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***Full Length Article***

# Effects of Metals Mixture on the Growth and their Bio-accumulation in Juvenile Major Carps

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## ABSTRACT

Effects of waterborne and dietary metal mixtures (MMs) of copper (Cu), cadmium (Cd), zinc (Zn), nickel (Ni) and cobalt (Co) on the growth and their accumulation in fish, *Catla catla, Labeo rohita* and *Cirrhina mrigala* were examined under controlled laboratory conditions for 12 weeks. The exposure of MM, either waterborne or dietary, showed significant impacts on the growth of all the three fish species to exhibit significantly variable responses toward feed intakes, weight and length increments, and FCE. Dietary treatments caused significantly better feed intake and FCE than the fish exposed to waterborne treatments. Fish were found significantly more susceptible to waterborne than dietary MM treatments. Exposure of both waterborne and dietary MMs caused significantly variable accumulation of all metals in the fish body that followed: Zn>Ni>Cu=Cd>Co. Among the three fish species, *C. mrigala* showed significantly higher amassing of metals (21.79±9.74 µg $g^{-1}$), followed by that in *C. catla* (18.26 µg $g^{-1}$) and *L. rohita* (17.33 µg $g^{-1}$). Dietary intake of MM caused significantly higher accumulation of all metals than that of waterborne treatments. Accumulation of metals in fish body followed the order: liver>kidney>gills>skin>muscle>fins>bones. Both waterborne and dietary MMs caused significant toxicity to the fish in a synergistic/antagonistic way. Significant interactions among Cu, Cd, Zn, Ni and Co, in a mixture form, predict their competitive uptake that fluctuated significantly between waterborne and dietary treatments to cause significant distribution in various organs of three fish species. 

**Key Words:** Metals mixture; Fish growth; Bioaccumulation; Organs

## INTRODUCTION

An interest to elucidate the effect of individual metals and their mixture on fish is increasing in the world due to their harmful effects on the environment and human health. Most studies have evaluated the toxicity of aquatic pollutants to the fish and their environments exposed to individual metals (Ali *et al*., 2003; Gupta & Srivastava, 2006; Naz *et al*., 2008; Naeem *et al*., 2011; Azmat *et al*., 2012). However, aquatic ecosystems are usually exposed, simultaneously, to a mixture of metals that can exhibit significant interactions among them (Vosyliene & Jankaite, 2006; Bu-Olayan & Thomas, 2008; Hussain *et al*., 2010).

Fish can act as bio-indicator of metals effect in an aquatic ecosystem (Jabeen *et al*., 2012) that can be supported by studying the growth potentials and their ability to bio-accumulate metals during chronic exposure of MMs. Metals include both essential and non-essential having no known biological role such as $Cd^{2+}$, $Hg^{2+}$, $Ag^{2+}$ and $Sn^{2+}$. All metals become detrimental to the organisms at a particular level of exposure, either waterborne or dietary intakes. Aquatic organisms have specific ability to regulate essential metals up to a certain concentration however this ability is disturbed under continual long term exposure to initiate metals bio-accumulation in the body beyond permissible limits (Jabeen *et al*., 2012).

Zinc is an essential metal while its persistent existence in water may become toxic to the fish due to its biological non-degradability (Hogstrand *et al.*, 1994). Cadmium needs particular attention due to its menace to cause high toxicity to the aquatic biota (Barber & Sharma, 1998) and human life (Vanderpool & Reeves, 2001). Toxic responses of various fish species exposed to waterborne concentration of metals are well documented while little research has been done to elucidate the toxic effects of dietary metals and their mixtures despite diet being an important route to contaminate fish in natural aquatic ecosystems (Jabeen *et al*., 2012). Metallic ion exposure can modify the fish metabolism and physiology leading to irreparable damage to their behavior (Petrauskiene, 1999), growth (Ali *et al*., 2003) and reproduction (James *et al*., 2003). Metals are also known to induce genetic alterations and teratogenesis in fish (Bagdonas & Vosyliene, 2006).

Metals toxicity may affect all phases of reproduction, growth and survival of fish. Metals viz. Cu, Cd, Zn, Ni and Co are the common pollutants of the rivers in Punjab province entering with untreated municipal and industrial waste waters causing adverse effects on the indigenous fish

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fauna. Therefore, conservation of indigenous cyprinids viz. *C. catla, L. rohita* and *C. mrigala* in their natural habitats make it necessary to determine their growth potentials and ability to bio-accumulate metals during chronic exposure of waterborne and dietary MMs. This will help in formulating strategies for sustainable conservation of indigenous fish species and to predict possible impacts of persistent metal's pollution in the aquatic habitats of Pakistan.

## MATERIALS AND METHODS

**Fish species:** Juvenile major carps viz. *C. catla, L. rohita* and *C. mrigala* of average weights 7.00, 7.50 and 6.40 g, fork lengths 84.40, 87.40 and 77.20 mm, and total lengths of 85.60, 89.60 and 80.20 mm, respectively were grown in glass aquaria containing 50 L water with three replications for each treatment by following RCBD design. Prior to growth trials, fish were acclimatized to laboratory conditions for two weeks. Pure chloride compounds (Aldrich, USA) of metals (Cu, Cd, Zn, Ni & Co) were used to prepare the desired MM concentrations on metallic ion basis. Fish were fed on crumbled feed (32.50% Digestible Protein & 3 Kcal $g^{-1}$ Digestible Energy) twice daily throughout growth period of 12 weeks.

**Waterborne metal exposure growth trails:** Juvenile 120-day old major carps viz. *C. catla, L. rohita* and *C. mrigala* were grown, separately, in glass aquaria containing sub-lethal concentrations ($1/3^{rd}$ of $LC_{50}$) of MM. Ten fish of each species, with three replications, were grown, separately, under sub-lethal metal mixture concentrations of 20.97, 22.10 and 22.02 mg $L^{-1}$ for *C. catla, L. rohita* and *C. mrigala*, respectively (Javed & Yaqub, 2008). At the beginning and end of each test, water samples were taken and analyzed for the corresponding metal concentrations by following the methods described in APHA (1998) through Atomic Absorption Spectrophotometer (Analyst 400 Perkin Elmer, USA). Fish were fed the diet, to satiation, twice a day at 10:00 am and 4:00 pm.

**Dietary metal exposure growth trails:** The separate groups (n=10) of *C. catla, L. rohita* and *C. mrigala* of above mentioned average weights and lengths were grown in clean metal free water. However, they were fed the diets, twice a day to satiation, containing sub-lethal MM concentrations of 63.62, 71.36 and 63.63 µg $g^{-1}$, respectively (Javed & Yaqub, 2008) for 12 weeks with three replications for each growth trail. The control fish were grown under metal free water and diets for comparison. The growth of each species of fish was monitored in terms of increase in wet weights, fork and total lengths, condition factor, feed intake and feed conversion efficiency on weekly basis.

**Data analyses:** Data were confirmed for normality of distribution and variance homogeneity. Analysis of variance and comparison of mean values were performed to find out significant differences among variables (Steel *et al*., 1996).

## RESULTS AND DISCUSSION

**Fish growth:** Exposure of both waterborne and dietary MMs caused minimum growth in all the three fish species than that of control. Dietary exposure of MM to *C. catla*, *L. rohita* and *C. mrigala* gave significantly ($p<0.05$) higher average weights of 6.15, 7.15 and 7.51 g, than that of waterborne exposed fish with the average increments of 1.49, 1.24 and 1.39 g, respectively (Table I). Among waterborne MM exposed fish, *L. rohita* had significantly higher feed intake of 2.85 g, followed by that of *C. mrigala* and *C. catla* with the average feed intakes of 2.40 and 2.39 g respectively. Dietary MM treatments also exerted significant impacts on both feed intakes and FCE with the mean values of 10.43 g and 76.87% observed for *C. mrigala* and *C. catla*, respectively (Table I).

The effects of waterborne and dietary metal mixtures varied significantly in their toxicity on the fish (Vosyliene & Jankaite, 2006; Bu-Olayan & Thomas, 2008). Sub-lethal concentrations of MM caused adverse impacts on fish growth and health as evident from fish condition factor (Table I). The exposure of MM, either waterborne or dietary, showed significant impacts on the growth of all the three fish species to exhibit significantly variable responses toward feed intakes, weight and length increments, and FCE also (Naeem *et al*., 2011). Most studies of the effect of metals on fish address exposure to a single metal. Polluted water bodies, however, usually contain elevated levels of metals in a mixture form (Jabeen *et al*., 2012). Therefore, the results obtained for exposure to a single metal in laboratory studies are hardly comparable with those from natural conditions. To explain the interactions among various metals it is necessary to compare their effects in mixture forms that may become antagonistic, additive or synergistic (Marr *et al*., 1998). Three fish species exhibited significantly variable sensitivity to both waterborne and dietary MM treatments that shows species specificity to

**Table I: Growth performance of metals mixture exposed fish**

| Growth parameters | *Catla catla* | | | *Labeo rohita* | | | *Cirrhina mrigala* | | |
|---|---|---|---|---|---|---|---|---|---|
| | Waterborne MM | Dietary MM | Control | Waterborne MM | Dietary MM | Control | Waterborne MM | Dietary MM | Control |
| Bodyweight (g) | 1.49±0.04c | 6.15±0.34b | 31.22±1.43a | 1.24±0.04c | 7.15±0.28b | 32.39±0.67a | 1.39±0.08c | 7.51±0.01b | 35.01±1.01a |
| Fork length (mm) | 3.86±0.66c | 5.93±0.59b | 16.13±2.11a | 4.56±1.01c | 8.57±1.03b | 17.87±2.11a | 4.83±0.81c | 7.54±1.12b | 19.67±1.65a |
| Total length (mm) | 3.91±0.31c | 8.01±1.01b | 18.50±2.01a | 4.59±0.05c | 9.04±0.01b | 16.12±0.01a | 4.45±0.24c | 7.83±1.01b | 18.40±2.01a |
| Fish condition factor | 1.99±0.13c | 2.21±0.01b | 2.85±0.01a | 2.16±0.01c | 2.58±0.11b | 3.19±0.01a | 2.39±0.21c | 2.66±0.10b | 2.99±0.01a |
| Average feed intake (g) | 2.39±0.13c | 8.00±0.05b | 35.67±0.01a | 2.85±0.01c | 9.85±0.01b | 35.88±0.01a | 2.40±0.33c | 10.43±0.09b | 36.74±0.01a |
| Average FCE (%) | 62.23±1.11c | 76.87±2.12b | 87.52±2.21a | 43.51±3.04c | 72.59±3.00b | 90.27±4.66a | 58.16±3.78c | 72.00±5.76b | 95.29±4.22a |

FCE= weight increment /feed intakex100; Similar letter means for each species of fish are statistically non-significant at p<0.05

**Table II: Accumulation of metals (µg $g^{-1}$) in three fish species exposed to metal mixture at sub-lethal concentrations**

| Treatments | Fish Organs | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Kidney** | **Liver** | **Skin** | **Muscles** | **Fins** | **Gills** | **Bones** | ***Overall means** |
| | **Metals × Organs** | | | | | | | |
| **Control Fish** | | | | | | | | |
| Copper | 20.18±5.43a | 22.03±1.60a | 21.39±5.44a | 17.06±5.76b | 5.78±1.59d | 13.6±1.98c | 4.71±1.37d | 14.96±7.23 c |
| Cadmium | 20.77±5.56b | 24.92±2.58a | 9.61±5.03d | 6.64±1.74ef | 5.57±1.57fg | 14.24±1.08c | 3.31±1.36g | 12.15±8.16 d |
| Zinc | 28.17±2.22b | 42.59±7.81a | 25.22±1.47c | 25.42±2.89c | 16.18±2.20d | 28.14±6.73b | 13.13±6.44e | 25.55±9.54 a |
| Nickel | 20.60±7.71b | 34.03±8.94a | 20.33±3.86b | 10.81±2.91d | 14.18±2..88c | 21.05±8.04b | 5.20±2.24e | 18.02±9.20 b |
| Cobalt | 4.06±1.30cd | 6.10±2.17bc | 8.51±5.20a | 4.26±1.51cd | 5.40±2.40cd | 8.61±2.88ab | 3.39±1.37d | 5.76±2.10 c |
| **Treated Fish** | | | | | | | | |
| Copper | 28.56±5.72a | 29.42±16.23a | 23.94±6.98bcd | 20.86±8.46d | 6.57±2.37ef | 21.11±2.75cd | 5.46±1.29f | 17.89±9.70 c |
| Cadmium | 31.85±13.06b | 36.37±5.12a | 13.38±6.33d | 8.96±3.41ef | 5.59±1.35fg | 18.72±1.47c | 4.49±1.01g | 17.01±12.60 c |
| Zinc | 35.90±3.11b | 52.61±9.58a | 29.27±2.18c | 22.73±9.43d | 17.50±2.05ef | 31.59±1.94c | 13.98±8.66f | 29.08±12.96 a |
| Nickel | 27.02±9.05bc | 44.53±13.93a | 24.59±1.41c | 12.86±3.95e | 17.32±4.36d | 27.57±10.68bc | 6.45±2.85f | 22.91±12.30b |
| Cobalt | 5.93±1.33df | 9.29±3.37bcd | 8.83±5.50cd | 5.90±2.51de | 5.41±1.70ef | 10.61±4.06abc | 4.52±1.01f | 7.21±2.32 d |
| **Species × Organs** | | | | | | | | |
| **Control Fish** | | | | | | | | |
| *Catla catla* | 15.70±8.79c | 25.71±11.28a | 16.40±7.80bc | 11.73±8.73d | 9.64±5.16e | 16.91±9.03b | 5.36±3.88f | 14.49±6.47b |
| *Labeo rohita* | 18.99±8.60b | 23.00±11.50a | 17.37±7.38cd | 11.99±7.24e | 9.51±4.61f | 16.53±6.34d | 4.28±1.78g | 14.52±6.32b |
| *Cirrhina mrigala* | 21.58±9.32b | 29.10±18.16a | 17.27±8.16cd | 14.80±8.72e | 9.10±6.00f | 17.94±8.01c | 8.20±6.76f | 16.85±7.22a |
| **Treated Fish** | | | | | | | | |
| *Catla catla* | 22.63±11.25b | 35.86±14.36a | 17.93±8.65c | 12.55±6.52d | 9.738±2.56d | 23.77±8.56b | 5.467±1.23e | 18.26±10.23b |
| *Labeo rohita* | 25.34±12.56b | 29.67±12.58a | 21.81±11.26c | 11.21±8.29e | 9.872±3.11e | 17.95±10.31d | 5.473±1.42f | 17.33±8.85c |
| *Cirrhina mrigala* | 29.59±14.85b | 37.80±15.26a | 20.26±10.58de | 19.04±7.56e | 11.83±2.98f | 24.04±11.25c | 10.01±2.34f | 21.79±9.74a |
| **Source × Organs** | | | | | | | | |
| **Control Fish** | | | | | | | | |
| Waterborne | 17.53±9.16a | 24.23±10.34a | 16.21±7.28a | 12.30±8.25a | 9.00±4.97a | 16.27±6.72a | 5.28±4.73a | 14.40±6.19 b |
| Dietary | 19.70±8.94a | 27.63±15.01a | 17.79±8.10a | 13.63±8.34a | 9.83±5.50a | 17.98±8.70a | 6.60±4.86a | 16.16±6.92 a |
| **Treated Fish** | | | | | | | | |
| Waterborne | 23.81±13.56b | 32.36±16.93a | 19.63±9.19c | 14.79±11.21d | 10.60±6.46e | 21.50±9.16bc | 6.83±5.31f | 18.50±8.59 b |
| Dietary | 27.90±12.69b | 36.52±19.51a | 20.38±8.86 d | 13.74±10.85e | 10.36±6.08f | 22.34±8.91cd | 7.131±5.60g | 19.76±10.31 a |

Mean±SD; Means with same letters in a single row and overall means in *column for treated and control fish are statistically non-significant at $p < 0.05$

these metals at different concentrations (Rauf *et al*., 2009). The MM stress caused significant effects on fish appetite that resulted in to significant change in FCE of three fish species (Hayat *et al*., 2007). Significant changes in the growth, feed intake and body ion regulation have also been reported in Cd, Cu and Zn exposed rainbow trout (McGeer *et al*., 2000).

**Accumulation of metals in fish:** Exposure of MMs to the fish caused significantly higher amassing of Zn (29.08 µg $g^{-1}$), followed by that of Ni, Cu, Cd and Co with the mean concentrations of 22.91, 17.89, 17.01 and 7.21 µg $g^{-1}$, respectively (Table II). Among the three fish species, *C. mrigala* accumulated significantly higher quantity of metals (21.79 µg $g^{-1}$) than that of *C. catla* and *L. rohita* with the average concentrations of 18.26 and 17.33 µg $g^{-1}$, respectively. Dietary MM treatments caused significantly higher average accumulation of metals (19.76 µg $g^{-1}$) than that of waterborne treatments (18.50 µg $g^{-1}$).

Lemus and Chung (1999) observed concentration based accumulation of copper in *Petenia kraussii*. Three fish species showed significantly higher accumulation of zinc in their body organs, while Co amassing was significantly lowest. Gupta and Srivastava (2006) exposed *Channa punctatus* to three concentrations of zinc (10, 15 & 25 mg $L^{-1}$) that resulted in significant increase of this metal in fish body. Exposure of waterborne and dietary Cu + Cd + Zn + Ni + Co mixtures caused significantly higher accumulation of all metals in fish liver, kidney and gills (Javed & Abdullah, 2004). Therefore, the interactions among Cu, Cd, Zn, Ni and Co were related to their competitive uptake from the treatments (waterborne or dietary) as certain metals can affect the accumulation of other metals in fish (Ribeyre *et al*., 1995). Generally, fish muscle and bones showed significantly least tendencies to accumulate metals that might have been taken up by the fish through gills, body surface or by ingestion of contaminated feed (Phillips & Rainbow, 1994). However, which route is imperative depends upon the prevailing conditions of an environment. Depledge *et al*. (1994) reported concentration based uptake of essential (Cu, Ni & Zn) and nonessential (Cd & Pb) metals through the gills of zebra fish.

The present results demonstrated the complexity of the uptake processes in fish during exposure of MMs at sub-lethal toxicity concentrations. The interactions among Cu, Cd, Zn, Ni and Co predicting metals uptake and accumulation to cause significantly variable toxicity to the three fish species. Kidney acts as a critical target organ for the accumulation of metals also. The observed data suggest that *C. mrigala* could act as a suitable monitoring fish to study the bioavailability of metals in the aquatic ecosystem (Palaniappan & Karthikeyan, 2009). Fish gills were also very susceptible to waterborne metals that could lead not only to osmotic imbalance, but may also impair respiratory functions (Reader *et al*., 1989). The present study reveals that interactions among Cu, Cd, Zn, Ni and Co, in a mixture form, were related to their competitive uptake from water or diet that caused significantly variable distribution of all these metals in fish body organs. Comparative studies demonstrated that the effects of MMs may differ by their toxicity to living organisms from the effects of single components and mixtures of different metals are characterized by some antagonistic effects (Bagdonas & Vosyliene, 2006), while others are by synergetic ones (Cicik & Karayakar, 2004).

## CONCLUSION

Dietary intake of MM caused significantly better feed intake and FCE than the fish exposed to waterborne mixture of metals. All the three fish species showed significantly higher susceptibility to waterborne than dietary MM. Exposure of MMs caused significant accumulation of Zn>Ni>Cu=Cd>Co in the fish body. However, *C. mrigala* showed significantly higher tendency than *C. Catla* and *L. rohita* to accumulate metals in their body organs. Accumulation of metals in fish body followed the order: liver>kidney>gills>skin>muscle>fins>bones that caused synergistic/ antagonistic toxicity to the fish.

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