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RESEARCH ARTICLE

Assessment of Heavy Metals in the Fish Collected from the River Ravi, Pakistan

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ABSTRACT

The toxicity of heavy metals viz. aluminium (Al), arsenic (As), barium (Ba), chromium (Cr), nickel (Ni) and zinc (Zn) in fish at three main public fishing sites of the river Ravi viz. Shahdara bridge, Baloki headworks and Sidhnai barrage has been studied from June, 2009 to May, 2010. The concentrations of heavy metals in the body organs (gills, liver, kidney, intestine, reproductive organs, skin, muscle, fins, scales, bones, fats) of three fish species viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* were determined. The present results reveal that the toxicity of metals fluctuated significantly in fish at all the three sampling stations with season. The fish samples collected from all the three sampling stations had significantly higher aluminium and zinc. However, the fish at Sidhnai barrage showed significantly lower metallic toxicity, followed by that at Baloki headworks and Shahdara bridge. Significantly higher metals were observed in fish liver, followed by that of kidney, gills, intestine, reproductive organs, skin, scales, fins, bones, muscle and fats. The accumulation of metals in carnivorous fish body organs showed significantly direct dependence on the metallic toxicity of herbivorous cyprinids. Fish liver and kidney showed significantly higher abilities for the accumulation of all metals while accumulations were lowest in fish muscle and fats. The health status of river Ravi at three main public fishing sites viz. Shahdara bridge, Baloki headworks and Sidhnai barrage, with respect to eco-toxicity of Al, As, Ba, Cr, Ni and Zn was above the recommended permissible standards.

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INTRODUCTION

Heavy metals discharges into the aquatic ecosystems, through domestic sewage and industrial effluents have severe environmental and ecological impacts (Gagnaire *et al*., 2004; Naeem *et al*., 2010; Abu-Darwish *et al*., 2011). The contamination of freshwaters due to several harmful substances, like metals, through inputs from anthropogenic sources viz. industrial and agricultural activities, domestic sewage, groundwater leaching and runoffs from agriculture has devastating effects on animals including fish (Donohue *et al*., 2006). Rivers running through urban areas have also been faced water quality issues due to discharge of untreated domestic sewage, municipal wastes and industrial effluents into them leading to increase the metallic toxicity in the river waters (Venugopal *et al*., 2009; Sekabira *et al*., 2010).

In the aquatic ecosystems, fish are considered the important indicators of heavy metal enrichment of the aquatic ecosystems (Gernhofer *et al*., 2001). In an aquatic ecosystem, metals are transferred to the fish through food chain that could ultimately affect the health of people consuming this fish. Heavy metals are known for their persistent toxicity and tendency to bio-accumulate in aquatic ecosystems (Miller *et al*., 2002). Therefore, metals eco-toxicity of the aquatic ecosystems has become a major health concern over the years (Mendil *et al*., 2010; Shah *et al*., 2010). Heavy discharge of enormous metallic compounds into the river systems of Pakistan has adversely affected the freshwater fisheries especially in the river Ravi (Rauf, 2009). This has attributed to bio-accumulation and biological magnification of metals in fish even at low concentration levels of various metals in the river Ravi. Therefore, the present investigation was conducted to determine the heavy metals (Al, As, Ba, Cr, Ni and Zn) toxicity gradient and multivariate relationships for the flow of metals in the river Ravi aquatic ecosystem at three main public fishing sites viz. Shahdara bridge, Baloki headworks and Sidhnai barrage.

MATERIALS AND METHODS

Three main public fishing points viz. Shahdara bridge, Baloki headworks and Sidhnai barrage were selected at the river Ravi stretch to monitor the year-round variations of aluminium (Al), arsenic (As), barium (Ba), chromium (Cr), nickel (Ni) and zinc (Zn) toxicity in the fish. Each sampling station was divided into two sub-stations viz. upstream and downstream for the collection of fish samples. Fish samples were collected on monthly basis for one year (from June, 2009 to May, 2010). Three herbivorous cyprinids including *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* while those of carnivore fish species were *Rita rita*, *Mystus sperata* and *Wallago attu*. Sampled fish were dissected and their organs viz. gills, liver, kidney, intestine, reproductive organs, skin, muscle, fins, scales, bones and fats isolated for the determination of heavy metals. The samples were digested with concentrated nitric acid and perchloric acid (3:1, v/v) until a clear and transparent solution was obtained. The digested samples were diluted to make the volume by using the double distilled water in 500 ml volumetric flask. After wet digestion, samples of fish organs were analyzed for Al, As, Ba, Cr, Ni and Zn concentrations according to A.P.H.A. (1998) through Atomic Absorption Spectrometry (Perkin Elmer, AAnalyst 400).

Data Analysis: The data on different variables, obtained from the experiment, were statistically analyzed by using SPSS 10.1 computer program. Analysis of variance and Tukey's/ Student Newman-keul tests were performed to findout statistical differences among various parameters at $p<0.05$. Regression and Correlation analyses were also computed to establish relationships among various parameters (Steel *et al*., 1996). MS Office package was employed for graphical presentation of the data.

RESULTS

Fish organs of both herbivorous cyprinids and carnivore fish species showed significantly variable tendencies to accumulate aluminium, arsenic, barium, chromium, nickel and zinc. All fish species collected from three sampling stations had significantly higher Al, followed by Zn, Cr, Ni, As and Ba in their organs.

Aluminium: The carnivore fish species had significantly ($p<0.05$) higher mean aluminium in their body organs than that found in herbivorous fish. In both herbi– and carnivore fish, the liver showed the mean maximum aluminium concentrations of 152.66±19.60 and 169.83±11.30 μgg^{-1} respectively, followed by that in kidney and gills. Both herbi- and carnivore species exhibited significantly lowest aluminium contents of 58.02±17.43 and 69.23±18.28 μgg^{-1}, respectively in their fats. However, the ability of fish organs like kidney, liver and gills to accumulate aluminium in both types of fish species remained statistically significant (Table 1 and 2).

Arsenic: The mean arsenic concentrations in the body organs of carnivore fish were significantly higher than that observed in herbivorous fish species. Both herbi– and carnivore fish species accumulated significantly higher arsenic in their liver as 3.22±0.84 and 4.41±1.07 μgg^{-1}, closely followed by the accumulations in kidney with the mean arsenic concentrations of 3.17±0.84 and 4.28±1.10 μgg^{-1}, respectively. However, the difference between fish liver and kidney, for the accumulation of arsenic, remained statistically non-significant. The fats in both herbi– and carnivore fish species exhibited significantly lowest ability to concentrate arsenic in their bodies (Table 1 and 2).

Barium: Barium accumulation was significantly higher in both liver and kidney, with non-significant difference, of herbivorous fish while carnivore fish had significantly higher barium (0.40±0.11 μgg^{-1}) in their liver, followed by that in kidney and gills with the mean concentrations of 0.37±0.10 and 0.34±0.11 μgg^{-1}, respectively. Both herbi– and carnivore fish species showed significantly minimum ability to bio-accumulate barium in their fats. However, these accumulations were significantly higher in carnivorous fish species (Table 1 and 2).

Chromium: The chromium contents in the liver of both herbi– and carnivore fish species were significantly higher as 6.31±1.48 and 9.07±1.35 μgg^{-1} respectively, followed by that in kidney and gills. All the three carnivore species showed significantly higher chromium in their body organs than that of herbivorous species (Table 1 and 2).

Nickel: The uptake and accumulation of nickel in the liver of both herbi– and carnivore fish species were significantly higher, followed by that in kidney and gills with statistically significant differences (Table 1). The fats in both herbi- and carnivore species had significantly least nickel contaminations of 1.04±0.36 and 1.72±0.65 μgg^{-1}, respectively (Table 2). The tendency of carnivore fish to bio-accumulate nickel in their body organs was significantly higher than that observed in herbivore fish.

Zinc: Both herbi– and carnivore species of fish exhibited significant tendencies to bio-accumulate zinc in their liver, with the concentrations of 84.77±26.23 and 124.79±11.91 μgg^{-1}, respectively, followed by the accumulation of this metal in kidney and gills with statistically significant differences (Table 1). The fats in both herbi– and carnivore fish species had significantly least amounts of zinc as 24.10±9.89 and 41.45±8.53 μgg^{-1}, respectively (Table 2). The body organs of carnivore fish exhibited significantly higher tendency to bio-accumulate zinc (80.08±27.43 μgg^{-1}) than that of herbivorous fish having the mean zinc contamination of 51.64±19.75μgg^{-1}.

Both herbi– and carnivore fish species at Shahdara bridge were found significantly contaminated with aluminium and arsenic, followed by the fish collected from Baloki headworks and Sidhnai barrage (Table 3). The fish at Shahdara bridge was significantly contaminated with barium and chromium, followed by the fish sampled from the Baloki headworks and Sidhnai barrage. However, there was non-significant difference between Shahdara bridge and Baloki headworks to cause barium toxicity in the fish body. Nickel contamination was significantly higher in carnivore fish at Baloki headworks while herbivorous fish at Shahdara bridge

Table 1: Metal concentration (µgg^{-1}) of metals in the body organs of fish collected from the river Ravi

Metals	Fish Types	Metal concentrations in fish body organs (µgg^{-1})							
		Gills	Liver	Kidney	Intestine	Reproductive Organs	Skin	Muscle	Fins
Aluminium	Herbivore	128.14±17.01c	152.66±19.60a	145.58±17.52b	126.70±12.38c	116.36±16.64d	105.20±14.91ef	79.94±12.59h	100.26±9.53f
	Carnivore	145.64±10.04c	169.83±11.30a	160.67±11.10b	134.48±14.13d	126.03±11.15ef	117.88±14.04f	92.94±14.52j	108.27±12.80hg
Arsenic	Herbivore	2.79±0.80b	3.22±0.84a	3.17±0.84a	2.92±0.85ab	2.82±0.86b	2.62±0.84c	1.82±0.69f	2.47±0.88d
	Carnivore	3.96±1.06bc	4.41±1.07a	4.28±1.10a	4.04±1.06b	3.92±1.04bc	3.75±1.03c	2.70±0.85e	3.65±1.04c
Barium	Herbivore	0.24±0.08b	0.29±0.09a	0.27±0.09a	0.24±0.09b	0.21±0.08c	0.18±0.07d	0.11±0.08f	0.17±0.06d
	Carnivore	0.34±0.11c	0.40±0.11a	0.37±0.10b	0.33±0.10c	0.31±0.09c	0.28±0.10d	0.17±0.08f	0.25±0.08de
Chromium	Herbivore	3.69±1.44 c	6.31±1.48 a	5.54±0.98b	3.05±1.22cd	2.94±1.28d	3.27±0.50c	1.38±0.42h	2.24±0.94f
	Carnivore	6.70±1.37c	9.07±1.35 a	8.18±1.27b	5.80±1.28d	5.66±1.68d	4.82±0.89e	2.32±0.59i	3.90±0.76g
Nickel	Herbivore	3.07±0.74b	4.66±0.72 a	4.76±0.95a	2.49±1.04c	2.35±0.99d	2.14±0.89e	1.60±0.59h	1.95±0.68f
	Carnivore	6.01±1.63c	7.28±1.65 a	6.80±1.72b	5.35±1.54d	5.09±1.54e	3.97±1.29f	2.58±1.03g	4.00±1.51f
Zinc	Herbivore	65.20±19.41c	84.77±26.23a	78.95±22.41b	62.72±20.38c	57.58±16.90d	45.62±14.82e	29.60±9.85h	39.06±13.84f
	Carnivore	101.66±9.57c	124.79±11.91a	117.84±20.22b	93.61±9.14d	86.95±8.06e	74.27±7.11f	48.47±5.74i	66.11±8.58g

Means with similar letters in a single row and their respective means in a column are statistically similar at P<0.05.

Table 2: Mean concentration (µgg^{-1}) of metals in the scales, bones and fats of fish

Metals	Fish Types	Metal concentrations in fish body organs (µgg^{-1})		
		Scales	Bones	Fats
Aluminium	Herbivore	105.20±10.75ef	90.71±14.34g	58.02±17.43h
	Carnivore	113.32±13.39g	101.27±13.34i	69.23±18.28k
Arsenic	Herbivore	2.57±0.82cd	2.14±0.76e	1.13±0.64f
	Carnivore	3.69±1.05c	3.09±0.79d	1.87±1.04f
Barium	Herbivore	0.19±0.07d	0.14±0.07e	0.06±0.06g
	Carnivore	0.28±0.09d	0.22±0.08e	0.11±0.08g
Chromium	Herbivore	2.82±0.76e	1.91±0.65g	0.93±0.33i
	Carnivore	4.20±0.64ef	3.48±0.50gh	1.64±0.60j
Nickel	Herbivore	2.01±0.61fg	1.87±0.58g	1.04±0.36i
	Carnivore	4.12±1.51f	3.52±1.33g	1.72±0.65h
Zinc	Herbivore	43.10±13.58e	37.34±13.56g	24.10±9.89i
	Carnivore	69.57±6.13g	56.15±6.39h	41.45±8.53j

Means with similar letters in a single row and their respective means in a column are statistically similar at P<0.05.

Table 3: Metal concentration (µg g^{-1}) of metals in the bodies of herbivore and carnivorous fish collected from three sampling stations of the river Ravi

Metals	Fish species	Sampling Stations		
		Shahdara bridge	Baloki headworks	Sidhnai barrage
Aluminium	Herbivore	121.35±7.65a	112.03±10.65b	96.29±5.57c
	Carnivore	131.90±8.42a	115.91±12.62b	117.52±5.94b
	Herbivore	3.11±0.49a	2.46±0.66b	1.98±0.75c
Arsenic	Carnivore	4.61±0.83a	3.27±0.81b	2.86±0.42c
	Herbivore	0.25±0.04a	0.21±0.03b	0.11±0.05c
Barium	Carnivore	0.33±0.05a	0.32±0.06a	0.18±0.06b
	Herbivore	3.74±0.44a	2.47±0.23c	3.08±0.86b
Chromium	Carnivore	5.94±0.40a	4.48±0.39b	4.79±0.65b
	Herbivore	1.94±0.27c	2.62±0.36b	3.07±0.68a
Nickel	Carnivore	4.26±0.77b	5.90±1.03a	3.60±0.78c
	Herbivore	71.89±5.97a	38.54±5.95c	44.50±6.99b
Zinc	Carnivore	84.93±3.99a	78.61±5.22b	76.69±7.28b

Single row means are non-significant at P<0.05.

exhibited higher nickel contamination. Zinc contamination was significantly higher (71.89±5.97 µgg^{-1}) in herbivorous fish at Shahdara bridge, followed by the contamination levels of 44.50±6.99 and 38.54±5.97 µgg $^{-1}$ recorded in the fish sampled from Sidhnai barrage and Baloki headworks, respectively. However, all these contamination levels were significantly variable. Carnivore fish were significantly more contaminated with zinc at Shahdara bridge (84.93±3.99 µgg^{-1}), followed by the contamination levels of 78.61±5.22 and 76.69±7.28 µgg^{-1} in the fish at Baloki headworks and Sidhnai barrage, respectively. However, there was non-significant difference between Baloki headworks and Sidhnai barrage to cause zinc contamination in carnivore fish. Carnivorous fish exhibited significantly higher tendency to bio-accumulate zinc in their body than that of herbivorous fish (Table 3). The regression equations computed reveal highly significant and direct dependence of all metals, except nickel, bio-accumulation in carnivore fish body on the extent of metallic ions in the body organs of herbivorous cyprinids This shows tissue specific bio-accumulation of metals in the bodies of carnivore fish depending upon their probable food i.e. herbivorous cyprinids in the river Ravi aquatic ecosystem (Table 4).

DISCUSSION

Heavy metals are the serious aquatic pollutants and their uptake and accumulation in the aquatic ecosystems, beyond safe limits, would cause direct consequences to the aquatic food chain and ultimately to the man (Rauf and Javed, 2007). The present investigation reveals that the mean annual toxicity of Al, As, Ba, Cr, Ni and Zn in the river Ravi at three main public fishing sites viz. Shahdara bridge, Baloki headworks and Sidhnai barrage fluctuated significantly. Heavy metals are persistent in surface waters in the form of colloidal, particulate and dissolved forms and rivers are known as the dominant pathway for the transport of metals (Miller *et al*., 2003). In the body organs of both herbi- and carnivorous fish species, the accumulation of Al, As, Ba, Cr, Ni and Zn were significantly higher in liver than that of other body organs. However, fish kidney and gills have also been associated with the higher bio-accumulation of metals in the fish body. The mechanism of trace metals bio-accumulation in fish is complex and diversified, varying with their chemistry, mode of action and metal species (Louma, 1983). The toxic levels of different metals found in both herbi- and carnivorous fish species were directly dependent upon the metallic eco-toxicity of the river Ravi, the ecological needs, metabolism and feeding patterns of various fish species sampled during this study period. Bioaccumulation of metals refers the amount consumed by an organism. Fish gills as a site of metallic ion entry can enhance lesions and ultimately cause damage to the gills (Bols *et al*., 2001). All the organs of both herbi- and carnivorous fish species showed significantly variable accumulation of metals with the sequence of liver > kidney > gills > intestine > reproductive organs > scales > fins > bones > muscle > fats. Fish liver and kidney accumulated significant quantities of all metals while

Table 4: Relationships between herbivorous and carnivore fish for the uptake and accumulation of metals in their bodies

Metal	Mean body concentrations (μgg^{-1})		Regression Equation	r	R^2
	Herbivore fish (x)	Carnivore fish (y)	y= a + bx		
Aluminium	109.89±12.67	121.78±8.80	Carnivorous fish = 50.08 + 0.65 (Herbivorous fish) (0.10)$^{P<0.001}$	0.7460	0.5565
Arsenic	2.52±0.57	3.58±0.92	Carnivorous fish = 0.93 + 1.05 (Herbivorous fish) (0.14)$^{P<0.001}$	0.8008	0.6412
Barium	0.19±0.07	0.28±0.08	Carnivorous fish = 0.09 + 1.02 (Herbivorous fish) (0.11)$^{P<0.001}$	0.8509	0.7240
Chromium	3.10±0.64	5.07±0.77	Carnivorous fish = 2.61 + 0.79 (Herbivorous fish) (0.12)$^{P<0.001}$	0.7592	0.5763
Nickel	2.54±0.57	4.59±1.18	Carnivorous fish = 3.57 + 0.40 (Herbivorous fish) (0.33)$^{N.S.}$	0.2026	0.0411
Zinc	51.64±17.79	80.08±4.31	Carnivorous fish = 66.84 + 0.26 (Herbivorous fish) (0.061)$^{P<0.001}$	0.6039	0.3647

(Single row means are non-significant at P<0.05).

these accumulations were significantly lowest in muscle and fats. Fish kidney as its role to detoxify metals has also accumulated significant amounts of heavy metals (Vinodhini and Narayanan, 2008). The differences for the metals accumulation tendencies of various fish organs are basically attributed to the differences in physiological function of each organ in fish body (Karuppasamy, 2004). The function and ability of specific organs to regulate metals are the factors to affect the accumulation differences in various tissues (Murugan *et al*., 2008). The differences in various tissues for the accumulations of Al, As, Ba, Cr, Ni and Zn might be the result of their capacity to induce metal-binding proteins such as metallothioneins (Canli and Atli, 2003). The metallic ions would become biologically magnified when taken up by the fish from the polluted waters and start accumulation in fish tissues. Such uptakes are significantly pronounced in fish liver, gills, stomach, kidney and other organs depending upon the exposed concentration and species of metal (Fabris *et al*., 2006).

Fish liver, kidney and gill tissues showed significant ability to accumulate metals as observed during present investigation (Ahmad and Bibi, 2010). Bervoest *et al*. (2001) reported higher accumulation of copper in the liver of *Gasterosteus aculeatus* that followed the order: liver> kidney> gills> intestine. The fish fats showed significantly least Al, As, Ba, Cr, Ni and Zn concentrations than all the body organs. Ekmekci *et al*. (2000) studied heavy metal accumulation in the liver and fatty tissues of fish and showed that accumulation of metals in liver was higher than the adipose tissues. The mean concentrations of metals in the organs of both herbi- and carnivorous fish species were in the order of Al > Zn > Cr > Ni > As > Ba. The exposure of metallic ions beyond the permissible limits, in the aquatic ecosystem (Jabeen, 2011) has resulted in the accumulation of higher quantities of metals in herbivorous fish species. However, significant escalation of metals in carnivorous fish species showed bio-magnification of all these metals further up the trophic level (Eimers *et al*., 2001).

Conclusions

The present research work reveals that the health status of river Ravi at three main public fishing sites viz. Shahdara bridge, Baloki headworks and Sidhnai barrage, with respect to Al, As, Ba, Cr, Ni and Zn toxicity in fish was significantly higher that showed variable accumulation patterns of these metals in fish body organs. However, fish liver, gills and kidney showed significantly higher tendencies to bio-accumulate all metals. This investigation also reveals significantly direct dependence of metallic toxicity of carnivorous fish on the extent of metals in herbivorous cyprinids. This shows tissue specific bio-accumulation of metals in the bodies of carnivore fish was depending upon their probable food i.e. herbivorous cyprinids in the river Ravi aquatic ecosystem. The potential of metallic toxicity danger may become more severe in future depending upon the extent of industrial and domestic wastewater influx into the river Ravi due to man-made activities in the adjacent areas.

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