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Sublethal Copper Exposure Effect On Protein Metabolism In Fish, Oreochromis Mossambicus (Peters)

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ABSTRACT

The natural aquatic systems have been extensively contaminated with heavy metals released from domestic, industrial and other man-made activities. Among all types of pollution, aquatic pollution is of greater concern as each and every kind of the life depends on water. Pollution of the aquatic environment generally causes changes in the physiological and structural aspects of the inhabitant organisms, particularly the fishes. Heavy metals cause several ill effects to aquatic organisms and environment. In the present study, Oreochromis mossambicus were exposed to sublethal concentrations (1/16, 1/12, 1/8 and 1/4th of 96 hours LC50 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 proteins, Free Aminoacids, Aspartate amino transferase (AST) and Alanine amine transferase (ALT) levels in two different tissues such as Gills and Kidney were studied. Decreased tendency was observed in protein levels and an increased tendency was observed in Free Aminoacids, AST and ALT levels in all the vital tissues of fish exposed to Copper Sulphate over control. Proteins gradually decreased, Amino acids, AST and ALT levels gradually increased with increased exposure period and the increase was observed to be directly proportional to increased sublethal concentrations.

Keywords : Oreochromis mossambicus, Copper Sulphate, Proteins, Amino Acids, AST ALT.

I. INTRODUCTION

E Water is one of the most valuable natural resources and which serve as habitat for various aquatic fauna and flora. It is generally believed that every water body is capable of accepting certain minimum amount of pollution without any adverse effect on itself due to natural biological cycles and self-purification capacity. The quality of water is of vital concern for the mankind since it is directly link with human welfare (Kumar, 2004). The natural aquatic systems have been extensively contaminated with heavy metals released from domestic, industrial and other man-made activities. Among all types of pollution, aquatic pollution has a superior concern as each and every kind of the life depends on water. Pollution of the aquatic environment generally causes changes in the physiological and structural aspects of the aquatic organisms, such as fishes and other vertebrates (Anitha Kumari and Sreeramkumar,1997; Velez and Montoro, 1998; Anand Pratap Singh et al, 2010).

Metals such as copper (Cu), zinc (Zn), and iron (Fe) are essential elements playing vital roles in biological systems. However, the essential elements can also be detrimental to living organism at high concentrations (Sfakianakis et al, 2015). Copper occurs naturally in soil and water and it plays an essential role in a variety of metabolic activities. It is a component of many enzymatic and structural proteins, including cytochrome oxidase, and ceruloplasmin. According to (WHO, 1998) sources of water pollution occur due to mining, industrial discharges, and copper-based pesticides. Toxicity of Copper to fish and its bioavailability in water vary with physico-chemical parameters of water (Di Giulio and Meyer, 2008).

Fishes are suitable bio-indicators of aquatic environment. Heavy metal contamination of aquatic environment adversely effect the fish physiological activities, consumption of these fishes as food is significant risk to human health. Metals are well-known inducers of oxidative stress, and assessment of oxidative damage and antioxidant defences in fish can reflect metal contamination of the aquatic environment (Livingstone et al, 2003). The most important factors like imperishable nature and bioaccumulation of heavy metals are polluting the sediment and fauna of aquatic habitat. (Forstner et al, 1983). Inorganic and organic component of aquactic environment contaminated due to different substances like heavy metals flammable and rotting substances, dangerous wastes, explosives and petroleum products, Phenol and textile dyes (Ghosh, M. et al, 2005; Gad, N. S et al, 2008; Jadhav et al, 2010).

The total protein is one of the essential ingredients in cells and tissues playing an essential role in normal physiology of organisms. An extreme proteolysis to supplement aminoacids to be converted to ketoacids as precursors for the maintenance of carbohydrate metabolism to convene the energy requirement elicited by heavy metal stress has been reported in *Channa punctatus* and *Heteropneustes fossilis* exposed to copper (Masthan, 2008; Dhanraj Balbhim Bhure et al, 2011).

Glutamate oxaloacetate transaminase (GOT) and Glutamate pyruvate transaminase (GPT) are cellular metabolic enzymes usually found in small concentrations in plasma derived probably from the regular physiological shedding of cells (Schmidt, et al, 1974). Liberation of these enzymes in to the blood stream is a injurious effect of the heavy metals inducing degenerative necrotic changes and hypofunction of liver (Tietz, 1990; Campbell, et al, 1984). These are hence reliable indicators of heavy metal induced hepatotoxicity in organisms and magnitude of aquatic pollution and increase in GOT and GPT activity has been reported in *Cyprinus carpio* on exposure to cadmium and curacron substances (De Smet et al, 1990; Baby Joseph, et al, 2011).

Biochemical changes by fenvalerate in freshwater fish of *Channa puntctatus* have been studied by (Tilak et al, 2003). Proteins are important organic substances required by organisms in tissue building and play an important role in energy metabolism (Yeragi et al, 2003). The concentration of free amino acid and the activities of proteases were increased in gill, liver, and kidney of carp *Cyprinus carpio* exposed to cadmium due to proteolysis (De Smet et al, 2000 and Bais et al, 2012). The purpose of present investigation is to determine the effect of copper sulphate on protein content, Free amino acids, AST and ALT levels of certain important tissues, i.e. Gills and Kidneys of fish (*Oreochromis mossambicus).*

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MATERIALS AND METHODS:

The fish, *Oreochromis mossambicus* weighing 12- 14 grams 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 10-15 days. 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 27 ± 1°C and water in the aquariums was replaced by fresh water every day. Before 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 exposure. Biochemical parameters were estimated in two tissues like Gills and Kidneys by exposing 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). Total Proteins were estimated by Bradford method (1976). Amino acids were estimated by Moore and Stein method (1954). AST (Aspartate Aminotransferase) and ALT (Alanine Aminotransferase) levels were estimated by Bergmeyer method (1965).

RESULT AND DISCUSSION:

After exposing the fish, *Oreochromis mossambicus* to different sublethal concentration 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), the protein content, Free amino acids, AST and ALT levels in Gills and kidneys of *Oreochromis mossambicus* fish were studied and the results were statically analyzed. The variations in levels of Protein content, Free aminoacids, AST and ALT in different tissues given in figures (Figure 1 to 8) in terms of Mean with Standard Error values over control.

The observation is made in both control and $CuSO_4$ exposed fish. At the end of the experiment the protein levels were decreased in two tissues. The order of decrease in two different tissues when exposed to sublethal concentrations was observed as Gills > Kidney of fish. The decrease in protein levels may be due to metabolic stress and proteolysis under toxic exposure of fish. Decrease of protein levels was more at higher concentrations of $CuSO_4$ (12mg/L) and higher durations (40 days). Similar results were observed by Mastan, 2008, studied the impact of (5mg/l) Copper on the protein level of the certain tissues of *Heteropneustes fossilis* after 30 days, 60 days, 90 days of treatment. There was a steady and considerable decline in the protein level.

The amino acids may be utilized for ATP production in two different ways. They could be converted to keto acid via transaminase and then fed to the TCA cycle. Alternatively they could be channeled into gluconeogenic pathway. Oxidation in Kreb's cycle meets the higher energy demands under copper sulphate toxic impact. Amino acids are essential intermediate substances in the process of protein synthesis and its degradation products appear in the from of various nitrogenous compounds (Dange and Masurekar 1985). The present study revealed that, free amino acids levels in two different tissues like Gills and Kidneys were observed under sublethal exposure of $CuSO_4$. The calculated values of Free Amino acid along with mean and Standard Error over the control were given in Figure 3 and 4. There was a significant increase in Free Amino acid levels in two tissues at all durations and in all sublethal doses were observed. The order of increase in two different tissues when exposed to sublethal concentrations was observed as Kidney > Gills of fish. Similar result was observed by Tomar et al, (2015), the total free amino acid content showed a significant increase in the tissues of *Rita rita* fish exposed to different concentrations of copper sulphate. From the result, it is inferred that the increased free amino acid can be utilized for energy production (ATP) by feeding them into the TCA cycle through aminotransferase reaction.

The Aspartate aminotransferase (AST) levels in two different tissues like Gills and Kidneys were observed under sublethal exposure of $CuSO_4$. The calculated values of AST along with mean and Standard Error over the control were given in Figure 5 and 6. There is a significant increase in AST levels in all tissues at all durations and in all sublethal concentrations were observed. The order of increase in two different tissues when exposed to sublethal concentrations was observed as Kidney > Gills of fish. Similarly, Mckim et al, (1970) found that sublethal concentration of copper caused significant increase of ALT of *Salvelunus farttinalis* after 6 and 21 days of exposure.

The Alanine aminotransferase (ALT) levels in two different tissues like Gills and Kidneys were observed under sublethal exposure of $CuSO_4$. The calculated values of ALT along with Mean and Standard Error over the control were given in Figure 7 and 8. There was a significant increase in ALT levels in all tissues at all durations and in all sublethal concentrations. The order of increase in different tissues when exposed to sub lethal concentrations was observed as Kidney > Gills of fish.

The present study indicates that $CuSO_4$ caused variations in biochemical parameters of *Oreochromis mossambicus* might be caused by intoxication of heavy metal. It is concluded that the using of Copper sulphate should be minimize and should create an awareness among the people about the toxicity of Copper Sulphate on animals over and above on human. Since majority of heavy metals are released cumulatively and frequently, through the industrial and human activities. Heavy metal residues are known to bioaccumulate in the tissues of fish and other animals, and transfer via food chain to the human body, they grave risk to the health of the people who consume these fish seems to be extensively. There is necessitate to protect the people from undue exposure to the heavy metals through the food chain cannot be over emphasized.

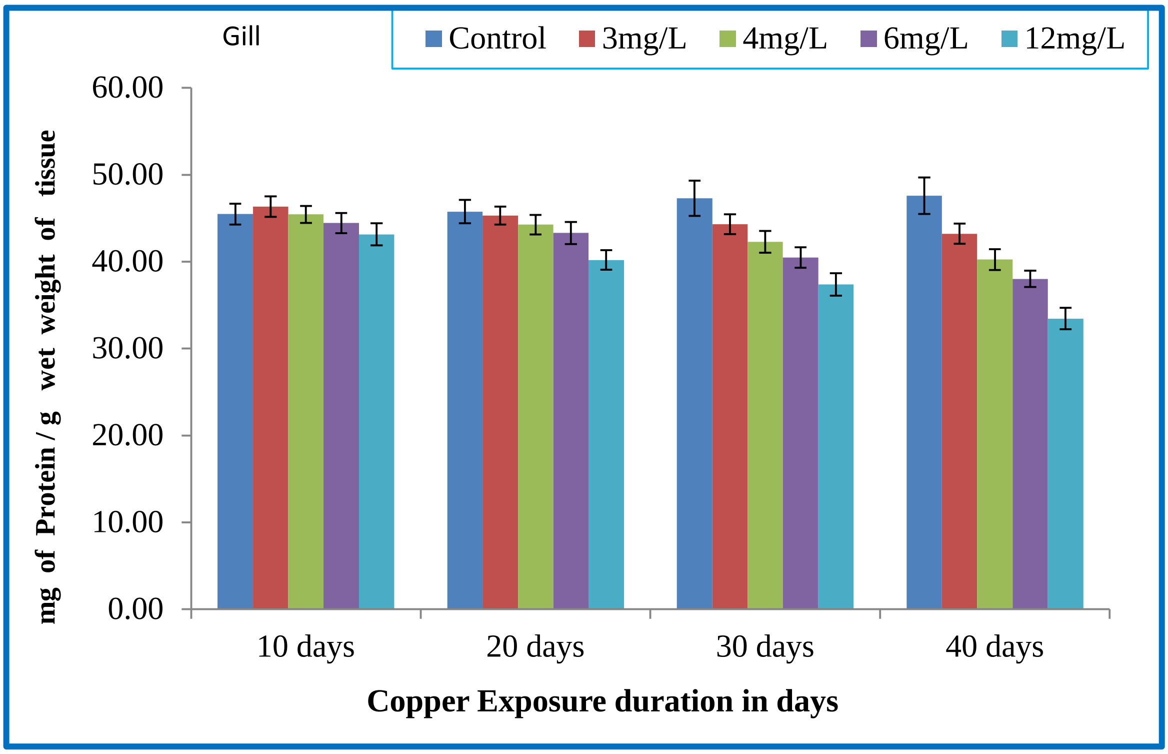

Figure 1. Protein content in Gill after exposure to sublethal concentrations of Copper compared to control (Mean ± SE)

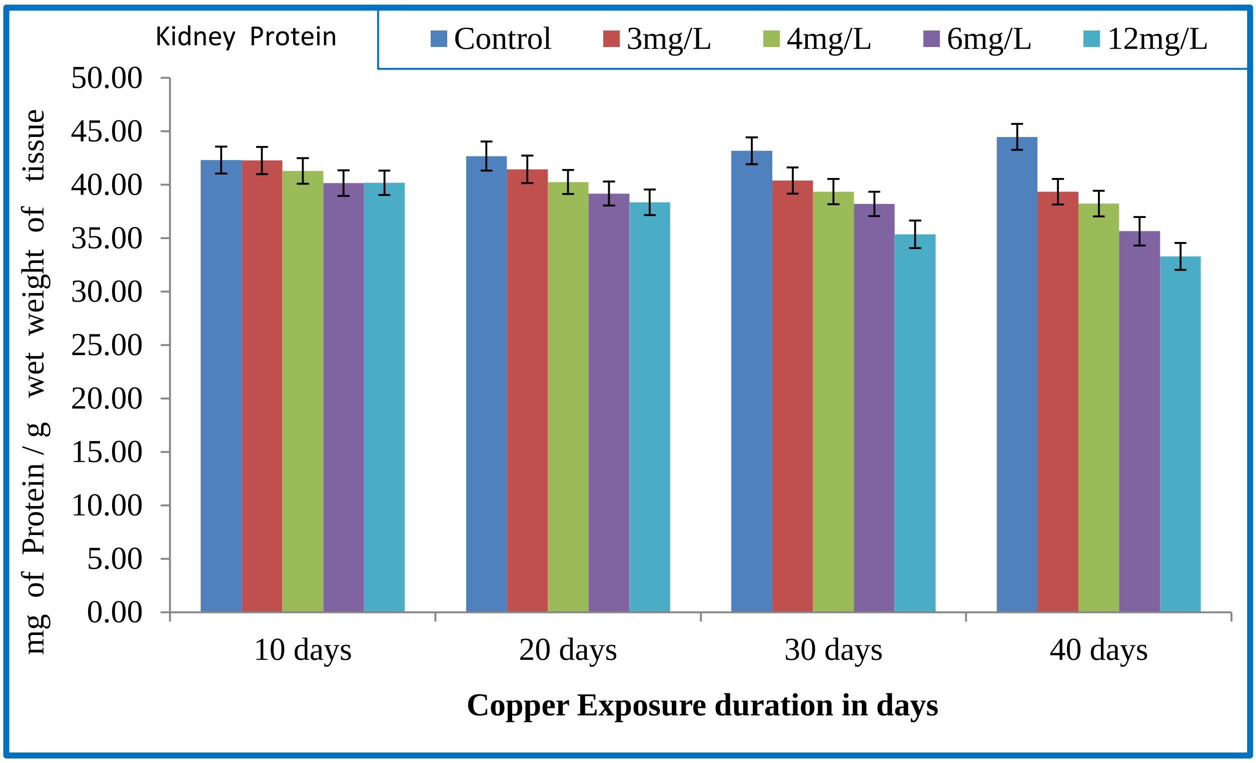

Figure 2. Protein content in Kidney after exposure to sublethal concentrations of Copper compared to control (Mean ± SE)

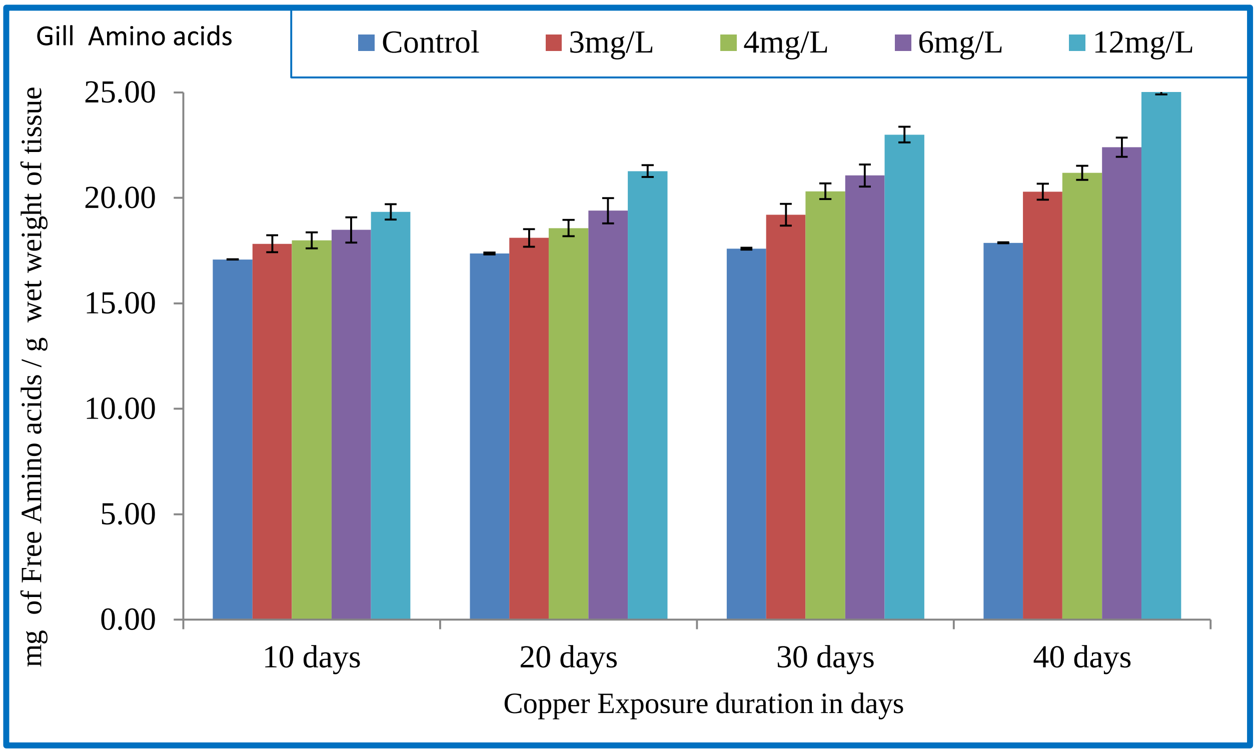

Figure 3. Free Amino acid content in Gills after exposure to sublethal concentrations of Copper compared to control (Mean ± SE)

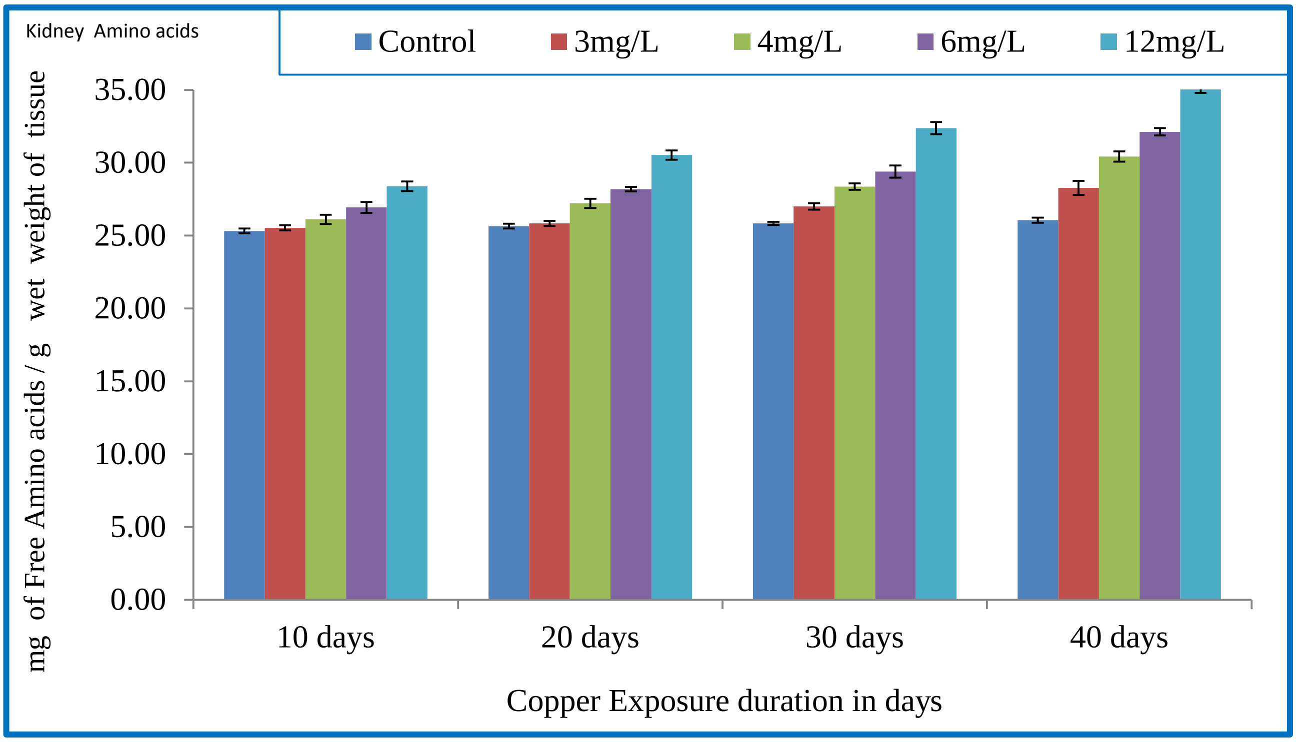

Figure 4. Free Amino acid content in Kidney after exposure to sublethal concentrations of Copper compared to control (Mean ± SE)

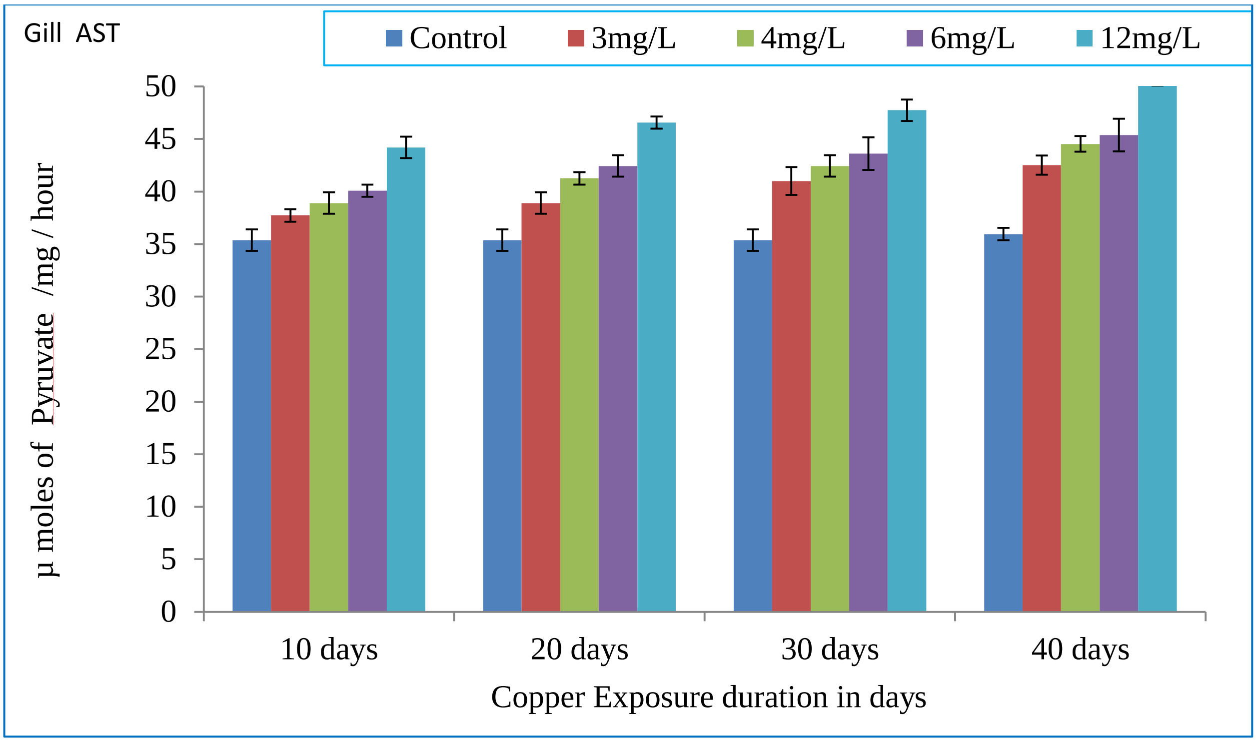

Figure 5. AST level in Gills after exposure to sublethal concentrations of Copper compared to control (Mean ± SE)

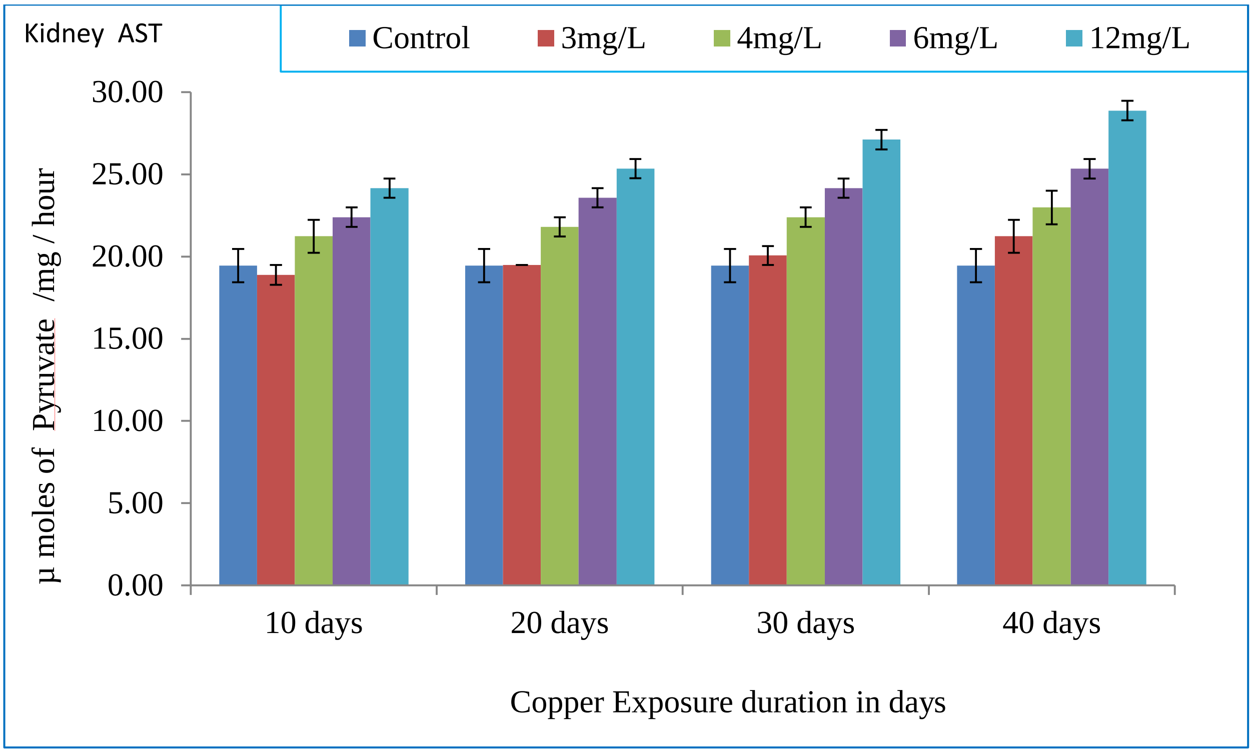

Figure 6. AST levels in Kidney after exposure to sublethal concentrations of Copper compared to control (Mean ± SE)

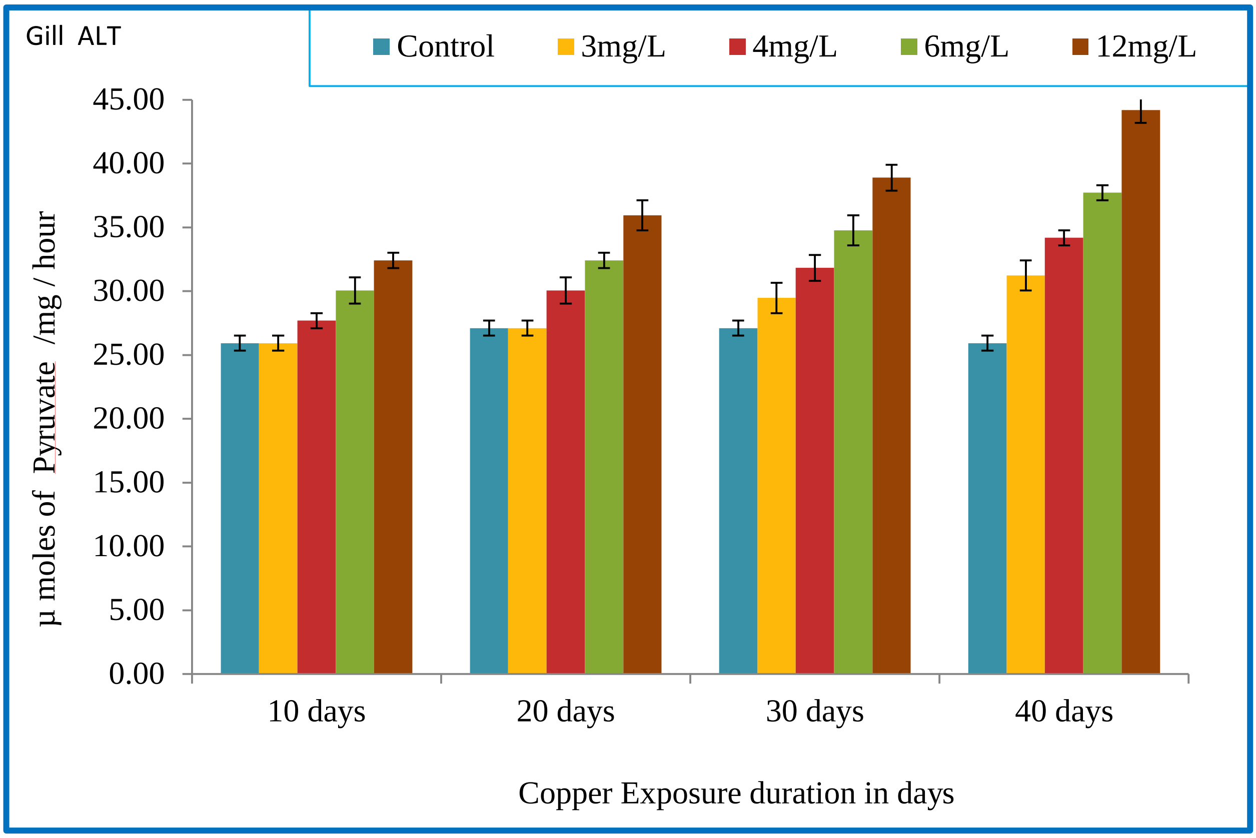

Figure 7. ALT level in Gills after exposure to sublethal concentrations of Copper compared to control (Mean ± SE)

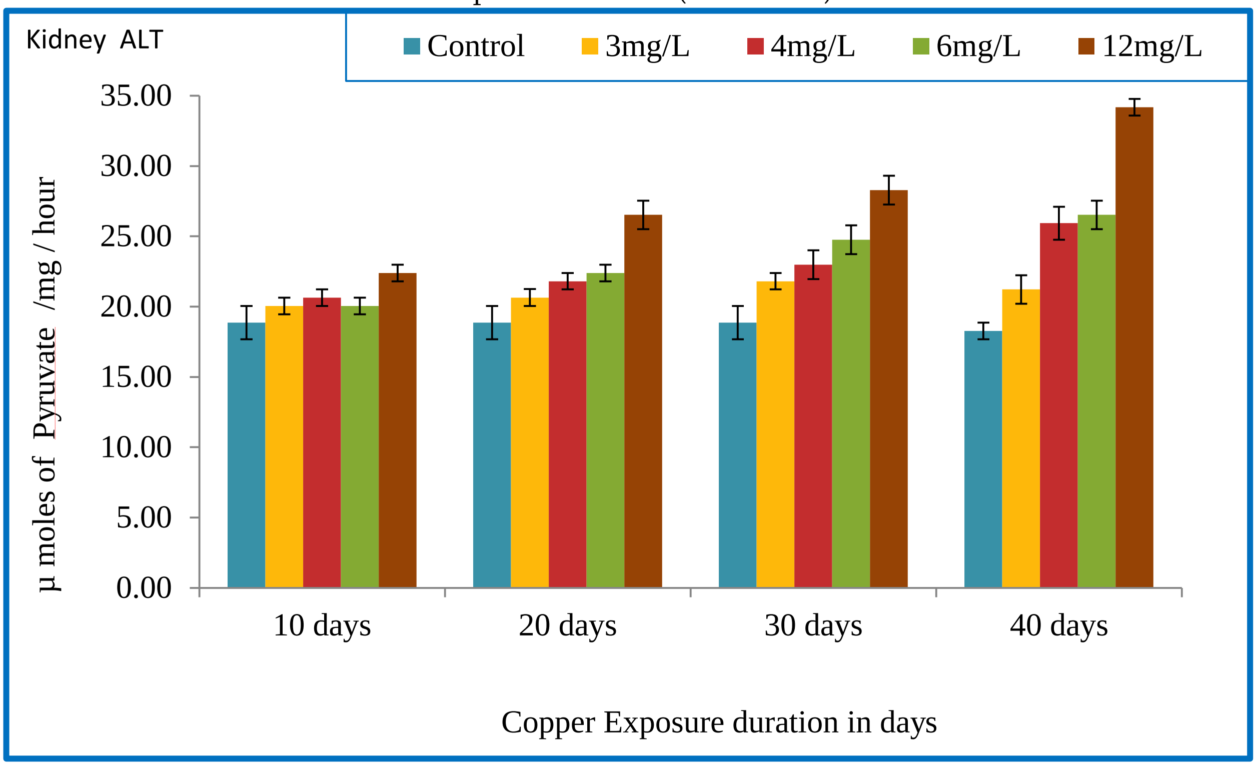

Figure 8. ALT level in Kidney after exposure to sublethal concentrations of Copper compared to control (Mean ± SE)

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