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Heavy metals bioaccumulation in muscle tissues of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* exposed to Chakbandi drain water concentrations in glass aquaria

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Abstract

This project was planned to study the heavy metals bioaccumulation in muscle tissues of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* exposed to Chakbandi drain water concentrations in glass aquaria. In this study, Chakbandi drain's composite water were collected from the selected sites in the month of April, May and June, 2016 and applied to fingerling's of three fish species i.e. *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* under laboratory conditions in glass aquaria. After determining the LC_{50}, the sub-lethal dilutions i.e. 20%, 25%, 30%, 35% and 40% of drain water were tested for three month's acute toxicity trial. After three months, muscle tissues of all the three fish species were taken by dissecting the control and experimental fishes for heavy metals bioaccumulation studies. Bioaccumulation of Cu, Cr, Mn, Ni, Cd, Co, Sn, Hg, Zn and Pb was detected in all the drain water treated fish species. Among all the treated fish species *Cirrhinus mrigala* showed the maximum bioaccumulation of all the selective heavy metals as compared to *Labeo rohita* and *Catla catla*. Higher level of bioaccumulation of Mn, Cu and Pb was detected in the muscle tissues of *Cirrhinus mrigala* as compared to other treated fish species. Conclusively, bioaccumulation of heavy metals in fish species is reliable tool for the assessment of environmental pollution. The findings of this study are helpful as an early warning for environmental monitoring strategies and for the evaluation of eco-toxicological impacts of pollutions.

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Introduction

Water is very important and fundamental natural resource that covers the 70% of earth surface and without it, survival of life is not possible. The majority of human activities are involved for polluting fresh water bodies. It is estimated that approximately 1.5 billion people are living without safe drinking water globally. There are about 5 million deaths per year in the world which is due to water borne diseases (Qureshi *et al.*, 2015).

Now a day, aquatic pollution has become the burning issue and alarming problem in Pakistan as domestic sewage and industrial contaminants which contain bulk of toxic chemical compounds, including especially the heavy metals that are constantly discharged into aquatic environments. These heavy metals have drastically toxic effects on aquatic organisms carps (Javed, 2005; Hayat *et al.*, 2007) causes the reduction of haemoglobin that effect the oxygen binding capacity in these aquatic organisms (Bonga, 1997; Ruane *et al.*, 1999; Adeyemo, 2005).

A wide variety of domestic and industrial pollutants directly or indirectly are affecting DNA and causing genotoxicity in aquatic organisms especially in fishes (Shakir *et al.*, 2015; Sultana *et al.*, 2016). Impacts of these chemical compounds can drastically lead to abnormal physiological activities and cause detrimental effects on growth, development, reproduction and behavior in aquatic organisms (Lee & Peart, 2000; Ginebreda *et al.*, 2014). The detrimental genotoxic effects are considered the endpoints for the assessment of pollution related toxicity (Bolognes & Cirillo, 2014). Fish are selected as a model organism in genotoxicological studies due to their sensitivity as bio-indicator for water quality assessment. Hence, fish can highlight the detrimental effects of new chemicals that are discharged into the aquatic environment (Gupta *et al.*, 2017; Sabullah *et al.*, 2015) and can respond to toxic pollutants in the similar way as higher vertebrates (Al-Sabti & Metcalfe, 1995).

Domestic and municipal wastes and Industrial effluents of Faisalabad district are discharged into River Chenab from different directions through a drainage system especially the zigzag Chakbandi drain.

Contaminated water includes huge number of chemical compounds from different types of industries including chemical, textiles, soap, pharmaceutical, tanneries and leather and sugar mills etc. which are sufficient to alter the water quality parameters of River Chenab. This devastating alteration in water quality parameters is the main reason for eradication of the aquatic populations of many peculiar and native fish species including Indian major carps i.e. *Catla catla, Labeo rohita* and *Cirrhinus mrigala* etc. These Indian major carps are widely cultured in the farms and even harvested from the Rivers of the province Punjab.

The current study has been planned to assess the bioaccumulation of heavy metals in the muscle tissues of *Catla catla*. *Labeo rohita* and *Cirrhinus mrigala* exposed to Chakbandi drain water concentrations in glass aquaria.

Materials and methods

Experimental model

The fish fingerlings (11-13g) of three fish species i.e., *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala* were procured alive from Government Fish Seed Hatchery, Satiana Road, Faisalabad and shifted in plastic bags containing fresh water filled with oxygen to Fisheries Research Laboratory, Department of Zoology, Government College University, Faisalabad. The fingerlings of fish were bathed for 2-3 minutes in 0.1% $KMNO_4$ to sterilize before acclimatization.

Acclimatization and maintenance

The process of acclimatization of fish was carried out in Fisheries Research Laboratory for two weeks in glass aquaria of 30cm x 60cm x 44.5cm in size filled with dechlorinated and aerated tap water before the start of experiment. All the experimental glass aquaria were washed thoroughly with tap water before use. Twenty fish of each fish species were kept in each glass aquaria for acclimatization. The fish fingerlings were fed the commercial artificial feed @ 3-4% of wet body weight during the acclimatization period. Proper aeration was supplied continuously to all the glass aquaria with electric air pump (China). The water in the glass aquaria was replaced with fresh water after every three days regularly.

Experimental site selection and water sampling

This project was planned to study the exposure effects of domestic waste and industrial effluents on water quality parameters and fish under laboratory conditions. Faisalabad the so called "Manchester" of Pakistan has plastic, leather, textile, dying, printing, finishing, seizing industries, sugar mills and a large number of factories that release the variety of industrial discharges and municipal wastes into River Chenab through Chakbandi drain at Moza Thatta Muhammad Shah (Ahmad Wala) at latitude 31.570° and longitude 72.534°. Five sites of Chakbandi drain were selected for water sampling. These sites were namely, Akbar Abad Chowk Faisalabad, Bawa Chak Faisalabad, Chak- 188 J. B., Chak Sial 194 and Moza Thatha Muhammad Shah (End point of the drain entering into River Chenab) along the stretches of Chakbandi drain. This drain, almost span more than 160km of distance before joining the river Chenab. Water sampling were done from five selected sites along the length of drain by depth, centre, right and left banks. Representative water samples from each selected site were collected in pre-washed polypropylene bottles with polyethylene caps from the five selected sites.

Determination of 96h LC_{50} values

LC_{50} values for three fish species were determined separately according to their resistance and sensitivity to drain water concentrations. The period for LC_{50} determination was 96 hours (short term) and mortality data was recorded for this duration. In each glass aquarium 10 number of fishes of each selected fish species were released after two days (24 hrs) starvation. Fish mortality % age was recorded against each drain water concentration during 96h test duration.

Experimental setup

In this study, after the determination of LC_{50} Chakbandi drain's composite water concentration of five selected sites was prepared. The dead fish was immediately removed from the test tank. The five sub-lethal dilutions (20%, 25%, 30%, 35%, 40%,) of this composite water concentration was applied to fingerlings approximately 11- 13g of three fish species i.e. *Catla catla, Labeo rohita* and *Cirrhinus mrigala* under laboratory conditions in glass aquaria.

All the three fish species were distributed into two groups, one as sub-lethal exposure group (experimental) which was further subdivided into five sublethal concentration (20-40%) and other as control group (Table 1). 30 fish of the three fish species were distributed equally into 18 glass aquaria (80L) by maintaining the stocking density of about 2.3L per fish (11-13g). The percentage concentrations of drain water were prepared on the basis of volume to volume (*v/v)* ratio.

Table 1. Sub-lethal drain water concentration (%) applied to different fish species in glass aquaria.

Drain water concentrations	No. of *C. catla*	No. of *L. rohita*	No. of *C. mrigala*
Control water	10	10	10
DW- 1 (20%)	10	10	10
DW- 2 (25%)	10	10	10
DW- 3 (30%)	10	10	10
DW-4 (35%)	10	10	10
DW- 5 (40%)	10	10	10

Water analysis

Water concentrations were collected and analyzed fresh immediately after sampling for determining temperature, pH and dissolved oxygen at the site. The water was preserved (0.8ml of H_2SO_4/L) in sampling bottles for the determination of nitrates and trace elements. All the samples were brought to the laboratory and kept in the refrigerator at a temperature of 4°C until the analysis. Rest of water quality parameters (WQPs) and selected heavy metals were analyzed in Research laboratory, Department of Zoology, Government College University, Faisalabad, described by Environmental Protection Agency (EPA) of Pakistan.

Digestion of water samples

The preserved water sample (100ml) was taken and evaporated in the volumetric flask on hot plate within fume hood till volume remained 20ml. After cooling it, 5ml of HNO_3 (55%) and 10ml of per chloric acid (70%) were added to the sample. This mixture was evaporated on a hotplate till the brown fumes were changed into the dense white fumes of per chloric acid. Then sample was cooled slowly and diluted to 100ml of double de-ionized water and then filtered the samples.

Digestion of fish muscle samples

The fish muscle samples from each fish species of the respected drain water concentration were obtained by dissecting it at the end of exposure, dried in filter paper, pack in polyethylene bags and stored at -20°C before the analysis. One gram fish muscle sample was taken and digested it with 5ml of per chloric acid and 15ml of nitric acid. Placed the sample on hotplate until brown fumes ceased to evolve then cool the sample at room temperature and finally diluted it with 50ml of distilled water and filtered it with whatmann filter paper.

Atomic absorption spectrometry

Digested fish muscle and water sample were aspirated into an atomic absorption spectrophotometer (Hitachi polarized Zeeman Atomic Absorption Spectrophotometer AAS, 2000 series) by using an air acetylene flame (APHA, 1998; Authman *et al.*, 2015). The selected heavy metals analyzed were Copper (Cu), Chromium (Cr), Ferrous (Fe), Manganese (Mn), Nickel (Ni), Cadmium (Cd), Cobalt (Co), Stannous (Sn), Mercury (Hg), Zinc (Zn) and Lead (Pb).

Statistical analysis

Analyses of variance (ANOVA), mean and standard error were calculated through SPSS. The means were compared by using Duncan's Multiple Range test (DMR-test). Probability values of $p<0.05$ were considered significant. Microsoft excel (2010) was used for graphic representation and Simple regression plots.

Results

Copper (Cu) accumulation

Maximum mean Copper values were detected in *Cirrhinus mrigala* (0.49±0.05) and minimum in *Catla catla* (0.32±0.03). In the muscle tissues of *Catla catla*, the mean Copper accumulation values were maximum from the drain water concentration DW-2 (0.48±0.06) and minimum from DW-1 (0.20±0.01). In *Catla catla*, the controlled fish showed non-significantly ($p>0.05$) different accumulation of Copper values (0.02±0.00) as compared to experimental fish.

In the muscle tissues of *Labeo rohita*, the maximum mean Copper accumulation values were detected from the drain water concentration DW-2 (0.58±0.12) and minimum from DW-1 (0.23±0.01). The controlled *Labeo rohita* showed non-significantly ($p>0.05$) different accumulation of Copper values (0.01±0.00) compared to all drain water concentration exposed fish. In the muscle tissues of *Cirrhinus mrigala*, the maximum mean Copper accumulation values were detected from the drain water concentration DW-2 (0.67±0.09) and minimum from DW-1 (0.27±0.03). In *Cirrhinus mrigala*, the controlled fish showed non-significantly ($p>0.05$) different accumulation of Copper values (0.03±0.00) as compared to experimental fish (Table 3).

Chromium (Cr) accumulation

Maximum mean Chromium accumulation values were recorded from *Cirrhinus mrigala* (0.38±0.03) and minimum from *Catla catla* (0.30±0.02). In the muscle tissues of *Catla catla*, the mean Chromium accumulation values were maximum from the drain water concentration DW-2 (0.38±0.02) and minimum from DW-5 (0.17±0.01). In *Catla catla*, the controlled fish showed non-significantly ($p<0.05$) different accumulation of Chromium values (0.02±0.00) as compared to experimental fish. In the muscle tissues of *Labeo rohita*, the mean Chromium accumulation values were maximum from the drain water concentration DW-2 (0.44±0.02) and minimum DW-1 (0.20±0.01). The controlled *Labeo rohita* showed non-significantly ($p>0.05$) different accumulation of Chromium values (0.02±0.00) compared to all drain water concentration exposed fish. In the muscle tissues of *Cirrhinus mrigala*, the mean Chromium accumulation values were maximum from the drain water concentration DW-2 (0.47±0.02) and minimum from DW-1 (0.25±0.02) (Table 2). In *Cirrhinus mrigala*, the controlled fish showed non-significantly ($p>0.05$) different accumulation of Chromium values (0.03±0.00) as compared to experimental fish (Table 3).

Table 2. Analysis of variance (mean squares) of heavy metals accumulated in skeletal muscle tissues of all the three fish species.

Source of variation	Degrees of freedom	Mean squares									
		Cu	Cr	Mn	Ni	Cd	Co	Sn	Hg	Zn	Pb
Species (S)	2	0.1107**	0.02328*	0.19333**	0.01622NS	0.03502*	0.03040**	0.0491**	0.05028**	0.03474**	0.16982**
Treatment (T)	5	0.1610**	0.06734**	0.09508**	0.15438**	0.16560**	0.02510**	0.1999**	0.09332**	0.03924**	0.11982**
S x T	10	0.0134NS	0.00122NS	0.00902NS	0.00583NS	0.00397NS	0.00157	0.0074**	0.01124	0.00321	0.00743*
Error	36	0.0114	0.00514	0.00428	0.00992	0.01022	0.00353	0.0020	0.00536	0.00240	0.00320
Total	53										

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01)

Table 3. DMR-test showing the comparison of mean (±SE) of heavy metals accumulated in skeletal muscle tissues of all the three fish species.

	Cu	Cr	Mn	Ni	Cd	Co	Sn	Hg	Zn	Pb
C. catla	0.32±0.03b	0.30±0.02b	0.34±0.02c	0.28±0.03a	0.32±0.03b	0.24±0.01b	0.28±0.03c	0.25±0.02b	0.21±0.01b	0.27±0.02c
L. rohita	0.38±0.04b	0.35±0.03ab	0.41±0.03b	0.34±0.04a	0.38±0.04ab	0.30±0.02a	0.35±0.04b	0.30±0.03b	0.26±0.02a	0.36±0.03b
C. mrigala	0.49±0.05a	0.38±0.03a	0.56±0.04a	0.34±0.05a	0.41±0.05a	0.33±0.02a	0.40±0.05a	0.37±0.04a	0.30±0.02a	0.48±0.04a

Continued.....

C. catla	Control water	0.02±0.00g	0.02±0.00g	0.05±0.00g	0.03±0.01g	0.02±0.00g	0.03±0.00g	0.03±0.00g	0.02±0.00g	0.04±0.01g	0.02±0.00g
	DW-1	0.20±0.01f	0.17±0.01f	0.24±0.02f	0.16±0.01f	0.19±0.02f	0.19±0.04f	0.19±0.01f	0.19±0.02f	0.18±0.02f	0.19±0.01f
	DW-2	0.48±0.06c-f	0.38±0.02c-f	0.48±0.04c-f	0.43±0.03c-f	0.45±0.04c-f	0.28±0.01c-f	0.42±0.01c-f	0.38±0.02c-f	0.25±0.01c-f	0.32±0.02c-f
	DW-3	0.37±0.02b-e	0.28±0.04b-e	0.33±0.02b-e	0.29±0.08b-e	0.44±0.06b-e	0.25±0.03b-e	0.37±0.03b-e	0.26±0.04b-e	0.18±0.03b-e	0.39±0.03b-e
	DW-4	0.27±0.03f	0.33±0.07f	0.32±0.02f	0.22±0.05f	0.22±0.01f	0.22±0.02f	0.16±0.01f	0.18±0.01f	0.18±0.01f	0.21±0.01f
	DW-5	0.31±0.01ef	0.34±0.02ef	0.35±0.02ef	0.32±0.03ef	0.29±0.01ef	0.25±0.02ef	0.27±0.02ef	0.26±0.02ef	0.24±0.01ef	0.26±0.02ef
L. rohita	Control water	0.02±0.01g	0.02±0.00g	0.05±0.01g	0.03±0.00g	0.02±0.00g	0.04±0.00g	0.02±0.00g	0.02±0.00g	0.04±0.00g	0.02±0.00g
	DW-1	0.23±0.01ef	0.20±0.01ef	0.28±0.02ef	0.14±0.01ef	0.23±0.01ef	0.24±0.04ef	0.23±0.01ef	0.15±0.01ef	0.16±0.01ef	0.22±0.01ef
	DW-2	0.58±0.12 bcd	0.44±0.02 bcd	0.55±0.03 bcd	0.52±0.02 bcd	0.51±0.06 bcd	0.36±0.02 bcd	0.54±0.02 bcd	0.46±0.02 bcd	0.39±0.04 bcd	0.44±0.02b cd
	DW-3	0.45±0.02b	0.33±0.05b	0.41±0.02b	0.34±0.08b	0.55±0.08b	0.31±0.02b	0.41±0.03b	0.32±0.01b	0.23±0.05b	0.53±0.06b
	DW-4	0.29±0.03def	0.36±0.08def	0.35±0.02def	0.28±0.07def	0.26±0.03def	0.24±0.04def	0.16±0.02def	0.20±0.03def	0.22±0.03def	0.27±0.02def
	DW-5	0.33±0.02 c-f	0.41±0.03 c-f	0.44±0.04 c-f	0.44±0.04 c-f	0.34±0.02 c-f	0.36±0.02 c-f	0.39±0.05 c-f	0.36±0.02 c-f	0.30±0.02 c-f	0.33±0.02c-f
C. mrigala	Control water	0.03±0.00g	0.03±0.00g	0.05±0.01g	0.03±0.01g	0.02±0.00g	0.04±0.01g	0.03±0.00g	0.02±0.00g	0.05±0.01g	0.02±0.00g
	DW-1	0.27±0.03c-f	0.25±0.02c-f	0.37±0.04c-f	0.21±0.02c-f	0.25±0.02c-f	0.24±0.01c-f	0.24±0.02c-f	0.23±0.01c-f	0.25±0.01c-f	0.31±0.04c-f
	DW-2	0.67±0.09 bc	0.47±0.02 bc	0.66±0.03 bc	0.58±0.02 bc	0.47±0.12 bc	0.38±0.02 bc	0.65±0.04 bc	0.41±0.08 bc	0.44±0.04 bc	0.46±0.05b c
	DW-3	0.53±0.04bc	0.38±0.02a	0.72±0.09a	0.32±0.09a	0.62±0.10a	0.38±0.02a	0.54±0.02a	0.53±0.12a	0.27±0.05a	0.72±0.04a
	DW-4	0.34±0.04a	0.34±0.06bc	0.47±0.04bc	0.20±0.04bc	0.30±0.03bc	0.27±0.04bc	0.22±0.01bc	0.24±0.02bc	0.25±0.02bc	0.46±0.04b c
	DW-5	0.65±0.15bc	0.45±0.06bc	0.60±0.04bc	0.37±0.12bc	0.43±0.09bc	0.37±0.08bc	0.34±0.04bc	0.43±0.03bc	0.30±0.03bc	0.47±0.05b c

Means sharing similar letter within a cell are statistically non-significant (P>0.05)

DW-1 = Drain water concentration 1, DW-2 = Drain water concentration 2, DW-3 = Drain water concentration 3,

DW-4 = Drain water concentration 4,

DW-5 = Drain water concentration 5

Manganese (Mn) accumulation

Maximum mean Manganese values were accumulated by the fish species *Cirrhinus mrigala* (0.56±0.04) and minimum in *Catla catla* (0.34±0.02). In the muscle tissues treated with drain water concentrations in aquaria showed the highly significant (p<0.01) differences in different fish species and drain water concentrations but remained non-significant (p>0.05) with respect to species and drain water concentration interaction (Table 2). Maximum mean Manganese values were accumulated by the fish species *Cirrhinus mrigala* (0.56±0.04) and minimum in *Catla catla* (0.34±0.02).

In the muscle tissues of *Catla catla*, the mean Manganese accumulation values were maximum from the drain water concentration DW-2 (0.48±0.04) and minimum from DW-1 (0.24±0.02). In *Catla catla*, the controlled fish showed non-significantly (p>0.05) different accumulation of Manganese values (0.05±0.01) as compared to experimental fish. In the muscle tissues of *Labeo rohita*, the mean Manganese accumulation values were maximum from the drain water concentration DW-2 (0.55±0.03) and minimum from DW-1 (0.28±0.02). The controlled *Labeo rohita* showed non-significantly (p>0.05) different accumulation of Manganese values

(0.05±0.01) compared to all drain water concentration exposed fish. In the muscle tissues of *Cirrhinus mrigala*, the mean Manganese accumulation values were maximum from the drain water concentration DW-3 (0.72±0.01) and minimum from DW-1 (0.37±0.05). In *Cirrhinus mrigala*, the controlled fish showed non-significantly ($p>0.05$) different accumulation of Manganese values (0.05± 0.01) as compared to experimental fish (Table 3).

Nickel (Ni) accumulation

Nickel accumulation by fish muscle tissues treated with drain water concentrations in aquaria showed the non-significant ($p<0.01$) differences in different fish species and drain water concentrations interactions while remained highly significant ($p>0.01$) with respect to drain water concentration (Table 2). Maximum mean Nickel accumulations were recorded in *Cirrhinus mrigala* (0.34±0.05) and minimum in *Catla catla* (0.28±0.03). In the muscle tissues of *Catla catla*, the mean Nickel accumulation values were maximum from the drain water concentration DW-2 (0.43±0.03) and minimum from DW-1 (0.16±0.01). In *Catla catla*, the controlled fish showed non-significantly ($p>0.05$) different accumulation of Nickel values (0.03±0.01) as compared to experimental fish. In the muscle tissues of *Labeo rohita*, the mean Nickel accumulation values were maximum from the drain water concentration DW-2 (0.52±0.02) and minimum from DW-1 (0.14±0.01). The controlled *Labeo rohita* showed non-significantly ($p>0.05$) different accumulation of Nickel values (0.03±0.01) compared to all drain water concentration exposed fish. In the muscle tissues of *Cirrhinus mrigala*, the mean Nickel accumulations were maximum from the drain water concentration DW-2 (0.58±0.02) and minimum from DW-4 (0.20±0.04). In *Cirrhinus mrigala*, the controlled fish showed non-significantly ($p>0.05$) different accumulation of Nickel values (0.03±0.01) as compared to experimental fish (Table 3).

Cadmium (Cd) accumulation

Cadmium accumulation by fish muscle tissues treated with drain water concentrations in aquaria showed the significant ($p<0.05$) differences in different fish species and highly significant ($p<0.01$) in drain water concentrations while remained non-significant ($p>0.05$) with respect to species and drain water concentration interaction (Table 2). Maximum mean Cadmium accumulations were recorded in *Cirrhinus mrigala* (0.41±0.05) and minimum in *Catla catla* (0.32±0.03). In the muscle tissues of *Catla catla*, the mean Cadmium accumulation values were maximum from the drain water concentration DW-2 (0.45±0.04) and minimum from DW-1 (0.19±0.02). In *Catla catla*, the controlled fish showed significantly ($p<0.05$) different accumulation of Cadmium values (0.02±0.00) as compared to experimental fish. In the muscle tissues of *Labeo rohita*, the mean Cadmium accumulations were maximum from the drain water concentration DW-3 (0.55±0.08) and minimum from DW-1 (0.23±0.01). The controlled *Labeo rohita* showed significantly ($p<0.05$) different accumulation of Cadmium values (0.02±0.00) compared to all drain water concentration exposed fish. In the muscle tissues of *Cirrhinus mrigala*, the mean Cadmium accumulations were maximum from the drain water concentration DW-3 (0.62±0.10) and minimum from DW-1 (0.25±0.02). In *Cirrhinus mrigala*, the controlled fish showed significantly ($p<0.05$) different accumulation of Cadmium values (0.02±0.00) as compared to experimental fish (Table 3).

Cobalt (Co) accumulation

Maximum mean Cobalt accumulations were accumulated by the fish species *Cirrhinus mrigala* (0.33±0.02) and minimum from *Catla catla* (0.24±0.01). In the muscle tissues of *Catla catla*, the mean Cobalt accumulations were maximum from the drain water concentration DW-2 (0.28±0.01) and minimum from DW-1 (0.19±0.04). In *Catla catla*, the controlled fish showed significantly ($p<0.05$) different accumulation of Cobalt values (0.03±0.00) as compared to experimental fish. In the muscle tissues of *Labeo rohita*, the mean Cobalt accumulations were maximum from the drain water concentration DW-2 and DW-5 (0.36±0.02) and minimum from DW-1 and DW-4 (0.24±0.01).

In the muscle tissues of *Cirrhinus mrigala*, the mean Cobalt accumulations were maximum from the drain water concentration DW-2 and DW-3 (0.38±0.02) and minimum from DW-1 (0.24±0.01).

In *Cirrhinus mrigala*, the controlled fish showed significantly ($p<0.05$) different accumulation of Cobalt values (0.04±0.01) as compared to experimental fish (Table 3).

Stannous (Sn) accumulation

Stannous accumulation by fish muscle tissues treated drain water concentrations in aquaria showed the highly significant ($p<0.01$) differences in different fish species and drain water concentrations and species and drain water concentration interaction (Table 2). Maximum mean Stannous accumulation values were recorded in *Cirrhinus mrigala* (0.40±0.05) and minimum in *Catla catla* (0.28±0.03). In the muscle tissues of *Catla catla*, the mean Stannous accumulation were maximum from the drain water concentration DW-2 (0.42±0.01) and minimum from DW-4 (0.16±0.01). In *Catla catla*, the controlled fish showed significantly ($p<0.05$) different accumulation of stannous values (0.03±0.00) as compared to experimental fish. In the muscle tissues of *Labeo rohita*, the maximum mean Stannous accumulation values were recorded from drain water concentration DW-2 (0.54±0.02) and minimum from DW-4 (0.16±0.02). The controlled *Labeo rohita* showed significantly ($p<0.05$) different accumulation of Stannous values (0.02±0.00) compared to all drain water concentration exposed fish. In the muscle tissues of *Cirrhinus mrigala*, the maximum mean Stannous accumulation values were detected from the drain water concentration DW-2 (0.65±0.04) and minimum from DW-4 (0.22±0.01). In *Cirrhinus mrigala*, the controlled fish showed significantly ($p<0.05$) different accumulation of Stannous values (0.03±0.00) as compared to experimental fish (Table 3).

Mercury (Hg) accumulation

Mercury accumulation in fish muscle tissues treated with drain water concentrations in aquaria showed the highly significant ($p<0.01$) differences in different fish species and drain water concentrations while remained non-significant ($p>0.05$) with respect to species and drain water concentration interaction (Table 2). Maximum mean mercury accumulation values were recorded in *Cirrhinus mrigala* (0.37 ± 0.04) and minimum in *Catla catla* (0.25±0.02). In the muscle tissues of *Catla catla*, the mean mercury accumulation was maximum from the drain water concentration DW-2 (0.38±0.02) and minimum from DW-4 (0.18±0.01). In *Catla catla*, the controlled fish showed significantly ($p<0.05$) different accumulation of Mercury values (0.0±0.00) as compared to experimental fish. In the muscle tissues of *Labeo rohita*, the mean mercury accumulation values were maximum from the drain water concentration DW-2 (0.46±0.02) and minimum from DW-1 (0.15±0.01). In the muscle tissues of *Cirrhinus mrigala*, the mean mercury accumulation values were maximum from the drain water concentration DW-3 (0.53±0.12) and minimum from DW-1 (0.23±0.01). In *Cirrhinus mrigala*, the controlled fish showed significantly ($p<0.05$) different accumulation of Mercury values (0.02±0.00) as compared to experimental fish (Table 3).

Zinc (Zn) accumulation

Zinc accumulation by fish muscle tissues treated with drain water concentrations in aquaria showed the highly significant ($p<0.01$) differences in different fish species and drain water concentrations while remained non-significant ($p>0.05$) with respect to species and drain water concentration interaction (Table 2). Maximum mean Zinc accumulation values were recorded in *Cirrhinus mrigala* (0.30±0.02) and minimum in *Catla catla* (0.21±0.01). In the muscle tissues of *Catla catla*, the maximum mean Zinc accumulation values were detected from the drain water concentration DW-2 (0.25±0.01) and minimum from DW-4 (0.18±0.01). In *Catla catla*, the controlled fish showed significantly ($p<0.05$) different accumulation of Zinc values (0.04±0.01) as compared to experimental fish. In the muscle tissues of *Labeo rohita*, the maximum mean Zinc accumulation values were detected from the drain water concentration DW-2 (0.39±0.04) and minimum from DW-1 (0.16±0.01).

The controlled *Labeo rohita* showed significantly ($p<0.05$) different accumulation of Zinc values (0.04±0.00) compared to all drain water concentration exposed fish. In the muscle tissues of *Cirrhinus mrigala*, the maximum mean Zinc accumulation values were detected from the drain water concentration DW-2 (0.44±0.04) and minimum from DW-1 (0.25±0.01). In *Cirrhinus mrigala*, the controlled fish showed significantly ($p<0.05$) different accumulation of Zinc values (0.05±0.01) as compared to experimental fish (Table 3).

Lead (Pb) accumulation

Lead accumulation by fish muscle tissues treated with drain water concentrations in aquaria showed the highly significant ($p<0.01$) differences in different fish species and drain water concentrations and significant ($p<0.05$) with respect to species and drain water concentration interaction (Table 2). Maximum mean Lead accumulation values were detected in *Cirrhinus mrigala* (0.48±0.04) and minimum in *Catla catla* (0.27±0.02). In the muscle tissues of *Catla catla,* the maximum mean Lead accumulation were detected from the drain water concentration DW-3 (0.39±0.03) and minimum from DW-1 (0.19±0.01). In *Catla catla*, the controlled fish showed significantly ($p<0.05$) different accumulation of Leasd values (0.02±0.00) as compared to experimental fish. In the muscle tissues of *Labeo rohita*, the maximum mean Lead accumulation were detected from the drain water concentration DW-3 (0.53±0.06) and minimum from DW-1 (0.22±0.01). The controlled *Labeo rohita* showed significantly ($p<0.05$) different accumulation of Lead values (0.02±0.00) compared to all drain water concentration exposed fish. In the muscle tissues of *Cirrhinus mrigala*, the maximum mean Lead accumulation were detected from the drain water concentration DW-3 (0.72±0.01) and minimum from DW-1 (0.31±0.04). In *Cirrhinus mrigala*, the controlled fish showed significantly ($p<0.05$) different accumulation of Lead values (0.02±0.00) as compared to experimental fish (Table 3).

Graphical representation

The bar graph represents the mean heavy metals bioaccumulation in all the three fish species. Bioaccumulation of Cu, Cr, Mn, Ni, Cd, Co, Sn, Hg, Zn and Pb was detected in all the three treated fish species. Among all the three treated fish species *Cirrhinus mrigala* showed the maximum mean bioaccumulation of all the selective heavy metals as compared to *Labeo rohita* and *Catla catla*. Higher level of bioaccumulation of Mn, Cu and Pb was detected in the muscle tissues of *Cirrhinus mrigala* as compared to other treated fish species. Whereas *Catla catla* shows the low level of bioaccumulation of all the selective heavy metals when compared with *Labeo rohita* and *Cirrhinus mrigala*.

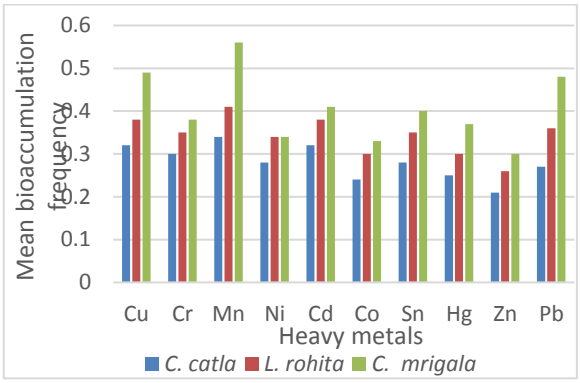

Fig. 1. Mean bioaccumulation of heavy metals in the muscle tissues of all the three fish species.

Discussion

In the present study, the bioaccumulation of selected heavy metals in fish muscle tissues were identified from five different drain water concentrations i.e. 20%, 25%, 30%, 35% and 40%, respectively, Copper bioaccumulation by fish muscle tissues in drain water samples showed the highly significant ($p<0.01$) differences in different fish species and drain water concentrations. Maximum mean Copper bioaccumulation were recorded from *Cirrhinus mirgala* (0.49±0.05) and minimum from *Catla catla* (0.32±0.03) which shows the significant ($p<0.05$) results. The present study is in agreement with Moody *et al.* (2013)'s findings who determined the order of heavy metals accumulation in fishes was Cr >Pb>Cd and their quantity was above the recommended level except for cadmium and unfit for human utilization.

Rauf *et al*., (2009); Cid *et al*., (2001) also reported that heavy metal detection in a fish is an important tool because it will further decide about the possible risks and health concerns for human who feed on the fish of polluted water system. Heavy metals such as Cu, Cd, Zn, Hg, Pb and their derivatives are hazardous for human health. Sabullah *et al*., (2015).

Chromium bioaccumulation by fish muscle tissues treated with drain water samples in aquaria showed the highly significant ($p<0.01$) differences in different fish species and drain water concentrations but remained non-significant ($p>0.05$) with respect to species and drain water concentration interaction. Maximum mean Chromium values were recorded from *Cirrhinus mirgala* (0.38±0.03) and minimum from *Catla catla* (0.30±0.02) which shows the significant results. Heavy Metals such as Pb, Cr, Hg etc. produce chronic poisoning in aquatic life. The findings of Authman *et al*., (2015); Sultana *et al*., (2016) are in line with present study's findings as they reported that these heavy metals naturally exist in very little amount of water and heavy metals such as Cu, Cd, Hg, Zn and Pb are the most important pollutants that are directly or indirectly effecting the fish health.

Manganese bioaccumulation by fish muscle tissues treated with drain water samples in aquaria showed the highly significant ($p<0.01$) differences in different fish species and drain water concentrations but remained non-significant ($p>0.05$) with respect to species and drain water concentration interaction. Maximum mean Manganese values were recorded from *Cirrhinus mirgala* (0.56±0.04) and minimum from *Catla catla* (0.34±0.02) which shows the significant ($p<0.05$) results. The bioaccumulation of Cr, Cd and Mn in greater concentration than those permissible for human consumption by the WHO standards are the most alarming results that reflect the conformity of pollution in the aquatic environment. Shakir *et al*., (2015) and Sultana *et al*., (2016) also supported present study's findings.

Nickel bioaccumulation by fish muscle tissues treated with drain water samples in aquaria showed the highly significant ($p<0.01$) differences in different fish species and drain water concentrations but remained non-significant ($p>0.05$) with respect to species and drain water concentration interaction. Maximum mean Nickel values were recorded from *Cirrhinus mirgala* (0.34±0.05) and minimum from *Catla catla* (0.28±0.03) which shows the significant ($p<0.05$) results. Overall in the muscle tissues of Indian major carps, the mean Cadmium values were maximum from the drain water concentration-3 (0.54±0.05) and minimum from drain water concentration-1 (0.22±0.01). Maximum mean Cadmium values were recorded from *Cirrhinus mirgala* (0.41±0.05) and minimum from *Catla catla* (0.32±0.03) which showed the significant ($p<0.05$) results. Maximum mean Cobalt values were recorded from *Cirrhinus mirgala* (0.33±0.02) and minimum from *Catla catla* (0.24±0.01) which shows the significant ($p<0.05$) results. Overall in the muscle tissues of Indian major carps, the mean Stannous values were maximum from the drain water concentration-2 (0.53±0.04) and minimum from drain water concentration-4 (0.18±0.01). Maximum mean Stannous values were recorded from *Cirrhinus mirgala* (0.40±0.05) and minimum from *Catla catla* (0.28±0.03) which shows the significant ($p<0.05$) results.

Mercury bioaccumulation in fish muscle tissues treated with drain water samples in aquaria showed the highly significant ($p<0.01$) differences in different fish species and drain water concentrations but remained non-significant ($p>0.05$) with respect to species and drain water concentration interaction. Maximum mean Mercury values were recorded from *Cirrhinus mirgala* (0.37±0.04) and minimum from *Catla catla* (0.25±0.02) which shows the significant ($p<0.05$) results. Authman *et al*., (2015) reported that the heavy metals such as mercury influence the vital operations and reproduction of fish and induce pathological changes supporting present study's findings.

Zinc accumulation by fish muscle tissues treated with drain water samples in aquaria showed the highly significant ($p<0.01$) differences in different fish species and drain water concentrations but remained non-significant ($p>0.05$) with respect to species and drain water concentration interaction (Table 4.7).

Maximum mean Zinc values were from *Cirrhinus mirgala* (0.30±0.02) and minimum from *Catla catla* (0.21±0.01) which shows the significant ($p<0.05$) results. Lead accumulation by fish muscle tissues treated with drain water samples in aquaria showed the highly significant ($p<0.01$) differences in different fish species and drain water concentrations but remained non-significant ($p>0.05$) with respect to species and drain water concentration interaction. Maximum mean Lead levels were recorded from *Cirrhinus mirgala* (0.48±0.04) and minimum from *Catla catla* (0.27±0.02) which shows the significant ($p<0.05$) results. Bioaccumulation of the metals even in trace amount in fish tissues can cause potential danger to human health when consumed on regular basis (Noor & Zutshi, 2016; Junianto *et al*, 2017).

The results of present study reflected the significant ($p<0.05$) response of fish species by the exposure of different drain water concentrations in glass aquaria. The accumulation of heavy metals in muscle tissues of different fish species showed highly significant ($p<0.01$) variations. The order of accumulation of heavy metals in muscle tissues of fish species was as: *Cirrhinus mirgala* > *Labeo rohita* > *Catla catla*.

It means, *Cirrhinus mirgala* showed maximum sensitivity and low resistance for the accumulation of heavy metals as compared to *Labeo rohita* and *Catla catla*. In the same context, *Catla catla* showed maximum resistance and low sensitivity for the accumulation of heavy metals than other two fish species. Heavy metal concentrations in the tissue of freshwater fish vary considerably among different studies (Hayat *et al*., 2007; Naz & Javed, 2013; Jamdade & Gawande, 2017). All the treated fish species showed the maximum bioaccumulation of heavy metals from DW-5 and minimum from DW-1 which shows that drain water concentration was directly influenced the bioabsorbance of heavy metals. As DW-5 has 40% drain water concentration as compared to DW-1 that contains 20% drain water concentration. Hence, increased concentration of drain water elevates the bioaccumulation of heavy metals. Moreover, fish sensitivity and fish resistance play a very important role for the determination of heavy metals absorbance.

These findings are in accordance with the findings of several workers such as Ambreen *et al*., (2015); Shakir *et al*., (2015); Noor & Zutshi, (2016) and Sultana *et al*., (2016). Results of present study confirm that *Cirrhinus mirgala* shows more bioaccumulation of selected heavy metals in the muscle tissues when compared with *Labeo rohita* and *Catla catla*.

Conclusion

Bioaccumulation of heavy metals in the muscle tissues of fish species is reliable tool for the assessment of environmental pollution. The findings of this study are helpful as an early warning for environmental monitoring strategies and for the evaluation of eco-toxicological impacts of pollutions.

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