Journal of Animal & Plant Sciences, 33(1): 2023, Page: 201-210
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2023.1.0610

ASSESSMENT OF HEAVY METALS CONTENT IN ORGANS OF EDIBLE FISH SPECIES OF RIVER RAVI IN PAKISTAN

J. Shafi*, Z. S. Mirza, N. Kosour and M. Zafarullah

Fisheries Research and Training Institute, Manawan, Lahore, Pakistan
*Corresponding author's Email: javairiamalik@gmail.com

ABSTRACT

Present study was conducted to investigate the content of toxic heavy metals; lead, cadmium, manganese, nickel and zinc in organs of edible fish of River Ravi. Samples of *Labeo rohita*, *Cyprinus carpio*, *Gibelion catla* and *Cirrhinus mrigala* were captured from two sites at River Ravi i.e. Kala Khatai and Head Balloki. Zinc and lead content was higher in organs of fish specimens collected from Head Balloki than those collected from Village Kala Khatai. Higher average cadmium content was detected in most of the organs of fish collected from Head Balloki than those of Village Kala Khatai. However, higher average manganese content was found in examined organs of fish captured from Village Kala Khatai than those of Head Balloki. Lead and cadmium content in muscles of fish collected from Head Balloki was higher than acceptable level proposed by European Commission (0.2 µg/g and 0.05 µg/g, respectively). Hepatosomatic index of fish species (except *L. rohita*) collected from Head Balloki was lower than those of Village Kala Khatai. Study has led to the conclusion that edible fish of River Ravi is contaminated with toxic metals and contamination level is higher in fish at Head Balloki as compared to that observed at Village Kala Khatai.

Keywords: Bioaccumulation, health hazards, pollution load, River Ravi

Published first online September 20, 2022 Published final February 22, 2023

INTRODUCTION

Fish and fish products have been considered as an important source of high quality animal proteins in healthy human diet that not only supply all the essential amino acids required but is also highly digestible (Ruxton, 2011; Perschbacher and Stickney, 2016). In developing countries where most of the population has to cope with undernutrition, fish provides an invaluable source of high quality proteins, vitamins, minerals and omega-3 fatty acids (Medeiros *et al.*, 2012). However, the dietary benefits of fish can be offset by presence of toxicants in its edible parts. As fish are at the highest trophic level in aquatic ecosystems, they easily bioconcenterate toxic materials from water, food and bottom sediments.

Polluting effects of heavy metals on aquatic ecosystems and aquatic life have long been known (Avigliano *et al.*, 2015). In aquatic environments, heavy metal pollution results from geological erosion, dry/ wet deposition from atmosphere and discharge of urban, industrial and agricultural waste products in natural water bodies. Increasing industrialization and metamorphic activities have led to increasing discharge of heavy metals in water reservoirs of Pakistan (Waseem *et al.*, 2014; Shafi *et al.*, 2018). Pollution of our aquatic ecosystems due to heavy metals not only causes deterioration of water quality for sustainable fisheries but also endangers human health due to deposition of toxic metals in edible fish (Jomova and Valko, 2011; Morais *et al.*, 2012).

River Ravi, one of six major Rivers of Indus System, receives untreated water discharge of several waste water carrying drains during its course of flow through Lahore. Village Kala Khtai near Narang Mandi Town is located about 15 km upstream of Ravi Siphon and River at this site is considered as relatively unpolluted due to absence of any significant source of pollution. During its course of flow through Lahore, the River receives polluted industrial and sewage water through six primary drains and two waste water carrying tributaries namely Hudiara Drain and Deg drain. The result of disposal of such huge pollution load is that the river appears like a waste water carrying drain at Head Balloki especially during low flow season. River water at Head Balloki is, therefore, considered as highly polluted (Shakir *et al.*, 2013; Shafi *et al.*, 2018). Although earlier studies have assessed the metal contamination in fish species of River Ravi, most of them were carried out in previous decade. Regular monitoring of our natural water bodies and their fish biota is vital to determine the pollution status of our natural reservoirs. However, there is a lack of up-to-date data on toxic metals contents in fish of River Ravi. The aim of this research work was, therefore, to assess the toxic heavy metals content in organs and tissues of fish species collected from River Ravi at Village Kala Khatai in Narang Mandi and Head Balloki.

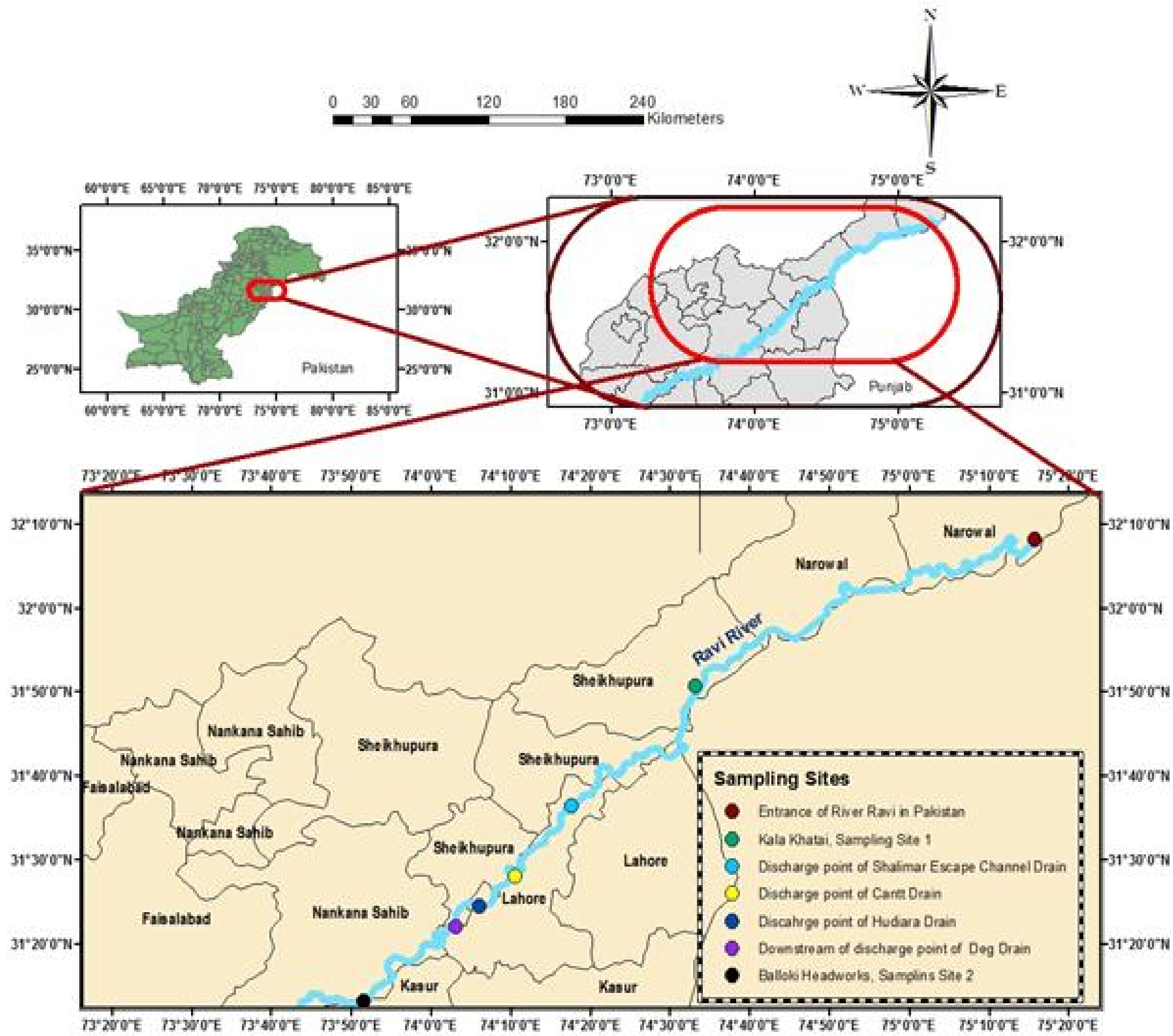

Figure 1: Map of River Ravi in Pakistan showing two sampling sites and discharge points of major waste water drains in River Ravi.

MATERIALS AND METHODS

Description of the Study Area: Fish samples were collected from River Ravi at Village Kala Khatai (32°08'11.6000N, 75°15'47.7000"E; upstream of discharge points of waste water carrying primary drains and tributaries) located in Narang Mandi Town and Head Balloki (31°13'09.9000"N, 73°51'29.7600"; downstream of discharge points of waste water carrying primary drains and tributaries). A map of sampling sites and discharge points of waste water carrying drains in River Ravi is presented in Figure 1.

Fish Species: Fish specimens of *Labeo rohita*, *Catla catla*, *Cyprinus carpio* and *Cirrhinus mrigala* were captured from two sampling sites at River Ravi with the aid of drag and seine net ~~(Table 1)~~ in February 2016. Collected fish specimen were slaughtered on ice, washed, stored in ice boxes and immediately transported to laboratory. Morphometric features of fish samples (weight and total length) were recorded and specimens were kept frozen at -20 °C till further analysis.

Chemicals & Equipment: All the chemicals used for sample digestion and analysis were of analytical reagent grade. All dilutions were made with ultrapure deionized water prepared using Siemens's water purification system (2001-D/60 Ultraclear, Seimens, Germany). Nitric acid (HNO_3, > 65%) and hydrogen peroxide were supplied by Sigma Aldrich, Steinheim, Germany. Multielement mixed standard solutions of heavy metals were purchased from Perkin-Elmer, Norwalk, CT, USA. Perkin Elmer's ICP-OES (Optima 7000 DV, Perkin Elmer, Inc., Norwalk, CT, USA) was used for analysis of heavy metals in fish organs. Operating conditions of ICP-OES used in the present study have been described in Table 1.

Table 1: Operating conditions of ICP-OES for analysis of heavy metals.

Detection Wavelength	Cd	228.802 nm
	Pb	405.781 nm
	Mn	257.610 nm
	Ni	231.604 nm
	Zn	206.200 nm
Purge gas	Nitrogen, 99.999% pure	
Auxiliary gas flow	0.2 L/min	
Nebulizer gas flow	0.8 L/min	
Peristaltic pump flow rate	2.5 ml/ min	
RF power	1300 watts	
Replicates	2	
Delay time	60 sec	
Spray chamber	Ryton Double Pass Scott type	
Nebulizer	Gem Tip Cross Flow II	
Injector	Alumina, 2.0 mm ID	
Sample tubing	0.76 mm ID	
Drain tubing	1.14 mm ID	

Sample Preparation: All the glassware used for the preparation and storage of fish organ-based samples was rinsed with acid to prevent metal contamination. Concentration of heavy metals; cadmium (Cd), lead (Pb), nickel (Ni), manganese (Mn) and zinc (Zn) was determined in liver, kidney, gills, muscles and skin of fish samples. Frozen fish specimens were thawed and dissected using stainless steel tools. Liver and kidney were weighed (wet weight) as whole organs while about 10 g of muscles and skin each and 5 g of gills was accurately weighed. Muscles, skin, gills, liver and kidney were digested according to the method described by FAO/SIDA, 1983. Prior to digestion, samples were dried to constant weight at 105 °C to determine the dried weight of organs. Dried organs were grinded with the aid of a pestle and mortar and transferred quantitatively to 100 mL glass beakers. Freshly prepared mixture of nitric acid: hydrogen peroxide (1:1, 10 mL) was added to each beaker. Beakers covered with watch glass were left undisturbed for one hour followed by gentle boiling to reduce volume to about 3 ml. Digested samples were allowed to cool, diluted to 25 mL with deionized water and filtered through 0.45 µm membrane filters.

Sample Analysis: Digested samples of fish organs were analysed for Ni, Mn, Zn, Cd and Pb by ICP-OES (Perkin Elmer; Optima 7000 DV). For quantification, multi element mixed standard solutions were used for the preparation of working standard solutions of metal of interest. Metal concentrations are expressed as µg/g wet weight and dry weight of fish organs.

Statistical Analysis of Data: One-way analysis of variance was used to identify significant differences in metals content in different organs of a fish species collected from each site. Fisher's Least Significant Difference (LSD) test was used to find the differences between pairs of means. Pearson's correlation analysis was carried out to find any significant correlation between metals bioaccumulated in fish liver. All statistical analysis was carried out thorough the use of Minitab-17 software.

RESULTS

Data on moisture content of fish organs, condition factor and hepatosomatic index of fish samples has been presented in Table 2. Results of average metal contents in organs of different fish samples collected from River Ravi at Village Kala Khatai and Head Balloki are presented in Table 3 and Table 4 respectively along with statistical analysis. Significant differences for Mn and Zn content in various organs of a fish species were found. Earlier investigations on assessment of heavy metals content in fish organs have either reported the results on wet weight or dry weight basis. In present study, results based on both wet and dry weight of fish organ have been presented for comparison with prior studies.

Moisture Content: Moisture content of liver, kidney, gills and muscles was found to be in the range of 79%-81% in most of the fish samples. However, in the case of fish skin, moisture content ranged from 72%-74%.

Condition Factor (CF): Condition factor (CF) ranged from 1.17 ± 0.11 to 1.36 ± 0.16 in fish species collected from Village Kala Khatai and from 1.26 ± 0.14 to 1.60 in fish samples at Head Balloki. Highest condition factor (1.69 ± 0.13) was found for mrigal collected from Head Balloki.

Hepatosomatic Index (HSI): For rohu, mrigal and catla collected from Village Kala Khatai, HSI was calculated to be 0.12 ± 0.04, 0.29 ± 0.16 and 0.41 ± 0.24 respectively. For rohu, catla, mrigal and common carp collected from Head Balloki, HSI was found to be 0.20, 0.10, 0.18 ± 0.06 and 0.26 ± 0.05 respectively.

Cadmium (Cd): Highest cadmium contents were found in liver of catla collected from Village Kala Khatai and Head Balloki (0.29 µg/g and 0.33 µg/g respectively). Average Cd content in organs of fish at Head Balloki was higher than that of fish at Village Kala Khatai.

Lead (Pb): Lead was detected in liver, kidney and muscles (0.547 µg/g, 0.612 µg/g, 0.402 µg/g respectively) of only one sample of catla collected from Village Kala Khtai. Liver, kidney, muscles and skin of most of the samples collected from Head Balloki showed accumulated Pb contents. This toxic metal was not found in gills of any fish species. For liver, kidney, muscles and skin, collective Pb content was 659%, 668%, 115% and

Table 2. Morphometric features, condition factor, HI index and organ's moisture content (Mean ± SD) of fish samples collected from two locations at River Ravi

Sampling Site	Fish species	Weight (g)	Length (cm)	Moisture content (%)					Condition factor	HI index
				Liver	Kidney	Gills	Muscles	Skin		
Village Kala Khatai	*L. rohita*	820-997	40-44	80.56 ± 1.89	82.02 ± 2.29	79.36 ± 0.45	80.24 ± 0.95	73.15 ± 0.87	1.17 ± 0.11	0.12 ± 0.04
	C. mrigala	507-690	36-39	80.75 ± 0.61	81.33 ± 0.52	79.81 ± 1.59	80.18 ± 0.65	72.10 ± 0.44	1.20 ± 0.14	0.29 ± 0.16
	G. catla	23-52	158-2186	80.26 ± 1.62	80.34 ± 1.55	80.06 ± 0.17	79.23 ± 1.37	73.04 ± 0.64	1.36 ± 0.16	0.41 ± 0.24
Head Balloki	*L. rohita*	1273	43	79.03	80.82	81.18	79.94	73.38	1.60	0.200
	G. catla	1238	44	78.69	78.35	77.41	81.3	72.72	1.45	0.100
	C. mrigala	619-702	36-40	79.33 ± 1.55	80.54 ± 1.09	81.21 ± 1.37	81.48 ± 0.29	73.24 ± 0.95	1.69 ± 0.13	0.18 ± 0.06
	C. carpio	849-1158	38-40	79.45 ± 0.98	79.64 ± 1.07	81.00 ± 0.77	81.00 ± 0.65	73.31 ± 1.12	1.26 ± 0.14	0.26 ± 0.05

Table 3. Metal concentration (µg/g) in organs of fish species collected from Village Kala Khatai (Values are mean ± SD).

Fish Species	Organ	Cd		Pb		Ni		Mn		Zn	
		FW*	DW**	FW	DW	FW	DW	FW	DW	FW	DW
L. rohita	Liver	0.12 ± 0.02^{a}	0.6 ± 0.14^{a}	ND^{b}	ND^{b}	ND	ND	2.02 ± 1.05^{b}	10.21 ± 4.77^{b}	46.7 ± 13.80^{a}	246.67 ± 100.26^{a}
	Kidney	0.07 ± 0.02^{a,}	0.41± 0.14^{a}	ND^{b}	ND^{b}	ND	ND	0.61 ± 0.07^{b}	3.44 ± 0.6^{b}	21.79 ± 4.52$^{b, c}$	124.83 ± 43.34$^{b, c}$
	Gills	0.12 ± 0.10^{a}	0.56 ± 0.48^{a}	ND^{b}	ND^{b}	ND	ND	30.32 ± 4.5^{a}	146.90 ± 21.51^{a}	141.94 ± 29.28^{b}	688.51 ± 145.0^{b}
	Muscles	0.06 ± 0.00^{a}	0.26 ± 0.02^{a}	ND^{b}	ND^{b}	ND	ND	0.47 ± 0.22^{b}	1.60 ± 1.16^{b}	4.88 ± 0.44^{c}	22.19 ± 5.32^{c}
	Skin	0.06 ± 0.00^{a}	0.22 ± 0.02^{a}	ND^{b}	ND^{b}	ND	ND	0.25 ± 0.03^{b}	0.92 ± 0.13^{b}	18.0 ± 0.46^{c}	67.13 ± 3.8^{c}
C. mrigala	Liver	0.12 ± 0.11^{a}	0.61 ± 0.59^{a}	ND^{b}	ND^{b}	ND	ND	0.769 ± 0.67^{b}	3.92 ± 3.50^{b}	9.12 ± 7.92$^{b, c}$	46.58 ± 40.45$^{b, c}$
	Kidney	0.17 ± 0.11^{a}	0.90 ± 0.56^{a}	ND^{b}	ND^{b}	ND	ND	0.959 ± 0.13^{b}	5.13 ± 0.6^{b}	12.99 ± 1.2^{b}	69.56 ± 5.24^{b}
	Gills	0.17 ± 0.01^{a}	0.86 ± 0.03^{a}	ND^{b}	ND^{b}	ND	ND	15.48 ± 1.09^{a}	76.76 ± 3.7^{a}	21.92 ± 2.05^{a}	109.16 ± 14.6^{a}
	Muscles	0.06 ± 0.00^{a}	0.28 ± 0.03^{a}	ND^{b}	ND^{b}	ND	ND	0.42 ± 0.13^{b}	1.54 ± 0.19^{b}	3.71 ± 0.44^{c}	18.73 ± 2.4^{c}
	Skin	0.08 ± 0.02^{a}	0.28 ± 0.08^{a}	ND^{b}	ND^{b}	ND	ND	0.42 ± 0.18^{b}	1.53 ± 0.68^{b}	8.35 ± 2.94$^{b, c}$	30.02 ± 10.88$^{b, c}$
G. catla	Liver	0.29 ± 0.40^{a}	1.57 ± 2.2^{a}	0.18 ± 0.32^{a}	0.85 ± 1.5^{a}	ND	ND	4.97 ± 5.48^{b}	26.33 ± 30.33^{b}	91.93 ± 49.25$^{a, b}$	531.57 ± 113.03$^{a, b}$
	Kidney	0.21 ± 0.18^{a}	1.11 ± 0.97^{a}	0.204 ± 0.35^{a}	0.95 ± 1.65^{a}	ND	ND	4.49 ± 5.48^{b}	23.60 ± 28.94^{b}	70.04 ± 41.99$^{a, b}$	227.67 ± 97.87$^{a, b}$
	Gills	0.15 ± 0.01^{a}	0.75 ± 0.08^{a}	ND^{a}	ND^{a}	ND	ND	21.20 ± 4.98^{a}	106.25 ± 24.54^{a}	118.3 ± 96.28^{a}	768.2 ± 211.62^{a}
	Muscles	0.06 ± 0.00^{a}	0.22 ± 0.00^{a}	0.37 ± 0.34^{a}	1.74 ± 1.68^{a}	ND	ND	0.44 ± 0.12^{b}	1.86 ± 1.05^{b}	6.45 ± 2.45^{b}	39.94 ± 3.63^{b}
	Skin	0.06 ± 0.00^{a}	0.21± 0.02^{a}	ND^{a}	ND^{a}	ND	ND	0.67 ± 0.39^{b}	2.77 ± 1.03^{b}	12.57 ± 3.10^{b}	48.52 ± 7.89^{b}

*: Fresh weight; **: Dry weight; ND: Not detected; For each fish species, means with similar superscripts in one column are statistically similar at $P>0.05$, When analyte was not detected (ND), its content was assumed to be 0.00 µg/g for post hoc analysis.

Table 4. Metal concentration (µg/g) in organs of fish species collected from Head Balloki (Values are mean ± SD***)

Fish Species	Organ	Cd		Pb		Ni		Mn		Zn	
		FW*	DW**	FW	DW	FW	DW	FW	DW	FW	DW
L. rohita	Liver	0.037	0.175	0.576	2.747	ND	ND	0.10	0.46	13.00	62.01
	Kidney	0.094	0.491	0.822	4.288	ND	ND	0.26	1.37	23.09	120.42
	Gills	0.218	1.160	ND	ND	ND	ND	18.81	99.96	79.81	424.18
	Muscles	0.067	0.312	0.166	0.767	ND	ND	0.35	1.73	10.58	36.22
	Skin	0.065	0.246	0.215	0.809	ND	ND	0.07	0.28	26.42	99.26
G. catla	Liver	0.328	1.538	ND	ND	ND	ND	10.36	48.64	197.12	925.11
	Kidney	0.162	0.750	ND	ND	ND	ND	1.74	8.06	75.89	350.58
	Gills	0.161	0.711	ND	ND	ND	ND	15.26	67.55	200.84	888.94
	Muscles	0.051	0.191	ND	ND	ND	ND	0.45	1.86	5.90	31.54
	Skin	0.058	0.212	ND	ND	ND	ND	0.54	1.98	14.03	51.44
C. mrigala	Liver	0.10 ± 0.02$^{b, c}$	0.47 ± 0.13$^{b, c}$	1.48 ± 0.15^{a}	7.16 ± 0.85^{a}	ND	ND	0.91 ± 0.22^{b}	4.44 ± 1.21^{b}	31.02 ± 7.56^{a}	149.63 ±33.36^{a}
	Kidney	0.12 ± 0.03^{b}	0.64 ± 0.13^{b}	1.32 ± 0.23^{a}	6.86 ± 1.52^{a}	ND	ND	0.99 ± 0.09^{b}	5.06 ± 0.21^{b}	17.37 ± 0.38^{b}	89.54 ± 6.73^{b}
	Gills	0.18 ± 0.02^{a}	0.96 ± 0.2^{a}	ND^{c}	ND^{c}	ND	ND	11.39 ± 1.99^{a}	61.26 ±14.99^{a}	20.73 ± 1.46^{b}	110.99 ± 14.92^{b}
	Muscles	0.08 ± 0.03^{c}	0.35 ± 0.06^{c}	0.44 ± 0.03^{b}	1.78 ± 0.68^{b}	ND	ND	0.18 ± 0.03^{b}	0.97 ± 0.15^{b}	4.10 ± 0.37^{b}	22.16 ± 2.35^{b}
	Skin	0.08 ± 0.00^{c}	0.32 ± 0.05^{c}	0.013 ± 0.015^{c}	0.032 ± 0.06^{c}	ND	ND	0.16 ± 0.05^{b}	0.62 ± 0.19^{b}	21.65 ± 6.41^{c}	80.77 ± 22.88^{c}
C. carpio	Liver	0.11 ± 0.04$^{b, c}$	0.54 ± 0.22$^{b, c}$	0.71 ± 0.63^{a}	3.47 ± 3.12^{a}	ND	ND	0.62 ±0.62^{b}	3.05 ± 3.07^{b}	56.28 ± 23.34^{b}	270.95 ± 101.62^{b}
	Kidney	0.25 ± 0.09^{a}	1.22 ± 0.49^{a}	0.83 ± 0.14^{a}	4.12 ± 0.89^{a}	ND	ND	0.50 ± 0.22^{b}	2.45 ± 1.13^{b}	88.98 ± 51.40^{b}	441.23 ± 269.99^{b}
	Gills	0.13 ± 0.01^{b}	0.68 ± 0.04^{b}	ND^{b}	ND^{b}	ND	ND	6.53 ± 1.03^{a}	34.32 ± 4.20^{a}	282.31±205.03^{a}	1467.69± 1058.92^{a}
	Muscles	0.06 ± 0.00^{b}	0.30 ± 0.04^{b}	0.41 ± 0.13$^{a, b}$	2.18 ± 0.72$^{a, b}$	ND	ND	0.11 ± 0.03^{b}	0.47 ± 0.07^{b}	9.68 ± 5.20^{b}	58.78 ± 7.19^{b}
	Skin	0.07 ± 0.12^{b}	0.29 ± 0.04^{b}	0.17 ± 0.05^{b}	0.65 ± 0.21$^{a, b}$	ND	ND	0.091 ± 0.04^{b}	0.34 ± 0.14^{b}	23.9 ± 8.35^{b}	75.50 ± 49.30^{b}

*: Fresh weight; **: Dry weight; ***: Where SD is not mentioned, it couldn't be calculated due to availability of a single fish sample;
ND: Not detected; For each fish species, means with similar superscripts in one column are statistically similar at $P>0.05$; When analyte was not detected (ND), its content was assumed to be 0.00 µg/g for post hoc analysis.

Table 5: Maximum permissible content of heavy metals in fish muscles (µg/g, wet weight).

Standard	Metal					Reference
	Cd	Pb	Ni	Mn	Zn	
WHO (1989)	1.0	2.0	---	1.0	100.0	(Mokhtar *et al.*, 2009)
EC	0.05	0.2	---	---	---	(Commission, 2005)
UK	0.2	2.0	---	---	50.0	(Jones, 1997)
USFDA	---	---	70.0-80.0	---	---	(USFDA, 1993)

76% higher in fish of Head Balloki than those of Village Kala Khatai. Highest Pb content was 1.48 µg/g detected in liver of mrigal collected from Head Balloki and lowest content (0.18 µg/g) was found in liver of catla captured from Village Kala Khatai.

Nickel (Ni): Nickel was not found in any fish species collected from Village Kala Khatai or Head Balloki.

Manganese (Mn): Manganese content was significantly higher in gills of all fish samples when compared with other organs *(P*<0.05*)*. As compared to Pb and Cd, average Mn content was higher in kidney, gills, muscles and skin of fish samples captured from Village Kala Khatai than those of Head Balloki. Highest Mn content was found in gills of rohu at both sampling sites (30.32 µg/g and 18.81 µg/g respectively).

Zinc (Zn): Zinc content was significantly higher in gills of all fish species as compared to other organs (*P<0.05)*. Highest Zn content was found in gills of rohu (146.90 µg/g) captured from Village Kala Khatai and common carp (282.31 µg/g) collected from Head Balloki.

DISCUSSION

Due to its tendency to accumulate toxic materials, fish has been considered as a sensitive marker of river health and potential health hazards that are posed to human health through consumption of contaminated fish (Authman *et al.*, 2015). It has been reported that aquatic biota can accumulate the toxic metals up to 1,000,000 times of their concentration present in water column (USEPA, 1992). Consumption of contaminated fish can pose considerable threat to human health due to toxicity of these metals at elevated concentration (Zahra *et al.*, 2017).

In present study, accumulation pattern of metals in fish organs was found to vary with metal type and fish species. In general, kidney, liver and gills showed the higher concentration of heavy metals than muscles and skin. These findings are in accordance with those of Squadrone *et al.* (2013), El-Moselhy *et al.* (2014) and Mahboob *et al.* (2016) who also reported higher metal bioaccumulation in fish liver, kidney and gills. These fish organs show higher metal content because gills remain in direct contact with surrounding water and can easily absorb toxic metals from polluted water while kidney & liver are involved in excretion and detoxification of metals respectively. In present study, it has been found that average content of toxic heavy metals was higher in fish species collected from River Ravi at Head Balloki (polluted site of the River) than those collected at Village Kala Khatai (relatively unpolluted site of the River). Earlier investigations have also reported higher metal bioaccumulation in fish collected from River Ravi at Balloki Headworks than those collected from less polluted sites of the River (Rauf *et al.*, 2009; A. Shakir *et al.*, 2013).

Table 6: Correlation matrix for heavy metals in fish organs.

Heavy metal	Correlation	Heavy metal Cd	Pb	Mn
Liver				
Pb	*r*	-.316		
	Sig.	.217		
Mn	*r*	0.850	-.354	
	Sig.	0.000	.164	
Zn	*r*	0.424	-.166	.741
	Sig.	0.090	.523	.001
Kidney				
Pb	*r*	-.072		
	Sig.	.783		
Mn	*r*	.608	-.266	
	Sig.	.010	.302	
Zn	*r*	.421	.005	.203
	Sig.	.092	.984	.433
Gills				
Pb	*r*	---*	---*	
	Sig.	---	---	
Mn	*r*	-.055	---	
	Sig.	.835	---	
Zn	*r*	-.320	---	-.175
	Sig.	.211	---	.501
Muscles				
Pb	*r*	.306		
	Sig.	.232		
Mn	*r*	-.444	-.540	
	Sig.	.074	.025	
Zn	*r*	-.108	.317	-.260
	Sig.	.681	.215	.314
Skin				
Pb	*r*	.058		
	Sig.	.825		
Mn	*r*	-.104	-.508	
	Sig.	.690	.038	
Zn	*r*	.227	.478	-.493
	Sig.	.381	.052	.044

r: Pearson Correlation; Sig.: Significance (two tailed); * Pb was not found in gills of any fish species

Average metals contents in different organs of fish collected from two sites of River Ravi have been presented in Figure 2. Distribution of Cd, Pb, Mn and Zn in fish muscles collected from two sampling sites at River Ravi has been presented in Figure 3 along with the maximum permissible limit of these metals in fish edible portions. In general, fish liver shows higher content of Cd compared to muscle and skin. However, we found liver Cd content to be the lowest in one sample of rohu collected from Head Balloki. It could be the result of interactions of Cd with other toxicants that may not have been investigated in the present study. Catla showed higher tendency to accumulate Cd than rohu and mrigal. Content of Cd in muscles of fish collected from both sampling sties was found to be less than the maximum permissible limits suggested by WHO (1.0 µg/g) and UK (0.2 µg/g). However, average Cd content was higher than the limit (0.05 µg/g) proposed by European Commission in muscles of all the fish samples collected from two sampling sites (Table 5).

Average content of Pb in liver, kidney, muscles and skin of fish collected from Head Balloki was 83.19%, 84.40%, 38.39% and 9.47% higher respectively than that collected from Village Kala Khatai (Figure 2). Although earlier investigations (Yousafzai *et al.*, 2012; El-Moselhy *et al.*, 2014; Shovon *et al.*, 2017; Bawuro *et al.*, 2018) have reported accumulation of Pb in gills of different fish species including carps, this metal was not detected in gills of any fish species in present study. Gills are directly involved in exchange of metals from surrounding water and accumulation of a metal in fish gills is an indicator of its concentration in surrounding water (Shovon *et al.*, 2017). According to present study, the direct route of Pb intake via gills seems not to be significant in the case of fish species collected from River Ravi due to integration of prevailing biotic and abiotic factors. Lead level in muscles of all the collected fish samples was higher than the proposed limit of 0.2 µg/g. It is noteworthy that all the fish specimens captured from Head Balloki were contaminated with Pb while it was detected in only one sample of catla collected from Village Kala Khatai.

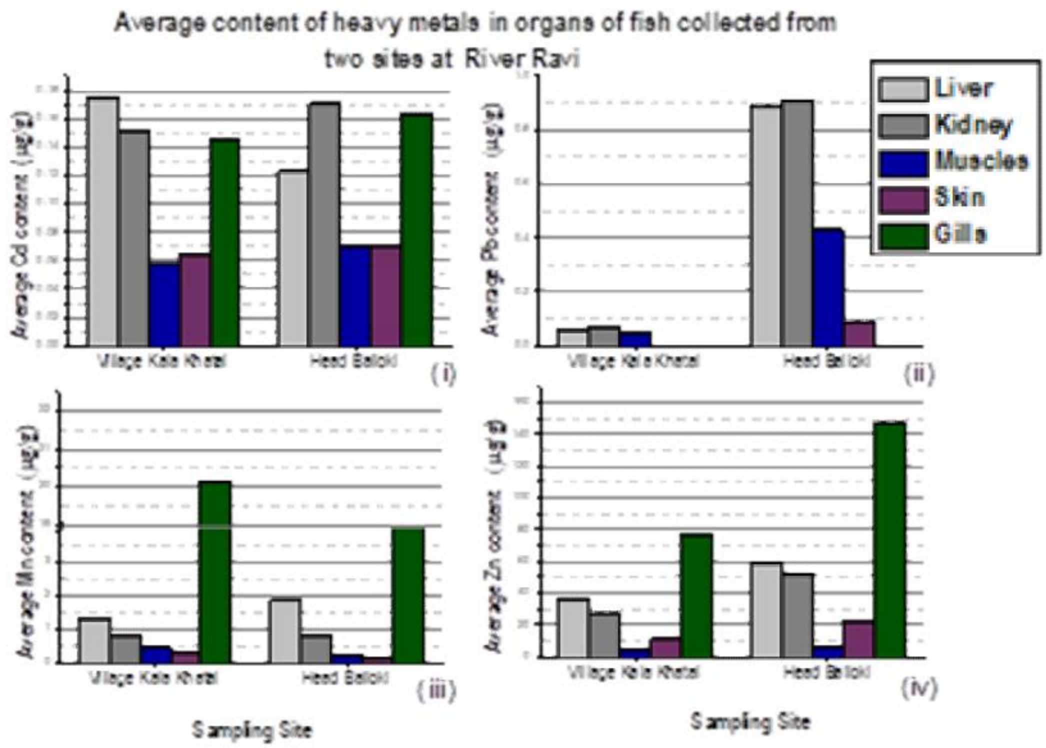

Figure 2: Average content of (i) Cd, (ii) Pb, (iii) Mn and (iv) Zn in different organs of fish collected from two sampling points at River Ravi.

Table 7: Comparison of heavy metals content (µg/g) in herbivorous fish species collected from Rivers in Pakistan.

Fish Species	Sampling Location	Heavy Metal (µg/g)					Reference
		Cd	Pb	Ni	Mn	Zn	
Labeo rohita	River Ravi, Head Balloki	0.067	0.166	ND	*0.35*	*10.58*	Present study
Cirrhinus mrigala		0.08	0.013	ND	0.16	21.65	
Gibelion catla		0.51	ND	ND	0.45	5.90	
Cyprinus carpio		0.06	0.41	ND	0.11	9.68	
Gibelion catla	River Ravi, Siphon	0.03	0.19	0.37	2.62	22.68	(Shakir *et al.*, 2013)
Gibelion catla	River Ravi, Head Balloki	0.04	2.05	0.79	5.23	37.35	
Labeo rohita	River Ravi, Head Balloki	---	---	0.74	---	51.84	(Tabinda *et al.*, 2013)

Cirrhinus mrigala		---	---	1.37	---	61.74	
Gibelion catla		---	---	0.38	---	34.02	
Labeo rohita	River Sutlej, Sulemanki	---	24.6	---	---	1.68	(Tabinda *et al.*, 2013)
Cirrhinus mrigala	Headworks	---	29.9	---	---	1.76	
Gibelion catla		---	24.9	---	---	1.60	
Cyprinus carpio	Sardaryab tributary, KPK	---	0.0	---	---	0.018	(Yousafzai *et al.*, 2017)
Labeo rohita		---	0.0	---	---	0.02	
Labeo rohita	River Indus	---	1.94	8.87		57.6	(Iqbal *et al.*, 2017)
Labeo rohita	River Chenab, Nullah Aik	0.89	4.35	---	---	---	
Cirrhinus reba	and Palkhu	2.09	1.04	---	---	---	(Qadir and Malik, 2011)
Cyprinus carpio	River Shah Alam,	---	ND	---	0.020	0.060	(Khan *et al.*, 2012)
Cirrhinus mrigala	Charsadda, KP	---	ND	---	0.049	0.082	

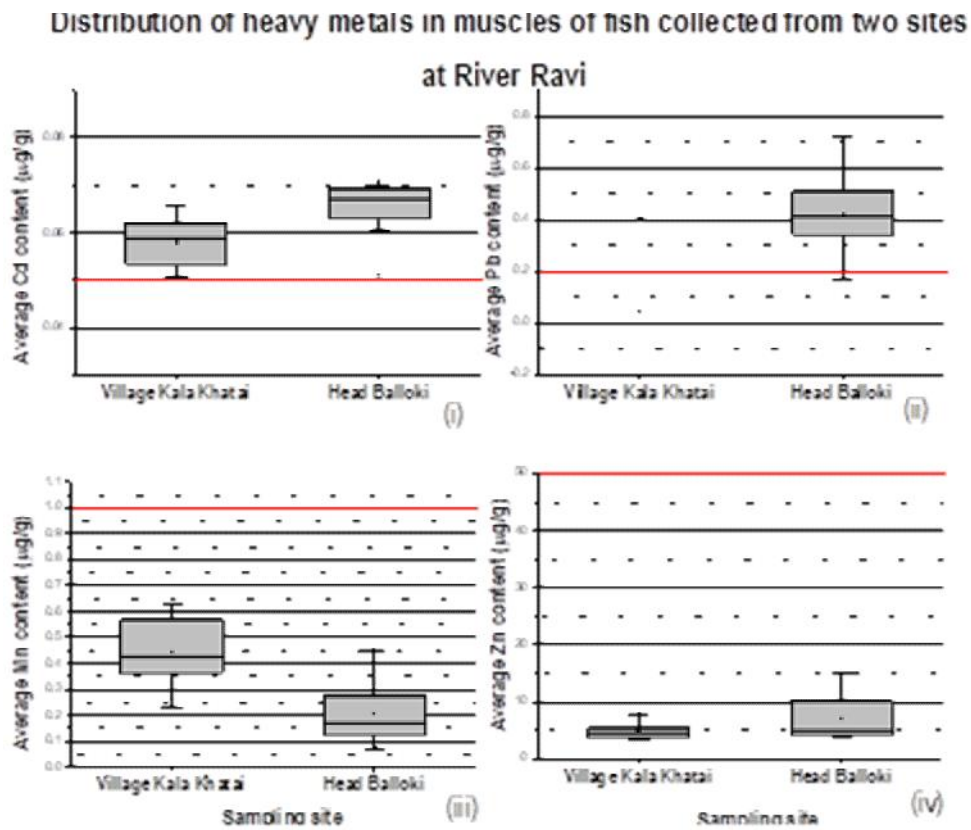

Figure 3: Distribution of (i) Cd (ii) Pb, (iii) Mn and (iv) Zn in fish muscles (Red line indicates maximum permissible limit proposed by EC for metals in fish edible parts)

Nickel was not found in any organ of any fish species. These results are in agreement with one of our earlier study (Shafi *et al.*, 2018) according to which Ni was not found in River Ravi water at most of the investigated sites. Lead was not found in most of the fish samples collected from Village Kala Khatai. Contents of Zn and Mn both of which are essential metals were found to be significantly higher in gills of all fish species than other organs. These findings are in accordance with those of El-Moselhy *et al.* (2014) and Rajkowska & Protasowicki (2013) who also reported higher accumulation of Zn and Mn in fish gills than kidney and liver. However, Jabeen *et al.* (2012) have reported higher Zn content in fish liver (84.77±26.23 µg/g) than kidney (78.95±22.4 µg/g) and gills (65.20±19.4 µg/g). In general, Zn and Mn content were higher in liver and kidney than muscles and skin for all fish species. Content of Mn and Zn was less than the maximum proposed limits of 1 µg/g and 50 µg/g respectively.

Bioaccumulation of metals in fish organs is affected by interaction with other heavy metals. A correlation matrix of average metals content in organs of collected fish samples is presented in Table 6. It is interesting to note that a strongly positive correlation was found for Mn with Cd and Zn: *r* = .850 (Sig. 0.00) and .741 (0.001) respectively in liver. A comparison of heavy metals content in muscles of fish of River Ravi as determined in present and prior studies has been presented in Table 7.

Condition factor has been used as an indicator of fish health and decreases in response to environmental or feeding stress. In present study, it was interesting to note that although fish samples collected from Head Balloki showed higher metals content, their condition factor was found to be higher than those collected from Village Kala Khatai which is a relatively unpolluted site of River Ravi. Kasimoglu (2014) has also reported a negative correlation between fish condition factor and content of different heavy metals in fish muscles.

As liver plays the central role in detoxification of toxic substances, hepatosomatic index (HSI) is the most commonly used index in fish toxicology studies (Çiftçi *et al.*, 2015). In line with exposure to metal toxicants, HSI for fish samples collected from Head Balloki was lower than those of Village Kala Khatai indicating smaller liver size in fish with higher bioaccumulation of heavy metals. Ciftci *et al.* (2015) found that exposure to Zn and Cd reduced HSI in *Oreochromis niloticus.* Bervoets *et al.* (2013) found negative correlation between HSI and accumulated cadmium content in examined fish species.

Conclusion: Detection of high content of Pb, Cd and Zn in fish collected from River Ravi at Head Balloki indicates the detrimental effect of waste water discharge on the River ecosystem. Presence of Pb and Cd in fish edible parts in level higher than the proposed limits marks the alarming situation that human population can be exposed to the toxic effects of these metals upon consumption of contaminated fish. In future, there is the dire need to take mitigation steps to reduce pollution load to the River. In addition, regular assessment of toxic metals in organs of fish biota of our natural water reservoirs is essential for human health risk assessment through fish consumption.

Disclosure of funding/ conflict of interest: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

Authman, M.M., M.S. Zaki, E.A. Khallaf and H.H. Abbas (2015). Use of fish as bio-indicator of the effects of heavy metals pollution. J. Aquac. Res. Dev. 6(4): 1-13.

Avigliano, E., N.F. Schenone, A.V. Volpedo, W. Goessler and A.F. Cirelli (2015). Heavy metals and trace elements in muscle of silverside (*Odontesthes bonariensis*) and water from different environments (Argentina): Aquatic pollution and consumption effect approach. Sci. Total Environ. 506: 102-108.

Bawuro, A., R. Voegborlo and A. Adimado (2018). Bioaccumulation of heavy metals in some tissues of fish in lake Geriyo, Adamawa state, Nigeria. J Environ Public Health. 2018: 1-7. doi:https://doi.org/10.1155/2018/1854892

Bervoets, L., D. Knapen, M. De Jonge, K. Van Campenhout and R. Blust (2013). Differential hepatic metal and metallothionein levels in three feral fish species along a metal pollution gradient. PLoS One, 8(3): e60805.

Çiftçi, N., Ö. Ay, F. Karayakar, B. Cicik and C. Erdem (2015). Effects of zinc and cadmium on condition factor, hepatosomatic and gonadosomatic index of *Oreochromis niloticus*. Fresenius Environ. Bull. 24: 1-4.

European Commission (2005). Commission regulation (EC) No. 78/2005 of 19 january 2005, amending regulation (EC) No. 466/2001 as regards heavy metals. Official J. The European Union, L 16: 43-45.

El-Moselhy, K.M., A. Othman, H. Abd El-Azem and M. El-Metwally (2014). Bioaccumulation of heavy metals in some tissues of fish in the Red Sea, Egypt. Egypt. J. Basic Appl. Sci. 1(2): 97-105.

FAO/SIDA (1983). Manual of methods in aquatic environment research. Part 9. Analyses of metals and organochlorines in fish (212, pp. 21-33): FAO Fisheries Technical Paper.

Iqbal, A., A.B. Tabinda, A. Yasar and Y. Mahfooz (2017). Heavy metal uptake and toxicity in tissues of commercially important freshwater fish (*Labeo rohita* and *Wallago attu*) from the Indus River, Pakistan. Pol. J. Environ. Stud. 26(2): 627-633.

Jabeen, G., M. Javed and H. Azmat (2012). Assessment of heavy metals in the fish collected from the River Ravi, Pakistan. Pakistan Vet. J. 32(1): 107-111.

Jomova, K. and M. Valko (2011). Advances in metal-induced oxidative stress and human disease. Toxicology, 283(2-3): 65-87.

Jones, J. (1997). Monitoring and surveillance of non-radioactive contaminants in the aquatic environment and activities regulating the disposal of wastes at sea, 1994. Aquatic Environment Monitoring Report No. 52.

Kasimoglu, C. (2014). The effect of fish size, age and condition factor on the contents of seven essential elements in *Anguilla anguilla* from Tersakan Stream Mugla (Turkey). J. Pollut. Eff. Cont. 2(2): 123-129.

Khan, B., H. Khan, S. Muhammad and T. Khan (2012). Heavy metals concentration trends in three fish species from Shah Alam River (Khyber Pakhtunkhwa province, Pakistan). Nat Env Sci. 3(1): 1-8.

Mahboob, S., S. Kausar, F. Jabeen, S. Sultana, T. Sultana, K. Al-Ghanim, B. Hussain, F. Al-Misned, and Z. Ahmed (2016). Effect of heavy metals on liver, kidney, gills and muscles of *Cyprinus carpio* and *Wallago attu* inhabited in the Indus. Braz. Arch. Biol. Technol., 59: 1-10.

Medeiros, R.J., L.M.G. Dos Santos, A.S. Freire, R.E. Santelli, A.M.C. Braga, T.M. Krauss and S.D.C. Jacob (2012). Determination of inorganic trace elements in edible marine fish from Rio de Janeiro state, Brazil. Food Control, 23(2): 535-541.

Mokhtar, M.B., A.Z. Aris, V. Munusamy and S.M. Praveena (2009). Assessment level of heavy metals in *Penaeus monodon* and *Oreochromis spp*. in selected aquaculture ponds of high densities development area. Eur. J. Sci. Res. 30(3): 348-360.

Morais, S., F.G. E Costa and M. De Lourdes Pereira (2012). Heavy metals and human health. In Oosthuizen, J. (Ed.), Environmental health-emerging issues and practice. IntechOpen. pp. 227-246.

Perschbacher, P.W. and R.R. Stickney. (2016). Tilapia in intensive co-culture. Wiley Blackwell; Chichester. 380 p.

Qadir, A. and R.N. Malik (2011). Heavy metals in eight edible fish species from two polluted tributaries (Aik and Palkhu) of the River Chenab, Pakistan. Biol. Trace Elem. Res. 143(3): 1524-1540.

Rajkowska, M. and M. Protasowicki (2013). Distribution of metals (Fe, Mn, Zn, Cu) in fish tissues in two lakes of different trophy in Northwestern Poland. Environ. Monit. Assess. 185(4): 3493-3502.

Rauf, A., M. Javed and M. Ubaidullah (2009). Heavy metal levels in three major carps (*Catla catla*, *Labeo rohita* and *Cirrhina mrigala*) from the River Ravi, Pakistan. Pakistan Vet. J. 29 (1.25b): 24-26.

Ruxton, C. (2011). The benefits of fish consumption. Nutr. Bull. 36(1): 6-19.

Shafi, J., Z. S. Mirza, N. Kosour and M. Zafarullah (2018). Assessment of water quality and heavy metals contamination of River Ravi in Pakistan. Pakistan J. Anal. Environ. Chem. 19(2): 169-180.

Shakir, A., A. S. Chaudhry and J. I. Qazi (2013). Impact of anthropogenic activities on physico-chemical parameters of water and mineral uptake in *Catla catla* from River Ravi, pakistan. Environ. Monit. Assess. 185(3): 2833-2842.

Shakir, H.A., J.I. Qazi and A.S. Chaudhry (2013). Monitoring the impact of urban effluents on mineral contents of water and sediments of four sites of the RiverRavi, Lahore. Environ. Monit. Assess. 185(12): 9705-9715.

Shovon, M., B. Majumdar and Z. Rahman (2017). Heavy metals (lead, cadmium and nickel) concentration in different organs of three commonly consumed fishes in Bangladesh. Fish. Aqua. J. 8(3): 1-6.

Squadrone, S., M. Prearo, P. Brizio, S. Gavinelli, M. Pellegrino, T. Scanzio, S. Guarise, A. Benedetto and M. Abete (2013). Heavy metals distribution in muscle, liver, kidney and gill of European catfish (*Silurus glanis*) from Italian Rivers. Chemosphere, 90(2): 358-365.

Tabinda, A. B., S. Bashir, A. Yasar and M. Hussain (2013). Metals concentrations in the Riverine water, sediments and fishes from River Ravi at Balloki Headworks. J Anim Plant Sci. 23: 76-84.

Tabinda, A.B., S. Bashir, A. Yasar and S. Munir (2013). Heavy metals concentrations in water, sediment and fish in River Sutlej at Sulemanki Headworks. Pakistan J. Zool. 45(6): 1663-1668.

USEPA. (1992). National study of chemical residues in fish : Volume i (EPA 823-r-92-008a). Office of Science and Technology, United States Environmental Protection Agency.; Washington.

USFDA (1993). Guidance document for nickel in shellfish. Drug Administration, US Department of Health Human Services, Public Health Service, Office of Seafood, Washington DC.

Waseem, A., J. Arshad, F. Iqbal, A. Sajjad, Z. Mehmood and G. Murtaza (2014). Pollution status of Pakistan: A retrospective review on heavy metal contamination of water, soil, and vegetables. Biomed Res. Int. 2014: 1-29.

Yousafzai, A.M., M. Siraj, A. Habib and D.P. Chivers (2012). Bioaccumulation of heavy metals in Common Carp: Implications for human health. Pakistan J. Zool. 44(2): 489-494.

Yousafzai, A.M., F. Ullah, F. Bari, S. Raziq, M. Riaz, K. Khan, U. Nishan, I.A. Sthanadar, B. Shaheen and M. Shaheen (2017). Bioaccumulation of some heavy metals: Analysis and comparison of *Cyprinus carpio* and *Labeo rohita* from Sardaryab, Khyber Pakhtunkhwa. Biomed Res. Int. 2017: 1-5.

Zahra, N. and I. Kalim (2017). Perilous effects of heavy metals contamination on human health. Pakistan J. Anal. Environ. Chem. 18(1): 1-17.