International Journal of Aquatic Biology (2015) 3(3): 135-148
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.NPAJournals.com

Original Article

Metal bioaccumulation levels in different organs of three edible fish species from the river Ravi, Pakistan

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Abstract: Metals bioaccumulation in five organs of *Cirrhinus mrigala*, *Labeo rohita* and *Catla catla* captured from three industrial and sewage polluted downstream sites (Shahdera = B, Sunder = C and Balloki = D) were compared with a non-industrial upstream site (Siphon = A) during high (post monsoon) and low (winter) flow seasons of river Ravi. Mean concentrations of metals were significantly higher in low flow than the high flow season. Pattern of metal accumulation in the studied organs was: Zn > Fe > Mn > Cu > Cr > Pb > Ni > Hg > Cd. Kidneys showed mostly greater metal bioaccumulation than intestines, hearts, eyes and gills. Among fish species, the highest concentrations (µg/g dry weight) of Cr (3.77), Zn (56.22), Mn (8.95), Ni (1.70) and Hg (1.60) and lowest of Pb (2.53) were detected in *C. mrigala* whereas Cu (7.19), Fe (62.11) and Pb (2.64) appeared higher while Zn (52.69), Mn (7.82) and Ni (1.41) with lowest concentrations in *C. catla*. In contrast, lower concentrations of Cd (0.15), Cr (3.16), Cu (7.06) and Fe (54.18) were recorded in *L. rohita*. Accumulation of the metals was significantly different in organs among the different sampling sites. Based on metals accumulation pattern, second downstream site (Sunder) identified as the most polluted site due to untreated industrial and municipal discharges. Measured elevated levels of metals concentrations in fish organs indicated potential health risks for the fish and the food chain.

Article history:
Received 2 February 2015
Accepted 21 March 2015
Available online 25 June 2015

Keywords:
River Ravi
Lahore
Urban and industrial effluents
Major carps
Metals contents of fish

Introduction

Like many other developing countries, disposal of untreated municipal and industrial effluents into natural waters is known to cause a serious damage to the water quality in Pakistan. Due to unmanaged and large-scale addition of wastewaters, water quality of rivers in densely populated cities and towns, especially during low-flow months of the year remains highly degraded (Bhatti and Latif, 2011; Shakir et al., 2013). Lahore being the second-largest city of Pakistan, comprises rapidly expanding population and industrial zones. The city represents one of the few major agricultural, industrial, and urbanization centers of Pakistan. Consequently, a large number of toxic chemicals and effluent-producing industries are located in and around Lahore. The Ravi River receives large quantities of untreated domestic sewage and industrial chemical pollutants from seven major municipal sewage outfalls, and two drains called Hudaira and Deg-Nullah when the river passes through Lahore (Shakir and Qazi, 2013; Shakir et al., 2013). Hudiara drain enters in Pakistan loaded with effluents of around 100 industries situated alongside the 55 km Indian side and more than 112 industries alongside 63 km of the Punjab, Pakistan. Deg-Nullah carries effluents from Kala Shah Kaku industrial complex, which has more than 149 industrial units. Some industries located along-sides Lahore-Sheikhupura road, also discharge their wastewater into this drain. The load of hazardous and untreated waste going into Ravi River from Lahore is about 728.75 tons per day.

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Also, about 1810 sources of domestic and industrial effluents are dumped in this river through pumping stations and urban drains (Saeed and Bahzad, 2006; Yasar et al., 2010).

While the toxic effects of heavy metals on freshwater animals are known for a long time, using biological methods has been recognized as great economic and sensitive ways to monitor metals in fresh water environment. Fish are widely used to evaluate the health of aquatic ecosystems because pollutants that build-up in the food chain can exert adverse effects and even death of the biotic components of the aquatic systems (Yousuf and El-Shahawi, 1999; Farkas et al., 2002). Studies carried out on various fish have shown that pollutants alter the physiological activities of the animals and biochemical parameters of their tissues (Sanchez et al., 2011; Yousafzai and Shakoori, 2011).

Previous studies on the river Ravi inhabitant fauna were restricted to one catch site, single flow season, single fish species or non-edible fish varieties, specific tissues or limited metal types (Javed and Hayat, 1998; Javaid and Mahmood, 2000; Javed, 2005; Nawaz et al., 2010; Jabeen et al., 2012). This study was therefore extended to investigate site-wise seasonal variations of various metal bioaccumulation in eyes, gills, heart, intestine and kidneys of three nutritionally and economically important carp fish species; *Catla catla* (thaila), *Labeo rohita* (rohu), *Cirrhinus mrigala* (mori) comprehensively. The bioaccumulation of metals in muscles and liver samples of same species already reported (Shakir et al., 2013; Shakir et al., 2014). Therefore, it was not included in this paper.

These three fresh water carp species, are regarded as important fish species in aquaculture and these are the most common inhabitants of the river Ravi of Pakistan and other water sources of sub-continent, South East Asia and China. These fish represent different parts of their habitat where *C. catla* is a surface feeder, *L. rohita* is a column feeder and *C. mrigala* is a bottom feeder. These fish are the most preferred species by the Pakistani consumers due to their size, flavour and taste. However, their food quality, safety, and market value can be affected by the level of pollutants in their inhabiting waters. Therefore, the aim of this study to monitor different organs of these fish species for metal bioaccumulation by capturing representative samples of fish from different sites during different flow seasons of the river.

Materials and methods

Sampling of fish species and dissection: The selected stretch of the river receives direct discharge of untreated urban and industrial effluents from the Lahore city. The upstream site, Siphon (31°41′N and 74°25′E) was characterized least polluted site as no point source of pollution was identified at or above this site. The first downstream site Shahdera (31°36′N and 74°18′E) receives discharges from three untreated municipal pumping stations. Solid waste damping on the banks of river with blackish water colouration associated with urbanized overcrowded towns were noted for this site. The second downstream site Sunder (31°21′N and 74°3′E) receives untreated effluents from Hudiara and Deg-Nullah loaded with pollutants of more than 212 and 112 industries, respectively as reported by Saeed and Bahzad (2006) and four municipal pumping stations. Inflows of polluted upstream domestic sewage water plus effluents of drains carrying industrial effluent together make the river segment a highly polluted site. Qadirabad link canal join river Ravi before Head Balloki, last downstream sampling site (31°13′N and 73°52′E). The industrial and urban effluent not discharge at or before this site. Brief descriptions of the study sites have been reported by Shakir and Qazi (2013) and Shakir et al. (2013).

All fish samples were netted from three polluted sites (Shahdera = B, Sunder = C and Balloki = D) and an upstream site A (Siphon) of the river Ravi. During low flow season, in winter (November- December 2009) and high flow season after monsoon (September- October, 2010). The metal accumulation was studied in eyes, gills, heart, intestine and kidneys of replicated samples of three fish species comprising *C. mrigala*, *L. rohita* and *C. catla*. Nine fish specimens for each of the three species of comparable size from each collection site during each flow period were saved from triplicate netting per site. The detailed fish size and sampling procedure have been described earlier by Shakir and Qazi (2013). Each fish specimen was washed with water before their transfer to separate polythene bags being placed in an ice box that was immediately transported to the laboratory for analysis. Each fish specimen was then identified based on Mirza (2003). In total two hundred and sixteen specimens of the three fish species were dissected under aseptic condition by using sterilized forceps, scissors and scalpel to incise and remove kidneys, heart, eyes, intestine, and gills. The removed organs were carefully washed with distilled water and stored in marked polythene bags at -20ºC until their laboratory analysis.

Acid digestion of the fish tissues: The frozen fish organs were thawed, rinsed in distilled water and dried on blotting paper. Then whole kidneys and heart, both eyes and homogenized portions of gills and intestines were shifted into individually labeled and pre-weighed dried glass vials. A known weight of each dried fish organ was acid digested according to Du Preez and Steyn (1992) with a slight modification made by Yousafzai and Shakooki (2008) and Shakir et al. (2013). To each sample, about 5 ml of nitric acid (55%) and 1 ml of perchloric acid (65%) were added as a first dose while working in a fume hood and the samples were then kept for overnight at room temperature. Next day, 5 ml of nitric acid and 4 ml of perchloric acid were added as a second dose to each flask containing a few glass beads to prevent bumping during heating of flasks on a hot plate in fume hood at 200-250ºC. Turning of dense brown fumes into white fumes escaping from the flask indicated completion of the digestion process. However, the mixture was evaporated until the mixture approached about 0.5 ml of volume. The sample within each flask was then cooled and diluted up to 20 ml with distilled water while rinsing the digestion flask. The diluted sample was filtered through Whatman filter paper No. 541 filter paper. The filtrate was stored in properly washed labeled vials at 4ºC until the metal concentrations analyses.

Standard solutions and metal analysis by atomic absorption spectrophotometer (AAS): The diluted samples were analyzed for Cd, Cr, Cu, Pb and Ni using fast sequential atomic absorption spectrometer (Varian Spectra AA-240). Mn and Fe concentrations were determined with a Pye Unicam atomic absorption spectrophotometer whereas Hg and Zn were measured with a varian atomic absorption spectrophotometer (Varian AAS-1275). Different instruments were used in this study due to limitation of lamps. Single standard solutions of Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn (1000 µg/ml) were purchased from BDH (England). Different diluted working standard solutions were prepared stepwise from the stock solutions. Standard curves were prepared for different metals between working standard solutions concentrations verses their corresponding absorbances. The accuracy of the AAS was checked after each 10 samples by feeding diluted working standard solution of the respective element as a reference sample. Samples that were over calibrated were further diluted. The absorbance of samples were calibrated against their relevant standard curves to find out the concentration of metals present in the analyzed samples. Metal concentrations were expressed in µg/g of dried fish organs.

Statistical analysis: Basic descriptive statistics of metal concentrations in organs of different fish species was performed using Microsoft Excel. The General Linear Model in Minitab-16 software was used to the statistically compare the sampling sites, flow seasons, fish species, fish tissues and 2 or 3 way interactions for each metal concentration. The effect of these factors were declared highly significant at $P<0.001$, very significant at $P<0.01$, significant at $P<0.05$ and non-significant at $P>0.05$.

Results

Mean Cd, Cr, Cu, Fe, Pb, Zn, Mn, Ni and Hg concentrations and their respective standard deviations of dried samples of eyes, gills, heart, inte-

Table 1. Means ± SD (µg/g dry weight) of metals concentrations in different organs of the sampled fish species sampled from site A (Siphon) during Low and High flow season.

Fish Species	Metals	Eyes		Gills		Heart		Intestine		Kidneys	
		Low	High	Low	High	Low	High	Low	High	Low	High
Cirrhinus mrigala	Cd	0.09±0.01	0.03±0.00	0.06±0.00	0.04±0.00	0.07±0.00	0.05±0.00	0.08±0.00	0.04±0.00	0.13±0.04	0.08±0.03
	Cr	1.4±0.01	1.12±0.04	1.74±0.04	0.98±0.01	1.58±0.04	1.39±0.06	1.82±0.02	1.46±0.07	1.98±0.02	1.58±0.05
	Cu	5.24±0.10	4.42±0.06	4.38±0.08	3.76±0.05	6.13±0.11	5.69±0.08	5.86±0.11	6.09±0.08	6.93±0.13	6.21±0.13
	Fe	36.55±1.36	32.04±1.79	29.76±1.11	24.35±1.36	35.24±1.31	39.51±2.21	51.95±2.29	45.92±2.57	56.99±2.56	48.45±2.71
	Pb	0.31±0.03	0.24±0.05	0.26±0.02	0.18±0.04	0.44±0.05	0.37±0.08	0.47±0.05	0.27±0.06	0.41±0.03	0.28±0.06
	Zn	35.08±2.23	29.94±3.54	31.92±2.03	25.66±3.04	41.41±2.63	35.34±4.18	44.84±2.85	38.27±4.53	51.70±3.28	41.65±4.93
	Mn	3.39±0.03	3.31±0.03	3.08±0.03	3.01±0.03	4.00±0.04	3.90±0.04	4.33±0.04	4.23±0.04	4.99±0.05	4.87±0.05
	Ni	0.53±0.03	0.42±0.03	0.48±0.03	0.36±0.02	0.61±0.03	0.46±0.03	0.65±0.04	0.54±0.03	0.69±0.04	0.62±0.04
	Hg	0.15±0.05	0.10±0.02	0.13±0.04	0.14±0.02	0.17±0.05	0.14±0.02	0.22±0.07	0.22±0.03	0.19±0.06	0.16±0.02
Labeo rohita	Cd	0.07±0.01	0.04±0.01	0.09±0.01	0.05±0.02	0.06±0.00	0.04±0.00	0.07±0.00	0.03±0.01	0.08±0.00	0.05±0.01
	Cr	1.83±0.11	1.69±0.12	1.33±0.12	1.29±0.17	1.79±0.06	1.2±0.07	1.83±0.06	1.15±0.06	1.99±0.06	1.27±0.07
	Cu	5.09±0.07	4.17±0.10	4.25±0.06	3.55±0.09	5.96±0.09	5.36±0.13	5.99±0.35	5.74±0.14	6.74±0.10	6.04±0.15
	Fe	37.52±1.00	35.5±1.49	30.55±0.81	26.98±1.13	46.18±0.96	41.78±0.83	53.33±1.42	50.88±2.14	58.24±4.32	53.68±2.26
	Pb	0.35±0.07	0.27±0.06	0.30±0.06	0.19±0.04	0.50±0.11	0.40±0.08	0.53±0.11	0.30±0.06	0.46±0.10	0.31±0.06
	Zn	29.48±1.03	27.73±1.34	26.82±0.94	23.76±1.15	34.80±1.21	32.73±1.58	37.68±1.31	35.44±1.71	43.44±1.52	38.57±1.86
	Mn	3.38±0.04	2.50±0.11	3.08±0.04	2.27±0.10	3.99±0.05	2.95±0.14	4.32±0.05	3.19±0.15	4.98±0.06	3.68±0.17
	Ni	0.56±0.05	0.48±0.04	0.51±0.04	0.41±0.04	0.64±0.05	0.53±0.05	0.69±0.06	0.62±0.06	0.73±0.06	0.70±0.06
	Hg	0.12±0.01	0.11±0.01	0.10±0.01	0.14±0.01	0.13±0.01	0.14±0.01	0.16±0.01	0.22±0.01	0.14±0.01	0.16±0.01
Catla catla	Cd	0.05±0.01	0.03±0.00	0.08±0.02	0.06±0.02	0.09±0.02	0.05±0.01	0.12±0.02	0.09±0.03	0.15±0.04	0.11±0.04
	Cr	1.52±0.06	1.23±0.07	1.57±0.05	1.12±0.05	1.81±0.06	1.5±0.50	1.85±0.06	1.43±0.48	2.01±0.07	1.58±0.53
	Cu	5.42±0.17	4.38±0.05	4.53±0.14	3.73±0.04	6.35±0.20	5.64±0.06	6.06±0.19	6.03±0.07	7.18±0.23	6.35±0.07
	Fe	38.28±2.18	37.12±1.73	31.17±1.77	28.21±1.31	56.91±2.37	45.78±2.13	54.41±3.10	53.2±2.48	69.21±3.05	56.14±2.62
	Pb	0.33±0.07	0.32±0.06	0.28±0.06	0.23±0.05	0.48±0.10	0.47±0.12	0.50±0.13	0.35±0.07	0.44±0.10	0.36±0.07
	Zn	28.38±1.87	22.83±1.57	25.82±1.70	19.57±1.35	33.50±2.21	26.95±1.85	36.28±2.40	29.18±2.01	41.82±2.76	31.76±2.18
	Mn	3.50±0.15	1.92±0.11	3.18±0.13	1.75±0.10	4.13±0.17	2.27±0.14	4.47±0.19	2.46±0.15	4.89±0.20	2.84±0.17
	Ni	0.57±0.02	0.42±0.02	0.51±0.02	0.35±0.02	0.65±0.02	0.46±0.03	0.69±0.02	0.54±0.03	0.74±0.03	0.61±0.03
	Hg	0.13±0.01	0.10±0.01	0.11±0.01	0.13±0.01	0.14±0.01	0.14±0.01	0.18±0.01	0.22±0.01	0.16±0.01	0.15±0.01

Table 2. Means ± SD (µg/g dry weight) of metals concentrations in different organs of the sampled fish species sampled from site B (Shahdera) during Low and High flow season.

Fish Species	Metals	Eyes		Gills		Heart		Intestine		Kidneys	
		Low	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**
Cirrhinus mrigala	**Cd**	0.18±0.02	0.05±0.02	0.14±0.02	0.1±0.01	0.15±0.01	0.12±0.01	0.14±0.01	0.11±0.01	0.19±0.01	0.14±0.01
	Cr	2.43±0.05	2.03±0.05	2.99±0.09	2.4±0.06	3.26±0.07	2.89±0.07	3.34±0.07	2.1±0.14	4.18±0.09	3.29±0.12
	Cu	6.48±0.13	5.15±0.35	6.31±0.46	5.3±0.33	7.83±0.16	6.22±0.32	8.51±0.17	7.69±0.08	10.26±0.21	9.08±0.39
	Fe	42.78±3.33	36.7±2.07	49.37±3.80	41.07±2.31	57.87±3.92	51.26±3.65	64.26±3.14	57.96±3.27	75.77±2.56	69.82±3.94
	Pb	2.19±0.16	1.75±0.09	1.83±0.13	1.36±0.07	2.88±0.21	1.86±0.09	2.02±0.14	1.79±0.09	2.16±0.11	2.05±0.15
	Zn	43.49±2.07	39.35±2.27	37.27±1.78	33.73±1.95	57.76±2.75	50.29±2.90	55.58±2.65	43.19±2.49	77.54±3.70	57.99±3.35
	Mn	4.87±0.03	4.31±0.08	5.17±0.03	3.92±0.07	6.37±0.03	5.09±0.09	7.91±0.04	6.00±0.11	8.38±0.04	6.36±0.12
	Ni	0.61±0.03	0.65±0.03	0.65±0.03	0.60±0.03	0.80±0.04	0.83±0.04	0.85±0.04	0.78±0.03	0.92±0.04	0.95±0.04
	Hg	0.34±0.06	0.28±0.08	0.32±0.06	0.19±0.05	0.50±0.09	0.34±0.09	0.69±0.12	0.33±0.09	0.54±0.09	0.42±0.11
Labeo rohita	**Cd**	0.12±0.02	0.08±0.01	0.1±0.00	0.09±0.01	0.12±0.00	0.11±0.01	0.11±0.00	0.1±0.01	0.15±0.00	0.13±0.01
	Cr	2.65±0.05	1.89±0.03	3.04±0.05	2.13±0.04	3.34±0.06	2.46±0.03	3.64±0.06	2.64±0.04	4.55±0.08	3.22±0.04
	Cu	6.05±0.06	4.82±0.10	5.62±0.21	5.51±0.12	7.31±0.07	5.83±0.12	7.94±0.08	7.58±0.16	9.58±0.09	9.24±0.20
	Fe	46.13±2.14	39.84±2.73	41.09±1.82	38.11±3.23	51.28±2.27	47.07±4.14	68.08±4.07	56.61±1.93	68.28±4.73	59.19±2.32
	Pb	2.28±0.13	1.84±0.17	1.91±0.11	1.43±0.13	3.00±0.18	1.96±0.18	2.11±0.12	1.89±0.17	2.28±0.21	2.14±0.13
	Zn	42.22±1.06	38.34±1.16	36.19±0.91	32.86±0.99	56.08±1.41	49.00±1.48	53.97±1.35	42.08±1.27	75.28±1.89	56.50±1.71
	Mn	4.88±0.04	5.53±0.10	5.18±0.04	5.03±0.09	6.38±0.06	6.53±0.12	7.93±0.07	7.69±0.14	8.40±0.07	8.15±0.15
	Ni	0.65±0.07	0.64±0.07	0.70±0.07	0.60±0.07	0.85±0.09	0.83±0.09	0.91±0.10	0.78±0.09	0.98±0.10	0.94±0.10
	Hg	0.31±0.02	0.26±0.03	0.29±0.02	0.18±0.02	0.46±0.03	0.31±0.03	0.62±0.04	0.30±0.03	0.49±0.03	0.39±0.04
Catla catla	**Cd**	0.09±0.01	0.07±0.01	0.12±0.01	0.08±0.01	0.13±0.02	0.09±0.01	0.16±0.02	0.12±0.03	0.21±0.03	0.15±0.03
	Cr	2.57±0.05	2.19±0.07	2.95±0.05	2.46±0.08	3.24±0.06	2.84±0.09	3.53±0.06	3.05±0.10	4.41±0.08	3.73±0.12
	Cu	6.05±0.05	4.85±0.05	5.72±0.20	5.54±0.06	7.31±0.51	5.86±0.50	7.94±0.51	7.62±0.47	9.58±0.46	9.28±0.55
	Fe	47.6±3.76	39.56±1.73	52.76±1.59	44.27±1.94	58.37±2.21	53.25±2.71	62.03±4.59	50.47±4.41	75.65±4.33	63.25±5.88
	Pb	2.28±0.20	1.73±0.22	1.91±0.17	1.35±0.17	3.01±0.27	1.84±0.23	2.11±0.19	1.78±0.22	2.14±0.19	2.14±0.27
	Zn	43.91±1.60	35.97±1.45	37.64±1.37	30.83±1.24	58.33±2.12	45.97±1.85	56.13±2.04	39.48±1.59	72.31±2.63	53.00±2.13
	Mn	3.85±0.04	4.06±0.09	4.09±0.05	3.69±0.08	5.03±0.06	4.79±0.10	6.25±0.07	5.65±0.12	6.62±0.07	5.98±0.13
	Ni	0.79±0.02	0.68±0.02	0.85±0.02	0.64±0.02	1.03±0.03	0.88±0.03	1.10±0.03	0.82±0.03	1.19±0.03	1.00±0.03
	Hg	0.30±0.03	0.30±0.03	0.28±0.02	0.20±0.02	0.44±0.04	0.35±0.03	0.60±0.05	0.34±0.03	0.47±0.04	0.44±0.04

Table 3. Means ± SD (µg/g dry weight) of metals concentrations in different organs of the sampled fish species sampled from site C (Sunder) during Low and High flow season.

Fish Species	Metals	Eyes		Gills		Heart		Intestine		Kidneys	
		Low	High	Low	high	Low	High	Low	High	Low	high
Cirrhinus mrigala	**Cd**	0.36±0.03	0.2±0.018	0.29±0.03	0.18±0.02	0.38±0.04	0.25±0.02	0.4±0.04	0.28±0.03	0.49±0.05	0.34±0.03
	Cr	7.67±0.16	3.28±0.16	6.86±0.14	3.91±0.47	8.62±0.18	5.64±0.17	10±0.21	4.27±0.20	10.38±0.22	4.95±0.23
	Cu	7.46±0.05	5.82±0.09	7.99±0.63	6.67±0.11	8.41±0.49	7.7±0.12	12.79±0.08	11.03±0.18	11.85±0.07	9.64±0.90
	Fe	73.13±2.16	61.44±2.06	66.24±1.95	54.8±2.72	70.22±2.97	64.25±2.15	85.6±2.53	77.5±2.59	97.43±5.06	80.32±2.69
	Pb	4.08±0.07	3.97±0.21	4.71±0.08	3.59±0.19	4.92±0.26	4.43±0.07	6.23±0.10	5.11±0.27	6.28±0.40	5.94±0.31
	Zn	82.04±8.05	56.27±2.65	74.63±7.32	54.26±2.56	96.84±9.50	67.90±3.20	104.86±10.29	69.35±3.27	120.89±11.86	79.96±3.77
	Mn	21.67±1.32	10.96±1.73	17.58±1.07	9.97±1.58	26.98±1.64	12.93±2.05	22.73±1.39	15.24±2.41	28.49±1.74	16.15±2.55
	Ni	3.78±0.03	2.89±0.14	4.09±0.03	2.65±0.13	5.11±0.03	3.28±0.16	5.36±0.04	3.51±0.17	5.88±0.04	4.29±0.21
	Hg	3.77±0.14	2.78±0.11	1.83±0.07	1.28±0.05	4.07±0.15	3.00±0.12	3.95±0.15	3.00±0.12	5.70±0.22	4.14±0.16
Labeo rohita	**Cd**	0.26±0.00	0.19±0.01	0.21±0.00	0.17±0.01	0.27±0.00	0.24±0.01	0.29±0.00	0.2±0.06	0.38±0.04	0.29±0.05
	Cr	6.63±0.65	3.37±0.19	4.13±0.14	2.99±0.17	5.19±0.18	3.75±0.22	6.03±0.21	4.4±0.25	6.26±0.21	5.1±0.29
	Cu	7.08±0.04	5.87±0.12	6.93±0.46	6.72±0.14	8.03±0.88	7.77±0.16	12.13±0.07	11.13±0.22	11.24±0.07	8.71±0.18
	Fe	65.8±2.42	56.45±0.88	59.61±2.19	47.6±0.74	64.19±6.08	59.03±0.92	77.03±2.83	71.21±1.11	79.67±4.54	73.79±1.15
	Pb	4.00±0.23	3.37±0.21	4.62±0.27	3.05±0.19	4.34±0.25	4.18±0.26	6.11±0.35	4.34±0.27	5.92±0.40	5.04±0.32
	Zn	62.61±1.45	59.74±1.21	56.96±1.32	57.60±1.16	73.91±1.72	72.08±1.46	80.03±1.86	73.62±1.49	92.27±2.14	84.88±1.72
	Mn	17.70±1.15	12.03±1.35	14.36±0.93	10.95±1.23	22.03±1.43	14.20±1.59	18.56±1.20	16.73±1.88	23.27±1.51	17.73±1.99
	Ni	3.18±0.10	2.13±0.14	3.44±0.11	1.96±0.13	4.30±0.13	2.42±0.16	4.51±0.14	2.59±0.17	4.95±0.15	3.17±0.21
	Hg	3.56±0.10	2.73±0.11	1.73±0.05	1.26±0.05	3.85±0.10	2.94±0.12	3.74±0.10	2.94±0.12	5.39±0.14	4.06±0.14
Catla catla	**Cd**	0.33±0.03	0.23±0.04	0.29±0.02	0.18±0.02	0.35±0.03	0.25±0.03	0.37±0.03	0.27±0.03	0.55±0.04	0.39±0.04
	Cr	6.54±0.07	3.68±0.14	5.92±0.07	3.26±0.12	7.22±0.08	4.09±0.16	8.47±0.82	4.79±0.18	8.82±0.10	5.56±0.21
	Cu	7.99±0.50	6.01±0.23	7.49±0.57	6.89±0.27	8.13±0.42	7.96±0.31	13.69±1.0	11.4±0.64	12.68±1.00	10.93±1.34
	Fe	83.56±2.21	66.78±2.72	75.69±2.02	56.31±2.30	78.81±1.82	69.84±2.84	97.81±2.59	84.24±3.43	88.47±2.34	87.3±3.55
	Pb	4.36±0.23	4.23±0.43	4.89±0.26	3.83±0.39	5.25±0.53	4.59±0.21	5.46±0.30	5.45±0.55	6.43±0.31	6.33±0.64
	Zn	65.56±1.61	61.67±1.53	59.65±1.46	59.46±1.47	77.39±1.90	74.41±1.85	83.80±2.05	76.00±1.89	96.62±2.37	87.62±2.17
	Mn	16.48±1.37	11.73±0.93	13.37±1.11	10.67±0.85	20.51±1.70	13.84±1.10	17.28±1.44	16.31±1.30	21.66±1.80	17.28±1.38
	Ni	2.19±0.04	2.62±0.07	2.37±0.05	2.46±0.06	2.96±0.06	3.13±0.08	3.10±0.06	3.26±0.09	3.41±0.07	3.97±0.10
	Hg	3.67±0.11	2.81±0.06	1.79±0.05	1.30±0.03	3.97±0.12	3.03±0.06	3.85±0.12	3.03±0.06	5.55±0.17	4.18±0.09

Table 4. Means ± SD (µg/g dry weight) of metals concentrations in different organs of the sampled fish species sampled from site C (Sunder) during Low and High flow season.

Fish Species	Metals	Eyes		Gills		Heart		Intestine		Kidneys	
		Low	High	Low	High	Low	High	Low	High	Low	High
Cirrhinus mrigala	**Cd**	0.24±0.03	0.13±0.03	0.17±0.03	0.13±0.03	0.24±0.03	0.16±0.02	0.31±0.03	0.2±0.03	0.36±0.05	0.25±0.04
	Cr	4.05±0.61	2.75±0.09	4.36±0.17	2.85±0.34	5.30±0.35	3.16±0.48	6.46±0.26	3.03±3.03	5.95±0.56	3.43±0.13
	Cu	5.57±0.68	4.95±0.87	6.51±0.74	5.74±0.69	8.02±0.47	6.49±0.14	6.93±0.70	6.32±0.73	9.32±0.74	8.66±1.08
	Fe	59.46±3.2	40.55±3.14	56.75±4.58	44.4±5.32	67.9±3.69	51.48±5.82	70.6±4.67	52.13±3.02	83.41±5.15	76.02±4.42
	Pb	3.40±0.18	2.02±0.09	2.70±0.15	1.68±0.08	2.86±0.13	2.80±0.15	3.84±0.21	2.60±0.12	4.06±0.22	3.02±0.14
	Zn	57.30±2.32	39.46±2.41	48.54±1.97	43.38±2.65	69.33±2.81	60.34±3.68	67.52±2.73	48.60±2.97	71.53±2.90	63.92±3.90
	Mn	6.42±1.14	6.32±0.96	6.05±1.08	5.75±0.87	9.82±1.75	7.46±1.13	8.33±1.48	8.07±1.23	10.40±1.85	9.31±1.41
	Ni	1.39±0.05	1.14±0.04	1.28±0.04	0.97±0.04	1.41±0.01	1.43±0.05	1.52±0.05	1.47±0.05	1.74±0.058	1.68±0.06
	Hg	1.94±0.07	1.54±0.08	1.39±0.05	1.11±0.06	3.25±0.12	2.58±0.13	4.03±0.15	3.20±0.16	3.25±0.12	2.58±0.13
Labeo rohita	**Cd**	0.19±0.08	0.12±0.04	0.15±0.03	0.09±0.02	0.18±0.03	0.12±0.02	0.23±0.05	0.16±0.03	0.27±0.07	0.21±0.05
	Cr	3.69±0.48	2.55±0.67	3.34±0.52	2.47±0.47	3.89±0.40	2.65±0.20	3.03±0.15	2.99±0.39	5.43±0.49	3.46±0.43
	Cu	6.73±0.54	5.33±0.40	5.87±0.30	5.72±0.40	7.85±0.36	6.47±0.26	8.43±0.44	7.8±0.47	10.53±0.35	9.64±0.50
	Fe	51.42±3.73	47.25±3.48	43.35±4.03	39.07±4.13	55.88±5.87	51.79±4.97	64.14±6.26	60.46±4.83	78.79±4.64	70.27±4.86
	Pb	3.39±0.19	2.55±0.18	2.69±0.15	2.12±0.15	3.61±0.25	2.79±0.15	3.83±0.22	3.29±0.23	4.05±0.24	3.82±0.27
	Zn	68.84±1.77	42.53±1.68	58.31±1.50	46.75±1.85	83.29±2.14	65.02±2.57	81.11±2.08	52.37±2.07	85.94±2.20	68.89±2.72
	Mn	6.41±0.12	6.94±0.11	6.03±0.11	6.31±0.10	9.79±0.18	8.19±0.14	8.31±0.15	8.87±0.15	10.38±0.19	10.23±0.17
	Ni	1.27±0.08	0.92±0.41	1.17±0.07	0.78±0.34	1.28±0.08	1.15±0.51	1.39±0.08	1.19±0.52	1.58±0.10	1.35±0.599
	Hg	1.96±0.03	1.51±0.04	1.41±0.02	1.09±0.03	3.29±0.05	2.54±0.06	4.07±0.06	3.15±0.07	3.29±0.05	2.54±0.06
Catla catla	**Cd**	0.28±0.04	0.19±0.03	0.13±0.03	0.1±0.02	0.22±0.03	0.17±0.03	0.34±0.04	0.26±0.03	0.29±0.03	0.24±0.03
	Cr	3.77±0.36	2.53±0.34	3.04±0.52	2.75±0.44	3.98±0.59	3.63±0.52	3.13±0.44	2.96±0.09	3.55±0.39	3.13±0.35
	Cu	6.35±0.45	5.06±0.55	6.2±0.68	5.81±0.34	7.33±0.13	6.06±0.10	7.66±0.45	6.89±0.49	9.03±0.86	8.72±0.53
	Fe	66.35±2.92	60.69±2.88	58.53±5.30	53.61±2.54	76.12±3.63	70.8±3.36	65.56±3.94	63.38±0.01	84.5±3.01	78.55±3.73
	Pb	3.49±0.24	2.24±0.56	2.77±0.19	1.86±0.47	3.17±0.80	2.87±0.20	3.94±0.27	2.88±0.72	4.17±0.28	3.35±0.84
	Zn	58.21±1.99	42.68±1.14	49.31±1.69	46.92±1.25	70.43±2.41	65.26±1.74	68.59±2.35	52.56±1.40	72.66±2.49	69.14±1.84
	Mn	6.30±0.03	5.47±0.74	5.94±0.03	4.98±0.67	9.64±0.04	6.46±0.87	8.18±0.038	7.00±0.95	10.21±0.05	8.07±1.09
	Ni	1.15±0.03	1.11±0.03	1.06±0.03	0.94±0.02	1.16±0.03	1.38±0.03	1.26±0.033	1.43±0.03	1.43±0.04	1.63±0.04
	Hg	1.87±0.02	1.55±0.04	1.35±0.02	1.12±0.03	3.14±0.04	2.61±0.07	3.89±0.049	3.23±0.08	3.14±0.04	2.61±0.07

Table 5. Means of metals' concentrations (μg/g dry weight) for the sampling sites, flow seasons, fish species and fishes' organs with standard error of means (SEM).

	Metals								
	Cd	**Cr**	**Cu**	**Fe**	**Pb**	**Zn**	**Mn**	**Ni**	**Hg**
Sampling sites									
Site A: Siphon (Control)	0.07	1.53	5.44	43.53	0.35	33.41	3.50	0.56	0.15
Site B: Shahdera	0.12	2.98	7.07	53.15	2.03	48.41	5.80	0.82	0.38
Site C: Sunder	0.29	5.73	8.94	72.47	4.83	75.43	16.98	3.43	3.30
Site D: Head Balloki	0.21	3.58	7.07	61.47	3.06	60.62	7.72	1.29	2.47
SEM	0.002	0.015	0.021	0.195	0.015	0.178	0.054	0.007	0.005
Flow seasons									
High	0.14	2.78	6.68	53.84	2.31	49.18	7.40	1.37	1.37
Low	0.20	4.13	7.58	61.47	2.83	59.76	9.60	1.68	1.78
SEM	0.001	0.011	0.015	0.138	0.011	0.126	0.038	0.005	0.003
Fish Species									
Cirrhinus mrigala	0.18	3.77	7.14	56.67	2.53	56.22	8.95	1.70	1.60
Labeo rohita	0.15	3.16	7.06	54.18	2.54	54.49	8.73	1.46	1.55
Catla catla	0.18	3.44	7.19	62.11	2.64	52.69	7.82	1.41	1.57
SEM	0.001	0.013	0.018	0.169	0.013	0.154	0.047	0.006	0.004
Fishes organs									
Eyes	0.15	3.04	5.68	50.10	2.29	46.40	7.25	1.28	1.34
Gills	0.13	2.91	5.70	45.57	2.07	42.41	6.48	1.24	0.79
Heart	0.16	3.52	6.90	56.27	2.63	58.27	9.05	1.57	1.73
Intestine	0.19	3.64	8.30	64.12	2.80	57.11	9.17	1.65	1.92
Kidneys	0.23	4.16	9.06	72.22	3.07	68.16	10.55	1.88	2.09
SEM	0.002	0.017	0.024	0.218	0.017	0.199	0.060	0.008	0.005

Table 6. Means of metals' concentrations (μg/g dry weight) for the sampling sites, flow seasons, fish species and fishes' organs with standard error of means (SEM).

Metals	**Sampling sites (DF=3)**		**Flow seasons (DF=1)**		**Fish species (DF=2)**		**Fish organs (DF=4)**		**Site x season x species x tissues (DF=24)**	
	F value	**Significance level**	**F value**	**Significance level**	**F value**	**Significance level**	**F value**	**Significance level**	**F value**	**Significance level**
Cd	3761	P<0.001	1481	P<0.001	238	P<0.001	483	P<0.001	2.91	P<0.001
Cr	12839	P<0.001	7765	P<0.001	536	P<0.001	846	P<0.001	22.3	P<0.001
Cu	4527	P<0.001	1807	P<0.001	13.5	P<0.001	4121	P<0.001	2.5	P<0.001
Fe	3970	P<0.001	1529	P<0.001	575.8	P<0.001	2407	P<0.001	9.1	P<0.001
Pb	15624	P<0.001	1177	P<0.001	21.3	P<0.001	555	P<0.001	6.3	P<0.001
Zn	10056	P<0.001	3530	P<0.001	132	P<0.001	2643	P<0.001	1.3	P>0.05
Mn	12082	P<0.001	1669	P<0.001	166	P<0.001	736	P<0.001	0.71	P>0.05
Ni	31018	P<0.001	1785	P<0.001	556	P<0.001	1028	P<0.001	3.5	P<0.001
Hg	112684	P<0.001	7637	P<0.001	35.7	P<0.001	10149	P<0.001	0.37	P>0.05

DF= degree of freedom

stine and kidneys of each fish species are presented in Tables 1-4. Mean metals accumulation appeared to be the highest for site C and lowest for the site A whereas the sites B and D had higher metal contents than the upstream site A. The trend of the metal concentrations appeared to be significantly higher during the low than the high flow season. Highest contents of Zn followed by Fe, Mn, Cu, Cr, Pb, Ni, Hg and Cd appeared for the studied fish organs (Table 5). Metals bioaccumulation was significantly different (P<0.001) among sampling sites, flow seasons and fish organs (Table 6).

Cadmium (Cd): Highest mean Cd bioaccumulation was found at site C followed by the sites D, B and A, respectively. The Cd contents of the fish organs were found higher during low than the high flow periods of the river (Table 2). Among the fish species, lowest Cd bioaccumulation was recorded in *L. rohita* than *C. catla* and *C. mrigala*. Mean Cd accumulations pattern in the fish organs were in the order of: kidneys > intestine > heart > eyes > gills (Table 5). The highest Cd concentration of 0.55 ± 0.039 μg/g was found in kidneys of *C. catla* from site C during low flow season (Table 3) whereas the eyes of *C. mrigala* sampled from site A during high flow season showed the lowest Cd accumulation of 0.03 ± 0.003 μg/g (Table 1).

Chromium (Cr): Highest mean chromium bioaccumulation was recorded at site C than the sites D, B and A (Table 4). Effects of seasons appeared more during the low flow than high-flow season. The highest chromium accumulation was recorded in the organs of *C. mrigala* than *C. catla* and *L. rohita*. The accumulation pattern in fish organs was in the order of: kidneys > intestine > heart > eyes > gills (Table 5). The Cr accumulation ranged from 1.4 ± 0.012 μg/g to 10.38 ± 0.216 μg/g in *C. mrigala*. While in *L. rohita* the metal bioaccumulation ranged from 1.83 ± 0.109 μg/g to 6.26 ± 0.214 μg/g, in *C. catla*, it ranged from 1.23 ± 0.012 μg/g to 8.82 ± 0.100 μg/g.

Copper (Cu): The site C had the highest mean Cu bioacuumulation than the sites B, D and A. The mean Cu accumulation differed significantly between low and high-flow seasons (P<0.001). The species also showed significant variations where *C. catla* contained the highest Cu bioaccumulation than *C. mrigala* and *L. rohita*. The metal accumulation pattern in the fish organs was in the order of kidneys > intestine > heart > gills > eyes (Table 5). Mean Cu bioaccumulation in different organs of *C. mrigala* ranged from 4.38 to 6.93 μg/g and 3.76 to 6.21 μg/g at site A during low and high flow seasons, respectively. In contrast for sites B, C and D, the Cu concentration ranged from 6.31 to 11.16 and 5.15 to 9.08 μg/g; 7.42 to 12.79 and 5.82 to 11.03 μg/g; and 5.56 to 9.98 and 4.95 to 8.66 μg/g during low and high-flow seasons, respectively.

Iron (Fe): The sites differed for the mean Fe concentrations where the site C had the highest and upstream site A the lowest Fe accumulation. Also, Fe concentration was significantly greater during the low than high-flow season. The fish species also differed for the mean Fe concentration which was highest in *C. catla* followed by *C. mrigala* and *L. rohita*. The Fe bioaccumulation pattern in the fish organs was in the order of: kidneys > intestine > heart > eyes > gills (Table 5). The highest Fe concentration of 97.43 ± 5.060 μg/g was found in kidneys of *C. mrigala* that were caught from site C during the low-flow season whereas the gills of *C. mrigala* from site A showed the lowest Fe concentration of 24.35 ± 1.362 μg/g during the high-flow season (Table 1).

Lead (Pb): Similar to the trends for previous metals, the Pb contents differed between sites with highest Pb at site C followed by the sites D, B and A. The flow seasons also differed for the Pb content which was higher during the low than high-flow season. The metal bioaccumulation pattern in fish organs was in the order of kidneys > intestine > heart > eyes > gills. The fish species also differed for the Pb contents which were highest in *C. catla* and lowest in *C. mrigala* (Table 5). Higher Pb accumulation in the organs of *C. mrigala* was measured at site C where the Pb ranged from 4.08 ± 0.066 to 6.28 ± 0.401 μg/g and from 3.59 ± 0.189 to 5.94 ± 0.313 μg/g during the low and high-flow season, respectively (Table 3). In contrast, the lowest Pb accumulation ranging from

0.26 ± 0.022 to 0.47 ± 0.053 µg/g and from 0.18 ± 0.037 to 0.37 ± 0.077 µg/g during the low and high-flow seasons, respectively, were recorded in *C. mrigala* from site A (Table 1). The Pb contents in the organs of *C. catla* ranged from 0.28 ± 0.064 to 0.50 ± 0.126 µg/g and 0.23 ± 0.046 to 0.47 ± 0.117 µg/g at site A the low and high-flow seasons, respectively. The corresponding Pb accumulation in the fish sampled from site C ranged from 4.36 ± 0.229 to 6.43 ± 0.310 µg/g and 3.83 ± 0.385 to 6.33 ± 0.636 µg/g during low and high flow season, respectively. The mean Pb accumulation in organs of *L. rohita* ranged from 0.30 ± 0.061 to 6.11 ± 0.351 µg/g and from 0.19 ± 0.040 to 5.04 ± 0.318 µg/g during the low and high-flow season, respectively.

Zinc (Zn): The order of mean Zn bioaccumulation for sites was C > D > B > A where mean Zn contents differed significantly (*P*<0.001) for low and high-flow seasons (Table 6). The fish species differed significantly for Zn which was highest in *C. mrigala* and lowest in *C. catla*. The Zn accumulation pattern in fish organs was in the order of: kidneys > heart > intestine > eyes > gills (Table 5). Highest Zn concentration of 104.86 ± 10.287 µg/g was found in the intestines of *C. mrigala* than the intestines of *C. catla* (83.80 ± 2.052 µg/g) and *L. rohita* (80.03 ± 1.858 µg/g) during low flow season at site C (Table 3).

Manganese (Mn): Mean highest maganese accumulation was measured at site C. Then the metal content appeared in descending order at the sites D, B and A. The mean metal contents of the fishes' organs representing low flow to high-flow season differed significantly (*P*<0.001) from each other (Table 6). Among the three fish species, the *C. mrigala* had highest maganese accumulation. While *L. rohita* and *C. catla* showed the metal levels in descending order. The metal bioaccumulation pattern in fish organs was in order of: kidneys > intestine > heart > eyes > gills. The gills of *C. catla* showed lowest Mn accumulation (1.75 ± 0.104 µg/g) at site A during high flow season (Table 1).

Nickel (Ni): Among sampling sites, highest mean nickel bioaccumulation occurred at site C (3.43 µg/g). While up to 1.29, 0.82 and 0.56 µg/g of fishes' organ/tissues of Ni appeared for the sites D, B and A, respectively. Higher metal accumulation was recorded during low than high flow season, respectively (Table 5). Among the fish species, *C. mrgiala* showed highest bioaccumulation of Ni. While *C. catla* showed lowest concentration of the metal (Table 5). The Ni bioaccumulation pattern in the fishes' organs was in descending order: kidneys > intestine > heart > eyes > gills.

Mercury (Hg): Mean mercury (Hg) bioaccumulation measured highest at site C than D, B and A during low as well as high-flow seasons. Hg bioaccumulation pattern in the fishes' organs was the same observed by Ni. Highest mercury accumulation was recorded in *C. mrigala* than *C. catla* and *L. rohita* (Table 5). Mean Hg bioaccumulation in different organs of *C. mrigala* caught from different sites ranged from 0.13 ± 0.039 to 5.70 ± 0.216 µg/g and from 0.10 ± 0.015 to 4.14 ± 0.163 µg/g during low and high flow seasons, respectively. Hg accumulation in organs of *L. rohita* corresponding to low and high flow seasons ranged from 0.10 ± 0.005 to 5.39 ± 0.143 µg/g and from 0.11 ± 0.005 to 4.06 ± 0.143 µg/g. Whereas Hg bioaccumulation in organs of *C. catla* were recorded higher during low flow and ranged from 0.11 ± 0.007 to 5.55 ± 0.168 µg/g than high flows seasons when metal concentrations ranged from 0.10 ± 0.005 to 4.18 ± 0.087 µg/g.

Discussion

In the present study, metals' concentrations in fishes' organs varied significantly (*P*<0.001) among the selected sampling sites and flow seasons of the river Ravi. It is worth mentioning that means of total length and total wet body weight of same sampled specimen of each species (*C. mrigala*, *L. rohita*, *C. catla*) did not differ significantly (*P*>0.05) among sampling sites and flow seasons as already reported by Shakir and Qazi (2013). Site specific metals' accumulations in the fishes' organs sampled from the river Ravi have been reported by several workers (Javed, 2003; Nawaz et al., 2010; Jabeen et al., 2012; Shakir et al., 2013). Metals' bioaccumulation in tissues are related to change in feeding behaviour and physiological activities of fish species during different seasons (Farkas et al., 2000; Tekin-Ozan and Kir, 2007). Seasonal variation in metals' accumulation may be influenced by stream conditions, toxicants load, water chemistry and other environmental factors which affect the availability of different metals differently (Heiny and Tate, 1997).

Physico-chemical and ecological factors do influence the intensity of heavy metals uptake in animals. Avenant-Oldewage and Marx (2000) reported that physico-chemical parameters such as temperature, pH and total dissolved solids influence the availability of heavy metals. For the present studied sites, Shakir et al. (2013) reported along-stream increasing trend of ambient temperature with negative correction of dissolved oxygen and higher values of total dissolved solids, especially during low flow season of the river Ravi. Increasing temperature at downstream sampling sites leads to increase in metabolic rate. Thus, increased diffusion or active transport associated with higher rates of water movement across the gills, might have led greater amounts of metals uptake by the fish (Prosi, 1979).

The results showed that metals' accumulation in different organs of the fishes increased progressively at the downstream locations. Pattern of metals' bioaccumulation in fishes' organs with respect to sampling sites provided evidences of exposure to contaminated aquatic environment. It was documented that fish can absorb and bioaccumulate available metals directly from their surrounding environment via skin and gills or through the ingestion of contaminated water and food (Holliset al., 1999; Kotze et al., 1999; Qadir and Malik, 2011). Elevated level of metals in different fish tissues mainly originates from abiotic and biotic components of aquatic resources polluted by municipal sewage and industrial effluents (Mansour and Sidky, 2002; Van Aadt and Erdmann, 2004; Altindag and Yigit, 2005; Javed, 2006). Therefore, metals' bioaccumulation in different fish species of different trophic levels can be considered as an index of metal pollution in the aquatic bodies (Tawari-Fufeyin and Ekaye, 2007; Karadede-Akin and Unlu, 2007).

In the present study, Cd, Cr, Cu Fe, Pb, Zn, Mn, Ni and Hg bioaccumulations in different organs appeared significantly ($P<0.001$) different among selected fish species. Highest level of Zn and Fe, while lowest of Cd were recorded in the fishes' organs. The present study results are in line with Jabeen et al. (2012) that reported higher Zn content than As, Ba, Cr, Ni in different tissues/organs (gills, liver, kidneys, intestine, reproductive organs, skin, muscle, fins, scales, bones and fats) of *C. catla*, *C. mrigala* and, *L. rohita* caught from different sampling sites (Shahdara bridge, Balloki head works and Sidhnai barrage) of river Ravi, Pakistan. Higher Fe has been reported in various organs of fishes sampled from river Chenab, Pakistan as compared to Pb, Cd, Cr, Ni, Cu and Zn (Qadir and Malik, 2011).

Trace amount of Cd can cause anomalies such as reduction in development and growth rates (Hollis et al., 1999). During the present study, lowest concentration of Cd was detected in all organs as compared to the other metals. Such variations have been correlated by various workers with difference in uptake, absorption, storage, regulation, animals' age, geographical location, season and excretion abilities of given fish species (Al-Yousuf et al., 2000; Scerbo et al.,2005; Solhaug Jenssen et al., 2010). The metals' contents in different organs of the investigated fish species appeared several folds higher than their corresponding values in the waters (Shakir et al., 2013) as well as the level of water quality guidelines and proposed standards (NEQS,2000; WHO,2004; WWF, 2007; NSDWQ, 2008; USEPA, 2009). There is fairly high amount of Hg in different organs of fish species particularly sampled from site C. The authors' best knowledge, no specific source of mercury contamination in river Ravi reported. However, this might be related to untreated industrial effluents discharged through Hudiara and Deg-Nullah into river Ravi before this site. Hudiara drain received effluents from around 100 industries before leaving from India and then loaded with 112 industrial effluents from Pakistan side before joining with river Ravi. The Deg-Nullah also carries effluents of more than 149 industrial units (Saeed and Bahzad, 2006).

The metals' accumulation also significantly varied among different organs of the same fish. Highest metals' accumulation in kidneys than the other organs of sampled fish species might be associated to the fact that kidneys play a vital role in excretion. The higher Zn and other metal accumulation in studied fishes' kidneys might indicate maximum deloading capability of the sampled fishes under the prevailing condition. Murugan et al. (2008) reported that fish have a tendency to push zinc burden from muscles to other tissues like kidney, during metallic stress and this deloading is beneficial to consumers who use fish muscle for food. Varying levels of the metals' bioaccumulations in different organs of the fishes are attributed to differences in their physiological functions (Karuppasamy, 2004). Metals uptake from blood at tissue level is a biphasic process, which involves rapid adsorption or binding to the surface, followed by a slower transport into cell interior. Transport of different metals ions in to intracellular compartment may be facilitated by either diffusion of the metals ions across the cell membrane or by active transport of metals ions through binding with different specific carrier proteins. Presence of different metal binding proteins is an indication of toxic metal pollution in an aquatic environment (Hennig, 1986; Crist et al., 1988). Fish regulate metal ions through excretion via kidney and gills, however, such capacity of a tissue is directly related to the total amount of metal's accumulation in that specific tissue. Fish's ability to synthesize metal binding proteins is limited. When metabolic capabilities for excretion and binding the pollutants are exceeded from threshold limit, toxic effects results, unless the fish has an alternate way of detoxification (Kojima and Kagi, 1978; Cosson, 1994).

The present study highlights the metal accumulation greater in fish species dwelling downstream sites, indicating impairment of ambient water due to continuous discharges of untreated industrial and municipal effluents into the studied segment of the river. The studied organs of fish species sampled from site C (industrial area) showed highest bioaccumulation levels which are directly associated with the pollution level of this site due to discharge of untreated industrial effluents and urban sewage in comparison with non-industrial upstream site A. The water and sediment samples also showed highest concentration of metals for this site (Shakir et al., 2013). Measured concentrations of the metals in fish organs indicated potential health risks for the fish and the food chain. The accumulation intensity of studied metals in different organs of the economically important fishes as a consequence of municipal sewage and industrial effluents. These discharges are not only threatening the ecological integrity of aquatic resources but also putting the health of local population at risk. Therefore, contaminations due to heavy metals should be considered a priority concern and needs to be addressed urgently.

Acknowledgement

First author acknowledged to Higher Education Commission (HEC), Pakistan for funding under scholarship scheme "Indigenous Ph.D. 5000 Fellowship program" (Pin No. 074-1921-BM4-146) and "IRSIP" to support this research at University of the Punjab, Pakistan and Newcastle University, UK. Many thanks of Dr. Diky Ramdani from Newcastle University UK for his help in the statistical analysis.

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