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Metal Bioaccumulation Patterns in Major Carps during Acute Toxicity Tests

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ABSTRACT

Laboratory tests were conducted on juvenile major carps viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* to determine zinc (Zn), lead (Pb), nickel (Ni) and manganese (Mn) bio-accumulation patterns during acute toxicity LC_{50} and lethal test at constant water temperature, pH and total hardness. Significant direct relationships were observed among metal LC_{50} and lethal concentrations that were responded in terms of metal accumulation in fish body. The present findings also demonstrated that the metal contents of fish body before exposure had a direct impact on 96h LC_{50} values of respective heavy metal and the susceptibility of fish. The differences among fish species for their ability to accumulate different metals in their bodies appeared to be species specific. Regarding overall ability of fish to concentrate metals in their bodies, *C. mrigala* exhibited the highest tendency, followed by that of *L. rohita* and *C. catla*. However, the difference between *C. catla* and *L. rohita*, to accumulate metals in their bodies during 96 h LC_{50} tests was statistically non-significant. The ability of all the three fish species to concentrate manganese in its body was significantly maximum followed by that of zinc, nickel and lead, respectively. Lead accumulation in fish body was significantly less than that of other metals during 96-h lethal toxicity tests. However, the ability of fish to accumulate manganese and zinc and lead and nickel showed statistically non-significant differences during 96-h LC_{50} tests.

Key Words: Bio-accumulation; Acute; Major carps; Zinc; Lead; Nickel; Manganese

INTRODUCTION

Ominously increasing human population and establishment of industries in the urban areas of the Punjab province has resulted in the discharge of untreated industrial and sewage wastes, containing heavy metals and their compounds, into the rivers. This polluted water is causing serious effects on the aquatic life including fish as indigenous major carps are on the verge of extinction in the rivers (Ubaidullah *et al*., 2004; Rauf *et al*., 2009a,b). Unlike other types of contaminants, heavy metals can not be eliminated since they occur all over the world. Instead, efforts can be made to control the human activities that release them untreated into the aquatic environment. Fish have been used in scientific research for a long time, but less than other animals such as rats and mice. However, their use has been increased since 1960s. Fish represents the oldest and most diverse class of vertebrates, comprising around 48% of the known member species in the sub-phylum Vertebrata (Altman & Dittmer, 1972).

Ecotoxicology is an important scientific discipline dealing with to describe and predict the behavior of substances present in a specific environment and the response of biological systems and ultimately assessing the risks associated with different toxicants. By "behavior of substances" is meant their fate, toxicity and specificity of action, whereas the response of biological systems involves defense and adaptation, stress reaction and recovery. The sub-disciplines that deal with these aspects have developed relatively independently (Forbes & Forbes, 1994). Moreover, bridging levels of biological organization (from subcellular structures to the bio-sphere) and chemical organization (from elements to complex molecules) are the unresolved issues (Clements & Kiffney, 1994). Therefore, ecotoxicological research focuses on logical rationalization, historical explanation and mechanistic understanding (Cairns, 1990). So far, different studies have demonstrated the promise for unifying sub-disciplines e.g., bio-accumulation and toxicity (McKim & Schmieder, 1991; Javed & Mahmood, 2000; Abdullah *et al*., 2003), levels of biological organization (Pahl-Wostl & Ulanowicz, 1993; Javed, 2003; Ubaidullah *et al*., 2004) or levels of chemical organization (Birge & Cassidy, 1983; Kishino & Kobayashi, 1995; Warne & Hawker, 1995). However, a conceptual synthesis is still deficient.

In the acute toxicity test, juvenile fish are exposed for specified period of time e.g., 96-h to a range of toxicant concentrations in a static system. In aquatic ecotoxicology the term lethal is referred to causing death, or sufficient to cause death, while sub-lethal means the concentration or

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level that would not cause death; effect that is not directly lethal. However, acute coming speedily to a crises or end point; happening quickly. An acute effect could be sub-lethal. For fish, the term customarily is used for effects that occur within 4-7 days. It can also refer to the duration of exposure (e.g., an acute test). LC_{50} refers to a median lethal concentration; the concentration of a substance that is estimated to kill half of a group of organisms. However, the duration of exposure must be specified (Schreck & Moyle, 1990). A toxic effect is determined by a statistically significant decrease in the survival rate of fish exposed to the toxicant relative to the survival of fish in a control (i.e., toxicant is absent). Under normal circumstances, metals, which are mainly beneficial, indeed essential, such as zinc and copper, may become pollutants when present in excess by exhibiting toxic effects on organisms (Mason, 1991). Thus, the stress response of fish is comparable in many ways to that occurring in higher vertebrates. Therefore, effects of pollutants bioaccumulation on fish are evaluated by acute and chronic toxicity tests. The objective of this study was to investigate the accumulation of heavy metals in fish body during acute exposure.

MATERIALS AND METHODS

The concentrations of metals viz. zinc, lead, nickel and manganese were determined in the fish viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* (whole body) of three age groups before and after acute toxicity tests in the wet laboratory of Fisheries Research Farms, Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan. Fish stock (induced bred) of desired weight and age were obtained from the Fish Seed Hatchery, Faisalabad, Pakistan. They were brought to the wet laboratory and acclimated to laboratory conditions for 14 days. Fish were subjected to 12 h photoperiod using florescent light. Fish were fed with crumbled feed (35% digestible protein & 2.90 kcal g^{-1} digestible energy) during adaptations, but they were not fed during the last 24 h of adaptations and throughout the test duration.

All glassware and aquariums used in this experiment were washed with nitric acid and thoroughly rinsed with deionized water prior to use. Pure chloride compounds of metals viz. zinc ($ZnCl_2$), lead ($PbCl_2$), nickel ($NiCl_2$. $6H_2O$) and manganese ($MnCl_2$. $4H_2O$) were used in these experiments. Prior to each trial, all aquariums (70-L capacity) were filled with 50-L dechlorinated tap water of desired hardness (100 mg L^{-1}) and pH (7). However, temperature of water was maintained at 30°C by using automated heaters throughout the experimental period.

To maintain the pH value of the test mediums, NaOH (to increase pH) and HCl (to decrease pH) were added as required. However, to maintain the total hardness, the salts of $CaSO_4$ and $MgSO_4$ (to increase hardness), while ethylenediaminetetraacetic acid and its sodium salts (EDTA) were used to decrease water hardness.

Ten fish of each species were placed into separate aquaria for acclimation. In order to not stress the fish, the concentration of metals in the aquaria were increased gradually, with 50% test concentration being reached in 3.50 h and full toxicant concentration in 7 h. Each test was conducted with three replications for each species of fish. During all the trials, constant air was supplied to the test mediums with air pumped through a capillary system. For LC_{50} and lethal acute toxicity trials for each metal, the concentrations tested for three fish species, separately, were started from zero with an increment of 0.05 and 5 mg L^{-1} (as total concentration) for low and high concentrations, respectively.

Dead fish were weighed individually after being lightly blotted dry at the time of mortality observations. No mortality was observed among control fish. At the end of each test, water samples were taken and analyzed for the corresponding metal concentration by following the methods as described in S.M.E.W.W. (1989). The analytical data obtained confirmed that the determined zinc, lead, nickel and manganese concentrations in the test mediums coincided with the estimated data quite satisfactorily. The concentrations of each metal in the fish (whole body) before and after acute toxicity tests were determined (S.M.E.W.W., 1989). Statistical analyses were performed with SPSS 10.1 Computer program. Differences in metal toxicity means among species were analyzed by Analysis of Variance and Duncan's Multiple Range tests (Steel *et al*., 1996). Relationships among various parameters were determined from Pearson Correlation Coefficients.

RESULTS

Accumulation of metals in fish body during acute toxicity tests: Table I shows metal LC_{50} and lethal profiles of three fish species and age groups after acute exposures to metals during toxicity tests. For this purpose, whole body metal concentrations were considered during acute exposure.

Metal profiles of fish before exposure: Before metal exposure, three fish species showed significantly variable metal concentrations in their bodies (Table II). This significant variability of different metals in fish made the interaction (metals x species) statistically significant at $p<0.01$. Zinc, lead and manganese; zinc and lead; and lead and nickel contents of 30, 60 and 90-days age groups, respectively in three fish species before exposure showed non-significant differences. Mean zinc contents of 30, 60 and 90-days *C. mrigala* were non-significantly higher as 7.58, 13.06 and 14.95 $\mu g g^{-1}$ than that of *L. rohita* and *C. catla* as 6.97, 12.45 and 13.38 $\mu g g^{-1}$ and 6.72, 11.19 and 11.50 $\mu g g^{-1}$, respectively (Table II). The 60-day *C. mrigala* (5.42 $\mu g g^{-1}$) and *L. rohita* (4.16 $\mu g g^{-1}$) exhibited non-significant differences for their body nickel concentrations, while the mean metal concentration in *C. catla* (3.32 $\mu g g^{-1}$) was significantly lower than that of *C. mrigala*. The 30, 60

Table I: Mean LC_{50} and lethal concentrations of three fish age groups

Fish Species	LC_{50} concentrations (±SE) (mgL^{-1})			Lethal concentrations (±SE) (mgL^{-1})		
	30-day	60-day	90-day	30-day	60-day	90-day
Zinc						
Catla catla	20.66 ± 1.35 c*	23.30 ± 1.34 c	25.88 ± 1.28 c	35.90 ± 2.35 c	38.60 ± 2.48 c	39.25 ± 2.52 c
Labeo rohita	26.23 ± 1.35 b	28.36 ± 1.38 b	31.37 ± 1.70 b	41.75 ± 2.92 b	43.81 ± 2.84 b	49.36 ± 3.37 b
Cirrhina mrigala	40.58 ± 3.14 a	47.32 ± 3.30 a	56.67 ± 3.41 a	79.52 ± 6.45 a	85.63 ± 6.37 a	96.38 ± 7.68 a
Lead						
Catla catla	18.86 ± 1.82 c	25.77 ± 1.36 b	26.85 ± 1.64 c	37.75 ± 3.40 b	38.74 ± 2.46 c	43.74 ± 3.00 c
Labeo rohita	22.11 ± 1.66 b	27.20 ± 1.74 b	34.20 ± 1.80 b	38.80 ± 3.19 b	45.24 ± 3.26 b	52.36 ± 3.56 b
Cirrhina mrigala	32.68 ± 2.12 a	39.78 ± 2.15 a	46.10 ± 2.43 a	53.91 ± 4.11 a	62.29 ± 4.04 a	72.36 ± 5.25 a
Nickel						
Catla catla	11.83 ± 1.01 b	13.28 ± 1.10 b	18.99 ± 1.26 c	22.78 ± 1.77 c	25.25 ± 1.99 b	33.17 ± 2.68 c
Labeo rohita	22.01 ± 1.69 a	26.35 ± 2.20 a	29.40 ± 2.13 b	39.14 ± 3.05 b	50.32 ± 4.59 a	51.47 ± 3.88 b
Cirrhina mrigala	23.96 ± 2.40 a	27.62 ± 2.34 a	36.05 ± 2.05 a	52.07 ± 5.30 a	51.91 ± 4.22 a	55.86 ± 3.65 a
Manganese						
Catla catla	55.26 ± 3.67 c	64.67 ± 3.61 c	67.71 ± 3.94 c	92.03 ± 7.20 c	99.59 ± 6.52 c	107.72±6.83b
Labeo rohita	64.13 ± 3.39 b	71.15 ± 3.77 b	73.70 ± 3.64 b	99.82 ± 6.63 b	110.54 ±7.04b	108.74±6.44 b
Cirrhina mrigala	71.24 ± 3.87 a	76.23 ± 4.55 a	91.68 ± 4.65 a	110.43± 6.81a	123.21± 8.11a	142.13±9.66 a

*Means with same letters in a single column for each metals are statistically similar at $p<0.05$

Table II: Mean metals concentrations in three age groups of fish before and after acute exposure

	30-day		60-day		90-day	
	Before exposure	After exposure	Before exposure	After exposure	Before exposure	After exposure
Zinc						
Catla catla	6.72 ± 0.36 a	42.50 ± 1.23 b	11.19±1.01 a	84.59±6.97 b	11.50 ± 0.36 b	100.12 ± 0.21 b
Labeo rohita	6.97 ± 0.15 a	50.99 ± 2.23 a	12.45±1.65 a	110.35±2.13 a	13.38 ± 0.99 a	121.01 ± 2.32 a
Cirrhina mrigala	7.58 ± 0.28 a	52.16 ± 3.56 a	13.06±3.42 a	114.51±4.84 a	14.95 ± 0.89 a	123.05 ± 5.63 a
***Overall means**	**7.09 ± 0.44 B**	**48.55 ± 5.27A**	**12.23 ± 0.95 B**	**103.15 ± 16.21 A**	**13.28 ±1.73 B**	**114.73 ±12.69 A**
Lead						
Catla catla	2.16 ± 0.05 a	20.71 ± 1.36 b	4.37±1.13 a	57.36±1.15 c	6.15 ± 0.99 a	63.14 ± 2.63 c
Labeo rohita	2.29 ± 0.30 a	26.19 ± 1.98 a	4.82±1.93 a	66.30±5.32 bc	7.12 ± 0.09 a	69.12 ± 0.96 bc
Cirrhina mrigala	2.35 ± 0.15 a	28.14 ± 2.09 a	5.35±0.55 a	79.52±3.86 a	7.18 ± 0.87 a	83.16 ± 2.25 a
***Overall means**	**2.27 ± 0.10B**	**25.01 ± 3.85A**	**4.85 ± 0.49B**	**67.73 ± 11.15A**	**6.82 ± 0.58B**	**71.81 ± 10.28A**
Nickel						
Catla catla	2.76 ± 0.87 b	34.53 ± 2.69 b	3.32±0.77 b	84.28±3.58 b	7.23 ± 0.88 a	89.62 ± 3.69 b
Labeo rohita	3.58 ± 0.27 b	38.66 ± 2.63 a	4.16±0.05 ab	107.99±5.45 a	7.26 ± 0.25 a	116.13 ± 3.86 a
Cirrhina mrigala	4.69 ± 0.74 a	38.71 ± 3.00 a	5.42±1.57 a	113.77±5.66 a	7.26 ± 0.74 a	119.25 ± 6.66 a
***Overall means**	**3.68 ± 0.97B**	**37.30 ± 2.40A**	**4.30 ± 1.06B**	**102.01 ± 15.63A**	**7.25 ± 0.02B**	**108.33 ± 16.28A**
Manganese						
Catla catla	8.19 ± 0.21 a	61.61 ± 2.32 c	10.39±0.43b	158.79± 11.11 c	11.11 ± 0.23 b	160.12 ± 1.23 c
Labeo rohita	8.62 ± 0.36 a	67.39 ± 3.99 b	11.65±0.52 ab	168.42±42.43 bc	11.69 ± 0.58 b	168.88 ± 2.52 bc
Cirrhina mrigala	8.73 ± 0.69 a	79.15 ± 1.79 a	12.84±0.63 a	183.39±23.46 a	13.15 ± 0.36 a	186.15 ± 1.95 a
***Overall means**	**8.51 ± 0.29B**	**69.38 ± 8.94 A**	**11.63 ± 1.23B**	**170.20 ± 12.40A**	**11.98 ±1.05 B**	**171.72 ± 13.24A**

Means with same letters in a single column for each metals expect overall means are statistically similar at $p<0.05$

*****Overall means with capital letters in a single row for each age group are statistically different at $p<0.05$

Table III: Correlation coefficients among three age groups and metals in three fish species

Age groups		LC_{50}	Lethal	Before exposure
30-day	Lethal	0.989		
	Before Exposure	0.791	0.811	
	After Exposure	0.887	0.878	0.684
60-day	Lethal	0.999		
	Before Exposure	0.814	0.843	
	After Exposure	0.925	0.901	0.755
90-day	Lethal	0.999		
	Before Exposure	0.855	0.879	
	After Exposure	0.982	0.966	0.882

Critical Value (2-tail, 0.05) = +/- 0.51235

and 90-days *C. mrigala* had the highest mean manganese contents of 8.73, 12.84 and 13.15 μgg^{-1}, respectively followed by that of *L. rohita* and *C. catla*. The difference between *C. catla* and *C. mrigala* for 60 and 90-days fish was significant ($p<0.05$), while the same for 30-day fish was statistically at par.

Metal profiles of fish after exposure: After acute exposure, all the three fish species and age groups showed significant increase in their body metals concentration. The accumulation patterns of metals and three age groups in fish

body varied significantly ($p<0.001$) with significant interaction of metal x species. Final concentration of metals in the three fish species differed significantly. The 30, 60 and 90-days *C. catla* (42.50, 84.59 & 100.12 μgg^{-1}) had significantly less ability to concentrate zinc in its body than that of *L. rohita* (50.99, 110.35 & 121.01 μgg^{-1}) and *C. mrigala* (52.16, 114.51 & 123.05 μgg^{-1}). However, the difference between *L. rohita* and *C. mrigala* for this accumulation was statistically similar. Lead accumulation in *C. mrigala* was significantly higher than that of *L. rohita* and *C. catla.* Lead accumulations in 60 and 90-days *C. catla* and *L. rohita* did not vary significantly, while the same for 30-day was significant ($p<0.05$). *C. mrigala* exhibited significantly highest ability to concentrate nickel in its body, followed by that of *L. rohita* and *C. catla*. The 30, 60 and 90-days fish followed the similar trend for nickel accumulation in their bodies. The accumulation of manganese, during acute toxicity trials, in 30, 60 and 90-days *C. mrigala* were significantly higher than that of *L. rohita* and *C. catla* . However, 60 and 90-days *C. catla* and *L. rohita* showed non-significant ($p<0.05$) differences for their ability to concentrate this metal in their bodies (Table II).

Regarding overall ability to concentrate all metals in fish body before and after acute exposures, *C. mrigala* showed the highest tendency, followed by that of *L. rohita* and *C. catla* (Table II). However, before exposure the differences among 30-day *C. catla, L. rohita* and *C. mrigala* were statistically at par for zinc, lead and manganese. Mean ability of all the three fish species of different age groups to concentrate manganese in its body after acute exposure was significantly maximum, followed by that of zinc, nickel and lead, respectively. In all age groups of three fish species before and after acute exposures almost lead accumulation were significantly lesser than that of the other metals. However, before exposure the differences for the accumulation of zinc and nickel in 60 and 90-days fish were statistically non-significant at $p<0.05$ (Table II).

Correlation among LC_{50} and lethal concentrations for the three age groups, fish species and concentration of all metals before and after acute exposures showed highly significant correlation coefficients. Therefore, it is evident from the data that the species and age groups showing higher values of LC_{50} and lethal concentrations exhibited higher tendency to accumulate metals in their bodies during acute toxicity exposures that were directly dependent upon the concentration of metals in their bodies before exposure and hence appeared species specific for such accumulations (Table III).

DISCUSSION

The present investigation reveals that all three fish species showed highly significant differences for their tolerance limits (determined as LC_{50} & lethal responses) for the five metals. 96-h LC_{50} concentrations of metals also varied significantly among the three fish species with age. 30-day fish were more sensitive than that of 60 and 90-days to metallic ion concentrations in all tests. Giguere *et al.* (2004) reported that heavy metal concentration in fish increased with age that exerted significant impact on the tolerance limits of fish. The present investigation reveals that before exposure to zinc, the mean metal contents of *C. mrigala* were significantly higher than that of *L. rohita* and *C. catla*. However, *C. mrigala* and *L. rohita* showed non-significant differences for their body nickel concentrations which were significantly different to that of *Catla catla*. After acute exposure of metals, all the three fish species showed significant increase in their body metal concentrations. The accumulation patterns of metals in the fish varied significantly ($p<0.001$). These differences among fish species for their ability to accumulate different heavy metals in their bodies, during exposure period, appeared to be species specific as evident from the data regarding various metal profiles of three fish species that were variable before acute metal exposure experiments. Javid *et al.* (2007) reported that *C. catla* was more sensitive to nickel concentrations followed by that of *L. rohita* and *C. mrigala.* The profile of copper accumulation among tissues in rockfish showed dependence upon exposure time and metal concentration (Kim & Kang, 2004). Kim *et al.* (2004) reported an increased cadmium accumulation in fish tissues with exposure periods and concentration and metal accumulation in gill and liver increased linearly with the exposed time. Adhikari *et al.* (2006) reported linear relationship between increasing pH/alkalinity and decreasing accumulation of lead and chromium at variable exposure periods in *L. rohita*. However, they observed non-significant linear relationship for cadmium toxicity to the fish. Therefore, it is evident from the results that previous contents of metal in fish body (before exposure) had significantly direct impact on LC_{50} and lethal values of the respective metals in major carps. The fish having a higher concentration of metals before exposure exhibited significantly higher LC_{50} and lethal concentrations. All three species of fish showed direct relationships with metal concentrations in its body before exposure, LC_{50} and lethal concentrations and the accumulation of metals in fish body during acute exposure of metals. Therefore, this close relationship demonstrated that the previous body contents/accumulations of heavy metals in fish had a direct effect on 96-h LC_{50} values of respective heavy metal and the susceptibility of the fish and thus, showing the tendency of different fish species to concentrate metals in their bodies (Shah & Altindag, 2005). Bio-accumulation of various heavy metals in fish are related to several factors i.e., foraging behavior and feeding habits (Chen & Folt, 2000), trophic status, metal source, distance of the organism from the contamination source and the presence of other ions in the milieu (Giesy & Wiener, 1977) physico-chemical properties of the water (Wepener *et al*., 1992).

During the present investigation, it was observed that

the accumulation of metals in fish tissues increased with elevated metal's toxicity of water. There were significant differences in the survival rate of fish and tissue metal concentrations among the treatment groups. Olaifa *et al*. (2003) reported that fish exposed to sub-lethal levels of lead produced dose-dependent ($p<0.01$) increase in the concentration of lead in the liver and muscle of *Oreochromis mossambicus*. They also reported that the concentration of lead in fish tissues declined on transfer of fish to lead-free water. The recovery of fish was faster for those placed in lower concentrations of lead in higher concentrations. The concentration and distribution of Cr, Cu, Pb and Zn among the tissues of a freshwater fish, *Clarias gariepinus*, exposed to combined (composite) tannery effluent was investigated at two sub-lethal concentrations (2 & 6%) in static bioassay for 8 weeks by Gbem *et al*. (2001). The distributions of four metals in fish were of the order of Pb > Cr > Cu > Zn and the accumulation was found to be dose and time-dependent.

CONCLUSION

Direct relationships observed among metal concentrations, LC_{50} and lethal concentrations, different age groups and the accumulation of metals in fish body during acute exposure of metals demonstrated that the previous body contents/accumulations of heavy metals in fish had a direct impact on 96-h LC_{50} and lethal values of respective heavy metal and the susceptibility of fish. The differences among fish species and age groups for their ability to accumulate different heavy metals in their bodies, during metal exposure period, appeared to be species specific. Regarding overall ability to concentrate all metals in fish bodies, *C. mrigala* showed the highest tendency, followed by that of *L. rohita* and *C. catla.*

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