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Bio-accumulation of Lead in the Bodies of Major Carps (*Catla catla*, *Labeo rohita* and *Cirrhina mrigala*) during 96-h LC_{50} Exposures

ARSHAD JAVID[1], MUHAMMAD JAVED†, SAJID ABDULLAH† AND ZULFIQAR ALI
Department of Wildlife and Ecosystem, University of Veterinary and Animal Sciences, Lahore, Pakistan
†Department of Zoology and Fisheries, University of Agriculture, Faisalabad-38040, Pakistan
[1]Corresponding author's e-mail: arshadjavid@hotmail.com

ABSTRACT

Studies on the 96-h acute lead lethality for three fish species viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* and metal bio-accumulation in their bodies were performed during 96 h LC_{50} exposure in glass aquariums at room temperature. The 96 h LC_{50} and lethal concentration of lead varied significantly among three fish species. *C. catla* were found more sensitive to lead concentration followed by that of *L. rohita* and *C. mrigala.* The oxygen consumption by the fish increased significantly with concomitant increase in metal concentrations. Among three fish species, *L. rohita* accumulated significantly higher lead concentrations than that of *C. catla* and *C. mrigala*. Dissolved oxygen, electrical conductivity, chlorides and sodium showed negatively significant relationships with fish age, while pH, potassium and magnesium showed negatively non-significant relationship with fish age. However, calcium showed positively significant relationship with fish age. It was concluded that from the major carps *C. catla* is more susceptible to lead toxicity, while the *L. rohita* has the higher tendency to accumulate metallic ions.

Key Words: Lead; 96-h LC_{50}; Bio-accumulation; Major carps

INTRODUCTION

In aquatic ecosystems, the heavy metals have received considerable attention due to their toxicity and accumulation in biota (Javed & Hayat, 1999). In fish, the toxic effects of heavy metals may influence physiological functions, individual growth rates, reproduction and mortality (Woodward *et al*., 1994). In Pakistan, the water pollution has become a serious problem due to discharge of un-treated industrial effluents and domestic sewage, containing bulk quantities of toxic heavy metals, into the rivers (Javed, 2005). Trace metals are essential for normal physiological processes abnormally high concentration, however can be toxic to aquatic organisms (Javed, 2003).

Lead exists in several oxidation states, which are of environmental importance. The divalent form of Pb (II) is the stable ionic form present in the environment and is thought to the form in which most lead is bio-accumulated by aquatic organisms. Lead enters the aquatic environment through erosion and leaching from soil, lead-dust fall out, combustion of gasoline, municipal and industrial waste discharges, run-off water deposits from streets and other surfaces as well as precipitation (D.W.A.F., 1996). In natural water, the total lead concentrations generally range between 0.05 and 10.00 mg L^{-1} (Galvin, 1996). The lead is known to accumulate the tissues of fish including the bone, gills, kidneys, liver and scales (Dallas & Day, 1993). The present work was therefore, planned to study the "bio-accumulation of lead in the bodies of major carps during 96-h LC_{50} exposures" to develop strategies regarding sustainable conservation of these species in the riverine systems of the Pakistan.

MATERIALS AND METHODS

An experiment was conducted in glass aquariums of 70 L water capacity in the wet laboratory of Fisheries Research Farms, University of Agriculture, Faisalabad. Ten fish of each species (approximately 3 - 5 g weight) separately, were placed in each aquarium for acclimation. In order to avoid stress to the fish, the concentrations of metal in each aquarium was increased gradually and 50% of test concentration was maintained within 3.5 h and full toxicant concentrations in 7 h. Each test was conducted with three replications. Constant air was supplied to all the test mediums with an air pump through capillary system. Chemically pure chloride compound of lead was dissolved in distilled water and stock solutions were prepared for required metal dilutions. The metal concentrations for each fish species were started from zero with an increment of 0.05 mg L^{-1} and 5 mg L^{-1} (as total concentration) for low and high concentrations, respectively at room temperature. In each test trial, the observations of fish mortality and physico-chemical variables viz. water temperature, pH, total hardness, electrical conductivity, chlorides, calcium, magnesium, dissolved oxygen, total ammonia, sodium, potassium and carbon-dioxide were made at 12 h intervals for 96 h. Dead fish were weighed individually (after being lightly blotted dry) and their total lengths measured at the time of mortality observations. At the start and end of each test trial, water samples were taken from each aquarium and tested for metal concentrations through the methods described in S.M.E.W.W. (1989). The lead toxicity tests were performed on three fish species viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala*, separately for their 96-h LC_{50} and lethal concentrations at room temperature. The 96-h LC_{50} values and their 95% confidence intervals were estimated by using Trimmed Spearman Karber Method (Hamilton *et al*., 1977).

The data on different variables were analyzed statistically through Micro-computer by following Steel *et al.* (1996). Analysis of variance (Factorial Experiment) and Duncan's Multiple Range tests were performed to find-out statistical differences among variables under study. Regression analysis was also computed to find-out relationships among various parameters.

RESULTS

96 h LC_{50}. Analysis of variance on 96 h LC_{50} values of lead for three fish species showed highly significant variations among three fish species. *C. catla* appeared as the most sensitive species of fish to lead, followed by that of *L. rohita* and *C. mrigala* (Table I). The mean 96 h LC_{50} value of *C. catla* was 22.38 ± 3.24 mg L^{-1}, followed by that of *L. rohita* (32.70 ± 2.23 mg L^{-1}) and *C. mrigala* (47.00 ± 4.44 mg L^{-1}).

Increasing lead concentrations in water decreased the dissolved oxygen contents of test mediums. The maximum dissolved oxygen concentrations was recorded as 7.42 ± 1.24 mg L^{-1} at 0.05 mg L^{-1} lead concentration, while the same was lowest as 5.64 ± 2.21 mg L^{-1} at 40 mg L^{-1} lead concentration. Water temperature of test medium fluctuated between the lowest and highest values of 23.39 ± 4.42 and 33.33 ± 5.97°C at concentration gradients of 0.05 and 4.00 mg L^{-1}, respectively. However, the water temperature fluctuations were almost similar at various lead concentrations during this experiment. The maximum and minimum fluctuations in pH were 6.07 and 8.06 at 50 and 0.05 mg L^{-1} lead concentrations, respectively. Total ammonia at various lead concentrations showed variations between 0.78 and 1.58 mg L^{-1} at lead concentrations of 50.00 and 0.05 mg L^{-1}, respectively. The sodium contents of test medium increased with the lead contamination of 0.05 up to 4 mg L^{-1}. However, there was sharp decline in sodium contents of test medium at lead concentration of 20 mg L^{-1}. Potassium contents of water decreased at 20 mg L^{-1} lead concentrations and onwards. The lead concentrations i.e., 2 mg L^{-1} to 50 mg L^{-1} showed higher calcium contents in the test mediums. The magnesium contents were highest at lower lead concentrations from 0.05 to 30 mg L^{-1}. However, the magnesium of test medium decreased significantly from 30 mg L^{-1} lead concentration onwards. Total hardness of test medium was higher at lower level of lead concentration (Table II).

Table I. Comparison of Means for 96-h LC_{50} values of lead for three fish species

Fish species	96-h LC_{50}	Lethal Concentrations
Catla catla	22.38±3.24 c	32.94±5.59 c
Labeo rohita	32.70±2.23 b	42.42±4.24 b
Cirrhina mrigala	47.00±4.44 a	57.10±6.23 a

Means with similar letters in a single column are statistically non-significant at p<0.05

Accumulation in fish body. Table III shows the accumulation pattern of lead in fish body at different metal concentrations of test mediums. There were significant differences for accumulation of this metal at different concentrations of test mediums among three fish species viz. *C. catla*, *L. rohita* and *C. mrigala*. As the metal concentration of test medium increased, the accumulation pattern in fish body also increased significantly. However, among the three fish species *L. rohita* showed significantly higher tendency to accumulate lead as 41.37 ± 31.18 µg g^{-1} followed by that of 34.90 ± 30.27 and 35.12 ± 30.97 µg g^{-1} in *C. catla* and *C. mrigala*, respectively.

Regression studies. Table IV shows regression coefficients among various parameters during acute lethality trials with three fish species viz. *Cat. catla*, *L. rohita* and *C. mrigala*. Dissolved oxygen, electrical conductivity, chlorides and sodium showed negatively significant relationship with fish age, while pH, potassium and magnesium negative and non-significant relationship with fish age. However, calcium showed positively significant relationship with fish age. Fish age showed positively significant relationship with 96-h LC_{50} of metal. Metal concentrations of test medium showed inverse relationships with all physico-chemical characteristics of test medium except temperature, pH, total ammonia and total hardness. Relationship coefficients for electrical conductivity and sodium were highly significant but negative with metal concentrations in water. Water temperature had negative significant relationship with total ammonia contents. However, regression coefficients of total ammonia for dissolved oxygen and total hardness of water were positive but non-significant.

DISCUSSION

Present investigation revealed that 96-h LC_{50} concentrations of lead varied significantly among the three fish species viz. *C. catla*, *L. rohita*, *C. mrigala*. *C. catla* appeared as a more sensitive species that showed significantly lower LC_{50} value (20.00 mg L^{-1}), followed by that of *L. rohita* (30.00 mg L^{-1}) and *C. mrigala* (45.00 mg L^{-1}). However, lethal concentrations of lead for three fish species differed significantly also. Both *L. rohita* and *C. mrigala* had significantly higher lethal concentrations than those of *C. catla*.

Among the three fish species, considerable differences in sensitivity to metals have been reported. Salmonids are

Table II. Mean physico-chemistry of test medium during 96-h LC_{50} trials with three fish species

Trial Concentrations ($mg L^{-1}$)	Dissolved oxygen ($mg L^{-1}$)	Temperature (°C)	pH	Electrical conductivity ($mS cm^{-1}$)	Total Ammonia ($mg L^{-1}$)	Chlorides ($mg L^{-1}$)	Sodium ($mg L^{-1}$)	Potassium ($mg L^{-1}$)	Calcium ($mg L^{-1}$)	Magnesium ($mg L^{-1}$)	Total hardness ($mg L^{-1}$)
0.05	7.42±1.24	23.39±4.42	8.06±1.20	3.53±0.98	1.58±0.59	198.36±11.31	290.12±15.40	8.18±1.22	10.75±2.40	43.28±9.97	200.00±10.20
0.10	7.40±2.23	23.59±4.53	8.05±2.32	3.56±0.97	1.57±0.64	210.12±14.32	294.42±14.91	8.18±1.23	11.00±2.45	40.62±7.21	190.00±11.15
2.0	6.59±1.97	33.19±5.24	6.94±3.31	3.36±1.24	1.46±0.37	202.62±10.44	308.04±15.77	9.23±2.59	18.24±2.97	42.59±4.93	215.96±15.22
4.0	6.58±1.79	33.33±5.97	6.94±2.97	3.47±0.59	1.39±0.95	204.67±9.54	309.08±15.47	9.12±2.78	17.58±3.15	41.42±5.27	209.62±11.11
20.0	6.37±1.54	29.84±2.37	6.92±2.24	2.08±1.11	1.28±0.43	158.12±10.44	160.72±14.44	5.69±1.14	17.30±3.17	38.56±5.44	197.50±15.23
30.0	6.08±1.43	29.68±3.35	6.90±2.22	2.17±1.24	1.24±0.56	169.37±9.55	162.04±13.71	5.96±2.25	17.19±4.25	40.05±4.79	208.12±13.12
40.0	5.80±1.25	29.68±4.71	6.84±2.32	0.76±0.33	0.89±0.19	137.50±6.67	90.00±9.90	2.82±0.59	15.53±5.27	24.41±5.55	136.50±13.27
50.0	5.64±2.21	29.57±6.91	6.07±2.12	0.83±0.27	0.78±0.37	154.83±7.72	91.67±7.97	2.97±1.11	18.75±4.06	24.86±6.99	146.33±13.15

Table III. Accumulation of lead ($\mu g g^{-1}$) in fish body under variable metal concentrations

Species	Concentrations ($mg L^{-1}$)							Overall Means
	0.05	0.10	2.00	4.00	20.00	30.00	45.00	
C. catla	11.13±1.21 e	15.46±5.23 e	19.31±2.77 d	25.10±2.25 c	32.77±5.29 b	41.05±5.54 a	99.49±7.29 b	34.90±30.27
L. rohita	15.41±2.25 d	21.48± 6.43 c	25.99±3.35 b	28.61±2.79 b	44.29±6.43 a	46.69±3.33 a	107.16±11.11 c	41.37±31.18
C. mrigala	14.47±3.49 d	13.49±4.44 d	17.29±5.50 d	26.49±3.39 c	32.29±4.44 b	40.08±5.24 a	101.71±5.95 b	35.12±30.97

Means with same letters in a single row are statistically similar at $p<0.05$

Table IV. Regression coefficients among various parameters under study

	Fish age	Metal concentration	Temperature	Total ammonia
Age	-	0.884*	-	-
Dissolved oxygen ($mg L^{-1}$)	-0.793*	-0.419^{NS}	-0.890*	0.476^{NS}
Temperature (°C)	0.633^{NS}	0.255^{NS}	-	-0.821*
pH	-0.103^{NS}	0.302^{NS}	-	-
Electrical conductivity ($mS cm^{-1}$)	-0.903**	-0.952**	-	-
Total ammonia ($mg L^{-1}$)	-0.175^{NS}	0.119^{NS}	-	-
Chlorides ($mg L^{-1}$)	-0.846*	-0.890*	-	-
Sodium($mg L^{-1}$)	-0.816*	-0.946**	-	-
Potassium ($mg L^{-1}$)	-0.683^{NS}	-0.876*	-	-
Calcium ($mg L^{-1}$)	0.872*	0.629^{NS}	-	-
Magnesium ($mg L^{-1}$)	-0.686^{NS}	-0.711^{NS}	-	-
Total hardness ($mg L^{-1}$)	0.370^{NS}	0.109^{NS}	-	0.009^{NS}

* = Significant at $p<0.05$; ** = Significant at $p<0.01$; NS = Non-significant

generally more sensitive to high cadmium levels. Juvenile trout (*Orcorhynchus mykiss*) showed higher 48-h LC_{50} (Handy, 1992). At water hardness of 100 mg L^{-1} Ca^{2+}, carp fry and fingerlings (*Cyprinus carpio*) showed 96-h LC_{50} of 4.3 mg L^{-1} and 17.10 mg L^{-1} of cadmium, respectively (Suresh *et al*., 1993). Other fish characteristics, such as age, body size, feeding habit and sex can also be considered for variable LC_{50} of metals for different species of fish (Witeska *et al*., 1993). Therefore, it is important to consider the physico-chemical characteristics of the test medium along with biotic factors to know the mechanisms affecting LC_{50} concentrations of fish in toxicity tests.

Electrical conductivity, chlorides, sodium and potassium showed significantly inverse relationship with lead ions in water. According to Nussey *et al*. (2000), bio-accumulation of chromium, lead and manganese varied in different tissues of cyprinid fish (*L. ambratus*) depending on size, gender and season. Waterborne metals generally exhibited their highest toxicity to aquatic organism in soft water of low pH and low dissolved organic carbon (Nogami *et al*., 2000). This is because the hardness cations (Mg & Ca) compete with heavy metal cations for binding sites within the organism. During present investigations, water hardness had non-significant positive correlation coefficient with metallic ions in water. Davies (1992) reported negative correlation between pH and exchangeable zinc from water. Bio-accumulation of metals in fish is a function of metal bio-availability, which can vary with pH, uptake and toxicokinetics (Spry & Wiener, 1991).

The ammonia excretion by the fish decreased at higher metal concentrations. At higher metal concentrations, the dissolved oxygen contents of the test medium declined. This shows that high concentrations of metallic ions induced stress in the fish that resulted in more oxygen consumption and thus, dissolved oxygen concentrations of the test medium declined (Witeska *et al*., 1993). Boqomaov *et al*. (1991) reported an inverse relationship between pH and the concentrations of magnesium, iron, manganese and cobalt. Increase in water temperature enhanced the uptake of metals by the aquatic organism also (Jackson, 1988). During present investigation, pH of water showed positive but non-significant relationship with metallic ions. Temperature of water had non-significantly positive correlation coefficients with metallic ion in water.

Nussey *et al*. (2000) found that bio-accumulation of chromium, nickel and manganese varied significantly in different species of fish depending on size, gender and season. During this investigation as the concentrations of test medium increased the accumulation pattern of lead also increased significantly among the three fish species viz. *C. catla*, *L. rohita* and *C. mrigala*. *L. rohita* showed significantly higher lead accumulation, followed by that of *C. catla* and *C. mrigala*.

CONCLUSION

During 96 h LC_{50} trials the concentrations of lead varied significantly among the three fish species (major carps) with age. *C. catla* was found to be more vulnerable to lead toxicity, while *L. rohita* showed higher tendency of accumulating Pb^{2+}. The bio-accumulation of Pb^{2+} in fish body and the oxygen requirements of fish increased with concomitant increase in metal concentrations.

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