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Assessing Human Health Risk of Metal Accumulations in a wild carp fish from Selected Sites of a River Loaded with Municipal and Industrial Wastes

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ABSTRACT: This 4x2 factorial study compared the effect of four sites (A=Siphon as upstream, and B=Shahdera, C=Sunder and D= Balloki as 3 downstream sites) during low and high flow seasons of river Ravi on metal cconcentrations in muscles from *Cirrhinus (C) mrigala,* a bottom feeder carp. While weight and length of studied specimens did not differ significantly ($P>0.05$). Overall metal accumulations appeared in the order of calcium (Ca) > sodium (Na) > potassium (K) > magnesium (Mg) > zinc (Zn) > iron (Fe) > manganese (Mn) > copper (Cu) > chromium (Cr) > lead (Pb) > nickel (Ni) > cadmium (Cd). The sampling sites were significantly different ($P<0.001$) for all the metals except Mg. The metal accumulation pattern for sites was C > B > D > A, except Mg, Pb, Mn, Ni, Zn and Fe. The macro metal contents, except K and trace metals except Cd were significantly higher during low flow than the high flow season. The bioaccumulation of Cd, Cr and Mn were greater than those permissible for human consumption by the WHO standards. The most alarming results contradict the views of local fish consumers that riverine fish are more healthy and valuable than the pond fish. This fish species inhabiting the downstream sites was more polluted and may be a source of risk to consumer health. Regular monitoring is obligatory to evaluate eco-health of the river Ravi by choosing perhaps *C. mrigala* as a bio-indicator which might provide reliable measurements to frequently assess environmental quality of rivers.

Key words: *Cirrhinus mrigala,* Metal toxicity, River pollution, Human health risk

INTRODUCTION

It is unfortunate that human beings perform many activities without realizing that they cause pollution that can terribly ruin the natural resources and ultimately risk their own lives. Metal contamination is one of the serious concerns because it affects water bodies with implications for both fish and fish consuming humans. Like many other developing countries, Pakistan is critically facing water scarcity and quality issues for the last few decades. Rapidly increasing urbanization is the major cause of the emerging situation. For example the river Ravi while passing through the densely populated cities and towns gets contaminated with heavy loads of untreated wastewaters (Bhatti and Latif, 2011; Shakir *et al.,* 2013a,b). River Ravi receives enough rain water during monsoon season but the water flow decreases significantly in winter. Moreover, uninterrupted intrusion of untreated domestic as well as industrial effluents from Lahore, the second largest city of Pakistan, has been turning the river into a polluted drainage (Shakir and Qazi, 2013; Shakir *et al.,* 2013a, b, Tabinda *et al.,* 2013). Metal accumulation, especially in muscles as the most edible part, in contaminated water inhabiting fish can potentially threaten the health of many species at the top of the food chain. Metal concentrations exceeding the permissible limits in the aquatic environment would become detrimental to fish health and subsequently the health of people consuming contaminated fish meat (Wasi *et al.,* 2013). Exposure to metals is associated with a variety of adverse effects. Indeed, different metal pollutants can exert specific or wide-spread health effects. For instance, Lead termed as neurotoxic as it reduces mental development in children and causes cardiovascular diseases in adults (Liu *et al.,* 2008). While consumption of food contaminating cadmium leading to gastrointestinal problems, kidney and bone diseases (Beyersmann and Hechtenberg, 1997; Jarup *et al.,* 1998). Zinc high doses exposure has toxic effects on human health. The most important organ is the

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brain where zinc is significantly involved in cell death by acting on several molecular regulators, and cytotoxicity in consequence of ischemia or trauma involves the accumulation of free zinc (Plum *et al.*, 2010). The long term exposure of chromium can cause damage to kidney, liver and nerve tissues and termed as human carcinogens (Martin and Griswold, 2009). Several studies, in general, have shown that consumption of contaminated fish can cause damage to the liver, kidneys alongside digestive and nervous systems (Bervoets and Blust, 2003; Oyewale and Musa, 2006; Farombi *et al.*, 2007).

This study is a continuation of our previous reports in which we found higher levels of heavy metals in water, river bed sediments (Shakir *et al.*, 2013b) and muscles of *C. catla* (Shakir *et al.*, 2013a). *Cirrhinus mrigala* locally known as 'mori' is a bottom feeder and natural inhabitant of freshwaters of South East Asian countries. As the bottom feeder has a prolonged contact with the river bed sediments, it may accidentally consume sediments when digging in search of food. Consequently, the fish from metal polluted areas may attain higher concentrations of the contaminants in their muscles. Such metal uptake and accumulation may vary during different water flow periods at various sites of even a small stretch of the same river. Therefore, the present study investigated the effects of urban pollution loads on macro and micro metal uptake on their accumulation in the meat of *C. mrigala*. The study reports levels of Na, K, Ca, Mg, Cd, Cr, Cu, Pb, Zn, Mn, Ni and Fe in muscles of the bottom feeding fish caught from an upstream and three downstream sites during low and high flow seasons of a selected stretch of the river Ravi, Pakistan.

MATERIALS & METHODS

Four sites around river Ravi were selected on the basis of their increasing level of contamination due to industrial and domestic wastes as briefly described in Table 1.

A total of seventy two fish specimen of comparable size were sampled with the help of professional fishermen from four sampling sites during low (winter) and high (postmoon) flow seasons. Briefly, the fish samples were immediately sacrificed by following the local commercial procedures under ethical guidelines.

The selected fish specimens were washed with distilled water, kept in separate polythene bags placed on ice and immediately transported to the laboratory. Taxonomic identification of fishes collected was verified on the basis of morphometric characteristics up to the species level. Fish species were identified following regional identification key (Mirza, 2003). The wet weight of each fish after blot-drying excess water in the body and total length *i.e.*, from the tip of the snout to distal end of the caudal fin ray were recorded on the day of sampling. External surface of each fish specimen was wiped with 95 % ethanol soaked cotton swab. Then the fish specimens were dissected under aseptic condition by using sterilized forceps, scissors and scalpel to collect representative muscle samples which were then stored at -20^{o}C until their laboratory analysis for metals. The frozen fish muscle samples were freeze dried, ground and homogenized afterwards for the chemical analysis.

About 1g of each ground sample was mixed with 55 % 10 ml nitric acid in conical flasks at room temperature and the flasks were then placed on a hot plat at 200–250 °C for the acid digestion of fish muscles. Turning of dense brown fumes in to white fumes indicated completion of the digestion process. The evaporation was continued until the mixture attained a volume of around 0.5 ml. The digested samples were further processed as described earlier (Shakir *et al.*, 2013a, b). A Varian Vista-MPX CCD Inductively coupled plasma optical emission spectroscopy (ICP-OES Varian Inc, Australia) machine was calibrated by using the settings in Table 2 over

Table 1. Description of sampling sites and anthropogenic pressures on river Ravi, Pakistan

Site (river flow A-->D)	Coordinates	Pressure
A: Lahore Siphon	31° 41' N, 74° 25' E	Upstream site with no point source
B: Shahdera	31° 36'N, 74° 18' E	First downstream site receiving untreated municipal sewage effluent of Lahore city from three major pumping stations.
C: Sunder	31° 21'N, 74° 3'E	Second downstream site after an area receiving urban and industrial effluents from four major municipal pumping stations, and Hudiara and Deg Drain
D: Balloki headworks	31° 13' N, 73° 52' E	Third downstream site gets diluted effluents due to a relatively clean water fromQadirabad (Q.B.) Link Canal

the relevant concentration of individually certified standards (Table 2) and to prepare standard metal solutions, stock solutions of $Mg(NO_3)_2$, $Mn(NO_3)_2$, $Fe(NO_3)_2$, $Pb(NO_3)_2$ were used. Double distilled water was used throughout the study. All the glassware and containers were thoroughly cleaned with dilute acid solutions, finally rinsed with double distilled water for several times and air-dried prior to use. Macro and micro metal concentrations in the fish muscle samples were then recorded in milligram per kilogram of dry weight. However, for comparison of metal concentrations with the international standards being described mostly on wet weight basis the dry weight based values were converted to wet weight concentrations by using the following formula:

Wet weight concentration = (Dry weight concentration) x (100- % moisture content) /100)

The data were tested for normality by using Anderson-Darling test before further statistical analysis by using GLM procedures in Minitab software-16 to find the effects of site, season and site x season interaction on metal profiles and metal bioaccumulation in the sampled fish muscles. Effect of these factors were declared highly significant if $P<0.001$, very significant if $P<0.01$ and significant if $P<0.05$. Tukey's post-hoc test was used if there were more than two means to be compared at $P<0.05$. The data are represented as mean±SD metal contents in mg/kg of wet weight of fish muscles.

RESULTS & DISCUSSION

The mean wet body weight and total length of the sampled fish specimens ranged from 470 to 486g and 36.15 to 37.15cm, respectively and those did not differ significantly among sampling sites and flow seasons (Table 3). The effects of site, season and site x season interaction for the moisture content of the sampled muscles were not significant. All metals (Ca, Mg, K and Na) except 'K', were significantly different for two flow seasons ($P<0.05$). The metal analyses showed that site x season interaction for muscles of *C. mrigala* was non-significant ($P>0.05$), except for Ca. The metal values were highest at site C during low flow season with the order of Ca>P>Na>K>Mg (Table 4). Micro metals (Cd, Cr, Cu, Pb, Mn, Ni, Zn and Fe) were significantly different ($P<0.001$) among the sampling sites, while, site x season interaction showed highly significant effect ($P<0.001$) on Cr and Mn in the fish muscles. Increasing trends of metal accumulation in the fish muscles sampled from the downstream sites were recorded for both low and high flow seasons. Bioaccumulation levels of Cd, Cr and Cu showed a decreasing trend for the site as compared to the values obtained for the site C. Zinc bioaccumulation (60.38 mg/kg dry wt.) was highest while Cd (0.16 mg/kg dry wt.) appeared to be the lowest among all the samples of this study (Table 5). Muscles of *C. mrigala,* sampled from site C accumulated Cd for up to 529 %, Cr (381 %), Cu (96 %), Pb (1889 %), Mn (353 %), Ni (554 %), Zn (145 %) and Fe (79 %) of the corresponding metal levels of muscles samples from the upstream site A during low flow season on wet weight basis (Table 6). The Cr contents of the fish muscles were up to 416 and 324 % at site A, 964 and 854 % at site B, 2380 and 1278 % at site C and 664 and 554 % higher at site D during low and high flow seasons, respectively in reference to the permissible values for fish as a food proposed by WHO (2004). The alarming increase in Mn accumulation ranged from 6080 to 28190 % of that of the WHO standard.

Table 2. ICP-OES operational settings during analysis of the fish muscle samples

Parameter	Plasma (L/min)	Auxiliary gas (L/min.)	Mass flow controller MFC (L/min.)	Power (KW)	Pump (rpm)	Time (sec.)
Purge	22.5	2.25	0.9	0,0	0	15
Delay	22.5	2.25	0.0	0.0	0	10
Ignite	1.5	1.50	0.0	2.0	50	5
Run	15.0	1.50	0.9	1.2	7	5

Table 3. Biometric characteristics of *Cirrhinus mrigala* (Mori) sampled from different study sites during low and high flow seasons of the river Ravi, Pakistan

Parameter	Siphon		Shahdera		Sunder		Head Balloki		SEM and Significance		
	Low	High	Low	High	Low	High	Low	High	Sites	Seasons	Sites x Seasons
Total length (cm)	36.4a	36.2a	36.8a	36.6a	37.1a	37.1a	37.2a	36.8a	0.294	0.208	0.416
Wet weight (g)	486a	475a	470a	480a	481a	473a	468a	482a	18.09	12.79	25.59
Moisture (%)	75.6a	75.9a	75.0a	75.7a	72.3b	72.9b	75.2a	75.43a	0.27***	0.191	0.382

Means that do not share a letter in the same row wee significantly different ($P<0.05$). Here *** represent significance at $P<0.001$.

Table 4. Mean macro metal (mg/kg dry wt.) profiles of *Cirrhinus mrigala* (Mori) muscles sampled from different study sites during low and high flow seasons of the river Ravi, Pakistan

Minerals	Siphon		Shahdera		Sunder		Head Balloki		SEM and Significance		
	Low	High	Low	High	Low	High	Low	High	Sites	Seasons	Sites x Seasons
Ca	4438^{ef}	3494^{f}	10331^{b}	8260^{cd}	14356^{a}	9311^{bc}	7670^{d}	5472^{e}	156.28^{***}	110.51^{***}	221.02^{***}
Mg	666^{a}	580^{b}	670^{a}	567^{b}	696^{a}	603^{b}	678^{a}	607^{b}	6.14^{*}	6.14^{***}	8.68
K	2968^{ab}	2802^{a}	3798^{de}	3653^{d}	3912^{e}	3770^{de}	3381^{c}	3160^{bc}	94.26^{***}	66.66	133.31
Na	3207^{e}	2904^{de}	4613^{ab}	4306^{abc}	4877^{a}	4344^{abc}	4005^{bc}	3803^{cd}	89.34^{***}	63.17^{**}	126.34

Means that do not share a letter in the same row were significantly different (P<0.05). Here *, ** and *** represent significance at P<0.05, P<0.01 and P<0.001, respectively.

Table 5. Mean micro metal bioaccumulation (mg/kg dry wt.) in muscles of *Cirrhinus mrigala* (Mori) sampled from different study sites during low and high flow seasons of the river Ravi, Pakistan

Metals	Siphon		Shahdera		Sunder		Had Balloki		SEM and Significance		
	Low	High	Low	High	Low	High	Low	High	Sites	Seasons	Sites x Seasons
Cd	0.03^{bc}	0.03^{c}	0.08^{abc}	0.07^{bc}	0.16^{a}	0.11^{ab}	0.04^{bc}	0.04^{bc}	0.010^{***}	0.007	0.014
Cr	1.06^{fg}	0.88^{g}	2.13^{bc}	1.96^{cd}	4.48^{a}	2.54^{b}	1.54^{de}	1.33^{ef}	0.053^{***}	0.038^{***}	0.075^{***}
Cu	3.04^{f}	2.99^{f}	4.41^{bc}	4.09^{cd}	5.24^{a}	4.79^{b}	3.90^{de}	3.55^{e}	0.048^{***}	0.034^{***}	0.068
Pb	0.18^{e}	0.14^{e}	1.03^{de}	0.94^{cde}	3.16^{a}	2.64^{ab}	2.00^{bc}	1.36^{cd}	0.134^{***}	0.095^{*}	0.189
Mn	2.56^{e}	2.50^{e}	4.29^{cd}	3.23^{de}	10.22^{a}	6.70^{b}	6.40^{b}	4.99^{c}	0.157^{***}	0.111^{***}	0.223^{***}
Ni	0.37^{d}	0.29^{d}	0.46^{d}	0.44^{d}	2.12^{a}	1.52^{b}	1.00^{c}	0.78^{c}	0.040^{***}	0.028^{***}	0.056^{**}
Zn	27.94	24.57	34.78	31.96	60.38	43.52	38.28	34.97	2.363^{***}	1.671^{*}	3.342
Fe	27.31^{d}	21.83^{e}	31.44^{c}	30.63^{c}	43.24^{a}	42.11^{a}	39.36^{b}	38.87^{b}	0.418^{***}	0.295^{**}	0.591^{**}

Means that do not share a letter in the same row were significantly different (P<0.05).
Here *, ** and *** represent significance at P<0.05, P<0.01 and P<0.001, respectively

We examined the presence of macro and micro metals in fish muscles collected from four sites of the river Ravi during low and high water flow seasons to assess the potential risks for the fish consuming humans of this and other areas (Callan *et al.,* 2013). The Ca, Mg, K and Na contents of the fish muscles increased at downstream polluted sites when compared with the less polluted upstream site of this river. These results are in line with our previous investigation in which muscles of *C. catla* showed similar trend (Shakir *et al.,* 2013a). It has been reported that level of Na in naturally occurring fish species at site D (Balloki) was higher as compared to various farmed fish (Nawaz *et al.,* 2010). Excessive Na and K can impart a bitter taste to drinking water and could be hazardous for people with cardiac, hepatic and renal ailments (USEPA, 1999). However, there is no defining permissible level of these elements available for fish muscles and it is not clear if the elevated levels of the metals in fish muscles are harmful for fish itself and the fish consuming community (Swann, 2000). Therefore, it is difficult to comment on this aspect of the study. Laboratory scale studies may be conducted to determine threshold limits of these metals required for fish itself as well as consumer health. Chromium accumulation varied between 0.212±0.022 to 1.240±0.216 mg/kg of wet wt. in the muscle of *C. mrigala.* These levels were much higher than the standard permissible limits of 0.05 to 0.15 mg/kg for food fish (FEPA, 2003; WHO, 2004). The results are in agreement with those reported by Qadir and Malik (2011) and Shakir *et al.* (2013a) for the muscles of *Labeo rohita* and *Catla catla* sampled from river Chenab and river Ravi, Pakistan, respectively. In the present study, Cr accumulations differed significantly among the sampling sites and seasons. Comparable results have been reported by Jabeen and Chaudhry (2010) for *Cyrinus carpio* from the river Indus, Pakistan. Tabinda *et al.* (2013) reported that highest concentration of Cr was detected in tissues of *Cirrhina mrigala* followed by *Labeo rohita* and *Catla catla*. Chromium is widely used in industries and it is considered as a serious environmental toxicant. Unregulated disposal of chromium containing industrial effluents has led to the contamination of soil, sediment and water. Human exposure of Cr occurs by intake of contaminated food and water and breathing the contaminated air. Cr toxicity can lead to various disorders including allergic disease, liver damage, lung irritation and cancer and so high levels of the carcinogen in fish tissue are of prime concern (Holmes *et al.,* 2008). The toxic effect and bioaccumulation of Cr in fish is highly influenced by water hardness and presence and nature of organic matter in relation to the developmental stage of fish (Dara, 1995; USEPA, 1999). Higher concentration of Cr causes abnormal development of fish embryos, over production of mucous and blood serum, malfunction of liver and chromosomal aberrations. Chromium does not normally

Table 6. Mean±SD metal contents in mg/kg of wet weight of fish muscles alongside the permissible WHO standards (2004)

Metals	Siphon		Shahdera		Sunder		Head Balloki		Permissible levels of metals in fish for human consumption
	Low	High	Low	High	Low	High	Low	High	
Cd	0.007±0.002	0.007±0.004	0.020±0.007	0.017±0.002	0.044±0.015	0.030±0.007	0.010±0.002	0.010±0.002	0.007
Cr	0.258±0.020	0.212±0.022	0.532±0.001	0.477±0.121	1.240±0.216	0.689±0.040	0.382±0.140	0.327±0.087	0.05
Cu	0.740±0.019	0.722±0.011	1.101±0.242	0.995±0.077	1.450±0.030	1.299±0.002	0.968±0.081	0.872±0.018	30
Pb	0.044±0.016	0.034±0.009	0.257±0.151	0.229±0.231	0.875±0.554	0.716±0.399	0.338±0.029	0.334±0.167	2.0
Mn	0.624±0.032	0.618±0.041	1.071±0.030	0.786±0.092	2.829±0.270	1.816±0.501	1.588±0.668	1.226±0.107	0.01
Ni	0.090±0.003	0.070±0.016	0.115±0.039	0.107±0.012	0.589±0.142	0.412±0.166	0.248±0.027	0.192±0.007	-
Zn	6.809±1.702	5.931±0.840	8.685±2.343	7.776±1.527	16.71±2.97	11.80±7.702	9.501±2.656	8.592±4.799	50
Fe	6.670±0.080	5.269±1.062	7.851±1.334	7.452±0.087	11.97±1.018	11.42±0.051	9.769±0.100	9.550±1.270	50

accumulate in fish and hence low concentrations were reported even from the industrialized regions of the world (Carvalho *et al.,* 2005; Yilmaz *et al.,* 2007; Tabinda *et al.,* 2010). However, high Cr bioaccumulation in fish muscles in addition to higher level of the pollution in the ambient water could be due to disturbed physico-chemical profile of water at the same sampling sites (Shakir *et al.,* 2013b). It is important to note that presence of chromite deposits, tanning, corrosion control plating and pigment manufacturing units in close vicinity of the study area and along the Hudiara drain both in Indian and Pakistani sides of the river Ravi could have contributed to the greater levels of Cr and other metals in fish muscles (Saeed and Bahzad, 2006). In view of the higher levels of Cr than the WHO limits, it could be inferred that consumption of such fish could lead to health hazards in humans. Cadmium is deemed as metal being capable of producing chronic toxicity even when it is present at low concentration (Friberg *et al.,* 1971). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has recommended the provisional tolerable weekly intake (PTWI) of cadmium for up to 0.007 mg/kg body weight. In the present study, Cd accumulation in fish muscles ranged from 0.007±0.002 to 0.044±0.015 mg/kg wet body weight. Similar finding was reported by Rahman *et al.* (2012) for ten species sampled from the river Bangshi in Bangladesh. Nickel bioaccumulation in the muscles of *C. mrigala* was recorded for up to 0.589±0.142 mg/kg wet weight. Tabinda *et al.* (2013) reported Ni bioaccumulation for up to 1.99±0.1 mg/kg wet weight for the same species caught at a different time from head Balloki on river Ravi, Pakistan. The differences in the Ni bioaccumulation in the fish muscles can be attributed to seasonal variations of the river and the fish age. Nickel normally occurs at very low levels in environment and it can cause variety of pulmonary adverse health effects, such as lung inflammation, fibrosis, emphysema and tumours (Forti *et al.,* 2011). Since, nickel is a cumulative body poison so its presence in food must be considered seriously. In the present study, the level of manganese bioaccumulation in fish muscle ranged from 0.618±0.041 to 2.829±0.270 mg/kg which was much higher than that recommended WHO (2004) permissible limit of 0.01 mg Mn /kg in fish muscles for human consumption. High concentrations of manganese can interfere with the central nervous system of vertebrates by inhibiting dopamine formation as well as interfering with other metabolic pathways (Aschner *et al.,* 2007).

The present results showed that metal accumulation levels were mostly higher than the proposed acceptable limits for human consumption. The *C. mrigala* is a bottom living fish, therefore, sediments of the polluted river could be the major source of metal contamination in this fish (Shakir *et al.,* 2013b; Tabinda *et al.,* 2013). In addition, the studied metals were ubiquitous pollutants which could find their way into the river Ravi through discharge of effluents from various industries (Saeed and Bahzad, 2006; Shakir *et al.,* 2013b). The variability in metal bioaccumulation in fish species depends on age, size, and length of the fish (Linde *et al.,* 1998), ecological need, metabolism (Canli and Furness, 1993) and feeding habits (Romeo *et al.,* 1999; Chi *et al.,* 2007; Turan *et al.,* 2009).

CONCLUSIONS

The present study is a crucial research work at our chosen sites that provided an important insight into the level of different macro and trace metal accumulation in *C. mrigala* as an inhabitant of river Ravi, Pakistan. The study showed that the fish could be used as a pollution bio-indicator. Previous studies on metal contamination of fish species from river Ravi were limited especially in different flow seasons and sites, but the results obtained in this investigation have shown dangerous levels of metals in the fish muscles. The fish appeared to have been affected by the dumping of untreated urban and industrial pollutants into this important river which used to be regarded as a source of good quality water and fish for human consumption. This report is an important piece of work to estimate possible health hazards of the toxic metals through the consumption of the contaminated fish. The data may be taken as a base line against which any future pollution trends can be evaluated in order to design suitable remedial measures to prevent further contamination of the river Ravi and ultimately to protect the human health.

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