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Research Article

Metal accumulation patterns in livers of major carps from anthropogenically affected segment of the river Ravi, Pakistan

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Abstract

A replicated 4 x 2 x 3 factorial arrangement comprising 4 sampling sites (Siphon=A, Shahdera=B, Sunder=C and Balloki=D), 2 flow seasons i.e., high (post monsoon) and low (winter) and 3 fish species (*Cirrhinus* (*C*) *mrigala*, *Labeo* (*L*) *rohita* and *Catla* (*C*) *catla*) was applied for studying bioaccumulation of cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), zinc (Zn), manganese (Mn), nickel (Ni)and mercury (Hg) in livers of sampled fish from the river Ravi around Lahore. The order of metals bioaccumulation was Zn > Fe > Mn > Cu > Cr > Hg > Ni > Pb > Cd. While site wise order was C > D > B > A. The present investigation revealed that the toxicity of metals fluctuated significantly in fishes at all the sampling sites with season. The entire sampling sites showed higher metal concentrations in fish livers during low than the high flow season. Among the fish species, Cd, Cr, Cu, Zn, Mn, Ni and Hg accumulations were highest in *C. mrigala* while lowest in *C. catla*, excepting the Cr and Fe. Significantly elevated levels of all the investigated metals in livers of the sampled fishes from the polluted sites indicate differential response of the three fish species to the anthropogenically modified river segment and raise concerns about the alarming situation of the Ravi ecosystem and consequently the fish and its consumers' health.

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Keywords: Industrial and municipal effluents, Carp, metal bioaccumulation, Atomic absorption Spectrophotometer

Introduction

In developing countries, river water pollution has become a matter of concern over the last few decades (Rashid et al., 2012). Pakistan is also among such countries where aquatic resources are facing severe degradation from industrial, municipal and agriculture sources (UNIDO, 2000). From the long list of pollutants, natural aquatic systems may extensively be contaminated with heavy metals released from domestic, industrial and other anthropogenic activities. Heavy metals may have detrimental effects on the ecological balance of recipient environment (Farombi et al., 2007). All metals become toxic if their concentrations exceed the permissible levels (Wright and Welbourn, 2002). Metal concentrations in different organs are used as indices for highlighting the contaminations of fish and the aquatic medium (Farkas et al., 2002; Mendil et al., 2005). Heavy metal bioaccumulation in various tissues or organs of animals is derived from diet and/or prevalence of elevated levels of the pollutant in surrounding water (Nussey et al., 2000).

Rivers passing through urban areas have been facing water quality issues due to discharges of untreated domestic sewage, municipal wastes and industrial effluents. Consequently, like other recalcitrant pollutants, increasing levels of metallic toxicities have appeared in the influenced river water. Increasing levels of aquatic metallic toxicity is one of the consequences (Venugopal et al., 2009; Sekabira et al., 2010). The Ravi is highly polluted amongst the rivers in Pakistan and its fauna, especially the fishes have been drastically affected (Jabeen et al., 2012; Shakir et al., 2013a, b; Shakir and Qazi, 2013). Some incipient work has indicated heavy metal deposition in the fish (Javed,

2005; Rauf et al., 2009; Jabeen et al., 2012). However, information regarding bioaccumulation pattern of heavy metals in the fishes from the subject river before and after its passage through Lahore (the second largest city of Pakistan) are lacking. Owing to the fact that metal build up in food chains, importance of protection, management and restoration of aquatic resources concerning metals toxicants have been realized all over the world. Different fish species are used for evaluating health of aquatic ecosystems for metal toxicity because metal accumulation is faster than their excretion from these aquatic organisms (Farkas et al., 2002; Nakamura et al., 2005).

In present study, the carp species were sampled from three polluted downstream sites (Shahdera=B, Sunder=C and Balloki=D) during high (Post monsoon) and low (winter) flow seasons and compared with less polluted upstream site (Siphon=A).of river Ravi for hepatic accumulation of Cd, Cr, Cu, Fe, Pb, Mn, Ni, Zn, and Hg. Major carps (*C. mrigala, L. rohita* and *C. catla*) are native to Pakistan and other Asian countries. These fishes have also been introduced in many other countries as exotic species owing to their high nutritive value, good taste, high price and huge market demand. Objective of this study was to ascertain the extent of urban and industrial effluent stresses on the river Ravi inhabitant fish species. Hepatic metal bioaccumulation base line data presented in this paper will be helpful for river fish health management as well as food security assuring authorities.

Materials and Methods

Downstream part of the selected area of the river Ravi is contaminated by agricultural, industrial and municipal effluents of Lahore city. Four sites and two flow seasons were selected for sampling. For site A (Siphon): no urban and industrial effluent discharge was observed at or before this site to contaminate the river. Site B (Shahdera): receives sewage discharge while site C (Sunder) is affected by both domestic and industrial discharges. At site D (Bolloki), the river water carries mainly the pollutants received at sites B and C, however, it gets diluted by the feeding of the Qadir Abad (Q.B) link canal. Details of the same sampling sites have been described previously (Shakir and Qazi, 2013; Shakir et al. 2013a). The map of the study area has also been reported earlier (Shakir and Qazi, 2013; Shakir et al., 2013a).

Fish Sampling and measurements

Nine specimen of comparable sizes each of *C. mrigala*, *L. rohita* and *C. catla* were collected from triplicate netting per site during low (winter.) and high (post monsoon) flow seasons of the river Ravi. The mean total weight and total length were ranged from 645-657g and 39.47-40.23cm, 630-644g and 37.55-37.81cm, 627-642g and 36.57-37.01cm for *C. mrigala*, *L. rohita* and *C. catla*, respectively. There were no significant differences ($P>0.05$) in weight and length of fish specimen captured from selected sites and seasons (Shakir and Qazi, 2013).

Fish dissection to process liver samples

After dissection, liver of each specimen was removed carefully, washed with distilled water and stored in marked, separate polythene bag in freezer at -20 °C till further analysis. Frozen fish liver samples were thawed, rinsed in distilled water and paper blotted. Then each liver sample after wet weighing was shifted into labelled glass vial of known weight before dried in an oven at 105 °C. Weight of tissue and vial was determined following cooling in desiccator till the consistent weight was observed. Liver samples were digested according to the method of Du Preez and Steyn (1992) with slight modification made by Yousafzai and Shakooki (2008). Each dried known sample weight was shifted in to marked 250 ml volumetric flask for acid digestion. Following the addition of 5 ml of nitric acid (55 %), 1 ml of perchloric acid (65 %) the flasks were kept at room temperature for overnight. Next day to each flask, 5 ml of 55 % nitric acid and 4 ml of 65 % perchloric acid were added as a second dose. During this process, few glass beads were added to reduce vigorous boiling. The flasks were then placed on hot plate to digest the tissues and evaporate the fluid at 200-250 ºC till the conversion of the dense brown fumes into white fumes which indicated completion of the digestion process. The clear mixture was further evaporated down to 0.5 ml. Each digested sample was cooled, diluted to 20 ml with distilled water by properly rinsing the digestion flasks and filtered through Whatman filter paper No. 541. The filtrate was stored in properly washed labelled vials until the metal concentration was determined by atomic absorption spectrophotometer.

All prepared samples were analyzed for Cd, Cr, Cu, Pb and Ni by using Fast Sequential Atomic Absorption Spectrophotometer (Varian Spectra AA-240). Mn and Fe concentrations were determined using Pye Unicam Atomic absorption spectrophotometer while the Hg and Zn were measured using variant atomic absorption spectrophotometer (variant AAS-1275).

For each element determined, single standard solutions (1000 µg/ml; 99.9 % purity) were purchased from BDH

(England). Different working standard solutions were then prepared by diluting the stock solution (1000 µg/ml). Standard curves were prepared between working standard solution concentrations verses their corresponding absorbance. Optical density (OD) of liver samples were calibrated against the standard curves to find out the concentration of a given metal present in the analyzed samples. Metal concentrations were expressed in µg/g dry weight of liver sample.

Statistical analysis

The metal bioaccumulation data were statistically analysed by using general linear model in Minitab-16 software to find the effect of either site, flow season, fish species and site x flow season interactions on each fish and liver parameter. The effect of these factors were declared highly significant at P <0.001, very significant at P<0.01 and significant at P<0.05. Turkey's post-hoc test was used when more than two least square means were required to be compared at P< 0.05.

Results

Mean weights ranged from 636 to 650 g and 650 to 665 g for *C. mrigala;* 627 to 649 g and 634 to 647 g for *L. rohita,* and 621 to 641 g and 633 to 643 g for *C. catla* during high and low flow seasons, respectively. Mean total lengths ranged from 39.5 to 40.3 cm and 39.5-40.2 cm in *C. mrigala;* 37.7 to 38.1 cm and 37.4 to 39.5 cm in *L. rohita,* and 36.5 to 36.8 cm and 36.7 to 37.2 cm in *C. catla* during low and high flow seasons, respectively. Means of total length and total wet weight of the sampled specimen of each species did not differ significantly (P>0.05) among the sampling sites and flow seasons (Shakir, 2013; Shakir and Qazi, 2013). Conversely, mean metal (Cd, Cr, Cu, Fe, Pb, Zn, Mn, Ni and Hg) concentrations in dried liver samples were significantly (P<0.001) different among the sampling sites, flow seasons and the fish species (Table 1). The mean (µg/g dry weight) highest Cd (0.36), Cr (6.14), Cu (11.03), Fe (86.69), Pb (0.88), Zn (88.45), Mn (17.94), Ni (4) and Hg (4.33) were found at site C when data were pooled to determine the effect of sites on metal bioaccumulation. At site A, lowest concentrations of the corresponding metals were recorded as 0.06, 1.56, 5.80, 49.01, 0.29, 41.32, 4.15, 0.64 and 0.19 µg/g (Table 1). Liver of *C. mrigala* from polluted site C accumulated higher (%) Cd (411 and 417), Cr (392 and 352), Cu (81 and 101), Fe (86 and 95), Pb (245 and 332), Zn (134 and 71), Mn (421 and 205), Ni (768 and 572) and Hg (2021 and 2082) than the corresponding values for site A (less polluted) during low and high flow seasons, respectively (Table 2). While liver of *L. rohita* sampled from site C showed (%) Cd (457), Cr (195), Cu (68), Fe (68), Pb (185), Zn (112), Mn (327), Ni (577) and Hg (2578) higher than the respective values for the site A during low flow season. Whereas differences for the corresponding metal contents of the livers of *L. rohita* were 900, 280, 115, 84, 123, 96, 343, 336 and 1922 % the values of samples representing site A higher during high flow season. (Table 2). The livers of *C. catla* sampled from site C, bioaccumulated Cd, Cr, Cu, Fe, Pb, Zn, Mn, Ni and Hg for up to 500 and 460 %, 306 and 232 %, 78 and 110 %, 54 and 83 %, 203 and 223 %, 131 and 146 %, 295 and 459 %, 366 and 528 %, and 2380 and 2106 % of the samples from site A during low and high flow seasons, respectively (Table 2).

Discussion

In the present study, higher metal accumulation in livers of all the sampled fish species appeared in line with the findings of Jabeen et al. (2012) who reported higher accumulations of Al, As, Ba, Cr, Ni and Zn in livers of both herbivorous and carnivorous fish species sampled from the river Ravi. Comparable results of metal bioaccumulation in different tissues and organs of different fish species have been reported by various workers (Canli and Kalay, 1998;. Avenant-Oldewage and Marx, 2000; Qadir and Malik, 2011). Yilmaz et al. (2007) reported higher accumulation of metals in livers and gills of Leuciscus cephalus and Lepornis gibbosus. Likewise Alhashemi et al. (2011) documented high mean metal bioaccumulation in livers and gills of B. grypus. During present study, the range of Zn in livers of *C. mrigala, L. rohita and C. catla* were measured within the range reported in the literature (Table 3). Andres et al. (2000) reported that the Zn levels of about 50 mg/Kg wet weight were found in livers of four sampled fish species captured from the Lot river polluted by effluents from a Zn –mineral-processing plant in France. Copper have important role in biological system and the low levels found in the present study (4.93 – 11.90 µg/g dry weight) are in agreement with its homeostatic control (Fallah et al., 2011; Weber et al., 2013).

Higher bioaccumulation of metals in the fishes liver may be related to the fact that liver play very important role in process of metal detoxification. Metals get bounded with specific metal induced polypeptides known as metallothioneins (MTP) in liver. Levels of MTP increased in fish following exposure to metals (Cosson, 1994; Jezierska and Witeska, 2000). MTP have high affinities for binding with metals and regulate or detoxify the metal ions (Kojima and Kagi, 1978).

Table 1 Mean metal concentrations (µg/g dry weight of liver) for sampling sites, flow seasons and fish species with standard error of means (SEM) and significance.

Metals									
	Cd	**Cr**	**Cu**	**Fe**	**Pb**	**Zn**	**Mn**	**Ni**	**Hg**
Sampling sites									
Site A: Siphon (Control)	0.06^{d}	1.56^{d}	5.80^{d}	49.01^{d}	0.29^{d}	41.32^{d}	4.15^{d}	0.64^{d}	0.19^{d}
Site B: Shahdera	0.14^{c}	3.29^{c}	9.13^{b}	63.08^{c}	0.39^{c}	56.40^{c}	6.34^{c}	0.93^{c}	0.44^{c}
Site C: Sunder	0.36^{a}	6.14^{a}	11.03^{a}	86.69^{a}	0.88^{a}	88.45^{a}	17.94^{a}	4.00^{a}	4.33^{a}
Site D: Head Balloki	0.23^{b}	4.51^{b}	8.65^{c}	74.37^{b}	0.56^{b}	67.12^{b}	8.82^{b}	1.47^{b}	4.01^{b}
SEM and Significance	0.004^{***}	0.029^{***}	0.056^{***}	0.521^{***}	0.007^{***}	0.464^{***}	0.128^{***}	0.019^{***}	0.014^{***}
Flow seasons									
High	0.16^{b}	3.14^{b}	8.13^{b}	64.73^{b}	0.45^{b}	57.52^{b}	8.25^{b}	1.60^{b}	1.97^{b}
Low	0.23^{a}	4.61^{a}	9.17^{a}	71.84^{a}	0.61^{a}	69.13^{a}	10.38^{a}	1.92^{a}	2.51^{a}
SEM and Significance	0.003^{***}	0.020^{***}	0.039^{***}	0.368^{***}	0.005^{***}	0.328^{***}	0.091^{***}	0.014^{***}	0.010^{***}
Fish species									
Cirrhinus mrigala	0.23^{a}	4.42^{a}	8.95^{a}	66.58^{b}	0.51^{b}	65.49^{b}	9.81^{a}	1.97^{a}	2.27^{a}
Labeo rohita	0.19^{b}	3.33^{c}	8.69^{b}	66.79^{b}	0.54^{a}	63.23^{a}	9.58^{a}	1.69^{b}	2.21^{b}
Catla catla	0.18^{b}	3.87^{b}	8.31^{c}	71.48^{a}	0.54^{a}	61.24^{c}	8.56^{b}	1.63^{c}	2.24^{ab}
SEM and Significance	0.003^{***}	0.025^{***}	0.048^{***}	0.451^{***}	0.006^{**}	0.402^{***}	0.111^{***}	0.017^{***}	0.012^{**}

Mean in column share a letter are non significant (P>0.05). Here *, **, *** represent significance at P<0.05, P<0.01 and P<0.001 respectively.

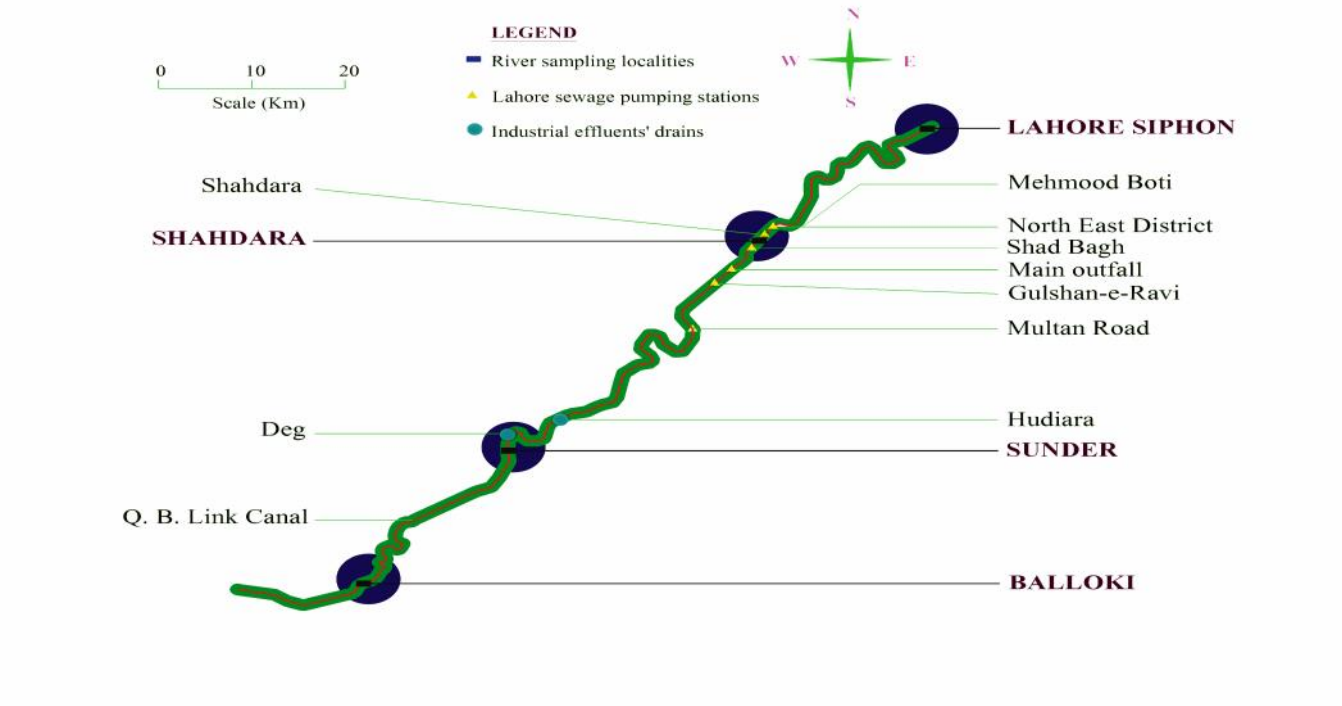
Table 2 Mean concentrations (µg/g dry weight) of metal bioaccumulation in liver of carps sampled from different sites of the river Ravi.

Fish species	Element	A		B		C		D		SEM with significance		
		Low	High	Low	High	Low	High	Low	High	Site	Season	Site x Season
Cirrhinus mrigala	Cd	0.09^{de}	0.06^{e}	0.23^{c}	0.13^{d}	0.46^{a}	0.31^{b}	0.32^{b}	0.23^{c}	0.008^{***}	0.005^{***}	0.011^{***}
	Cr	1.88^{g}	1.30^{h}	3.67^{e}	2.24^{f}	9.25^{a}	5.88^{c}	6.77^{b}	4.38^{d}	0.036^{***}	0.026^{***}	0.051^{***}
	Cu	6.47^{e}	5.23^{f}	11.16^{ab}	8.15^{d}	11.72^{a}	10.52^{bc}	9.98^{c}	8.39^{d}	0.138^{***}	0.098^{***}	0.196^{***}
	Fe	48.30^{e}	43.34^{e}	69.83^{bc}	64.37^{cd}	89.73^{a}	84.33^{a}	72.70^{b}	60.07^{d}	0.889^{***}	0.629^{***}	1.257^{**}
	Pb	0.29^{fg}	0.19^{g}	0.48^{d}	0.31^{ef}	1.00^{a}	0.82^{b}	0.62^{c}	0.39^{de}	0.016^{***}	0.012^{***}	0.023^{*}
	Zn	48.80^{de}	44.12^{e}	60.49^{c}	54.73^{cd}	114.11^{a}	75.47^{b}	70.73^{b}	55.44^{cd}	1.216^{***}	0.860^{***}	1.719^{***}
	Mn	4.71^{e}	4.60^{e}	7.27^{cd}	5.51^{de}	24.55^{a}	14.01^{b}	9.02^{c}	8.79^{c}	0.283^{***}	0.200^{***}	0.400^{***}
	Ni	0.65^{e}	0.58^{e}	0.86^{d}	0.88^{d}	5.64^{a}	3.90^{b}	1.64^{c}	1.57^{c}	0.019^{***}	0.013^{***}	0.026^{***}
	Hg	0.24^{e}	0.17^{e}	0.45^{d}	0.47^{d}	5.09^{a}	3.71^{c}	4.49^{b}	3.57^{c}	0.032^{***}	0.023^{***}	0.045^{***}
Labeo rohita	Cd	0.07^{f}	0.03^{f}	0.13^{e}	0.12^{e}	0.39^{a}	0.30^{b}	0.25^{c}	0.18^{d}	0.007^{***}	0.005^{***}	0.010^{**}
	Cr	1.89^{d}	1.05^{e}	4.00^{b}	2.76^{c}	5.57^{a}	3.99^{b}	4.28^{b}	3.12^{c}	0.059^{***}	0.041^{***}	0.083^{**}
	Cu	6.29^{d}	4.93^{e}	9.20^{bc}	8.99^{c}	10.54^{a}	10.61^{a}	9.61^{b}	9.36^{bc}	0.082^{***}	0.058^{***}	0.117^{***}
	Fe	49.58^{d}	42.02^{e}	63.91^{c}	52.87^{d}	83.54^{ab}	77.48^{b}	84.72^{a}	80.24^{ab}	0.968^{***}	0.684^{***}	1.368
	Pb	0.34^{ef}	0.30^{f}	0.46^{d}	0.37^{e}	0.97^{a}	0.67^{b}	0.69^{b}	0.51^{c}	0.008^{***}	0.006^{***}	0.011^{***}
	Zn	41.00^{e}	40.86^{e}	58.73^{c}	53.33^{d}	87.09^{a}	80.12^{b}	84.97^{a}	59.75^{c}	0.439^{***}	0.310^{***}	0.620^{***}
	Mn	4.70^{e}	3.47^{f}	7.28^{d}	7.07^{d}	20.05^{a}	15.38^{b}	9.00^{c}	9.65^{c}	0.182^{***}	0.129^{***}	0.257^{***}
	Ni	0.70^{d}	0.66^{d}	0.92^{d}	0.88^{d}	4.74^{a}	2.88^{b}	1.49^{c}	1.26^{c}	0.053^{***}	0.037^{***}	0.075^{***}
	Hg	0.18^{f}	0.18^{f}	0.41^{e}	0.43^{e}	4.82^{a}	3.64^{c}	4.54^{b}	3.51^{d}	0.019^{***}	0.014^{***}	0.027^{***}
Catla catla	Cd	0.07^{fg}	0.05^{g}	0.13^{de}	0.10^{ef}	0.42^{a}	0.28^{b}	0.21^{c}	0.16^{d}	0.006^{***}	0.004^{***}	0.009^{***}
	Cr	1.92^{e}	1.31^{f}	3.88^{c}	3.19^{d}	7.79^{a}	4.35^{b}	4.39^{b}	4.09^{bc}	0.051^{***}	0.036^{***}	0.072^{***}
	Cu	6.70^{e}	5.18^{f}	8.8^{c}	8.51^{c}	11.9^{a}	10.87^{b}	7.67^{d}	6.89^{e}	0.046^{***}	0.033^{***}	0.065^{***}
	Fe	60.5^{d}	50.21^{e}	69.1^{c}	58.38^{d}	93.39^{a}	91.66^{a}	76.68^{b}	71.81^{bc}	0.845^{***}	0.598^{***}	1.195^{**}
	Pb	0.33^{ef}	0.26^{f}	0.41^{de}	0.32^{f}	1.00^{a}	0.84^{b}	0.68^{c}	0.47^{d}	0.012^{***}	0.009^{***}	0.017^{***}
	Zn	39.40^{f}	33.65^{g}	61.08^{d}	50.03^{e}	91.20^{a}	82.71^{b}	71.85^{c}	59.97^{d}	0.520^{***}	0.368^{***}	0.735^{***}
	Mn	4.73^{e}	2.68^{f}	5.74^{e}	5.19^{e}	18.67^{a}	14.99^{b}	8.86^{c}	7.61^{d}	0.186^{***}	0.131^{***}	0.263^{***}
	Ni	0.70^{g}	0.57^{h}	1.12^{e}	0.93^{f}	3.26^{b}	3.58^{a}	1.35^{d}	1.52^{c}	0.011^{***}	0.008^{**}	0.016^{***}
	Hg	0.20^{f}	0.17^{f}	0.39^{e}	0.50^{e}	4.96^{a}	3.75^{c}	4.34^{b}	3.60^{d}	0.017^{***}	0.012^{***}	0.025^{***}

Sites = A= Siphon (upstream); B=Shahdera; C=Sunde; and D=Head balloki; with two seasons (Low and High flow).

Mean in column share a letter are non significant (P>0.05). Here *, **, *** represent significance at P<0.05, P<0.01 and P<0.001 respectively

Fig. 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)



This metal-accumulating ability allowing the liver to accumulate high levels of metal pollutants from the environment (Karadede and Unlu, 2000; Usero et al., 2003). Metal bioaccumulation in liver of carps has been considered as indicative of storage of sequesting products in this organ (Gbem et al. 2001; Yousafzai et al. 2009). Since blood passes through liver before reaching the systemic circulation and liver removes toxicants from the blood before excrete these into bile or biotransform them. Liver has multifunctional role in storage and detoxification of toxicants. Accumulation of metals in fish liver proved that this organ is well reflective of aquatic metal pollution. Ability of a given tissue or organ to either regulate or accumulate metals can be directly related to the total amount of metal loads. However, fish have limited ability for MTP synthesis (Brown and Parsons, 1978). When metabolic capabilities for excretion and binding the pollutants are exceeded from threshold limit, toxic effects will results, unless the fish has an alternate way of detoxification. In scaly fish species, the alternate detoxification process may be calcification as suggested by Simkiss (1977). In the present study, it is worth mentioning that sampled fish species were scaly and higher contents of the metals in scales (unpublished data) might had been playing a role in the detoxification by calcification and had made survival of the sampled fish species in the otherwise highly polluted river water.

Trend of changes in the metal bioaccumulation appeared responsive to the downstream locations. The mean metal concentrations of the fish livers were in the order of; site C >site D > site B > site A. This trend was expected owing to the fact that most of the untreated industrial effluents of Hudiara and Deg Nullah are discharged between site B and C. The Hudiara drain enters the river loaded with pollutants of around 212 industries. While the Deg Nullah carries effluents from Kala Shah Kaku industrial complex, which has more than 149 industrial units. Some industries on Lahore-Sheikhupura road also discharge their wastewater into the drain (Saeed and Bahzad, 2006). Reduction in metal bioaccumulation at site D might be related to the dilution of the river pollutants due to joining of Q. B. link canal between site C and D. The metal bioaccumulation profiles indicated that the fish health is under strong negative effects of the pollutant loads and warrants for quick measures to be taken for controlling the polluting sewage entrance to the river. Moreover these levels of hepatic metal contents might be considered indicative of long term pollution of the water resources.

Conclusively, the livers of the reported fish species revealed noticeable fluctuations in the metal accumulations as a function of domestic and industrial effluents. As bioaccumulation response trend for a given metal, mostly responded to the effluent loads. The effects of water pollution appeared milder for the first and the last downstream locations and were intense for the second downstream locations as compared to the upstream site A. From these observations it can be safely arrived at the conclusion that the urban pollutant loads translated through untreated domestic and industrial effluents of the city Lahore exerted drastic cumulative negative effects on the fish health. However, in the recent past, the negative effects appeared to be mitigated to lesser extent at about 65 km downstream. The much higher metal bioaccumulation levels at the second last sampling station were albeit drastic than the values obtained for the upstream collection point. These observations clearly demonstrate that the river's study segment is approaching risky level for the inhabitation of various fauna. Prompt formation of strict regulations and their effective application for the urban effluents is warranted for rehabilitating the river Ravi in conjunction with environmental and public health prospective.

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