



Bioaccumulation of metals in selected cultured fish species and human health risk assessment: a study in Mymensingh Sadar Upazila, Bangladesh

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Accepted: 19 April 2021 / Published online: 28 April 2021

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Abstract

This paper aims to measure the metals (Fe, Cu, Cr, Co, As, Zn, Hg, and Pb) concentration and assesses human health risks posed by these eight metals in the five most available cultured fish species (*Pangasius pangasius*, *Oreochromis niloticus*, *Heteropneustes fossilis*, *Anabustudineus*, *Clarias batrachus*) collected from the Mymensingh Sadar Upazila. Both fishes and three fish feeds were analyzed using energy dispersive X-ray fluorescence (EDXRF). In fish feeds, the concentrations of metals were detected in the following order Fe > Zn > Cu > Hg > Pb. Besides, Hg concentration in three feeds samples was found higher than the maximum residue limit. Fe, Cu, Cr, Co, As, Zn, Hg, and Pb concentration in fishes were 82.45 to 104.55, 24.47 to 32.88, 2.62 to 6.73, < 0.28, < 0.41, 70.01 to 96.56, 0.57 to 1.07, and 0.34 to 0.49 µg/g respectively as dry weight basis. Health Risk Index (HRI) was observed > 1 both for adults and children and maximum HRI values for Pb was found in both *P. pangasius* and *C. batrachus* fish species. Additionally, PCA explained 73% variation in data and CA comprised the similarity within sampling stations. Pearson correlation implied a strong association amongst the trace metals. Finally, the accumulation of metals tends to the massive health hazard that should be mitigated by adopting several measures on fish feeds.

Keywords Cultured fishes · Fish feeds · Health risk · Metal pollution · Multivariate analysis

1 Introduction

Bangladesh has tremendous fishing and aquaculture potential because of its abundant inland waters and river systems. The country is recognized as one of the most attractive areas for fisheries in the world and third in inland fish production (Shamsuzzaman et al. 2017). Bangladesh's fisheries sector is mainly divided into three sub-sectors: inland capture, inland culture, and marine fisheries. A

significant contribution of 3.57% of the national Gross Domestic Product (GDP) and more than one-fourth (25.30%) to the agricultural of GDP is played by the fishing sector in the national economy (Department of Fisheries. 2018). In freshwater systems, fish is one of the aquatic foodstuffs that humans consume and also provide a good indicator of trace element pollution (Rashed. 2001). As fish are often at the top of aquatic food webs and may concentrate large amounts of metals from the water and sediments, through this process, heavy metals can easily enter the human body (Mansour and Sidkey. 2002). Fishes have been considered a significant source of proteins for human food consumption in the last several years. Consequently, the human body is likely to be enriched with a greater degree of heavy metal concentration (Ali and Khan 2018). In Bangladesh, fish farmers gradually use manufactured feeds rather than farm-made feeds (Mahmud et al. 2012). Fish feeds are commonly manufactured from rice bran, soya meal, flour, fish meal, shrimp meal, beef liver, silkworms, and earthworms (Asaduzzaman et al. 2006).

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Tannery solid wastes are also used as poultry and fish feeds in Bangladesh (Hossain et al. 2007). Due to certain anthropogenic activities and feeding fish feeds containing toxic metals, the closed water sources are polluted with high metals as various feedstuffs are generated with solid tannery waste as raw materials (Patwardhan 2013). Subsequently, intensive anthropogenic activities and natural sources can have an effect on the local aquatic environment, increasing the heavy metal contents in sediments and water and contributing to the associated ecological damage (Ogundele and Ayeku 2020; Alam et al. 2020). Some commercial fish feeds of Bangladesh have also been found to have heavy metals burden (Kundu et al. 2017). Heavy metals are ingested on the human body through the consumption of fish. In recent years, the science community has become profoundly responsive to human health's potential threats arising from the fishes exposed to toxic metals (Griboff et al. 2017). The ingestion of metal-contaminated fish and other foods polluted with chemical and organic contaminants from aquatic ecosystems can endanger human life (Aderinola et al. 2009). Some feeds contain substantial concentrations of contaminants, including heavy metals. Many of which can bio-accumulate and bio-concentrate in fish could harm humans (Maule et al. 2007; Indrajit et al. 2011). Trace elements (cobalt, copper, chromium, manganese, and nickel) are necessary for humans in minute amounts while heavy metal is carcinogenic or toxic, affecting the central nervous system (by mercury, lead, and arsenic), the kidneys or liver (by mercury, lead, cadmium, and copper) or skin, bones, or teeth (by nickel, cadmium, copper, and chromium) (Bhattacharya et al. 2007). Heavy metals exposure in human health causes dysfunctioning urinary organ and skeletal injury, medical speciality disturbances, endocrine disturbance, vessel disease, and cancer effects (Renjeri et al. 2019). Besides, an excessive amount of Hg, As, Pb, and Cd elements could be detrimental to the living cells, and prolonged exposure to the body can lead to illness or death. (Azaman et al. 2015).

Fish supplies comparatively cheap, and it is a readily available source of nutrients in several low and middle-income countries where diets are depending heavily on a narrow range of staple foods (Reksten et al. 2020). Commercial fish species are very cheap sources of protein, and the accumulation of metals in fish tissues act as a medium through which metals are ingested in the human body after consumption. Heavy metal contamination of the food chain is well documented in Bangladesh (Islam et al. 2014). Metals deposited into the aquatic environment may accumulate in the food chain and pose carcinogenic and other adverse effects on human health due to biomagnification over time (Malik et al. 2009). The excessive intake of heavy metal in small quantities contributes to the

dysfunction of a variety of physiological and biochemical processes such as thalassemia, dermatitis, brain and kidney damage and cancer, which are known health risks linked to heavy metal exposure (Hu 2002; Tripathi et al. 2001). Heavy metals tend to accumulate in advanced organisms through bio-magnification effects in the food chain and accumulate in the human tissues to pose chronic toxicity (Qiao-qiao et al. 2007). The significant adverse effects of exposures to these heavy metals, even at low concentrations, are responsible for neurotoxicity and carcinogenicity (Jomova and Valko. 2010). This paper aims to determine the concentration of selected eight metals in five commercial fish species tissues (flesh), which are very available in Bangladesh and also assess the probable health risk due to the consumption of those fishes collected from the study area.

2 Materials and methods

2.1 Study area and sampling procedure

The study area (shown in Fig. 1) is located (latitude 24.38° N to 24.54° N and longitude 90.11° E to 90.31° E) in the Mymensingh Division of Bangladesh. Fish were sampled from this area because it is one of the concentrated aquaculture regions in Bangladesh. Fish were selected for sampling based on the species significance to local food habits and availability in the Bangladesh fish market. Five fish species (three of each) and fifteen fish samples (Table 1) were collected from five different fishing zones by the Multi-stage cluster method during the months of June to July 2019. Fishes were captured by using a seine net with the help of local fisherman and all the samples were in the mature stage. The samples were immediately placed in air-sealed plastic bags and kept in an icebox (Pinnacle TPX 60,091, Tokyo Plast Int., India) running at or below 40° F before further analysis laboratory of the Chemistry Division at Atomic Energy Center, Dhaka, Bangladesh. Sample collection time and fish growth stage are depicted in Table 1.

2.2 Sample preparation and analysis

All the samples were prepared in the laboratory of the Chemistry Division of Atomic Energy Center, Dhaka, Bangladesh. Collected fish were washed multiple times with deionized water. Fish collected from the study area were cut into small pieces (2–4 cm) with the stainless steel knife, separated into flesh, and all the samples, including fish feed, were dried in an oven at 80°C until a constant weight was obtained (24–48 h in most cases). All dried samples were ground as powder by a carbide mortar and

Fig. 1 Sampling location of the study area

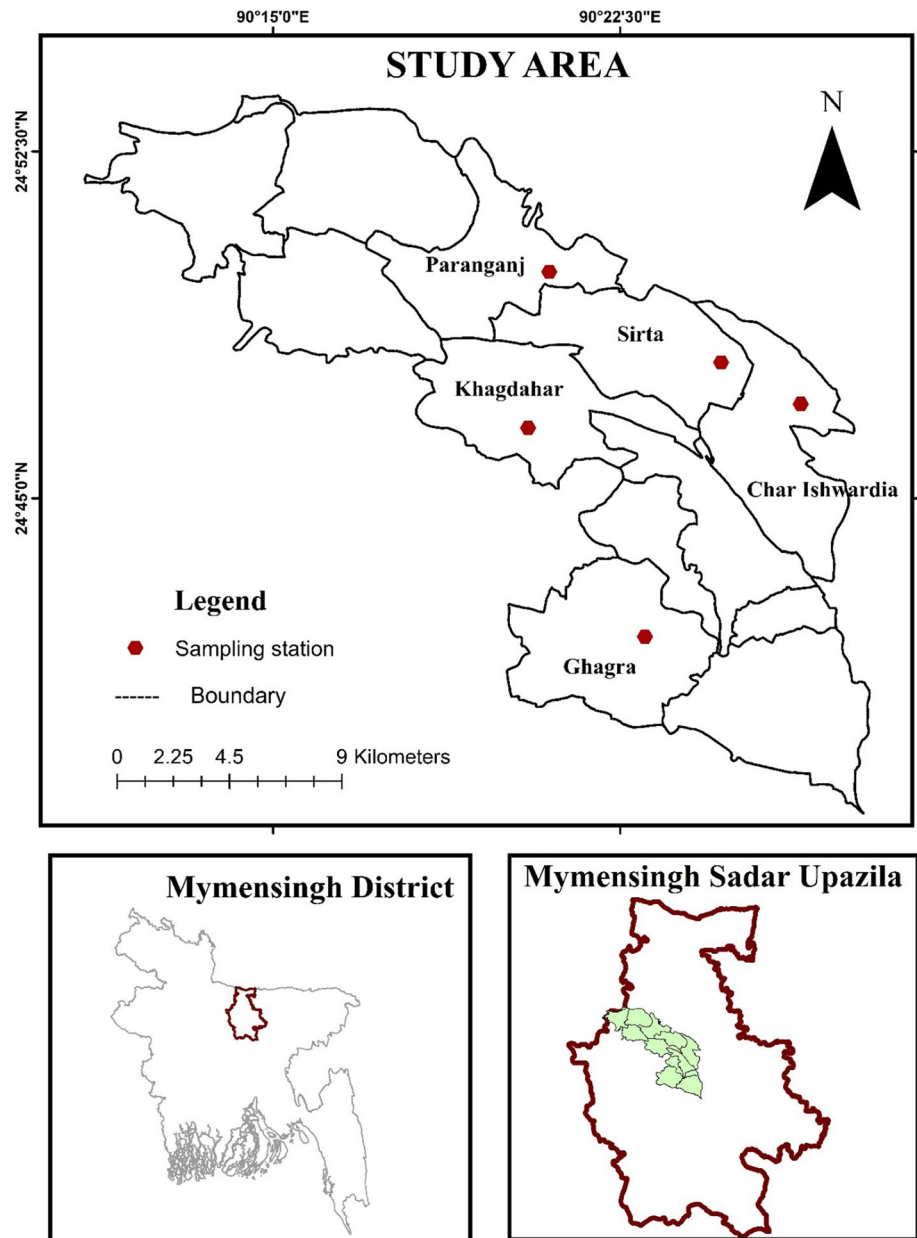


Table 1 The biological background information of fishes

SL. No	Scientific Name	EnglishName	Local Name	No. of samples	Length ± SE* (cm)	Sample time	Fish growth stage
01	<i>Pangasius pangasius</i>	Yellowtail catfish	Pangus	3	43.500 ± 1.442	June, 2019	Mature
02	<i>Oreochromis niloticus</i>	Indian tilapia	Tilapia	3	25.267 ± 0.698	July, 2019	Mature
03	<i>Heteropneustes fossilis</i>	Stinging catfish	Shing	3	22.640 ± 0.957	July, 2019	Mature
04	<i>Clarias batrachus</i>	Climbing perch	Magur	3	26.630 ± 2.295	July, 2019	Mature
05	<i>Anabustudineus</i>	Walking catfish	Koi	3	18.2 ± 1.345	June, 2019	Mature

SE* standard error

preserved in a plastic vial with an identification mark inside a desiccator. A 10-mm pellet with approximately 10 tons of hydraulic pressure was used to make by the pellet maker

(Specac Ltd., UK) for 2–3 min for processing pellets for analyses using a sample of 0.1 g dried powdered materials. The pellets were then stored in desiccators for further

experiments via X-ray Fluorescence Spectrometer (XRF) (Epsilon 5, PANalytical, The Netherlands). Pellets were loaded for 1000 s to complete the analysis in the X-ray excitement chamber. A system software package controlled the irradiation of the sample. The concentration of Iron (Fe), Chromium (Cr), Copper (Cu), Cobalt (Co), Arsenic (As), Zinc (Zn), Lead (Pb), and Mercury (Hg) in fifteen fishes and three feeds sample were measured.

Tuna fish Homogenate (IAEA-350) was used to construct the calibration curve for the elemental analysis in fish and feed samples, whereas CRM DORM-2 (National research council, Canada) was used to verify the accuracy of the calibration curve as well as to validate the analytical method. Moreover, it can be supplemented that EDXRF is a multi-elemental measurement technique and it can detect a range of elements at a time. In the present study, the model that is used can detect elements from K to Mo. So when a calibration curve has been constructed that can detect all the elements within this range (K to Mo) even though the standard reference material (SRM) may not contain those elements, thus the use of DORM-2 is justified. The results obtained for the elements and the standard values for their corresponding elements are shown in Table 2 where the values were found to be in good relation with the standard values.

2.3 Quality control and quality assurance measures

The samples were prepared and analyzed to ensure correct and reliable analytical data following a rigorous quality assurance/quality control (QA / QC) system. Prior to use, the disinfection equipment and sample containers were pre-cleaned with deionized water, and 100% Acetone (CH₃)₂CO was used for disinfection. Before being used for dissection, the stainless-steel scissor and knife were washed with 100% Acetone (CH₃)₂CO per flesh sample *Clariasbatrachus e.* Sterile laboratory gloves and nose masks were used throughout the experimental session to avoid hand contamination of the samples. All readings were taken in triplicates to minimize errors. All analytical values were exported from the Laboratory Information Management System (LIMS) to Microsoft Office 365 Excel 2013 version 15.0 to calculate means and standard

deviations (SD). Statistical analyses were performed using GraphPad Prism 8.3.0 and Stata 13. After that, all the statistical analyses were rechecked by SPSS 16.

While constructing the calibration curve, Tuna homogenates were used as a commercially available fish standard. The following curves were constructed by plotting the sensitivities of the elements using the atomic number as a function. Subsequently, certified referenced materials were used to check the accuracy and precision of the constructed calibration curve (Hasan et al. 2020). The obtained result indicates that the proposed method is valid for determining the heavy metals in the fish and fish feed samples. The recoveries ranged from 87.66 to 108.27% and the resulted relative error and coefficient of variance were found less than 10% for most of the considered metals.

2.4 Health risk estimation

2.4.1 Metal pollution index (MPI)

The cumulative metal (Fe, Cu, Zn, Hg & Pb) concentrations contained on fish samples were determined by the Metal Pollution Index (MPI). The geometrical mean of the concentration of all metals on feeds and fishes was determined using the MPI index (Ureso et al. 1997). For computing, the MPI Eq. (1) is used

$$MPI \left(\mu \frac{g}{g} \right) = \sqrt[n]{Cf1 \times Cf2 \times Cf3 \times \dots \times Cfn} \quad (1)$$

where Cf_n = concentration of metal in n the sample.

2.4.2 Daily intake of metal (DIM)

DIM's daily intake was estimated to simulate the daily sorting of metals in the body system of established individual bodyweight. To calculate daily metal intake Eq. (2) (Islam et al. 2017) are used

$$\text{Daily intake of metal (DIM)} = \frac{C_{\text{metal}} \times D_{\text{fishintake}} \times K_{\text{conversionfactor}}}{B_{\text{average body weight}}} \quad (2)$$

where C_{metal} is the metal concentration in fishes ($\mu\text{g/g}$), $SD_{\text{fish intake}}$ is the Daily intake of fishes (g/persons/day), $B_{\text{average body weight}}$ is the average body weight (65 kg used

Table 2 Comparison between experimental results and certified values (mg kg^{-1} , dry weight, DORM-2)

Element	Results obtained	Certified values	Relative error (%)	CV (%)	Recovery (%)
As	16.87	18.0	5.89	5.84×10^{-5}	93.72
Cr	30.07	34.70	9.34	3.8×10^{-4}	86.66
Pb	0.071	0.065	- 7.69	4.04×10^{-4}	109.23
Hg	4.87	4.64	- 4.56	4.08×10^{-4}	104.95
Co	0.19	0.182	1.89	3.43×10^{-4}	104.40

for this study for adult and 32.7 kg for children (Wang et al. 2005), $K_{\text{conversion factor}} = 0.1455$, based on the moisture content of the samples and their dry weight.

The mean current consumption of fishes in Bangladesh was 53 g/person/day as reported by the Department of Fisheries (DoF) in the Department of Fisheries Report (2015), though 60 g/person/day of fish intake by humans is considerable.

2.4.3 Health risk index (HRI)

HRI > 1 in any substance that is known as detrimental to human health. The daily metal intake (DIM) in any food and the oral reference dose (Rf_D) are used to estimate HRI values. The oral Rf_D is known to be an empirical approximation transmitted to humans by oral regular, which is not expected to be life-length threatening, including vulnerable subgroups (US-EPA IRIS. 2006). The health risk index for Fe, Cu, Zn, Hg, and Pb were calculated using Eq. (3) (Cui et al. 2004).

$$HRI = DIM/Rf_D \quad (3)$$

The value of oral Rf_D for Fe, Cu, Zn, Hg, and Pb are 0.7, 0.04, 0.3, 0.00016, and 0.004 mg/Kg/ day, respectively (USEPA 2006, 2010).

2.5 Non-carcinogenic health hazard

The target hazard quotient (THQ) estimation method indicates human health risk caused by exposure to pollutants. The risk-based concentration table of the USEPA Region III (USEPA 2011) is used to calculate the target hazard quotient (THQ) by Eq. (4).

$$THQ = \frac{EF \times ED \times FIR \times Cf \times CM}{WAB \times ATn \times RfD} \times 10^{-3} \quad (4)$$

where EF = the exposure frequency (365 days), ED = the exposure duration (30 years for noncancer risk as used USEPA 2011), FIR = the fish ingestion rate (62.58 g/person/day; DoF (Department of Fisheries) 2018), Cf = the conversion factor (0.208) to convert fresh weight (F_w) to dry weight (D_w) considering 79% of moisture content in fish, CM = the metal concentration in fish, WAB = the average body weight (70 kg), ATn = the average exposure time for non-carcinogens ($EF \times ED$) ($ATn = 365 \times 30 = 10,950$ days) as used in characterizing noncancer risk (USEPA. 2011), and Rf_D = the oral reference dose of metals.

2.6 Hazard index

Hazard Index (HI) is used to assess the risk of human health due to metal contamination (USEPA 1989), which is the sum of the Target Hazard Quotients (THQ) of

metals (USEPA 2011). The Hazard Index is calculated for cultured fish samples by Eq. (5).

$$HI = \sum THQ \\ = THQ(Fe) + THQ(Cu) + THQ(Zn) + THQ(Hg) \\ + THQ(Pb) \quad (5)$$

where HI is the Hazard Index, THQ (Fe) is the target hazard quotients for Fe intake and others.

2.7 Carcinogenic risk

For carcinogenic risk measurement, Target Cancer Risk (TR) is used. The method of estimating TR is also given in the USEPA Region III Risk-Based Concentration Table (USEPA 2011). The model for TR was followed by Eq. (6).

$$TR = \frac{EF \times ED \times FIR \times Cf \times CM \times CPSo}{WAB \times ATn \times RfD} \times 10^{-3} \quad (6)$$

where CPSo is the Carcinogenic potency slope.

In this research work, the concentration of lead was found as a carcinogenic, and its effects were calculated by using the Oral Carcinogenic potency slope (i.e., CPSo), which has been reported by the Integrated Risk Information System (USEPA., 2010). The CPSo value for lead (Pb) (8.5×10^{-3} mg/kg/day) and arsenic (As) (1.5×10^{-3} mg/kg/day) were used respectively for the study.

3 Results

3.1 Metal concentration in feeds

Table 3 represents the metal concentration in fish feeds samples. The concentration of iron (Fe), copper (Cu), and lead (Pb) is 336.60, 62.48 and 0.48 $\mu\text{g/g}$, respectively, in fish feeds samples. Hg concentration exceeded the safe limit in all fish feeds samples (EU 2008). The chromium (Cr) concentration was found in the Paragon feed (9.02 $\mu\text{g/g}$), while the other two, Mega feed and Fresh feed, were not unrolled where values were < 0.41 $\mu\text{g/g}$. The concentration of Zn in Mega, paragon, and fresh feeds are 143.24 $\mu\text{g/g}$, 94.90 $\mu\text{g/g}$, 163.47 $\mu\text{g/g}$ respectively. In opposition, As and Co were not detectable in any of the feed samples measured where the concentrations of them were < 0.41 $\mu\text{g/g}$ and < 0.28 $\mu\text{g/g}$, respectively.

3.2 Metal concentration in fish species

Table 4 illustrates the eight metal concentrations of (Fe, Cu, Cr, Co, Zn, As, Hg & Pb) in fish species samples,

Table 3 Metal concentration ($\mu\text{g/g}$) in fish feeds

Metals	Mega	Paragon	Fresh	Standard error	Acceptance safety limit
Fe	199.25	197.87	336.60	46.01	500 (WHO)
Cr	< 0.41	9.02	< 0.41	N/A	5.00 (EU 2008)
Co	< 0.28	< 0.28	< 0.28	N/A	
Cu	27.12	47.09	62.48	10.24	100 (WHO)
Zn	143.24	94.90	163.47	20.34	
As	< 0.41	< 0.41	< 0.41	N/A	1.00 (EU 2008)
Hg	0.91	1.23	1.21	0.10	0.05 (EU 2008)
Pb	0.37	0.47	0.48	0.04	5.00 (EU 2008)

Table 4 Metal concentration ($\mu\text{g/g}$, dry weight) in fish samples collected from Mymensingh Sadar Upazila Fisheries Zone

Metals		<i>Pangasius pangasius</i>	<i>Oreochromis niloticus</i>	<i>Heteropneustesfossilis</i>	<i>Anabustestudineus</i>	<i>Clariasbatrachus</i>
Fe	Mean	90.37	98.38	92.12	82.45	104.55
	SD	11.37	12.70	9.93	8.72	6.13
Co	Mean	< 0.28	< 0.28	1.79	1.49	3.01
	SD	–	–	2.62	2.10	2.37
Cr	Mean	5.44	2.62	4.81	6.73	< 0.41
	SD	4.48	3.83	3.81	6.21	–
Cu	Mean	30.98	28.63	24.47	32.88	30.44
	SD	4.04	1.49	4.18	15.23	6.37
Zn	Mean	77.35	94.56	70.01	83.16	96.56
	SD	5.46	15.35	8.32	3.15	9.23
As	< 0.41	< 0.41	< 0.41	< 0.41	< 0.41	
Hg	Mean	1.07	0.97	0.57	0.74	0.88
	SD	0.13	0.21	0.09	0.08	0.06
Pb	Mean	0.48	0.34	0.45	0.35	0.49
	SD	0.10	0.05	0.09	0.05	0.11

Safe limit_a for Fe, Co, Cr, Cu, Zn, As, Hg and Pb are 100, 0.01, 0.15, 30, 40, 1.4, 0.5 and 0.5 respectively. Safe limit_b for As, Hg and Pb are 2, 0.5 and 0.5 respectively

which were analyzed in the laboratory. The mean concentration of Fe in all fish species was lower than the permissible limit 100 $\mu\text{g/g}$ (FAO/WHO 1989), excepting the *C.batrachus* fish species (104.55 \pm 6.13 $\mu\text{g/g}$). The mean concentrations of Zn were in the range of 70.01 \pm 8.32 to 96.56 \pm 9.23 $\mu\text{g/g}$. The lowest concentration of Hg, 0.57 \pm 0.09 $\mu\text{g/g}$ was measured in *H.fossilis* while the highest concentration, 1.07 \pm 0.13 $\mu\text{g/g}$ was found in *P.pangasius*. The concentration of Co was not detected in *P.pangasius*, and in *O.niloticus* fish species, Co concentration (< 0.28 $\mu\text{g/g}$) is the same. The mean concentrations of Co were found in other fish species but exceeded the permissible limit, which is 0.01 $\mu\text{g/g}$. The mean concentrations of Cr in all fish species ranged from 2.62 to 6.73 $\mu\text{g/g}$, with the highest concentration in *A.testudineus* fish species. The mean values of Pb were 0.48 \pm 0.10 $\mu\text{g/g}$ in *P.pangasius*, 0.34 \pm 0.05 $\mu\text{g/g}$ in *O.niloticus*, 0.45 \pm 0.09 $\mu\text{g/g}$ in *H.fossilis*,

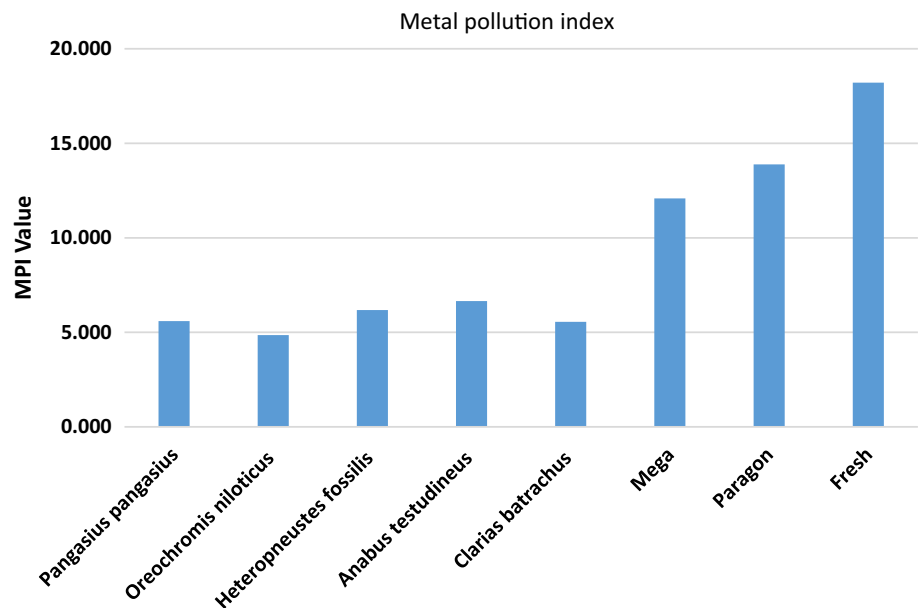
0.35 \pm 0.05 $\mu\text{g/g}$ in *A.testudineus*, and 0.49 \pm 0.11 $\mu\text{g/g}$ in *C.batrachus*.

Safety limit^a: Allowable metal concentration in Fishes by FAO/WHO (1989); Safety limit^b: Maximum levels of metals in crustaceans by EC (European communities), EC regulation on. 1881/2006 (EC 2006).

3.3 Health risk estimation

3.3.1 Metal pollution index (MPI)

A higher value of MPI indicates more significant aggregate metal accumulation in the sample, which determines the health risks of human beings through metal toxicity. The value of MPI varied from 4.85 to 6.65 $\mu\text{g/g}$ for fish samples and 12.09–18.21 $\mu\text{g/g}$ for fish feeds. Figure 2 illustrates MPI values for fish and fish feed in which *A.testudineus* fish species showed the highest Metal Pollution index

Fig. 2 MPI value for fishes and fish feeds

(6.657 $\mu\text{g/g}$) whereas *O.niloticus* fish species demonstrated the lowest (4.86 $\mu\text{g/g}$). The second highest value was in *H.fossilis* (6.18 $\mu\text{g/g}$) fish species. Otherwise, feed samples were ranked by MPI, the highest value was in Fresh feed (18.21 $\mu\text{g/g}$), and the values of MPI in Mega and Paragon feed were 12.09 and 13.88 $\mu\text{g/g}$ respectively.

3.3.2 Daily intake of metal (DIM)

In this study, the DIM value was calculated both for adults and children. Table 5 reveals the Daily Intake of Metals (DIM) for adult and children consumers, in which daily Fe, Cu, Zn, Hg, Pb intake ranged are 9.78–12.40 $\mu\text{g/kg}$, 2.9–3.9 $\mu\text{g/kg}$, 9.17–11.45 $\mu\text{g/kg}$, 0.06–0.13 $\mu\text{g/kg}$, 0.04–0.06 $\mu\text{g/kg}$ body weight respectively. For children consumers, all the daily intake of metal values were higher than the DIM value of adult consumers. This study revealed that the highest DIM value for Cu was found in *C.batrachus*, both adults and children (12.40 and 24.66 $\mu\text{g/kg}$, respectively). The maximum intake of Fe was found through the consumption of *C.batrachus*, both adult and children. The DIM value of Fe was the highest, and other parameter sequences order were $\text{Zn} > \text{Cu} > \text{Hg} > \text{Pb}$, respectively.

3.3.3 Health risk index (HRI)

For adults and children, the Health risk index (HRI) was calculated by dividing the Daily intake of metal and Reference Dose (Rf_D). Table 6 indicates that the concentration of metals for both adults and children had crossed the safe value of HRI for many folds. HRI for Fe exceeded the safe limit in adults; its value was 13–17 folds and 27–35 folds

for children. HRI value for Hg was very high in all the fish species where it was higher than 1. The sequences of metals from higher to lower that exceeded the safe limit of HRI were $\text{Hg} > \text{Cu} > \text{Zn} > \text{Fe} > \text{Pb}$ for adults and children. The HRI was maximum for Hg and minimum for Pb in all the measured samples for adults and children. However, the maximum HRI value for Pb was present in *P.pangasius* and *C.batrachus* species (14.41 for adults and 28.65 for children).

3.3.4 Target hazard quotient (THQ)

Non-carcinogenic effects were determined to assume the consumption of the detected metals in contaminated fish species. Table 7 indicates the results of the target hazard quotient (THQ). The acceptable risk level for THQ is 1 (USEPA 2011). If $\text{THQ} < 1$ for all individual metals, which indicates having non-carcinogenic effects. In this research work, the values of THQ for all detected metals in five fish species are less than 1, expecting Hg. The highest THQ value for Hg (2.00) was in *P.pangasius* fish species, and the THQ sequences of Hg in other fish species were *O.niloticus* $>$ *C.batrachus* $>$ *A.testudineus* $>$ *H.fossilis* respectively.

3.3.5 Hazard index (HI)

The hazard index is the summed-up score of all detected metals in the specific samples. The higher magnitude of the HI indicates higher risks for consumers. Figure 3 reveals that *P.pangasius* sample had the highest HI value (2.23), whereas the lowest value (1.26) was calculated in *H.fossilis* fish samples. On the other hand, HI values for *P.*

Table 5 Daily intakes of metals (DIM) in $\mu\text{g}/\text{kg}$ both Adult and children consumers through fish consumption from the sampling stations

Metals	<i>Pangasius pangasius</i>		<i>Oreochromis niloticus</i>		<i>Heteropneustes fossilis</i>		<i>Anabustudineus</i>		<i>Clarias batrachus</i>	
	Adults	Children	Adults	Children	Adults	Children	Adults	Children	Adults	Children
Fe	10.72	21.31	11.67	23.20	10.93	21.73	9.78	19.44	12.40	24.66
Cu	3.67	7.30	3.40	6.75	2.90	5.77	3.90	7.75	3.61	7.18
Zn	9.18	18.24	11.22	22.30	8.31	16.51	9.87	19.61	11.46	22.77
Hg	0.13	0.25	0.11	0.23	0.07	0.13	0.09	0.17	0.10	0.21
Pb	0.06	0.11	0.04	0.08	0.05	0.11	0.04	0.08	0.06	0.12

Table 6 Health Risk Index for both adults and children

Metals	<i>Pangasius pangasius</i>	<i>Oreochromis niloticus</i>	<i>Heteropneustes fossilis</i>	<i>Anabustudineus</i>	<i>Clarias batrachus</i>
<i>Adults</i>					
Fe	15.32	16.67	15.61	13.97	17.72
Cu	91.87	84.91	72.58	97.51	90.29
Zn	30.59	37.40	27.69	32.88	38.18
Hg	1274.18	1144.86	676.24	873.18	1046.04
Pb	14.36	10.00	13.23	10.32	14.47
<i>Children</i>					
Fe	30.44	33.14	31.04	27.78	35.22
Cu	182.62	168.79	144.28	193.84	179.48
Zn	60.80	74.33	55.03	65.37	75.90
Hg	2532.77	2275.72	1344.21	1735.68	2079.28
Pb	28.53	19.87	26.29	20.52	28.77

Table 7 Target Hazard Quotients (THQ) for different metals from the consumption of fish species collected from the study area

Metals	Target hazard quotients (THQ)				
	<i>Pangasius pangasius</i>	<i>Oreochromis niloticus</i>	<i>Heteropneustes fossilis</i>	<i>Anabustudineus</i>	<i>Clarias batrachus</i>
Fe	0.02	0.03	0.02	0.02	0.03
Cu	0.14	0.13	0.11	0.15	0.14
Zn	0.05	0.06	0.04	0.05	0.06
Hg	2.00	1.80	1.06	1.37	1.64
Pb	0.02	0.02	0.02	0.02	0.02

pangasius, *A. testudineus*, and *O. niloticus* were 1.89, 1.61 and 2.03, respectively. The sequences of HI in fishes from higher to lower order was: *P. pangasius* > *O. niloticus* > *C. batrachus* > *A. testudineus* > *H. fossilis*.

3.4 Target cancer risk

The target cancer risk was calculated for the intake of Pb and As. The acceptable level for cancer risk ranges from

10^{-4} to 10^{-6} , where the risk of cancer is considered negligible below 10^{-6} and above 10^{-4} is unacceptable (USEPA 1989, 2011). Table 8 represents the target cancer risk, which points out that TR values for Pb ranged from 1.33×10^{-4} to 1.93×10^{-4} in the measured fish species, while the cancer risk for As was not detected due to low concentration ($< 0.41 \mu\text{g}/\text{kg}$). The values of TR for Pb in *P. pangasius*, *O. niloticus*, *H. fossilis*, *A. testudineus*, and *C. batrachus* fish species were 1.91×10^{-4} , 1.33×10^{-4} ,

Fig. 3 Hazard index (HI) for the consumption of five fish species collected from the study area

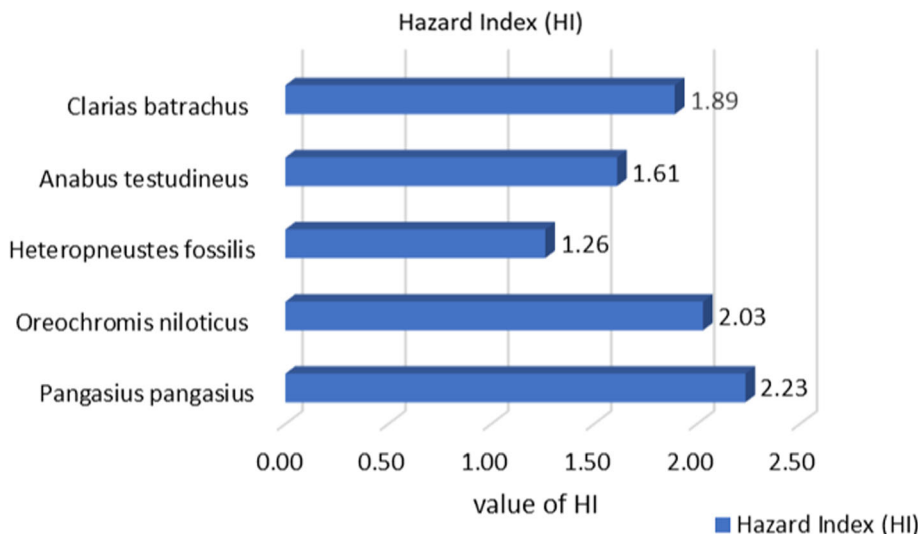


Table 8 Target cancer risk for studied fishes

Metals	<i>Pangasius pangasius</i>	<i>Oreochromis niloticus</i>	<i>Heteropneustesfossilis</i>	<i>Anabustestudineus</i>	<i>Clariasbatrachus</i>
Pb	1.91E-04	1.33E-04	1.76E-04	1.37E-04	1.93E-04
As	ND	ND	ND	ND	ND

ND not detected

1.76×10^{-4} , 1.37×10^{-4} , and 1.93×10^{-4} , respectively. However, The result of target cancer risk suggests that five commercial cultured fish species were capable of carcinogenic risk.

3.5 Principal component analysis of the metal parameters

PCA analysis was applied to determine the association between the parameters with the principal components. Eigenvalue was more significant than 1 were considered to demarcate the principal components. The scree plot of the PCA is shown in Fig. 4. Therefore, 5 principal components were derived from the analysis. These five components explained 73% of the variation in the data. Amongst the metal parameters, PC1 explains the highest 21% of the variation, whereas PC4 and PC5 explain 0.11% of the total variation. Cu and Co had the highest positive relation with PC1, while Pb and Fe had the highest negative association with PC1. Besides, Zn and Sr made the highest positive relation with PC2, whereas Cr and Rb had the highest negative association. The association between the parameters and PC2 follows the order Zn > Sr > Fe > Co > Cu > Se > Ca > Rb > Cr from strongly positive to strongly negative, respectively. Moreover, Se and Hg had

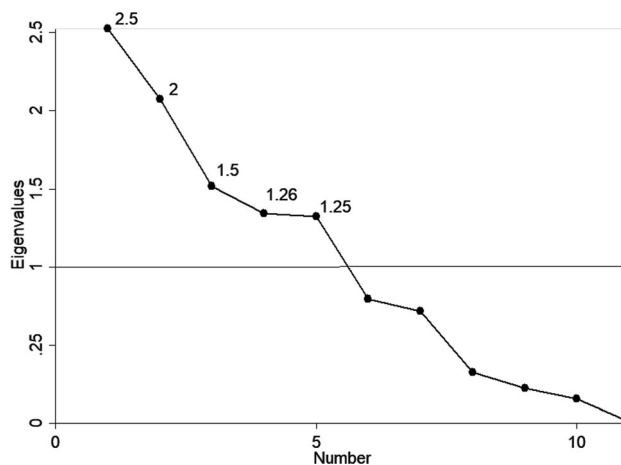


Fig. 4 Scree plot of the PCA

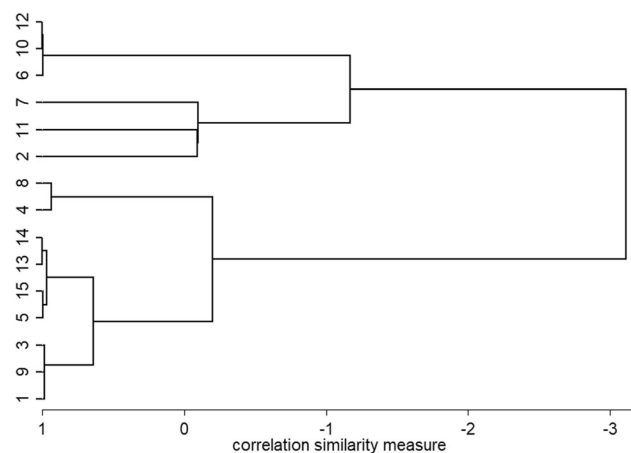
the highest positive association with PC3, whereas Rb, Zn, and Cu had the highest negative relation. In PC4, most of the variables were found to have a positive association except for Zn, Se, and Rb. Furthermore, Se and Co showed a strong negative relation with PC5, respectively. On the contrary, Cr, Rb, and Sr illustrated strong positive associations, respectively. Table 9 depicts the PCA analysis of metal parameters.

Table 9 PCA analysis of metal parameters

Parameters	PC1	PC2	PC3	PC4	PC5
Ca	0.41	-0.18	0.09	0.40	0.10
Cr	-0.14	-0.45	0.18	0.10	0.47
Fe	-0.47	0.11	-0.26	0.43	-0.06
Co	0.41	0.03	-0.04	0.52	-0.23
Cu	0.45	-0.08	-0.43	0.05	-0.10
Zn	-0.03	0.59	-0.29	-0.16	0.12
Se	-0.06	-0.13	0.51	-0.15	-0.46
Rb	-0.01	-0.33	-0.29	-0.12	0.47
Sr	0.08	0.42	0.22	0.20	0.37
Hg	0.05	0.28	0.47	0.22	0.31
Pb	-0.47	-0.10	-0.12	0.48	-0.15
Eigenvalue	2.52	2.07	1.51	1.34	1.32
Cumulative variance	0.23	0.41	0.55	0.68	0.80
Total variance	0.21	0.17	0.13	0.11	0.11

3.6 Hierarchical cluster analysis over the sampling stations

Correlational agglomerated cluster analysis was applied to examine the similarity and uniformity within the sampling stations based on metal values. The dendrogram is shown in Fig. 5 was resulted from the average linkage. Sampling stations 5,15,13 and 14 showed uniformity amongst the sampling sites. Besides, 1,9 and 3 sampling stations made a cluster which was further made a sub-cluster with the 5,15,13 and 14 stations. After that, 6, 10, and 12 sampling stations were found to have a strong association within them. The notable heterogeneity was found from the 2, 11, and 7 sampling stations. Each of the stations individually revealed a weak association. Figure 5 shows the dendrogram of cluster analysis using average linkage.

**Fig. 5** Dendrogram using average linkage

3.7 Pearson correlation matrix within trace metals accumulated in fishes

Pearson correlation matrix determined the degree of association within fishes regarding metal accumulation. Ca had a strong positive relation with Co and Ni at 95% significant level while Fe and Zn had a strong negative association with Ca. Besides, Cr was found to have a moderate positive relation with Mn and Rb, whereas it shows a negative relation with Zn and Co at 0.05 significant level. Conversely, Mn did not have a notable positive association with these trace metals, while it had a moderate negative association with Co and Hg. After that, Fe had a significant positive relation with Pb. Also, Co seems to have a lower to moderate positive correlation with Ni, Co, and Hg. Ni had a positive association with As and Pb. On the contrary, Cu negatively correlated with Pb and Hg. Similarly, Zn positively associated with Sr. As was observed to have negatively associated with the maximum of the metals. Furthermore, As was positively associated with Ni and Ca. Then, Se was negatively correlated with Zn. Rb had negatively related with the maximum of the metals, while with Cr, it was positively correlated. Hg was positively associated with Ni. All the metals had remained at 95% significant level. Table 10 shows the correlation matrix within accumulated metals in fish species. Besides, comparatively toxic metals in fish species and fish feeds were taken into consideration. Table 11 shows the association of accumulated metals between fishes and fish feeds. Only one association was found to have a significant level. The table reveals no significant relation of one particular metal of fish feeds over the other metals in fish species. The specific metal from fish feeds might cause bioaccumulation of that metal in the fish species.

4 Discussion

The bioaccumulation of metal in cultured fish depends on numerous factors; among them, fish feeds act as relevant sources. In an aquatic environment, metal toxicity can be affected by the length and weight of fishes (Nsikak et al. 2007). Fish feeds are the primary sources of metal accumulation in the flesh of studied fish. Therefore, it is assumed that metals accumulated in the fish are mainly from fish feeds because there are no other anthropogenic sources of pollution in the study area of the cultured fishing zone. Besides, Ali and Khan (2018) also stated that, Possible factors have an associate influence on fish bioaccumulation, corresponding to fish feed groups. This is in agreement with the findings of Sabbir et al. 2018. They reported the Fish feeds could be a source of metal

Table 10 Pearson Correlation matrix of metals found in fishes

	Ca	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Rb	Sr	Hg	Pb
Ca	1.00													
Cr	0.19*	1.00												
Mn	0.12	0.45*	1.00											
Fe	-0.31*	-0.03	-0.19*	1.00										
Co	0.50*	-0.25*	-0.45*	-0.12	1.00									
Ni	0.62*	0.30*	0.13	0.34*	0.26*	1.00								
Cu	0.36*	-0.24*	-0.26*	-0.34*	0.49*	-0.22*	1.00							
Zn	-0.28*	-0.49*	-0.17	0.20*	-0.14	-0.34*	0.02	1.00						
As	0.25*	-0.08	0.17	0.23*	-0.18	0.42*	-0.23*	0.12	1.00					
Se	-0.08	-0.07	-0.11	-0.10	-0.08	0.08	-0.20*	-0.40*	-0.07	1.00				
Rb	-0.03	0.33*	-0.11	0.06	-0.16	-0.17	0.23*	-0.23*	-0.19	-0.19*	1.00			
Sr	0.16	-0.09	-0.29*	0.04	0.03	-0.04	-0.06	0.42*	-0.18	-0.03	-0.13	1.00		
Hg	-0.02	-0.03	-0.41*	-0.08	0.19*	-0.01	-0.27*	0.07	-0.28*	0.00	-0.10	0.42*	1.00	
Pb	-0.24*	0.19*	-0.03	0.81*	-0.16	0.45*	-0.30*	-0.19*	0.13	0.00	-0.07	-0.13	-0.10	1.00

*Significant level at 0.05

contamination of the fish body in the aquaculture system and the mean concentration of Pb, Cd, Mg, As and Cr for different fish feed collected from Khulna, Satkhira and Bagherhat districts of Bangladesh were 8.49 ± 3.66 , 0.29 ± 0.8 , < 0.02 , < 0.05 , and 8.57 ± 3.47 mg/kg respectively. Kundu et al., 2017 evaluated the metal concentration in four fish feeds and metal burden in cultured *Oreochromis niloticus* fish species in Bangladesh and the study found was reveal that the concentration of Cu, Pb, and Cd in fish feeds was 22.618 to 38.480 mg/kg, 7.671 to 12.232 mg/kg, and 8.082 to 9.771 mg/kg respectively. The observations from Anhwange et al., 2012 on metal contents on two synthetic fish feeds namely Multi, and Coppen feeds in Nigeria whereas the concentration of Cd, Cu, Fe, Ni, Pb and Zn in Multi feed are 30.00 µg/kg, 157.00 µg/kg, 2196.00 µg/kg, 92.00 µg/kg, 348.00 µg/kg and 1209.00 µg/kg respectively while the concentration of Cd, Cu, Fe, Ni, Pb and Zn in Coppen feed are Cd, Cu, Fe, Ni, Pb, and Zn are 20.00 µg/kg, 204.00 µg/kg, 2435.00 µg/kg, 8.00 µg/kg, 375.00 µg/kg and 3324.00 µg/kg respectively. Onsanti et al. 2012 measured mercury concentration in three types of fish feeds (dried pellet feed, forage fish, and fish viscera), about Mercury and stable isotope signatures in caged marine fish and fish feed in Fujian coastline, China. The recorded result of onsanti et al. showed that tuna viscera contained the highest level of mercury (0.20 µg/g THg and 0.13 µg/g MeHg), with pellet feed containing the lowest level (0.05 µg/g THg and 0.01 µg/g MeHg). Besides, Hu et al. (2016) measured that the mean value of Cd in marine fish, crustaceans and mollusks were 0.023 (0.002–0.223) mg/kg, 0.155 (0.002–1.90) mg/kg and 0.460 (0.002–3.06) mg/kg respectively and, the mean content of Pb in marine fish, crustaceans and mollusks were 0.08 (0.02–0.223) mg/kg, 0.08 (0.02–1.04) mg/kg and 0.30 (0.02–2.63) mg/kg respectively in the coastline China.

Fish feeds in the present study reveal that the concentration of iron (Fe), copper (Cu), and lead (Pb) in three types of feed samples containing lower than safety limits 500, 100, and 5.00 µg/g, respectively. Arsenic concentration in the present study was below the detection limit. Zn standards were not available as permitted standards. Hg concentrations in studied three types of feeds (mega, paragon and fresh) are higher than the acceptable standard limit which were 0.91 µg/g, 1.23 µg/g and 1.21 µg/g in mega, paragon and fresh feeds respectively whereas the concentration of Cr in paragon feed sample is 9.02 µg/g which are also very high from accepted safety limit. It was alarming to note that all feed samples collected for the present study contain metals close to the standard limit, and few are higher than the safety limit.

Table 11 Pearson Correlation matrix of metals in fishes and fish feeds

	Cr (F)	Zn (F)	Cu (F)	Pb (F)	Co (F)	Hg (F)	Cr (FF)	Cu (FF)	Zn (FF)	Pb (FF)	Co (FF)	Hg (FF)
Cr (F)	1.00											
Zn (F)	− 0.73	1.00										
Cu (F)	0.16	0.42	1.00									
Pb (F)	0.18	0.71	0.88*	1.00								
Co (F)	0.19	0.06	0.04	0.76	1.00							
Hg (F)	0.14	− 0.65	0.07	0.12	0.75	1.00						
Cr (FF)	0.16	0.48	0.59	− 0.51	0.60	0.23	1.00					
Cu (FF)	0.11	0.13	− 0.69	0.71	0.45	− 0.12	0.11	1.00				
Zn (FF)	0.37	− 0.18	− 0.84	0.52	− 0.12	0.03	− 0.74	0.25	1.00			
Pb (FF)	− 0.21	0.35	− 0.34	0.14	− 0.08	0.01	0.50	0.70	0.18	1.00		
Co (FF)	0.24	0.12	0.22	− 0.23	0.14	0.13	0.03	0.04	0.01	0.32	1.00	
Hg (FF)	0.12	− 0.88	0.43	0.08	0.34	0.19	0.32	0.23	0.24	0.32	0.12	1.00

*Significant level at 0.05, *F* Fishes, *FF* Fish feeds

Table 12 Comparison of metal content in fish flesh samples (µg/g) observed by different authors values

SampleNo	Description	Fe	Cu	Cr	Co	Zn	As	Hg	Pb	References
01	Mymensingh SadarUpazila, Bangladesh	93.57	29.48	ND-6.73	ND-3.01	84.33	< 0.41	0.85	0.42	Present study
02	Kutubdia Channel, Bangladesh	–	3.26	1.74	–	27.45	< 0.41	–	3.116	Rahman et al. (2019)
03	Taihu Lake, Chine	–	0.21	0.34	–	–	–	–	0.61	Rajeshkumar et al. (2018)
04	Red Sea, Egypt	3.68	0.29	–	–	3.22	–	–	0.438	El-Moselhy et al. (2014)
05	Turag river, Bangladesh	–	22.585	0.75	–	–	–	–	–	Mandal and Ahmed (2014)
06	Dhaleshwari river, Bangladesh	–	18.23	1.37	–	–	–	–	15.08	Ahmed et al. (2012)
07	Wadi hanifah, Saudi Arabia	–	1080 ppb	230 ppb	–	–	–	3.1 ppb	39.7 ppb	Abdel-Baki et al. (2011)
08	Taihu Lake, Chine. (4 species)	–	ND-1.89	ND-0.39	–	16–130	–	–	0.003–0.021	Qiao-qiao (2007)
09	Kolleru Lake, India	–	–	11	–	–	–	–	1.84	Sekhar et al. (2003)

In Bangladesh, where malnutrition remains a significant development challenge, fish is an irreplaceable animal-source food in the diet of millions (Bogard et al. 2015). Fish is one of the most important sources of protein, but the ingestion of metal from anthropogenic and other sources, especially in the case of cultured fish species, can caused human health risk. Metal in fish flesh causes a severe health risk to humans (Onyia et al. 2010). Rahman et al. (2016) identify metal pollution of water and fishes in Balu and Brahmaputra rivers of Bangladesh where the Cr

concentration of Balu river three fish species (*Mastacem belusarmatus*, *Channapunctatus*, and *Mystusvittatus*) are 0.72 mg/kg, 0.76 mg/kg and 0.51 mg/kg respectively and Cr concentration of Brahmaputra river three fish species (*Mastacem belusarmatus*, *Channapunctatus*, and *Mystusvittatus*) are 0.19 mg/kg, 0.17 mg/kg, 0.22 mg/kg respectively. Raknuzzaman et al. 2016 conduct a study about trace metal contamination in commercial fish in the coastal area of Bangladesh where Cr concentration range 0.15–2.2 mg/kg.

The present study finding showed that the concentration of Cr, Co, and Zn for all fish species was above the higher limit from the recommended value of FAO and WHO (1989). Cu concentration is high in *Pangasius pangasius*, *Oreochromis niloticus*, Hg concentration is high in *Pangasius pangasius*, *Oreochromis niloticus*, *Anabustestudineus* and *Clariasbatrachus*. Copper high doses cause anemia, liver and kidney damage, and stomach and intestinal irritation and high levels of chromium, which can irritate skin and can produce ulcers; cause liver, kidney, circulatory and nerve tissue damage (Mandal and Ahmed 2014). Pb concentration is very close to the standard limit in *Pangasius pangasius* and *Heteropneustes fossilis* fish species. In humans, the exposure of Lead can inhibit the hemoglobin synthesis; distort the cardiovascular system (Ogwuegbu and Muhanga 2005).

Given human health risk, MPI values for fish and fish feed in which *A.testudineus* fish species showed the highest Metal Pollution index. MPI values ranged from 2.38 to 4.89 $\mu\text{g/g}$ in the freshwater prawn (*Macrobrachium rosenbergii*) of Bangladesh (Islam et al. 2017). The highest DIM value for Cu was found in *C.batrachus*, both adults and children. The DIM value of Fe was the highest, and other parameter sequences order were $\text{Zn} > \text{Cu} > \text{Hg} > \text{Pb}$, respectively. Zhong et al. (2018) identified the values of estimated daily intake for both adult and children in ten fish species in the central and eastern North China whereas the estimated daily intake of Cu, Pb, As, Cr and Zn for adult were 0.37, 0.13, 0.11, 2.24 and 6.36 respectively while the estimated daily intake of Cu, Pb, As, Cr and Zn for children were 0.70, 0.24, 0.21, 4.22 and 11.97 respectively.

Maximum HRI values for Pb was present in both *P. pangasius* and *C. batrachus* fish species. Furthermore, the highest HI value (2.23) recorded for this study in *P. pangasius* fish species whereas the lowest value (1.26) was calculated in *H.fossilis* fish samples. Akoto et al. (2014) calculated HRIs of Fe, Cu, Pb and Zn in muscles of fish samples from the Fosu Lagoon, Ghana and the HRI value were 1.90×10^{-3} , 7.25×10^{-4} , 8.69×10^{-2} and 9.17×10^{-4} respectively. Ahmed et al. (2015) estimated hazard index (HI) for different metals from the consumption of three fish species collected from Buriganga River, Bangladesh, and the HI values of *Ailiacoila*, *Gagatayous-soufi* and *Mastacembelus pancalus* were 5.10×10^{-1} , 4.49×10^{-1} and 1.03×10^{-1} respectively. The carcinogenic risk also showed that the examined fish species have carcinogenic effects through the consumption of the selected fish species.

5 Conclusions

The study demonstrated the concentration of metal in fish feeds and determined the metal concentration in the selected commercial fish flesh. However, the result of the present investigation indicates that the metal concentration in fish feeds may be responsible for bioaccumulation of metal in the fish flesh but future research work about fish feeds and fish metal accumulation considering other factors such as time, growth of fish and size of fish helps to understand about accumulation process and concentration. Health-related diseases may cause for consumption of the fishes with high degrees of metals. Amongst the measured fish species, *Heteropneustes fossilis* was found to have a lower accumulation of metals. According to the indices result, this fish species likely to be safer compared to other fish species. In contrast, *Clariasbatrachus* was observed to have a high contamination rate comparatively. As samples were collected from homogenous areas in different fishing zone, the degree of contamination might not vary significantly within the study area. The study provides useful tools for future ecotoxicological studies and the safety of consumers and helps the decision-maker implement rules and guidelines about the manufacture of commercial fish feed for fisheries. In developing countries, cultured fish are a regular part of the diet for most people, and from the consumer safety perspective, regular investigation and monitoring of related institutions and authorities are strictly required. A better aquaculture management system should be established for the conservation of the fish resources for the next generation, and more attention should be given on the manufacturing environment, and health-friendly fish feeds.

Acknowledgements The authors would like to thank the authority of Bangladesh Atomic Energy Commission (BAEC) for providing their lab facilities and guidelines.

Authors' contributions PG initiated and contributed to the preliminary concept of the study under the supervision of ZA and BB. Afterward, PG collected the primary data from the study area and completed the laboratory analysis according to the instruction of SA. Meanwhile, YNJ provided suggestions and guidelines. Statistical analyses were done by RA. Subsequently, RA and ZA completed the final draft and revised it critically. Finally, all the authors approved this final version.

Funding The study did not receive any funding.

Declaration

Conflict of interest The authors declare that they do not have any competing interests.

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