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Bioaccumulation of Some Potentially Toxic Heavy Metals in Freshwater Fish of River Shah Alam, Khyber Pakhtunkhwa, Pakistan

Hazrat Ali[1,2,*], Ezzat Khan[1,*] and Muhammad Jamal Nasir[3]

[1]*Department of Chemistry, University of Malakand, Chakdara 18800, Dir Lower, Khyber Pakhtunkhwa*

[2]*Green & Environmental Chemistry, Ecotoxicology and Ecology Laboratory, Department of Zoology, University of Malakand, Chakdara 18800, Dir Lower, Khyber Pakhtunkhwa*

[3]*Department of Geography, University of Peshawar, Peshawar, Khyber Pakhtunkhwa*

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Authors' Contributions
HA and EK conceived and designed the study. HA collected, prepared and analyzed the samples. HA analyzed the data and wrote the manuscript. MJN contributed to data analysis. EK supervised the research work.

Key words
Bioaccumulation, Fish, Heavy metals, River Shah Alam, Sediments, Water.

ABSTRACT

The present study was designed to assess the concentrations of Cr, Ni, Cd and Pb in water, sediments and different fish species of River Shah Alam, a tributary of River Kabul in Khyber Pakhtunkhwa, Pakistan. The different environmental samples were collected from the river, downstream the city of Peshawar, and analyzed for the selected heavy metals by atomic absorption spectrophotometry. Heavy metal accumulation was studied in muscles of six fish species collected from the river. Heavy metal accumulation was also studied in other tissues such as skin, gills, liver and kidneys of two fish species *i.e.*, *Clupisoma naziri* and *Mastacembelus armatus*. Cr concentrations in fish muscles ranged from 30.5±41.9 to 70.8±12.1 mg kg^{-1} wet weight, Ni from 16.7±10.2 to 103.5±114.1 mg kg^{-1} wet weight, Cd from 1.1±0.18 to 2.5±0.21 mg kg^{-1} wet weight and Pb from 29.7±18.3 to 97.7±95.4 mg kg^{-1} wet weight. Metal concentrations in muscle samples of the different six fish species showed random variations. Inter-species differences were statistically not significant, most probably due to more variance within samples. Comparative metal accumulation in the five different tissues of *C. naziri* and *M. armatus* did not show a consistent trend across the studied tissues.

INTRODUCTION

Heavy metals released into the environment from a variety of natural and anthropogenic sources. Vehicle traffic is among the major anthropogenic sources of heavy metals such as Cr, Zn, Cd and Pb (Ferretti *et al*., 1995). Environmental monitoring of river water is important for protecting aquatic life (Ebadi and Hisoriev, 2017). Assessment of heavy metal accumulation in fish is important from public health point of view because of their consumption by humans (Ali *et al*., 2017).

Cd and Pb are biologically non-essential heavy metals. Cd is among the most toxic heavy metals (Bernard, 2008). A recent study conducted in China on a large adult population has found an association between blood Pb levels and an increased prevalence of cardiovascular diseases (CVD) (Chen *et al*., 2017). Apart from affecting the fish consumers, heavy metals also affect the exposed fish. Exposure of fish to heavy metals disrupts secretion of reproductive hormones and causes pathological changes (Ebrahimi and Taherianfard, 2011). Cr causes biochemical, hematological and behavioral changes in fish (Aslam and Yousafzai, 2017). It has been found that exposure to Cr changed locomotor behavior in the fish *Gambusia affinis*. It also caused adverse effects such as morphological changes, necrosis and periodic hemorrhages in the gills of the exposed fish (Begum *et al*., 2006). It has been reported that exposure to Pb at sublethal concentrations has a potential immuno-suppressor effect on tilapia fish (*Oreochromis mossambicus*) (Kaya *et al*., 2013).

Industrialization and urbanization as well as rapid population growth and economic development have caused deterioration of environmental quality and contamination of freshwater ecosystems with various toxic chemicals including heavy metals. Rivers are freshwater ecosystems, which are much vulnerable to environmental changes and pollution. River Shah Alam is a severely polluted tributary of River Kabul (Khan *et al*., 1999), which receives wastewaters from different industries including distilleries, ghee mills, paper mills, sugar mills, tanneries, and textile mills (Khan *et al*., 2011). The present study was designed to assess the concentrations of four heavy metals *i.e.,* Cr, Ni, Cd and Pb in the water, sediments and different

* Corresponding authors: hazrataliuom@gmail.com; hazratali@uom.edu.pk; ekhan@uom.edu.pk
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freshwater fish of River Shah Alam, Khyber Pakhtunkhwa, Pakistan. Accumulation of the selected heavy metals was studied in muscles because this tissue is the edible part of the fish and is the most important from human health point of view. However, comparative metal accumulation was also studied in different tissues of two fish species *i.e., Clupisoma naziri* and *Mastacembelus armatus*.

MATERIALS AND METHODS

River Shah Alam is a tributary of River Kabul, and receives sewage from Peshawar city through Ganda Erab and Budni Nalla and from 30 villages in the surroundings. Therefore, River Shah Alam is considered as a more polluted river (Khan *et al.*, 2012). A map of the study area showing the sampling site is given in Supplementary Figure S1.

Water, sediment and fish samples were collected at the sampling site (34°04′04″N and 71°38′54″E) on River Shah Alam. This sampling site was selected because it is downstream to the point where Budni Nalla, carrying polluted water from Peshawar, joins River Shah Alam. The environmental samples were collected according to Csuros and Csuros (2002) and transported to the laboratory with proper care and arrangements. Water samples were prepared according to Malik and Maurya (2014), sediment samples were digested according to Jan *et al.* (2010) while the fish samples were digested according to Javed and Usmani (2016) with modifications. A 50 mL water sample was acidified with 5 mL concentrated HNO_3 and evaporated at 80°C on a hot plate until its volume was reduced to 50 mL. After filtration, the water sample was taken in a 50 mL volumetric flask. Sediment samples were air dried and passed through a less than 2 mm sieve. A 0.5 g dried and finely powdered sediment sample was digested with 10 mL *aqua regia* ($HCl:HNO_3$, 3:1 ratio). Fish muscle samples were taken from the dorsal side, behind the dorsal fin. A 1.0 g muscle sample (wet weight basis) was digested with a 10 mL mixture of HNO_3 and $HClO_4$, 3:1 ratio. Digestion was carried out at 80°C on a hot plate. The four selected heavy metals *i.e.,* Cr, Ni, Cd and Pb were quantified in the prepared samples with Flame Atomic Absorption Spectrophotometer (Perkin-Elmer Model No. 2380). Quality assurance tests were done to establish the reliability of metal analysis (Supplementary Table I).

Results are shown as mean±standard deviation, from three independent experiments. Data were analyzed with SPSS version 16. Mean metal concentrations at different study sites were compared using One-Way ANOVA (Tukey Test). A *p*-value of 0.05 was considered for statistical significance.

RESULTS AND DISCUSSION

Table I shows the concentrations of the four heavy metals in the water and sediments of River Shah Alam. The concentrations of these metals in both water and sediments followed the order: Ni > Cr > Pb > Cd. Previously, Khan *et al*. (2011) have reported the concentrations (mg L^{-1}) of heavy metals in water of River Shah Alam as: Cr (0.02), Ni (0.65), Cd (0.03), and Pb (0.09), which are lower than the current levels. Usman *et al*. (2017) have reported Cr, Cd and Pb concentrations of 0.04-2.0, 0.2-0.69 and 1.0-1.2 mg L^{-1}, respectively in water of River Shah Alam, which are higher than our reported concentrations. Tabinda *et al.* (2013) have reported Cr and Ni concentrations of 0.16 ± 0.89 and 0.04 ± 0.03 mg L^{-1}, respectively in water from River Sutlej, Pakistan, which are lower than our reported concentrations. Ebadi and Hisoriev (2017) have reported Cr, Cd and Pb concentrations of 2.1-4.6, 0.01-0.59 and 1.9-4.2 mg L^{-1}, respectively in water from different sites of Tajan River, Iran; these values are also higher than our reported concentrations.

Table I.- Concentrations (mean ± SD, n=3) of four potentially toxic heavy metals in water and sediments of River Shah Alam, Khyber Pakhtunkhwa, Pakistan.

Metal	Concentration in water (mg L^{-1})	Concentration in sediments (mg kg^{-1} dry weight)
Cr	0.80 ± 0.32	47.0 ± 20.9
Ni	2.4 ± 1.0	159.3 ± 150.8
Cd	0.05 ± 0.02	4.4 ± 0.42
Pb	0.44 ± 0.15	36.3 ± 19.1

Tabinda *et al*. (2013) have reported Cr and Ni concentrations of 35.0 ± 7.3 and 21.8 ± 5.2 ppm respectively in sediments from River Sutlej, Pakistan, which are lower than our reported concentrations. Miri *et al*. (2015) have reported Cd, Ni and Pb concentrations of 0.22-5.8, 10.0-17.6 and 5.6-14.9 ppm, respectively in sediments of Chabahar Bay, Iran, which are also lower than our reported concentrations. Metal concentrations in both water and sediments depend on several factors and fluctuate from site to site due to their variable natural and anthropogenic sources. The anthropogenic sources of the selected heavy metals in the study area are agricultural run-off, domestic sewage and industrial effluents.

Table II shows metal concentrations in muscles of the different fish species. Metal concentrations in muscle samples of the different six fish species showed random variations. A high degree of randomness in heavy metal distribution has also been reported in the muscles of different six fish species from five freshwater lakes of Pakistan (Tariq *et al*., 1991). Inter-species differences were statistically not significant, most probably due to more variance within samples. Table III shows a comparison of concentrations of the four potentially toxic heavy metals in muscles of fish reported by the present study with those reported in fish from some other freshwater bodies.

Our reported heavy metal concentrations in fish muscles are generally higher than or comparable to those reported by the above-mentioned studies. However, our reported heavy metal concentrations are lower than those reported by Siraj *et al*. (2014) in muscles of fish collected from River Kabul near Nowshera. Similarly, Ahmad *et al*. (2015) have reported heavy metal concentrations, much higher than our reported values, in muscles of different edible fish species from River Kabul. Thus, our reported concentrations are lower than those reported by these two studies from River Kabul, Pakistan, the most relevant river for River Shah Alam. As mentioned in the introduction, River Shah Alam is a tributary of River Kabul; both are relatively polluted water bodies in the province because they receive industrial effluents, domestic sewage and agricultural run-off from Peshawar and adjacent areas.

Table II.- Metal concentrations (mean ± SD, n = 3) in muscles of different fish species from River Shah Alam, Khyber Pakhtunkhwa, Pakistan.

Fish species	Metal concentration (mg kg^{-1} wet weight) in muscles			
	Cr	Ni	Cd	Pb
Barilius vagra	40.2 ± 39.4	58.3 ± 39.9	1.9 ± 0.94	29.7 ± 18.3
Clupisoma naziri	31.0 ± 28.5	103.5 ± 114.1	2.5 ± 0.21	62.7 ± 51.9
Glyptothorax cavia	30.5 ± 41.9	78.0 ± 53.2	1.5 ± 0.52	57.2 ± 49.5
Glyptothorax punjabensis	32.2 ± 21.0	99.7 ± 44.3	2.0 ± 0.76	40.5 ± 30.1
Mastacembelus armatus	57.5 ± 12.5	72.7 ± 93.0	1.4 ± 0.28	97.7 ± 95.4
Notopterus chitala	70.8 ± 12.1	16.7 ± 10.2	1.1 ± 0.18	51.0 ± 46.5

Table III.- Comparison of concentrations of the four potentially toxic heavy metals in muscles of fish reported by the present study with those reported in fish from some other freshwater bodies.

Country	Freshwater body	Fish species	Concentration (µg g^{-1})				Reference
			Cr	Ni	Cd	Pb	
Bangladesh	Buriganga river	*Puntius ticto* ww	5.5 ± 1.5	1.6 ± 0.27	—	—	Ahmed *et al.* (2016b)
		Puntius sophore ww	4.3 ± 1.4	1.2 ± 0.30	—	—	
		Puntius chola ww	3.6 ± 1.6	1.0 ± 0.52	—	—	
		Labeo rohita ww	18.8 ± 1.7	6.6 ± 0.24	—	—	
		Glossogobius giuris ww	5.1 ± 1.0	0.73 ± 0.19	—	—	
India	River Ganga	*Mastacembelus armatus* ww	19.7 ± 1.1	4.6 ± 0.9	4.4 ± 0.9	30.3 ± 6.4	Pandey *et al.* (2017)
Pakistan	Tarbela Lake	*Catla catla*	0.17 ± 0.03	0.26 ± 0.06	—	0.16 ± 0.04	Ahmed *et al.* (2016a)
		Cyprinus carpio	0.23 ± 0.07	0.22 ± 0.05	—	0.23 ± 0.03	
		Tor putitora	0.18 ± 0.02	0.18 ± 0.02	—	0.18 ± 0.02	
Pakistan	River Swat	*Oncorhynchus mykiss*	0.23 ± 0.06	0.19 ± 0.02	—	0.16 ± 0.04	Ahmed *et al.* (2016a)
Pakistan	River Kabul	*Wallago attu*	533.3 ± 206.1	106.7 ± 6.8	68.0 ± 15.0	599.3 ± 188.3	Ahmad *et al.* (2015)
		Cyprinus carpio	489.0 ± 49.7	74.7 ± 17.3	53.3 ± 2.9	226.3 ± 222.2	
		Labeo dyocheilus	647.3 ± 105.1	117.7 ± 33.5	66.7 ± 8.5	528.7 ± 236.4	
Pakistan	River Kabul	*Aorichthys seenghala* ww	565.3 ± 148.7	94.7 ± 33.3	60.7 ± 17.2	350.7 ± 37.2	Siraj *et al.* (2014)
		Ompok bimaculatous ww	703.0 ± 125.3	135.0 ± 52.6	71.7 ± 12.1	407.0 ± 126.6	
Pakistan	River Sutlej	*Cirrhina mrigala*	2.0 ± 0.74	1.3 ± 0.67	—	—	Tabinda *et al.* (2013)
		Labeo rohita	1.8 ± 0.84	1.2 ± 0.50	—	—	
		Catla catla	1.5 ± 0.49	1.1 ± 0.72	—	—	
Pakistan	Freshwater lakes	Six fish species ww	0.12-2.3	0.14-2.7	0.02-1.2	0.06-4.1	Tariq *et al.* (1991)
Pakistan	River Shah Alam	Six fish species ww	30.5-70.8	16.7-103.5	1.1-2.5	29.7-97.7	Present study

ww, wet weight.

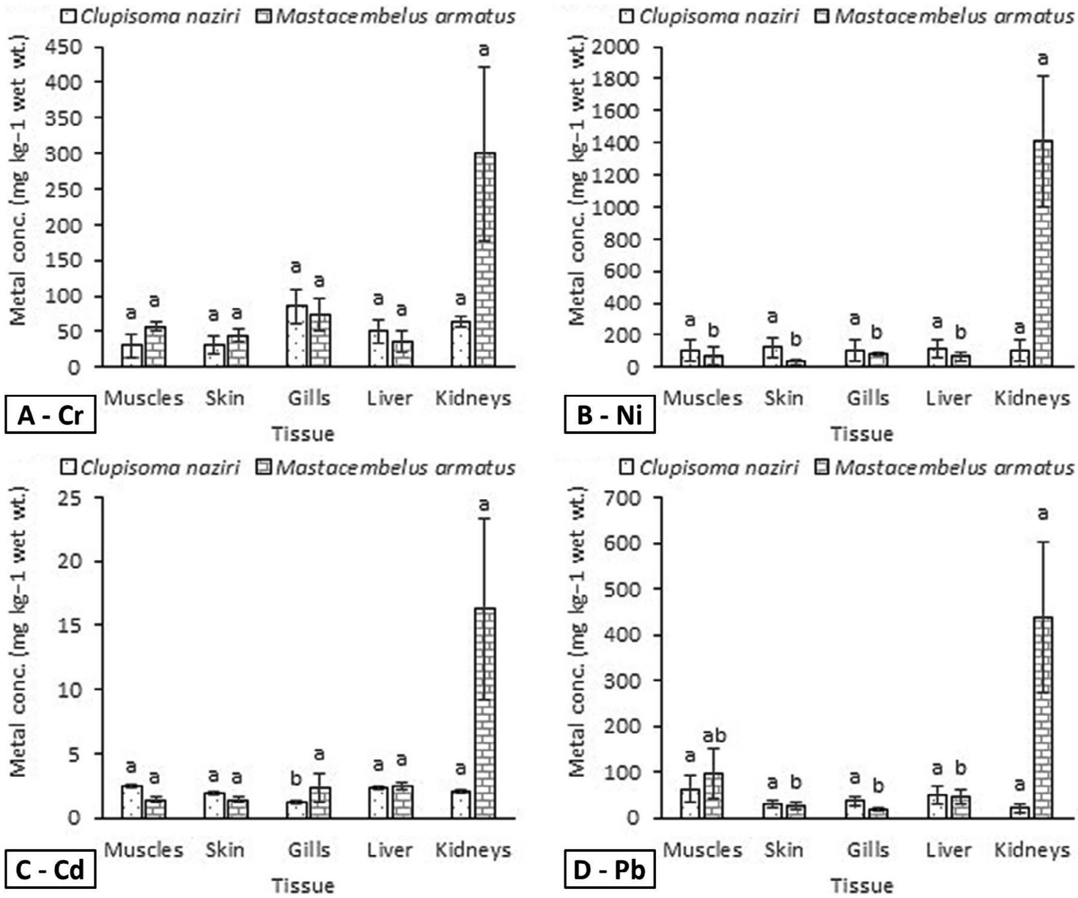

Fig. 1. Comparative accumulation of heavy metals in different tissues of *Clupisoma naziri* and *Mastacembelus armatus*.

Apart from assessment of heavy metal levels in fish muscles from natural water bodies such as rivers and lakes, some studies have investigated levels of heavy metals in fish from commercial fish farms. For example, Nawaz *et al*. (2010) have reported Cd concentrations in fish muscles as 0.40 and 0.35 µg g^{-1} in edible and non-edible fish respectively from River Ravi, Pakistan while 0.45 µg g^{-1} in edible farmed fish from fish farms at Manawan, Lahore. Their reported Pb concentrations in fish muscles were 2.7 and 2.1 µg g^{-1} in edible and non-edible fish, respectively from River Ravi, Pakistan while 3.0 µg g^{-1} in edible farmed fish from fish farms at Manawan, Lahore.

Figure 1 shows the comparative accumulation of the four heavy metals in different tissues of *C. naziri* and *M. armatus*. Among fish tissues, usually higher metal accumulation is observed in metabolically active tissues such as liver, kidneys and gills as compared to muscles. In this study, metal concentrations in different tissues of the two studied fish species followed the following order; for *C. naziri*: Cr, gills > kidneys > liver > skin = muscles; Ni, skin > liver > gills > kidneys > muscles; Cd, muscles > liver > kidneys > skin > gills; Pb, muscles > liver > gills > skin > kidneys; for *M. armatus*: Cr, kidneys > gills > muscles > skin > liver; Ni, kidneys > gills > muscles > liver > skin; Cd, kidneys > liver > gills > muscles ≅ skin; Pb, kidneys > muscles > liver > skin > gills.

Rauf *et al*. (2009) have reported highest Cd and Cr accumulation in liver among six fish tissues for three major carps from River Ravi, Pakistan. Mohammadi-Rouzbahani (2017) has also reported gills and liver as the target tissues for accumulation of heavy metals in two fish species from the Persian Gulf, Iran. Khan *et al*. (2012) have reported heavy metal concentrations in different fish tissues as: liver ≥ gills > muscles. Ahmed *et al*. (2016a) have found higher heavy metal accumulation in liver and skin than in gills and muscles.

Yousafzai and Shakoori (2008) have reported heavy metals concentrations (µg g^{-1} wet weight) in gills of the freshwater fish *Tor putitora* at control, polluted site 1 and polluted site 2 respectively of River Kabul, Pakistan as: Cr (5.3±0.18, 6.6±0.07 and 6.0±0.38); Ni (53.3±8.4, 128±8.8 and 133±7.3); Pb (219.3±31.4, 313.7±29.9 and 321±9.8).

These values are lower (Cr), comparable to (Ni) and higher than (Pb) our reported metal concentrations in gills of *C. naziri* and *M. armatus*.

CONCLUSIONS

Metal concentrations in muscle samples of the different six fish species showed random variations. A high degree of randomness in heavy metal distribution has also been reported in the muscles of different fish species from other freshwater bodies of Pakistan. Inter-species differences were statistically not significant, most probably due to more variance within samples.

Comparison with similar studies done in Pakistan shows that heavy metal levels reported in fish muscles by this study are generally higher than or comparable to those reported from other neighborhood rivers. However, our reported concentrations are lower than those reported by two studies from River Kabul, Pakistan, the most relevant river for River Shah Alam. As mentioned in the introduction, River Shah Alam is a tributary of River Kabul; both are relatively polluted water bodies in the province because they receive industrial effluents, domestic sewage and agricultural run-off from Peshawar and adjacent areas. Regarding tissue-specific metal accumulation, the five different tissues of *C. naziri* and *M. armatus* did not show a consistent trend across the studied tissues.

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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/20171117161138

Statement of conflict of interest

The authors declare no conflict of interest.

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