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Temporal Metal Bioaccumulation in Tissues of *Labeo rohita* and *Cyprinus carpio* from Indus River, Pakistan

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The concentrations of Pb, Fe, Ni, Cr, Cu, Zn and As in tissues of two freshwater fish species (*C. carpio* and *L. rohita*) caught from Chashma barrage, Indus river, (Pakistan) during winter, spring, summer and autumn seasons of 2013-14 were measured by atomic absorption spectrometry. Metal levels varied depending on the type of tissue, season and fish species. Mean metal concentrations in tissues of *L. rohita* were in the following ranges (μg g^{-1}): Pb 1.12-3.24, As 0.38-0.83, Ni 4.54-6.72, Cu 5.43-8.27, Cr 0.77-3.20, Fe 29.66-62.81 and Zn 63.35-110.99 and metal concentration measured in tissues of *C. carpio* were in the following ranges (μg g^{-1}): Pb 1.27-2.52, As 0.54-1.02, Ni 1.90-2.97, Cu 3.95-12.21, Cr 0.76-1.88, Fe 25.58-60.71 and Zn 69-315.98. Bioaccumulation level of Zn was highest and As was lowest in both fish species. Highest metal levels were found in winter season and lowest in summer season. A statistically significant difference ($p < 0.05$) was observed among different seasons for Ni, Fe, Cu and Zn concentration in *L. rohita* and Pb, Ni and Fe concentration in *C. carpio*. There was statistically significant difference ($p < 0.05$) among the tissues for As and Pb concentration in *L. rohita* and As, Cu and Zn concentration in *C. carpio*. Highest metal levels were observed in liver followed by gills, skin and muscles. The concentrations of analyzed metals in muscles of both fish species were found within international permissible limits of WHO and EPA and both fish species are safe for human consumption.

Keywords: Metals, Indus river, *Labeo rohita*, *Cyprinus carpio*, Fish.

INTRODUCTION

Overproduction due to rapid rise in human population has brought huge amount of waste in the surrounding environment. This has affected self renewal capacity of the nature which has polluted the environment. Different types of contaminants present in air and soil eventually goes to water and pollute it. Combination of various toxic organic wastes with heavy metals or formation of more complex compounds may pose more toxic effect on river ecosystems [1]. Among the pollutants heavy metals are important as these are toxic, non degradable, persistent and bioaccumulate along the food chain [2]. Their contamination level is dependent on type of pollutant, target fish species, location of sampling points, trophic level and specific feeding mode. Various natural and anthropogenic sources of heavy metals are contaminating aquatic ecosystems causing severe health hazards. Sources of heavy metal pollution in fluvial environment are geologic weathering of rocks, atmospheric deposition and discharge of urban, industrial and agricultural wastes [3]. Heavy metals move along food chain starting from primary producers and several types of consumer organisms and at last into fish. Accumulation of heavy metals in aquatic environment can damage entire food chain and can cause carcinogenic and other diseases in fish as well as human being.

Fish is extensively studied as good indicator of river ecosystem health and aquatic metal pollution. In the aquatic food web, fish is present at the upper tropic level and accumulate higher amount of metals from surrounding water. Fish has important role in human nutrition due to their high protein content. Entry of heavy metals into fish occurs through different routes *i.e.*, water, skin, gills, food and non-food particles. Blood of fish carry these metals to various parts of its body. Heavy metals accumulate in different tissues in different concentrations. Metal distribution in different fish tissues is dependent on their mode of exposure, *i.e.,* metal contaminated food or water and so fish can be used as a pollution indicator [4]. Main organ of heavy metal accumulation, their biotransformation and excretion in fish is liver [5]. Assessment of metal pollution in fish is vital with respect to fish biology and health of human beings. Therefore various studies have focused on bioaccumulation of metals in freshwater fish [6,7].

Indus, being the backbone of agricultural and food production in Punjab and Sindh, supports many heavy industries and also plays an important role in supplying potable water in Pakistan. At the present time most of the population of Pakistan, in one way or the other, depends on the goods (drinking, domestic, industrial and irrigation waters and fisheries) and services (sewage and industrial effluent discharge, drainage, recreation, wetlands sustaining large populations of ecologically important migratory birds) provided by the Indus. It receives untreated industrial and agricultural wastes from nearby cities and agricultural areas. This is impacting natural assimilating capacity of Indus river in addition to its reduced flow [8]. Therefore the current study was done to determine metal concentration in different tissues of two fish species (*Labeo rohita* and *Cyprinus carpio*) on seasonal basis from Chashma barrage of Indus river. Furthermore this study was attempted to verify that selected fish species could also be used as environmental indicator for large scale study about aquatic ecosystem health quality.

EXPERIMENTAL

Chashma barrage is internationally recognized as Ramsar site on Indus river. It is the main source of fish and water for irrigation. *L. rohita* and *C. carpio* are common fish species of river Indus. Sampling of both fish species was carried out during winter, spring, summer and autumn seasons of 2013-14. Five to six samples of each fish species were bought from local fisherman at each sampling time. Fish samples were taken in precleaned plastic bags and transported in cooling boxes to the laboratory. Each fish sample was quickly dissected to remove various tissues including muscle, gill, liver and skin with the help of stainless steel utensils and cleaned many times with de-mineralized pure water. Then fish samples were subjected to drying at 105 °C for 24 h in an oven until they attain a constant weight and stored at -20 °C prior to analysis [1].

For digestion, 0.5 g powdered sample of fish tissue was mixed with 4 mL of conc. nitric acid and 2.5 mL concentrated sulphuric acid. Initial vigorous reaction starts and at last it become moderate. This mixture was then placed on a hot plate and heated slowly. About 3 to 4 drops of hydrogen peroxide were added and this process was repeated at the point when solution became clear. Then mixture was further heated for period of additional 20 min at 150 °C. Then it was allowed to cool at room temperature [9]. The digested sample was diluted with de-ionized water and filtered quantitatively through membrane filter paper (pore size 0.45 μm) into a 50 mL volumetric flask. Samples were analyzed for Pb, Fe, Ni, Cr, Cu, Zn and As by Perkin Elmer atomic absorption spectrophotometer model 3100.

RESULTS AND DISCUSSION

Heavy metal concentrations were determined in standard reference material DORM-2 (dogfish muscle) to evaluate the accuracy and precision of our method. Heavy metal recovery rates were in good agreement with reference material and were 96.25 for chromium, 95 for arsenic, 97.44 for copper, 98.97 for nickel, 95.78 for iron, 104.69 for zinc and 101.54 for lead as shown in Table-1. In current study, the concentrations of Cr, Fe, Ni, As, Zn, Pb and Cu were investigated in different tissues (gills, skin, liver and muscle) of *C. carpio* and *L. rohita* and summarized in Tables 2 and 3.

TABLE-1
COMPARISON OF CONCENTRATIONS OF CERTIFIED REFERENCES MATERIAL DORM-2 (mg kg^{-1} AS DRY wt, n = 3) VALUES TO OUR ANALYZED VALUES

Element	Certified value	Detected value	Recovery (%)
Cu	2.34 ± 0.16	2.28 ± 0.19	97.44
Fe	142 ± 10	136 ± 9	95.78
Ni	19.4 ± 3.1	19.2 ± 1.3	98.97
Pb	0.065 ± 0.007	0.066 ± 0.010	101.54
Zn	25.6 ± 2.3	26.8 ± 2.0	104.69
As	18 ± 1.1	17.1 ± 0.23	95.00
Cr	34.7 ± 5.5	33.4 ± 2.5	96.25

The total Pb concentration in *L. rohita* was found higher than in *C. carpio*, but Pb concentration was higher in muscle tissues of *C. carpio* comparatively (Fig. 1A). In *L. rohita*, gills were major organ of Pb accumulation (3.24 μg g^{-1}) followed by liver (2.03 μg g^{-1}), skin (2.17 μg g^{-1}) and muscles (1.12 μg g^{-1}), respectively. In *C. carpio*, gills accumulated the highest Pb concentration (2.52 μg g^{-1}) followed by liver (1.88 μg g^{-1}) and skin (1.74 μg g^{-1}). Arsenic concentration was found higher

TABLE-2
MEAN CONCENTRATIONS (μg/g) OF METALS IN TISSUES OF *Cyprinus Carpio* FROM CHASHMA BARRAGE OF INDUS RIVER

Organs	Seasons	Pb	As	Ni	Cu	Cr	Fe	Zn
Gills	Spring	2.90	1.21	2.26	12.23	1.85	39.90	278.3
	Summer	1.60	0.77	1.13	4.26	0.84	20.09	115.6
	Autumn	2.55	1.09	2.26	8.06	1.83	32.60	345.2
	Winter	3.01	1.01	3.12	16.42	2.27	83.70	524.8
Muscles	Spring	1.40	0.61	1.98	4.21	0.77	29.1	59.6
	Summer	0.77	0.28	0.91	2.85	0.59	10.2	29.4
	Autumn	1.34	0.52	1.79	3.74	0.69	23.2	88.7
	Winter	1.58	0.76	2.91	5.01	0.98	39.8	98.3
Liver	Spring	1.85	0.77	2.26	13.88	1.23	51.23	158.5
	Summer	1.21	0.34	1.44	4.43	0.97	35.7	82.7
	Autumn	2.02	0.64	2.86	10.29	1.71	61.3	164.6
	Winter	2.45	0.86	5.32	20.23	2.61	94.6	319.2
Skin	Spring	1.95	1.02	2.81	4.23	1.89	34.60	110.2
	Summer	0.89	0.73	1.15	3.01	1.21	19.9	32.9
	Autumn	1.64	0.81	1.89	3.49	2.02	22.76	106.7
	Winter	2.47	1.35	3.14	6.19	2.41	61.34	178.2

TABLE-3
MEAN CONCENTRATIONS (μg/g) OF METALS IN TISSUES OF *Labeo rohita* FROM CHASHMA BARRAGE OF INDUS RIVER

Organs	Seasons	Pb	As	Ni	Cu	Cr	Fe	Zn
Gills	Spring	3.22	0.56	5.31	6.21	2.61	72.34	151.6
	Summer	1.92	0.42	5.00	5.01	1.89	31.18	32.2
	Autumn	2.71	0.55	7.34	7.31	2.27	37.23	57.77
	Winter	5.12	0.61	9.23	10.13	3.15	110.5	202.4
Muscles	Spring	1.13	0.42	4.13	4.49	0.92	29.51	67.78
	Summer	0.89	0.21	2.67	3.02	0.47	19.21	10.2
	Autumn	1.02	0.30	6.14	5.78	0.71	22.12	60.2
	Winter	1.43	0.59	5.21	8.42	0.98	47.8	115.2
Liver	Spring	1.98	0.81	7.22	7.23	3.65	39.03	89.2
	Summer	1.89	0.61	4.01	6.61	1.13	27.43	25.6
	Autumn	2.01	0.89	4.99	7.45	2.05	41.42	115.2
	Winter	2.23	1.01	7.42	11.78	5.95	95.12	168.32
Skin	Spring	2.87	0.93	3.67	5.04	1.65	46.2	104.3
	Summer	1.56	0.35	3.02	4.14	1.13	27.45	14.53
	Autumn	1.77	0.89	6.12	6.59	1.71	34.78	67.78
	Winter	2.48	1.12	6.18	9.91	2.25	46.27	157.52

in all of the tissues of *C. carpio* compared to *L. rohita* but the arsenic concentration was higher in liver of *L. rohita* compared to *C. carpio* (Fig. 1B). In *L. rohita*, liver accumulated highest amount of As (0.83 μg g^{-1}) followed by skin (0.82 μg g^{-1}), gills (0.54 μg g^{-1}) and muscles (0.38 μg g^{-1}). In this study, *L. rohita* accumulated higher Ni levels compared to *C. carpio* (Fig. 1C). In *L. rohita*, gills were the main tissues for Ni accumulation in winter. In *L. rohita*, gills and liver (6.72 μg g^{-1} and 5.91 μg g^{-1}, respectively) accumulated highest amount of Ni while lowest Ni level was found in muscles (4.54 μg g^{-1}). In *C. carpio*, highest Ni concentration was found in liver (2.97 μg g^{-1}) followed by skin (2.25 μg g^{-1}), gills (2.19 μg g^{-1}) and muscles (1.9 μg g^{-1}).

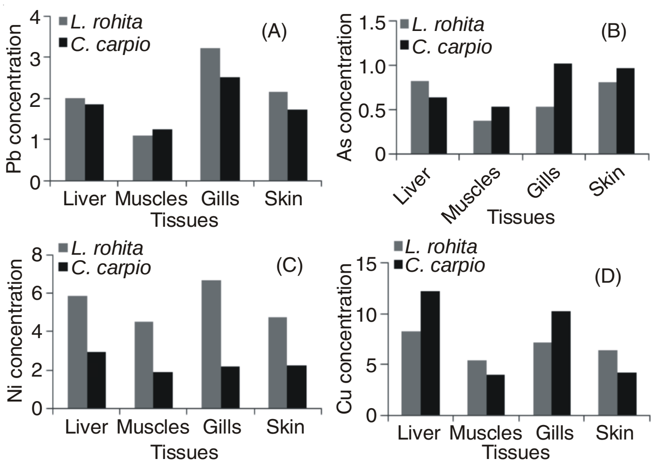

Fig. 1. Mean heavy metal contents (μg g^{-1}) of Pb (A), As (B), Ni (C) and Cu (D) in different tissues of *C. carpio* and *L. rohita*

Chromium concentration was higher in *L. rohita* than in *C. carpio* (except in skin). The lowest and highest Cr concentration in liver tissue was found to be 1.13-5.95 μg g^{-1} in *L. rohita* and 0.97-2.61 μg g^{-1} in *C. carpio*, respectively. However, Cr concentration in gills was in the range of 1.89-3.15 μg g^{-1} and 0.84-2.27 μg g^{-1} in both fish species, respectively (Tables 1 and 2). In this study, Cu concentrations in liver and gills of *C. carpio* were higher than in *L. rohita* (Fig. 1D). Highest Cu levels were observed in liver and gills (8.27 and 7.17 μg g^{-1}, respectively) of *L. rohita* and lowest in muscles (5.43 μg g^{-1}) whilst Cu accumulation in liver (12.21 μg g^{-1}) of *C. carpio* was highest, followed by gills (10.24 μg g^{-1}), skin (4.23 μg g^{-1}) and muscles (3.95 μg g^{-1}). The main range of Fe concentration was 25.58-60.71 μg g^{-1} in *C. carpio* and 29.66-62.81 μg g^{-1} in *L. rohita*. The total Fe concentration was higher in *L. rohita* compared to *C. carpio* but liver of *C. carpio* accumulated higher amount of Fe (Fig. 2B). Gills (62.81 μg g^{-1}) were main organs for Fe accumulation in *L. rohita* and liver in *C.carpio* (60.71 μg g^{-1}). Muscle tissues of *L. rohita* (29.66 μg Fe g^{-1}) and *C. carpio* (25.58 μg Fe g^{-1}) accumulated lowest amount of Fe. The liver of *L. rohita* was found as target organ for Fe accumulation in winter.

The mean range of Zn was 69-315.98 μg g^{-1} in *C. carpio* and 63.35-110.99 μg g^{-1} in *L. rohita*. Zinc concentration was found higher in all the tissues of *C. carpio* compared to *L. rohita* (Fig. 2C). The gills of *L. rohita* accumulated highest Zn concentration (110.99 μg g^{-1}) followed by liver (99.58 μg g^{-1}), skin (86.03 μg g^{-1}) and muscles (63.35 μg g^{-1}), respectively. Gills (315.98 μg g^{-1}) of *C. carpio* were major organ of Zn accumulation, followed by liver (181.25 μg g^{-1}), skin (107 μg g^{-1}) and muscles (69 μg g^{-1}).

Heavy metals accumulated at different levels and were distinguishable from each other in different tissues of *C. carpio* and *L. rohita*. All metal levels were not high in a single type of fish. While *C.carpio* had the highest level of arsenic, copper, zinc and *L. rohita* had the highest level of lead, chromium, nickel and iron (Tables 2 and 3). Mean heavy metal levels found in different tissues oh species yielded the following order: Zn > Fe > Cu > Ni > Pb > Cr > As.

Lead is non-essential metal which is toxic and can cause neurotoxicity and carcinogenic effects on human beings and aquatic biota [10]. Highest Pb concentration was recorded in gills of *L. rohita* in winter, which may be due to higher amount of Pb present in ambient water and accumulation of Pb across gill filaments by *L. rohita*. Previously, similar levels of Pb (1.73 μg g^{-1}) were observed in muscles of freshwater fish in Indus river [11]. Our findings showed that lead levels in muscles of studied fish species are within the WHO and EPA prescribed limits for human consumption [12,13]. Lead was also found

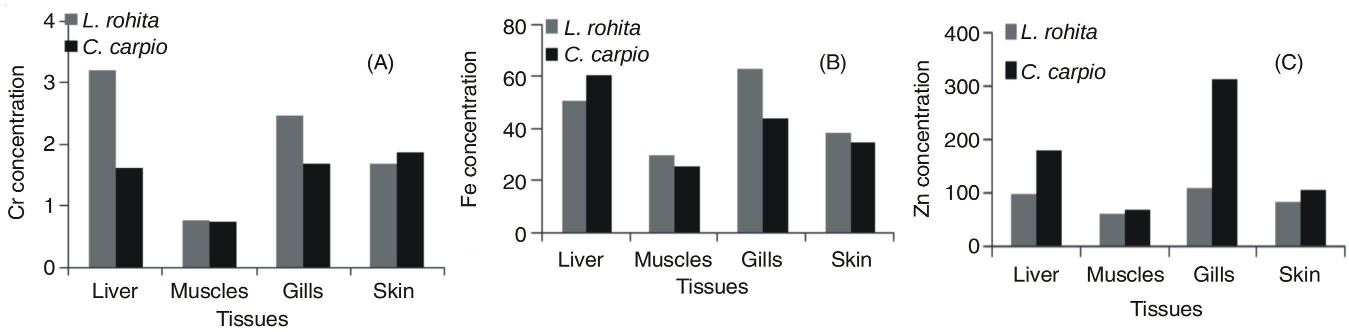

Fig. 2. Mean heavy metal contents (µg g^{-1}) of Cr (A), Fe (B) and Zn (C) in different tissues of *C. carpio* and *L. rohita*

to be highly accumulated in liver as reported by Ikem *et al.* [14] for *Micropterus salmoides*. Literature showed that Pb bio-accumulated poorly in muscles [15,16] which is in agreement with the findings of this study in which muscles accumulated least amount of lead as compared to other tissues.

Arsenic is a toxic metal and it disrupts endocrine even at very low concentrations [17]. Levels of As in gills (1.02 µg g^{-1}) and liver (0.65 µg g^{-1}) of *C. carpio* in present study were higher than As values in liver (0.04 µg g^{-1}) and gills (0.03 µg g^{-1}) of same species collected in the Salek lakes of Slovenia [18]. Liver was main As accumulating tissue in both the species in winter season. It was found that muscles accumulated lowest concentration of As than other tissues of *C. carpio* and L. *rohita* which is similar to the findings of Mason *et al.* [19] for *Tribolodon bakonensis*. In contrast to our findings, De Rosemond *et al.* [20] reported higher levels of As 0.42-2.25 µg g^{-1} in liver and 0.57 to 1.15 µg g^{-1} in muscle tissues of five freshwater fish species. Mol *et al.* [21] reported comparatively lower As concentration (0.164-0.279 µg g^{-1}) in muscles of freshwater fish species from Ataturk dam lake, Euphrates, Turkey as compared to our study.

Nickel is essential mineral for animals and human beings. Its high intake can cause various respiratory pathological effects in lungs of human beings [22]. In this study, liver was found as target organ for Ni accumulation. Liver was also reported to as high Ni accumulating tissue by Ayas *et al.* [23]. Nickel was found to be highly accumulated in gills of *Channa punctatus* as compared to liver [24]. The maximum permissible level of EPA for Ni is 27 µg g^{-1} [13]. Thus the Ni levels in muscle tissues of both fish species was far below the recommended limit. Ozparlak *et al.* [25] found similar Ni levels (0.91-4.01 µg g^{-1}) in *Alburnus akili* from Beysehir Lake, Turkey.

Copper is an important component of several enzymes and has important role in hemoglobin synthesis [26]. Our results showed that highest copper levels (8.27 µg g^{-1} in *L. rohita* and 12.21 µg g^{-1} in *C. carpio*) were present in liver compared to other tissues which are in agreement with the observations of many authors who reported that liver is the target organ for Cu accumulation compared to other fish tissues [13,27]. High Cu levels in liver tissue can cause loss of its homeostatic regulatory control [28]. In contrast to our findings, Danabas *et al.* [29] reported high Cu levels in muscles and gills than liver of *Capoeta trutta*.

Chromium acts as a key element in glucose metabolism. Chromium usually has fewer tendencies to accumulate in fish tissues and published data from industrialized countries depicts lower Cr concentration in fish. Findings of this study showed lower concentration range of Cr in muscles of *L. rohita* (0.47-0.98 µg g^{-1}) and *C. carpio* (0.59-0.98 µg g^{-1}) compared to Cr concentration range (0.47-2.07 µg g^{-1}) reported by Rahman *et al.* [9] in fish muscles of Bangshi river, Bangladesh. Chromium concentrations determined in muscles of both fish species was far below the EPA standard value of 4.4 µg g^{-1} [13]. Highest Cr concentration was recorded in liver of *L. rohita* and skin of *C. carpio* in winter. This result was in contrast to findings of Vaseem and Banerjee [30]. They found gills as main target organ for Cr in *L. rohita*. Similar to findings of our study Tekin-Ozan and Kir [31] also found that Fe concentration was highest in liver and lowest in muscles of *C. carpio* of Beysehir Lake (Turkey).

Zinc is known as an essential mineral for living organisms. It is well documented that it has the ability to bio-accumulate in fatty tissues of fish and affects their reproductive physiology [9]. Our results showed that gills were target organs for Zn accumulation and muscles accumulated least Zn concentration in both fish species. Literature showed a highly variable Zn levels in freshwater fish of different countries. Yohannes *et al.* [32] reported that a mean range of Zn was 3.67-5.30 µg g^{-1} in muscles and 13.51-62.33 µg g^{-1} in liver of various freshwater fish species from Lake Awassa, Ethiopia which were much low as compared to present study. In another study, a mean range of Zn 54.09 -367.39 µg g^{-1} as per dry weight was found in fish from the Yangtze river and Taihu Lake, China which was in agreement to results of present study [33]. In contrast, in a study Zn concentration of 2.10 µg g^{-1} was found in muscles of freshwater fish from Indus river [11] which was lower than findings of this study.

Our results showed that metals levels in muscle tissues of both fish species were low compared to maximum allowable international limits. *L. rohita* is more contaminated with different heavy metals as compared to *C. carpio*. This difference may be due to differences in their metabolic rates, ecological needs and feeding habits. In both the fish species, liver and gills were found to be more concentrated by metals than other muscle tissue. Similarly in a study carried out in *Squalius cephalus* (L., 1758) in Yamula Dam Lake by Duman and Kar [13] high concentration of heavy metals in liver and gills were observed compared to other tissues. The reason for high metal content in liver can be synthesis of metallothionein enzyme in liver which has the capacity to bind metals. High metal contamination of gills is due to their direct exposure to water and sticky mucus secretion of gills which bind metals with

TABLE-4
MAXIMUM RECOMMENDED HEAVY METALS LEVELS IN FISH MUSCLES (μg/g)

	Cu	Cr	Pb	Fe	Zn	As	Ni	Ref.
WHO 1989	30	–	2	100	100	–	–	[12]
EPA	54	4.1	2.7	–	410	–	27	[13]

them. Muscles are not in direct contact with the heavy metals from the outside and are completely covered by the skin, which helps the fish to prevent the penetration of the trace metals in many ways. Furthermore muscle is not an active center for metal detoxification and so there is low transport of metals from other fish tissues to muscles [27].

Seasonal difference in metal distribution in fish species: Highest mean seasonal concentrations of studied metals were observed in all tissues of both freshwater fish species in winter than the other seasons. The lowest mean seasonal concentrations of all studied metals were observed in summer (Figs. 1 and 2). Main reason for low metal content in summer might be rainy season, due to which high water flow of Indus river dilutes metal amounts. In winter water flow was lowest, so metal levels were found highest in this season. It was found that the ratio between the minimum and maximum seasonal concentrations of zinc was highest and least for As in *L. rohita* and *C. carpio*, respectively.

Human health risks due to fish consumption: The standard threshold values of heavy metal concentrations in Table-4 were selected to determine the human health risks due to consumption of fish from this study area. Levels of Pb, As, Ni, Cu, Cr, Fe and Zn in muscles of both fish species were found lower compared to threshold values recommended by WHO [12] and EPA [13]. After all, *C. carpio* and *L. rohita* were found to be safe for human consumption and may not pose significant health risks to local people.

Conclusion

It is found that metals concentration in both fish species varied according to season, type of tissue and fish species. Highest metals concentrations were found in fish samples during winter season due to reduced water flow but lowest concentration of metals were found in summer due to rainy season. Although metal levels in liver and gills were found higher than muscles of both fish species but metal levels in muscles were within safe limits of international food standards. Therefore, no public health hazard is expected by consumption of these fish species.

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