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RESEARCH ARTICLE

TOXICITY EFFECT OF COPPER SULPHATE ON PROTEIN ALTERATIONS IN ESTUARINE FISH, *MUGIL CEPHALUS*

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ABSTRACT

Heavy metals are common pollutants of estuarine ecosystems where they induce adverse effects on the aquatic biota. Copper is one of the most toxic heavy metal to fish and consumption of fish after copper treatment in water may pose a serious risk to human health. Fishes are generally used as pollution indicators in water quality management. Chronic effects include reduced growth, shorter lifespan, reproductive problems, reduced fertility and behavioral changes. Estuarine fish, *Mugil cephalus* is an important fin fish species of Uppanar estury in Cuddalore region having good nutritional values. Fishes living in close association with the sediment may accumulate copper sulphate. In the present observation, the toxic effects of the copper sulphate LC_{50} 35 µg/L ($CuSO_4$) on (10% and 30%) two sublethal concentrations of total protein in gill, liver and muscles tissues of the estuarine fish, *Mugil cephalus* were estimated during the periods of 5, 10 and 15 days exposure. There is decreased in all tissues on comparison with control. The results indicated the toxic nature of the heavy metal copper sulphate.

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INTRODUCTION

Copper sulphate is frequently used as an algaecide in commercial and recreational fish ponds to control growth of phytoplankton and filamentous algae, and to control certain fish diseases (Tucker and Robinson, 1990). Conversely, above a particular concentration, copper is toxic to fishes including such cultured species as salmonids, cyprinids and catfishes (Wurts and Perschbacher, 1994). Consequently, treating plankton with copper compounds might lead to copper bioaccumulation reaching a toxic stage in fish. The toxic effect of copper is correlated to its aptitude for catalyzing oxidative reactions, leading to the production of reactive oxygen species (Lopes *et al.,* 2001). Fishes are superlative organisms to examine aquatic systems because they engage positions towards the zenith of food pyramids and could, consequently, imitate effect of heavy metals on other organisms including human beings as well as direct stresses on themselves (Vander *et al.,* 2003). Pollution of the natural environment by heavy metals is a worldwide problem because these metals are indestructible and when they exceed a certain concentration, most of them have toxic effects on living organisms (Alam *et al.,* 2002).

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Fish species are widely used to biologically monitor variation in environmental levels of anthropogenic pollutants (Vinodhini, and Narayanan, 2008; Palaniappan and Karthikeyan, 2009). Heavy metals enter the aquatic environment by atmospheric deposition, by weathering from the geological matrix, or from anthropogenic sources, such as industrial discharge, sewage, agricultural waste, and mining wastes (Ebrahimpour and Mushrifah, 2010). Copper pollution appears in the aquatic environment from natural and anthropogenic sources such as mine washing, agricultural leaching and direct application as algaecide and molluscicide. Moreover, liver is known to be the primary organ for copper storage in fish (Trivedi *et al.*, 2012). The gill is the first tissue contacting with the contaminants in the water. Due to its large surface area and the small diffusion distance between the water and blood, the gills are primarily affected by contaminants such as metals. In general, the gill cells respond rapidly to various chemicals to overcome physiological impairment or tissue damage, and chemicals may have a negative effect on the overall gill function, enhancing fish susceptibility to toxic compounds and potentially leading to fish mortality (Demir *et al.,* 2016). The gill is the main place for copper uptake, and because it has a constant and direct contact with the external environment (Atabati *et al*., 2015). The copper sulphate is worldwide used as an algaecide and a fungicide in aquaculture and agriculture (Lasiene *et al*., 2016).

In the aquaculture industry, copper sulphate is used as a therapeutic chemical for various ectoparasitic and bacterial infections. It is reducing the incidence of fish parasites such as protozoa, trematodes, and external fungi and bacteria. It also inhibits growth of bacteria (Nouh and Selim, 2013; Lasiene *et al*., 2016).

MATERIALS AND METHODS

The estuarine fish, *Mugil cephalus* were collected from Uppanar estury on the Cuddalore area and were brought to the laboratory in large plastic troughs and acclimatized for one week. Healthy, fishes having equal size (length 10 to 15 cm) and weight (50 to 100 g) were used for experimentation. Stock solution of copper copper sulphate ($CuSO_4$ + 5 H_2O) was prepared by dissolving appropriate amount of salt in distilled water. The physico-chemical characteristic of test water have analyzed regularly during the test periods following the standard method describe by APHA (1998). Batches of 10 healthy fishes were exposed to different concentrations of insecticide copper sulphate to calculate the medium lethal concentration LC_{50} value (35 µg/L) using probit analysis Finney method (1971). The fishes (Four groups) were exposed to two sublethal concentrations ($1/10^{th}$ and $1/30^{th}$ µg/L) of copper sulphate for 5, 10 and 15 days respectively. Another group was maintained as control. At the end of each exposure period, fishes were sacrificed and tissues such as gill, liver and muscle were dissected and removed. The tissues (10 mg) were homogenized in 80% methanol, centrifuged at 3500 rpm for 15 minutes and the clear supernatant was used for the analysis of total proteins. Total protein concentration was estimated by the method of Lowry (1951).

RESULTS

The changes in the total protein of gill, liver and muscles of estuarine fish, *Mugil cephalus* exposed to sublethal concentrations of copper sulphate were studied along with control fish. The data was supported by various statistical analyses and the standard deviation of the mean was calculated. Estuarine fish, *Mugil cephalus* treated with 10% sublethal concentration of copper sulphate showed a decreasing trend in the gill protein (Table 1 and Fig. 1). The control gill protein values were recorded 6.23, 6.36 and 6.39 mg/g respectively to 5, 10 and 15 days of exposure periods. The 10% sublethal concentration of gill protein values were noted from 5.85, 4.70, 4.01 and the 30% sublethal concentration values were recorded from 5.12, 4.24 and 3.67 mg/g respectively. Fish *Mugil cephalus* treated with sublethal concentration of copper sulphate on 10% & 30% showed a decreasing trend in the liver protein when compared to control (Table 1 and Fig. 2). Liver protein values of control fish were estimated from 8.68, 8.61 and 8.68 mg/g. The 10% sublethal concentrations of liver protein content were noted from 6.67, 5.66 and 4.83 followed by the 30% sublethal concentration values 6.21, 5.33 and 4.55 mg/g respectively, during the 5, 10 and 15 days of exposure periods.

Estuarine fish *Mugil cephalus* treated with sublethal concentrations of heavy metal copper sulphate on 10% & 30% showed a decreasing trend in the muscle protein when compared to control (Table 1 and Fig. 3). Muscle protein values of control fish were estimated from 9.35, 9.33 and 9.36 mg/g.

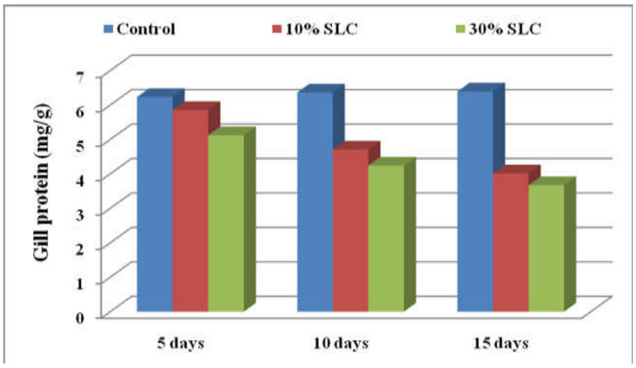

Fig. 1. Total protein content (mg/g) in gill tissues of estuarine fish, *Mugil cephalus* exposed to 10% and 30% sublethal concentrations of copper sulphate

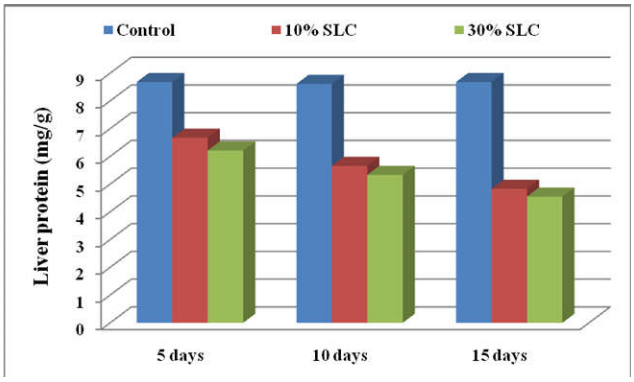

Fig. 2. Total protein content (mg/g) in liver tissues of estuarine fish, *Mugil cephalus* exposed to 10% and 30% sublethal concentrations of copper sulphate

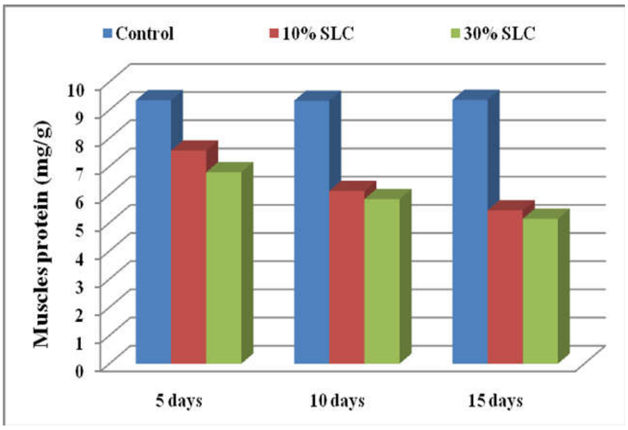

Fig. 3. Total protein content (mg/g) in muscles tissues of estuarine fish, *Mugil cephalus* exposed to 10% and 30% sublethal concentrations of copper sulphate

Table 1.Total protein content (mg/g) in tissues of estuarine fish, *Mugil cephalus* exposed to 10% and 30% sublethal concentrations of copper sulphate (Means ± SD N=3)

Exposure	Treatment	Gill	Liver	Muscles
5 days	Control	6.23 ± 0.11	8.68 ± 0.09	9.35 ± 0.15
	10% SLC	5.85 ± 0.08	6.67 ± 0.19	7.56 ± 0.19
	30% SLC	5.12 ± 0.07	6.21 ± 0.16	6.79 ± 0.18
10 days	Control	6.36 ± 0.29	8.61 ± 0.26	9.33 ± 0.22
	10% SLC	4.70 ± 0.11	5.66 ± 0.31	6.13 ± 0.13
	30% SLC	4.24 ± 0.09	5.33 ± 0.12	5.84 ± 0.11
15 days	Control	6.39 ± 0.11	8.68 ± 0.32	9.36 ± 0.15
	10% SLC	4.01 ± 0.15	4.83 ± 0.18	5.44 ± 0.18
	30% SLC	3.67 ± 0.19	4.55 ± 0.25	5.14 ± 0.08

Values are mean three observations ± SD

In the 10% sublethal concentration of muscle protein values were recorded from 7.56, 6.13 and 5.44 followed by the 30% sublethal concentration of muscle protein values from 6.79, 5.84 and 5.14 mg/g respectively during the period of 5, 10 and 15 days exposure. Decreased in the gill, liver and muscles tissues of total protein levels as observed in different sublethal concentrations when compared to control. The maximum decrease of protein content were observed in the tissue of gill, liver and muscles of fish *Mugil cephalus* exposed to 30% sublethal concentration of copper sulphate reared for 15 days.

DISCUSSION

The changes in the biochemical compositions of an organ due to heavy metal stress indicate the change in activity of an organism. It reflects light on the utilization of their biochemical energy to counteract the toxic stress. Heavy metal salts affect the metabolism of the estuarine fish, *Mugil cephalus*. Alterations in metabolic processes, following exposure to heavy metal stress have been always used as an indicator of stress. But there is a vast difference in the pattern & metal induced physiological alterations from metal to metal and animal to animal. Protein content in the tissue of animal is an important essential organic constituent which plays a vital role in the cellular metabolism. All enzymes are proteins in nature and they control sub cellular functions and accelerate the rate of metabolic action in the body of organism.

In present study, fish *Mugil cephalus* of the protein contents in the selected tissues was decreased in chronic concentration of copper sulphate as compared to control. The result of the present study shows reduced level of protein in liver, gills and kidney similar to the observation recorded by Mastan (2008), Kumar and Gopal (2001) on depletion level of protein in different organs of fish *C. punctatus* under the stress of copper and distillery effluents. Dinodia *et al.* (2002) also carried out effect of cadmium toxicity on fresh water species *Labeo rohita*, *Cirrhinus mrigala* and *Cyprinus carpio* as evidenced by reduction in the body tissue and residual protein in all the fish species after 45 days of exposure, which may be due to dysfunction of several physiological and biochemical processes in the body. According to Abel, (1974) the decrease of protein may be due to alterations of membrane permeability. The depletion in the protein content was reported from the muscles of fish, *Clarias batrachus* after treatment with pesticide by the Yagana Bano *et al*. (1981). Nagabhushanm and Kulkarni, (1979) studied variation in protein metablism in *Barytelephusa cunicularis*. Mahajan and Zambare, (2001) reported decrease in protein contents in the fresh water bivalve *Corbicula striatella* after heavy metal stress most of the time. Kumar *et al.* (2012) reported that sodium arsenide decreased in the concentration of protein in catfish *Clarius batractus.* In the present study in total protein content in the gill, liver and muscle tissues of estuarine fish, *Mugil cephalus* were declined when compared to control.

Conclusion

The present study indicates that presence of low concentration of heavy metal copper sulphate in the water is toxic to fishes and alters the protein of the fish tissues. The results indicate that the usage of the copper sulphate in the agriculture fields may be a threat to aquatic fauna and flora as well as humans. Therefore, the information obtained may be useful for management and monitoring of agricultural insecticide contamination in aquatic ecosystem.

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