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TOXICITY OF COPPER ON THE PROTEIN CONTENTS OF CERTAIN TISSUES OF FRESH WATER FISH, CLARIAS BATRACHUS (LINN)

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ABSTRACT

Discharge of heavy metals into aquatic environment from various sources even below permissible levels, creates health hazards in aquatic organisms. The persistence and ubiquitous nature of these pollutant compounds coupled with their tendency to accumulate in organisms ultimately produce toxic reaction in aquatic biota especially, fish. The present study deals with the toxicity of copper as ($CuSO_4$), as a component of industrial waste and its effect on tissue protein at 24, 48, 72 and 96h. The LC50 values were found at 180 ppm, 88 ppm, 40 ppm and 20 ppm for 24, 48, 72 and 96h respectively. The estimated protein concentration in the tissues-gills, liver, kidney, ovary and testis were found to be reduced during the exposure periods. Maximum reduction in protein level in the tissues was found at 96h.

KEYWORDS: Heavy metal, Gills, Liver, kidney, testis, ovary, Clarias batrachus.

INTRODUCTION

Some of heavy metals are essential to living organisms and they are commonly found in natural waters but high concentrations and accumulation of them may become so toxic. Copper is a necessary metal with a recognized biological role and like other heavy metals, it is potentially toxic at high concentrations. The mode of action of toxicants and cause for death of poisoned aquatic animals is better understood from biochemical investigations besides mortality studies. Since the stress condition caused alteration in metabolic cycles, it is

necessary to understand the significance of these variations in the organic contents of tissues. Biomarkers of oxidative stress and heavy metal levels as indicator of environmental pollution in African (*Clarias gariepinus*) from Nigeria Ogun River (Farombi *et al* 2007). Alterations in biochemical composition have been studied by many workers. Proteins are basic molecules to any living system. In cells they function as enzymes, structural materials, lubricants and carrier molecules. Carbohydrates play a structural role as well acts as a reservoir of chemical energy to be increased or decreased according to organisms need. Glycogen in the tissue is also considered to be the immediate source of energy to adapt to the environmental conditions. Several workers have reported the impact of various heavy metals on the carbohydrate metabolism of different aquatic organisms (Kharat *et al*, 2009). Heavy metal copper is an osmoregulatory toxicant in gibel carp, *Carassius auratus* causing Na loss and glycogen depletion in liver (Boeck, 2010). (Okacha and Adedeji 2011) overview of cadmium toxicity in fish. Among heavy metals, Copper, a group 1B metal, is also being used in industries like organic chemicals, electroplating, iron and steel works, electrical works, antifouling paints, pulp and paper industries, pesticides, fungicides and automobile industries. Even though copper is an essential element in low concentrations, it is discharged into the freshwater environments in higher concentrations as an industrial effluent and severely affects the freshw ater fauna, especially fishes (Lodhi *et al*., 2011). Reports are available on the toxicity of copper on carbohydrate metabolism in certain tissues of freshwater mussel, *Lamellidens marginalis* (Satyaparmeshwar *et al*., 2011). Copper is also shown to inhibit carbohydrate level in snails (Patil *et al*., 2011). The mode of action of toxicants and cause for death by poisoning of aquatic animals is better understood from biochemical investigations besides mortality studies. Since the stress condition caused alteration in metabolic cycles, it is necessary to understand the significance of these variations in the organic contents of tissues. Proteins are basic molecules to any living system. In cells they function as enzymes, structural materials, lubricants and carrier molecules. Copper has been reported as an osmoregulatory toxicant in gibel carp, *Carassius auratus* causing Na loss and glycogen depletion in liver (Boeck, 2010). Khalid Saraf-Eldeen and Nassr-Allah Abdel-Hamid, (2011), reported alterations in protein patterns on fish exposed to $CuSO_4$. The present study deals with the toxicity of copper (as $CuSO_4$) on the protein levels of gills, liver, kidney, gonads (ovary and testis) of freshwater fish, *Clarias batrachus*, after exposure for 24, 48, 72 and 96h.

MATERIALS AND METHODS

Adult and live fish *Clarias batrachus* were collected from the farm Patra and Bhadbhada Bhopal brought to the laboratory, cleaned by using 0.1% $KMno_4$ to avoid dermal infection. Only healthy fishes (Length: 12-15cm, Weight: 50-60g) were taken for experiment. Fishes were acclimatized in glass aquaria for 15 days and were fed with fish food (earthworms) and water in the aquaria was replaced by freshwater at every 24h. Stock solution of Copper sulphate was prepared by dissolving appropriate amount of $CuSO_4$ as Cu salt in distilled water. The fish *Clarias batrachus* were exposed to Cu (as $CuSO_4$) to know the acute toxicity at 24, 48, 72 and 96h. For selection of test concentration, some pilot tests were carried out. The range of concentration was selected between 0 to 100% mortality. In order to maintain the concentration of copper, the water in the aquaria was changed every 24h during the exposure. The mortality rate of *Clarias batrachus* was recorded at 24, 48, 72 and 96h exposure to the heavy metal. The percentage for corrected mortality was calculated using the Abbott's formula (1952).

$$\text{Corrected mortality (\%)} = \frac{\text{Percentage living in control - Percentage liv ing in treatment}}{\text{Percentage living in control}} \times 100$$

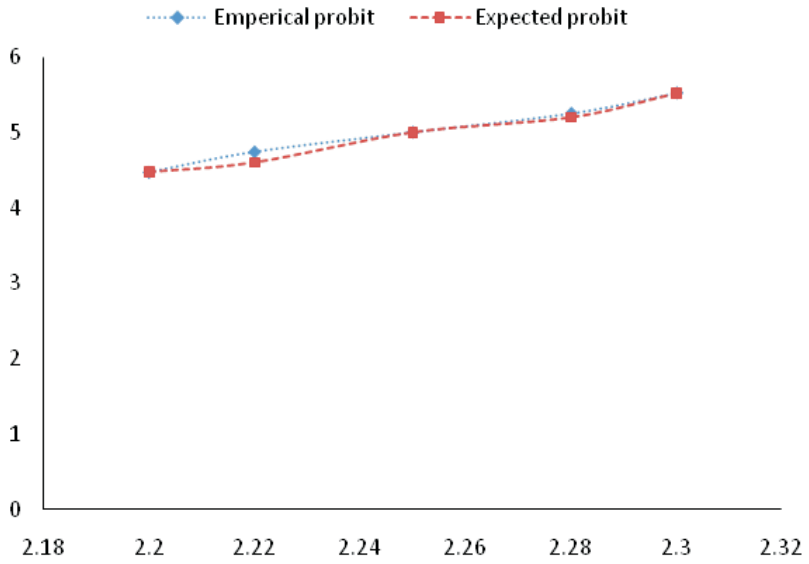
The corrected mortality data was analyzed to determine the LC50 values for 24, 48, 72 and 96h. and were calculated by probit analysis method (Finney, 1971).For studying the protein levels in the gills, liver, kidney and gonads, fishes were divided in two groups as control and experimental .After exposure both control and experimental fishes were sacrificed. The fishes were dissected and gills, liver, kidney and gonads were processed for protein estimation (Lowry *et al.*, 1951).

RESULTS

Copper sulphate toxicity The mean LC50 values of Copper sulphate toxicity for 24 (Fig.1A), 48 (Fig. 1B), 72 (Fig. 1C), 96 (Fig. 1D) h of exposure were estimated as 180 ppm, 88 ppm, 40 ppm and 20 ppm respectively (Table 1, Fig. 1). The observed data of present study indicate that the fish *Clarias batrachus* survived well from 1 to 173 ppm for 24h, 1 to 81 ppm for 48h, 1 to 31 ppm for 72h, 1 to 11 ppm for 96h of exposure.

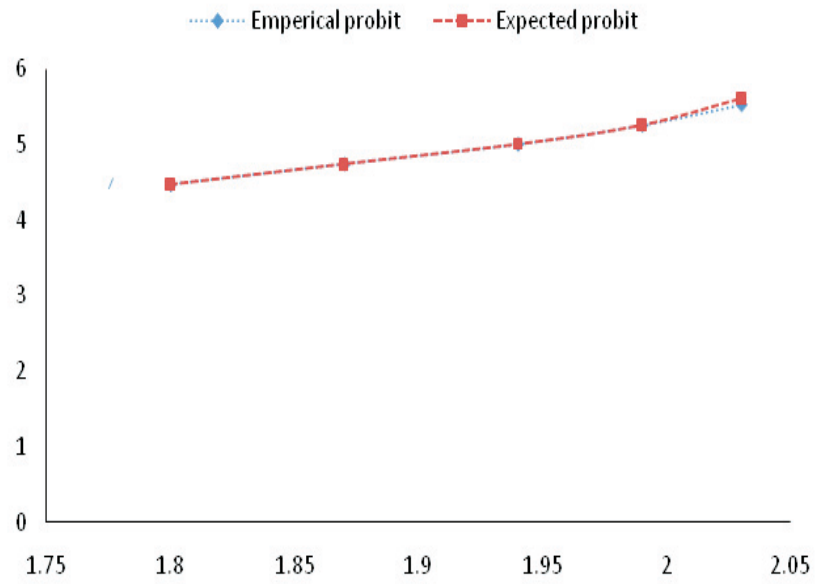
Protein Content

Level of protein from control and exposed tissues of fish are presented in Table 2. A significant reduction in protein levels in all tissues were observed as compared to the controlled fishes. In the gills of control fishes, the protein content was



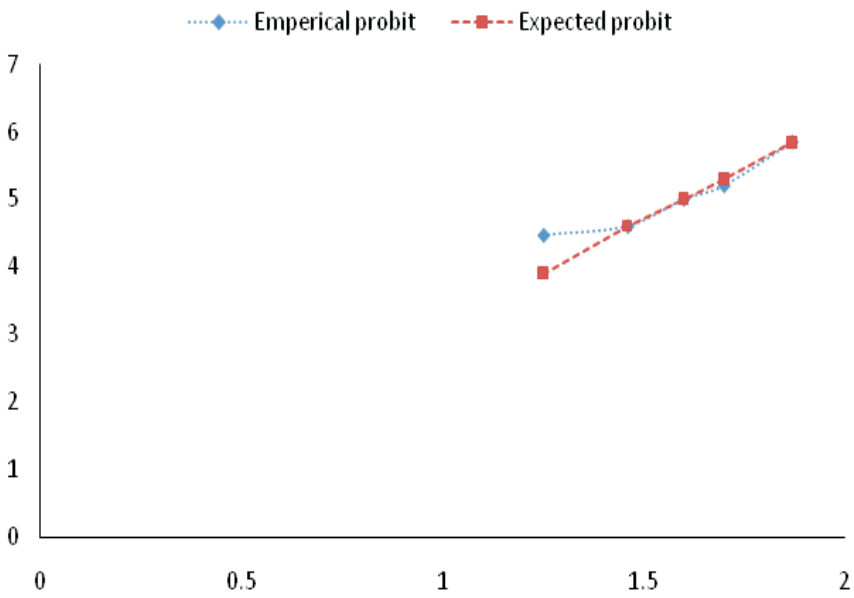
Log of concentration.....

Figure 1A: Emperical and expected probit lines for *Clarias batrachus* exposed to $CuSO_4$ showing LC50 values at 24h.



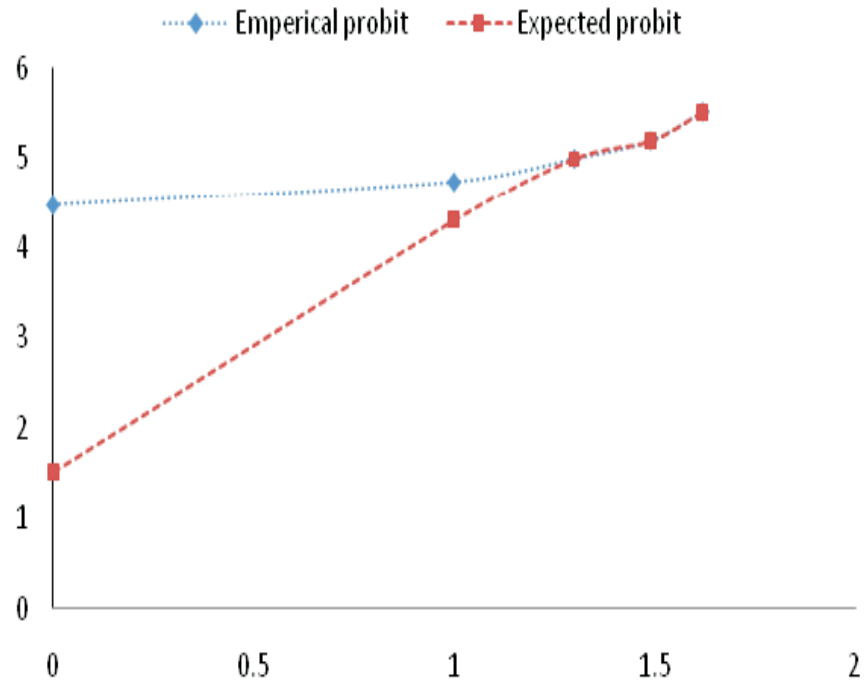
Log of concentration.....

Figure 1B: Emperical and expected probit lines for *Clarias batrachus* exposed to $CuSO_4$ showing LC50 values at 48h.



Log of concentration.....

Figure 1C: Emperical and expected probit lines for *Clarias batrachus* exposed to $CuSO_4$ showing LC50 values at 72h.



Log of concentration.....

Figure 1D: Emperical and expected probit lines for *Clarias batrachus* exposed to $CuSO_4$ showing LC50 values at 96h.

Table 1: LC50 values, calculated and observed, for freshwater fish *Clarias batrachus*, after exposure to $CuSO_4$ for a period of 24, 48, 72 and 96h.

Exposure period (h.)	LC50 values (ppm)	Regression equation: Y'=(y-bx)+bx M1 M2	Chi-square	Variance	Fiducial limits upto 95% confidence M1	M2
24	180	9.779977x - 17.003971	0.066881	0.000346	2.213541	2.286458
48	88	4.742416x - 4.158020	4.541213	0.0014843	1.854395	2.005404
72	40	2.090739x - 1.768394	0.387453	0.008330	1.397109	1.75489
96	20	3.445121 + 1.250568x	0.328928	0.166447	0.282360	1.881639

Table 2: Changes in protein levels in different tissues of *Clarias batrachus* after 24, 48, 72 and 96 h exposure to $CuSO_4$.

Organs	Control	Experimental			
		24 h. (180ppm)	48 h. (88 ppm)	72 h. (40 ppm)	96 h. (20 ppm)
Gills	19.20 ± 0	12.06 ±0.12 (-37.16%)*	10.96 ±0.43 (-42.91%)*	8.74 ± 0.66 (-54.49%)*	6.68 ± 0.88 (-65.20%)*
Liver	16.34 ± 0.66	10 ± 0.81 (-38.77%)**	9.84 ± 0.59 (-39.77%)**	7.79 ± 0.38 (-52.32%)*	5.88 ± 0.67 (-64.01%)*
Kidney	14.60 ± 0.20	11.11 ± 0.67 (-23.87%)*	10.16 ±0.66 (-30.38%)*	7.94 ± 0.59 (-45.61%)*	6.20 ± 0.45 (-57.53%)*
Ovary	16.02 ± 0.31	10.95 ± 0.36 (-31.64%)*	10.00 ±0.80 (-35.57%)*	9.53 ± 0.29 (-40.51%)*	7.15 ± 0.44 (-55.36%)*
Testis	14.76 ± 0.43	12.06 ± 0.39 (-18.29%)***	9.85 ± 0.51 (-33.28%)*	8.42 ± 0.59 (-42.95%)*	6.68 ± 0.60 (-54.76%)*

Each value is the mean (X±SD) of three estimations; Values in the parenthesis indicate percent changes over control; [* $p<0.001$, ** $p<0.01$, *** $p<0.05$]; Highly significan; **significant; ***non-significant.

19.20 mg /100 mg of wet weight of tissue, which was reduced to12.06 mg, 10.96mg, 8.74 mg and 6.68 mg at 24h, 48h, 72h and 96h respectively. This showed a highly significant reduction ($p<0.001$) of (-37.16%), (-42.91%), (-54.49%) and (-65.20%) at 180 ppm, 88 ppm, 40 ppm and 20 ppm respectively, as compared to the controlled values. In the liver of control fish, protein concentration of 16.34 mg /100 mg of wet weight was found which declined to 10mg, 9.84 mg, 7.79 mg, and 5.88 mg at 24h, 48h, 72h and 96h respectively. Here, a significant reduction ($p<0.01$) of (-38.77%), (-39.77%) occurred at 24 and 48h respectively whereas a highly significant reduction ($p<0.001$) of (-52.32%) and (-62.01%) at 72 and 96h was observed respectively. In control fishes, the protein content in kidney was 14.60 mg /100 mg. After an exposure to 180ppm, 88ppm, 40ppm and 20ppm for 24, 48, 72 and 96h, the protein content was reduced to 11.11mg, 10.16mg, 7.94mg and 6.20mg respectively. A highly significant reduction ($p<0.001$) of (-23.87%), (-30.38%), (-45.61%) and (-57.53%) occurred at all the four concentrations respectively. In controlled fishes, the protein content in ovary was 16.02mg /100mg which was gradually reduced to 10.95mg, 10mg, 9.53mg, and 7.15mg at 24, 48, 72 and 96h exposure to $CuSO_4$. A highly significant reduction ($p<0.001$) of (-31.64%), (-35.57%), (-40.51%) and (-55.36%) occurred respectively. In the testis of controlled fishes, 14.76 mg /100mg wet weight was found reduced to 12.06mg, 9.85 mg, 8.42

mg and 6.68 mg was found at 180 ppm, 88 ppm, 40 ppm and 20 ppm respectively with a highly significant reduction ($p<0.001$) of (-33.28%), (-42.95%) and (-54.76%) at 48, 72 and 96h respectively and a non-significant reduction of (- 18.29%) at 24h. During this acute toxicity test, gills were the most affected followed by liver, kidney, ovary and testis. Minimum reduction in the tissue protein level occurred at 24h and maximum reduction occurred at 96h indicating that % reduction is related with exposure period.

DISCUSSION

Heavy metals like zinc and copper enter the aquatic ecosystem through a wide spectrum of natural source such as volcanic activities, erosion and anthropogenic ones including industrial wastes as well as a leakage and get further biomagnified in the food chain. Not only in India, but globally, pollution is a scare word. Heavy metals are natural components of earths' crust. Large doses of these heavy metals can enter the water and thus affect the aquatic organisms. In the present study, the toxicity of Cu increases with increasing exposure time, at 24, 48, 72, 96h. recorded at 180, 88, 40, 20ppm respectively. A reduction in the protein level of all the tissues was found at all the exposure periods. Similar results were obtained by Emad *et al*., (2005) Hatai and Subhasis (2005). Muley *et al.* (2007) reported reduction in protein, glycogen and lipid in tissues of freshwater fish *Labeo rohita* induced by heavy metals from electroplating industry. Shoba *et al*. (2007), studied biochemical changes in freshwater fish, *Catla catla* on exposure to heavy metal toxicant cadmium chloride. Mastan, (2008) studied changes in protein levels of certain tissues of freshwater fish *Heteropneustes fossilis* induced by copper. Initally a decrease at 24h may be observed due to Cu stress. But this decrease continued with an increase in exposure period i.e., 48, 72 and 96h. The alteration in the tissue protein, in the present study suggests disturbance in the physiological activity. Decrease in the level of tissue protein may be due to excessive proteolysis to overcome the metabolic stress, as deposited protein in the cytoplasm can easily be used to replace the loss of proteins that occur during physiological stress (Patil, 2011). These alterations may be due to utilization of amino acids through transamination and deamination which might have supplied necessary keto acids to act as precursors for the maintenance of carbohydrate metabolism to meet the energy requirements during copper stress (Palanisamy *et al.*, 2011). Cu also induces alterations in other biochemical compositions. (Reddy *et al.*, 2008; Fatma and Nahed, 2008). Apart from Cu, other heavy metals and pollutants like pesticides also alter the biochemical composition of different organs. Martin and Arevoli (2008) reported biochemical alterations induced by mercuric chloride in freshwater fish, *Catla catla*.

Insecticide like Monocrotophos also induced reduction in protein levels in fish *Tilapia mossambica* (Remia *et al*., 2008). The contamination of heavy metals is a serious threat to aquatic organisms because of their toxicity, long persistence, bioaccumulation and biomagnifications in the food chain. Toxicity of heavy metals is time dependant and on nature of heavy metal. The present study reveals that copper has a tangible effect on the protein level of certain tissues of fresh water fish, *Clarias batrachus*, which may cause severe to fatal physio-metabolic dysfunction.

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