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Studies on Acute and Lethal Toxicities of Iron and Nickel to the Fish

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Abstract: Laboratory tests were conducted on three fish species viz. *Catla catla, Labeo rohita* and *Cirrhina mrigala* to determine their sensitivity to iron and nickel for 96-hr LC_{50} and lethal toxicity. These tests were performed with three age groups of each fish species at water temperature of 30°C with pH 7 and total hardness of 100 mg L^{-1}. The 96-hr LC_{50} concentrations of both iron and nickel varied significantly among the three fish species with age. 30-day fish were more sensitive to metals toxicity, followed by that of 60 and 90 day fish, respectively. Among the three fish species, *Catla catla* were more sensitive to nickel while *Cirrhina mrigala* showed highest sensitivity to iron. Lethal iron concentrations for all the three fish species ranged between 72.20 mg L^{-1} (for 30 day *Labeo rohita*) and 164.65 mg L^{-1} (for 90-day *Catla catla*). However, nickel concentrations fluctuated between 20.20 and 50.11 mg L^{-1} for 30-day *Catla catla* and 60 day *Cirrhina mrigala*, respectively. The 96-hr LC_{50} nickel concentrations in three fish species varied significantly within age groups. However, the difference between 60 and 90 day old fish was statistically non-significant. Among the three fish species, *Catla catla* appeared significantly more sensitive than that of *Labeo rohita* and *Cirrhina mrigala* to nickel contaminations. The 96-h LC_{50} concentrations of both metals for all the three fish species were positively and significantly dependent upon fish age. The regressions of water temperature on 96-hr LC_{50} concentrations of both the metals were negatively significant. However, water pH, hardness, ammonia, sodium and potassium showed significantly direct relationships with 96-hr LC_{50} concentrations of fish. The ammonia concentrations of test medium increased while dissolved oxygen decreased at high levels of toxicants. At higher 96-hr LC_{50} values of iron, dissolved oxygen decreased significantly in the test medium.

Key words: Acute, lethal, toxicity, 96-hr LC_{50}, iron, nickel, fish

INTRODUCTION

Rapid industrialization in the province of Punjab has created a lot of problems including contamination of natural waters with metals due to dumping of untreated wastes in the aquatic habitats, causing deleterious effects on fish (Javed, 2004). The accumulation of metals in an aquatic environment has direct consequences to man and to the ecosystem also. Interest in the metals, which are required for metabolic activities in organism, lies in the narrow "window" between their essentiality and toxicity (Fatoki *et al*., 2002). Fish accumulate metals in its tissue from polluted environment. Metal distribution between different tissues of fish varies depending on the sources of uptake, diet and/or water borne exposure (Kraal *et al*., 1995). 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 iron to the levels several orders of magnitude above those found in their environment (Abdullah *et al*., 2003). Determination of water quality criteria for aquatic life is similar to the solving of most biological problems, in which experimental data are obtained under controlled laboratory conditions in order to predict effects that might occur under natural conditions. Iron, zinc, lead, nickel and manganese are the most common pollutants of the rivers of Punjab province, entering them with industrial and municipal waste waters (Javed and Mahmood, 2001). Althoough, trace metals are essential for normal physiological process, abnormally high concentrations can be toxic to aquatic organisms (Javed, 2003). Heavy metals being non-biodegradable primarily necessitate knowledge on their uptake, distribution and persistence in tissues of organisms (Lewis *et al*., 2004). Egg hatchability and hatchling time are more sensitive indicators of toxicity than "standard" end points, like mortality and growth (Pyle *et al*., 2002). No work has been done on the determination of tolerance limits of major carps viz. *Catla catla, Labeo rohita* and

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Cirrhina mrigala against iron and nickel toxicities. Therefore, present work was planned to investigate the comparative sensitivity of iron and nickel by means of toxicity tests (both LC_{50} and lethal) of three fish species for different age groups.

MATERIALS AND METHODS

Iron and nickel toxicity tests on three fish species viz. *Catla catla, Labeo rohita* and *Cirrhina mrigala* were conducted in 50 L glass aquaria in clean water. Ten fish were placed into each aquarium for acclimation. In order to not stress the fish, the metal concentrations in aquarium were increased gradually, 50% test concentration being reached in 3.50 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 air pump through capillary system. Chemically pure chloride compounds of iron and nickel were dissolved in distilled water and stock solutions prepared for required metal dilutions. The hardness, pH and electrical conductivity of water were adjusted. 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. In each trial, the observations of fish mortality and physico-chemical variables of the test medium viz. temperature, pH, total hardness, dissolved oxygen, 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 measured (total length) at the time of mortality observations. No mortality was observed among control fish. At the end of each test, water samples were taken from the aquarium and tested for metal concentrations (Anonymous, 1989). The analytical data obtained confirmed that the determined iron and nickel concentrations coincided with the estimated data quite satisfactorily. Iron and nickel toxicity tests were performed with three age groups of fish viz. 30, 60 and 90 day having following mean total lengths and weights:

Age group	Fish species	Total length (mm)	Weight (g)
(30 day)	*Catla catla*	43.37±4.44	1.2175±0.45
	Labeo rohita	21.87±2.17	0.1862±0.08
	Cirrhina mrigala	18.32±1.25	0.4203±0.05
(60 day)			
	Catla catla	45.32±1.85	1.4110±0.04
	Labeo rohita	28.25±2.36	0.2650±0.02
	Cirrhina mrigala	42.87±2.69	0.6737±0.14
(90 day)			
	Catla catla	52.25±3.59	1.6900±0.29
	Labeo rohita	30.00±1.05	1.6500±0.39
	Cirrhina mrigala	43.28±2.33	1.0500± 0.20

The LC_{50} and lethal levels, as total metal concentrations, were estimated for three fish age groups viz. 30, 60 and 90 day at temperature of 30°C with pH 7 and 100 mg L^{-1} total hardness of water. The 96-hr LC_{50} values and their 95% confidence intervals were estimated by using trimed spear man-Karber method (Hamilton *et al*., 1977). Physico-chemical constituents of test mediums were also analyzed (Anonymous, 1984). The data obtained from this experiment were subjected to statistical analysis (Steel and Torrie, 1986) through micro-computer. Analysis of variance and Duncan's multiple range tests were performed to find-out statistically significant differences among parameters under study. Correlation and regression analyses were performed to find-out relationships among various parameters also.

RESULTS

Iron 96-hr LC_{50} for fish: The present data shows that calculated 96-hr LC_{50} values of three fish species varied within the range of 50.56±1.99-115.38±3.11 mg L^{-1}. The lowest 96-hr LC_{50} was recorded for 30 day *Labeo rohita* while the highest one for 90-day *Catla catla*. The 95% confidence intervals of 96-hr LC_{50} for 60 and 90 day *Catla catla* were almost similar (Table 1). Table 2 shows that 96-hr LC_{50} concentrations varied significantly ($p<0.05$) among three fish species with age. Thirty day fish was significantly more sensitive to iron contamination than that of 60 and 90 day fish. The 96-hr LC_{50} concentrations of iron increased with fish age. However, there was non-significant difference between 60 and 90 day old fish. *Labeo rohita* were significantly more sensitive to iron toxicity than that of *Cirrhina mrigala* and *Catla catla.*

Nickel 96-hr LC_{50} for fish: The mean nickel 96-hr LC_{50} concentrations varied significantly within the fish of three age groups as minimum of 6.00±0.25 mg L^{-1} for 30 day *Catla catla* and a maximum mean value of 38.80±2.15 mg L^{-1} for 90 day *Cirrhina mrigala*. Lethal concentrations of nickel for fish ranged between 20.20 and 50.11 mg L^{-1} (Table 1). The 96-hr LC_{50} nickel concentrations in three fish species varied significantly within age groups. However, the difference between 60 and 90 day old fish was statistically non-significant. Among the three fish species, *Catla catla* were significantly more sensitive than that of *Labeo rohita* and *Cirrhina mrigala* to nickel (Table 2).

Dependence of 96-hr LC_{50} of metals on fish age: The 96-hr LC_{50} concentrations of both metals for all the three fish species were positive and significantly dependent upon fish age i.e., with the increase in fish age the 96-hr

Table 1: Calculated 96-hr LC_{50} as total iron and nickel concentrations for three fish species

Age group	Fish species	96-hr LC_{50} (mg L^{-1})	95 % confidence interval (mg L^{-1})	Lethal concentration (mg L^{-1})
Iron				
30 day	*Catla catla*	92.11±2.26	86.50-97.72	130.32
	Labeo rohita	50.56±1.99	45.62-55.50	72.20
	Cirrhina mrigala	77.95±3.66	68.86-87.04	83.16
60 day	*Catla catla*	110.02±2.91	102.79-17.25	162.28
	Labeo rohita	48.26±3.14	47.42-62.40	80.20
	Cirrhina mrigala	80.15±1.35	91.62-104.38	150.16
90 day	*Catla catla*	115.38±3.11	109.45-121.31	164.65
	Labeo rohita	56.20±2.34	50.62-62.01	75.66
	Cirrhina mrigala	100.05±4.01	90.09-10.01	154.60
Nickel				
30 day	*Catla catla*	6.00±0.25	5.38-6.62	20.20
	Labeo rohita	20.20±1.11	17.44-22.96	30.25
	Cirrhina mrigala	23.47±2.01	18.48-28.46	40.11
60 day	*Catla catla*	11.52±2.05	6.43-16.61	19.88
	Labeo rohita	24.94±3.61	15.97-33.91	40.02
	Cirrhina mrigala	34.80±2.25	29.21-40.39	50.11
90 day	*Catla catla*	20.15±1.64	16.08-24.22	30.36
	Labeo rohita	27.00±2.00	22.03-31.97	40.28
	Cirrhina mrigala	38.80±2.15	32.96-43.64	50.05

Table 2: Metals 96-hr LC_{50} responses of three age groups of fish

Fish age	Iron LC_{50}	Nickel LC_{50}	Fish species	Iron LC_{50}	Nickel LC_{50}
30 day	73.54±21.12b	16.56±9.29b	***Catla catla***	105.84±12.18a	12.56±7.13c
60 day	79.98±30.88a	23.75±11.68a	***Labeo rohita***	51.67±4.08c	24.05±3.49b
90 day	90.54±30.71a	28.65±9.43a	***Cirrhina mrigala***	86.05±12.17b	32.36±7.95a

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

Table 3: Dependence of 96-hr LC_{50}, concentration of three fish species on their age

Species	Regression equation			r	R^2
Catla catla					
	Iron LC_{50}	=	82.57+0.388** (Fish age)	0.955	0.9120
	Nickel LC_{50}	=	-1.59+0.235** (Fish age)	0.992	0.9841
Labeo rohita					
	Iron LC_{50}	=	48.25+0.094** (Fish age)	0.954	0.9101
	Nickel LC_{50}	=	17.25+0.113** (Fish age)	0.975	0.9506
Cirrhina mrigala					
	Iron LC_{50}	=	69.90+0.368* (Fish age)	0.905	0.8190
	Nickel LC_{50}	=	17.03+0.255** (Fish age)	0.964	0.9293

* = Significant at $p<0.05$; ** = Significant at $p<0.01$; r = correlation coefficient; R^2 = coefficient of determination

Table 4: Physico-chemistry of the mediums during 96-hr LC_{50} toxicity tests with three fish species

Age group	Fish species	Temperature (°C)	pH	Total hardness (mg L^{-1})	Dissolved oxygen (mg L^{-1})	Ammonia (mg L^{-1})	Sodium (mg L^{-1})	Potassium (mg L^{-1})	CO_2 (mg L^{-1})
IRON									
30 day	*Catla catla*	30.22±0.34	7.01±0.01	102.35±0.37	4.85±0.50	1.20±0.07	201.00±25.14	10.21±2.34	Nil
60-day	*Catla catla*	30.21±0.03	7.02±0.03	100.22±0.35	4.68±0.45	1.34±0.08	307.83±31.48	13.47±4.19	Nil
90-day	*Catla catla*	30.30±0.01	7.08±0.01	103.52±0.40	4.31±0.41	1.62±0.06	311.52±30.25	12.22±3.68	Nil
30-day	*Labeo rohita*	30.24±0.02	7.04±0.01	99.91±0.32	5.11±0.52	1.05±0.05	240.33±22.35	9.74±2.56	Nil
60-day	*Labeo rohita*	30.21±0.03	7.02±0.02	100.22±0.31	5.00±0.20	1.40±3.00	380.31±16.17	5.88±1.06	Nil
90-day	*Labeo rohita*	30.25±0.01	7.00±0.02	105.15±0.40	4.77±0.17	1.70±3.11	221.01±16.45	7.25±2.00	0.01
30-day	*Cirrhina mrigala*	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	Nil
60-day	*Cirrhina mrigala*	30.21±0.03	7.02±0.02	100.22±0.35	4.68±0.45	1.34±0.75	307.83±31.48	13.47±4.19	Nil
90-day	*Cirrhina mrigala*	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	0.01
NICKEL									
30 day	*Catla catla*	30.12±0.29	6.95±0.01	99.32±0.32	5.01±0.15	0.55±0.33	144.00±8.67	5.02±1.24	Nil
60 day	*Catla catla*	30.01±0.27	7.02±0.01	100.66±0.33	6.37±0.49	1.63±0.69	160.72±16.09	5.69±0.41	Nil
90 day	*Catla catla*	29.67±0.28	7.05±0.02	102.12±0.35	6.38±0.43	1.50±0.56	162.35±14.38	5.68±0.42	Nil
30 day	*Labeo rohita*	30.21±0.30	6.98±0.01	100.02±0.35	5.28±0.40	1.25±0.51	152.00±20.31	5.82±1.00	0.12
60 day	*Labeo rohita*	30.01±0.31	7.02±0.01	100.66±0.31	6.88±0.43	1.24±0.58	162.04±20.00	5.96±0.40	Nil
90 day	*Labeo rohita*	29.65±0.28	7.05±0.03	101.00±0.32	6.90±0.34	1.30±0.48	140.95±18.94	5.97±0.57	0.01
30 day	*Cirrhina mrigala*	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	0.01
60 day	*Cirrhina mrigala*	30.01±0.31	7.02±0.01	100.66±0.30	5.64±0.17	0.89±0.35	90.00±9.01	2.88±0.30	0.09
90 day	*Cirrhina mrigala*	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	0.03

Table 5: Relationships between metals toxicity (y) and physico-chemical variables (x) of the test medium

Metal	Regression equation (y = a + bx)	r	R^2
Iron			
	96-hr LC_{50} of fish (y) = 30.20 – 0.0014* (Water temp.)	- 0.692	0.4788
	= 7.01 + 0.0001* (pH)	0.565	0.3192
	= 104.81 + 0.032* (Hardness)	0.537	0.2884
	= 5.48 – 0.009** (D. O.)	- 0.790	0.6241
	= 1.19 + 0.002* (Ammonia)	0.502	0.2520
	= 256.30 + 0.184^{NS} (Sodium)	0.179	0.0320
	= 3.28 + 0.087** (Potassium)	0.826	0.6823
Nickel			
	96-hr LC_{50} of fish (y) = 30.15 – 0.0011* (Temp.)	- 0.614	0.3770
	= 6.97 + 0.002* (pH)	0.611	0.3733
	= 99.76 + 0.037* (Hardness)	0.654	0.4277
	= 6.04 – 0.013* (D.O.)	- 0.545	0.2970
	= 1.06 + 0.002* (Ammonia)	0.641	0.4109
	= 178.61 + 2.061* (Sodium)	0.608	0.3696
	= 6.46 + 0.075^{NS} (Potassium)	0.523	0.2735

* = Significant at $p < 0.05$; ** = Significant at $p < 0.01$; r = correlation coefficient; R^2 = coefficient of determination

LC_{50} values of metals increased. The high values of R^2 (coefficient of determination) for all the regression equations computed for the relationship of metal's toxicity with age predict highly reliable regression models (Table 3).

Physico-chemical variables of the test medium: The mean values for physico-chemical variables of the test mediums used during 96-hr toxicity tests are presented in Table 4. Table 5 shows the dependence of fish 96-hr LC_{50} of iron and nickel on physico-chemical variables of the test medium. The regression equations reveal negative regression of water temperature on 96-hr LC_{50} concentrations of both iron and nickel. However, water pH, hardness, ammonia and potassium showed direct relationships with 96-hr LC_{50} concentrations of fish. The ammonia concentrations of test medium increased while dissolved oxygen decreased at high levels of toxicity. At higher 96-hr LC_{50} concentrations of iron, dissolved oxygen decreased significantly. The 96-hr LC_{50} of nickel for the fish showed positively significant correlation with sodium and potassium.

DISCUSSION

The present investigation reveals that 96-hr LC_{50} concentrations of iron and nickel varied significantly among the three fish species with age. Thirty day fish exhibited more sensitivity than 60 and 90 day fish to metallic ion concentrations. Among the three fish species, *Catla catla* were the most sensitive to nickel toxicity, followed by *Labeo rohita* and *Cirrhina mrigala*. *Cirrhina mrigala* exhibited highest tolerance to nickel. *Labeo rohita* had the lowest 96-hr LC_{50} to iron while the same was maximum for *Catla catla*. Amongst fish species, considerable differences in sensitivity to metals have been reported. Salmonids are generally sensitive to high cadmium levels. Juvenile trout (*Orcorhynchus mykiss*) have higher 48-hr LC_{50} (Handy, 1992). In water hardness of 100 mg L^{-1} Ca^{2+}, carp fry and fingerling (*Cyprinus carpio*) had the 96-hr LC_{50} of 4.3 and 17.10 mg L^{-1} of cadmium, respectively (Suresh *et al*., 1993). Other fish characteristics, such as age, body size, feeding habits and sex can also be considered for variable LC_{50} of metals for different species of fish (Witeska and Jezierska, 2003). Therefore, it is important to consider the physico-chemical characteristics of test medium along with biotic factors to know the mechanisms affecting LC_{50} concentrations of fish in toxicity tests. During present investigation, total hardness showed significantly direct correlation with 96-hr LC_{50} concentration of metals for fish as these values escalated with increasing hardness of test medium. Zinc toxicity to rainbow trout showed positively significant dependence upon hardness and pH of water (Hansen and Welsh, 1996). At 8°C trout was less sensitive than that of at 12°C. Environmental conditions such as oxygen concentration, temperature, hardness, salinity and presence of other metals may modify metal's toxicity to fish. Hypoxic conditions, temperature increase and acidification usually render the fish more susceptible to intoxication (Witeska and Jezierska, 2003).

The ammonia excretion by the fish increased significantly at higher concentrations of metals. An inverse relationship between pH and the concentration of mobile magnesium, iron, manganese and cobalt has been reported (Boqomazov *et al*., 1991). Increase in water temperature enhanced the uptake of metals by the aquatic organisms also (Jackson, 1988). Water-borne metals generally exhibit their greatest toxicity to aquatic organisms in soft waters of low pH (Penttinen *et al*., 1995). This is because, the hardness cat-ions (Mg and Ca) compete with heavy metal cat-ions for binding sites within the organism. Acidic water, with high concentration of hydrogen ions, may also compete with metal cat-ions for binding sites in the same manner as magnesium and calcium in hard waters. A negative correlation between pH and exchangeable zinc in water has been reported (Davies, 1992). Bioaccumulation of metals in fish is a function of metal bioavailability which can vary with pH, uptake and toxicokinitics (Spry and Wiener, 1991). Mercury uptake enhanced by increasing water temperature, reduced salinity and pH, at increased levels of zinc and cadmium in water (Eisler, 1985). Zinc toxicity to aquatic organisms was the highest at conditions of low pH, alkalinity, dissolved oxygen and elevated temperature (Weatherley *et al.*, 1988). Heavy metal toxicity may also decrease the oxygen consumption by the fish (Sarkar, 1999) while water hardness had a significantly positive effect on heavy metals toxicity (Rathore and Khangranot, 2003).

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