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Studies on the effects of copper sulphate on liver protein content of an air-breathing fish *Clarias batrachus* (Linn.)

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Abstract : Among the various toxic pollutants, heavy metals are particularly severe in their action due to tendency of bio-magnification in the food chain. The global heavy metal pollution of water is a major environmental problem. With the advent of agricultural and industrial revolution, most of the water sources are becoming contaminated. Natural waters are often contaminated by untreated wastes of industrial, technological and agricultural origin containing various metallic compounds. Heavy metals due to their bio-accumulative and non biodegradable properties constitute a core group of aquatic pollutants. The direct or indirect bioaccumulation of metals in organisms in contaminated water is of great significance because it affects the food chain. In present investigation, the effect of copper sulphate on freshwater fish *Clarias batrachus* (Linn) has been shown. It was found that the biochemical parameters shown gradual decrease level in tissues exposed by copper sulphate.

Keywords : copper sulphate, liver, protein, fish, *Clarias batrachus.*

INTRODUCTION

The modern civilization, industrialization, urbanization and increased population have led to fast degradation of our aquatic environment. Water is polluted by domestic sewage, animal and industrial wastes. In addition, various chemical compounds like heavy metals pesticides, acaricides, fungicides, herbicides, algaecides and other chemical substances are used for agricultural production. These compounds load off the waterways with various chemicals that alter the nature of the water.[1]

Heavy metals are being utilized in a variety of ways in industries, agriculture and food processing and household in many forms. Metals are unique environmental and industrial pollutants in the sense that they are neither created nor destroyed by human beings but are only transported and transformed into various products.[2] Heavy metals play a vital role in the growth and development of plants. They may act as co-factors of some enzymes and help in the formation of intermediate metabolites. The primary anthropogenic sources of heavy metals are point sources such as mines, foundries, smelters, and coal-burning power plants, as well as diffuse sources such as combustion by-products and vehicle emissions. When excess amount of metals are absorbed by plants and animals, toxic effects are produced resulting in impairment of growth, inhibition of respiration and abnormalities in cell division and the extent of injury depends on the concentration of the metals. The heavy metals move from fields to ponds, lakes, rivers, estuaries and finally to sea, where they adversely affect the growth and survival of the non target species like fish and other aquatic animals.[3]

Among the various toxic pollutants, heavy metals

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are particularly severe in their action due to tendency of bio-magnification in the food chain. The global heavy metal pollution of water is a major environmental problem. With the advent of agricultural and industrial revolution, most of the water sources are becoming contaminated. Natural waters are often contaminated by untreated wastes of industrial, technological and agricultural origin containing various metallic compounds. Heavy metals due to their bio-accumulative and nonbiodegradable properties constitute a core group of aquatic pollutants. The direct or indirect bioaccumulation of metals in organisms in contaminated water is of great significance because it affects the food chain.[4,5]

For the past few decades, considerable attention had been focused on the fate of metals and their derivatives in the aquatic environment. (Environment Agency warning over *Clarias* Catfish found in River Tonge, Bolton. The Bolton News. 19 July 2017). The impact of heavy metals on aquatic animals can be evaluated in terms of toxicity and physiological alterations on aquatic animals. Various investigators studied intrusion of heavy metals and their salts in terrestrial and aquatic environments, and their toxicity to aquatic system. The heavy metal contaminations resulted in epidemic diseases such as "Minamata"[6] and "Itai Itai"[7]. The heavy metals were reported to cause massive fish kills and other aquatic animals.[8-13]

Heavy metals such as copper, lead, etc. are naturally occurring substances in the Earth's crust. Some are needed by humans in trace amounts to maintain optimum body function, whilst others such as mercury, arsenic, and cadmium can be harmful. All metals can be toxic if levels are too high.

Copper is one of 26 essential trace elements occurring naturally in plant and animal tissues and its availability is influenced by physico-chemical, hydrodynamic and biological factors. It makes its way into the receiving waters by extensive use in agriculture apart from usage in various industries like textile, tanneries, paints, battery, laundry, photography, copper ware and piping for water distribution systems. Copper ions are quite toxic to fish at various functional levels when environmental concentrations are increased.[14-16]

MATERIALS & METHODS

The fish were procured live from the local fish market with the help of fishermen. They were brought to the laboratory in wide mouthed large earthen pots half filled with natural water and covered with mosquito net. Every effort was taken to give least stress to the fish during transportation. They were first thoroughly washed and then rinsed in 0.1% $CuSo_4$ solution to remove any sort of dermal infections. Thereafter, healthy fish of an. average length (12-16 cm) and weight (80-100g) were transferred one by one with the help of small hand net to 40 litre rectangular glass aquaria for acclimation to various laboratory conditions for a fortnight.

The fish were not supplied any food for the first three days of acclimation. The fish were adjusted to natural photoperiod and ambient temperature. Running tap water was used in all the experiments and no aeration was done. They were fed with chopped goat liver every day Ad libitum at 11. 00 A.M. sharp. The feeding was stopped 24 hr before the start of experiment (Static acute bioassay) and no food was supplied to them during the period of experimentation. However, the feeding schedule was strictly followed during chronic experiments to avoid starvational effects.

TISSUE BIOCHEMISTRY

At the end of exposure period (day 30) the fish were anaesthetized with 1:4000 MS 222 (tricane methane-sulfonate, sandoz) for two minutes. The liver, muscle and gonads were quickly dissected out. Weighed and processed fur the quantitative estimation of total protein.

ESTIMATION OF TOTAL PROTEIN

The Folin-Lowry Method was followed for the estimation of total protein of liver, muscle and gonads. Tissues were homogenised in TCA buffer and then centrifuged at 3000 rpm for 10 minutes. The TCA precipitate the protein. After centrifugation the white precipitate so formed was analysed for the estimation of total proteins.

To 1 ml of the test solution was added 5 ml of alkaline solution (2% Na_2CO_3 in 0.1N NaOH + 0.5% $CuSO_4$ in 1% Na-K tartarate in equal volume). It was mixed thoroughly and allowed to stand at room temperature for 10 minutes. Thereafter 0.5 ml of diluted Folin-Ciocalteau reagent, which gives a coloured complex on reaction with protein, was added rapidly with immediate mixing. After 30 minutes rite optical densities were noted against the appropriate blank at 750 nm with the help of spectrophotometer (Spectronic-20).

Bovine serum albumin was used as standard in this test. After preparing a standard curve protein concentration of the test solution was estimated.

RESULTS AND DISCUSSION

In present investigation, the effect of copper sulphate on freshwater fish *Clarias batrachus* (Linn) has been shown. It was found that the biochemical parameters shown gradual decrease level in tissues exposed by copper sulphate.

Total protein

The changes observed in protein content exposed by copper sulphate on *Clarias batrachus*. It can be expressed as mg of protein/gram wet weight of tissue from control to treated set at 24 hr, 48hr, 72hr and 96 hours.

Freshwater catfishes treated with copper sulfate showed decline protein range from control set to 96 hours as in muscle 50.90+ to 30.16+2.30, in gills 60.05+2.16 to 31.30+1.52, in liver 65.05+1.71 to 42.40+1.19, in Gonads 55.49+1.71 to 42.36+1.19 (Table 1 and Fig 1).

Table 1- Levels of protein content in different tissues *of Clarias batrachus* exposed to copper sulphate

Sl. No.	Tissue	Control	Experimental			
			24 hrs.	48 hrs.	72 hrs.	96 hrs.
1.	Muscle	97.23±2.06	90.50±2.10	84.20±2.07	78.56±0.30	75.14±2.29
2.	Gills	80.40±2.30	74.52±1.19	71.49±1.20	67.55±1.90	62.30±1.52
3.	Liver	65.05±1.02	58.25±1.55	55.50±1.18	51.03±1.40	47.20±1.47
4.	Gonads	90.50±1.80	87.80±1.11	77.28±1.56	69.06±1.06	65.40±1.18

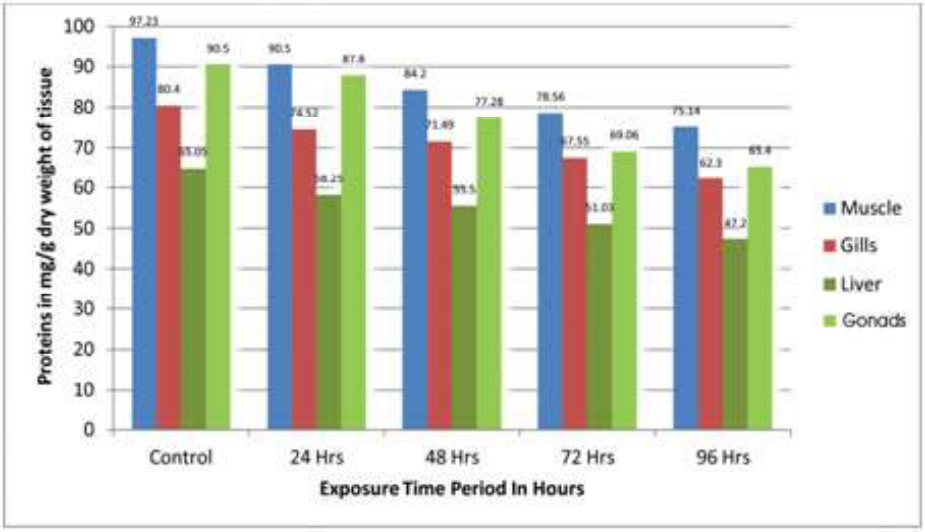

Fig- 1 – Levels of protein content of *Clarias batrachus* exposed to copper sulphate

Protein is most characteristic organic compound found in the living cell while the protoplasm of the cell is composed of protein. They play vital role in the process of interaction of cellular medium. During presents investigation increase in muscle and liver protein content of *Clarias batrachus* had been observed during 24 hours and 48 hours respectively after exposure to sub-lethal concentration of copper sulphate gonads also showed increased protein content during 48 hours. Increase in protein content occurs due to anaerobic metabolism which contribute major role in this situation. Proteins are main component for the building blocks of animal body.

In present investigation the total proteins were found progressively depleted in all the tissues throughout the exposed time. The percent responses appeared tissue-specific and the magnitude of depletion was more marked in the tissues of fish exposed to mercury chloride. The bio-accumulative heavy metal copper with biologically active constituents of the body such as lipids and proteins.

Another reason for increase in protein content is due

to increase in protein synthesis by increase the enzyme activity involved in protein synthesis. Toxic stress like copper sulphate treatment to the fish causes increase in the rate of protein synthesis. In experimentation, after 96 hours the decline in protein content was observed. This fall attributed to the constantly increasing contact of the copper sulphate with the bio-system which ultimately resulted in protein breakdown.

From the above results, it is clear that copper sulphate is very toxic and it lowers the rate of energy metabolic levels forcing the proposed fish to adapt to a sustained metabolic level, but shifting the gears of energy trapping from one segment to the other. The copper sulphate intoxicated proposed fish cannot rise to the high energy demands if necessary as there is limited energy production. Increasing fluoride excretion vit. C intake reduces its burden. Concluded that vit. C are useful in regulating fluoride induced improve the antioxidant status.

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