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Effect of *Moringa* diets on metal accumulation in *C. carpio*

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Abstract
The effect of *Moringa* leaf supplementation on the reduction of copper toxicity in fish *Cyprinus carpio*. The gill tissue elicited the highest copper accumulation followed the liver in *C. carpio*. Copper accumulation was significantly (P<005) high in (MO) group and it gradually decreased with an increasing the levels in (M_1 –M_4) groups. The maximum reduction of copper accumulation in tissues occurred with the supplementation of 30% (M_2) *Moringa* diet followed by 40% (M_3) and, 50% (M_4) and 20% M_1) *Moringa* diet respectively in both size groups *C. carpio*. The elimination of accumulated copper through feces and water increased with increasing the dietary levels of *Moringa* diet in small and large size *C. carpio.*

Keywords: *Moringa*, copper, *Cyprinus carpio*, size groups, elimination

1. Introduction

The aquatic ecosystem is contaminated by variety of pollutants. Copper is one of the most important pollutants which affect fish health and growth especially in an intensive system. Copper is extremely toxic to fish and cause tissue damages in gills and hematopoietic organs. Gills are the primary target organ for the toxic action of copper (Mazon *et al.*, 2002 ; Figueiredo – Femandeset *et al*., 2007) [2, 3]. Copper are absorbed through gills and gastrointestinal track by fish organisms, free of the uptake way they are chiefly accumulated in metabolically active tissues such as liver and kidney. Liver acts in the transformation of basic nutrients and in detoxification on storage of toxic materials. The interference in the gas exchange, nitrogenous waste excretion, acid-base and ionic stability due to the change in water pH cause stress in fish affecting its body physiology and growth (Roja Sadat Jalali Mottahari *et al*., 2013) [4]. Hence reduction of toxic copper in aquatic systems / organisms by some acceptable methods is the need of the hour. The most widely used technique for removal of toxic elements involves the process of neutralization and metal hydroxide precipitation (Hamesh and Mahadeva Swamy, 1994) [5]. The complete removal or reduction of elements in polluted environments and fish body is of utmost importance (James *et al*., 1998, James and Sampath, 2003) [8, 10]. Researchers have reported the therapeutic effects of *Moringa* leaf as a growth promoter, and booster of immune system in animals including fishes (Fahey *et al.,* 2005) [12]. So far *Moringa* leaf is known for its nutritive value only and its role in alleviating metal toxicity in fishes and other cultivate organisms are completely unexplored. The aim of the present study was the effect of *Moringa* leaf on the reduction of copper toxicity in fish *Cyprinus carpio.*

2. Materials and Methods

For the experiment, active and healthy juveniles of *C. carpio* were collected for the acclimation tank and starved for 24hr prior to the commencement of the experiment. They were divided in to six groups and maintained with chosen sub lethal levels a copper. Group one served as control and reared in freshwater and fed with control diet (c) Test animals belonging to 2nd - 6th groups were exposed to 0.5 ppm copper. Among the copper exposed group 2nd was fed with control diet (Mo), however, 3rd (M1), 4th (M2) 5th (M3) and 6th(M4) groups were fed with 0, 20, 30, 40, and 50% *Moringa* leaf meal diets respectively. Triplicates were maintained for corresponding experimental diets. The experiment was estimated at an intervals of 20 days. The liver, and gill of the animals were dissected out and they were analysed copper accumulation to acid digestion following the method of FAO (1975). The copper concentration was estimated in following method Atomic Absorption Spectrophotometer (AAS) for metal analysis (APHA, 1993).

3. Results

The gill tissue elicited the highest copper accumulation followed the liver in *C. carpio*. Copper accumulation was significantly (P<005) high in (MO) group and it gradually decreased with an increasing the levels in (M_1 –M_4) groups. The maximum reduction of copper accumulation in tissues occurred with the supplementation of 30% (M_2) *Moringa* diet followed by 40% (M_3) and, 50% (M_4) and 20% M_1) *Moringa* diet respectively in both size groups *C. c arpio* (Fig.3and 5) Two – way ANOVA test revealed that the tissue copper accumulation decreased was significantly related to levels *Moringa* diets Duncan multiple range test revealed that, metal accumulation of liver and gill tissues was completely recovered from copper toxicity, on day 80 in *C. carpio* received M_2 diets as compared to other diets.

The elimination of accumulated copper through feces and water increased with increasing the dietary levels of *Moringa* diet in small and large size *C. carpio*. A positive correlation co-efficient. A positive correlation Co-efficent was obtained for the relationship between the supplementation of dietary *Moringa* and elimination of copper through feces and it was statistically significant (P<0.05) (Fig .3-6).

Table 1: Effect of dietary *Moringa* diets on metal accumulation in liver and gill tissues (µg Cu g^{-1} wet tissue) of small size *Cyprinus carpio* exposed to sublethal level of copper as a function of time. Each value is the mean $(\bar{X} \pm SD)$ of three observations.

Exposure period (days)	Control	Experimental diets				
		M0	M1	M2	M3	M4
Liver						
0	ND	ND	ND	ND	ND	ND
20	ND	^{ab}0.46 ± 0.02	^{a}0.38 ± 0.04	^{bc}0.30 ± 0.05	^{bc}0.27 ± 0.04	^{c}0.24 ± 0.05
40	ND	^{a}0.60 ± 0.04	^{b}0.29 ± 0.03	^{bc}0.25 ± 0.02	^{c}0.21 ± 0.06	^{c}0.19 ± 0.03
60	ND	^{a}0.73 ± 0.06	^{b}0.22 ± 0.04	^{b}0.21 ± 0.04	^{b}0.16 ± 0.04	^{b}0.16 ± 0.04
80	ND	^{a}0.96 ± 0.07	^{b}0.17 ± 0.02	^{b}0.14 ± 0.03	^{b}0.13 ± 0.04	^{b}0.13 ± 0.02
Gill						
0	ND	ND	ND	ND	ND	ND
20	ND	^{d}1.07 ± 0.07	^{a}0.95 ± 0.07	^{b}0.81 ± 0.07	^{c}0.70 ± 0.06	^{c}0.61 ± 0.05
40	ND	^{d}1.28 ± 0.05	^{a}0.86 ± 0.06	^{b}0.70 ± 0.06	^{b}0.63 ± 0.05	^{c}0.49 ± 0.04
60	ND	^{e}1.52 ± 0.04	^{a}0.67 ± 0.05	^{b}0.58 ± 0.04	^{c}0.43 ± 0.04	^{d}0.34 ± 0.05
80	ND	^{b}1.96 ± 0.03	^{a}0.49 ± 0.03	^{b}0.25 ± 0.05	^{bc}0.21 ± 0.02	^{c}0.16 ± 0.04

Values (mean ±SD) with different superscript in the same row are significantly different (P<0.05)

Table 2: Effect of dietary *Moringa* diets on metal accumulation in liver and gill tissues (µg Cu g^{-1} wet tissue) of large size *Cyprinus carpio* exposed to sublethal level of copper as a function of time. Each value is the mean $(\bar{X} \pm SD)$ of three observations.

Exposure period (days)	Control	Experimental diets				
		M0	M1	M2	M3	M4
Liver						
0	ND	ND	ND	ND	ND	ND
20	ND	^{a}0.41 ± 0.05	^{ab}0.38 ± 0.07	^{ab}0.32 ± 0.06	^{bc}0.30 ± 0.05	^{c}0.28 ± 0.05
40	ND	^{a}0.47 ± 0.04	^{b}0.33 ± 0.02	^{b}0.28 ± 0.07	^{b}0.26 ± 0.08	^{b}0.23 ± 0.07
60	ND	^{a}0.50 ± 0.06	^{b}0.24 ± 0.04	^{b}0.21 ± 0.04	^{b}0.20 ± 0.07	^{b}0.19 ± 0.06
80	ND	^{a}0.52 ± 0.07	^{a}0.20 ± 0.08	^{a}0.15 ± 0.06	^{a}0.14 ± 0.03	^{a}0.13 ± 0.04
Gill						
0	ND	ND	ND	ND	ND	ND
20	ND	^{c}0.72 ± 0.04	^{c}0.11 ± 0.02	^{a}0.98 ± 0.07	^{a}0.89 ± 0.07	^{b}0.76 ± 0.06
40	ND	^{d}0.94 ± 0.03	^{d}0.10 ± 0.06	^{a}0.87 ± 0.06	^{b}0.71 ± 0.05	^{c}0.58 ± 0.04
60	ND	^{e}1.27 ± 0.05	^{a}0.88 ± 0.05	^{b}0.69 ± 0.08	^{c}0.54 ± 0.04	^{d}0.42 ± 0.05
80	ND	^{b}1.32 ± 0.02	^{a}0.67 ± 0.07	^{b}0.32 ± 0.04	^{c}0.21 ± 0.04	^{c}0.30 ± 0.03

Values (mean ±SD) with different superscript in the same row are significantly different (P<0.05)

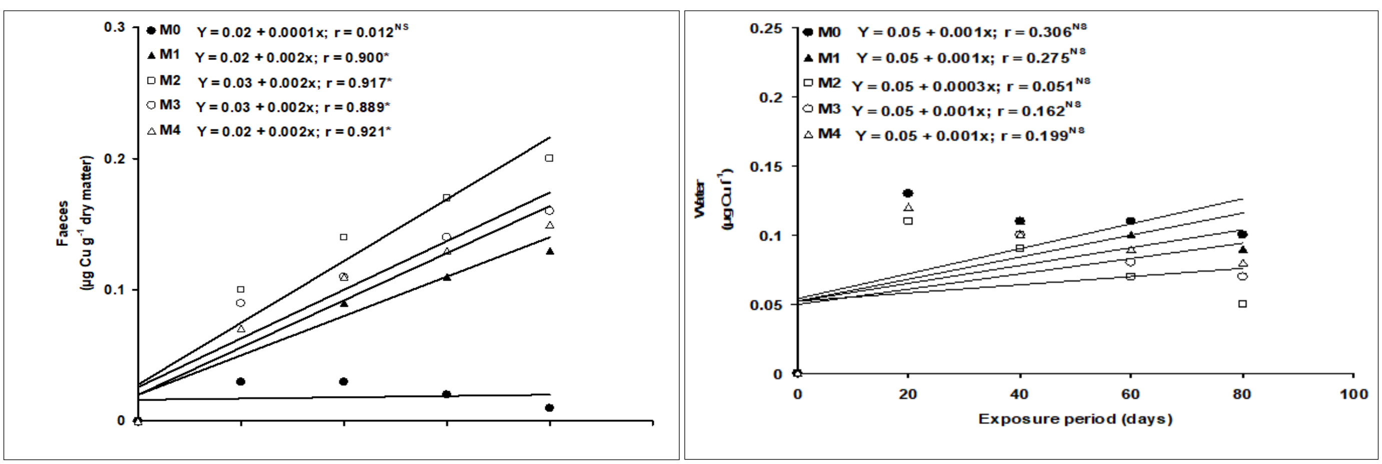

Fig 1: Regression lines for dietary *Moringa* diets on metal accumulation in faeces and water in small size *Cyprinus carpio* exposed to sublethal level of copper as a function of time. * p < 0.01; NS – Non-significant

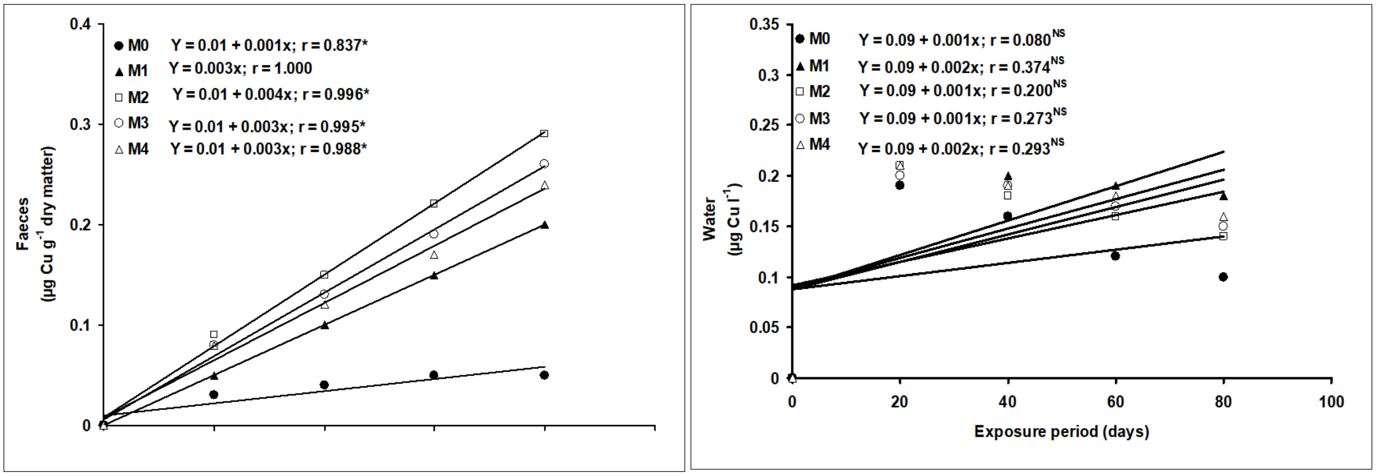

Fig 2: Regression lines for dietary *Moringa* diets on metal accumulation in faeces and water in large size *Cyprinus carpio* exposed to sublethal level of copper as a function of time. * P < 0.01; NS – Non-significant

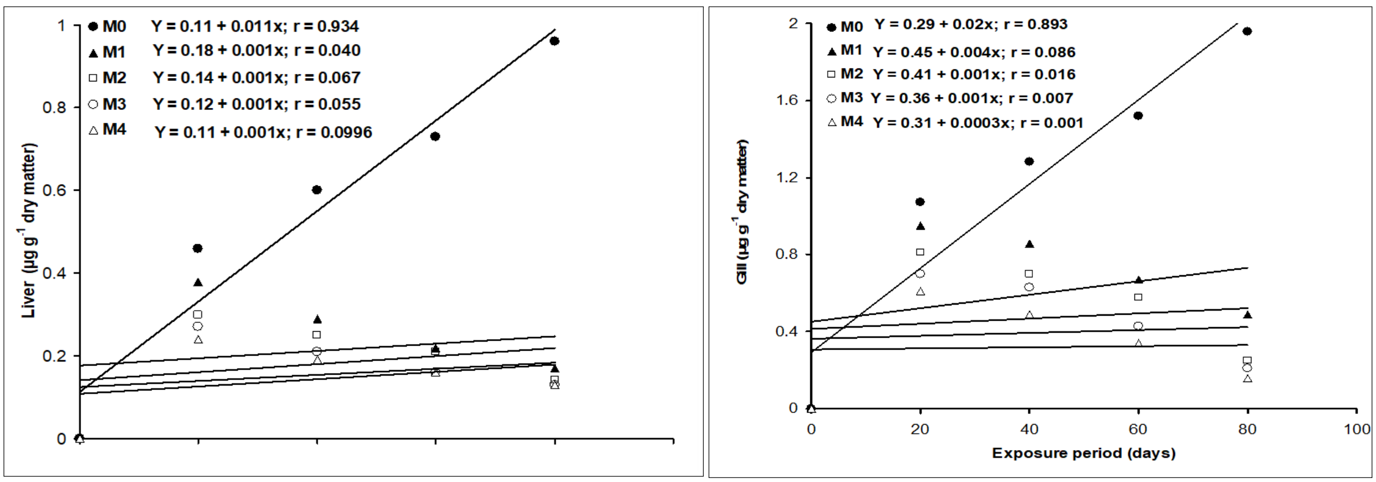

Fig 3: Regression lines for dietary *Moringa* diets on metal accumulation in gill and liver tissues of a small size *Cyprinus carpio* exposed to sublethal level of copper as a function of time. * p < 0.01; NS – Non-significant

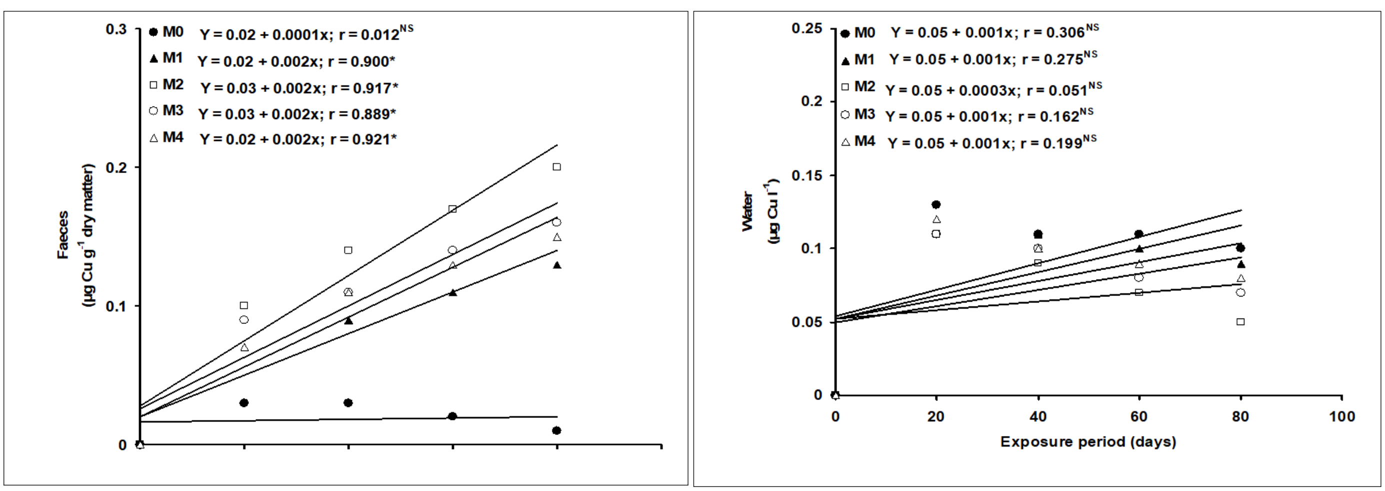

Fig 4: Regression lines for dietary *Moringa* diets on metal accumulation in faeces and water in small size *Cyprinus carpio* exposed to sublethal level of copper as a function of time. * p < 0.01; NS – Non-significant

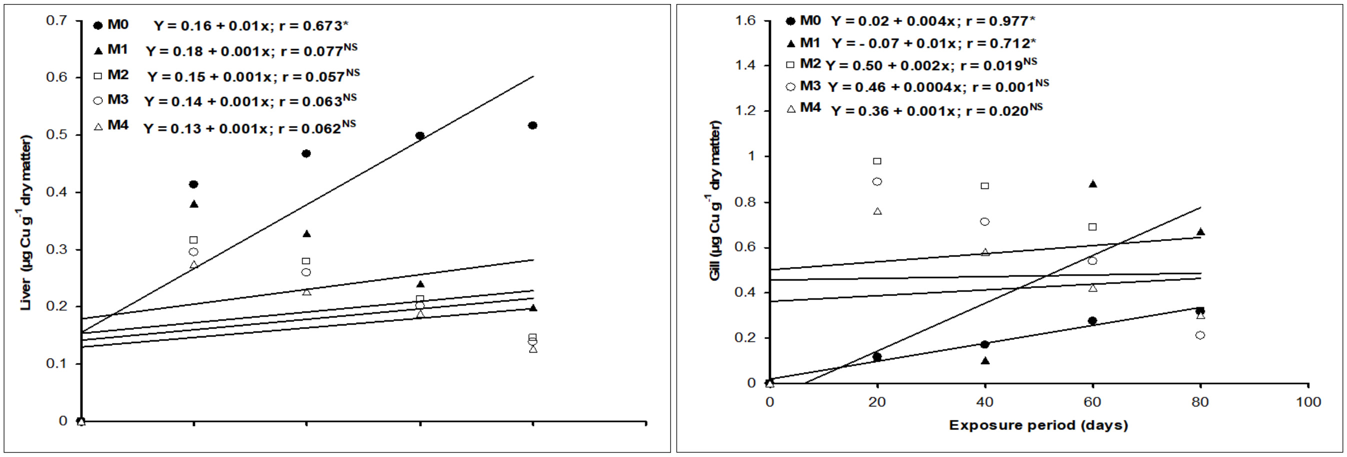

Fig 5: Regression lines for dietary *Moringa* diets on metal accumulation in gill and liver tissues of a large size *Cyprinus carpio* exposed to sublethal level of copper as a function of time. * P < 0.01; NS – Non-significant

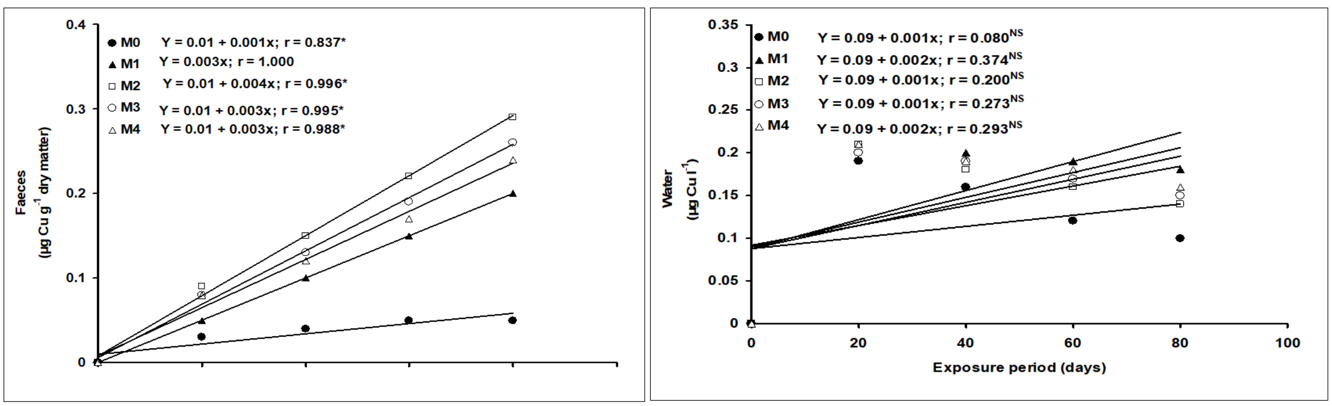

Fig 6: Regression lines for dietary *Moringa* diets on metal accumulation in faeces and water in large size *Cyprinus carpio* exposed to sublethal level of copper as a function of time. * P < 0.01; NS – Non-significant

4. Discussion

The present study revealed that copper accumulation was significantly ($P<0.05$) high in *Moringa* free diet and it was gradually decreased with an increasing the *Moringa* levels in $M_1 - M_4$ groups. The elimination of accumulated copper from body tissues through feces increased with increasing the *Moringa* levels in the diet. Lanno *et al.* (1985) [13] observed that, supplementation of ascorbic acid at 2000 mg Kg^{-1} diet resulted in decreased copper accumulation in tissues of copper exposed rainbow trout, *Salmo gairdneri*. James and Sampath (1999) [9] reported that addition of ion-exchanging agent, Zeolite to cadmium contaminated media significantly reduced the cd level in water and fish body (metal elimination through feces) which reduced the cd toxicity and improved biochemical and growth parameters in *O. mossambicus*. James *et al.*, (1992) [1] studied utilization of *Eichornia crassipes* for the reduction of mercury toxicity on food transformation in *Heteropneustes fossilis*. They reported that *E. crassipes* removed the considerable amount of mercury from the test medium and there by indirectly reduced the mercury toxicity of *H. fossilis*. James *et al.* (2009) [9] found that, copper exposed *C. mrigala* when feed *spirulina* supplemented diet might have eliminated the accumulated copper through faeces, which supports the present investigation. The leaf of *Moringa* being a good source of vitamins and amino acids, it has some medicinal uses (Makkar and Becker, 1999; Francis *et al.*, 2002) [6, 7]. Sunisa *et al.* (2012) [14] reported that *M. oleifera* diet reduced the lead (pb) toxicity *Puntius altus* and which inturn improved the degenerated organs (liver, gill, kidney) of pb exposed fish. The present investigation revealed that, dietary *Moringa* can reduce the toxic effect of copper in tissues.

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