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"*BIOACCUMULATION LEVEL DUE TO COPPER TOXICITY ON FRY OF FRESH WATER EDIBLE FISH, PANGASIANODON HYPOPHTHALMUS*"

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Abstract

Heavy metals are metallic elements which are having high atomic weight and greater density (at least 5 times than water) or 5 g/cm Eisler *et al*. (1979). They are like aluminum, antimony, arsenic, beryllium, cadmium, cesium, chromium, cobalt, copper, gallium, iron, lead, manganese, nickel, platinum, gold, tellurium, thorium, uranium, vanadium and zinc. In animals accumulation and effects generally are in the order of Fe > Zn > Mn > Cu > Ni > Pb > Cd > Cr Canli *et al.* (1997). Copper accumulation levels were in the order of liver ≤ gills ≤ kidneys in fish Coho salmon and common carp as reported by Buckley *et al.* (1982). These metals alter, remove or impair the production of specific molecules needed in the body. WHO review them regularly in every aspect. 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.

Keywords: Accumulation, Urbanization, Industra;ization, Sublethal Concentration.

1. INTRODUCTION

The main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury, arsenic and copper. Less developed countries emit more as it is inevitable due to rapid industrialization globally. It was noticed that the aquatic environment serves as a major sink for various metal ions which are formed as byproducts of different industrial activities De Boeck *et al*. (2004b). Pollutants of heavy metals have been identified as stable and non-biodegradable compounds which accumulate in the tissue of aquatic organisms causing physiological disorders and mortality Di Giulio *et al*. (1989). Dandapat *et al*. (1999) reported that copper accumulation in fishes increases with increasing concentration of metals. In general, higher animals such as fish tend to show relatively more sophistication in this regard compared to aquatic invertebrates. S.C.Deb and Santra (1997) observed the relative accumulation range of the heavy metals are in the order of liver > brain > intestine > muscle > ovary > bone etc., of some fresh water fish.

Heavy metals if accumulated in vital organs of human body such as in heart, brain, kidney, intestine and liver may create disturbances in the cell to cell communication occurring between inflammatory mediators, nerve cells or hormones. Nutritional health requires heavy metals such as copper, chromium, iron and zinc. Heavy metals also displace vital nutritional minerals from their proper place in the body. They are non-toxic unless taken in large amounts. Other heavy metals such as mercury, arsenic, lead are toxic even at low levels.

James and Sampath (2000) reported that copper accumulation in the fish linearly increased with increase in exposure period up to 140 days and also with increase in tested copper concentration. Mc Geer *et al.* (2003) reported that the bio-accessibility, bioavailability and bioaccumulation properties of inorganic metals in soil, sediments and aquatic systems are complex causing bio-magnifications through food chain. Sonoko *et al.* (2007) reported that heavy metals exert inhibitory effects on sperm production of cat fish i.e. *Pangasianodon hypophthalmus by* copper or copper-binding protein in testis thereby affecting spermatogenesis.

After reviewing the literature, it can be concluded that there are no investigations on bioaccumulation of metals especially copper on fish fry of *Pangasianodon* hypophthalmus at LC_{50} value of 0.4578 ppm. Therefore, an attempt has been made to study the bioaccumulation of copper at different intervals of time by exposing the fry to sublethal concentrations of copper i.e., 0.0915 ppm for 30 days.

2. MATERIAL AND METHODOLOGY

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 ($CuSO_4.5H_2O$). The stock solution was prepared by dissolving copper sulphate (1%) in distilled water. 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 at various concentrations of metal the fish fry were exposed to a sublethal concentration of the metal for a period of 30 days. The sublethal concentration 0.0915 ppm was calculated as $1/5^{th}$ 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^{o} C for 48 hrs. The graphite crucibles and the glass vials used for all these experiments were washed carefully and rinsed with metal-free water and air dried. The dried tissue was finely powdered with motor and pestle and transferred to dry and clean glass vial and kept in desiccators for further analysis.

3. ANALYSIS OF COPPER

The analysis of metal content was carried out with the dried tissue powder. A known quantity of the tissue powder was kept in muffle furnace at a temperature of 600^{0} C for about 4-5 hrs to make into ash (George and Kureishy, 1979; Prabhakara Rao *et al*., 1986). The dry ash obtained was dissolved in a known amount of 0.01N HNO_3. The final clean and colorless solution was used for metal estimation with ICP-MS (Agilent Technologies).

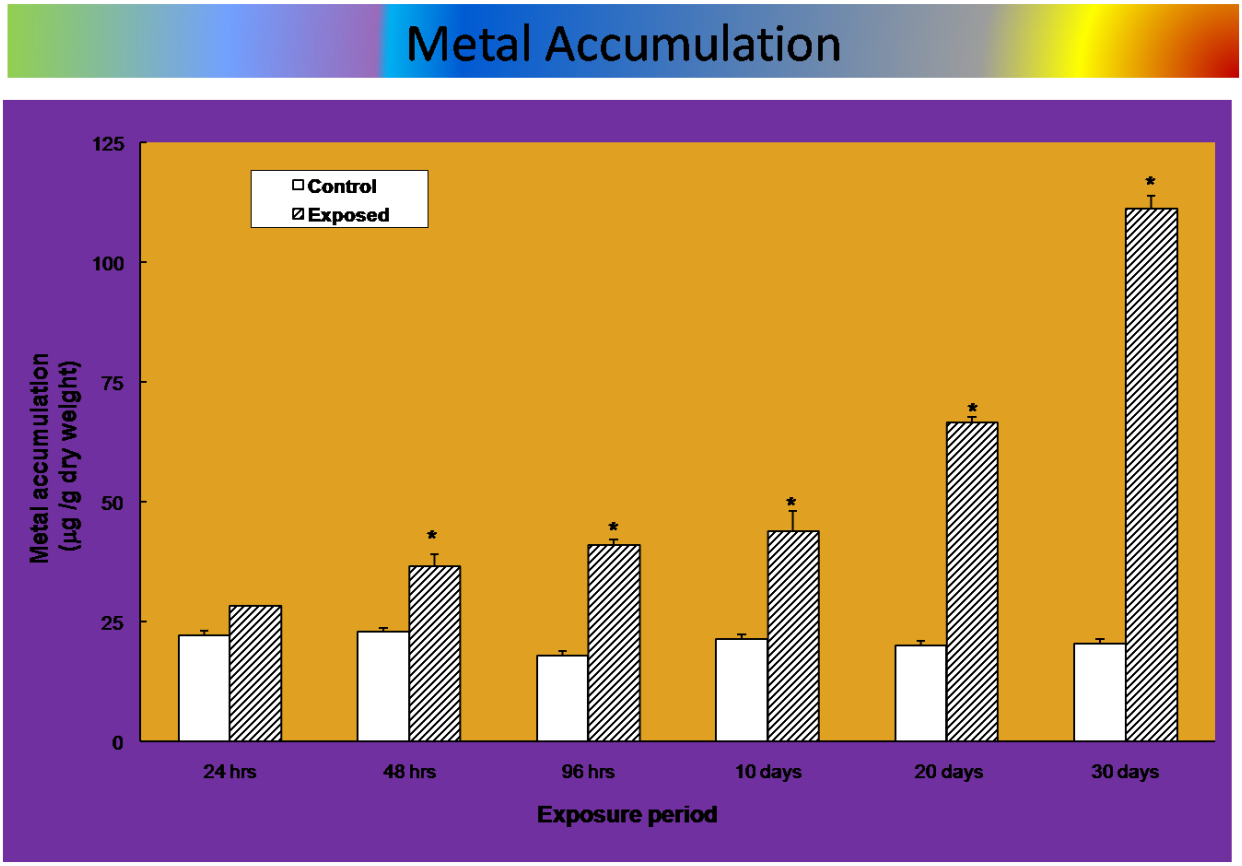
4. STATISTICS

The accumulation experiments were repeated for 3 times and each of the samples was analyzed in triplicates. The mean value and standard deviation were calculated at each interval. The significant differences in metal content between control and exposed group was made using "One-way ANOVA with Bonferronis post test using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com".

RESULTS

The results on accumulation of copper in fry of *Pangasianodon hypophthalmus* exposed to sublethal concentration i.e., 0.0915 ppm are presented in Table and Figure. The data indicated that there was a gradual increase in the accumulation of copper in the exposed fry when compared to the control at all the time intervals. A significant increase ($P < 0.05$) in the accumulation was noticed at all the time intervals over their controls. Maximum accumulation of metal was observed at 30 days (111.2 µg/gm dry weight) while the lowest accumulation occurred at 24 hrs which is (28.31 µg/gm dry weight) on exposure to sublethal concentration. Lowest concentration of copper was observed in control fry at 96 hrs i.e., 17.94 µg/gm dry weight. The lower values of 24 hrs and 48 hrs observed in exposed fish fry indicate less capacity of tissue accumulation. The accumulation of copper increased with increase in time. But more accumulation was seen on long term exposure in exposed fish.

A significant increase (P<0.05) in metal accumulation ranging from 28.31µg/gm(24hrs) to 111.2µg/gm(30days) can be seen with lowest of 17.94µg/gm at 96hrs.



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DISCUSSION AND CONCLUSION

From the results, it is evident that *Pangasianodon hypophthalamus* fry accumulated the metal on exposure to sublethal concentration of copper. In the present investigation, the accumulation of metal increased with increasing exposure period. Similar results were noticed in several fishes. Karakoc (1999) reported increase in copper

accumulation with increasing concentration of the metal in *Tilapia nilotica*. Mausumi *et al*. (2009) also noticed an increase in accumulation of copper with increasing concentration of the metal in *Cirrhinus mrigala*. Surya kumari (2008) reported that there was a gradual increase in cadmium metal accumulation in the exposed fry of *Labeo rohita* and *Cyprinus carpio* over their controls at all the time intervals, starting from 24 hrs to 20 days. Patil *et al.* (2010) studied the bioaccumulation of cadmium in the fresh water fish *Labeo rohita* and found that the rate of accumulation was increasing with increase in concentration and is time dependent. Accumulation of copper occurred in the whole body. The concentrations of essential mineral nutrients in organisms tend to be highly regulated compared to nonessential elements. The mechanisms of reducing metal accumulation and toxicity vary with the organism and include inhibiting uptake, detoxification, storage and increased elimination. Similarly copper accumulation might also have taken in the whole body of *Pangasianodon* fish fry on exposure to sublethal concentration of metal for 30 days whereas there was not much change in copper accumulation in control from 24 hrs to 30 days.

Fish can also utilize metallothionein to detoxify and store metals (Mason and Jenkins, 1995). The enhanced elimination of copper was also well documented (Grosell *et al.*, 1997). There might be reduced uptake of metal due to metalloionein or enhanced elimination of copper in fry of *Pangasianodon hyophthalmus* in the present investigation. This might be the reason for the decrease of metal accumulation on 10^{th} day of exposure thereby showing a percent decrease of only 51.37.

A comparison of the metal accumulation between various intervals of time is presented in Table and Figure. The lower values of 24 hrs and 48 hrs indicate less capacity of tissue accumulation. The accumulation of copper increased with increasing time on long time exposure up to 30 days. The values of bioaccumulation in *Pangasianodon hypophthalmus* fry indicates that in the exposed fry the metal concentration was not much upto 96 hrs (56.15%) but during longer duration i.e., 30 days, the accumulation was 86.76%. Hence the maximum accumulation was found on long-term exposure in the fry of *Pangasianodon hypophthalmus.* The metal accumulation observed in the present investigation may cause oxidative stress in fish fry leading to alteration of oxygen consumption, biochemical composition, growth and lipid peroxidation products which are studied in the subsequent experiments.

Table

Metal accumulationin *Pangasianodon hypophthalmus* fry exposed to 0.0915ppm of copper. Each value represents the Mean ± Standard Deviation. The values in the parenthesis 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 (µg/gm dry Wt.)	22.09 ± 2.51	22.79 ± 2.731	17.94 ± 4.19	21.33 ± 1.144	20.01 ± 2.651	20.33 ± 4.73

Exposed (µg/gm dry Wt.)	28.31 ± 2.731 (21.97)	36.56 ± 3.719* (37.66)	40.92 ± 2.316* (56.15)	43.87 ± 2.302* (51.37)	66.59 ± 5.155* (69.96)	111.2 ± 2.267* (86.76)

ONE WAY ANOVA

Source of Variation	Degrees of freedom	Sum of squares	Mean Square	F Value
Treatments (between columns)	11	24360	2215	221.9
Residuals (with in Columns)	24	239.5	9.979	
Total	35	24600		

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