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Original article

Human health risk assessment for exposure to heavy metals in finfish and shellfish from a tropical estuary

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ABSTRACT

This investigation measured the extent of heavy metal concentrations (Cr, Pb, Fe, Zn and Ni) in nine fin-fish and shellfish species from a tropical estuary to determine the degree of pollution and evaluate the human health risk from the exposure to heavy metals through consumption of fish. The hierarchy of mean concentrations (mg/kg) of the heavy metals were: Zn (398.47) > Fe (83.015) > Cr (0.906) > Ni (0.233) > Pb (0.128). The metal concentrations in the edible tissues were ranked in the following sequence: *A. bato* > *C. cirrhosus* > *A. grammepomus* > *M. dobsoni* > *P. paradiseus* > *O. pama* > *M. rosenbergii* > *N. smithi* > *S. phasa*. The results demonstrated that the concentration of Cr and Zn was considerably higher than the guideline values of FAO. Estimated daily intake (EDI), hazard quotient (HQ), hazard index (HI), and cancer risk (CR) values were within the acceptable threshold for both adults and children. However, the EDIs of local consumers in the research area for both adults and children were lower than the recommended daily amount (RDA), following the order of Zn > Fe > Cr > Ni > Pb. The HQs of the elements followed the order of Zn > Cr > Fe > Pb > Ni. The investigated HI was not exceeding the recommended limit.

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1. Introduction

Heavy metals (HMs) in the aquatic ecosystem are one of the most important concerns globally because of their potentially harmful effects and bioaccumulation in aquatic ecosystems (Hossain et al., 2022; Kahal et al., 2020). Thus, it can damage aquatic species diversity and ecosystems (Ayanda et al., 2019; Qian et al., 2020). The concern is becoming increasingly severe worldwide, particularly in developing countries such as Bangladesh (Hossain et al., 2019). Pollution by HMs in natural water bodies adversely affects aquatic life and carries many environmental risks and concerns (Alahabadi and Malvandi, 2018; Ullah et al., 2019). Furthermore, the availability of HMs in fish weakens the consumer's health and interrupts the activities of beneficial compounds of the human body (Mahboob et al., 2020). Therefore, the increasing degree of pollution in the aquatic ecosystems is a threat to both fish and human health (Ustaoğlu and Islam, 2020; Yang et al., 2020).

HMs can occur through natural and anthropogenic activities in the estuarine environment (Liu et al., 2020; Siddiqui and Pandey, 2019). Anthropogenic activities include wastewater discharge,

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industrial wastewater, agricultural and coastal land runoff, while physical and chemical weathering of rocks, erosion and atmospheric deposition are considered as natural activities (Kang et al., 2019; Qu et al., 2018). In addition, any changes in environmental conditions such as pH, oxidation–reduction potential (Eh), salinity, and organic matter, then heavy metals can be dissolved into water and fixed into sediments which create secondary contamination (Islam et al., 2015; Lee et al., 2017; Nour et al., 2018). Sea temperature also affects the biological production rate, hence, the impact on fish abundance, distribution and migration pattern (Habib, 2010; Islam et al., 2020). Some industrial sources such as petroleum combustion, nuclear power stations, high voltage lines, coal burning in power plants, metal processing in refineries, plastics, textiles, microelectronics, wood preservation, and paper processing plants are also responsible for HMs discharges (Akindele et al., 2020).

Fish and shellfish are a significant part of the human diet, particularly in coastal areas (Ahmed et al., 2019a; Ali et al., 2020; Hossain et al., 2019) and on smaller islands, providing all the essential elements necessary for life processes in a balanced way (El-Sorogy et al., 2018). Furthermore, shellfish, particularly shrimps, is also the main exportable item in countries such as Bangladesh and is therefore closely linked to the country's economy (Hossain et al., 2019; Islam et al., 2016). Therefore, it is important to investigate the heavy metal levels in these organisms in order to assess whether the concentration is within the permissible levels and the potential to pose any health risks to consumers (Ali et al., 2019; Lee et al., 2019). Subsequently, fish have often been considered the most suitable organisms among aquatic ecosystem bioindicators because they occupy a high trophic level and are an essential food source for human population (Ahmed et al., 2019a Zhu et al., 2020). Therefore, metal contents in fish tissues and organs indicate the levels of metals in water and their accumulation in food chains (Ali et al., 2019; Liu et al., 2020).

Karnaphuli River in Bangladesh is the largest industrial River in Chittagong (the second most populous city in Bangladesh with nearly four million people) and maritime port area. The Karnaphuli River estuary plays an important role in breeding, feeding, and nursery ground for various aquatic species alongside the Bay of Bengal. As the estuary is located in the catchment area of a heavily industrially developed area, Chattagram port city, it has been polluted receiving discharges from multiple point and non-point sources e.g., solid waste from household and commercial activities, effluents from textile and cement industries, ship recycling, oil refineries, tanneries, spinning mills, paint manufacturing and dyeing plants, paper and rayon mills, cotton mills, ships, steel and engineering factories, fertilizer and other chemical industries (Ali et al., 2016). These solid and liquid wastes contain almost all of the hazardous metals and metalloids, including arsenic (As), chromium (Cr), cadmium (Cd) and lead (Pb) (Ahmed et al., 2019a).

Several studies have attempted to assess the status of heavy metal contamination from Bangladesh's River and estuarine environment (Ahmed et al., 2019b; Ali et al., 2018; Bhuyan et al., 2017; Hossain et al., 2019; Islam et al., 2017). A few studies have been conducted on the Karnaphuli River with limited metal analysis, and most of them are only in sediment and water (Ahmed et al., 2019a; Ali et al., 2016). Moreover, that River is potential for many aquatic species for their breeding, feeding and nursery ground purposes (Ahmed et al., 2019a). But there has been no limited investigation conducted on the Karnaphuli River estuary to assess the extent of heavy metal concentration in finfish and shellfish. Therefore, the present study was performed to determine the degree of heavy metal pollution in Karnaphuli River estuary, their sources and assess health risk for humans through fish and shellfish consumption.

2. Materials and methods

Ethical statement

Fish and shellfish samples were collected from the study area/ stations. Those species are not on the IUCN list of endangered or protected species. The permission was taken from Research Cell, Noakhali Science and Technology University to catch specimens. The letter number is RCNSTU/2019/82.

2.2. Description of the study area

The study area is located on the Karnaphuli River, passing through Chittagong City, near the Bay of Bengal. This River is Chittagong 's largest River, native to the Lushai hills in India's Mizoram State. The finfish and shellfish samples were collected from various Karnaphuli River estuary stations (Fig. 1).

2.3. Sample collection

Nine finfish and shellfishes were selected for assessing their metal levels. The studied species were selected based on their habitat preferences (e.g., surface, mid or bottom), feeding habits (e.g., carnivores/ herbivores/omnivores) and economic importance. A total of 27 samples of nine fish and shellfish species were collected from different stations of the Karnaphuli River estuary (marked as S1, S2, S3, S4, S5) (Table 1, Fig. 1) in August 2019. By storing them in an ice box, all samples were kept frozen, and transported to the laboratory as soon as possible. After measuring each fish's physical parameters (weight and length), the scales, bones, and viscera were removed to fillet. Next, they rinsed the samples with purified water to remove foreign particles. After that, the samples were dried in an oven at 60 °C for 48 h, grounded into powder, and homogenized.

2.4. Determination of heavy metal contents

The AAS (Model: AA-700, SHIMADZU, JAPAN) was used to analyze the collected finfish and shellfish samples using the standard analytical procedure. The analyses of the trace elements were carried out in the laboratory in the Bangladesh Council for Scientific and Industrial Research (BCSIR), Dhaka. Recommended clean powder-free latex gloves and lab coats were used to avoid contamination during handling of the samples. Chromic acid solution and distilled water were used to clean the glassware properly. The present study utilized analytical-grade chemicals and reagents. Blank determinations were used as a control for obtaining the correct instrument readings.

2.5. Sample preparation

In a Teflon vessel with a mix of 5 ml of hydrofluorinated-nitric-perchloric acid, a known amount (0.5 g) of dried powder was digested. The reaction blend was heated to dryness at 80 °C. The residue was dissolved in 3 ml (Merck, supra-pure) diluted hydrochloric acid. Then, the digested solution was filtered through a filter paper of Whatman No. 42 and diluted to 10 ml with 2% HCl, used for Atomic Absorption Spectrophotometer metal determination (AA-7000 SHIMADZU, Japan). Triple estimates were made, and the results were expressed as mg / kg or ppm dry.

2.6. Sample analysis

A spectrophotometer for atomic absorption was developed with a flammable condition, and observance absorption optimized for
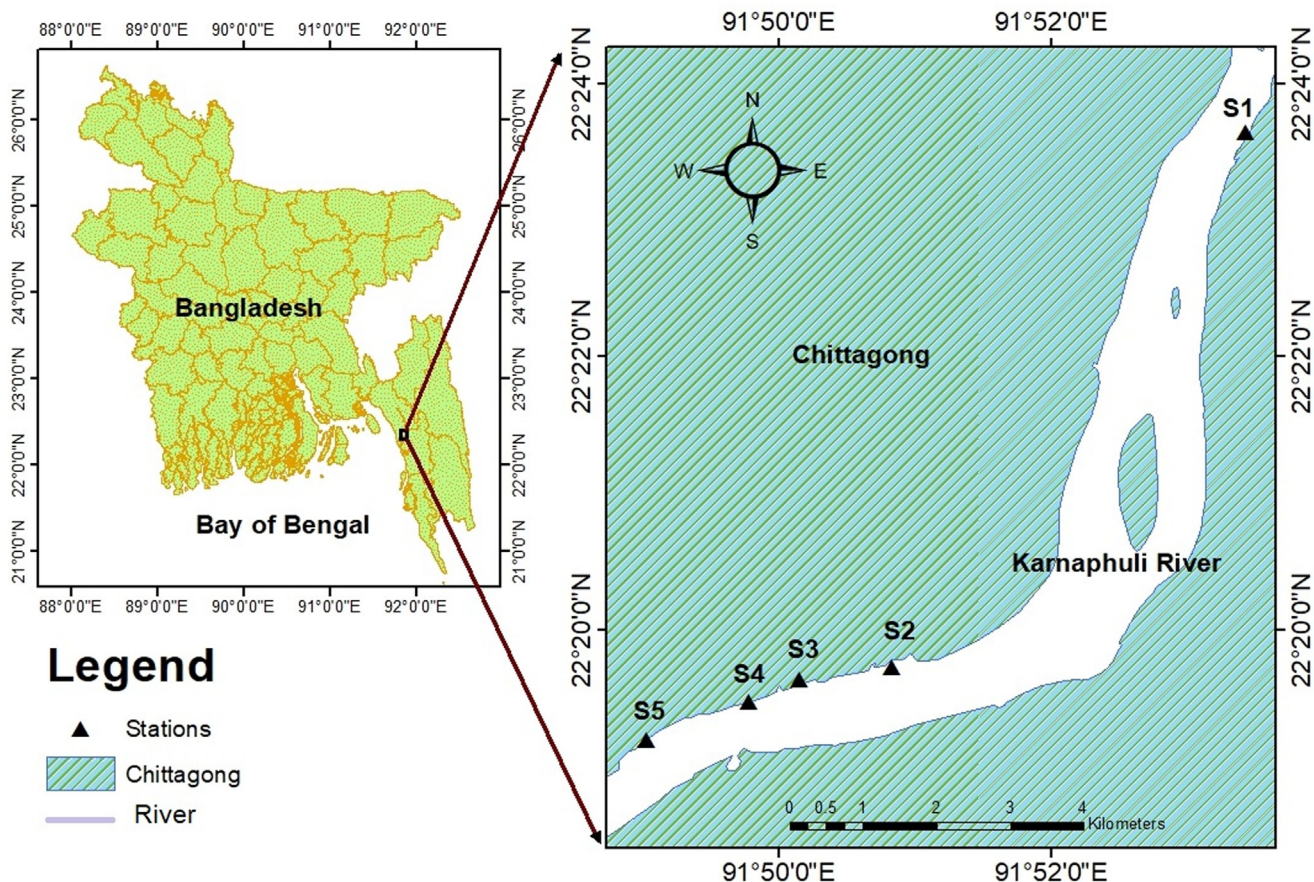
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Fig. 1. Sampling locations in the study area (Karnaphuli River estuary).

Table 1
Feeding habit, length & weight (mean ± SD) of fish and shellfish samples collected from Karnaphuli River estuary.

Local name	Scientific name	Feeding habit	Length (cm)	Weight (gm)
Poa	*Otolithoides pama*	Carnivorous	22.9 ± 1.714	83.68 ± 11.332
Baila	*Awaous grammepomus*	Omnivorous	23.93 ± 0.665	111.37 ± 23.11
Faisa	*Setipinna phasa*	Omnivorous	19.6 ± 0.565	36.816 ± 3.152
Taposi	*Polynemus paradiseus*	Carnivorous	18.0 ± 0.707	34.77 ± 3.282
Mrigal	*Cirrhinus cirrhosus*	Herbivorous	30.26 ± 1.934	249.7 ± 48.903
Chiring	*Apocryptes bato*	Carnivorous	10.0 ± 0.0816	6.276 ± 1.472
Golda chingri	*Macrobrachium rosenbergii*	Omnivorous	15.0 ± 0.408	26.706 ± 2.727
Gosha chingri	*Metapenaeus dobsoni*	Omnivorous	6.6 ± 0.993	1.973 ± 0.977
Daga Shamuk	*Neritina smithi*	Herbivorous	2.2 ± 0.081	6.486 ± 0.564

testing. Then in AAS (AA-7000 Model SHIMADZU) the blanks (de-monized water), standards, sample blank and samples were sucked into the flame. The curves of calibration for concentration vs. absorption. Data were statistically analyzed using the least square method to fit a straight line. There was also a blank reading, and necessary corrections were made in calculating the concentration of different elements. The wavelength and instrumental detection limit (mg/l) for different heavy metals are presented in Table 2.

2.7. Quality control

All instruments, glassware, and plastics were washed in a nitric acid solution for 15 min before being rinsed with deionized water. Throughout the study, analytical grade reagents (Merck; Germany) and de-ionized water were used for analysis. The certified refer-

Table 2
Wavelength and instrumental detection limit (mg/l) for different heavy metals.

Elements	Wavelength(nm)	Instrumental detection limit(mg/L)
Pb	217.0	0.013
Cr	357.9	0.1
Ni	232.0	0.01
Zn	213.9	0.1
Fe	248.3	0.1

ence material (CRM 320, Merck KGaA, Darmstadt, Germany) was used for validation and accuracy. In the applied analytical method, the recovery rate of the studied metals ranged from 72 to 105 percent. Furthermore, contamination had no effect on the analytical process, and the relative standard deviation (RSD) for all tests was 10%0.5.

2.8. Human health risk assessment

2.8.1. Metal pollution index (MPI)

The MPI was calculated following (Usero & González-Regalado, 1996) the equation 1 (Abdel-Khalek et al., 2016). The equation is as follows:

$$\text{MPI}= (CM_1 \ \times \ CM_2 \ \times \ CM_3 \times .... \times CM_n) \ ^{1/n} \tag{1}$$

where, CM_1 is the value of concentration of first metal, CM_2 refers second concerned metal, CM_3 the metal and CM_n the nth metal (mg/kg dry wt) in the tissue sample of a certain species.

2.8.2. Estimated daily intake (EDI) and lifetime daily dose (LADD)

The estimated daily intake (EDI) value was estimated by using the equation (USEPA, 2008; Varol et al., 2017; Wei et al., 2014).

$$EDI = \frac{Cn \times IGr}{Bwt} \tag{2}$$

where, Cn is the level of metal in the fish tissues (mg/kg dry-wt); IGr is the suitable rate of consumption (55.5 g/day for adults and 52.5 g/day for children) (USEPA, 2008; Vu et al., 2017); Bwt refres the body weight (70 kg for adults and 15 kg for children) (USEPA, 2008).

2.8.3. Hazard quotient (HQ)

THQ was estimated by the ratio of EDI and oral reference dose (RfD). The value of ratio < 1 indicates a non-significant risk effect (Abtahi et al., 2017). The THQ calculated followed by (Baki et al., 2018; Heshmati et al., 2018).

$$HQs = \frac{Ed \times Ep \times EDI}{At \times RfD} \times 10^{-3} \ (3).$$ where Ed indicates the total exposure duration in the whole life (65 years) (USEPA, 2008), Ep indicates exposure frequency (365 days/ year) (Ahmed et al., 2015), and it indicates the average time for the non-carcinogenic element (Ed × Ep).

2.8.4. Hazard index (HI)

Hazard index (HI) was calculated for the multiple elements (Hg, As, Mn, and Cr) found in the fish samples and the equation is as follows (Hossain et al., 2019; Xiao et al., 2018).

$$HI = \sum_{i=k}^{n} THQ \tag{4}$$

where, THQs refers the estimated risk value for each element. When the HI value is greater than 10, the non-carcinogenic risk effect for exposed consumers is considered high (Dadar et al., 2017; Fakhri et al., 2017).

2.8.5. Cancer risk (CR)

The cancer-causing risk is assessed for the impact of exposure to the substantial pollutants that may develop cancer over a lifetime period. The risk limit is 10–6 to 10–4 (FAO, 2014; USEPA, 2000; Yin et al., 2015), which is standard and acceptable. Once CRs are higher than reported range (10–4), chances are higher of cancer-causing risk effect (Hu et al., 2017; USEPA, 2010). The CR is assessed from the method of slope factor as follows (Zhu et al., 2020).

$$CR = \frac{Ed \times Ep \times EDI \times CSF}{AT} \times 10^{-3} \ (5).$$ where CSF indicates oral slope factor of specific pollutant (mg/kg-day) (USEPA, 2000).

2.8.6. Statistical analysis

Statistical analyses such as cluster analysis (CA), correlation matrix (CM), PCA were performed by using the program, called PAST (PAleontological Statistics, Ver. 2.1). The CA of metals based on arbitrary Bray–Curtis similarity values was performed to plot elements in a separate cluster, thereby differentiating the contamination status of the samples. The CM and PCA were used to determine the correlation between heavy metals in order to identify potential metal sources. The mean values and standard deviations of the heavy metal concentrations in finfish and shellfish samples were calculated. Other calculations were performed by Microsoft Excel 2016.

3. Results

3.1. Concentrations of heavy metals

Concentrations of five heavy metals Cr, Pb, Fe, Zn, and Ni in fish tissues of six finfish and three shellfish species from Karnaphuli River were presented in Table 3. The mean heavy metal concentration of Cr, Pb, Fe, Zn and Ni in the tissues of fish and shellfish species was 0.906 ± 0.052 (0.059 to 1.605 mg/kg); 0.128 ± 0.032 (0.025 to 0.385 mg/kg); 83.015 ± 1.166 (42.13 to 133.85 mg/kg); 398.47 ± 1.154 (31.543 to 1458.68 mg/kg) and 0.233 ± 0.026 mg/kg (0.023 to 0.355 mg/kg) mg/kg respectively. In this present study, the ranking order of mean concentrations of the heavy metals in the muscle of fish and shellfish collected from Karnaphuli River estuary were Zn > Fe > Cr > Ni > Pb.

The highest value of Cr was 1.605 ± 0.136 mg/kg found in *A. grammepomus* and the lowest value of Cr concentration was 0.059 ± 0.004 mg/kg) found in *N. smithi*. On the other hand, the highest amount of Pb was found in the fish sample of *C. cirrhosis* (0.385 mg/ kg) and the lowest amount of Pb was found in *M. rosenbergii* (0.025 mg/kg). In this study, *S. phasa* contains the highest Fe (133.85 ± 1.854 mg/kg), whereas *N. smithi.* contains the lowest value of Fe concentration (42.13 ± 1.54 mg/kg). Additionally, the highest and lowest amount of Zn was found in *A. bato* (1458.68 mg/kg as dry wt.) and *O. pama* (31.543 mg/kg), respectively. The highest amount of Ni was found in the shellfish sample of *N. smithi* (0.355 mg/kg as dry wt.), the lowest amount of Ni was found in the fish sample of *C. cirrhosis* (0.023 mg/kg).

The estimated MPIs varied from 1.16 mg/kg to 4.72 mg/kg with the mean of 2.71 mg/kg (Fig. 2). The maximum MPI value (4.72 mg/ kg) was linked to *A. bato*, followed by *C. cirrhosis* (3.94 mg/kg), *A. grammepomus* (3.19 mg/kg), *M. dobsoni* (2.67 mg/kg), *P. paradiseus* (2.64 mg/kg), *O. pama* (2.62 mg/kg), *M. rosenbergii* (2.24 mg/kg), *N. smithi* (1.22 mg/kg) and *S. phasa* (1.16 mg/kg) due to greater concentration level.

3.2. Sources identification

Table 4 presents the interrelations among metals as a Pearson correlation matrix. Pb displays a significant relationship with Ni that could have been originated from anthropogenic sources like industrial effluents from various industries such as printing, dyeing, and textile around Chittagong City. Other metals have no significant correlation between them. That means they probably come from different sources. If there is any significant relationship between two metals with the same pollution level, they release from the same source, mutual dependence, and individual character through River flow.

PCA is the simplest of the eigenvector-based multivariate analysis presented in Fig. 3. In the Table 5, the result with eigenvalue, the variance of 100%, and cumulative variance for each factor is summarized. PC1 has the highest eigenvalue of 2.275. It suggests that PC1 is the most prevalent factor, and it contributes 45.502% variance having a high loading on Zn 0.552, which is not significant. PC2 contributes 28.019% of the total variance and has a high load on Cr 0.6862, which is significant. PC2 has an eigenvalue of 1.401. PC3 contributes 13.844% of total variance having a high load on Cr (0.6574) which is less significant. This factor has an eigenvalue of 0.692. PC4 has an eigenvalue of 0.436. It contributes

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Table 3

Mean concentrations (mg/kg dry wt.) and standard deviations (SD) of analyzed metals.

Species name	Heavy metals (mean ± SD) mg/kg				
	Cr	Pb	Fe	Zn	Ni
O. pama	1.525 ± 0.049	0.14 ± 0.028	80.846 ± 0.738	31.543 ± 1.275	0.225 ± 0.007
A. grammepomus	1.605 ± 0.136	0.183 ± 0.208	50.42 ± 1.285	393.93 ± 0.557	0.057 ± 0.004
S. phasa	0.09 ± 0.001	0.028 ± 0.001	133.85 ± 1.854	36.108 ± 1.15	0.17 ± 0.197
P. paradiseus	1.036 ± 0.005	0.165 ± 0.02	65.643 ± 1.458	33.24 ± 0.98	0.345 ± 0.007
C. cirrhosus	0.71 ± 0.016	0.385 ± 0.007	103.8 ± 0.469	1457.2 ± 2.16	0.023 ± 0.001
A. bato	0.709 ± 0.014	0.086 ± 0.008	103.27 ± 1.34	1458.7 ± 1.42	0.255 ± 0.007
M. rosenbergii	1.17 ± 0.212	0.025 ± 0.002	84.2 ± 1.09	70.34 ± 1.26	0.325 ± 0.007
M. dobsoni	1.25 ± 0.036	0.056 ± 0.008	82.98 ± 0.72	69.05 ± 0.41	0.34 ± 0.000
N. smithi	0.059 ± 0.004	0.086 ± 0.008	42.13 ± 1.54	36.15 ± 1.178	0.355 ± 0.007
Range	0.059–1.605	0.025–0.385	42.13–133.85	31.54–1458.68	0.023–0.355
Mean ± std	0.906 ± 0.052	0.128 ± 0.032	83.015 ± 1.166	398.47 ± 1.154	0.233 ± 0.026
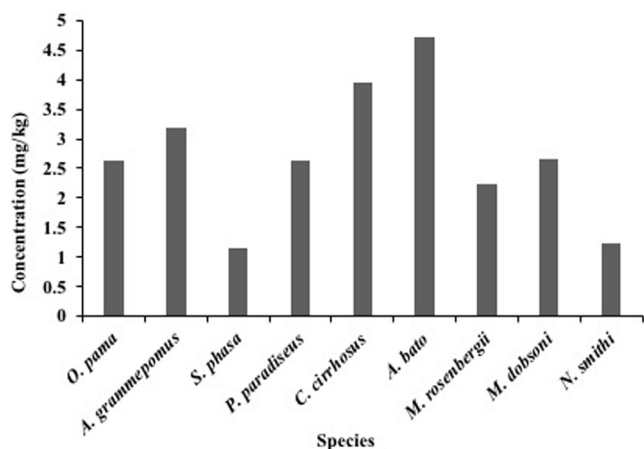
Fig. 2. Metal Pollution Index (MPI) of studied metals in the Karnaphuli River estuary.

8.710% variance with high loading on metal Zn 0.7429, which is significant. PC5 contributes 3.924% of the total variance, having a high load on Pb (0.6840) which is significant. This factor has an eigenvalue of 0.196.

The Ward-Linkage method was used with Euclidean distance to execute hierarchical cluster dendrograms, resulting in three distinct clusters (Fig. 4). Cluster 1 contains Fe and Zn, while Cluster 2 contains Cr, Pb, and Ni. The metals associated with Cluster 1 could have come from anthropogenic activities such as chemical factories, the discharge of industrial effluents from diverse industries such as printing, dyeing, oil refineries, and textile around KEPZ. The metals, Cr, Pb, and Ni linked to cluster 2 could have been associated with the textile industry, fertilization, dying, tanning industries and oil leaking from the boats/ships in the study site.

3.3. Health risk evaluation

3.3.1. Estimated daily intake (EDI)

The studied EDI of two concerned age groups, adults and children, is presented and summarized in Table 6. For intake, the

results for adults and children in the study area were lower the recommended daily allowance (RDA), following the descending order of Zn > Fe > Cr > Ni > Pb. EDIs, lower than RDA, indicated that the targeted groups of people could experience a possible lower health effect.

3.3.2. Hazard quotient (HQ) and hazard index (HI) for noncarcinogenic risk

THQs in the adult group induced to Cr, Pb, Fe, Zn and Ni were 0.0002, 2.0E-05, 0.0002, 0.001 and 2.0E-06, respectively, while the values for children were 0.0011, 0.0001, 0.001, 0.004 and 5.0E-05, respectively (Table 7). In addition, the rank of the THQs of the studied metals was as follows: Zn > Cr > Fe > Pb > Ni. Children were 5–6 times more vulnerable than adults for the cumulative state of HI. However, the examined HI was not exceeded the suggested boundary (Table 7).

3.3.3. Cancer risk (CR)

Exposure of CR was calculated only for specific metals (Table 8). The CR values of Cr, Pb and Ni were ranged from 2.34E to 08 – 6.05E-07, 1.68E-10 – 3.0E-09 and 3.1E-08 – 5.0E-07 respectively in adults and 1.03E-07 – 3.0E-06, 7.43E-10 – 1.0E-08 and 1.37E-07 – 2.1E-06 in children. The findings suggested that children were more susceptible to higher CRs than adults. Despite this, the CR values for both age groups were far away from the risk, as the standard range is 10−6 to 10−4.

4. Discussion

4.1. Heavy metals concentration and source identification

In the present study, fish and shellfishes were collected from different stations and the concentration of metals follows the order as: Zn (398.47) > Fe (83.015) > Cr (0.906) > Ni (0.233) > Pb (0.128). *A. grammepomus* had the highest concentration of Cr, whereas *C. cirrhosus* exhibited the maximum level of Pb and Zn, and *N. smithi* had the highest concentration of Ni. *S. phasa* has shown the highest concentration of Fe and *A. bato* has shown the highest concentration of Zn. The metal concentrations in the edible tissues were

Table 4

Pearson correlation matrix of studied metals of the Karnaphuli River estuary.

	Cr	Pb	Fe	Zn	Ni
Cr	1				
Pb	0.14658	1			
Fe	−0.32004	−0.057289	1		
Zn	−0.10581	0.58233	0.33631	1	
Ni	−0.1188	**−0.6796***	−0.28604	−0.52413	1

Note: *p < 0.05 is significant (Bold value represents significant correlation).

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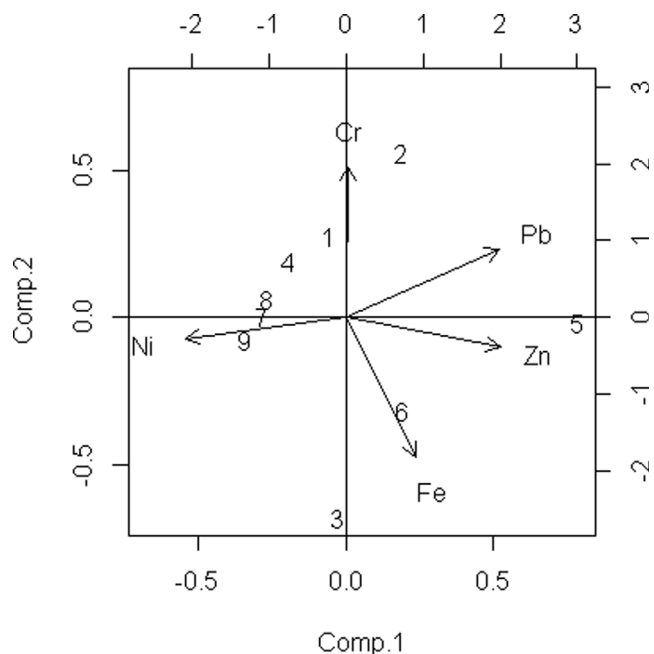Journal of King Saud University – Science 34 (2022) 102035

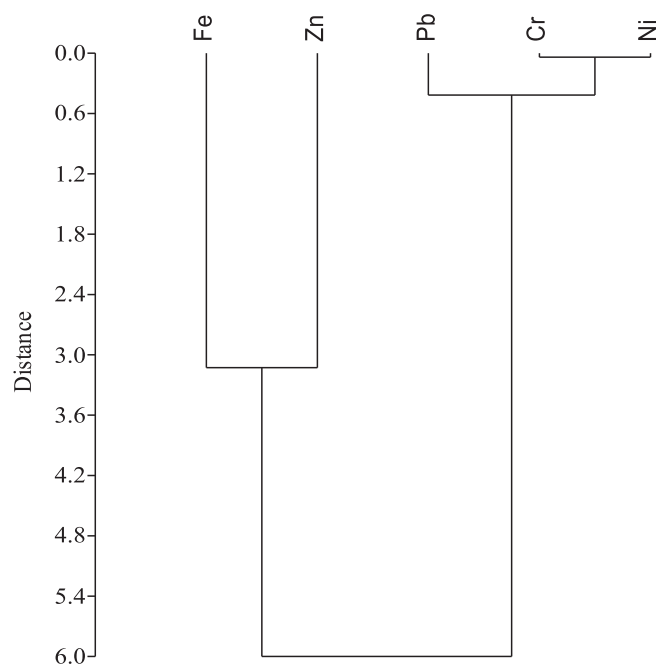**Fig. 3.** Principal component analysis of studied metals in the Karnaphuli River estuary.

Fig. 4. Hierarchical cluster (Dendrogram) of heavy metals (Variables) (Ward-Linkage).

ordered: *A. bato > C. cirrhosus > A. grammepomus > M. dobsoni > P. paradiseus > O. pama > M. rosenbergii > N. smithi > S. phasa* (Table 3). The data from the earlier studies exhibited that metal concentrations in fish and shellfish tissues extensively varied subject to the location and species (Table 9).

In the fish and shellfish species, except for *S. phasa, C. cirrhosus, A. bato*, and *N. smithi*, the mean Cr contents were greater than the recommendation value of 1.0 mg/kg (MOFL, 2014). Furthermore, all fish and shellfish species except *S. phasa* and *N. smithi* surpassed the FAO's recommended threshold of 0.15 mg/kg (FAO, 1983). The findings of this study were roughly 18 times greater than those of Copat et al., (2013), who found 0.049 mg/kg of Cr in the Eastern Mediterranean Sea. Furthermore, average Cr concentrations in this study were greater than in previous investigations (Islam et al., 2013; Leung et al., 2014) (Table 5). Cr in the diet affects lipid and glucose absorption in a significant way (Ahmed et al., 2015; Ali et al., 2018). Cr deficiency can cause slowed progress and denature lipid, protein, and glucose absorption (Calabrese et al., 1985). Excessive Cr consumption, on the other hand, might result in acute respiratory diseases (Ahmed et al., 2016; Forti et al., 2011), as well as damage of potential organs like the liver, lungs, and kidneys (Ali et al., 2020).

The recommended maximum permissible concentration limit for Fe is 100 mg/kg (WHO, 2000). Thus, the concentrations of Fe in the fish and shellfish samples analyzed did not exceed the maximum permissible limit (WHO, 2000), except *S. phasa* (133.85 mg/

kg). However, mean concentrations of Fe in all species were higher than fishes from Iskenderun Bay, Turkey (Türkmen et al., 2005). The FAO maximum guideline for Zn is 30 mg/kg (FAO, 1983). The rank denotes that the highest concentration of Zn (1458.68 mg/kg) was detected in *A. bato,* and *O. pama* represented the lowest Zn concentration level (31.54 mg/kg). However, the concentration level of Zn in the fish and shellfish species was mostly similar to the reported value of the Bangshi River (Rahman et al., 2012). Moreover, from Table 5, the average concentrations of Zn in all specimens were higher than other related studies (Ahmed et al., 2015; Copat et al., 2013). Thus, through the consumption of these organisms, these metals posed a threat. Toxicity due to excessive Zn intake causes electrolyte imbalance, nausea, anemia, and lethargy (Prasad, 1984). In this present study, Ni concentrations in Karnaphuli River water fish and shellfishes were below the established safe level of 5.5 mg/kg by Western Australian Food and Drug Regulations (Plaskett and Potter, 1979). The ranking order of mean concentrations of Ni in the muscle of fish and shellfish were, *N. smithi > M. dobsoni > P. paradiseus > M. rosenbergii > A. bato > O. pama > S. phasa > A. grammepomus > C. cirrhosus* (Table 3). However, the concentrations of Ni in the fish and shellfish species were similar to the reported value of (Copat et al., 2013), who found the range of Ni level as 0.011–0.327 mg/kg (ww). The concentration of Ni was reported in the range of 1.20– 6.10 mg/kg in fish species from the freshwater River of Bangladesh (Sharif et al., 1993), which exceeded the present study findings. Moreover,

Table 5
Principal component analysis of collected fish samples.

Variable	PC 1	PC 2	PC 3	PC 4	PC 5
Cr	0.0087	**0.6862***	0.6574	0.3035	0.0695
Pb	0.5491	0.3104	−0.3347	−0.14926	**0.6840***
Fe	0.2480	−0.6362	0.6161	0.0069	0.3925
Zn	0.5522	−0.1358	−0.1762	**0.74297***	−0.3057
Ni	−0.5761	−0.0979	−0.2127	0.57754	0.5288
Eigenvalue	2.275	1.401	0.692	0.436	0.196
Variance (%)	45.502	28.019	13.844	8.7101	3.924
Cumulative of Variance (%)	45.502	73.52	87.36	96.07	99.99

Note: *p < 0.05 is significant.

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Table 6
A comparison between recommended daily allowance (RDA) and estimated daily intake (EDI).

Element	Mean concentration (mg/kg)	Recommended daily allowance (mg/kg/person)	EDIs (mg/day per person)	
			Adult	Child
Cr	0.906	0.23	0.0007	0.003
Pb	0.128	0.25	0.0001	0.0004
Fe	83.015	—	0.066	0.2905
Zn	398.470	70	0.316	1.395
Ni	0.233	0.35	0.0002	0.0008

Table 7
Target hazard quotient (THQ) and Hazard Index (HI) for adult and child.

Species	THQ (Cr)		THQ (Pb)		THQ (Fe)		THQ (Zn)		THQ (Ni)		HI	
	Adult	Child	Adult	Child	Adult	Child	Adult	Child	Adult	Child	Adult	Child
O. pama	0.0004	0.0018	0.00	0.0001	0.0002	0.0009	0.000	0.0004	0.00	0.000	0.0006	0.0033
A. grammepomus	0.0004	0.0018	0.00	0.0002	0.0001	0.0005	0.001	0.0045	0.00	0.000	0.0016	0.008
S. phasa	0.000	0.0001	0.00	0.000	0.0003	0.0015	0.0001	0.0004	0.00	0.000	0.0004	0.002
P. paradiseus	0.0003	0.0012	0.00	0.0002	0.0002	0.0008	0.000	0.0004	0.00	0.000	0.0005	0.0027
C. cirrhosus	0.0002	0.0008	0.00	0.0003	0.0002	0.0012	0.0038	0.017	0.00	0.000	0.0044	0.0193
A. bato	0.0002	0.0008	0.00	0.0001	0.0002	0.0012	0.0038	0.017	0.00	0.000	0.0043	0.0192
M. rosenbergii	0.0003	0.0013	0.00	0.00	0.0002	0.001	0.0002	0.0008	0.00	0.0001	0.0007	0.0032
M. dobsoni	0.0003	0.0014	0.00	0.0001	0.0002	0.001	0.0002	0.0008	0.00	0.0001	0.0007	0.0035
N. smithi	0.00	0.0001	0.00	0.00	0.0001	0.0004	0.0001	0.0004	0.00	0.0001	0.0002	0.001
Mean	**0.0002**	**0.0011**	**0.00**	**0.0001**	**0.0002**	**0.001**	**0.001**	**0.004**	**0.00**	**0.00**	**0.0015**	**0.0069**

Table 8
Carcinogenic risk (CR) of Cr, Pb and Ni in analyzed sample.

Species	CR (Cr)		CR (Pb)		CR (Ni)	
	Adult	Child	Adult	Child	Adult	Child
O. pama	6.05E-07	2.67E-06	9.43E-10	4.0E-09	3.0E-07	1.0E-06
A. grammepomus	6.0E-07	3.0E-06	1.0E-09	5.0E-09	8.0E-08	3.0E-07
S. phasa	4.0E-08	2.0E-07	2.0E-10	8.0E-10	2.0E-07	1.0E-06
P. paradiseus	4.0E-07	2.0E-06	1.0E-09	5.0E-09	5.0E-07	2.0E-06
C. cirrhosus	3.0E-07	1.0E-06	3.0E-09	1.0E-08	3.1E-08	1.37E-07
A. bato	2.8E-07	1.24E-06	5.8E-10	2.55E-09	3.43E-07	1.52E-06
M. rosenbergii	4.64E-07	2.04E-06	1.68E-10	7.43E-10	4.38E-07	1.93E-06
M. dobsoni	4.96E-07	2.19E-06	3.7E-10	1.67E-09	4.58E-07	2.02E-06
N. smithi	2.34E-08	1.03E-07	5.8E-10	2.55E-09	4.78E-07	2.1E-06
Mean	4.0E-07	2.0E-06	8.71E-10	3.59E-09	3.14E-07	1.33E-06

Table 9
Comparison of heavy metal concentration in fish and shellfish muscle with the reported values in the literatures.

Sample area	Sample	Cr	Pb	Fe	Zn	Ni	Reference
Eastern Mediterranean Sea	Wet wt. (mg/kg)	0.0007–0.24	0.003–0.071	NA	3.418–7.625	0.01–0.327	Copat et al., (2013)
Pearl River Delta, China	Wet wt. (mg/kg)	0.20–0.65	0.03–8.62	NA	15.2–29.5	0.44–9.75	Leung et al., (2014)
Bangshi River	Dry wt. (mg/kg)	0.47–2.07	1.76–10.27	NA	42.83–418.1	0.69–4.36	Rahman et al., (2012)
Mediterranean and Aegean Sea (Turkey)	Wet wt. (mg/kg)	0.07–1.48	0.21–1.28	NA	3.51–53.5	0.03–1.72	Turkmen et al., (2009)
Iskenderun Bay, Turkey	Dry wt. (mg/kg)	0.07–6.46	0.09–6.95	0.82–27.35	0.60–11.57	0.11–12.88	Turkmen et al., (2005)
Hooghly River (India)	Dry wt. (mg/kg)	0–3.89	12.40–19.96	NA	12.13–44.74	2.20–3.69	De et al., (2010)
Rivers (Bangladesh)	Dry wt. (mg/kg)	NA	0.29–10.05	31.80–296.2	33.01–286.5	1.20–6.10	Sharif et al., (1993)
Buriganga River	Wet wt. (mg/kg)	1.59–16.05	0.47–4.55	NA	58.56–187.04	0.36–5.75	Ahmed et al., (2015)
Meghna River Estuary	Dry wt. (mg/kg)	0.62–1.19	2.76–4.63	NA	NA	NA	Ahmed et al., (2019b)
Karnaphuli River Estuary	**Dry wt. (mg/kg)**	**0.059–1.605**	**0.025–0.385**	**42.13–133.85**	**31.54–1458.6**	**0.023–0.355**	**Present Study**

Note: NA- not analyzed.

average concentrations of Ni in the present study area was lower than other recognized studies (De et al., 2010; Leung et al., 2014) (Table 5). Nickel occurs typically at very low levels in the environment, and it can cause a variety of pulmonary adverse health effects, such as lung inflammation, fibrosis, emphysema, and tumors (Forti et al., 2011).

The correlation analyses revealed that the sources of the metals in the corresponding specimens were perhaps similar (Table 5). According to Pearson correlation coefficients, values with a confidence level of 95 percent (significance level at $p = 0.05$), a significant correlation was found between Pb and Ni. Both of them probably have a common anthropogenic sources. It was found by various indices that the study site is moderately polluted by Cr and Zn. Table 9 shows that the heavy metal concentration of the Karnaphuli River estuary is higher than Eastern Mediterranean Sea (Copat et al., 2013). Heavy metal concentrations in the Rivers like Bangshi River and Buriganga River are higher than Karnaphuli River estuary. Though it was found that Cr and Zn moderately pol-

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lute the estuary, the degree of contamination by other metals is not so high in the study site. Till now, we can say the Karnaphuli River estuary doesn't contain any high contaminants, and the metal concentration doesn't possess an increased risk for humans, other animals, and the environment.

4.2. Human health risk evaluation

EDIs evaluate both the non-carcinogenic risk (THQ) and the carcinogenic risk (CR) of targeted metals ingestion in aquatic food-stuffs (Liu et al., 2018). Table 6 shows the EDIs of local people in the current study area for the selected brackish and shellfish species. In terms of intake, the results for adults and children were all below the recommended daily allowance (RDA) and in the following descending order: Zn > Fe > Cr > Ni > Pb. Because EDIs were lower than RDA, it was assumed that the targeted groups of persons would have a reduced health effect. For example, the results were significantly below the proposed tolerance level (Table 4). Given the increased buildup of metals in fish specimens, the long-term health implications would be considered (Ali et al., 2020).

The target hazard quotient (THQ) for ingestion of targeted specimens of fish and shellfish was shown in (Table 7). For THQ, the standard threshold limit is 1, recommended by USEPA (USEPA, 2011). The present study described that the THQ means of all fish and shellfish species were below 1, both for adults and children. None of these exceeded the guideline limit for the every metal in all specimens. However, the examined HI did not exceed the recommended limit, indicating that consumers (adults and children) would not suffer non-carcinogenic health effects from consuming selective fish and shellfish species.

In fact, humans are vulnerable to undergo the detrimental effects of combined pollutants simultaneously (Li et al., 2013a; Li et al., 2013b). Due to the lack of a certain level of concentration, THQ, and HI are still not measured a direct measure of risk alarm described by USEPA (USEPA, 1999). Cr, Pb, and Ni carcinogenic risk (CR) was calculated in (Table 8), due to the availability of the cancer-causing power slope factor for those metals. The CR values below 10^{-6} indicate a negligible health risk, while the 10^{-6}–10^{-4} range values are in the acceptable belt (Baki et al., 2018). CRs above 10^{-4} are likely to increase the likelihood of affecting carcinogenic risk (Hu et al., 2017; USEPA, 2010). All exposures to CR had been negligible in the study. Additionally, for both adults and children, CR of Pb was less than the negligible limit. The results also indicated that children are more vulnerable to exposure to CR than adults. Similar findings were consistent with previous literatures (Liu et al., 2018; Saha et al., 2016).

5. Conclusions

This study ranked the average concentrations of heavy metals in fish and shellfish muscle as follows: Zn > Fe > Cr > Ni > Pb. Results showed that Cr and Zn concentration was significantly higher than standard FAO recommended values. In *A. bato*, the mean Zn content was the highest, exceeding the maximum FAO recommendations. The mean Cr concentration in this study demonstrated the highest concentration level in *A. grammepomus*, which slightly exceeded Bangladesh 's guidelines on food safety. Because EDIs are lower than RDA, they imply that the targeted groups of people may have a lesser health effect. THQ was conducted on both adults and children and concluded that none of them had a non-carcinogenic effect on health. On the other hand, CR values for adults and children were less than the threshold limit (10–4), indicating that CR was negligible for all consumers. Pearson's correlation showed significant correlations between Pb and Ni, indicating

they had the same source, either natural or anthropogenic. Carcinogenic risk assessment, however, suggested that for the time being, local consumers were free from the risk of cancers; still, they may be affected in the future when fish from the studied region are consumed. As the levels of some metals (e.g., Cr, Zn) were found higher than the standard limit, continuous monitoring is suggested and untreated discharge of solid or liquid waste must be stopped.

6. Animal ethics

No field permit or ethics approval was needed since no rules and regulations were set by the University and/or local authorities.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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