Research Article

Bioaccumulation of Metals in Fishes' Scales – A Reliable Non-Lethal Assessor of Food Security

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Abstract | The aim of present study was to study bioaccumulation potential of selected metals (chromium (Cr), cadmium (Cd), nickel (Ni), copper (Cu), manganese (Mn), mercury (Hg), lead (Pb), zinc (Zn) and iron (Fe) in fish scales. A study was conducted on total 216 fish specimens, comprised of 3 fish species (*Catla catla* (thaila), *Labeo rohita* (rohu) and *Cirrhinus mrigala* (mori)) from river Ravi during two flow seasons at four sampling locations including upstream Lahore Siphon = A, Shahdera = B, Sunder=C and downstream Balloki headworks = D. All the metal contents in fish scales were highly significantly different ($P<0.001$) among sampling locations and flow seasons. Location-wise metal accumulation pattern was C > D > B > A. The highest concentrations (µg/g dry weight) of Cd (0.29), Cr (4.64), Cu (8.85), Fe (65.66), Mn (5.14), Hg (2.91), Ni (3.18), Pb (5.14) and Zn (72.16) were recorded at site C. Among the sampled fish species, *C. mrigala* showed highest potential of metals bioaccumulation than *L. rohita* and *C. catla.* Scales of the fishes caught during low flow season showed significantly ($P<0.001$) higher concentrations of metals than the high flow season. The variations in metals contents in fishes' scales were associated with variations in heavy metals contents in the environment during different seasons. These results revealed the potential of fish scales to depict the metal profile of water bodies without sacrificing the animals.

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Introduction

Water quality of major rivers is being spoiled speedily due to massive and untreated urban and industrial waste discharges. Such environmental exploitation has raised and the issue of food security significantly all over the world, especially in developing countries among the other issues (Khanna et al., 2007). Fishes exposure to water contaminated with metals does attain significant levels of different contaminants (Shakir et al., 2013a). Metals represent highly recalcitrant pollutants. It is well known that flesh as well as non-eatable organs do accumulate metals from water and sediments. The high metal contents in fishes not only cause detrimental effects on fish health but also disturb food chain. The fish specimens scarify first, in general, and then evaluate the metal profile in fishes. This metal profiling depicts contamination level in water body as well as in fishes. On the other hand, if the alternate procedure of metal contents determination is available without dissecting the fish specimens, it will facilitate the fish welfare.

Fish scales (calcified tissues) might be used as non lethal (alternate) means for assessing metals accumulation in fishes because scales of fishes are present most outside the body. The pollutants continuously incorporate in fishes scales during developmental stage because it constantly exposed with aquatic pollutants. Therefore, the scales can be used as potential specimen to express the metal profile in water body. Furthermore, fluctuation in temperature not cause any detrimental impact on preservation, storage and transportation of fish scales for evaluation of metals' profile. This approach also facilitate during food security survey in remote areas. Therefore, the main objective of present study was to verify accumulation potential of selected metals in fish scales of the *C. mrigala* (bottom feeder), *L. rohita* (column feeder) and *C. catla* (surface feeder) sampled from four sampling locations during low and high flow seasons of river Ravi.

Materials and Methods

The selected study area of river Ravi from Lahore Siphon to Balloki Headworks receives discharge of untreated industrial effluents and urban sewage during its passage through Lahore (the second biggest city of Pakistan). The first upstream sampling location Lahore Siphon (A) had no point source and it was located at coordinates 31° 41′ N, 74° 25′ E. The downstream location B, Shahdera (31° 36′N, 74° 18′ E) was receiving untreated urban sewage from three major pumping stations while the downstream location C, Sunder (31° 21′N, 74° 3′E) was receiving municipal and industrial effluents from four major pumping stations and two drains called Hudiara and Deg. The last sampling location, Balloki Headworks (31° 13′ N, 73° 52′ E) was receiving diluted effluents due to a relatively clean water joining from the Qadirabad Link Canal (Figure 1).

Total 216 specimens comprising of nine fish specimens for each of the three species *viz., C. Catla, L. rohita* and *C. mrigala* of comparable size range representing each location and low (winter) and high (post monsoon) flow seasons were collected. After immediate transportation of sampled specimens to the laboratory, each fish specimen's identification was verified (Mirza, 2003).

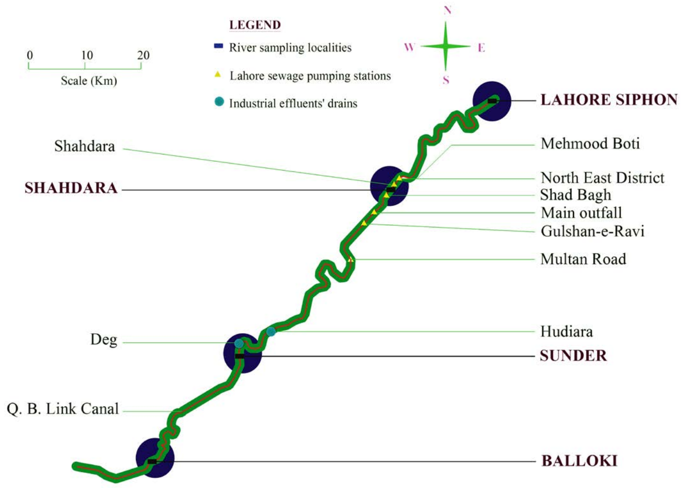

Figure 1: *Map of the Ravi River Lahore showing the four sampling sites around major urban pollution inlets of Lahore (Shakir et al., 2013a, Shakir and Qazi, 2013).*

The scales of total 216 specimens of 3 fish species were removed from the second row of left side, just above the lateral line, directly under the anterior ray of dorsal fin with the help of tweezers. These scales were then gently rinsed, cleaned with distilled water and shifted in labelled polythene bags which were stored at -20 °C till further use. Frozen fishes scale samples were thawed, rinsed in distilled water and blotted on blotting paper. Known weight of scales were shifted into respective labeled pre-weighed glass vials and kept in an oven at 105 °C till constant dried weight was achieved for a given sample. For fish scale digestion, 1g dried fish scale samples were incubated with 5 ml nitric acid (55%) and 1 ml perchloric acid (65%) for overnight at room temperature. Then, next day, 5 ml nitric acid and 4 ml perchloric acid were added to each flask following the addition of few glass beads in each flask to prevent pumping. The further acid digestion procedure was proceeded as reported by Shakir (2013).

All the prepared scale samples were analyzed for the metals such as Cd, Cr, Cu, Pb and Ni by using Fast Sequential Atomic Absorption Spectrometer (Varian Spectra AA-240). While Mn and Fe concentrations were determined using Pye Unicam Atomic absorption spectrophotometer. Whereas the Hg and Zn were measured using variant atomic absorption spectrophotometer (variant AAS-1275) . Metal accumulation in scales samples were statistically analysed by using Minitab-16 software (General linear model).

Table 1: *Mean (µg/g dry weight) of metal bioaccumulation in scales of fish specimens sampled from different sites during low and high flow seasons of the river Ravi.*

Metals	Cd	Cr	Cu	Fe	Pb	Zn	Mn	Ni	Hg
Sampling sites									
Site A: Siphon (Control)	0.09d	1.32d	4.87c	42.99d	0.30d	31.73d	3.36d	0.52d	0.17d
Site B: Shahdera	0.12c	2.90c	6.12b	44.76c	1.60c	47.86c	5.72c	0.76c	0.35c
Site C: Sunder	0.29a	4.64a	8.85a	65.66a	5.14a	72.16a	14.75a	3.18a	2.91a
Site D: Head Balloki	0.19b	3.34b	6.28b	59.03b	3.27b	55.53b	7.42b	1.14b	2.45b
SEM and Significance	0.004***	0.030***	0.057***	0.466***	0.033***	0.373***	0.108***	0.015***	0.009***
Flow seasons									
High	0.15b	2.44b	6.20b	47.65b	2.18b	48.88b	7.01b	1.28b	1.29b
Low	0.20a	3.66a	6.85a	58.57a	2.98a	54.77a	8.62a	1.53a	1.65a
SEM and Significance	0.003***	0.021***	0.041***	0.330***	0.023***	0.264***	0.076***	0.011***	0.006***
Fish species									
Cirrhinus mrigala	0.17ab	3.21a	6.91a	51.19b	2.53b	53.47a	8.19a	1.57a	1.49a
Labeo rohita	0.16b	2.85c	6.22c	52.16b	2.54b	51.73b	8.04a	1.35b	1.45b
Catla catla	0.18a	3.09b	6.45b	55.99a	2.66a	50.27c	7.20b	1.30c	1.47ab
SEM and Significance	0.003**	0.026***	0.050***	0.404**	0.028**	0.323***	0.093***	0.013***	0.008**

*Mean in column sharing a letter did not significantly different (P>0.05). Here *, **, *** on standard error of means (SEM) represent significance at P<0.05, P<0.01 and P<0.001, respectively.*

Results and Discussion

The mean wet body weight and total length did not significantly different (P>0.05) for fish specimen captured from selected location during both flow seasons and were ranged from 636-665 g and 39.46-40.31 cm, 627-647 g and 37.44-38.09 cm and 621-643 g and 36.48-37.22 cm for *C. mrigala, L. rohita* and *C. catla,* respectively. The metals accumulation in scale samples significantly varied among different locations, flow seasons and fish species. Location-wise metal accumulation pattern was C > D > B > A. The lowest concentrations (µg/g) of the metals were recorded at site A in the order of Fe (42.99), Zn (31.73) > Cu (4.87) > Mn (3.36) > Cr (1.32) > Ni (0.52) > Pb (0.30) > Hg (0.17) and Cd (0.09) (Table 1). Jangu and Brraich (2014) reported that metals have high potential to be bioaccumulated in the scales of fishes. The metal contamination pattern in water, sediment and fish muscles for the same sampling sites and flow seasons were also revealed similar trend which were earlier reported by Shakir et al. (2013a, b). The variation of metal accumulation at different sites was reported by other researchers (Javed, 2003; Jabeen and Chaudhry, 2010a, b). All metal concentrations were significantly (P<0.001) higher during low flow than high flow season. Overall metal accumulation (µg/g dry weight) levels was; Fe (58.57 and 47.65) > Zn (54.77 and 48.88) > Mn (8.62 and 7.01) > Cu (6.85 and 6.20) > Cr (3.66 and 2.44) > Pb (2.98 and 2.18) > Hg (1.65 and 1.29) >Ni (1.53 and 1.28) > Cd (0.20 and 0.15) during low and high flow season, respectively when data were pooled to assess the impact of season on metal accumulation in fishes scales (Table 1). The variation in metal profile during different seasons might be associated with changing feeding behaviour of different fish species (Farkas et al., 2000). In present study, the bottom feeder *(Cirrhius mrigala)* showed highest Cr, Cu, Zn, Mn, Ni and Hg accumulation than column (*Labeo rohita)* and surface (*Catla catla)* feeder (Table 2) . While *Catla catla* showed highest accumulation of remaining studied metals. The trend of change in bioaccumulation of metals in scales of different fish species may be associated with differences in species' trophic level in natural water bodies, uptake and absorption abilities of the fishes as reported by different researchers (Kotze, 1997; Kotze et al., 1999; Solhaug et al., 2010). The metal concentrations were found in the scales higher than the muscles of same sampled fish species (Shakir et al., 2013a). Similar results were reported by Rashed (2001) that Co, Cr, Ni and Sr concentrations in scales of *Tilapia nilotica* (tilapia) than muscles, intestine, stomach, vertebral column and liver. Teodorof et al. (2009) reported highest Cd, As, Pb and Zn bioaccumulation in scales than

Table 2: *Mean concentrations (µg/g dry weight) of metal bioaccumulation in scale of carps sampled from different sites of the river Ravi.*

Fish Species	Sites	Seasons	Metals								
			Cd	Cr	Cu	Fe	Pb	Zn	Mn	Ni	Hg
Cirrhinus mrigala	A	Low	0.10±0.008hijkl	1.32±0.012k	5.29±0.396gh	42.29±1.574ij	0.32±0.026h	38.51±2.446i	3.8±0.037ij	0.5±0.027l	0.2±0.060jk
		High	0.08±0.019jkl	1.18±0.040kl	4.74±0.063hi	37.95±2.123jk	0.23±0.049h	32.87±3.888j	3.71±0.039ij	0.51±0.032l	0.16±0.024k
	B	Low	0.15±0.021efgh	3.08±0.063efgh	7.08±0.731cd	52.21±6.274fg	1.90±0.140f	51.33±2.446de	6.71±0.035ef	0.72±0.032ij	0.43±0.074h
		High	0.12±0.018ghijk	2.21±0.167j	6.90±0.449cd	33.21±1.871k	1.22±0.060g	46.45±2.681fgh	4.83±0.088ghi	0.71±0.030ijk	0.31±0.084hij
	C	Low	0.35±0.038a	7.36±0.153a	8.98±0.054ab	75.71±2.233b	5.85±0.094a	90.05±8.835a	19.25±1.173a	4.55±0.030a	3.40±0.129a
		High	0.26±0.037b	3.09±0.146efgh	8.24±0.376b	57.79±7.262ef	4.53±0.238b	64.05±3.020c	12.28±1.942d	3.04±0.149c	2.53±0.100f
	D	Low	0.19±0.025cde	4.67±0.196c	7.38±0.90c	61.84±2.27de	3.70±0.20c	53.29±2.158de	7.91±1.406e	1.23±0.041f	2.74±0.102cd
		High	0.16±0.024efg	2.75±0.382hi	6.66±0.88cde	48.51±3.35gh	2.51±0.12e	51.20±3.127def	7.08±1.075ef	1.26±0.045f	2.18±0.109g
Labeo rohita	A	Low	0.12±0.022ghij	1.99±0.395j	4.85±0.071hi	43.41±1.154hij	0.36±0.076h	32.36±1.128j	3.79±0.045ij	0.53±0.044kl	0.15±0.007k
		High	0.07±0.028kl	0.89±0.136l	4.47±0.107i	42.04±1.768ij	0.26±0.053h	30.44±1.467j	2.80±0.128jk	0.58±0.051ijkl	0.16±0.008k
	B	Low	0.15±0.029efghi	3.36±0.059de	5.67±0.054fg	56.71±4.494ef	1.98±0.115f	49.84±1.251efg	6.73±0.058ef	0.77±0.081hi	0.39±0.023hi
		High	0.10±0.024hijkl	2.81±0.182ghi	5.17±0.382ghi	52.43±4.823fg	1.28±0.118g	45.26±1.369gh	6.19±0.115fg	0.71±0.078jk	0.29±0.031ij
	C	Low	0.26±0.035b	4.44±0.152c	8.81±0.427b	68.12±2.502c	5.74±0.329a	68.73±1.596bc	15.72±1.018b	3.83±0.118b	3.22±0.085b
		High	0.22±0.010bcd	3.18±0.183efg	8.62±0.174b	53.91±3.619fg	3.84±0.243c	67.99±1.373bc	13.48±1.511cd	2.25±0.151e	2.48±0.103f
	D	Low	0.22±0.03bcd	3.2±0.413efg	6.57±0.301de	52.11±3.853fg	3.69±0.204c	64.01±1.642c	7.89±0.146e	1.13±0.068fg	2.77±0.043c
		High	0.17±0.04def	2.91±0.296fghi	5.64±0.223fg	48.52±4.459gh	3.17±0.221d	55.18±2.179d	7.77±0.129e	1.01±0.447g	2.14±0.050g
Catla catla	A	Low	0.09±0.025jkl	1.35±0.045k	5.17±0.162ghi	48.29±1.221gh	0.35±0.077h	31.16±2.057j	3.92±0.162ij	0.53±0.018jkl	0.17±0.011k
		High	0.06±0.012l	1.19±0.398kl	4.7±0.051hi	43.96±2.048hi	0.35±0.077h	25.06±1.723k	2.16±0.128k	0.50±0.027l	0.16±0.008k
	B	Low	0.13±0.024fghij	3.26±0.058def	6.67±0.680cde	38.21±1.417ijk	1.98±0.175f	51.84±1.887de	5.30±0.058gh	0.93±0.022gh	0.37±0.032hi
		High	0.10±0.025ijkl	2.67±0.084i	5.21±0.712gh	35.79±1.566k	1.21±0.152g	42.46±1.708hi	4.55±0.099hi	0.75±0.024hi	0.33±0.030hi
	C	Low	0.39±0.048a	6.31±0.072b	9.61±0.469a	86.51±2.289a	6.07±0.280a	71.97±1.762b	14.63±1.215bc	2.64±0.050d	3.31±0.100ab
		High	0.26±0.048b	3.47±0.132de	8.83±0.342b	51.94±2.115fg	4.82±0.485b	70.19±1.740b	13.14±1.046d	2.78±0.072d	2.55±0.053ef
	D	Low	0.24±0.029bc	3.6±0.322d	6.14±0.110ef	77.47±5.142b	3.80±0.258c	54.13±1.852de	7.76±0.036e	1.02±0.027g	2.65±0.033de
		High	0.18±0.027def	2.89±0.321fghi	5.28±0.537gh	65.74±3.118cd	2.78±0.698de	55.38±1.475d	6.13±0.828fg	1.22±0.028f	2.20±0.055g

Mean±Standard Deviation in column represent different alphapat(s) are significantly different (P<0.05).

different tissues of *Cyrinus carpio, Hypophthalmichthys molitrix, Aristichthys nobilis* and *Ctenopharyngodon idella*. Farrell et al. (2000) reported significant higher metals contents in scales in comparison with muscles of Arctic Graying. Higher concentration of metals in scales might be due to direct contact with contaminated water and river bed sediment. Several pollutants in water, river bed sediment and fish species were exceeded from threshold limit in the studied stretch proposed by National Environmental Quality Standards and World Health Organization as reported by Shakir et al. (2013a, b). These pollutants can cause fish death unless the fish has an alternate way of detoxification. Calcification is one of the important detoxification mechanism (Simkiss, 1977). The survival of fish species in the presence of high metals accumulation in select study area of river Ravi might be associated with detoxification of these metals by calcification. Different studies showed the feasibility of fish scale as a biosorbent (Mustafiz, 2003; Prabu et al., 2012). Several researchers described that normally a considerable portion of protein (collagen) containing sulfhydryl rich peptides (phytochelatines) was present in fish scale (Say et al., 2001; Mustafiz, 2003).

Conclusions and Recommendations

It is demonstrated from the present study that metal accumulation in fish scale is promising as a non lethal sensitive method for measuring aquatic pollution. Periodic monitoring of metals levels in fish scales without their scarification give better idea about water body and health of marketable fishes. This technique helps to ensure safety of consumers in the area. From the present study, it is also noteworthy revealed that the efficacy of these nonlethal techniques was highly variable across fishes of different trophic levels and likely depends on species-specific life-history characteristics. Further research is needed for determination of storage capacity of scales and lethal doses.

Novelty Statement

Fish scales might be used for assessing metals accumulation profile in fishes without sacrificing the animals.

Author's Contribution

Hafiz Abdullah Shakir: Performed the experiments and drafted the manuscript.
Javed Iqbal Qazi: Designed and planned the study and revised the manuscript.
Abdul Shakoor Chaudhry, Muhammad Irfan, Muhammad Khan and Saima Shahzad Mirza: Revised the manuscript
Shaukat Ali: Analyzed the data.

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