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# Evaluation of Acute Toxicity of Metals Mixture and Bioaccumualtion in Freshwater Fish

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**Abstract** A study was carried out to investigate acute toxicity (96-hr $LC_{50}$ and lethal concentrations) of waterborne metal mixture (Fe+Ni) for the fish, *Catla catla*, *Labeo rohita*, *Cirrhina mrigala*, *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix*. The degree of bioaccumulation of metals in fish body organs viz. gills, liver, kidney, fins, bones, muscle and skin at both 96-hr $LC_{50}$ and lethal concentrations were also determined. Mortality upon the 90-day old fish species was used as a criterion of toxicity during these experiments. With three replications for each test dose, the tests were performed, separately, at constant temperature (30℃), pH (7) and hardness (200 mg/L) of water. Against metals mixture (Fe+Ni), the overall sensitivity of five fish species, determined in terms of $LC_{50}$ and lethal concentrations varied significantly. Regarding overall sensitivity of five fish species, Hypophthalmichthys molitrix were significantly more sensitive to metals mixture, followed by that of *Labeo rohita*, *Ctenopharyngodon idella*, *Catla catla* and *Cirrhina mrigala*. The concentration of both metals in fish body organs were determined by using Atomic Absorption spectrophotometer. Fish kidney exhibited significantly higher ability to amass both these metals during acute exposure of metal mixture. Accumulation of the metals in fish body followed the general order: kidney>liver>skin>gills>fins>muscles>bones. During both 96-hr $LC_{50}$ and lethal concentrations fish showed significantly higher accumulation of iron than that of nickel in their body.
**Keywords** Acute toxicity, Bioaccumulation, Metal mixture, Fish, Fe, Ni

## Introduction

Aquatic pollution with metals is a worldwide ecological problem. Among different pollutants, heavy metals are distinctive in their action due to their non-biodegradability (Javed and Abdullah, 2006), susceptibility of bio-magnification in food chain (Khare and Singh, 2002) and their consequences on the environmental stability of the beneficiary aquatic body and variety of aquatic organisms (Farombi et al., 2007). Due to damaging effects of metals to aquatic flora and fauna, it is crucial to observe their toxicity to the key edible species, because this will present a warning sign of sequential and spatial level of the process, as well as assessment of possible impacts of metal on the human health (Fernandes et al., 2007).

Quantitative factor involving survival and mortality of fish are used to estimate the effects caused due to acute toxicity of different toxicants for the fish (Azmat et al., 2012) and to assess the sensitivity of various fish species against metal's toxicity (Naz et al., 2012 ). Fish is more susceptible to metals accumulation when the concentration of metals exceeds the permissible limits in the aquatic environment (Rauf et al., 2009) and serious health problems were induced due to consumption of the metals contaminated fish by humans. Fish can uptake metals via gut, skin and gills; however which course is more crucial is based upon existing environmental conditions (Javed, 2012a).

However, metals amassing in tissues reveal the past exposure of fish via water or food that can work as bio-indicator of metallic pollution of the atmosphere (Jabeen et al., 2012). Different organs of the fish take up heavy metals due to the affinity between them. During this process, these heavy metals are accumulated at different levels in different body organs of the fish (Javed, 2012b; Bervoets et al., 2001).

Accumulation of heavy metals in fish is generally based upon concentration of water in sambient water and period of exposure, however many other factors such as pH, water salinity, hardness and temperature, size and age, ecological needs, life cycle, capture season and feeding habits of fish also play considerable role in accumulation of metals (Naz et al., 2012). Studies conducted on numerous fishes showed that heavy metals amend the biochemical parameters and physiological activities both in blood and tissues (Basa and Usha Rani, 2003; Javed, 2012a). The toxic effects of heavy metals have been reviewed (Naz and Javed, 2012), including bioaccumulation (Javed, 2012b; Vinodhini and Narayanan, 2008). Iron is the most vital element for hemoglobin and myoglobin development in fish as well as it also plays a fundamental role for the growth of aquatic organisms. Unfortunately, increased industrial effluents polluted the natural ecosystem and it enhanced at momentous contamination stage (Hussain et al., 2011). Nickel, a grey-listed metal (Mason, 1996), is an element of less importance for numerous animal species (Phipps et al., 2002), and if it is existed in large deposits, then the endurance, augmentation, behavior, and reproduction of aquatic animals are affected (Wong et al., 1993).

The insecure concentrations of heavy metals in our riverine systems and their negative effects on fish require conducting this research project to observe the toxic impacts of iron and nickel mixture on the most cultured fish species of Pakistan viz. *Catla catla*, *Labeo rohita*, *Cirrhina mrigala*, *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix*.

## 1 Results and Discussion

### 1.1 Acute toxicity of metals mixture to the fish

Large deposits of heavy metals found in the earth's crust, air, water and food impose negative impacts on aquatic life (Naz and Javed, 2012) as these metals cause serious health problems in animal and humans, directly as well as indirectly (Azmat and Javed, 2011). However, the individual metals do not induce toxicity in the natural aquatic environment but they become hazardous when present in form of mixture (Naz et al., 2012).

Testing of five fish species for their 96-hr $LC_{50}$ and lethal concentrations of metal mixture with 95% confidence interval are shown in Table 1. The differences among all the five fish species, for their ability to tolerate metal mixture in terms of 96-hr $LC_{50}$ and lethal concentrations, varied significantly. Regarding overall sensitivity of five fish species, Hypophthalmichthys molitrix were significantly more sensitive to metals mixture, followed by that of *Labeo rohita*, *Ctenopharyngodon idella*, *Catla catla* and *Cirrhina mrigala*. Acute ($LC_{50}$) toxicity trails were performed on major carps (*Catla catla*, *Labeo rohita* and *Cirrhina mrigala*) exposed to metals mixture (Fe+Ni+Pb+Zn+Mn) by Naz and Javed (2012). They found that, on observing overall sensitivity of three fish species, *Labeo rohita* depicted significantly least sensitivity to metal mixtures with mean $LC_{50}$ and lethal concentrations of 81.73±12.73 and 128.80 ±19.95 mg/L, respectively. Hua and Qixing (2009) demonstrated an experiment to predict the single and joint effects of metals (Cd and Zn) on grass carp (*Ctenopharyngodon idella*). The concluded on the basis of $LC_{50}$ (96-hr) values that acute toxicity of zinc was significantly lower than cadmium as the 96-hr $LC_{50}$ of cadmium and zinc were 26.86 and 33.14 mg/L, respectively. Acute methods are utilized to compare the ability of species having numerous developmental stages and diversified phylogenetic positions, when they are exposed to toxicants (Kazlauskiene et al., 2003; Abdullah and Javed, 2006). However, in nature, the toxicants and their mixtures have constant effect on various species during continuing exposure of their negligible concentration.

The relationships between $LC_{50}$ and lethal concentrations of five fish species against metal mixture were computed also by regression analysis. There existed highly significant correlations between 96-hr $LC_{50}$ and lethal concentrations of all the four fish species against metal mixture except for *Ctenopharyngodon idella* (Table 2). The higher values of coefficient of determination ($R^2$) for all the equations computed for

Table 1 Responses of five fish species for their 96-hr $LC_{50}$ and lethal concentrations (mg/L) of metals mixture (Fe+Ni)

| 96-hr | Fish species | | | | |
|---|---|---|---|---|---|
| | *Catla catla* | *Labeo rohita* | *Cirrhina mrigala* | *Ctenopharyngodon idella* | *Hypophthalmychthys molitrix* |
| $LC_{50}$ | 78.75±0.56 c | 96.99±0.72 a | 64.44±0.70 d | 89.80±0.77 b | 52.16±0.58 e |
| Lethal concentrations | 128.53±0.69 c | 140.98±0.46 a | 100.35±0.46 d | 135.25±1.42 b | 87.09±0.09 e |

Note: Means with similar letters in a single row are statistically non-significant at $p<0.05$

Table 2 Relationships between 96-hr $LC_{50}$ and lethal concentrations (mg/L) of mixture for five fish species

| | Means | | Regression Equation | r | $R^2$ |
|---|---|---|---|---|---|
| Fish Species | $LC_{50}$ (x) | Lethal Con. (y) | | | |
| *Catla catla* | 78.75 | 128.53 | Y=224.44-1.218 ($LC_{50}$) SE=0.047** | 0.999 | 0.998 |
| *Labeo rohita* | 96.99 | 140.98 | Y=93.42+0.490 ($LC_{50}$) SE=0.410** | 0.767 | 0.588 |
| *Cirrhina mrigala* | 64.44 | 100.35 | Y=79.52+0.323 ($LC_{50}$) SE=0.567* | 0.495 | 0.245 |
| *Ctenopharyngodon idella* | 89.90 | 135.25 | Y=84.86+0.561 ($LC_{50}$) $SE=1.452^{NS}$ | 0.360 | 0.130 |
| *Hypophthalmychthys molitrix* | 52.16 | 87.08 | Y=92.07-0.096 ($LC_{50}$) SE=0.086** | 0.743 | 0.552 |

Note: Con.=Concentration; r=Correlation coefficient; $R^2$=Coefficient of determination; *=Significant at $p<0.05$; **=Significant at $p<0.01$; $^{NS}$=Non-significant

all the fish species predict high reliability of these regression models. On exposing three fish species viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* to 96-hr $LC_{50}$ and lethal concentrations of metals, significantly direct relationships were examined between them (Azmat, 2011).

### 1.2 Uptake and accumulation of metals in fish body during acute exposure of mixture

Studies on metals bioaccumulation can present data to investigate aquatic toxicants. Hence, the routes of entry of different toxicants to different tissues/organs, their amount ingested and their retention level in each tissue can be evaluated. The background iron and nickel concentrations in body organs of 90-day *Catla catla*, *Labeo rohita*, *Cirrhina mrigala*, *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix*, before metal mixture exposure were determined and their values are presented in Table 3. However, during acute toxicity trails (96-hr $LC_{50}$ and lethal concentrations) the accumulation of both metals in form of mixture in body organs of five fish species increased significantly (Table 4). Metals accumulation in kidney, liver, fins, bones, muscles and gills occurs, when different metals enter in the fish body by means of water and feed intakes (Rauf et al., 2009). The 96-hr $LC_{50}$ exposure of Fe+Ni mixture caused significantly maximum mean accumulation of iron (258.62±279.42 $\mu g g^{-1}$) in body organs of *Cirrhina mrigala*, followed by that of *Hypophthalmichthys molitrix*, *Labeo rohita*, *Ctenopharyngodon idella* and *Catla catla* (Table 4). The uptake of essential and non-essential metals as well as their accumulation by the fish is related. In contrast to it, the metals accumulation patterns and

Table 3 Metal concentrations (μgg$^{-1}$) in control fish used during acute toxicity trials.

| Metals | Fish species | Organs | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Kidney | Liver | Skin | Muscle | Fins | Gills | Bones |
| Fe | *C. catla* | 17.47±0.28 c | 13.12±1.17 c | 12.78±2.34 e | 23.03±2.23 d | 8.22±0.81 d | 14.97±2.24 e | 13.41±0.39 e |
| | *L. rohita* | 10.69±1.19 e | 7.40±0.87 d | 16.52±6.20 d | 31.81±0.85 a | 24.68±2.11 b | 25.36±1.58 d | 35.23±2.37 a |
| | *C. mrigala* | 35.77±1.63 a | 33.40±2.20 a | 27.19±0.56 b | 24.07±0.12 c | 33.79±0.24 a | 30.96±0.67 b | 25.60±0.40 d |
| | *C. idella* | 11.51±1.39 d | 33.39±1.20 a | 37.05±0.36 a | 1.45±0.05 e | 21.80±0.64 c | 27.08±1.21 c | 27.39±0.43 b |
| | *H. molitrix* | 30.24±0.16 b | 32.32±1.02 b | 25.45±0.14 c | 27.21±0.10 b | 24.83±0.58 b | 35.85±0.50 a | 26.10±0.51 c |
| Ni | *C. catla* | 11.17±0.02 c | 13.59±0.05 c | 1.50±0.07 c | 2.27±0.01 d | 3.11±0.01 e | 5.78±0.06 d | 7.21±1.57 d |
| | *L. rohita* | 27.57±1.41 a | 20.24±3.44 a | 8.03±0.69 a | 9.67±1.15 b | 6.44±1.15 c | 10.36±0.71b | 11.55±1.70 c |
| | *C. mrigala* | 12.16±2.90 b | 14.13±1.47 b | 8.25±0.53 a | 11.23±0.33 a | 7.18±0.56 b | 7.91±1.27 c | 12.40±0.44 b |
| | *C. idella* | 9.74±3.44 d | 7.03±1.21 e | 5.76±1.11 b | 5.17±0.51 c | 8.47±0.79 a | 13.99±1.02 a | 15.36±0.20 a |
| | *H. molitrix* | 11.28±1.91 c | 8.55±1.70 d | 8.88±0.74 a | 5.20±0.84 c | 5.40±0.82 d | 4.24±1.48 e | 4.28±0.06 e |

Table 5 Mean values for physico-chemical variables of the test and control media

| | Total ammonia (mg/L) | Electrical conductivity (m/Scm) | Calcium (mg/L) | Sodium (mg/L) | Dissolved oxygen (mg/L) | Potassium (mg/L) | Total hardness (mg/L) | Temperature (°C) | pH | Magnesium (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|
| $LC_{50}$ | 1.49±0.43 | 2.85±0.14 | 13.359±2.87 | 295.91±0.54 | 4.99±0.22 | 7.25±0.45 | 224.17±2.32 | 30.32±0.84 | 7.24±0.02 | 47.69±2.11 |
| Lethal concentration | 1.44±0.37 | 2.64±0.37 | 13.68±1.67 | 290.16±15.90 | 4.88±0.39 | 7.94±0.86 | 223.50±3.37 | 30.35±0.43 | 7.24±0.02 | 47.31±1.23 |
| Control | 1.30±0.85 | 2.74±0.55 | 13.00±0.25 | 221.00±5.56 | 5.16±0.26 | 7.50±0.20 | 221.00±0.22 | 30.00±0.12 | 7.24±0.03 | 47.11±1.28 |

their toxic effects in several fish species fluctuate significantly (Luoma and Rainbow, 2005; Abdullah et al., 2011). Activities of fish like feeding and swimming drastically reduce and they grew weaker as the exposure time was prolonged. This is particularly noticed in test organisms in higher concentrations.

Mean iron accumulation in five fish species was significantly higher in kidney (335.05±302.02 μgg$^{-1}$), followed by that in gills, liver, skin, bones, muscle and fins. Due to waterborne and dietary exposure of metal mixture (Cu+Cd+Zn+Ni+Co) to fish, the kidney, gills and liver demonstrated significantly higher accumulation of all metals (Javed and Abdullah, 2004). Nickel accumulation was significantly maximum (143.90± 136.92 μgg$^{-1}$) in *Cirrhina mrigala* and that of minimum was exhibited by *Labeo rohita*. The effects of toxicants in terms of level of toxicity and accumulation varied from species to species (Luoma and Rainbow, 2005). Gupta and Srivastava (2006) investigated the accumulation level of zinc in *Channa punctatus* exposed to three concentrations of zinc (10 mg/L, 15 mg/L, and 25 mg/L). They found significant increase of this metal in fish body. Fish kidney showed significantly highest tendency for the accumulation of 194. 44±110.98 μgg$^{-1}$ nickel while fish bones exhibited the least tendency for these accumulations. An exposure of metals mixture (Cu+Cd+Zn+Ni+Co) to major carps caused significantly higher accumulation of theses metals in their body. However, these accumulation were significantly minimum in fish bones (Javed , 2012b).

During lethal exposure of Fe+Ni mixture, *Cirrhina mrigala* accumulated significantly higher concentrations of both iron and nickel in its body. Among the fish organs, kidney showed significantly higher tendency for the accumulation of both iron (517.20± 362.62 μgg$^{-1}$) and nickel (323.57±136.58 μgg$^{-1}$). Azmat et al. (2012) pointed out kidney as an appropriate indicator of metal contamination in major carps. Water-borne metals can be elated to various organs to stimulate cellular and histopathological amendments that lead to genetic alteration in animals (Tkatcheva et al., 2000). Metals bio-accumulation is dependent upon their route of uptake, concentration,

storage, availability and mechanisms of excretion in animals (Vijver et al., 2004). The exposure of this mixture resulted in significantly higher iron accumulation in fish body than that of nickel. Palaniappan and Karthikeryan (2009) conducted a study to investigate the bio-accumulation as well as the depuration of chromium, individually or in mixture form with nickel in the body organs of *Cirrhinus mrigala*. Their results indicated that the level of accumulation of chromium in all fish organs was significantly lower than that of nickel. Synergistic relationship was developed between these metals as the mixture of Ni+Cr was more toxic to the fish than the effect of these metals alone.

### 1.3 Physico-chemical parameters

Due to various types of biological and physical pollutants that come out from several industrial and agricultural sources has greatly influenced the water quality (Andhale and Zambare, 2011). The data on physico-chemical parameters of the test media estimated during these tests and control treatments revealed that the mean values of total ammonia, electrical conductivity and sodium were higher than that of control media. However, the treated fish mediums had lower concentrations of dissolved oxygen than mediums used for control fish. The concentrations of calcium, potassium and magnesium in both mediums used for treated and control fish remained almost same during acute toxicity trails (Table 5). Dissolved oxygen of contents of the media decreased significantly due to enhanced excretion of ammonia by the fish. Naz et al. (2012) investigated during their study on the acute toxicity of metals mixture to fish (*Catla catla*, *Labeo rohita*, *Cirrhina mrigala*, *Ctenopharyngoden idella* and *Hypophthalmichthys molitrix*) that increase in ammonia excretion caused significant decrease in dissolved oxygen contents of the water media.

## 2 Conclusion

Regarding overall sensitivity of fish species, *Labeo rohita* were significantly least sensitive towards $LC_{50}$ and lethal concentrations of metals mixture. While concerning overall bioaccumulation patterns of iron and nickel in fish body, significant differences were observed for accumulation of metals. However, significantly higher accumulation was shown by iron as compared to that of nickel. Fish kidney appeared as an organ that showed significantly higher tendency of the accumulation of both nickel and iron.

## 3 Materials and Methods

Fingerlings of *Catla catla*, Labeo rohita, *Cirrhina mrigala*, *Ctenopharyngoden idella* and *Hypophthalmichthys molitrix* of average weights of 3.28 g, 5.60 g, 4.46 g, 4.03 g, 3.85 g, fork lengths of 56.04 mm, 74.05 mm, 71.88 mm, 69.31 mm and 64.06 mm and total lengths of 65.84 mm, 84.11 mm, 81.51 mm, 80.00 mm and 74.91 mm, respectively were collected from local Fish Seed Hatchery, Faisalabad, Pakistan. Fish species were kept under laboratory conditions for 14 days for acclimation prior to start of this experiment. The laboratory photoperiod was 12 hours by using fluorescent light. Fish species were fed with pelleted feed having 35% digestible protein and 2.90 Kcal/g digestible energy and then 70% water was renewed every day. Feeding was hovering 24 hour earlier during this mortality experiment for all fish species. Glass aquaria of 60 liter water capacity were used in this experiment. Fresh air was supplied to the each aquaria through air pump fitted with capillary system. Pure chloride compounds of iron and nickel (Aldrich, USA) were used as the metal toxicant in this study. Desired concentration of metal mixture was prepared by dissolving an appropriate volume of stock solution in tap water. Fish species were exposed to metal mixture concentration of 0, 5 mg/L, 10 mg/L, 20 mg/L, 25 mg/L, 30 mg/L, 35 mg/L, 35 mg/L, 40 mg/L, 45 mg/L, 50 mg/L, 55 mg/L, 60 mg/L, 65 mg/L, 70 mg/L, 75 mg/L, 80 mg/L, 85 mg/L, 90 mg/L, 95 mg/L, 105 mg/L, 120 mg/L, 125 mg/L, 130 mg/L and 135 mg/L for 96 hr for determination of their tolerance limits. The concentrations were maintained within 3.5 hr and full toxicant concentrations in 7 hr. Each fish species was experienced, separately, for this acute toxicity experiment against

binary mixture of iron and nickel at constant temperature (300℃) total hardness (225 mg/L) and pH (7). Fish mortality observations were made twice a day. The metal mixture (Fe+Ni) concentrations for each fish species were started from zero with an increment of 0.05 mg/L and 5 mg/L for low and high concentrations, respectively for $LC_{50}$ and lethal concentrations. No mortality of fish was observed in control media. Fish mortality data obtained against each concentration during 96 hr test duration. Each test dose was tested in triplicate. The acute toxicity bioassay method based on standard method was determined to evaluated 96-hr and $LC_{50}$ and lethal concentrations of metal mixture for five fish species. The 96-hr and $LC_{50}$ and lethal concentrations of Fe+Ni mixture for each fish species as calculated by using probit analysis method (Ezeonyejiaku and Obiakora, 2011). Regression analyses were performed to find out relationship between $LC_{50}$ and lethal concentrations for each fish species.

The metals in fish body organs were also determined after acute toxicity tests by following the method of S.M.E.W.W. (1989). The fish were dissected and different organs viz. gills, liver, kidney, fines, bones, muscles and skin were taken from the experimental and metal free (control) aquaria separately and all the organs were washed with distilled water. Fish organs were digested in $HNO_3$ and $HCLO_4$ (3:1 V/V) by following S.M.E.W.W. (1989). The final acid digested extract was analyzed for metal mixture (Fe+Ni) by using Atomic Absorption Spectrophotometer (Analyst-400, Perkin Elmer).

Water temperature, pH and dissolved oxygen of the test media were determined twice a day by using digital meters, viz. HANNA HI-8053, HI-8733, HI-8520, HI-9146, respectively. However, total hardness, total ammonia, chlorides, sodium and potassium concentrations in each test medium were determined by the methods of A.P.H.A. (1998). Water temperature was maintained at 30oC by using automatic heaters. The pH of the test media was maintained by adding NaOH and HCl to increase and decrease pH, respectively. In order to maintain the total hardness of water, salts of $MgSO_4$ and $CaSO_4$ were added to increase the hardness while EDTA was used to decrease the water hardness. Data obtained from this acute toxicity tests were analyzed and all the results were expressed as mean±S.D. The results were evaluated using analysis of variances and Tukey's/ Student Newnan-Keul tests (Steel et al., 1996).

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Table 4 Accumulation patterns of metals (μgg$^{-1}$) in fish organs during 96-hr $LC_{50}$ and lethal concentrations exposure of metal mixture (Fe+Ni)

| Metals | Fish species | Organs | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Kidney | Liver | Skin | Muscle | Fins | Gills | Bones | *Means |
| $LC_{50}$ | | | | | | | | | |
| Fe | *C. catla* | 114.89±0.23 c | 115.47±2.41 d | 464.80±1.88 a | 54.02±2.4 bcd | 48.43±9.75 b | 2.02±2.49 e | 18.21±0.48 e | 116.84±159.49 c |
| | *L. rohita* | 112.01±0.54 c | 352.87±1.72 b | 291.56±1.13 b | 284.68±3.15 a | 56.31±1.09 b | 121.34±2.24 d | 219.47±2.41 ab | 205.46±110.81 b |
| | *C. mrigala* | 602.92±4.02 b | 487.08±5.64 a | 8.02±1.04 e | 19.84±2.48 cd | 54.40±2.54 b | 572.91±3.65 a | 65.17±5.01 de | 258.62±279.42 a |
| | *C. idella* | 123.11±1.69 c | 96.58±2.75 d | 164.78±1.09 c | 10.43±1.46 d | 116.90±1.50 a | 259.81±1.79 c | 86.38±1.81 cde | 122.57±76.62 c |
| | *H. molitrix* | 722.33±2.52 a | 182.83±1.77 c | 19.29±1.77 de | 51.04±1.85 bcd | 56.33±1.28 b | 399.72±3.13 b | 125.34±2.52 b | 222.41±255.13 b |
| | Overall means | 335.05±302.0 a | 246.97±167 b | 189.69±192.8 c | 84.00±113.78 d | 66.48±28.37 d | 271.16±225.0 b | 102.91±75.76 d | |
| Ni | *C. catla* | 326.67±2.89 a | 191.67±2.89 c | 27.08±0.41 e | 51.80±0.64 c | 49.22±0.84 c | 96.20±1.12a | 14.25±0.76 d | 108.13±113.22 c |
| | *L. rohita* | 48.33±1.91 b | 80.00±1.00 d | 58.92±2.15 d | 60.37±3.90 b | 39.92±1.61 e | 31.92±0.09 d | 113.05±2.68 a | 61.79±27.45 e |
| | *C. mrigala* | 156.67±2.89 c | 430.91±0.19 a | 163.50±1.25 d | 41.23±1.26 d | 104.79±0.95 a | 84.33±2.69 b | 25.83±3.25 c | 143.90±136.92 a |
| | *C. idella* | 282.22±2.55 b | 207.33±2.52 b | 74.20±2.48 c | 83.63±2.65 a | 61.00±2.00 b | 74.16±3.21 c | 59.63±2.23 b | 120.31±88.12 b |
| | *H. molitrix* | 158.33±1.67 c | 28.44±2.22 e | 298.14±1.98 a | 24.75±1.33 e | 44.10±1.65 d | 32.46±0.61 d | 1.37±0.06 e | 84.07±107.23 d |
| | Overall means | 194.44±110.98 a | 187.67±155.33 b | 124.37±109.54 c | 52.36±21.96 f | 59.98±26.23 e | 63.82±29.90 d | 42.83±44.83 g | |
| Lethal concentrations | | | | | | | | | |
| Fe | *C. catla* | 214.33±1.54 e | 240.83±2.37 c | 532.67±5.51 a | 116.97±0.60 b | 110.90±1.44 e | 82.37±2.41 e | 2.96±1.20 e | 185.86±172.55 e |
| | *L. rohita* | 328.33±3.82 c | 517.42±2.02 b | 387.55±2.41 b | 387.54±2.05 a | 128.09±1.45 c | 305.55±0.69 d | 351.33±1.16 a | 343.69±117.09 b |
| | *C. mrigala* | 995.67±1.03 a | 532.61±1.49 a | 37.37±0.83 e | 385.28±1.28 a | 167.57±0.91 b | 919.87±3.59 a | 105.83±1.61 d | 449.17±386.87 a |
| | *C. idella* | 232.68±1.69 d | 194.26±2.75 e | 308.82±1.09 c | 27.69±1.46 d | 296.04±1.50 a | 409.37±1.79 c | 212.66±1.81 c | 240.22±118.70 d |
| | *H. molitrix* | 815.00±3.31 b | 228.33±3.8 d | 102.89±1.95 d | 106.91±1.21 c | 114.99±1.95 d | 466.27±2.41 b | 289.85±1.63 b | 303.46±119.27 c |
| | Overall means | 517.20±362.62 a | 342.69±159.0 c | 273.86±203.89 d | 204.88±169.2 e | 163.52±77.3 g | 436.68±307.34 b | 192.52±140.13 f | |
| Ni | *C. catl* | 462.50±3.31 a | 212.96±2.31 c | 155.08±1.13 d | 119.85±2.23 d | 85.72±1.52 e | 232.41±1.55 a | 111.43±1.51 d | 197.13±128.72 d |
| | *L. rohita* | 121.57±1.48 e | 123.44±1.79 e | 150.38±2.00 e | 104.27±2.73 e | 166.30±2.73 c | 153.38±3.17 c | 231.17±0.76 a | 150.07±41.77 e |
| | *C. mrigala* | 340.71±3.57 c | 586.66±1.67 a | 244.42±1.28 b | 219.05±1.65 b | 259.65±3.05 a | 220.06±1.35 b | 134.72±1.94 c | 286.47±145.79 a |
| | *C. idella* | 427.67±1.53 b | 371.66±1.09 b | 172.89±1.67 c | 185.37±1.37 c | 156.95±2.35 d | 106.93±2.86 d | 140.58±0.76 b | 223.15±124.19 c |
| | *H. molitrix* | 265.41±0.96 di | 145.25±2.46 d | 538.05±2.92 a | 414.67±2.08 a | 263.85±1.30 b | 152.03±2.27 c | 105.07±1.19 e | 269.19±157.86 b |
| | Overall means | 323.57±136.58 a | 287.99±193.19 b | 252.17±164.21 c | 208.64±124.37 d | 186.50±75.45 e | 172.96±52.28 f | 144.60±50.67 g | |

Comparison of means

| Metals | Mean accumulation in fish body (μgg$^{-1}$) | |
|---|---|---|
| | * $LC_{50}$ | * Lethal concentrations |
| Fe | 185.18±194.36 a | 304.48±236.36 a |
| Ni | 103.64±98.73 b | 225.20±127.60 b |

Note: Means with the same letters in a single row and * column are statistically similar at $p<0.05$; *C. catla=Catla catla*; *L. rohita=Labeo rohita*; *C. mrigala=Cirrhina mrigala*; *C. idella=Ctenopharyngodon idella*; *H. molitrix=Hypophthalmichthys molitx*