STUDIES ON 96-HR LC_{50} AND LETHAL TOXICITY OF METALS TO THE FISH CIRRHINA MRIGALA

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The rivers that are potential breeding grounds of *Cirrhina mrigala* are subject to heavy metals pollution through industrial effluents and domestic sewage. In present investigation, laboratory experiments were conducted to study the acute toxicity of metals viz. iron, zinc, lead, nickel and manganese on the three age groups viz. 30-, 60- and 90-day of *Cirrhina mrigala*. Data obtained from the acute toxicity tests were evaluated by using the Probit Analysis Statistical Method. The study includes the determination of 96-hr LC_{50} and lethal toxicity to the fish. The tests were performed, separately, at constant temperature (30 ^{o}C), pH (7) and hardness (100 mgL^{-1}) of water. Physico-chemical variables viz. dissolved oxygen, total ammonia, sodium, potassium and carbondioxide were also studied during the experiment. The 96-hr LC_{50} and lethal concentrations of all metals varied significantly in fish. This fish showed significantly highest sensitivity (determined LC_{50} as 29.55 ± 5.39 mg L^{-1}) against nickel, followed by that of lead (39.51 ± 5.84 mg L^{-1}), zinc (48.41 ± 6.79 mg L^{-1}), manganese (79.97 ± 8.48 mg L^{-1}) and iron (86.24 ± 16.87 mg L^{-1}). The responses of three fish age groups in terms of LC_{50} and lethal concentrations were statistically significant also. Among the three fish age groups, 90-day fish showed significantly higher tolerance against all metals than that of 60- and 30-day fish. The ammonia excretion by the fish increased significantly with increase in metals concentrations of the mediums. However, carbondioxide contents of the test medium increased at higher metals concentrations.
Key words: *Cirrhina mrigala*, 96-hr LC_{50}, lethal, iron, zinc, lead, nickel, manganese

INTRODUCTION

Ecotoxicology is a young scientific discipline concerned with describing and predicting the behavior of substances in an environment and the response of biological systems and, ultimately assessing the risks associated with emissions. 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 various aspects have developed relatively independently (Forbes and Forbes, 1994). 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 and Dittmer, 1972). From the surrounding water, fish may absorb dissolved heavy metals that may accumulate in various tissues and organs and even be biomagnified in the food-chain/ web. In the absorption process there are four possible routes of metals to enter in a fish: the food ingested; simple diffusion of the metallic ions through gill pores; through drinking water; and by skin adsorption (Sindayigaya *et al.*, 1994). Amongst fish species, considerable differences in sensitivity to metals have been reported. Salmonids are generally sensitive to high cadmium levels (Suresh *et al.*, 1993). Carp (*Cyprinus carpio*) and *Catla catla* have the ability to accumulate and concentrate cadmium and iron to the levels several orders of magnitude above those found in their environment (Cinier *et al.*, 1999; Abdullah *et al.*, 2003). In the acute toxicity test, juvenile fish are exposed to a range of toxicant concentrations in a static system for 96-hr. 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. with out toxicant). 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 organism (Mason, 1991). Presently no work is available on the tolerance limits of *Cirrhina mrigala* against iron, zinc, lead, nickel and manganese in Pakistan. Therefore, present project was planned to study the toxicity (both LC_{50} and lethal) of these selected heavy metals to the fish.

MATERIALS AND METHODS

Fingerling *Cirrhina mrigala* selected for metals toxicity tests were obtained from the Fish Seed Hatchery, Faisalabad and acclimatized at the wet laboratory of Fisheries Research Farms, Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan, for two weeks. All glassware and aquariums used in this experiment were washed and thoroughly rinsed with deionized water prior to use. To start each trail, all aquariums (80 liter capacity) were filled with 60-liter decholorinated tap water. Chemically pure chloride compounds of metals were dissolved in deionized water for the preparation of desired stock solutions.

In order to avoid the sudden stress to fish, the concentrations of metals in aquariums were increased gradually, 50% test concentration being reached in three and half hours and full toxicant concentration in seven hours. Each test was conducted with three replications for each metal. During all the trails constant air was supplied to all the test mediums with an air pump through capillary system. Both for LC_{50} and lethal acute toxicity trails, for each metal, the concentrations tested for fish 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. In each trail, the observations of fish mortality, temperature, pH, total hardness, dissolved oxygen, total ammonia, sodium, potassium and carbondioxide were made at 12-hour intervals during 96-hours determination of LC_{50} and lethal concentrations (100% mortality) for the fish. No mortality was observed among control fish. At the end of each test, water samples were taken from the aquariums and analyzed for corresponding metal concentrations through the methods described in S.M.E.W.W. (1989). The data obtained from analyses confirmed that the determined iron, zinc, lead, nickel and manganese concentrations coincided with the estimated data. The 96-hr LC_{50}, lethal values and their 95% confidence intervals were estimated by using Static Bioassay Probit Analysis Method. Physico-chemistry of test mediums were determined by following A.P.H.A. (1998). Differences in metals toxicity towards fish were analyzed by Analysis of Variance and Duncan's Multiple Range tests by following Steel *et al.* (1996).

RESULTS

Acute toxicity tests

Cirrhina mrigala of three age groups viz., 30-, 60- and 90-day were tested for their 96-hr LC_{50} and lethal concentrations of iron, zinc, lead, nickel and manganese, separately. During these toxicity tests water temperature (30 °C), pH (7) and total hardness (100 mgL^{-1}) were kept constant.

The fish had the mean lowest iron LC_{50} concentration of 63.64 ± 4.64 mgL^{-1} for 30-day age group, followed by that of 60- and 90-day that had the average values of 86.97 ± 5.84 and 104.17 ± 5.47 mgL^{-1} respectively. The differences among three fish age groups for LC_{50} values were statistically significant. Lethal concentrations of 90-, 60- and 30-, day fish varied significantly as 167.45 ± 10.03, 157.19 ± 10.63 and 115.41 ± 8.80 mgL^{-1} respectively. However, the differences between 90- and 60-day age groups were statistically non-significant (Table 1).

90-day *Cirrhina mrigala* showed significantly highest mean zinc LC_{50} value of 56.67 ± 3.41 mgL^{-1}, followed by that of 60- and 30-day with the mean LC_{50} values of 47.32 ± 3.30 and 40.58 ± 3.14 mgL^{-1} respectively. However, the lethal response of fish to this metal exhibited non-significant differences between 30- and 60-day age groups which were significantly lower than 90-day fish (Table 1).

The differences among 30-, 60- and 90-day fish age groups for their responses to lead 96-hr LC_{50} concentrations were statistically significant at $p<0.05$. 30-day *Cirrhina mrigala* showed significantly highest sensitivity to lead with the mean LC_{50} value of 32.68 ± 2.12 mg L^{-1}, followed by that of 39.78 ± 2.15 and 46.10 ± 2.43 mg L^{-1} for 60- and 90-day respectively. The differences among all the three fish age groups, for their responses towards lethal concentrations, were statistically significant at $p< 0.05$ also (Table 1).

Nickel mean 96-hr LC_{50} concentrations varied significantly among 30-, 60- and 90-day fish age groups with the mean values of 23.96 ± 2.40, 27.62 ± 2.34 and 36.05 ± 2.05 mgL^{-1} (Table 1). However, lethal concentrations varied non-significantly between 30- and 60-day fish as 52.07± 5.30 and 51.91 ± 4.22 mgL^{-1} respectively while the same for 90-day age group remained significantly maximum as 55.86 ± 3.65 mgL^{-1}.

96-hr LC_{50} value of manganese were recorded as 71.24 ± 3.87 mgL^{-1} for 30-day fish (Table 1) while the same for 60- and 90-day fish were recorded as 76.23 ± 4.55 and 91.68 ± 4.65 mgL^{-1} respectively. However, for 30-, 60- and 90-day age groups, lethal concentrations were recorded as 110.43 ± 6.81, 123.21 ± 8.11 and 142.13 ± 9.66 mgL^{-1} respectively.

The responses of three fish age groups and five metals towards LC_{50} and lethal concentrations were statistically significant. Among the three age groups, 90-day fish showed significantly higher tolerance against all metals than that of 60- and 30-day fish. *Cirrhina mrigala* showed significantly highest tolerance (determined as 96-hr LC_{50} and lethal) against iron, followed by that of manganese, zinc, lead and nickel (Table 1).

Table 1. Calculated 96-hr LC_{50} and lethal concentrations (±SE) of metals for *Cirrhina mrigala*

Metals	Fish age groups	Mean 96-hr LC_{50} values (mgL^{-1})	95 % confidence intervals (mgL^{-1})	Mean lethal concentrations (mgL^{-1})
Iron	30-day	63.64 ± 4.64 c	52.27 – 72.00	115.41 ± 8.80 b
	60-day	86.97 ± 5.84 b	72.48 – 97.21	157.19 ± 10.63 a
	90-day	104.17 ± 5.47 a	90.53 – 113.79	167.45 ± 10.03 a
Zinc	30-day	40.58 ± 3.14 c	32.99 – 46.28	79.52 ± 6.45 b
	60-day	47.32 ± 3.30 b	39.30 – 53.27	85.63 ± 6.37 b
	90-day	56.67 ± 3.41 a	48.68 – 63.22	96.38 ± 7.68 a
Lead	30-day	32.68 ± 2.12 c	27.32 – 36.53	53.91 ± 4.11 c
	60-day	39.78 ± 2.15 b	34.47 – 43.70	62.29 ± 4.04 b
	90-day	46.10 ± 2.43 a	40.20 – 50.68	72.36 ± 5.25 a
Nickel	30-day	23.96 ±2.40 b	18.07 – 28.41	52.07 ± 5.30 b
	60-day	27.62 ±2.34 b	21.61 – 31.74	51.91 ± 4.22 b
	90-day	36.05 ±2.05 a	30.80 – 39.71	55.86 ± 3.65 a
Manganese	30-day	71.24 ± 3.87 c	61.96 – 78.34	10.43 ± 6.81 c
	60-day	76.23 ± 4.55 b	64.71 – 84.28	123.21 ± 8.11 b
	90-day	91.68 ± 4.65 a	80.17 – 100.25	142.13 ± 9.66 a

Means with same letters in a single column / age group are statistically similar at $p< 0.05$

COMPARISON OF MEANS

	Mean Metals concentrations (mgL^{-1}±SD) for *Cirrhina mrigala*		
Age groups	**30-day**	**60-day**	**90-day**
LC_{50} concentrations	47.29±19.33 c	55.94±23.65 b	66.98±27.27 a
Lethal concentrations	82.47±28.24 c	96.57±40.97 b	105.48± 43.65 a

Metals	Iron	Zinc	Lead	Nickel	Manganese
LC_{50} concentrations	86.24±16.87a	48.41±6.79c	39.51±5.84d	29.55±5.39 e	79.97±8.48 b
Lethal concentrations	147.32± 24.04a	86.73±6.48c	62.56±7.23d	52.96±1.87e	124.65±13.34b

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

Physico-chemistry of test mediums during acute toxicity tests with fish

Table 2 shows dissolved oxygen, total ammonia, sodium, potassium and carbondioxide concentrations of the test mediums used during metals viz. iron, zinc, lead, nickel and manganese, acute toxicity trials with *Cirrhina mrigala* of 30-, 60- and 90-day age groups.

All the test mediums showed significant differences for dissolved oxygen, total ammonia, sodium, potassium and carbondioxide contents. Control medium had significantly higher dissolved oxygen contents of 6.29 ± 0.12 mgL^{-1} than those used for five metals during toxicity trials. Zinc medium showed significantly highest mean dissolved oxygen concentration, followed by that of manganese, lead, iron and nickel mediums. Excretions of ammonia in 90-day fish were higher than that of 60- and 30-day age groups. *Cirrhina mrigala* showed significantly higher ammonia excretion under iron toxicity, followed by that of manganese, nickel, lead and zinc. Iron test mediums showed significantly higher sodium and potassium contents of 261.22 ± 41.10 and 12.05 ± 1.23 mgL^{-1} respectively than rest of the metals during acute toxicity trials. Significantly maximum carbondioxide contents were recorded as 2.51 ± 0.40 mgL^{-1} during iron toxicity trail, followed by that of lead, zinc, nickel, manganese and control mediums. However, the difference between nickel and manganese test medium was statistically non-significant (Table 2).

DISCUSSION

Acute toxicity, determined as 96-hr LC_{50} concentrations of metals, varied significantly among three age groups. The present investigation reveals that 96-hr LC_{50} and lethal concentrations of five metals viz. iron, zinc, lead, nickel and manganese varied significantly among three age groups. 30-day fish were more sensitive than that of 60- and 90-day to metallic ion concentrations in all the tests. Giguere *et al.* (2004) reported that heavy

Table 2. Mean (±SD) physico-chemistry of test mediums during 96-hr acute toxicity trails with three fish age groups of *Cirrhina mrigala* at constant temperature, pH and total hardness of water

Metals	Fish Age	Temperature ºC	pH	Total hardness mgL^{-1}	Dissolved oxygen mgL^{-1}	Total Ammonia mgL^{-1}	Sodium mgL^{-1}	Potassium mgL^{-1}	CO_2 mgL^{-1}
Iron									
	30-day	30.30 ±0.05	7.08 ± 0.03	100.01 ± 0.37	4.95 ± 0.16	1.02 ± 2.10	245.67 ± 24.68	11.33 ± 3.12	2.12 ± 0.13
	60-day	30.20 ± 0.03	7.01 ± 0.02	100.10 ± 0.35	4.68 ± 0.45	1.34 ± 0.75	307.83 ± 31.48	13.47 ± 4.19	2.92 ± 0.15
	90-day	29.94 ± 0.01	7.00 ± 0.01	101.35 ± 0.37	4.35 ± 0.36	1.45 ± 0.66	230.16 ± 26.68	11.35 ± 3.45	2.48 ± 0.11
					4.66±0.30 d	**1.27±0.22 a**	**261.22±41.10 a**	**12.05±1.23 a**	**2.51±0.40 a**
Zinc									
	30-day	30.01 ± 0.03	7.02 ± 0.01	99.48 ± 0.32	5.48 ± 0.44	0.28 ± 0.41	94.54 ± 4.12	2.99 ± 0.68	2.05 ± 0.03
	60-day	29.96 ± 0.22	7.01 ± 0.03	99.91 ± 0.30	5.20 ± 0.24	0.29 ± 0.11	101.90 ± 11.58	3.93 ± 1.30	2.07 ± 0.18
	90-day	29.59 ± 0.40	7.08 ± 0.04	100.25 ± 0.34	5.00 ± 0.26	0.37 ± 0.25	102.33 ± 9.25	3.87 ± 1.03	2.10 ± 0.07
					5.23±0.24 b	**0.31±0.05 f**	**99.59±4.38 b**	**3.60±0.53 d**	**2.07±0.02 c**
Lead									
	30-day	30.10 ± 0.31	7.03 ± 0.02	99.66 ± 0.32	5.10 ± 0.25	0.62 ± 0.20	104.02 ± 4.65	3.64 ± 0.23	2.10 ± 0.09
	60-day	30.02 ± 0.26	7.04 ± 0.02	101.09 ± 0.34	4.90 ± 0.15	0.45 ± 0.52	96.50 ± 4.29	4.27 ± 1.39	2.32 ± 0.48
	90-day	30.15 ± 0.34	7.08 ± 0.04	101.35 ± 0.38	4.81 ± 0.14	0.62 ± 0.44	101.51 ± 5.24	4.40 ± 1.20	2.11 ± 0.14
					4.94±0.15 c	**0.56±0.10 e**	**100.68±3.83 b**	**4.10±0.41 c**	**2.18±0.12 b**
Nickel									
	30-day	30.57 ± 0.34	7.01 ± 0.01	99.80 ± 0.30	4.85 ± 0.15	0.31 ± 0.25	77.77 ± 11.22	2.55 ± 0.42	2.01 ± 0.02
	60-day	30.11 ± 0.31	7.02 ± 0.01	100.16 ± 0.30	4.64 ± 0.17	0.89 ± 0.35	90.00 ± 9.01	2.88 ± 0.30	1.09 ± 0.35
	90-day	30.04 ± 0.29	7.04 ±0.03	101.28 ± 0.34	4.38 ± 0.15	1.20 ± 0.33	92.34 ± 8.99	3.11 ± 0.25	1.03 ± 0.05
					4.62±0.23 d	**0.80±0.40 d**	**86.70±7.82 c**	**2.85±0.28 e**	**1.38±0.55 d**
Manganese									
	30-day	30.45 ± 0.25	6.99 ± 0.01	98.25 ± 0.25	5.43 ± 0.13	0.95 ± 0.75	86.52 ± 4.64	3.75 ± 0.40	1.44 ± 0.41
	60-day	30.06 ± 0.27	7.03 ± 0.01	101.06 ± 0.31	5.19 ± 0.14	1.23 ± 0.86	87.66 ± 4.65	4.69 ± 1.60	1.33 ±0.60
	90-day	29.11 ± 0.34	7.04 ± 0.02	100.05 ± 0.32	4.21 ± 0.12	1.20 ± 0.76	88.15 ± 5.58	4.70 ± 1.50	1.00 ± 0.60
					4.94±0.65 c	**1.13±0.15 b**	**87.44±0.84 c**	**4.38±0.54 c**	**1.26±0.23 d**
Control									
	30-day	30.05 ± 0.25	6.98 ± 0.01	98.25 ± 0.26	6.43 ± 0.22	0.95 ± 0.15	100.26 ± 2.32	4.13 ± 0.40	0.58 ± 0.21
	60-day	30.01 ± 0.13	7.01 ± 0.03	101.01 ± 0.25	6.23 ± 0.13	0.96 ± 0.12	102.26 ± 4.00	4.69 ± 0.35	0.69 ±0.11
	90-day	29.91 ± 0.21	7.03 ± 0.02	101.05 ± 0.35	6.21 ± 0.11	0.99 ± 0.23	112.36 ± 2.36	5.11 ± 0.28	0.88 ± 0.18
					6.29±0.12 a	**0.97±0.02 c**	**104.96±6.49 b**	**4.64±0.49 b**	**0.72±0.15 e**

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

metal concentration in fish increased with age that exerted significant impact on the tolerance limits of fish. Early-life stage of fathead minnows were most sensitive to nickel among the species for which toxicological endpoints could be determined (i.e. fathead minnows, rainbow trout and white suckers). However, Smet and Blust (2001) observed 100 percent mortality in *Cyprinus carpio* after 21-29 days of exposure to 20 mgL^{-1} cadmium. The susceptibility of fish to a particular heavy metal is very important factor for LC_{50} values. The fish that is highly susceptible to toxicity of one metal may be less or non-susceptible to the toxicity of another metal at the same concentration of that metal (Das and Banerjee, 1980). During present investigation, 96-hr LC_{50} and lethal values of iron for *Cirrhina mrigala* were maximum while the same was minimum for nickel. Leblond and Hontela (1999) studied the acute toxicity of mercury, zinc and cadmium in rainbow trout and reported that fish was more susceptible to mercury, followed by that of zinc and cadmium. Chinni and Yallapragda (2000) carried out acute toxicity tests with metals (Pb, Zn, Cd and Co) on *Penaeus indicus* post larvae. The resulting 96-hr LC_{50} values showed that copper was the most toxic metal, followed by that of cadmium, zinc and lead. LC_{50} values for copper, cadmium, zinc and lead were 2.535, 3.119, 6.223 and 7.223 mgL^{-1} respectively. Pandey *et al.* (2005) conducted 96-hr acute toxicity tests in flowthrough systems to determine the lethal toxicity of mercuric chloride and malathion to air breathing teleost, *Channa punctatus.* They reported that mercuric chloride was more toxic than malathion. It was also observed that mortality rates were dose and dose-time-dependent.

In water with calcium hardness of 100 mg L^{-1}, carp (*Cyprinus carpio*) fry and fingerling have cadmium 96-hr LC_{50} of 4.3 and 17.10 mg L^{-1} respectively (Suresh *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. During present investigation, significantly maximum ammonia excretion by the fish was observed at higher concentrations of metals (manganese test medium). At higher concentrations of metals, the carbondioxide contents of the test mediums also increased significantly. This shows that high concentrations of metallic ions induced stress in the fish that resulted in significantly more carbondioxide liberation in water through respiration and thus, carbondioxide concentrations of the test medium increased. Environmental conditions such as oxygen concentration, temperature, hardness, salinity and presence of other metals may modify metal toxicity to the fish. Hypoxic conditions, temperature, increase and acidification usually render the fish more susceptible to intoxication while increase in mineral contents (hardness and salinity) reduces metal toxicity (Witeska and Jezierska, 2003). Acute toxicity testing of ammonia on swimming and resting rainbow trout revealed that resting fish was significantly more sensitive (32.38 ± 10.81 mgL^{-1}) than that of swimming fish (207.00 ± 21.99 mgL^{-1}). Sodium has been associated with decreased copper toxicity in fathead minnows at concentrations greater than 1nN (23.8 mgL^{-1}). However, toxicity tests conducted with sodium concentrations of 2nN (47.5mgL^{-1}) were associated with a two fold decrease in copper toxicity (Erickson *et al.* 1996). However, the results of present investigation are in confirmatory to their findings. Iron test mediums showed significantly higher sodium contents which results decreased sensitivity (higher LC_{50} values) of iron to fish than rest of the metals.

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