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Bioaccumulation of Cu and Zn in *Schizothorax plagiostomus* and *Mastacembelus armatus* from River Swat, River Panjkora and River Barandu in Malakand Division, Pakistan

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Authors' Contribution
HA and II conceived and designed the study. HA, WA, KU, FA, SA, IU, IA, AA and MAS collected, prepared and analyzed the samples. HA analyzed the data and wrote the manuscript. HA supervised the research work.

Key words
Bioaccumulation, Heavy metal, *Mastacembelus armatus, Schizothorax plagiostomus.*

ABSTRACT

Contamination of aquatic ecosystems with heavy metals leads to accumulation of these elements in aquatic biota including fish. Bioaccumulation of heavy metals in fish is of public health concern because fish are an important part of human diet. The present study was conducted to investigate the bioaccumulation of two heavy metals, Cu and Zn, in muscles of two economically important fish species, an herbivorous fish *Schizothorax plagiostomus* and a predatory fish *Mastacembelus armatus*, from three rivers of Malakand Division, Pakistan. Concentrations of Cu and Zn were determined in fish samples by atomic absorption spectrophotometer. Highest Cu concentration of 4.52 ± 0.24 mg kg^{-1} wet weight was observed in muscles of *M. armatus* at Chakdara on River Swat while highest Zn concentration of 18.00 ± 1.88 mg kg^{-1} wet weight was observed in muscles of *S. plagiostomus* at Daggar on River Barandu. A site wise comparison of both Cu and Zn concentrations in muscles of the study fish generally showed no significant differences among different sites on the rivers. However, in some cases, metal concentrations showed significant increase down the stream. Similarly, a comparison of metal concentrations in muscles of the two species generally did not show significant differences between the two species. Zn concentrations in fish muscles were higher than Cu concentrations in all cases. Bioaccumulation factor (BAF) values for Cu were in the order of kidneys > muscles > liver > gills > skin while for Zn the order was skin > kidneys > liver > gills > muscles.

INTRODUCTION

Environmental pollution is one of the major challenges faced by human society in the 21st century (Ali and Khan, 2017). Heavy metal pollution in aquatic ecosystems has become an issue of global concern due to industrialization (Javed and Usmani, 2016). Heavy metals are released to the environment from different natural and anthropogenic sources. Due to their non-biodegradable and persistent nature, they accumulate in the environment and subsequently accumulate in biota. Accumulation of toxic heavy metals in biota leads to contamination of the food chains/webs with consequent risk to wildlife and human health. Aquatic animals are at more risk from toxic heavy metals in their habitats because they cannot escape from such contamination. Monitoring of heavy metal contamination in fish is important because fish constitute an important food source for humans. In routine monitoring of environmental contamination, fish muscle is the main tissue of interest due to their consumption by humans (Siraj *et al*., 2014, 2016; Ambreen *et al*., 2015). Fish are important bioindicators of water quality (Hussain *et al*., 2016).

Fish consumption is recommended in human diet because they are a good source of proteins and unsaturated fatty acids. However, due to contamination of aquatic ecosystems with toxic heavy metals, it becomes necessary

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to monitor contents of these toxic elements in fish. Scientific interest from environmental point of view in essential heavy metals such as Mn, Fe, Cu and Zn is due to the narrow window between their essentiality and toxicity (Yousafzai and Shakoori, 2008). Essential heavy metals are required for proper functioning of life processes but either deficiency or excess of these metals in the body creates health problems (Tabinda *et al*., 2010, 2013). Although, Cu and Zn are essential elements but their excessive levels in fish can pose health risk to the consumer human population and to the exposed fish themselves. Excess of Cu in the body is associated with damage of the liver (Javed and Usmani, 2016). The Provisional Maximum Tolerable Daily Intake (PMTDI) of Cu and Zn given in Codex Alimentarius Commission are 0.05-0.5 mg/kg bw and 0.3-1 mg/kg bw, respectively (FAO/WHO, 2011). Some studies have investigated the effects of Cu on different biochemical parameters of fish (*e.g.,* Mutlu *et al*., 2016). In fish, Cu can induce respiratory distress (Javed and Usmani, 2011) and interferes with osmoregulation; death is caused by tissue hypoxia associated with disrupted ATP synthesis (Eisler, 1998). Cu pollution in natural waters may severely damage the gills and cause histological changes in liver and kidneys (Javed and Usmani, 2013). It has been reported that exposure to Cu induced DNA damage in the gill cells of the freshwater mussels (*Anodonta anatina*) and the genotoxic effects were more at an exposure dose of 120 µg L^{-1} (Sohail *et al*., 2017). Excess Zn is teratogenic to embryos of frogs and fish (Eisler, 1993).

Riverine pollution has been reported as one of the possible reasons for the rapid population decline of the endangered freshwater fish *Tor putitora* in River Kabul, Pakistan (Yousafzai and Shakoori, 2009). Recently, marble industry effluents were associated with lower species abundance and diversity in River Barandu, Pakistan (Mulk *et al*., 2016). In Pakistan, the major sources of heavy metals in the aquatic environment are agricultural runoff, domestic sewage and industrial effluents (Hussain *et al*., 2014). The aim of the present research work was to investigate the bioaccumulation of two heavy metals Cu and Zn in muscles of two economically important fish species, *Schizothorax plagiostomus* and *Mastacembelus armatus* from three rivers of Malakand Division, Pakistan. We selected fish muscles for analysis of the heavy metals because muscles are the edible part of fish. Accumulation of toxic chemicals in edible tissues of fish is of public health concern. Fish skin is also mostly consumed with muscles and is therefore important on bioaccumulation point of view (Yousafzai *et al*., 2010). However, fish muscles are usually analyzed for determining the levels of contaminants (Mortuza and Al-Misned, 2015).

MATERIALS AND METHODS

Study area

The study area of this research work included three rivers of Malakand Division *i.e.,* River Swat, River Panjkora and River Barandu. River Swat and River Panjkora meet at Bosaq Bridge, Sharbatti. Regarding ichthyofaunal diversity, River Swat has relatively more diversity compared to River Panjkora (Ullah *et al*., 2014). Discharge of effluents causes heavy metal contamination in River Panjkora as observed from slight increase in heavy metal concentrations downstream (Ahmad *et al*., 2014). River Barandu is also being polluted by effluents from marble industries in Buner (Khan *et al*., 2012).

Water samples

Water samples were collected according to Csuros and Csuros (2002) and transported to the laboratory in pre-cleaned polyethylene bottles. After filtration through a Whatman filter paper, a 50 mL sample was taken in a 100 mL beaker and acidified with 5 mL concentrated (65%) HNO_3. After acidification, the water samples were heated on a hot plate at 80°C until the volume was reduced to 50 mL (Malik and Maurya, 2014). These water samples were analyzed for Cu and Zn by Flame Atomic Absorption Spectrophotometer (Perkin-Elmer Model No. 2380).

Fish samples

This study investigated bioaccumulation of Cu and Zn in a herbivorous snow carp, *Schizotorax. plagiostomus* and a carnivorous *Mastacembelus armatus*. These fish species were selected because of their commercial importance in the study area. Furthermore, these species have different feeding habits. Dietary habits may affect metal bioaccumulation in different fish species (Papagiannis *et al*., 2004). Therefore, one aim of the study was to compare metal bioaccumulation in two fish species with different feeding habits.

Three samples each of *S. plagiostomus* and *M. armatus* (where available) were collected at each pre-selected site on River Swat, River Panjkora and River Barandu. Fish muscles were collected according to Rosseland *et al.* (2017). Skin-free muscle samples were taken from dorsal side behind the dorsal fin with the help of clean knife and scissors. In case of tissue-specific metal bioaccumulation study of *M. armatus*, other tissues *i.e.,* gills, skin, liver and kidneys were also separated. Fish samples were digested according to Javed and Usmani (2016) with modifications. A sample of 1 g (wet weight basis) was taken in a clean beaker. To this sample, 7.5 mL HNO_3 (65%) and 2.5 mL $HClO_4$ (70%) were added and heated on a hot plate at 80°C. The sample was heated until the evolution of brown fumes of NO_2 ceased and a clear (yellow) solution was obtained. The solution was cooled at room temperature and then filtered through a Whatman filter paper. The filtrate was diluted to 50 mL with analyte-free water. The heavy metals Cu and Zn were determined in these samples with Flame Atomic Absorption Spectrophotometer (Perkin-Elmer Model No. 2380).

For quality control check was performed in order to assure the accuracy of the metal analysis. The recoveries were obtained for the analyzed metals from reference materials (standards) (Table I).

Table I.- Recoveries obtained for the analyzed metals from reference materials.

Metal	Concentration (ppm)		Recovery (%)
	Measured	Standard	
Cu	4.998	5.000	99.96
Zn	0.997	1.000	99.70

Statistical analysis

Metal concentrations are shown as mean ± standard deviation with n = 3. Experimental data were analyzed with IBM SPSS Statistics 23. Mean metal concentrations at different river sites were compared using One-Way ANOVA (Tukey Test) while mean metal concentrations of the two fish species were compared using Independent-Samples T Test. *P* value of 0.05 was considered for statistical significance.

RESULTS AND DISCUSSION

Concentrations of Cu and Zn in muscles of *S. plagiostomus* and *M. armatus* collected from different sites of River Swat, River Panjkora and River Barandu are given in Table II. Cu and Zn concentrations in muscles of *S. plagiostomus* at different sites of River Swat did not show significant differences (Table II). However, Cu concentration in muscles of *M. armatus* (4.52 ± 0.24 mgkg^{-1} ww) was significantly higher than that of *S. plagiostomus* (1.47 ± 0.64 mg kg^{-1} ww) at Chakdara site. In case of River Panjkora, Cu and Zn concentrations in muscles of *S. plagiostomus* increased down the stream (Table II). Furthermore, Zn concentration in muscles of *M. armatus* (7.28 ± 1.71 mg kg^{-1} ww) was significantly higher than that of *S. plagiostomus* (3.30 ± 1.26 mg kg^{-1} ww) at Khazana site.

Table II.- Cu and Zn concentrations in muscles of *Schizothorax plagiostomus* and *Mastacembelus armatus* collected from River Swat, River Panjkora and River Barandu.

Site	Metal concentration (mg kg^{-1} wet weight)			
	Cu		Zn	
	S. plagiostomus	*M. armatus*	*S. plagiostomus*	*M. armatus*
River Swat				
Khwazakhela	1.35 ± 0.52 ^{a}	NA	12.12 ± 2.19 ^{a}	NA
Kabal	1.07 ± 0.36 ^{a}	0.62 ± 0.10	9.45 ± 1.15 ^{a}	8.95 ± 3.54
Chakdara	1.47 ± 0.64 ^{a}	4.52 ± 0.24**	7.53 ± 3.48 ^{a}	12.35 ± 1.48
Batkhela	2.05 ± 1.00 ^{a}	NA	12.80 ± 1.08 ^{a}	NA
River Panjkora				
Khall	0.93 ± 0.50	NA	2.72 ± 2.36 ^{b}	NA
Khazana	2.18 ± 0.46	3.92 ± 2.77	3.30 ± 1.26 ^{b}	7.28 ± 1.71*
Shagukas	2.72 ± 1.15	NA	10.32 ± 2.99 ^{a}	NA
River Barandu				
Toor Warsak	2.20 ± 0.74 ^{b}	1.67 ± 1.50	16.38 ± 4.23	11.98 ± 2.69
Elai	2.48 ± 0.39 ^{ab}	1.13 ± 0.80	10.17 ± 4.79	11.58 ± 2.50
Daggar	2.02 ± 0.33 ^{b}	1.83 ± 1.24	18.00 ± 1.88	13.12 ± 3.39
Dewana Baba	4.05 ± 0.91 ^{a*}	2.38 ± 0.41	12.08 ± 3.83	12.27 ± 4.20

NA, not available (species sample); Results are shown as mean ± standard deviation (n = 3). Mean values in a column with same superscript letter are not significantly different at p ≤ 0.05 (One Way ANOVA, Tukey Test). ** Means of the two species significantly different at p ≤ 0.01 (Independent-Samples T Test).

The trend of increase down the stream in concentrations of both metals in muscles of *S. plagiostomus* is shown in Figure 1. Ullah *et al*. (2016) have also reported higher concentrations of heavy metals in three fish species from River Panjkora at Timergara compared to those at Khall. In case of River Barandu, Cu and Zn concentrations in muscles of the two fish species did not show significant differences at different river sites except Cu concentrations in muscles of *S. plagiostomus*. However, Zn concentrations were higher than Cu at all sites. Furthermore, at Dewana Baba site, Cu concentration in muscles of *S. plagiostomus* (4.05 ± 0.91 mg kg^{-1} ww) was significantly higher than that of *M. armatus* (2.38 ± 0.41 mg kg^{-1} ww). At other river sites, Cu as well as Zn concentrations in muscles of the two fish species did not differ significantly. Zn concentrations in field collections of biota are extremely variable and difficult to interpret (Eisler, 1993).

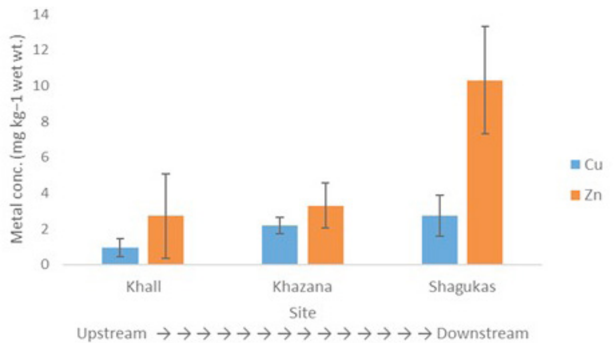

Fig. 1. Cu and Zn concentrations (mg kg^{-1} wet weight) in muscles of *Schizothorax plagiostomus* at different sites of River Panjkora.

Copper (Cu)

In this study, Cu concentrations in muscles of *S. plagiostomus* and *M. armatus* from River Swat ranged from 1.07 ± 0.36 to 2.05 ± 1.00 mg kg^{-1} ww and 0.62 ± 0.10 to 4.52 ± 0.24 mg kg^{-1} ww, respectively. Corresponding Cu concentrations from River Panjkora ranged from 0.93 ± 0.50 to 2.72 ± 1.15 mg kg^{-1} ww and 3.92 ± 2.77 mg kg^{-1} ww respectively while corresponding Cu concentrations from River Barandu ranged from 2.02 ± 0.33 to 4.05 ± 0.91 mg kg^{-1} ww and 1.13 ± 0.80 to 2.38 ± 0.41 mg kg^{-1} ww, respectively. Ngelinkoto et al. (2014) have reported highest Cu concentration of 15.7 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 Cu concentration of 41.36 ± 0.38 mg kg^{-1} dw in muscles of *M. armatus* collected from an effluent-loaded canal in India. A comparison of our results with those of the above two studies shows that our reported Cu concentrations are less than those of the above mentioned studies.

Zinc (Zn)

In this study, Zn concentrations in muscles of *S. plagiostomus* and *M. armatus* from River Swat ranged from 7.53 ± 3.48 to 12.80 ± 1.08 mg kg^{-1} ww and 8.95 ± 3.54 to 12.35 ± 1.48 mg kg^{-1} ww, respectively. Corresponding Zn concentrations from River Panjkora ranged from 2.72 ± 2.36 to 10.32 ± 2.99 mg kg^{-1} ww and 7.28 ± 1.71 mg kg^{-1} ww, respectively while corresponding Zn concentrations from River Barandu ranged from 10.17 ± 4.79 to 18.00 ± 1.88 mg kg^{-1} ww and 11.58 ± 2.50 to 13.12 ± 3.39 mg kg^{-1} ww, respectively. Ngelinkoto *et al*. (2014) have reported highest Zn concentration of 45.3 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 Zn concentration of 186.19 ± 0.12 mg kg^{-1} dw in muscles of *M. armatus* collected from an effluent-loaded canal in India. A comparison of our results with those of the above two studies shows that our reported Zn concentrations are less than those of the above mentioned studies.

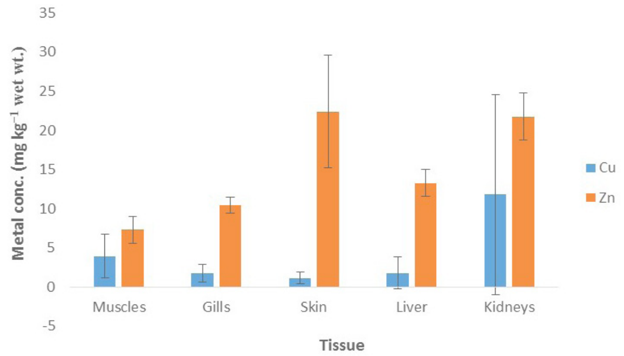

Fig. 2. Cu and Zn concentrations (mg kg^{-1} wet weight) in different tissues of *Mastacembelus armatus* collected at Khazana on River Panjkora.

Tissue-specific bioaccumulation of Cu and Zn in M. armatus

We also determined Cu and Zn concentrations in five different tissues of *M. armatus* collected at Khazana site on River Panjkora. Results of such tissue-specific bioaccumulation of the two heavy metals in *M. armatus* are shown in Figure 2. From Figure 2, it can be seen that the order of accumulation of Cu was: kidneys > muscles > liver > gills > skin while for Zn the order was: skin > kidneys > liver > gills > muscles. Among fish tissues, liver accumulates relatively more quantities of heavy metals (Vinodhini and Narayanan, 2008).

In our study, liver was at intermediate position in concentrations of Cu and Zn. Recently, Ullah et al. (2016) have reported the order of bioaccumulation of heavy metals in different tissues of three fish species from River

Table III.- Bioaccumulation factor (BAF) values of Cu and Zn in different tissues of *Mastacembelus armatus* at Khazana on River Panjkora.

Heavy metal	Conc. in water (mg L^{-1})	BAF				
		Muscles	Gills	Skin	Liver	Kidneys
Cu	0.022	178.18	78.64	50.91	80.91	535.91
Zn	0.022	330.91	474.09	1017.27	603.64	987.73

Panjkora, Pakistan as: liver > kidney > muscles > gills. Kidney is the principal tissue for the storage of metals and the main excretion route for toxicants (Palaniappan and Karthikeyan, 2009). Malik *et al*. (2014) have also 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.

In a recent study, Rosseland *et al*. (2017) have reported Cu concentrations of 2.5-5.2 and 7-1697 µg g^{-1} dw in gills and liver, respectively of *M. armatus* from Lake Phewa, Nepal. The corresponding Zn concentrations in gills and liver were 93-189 and 38-213 µg g^{-1} dw, respectively. Our reported Cu and Zn concentrations in gills and liver of *M. armatus* are less than those reported by the above mentioned study. Yousafzai and Shakoori (2008) have reported Cu concentrations of 44.70 ± 3.39, 67.70 ± 2.38 and 76.70 ± 4.82 µg g^{-1} ww in gills of *Tor putitora* collected from control and two polluted sites (site 1 and site 2), respectively of River Kabul, Pakistan. Their reported corresponding Zn concentrations were 1993.90 ± 126.70, 2124.90 ± 43.81 and 2414.00 ± 70.08 µg g^{-1} ww. Our reported Cu and Zn concentrations in gills are far less than those reported by the above mentioned study. The reason for low concentrations of the two heavy metals in different tissues of *M. armatus* in our study may be the fact that River Panjkora is less polluted than River Kabul.

Bioaccumulation factor

Bioaccumulation factor (BAF) is an index of the degree of accumulation of a contaminant in an organism relative to its environment. It is calculated by the following equation (Mortuza and Al-Misned, 2015):

$$BAF = \frac{Conc. of\ metal\ in\ organism\ tissue}{Conc. of\ metal\ in\ abiotic\ medium}$$

The BAF values of Cu and Zn in different tissues of *M. armatus* are given in Table III. From Table III, it is seen than BAF values of Zn are greater than those of Cu. Javed and Usmani (2013) have studied bioaccumulation of heavy metals in *M. armatus* collected from a rivulet at Kasimpur, Aligarh, India. Their reported BAF values of Cu in muscles, gills, skin, liver and kidneys of *M. armatus* were 48.09, 232.41, 42.17, 315.89, and 204.52, respectively while corresponding BAF values of Zn were 620.63, 1831.10, 560.36, 5806.50, and 1170.93. These results are comparable to our reported BAF values of Cu and Zn in different tissues of *M. armatus*.

CONCLUSIONS

The present study investigated the bioaccumulation of two heavy metals, Cu and Zn, in muscles of two economically important fish species *S. plagiostomus* and *M. armatus* from three rivers of Malakand Division, Pakistan. Generally, the concentrations of the two heavy metals in muscles of the study fish did not differ significantly at different sites of the rivers. However, in some cases Cu and Zn concentrations showed significant differences at different sites of the rivers. For example, in case of River Panjkora, Zn concentrations and in case of River Barandu Cu concentrations (except at Daggar) in muscles of *S. plagiostomus* increased down the stream. Regarding feeding habits of the two fish species, generally Cu and Zn concentrations did not show significant differences between the two species. However, in two cases, *M. armatus* accumulated more metals while in one case, *S. plagiostomus* showed more bioaccumulation of Cu. Thus, no clear trend could be noted in bioaccumulation of Cu and Zn regarding feeding habits of the study fish.

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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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