

Decrease in Energy Level Due to Copper Toxicity on Fry of Fresh Water Edible Fish, *Pangasianodon Hypophthalmus*

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Abstract: Cheapest readily available and important animal protein is fish, which is involved in socio-economic, ecological, health and aesthetic sense of man. As India is blessed with abundant water bodies, there is enormous potential for blue revolution to expand further. Due to rapid urbanization and industrialization copper in particular acted as one of the environmental contaminant, and is both an essential micronutrient as well as toxicant if present in more than required amounts especially in fish. Bioaccumulation studies show that *Pangasianodon hypophthalmus* fry accumulated the metal on exposure to sublethal concentration of copper. It was also found that the accumulation of the metal increased with increasing exposure period. Due to this energy levels from food of control fry showed an increase with increasing time. However, there was a significant decrease ($P < 0.05$) in exposed fish fry over their respective controls from 96 hrs to 30 days. Initially, a slight decrease was observed up to 48 hrs in exposed fry but later the highest (23.3%) decrease was noticed at 20 days. On 30 days of exposure a decrease of 22.65% was observed. The present investigation provides the base-line data in monitoring copper or metal-induced stress on fresh water fish fry in general and *Pangasianodon hypophthalmus* fry in particular.

Keywords: Essential micronutrient, Bioaccumulation, Environmental contaminant, urbanization, industrialization and base-line data

1. Introduction

Among all the xenobiotic substances, the highly toxic and non-biodegradable substances are heavy metals. The macromolecules of living organisms which are mostly polymeric forms constitute proteins, nucleic acids, lipids and carbohydrates. Proteins are the building units of the cells and body. Carbohydrates are the main instant energy source and play a key role in cell to cell recognition. Lipids are the stored food materials in the cell. They also serve as metabolic intermediates, fuels, part of enzymes and structural frame work of RNA and DNA. De-Burlin, (1976) reported that alteration in the carbohydrate metabolism is the most important biochemical lesion arising by the action of toxic compounds. Heavy metals, as pollutants of the aquatic environment, constitute a major hazard because of their toxicity, persistence in environment and affect the organisms by interacting with biochemical molecules and form metal-ion complexes. Flower and Gould, (1988) noticed that these metal complex formations disturb the physiological and biochemical mechanisms. According to Giesy *et al.* (1988), the metal ions disturb the energy expenditure of the cell. Muley *et al.* (2007) observed the impact of industrial effluents on the biochemical composition of fresh water fish *Labeo rohita* and found that the industrial effluents at sub lethal and lethal concentrations altered the biochemical composition (glycogen, protein and lipid) of the various organs of test fish, due to utilization of biochemical energy to counteract the toxic stress caused due to heavy metals present in effluents.

2. Method

The fish fry of almost the same size (2.8 - 3.0 cm) were collected from the local vendors of Eluru fish market, Andhra Pradesh, India. These were acclimatized to the laboratory conditions in plastic troughs, containing tap water with aeration for 48 hrs. Crowding was avoided during maintenance of the fish fry in the laboratory. Care was taken

while handling the fry to avoid any damage. The pH and temperature were maintained at 7 and 29 ± 2 respectively. The experimental media were changed daily in line with respective concentrations of copper and parallel controls. The fish fry were kept in the plastic troughs of 50 L capacity and each trough contains 40 liters of water. The medium was renewed daily. The fish fry were fed with rice bran and wheat bran in the ratio of 2:1 two times daily almost about 10% of the body weight. Excess feed was siphoned out while exchanging the water. The feed was stopped at least six hours before sacrifice of the animal.

The metal salt used for the experiments was copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$). The stock solution was prepared by dissolving copper sulphate (1%) in distilled. The metal effect on the fry, and thereby the survival was studied initially by exposing the individuals to increasing concentrations of the metal up to a determined point by "static renewal bioassay method" (APHA, 2000).

For conducting Bioaccumulation essay and thereby energy at various concentrations of metal the fish fry were exposed to a sub lethal concentration of the metal for a period of 30 days. The sub lethal concentration 0.0915 ppm was calculated as $1/5^{\text{th}}$ of the LC_{50} value for 96 hrs. Parallel controls were maintained along with the experiment without the metal. Both control and exposed samples were carefully collected at intervals of 24 hrs, 48 hrs, 96 hrs, 10 days, 20 days, and 30 days. Feeding was stopped at least six hours before sacrifice of the fish fry. At each interval a batch of 30 fish fry were isolated and kept in an oven at 60°C for 48 hrs. The dried tissue was finely powdered and transferred to dry and clean glass vial and kept in desiccators for further analysis.

Biochemical analysis: The analysis of biochemical constituent's namely total carbohydrates, total proteins and total lipids was carried out with dry tissue of control and

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exposed animals. The different biochemical constituents were estimated in fish fry samples.

Estimation of total energy levels: Total energy levels were estimated by following the conversion formula enunciated by Graney and Giesy, (1986) taking into account the total amounts of carbohydrates, proteins and lipids. The conversion factors used were 4.2, 4.2 and 9.3 cal/mg for carbohydrates, proteins and lipids respectively.

Statistics: The experiment was repeated for three times and each assay was done in triplicate. The mean value and standard deviation were calculated at each interval and also for each concentration. The significant differences in Biochemical components along with energy level between control and exposed group was made using "One-way ANOVA with Bonferronis post test was performed using GraphPad Prism version 5.00 for Windows, Graph Pad

Software, San Diego California USA, www.graphpad.com". The percent increase or decrease over their respective controls was also calculated. The values for exposed fry were compared with controls by using one-way ANOVA.

3. Results

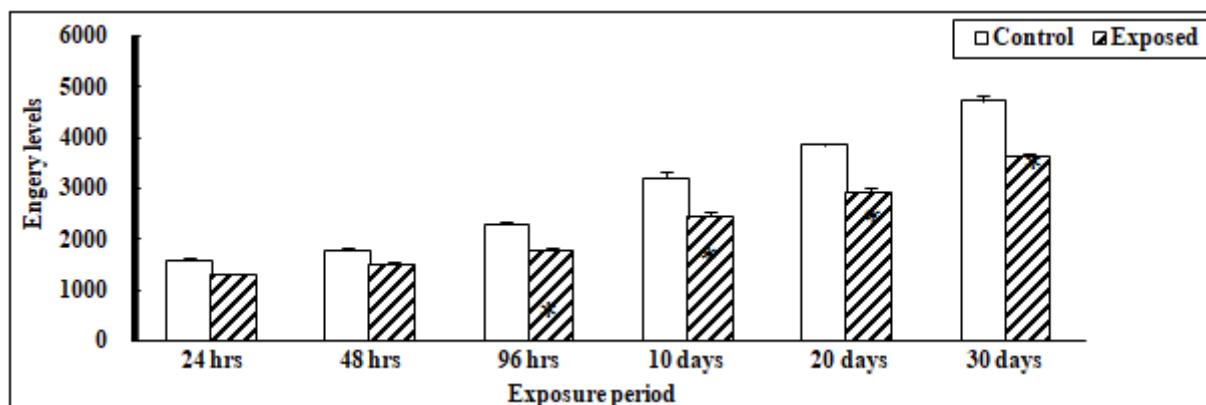
The results of copper toxicity and there by changes in energy levels were presented in Table & Figure given below.

Energy levels ratio of *Pangasianodon hypophthalmus* fry exposed to 0.09156 (ppm) of copper. Each value represents the mean ± Standard Deviation. The values in the parentheses represent percent decrease over their respective controls. *Significantly different from their respective controls at P < 0.05.

Groups	Exposure period					
	24 hrs	48 hrs	96 hrs	10 Days	20 Days	30 Days
Control (Cal/mg)	1592± 58.42	1782± 37.91	2303± 56	3210± 122.7	3858± 52.73	4722± 132.4
Exposed (Cal/mg)	1308± 27.31* (17.839)	1514± 42.21* (15.039)	1801± 42.19* (21.797)	2482± 48.58* (22.679)	2959± 51.16* (23.302)	3652± 34.44* (22.659)

One Way ANOVA

Source of Variation	Degrees of freedom	Sum of squares	Mean Square	F Value
Treatments (between columns)	11	116700000	10610000	2365
Residuals (with in Columns)	96	430700	4486	
Total	107	117100000		



The energy level values of *Pangasianodon hypophthalmus* fry when exposed to sub lethal concentration of copper in general, of control fry increased with increasing time except at 48 hrs and 30 days. However, there was a significant decrease ($P < 0.05$) in energy levels of exposed fish fry over their respective controls at all intervals. A maximum decrease in energy levels was observed at 20 days which was 23.30%. A minimum decrease of 15.03% was observed at 48 hrs. However, the respective percent decrease in energy levels between control and exposed fish fry at 24 hrs, 96 hrs, 10 days, and 30 days were 17.83%, 21.79%, 22.67%, 22.65% respectively.

4. Discussion

According to Giesy *et al.* (1988), the metal ions disturb the energy expenditure of the cell. A steady decrease in energy levels was observed in exposed fry of the present

investigation corroborate well with that of Giesy *et al.* (1988). However, the observed decrease (23.30%) was marginally more in 20 days exposure period. The decrease in the energy levels in exposed fish might be due to the utilization of energy by the fish fry to overcome the stress caused in the body. The differential responses observed at 30 days indicate the possible operation of compensatory mechanism to mitigate the stress caused by copper toxicity or the fish fry might have developed a mechanism either to detoxify the effect of the toxicant. Findings also suggest the aqua farmers to use clean, metal free and un-polluted water both for hatchery and farming of these fresh water fish to ensure better growth and survival for sustainable aquaculture.

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