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RESEARCH ARTICLE

# A COMPARATIVE ASSESSMENT OF TRACE METAL ACCUMULATION IN *OREOCHROMIS MOSSAMBICUS* AND *LABEO ROHITA* EXPOSED TO PLANT NUTRIENT LIBRELTM

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**ABSTRACT**

Metal pollution from multifarious sources like effluents from industries, agricultural runoff and untreated sewage system has adverse effects on aquatic ecosystem. Metallo-pesticides, including insecticides, fungicides, and herbicides are also known to contain various metals that can increase metal accumulation. The presence of metal pollutant in fresh water is known to disturb the delicate balance of the aquatic systems. Fish are often at the top of the aquatic food chain and may concentrate large amounts of some metals from the water. The study correlated the level of metal ions (Fe, Cu, Zn, Mn) in tissues and in water in a time dependent as well as dose dependent manner in both the species of teleost (O.mossambicus and L.rohita). Further Bioconcentration factor added metal based affinity towards tissue. Hence, the study gears up and proves that among both the fishes tested on exposure to micronutrient mixture, O.mossambicus was found to be more sturdy i.e it was able to with stand more metal load compared to L.rohita in a time dependent manner.

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## INTRODUCTION

The pollution of the aquatic environment with trace heavy metals has become a worldwide problem during recent years because they are indestructible and most of them have toxic effects on organisms (MacFarlane and Burchett, 2000). Among environmental pollutants, metals are of particular concern due to their potential toxic effect and ability to bio accumulate in aquatic ecosystems (Censi *et. al*., 2006). The presence of heavy metals in aquatic ecosystems is the result of two main sources of contamination; natural processes and anthropogenic activities (Goullé *et al*., 2012; Moore and Attar, 2011). Metal pollution from multifarious sources like effluents from industries, agricultural runoff and untreated sewage system has adverse effects on aquatic ecosystem. Intensification of agriculture practices to meet the demand has resulted in increased release of a wide range of agrochemical compounds to the environment (Desai and Parikh, 2013). Metallo-pesticides, including insecticides, fungicides, and herbicides are also known to contain various metals that can increase metal accumulation. (Senesi *et al.*, 1999; Nicholson *et al.*, 2003; Yabe *et al.*, 2012). Metal concentration in aquatic ecosystems are usually monitored by measuring their concentrations in water, sediments and biota (Ergul *et al.*, 2008) which generally exist in trace levels in water and attain considerable concentration in sediments and biota (Unlu *et al.*, 2008). These pollutants when compared with other types of aquatic pollution are less visible but its effects on the ecosystem and humans are intensive and very extensive due to their toxicity and their ability to accumulate in the aquatic organisms (Edem *et al*., 2008). The presence of metal pollutant in fresh water is known to disturb the delicate balance of the aquatic systems. Fish are often at the top of the aquatic food chain and may concentrate large amounts of some metals from the water (Mansour and Sidky, 2002).They are notorious for their ability to accumulate the metals in their muscles. Any of these metals can destroy life when they concentrate in the body above acceptable levels. Such a contaminated fish can cause health hazards when they enter into the human body through consumption (Ozuni et al., 2010). Hence, there is a need to carefully screen to ensure the unnecessary high level of some toxic trace metals that are being transferred to humans through fish consumption (Adeniyi and Yusuf, 2007).

Hence this study is geared towards determining the accumulation of the trace metals in the fish tissues as well as in the water with the view to establish the comprehensive evaluation of metals in various tissues i.e. liver, kidney, gills and muscles.

## MATERIALS AND METHODS

Healthy *O. mossambicus* and *L. rohita*, were collected from local fresh water bodies of Baroda district and acclimatized at laboratory conditions for 10 days. Fishes were maintained in 25 ± 2 C, pH 7.4 ± 0.05, dissolved oxygen 8 ± 0.3 mg/L, and total hardness 188 mg/L CaCO3 with a 12:12 light: dark photoperiod accordance with the Guidelines of A.P.H.A., A.W.W.A., W.P.C.F (Jomova and Valko, 2011). Fishes were daily supplied with commercial food during acclimation and experimental period. Acclimatized fishes were exposed to water containing the test chemical at the concentration of 300 mg/L (1/20th of LC50 value) for 45 days at the semi static

system. The Trace element mixture used was a commercial formulation of Librel TMX, Chelated Micronutrient mixture (Nutrient % by Wt. Min., Zn-4.0, Mn-0.5, Cu-0.3, Fe-2.0 and B-0.5). No test chemical was put into the aquarium containing the control fish. The water in the control and metal containing aquarium was renewed every day in order to maintain the concentration of the test mixture. At each interval of 15, 30 and 45 days of long-term exposure, fish were sampled from each group for determination of metals (Zn, Fe, Cu and Mn) in different organs (gills, liver, muscle and kidney). Water samples at every intervals were prepared using the method of APHA (1995) and different fish tissues were digested after drying according to the method described in APHA 3111B (Direct Acetylene Flame Method). The levels of Fe, Cu, Zn and Mn in digests as water were determined using atomic absorption spectrophotometer. Bioaccumulation factor was calculated according to using the following equation:

***Bio-concentration factor (BCF) =Concentration of M in dry fish tissue (mg/kg)***

***Concentration of M in water (mg/L)***

***MPI = (CF1x CF2 x----- CFn)1/n***

Where, *Cfn* is the contents for the metal n in the sample (Usero *et al.,* 1997). Average concentrations and standard deviations were calculated for each element, tissues and fish species. The significance levels of the differences between element concentrations in the studied fish organs and between experimental groups were determined using the Mann-Whitney (Sokal and Rohlf, 1987) test. Heavy metal contents determined in water and fish tissue samples were evaluated statistically using analysis of correlation by SPSS (version number-21) statistical package. The statistical analyses were determined as 0.05.

***Statistical analysis***

The values of protein content were statistically calculated using one way ANOVA and post hoc Dunnetts's t test was done to find the significance alterations if any between control and different exposure groups using SPSS software (version 21).

## RESULTS

The result of metals determined in the water at different exposure periods are presented in Table I-VII along with the standard values. They were in the order Fe (18.83 mgL-1) >Zn(6.34 mgL-1) >Mn (4.29 mgL-1) >Cu (0.7 mgL-1).

The alterations in the trace metal concentrations in water and tissue of *O. mossambicus* and *L. rohita* were determined and their means and S.D. are presented in Table (I-IV). Time dependent increase in the metal content of the tissues as well as water was observed. Amongst two fish species, *L. rohita* exhibited significantly higher ability to amass metals than *O. mossambicus*. The order of pattern of accumulation of metals in the tissues was liver>gills>kidney>muscle. Fe exhibited highest concentration in liver of both the fishes. Individual metal concentration assessment exhibited higher values of Fe in *L. rohita* (2108.20±771.67 mg/Kg) than *O. mossambicus* 1589.20±45.30 mg/Kg). Organ wise concentration that tracked the order for Fe was: L>G>K>M. The second highest trace metal in order was Zn in both the fishes, where *L.rohita (*184±4.35 mg/Kg) revealed higher concentration compared to *O. mossambicus* (155.3±2.33mg/Kg). Organ wise accumulation that followed the order for Zn concentration was K>G>M>L. Next in the order was Cu, and it was *L. rohita* (75.04±10.60 mg/Kg) which exhibited higher concentration compared to *O. mossambicus* (60.00±3.17 mg/Kg). Organ wise accumulation that followed the order for Cu accumulation was: L>G>M>K. Mn exhibited the least concentration, where *L. rohita* (131±3.60 mg/Kg) accumulated higher Mn compared to *O. mossambicus* 3.50±11.77 mg/Kg). Organ wise accumulation that followed the order for Mn accumulation was: M>L>K>G. To have an insight for the interspecific differences Mann-whitney test was performed (Table VI).

Concentration of trace metals (mean and standard deviations) in the tissues of both the teleost fish over the period of time are presented in Table I-IV where all trace element showed altered affinities in the studied fish tissues and did not differ significantly between the tissues except kidney. The overall relationship among the various elements was calculated by Pearson correlation coefficient and data is presented in the form of a matrix (Table VI&VII).

In water, all metals showed positive correlation with the other metals. Metal concentration for inter tissue correlation for *O. mossambicus* gills, Zn showed positive correlation with other three metals; while other three metals showed negative correlation with each other. In the Liver of showed negative correlation with all the other three metal ions, while Zn, Fe and Mn showed positive correlation with each other. In muscle Cu and Mn showed positive with each other as well as with Fe and Zn, on the other hand Zn and Fe showed a negative correlation with each other.

**Table I** Concentration of Zn in water and selected tissues of fish exposed to sub lethal concentration of plant nutrient (*n = 6*).

| Tissues | *O. mossambicus* | | | | *L. rohita* | | | |
|---|---|---|---|---|---|---|---|---|
| | Days of Exposure | | | | Days of Exposure | | | |
| | Control | 15 days | 30 days | 45 days | Control | 15 days | 30 days | 45 days |
| Gills | 10.62 ± 1.05 | 50.00 ± 7.37 | 66.1 ± 11.90 | 80.2 ±10.09 | 11.33 ± 0.99 | 40.81± 7.41 | 40.72 ± 4.71 | 41.43 ±1.83 |
| Liver | 1.33 ±0.09 | 15.99 ±2.69 | 60.54 ±9.60 | 126.88 ±5.94 | 5.22 ± 2.81 | 19.93 ± 3.66 | 57.60 ± 2.46 | 142.32 ± 3.08 |
| Muscle | 0.65 ±0.81 | 6.52 ±1.014 | 7.80 ±2.091 | 28.85 ±2.15 | 0.36 ± 0.54 | 2.64 ± 1.85 | 22.69 ± 2.51 | 61.91 ±3.75 |
| Kidney | 10.66 ± 0.99 | 55.33 ± 2.8 | 82.33 ±1.00 | 155.3 ± 2.33 | 12.33 ±1.36 | 36.15 ±3.19 | 99.32 ±5.22 | 184 ±4.35 |

*Values are presented in Means ± S.D.*

**Table II** Concentration of Fe in water and selected tissues of fish exposed to sub lethal concentration of plant nutrient (*n = 6*).

| Tissues | *O. mossambicus* | | | | *L. rohita* | | | |
|---|---|---|---|---|---|---|---|---|
| | Days of Exposure | | | | Days of Exposure | | | |
| | Control | 15 days | 30 days | 45 days | Control | 15 days | 30 days | 45 days |
| Gills | 4.13 ± 0.19 | 3.47 ±0.13 | 385.5 ±0.10 | 850.25 ±111.54 | 2 ± 1 | 5 ± 5 | 151.41 ±26.06 | 330.50 ±28.30 |
| Liver | 16.49 ±20.92 | 93.16 ±8.34 | 642.86 ±140.95 | 1589.2 ±45.30 | 41.44 ± 41.44 | 178.40 ±9.66 | 1142.073 ±99.77 | 2108.20 ±771.67 |
| Muscle | 1.55 ±1.18 | 7.28 ±1.27 | 24.25 ±3.45 | 121.7 ±23.50 | 0.14 ± 0.16 | 0.87 ± 0.42 | 10.53 ± 0.72 | 51.77 ± 2.93 |
| Kidney | 14.33 ±2.98 | 47.33 ±2.87 | 88.95 ±3.22 | 149.22 ±5.98 | 12.33 ±1.22 | 53.21 ±2.33 | 99.23 ±5.3 | 168.83 ±7.06 |

*Values are presented in Means ± S.D.*

**Table III** Concentration of Cu in water and selected tissues of fish exposed to sub lethal concentration of plant nutrient (n = 6).

| Tissues | *O. mossambicus* | | | | *L. rohita* | | | |
|---|---|---|---|---|---|---|---|---|
| | Days of Exposure | | | | Days of Exposure | | | |
| | Control | 15 days | 30 days | 45 days | Control | 15 days | 30 days | 45 days |
| Gills | 0.77 ±0.69 | 3.58 ±0.92 | 27.11 ±8.18 | 61.4 ±7.09 | 0.21 ± 0.11 | 0.97 ± 1.22 | 33.04 ± 20.24 | 46.01 ±6.10 |
| Liver | 2.58 ±0.15 | 6.26 ±0.20 | 59.25 ± 9.04 | 60.04 ±10.60 | 0.11 ± 0.18 | 1.48 ±0.76 | 15.75 ± 4.15 | 75.00 ± 3.17 |
| Muscle | 1.06 ±1.17 | 5.11 ±1.31 | 19.93 ±2.46 | 41.04 ±4.89 | 0.14 ± 0.16 | 0.92 ± 0.66 | 2.53 ± 0.64 | 27.94 ± 2.35 |
| Kidney | 1.22 ± 0.82 | 9.22 ±0.98 | 19.23 ±2.33 | 25.33 ± 1.98 | 0.99 ± 0.19 | 15.32 ±0.33 | 20.32 ±1.22 | 27.52 ±2.03 |

*Values are presented in Means ± S.D.*

**Table IV** Concentration of Mn in water and selected tissues of fish exposed to sub lethal concentration of plant nutrient (*n = 6*).

| Metals | *O. mossambicus* | | | | *L. rohita* | | | |
|---|---|---|---|---|---|---|---|---|
| | Days of Exposure | | | | Days of Exposure | | | |
| | Control | 15 days | 30 days | 45 days | Control | 15 days | 30 days | 45 days |
| Gills | 0.95 ±0.61 | 6.99 ±2.08 | 10.37 ± 14.61 | 29.58 ±0.89 | 0.02 ± 0.02 | 0.34 ± 0.22 | 5.93 ± 1.39 | 15.52 ± 3.68 |
| Liver | 1.55 ±0.69 | 30.17 ±15.53 | 67.07 ±6.60 | 115.55 ±5.011 | 5.22 ± 1.15 | 15.96 ± 4.10 | 32.83 ± 6.21 | 64.86 ± 3.64 |
| Muscle | 2.25 ±0.99 | 9.7 ±1.50 | 23.50 ±11.77 | 43.50 ±1.52 | 37.06 ± 17.69 | 62.70 ± 3.60 | 99.00 ± 3.50 | 131 ± 2.51 |
| Kidney | 5.32 ±0.87 | 15.33 ±2.39 | 25.38 ± 4.23 | 35.39 ±5.65 | 3.23 ±0.99 | 9.33 ±1.33 | 29.33 ±2.33 | 38.93 ±3.98 |

*Values are presented in Means ± S.D.*

In kidney, a positive correlation amongst Zn, Fe and Cu and a negative correlation of MN towards all the three was reported. Whereas, in *L. rohita*: Zn showed negative correlation with other three metals; while other three metals showed positive correlation with each other.

In liver muscle and kidney Zn, Fe and Mn showed positive correlation with each other while negative correlation of these three metals was seen with Cu. In order to estimate the toxicity of trace metals accumulated in the experimental set up the BCF for each trace metal was calculated (Fig. II). The order of BCF for trace metals was Cu>Fe>Mn>Zn for both the fishes. However, when tissue BCF was compared the order was for *O. mossambicus* and *L. rohita*

- Cu: liver>gills>muscle>kidney
- Fe: liver>gills>kidney>muscle
- Zn: liver>kidney>gills>muscle

Except for Mn which differed in both the fishes. For *O. mossambicus* it was liver>muscle>kidney>gills and *L. rohita* muscle>liver>kidney>gills

**Table V** Time dependent significance of differences in the concentrations of metals in the organs of fish species exposed to plant nutrient

| Samples | *O. mossambicus* | | | *L. rohita* | | |
|---|---|---|---|---|---|---|
| | Days of Exposure | | | Days of Exposure | | |
| | 15 days | 30 days | 45 days | 15 days | 30 days | 45 days |
| | | | **Zn** | | | |
| **Gills** | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 |
| **Liver** | ns | 0.050 | 0.046 | 0.050 | 0.050 | 0.050 |
| **Muscle** | 0.050 | 0.050 | 0.050 | ns | 0.050 | 0.050 |
| **Kidney** | 0.01 | 0.01 | 0.01 | 0.01 | 0.050 | 0.01 |
| | | | **Fe** | | | |
| **Gills** | 0.050 | 0.050 | 0.050 | ns | 0.050 | 0.050 |
| **Liver** | 0.050 | 0.050 | 0.050 | ns | 0.050 | 0.050 |
| **Muscle** | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 |
| **Kidney** | 0.01 | 0.01 | 0.01 | 0.01 | 0.050 | 0.01 |
| | | | **Cu** | | | |
| **Gills** | 0.050 | 0.050 | 0.050 | ns | 0.050 | 0.050 |
| **Liver** | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 |
| **Muscle** | 0.050 | 0.050 | 0.050 | ns | 0.050 | 0.050 |
| **Kidney** | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 | 0.01 |
| | | | **Mn** | | | |
| **Gills** | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 |
| **Liver** | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 |
| **Muscle** | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 |
| **Kidney** | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 | 0.01 |

*Values presented are the significance levels obtained using Mann-Whitney U*

**Table VII** Interwater and inter tissue Pearson Correlation of *O.mossambicus*

| | | W-Zn | W-Cu | W-Fe | W-Mn | G-Zn | G-Fe | G-Cu | G-Mn | L-Zn | L-Fe | L-Cu | L-Mn | M-Zn | M-Fe | M-Cu | M-Mn | K-Zn | K-Fe | K-Cu | K-Mn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| W-Zn | PC | 1 | .713 | .977 | .989 | | | | | | | | | | | | | | | | |
| | Sig. | | .495 | .136 | .095 | | | | | | | | | | | | | | | | |
| W-Cu | PC | .713 | 1 | .846 | .601 | | | | | | | | | | | | | | | | |
| | Sig. | .495 | | .359 | .589 | | | | | | | | | | | | | | | | |
| W-Fe | PC | .977 | .846 | 1 | .935 | | | | | | | | | | | | | | | | |
| | Sig. | .136 | .359 | | .231 | | | | | | | | | | | | | | | | |
| W-Mn | PC | .989 | .601 | .935 | 1 | | | | | | | | | | | | | | | | |
| | Sig. | .095 | .589 | .231 | | | | | | | | | | | | | | | | | |
| G-Zn | PC | | | | | 1 | -.905 | .827 | .557 | | | | | | | | | | | | |
| | Sig. | | | | | | .279 | .380 | .624 | | | | | | | | | | | | |
| G-Fe | PC | | | | | -.905 | 1 | -.511 | -.151 | | | | | | | | | | | | |
| | Sig. | | | | | .279 | | .659 | .903 | | | | | | | | | | | | |
| G-Cu | PC | | | | | .827 | -.511 | 1 | .927 | | | | | | | | | | | | |
| | Sig. | | | | | .380 | .659 | | .244 | | | | | | | | | | | | |
| G-Mn | PC | | | | | .557 | -.151 | .927 | 1 | | | | | | | | | | | | |
| | Sig. | | | | | .624 | .903 | .244 | | | | | | | | | | | | | |
| L-Zn | PC | | | | | | | | | 1 | 1.000** | -.704 | .827 | | | | | | | | |
| | Sig. | | | | | | | | | | .002 | .502 | .380 | | | | | | | | |
| L-Fe | PC | | | | | | | | | 1.000** | 1 | -.702 | .826 | | | | | | | | |
| | Sig. | | | | | | | | | .002 | | .504 | .382 | | | | | | | | |
| L-Cu | PC | | | | | | | | | -.704 | -.702 | 1 | -.981 | | | | | | | | |
| | Sig. | | | | | | | | | .502 | .504 | | .123 | | | | | | | | |
| L-Mn | PC | | | | | | | | | .827 | .826 | -.981 | 1 | | | | | | | | |
| | Sig. | | | | | | | | | .380 | .382 | .123 | | | | | | | | | |
| M-Zn | PC | | | | | | | | | | | | | 1 | -.828 | .089 | .464 | | | | |
| | Sig. | | | | | | | | | | | | | | .379 | .943 | .693 | | | | |
| M-Fe | PC | | | | | | | | | | | | | -.828 | 1 | .485 | .112 | | | | |
| | Sig. | | | | | | | | | | | | | .379 | | .678 | .928 | | | | |
| M-Cu | PC | | | | | | | | | | | | | .089 | .485 | 1 | .923 | | | | |
| | Sig. | | | | | | | | | | | | | .943 | .678 | | .251 | | | | |
| M-Mn | PC | | | | | | | | | | | | | .464 | .112 | .923 | 1 | | | | |
| | Sig. | | | | | | | | | | | | | .693 | .928 | .251 | | | | | |
| K-Zn | PC | | | | | | | | | | | | | | | | | 1 | 1.000** | 1.000** | 1.000** |
| | Sig. | | | | | | | | | | | | | | | | | | .000 | .000 | .000 |
| K-Fe | PC | | | | | | | | | | | | | | | | | 1.000** | 1 | 1.000** | 1.000** |
| | Sig. | | | | | | | | | | | | | | | | | .000 | | .000 | .000 |
| K-Cu | PC | | | | | | | | | | | | | | | | | 1.000** | 1.000** | 1 | 1.000** |
| | Sig. | | | | | | | | | | | | | | | | | .000 | .000 | | .000 |
| K-Mn | PC | | | | | | | | | | | | | | | | | 1.000** | 1.000** | 1.000** | 1 |
| | Sig. | | | | | | | | | | | | | | | | | .000 | .000 | .000 | |

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

**Table VII:** Interwater and inter tissue Pearson Correlation of *L.rohita*

| | | W-Zn | W-Fe | W-Cu | W-Mn | G-Zn | G-Fe | G-Cu | G-Mn | L-Zn | L-Fe | L-Cu | L-Mn | M-Zn | M-Fe | M-Cu | M-Mn | K-Zn | K-Fe | K-Cu | K-Mn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| W-Zn | PC | 1 | .989 | .977 | .933 | | | | | | | | | | | | | | | | |
| | Sig. | | .095 | .136 | .234 | | | | | | | | | | | | | | | | |
| W-Fe | PC | .989 | 1 | .935 | .976 | | | | | | | | | | | | | | | | |
| | Sig. | .095 | | .231 | .140 | | | | | | | | | | | | | | | | |
| W-Cu | PC | .977 | .935 | 1 | .835 | | | | | | | | | | | | | | | | |
| | Sig. | .136 | .231 | | .370 | | | | | | | | | | | | | | | | |
| W-Mn | PC | .933 | .976 | .835 | 1 | | | | | | | | | | | | | | | | |
| | Sig. | .234 | .140 | .370 | | | | | | | | | | | | | | | | | |
| G-Zn | PC | | | | | 1 | -.986 | -.999* | -.985 | | | | | | | | | | | | |
| | Sig. | | | | | | .108 | .033 | .110 | | | | | | | | | | | | |
| G-Fe | PC | | | | | -.986 | 1 | .975 | .942 | | | | | | | | | | | | |
| | Sig. | | | | | .108 | | .141 | .218 | | | | | | | | | | | | |
| G-Cu | PC | | | | | -.999* | .975 | 1 | .993 | | | | | | | | | | | | |
| | Sig. | | | | | .033 | .141 | | .077 | | | | | | | | | | | | |
| G-Mn | PC | | | | | -.985 | .942 | .993 | 1 | | | | | | | | | | | | |
| | Sig. | | | | | .110 | .218 | .077 | | | | | | | | | | | | | |
| L-Zn | PC | | | | | | | | | 1 | .933 | -.874 | .998* | | | | | | | | |
| | Sig. | | | | | | | | | | .234 | .323 | .045 | | | | | | | | |
| L-Fe | PC | | | | | | | | | .933 | 1 | -.990 | .956 | | | | | | | | |
| | Sig. | | | | | | | | | .234 | | .089 | .189 | | | | | | | | |
| L-Cu | PC | | | | | | | | | -.874 | -.990 | 1 | -.906 | | | | | | | | |
| | Sig. | | | | | | | | | .323 | .089 | | .278 | | | | | | | | |
| L-Mn | PC | | | | | | | | | .998* | .956 | -.906 | 1 | | | | | | | | |
| | Sig. | | | | | | | | | .045 | .189 | .278 | | | | | | | | | |
| M-Zn | PC | | | | | | | | | | | | | 1 | .672 | -.992 | .990 | | | | |
| | Sig. | | | | | | | | | | | | | | .531 | .080 | .090 | | | | |
| M-Fe | PC | | | | | | | | | | | | | .672 | 1 | -.574 | .770 | | | | |
| | Sig. | | | | | | | | | | | | | .531 | | .611 | .441 | | | | |
| M-Cu | PC | | | | | | | | | | | | | -.992 | -.574 | 1 | -.965 | | | | |
| | Sig. | | | | | | | | | | | | | .080 | .611 | | .170 | | | | |
| M-Mn | PC | | | | | | | | | | | | | .990 | .770 | -.965 | 1 | | | | |
| | Sig. | | | | | | | | | | | | | .090 | .441 | .170 | | | | | |
| K-Zn | PC | | | | | | | | | | | | | | | | | 1 | 1.000** | 1.000** | 1.000** |
| | Sig. | | | | | | | | | | | | | | | | | | .000 | .000 | .000 |
| K-Fe | PC | | | | | | | | | | | | | | | | | 1.000** | 1 | 1.000** | 1.000** |
| | Sig. | | | | | | | | | | | | | | | | | .000 | | .000 | .000 |
| K-Cu | PC | | | | | | | | | | | | | | | | | 1.000** | 1.000** | 1 | 1.000** |
| | Sig. | | | | | | | | | | | | | | | | | .000 | .000 | | .000 |
| K-Mn | PC | | | | | | | | | | | | | | | | | 1.000** | 1.000** | 1.000** | 1 |
| | Sig. | | | | | | | | | | | | | | | | | .000 | .000 | .000 | |

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

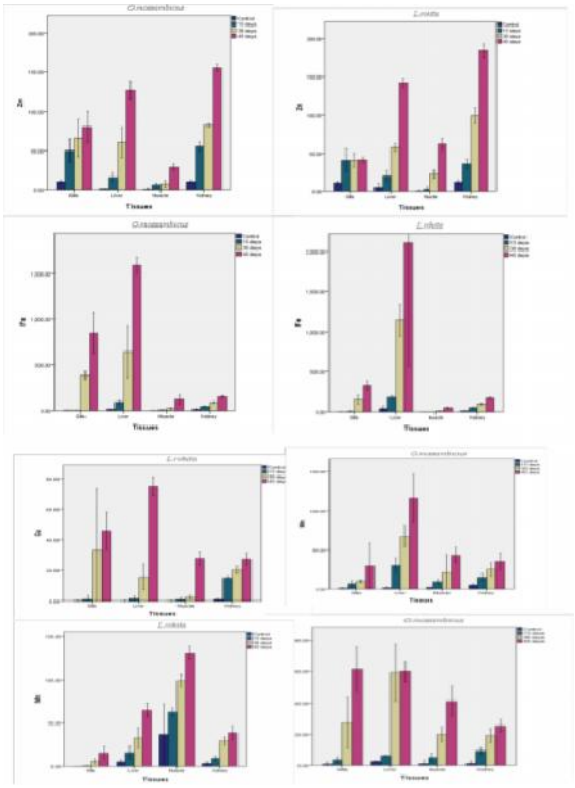

**Figure 1** Average concentration of various trace metals in the tissues of *Orechromis mossambicus* and *Labio rohita* exposed to plant nutrient Liberal. (a) zn concentration (mg/kg) in the tissues of *Orechromis mossambicus* (b) zn concentration (mg/kg) in the tissues of *Labio rohita* (c) Fe concentration (mg/kg) in the tissues of *Orechromis mossambicus* (d) Fe concentration (mg/kg) in the tissues of *Labio rohita* (e) )Cu concentration (mg/kg) in the tissues of *Orechromis mossambicus* (f) Cu concentration (mg/kg) in the tissues of *Labio rohita* (g) Mn concentration (mg/kg) in the tissues of *Orechromis mossambicus* (h) Mn concentration (mg/kg) in the tissues of *Labio rohita.*

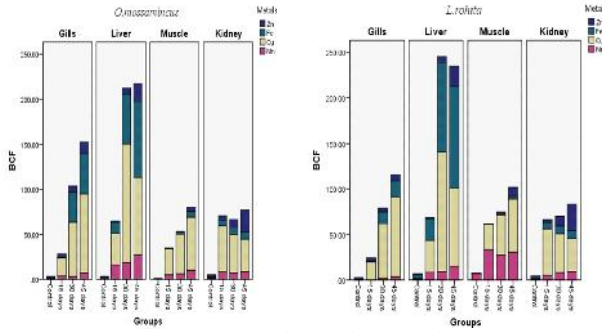


**Figure 2** Bio concentration factor of tissues of *Orechromis mossambicus* and *Labio rohita* exposed to plant nutrient Liberal. (i)BCF of tissues of *Orechromis mossambicus* (j) BCF of the tissues of *Labio rohita*

## DISCUSSION

The results of the present study strongly revealed that Librel exposure has resulted into time Dependent accumulation of metals in teleost. Although both the fishes showed the accumulation of the metals in tissues the most astonishing fact was that no mortality was reported during the experiment, presumably due to their function as co-factors for the activation of a number of enzymes and regulated to maintain a certain homeostatic status in fish. The results of several studies of metal accumulation in fish living in polluted waters showed that considerable amounts of metals may be deposited in fish tissues without causing mortality (Jezierska and Witeska, 2001, Jezierska and Witeska 2006, Nimick et al., 2007, Akan et al., 2012, Naz and javed, 2013). In the present study of the two fishes, *L. rohita* was found to have more affinity to accumulate metals compared to *O. mossambicus*, which are in agreement with the comparative studies done by Voigt, 2004 Javed 2005) who observed significantly higher accumulation of metals in *L. rohita* than *C. mrigala* and *C. catla.* Moreover, a variety of species of fish from the same water body are also reported to accumulate different amounts of metals (Senthilkumar and Sajwan, 2007; Sajwanm *et al*., 2008). Metal distribution in various organs is time-associated (Eggleton and Thomas, 2004).

The effect of time on metal distribution within the organism is a complex issue due to different affinity of various metals to the tissues of various fish species. In the current study both fishes showed a time dependent significant increase in the metal concentration in all the tissues. These differences result from different affinity of metals to fish tissues, different uptake, deposition and excretion rates (Giguere *et al.,* 2004). Of all the tissues, liver has shown the highest level of accumulation of metals followed by gills while kidney and muscle have shown the least level of accumulation. Significantly higher levels of all metals in fish liver can be related to the binding of metals to metallothionein that provide detoxification mechanism (sensi *et al.,* 2010). As reported by Szarek-Gwiazda *et al.,* (2006) that along with the species specificity the trace metal concentration and their binding capacity vary with tissues too. Liver accumulates high concentrations of metals, irrespective of the uptake route (Tsai *et al.,* 2013).

The liver is considered a good monitor of water pollution with metals since their concentrations accumulated in this organ are often proportional to those present in the environment (Dural *et al.,* 2006).Next in order of accumulation were the gills which are in direct contact with water and as the gill surface is negatively charged it provides a potential site for gill-metal interaction for positively charged metals. As reported earlier fish can absorb ions through gills, since they have special salt secreting cells and are involved in the secretion of metals, probably via the secretion of mucus, but when the metal accumulation crosses the excretion threshold limit bioaccumulation exceeds the excretion level. Hence the second highest metal burden observed in the gills of *O. mossambicus* and *L. rohita* is self-explanatory mechanism of metal accumulation. Our results are in agreement with earlier reported metal accumulation in tissues of freshwater fish *C. gariepinus* (Kusemiju *et al.,* 2012) *O.niloticus* (Al-Nagaawy, 2008) *in O.mossambicus* (Naigaga, 2002) in *Tor putitora* (Shakoori, and Yousafzai, 2006). Metal concentrations in the kidneys rise slower than in liver, hence the low concentration of ions in the kidney is the present work is well justified. Kidney is also one of the active metabolically important organs next to liver. Metal uptake and binding has been reported to increase with increase in the metabolic rate (Green & Knutzen, 2003 and Voigt, 2004). The present data corroborates with the studies of metal accumulation in the kidney of *O. niloticus* (Abdel- Baki *et al.,* 2011), *Onchorynchus mykiss* of *Carassius auratus and Cyprinus carpio* respectively (Boeck *et al.,* 2004).

Muscle tissue exhibited the least accumulation of all the tissues, This result are in agreement with many authors who reported that muscles is not an active organ in accumulation of most heavy metals (Yilmaz *et al.*, 2007 and Kraiem 2007, Khalil and Faragallah, 2008;). Thus, plant nutrient exposure in the present study has probably led to the increase in the metabolic rate particularly for the metabolically important tissues such as liver. In the present study the order of accumulation of metals in water was found to be Fe >Zn>Mn>Cu.The mean concentration of Cu recorded in water in this investigation was below permissible limits, while the mean concentration of Zn, Mn and Fe were above limits (WHO, 2005). This higher concentration could be linked to the presence of synergistic or additive effects other metals. Metals are non-biodegradable, and once they enter the aquatic environment, bioconcentration may occur in fish tissue by means of metabolic and bio sorption processes (Yousafzai and Shakoori, 2008; Kaoud and El-Dahshan, 2010). From an environmental point of view, bio concentration is important because metal ions usually occur in low concentrations in the aquatic environment and subtle physiological effects go unnoticed until gross chronic reactions (e.g. changes in populations' structure, altered reproduction, etc.) become apparent.

BCF in the present study has revealed alterations in the tissue specific and metal specific responses. Overall the BCF of liver tissue has resulted into the highest affinity for Cu, Fe and Zn, Whereas BCF of gills showed affinity for Cu and Fe, kidney showed affinity for Fe and Zn and muscle had affinity for Mn. Fish liver as a major detoxifying and storage organ differed from the concentrations detected in the gills, kidney and muscles. Significantly higher levels of all metals in fish liver can be related to the binding of metals to metallothionein that provide detoxification mechanism (sensi *et al.*, 2010). Species difference in heavy metals bio concentration is linked to difference in feeding habits and behaviour of the species (Altindag and Yigit, 2005). The variability observed in the fish species is a reflection o

f different thresholds of metals which are a function of homeostasis. The thresholds of metals in fish can be considered as the concentration level where the metal starts to interfere with the variable physiology of the fish species in such manner that once a particular level of the metal has been sequestered in the body, equilibrium is established between the fish burden and the ambience. The high bioaccumulation factor for Cu and Fe suggests that the concentration of these metal ions serves as a harborage or the fish species have poor mechanisms for digesting and eliminating these heavy metals. Simkiss & Taylor (1989) in their studies have proposed various pathways of metal accumulation by aquatic organisms either through lipid permeation, complex permeation, and carrier mediated, through ion channels, ion pumps or endocytosis. The high metal concentration in the tissues and water reported in the present studies thus suggest that possibly few of the mechanism might be working simultaneously, however at this point it is difficult to conclude the exact mechanism for metal accumulation. Moreover depuration studies were not done and perhaps it will throw more light to validate our data. As there was no mortality reported in the present studies, possibly the fishes are having an inborn mechanism to counteract the potential toxicity of the metals in the tissues, by synthesizing the stress proteins as they are assumed to play a role in the detoxification of heavy metals.

## CONCLUSION

The overall results from the metal accumulation studies have proved that the metal accumulation was time dependent, species specific and organ specific. Moreover, of the two species it was observed that *O.mossambicus* was able to withstand the metal load more compared to *L.rohita*.

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