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# Studies on Acute Toxicity of Metals to the Fish, *Catla catla*

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**Abstract:** Acute toxicity of metals viz., iron, zinc, lead, nickel and manganese to the fish, *Catla catla* has been studied. These tests included the determination of 96 h $LC_{50}$ and lethal toxicity of heavy metals to the fish. These tests were performed, separately, at constant temperature, pH and hardness of 30°C, 7 and 100 mg $L^{-1}$ respectively. Three fish age groups viz. 30, 60 and 90 day were tested for their sensitivity to metals toxicity. The impacts of physico-chemical variables were also studied towards sensitivity of fish to metals toxicity. The 96 h $LC_{50}$ and lethal concentrations of all metals varied significantly in fish. This fish showed significantly highest tolerance (determined as $LC_{50}$) against iron, followed by that of manganese, lead, zinc and nickel. However, non-significant differences for 96 h $LC_{50}$ tolerance limits towards zinc and lead were found. Among the three fish age groups, 30 day fish were more sensitive to metals toxicity, followed by that of 60 and 90 day respectively. The responses of three fish age groups and five metals 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. The ammonia excretion by the fish increased, significantly, with concomitant increase in metal concentrations of the mediums while dissolved oxygen content of the test medium decreased at higher metal concentrations. Sodium had slight protective effect against metals toxicity.

**Key words:** *Catla catla*, 96 h $LC_{50}$, lethal, iron, zinc, lead, nickel, manganese

## INTRODUCTION

Heavy metals have long been recognized as serious pollutants of the aquatic environment they cause serious impairment in metabolic, physiological and structural system when present in high concentrations (Javed, 2003). Heavy metals may affect organism directly by accumulating in their body or indirectly by transferring to the next trophic level of the food chain (Unlu and Gumgum, 1993). Trace metals are introduced into the environment by a wide spectrum of natural and anthropogenic sources. Metals are non-biodegradable and once they enter the environment, bio-concentration may occur in fish tissue by means of metabolic and bio-absorption processes (Hodson, 1988; Hogstrand and Haux, 1991). 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 for metals to enter 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). A decrease in oxygen consumption and ammonia-N excretion is observed in *Penaeus indicus* post larvae with increasing concentration of lead (Chinni *et al*., 2002). Elevated nickel concentrations in aquatic systems are associated with steel manufacture (Krantzberg and Boyed, 1992), electrical battery manufacture (Greenwood and Earnshaw, 1984), pesticide formulations (Galvin, 1996) and metal mining (Rutherford and Mellow, 1994; Nriagu *et al*., 1998). Acute toxic effect occurs in two stages, immediate and delayed. Initial symptoms of over exposure are dizziness and shortness of breath; the delayed effects (10-36 h) are bluish discoloration of the skin and death. Previously, no work has been done on the determination of tolerance limits of *Catla catla* against iron, zinc, lead, nickel and manganese. Therefore, present study was planned to study the toxicity (both $LC_{50}$ and lethal) of heavy metals to the fish.

## MATERIALS AND METHODS

Metal toxicity tests were conducted in the laboratory conditions. Juvenile *Catla catla* selected for this study were obtained from the Fish Seed Hatchery, Faisalabad. They were brought to the laboratory and acclimatized for 14 days. All glassware and aquariums used in this experiment were washed and thoroughly rinsed with deionized water prior to use. Prior to each trail, all

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aquariums (60 L) capacity, were filled with 50 L of dechlorinated tap water. Water quality characteristics in the experimental aquariums were determined (APHA, 1998). Chemically pure chloride compounds of metals were dissolved in deionized water for the preparation of desired stock solutions.

Ten fish were placed in to each aquarium for acclimation. In order to not stress the fish, the concentration of metals in aquariums 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 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 *Catla catla*, 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 trail, the observations of fish mortality, temperature, pH, total hardness, dissolved oxygen, total ammonia, sodium, potassium and carbondioxide were made at 12 h intervals during 96 h 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 aquarium and analyzed for corresponding metal concentrations through the methods described in APHA. and AWWA. (1989). The analytical data obtained confirmed that the determined iron, zinc, lead, nickel and manganese concentrations coincided with the estimated data. The 96 h $LC_{50}$ and lethal values and their 95% confidence intervals were estimated by using Probit analysis. Physico-chemistry of test mediums were analyzed by following APHA (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:** Three age groups (30, 60 and 90 day) of *Catla catla* were tested for their 96 h $LC_{50}$ and lethal concentrations for iron, zinc, lead, nickel and manganese concentrations, separately. These toxicity tests were conducted at constant water temperature, pH and total hardness of 30°C, 7 and 100 mg $L^{-1}$, respectively.

**Iron:** The fish exhibited the highest mean iron $LC_{50}$ concentration of 114.67±4.65 mg $L^{-1}$ for 90 day fish, followed by that of 60 and 30 day that had the average values of 104.17±5.47 and 91.68±4.65 mg $L^{-1}$ respectively. The differences among three fish age groups for $LC_{50}$ values were statistically significant. Lethal concentrations of 30, 60 and 90 day fish varied significantly as 142.13±9.66, 167.45±10.03 and 169.27±9.26 mg $L^{-1}$, respectively. However, the differences between 60 and 90 day age groups were statistically non-significant (Table 1).

**Zinc:** The mean zinc $LC_{50}$ concentrations varied significantly within three fish age groups. Thirty day *Catla catla* showed significantly highest sensitivity to zinc ($LC_{50}$ of 20.66±1.35 mg $L^{-1}$), followed by that of 60 and 90 day with the mean $LC_{50}$ values of 23.30±1.34 and 25.88±1.28 mg $L^{-1}$ respectively. However, the lethal response of fish to this metal exhibited non-significant differences between 60 and 90 day age groups which were significantly higher than that of 30 day fish (Table 1).

**Lead:** The difference between 90 and 60 day fish age groups for their responses to lead 96 h $LC_{50}$ concentrations was statistically non-significant. 30 day *Catla catla* showed significantly highest sensitivity to lead with the mean $LC_{50}$ value of 18.66±1.82 mg $L^{-1}$,

Table 1: Calculated 96 h $LC_{50}$ and lethal concentrations (±SE) as total iron, zinc, lead, nickel and manganese for *Catla catla*

| Metals | Age groups of fish | 96 h $LC_{50}$ (mg $L^{-1}$) values | 95 % confidence interval (mg $L^{-1}$) | Lethal concentrations (mg $L^{-1}$) | Overall means ± SD of $LC_{50}$ (mg $L^{-1}$) for three age groups |
|---|---|---|---|---|---|
| Iron | 30 day | 91.68±4.65c | 80.17-100.25 | 142.13±9.66b | |
| | 60 day | 104.17±5.47b | 90.53-113.79 | 167.45±10.03a | 104.06±10.32a |
| | 90 day | 114.67±4.65a | 103.81-123.36 | 169.22±9.26a | |
| Zinc | 30 day | 20.66±1.35c | 17.30-23.02 | 35.90±2.35b | |
| | 60 day | 23.30±1.34b | 19.98-25.68 | 38.60±2.48a | 23.21±2.48c |
| | 90 day | 25.88±1.28a | 22.49-28.13 | 39.25±2.52a | |
| Lead | 30 day | 18.86±1.82b | 13.91-21.97 | 37.75±3.40b | |
| | 60 day | 25.77±1.36a | 22.34-28.23 | 38.74±2.46b | 23.78±3.79c |
| | 90 day | 26.85±1.64a | 22.72-29.78 | 43.74±3.00a | |
| Nickel | 30 day | 11.83±1.01b | 9.29-13.59 | 22.78±1.77b | |
| | 60 day | 13.28±1.10b | 10.42-15.19 | 25.25±1.99b | 14.73±3.11d |
| | 90 day | 18.99±1.26a | 15.97-21.35 | 33.17±2.68a | |
| Manganese | 30 day | 55.26±3.67b | 45.89-61.93 | 92.03±7.20c | |
| | 60 day | 64.67±3.61a | 55.75-71.27 | 99.59±6.52b | 64.26±6.21b |
| | 90 day | 67.71±3.94a | 58.08-74.84 | 107.72±6.83a | |

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

followed by that of 25.77±1.36 and 26.85±1.64 mg $L^{-1}$ for 60 and 90 day, respectively. The differences among all the three fish age groups, for their responses towards 96 h $LC_{50}$ and lethal concentrations, were statistically significant at $p<0.05$ (Table 1).

**Nickel:** The mean 96 h $LC_{50}$ and lethal concentrations of nickel varied non-significantly between 30 and 60 day fish with the mean values of 11.83±1.01 and 13.28±1.10 mg $L^{-1}$ and 22.78±1.77 and 25.25±1.99 mg $L^{-1}$, respectively (Table 1). However, 90-day *Catla catla* were significantly more tolerant towards nickel toxicity for both $LC_{50}$ and lethal concentrations as 18.99±1.26 and 33.17±2.68 mg $L^{-1}$, respectively.

**Manganese:** The lowest mean 96 h $LC_{50}$ value of 55.26±3.67 mg $L^{-1}$ was recorded for 30 day fish (Table 1) while the same for 60 and 90 day were 64.67±3.61 and 67.71±3.94 mg $L^{-1}$, respectively. Both 60 and 90 day age groups were statistically at par for their $LC_{50}$ manganese concentrations. However, 30, 60 and 90 day age groups exhibited significant differences for their lethal concentrations as 92.03±7.20, 99.59±6.52 and 107.72±6.83 mg $L^{-1}$, respectively.

The responses of three fish age groups and five metals 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. *Catla catla* showed significantly highest tolerance (determined as 96 h $LC_{50}$) against iron, followed by that of manganese, lead, zinc and nickel. However, this species of fish showed non-significant differences for 96 h $LC_{50}$ tolerance limits towards zinc and lead (Table 1).

**Physicochemistry 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 iron, zinc, lead, nickel and manganese acute toxicity trials with *Catla catla* of 30, 60 and 90 day age groups. During these trials, mean water temperature, pH and total hardness have been fixed at 30°C, 7 and 100 mg $L^{-1}$, respectively.

All the test mediums showed significant differences for dissolved oxygen contents, total ammonia, sodium, potassium and carbondioxide. Control medium had significantly higher dissolved oxygen contents than those used for five metals during toxicity trials. Nickel medium showed significantly highest mean dissolved oxygen

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

| Fish age group | 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 | 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 | 2.01±0.11 |
| 60 day | 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 | 1.99±0.22 |
| 90 day | 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 | 2.16±0.15 |
| | | | | **4.61±0.28f** | **1.39±0.21b** | **273.45±56.60a** | **11.97±2.00a** | **2.05±0.10c** |
| Zinc | | | | | | | | |
| 30 day | 30.12±0.03 | 7.01±0.01 | 100.11±0.31 | 5.11±0.35 | 1.37±0.23 | 105.22±14.75 | 4.34±1.23 | 2.02±0.01 |
| 60 day | 29.66±0.22 | 7.05±0.02 | 99.65±0.30 | 5.08±0.10 | 1.63±0.69 | 125.00±2.06 | 3.84±0.47 | 2.04±0.06 |
| 90 day | 30.01±0.22 | 7.07±0.04 | 102.05±0.36 | 4.99±0.20 | 1.75±0.55 | 120.40±8.65 | 3.70±0.88 | 2.03±0.02 |
| | | | | **5.06±0.11c** | **1.58±0.19a** | **116.87±9.41c** | **3.96±0.45cd** | **2.03±0.11c** |
| Lead | | | | | | | | |
| 30 day | 30.04±0.39 | 6.93±0.01 | 101.45±0.40 | 5.03±0.20 | 0.79±0.33 | 101.54±8.45 | 2.87±1.00 | 2.00±0.06 |
| 60 day | 30.03±0.35 | 7.01±0.01 | 102.05±0.41 | 4.95±0.19 | 1.55±0.52 | 103.75±2.91 | 2.62±0.11 | 2.55±0.19 |
| 90 day | 30.04±0.32 | 7.08±0.03 | 102.23±0.36 | 4.77±0.15 | 1.56±0.44 | 107.11±3.65 | 2.95±0.14 | 2.16±0.22 |
| | | | | **4.92±0.16cd** | **1.30±0.40bc** | **104.13±2.75d** | **2.81±0.20f** | **2.24±0.28b** |
| Nickel | | | | | | | | |
| 30 day | 30.12±0.29 | 6.95±0.01 | 99.32±0.32 | 5.61±0.15 | 0.55±0.33 | 144.00±8.67 | 5.02±1.24 | 3.11±0.46 |
| 60 day | 30.08±0.27 | 7.04±0.01 | 100.66±0.33 | 5.37±0.49 | 1.63±0.69 | 160.72±16.09 | 5.69±0.41 | 2.15± 0.19 |
| 90 day | 29.67±0.28 | 7.05±0.02 | 102.12±0.35 | 5.08±0.43 | 1.50±0.56 | 162.35±14.38 | 5.68±0.42 | 2.11±0.21 |
| | | | | **5.35±0.24b** | **1.23±0.54c** | **155.69±12.79b** | **5.46±0.36b** | **2.46±0.52a** |
| Manganese | | | | | | | | |
| 30 day | 30.04±0.32 | 6.98±0.01 | 99.25±0.30 | 4.98±0.12 | 0.37±0.40 | 84.52±5.68 | 2.98±0.23 | 1.02±0.09 |
| 60 day | 30.01±0.31 | 7.02±0.01 | 100.98±0.31 | 4.62±0.12 | 1.87±0.71 | 99.75±2.62 | 2.94±0.34 | 1.04±0.25 |
| 90 day | 29.64±0.31 | 7.05±0.04 | 102.15±0.34 | 4.35±0.11 | 1.89±0.67 | 99.11±3.36 | 2.99±0.24 | 1.12±0.12 |
| | | | | **4.65±0.36ef** | **1.38±0.79bc** | **94.46±13.82e** | **2.97±0.37ef** | **1.06±0.06d** |
| Control | | | | | | | | |
| 30 day | 30.03±0.32 | 6.99±0.01 | 99.95±0.41 | 6.58±0.15 | 0.17±0.10 | 96.52±2.68 | 3.99±0.21 | 0.69±0.09 |
| 60 day | 30.01±0.35 | 7.01±0.01 | 100.22±0.33 | 6.32±0.16 | 0.87±0.11 | 100.50±3.62 | 4.26±0.14 | 0.63±0.15 |
| 90 day | 29.99±0.36 | 7.01±0.02 | 101.15±0.36 | 6.35±0.15 | 0.89±0.17 | 99.11±2.36 | 5.31±0.14 | 0.69±0.16 |
| | | | | **6.42±0.26a** | **0.64±0.38d** | **98.71±4.20de** | **4.52±0.66c** | **0.67±0.11e** |

Means with same letter(s) in a single column for each variable are statistically similar at $p<0.05$

concentration, followed by that in zinc, lead, manganese and iron mediums. Excretions of ammonia in 90 day fish were higher than that of 60 and 30 day age groups. *Catla catla* showed significantly higher ammonia excretion under zinc toxicity, followed by that of iron, manganese, lead and nickel. Iron test mediums showed significantly higher sodium and potassium contents than the other metals during toxicity trials. Maximum carbondioxide contents were recorded as 2.46±0.52 mg $L^{-1}$ during nickel toxicity trail, followed by that of lead, iron, zinc, manganese and control mediums as 2.24±0.28, 2.05±0.10, 2.03±2.11, 1.06±0.06 and 0.67±0.11 mg $L^{-1}$, respectively.

## DISCUSSION

Acute toxicity, determined by 96 h $LC_{50}$ concentrations of metals, varied significantly among three age groups. Thirty 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 metal concentration in fish increased with age that exerted significant impact on the tolerance limits of fish. The susceptibility of fish to a particular heavy metal is a 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. During present study, 96 h $LC_{50}$ values of iron for *Catla catla* was the maximum. Salmonids are generally sensitive to high cadmium levels. Juvenile trout (*Orcorhynchus mykiss*) have higher 48 h $LC_{50}$ (Handy, 1992). 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. Sprague (1969) observed variability in acute toxicity even in a single species and single toxicant depending on fish size, age and condition of the test species along with experimental factors. Gupta *et al.* (1981) reported that the differences in acute toxicity may be due to changes in water quality and test species. Chinni and Yallapragda (2000) carried out acute toxicity tests with metals (Pb, Zn, Cd and Co) on *Penaeus indicus* post larvae. The resulting 96 h $LC_{50}$ 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 mg $L^{-1}$, respectively. Pandey *et al.* (2005) conducted 96 h acute toxicity tests in flow- through 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 hardness of 100 mg $L^{-1}$ $Ca^{2+}$, carp fry and fingerling (*Cyprinus carpio*) have a cadmium 96 h $LC_{50}$ of 4.3 and 17.10 mg $L^{-1}$, 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 *et al.*, 1995). 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, the ammonia excretion by the fish increased significantly at higher concentrations of metals. At higher concentrations of metals, the dissolved oxygen contents of the test mediums decreased significantly. This shows that high concentrations of metallic ions induced stress in the fish that resulted in significantly more oxygen consumption and thus, dissolved oxygen concentrations of the test medium declined. 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) reduce 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 mg $L^{-1}$) than that of swimming fish (207.00±21.99 mg $L^{-1}$). Additionally it was also found that increased water hardness (calcium) ameliorates ammonia toxicity in fish living in high pH water (Wicks *et al.*, 2002). Sodium has been associated with decreased copper toxicity in fathead minnows at concentrations greater than 1 nN (23.8 mg $L^{-1}$), however, toxicity tests conducted with sodium concentrations of 2 nN (47.5 mg $L^{-1}$) were associated with a two fold decrease in copper toxicity (Erickson *et al.*, 1996). During this study iron test mediums showed significantly higher sodium contents resulted decreased sensitivity (higher $LC_{50}$ values) of iron to fish than rest of the metals.

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