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Bioaccumulation of Selected Toxic Heavy Metals in *Mastacembelus armatus* from Three Rivers of Malakand Division, Pakistan

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Authors' Contribution
EK and HA conceived and designed the study. HA collected, prepared and analyzed the samples. HA analyzed the data and wrote the manuscript. EK supervised the research work.

Key words
Bioaccumulation, Environmental pollution, Health risk, Heavy metals, *Mastacembelus armatus*

ABSTRACT

Contamination of water bodies with toxic heavy metals is a genuine environmental problem. Bioaccumulation of toxic heavy metals in fish poses a potential health risk to fish consumers including humans. The aim of present research work was to study the bioaccumulation of Cr, Ni, Cd and Pb in the carnivorous fish *Mastacembelus armatus* at different sites of three rivers in Malakand Division, Pakistan. The study also investigated tissue-specific accumulation of these metals in *M. armatus* at one site of River Panjkora. Concentrations of Cr, Ni, Cd and Pb in the fish muscles ranged from 10.2 ± 3.5 to 29.8 ± 17.3, 24.7 ± 13.1 to 104.5 ± 27.1, 0.77 ± 0.17 to 2.4 ± 0.12 and 7.5 ± 5.3 to 75.2 ± 41.0 mg kg^{-1} wet weight, respectively. The order of metal accumulation in different tissues of *M. armatus* was: kidneys > liver ≅ skin > muscles > gills. Bioaccumulation factor (BAF) values of the metals in muscles of *M. armatus* were in the order: Cr > Pb > Ni > Cd. BAF values show that these metals are accumulated in the fish tissues and may pose a potential health risk to regular consumers.

INTRODUCTION

Environmental pollution is one of the environmental challenges faced by human society (Ali and Khan, 2017). Heavy metals are defined as "naturally occurring metals having atomic number (Z) greater than 20 and an elemental density greater than 5 g cm^{-3}" (Ali and Khan, 2018b). A rapid increase in pollution of waters by metals is promoted by industrial and agricultural development. After release into the environment, heavy metals do not biodegrade, but rather accumulate in the environment and living organisms (Stankovic *et al.*, 2014). Even the essential heavy metals can also be harmful to biota and cause adverse health effects at high concentrations (Mahboob *et al.*, 2014; Stankovic *et al.*, 2014). At high concentrations, toxic heavy metals can generate reactive oxygen species (ROS) and thus induce oxidative stress (Waheed *et al.*, 2014). Contamination of freshwater fish with toxic heavy metals is an issue of environmental, ecological and economic importance and has scope for public health (Ali and Khan, 2018a). Since fish muscles are the primary edible part, they can cause serious health risks to fish consumers (Waheed *et al.*, 2014). Fish dwelling in contaminated waters should be used in diet with caution (Javed and Usmani, 2016).

Exposure to Cr causes oxidative stress with subsequent possible cellular and molecular damages such as genotoxic and carcinogenic effects (Junaid *et al.*, 2016). Exposure to Ni causes hematological effects in animals and humans. Its many harmful effects are due to its interference with the metabolism of essential metal ions like Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Cu^{2+} and Zn^{2+} (Cempel and Nikel, 2006). Exposure to Cd at low levels initially causes renal tubular dysfunction, and finally ends in itai-itai disease (Nogawa, 1984). There is also an association between such an exposure and increased risk of osteoporosis (Alfvén *et al.*, 2000). Cadmium has endocrine disruptive effects and affects synthesis of sexual steroids even at quite low concentrations (Knazicka *et al.*, 2015). Lead is a toxic metal and its extensive use has created environmental contamination and health problems throughout the world (Rehman *et al.*, 2015). Heavy metal pollution in water bodies also has adverse effects on the aquatic organisms. It has been reported that heavy metals-containing wastewater in river has induced stress in the fish *Channa punctatus*, making them weak and susceptible

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to diseases (Javed and Usmani, 2015).

In Pakistan, water pollution has become a serious problem with rapidly increasing industrialization. Industrial effluents as well as domestic sewage are continuously discharged into rivers. These discharges contain toxic heavy metals. Such discharges into Pakistani rivers have adversely affected freshwater fisheries (Javed, 2005). Industrially polluted waters are currently discharged into water bodies without pretreatment (Rehman *et al*., 2008). It has been reported that aquatic chemical pollution in River Kabul is most probably one of the major causes of the rapid population decline of the endangered freshwater fish *Tor putitora* in the river (Yousafzai and Shakoori, 2011). In a recent study (Ali *et al*., 2017), bioaccumulation of the essential heavy metals Cu and Zn was investigated in *Schizothorax plagiostomus* and *Mastacembelus armatus* from the study area. The aim of the present research work was to study the bioaccumulation of four toxic heavy metals, Cr, Ni, Cd and Pb in muscles of the economically important fish species *Mastacembelus armatus* in three rivers of Malakand Division, Pakistan. This fish species was selected because it is commercially important and preferred by consumers in the study area. Tissue-specific bioaccumulation of the selected heavy metals was also studied in *M. armatus* at Khazana on River Panjkora. We selected muscle tissue for quantification of metals because this is the tissue of main interest for routine monitoring of environmental metal contamination (Yousafzai *et al*., 2012) and is the most important in terms of human consumption.

MATERIALS AND METHODS

Study area and fish species

This research work was conducted along River Swat, River Panjkora, and River Barandu in Malakand Division of Khyber Pakhtunkhwa province of Pakistan. The study fish, tire-track spiny eel or zig-zag eel (*Mastacembelus armatus*), a common fish species of the Indian sub-continent, is an economically important fish.

Sample collection, preparation and analysis

Three specimens of *M. armatus* were collected at each sampling site on the three rivers. Thus, twenty-one individuals of the fish were collected in total from all sites in the study area. The collected fish were transported to the laboratory in ice on the same day. Samples were prepared for metal analysis through wet digestion method using concentrated acids HNO_3, and $HClO_4$. Fish samples were processed on wet weight basis. Muscle tissue samples, free of skin, were taken from behind the dorsal fin, above the lateral line; also recently reported by Rosseland *et al*. (2017). For tissue-specific bioaccumulation study, tissues were separated by fish dissection. Water samples were prepared according to Malik and Maurya (2014), while the fish samples were digested according to Javed and Usmani (2016) with modifications: For acid digestion, 1.0 g sample of fish tissue was placed in a 100 mL beaker, 7.5 mL HNO_3 and 2.5 mL $HClO_4$ were added to it. The sample was heated on a hot plate at 80C until the evolution of brown fumes of NO_2 ceased and a clear yellow solution was obtained. The solution was allowed to cool at room temperature and then filtered through a 0.45 µm filter paper. The filtrate was diluted to a final volume of 50 mL with analyte-free water. The selected heavy metals were determined in the prepared samples by Flame Atomic Absorption Spectrophotometer (Perkin-Elmer Model No. 2380). Quality control check was performed in order to assure the accuracy of the metal analysis (Supplementary Table I).

Statistical analyses

For each experimental analysis, three samples i.e., three individual fish specimens were used. Results are shown as mean ± standard deviation (SD). Results were analyzed with the statistical software SPSS version 23.0. Different statistical tests such as analysis of variance (ANOVA), and correlation were used for comparing and analyzing the results. *P* value of 0.05 was considered for statistical significance.

RESULTS AND DISCUSSION

The concentrations of the four heavy metals in muscles of the study fish species from River Swat, River Panjkora and River Barandu are shown in Table I. One Way ANOVA (Tukey test) was used to compare concentrations of heavy metals at different sites on River Barandu. In most cases, the differences were statistically not significant, most probably due to small sample size and large standard deviation values in the results. The reason for large standard deviation values was the use of three individual fish specimens for each analysis instead of pooling tissues from three specimens and then taking three composite samples from the pooled tissues. Therefore, generally, we could not observe clear trends in concentrations of the selected heavy metals along river sites. However, in some cases, a clear trend could be seen. For example, in case of Ni bioaccumulation in the muscles of the study fish species, an increase was seen in concentration from upstream to downstream (Fig. 1).

Chromium concentrations found in this study in muscles of *M. armatus* ranged from 11.3 ± 0.85 to 29.8 ± 17.3 mg kg^{-1} ww from River Swat, 1280.2 ± 600.0 from

River Panjkora while 10.2 ± 3.5 to 21.8 ± 10.6 from River Barandu. Siraj *et al*. (2014) have reported bioaccumulation of heavy metals in two fish species, *Aorichthys seenghala* and *Ompok bimaculatus* near Nowshera on River Kabul, Pakistan. Their reported Cr concentrations in muscles of these two fish are 565.3 ± 148.7 and 703.0 ± 125.3 µg g^{-1} ww respectively. Ngelinkoto *et al*. (2014) have reported highest Cr concentration of 8.6 mg kg^{-1} ww in muscles of some fish species from Kwilu-Ngongo River, Congo. Javed and Usmani (2011) have reported Cr concentration of 1.0 ± 0.56 mg kg^{-1} dw in muscles of *Clarias gariepinus* collected from Rasalganj fish market, Aligarh, India. They did not detect Cr in muscles of two other fish, *Channa punctatus* and *Labeo rohita* in the same collection. A comparison of our results with these studies shows that Cr concentrations from our study are lower than those of the first mentioned study and higher than those of the latter two studies. Cr concentration in muscles of *M. armatus* at Khazana site of River Panjkora (1280.2 ± 600.0 mg kg^{-1} ww) was unusually high and was beyond expectation.

Table I. Heavy metal concentrations in muscles of *Mastacembelus armatus* collected from three rivers of Malakand Division.

Site	Metal concentration (mg kg^{-1} wet weight)			
	Cr	Ni	Cd	Pb
River Swat				
Kabal	29.8 ± 17.3	34.2 ± 20.1	0.77 ± 0.17	32.0 ± 34.0
Chakdara	11.3 ± 0.85	24.7 ± 13.1	1.4 ± 0.62	75.2 ± 41.0
River Panjkora				
Khazana	1280.2 ± 600.0	39.2 ± 27.3	2.4 ± 0.12	40.2 ± 28.0
River Barandu				
Toor Warsak	10.2 ± 3.5	25.5^{b} ± 15.1	1.4 ± 0.29	19.0 ± 1.1
Elai	21.8 ± 10.6	32.2^{b} ± 5.1	0.83 ± 0.10	28.8 ± 12.3
Daggar	12.3 ± 4.8	38.7^{b} ± 23.7	1.2 ± 0.33	16.3 ± 14.6
Dewana Baba	10.3 ± 4.0	104.5^{a} ± 27.1	1.2 ± 0.53	7.5 ± 5.3

Results are shown as mean ± standard deviation (n = 3). Mean values in a column with different superscript letters are significantly different at $p \leq 0.05$ (One Way ANOVA, Tukey Test).

Nickel concentrations found in this study in muscles of *M. armatus* ranged from 24.7 ± 13.1 to 34.2 ± 20.1 mg kg^{-1} ww from River Swat, 39.2 ± 27.3 from River Panjkora while 25.5 ± 15.1 to 104.5 ± 27.1 from River Barandu. Siraj *et al*. (2014) have reported bioaccumulation of Ni in *Aorichthys seenghala* and *Ompok bimaculatus* collected near Nowshera from River Kabul, Pakistan. Their reported Ni concentrations in muscles of these two fish are 94.7 ± 33.3 and 135.0 ± 52.6 µg g^{-1} ww respectively. Previously, Ahmad *et al*. (2014) have not detected Ni in muscles of *Schizothorax plagiostomus* collected from three different sites on River Panjkora. Ngelinkoto *et al*. (2014) have reported highest Ni concentration of 6.4 mg kg^{-1} ww in muscles of some fish species from Kwilu-Ngongo River, Congo. In a recent study, Javed and Usmani (2016) have reported Ni concentration of 59.0 ± 0.06 mg kg^{-1} dw in muscles of *M. armatus* collected from a thermal power plant effluent-polluted canal in Aligarh, India. In another study, Javed and Usmani (2011) have reported Ni concentrations of 34.1 ± 1.4, 21.0 ± 1.7 and 10.8 ± 2.0 mg kg^{-1} dw in muscles of *Channa punctatus*, *Clarias gariepinus* and *Labeo rohita* collected from Rasalganj fish market, Aligarh, India. A comparison of our results with these studies shows that our reported concentrations are lower than those of the first mentioned study except Ni concentration in muscles of *M. armatus* at Dewana Baba site of River Barandu (104.5 ± 27.1 mg kg^{-1} ww). Furthermore, our reported Ni concentrations are higher than that of Ngelinkoto *et al*. (2014) and comparable to those of the latter two studies.

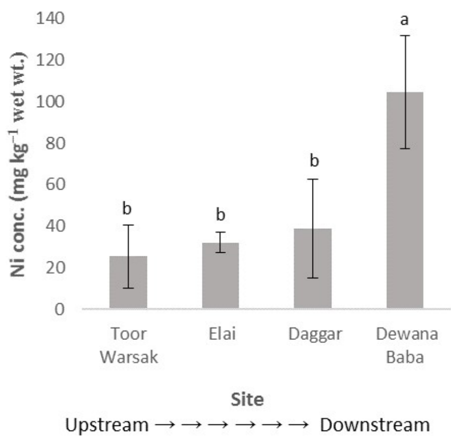

Fig. 1. Ni concentrations (mg kg^{-1} wet weight) in muscles of *Mastacembelus armatus* at different sites of River Barandu.

Cadmium concentrations found in this study in muscles of *M. armatus* ranged from 0.77 ± 0.17 to 1.4 ± 0.62 mg kg^{-1} ww from River Swat, 2.4 ± 0.12 from River Panjkora while 0.83 ± 0.10 to 1.4 ± 0.29 from River Barandu. Siraj *et al*. (2014) have reported bioaccumulation of Cd in *Aorichthys seenghala* and *Ompok bimaculatus* collected near Nowshera from River Kabul, Pakistan. Their reported Cd concentrations in muscles of these two fish are 60.7 ± 17.2 and 71.7 ± 12.1 µg g^{-1} ww respectively.

Previously, Ahmad *et al*. (2014) have not detected Cd in muscles of *Schizothorax plagiostomus* collected from three different sites on River Panjkora. A comparison of our results with these studies shows that our reported Cd concentrations are very low as compared to those of the first mentioned study.

Lead concentrations found in this study in muscles of *M. armatus* ranged from 32.0 ± 34.0 to 75.2 ± 41.0 mg kg^{-1} ww from River Swat, 40.2 ± 28.0 from River Panjkora while 7.5 ± 5.3 to 28.8 ± 12.3 from River Barandu. Siraj *et al*. (2014) have reported bioaccumulation of Pb in *Aorichthys seenghala* and *Ompok bimaculatus* collected near Nowshera from River Kabul, Pakistan. Their reported Pb concentrations in muscles of these two fish are 350.7 ± 37.2 and 407.0 ± 126.6 µg g^{-1} ww respectively. Previously, Ahmad *et al*. (2014) have reported Pb concentrations of 0.01, 0.03 and 0.09 µg g^{-1} in muscles of *Schizothorax plagiostomus* at upstream, sewage-release and downstream sites respectively on River Panjkora. Ngelinkoto *et al*. (2014) have reported highest Pb concentration of 6.0 mg kg^{-1} ww in muscles of some fish species from Kwilu-Ngongo River, Congo. A comparison of our results with these studies shows that our reported Pb concentrations are lower than those of the first mentioned study and higher than those of the latter two studies.

The relatively high accumulation of Pb in muscles of the study fish from River Swat and River Panjkora may be due to discharge of this metal into the river water from different anthropogenic sources such as industrial effluents, combustion of fossil fuels and application of phosphate fertilizers. The relatively high accumulation of Ni in the fish downstream in case of River Barandu may be due to discharge of marble industry effluents into the river water. However, release of different heavy metals into natural waters from natural sources especially from weathering of mafic and ultramafic rocks is also very important.

M. armatus is a carnivorous fish and may accumulate higher concentrations of potentially toxic heavy metals from its diet. Afrin *et al*. (2015) have reported highest concentrations of heavy metals i.e., Cr, Cd and Pb in *M. armatus* among three available fish species from River Turag, Bangladesh. Ahmad *et al*. (2014) have reported that presently, the water and fish of River Panjkora are safe for human consumption. Likewise, recently, Ullah *et al*. (2016a) have reported that River Panjkora is less polluted compared to other rivers in Pakistan. However, they have communicated that presently, the river is facing sewage burden and the continued pollution from such discharge in the river may ultimately lead to serious concerns in the future. River Barandu is also being polluted by effluents from marble industries in Buner (Khan *et al*., 2012). Recently, Mulk *et al*. (2016) have found decreased ichthyofaunal diversity near marble industry effluents in River Barandu.

Table II. Correlation of the selected heavy metals in different tissues of *Mastacembelus armatus* collected at Khazana site from River Panjkora.

Tissue	Metal	Pearson Correlation Coefficient			
		Cr	Ni	Cd	Pb
Muscles	Cr	1			
	Ni	0.794	1		
	Cd	− 0.992	−0.713	1	
	Pb	−0.757	−0.998*	0.671	1
Gills	Cr	1			
	Ni	−0.260	1		
	Cd	0.079	0.942	1	
	Pb	0.219	0.885	0.990	1
Skin	Cr	1			
	Ni	−0.526	1		
	Cd	−0.587	0.997*	1	
	Pb	−0.816	0.921	0.947	1
Liver	Cr	1			
	Ni	0.820	1		
	Cd	0.827	0.355	1	
	Pb	0.956	0.616	0.955	1
Kidneys	Cr	1			
	Ni	−0.382	1		
	Cd	−0.705	0.924	1	
	Pb	−0.740	0.904	0.999*	1

*: Correlation is significant at the 0.05 level (2-tailed).

Tissue-specific bioaccumulation of HMs in Mastacembelus armatus

The concentrations of the four heavy metals in different tissues of *M. armatus* are shown in Figure 2. From Figure 2, it is seen that the order of metal accumulation in different tissues of the fish is: muscles > kidneys > skin > liver > gills for Cr; kidneys > liver > skin > muscles > gills for Ni; kidneys > liver = skin > muscles > gills for Cd and finally kidneys > liver ≅ skin > muscles > gills for Pb. Thus generally, the order of metal accumulation is: kidneys > liver ≅ skin > muscles > gills except for Cr where muscles have the highest accumulation. The general pattern of heavy metal accumulation observed in this study can be expected because kidneys and liver are metabolically active tissues and accumulate comparatively higher concentrations of toxic heavy metals for storage, sequestration and detoxification. It has been reported that among fish tissues, liver accumulates relatively more quantities of heavy metals (Vinodhini and Narayanan, 2008). Rauf *et al.* (2009) have reported higher accumulation of Cd and Cr in fish liver and minimum accumulation in gills. Similarly, Javed and Usmani (2013) have observed highest heavy metal load in liver while least in skin of *M. armatus* collected from a rivulet at Kasimpur, Aligarh, India. Ullah *et al.* (2016b) have reported tissue-specific bioaccumulation of heavy metals in three fish species from River Panjkora, Pakistan. They have observed the order of heavy metal bioaccumulation in different tissues as liver > kidneys > muscles > gills. Malik *et al.* (2014) have reported relatively higher concentrations of heavy metals in liver, kidneys and gills compared to those in skin and muscles of four edible fish species i.e., *Tor putitora*, *Cirrhinus mrigala*, *Labeo calbasu* and *Channa punctatus* from Rawal Lake Reservoir, Pakistan.

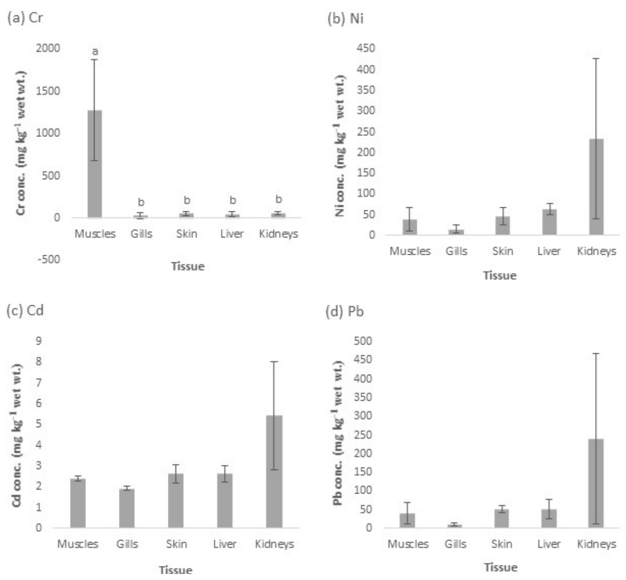

Fig. 2. Heavy metal concentrations (mg kg^{-1} wet weight) in different tissues of *Mastacembelus armatus* at Khazana site on River Panjkora.

In a previous study, Javed and Usmani (2013) have investigated the bioaccumulation of heavy metals in different tissues of *M. armatus* collected from a rivulet at Kasimpur, Aligarh, India. Their reported Ni concentrations in gills, liver, kidneys, muscles and skin were 200.0 ± 1.7, 450.0 ± 0.06, 149.3 ± 0.50, 59.0 ± 0.09 and 45.1 ± 0.02 mg kg^{-1} dw respectively. Our reported Ni concentrations in different tissues of *M. armatus* are comparable with these results. In a recent study, Rosseland *et al.* (2017) have reported Cr, Ni, Cd and Pb concentrations in gills and liver of *M. armatus* from Lake Phewa, Nepal. They have reported that in gills, the concentrations of these metals were 0.7-1.0, 2.3-3.8, 0-1.3 and 0.3-0.6 µg g^{-1} dw respectively while in liver the corresponding metal concentrations were 0.1-0.5, 0-0.2, 0.1-2.5 and 0-0.8 µg g^{-1} dw respectively, which are lower than our reported metal concentrations. Yousafzai and Shakoori (2008) have studied bioaccumulation of some heavy metals including Cr, Ni and Pb in gills of *Tor putitora* at three sites of River Kabul, Pakistan. Their reported Cr concentrations in gills at a reference site and two polluted sites, site 1 and site 2, were 5.3 ± 0.18, 6.6 ± 0.07 and 6.0 ± 0.38 µg g^{-1} ww respectively. The corresponding Ni concentrations were 53.3 ± 8.4, 128.0 ± 8.8 and 133.0 ± 7.3 µg g^{-1} ww respectively while the corresponding Pb concentrations were 219.3 ± 31.4, 313.7 ± 29.9 and 321.0 ± 9.8 µg g^{-1} ww respectively. A comparison of our results with these values shows that our reported Cr concentration in gills is higher than these values while our reported Ni and Pb concentrations in gills are lower than these values.

Correlation analysis was performed to check the correlation among the selected heavy metals in different tissues of *M. armatus*. Results of this analysis are shown in Table II. By looking at this table, it can be seen that significant correlations were found between Pb and Ni in muscles (R = −0.998), between Cd and Ni in skin (R= 0.997) and between Pb and Cd in kidneys (R = 0.999).

Calculation of bioaccumulation factor (BAF)

The bioaccumulation of the selected heavy metals in different tissues of *M. armatus* was also quantified by calculating the bioaccumulation factor (BAF). This factor is the ratio of the concentration of a heavy metal in a given tissue of a fish to the concentration of that heavy metal in the water in which the fish dwells. It is calculated by using the following equation (Javed and Usmani, 2013):

$$BAF = \frac{Concentration\ of\ HM\ in\ fish\ tissue\ (mg\ kg^{-1}\ dry\ weight)}{Concentration\ of\ HM\ in\ water\ (mg\ L^{-1})}$$

Table III gives BAF values of the selected heavy metals in different tissues of *M. armatus* collected from Khazana site on River Panjkora. Concentrations of the four heavy metals were determined in the water of River Panjkora at this site and are given in Table III. Concentrations of heavy metals in different tissues on wet weight basis were converted to their concentrations on dry weight basis assuming moisture content as 75% of wet weight, as considered by Ikemoto *et al.* (2008) for Cd, Pb and Hg in an aquatic food web study in the Mekong Delta, South Vietnam. In a previous study, Javed and Usmani (2013) have reported BAF values of some heavy metals including Ni in different tissues of *M. armatus* collected from a polluted rivulet at Kasimpur, Aligarh, India. Their reported BAF values of Ni in muscles, gills, skin, liver and kidneys of this fish were 491.5, 1666.7, 375.5, 3749.7 and 1244.4 respectively. Comparing these values with those in Table III shows that all these values are higher than those in our study except for BAF in kidneys. Javed and Usmani (2013) have reported high BAF values in liver for all the studied heavy metals. In our study, we found highest BAF values in kidneys for all the selected heavy metals except Cr, whose BAF value was highest in muscles.

Table III. Bioaccumulation factor (BAF) values of the selected heavy metals in different tissues of *Mastacembelus armatus* at Khazana site on River Panjkora.

Heavy metal	Conc. in water (mg L^{-1})	BAF				
		Muscles	Gills	Skin	Liver	Kidneys
Cr	0.367	13839.7	311.7	582.0	520.8	668.5
Ni	0.594	264.1	104.6	312.0	429.8	1573.0
Cd	0.041	234.2	188.3	256.6	256.6	529.8
Pb	0.227	707.8	182.0	910.5	907.5	4228.4

CONCLUSIONS

Results of the study showed that metal concentrations in muscles of the study fish from the three rivers were comparable. Generally, clear trends could not be observed for metal concentrations at different sites of the rivers when going from upstream to downstream. However, Ni concentrations in fish muscles at different sites on River Barandu show a clear pattern of increase downstream. This may be due to contamination of the river water by wastewaters from marble industries. Tissue-specific accumulation in different tissues of *M. armatus* showed the order as: kidneys > liver ≅ skin > muscles > gills. BAF values of the metals in different tissues of *M. armatus* show that these metals are accumulated from the river in the fish muscles and may pose a potential health risk to regular consumers.

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Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20170708190719

Statement of conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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