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Determination of heavy metals in fresh water fish species of the River Ravi, Pakistan compared to farmed fish varieties

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Abstract The untreated industrial and sewage wastes arising from industries and metropolitan activities make their passage to the River Ravi, Pakistan, where Balloki Headworks is one of the major sites of effluent concentration. This study was designed to evaluate the concentration of various toxic elements in fishes of that area compared to a nearby fish farm. The concentrations of heavy metals, such as As, Cd, Cu, Pb, Hg, and Zn, and electrolytes Ca, K, and Na were determined in different edible and non-edible fresh water fish varieties. Fish samples were collected from two selected sites and were analyzed for aforementioned elements. Higher levels of As (35.74–45.33 ppm), Cd (0.35–0.45 ppm), Pb (2.1–3.0 ppm), Hg (83.03–92.35 ppm) while normal levels of Zn (37.85–40.74 ppm) and Cu (1.39–2.93 ppm) were observed. Mercury, higher levels of which trigger cough, impairment of pulmonary function, and psychotic reactions, was significantly higher in all studied categories. At the sites under study, there has been observed alarming levels of toxic metals which are needed to be monitored regularly.

Keywords Heavy metals · Pakistani fish · River Ravi · Atomic absorption spectroscopy

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Introduction

The rapid industrial growth has resulted in an increasing production and usage of toxic chemicals such as trace elements in Pakistan. Due to the strategic location as one of the agriculture, industry, and urbanization centers of Pakistan, a large number of toxic chemicals and effluent-producing industries are located in and around Lahore. With the non-availability of scavenging phenomenon in the system and absence of treatment plants for industrial and metropolis wastes, stable and persistent toxic elements and heavy metals get their way into the River Ravi and contaminate the aquatic environment. The River Ravi originates from India and enters Pakistan moving along the Indo-Pak border after passing through Lahore and allied industrial zones; it joins River Chenab, at Shorekot (a garrison city), and finally joins the River Indus (Fig. 1).

The toxicants enter the food chain through edible commodities and cause severe health hazards in that area. Although some metals like Zn, Cu, Fe, and Mn as part of a metabolic system work as enzyme cofactors, they are essential elements for normal cellular functioning, but their higher quantities are toxic for the cell. Other elements like Na, K, and Ca are important for maintaining important cellular functioning (Farkas et al. 2000). On the other hand, As, Cd, Cu, Hg, Pb, and Zn may cause toxic effects even at low levels under certain conditions, thus implying a need for analytical monitoring of inhabitants like fish (Cohen et al. 2001). The chemical/biological, ecological, and toxicological approaches are used as pollution bio-indicators of the water environment. The value of biological methods to monitor metals within the fresh water environment has been considered of great importance, offering an economic and sensitive way of analysis. Fish are useful organisms to study metal contamination, because they explore freely the different trophic levels of the aquatic ecosystem or microbasin (Swaibuh Lwanga et al. 2003; Vives et al. 2006).

For decades, fish species as bio-indicators of heavy metal pollution has been studied by many researchers in a number of countries, including Turkey (Canli et al. 2001; Tuzen 2003; Altindag and Yigit 2005, Fidan et al. 2008), Brazil (Vives et al. 2006), Italy (Boncompagni et al. 2003; Storelli et al. 2005), USA (Heiny and Tate 1997; Gale et al. 2004), Saudi Arabia (Ashraf et al. 2006), Portugal (Leal et al. 1997), Libya (Voegborlo et al. 1999), Uganda (Swaibuh Lwanga et al. 2003), etc. In Pakistan, a study was previously carried out for the determination of some heavy metals from fresh water fish catch from the River Indus (the longest river in Pakistan and the 21st largest river in the world in terms of annual flow, also considered the lifeline of Pakistan). In that study, Arain and colleagues observed variable levels of As, Cd, and Pb in certain fish varieties of Jamshoro area (Arain et al. 2007). In another study, Javed estimated the level of different metals in fish collected from five different locations in the River Ravi. He reported that fish at Balloki accumulated higher values of Fe and Ni as compared to other locations. He also reported fish liver and kidney as the highest depository of metal contaminants in River Ravi fish (Javed 2005).

Previous studies were either limited to one catch site or were limited to specific metals or fish species; non-edible fish varieties and/or control sites were rarely studied. Also, previous studies were unable to demonstrate species-specific uptake of heavy metals in fish. This study was therefore designed to evaluate the concentration of various toxic elements in fishes of reportedly higher metal concentration area, compared to a nearby fish farm where fish are grown in controlled conditions. The purpose of the study was to determine levels of heavy metals, such as As, Cd, Cu, Pb, Hg, and Zn, and electrolytes Ca, K, and Na in different edible and non-edible fresh water fish varieties. As the concentration of metals in fish muscle remains almost constant throughout the life span of fish (Rashed 2001), this study gives direct measure to monitor metal contamination affecting human life by analyzing fish muscle (edible part). This study provides insight into the level of different heavy metals and micronutrients in fresh water fishes of Pakistan and is an invaluable research work to analyze fish of the River Ravi as pollution bio-indicators.

Materials and methods

Study sites

We selected Head Balloki point at the River Ravi which is ahead of allied industrial zones, almost 60 km southwest (Fig. 1 map location A), for collection of naturally occurring fish species. For comparison, we collected fish samples from Experimental Fish Farms, Department of Fisheries, Government of Punjab, at Manawan, almost

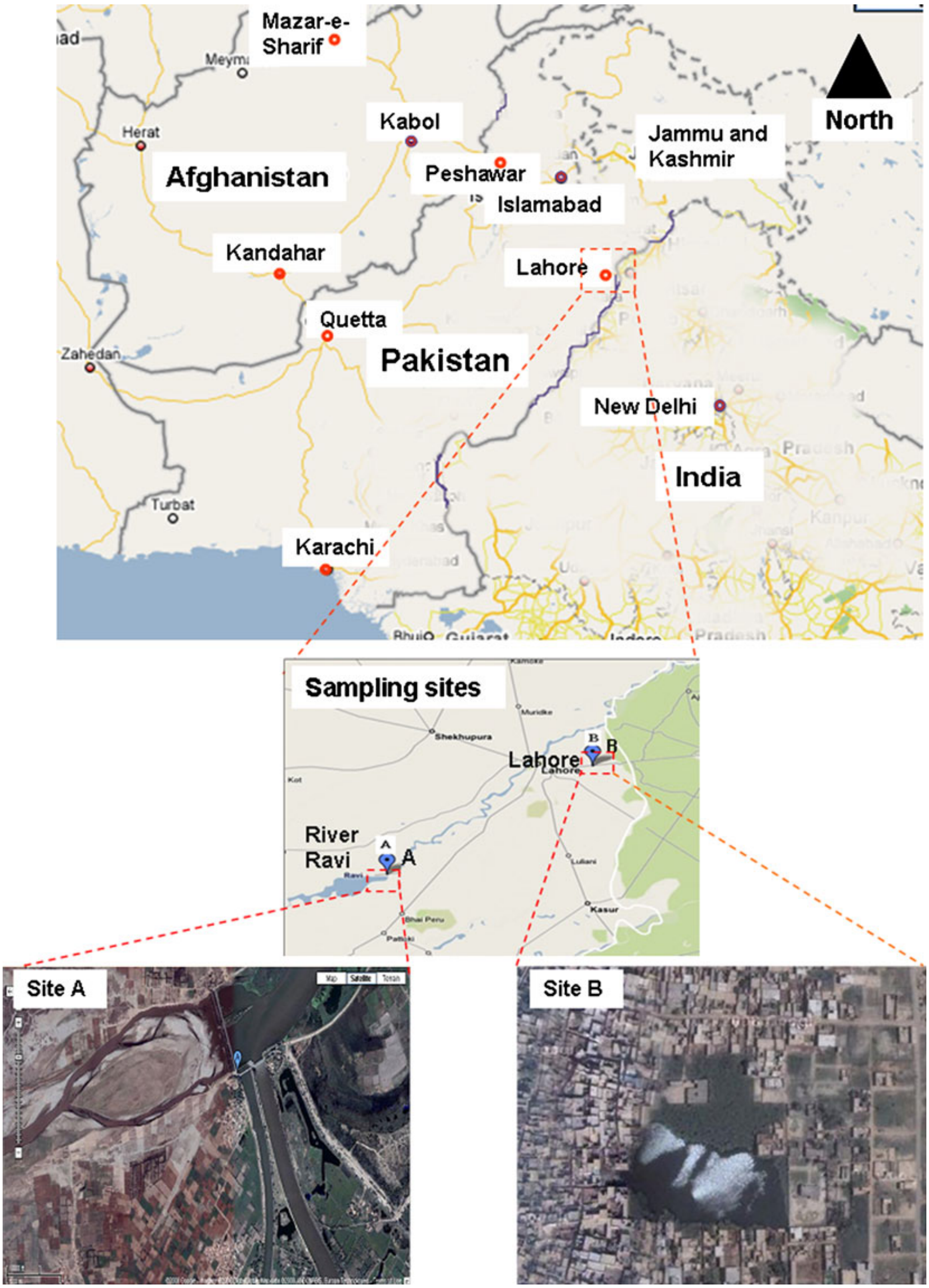

Fig. 1 Map of Pakistan with locations marked *A* for Head Balloki at the River Ravi and *B* for fish farms at Manawan. The map was taken from Google Earth (http://earth.google.com) and adjusted using Adobe Photoshop and MS PowerPoint

20 km east, a suburb of Lahore (Fig. 1 map location B). To check whether the metal uptake is an environmental concern for all fish varieties, we also compared edible fish with some non-edible fish of the aforementioned area. Samples were taken during year 1999 from both study sites; the catch locations were quite rural.

Sampling procedure

Freshwater fish species *Oreochromis mossambica* (tilapia), *Channa punctata* (doula), *Labeo rohita* (raho), *Cirrhinus mrigala* (mori), *Wallago attu* (malhi), *Notopterus notopterus* (pari), *Labeo calbasu* (kalbanse), *Labeo gonius* (sireha), *Mystus vittatus* (kainghra), *Colisa fasciata* (kanghi), *Puntius ticto* (chudda), *Labeo boga* (bhangan), *Xenetodaon cancila* (kan), *Catla catla* (thaila), and *Cyprinus carpio* (gulfam) were obtained seasonally from two different spots, as mentioned in Fig. 1. The samples were transported to the laboratory in ice on the same day. Species identification, number, size, and weight of each fish species were recorded. All fish samples were kept at −30°C until analysis.

Analytical procedures and analysis

Fish muscles were chopped to approximately 10-g pieces. Whole-body muscles were digested using wet digestion method according to the procedure described by Ashraf and Jaffar (1988) with minor modifications. Briefly, the muscles were introduced into a long neck digestion flask to the sliced fish muscles. AnalR grade H_2SO_4 was added and heated in a flask at low temperature for 30 min. After 2 h of digestion, hydrogen peroxide was added until the solution becomes transparent. Contents were concentrated to 2 ml by heat evaporation and diluted to 20 ml with demineralized water and used for analytical purpose. Demineralized water was used throughout the study. All the plastic sand glassware were washed in nitric acid for 15 min and rinsed with deionized water before use. Calibration standard solutions were made by stepwise dilution of the stock solution. High purity argon was used as inert gas wherever applicable. The metal analyses of samples As, Cd, Cu, Pb, Hg, and Zn were carried out by using a Varian atomic absorption spectrophotometer model 1275 equipped with variable lamps for different elements; details of experimental conditions are given in Table 1. A Corning 410 flame photometer was used for electrolyte estimation. All analytical experiments were performed two to three times to check the reproducibility of analytical procedure and machines. Every fish species were analyzed in triplicate and values are mentioned as mean ± SD as error bars.

Statistical analysis

Analysis of variance (ANOVA) was run for all the collected data for edible fish from two different locations using SAS (version 8.02) and SPSS (11.5 version) computer programs as mentioned by Fidan and colleagues (2008). Mean values of each parameter were compared using Fisher's protected least significant difference at 5% significance level (Table 2).

Table 1 Brief description of experimental conditions used for analysis

Mineral	Instrument/current	Detection limit (ppm)	Spectral bandpass (nm)	Fuel	Support	Wavelength (nm)
As	Varian AAS—1275/EDL	0.001	1.0	Acetylene	Nitrous oxide	197.3
Cd	Varian AAS—1275/EDL or 3.5 mA	0.002	0.5	Acetylene	Air	228.8
Cu	Varian AAS—1275/3.5 mA	0.003	0.5	Acetylene	Air	324.8
Pb	Varian AAS—1275/EDL	0.001	1.0	Acetylene	Air	217.0
Hg	Varian AAS—1275/EDL	0.01	0.5	Acetylene	Air	253.7
Zn	Varian AAS—1275/5 mA	0.002	1.0	Acetylene	Air	213.9
Ca	Corning 410 flame photometer	0.05	–	–	–	–
K	Same as above	0.01	–	–	–	–
Na	Same as above	0.01	–	–	–	–

Table 2 Mean squares and degrees of freedom obtained from analysis of variance table for metal components and its concentration in edible fish from two locations

Source of variation	*df*	S.S.	M.S.S.	*F* ratio (calculated)	F ratio (tabulated)	
					5%	1%
Between metal	8	14,870.2	1,858.7	270.1*	3.73	6.84
Within metal (error)	7	48.17	6.881			
Total SS	15					

*Highly significant

df degrees of freedom, *S.S.* sum of squares, *M.S.S.* mean sum of squares

Results and discussion

Estimation of the levels of various elements in different fish species as a measure of environmental pollution has been of great concern over decades. A variable range of different metal concentrations has been observed by various researchers worldwide (Ashraf et al. 2006). In this study, the level of different metals As, Cd, Cu, Pb, Hg, and Zn and electrolytes Ca, K, and Na was determined in edible and non-edible fishes occurring in a natural habitat and was compared to some farmed fish varieties. Average data showed that heavy metal concentration was higher at location A, whereas electrolyte concentration was observed higher in location B. Calculated value of metal concentration and electrolyte availability was found significantly different among edible fish at these locations. These data suggest that feeding habit and availability of nutrient are major factors responsible for the quality of fish growth and their yield. The individual metals are discussed as follows.

Arsenic

It was observed that the level of As remained very high as compared to recommended values. In edible fish species, the level was independent of fish variety as well as sampling spot and remained almost in the same range although there was observed great difference in catch weight. The level of As in edible fish of site A averaged 45.32 μg g^{-1} dry weight basis with a minimum of 40.9 μg g^{-1} in *C. punctata* and *L. rohita* and a maximum of 66.5 μg g^{-1} in *W. attu*. In non-edible fishes, the value ranged from 31.3 μg g^{-1} in *M. vittatus* to 57 μg g^{-1} in *L. boga* with an average value of 35.74 μg g^{-1}. The level of As in edible fish of site B averaged 45.18 μg g^{-1} dry weight basis with a minimum of 39.0 μg g^{-1} in *C. mrigala* and a maximum of 55.7 μg g^{-1} in *L. rohita* (Fig. 2b). Statistical analysis revealed that As concentration was significantly higher in non-edible fishes of site A compared to other metals' catch locations (Table 3). The recent reports about Pakistan revealed that the arsenic level in ground water, in a nearby area to the sites under study, was 32–47 μg L^{-1} (Farooqi et al. 2007) and in soil it ranged 6–19 μg g^{-1} (Farooqi et al. 2009). Previously, the level of As has been observed in different species and the highest level of As has been recorded in Italy; reported level of total arsenic in skate (*Raje* spp.) ranged from 14.4 to 61.5 μg g^{-1}, present as organic compounds (Storelli and Marcotrigiano 2000), while the level of As in shark muscle has been observed to be 20 μg g^{-1} of dry weight (Storelli et al. 2003).

Mercury

Very high levels of mercury, another toxic metal, were observed in all fish varieties. In this case, highest levels are present in naturally occurring fishes with highest value of 126.3 μg g^{-1} in *N. notopterus* with a group average of 92.35 μg g^{-1}. The average level in non-edible fishes of site A was 84.68 μg g^{-1} with minimum concentration of 28.4 μg g^{-1} in *X. cancila*. Similarly, the levels of Hg in edible farmed fishes are in the same range of the latter group with an average of 83.03 μg g^{-1}. The reason of this higher level of Hg is not known to date (Fig. 2b). Level of Hg was significantly higher at both catch locations in both

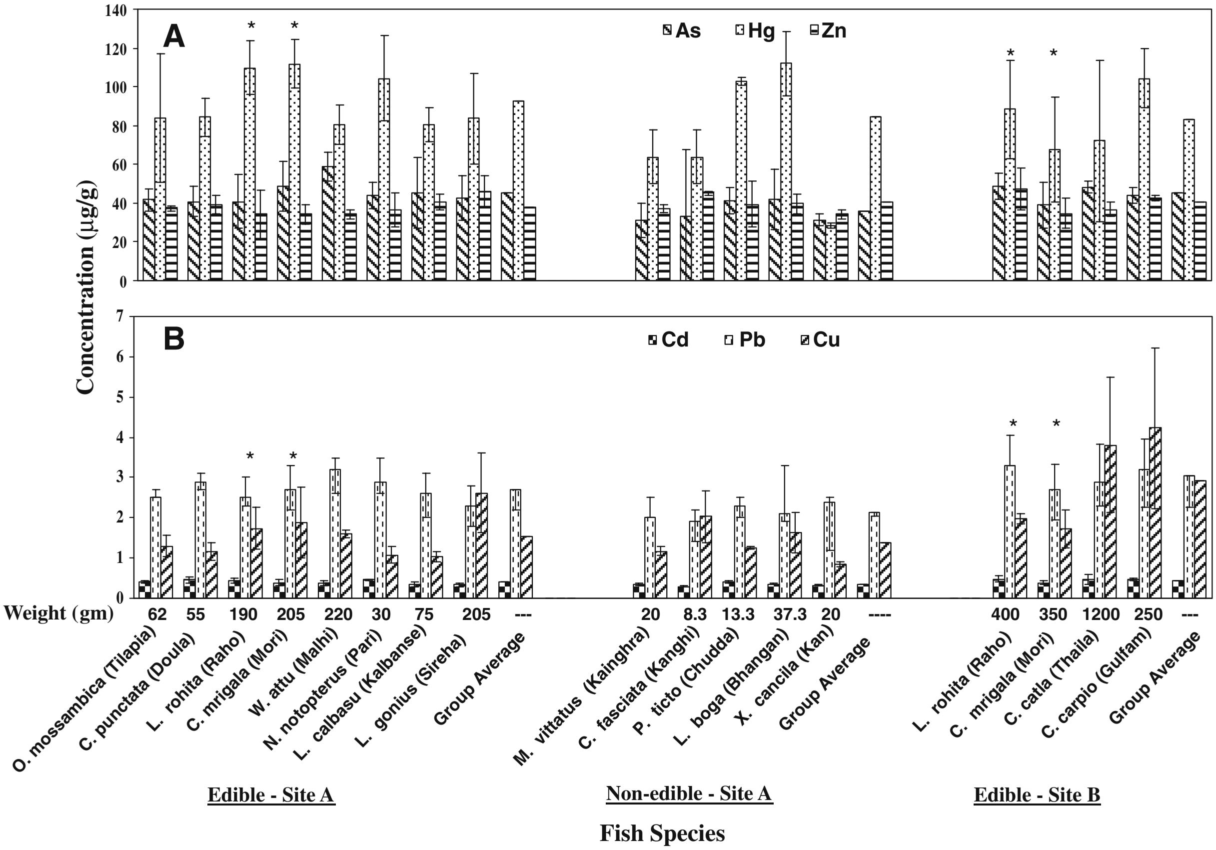

Fig. 2 Species-specific heavy metal concentrations. **a** Concentration (μg g^{-1} dry weight) of arsenic, mercury, and zinc in fish species from two locations. Fishes are separated on edible and non-edible varieties. **b** Concentration (μg g^{-1} dry weight) of cadmium, lead, and copper in fish species. Data shown as mean ± SD was based on observations of triplicate independent experiments. Fish species and corresponding catch weight for *upper* and *lower panel*s are mentioned at the *bottom* of the graph. Fish species common to both locations are marked with a *star* (*) for direct comparison

edible and non-edible fishes compared to other metals (Table 3). An estimated level of 0.66 μg g^{-1} of Hg was observed in Libya (Voegborlo et al. 1999), while the level was 3.07 μg g^{-1} of Hg observed in swordfish of USA, whereas the Food and Drug Administration (FDA) reported approximately 9.0 μg g^{-1} of Hg as a harmful level for human health (http://www.mercuryfacts.org).

Zinc

The level of zinc was almost similar in all fish varieties; in the group of edible fishes at Head Balloki (site A), average value of Zn was 37.85 μg g^{-1} with a minimum of 34.2 μg g^{-1} in *C. mrigala* and maximum level of 54.0 μg g^{-1} was observed in *L. gonius*. While in non-edible fishes at the same place, the average value was 40.74 μg g^{-1} with minimum value of 34.4 μg g^{-1} in *X. cancila* to maximum of 46.3 μg g^{-1} in *C. fasciata*. The average zinc concentration in edible farmed fishes at site B was 40.33 μg g^{-1} with a minimum of 34.7 μg g^{-1} in *C. mrigala* and a highest of 58.9 μg g^{-1} in *L. rohita*, the most widely consumed fish in Pakistan (Fig. 2b). Statistically, level of Zn was significantly higher in edible and non-edible fishes of site A compared to other metals (Table 3). Previous reports about Pakistani fish mention a level of 2.10 μg g^{-1} of Zn as per dry weight (Tariq and Jaffar 1993). Previously,

Table 3 Statistical comparison of different metals

Metal	Statistical function	Edible fish—site A	Non-edible fish—site A	Edible fish—site B
As	*F* test	*	**	*
	LSD 0.05 alpha level	3.22	4.95	1.98
	CV%	2.85	3.54	1.24
	Average (μg g^{-1})	45.32	35.74	45.18
Hg	*F* test	**	**	**
	LSD 0.05 alpha level	10.87	23.1	18.88
	CV%	7.53	14.66	5.63
	Average (μg g^{-1})	92.35	84.68	83.03
Zn	*F* test	**	**	*
	LSD 0.05 alpha level	3.64	5.81	6.27
	CV%	4.51	2.32	2.45
	Average (μg g^{-1})	37.85	40.74	40.33
Cd	*F* test	*	**	*
	LSD 0.05 alpha level	0.26	0.31	0.16
	CV%	1.78	2.24	1.08
	Average (μg g^{-1})	0.4	0.346	0.45
Pb	*F* test	*	*	*
	LSD 0.05 alpha level	0.34	0.46	0.34
	CV%	2.14	1.86	1.87
	Average (μg g^{-1})	2.7	2.14	3.03
Cu	*F* test	*	**	**
	LSD 0.05 alpha level	0.35	0.73	2.14
	CV%	2.28	3.15	4.32
	Average (μg g^{-1})	1.55	1.39	2.93
Ca	*F* test	**	*	*
	LSD 0.05 alpha level	1.58	1.15	0.72
	CV%	3.89	2.28	1.4
	Average (mg g^{-1})	3.34	2.71	4.24
K	*F* test	NS	*	*
	LSD 0.05 alpha level	0.22	0.89	0.8
	CV%	1.18	2.71	2.1
	Average (mg g^{-1})	2.48	2.31	2.49
Na	*F* test	*	**	NS
	LSD 0.05 alpha level	1.73	0.68	0.39
	CV%	2.25	2.93	1.1
	Average (mg g^{-1})	3.20	3.04	2.48

F test, LSD with 95% confidence, CV percent, and average metal concentration are mentioned for every metal in edible and non-edible fish varieties of site A and edible fishes of site B

NS non-significant, *significant, **highly significant, *LSD 0.05* least significant difference at 5%

some research reports reveal a highly variable level of Zn in fishes of different countries. In the USA, according to one report, a maximum level of 173 μg g^{-1} of Zn as per dry weight was observed (Gale et al. 2004). In Brazil, the level was observed to be 3.36 μg g^{-1} of Zn as per dry weight (Vives et al. 2006). In a report regarding level of Zn in Saudi Arabian fish, 16.15 μg g^{-1} of Zn as per dry weight was observed (Ashraf et al. 2006).

Cadmium

In the case of cadmium, the level of Cd was observed highest among edible fishes of site B with a mean value of 0.48 ppm averaging about 0.45 ppm. It was observed that *L. rohita* and *C. catla*, the two most liked fishes in Pakistan, contained highest levels of Cd (Fig. 2a). Statistically, level of Cd was significantly higher in non-edible fishes of site A compared to other metals (Table 3). In Pakistan, previously, 1.03 μg g^{-1} of Cd as per dry weight basis was observed (Tariq and Jaffar 1993). It is interesting to note that the amount of Cd in non-edible fishes is lesser as compared to that of edible fishes. Levels of Cd in fishes analyzed in this study were higher than reported values of Cd in the USA, where an estimated level of 0.23 μg g^{-1} of Cd as per dry weight basis was observed (Gale et al. 2004). While in Egypt, the level of Cd was observed to be 0.01 μg g^{-1}. The rangeof Cd varied from country to country; in Saudi Arabian canned fish, the range was 0.16–0.22 μg g^{-1} as per dry weight basis (Ashraf et al. 2006).

Lead

The levels of Pb were higher in the edible fishes of site B compared to that of edible and non-edible fishes of site A, showing highest levels in *L. rohita* with highest levels of 4.40 μg g^{-1} with a group average of 3.03 μg g^{-1}. While non-edible fishes of site A showed intermediary levels of Pb, the lowest level was observed in *C. fasciata* with a level of 1.9 μg g^{-1} with a group average of 2.14 μg g^{-1} (Fig. 2a). Previously, some researchers observed a lower level of 1.73 μg g^{-1} of Pb as per dry weight in Pakistani fish (Tariq and Jaffar 1993), whereas two different reports by independent researchers exhibited Cd level in the US fish of 13.05 and 13.6 μg g^{-1} of Pb as per dry weight (Gale et al. 2004; Murphy et al. 1978). These values are higher than what is approved by US FDA (Ashraf et al. 2006).

Copper

Normal level of copper was observed in naturally occurring edible fish varieties of site A with a group average of 1.55 μg g^{-1}, with a highest of 3.6 μg g^{-1} in *L. gonius* and a minimum of 1.04 μg g^{-1} in *L. calbansu*. Similarly, the levels of Cu in non-edible fish species of site A averaged 1.39 μg g^{-1} ranging from 0.84 μg g^{-1} in *X. cancila* to 2.67 μg g^{-1} in *C. fasciata*. Among the farmed edible fishes of site B, the levels of Cu were found to be higher as compared to naturally occurring fish varieties. A group average level of 2.93 μg g^{-1} was observed with a minimum of 1.97 μg g^{-1} in *L. rohita* to a maximum value of 7.22 μg g^{-1} in *C. carpio* (Fig. 2a). The level of Cu was significantly higher in non-edible fishes of site A and fishes of site B (Table 3). In Pakistan, previously, some researchers reported a level of 1.10 μg g^{-1} of Cu as per dry weight of fish (Tariq and Jaffar 1993). Previously, some researchers reported 1.62 μg g^{-1} of Cu in US fish (Gale et al. 2004), while in Brazil tilapia fish 1.62 μg g^{-1} of Cu was determined (Vives et al. 2006). In Saudi Arabian fish, the maximum level of Cu was 2.26 μg g^{-1} as per dry weight of catch (Ashraf et al. 2006).

Calcium

Calcium, potassium, and sodium play essential roles in the body. In order to check the level of Ca, K, and Na in different fish varieties, we used a flame photometer. Due to the very important role of calcium in everyday diets, we investigated levels of calcium in different fishes to evaluate the contribution of fish calcium as dietary component. In fishes of natural origin, level of calcium was lower as compared to farmed fishes. Edible fishes of site A had 3.34 mg g^{-1} of Ca as dry weight average level with a minimum of 2.61 mg g^{-1} in *N. notopterus* and a maximum of 5.60 mg g^{-1} of Ca in *C. mrigala*. The level of Ca in non-edible fishes of site A was comparatively lower as compared to that of edible ones with a group average of 2.71 mg g^{-1} having a minimum of 2.31 mg g^{-1} in *X. cancila* and a maximum value of 4.0 mg g^{-1} in *M. vittatus* and *L. boga*. In edible farmed fishes of site B, the level of Ca was higher as compared to that of naturally occurring species. It was observed that the average level of Ca was 4.24 mg g^{-1} with a minimum of 3.91 mg g^{-1} in *C. catla* and maximum of 5.81 mg g^{-1} in *L. rohita* (Fig. 3). Statistically, the level of Ca was significantly higher in edible fishes of site A compared to other electrolytes (Table 3).

Potassium

Potassium is also the major cation in animal cells; thus, it is important in maintaining fluid and electrolyte balance in the body. We analyzed the level of potassium in fish that was almost similar in all three fish groups. In naturally occurring edible fishes at site A, average potassium level was

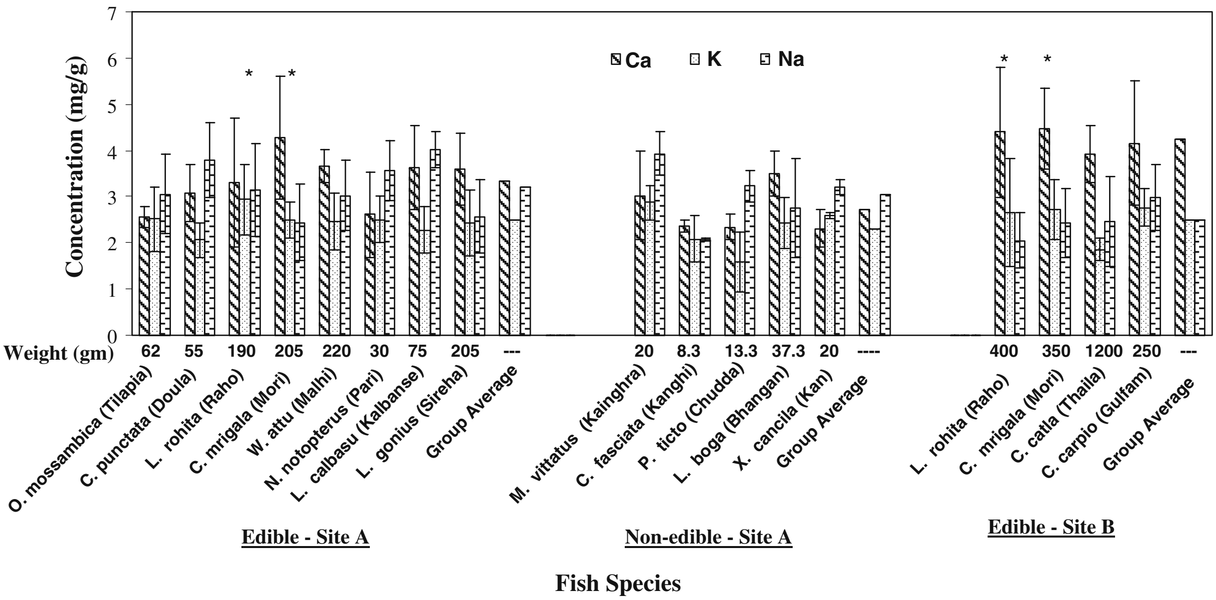

Fig. 3 Species-specific electrolytes concentration. Concentration (mg g^{-1} dry weight) of calcium, potassium, and sodium in fish species from two locations. Fishes are separated on edible and non-edible varieties. Data shown as mean ± SD was based on observations of triplicate independent experiments. Fish species and corresponding catch weight are mentioned at the *bottom* of the graph. Fish species common to both locations are marked with a *star* (*) for direct comparison

2.48 mg g^{-1} with a minimum of 2.06 mg g^{-1} in *C. punctata* and a maximum of 3.70 mg g^{-1} in *L. rohita*. In non-edible fishes of that area, the average level was 2.31 mg g^{-1} with a minimum of 1.58 mg g^{-1} in *P. ticto* and highest level of 3.24 mg g^{-1} in *M. vittatus*. In edible farmed fishes, the average K level was 2.49 mg g^{-1} with a minimum of 1.86 mg g^{-1} in *C. catla* and highest level of 3.83 mg g^{-1} in *L. rohita* (Fig. 3). Concentration of K was non-significant in edible fish varieties of site A compared to other electrolytes (Table 3).

Sodium

It was observed that the level of Na in naturally occurring fish species was higher as compared to farmed fishes, and collectively the level was normal as compared to standard values of Na in fish. It was observed that in edible fishes of site A the group average level of Na was observed to be 3.20 mg g^{-1} with lowest level of 2.44 mg g^{-1} in *C. mrigala* and highest level of 4.6 mg g^{-1} in *C. punctata*. In non-edible fishes of that area, a group average of 3.04 mg g^{-1} was observed with lowest level in 2.07 mg g^{-1} in *C. fasciata* and highest level of 4.4 mg g^{-1} in *M. vittatus*. On the other side, the level of Na in edible farmed fishes was comparatively lower than naturally occurring fishes. The group average sodium level was 2.48 mg g^{-1} with lowest level of 2.05 mg g^{-1} in *L. rohita* and highest level of 3.70 mg g^{-1} in *C. carpio* (Fig. 3). Concentration of K was highly significant in non-edible fishes of site A and non-significant in edible fish varieties of site B compared to other electrolytes (Table 3).

This study is an invaluable research work that provides an important insight into the level of different heavy metals and micronutrients in fresh water fishes of River Ravi, Pakistan as pollution bio-indicators. This work is an important piece of work to estimate possible health hazards by toxic metals which may happen in the future if the environment is not cleaned and protective and remedial measures are not taken. Anyhow, more extensive studies are needed to characterize fish varieties and studies are required to analyze the reasons of pollutants coming into the system.

Finally, at the sites under study, there has been observed alarming levels of some toxic metals like As, Cd, Pb, and Hg, while normal levels of Zn and Cu were observed which are needed to be monitored regularly. Previous studies on heavy metal contamination of fish species from River Ravi were limited, but the results obtained in this investigation show that some fish species contain dangerous levels of heavy metals. Further studies are required to evaluate the ecological significance of the fish catch sites all around the country as well as monitoring programs for assessment and management purpose.

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