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Original Research

Heavy Metal Uptake and Toxicity in Tissues of Commercially Important Freshwater Fish (*Labeo rohita* and *Wallago attu*) from the Indus River, Pakistan

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Abstract

The purpose of this study was to evaluate the accumulation of heavy metals in the liver, skin, gills, and muscles of two freshwater edible fish species (*Labeo rohita* and *Wallago attu*) collected from Taunsa barrage of the Indus River in Pakistan. Fish samples were collected on a seasonal basis and were analyzed by atomic absorption spectroscopy. Gills and liver accumulated relatively higher heavy metal concentrations. All fish organs accumulated the highest metal content in winter and the lowest in summer. Heavy metals accumulated in the order Fe>Zn>Ni>Cu>Pb>Cr>As in the body of *Labeo rohita* and the tissues with the abundance were liver>gills>skin>muscles. Similarly, the sequence of heavy metal accumulation in *Wallago attu* was Fe>Zn>Cu>Ni>Cr>Pb>As, and the targeted tissues were gills>liver>skin>muscles. Heavy metal bioaccumulation was different in both species. Fe was the highest and As was the least accumulated heavy metal in both of these fish species. The tissues of *Wallago attu* accumulated higher concentrations of Ni (83%), Cu (64%), Cr (50%), Fe (2.95%), and Zn (26%) compared to tissues of *Labeo rohita.* However, Pb (67%) and As (22%) accumulation in tissues of *Labeo rohita* were higher compared to their concentrations in tissues of *Wallago attu*. Overall metal burden was 10% higher in *Wallago attu* compared to *Labeo rohita*. Heavy metal concentration in fish tissues were compared with FAO threshold values.

Keywords: bioaccumulation of metals, *Labeo rohita*, *Wallago attu*, Indus River

Introduction

The deterioration of aquatic ecosystems as inflicted by heavy metals is looming large, hence the issue is getting momentum globally. Heavy metals are unabatedly released into aquatic ecosystems from anthropogenic sources such as mining of metals, urban and sewage wastes, industrial effluents, and metals by way of agricultural drainage, as well as natural sources such as geological weathering of rocks and atmospheric deposition [1]. Heavy metals are persistent, bio accumulative, and non-biodegradable in

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nature, so they dilute in water, settle in sediments, and enter aquatic organisms beyond measure, prompting spectacular ecological damage to each receiving body [2]. Having entered into an aquatic environment once, heavy metals follow the track of the food chain from lower to higher trophic levels. Fish at the top of aquatic food chain can let a large amount of heavy metals enter their bodies from the surrounding water [3]. Furthermore, fish serve as a good indicator of heavy metal pollution in freshwater bodies and help to estimate the risk potential of human fish consumption [4]. Various heavy metals show different affinities for different fish tissues as a result of different heavy metals concentrations in different tissues [5]. Hence, it is very important to determine metal concentrations in various tissues of commercial fish species and their risks to human health due to their consumption [6].

Heavy metals enter fish bodies through consumption of food contaminated with heavy metals or by adsorption through their gills, which consequently leads to metal levels in fish increasing many folds higher than the ambient levels. Fish remain invariably exposed to heavy metals in contaminated water, so it can be used as a bio-indicator of heavy metals in aquatic ecosystems [7]. Heavy metals show specific physiological behavior in fish tissues and so their accumulation varies in different fish species [8-9]. The Indus River, regardless of its importance in agricultural and fish food production in Punjab and Sindh, receives industrial effluents and the sewage from nearby communities and industrial areas, which is raising health problems among people [10]. The present study was done to analyze tissues of *Wallago attu* and *Labeo rohita* to assess possible health risks to human communities.

Experimental

Sampling Site

Water samples were collected in triplicate in polythene bottles from Taunsa barrage in each season to analyze heavy metals. About 10% HNO_3 was added to each sampling bottle, then samples were brought to a laboratory. The commonly found fish species *Labeo rohita* (Rohu) and *Wallago attu* (Mulle) were sampled over four seasons from Taunsa barrage, Indus River, Pakistan. In each season, five to seven fish samples of *Labeo rohita* and *Wallago attu* were caught from Taunsa barrage. Both species are cultivated at Taunsa barrage for commercial sale according to the choice of the local community. Fish samples were quickly washed with freshwater to remove mud before preserving them in polythene bags and transporting them in a cooling box to the Laboratory on the same day. The samples were stored at -20ºC prior to analysis. In the lab, fish muscles, the entire liver, and skin and gills tissues were dissected using a stainless steel knife. These were then washed with distilled water, dried on filter paper, packed in clean polythene bags, and kept at about -30ºC until further analysis [11].

Digestion

For digestion, 0.5 g powdered samples of fish tissue were taken in a digestion apparatus, then 2.5 ml concentrated sulphuric acid and 4.0 ml of conc nitric acid was added. Then an *initial vigorous reaction* appeared that was allowed to *subside.* The mixture was then heated slowly on a hot plate with the addition of three to four drops of hydrogen peroxide. This step was repeated several times till the solution become clear. After this, the mixture was further heated at 150ºC for an additional 20 minutes and allowed to cool at room temperature. The solution was filtered into a 50 ml volumetric flask and diluted to the mark with deionized water [12]. Metal concentrations in water and Fish samples were measured using a Perkin Elmer atomic absorption spectrophotometer model 3100.

Statistical Analysis

Significant differences were established at significance level of 0.05 using ANOVA. All statistical comparisons were performed using the SPSS 15.00 packaged software.

Results and Discussion

Heavy Metals Concentration of Water Samples

Concentrations of studied heavy metals in Indus water samples of varied over four seasons as follows: 0.032-0.072 mg L^{-1} for Cr, 1.273-1.98 mg L^{-1} for Fe, 0.126-0.229 mg L^{-1} for Ni, 0.149-0.271 mg L^{-1} for Cu, 0.194-0.293 mg L^{-1} for Zn, bdl-0.022 mg L^{-1} for As, and 0.082-0.251 mg L^{-1} for Pb (Table 1). Fe concentration was highest and that of As concentration was lowest in water samples. Metal accumulation followed the order: Fe>Zn>Cu>Ni>Pb>Cr, which was similar to the order of heavy metal accumulation in Indus water samples from Chashma barrage: Zn> Cu> Pb> Cr [10]. Metal concentrations were highest in winter and lowest in summer due to the dilution effect. There is a rainy season in summer that dilutes metal concentrations in Indus water, while in winter there is a very low flow rate of Indus metal concentrations that is highest in this season. According to WHO guidelines for drinking water, metal concentrations in water samples – such as Cr concentrations in autumn and winter, Pb concentrations in all seasons, and arsenic concentrations in spring and winter – exceeded the permissible limits. Concentrations of all other metals were within WHO limits (Table 1).

Metal Accumulation in Various Tissues of Fish Species

The mean concentrations of seven metals in various tissues of *Labeo rohita* and *Wallago attu* are given in Tables 2 and 3. The mean concentration ($\mu g g^{-1}$) of Fe in *Labeo rohita* varied between 87.99 and 177.28, while that

Table 1. Average Concentration of heavy metals in water of Taunsa Barrage

Taunsa	Spring	Winter	Summer	Autumn	Total	Average	WHO Limit
Cr	0.04^{A}	0.07^{A}	0.03^{A}	0.06^{A}	0.20	0.050	0.05
Fe	1.44^{B}	1.98^{D}	1.27^{A}	1.62^{C}	6.308	1.58	-
Ni	0.18^{A}	0.23^{B}	0.13^{A}	0.16^{A}	0.696	0.17	-
Cu	0.20^{B}	0.27^{C}	0.15^{A}	0.23^{B}	0.844	0.21	2
Zn	0.22^{A}	0.30^{B}	0.19^{A}	0.25^{B}	0.96	0.24	3
As	0.02^{A}	0.02^{A}	bdl	0.01^{A}	0.037	0.01	-
Pb	0.17^{B}	0.25^{C}	0.08^{A}	0.12^{A}	0.62	0.16	0.01

Different letters of same row indicate significant differences P<0.05 (ANOVA)

of Zn was 43.92-88.25, Ni 5.04-7.47, Cu 4.81-7.25, Pb 1.56-3.46, Cr 0.67-2.76, and As 0.46-0.96, whereas the respective heavy metal values ($\mu g g^{-1}$) for *Wallago attu* were recorded as 97.53-189.20, 45.42-124.5, 2.21-4.12, 5.39-16.75, 1.05-1.89,1.15-3.64, and 0.49-0.88. Mean heavy metal concentrations in tissues of *Labeo rohita* were Fe>Zn>Ni> Cu>Pb>Cr>As, while in tissues of *Wallago attu* they were in the order Fe> Zn>Cu>Ni>Cr> Pb>As. Except for Pb, all other metals distribution in both species was statistically significant.

Total concentrations of Fe were higher in all tissues of *Labeo rohita* than in those of *Wallago attu* (Tables 1 and 2). In *Labeo rohita*, the highest accumulation of Fe was in liver (177.28 $\mu g g^{-1}$), followed in winter by gills (163.55 $\mu g g^{-1}$), muscles (108.55 $\mu g g^{-1}$), and skin (87.99 $\mu g g^{-1}$). Besides, the measured concentrations of Fe

Table 2. Mean concentrations (μg/g) of metals in tissues of Labeo rohita from Taunsa Barrage of the Indus River.

Organs	**Seasons**	**Pb**	**As**	**Ni**	**Cu**	**Cr**	**Fe**	**Zn**
Gills	Spring	3.93^{C}	0.64^{B}	8.02^{B}	5.45^{A}	3.157^{A}	166.57^{B}	98.35^{B}
	Summer	2.34^{A}	0.48^{A}	4.46^{A}	4.39^{A}	1.97^{A}	119.75^{A}	45.5^{A}
	Autumn	3.31^{B}	0.63^{B}	5.54^{A}	6.41^{A}	2.77A	160.03^{B}	78.84^{B}
	Winter	4.24^{C}	0.70C	8.24^{B}	8.89^{B}	5.13B	207.86^{C}	130.32^{C}
	Average	**3.46**	**0.61**	**6.57**	**6.29**	**3.26**	**163.55**	**88.25**
Muscles	Spring	1.58^{A}	0.51^{B}	4.08^{A}	3.94^{A}	0.79^{A}	116.96^{A}	39.89^{A}
	Summer	1.29^{A}	0.29^{A}	3.36^{A}	2.85^{A}	0.41^{A}	89.78^{A}	32.1^{A}
	Autumn	1.44^{A}	0.35^{A}	6.8^{B}	5.07^{B}	0.61^{A}	100.41^{A}	46.1^{A}
	Winter	1.94^{B}	0.68^{B}	8.87^{B}	7.39^{B}	0.85^{A}	127.06^{A}	57.6^{A}
	Average	**1.56**	**0.46**	**5.78**	**4.81**	**0.67**	**108.55**	**43.92**
Liver	Spring	2.42^{A}	0.93^{B}	5.9^{A}	6.34^{A}	2.25^{A}	183.84^{A}	64.6^{A}
	Summer	2.19^{A}	0.7^{A}	5.56^{A}	5.8^{A}	1.63^{A}	131.19^{A}	44.8^{A}
	Autumn	2.45^{A}	1.02^{B}	8.16^{B}	6.54^{A}	1.96^{A}	153.95^{A}	87.6^{A}
	Winter	2.72^{A}	1.16^{B}	10.26^{B}	10.33^{B}	2.72^{A}	240.15^{B}	124.16^{B}
	Average	**2.45**	**0.95**	**7.47**	**7.25**	**2.14**	**177.28**	**80.29**
Skin	Spring	3.5^{B}	1.13^{B}	4.59^{B}	4.42^{A}	1.42^{B}	92.77^{B}	72.15^{B}
	Summer	1.9^{A}	0.4^{A}	2.97^{A}	3.63^{A}	0.97^{A}	57.84^{A}	31.27^{A}
	Autumn	2.16^{A}	1.02^{B}	6.82^{B}	5.78^{A}	1.47^{B}	82.06^{B}	49.89^{A}
	Winter	3.02^{B}	1.29^{B}	5.79^{B}	6.69^{A}	1.94^{C}	119.28^{B}	91.23^{B}
	Average	**2.65**	**0.96**	**5.04**	**5.13**	**1.45**	**87.99**	**61.14**

*Different letters of same column indicate significant differences P<0.05 (ANOVA)

Table 3. Mean concentrations (µg/g) of metals in tissues of Wallagu attu from Taunsa Barrage of the Indus River.

Organs	Seasons	Pb	As	Ni	Cu	Cr	Fe	Zn
Gills	Spring	3.97^{B}	0.37^{A}	3.82^{B}	12.56^{B}	1.43^{B}	191.65^{B}	119.34^{B}
	Summer	2.58^{A}	0.29^{A}	2.31^{A}	6.02^{A}	0.65^{A}	142.66^{A}	70.25^{A}
	Autumn	3.47^{B}	0.35^{A}	2.83^{A}	10.47^{B}	1.28^{B}	179.45^{B}	128.46^{B}
	Winter	4.53^{C}	0.41^{A}	4.51^{C}	19.04^{C}	1.61^{B}	243.03^{C}	179.95^{C}
	Average	**3.64**	**0.36**	**3.37**	**12.02**	**1.24**	**189.2**	**124.5**
Muscles	Spring	2.02^{B}	0.28^{A}	2.21^{B}	5.83^{B}	0.48^{A}	125.6^{A}	42.64^{A}
	Summer	1.49^{A}	0.17^{A}	1.17^{A}	3.47^{A}	0.51^{A}	88.25^{A}	29.26^{A}
	Autumn	1.97^{B}	0.21^{A}	2.46^{B}	4.874^{B}	0.56^{A}	112.05^{A}	47.95^{A}
	Winter	2.32^{C}	0.4^{A}	2.98^{B}	7.39^{C}	0.64^{A}	152.3^{A}	61.84^{A}
	Average	**1.95**	**0.27**	**2.21**	**5.39**	**0.55**	**119.55**	**45.42**
Liver	Spring	2.53^{A}	0.7^{A}	3.72^{A}	18.24^{B}	0.83^{A}	139.53^{A}	85.77^{A}
	Summer	2.28^{A}	0.52^{A}	3.41^{A}	7.13^{A}	0.66^{A}	123.97^{A}	58.05^{A}
	Autumn	2.83^{A}	0.86^{A}	4.31^{A}	15.25^{B}	1.25^{A}	149.45^{A}	106.35^{A}
	Winter	3.81^{B}	1.09^{A}	5.02^{A}	26.36^{B}	1.94^{B}	174.75^{A}	149.04^{B}
	Average	**2.86**	**0.79**	**4.11**	**16.75**	**1.17**	**146.93**	**99.8**
Skin	Spring	2.61^{A}	1.03^{B}	3.45^{A}	4.81^{B}	1.9^{B}	104.84^{A}	80.27^{B}
	Summer	2.09^{A}	0.28^{A}	3.26^{A}	3.53^{A}	0.61^{A}	51.19^{A}	47.45^{A}
	Autumn	3.14^{A}	0.96^{B}	4.02^{B}	4.31^{B}	1.18^{A}	73.95^{A}	60.45^{A}
	Winter	3.67^{A}	1.25^{B}	4.72^{B}	5.19^{B}	2.01^{B}	160.15^{B}	107.35^{B}
	Average	**2.88**	**0.88**	**3.86**	**4.46**	**1.43**	**97.53**	**73.88**

*Different letters of same column indicate significant differences $P<0.05$ (ANOVA)

in *Wallago attu* and *Labeo rohita* were higher than those reported in tissues of *Wallago attu* from Pakistan's Kabul River [13].

The total amount of Zn was higher in *Wallago attu* than in *Labeo rohita*. The gills of *Wallagu attu* were the major site for Zn accumulation (124.5m μgg^{-1}), followed by liver, skin, and muscles having mean metal concentrations of 99.80, 73.88, and 45.42 μgg^{-1}, respectively (Table 2). Zinc levels in muscles were higher than those reported in *Cyprinus carpio* captured from Ataturk Dam Lake [14] and Saricay in southwestern Anatolia [3]. Mean Zinc concentration was lower than FAO permissible levels [15].

The amount of Pb was higher in *Wallago attu* than *in Labeo rohita*. In *Wallago attu*, Pb concentration was highest in liver and gills (1.89 and 1.43 μgg^{-1}, respectively) and lowest in muscles (1.05 μgg^{-1}), while gills in *Labeo rohita* accumulated the highest amount of Pb (3.46 μgg^{-1}), followed by skin (2.65 μgg^{-1}), liver (2.45 μgg^{-1}), and muscles (1.56 μgg^{-1}). In liver, mean concentrations of Pb were lower than the lead concentrations (93.66-136.8 μgg^{-1}) as measured in the liver of *Tor putitora* from the Kabul River [16].

The measured quantity of As was found in both fish species, and the total As concentration was higher in *Labeo rohita* than in *Wallago attu*, but the muscles of *Wallago attu* accumulated higher As concentration than those of *Labeo rohita*. The mean values were 0.96, 0.95, 0.71, and 0.46 μgg^{-1} in skin, liver, gills, and muscles of *Labeo rohita* and in *Wallago attu*, with respective values of 0.79, 0.88, 0.36, and 0.49 μgg^{-1} (Tables 1 and 2). The highest concentration of As measured in *Wallago attu* liver was similar to the highest As concentration reported in the liver of *Oreochromis niloticus* and *Clarias gariepinus* from Lake Koka in Ethiopia [17].

Accumulation of Cr was higher in all tissues of *Wallago attu* than in tissues of *Labeo rohita*. In *Wallago attu*, the Cr concentration (Table 2) was higher in gills (3.64 μgg^{-1}), followed by skin (2.88 μgg^{-1}), liver (2.86 μgg^{-1}), and muscles (1.15 μgg^{-1}). Similarly, Cr concentration was also reported highest in gills and lowest in muscles of *Silurus glanis* from Italian rivers [18].

Ni accumulation was higher in *Labeo rohita* than in *Wallago attu* (Table 1 and 2). In *Labeo rohita*, Ni concentration was highest in liver and gills (7.47 and 6.57μgg^{-1}, respectively) and lowest in skin (5.04 μgg^{-1}), while in *Wallago attu*, liver accumulated the highest concentration of Ni (4.12 μgg^{-1}), followed by skin (3.86 μgg^{-1}), gills (3.37 μgg^{-1}), and muscles (2.21 μgg^{-1}).

Ni concentration (3.22 $\mu g g^{-1}$) was concluded in muscles of *Squalius cephalus* (L., 1758) from Yamula Dam Lake, Turkey, which was similar to the Ni concentration (3.39 $\mu g g^{-1}$) found in the *Wallago attu* of our study [19].

The mean range of Cu was 5.39-16.75 $\mu g g^{-1}$ in *Wallago attu* and 4.81-7.25 $\mu g g^{-1}$ in *Labeo rohita*. Collectively, it is inferred that the total amount of Cu was higher in *Wallago attu* than in *Labeo Rohita* (Tables 1 and 2). Compared separately, in *Wallago attu* Cu content was highest in liver and gills (16.75 and 12.02 $\mu g g^{-1}$, respectively) and lowest in skin (7.46 $\mu g g^{-1}$), while in *Labeo rohita* liver accumulated the highest Cu content (7.25 $\mu g g^{-1}$), followed by gills (6.29 $\mu g g^{-1}$), skin (5.13 $\mu g g^{-1}$), and muscles (4.81 $\mu g g^{-1}$). To substantiate the results, [20] also reported highest Cu levels (16.82 $\mu g g^{-1}$) in liver of *Capoeta trutta* captured from Keban Dam Lake in Turkey.

Seasonal Differences in Metal Accumulation

In *Labeo rohita,* no statistical differences ($p<0.05$) on a seasonal basis were observed for Cr, Fe, and Zn in muscles, Pb and Cr in liver, and Cu in skin tissues. Accumulation of Pb, Cr, and Zn was significantly higher in Gills, while Ni, Cu, and Fe accumulated at significantly higher levels in liver. Arsenic concentration was highest in skin. The accumulation of lead in spring and Ni concentration in autumn was significantly higher when metal concentrations were compared according to skin. Concentrations of all other metals were highest in winter in all tissues (Table 2).

In *Wallagu attu*, no statistical differences ($p<0.05$) on a seasonal basis were observed for Pb, Fe, and Zn in muscles, As in gills, As, Ni, and Fe in Liver, and Pb and Zn in skin tissues. Accumulation of Pb, Fe, and Zn was significantly higher in gills, while Ni and Cu accumulated significantly higher levels in liver. As and Cr concentrations were highest in skin. Accumulations of Pb in spring and Ni in autumn were significantly higher when metal concentrations were compared according to skin. Concentrations of all other metals were highest in winter season in all tissues (Table 3).

During the present study, the highest mean concentrations of all studied metals were found in winter, followed by autumn and spring. The lowest metal concentrations were detected in summer. It has been reported that physiological activities affect the rate of metal bioavailability of aquatic environments in different seasons [11]. In contrast to our findings, maximum mean metal concentrations of fish have been reported during spring and summer and the lowest in autumn and winter [21]. Feeding habits of fish species are strongly related to seasonal variations in accumulation patterns of metals [13]. In contrast to our findings, metal concentrations in fishes from the Seyhan River of Turkey rise from autumn to summer [22]. Stream conditions, contamination levels, water physico chemical properties, and other environmental factors play a role in seasonal variations in metal accumulation, which strongly affects heavy metal bioavailability [23].

We found that seasonal variation in detected heavy metals in fish species depends on metal concentrations in water samples and the amount of water flow in the Indus River. The high river flow of the Indus during the summer (monsoon) may dilute the heavy metal concentrations. Winter sees the lowest flow in the Indus, so it experiences elevated contamination levels of various metals. Results showed that fish organs accumulate the highest heavy metals in winter. It is found that in the pre-monsoon season, high temperatures help fish in their metaobolic activities so that metal accumulation remained high [24]. Higher metabolic rates utilize high oxygen concentrations, which decrease oxygen levels in blood and increases high accumulation of metal pollutants [25]. Elevated contaminant levels in the surrounding environment of fish can influence fish embryonic development during breeding season (pre-monsoon) of many fish species with high metabolic activities [26].

Human Health Implications from Consumption of Contaminated Fish

The mean heavy metal concentrations of *Labeo rohita* and *Wallagu attu* were used as an indicator for assessing human health risk. In Pakistan, average per capita consumption of freshwater fish is 21 g/person/day for a person of average body weight of 70 Kg.

Estimated daily intake of heavy metals was calculated using the following equation [27]:

$$EDI = \frac{C_{metal} * W_{fish}}{B_w}$$

…where EDI is estimated daily intake of metal by fish consumption for an adult, C metal is the concentration of heavy metal in a fish sample, W fish is daily fish consumption, and Bw is adult body weight.

Calculated EDI values of As, Cr, Pb, Ni, Cu, Zn, and Fe for *Labeo rohita* were 0.016, 0.023, 0.055, 0.202, 0.168, 1.54, and 3.80, and for *Wallago attu* were 0.017, 0.040, 0.077, 0.185, 0.421, 1.59, and 4.184, respectively. *Provisional tolerable daily intake (*PTDI) values set by the Joint FAO and WHO Expert Committee on Food Additives [28] for As, CR, Pb, Ni, Cu, Zn, and Fe ($mgkg^{-1}$ bw/day) are 0.021, 0.0233, 0.025, 0.035, 3.5, 7, and 5.6, respectively, which showed that no health risks were found for people consuming fish collected from Taunsa barrage. However, Cr and Fe EDI values in *wallago attu* were found to have enough potential to cause health risks in the future. In a nutshell, calculated values of the estimated daily intake (EDI) of all metals were found to be lower than the recommended ADI values.

Conclusions

This study has identified the concentrations of numerous heavy metals in different tissues of *Wallago*

attu and *Labeo rohita* collected from the Indus River. Carnivorous fish accumulated more heavy metal when juxtaposed with herbivorous fish. Heavy metal levels in non-edible tissues were higher than permitted levels of the metals by the FAO. In a few samples of muscle tissues, Zn concentrations in both fish species and Cr in *Wallago attu* were higher than the recommended FAO limits. Levels of most metals in edible tissues were within FAO threshold limits and these cannot pose a health risk to native human communities. However, it is recommended that Cr and Zn levels should be monitored in fish tissues on a regular basis and these can pose toxic effects in the future.

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