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Targeted accumulation of some heavy metals in Liver, Kidney, Gills and Muscles of *Labeo calbasu* inhibiting the freshwater riverine system

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Abstract

Riverine water is continuously polluted due to release of domestic and industrial wastes. Among them heavy metals pollution is major issue in aquatic habitats which cause toxicity to animal models. In present study, some heavy metals viz; copper, nickel, cadmium, chromium, lead and iron were recorded in water, sediments and organs of *Labeo calbasu* of canals system in Punjab Pakistan. Physiochemical parameters of water were also studied. Average values of temperature, pH, total hardness, DO, TDS, turbidity, BOD, alkalinity and conductivity were 15.26 ± 0.22 °C, 8.47 ± 0.45, 149.52 ± 10.00 mg/L, 3.08 ± 0.38, 232.10 ± 20.05, 45.44 ± 2.16 NTU, 2.46 ± 0.28 mg/L, 399.26-127.22 mg/L and 290.20±15.05 µScm−1 respectively. The level of metals in water was Pb > Cd > Fe > Cr > Ni > Cu and Pb > Ni > Cd > Fe > Cu > Cr in sediments. Significantly higher level of all metals was recorded in running water as compared to bank water. In fish organs, liver had higher level of metals followed by gills, kidney and muscle. The accumulation pattern of metals was Fe > Pb > Ni > Cu> Cr > Cd in all organs. Further, higher value of ALP and ASP value showed damaging effects of water pollution in fish organs. This study concluded that Pb level was highest in canal system of Punjab. Liver showed higher accumulation of all metals compared to other organs.

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Introduction

Industrial development is contaminating the aquatic environment with different pollutants and this situation is also alarming to developing countries like Pakistan (Hamid *et al.*, 2016; Khan *et al.*, 2015). Pollution through heavy metals is an irreversible and long lasting process that creates problems for aquatic animals (Asghar *et al.*, 2015; Khan, Qureshi, & Jabeen, 2017).

Fishery is very important part of economy and human food chain (Khan, Qureshi, & Jabeen, 2018; Khan *et al.*, 2020). *Labeo calbasu* (Hamilton, 1822) is a fresh water fish distributed in India, Bangladesh, Pakistan, Nepal, Myanmar, Burma, Thailand, Yunnan and South China (Ramasamy & Rajangam, 2016). It is famous as Kala rahu or dahi in Pakistan and kalbaus in Bangladesh (Rahman, Jo, Gong, Miller, & Hossain, 2008). It inhibits in all natural water bodies such as rivers, beels, haors, baors and ponds. From last decades, population of *L. calbasu* is continuously decreasing due to pollution of habitat and heavy metal pollution is one of them. Heavy metal pollution is destroying the natural habitat of this fish species.

It not only disturbs the habitat but also alters physiochemical parameters of water. Pollutants mixed with water cause drastic changes that effect directly and indirectly to biological equilibrium, mortality and disturb the activities of aquatic organisms (Khan *et al.*, 2018; Ong & Kamaruzzaman, 2009). Metals may enter in the food web by direct utilization of water or by feeding and assimilation in different hard and soft tissues of edible fish (Asghar *et al.*, 2018; Paquin *et al.*, 2003). These metals can reach toxic threshold and disturb biological balance (Agusa *et al.*, 2005; Raza, Javed, Qureshi, & Khan, 2017). For example: Nickel disturb embryo development, nephron functions and produce allergic reactions (Mandal & Suzuki, 2002). It can also cause cancer in lung and nasal cavity (Athar & Vohora, 1995; FDA, 1993). Excess of Iron weaken the immune responses and increase vulnerability to infection (Akoto, Bismark Eshun, Darko, & Adei, 2014). Cu can damage liver and kidney (Health & Services, 2000). Cd causes Proteinuria, Glucosuria, Osteomalacia, Aminoaciduria and Emphysema (Mahurpawar, 2015). Pb decreases haemoglobin synthesis, damage the neurobehavioral and psychosomatic roles, marginal neuropathy, indirectly effects on heart, renal tubular damage and reproductive problems (Brown & Kodama, 1986). Reviewing the toxicity of these metals, this study was designed to investigate the level of heavy metals in riverine water, sediments and fish organs.

Materials and methods

Physicochemical parameters

Dissolved oxygen (D.O) in water was measured on the spot with DO meter. pH, Temperature, total hardness, electro conductivity, alkalinity, biological oxygen demand, total dissolved solids (TDS) and turbidity were assessed by applying standard techniques (APHA 2005).

Sampling

Samples of water and sediments were collected from different depths of canals. Samples of water were collected with Vanguard Dorn Bottle Sampler and sediment samples with steel rod at depth of two feet. Water samples were stored in plastic bottles and acidified with analytical grade Nitric acid (HNO_3) at pH<2. Four sites were marked and *L. calbasu* was collected from each location in winter. Liver, gills, kidney and muscles were taken through dissection. Sediment and fish samples were stored in plastic bags and kept in icebox for transportation.

Metal concentrations

Separate samples of 2 g of each kidney, muscles, gills and liver were used for metal analysis. Fish samples were digested with pure Nitric acid at 100°C and dilute with deionized distilled water to required volume.

The samples of sediments were homogenized for composite state. These samples (5-7 g) were dried for 48 hours at 100°C in an oven. For removal of larger particles, the dried samples of sediments were passed through a standard screen. 1g sediment sample was moved to 100 mL quartz tube and on a hot plate; it was digest with concentrated HNO_3 and HCl (1:3 v/v). Then quartz tubes were then cooled. For concentration of Cd, Cu, Pb, Cr, Fe and Ni, the prepared samples were studied by using the Atomic Absorption Spectrophotometer. Results were expressed in mg/L and µg/g wet weight.

Activity of acid phosphatase and alkaline phosphatase

Activity of both enzymes ACP and ALP were measured according to Michell, Karnovsky, and Karnovsky (1970) and Estiarte *et al.* (2007). In common procedure, the reaction mixture was consisted of 0.7 ml sodium acetate buffer with pH 5. It was mixed with 0.25 ml nitro-phenyl phosphate. This mixture acted as substrate that was again mixed with 0.5 ml enzyme. All the mixture contents formed total volume of 1 ml. This mixture of reaction was incubated for 30 minutes at 37 ºC. To this mixture, 4 ml of 0.1 sodium hydro oxide was added and again incubated it in the incubator minutes for 30 at 37 ºC. For ALP, the solution for substrate was consisted of 0.5 ml buffer of glycine having pH 7.8, 10 mM 0.2 ml magnesium chloride 2 and 5 mM p. nitro-phenyl phosphate that have volume of 0.25. About 0.05 ml of enzyme was added and all mixture was equal to 1 ml. This mixture was then incubated for thirty minute at 37 ºC. The activities of ACP and ALP were observed by colour (yellow colour measurement) of p. nitro-phenol using a synergy Multi-Mode Micro plate Reader, (Winooski, VT, USA).

Statistical analysis

All readings and results were in mean and standard deviation of three replicates and comparison was made through Tukey's pair wise comparison test (Mini tab V.17). Heavy metals concentration was determined through standard curve method in Microsoft Excel (V.10).

Results

Average value of temperature was 15.26 ± 0.22 °C, and pH 8.47 ± 0.45. Total hardness was 149.52±10.00 mg/L and dissolved Oxygen 3.08 ± 0.38. Total dissolved solids (TDS) were 232.10±20.05. Turbidity of water was 45.44± 2.16. Biological oxygen demand (BOD) was 2.46 ± 0.28 mg/L. Alkalinity was 399.26-127.22 mg/L. Conductivity was 290.20 ± 15.05µScm^{-1} (Table 1).

Table 1. Physiochemical parameters of water.

Sampling season	Winter
Temperature	15.26±0.22
pH	8.47±0.45
Hardness	149.52±10.00
DO	3.08±0.38
Alkalinity	399.26±127.22
Biological oxygen demand (BOD)	2.46 ± 0.28
E.C	290.20±15.05
Total dissolved solids (TDS)	232.10±20.05
Turbidity	45.44±2.16

Ni in running water was 0.037 ± 0.0046 mg/L and 0.032±0.0008 mg/L in bank water, Fe 1.45±0.25 mg/L and 1.38±0.18 mg/L, Cd 0.52±0.056 mg/L and 0.36±0.018 mg/L, Cu 0.145±0.020 mg/L and 0.085±0.005 mg/L, Cr 0.079 ± 0.013 mg/L and 0.071± 0.0019 mg/L and Pb 0.32±0.04 and 0.30±0.06 mg/L in main stream and bank water respectively. Accumulation of Ni in the sediments of main stream was 182.44±8.52 µg/g and 162.32±10.14 µg/g in the bank of canal, Fe 12145±228.40 µg/g and 9191±266.51 µg/g, Cd 1.97±0.03 µg/g and 1.78±0.010 µg/g, Cu 31.48±1.22 µg/g and 28.42±1.44 µg/g, Cr 39.30±1.08 µg/g and 36.28±2.4 µg/g and Pb 136.82±8.50 and 117.20±8.45 µg/g in main stream and near banks of canal respectively. Accumulation of Ni in liver was 9.50±1.12 µg/g, 3.44±0.20 µg/g in gills, 4.07±0.50 µg/g in kidney and 1.72±1.00 µg/g in muscle. Fe in liver was 434.30±10.10 µg/g and 290.20±10.20 µg/g in gills. It was 213.25±12.20 µg/g in kidney and 70.50±5.50 µg/g in muscle of fish. Accumulation of Cd in liver was 4.29±0.50 µg/g and 1.12±0.1 µg/g in gills, 2.90±0.25 µg/g in kidney and 1.27±0.20 µg/g in muscle. Accumulation of Cu in liver was 6.30 ± 1.00µg/g and 5.70±0.04 µg/g in gills. It was 4.92±0.012 µg/g in kidney and 2.76±0.40 µg/g in muscle. Concentration of Cr in liver was 6.30±0.50 µg/g and 3.70±0.30 µg/g in gills, 5.55±0.35 µg/g in kidney and 1.57±0.50 µg/g in muscle. Concentration of Pb in liver was 12.60±0.50 µg/g and 12.67±1.20 µg/g in gills. It was 9.40±1.15 µg/g in kidney and 7.63±1.10 µg/g in muscle of fish (Fig.1). The concentration of ALP and ACP was 76.17 and 78.99 respectively which was higher than reference value (Fig.2).

Table 2. Accumulation level of metals (mg/L) in water.

Metal	Main stream	Bank	WHO standard
Ni	0.037±0.0046	0.032± 0.0008	0.07
Fe	1.45±0.25	1.38± 0.18	0.10
Cd	0.52±0.056	0.36±0.018	0.03
Cu	0.145±0.020	0. 085±0.005	2
Cr	0.079±0.013	0. 071±0.0019	0.05
Pb	0.32± 0.04	0.30±0.06	0.01

Discussion

The heavy metals concentration is increasing in Pakistan due to industries along with bank of rivers. A number of biological problems are rooted for water bodies from industrial and human waste (Khan, Qureshi, Jabeen, *et al.*, 2017). Heavy metals pollution is one of them which was analysed in term of accumulation in riverine system in Punjab. Since Pakistan has limited sources of water. Our cities are over populated and population increase pollutants which fall directly in to the rivers. The major causes of toxic waste in the river Ravi waste water from cities, agricultural and industrial waste material from different factories like electro plating factories, steel workshops, pulp and paper industries, medicine laboratories, scientific laboratories and surface overflow and municipal mess. For example, Lahore industries which located near river Ravi increase the level of iron (Fe) due to its discharge which was also recorded in this study. Public sewage could also be the source of heavy metals pollution. Some other factors like overpopulation, urbanization can produce huge wastes which could also be the source of contamination in ecosystem. High rainfall also brings large volume of waste with runoff water (Islam, Ahmed, Habibullah-Al-Mamun, & Hoque, 2015). Effluents of tanneries and wastes from manufacturing units, surgical instruments and sewage of the municipality are the chief source of pollution in river Ravi water. When water level was low in river the metals especially; Ni, Pb, Cr, Cu, Cd and Fe were higher than the acceptable limits of safe water.

Table 3. Accumulation of metals (µg/g) in sediments.

Metal	Main stream	Bank	WHO Standard
Ni	182.44±8.52	162.32± 10.14	30±1.0
Fe	12145±228.40	9191±266.51	7470±280
Cd	1.97±0. 03	1.78±0.010	0.6
Cu	31.48±1.22	28.42±1.44	29±1.0
Cr	39.30±1.08	36.28±2.4	69±1.0
Pb	136.82± 8.50	117.20±8.45	20

Fishes have great tendency to assimilate the heavy metals in their body organs such as gills, kidneys, scales, liver and muscles (Asghar, Quershi, Jabeen, Shakeel, & Khan, 2016). Fish are the part of food chain and through food chain heavy metals effect indirectly human health and cause different types of diseases. In this study, two sampling sites such as main stream and bank water were selected to record the water quality parameters and metal analysis in winter season. Mean values of temperature, pH, total hardness, DO, TDS, turbidity, BOD, alkalinity and conductivity were recorded. Mahmood (2003) reported recorded slightly different values of these physiochemical parameters in previous study. Our results recorded low temperature because water samples were collected early in the morning and in winter season and pH was high due to excess level of basic material like soaps detergents etc. Hardness was low than past study due to less quantity of chloride, calcium and magnesium in riverine water. In present study DO was low because low amount of river flora were present including aquatic plants.

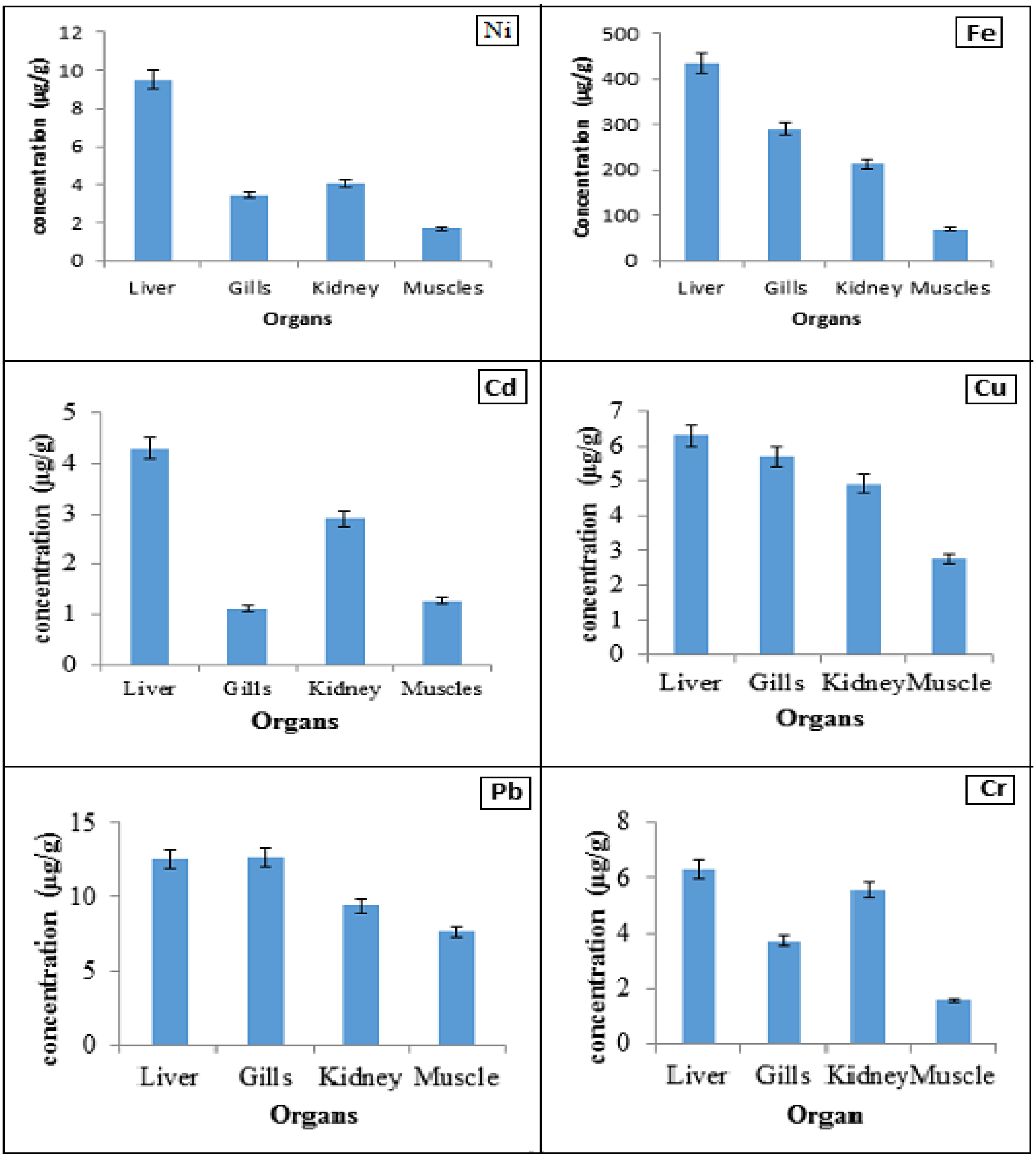

Fig. 1. Order of Bioaccumulation of some heavy metals in liver, kidney gills and muscles.

Almost all the studied metals except Cr were higher in sediments of main stream water. Values of these metals expect Cr indicated that the water of River Ravi was polluted with these metals. Tabinda, Bashir, Yasar, and Hussain (2013) recorded Fe, Zn, Cu, Cr, and Ni in sediments ranged between 1849.00-1863.00mg/L, 125.68-133.16mg/L, 38.4-47.93 mg/L, 48-51.85 mg/L and 23.5-25.21 mg/L in winter.

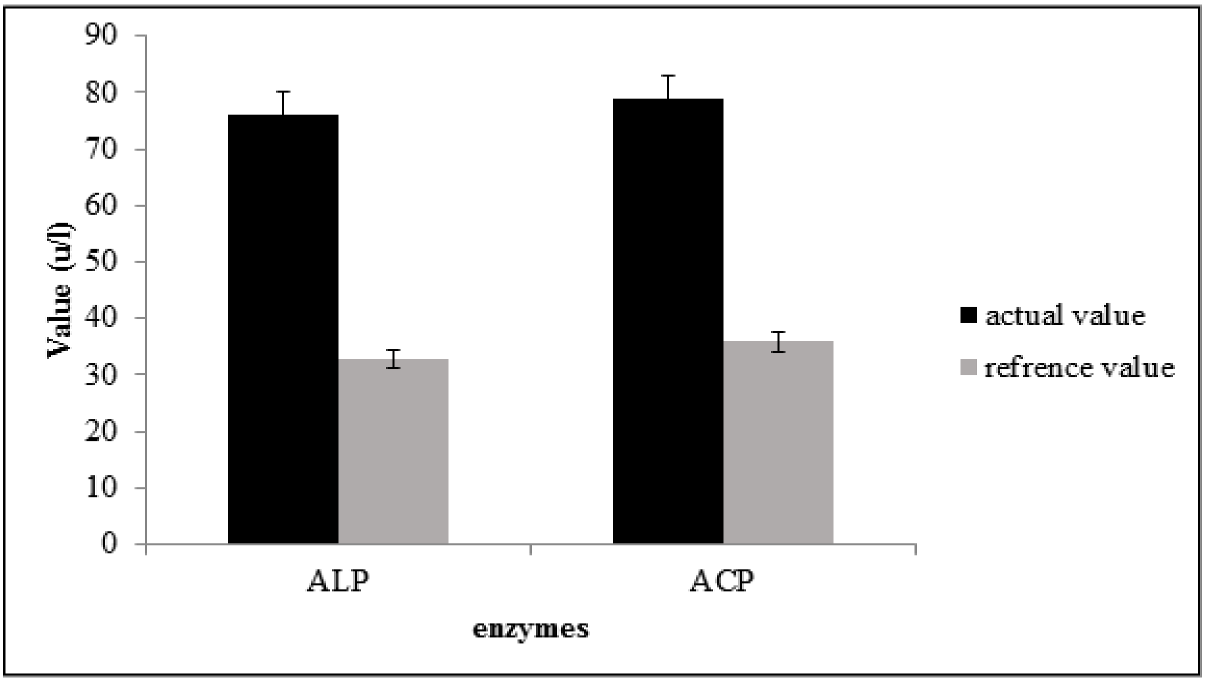

Fig. 2. Comparison of concentration of ACP and ALP with reference values.

The order was Fe > Zn > Cr > Cu > Ni. If we compare both studies, it can be concluded that the level of metals in present study was high than past because all waste of industries and cities sewage are now falling directly in river water (Theofanis, Astrid, Lidia, & Calmano, 2001).

In present study Ni accumulation pattern from higher to lower was: liver > kidney > gills > muscle, Cr liver > kidney > gills > muscle. Brraich and Jangu, (2015) evaluated higher concentration of Cr due to industrial wastes. Accumulation of Fe was: liver > gills > kidney > muscle, Cu liver > gills > Kidney > muscle. Accumulation of Cd was: liver > kidney > muscle > gills, Pb was gills > liver > kidney > muscle. The accumulation levels of metal was vary in different fish species and organs due to feeding system (Mormede & Davies, 2001), trophic status (Canli, Ay, & Kalay, 1998), territory pollution (Canli & Atli, 2003; Papagiannis, Kagalou, Leonardos, Petridis, & Kalfakakou, 2004) and accumulation in food web (Burger & Gochfeld, 2006).

In present study value of ALP and ACP was higher than reference value. When concentrations of these enzymes become high, it shows that animals were under metal stress. ACP enzyme is major marker indicate the stability of lysosomes. ALP is a poly functional enzyme. ALP enzyme activity can cause disturbance of membrane transport system(Atli & Canli, 2007; Karan, Vitorović, Tutundžić, & Poleksić, 1998). The specious sensitivity of Acid and Alkaline phosphatase revealed that these enzymes were used as bio indicator in metal pollution screening (Bindya Bhargavan & Mohammed Salih, 2008).

Conclusion

This study concluded that Pb level was highest in canal system of Punjab. Liver showed higher accumulation of all metals compared to other organs. The pattern of accumulation of metals was Fe > Pb > Ni > Cu > Cr > Cd in all fish organs. The results further revealed that the bioaccumulation of heavy metals in running water was significantly higher than standing water or bank water.

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