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Effect of Sublethal copper exposure on glycogen, glucose and total lipid levels in (muscle and liver) fish, *Oreochromis mossambicus* (peters)

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Abstract
From an ecotoxicological point of view, any substance which substantially modifies population and community characteristics must be considered dangerous. A toxic metal influences the biota if its concentration in the environment is above a certain level. Certainly some metals, dangerous at high concentrations, are essential to the biota at low concentrations. In the present study, *Oreochromis mossambicus* were exposed to sub lethal concentrations (1/16, 1/12, 1/8 and 1/4th of 96 h LC_{50} value) i.e. 3mg/L, 4mg/L, 6mg/L and 12mg/L of Copper Sulphate for four different exposure periods of 10, 20, 30 and 40 days. The Glycogen, Glucose, Lipid levels in two different tissues such as Muscle and Liver and in blood were studied. Decreased tendency was observed in Glycogen, Lipid levels and an increased tendency was observed in Blood glucose levels in two different tissues and blood of fish exposed to Copper Sulphate over control. Glycogen and Lipid levels gradually decreased, Blood glucose levels gradually increased with increased exposure period and the increase was observed to be directly proportional to increased sub lethal concentrations.

Keywords: *Oreochromis mossambicus*, copper sulphate, glycogen, glucose and lipids

Introduction

From an ecotoxicological point of view, any substance which substantially modifies population and community characteristics must be considered dangerous. Metals have a key role in chemical technology. However, the intrusion of indeterminable amounts into natural waters has caused ecological and biological changes that have yet to be comprehensively analyzed and evaluated. A toxic metal influences the biota if its concentration in the environment is above a certain level. Indeed some metals, dangerous at high concentrations, are essential to the biota at low concentrations (Ravera *et al.,* 1984) [1]. Copper is a transition metal with a high abundance in aquatic and terrestrial environments. Copper, as an essential nutrient, plays an important role in various functions in cellular biochemistry, especially as a cofactor for many enzymes and as a constituent of the non-enzymatic antioxidants ceruloplasm and the metallothioneins (Amiard *et al.,* 2006) [2]. It is known that physiological and biochemical parameters in fish blood and tissues could change when exposed to heavy metals and that these parameters are extremely sensitive to these elements (Sastry *et al.*, 1984) [3]. It has been found that Cd could change glycogen reserves and serum glucose levels in fish by affecting the activities of liver enzymes that have roles in the carbohydrate metabolism such as gluconeogenesis and glycolysis (Levesque *et al.*, 2002) [4]. Thus, it was argued that several biochemical parameters in fish blood and tissues could be used as an indicator of heavy metal toxicity (Toguyeni 1997) [5]. Because heavy metal contamination in an aquatic environment exerts an extra stress on fish, there must be several other changes in the fish metabolism when exposed to heavy metals (Heath *et al.,*1995) [6]. On the other hand, because glycogen reserves in the liver and muscle tissues of fish under stress are used as an emergency energy supply, changes in the glycogen levels in these tissues could indicate the health status of fish populations. It has been demonstrated that Cd might change glycogen reserves in fish via the endocrine system (Richard *et al.*, 1998) [7].

Materials and Methods

The fish, *Oreochromis mossambicus* weighing about 12±1 gram used in the present study, were collected from nearby pond. They were transported to the laboratory in oxygenated containers and treated with 0.1% $KMnO_4$ to avoid dermal infection and acclimatized to laboratory conditions for two weeks. The fishes were fed with commercial feed once a day at a rate of 2% of body weight both before and during the experiment. Temperature was maintained at 26 to 28°C and water was replaced by fresh water every day. Prior to starting the experiment, LC_{50} value was calculated by Finney Probit analysis method (1971) and the LC_{50} was obtained as 48 mg/L at 96 hours exposer period. Biochemical parameters were estimated in two tissues like muscle and liver by exposing the fishes to four sublethal concentrations of $CuSO_4$ i.e. 12 mg/L (1/4th of LC_{50}), 6 mg/L (1/8th of LC_{50}), 4 mg/L (1/12th of LC_{50}), and 3mg/L (1/16th of LC_{50}) for four different durations (10, 20, 30 and 40 days). Glucose was Estimated by Zarrow *et.al.* Method (1964) [8], Glycogen was Estimated by Kemp's method (1953) [9] and Lipids were Estimated by Bligh and Dyer method (1959) [10].

Results

After exposing the fish, *Oreochromis mossambicus* to different sublethal concentrations of Copper sulphate

($CuSO_4$), 12 mg/L (1/4th of LC_{50}), 6 mg/L (1/8th of LC_{50}), 4 mg/L (1/12th of LC_{50}), and 3mg/L (1/16th of LC_{50}) for four different durations (10, 20, 30 and 40 days) of exposure, The Glycogen, Glucose, Lipid levels in two different tissues such as Muscle and Liver and in blood of *Oreochromis mossambicus* fish were studied and the results were statically analyzed. The variations in levels of The Glycogen, Glucose, Lipid levels in different tissues and in blood were studied given in figures (Figure 1 to 5) in terms of mean with Standard error values over control. Glycogen is a polysaccharide that is the principal storage form of glucose in animal and human cells. Glycogen is found in the form of granules in the cytosol in many cell types. Hepatocytes (liver cells) have the highest concentration of it - up to 8% of the fresh weight in well fed state, or 100-120 g in an adult. In the muscles, glycogen is found in a much lower concentration (1% of the muscle mass), but the total amount exceeds that in liver. Small amounts of glycogen are found in the kidneys, and even smaller amounts in certain glial cells in the brain and white blood cells. Glycogen plays an important role in the glucose cycle. Present study revealed that, glycogen levels in different tissues like muscle, Liver, Gills and Kidneys were observed under sub lethal exposure of $CuSO_4$. At the end of experiment, levels of Glycogen in different tissues were significantly decreased. The order of decrease in different tissues when exposed to sub lethal concentrations was observed as Liver (31.92%) ($p < 0.001$) > Muscle (28.51%) ($p < 0.001$) of fish compared with control. Decrease of Glycogen levels was more at higher concentrations of $CuSO_4$ (12mg/L, 6mg/L, 4mg/L and 3mg/L) and Higher durations (40, 30, 20, and 10 days). Glucose is a monosaccharide of the aldohexose group. It is a necessary source of energy and carbon for most vertebrates including fish. Present study revealed that, glucose levels in blood were observed under sub lethal exposure of $CuSO_4$. Blood glucose levels in blood were increased up to 24.57% ($p < 0.001$). In biology, a lipid is a substance of biological origin that is soluble in nonpolar solvents. Lipids constitute the rich alternative energy reserve whose calorific value is twice as that of an equivalent weight of carbohydrates and proteins. Present study revealed that, lipid levels in different tissues like muscle and Liver were observed under sub lethal exposure of $CuSO_4$. At the end of experiment, levels of Lipids in different tissues were significantly decreased. The order of decrease in different tissues when exposed to sub lethal concentrations was observed as Liver (33.81%) ($p < 0.001$) > Muscle (31.71%) ($p < 0.001$) of fish compared with control. Decrease of lipids levels was more at higher concentrations of $CuSO_4$ (12mg/L, 6mg/L, 4mg/L & 3mg/L) and Higher durations (40, 30, 20 & 10 days).

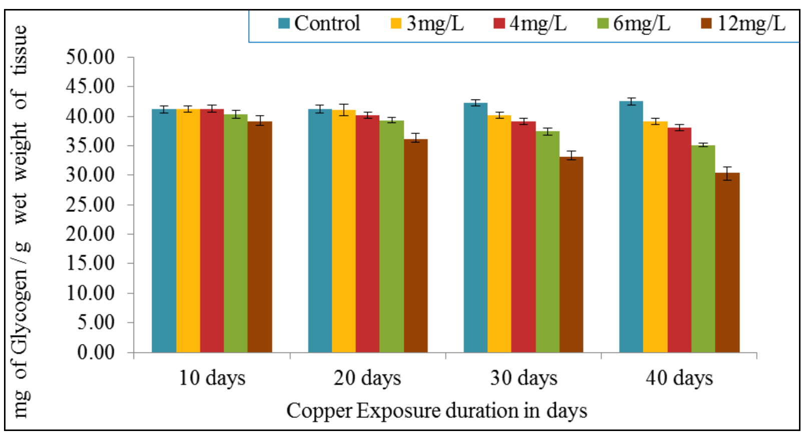

Fig 1: Glycogen content in fish muscle after exposure to sublethal concentrations of Copper compared to control (Mean ± SE) (n=10)

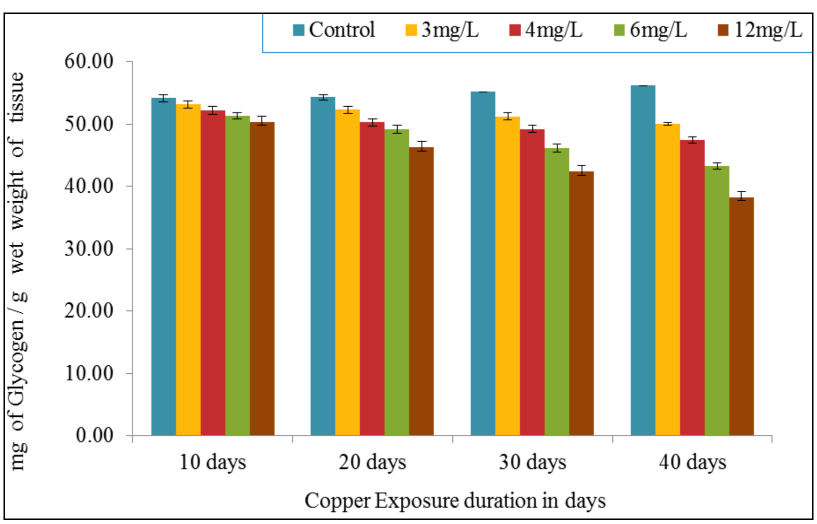

Fig 2: Glycogen content in fish Liver after exposure to sublethal concentrations of Coppercompared to control (Mean ± SE) (n=10)

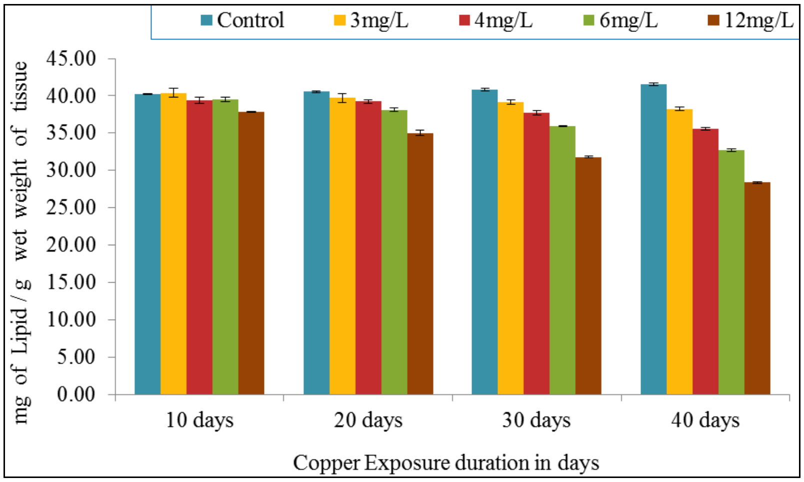

Fig 3: Lipid content in fish muscle after exposure to Sublethal concentrations of Copper compared to control (Mean ± SE) (n=10)

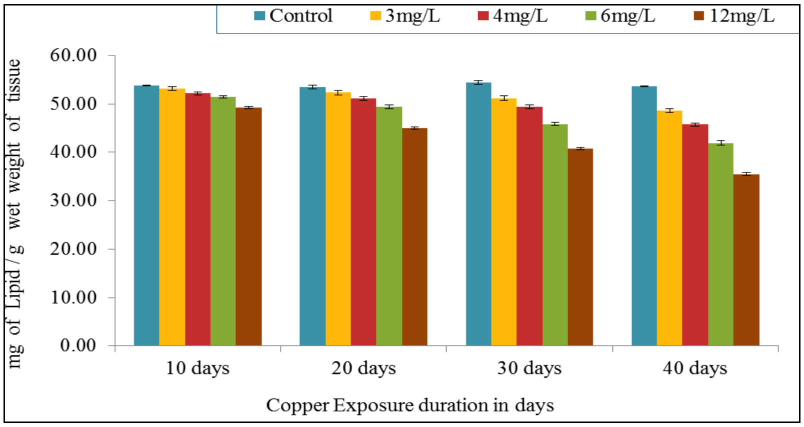

Fig 4: Lipid content in fish Liver after exposure to Sublethal concentrations of Copper compared to control (Mean ± SE) (n=10)

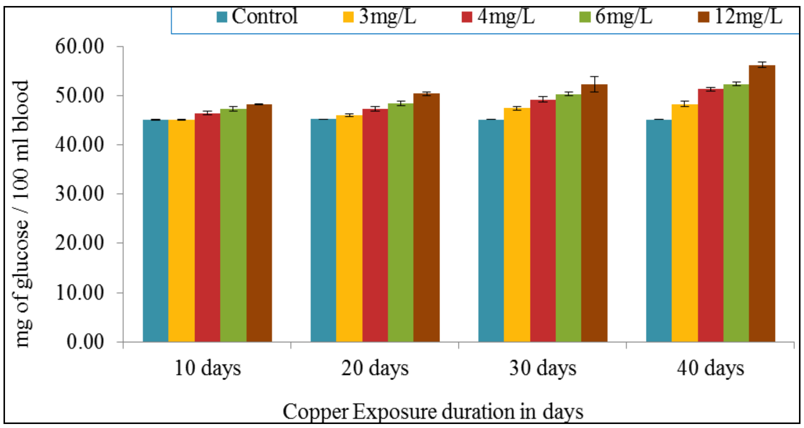

Fig 5: Glucose content in fish blood after exposure to Sublethal concentrations of Copper compared to control (Mean ± SE) (n=10)

Discussion

Present study revealed that, glycogen levels in different tissues like muscle and Liver were observed under sub lethal exposure of $CuSO_4$. At the end of experiment, levels of Glycogen in different tissues were significantly decreased. The order of decrease in different tissues when exposed to sub lethal concentrations was observed as Liver > Muscle of fish compared with control. Decrease of Glycogen levels was more at higher concentrations of $CuSO_4$ and Higher durations. Lomte and Sabhia Alam, (1984) [11] studied effect of Malathion on the biochemical components of prosobranch, Belamia bengalensis and reported that the decrease in glycogen under pesticidal stress. Glycogen plays an important role as a readily mobilized storage form of total free sugar in muscle (Stryer, 1988) [12]. The synchronized fall of carbohydrate level in the fish may be due to the expenditure of energy for the constant movements aided by muscular action (Maruthanayagam and Sharmila, 2004) [13] rapid utilization to meet the enhanced energy demand in toxicant treated fishes through glycolysis or HMP pathway (Caapon & Nicholas,

(1975) [14]. Depleted glycogen levels following chromium stress reported in *Cyprinus carpio. Var. Communis* by Ambrose *et al.* (1994) [15] under hypoxic conditions also supports this view.
Blood glucose levels in blood was increased up to 24.57% ($p < 0.001$) Vinodhini, R. and Narayanan, M., (2009) [16] reported that the blood of common carp showed significant increase in glucose during 32 days of heavy metal intoxication. This might be due to the vulnerable stress induced by the heavy metals resulted in hyperglycemia. Zikic *et al*., (1997) [17] observed increase tendency of blood glucose levels in the plasma of Carps (*Cyprinus Carpio. L.*) exposed to cadmium. Levesque *et al*., (2002) [18] also observed increase in blood glucose in yellow perch (*Perca flavescens*) chronically exposed to metals in the field. Almeida *et al*., (2001)[19] stated that Heavy metals increase the glucose content in blood, because of intensive glycogenolysis and the synthesis of glucose from extra hepatic tissue proteins and amino acids in their experiment Environmental cadmium exposure and metabolic responses of the Nile tilapia *Oreochromis niloticus.*
Lipids constitute the rich alternative energy reserve whose calorific value is twice as that of an equivalent weight of carbohydrates and proteins. Present study revealed that, lipid levels in different tissues like muscle and Liver were observed under sub lethal exposure of $CuSO_4$. At the end of experiment, levels of Lipids in different tissues were significantly decreased. The order of decrease in two tissues when exposed to sublethal concentrations was observed as Liver > Muscle of fish compared with control. Decrease of lipids levels was more at higher concentrations of $CuSO_4$ (12mg/L, 6mg/L, 4mg/L & 3mg/L) and Higher durations (40, 30, 20 & 10 days). In the sea bass, *L. calcarifer* the liver is the most sensitive indicator of physiological stress than the muscle tissue (Trendal and Prescott, 1989) [20]. The finding is in accord with the results obtained in *L. calcarifer*, although muscle and liver are the major energy stores. Lipids were found to be the primary source of energy under stress condition in *Penaeus duorarum* (Schafer, 1968) [21]. An increase in metal concentration and exposure duration resulted in the reduced level of lipid in *Oreochromis mossambicus* (Overstreet, 1988) [22].

Conclusion
The present investigation revealed that $CuSO_4$ caused changes in biochemical parameters of *Oerochromis mossambicus* might be caused by intoxication of heavy metal. It is concluded that the utilization of Copper sulphate have to be reduce and have a duty to create consciousness among the people about the toxicity of Copper Sulphate on animals and on human. Because majority of heavy metals are bioaccumulate in the tissues of fish and other animals, and transfer via food chain to the human bodies, they make threat to the health who consumes these fishes.

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