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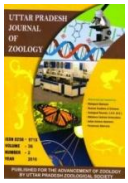

INFLUENCE OF COPPER SULPHATE ON GLYCOGEN AND TOTAL LIPID LEVELS IN GILL AND KIDNEYS OF FISH, *Oreochromis mossambicus* (PETERS)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Author RJ (Research Scholar) designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author GSD (Research Supervisor) guided and managed the analyses of the study. Both authors read and approved the final manuscript.

ARTICLE INFORMATION

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Short Research Article

ABSTRACT

Freshwater organisms face the challenge of being exposed to an assemblage of chemicals emitted by human activates. Thus, several biochemical parameters in fish organs could be used as indicators of heavy metal toxicity. In this study, *Oreochromis mossambicus* fish were exposed to sublethal concentrations (1/16, 1/12, 1/8 and 1/4th of 96 hours LC_{50} value), i.e. 3 mg/L, 4 mg/L, 6 mg/L and 12 mg/L of Copper Sulphate for different exposure periods of 10, 20, 30 and 40 days. The Glycogen level and Lipid level in the gills and kidneys were determined. The decreased Glycogen level and Lipid level in the two different organs of fish exposed to Copper Sulphate were observed when compared to control. Glycogen level and Lipid levels gradually decreased with increased exposure period, and the decrease was found to be directly proportional to increased sublethal concentrations. Thus it is concluded that the usage of Copper Sulphate must be reduced and to create an awareness among the people about the toxicity of $CuSO_4$ on animals & humans.

Keywords: *Oreochromis mossambicus*; copper sulphate; glycogen; lipids.

1. INTRODUCTION

Freshwater is facing many challenges of being exposed to a multitude of chemicals liquidated by humans [1]. Thus, several biochemical factors in fish organs might be used as indicators of heavy metal toxicity [2]. Because heavy metal spoliation in an aquatic environment exerts stress on fish, it leads to several other changes in the fish metabolism when exposed to heavy metals [3]. The primary water resources for living things are trenches, streams, ponds, lakes and rivers and other freshwater bodies.

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These water bodies are providing water for drinking, agricultural usage, cooling of industrial machines and for other domestic purposes. An undesirable change in the physical and chemical nature of water that causes an adverse effect on the life of man and local plants and animals are called water pollution [4]. Copper is a transition metal with a great quantity in aquatic and terrestrial environments. Copper, as an essential nutrient, it plays a vital role in different functions in cellular biochemistry, especially as a cofactor for many enzymes and as an integral of the non-enzymatic antioxidants ceruloplasmin and the metallothioneins [5].

The nutritional value of various species of fishes depends on their biochemical constituents such as carbohydrate, protein and lipids. These similar components can serve as sensitive indicators for discovering adverse effects, particularly the early events of contaminant injury because their modifications appear before the clinical symptoms produced by the toxicant [6]. It is therefore significant that potential effects of acute and chronic concentrations of a contaminant on proximate composition are determined and interpreted to explain the mechanism of pollutant action and possible ways to mitigate adverse effects [7].

The gills are the primary site of Copper uptake from the water, and a very slight amount of Copper accumulates in the gills after the initial exposure [8]. Copper is transferred from the gills to the blood and then transported to the liver where it is incorporated into ceruloplasmin transcuprein, and also is bound with albumin protein. It is then exported in these forms for use by other parts of the body.

The classic experimental tool of toxicology is animal testing. As of 2014, animal experiments provide information that is not available by other means about how substances function in living organisms. Fishes and their physiological changes are commonly used to assess the health of aquatic ecosystems. Also, they are helping as biomarkers of environmental pollution [9]. The organs like gills and kidneys of aquatic organisms may accumulate copper when they exposed to various toxic concentrations [10]. The copper can lead to redox reactions producing free radicals and, therefore, may cause many morphological and biochemical changes. It is familiar that changes in fish gill and kidneys are the most commonly predictable responses to environmental pollutants [11] (Figueiredo Fernandes et al., 2007). Also, kidneys are the main excretory organ of filtered toxicants and gills are the foremost target organs of aquatic pollutants, whey because gill is the main place for copper uptake because it has direct contact with the exterior environment. Therefore, this study was undertaken to examine the effect of different sublethal copper sulfate concentrations on glycogen and lipid levels in gills and kidneys of fish *Oreochromis mossambicus*.

2. MATERIALS AND METHODS

Oreochromis mossambicus weighing a mean of 12±1 gram was used for the present study. The 100 cultivable fish of both sexes were used for the current investigation. The fish were collected from Telangana state fisheries. They were transported to the laboratory in oxygenated containers and treated with 0.1% $KMnO_4$ to avoid dermal infection and acclimatised to laboratory conditions for 10 days. The fish were fed with a commercial feed once a day at a rate of 2% of body weight both before and during the experiment. The temperature was maintained at 27 to 28°C, and water was changed by fresh water every day. Before starting the experiment, the LC_{50} value was calculated by Finney Probit analysis method [12] and the LC_{50} was obtained as 48mg/L at 96 hours exposure period. Changes in biochemical parameters were estimated in gills and kidneys by exposing the fish to four sublethal concentrations of $CuSO_4$, i.e. 12 mg/L ($1/4^{th}$ of LC_{50}), 6 mg/L ($1/8^{th}$ of LC_{50}), 4 mg/L ($1/12^{th}$ of LC_{50}) and 3mg/L ($1/16^{th}$ of LC_{50}) for four different durations (10, 20, 30 and 40 days). Six fish of both sexes for each dosage and duration up to 40 days were used in the present experiment. Fish were exposed to the media that contained dissolved $CuSO_4$ in water and control fish exposed to the water without $CuSO_4$.

A glycogen level was estimated by Kemp and Heijninger method [13] and Lipid levels were estimated by Bligh and Dyer method [14]. The experimental data were analysed statistically by adopting valid statistical methods by Pillai and Sinha, [15], the standard deviation and probability test i.e. 't' test were calculated. The Student's 't'-test was carried out to know the levels of significance. The laboratory work was done in the fish physiology lab, Department of Zoology, University College of Science, Osmania University, Hyderabad.

3. RESULTS

The variation in levels of the Glycogen and Lipid in gills and kidney organs were studied and given in figures (Figs. 1-2), Fig. 1A shows the content of Glycogen in fish gills after exposure to sublethal concentrations of Copper Sulphate compared to control, Fig. 1B shows the content of Glycogen in fish kidneys after exposure to sublethal concentrations of Copper Sulphate compared to control, Fig. 2A

shows the levels of Lipid in fish gills after exposure to sublethal concentrations of Copper Sulphate compared to control and Fig. 2B shows the levels of Lipid in fish kidneys after exposure to sublethal concentrations of Copper Sulphate in terms of Mean with Standard error values over control.

Glycogen which is also called as animal starch is a polysaccharide that is the chief storage form of glucose in the animal as well as human tissues. Glycogen is found in the form of granules in the cytosol of many cells. The highest concentration of glycogen, up to 8% of the fresh weight in a well-fed state was found in hepatocytes. The little amount of glycogen found in the kidneys and this glycogen plays a vital role in the glucose cycle [16]. The current work revealed that glycogen content in different organs like Gills and Kidneys were identified under sublethal exposure of $CuSO_4$. At the end of the experiment, levels of Glycogen in two organs were significantly decreased in experimental fish compared to control. The order of decrease in two organs when exposed to sublethal concentrations was observed as Gills (24.25%, $p < 0.001$) > Kidney (19.39%, $p < 0.001$) of fish compared with control in gills more glycogen utilising due to toxicant stress. A decrease of Glycogen levels was more at higher duration (40 days) and a higher concentration of Copper Sulphate that is 12 mg/L.

The present investigation revealed that the levels of lipid in two organs, gills and kidneys were noticed to decrease under sublethal exposure of $CuSO_4$ and at the end of experimental periods. Lipid levels in the two organs were significantly decreased. The order of reduction in the two different organs when treated with sublethal concentrations of $CuSO_4$ was observed as Kidney (27.19%, $p < 0.001$) > Gills (21.34%, $p < 0.001$) of fish when compared to control fish organs. The Decrease in levels of lipid content was greater at higher concentrations of $CuSO_4$ that is 12mg/L and greater durations that is 40 days.

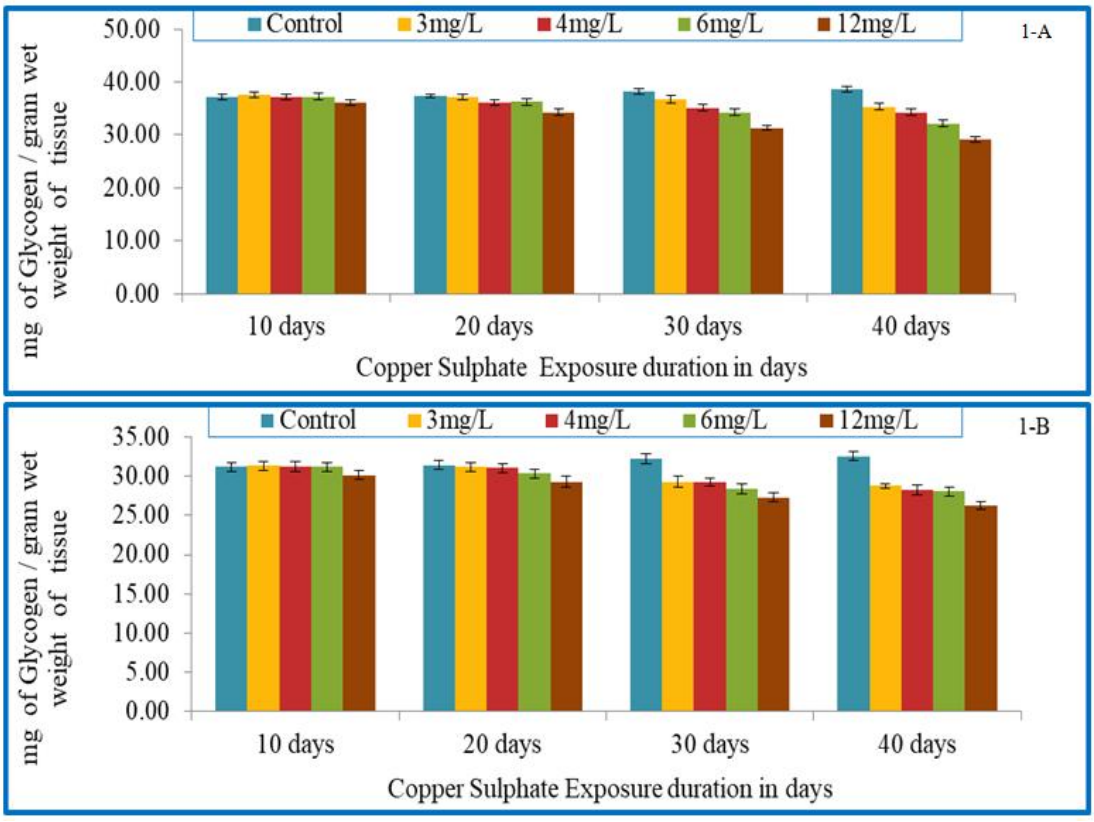

Fig. 1A. Content of Glycogen in fish gills after exposure to sublethal concentrations of Copper Sulphate compared to control (Mean ± SE) (n=6)
1B. Content of Glycogen in fish kidneys after exposure to sublethal concentrations of Copper Sulphate compared to control (Mean ± SE) (n=6)

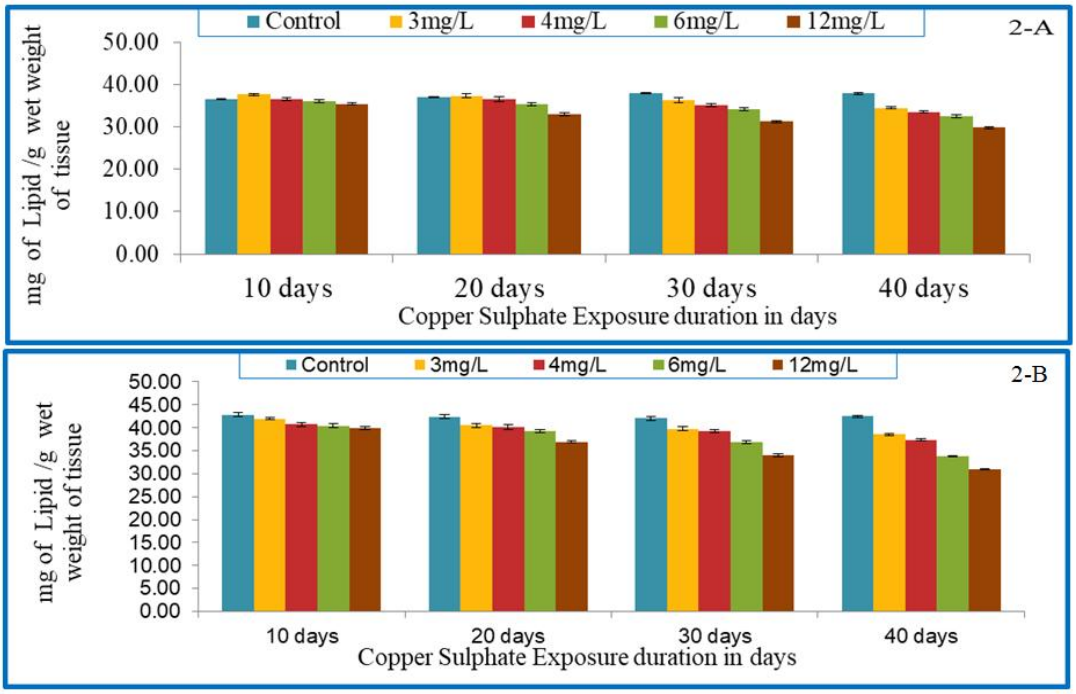

Fig. 2A. Levels of Lipid in fish gills after exposure to sublethal concentrations of Copper Sulphate compared to control (Mean ± SE) (n=6)
2B. Levels of Lipid in fish kidneys after exposure to sublethal concentrations of Copper Sulphate compared to control (Mean ± SE) (n=6)

4. DISCUSSION

At the end of the experimentation, Glycogen level in two organs were significantly reduced. A reduction of Glycogen content was more at higher concentrations of $CuSO_4$ and at higher duration. Glycogen plays an important role as a readily mobilized storage form of total free sugar in muscle [17]. [18], specified that "reduction in glycogen and its consequential depletion in tissues may be attributed to hypoxia since it increases carbohydrate consumption" under hypoxic conditions, the animals derive its energy from anaerobic breakdown of glucose, which is available to the cell of the animals by the increased glycogenolysis pathway [19,20,21]. Decrease in glycogen level probably due to its quicker break down for energy need of fish [22].

A lipid is a component which is biological in origin and insoluble in water but soluble in nonpolar solvents. Fats are the rich alternative energy reserve substances whose calorific value is double as that of an equivalent weight of proteins and carbohydrates. The present study indicates that the levels of lipid in two different organs like Gills and Kidneys were reduced under sublethal exposure of Copper Sulphate. At the end of the investigation, Lipid levels in two different organs were significantly declined. The order of reduction in two different organs when exposed to sublethal concentrations of Copper sulphate was observed as Kidneys > Gills of fish compared to control fish because after glycogen lipids are the second energy producers in animals. More amount of lipid was decreased at higher concentration of Copper sulphate that is 12mg/L and higher duration that is 40 days. Reduced level of lipid was observed with an increase in metal concentration and exposure duration in *Oreochromis mossambicus* fish [23]. [24], also described that the cuprous- ions may enhance lipid peroxidation by a metal to metal reaction reducing the ferric ions rather than by promoting propagation reaction in the Chinook salmon *Oncorhynchus tshawytscha*.

5. CONCLUSION

The present study revealed that Copper sulphate causes changes in Glycogen and lipid levels of *Oreochromis mossambicus* might be caused by intoxication of heavy metal. Thus it is concluded that the usage of Copper Sulphate must be reduced and to create awareness among the people about the toxicity of Copper Sulphate. Because, a majority of heavy metals bioaccumulate in the organs of fish and other

animals, and can be transferred via food chain to the human bodies, they make a threat to the health of human who consumes these fish.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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