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Monitoring trace metals in different tissues of *Cyprinus carpio* from the Indus River in Pakistan

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Abstract This replicated 4 × 2 factorial study investigated the bioaccumulation of selected metals (Mn, Pb, Zn, Hg and Cr) in four tissues (gills, liver, muscle and skin) of common carp (*Cyprinus carpio*) domiciled in two sites (upstream and downstream) of Indus River in Mianwali district of Pakistan. The data were statistically compared for the main effects of the site and fish organs and their interaction on the bioaccumulation pattern of these metals in fish organs at $P < 0.05$. It appeared that the fish sampled from downstream had higher trace metals than the fish from upstream. Significant differences between fish organs were observed for these trace metals ($P < 0.001$). The fish showed higher bioaccumulation of vital metals like Zn and lower bioaccumulation for the toxic metals like Pb. The gills had the highest metal load followed by liver, skin and muscles. High concentrations of Mn, Hg and Cr were observed in different fish organs as compared to the WHO and Federal Environmental Protection Agency standards for food fish. However, the mean concentration of Pb and Zn were under the permissible limits of food fish. It implies that higher levels of Mn, Hg and Cr in fish muscles would have detrimental effects on the health of fish consumers such as pregnant women, children and elderly people of this study area.

Keywords Metal uptake · Indus River pollution · Pakistan · *Cyprinus carpio*

Introduction

As metals are very persistent pollutants, they are accumulated in soil, water, sediments and, eventually, in the food chain (Radike et al. 2002; Reinecke et al. 2003; Cornelis et al. 2005; Swaileh and Sansur 2006). Therefore, it is essential to regularly monitor any potential contamination of the environment and its impact on food chains to ensure food quality and safety (Svobodová et al. 2002, 2004; Andreji et al. 2006a). One of the most closely monitored areas is the aquatic ecosystem where fish are regarded as the ultimate components of the food chain (Yilmaz 2006). Much attention has been paid to hazardous elements such as mercury, lead, cadmium and arsenic (Alam et al. 2002; Maffucci et al. 2005). These metals can bind with amino acids and SH groups of proteins and therefore can inhibit enzymes. As the metals can accumulate in organs such as liver, spleen, kid-

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neys and gonads (Spurný et al. 2002; Yilmaz 2006; Andreji et al. 2006b), it is necessary to examine their distribution patterns in an organism to understand their physiological, toxicological and hygienic effects. The distribution of metals in tissues of different fish such as perch, roach, silver bream, chub, smelt, tench, pike (Staniskiene et al. 2006) and chub (Spurný et al. 2002) has previously been described where fish were used as an indicator of water pollution.

In continuation to our earlier study with tilapia (Jabeen and Chaudhry 2009), the present study involved common carp (*Cyprinus carpio*) domiciled in the same sites of the Indus River which is one of the key water resources for the economy of Pakistan. As reported earlier by Jabeen and Chaudhry (2009), the increased water pollution of the Indus River due to its reduced water flow has resulted in the reduction of its natural assimilative capacity. It receives raw sewage from various sources during its flow through mainly the big cities in the form of untreated industrial wastewater and irrigation returns from the surrounding communities. The increased levels of contaminants from sewage, toxic compounds from industrial discharges and pesticides from irrigation returns have caused rise in water-borne diseases and decline in the number and diversity of fish and other aquatic species in Indus River. The increased pollution involving toxic trace metals and other hazardous substances cause their bioaccumulation in fish tissues. These trace metals are known for their toxic and carcinogenic, mutagenic and teratogenic properties. Therefore, this study was conducted in the Mianwali District of Pakistan along the stretch of the Indus River to monitor the impact of various neighbouring activities on the bioaccumulation of trace metals in *C. carpio*. The aim of this study was to assess the bioaccumulation of trace metals in different tissues of fish in order to improve nature management and human health. For this purpose, the bioaccumulation of Mn, Pb, Zn, Hg and Cr were investigated in the fish tissues like gills, liver, muscle and skin. *C. carpio* was selected as another experimental model because of its hardy and tolerant nature and adaptability to various conditions and habitats. As carp prefers stagnant or slow water flows, the metal loads of these fish can be studied easily. Because of the economic importance of fishing in the study area, the metal concentration in carp fish tissues were compared with those of *Oreochromis mossambicus* (Jabeen and Chaudhry 2009) and the international standards for similar fish in order to ascertain their suitability for human consumption.

Materials and methods

Study sites and their importance

The study was conducted at the same two sites (Chashma = downstream and Shebhaz Khel = upstream) of the Indus River in the Mianwali District of Pakistan as previously reported for *O. mossambicus* (Jabeen and Chaudhry 2009). However, the readers of this paper may like to read the description of this study area which is briefly described as follows. Mianwali is one of the north-western cities in the province of Punjab, Pakistan and covers an area of 5,840 km^2. The city is located at 32°34′60″ N and 71° 32′60″ E with an altitude of 211 m. This district is rich in minerals, argillaceous clay, coal, dolomite, fire clay, gypsum, limestone, salts, silica sand and rocks, which are excavated in commercial quantities. The district has extremely hot and cold weather where the summer temperature can range between 51°C in summer and −2°C in winter with an average rainfall of about 250 mm. The study sites were 40 km apart along the stretch of the Indus River in Mianwali District. Both sites were receiving domestic and municipal wastes and agricultural runoffs. Therefore, the study investigated the effect of metal pollution from these wastes on the selected trace metal profiles of gills, liver, muscles and skin components of *C. carpio*. Fish muscles and skins were selected as edible tissues for human consumption and the gills as the non-edible tissues which can accumulate metals and yet tolerate metal load. Therefore, the gills alongside liver were selected as indicators of chronic metal exposure as these tissues can help in the metabolism and perhaps detoxification of metals.

Fishing to collect fish samples

Fishing to collect representative samples was performed during late night towards the end of the wet season in October 2007 with the help of professional local fishermen as reported by Jabeen and Chaudhry (2009). Gill nets of about 1,200 cm long and 180 cm wide with a cork line at the top rope and the metal line with the ground nylon rope were used for fishing where one single net was shared between four fishermen on two wooden boats. Motor-driven boats were not used to minimise fish disturbance and unnecessary stress due to their noisy engines. The next morning, the total fish catches were harvested from three nets per site, and samples of live carp fish of similar size were transferred to large water buckets. The remaining fish were sold in the local market. The sampled fish were then immediately killed by using the concussive blow to the head (percussive stunning) of each selected fish. Twenty-seven samples of *C. carpio* by involving nine fish per net as replicates from each site, which were 54 in total, were then washed with Milli-Q water for removing salts and placed on ice. The fish samples were immediately transported to the laboratory where morphometric measurements by involving fresh dead weight (FDW), length, and width of each of these fish were carried out as described by Jabeen and Chaudhry (2009). Here the age of each fish sample was determined at later dates by counting the number of annual annuli on replicated scales dorsal to the lateral line under the microscope (Sheri and Parveen 1979).

Fish dissection and preservation

After morphometric measurements, each fish was dissected to collect the required organs and tissues. These organs were weighed individually and washed with Milli-Q water. Water adhering to the samples was removed by placing the samples on good quality contaminant-free filter papers and then transferred into properly marked sterilized polythene bags and stored in a freezer at −20°C for further analyses.

Transport and chemical analysis of samples

The frozen samples of fish tissues were carried to the UK by the prior authorisation of the Secretary of State for DEFRA under regulation 4 of Products of Animal Origin Regulation 2006 in November 2007. These samples were stored at −20°C on arrival but freeze dried and ground afterwards. These samples were digested in concentrated HNO_3 by using 1 g of freeze-dried sample in 10 ml of concentrated HNO_3 (trace metal grade, VWR Limited, UK) in digestion blocks at 80°C for 4 h. Each sample was evaporated to about 2 ml volume, cooled at room temperature, diluted to 10 ml with distilled water and filtered with Whatman filter paper 1. These samples were then analysed by inductively coupled plasma optical emission spectroscopy (ICP-OES Unicam 701) at Newcastle University, UK. The machine was calibrated over the relevant concentrations by using individually certified standards obtained from Sigma-Aldrich, UK. The trace metal concentrations in gills, liver, muscles and skins of fish were reported as milligrams per kilogram dry weight because dry weights rather than wet weights provide a more stable basis for comparison.

Statistical analysis

All data were statistically analysed by using Minitab software to test the main effects of the sampling site, fish organs and their interaction for each parameter of this study. These effects were declared significant if $P < 0.05$ and highly significant if $P < 0.01$. Tukey's test was used if there were more than two means to compare at $P < 0.05$. Pearson's correlation coefficient (r) was used to examine the relationship between differ-

Table 1 Mean length, width, fresh dead weight and weight of different organs as per cent of FDW of *Cyprinus carpio* at two sites (CH and SK) of Indus River

Parameters	CH	SK	CH versus SK	
			SE	*P* value
Length (cm)	34.97	33.45	2.14	0.786
Width (cm)	11.09	11.35	0.55	0.859
Age (years)	9.00	8.67	1.70	0.896
FDW (g)	633.3	568.3	82.11	0.851
Organs as per cent of FDW				
Muscles	65.25	62.91	1.45	0.535
Skin	5.99	6.39	0.29	0.762
Gills	2.93	3.31	0.24	0.559
Liver	0.32	0.38	0.05	0.980

Table 2 Mean concentrations (mg/kg DM) of trace metals in selected tissues of *Cyprinus carpio* from two sites (CH and SK) of Indus River

Trace metals	Gills		Liver		Muscle		Skin		SE and significance		
	CH	SK	CH	SK	CH	SK	CH	SK	Site	Organ	Site × organ
Mn	15.2	10.30	3.65	4.4	2.09	1.65	4.68	2.68	0.14***	0.22***	0.22***
Pb	3.8	3.74	1.9	0.61	1.52	1.08	2.08	1.83	0.24	0.35***	0.35
Zn	1,179	938.2	493.3	451	39.70	36.60	263.7	251.6	1.73***	2.45***	2.45***
Hg	1.89	1.76	4.7	5.85	8.72	4.98	2.02	4.08	0.61	0.86**	0.86
Cr	10.76	8.54	4.6	4.46	1.12	4.82	3.47	2.55	0.62	0.87***	0.87

** $P < 0.01$, *** $P < 0.001$

ent trace metals and then their correlations with different fish tissues.

Results

Table 1 presents the data on mean length, width, age, FDW and weight as percentage of FDW of different organs in *C. carpio* from each of the two sites of the Indus River. There was no statistically significant difference between the two sites for any of these fish parameters ($P > 0.05$), although the mean fish weight was numerically higher for Chashma (CH) than Shebhaz Khel (SK) site.

Table 2 shows the mean concentration of metals (mg/kg dry weight) in gills, liver, muscles and skin tissues at each of the two sites of the Indus River. Although the fish samples from these sites did not differ much in their length, width, weight and age, their tissues showed variable metal accumulations. All these metals showed highly significant differences between organs of fish ($P < 0.001$), whereas these metals except Mn and Zn showed non-significant differences ($P > 0.05$) for the site and the site × organ interactions (Table 2). The Mn concentration in different tissues ranged from 1.65 to 15.17 mg/kg dry weight, and its pattern of bioaccumulation among different tissues was in the order of gills > skin > liver > muscles at CH site and gills > liver > skin > muscles at SK site. Pb concentration was highest in gills, and its bioaccumulation order was gills > skin

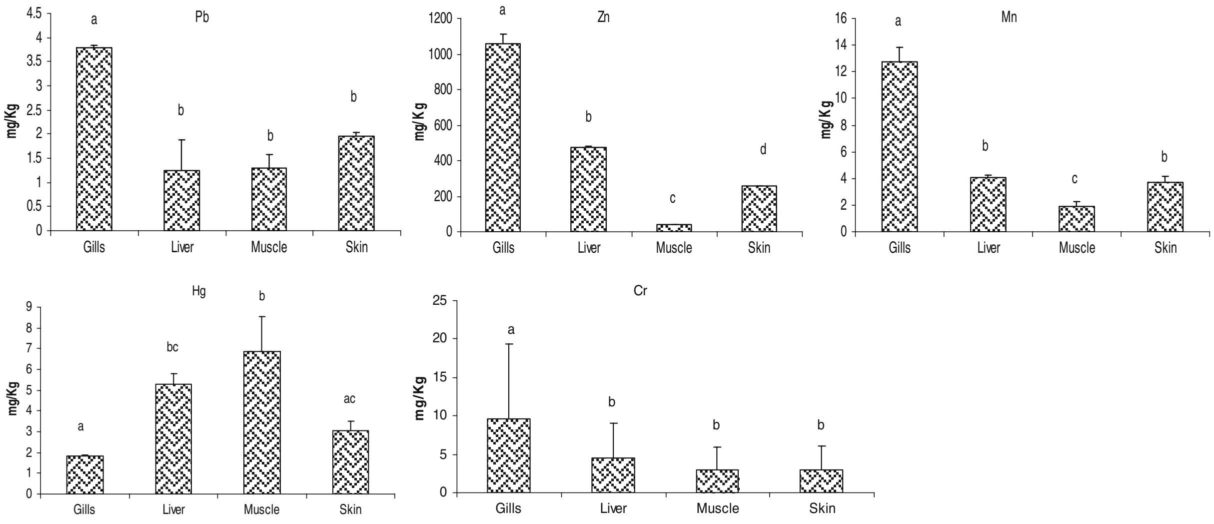

Fig. 1 Mean metal (Mn, Pb, Zn, Hg and Cr) concentrations (mg/kg DM) in gills, liver, muscle and skin of *Cyprinus carpio* (means as *columns showing different letters* differ significantly at $P < 0.05$, whereas standard errors of each mean are presented as *error bars*)

> liver > muscle at CH site and gills > skin > muscles > liver at SK site. The concentration of Pb in different fish tissues at both sites was 0.61–3.84 mg/kg dry weight. Zn was higher in gills and lower in muscles, and its bioaccumulation pattern was gills > liver > skin > muscles at both sites. Hg concentration among different tissues ranged from 1.76 to 8.72 mg/kg dry weight, and its pattern of bioaccumulation was muscles > liver > skin > gills at CH site and liver > muscles > skin > gills at SK site. Cr level was higher in gills where it ranged from 1.12 to 10.76 mg/kg dry weight and its order of bioaccumulation was gills > liver > skin > muscles at CH site and gills > muscles > liver > skin at SK site. When averaged over the two sites, the pattern of trace metal bioaccumulation in *C. carpio* from Indus River was gills > liver > skin > muscles. Figure 1 showed the statistical differences among bioaccumulation patterns of different metals in different tissues of *C. carpio* from the study area. The order of bioaccumulation of trace metals in different tissues of *C. carpio* from the Indus River was Zn > Mn > Cr > Hg > Pb. Table 3 shows the moisture contents in different tissues of *C. carpio* from Indus River. The moisture contents of gills, liver, muscles and skin tissues of fish was evaluated in order to enable the comparison of analytical results of metal concentrations with the already reported literature values on both dry and wet basis. The moisture content was generally lower in liver and skin tissues as compared to muscles and gills (Table 3). The moisture contents of muscles, gills, skin and liver were 78.69%, 77.19%, 66.95% and 58.63%, respectively.

Table 4 shows the inter-metal correlation coefficients (*r*) and the relevant probability values for different fish tissues. This study showed high

Table 3 Moisture content (%) in gill, liver, muscle and skin tissues of *Cyprinus carpio* sampled from two sites (CH and SK) of the Indus River

Tissues	CH	SK	Mean
Muscle	78.30	79.08	78.69
Gills	77.21	77.18	77.19
Liver	58.25	58.88	58.63
Skin	67.50	66.58	66.95

Table 4 Pearson's correlation coefficients (*r*) between metals in fish tissues and relevant *P* values

Metal	Mn	Pb	Zn	Hg
Pb	0.730			
P value	0.000			
Zn	0.952	0.696		
P value	0.000	0.000		
Hg	−0.483	−0.474	−0.526	
P value	0.017	0.019	0.008	
Cr	0.801	0.666	0.793	−0.335
P value	0.000	0.000	0.000	0.110

Table 5 Pearson's correlation coefficients (*r*) for different metals in various fish tissues and relevant *P* values

	Mn skin	**Mn gills**	**Mn muscles**
Mn gills	1.000		
P value	0.000		
Mn muscles	0.234	0.255	
P value	0.656	0.626	
Mn liver	−0.980	−0.979	−0.141
P value	0.001	0.001	0.790
	Pb skin	**Pb gills**	**Pb muscles**
Pb gills	0.410		
P value	0.419		
Pb muscles	0.603	−0.342	
P value	0.205	0.507	
Pb liver	0.765	0.779	0.239
P value	0.076	0.068	0.648
	Zn skin	**Zn gills**	**Zn muscles**
Zn gills	0.993		
P value	0.000		
Zn muscles	0.212	0.191	
P value	0.686	0.717	
Zn liver	0.964	0.961	0.400
P value	0.002	0.002	0.431
	Hg skin	**Hg gills**	**Hg muscles**
Hg gills	−0.531		
P value	0.278		
Hg muscles	−0.482	0.063	
P value	0.333	0.906	
Hg liver	0.453	−0.436	0.197
P value	0.367	0.388	0.708
	Cr skin	**Cr gills**	**Cr muscles**
Cr gills	0.996		
P value	0.000		
Cr muscles	−0.468	−0.516	
P value	0.349	0.295	
Cr liver	0.370	0.300	0.179
P value	0.470	0.564	0.735

Table 6 Pearson's correlation coefficients (*r*) between different trace metals in specific tissues of fish with relevant *P* values

	Mn skin	Pb skin	Zn skin	Hg skin
Pb skin	0.748			
P value	0.087			
Zn skin	0.991	0.735		
P value	0.000	0.096		
Hg skin	−0.991	−0.679	−0.992	
P value	0.000	0.138	0.000	
Cr skin	1.000	0.738	0.993	−0.993
P value	0.000	0.094	0.000	0.000
	Mn gills	**Pb gills**	**Zn gills**	**Hg gills**
Pb gills	0.544			
P value	0.265			
Zn gills	1.000	0.551		
P value	0.000	0.257		
Hg gills	0.528	−0.049	0.538	
P value	0.282	0.927	0.271	
Cr gills	0.997	0.572	0.996	0.470
P value	0.000	0.235	0.000	0.346
	Mn muscles	**Pb muscles**	**Zn muscles**	**Hg muscles**
Pb muscles	0.252			
P value	0.630			
Zn muscles	0.025	0.361		
P value	0.963	0.482		
Hg muscles	0.839	0.426	0.481	
P value	0.037	0.399	0.334	
Cr muscles	0.077	0.546	0.221	0.143
P value	0.885	0.262	0.673	0.788
	Mn liver	**Pb liver**	**Zn liver**	**Hg liver**
Pb liver	−0.894			
P value	0.016			
Zn liver	−0.965	0.861		
P value	0.002	0.028		
Hg liver	0.440	−0.587	−0.350	
P value	0.382	0.221	0.496	
Cr liver	−0.523	0.666	0.447	−0.129
P value	0.287	0.148	0.374	0.807

correlations for Mn with Pb, Zn and Cr; for Pb with Zn and Cr; and for Zn with Cr. Conversely, Hg showed negative correlations with all other metals. Table 5 shows correlations between different metals for various fish tissues. Mn in skin showed a significant correlation with Mn in gills. However, significant negative correlations were observed between Mn in skin and Mn in liver as well as Mn in gills and Mn in Liver. Non-significant correlations were observed among different fish tissues for Pb bioaccumulation. There was a significant correlation between Zn in skin and Zn in liver as well as Zn in gills and Zn in liver. For Hg, non-significant correlations were found among different tissues in fish. There was a significant correlation between Cr in skin and Cr in gills. Table 6 shows the correlation of different metals for the same tissue. In skin there were significant correlations between Mn, Zn and Cr. However, in skin there were strong negative correlations for Hg, Zn, Mn and Cr. Gills showed significant correlation for Mn, Zn and Cr. For muscles, no correlation was observed among different metals except for marginally significant correlation between Hg and Mn. For liver, there were significant negative correlations among Mn, Pb and Zn and a significant correlation between Pb and Zn.

Discussion

The metal concentration in fish tissues of this study was several folds higher than the corresponding levels in the Indus River water as well as the levels of water quality guide lines and standards by international organizations (UNEPGEMS 2006). These findings compared well with the previously reported values by Jabeen and Chaudhry (2009), who reported similar levels of bioaccumulation of metals in the fins, gills, scales, skin and muscle tissues of *O. mossambicus* from the same sites of the Indus River. However, the quality of Indus River water was considered suitable for the aquatic life (Jabeen and Chaudhry 2009). Concentrations of metals were highest at the downstream than the upstream site of this river. As expected, the pollutant levels were generally higher downstream than upstream as less human activities were noticed near the upstream site, and the fish, although not transfixed to a particular site, generally swim with the flow of water. The order of metal bioaccumulation (Zn > Mn > Cr > Hg > Pb) of this study was in agreement with the findings of Irwandi and Farida (2009), who reported higher Zn and lower Pb in marine fin fish in Langkawi Island of Malaysia.

Mn is an essential micronutrient (Dallas and Day 1993) and does not occur naturally as a metal in aquatic ecosystems, but it is found in various minerals and salts such as $MnCaCO_3$ (rhodocrosite), MnO_2 (pyrolusite) and $MnSiO_3$ (rhodonite) with oxides being the only important Mn-containing minerals that were mined (Galvin 1996). Mn concentration was considerably higher in liver than muscles presumably due to its function as a cofactor for the activation of many enzymes (Sures et al. 1999). Mn levels of 1.65–15.17 mg/kg DM in different fish tissues were dependent upon the sampling site and the organ type (Table 2). Mn can be taken up directly through the gills or indirectly from food and ingested sediments via the gut (Bendell-Young and Harvey 1986). High Mn levels in the gills of carp of this study indicated perhaps the ability of gills to accumulate Mn as previously reported for Tilapia by Jabeen and Chaudhry (2009). High Mn interferes with the central nervous system of vertebrates by inhibiting dopamine formation and also other metabolic pathways such as the disruption of Na regulation in fish which may ultimately cause fish deaths.

Pb belongs to the group of non-essential and toxic metals, which implies that it has no known function in biochemical processes (Adeyeye et al. 1996). It is acknowledged that human activities influence the Pb content of aquatic life including fish. Lead enters the aquatic environment through erosion and leaching from soil, lead-dust fallout, combustion of gasoline, municipal and industrial waste discharges, street runoffs and precipitation (DWAF 1996). Pb is known to induce reduced cognitive development and intellectual performance in children and increased blood pressure and cardiovascular diseases in adults (EC 2001). Pb poorly accumulates in fish muscle (Bradley and Morris 1986; Wagner and Boman 2003) which agreed well with the results of this study where mean muscle Pb concentrations were lower than those of the gills and skins but not liver. Pb profile in *C. carpio* varied between different tissues at each of these two sites (Table 2). The general order of Pb bioaccumulation in different tissues of *C. carpio* of this study agreed well with the findings that Pb in aquatic and terrestrial vertebrates localized more in hard tissues such as bone and teeth (Kurey 1991).

Although Zn is an essential element as it is carefully regulated by physiological mechanisms in most organisms (Eisler 1988), it is regarded as a potential hazard that can endanger both animal and human health. Therefore, information about the Zn concentrations in fish is important for both nature management and the consumption of fish (Amundsen et al. 1997). The Zn concentrations in fish tissues ranged from 36.6 to 1,179 mg/kg DM where Zn bioaccumulation in this study was higher in gills than muscles. The lower Zn concentration in muscles may be because the excessive Zn in muscles was transferred to other fish organs when exposed to Zn-contaminated system (Madhusudan et al. 2003). This deloading ability of fish has been reported to be advantageous to fish consumers (Murugan et al. 2008). Mercury is a highly toxic and the most closely monitored contaminant in fish. With the exception of occupational exposure, fish are acknowledged as the single largest source of mercury toxicity for human beings. Mercury is a metal found naturally in the environment, but human activities have greatly increased its atmospheric concentration, accounting for approximately 75% of its worldwide emissions. Anthropogenic sources of mercury in the environment include incinerators (municipal waste), coal-burning facilities (electrical generation), industrial processes (older methods for producing chlorine and caustic soda) and some consumer products (e.g. batteries, fluorescent lights, thermometers). The most worrying form of mercury for water quality is Hg^{2+} because it dissolves quickly in water and is consequently the most commonly found in the aquatic ecosystems. Chromium is widely used in industries such as tanning, corrosion control, plating, pigment manufacture and nuclear power, but it is considered as a serious environmental pollutant. Unregulated disposal of chromium-containing effluent has led to the contamination of soil, sediment and surface and ground waters. Exposure to chromium occurs by intake of contaminated food and water and breathing contaminated air. It leads to various disorders, including cancer, allergic disease, liver damage and lung irritation. Cr levels were found to be 1.12 to 10.76 mg/kg as enhanced metal levels in fish tissues could result in bio-magnification at each trophic level and carnivorous bottom feeders concentrate higher metal levels (Forstner and Wittmann 1981). As *C. carpio* is an omnivorous fish, it bio-accumulated high Cr levels from the River sediment and prey. High Cr levels in different tissues may be attributed to the chromite deposits in the study area (Jabeen and Chaudhry 2009). The correlation between different metals within the same fish tissue may have been due to the similar accumulation behaviour of trace elements in these fish (Kojadinovic et al. 2007). The significant correlations among most metals reflected a common source of occurrence and its subsequent accumulation in these fish tissues. These inter-metal correlations of this study are indicative of similar biogeochemical pathways for metal bioaccumulation in fish tissues. Pourang et al. (2005) also noted the inter-elemental correlations between the essential and non-essential elements in the muscle tissues of fishes from the Persian Gulf waters.

Table 7 Summary of water quality guidelines and standards by international organization or country

Geographic regions	WHO (guidelines)	European Union (standards)	Canada (guidelines)	Australia (guidelines)	New Zealand (guidelines)	Japan (standards)	USA (standards)	Pakistan (Indus River)[a]
Parameter⇓	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}
Lead	0.01	0.01	0.01	–	0.01	0.01	0	0.18
Manganese	0.5	0.05	0.05	0.5	0.5	0.05	0.05	0.02
Mercury	0.001	0.001	0.001	0.001	0.002	0.0005	–	–
Zinc	3			3		1	5	0.27–0.29
Chromium	0.05	0.05	0.05	–	0.05	0.05	0.1	0.06–0.14

Adapted from Water Quality for Ecosystem and Human Health, 2006 (prepared and published by the United Nations Environment Programme Global Environment Monitoring System (GEMS)/Water Programme)

[a] Jabeen and Chaudhry (2009)

Table 8 Comparison of the mean trace metals (mg/kg DM) in the muscles of two different fish species from the Indus River, Pakistan with those of other fish species from Indian subcontinent and the published data on maximum allowable metal contents in different fish for human consumption

Geographical areas/standards	Fish species	Mn	Pb	Zn	Hg	Cr	References
Indus River, Pakistan	*Cyprinus carpio*	1.65–2.09	1.08–1.52	36.6–39.7	4.98–8.72	1.12–4.82	This study
Indus River, Pakistan	*Oreochromis mossambicus*	6.8–6.94	2.87–3.6	27.42–66.2	2.24–2.69	25.4–33.7	Jabeen and Chaudhry (2009)
Southwest coast of India	*Lates calcarifer*		0.31–0.53	79.30–84.30			Rejomon et al. (2009)
Southwest coast of India	*Nemipterus japonicus*		0.23–0.45	61.00–73.40			Rejomon et al. (2009)
Southwest coast of India	*Caranx melampygus*		0.28–0.51	64.00–76.00			Rejomon et al. (2009)
Southwest coast of India	*Rastrelliger kanagurta*		0.34–0.56	24.40–37.40			Rejomon et al. (2009)
Southwest coast of India	*Cyanoglossus macrostomus*		0.32–0.53	26.40–38.10			Rejomon et al. (2009)
Off Cochin coastal waters	*Rastrelliger kanagurta*			14.99			Maheswari et al. (1997)
Off Mumbai coastal waters	*Lates calcarifer*		0.04	21.7			Asha and Vijayalakshmi (1999)
Gulf of Cambay India	Commercial fishes		1.09	38.24			Reddy et al. (2007)
Pakistan coastal waters	*Thunnus tonggol*		0.09	3.49			Jaffar and Ashraf (1988)
Pakistan coastal waters	*Thunnus thynnus*		0.08	1.27			Jaffar and Ashraf (1988)
Antarctic waters	Myctophid fishes		0.05–0.13	9.35–10.10			Honda et al. (1987)
Permissible levels of trace metals in fish for human consumption		0.01	2.0	50	0.14	0.05	[a]WHO (1985)
		0.05	2.0	75		0.15	[a]FEPA (2003)
			0.5	40	0.14		[a]FAO (1983)
			0.2–0.4				[a]EC (2001)

[a] Concentrations are in milligrams per kilogram wet weight; blank cells indicate that no citable information was available

High metal concentrations in gills and liver are related to the detoxification and excretion processes that take place in these organs. Furthermore, metals are bound in liver to specific polypeptides, i.e. metallothioneins (Jezierska and Witeska 2001), whereas metal concentrations in gills reflect the metal concentrations in water where the fish lives. The fish gills contained significantly higher Zn concentrations than muscles ($P < 0.001$). This pattern was similar to another study involving sea fish (Sidoumou et al. 2005) where higher Zn concentrations in gills than muscles were the result of high volumes of water being filtered through the gills. Higher mercury concentrations in muscles than other tissues was an indication that these fish were not contaminated with mercury from water. Data from earlier studies (Svobodová et al. 1995; Foster et al. 2000; Linde et al. 2004) indicated that the fish from heavily contaminated localities deposited Hg preferentially more in gills and liver, while in slightly contaminated areas, it was deposited preferentially in muscles. As more pollutant trace metal loads in fish tissues were found downstream than upstream, it could be inferred that high metal loads were not affecting the fish growth as there were no significant differences in the total weight of fish or the weights of their organs ($P > 0.05$) upstream and downstream. However, even the lower metal levels in different tissues might be affecting various metabolic pathways of fish and consequently consumer's health which needs to be investigated in further studies. At the moment there is no known reported case of metal poisoning arising from direct consumption of fish from this river, but the increased levels of these toxicants especially Hg could pose potential hazards to the local community.

Hazard level

Among the different metals analyzed, Pb and Hg are classified as toxic metals, which cause chemical hazards, and therefore maximum residual levels have been prescribed for human consumption by various agencies of food standards (WHO 1985; FAO 1983; EC 2001). Metal concentrations in the fish tissue of the present study given in dry weight (Table 2) were converted into wet weight basis by using the formula {Wet weight concentration = (Dry weight concentration) × (1 − moisture content/100)} for comparing with the existing food standards. Levels of Zn and Pb were well below the thresholds of concern, whereas the Mn, Hg and Cr levels were above the acceptable limits for human consumption (i.e. 50, 2, 0.01–0.05, 0.14 and 0.05 mg/kg wet weight for Zn, Pb, Mn, Hg and Cr, respectively) set by the WHO (1985) for fish (Table 7). A comparison with the European food standards (EC 2001) for fish (Pb 0.2–0.4 mg/kg wet weight) also showed that Pb was lower than the guidelines for the edible parts of the examined fish. Comparison with the Food and Agriculture Organisation (FAO) food standards also showed that Hg was higher than the acceptable limits for human consumption, whereas Pb and Zn were under acceptable limits (Table 8). Levels of Pb and Zn of this study were parallel with the findings of Rejomon et al. (2009), who studied the trace metal dynamics in fish from the southwest coast of India (Table 8). Pb and Zn levels were also comparable with the findings of Reddy et al. (2007) for commercial fishes from the Gulf of Cambay, India (Table 8). When we compared our results for Pb and Zn with the previous findings of Jaffar and Ashraf (1988) for fish from Pakistani coastal waters, it appeared that the Zn and Pb levels in the present study were higher, which could be attributed to the pollution of various types. Trace metals in present investigations were also comparable with our previous findings of metal uptake in *O. mossambicus* from the same sites of the Indus River (Jabeen and Chaudhry 2009). This study suggests that the carp fish was able to bioaccumulate different metals in its different tissues with variable intensity, and so it could be used alongside tilapia to monitor the metal pollution of the Indus River water and its impact on the fish consumers of this area.

Conclusions

Levels of Mn, Hg and Cr in different tissues of fish were found to exceed their permissible levels in food fish according to the international standards namely WHO, European Commission (EC) and Federal Environmental Protection Agency

(FEPA). However, the levels of Pb and Zn in fish muscles of this study were within the permissible limits in fish muscles for human consumption. The high levels of trace metals in *C. carpio* and specially Hg, which is a potential toxic metal, gives us cause for concern to community health issues as the communities depend on fish as a major protein source. The high trace metal levels in the fish tissues especially in the edible parts of fish would have detrimental effect on the health of the rural community of this study area. Although no such case of toxicity in humans due to fish consumption from Indus River has been reported, a potential danger may emerge in the future depending on the extent of the domestic sewage, industrial wastes and agricultural activities around the Indus River. Therefore, a very close monitoring of the source of metal loads in Indus River is needed to minimise the possible risks to the health of consumers involving children, elderly people and pregnant women.

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