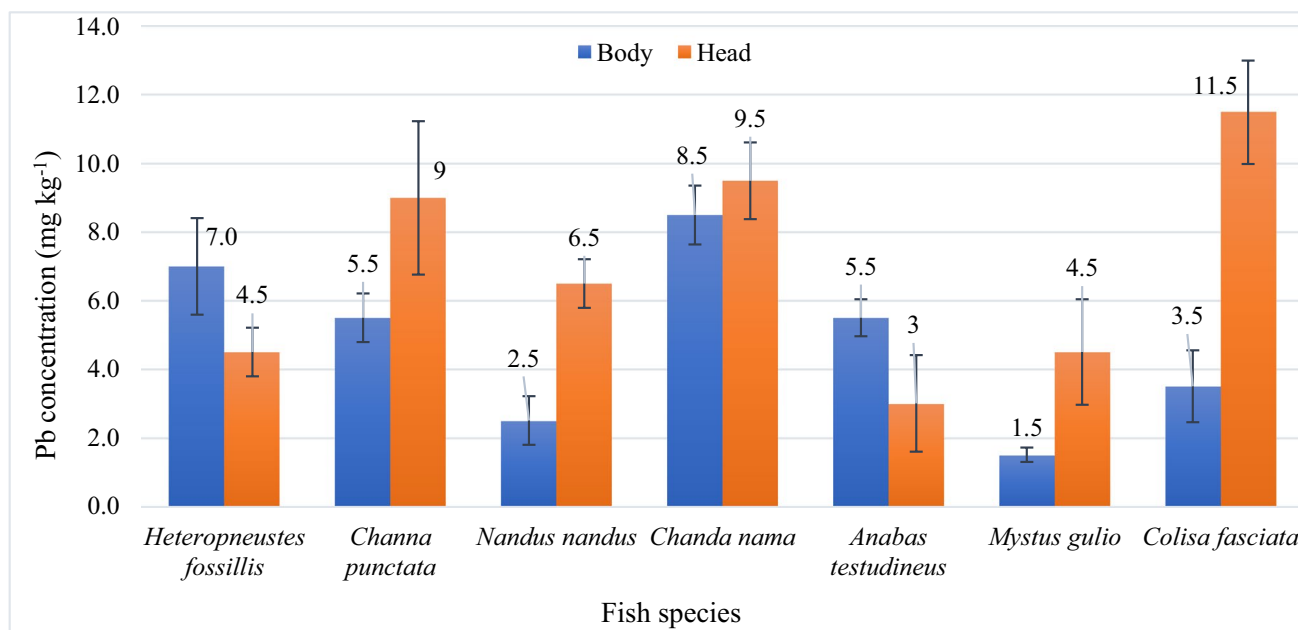


Fig. 3 Variation of lead concentration in the body and head of seven fish species ($n=6$)

and head, respectively. The recommended MPL value of Ni impurities provided by the FAO was 10 mg/kg [47], indicating that no samples crossed the maximum limit. Malik et al. [50] found a lower concentration (0.207–1.21 mg/kg) in the freshwater lake of Bhopal which was lower than these findings.

Zinc Concentration in Fish Samples

In all the fish samples, Zn concentration was highest among the four heavy metals. The Zn concentration in fish samples varied from 57.09 to 163.88 and 95.35–314.47 mg/kg in the body and head, respectively. The measured mean concentration of Zn in all the fish

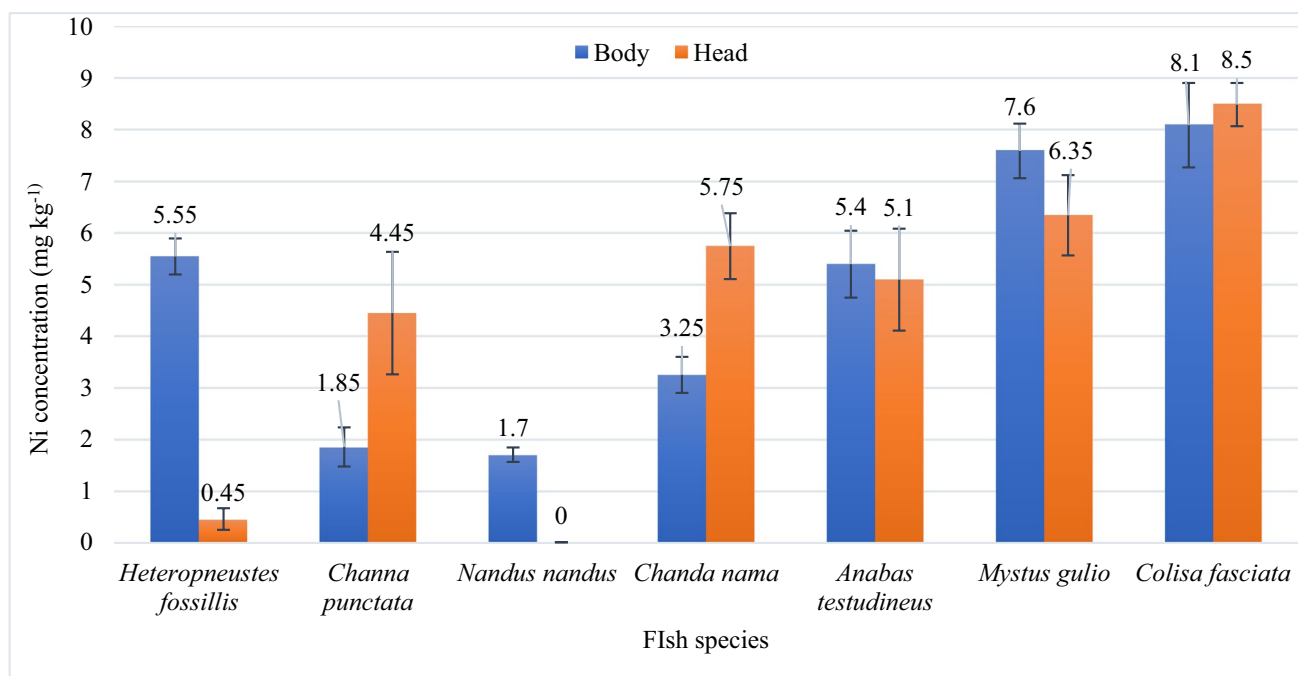


Fig. 4 Variation of nickel concentration in the body and head of seven fish species ($n=6$)

species is organized in the following order: *Chanda nama* (204.56 mg/kg) > *Channa punctata* (136.86 mg/kg) > *Colisa fasciata* (120.16 mg/kg) > *Nandus nandus* (107.21 mg/kg) > *Mystus gulio* (91.48 mg/kg) > *Heteropneustes fossilis* (90.40 mg/kg) > *Anabas testudineus* (76.34 mg/kg) (Fig. 5). Zinc concentration in this study

was higher than the WHO/FAO-approved maximum limit of 30 mg/kg [47] and this could be associated with anthropogenic activities, such as transportation activities, using chemicals and Zn-based fertilizers, oil wastes, and petrochemicals from the nearby welder and automobile mechanic workshops [51]. Bashar et al. [48] found

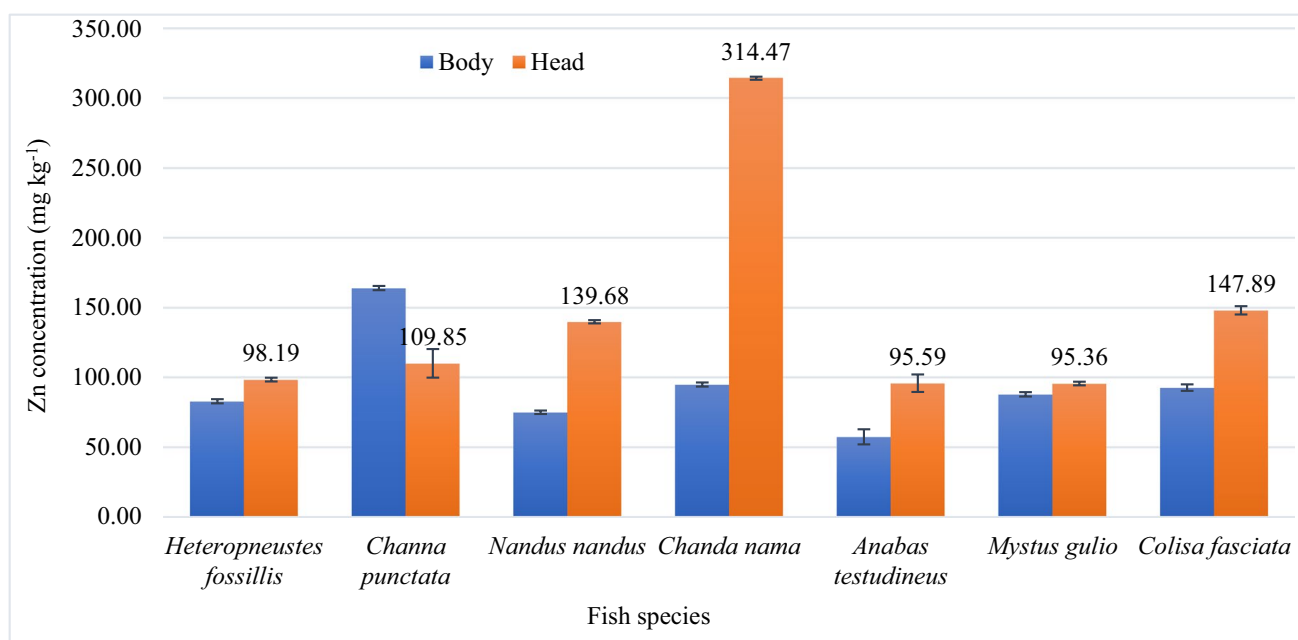


Fig. 5 Variation of zinc concentration in the body and head of seven fish species ($n=6$)

Table 4 Correlation matrix between the trace metals in seven fish species (body and head combined)

Heavy metals	Cr	Ni	Pb
Ni	0.518		
Pb	− 0.066	0.037	
Zn	− 0.407	− 0.051	0.840*

*Correlation was significant at $p \leq 0.05$ level (2-tailed)

a similar kind of Zn accumulation in fish samples of the Buriganga River, ranging from 29.7 to 338.5 mg/kg.

This study suggested that the Dhaleswari River has a very high frequency of Cr, Pb, and Zn pollution and this river will become uninhabitable for aquatic organisms very soon if this pollution trend is allowed to continue.

Correlation Matrix

The Pearson correlation matrix of analyzed metals of the fish samples is presented in Table 4. The statistical analysis showed a positive correlation ($r = 0.840$) between Pb and Zn which was significant at $p \leq 0.05$ level. Such correlation indicated that the sources and the characteristics of the metals of the corresponded specimens might be similar. However, no significant interaction was found between other metals.

A two-way ANOVA study displayed that heavy metal concentrations in seven fish species were significantly varied in the Dhaleswari River (Table 5). This suggests that heavy metal accumulation varied significantly from species to species and between the head and body of the same species.

Table 5 Two-way ANOVA for four trace metals in seven fish species

Source of variation	SS	df	MS	F	p-value	F crit
Two-way ANOVA (body)						
Fish species	9880.48	6	1646.747	102.998	2.06E-28**	2.266
Metals	109,614.30	3	36,538.110	2285.335	1.66E-58**	2.769
Interaction	54,614.99	18	3034.166	189.776	1.74E-43**	1.791
Within	895.33	56	15.988			
Total	175,005.10	83				
Two-way ANOVA (head)						
Fish species	29,754.92	6	4959.154	1360.043	9.36E-59**	2.266
Metals	292,860.30	3	97,620.100	26,772.220	2.44E-88**	2.769
Interaction	81,970.87	18	4553.937	1248.913	3.48E-66**	1.792
Within	204.19	56	3.646			
Total	404,790.3	83				

**Correlation was significant at $p \leq 0.01$ probability level

Table 6 Bioaccumulation factor (BAF) value in the body and head of seven fish species

Species	Sample type	Cr	Ni	Pb	Zn
<i>Heteropneustes fossilis</i>	Body	3460.95	1405.06	3111.11	6161.07
	Head	1447.17	113.92	2000	7322.15
<i>Channa punctata</i>	Body	643.19	468.35	2444.44	12,220.73
	Head	589.59	1126.58	4000	8191.65
<i>Nandus nandus</i>	Body	926.49	430.38	1111.11	5572.71
	Head	1500.77	0	2888.89	10,416.11
<i>Chanda nama</i>	Body	245.02	822.78	3777.78	7058.17
	Head	1179.17	1455.70	4222.22	23,450.41
<i>Anabas testudineus</i>	Body	17,871.36	1367.09	2444.44	4257.27
	Head	1401.23	1291.14	1333.33	7129.01
<i>Mystus gulio</i>	Body	520.67	1924.05	666.67	6532.44
	Head	1064.32	1607.59	2000.00	7110.37
<i>Colisa fasciata</i>	Body	14,938.74	2050.63	1555.56	6892.62
	Head	2212.86	2151.90	5111.11	11,027.59

Bioaccumulation Factor (BAF)

Bioaccumulation factors (BAFs) of heavy metals in the fish species of the Dhaleswari River are depicted in Table 6. BAF determines the numbers obtained from the fraction of fixed concentration in a specific organ, particularly in the body and head of a fish species and the surrounding environment quickly and effectively [26]. In this study, the highest value of BAF was recorded for Zn (23,450.41) in the head sample of *Chanda nama*, followed by Cr (17,871.36), Pb (5111.11), and Ni (2151.90). For particular species concern, the BAFs were organized in the following order with the range: *C. nama* > *A. testudineus* > *C. fasciata* > *C. punctata* > *N. nandus* > *H. fossilis* > *M. gulio*. However, the BAF values varied with body parts and species.

Data in Table 6 indicated that BAF values exceeded the limit value in most of the species, especially *C. nama*, *A. testudineus*, and *C. fasciata*, and thus, these species were in the highly bioaccumulative state. The fluctuation could be showed up because of various water valences related to the distinctive valence of the organisms that have fluctuated bioavailability [52].

Estimated Daily Intake (EDI)

Estimated daily intake determines dietary exposure of trace elements for the consumption of fish species in the daily diet of adult people in the study area. Data in Table 7 showed that the gross EDI values for Cr, Ni, Pb, and Zn were 0.157, 0.031, 0.04, and 0.825, respectively. According to the table, the determined values were lower than the maximum tolerable daily intake (MTDI) values; however, long period consumption of contaminated fish from the target area may cause serious health complications to the consumers [53]. This is probably due to the lower

Table 7 Comparison of estimated daily intake (EDI) from this study and recommended allowances for seven fish species

Fish species	Estimated daily intake (EDI)			
	Cr	Ni	Pb	Zn
<i>Heteropneustes fossilis</i>	0.016	0.003	0.006	0.090
<i>Channa punctata</i>	0.004	0.003	0.007	0.137
<i>Nandus nandus</i>	0.008	0.001	0.004	0.107
<i>Chanda nama</i>	0.005	0.004	0.009	0.204
<i>Anabas testudineus</i>	0.063	0.005	0.004	0.076
<i>Mystus gulio</i>	0.005	0.007	0.003	0.091
<i>Colisa fasciata</i>	0.056	0.008	0.007	0.120
Total	0.157	0.031	0.04	0.825
MTDI	0.23 ^b	0.35 ^b	0.25 ^a	70 ^a

^aCalculated from WHO [54]; ^bcalculated from Alipour et al. [55]

Table 8 Non-carcinogenic risk of heavy metals due to consumption of seven fish species

Fish species	Target hazard quotients (THQs)				Hazard index (Total)
	Cr	Ni	Pb	Zn	
<i>Heteropneustes fossilis</i>	5.25	0.148	1.62	0.296	7.31
<i>Channa punctata</i>	1.32	0.155	2.04	0.449	3.96
<i>Nandus nandus</i>	2.60	0.042	1.26	0.351	4.26
<i>Chanda nama</i>	1.52	0.221	2.53	0.670	4.94
<i>Anabas testudineus</i>	20.63	0.258	1.19	0.250	22.33
<i>Mystus gulio</i>	1.70	0.343	0.84	0.300	3.18
<i>Colisa fasciata</i>	18.36	0.408	2.11	0.394	21.26

Bolds are values > 1

consumption of fish by Bengali people. The gross EDI values for Cr, Ni, Pb, and Zn were 0.157, 0.031, 0.04, and 0.825.

Non-carcinogenic Risk

The target hazard quotient (THQ) and hazard index (HI) values of heavy metals (Cr, Ni, Pb, and Zn) in fish samples are presented in Table 8. The bold values were greater than 1 and indicated danger for human consumption. The THQ for four heavy metals was in descending order of Cr > Pb > Ni > Zn. The THQ of Cr and most of the Pb were higher than the permissible limit of 1. The high value indicates contamination of Cr in the Dhaleswari River. After continual consuming these contaminated fishes, humans might suffer from different non-carcinogenic diseases for increased Cr and Pb exposure. Taking one single metal (Cr) under consideration, the fish species were categorized in the following decreasing order: *Anabas testudineus* > *Colisa fasciata* > *Heteropneustes fossilis* > *Nandus nandus* > *Mystus gulio* > *Chanda nama* > *Channa punctata*. The highest THQ was found in *Anabas testudineus* (20.63) and the lowest THQ was recorded in *Channa punctata* (1.32). The THQ values for Ni and Zn were below the limit of 1, which indicated that these two metals were not hazardous.

In the case of HI (hazard index), the species followed the descending order of *Anabas testudineus* > *Colisa fasciata* > *Heteropneustes fossilis* > *Chanda nama* > *Nandus nandus* > *Channa punctata* > *Mystus gulio*. *Anabas testudineus* showed the highest HI value (22.33) among all. This high THQ and HI value of heavy metals in fish species might pose severe human health problems in Dhaleswari River area of Bangladesh.

Table 9 Carcinogenic risk of the heavy metals due to consumption of seven fish species

Species	Carcinogenic risk			Recommended limit
	Cr	Ni	Pb	
<i>Heteropneustes fossilis</i>	6.56e⁻⁰⁴	5.09e ⁻⁰⁴	4.88e ⁻⁰⁴	If CR < 10 ⁻⁶ , the cancer risk is negligible If CR > 10 ⁻⁴ , the cancer risk is high
<i>Channa punctata</i>	1.65 e⁻⁰⁴	5.35e ⁻⁰⁴	6.15e ⁻⁰⁴	
<i>Nandus nandus</i>	3.25e⁻⁰⁴	1.44e ⁻⁰⁴	3.82e ⁻⁰⁴	
<i>Chanda nama</i>	1.90e⁻⁰⁴	7.64e ⁻⁰⁴	7.64e ⁻⁰⁴	
<i>Anabas testudineus</i>	0.00258	8.91e ⁻⁰⁴	3.61e ⁻⁰⁴	
<i>Mystus gulio</i>	2.12e⁻⁰⁴	1.18e ⁻⁰⁴	2.55e ⁻⁰⁴	
<i>Colisa fasciata</i>	0.00229	1.41e ⁻⁰⁴	6.37e ⁻⁰⁴	

Bold values are greater than e⁻⁰⁴

Carcinogenic Risk

The carcinogenic risk (TR) was determined from the concentration of Cr, Pb, and Ni in fish species. The cancer risk value of Cr ranged from 6.56e⁻⁰⁴ to 0.00258, Ni ranged from 8.91e⁻⁰⁶ to 1.18e⁻⁰⁵, and Pb ranged from 2.55e⁻⁰⁵ to 7.64e⁻⁰⁵ (Table 9). The USEPA recommended range for cancer risk is between 10⁻⁶ and 10⁻⁴; a value higher than 10⁻⁴ is not safe for humans that could cause cancer [38]. Among the fish species, *Anabas testudineus* and *Colisa fasciata* showed a higher value (Cr) than 10⁻⁴ suggesting a high carcinogenic risk. Therefore, consumption of the present study fish species might expose the people around the Dhaleswari River area to lifetime cancer risk.

Conclusion

This study concentrated on determining the heavy metal accumulation (Cr, Pb, Ni, and Zn) in seven fish species of the Dhaleswari River and related risk assessment. The studied heavy metals (Cr, Pb, and Zn) indicated a higher concentration than FAO/WHO guideline values. The bioaccumulation factor (BAF) was also higher than the standard limit. The BAF value for Zn was found highest in *Chanda nama* fish species. The higher THQ values of Cr and Pb designated non-carcinogenic risk while the greater value of TR for Cr indicated carcinogenic risk on the health of people in the study area of Bangladesh. The species *Anabas testudineus* and *Colisa fasciata* showed the highest THQ and TR values. Although EDIs of all elements were less than the recommended daily allowance, possible bioaccumulation and health risk can still occur from continuous or high consumption of contaminated fishes. Moreover, the correlation analysis emphasized that the sources and the characteristics of the metals (Zn, Pb) of the corresponded specimens might be similar. The contamination of heavy metal in fish tissues of the Dhaleswari River must need serious attention from all the relevant parties to monitor and mitigate the situation.

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Author Contribution Md Wahiduzzaman and Zakia Parveen conceived and planned the experiments. Md Wahiduzzaman and Md Mahfuz Islam carried out the experiment, performed data analysis, and wrote the manuscript with inputs from other authors. Abdul Halim Farhad Sikder contributed to sample collection and manuscript preparation. This research was supervised by Zakia Parveen.

Data Availability Not applicable

Declarations

Consent to Participate The article does not contain any studies with human participants but fish samples from the Dhaleswari River were used for this study.

Consent for Publication We declare that this manuscript is original, has not been published before, and is not being currently considered for publication elsewhere.

Competing Interests The authors declare no competing interests.

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