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Nickel Bio-accumulation in the Bodies of *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* During 96-hr LC_{50} Exposures

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ABSTRACT

The experiment was conducted to study the 96-hr acute nickel lethality for three fish species viz. *Catla catla, Labeo rohita* and *Cirrhina mrigala* and metal bio-accumulation in their bodies during 96-hr LC_{50} exposure in glass aquariums at room temperature. The 96-hr LC_{50} and lethal concentrations of nickel varied significantly among the three fish species. *Catla catla* were more sensitive to nickel concentration followed by that of *Labeo rohita* and *Cirrhina mrigala*. The oxygen consumption by the fish increased significantly with concomitant increase in metal concentrations. Among the three fish species, *Labeo rohita* accumulated significantly higher nickel concentration than that of *Catla catla* and *Cirrhina 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.

Key Words: 96-hr LC_{50}; Nickel; Bio-accumulation; Major carps

INTRODUCTION

Fish represent the oldest and most diverse classes of vertebrates, comprising around 48% of the known member species in the sub-phylum Vertebrata. There are many scientific fields that use fish as models in research, including respiratory and cardiovascular research, cell culture, ecotoxicology, aging, pharmacological and genetic studies (Bolis *et al*., 2001). It is well documented that pollutants such as metals or organic compounds can be accumulated by aquatic biota. Bio-accumulation measurements refer to studies or methods monitoring the uptake and retention of pollutants like metals or biocides in organs and/or tissues of organisms, such as fish (Javed & Hayat, 1999).

Nickel (Ni) occurs as four basic ores namely, arsenide, laterite, silicate and sulphide (Galvin, 1996). Anthropogenic activities (i.e. mining, electroplating & steel plant operations) can result in nickel discharge into water and air (Galvin, 1996). Nickel ions tend to be soluble at pH < 6.5 forming mostly insoluble nickel hydroxides (Dallas & Day, 1993). In aquatic ecosystems, dissolved nickel concentrations are generally between 0.005 and 0.010 mg L^{-1} (Galvin, 1996). Nickel toxicity is generally low (Khangarot & Ray, 1990) but elevated concentrations can cause lethal effects in the aquatic ecosystems. Therefore, the present work was planned to study the "nickel bio-accumulation in the bodies of *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* during 96-hr LC_{50} exposures" to develop strategies regarding sustainable conservation of these species in the riverine systems of 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 concentration of metal in each aquarium was increased gradually and 50% of test concentration was maintained within 3.5 h and full toxicant concentration 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 nickel 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 carbondioxide 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 nickel toxicity tests were performed on three fish species viz. *Catla catla, Labeo rohita* and *Cirrhina mrigala*, separately for their 96-hr LC_{50} and lethal concentrations at room temperature. The 96-hr

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-hr LC_{50}. Analysis of variance on 96-hr LC_{50} values of nickel for three fish species showed highly significant variations among three fish species. *Catla catla* appeared as the most sensitive species of fish to nickel, followed by that of *Labeo rohita* and *Cirrhina mrigala* (Table I). The mean 96-hr LC_{50} values of *Catla catla* was 20.36 ± 2.21 mg L^{-1}, followed by that of *Labeo rohita* and *Cirrhina mrigala* as 30.10 ± 3.34 and 45.00 ± 5.72 mg L^{-1}, respectively.

Increasing nickel concentration in water decreased the dissolved oxygen contents of test mediums. The maximum dissolved oxygen concentration was recorded as 7.42 ± 1.24 mg L^{-1} at 0.05 mg L^{-1} nickel concentration, while the same was lowest as 5.64 ± 2.21 mg L^{-1} at 40 mg L^{-1} nickel 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 nickel 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} nickel concentrations, respectively. Total ammonia at various nickel concentrations showed variations between 0.78 and 1.58 mg L^{-1} at nickel concentration of 50.00 and 0.05mg L^{-1}, respectively. The sodium contents of test medium increased with the nickel contamination of 0.05 up to 4.00 mg L^{-1}. However, there was sharp decline in sodium contents of test medium at nickel concentration of 20.00 mg L^{-1}. Potassium contents of water decreased at 20 mg L^{-1} nickel concentration and onwards. The nickel 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 low nickel concentrations i.e. from 0.05 to 30 mg L^{-1}. However, the magnesium of test medium decreased significantly from 30 mg L^{-1} nickel concentration onwards. Total hardness of test medium was higher at lower level of nickel concentration (Table II).

Accumulation in fish body. Table III shows the accumulation pattern of nickel 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. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala.* As the metal concentration of test medium increased, the accumulation pattern in fish body also increased significantly. However, among the three fish species, *Labeo rothita* showed significantly higher tendency to accumulate nickel as 40.36 ± 31.20 µg g^{-1} followed by that of 34.04 ± 30.30 and 34.11 ± 31.20 µg g^{-1} in *Catla catla* and *Cirrhina mrigala,* respectively.

Table I. Analysis of variance on 96-hr LC_{50} values of nickel for three fish species

S.O.V.	Mean Square	F. Value	Probability
Nickel concentration	628.15	19.8988	p<0.01
Species	448.50	14.2078	p<0.01
Error	31.57	-	

Standard Error (S.E.) for concentrations = 3.2438
Standard Error (S.E.) for species = 2.2927

Comparison of means

Fish species	96-hr LC_{50}	Lethal Concentration
Catla catla	20.36±2.21 c	30.98±4.54 c
Labeo rohita	30.10±3.34 b	40.42±4.37 b
Cirrhina mrigala	45.00±5.72 a	55.01±5.23 a

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

Regression studies. Table IV shows regression coefficients among various parameters during acute lethality trials with three fish species viz. *Catla catla*, *Laboe rohita* and *Cirrhina mrigala*. Dissolved oxygen, electrical conductivity, chlorides and sodium showed negatively significant relationship with fish age, while pH, potassium and magnesium showed 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-hr 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 but 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 reveals that 96-hr LC_{50} concentrations of nickel varied significantly among the three fish species viz. *Catla catla, Labeo rohita* and *Cirrhina mrigala*. *Catla catla* appeared as a more sensitive species that showed significantly lower LC_{50} value (20.00 mg L^{-1}), followed by that of *Labeo rohita* (30.00 mg L^{-1}) and *Cirrhina mrigala* (45.00 mg L^{-1}). However, lethal concentrations of nickel for three fish species differed significantly also. Both *Labeo rohita* and *Cirrhina mrigala* had significantly higher lethal concentrations than those of *Catla catla*.

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

Trial Concentration (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 nickel (µg 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**	
Catla catla	10.12±1.27 e	14.46±5.23 e	18.31±2.77 d	24.10±2.23 c	31.75±5.27 b	41.05±5.54 a	98.49±9.24 b	34.04±30.30
Laboe rohita	14.41±2.24 d	20.46±6.42 c	24.97±3.34 b	27.61±2.79 b	42.29±4.41 a	46.64±4.43 a	106.16±10.1 c	40.36±31.20
Cirrhina mrigala	13.46±3.39 d	12.45±4.43 d	16.26±5.49 d	25.49±3.31 c	30.29±4.44 b	40.08±6.27 a	100.71±8.95 b	34.11±31.02

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	-0.175^{NS}	0.119^{NS}	--	--
Chlorides (mgL^{-1})	-0.846^{*}	-0.890^{*}	--	--
Sodium (mgL^{-1})	-0.816^{*}	-0.946^{**}	--	--
Potassium (mgL^{-1})	-0.683^{NS}	-0.876^{*}	--	--
Calcium (mg L^{-1})	0.872*	0.629^{NS}	--	--
Magnesium (mgL^{-1})	-0.686^{NS}	-0.711^{NS}	--	--
Total hardness (mgL^{-1})	0.370^{NS}	0.109^{NS}	--	0.009^{NS}

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

Among the three fish species, considerable differences in sensitivity to metals have been reported. Salmonids are generally more sensitive to high cadmium levels. Juvenile trout (*Orcorhynchus mykiss*) showed higher 48-hr LC_{50} (Handy, 1992). At water hardness of 100 mg L^{-1} Ca^{2+}, carp fry and fingerling (*Cyprinus carpio*) showed 96-hr 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 (Witestka *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 nickel ions in water. According to Nussey *et al*. (2000), bio-accumulation of chromium, nickel and manganese varied in different tissues of cyprinid fish (*Labeo ambratus*) depending on size, gender and season. Waterborne metals generally exhibited their highest toxicity to aquatic organisms 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 concentration of magnesium, iron, manganese and cobalt. Increase in water temperature enhanced the uptake of metals by the aquatic organisms also (Jackson, 1988). During present investigation, the pH of water showed positive but non-significant relationship with metallic ions. Temperature of water had non-significantly positive correlation coefficient 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 concentration of test medium increased, the accumulation pattern of nickel also increased significantly among the three fish species viz. *Catla catla, Labeo rohita* and *Cirrhina mrigala. Labeo rohita* showed significantly higher nickel accumulation, followed by that of *Catla catla* and *Cirrhina mrigala*.

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