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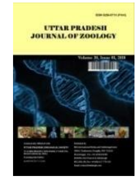

IMPACT OF SUBLETHAL CONCENTRATIONS OF COPPER (Cu) ON PROTEIN METABOLISM OF MOZAMBIQUE TILAPIA (*Oreochromis mossambicus*, PETERS, 1852)

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AUTHORS' CONTRIBUTIONS
This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Industries are the major sources of heavy metal pollution and it is released into water and soil. Heavy metals cause several negative effects to aquatic organisms and environment.
Aims: The present investigation aims to determine the effect of copper sulphate on protein content, free amino acids, AST and ALT levels in certain tissues, i.e. muscle and zliver of Mozambique tilapia, *Oreochromis mossambicus.*
Study Design: *Oreochromis mossambicus* weighing about 12- 14g used in the present study were procured from State fisheries culture tanks. They were transported to the laboratory in oxygenated containers and treated with KMnO4 to avoid dermal infection and acclimatized to laboratory conditions for 10 days.
Results and Discussion: In the present study, *Oreochromis mossambicus* was exposed to sublethal concentrations (1/16, 1/12, 1/8 and 1/4th of 96 h LC_{50} value) i.e. 3 mg/L, 4 mg/L, 6 mg/L and 12 mg/L of copper sulphate for four different exposure periods of 10, 20, 30 and 40 days. The proteins, free amino acids, Aspartate amino transferase (ASAT) and Alanine amine transferase (ALAT) levels in muscle and liver were studied. Decreased tendency was observed in protein levels and an increased tendency was observed in free amino acids, AST and ALT levels in all the vital tissues of fish exposed to copper sulphate over control.
Conclusion: Proteins gradually decreased with increased exposure period of sublethal concentrations. Amino acids, AST and ALT levels gradually increased with increased exposure period and the increase was observed to be directly proportional to the increased toxicant concentrations.

Keywords: *Oreochromis mossambicus*; copper sulphate; proteins; amino acids; AST and ALT.

1. INTRODUCTION

Water is an important natural resource which is essential for all living beings. The important water resources are ponds, lakes, rivers, streams, etc. These water bodies provide water for drinking, irrigation, cooling industrial machines, and for other domestic purposes.

Among all pollutants heavy metals are of special concern because these elements are generally released in small amounts into the environment by processes

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like weathering of rocks, volcanic eruptions etc. and their intake or exposure is necessary in trace amounts for good health. But, presently, there is a steady increase in their concentration in all habitats owing to mining, electroplating, paints and dye, battery making industries. The release is fast with the rapidly growing technology and heavy metal application in these industries. The two most important factors that contribute to the deleterious effects of heavy metals as pollutants are their indestructible nature through bioremediation unlike organic pollutants and their tendency to accumulate in environment especially in the bottom sediments of aquatic habitats in association with organic and inorganic matter [1]. The undesirable change in water that has harmful effect on the life of human and domestic plants and animals is called water pollution [2].

Copper occurs naturally within the environment. At low concentrations, it is an essential element both for plants and other organisms; however, large doses can be harmful. It is involved in diverse life processes, including cellular respiration, antioxidant defense, pigment formation, neurotransmitter production and peptide biosynthesis. Copper sulphate is a fungicide used to control bacterial and fungal diseases of fruit vegetable, nuts and field crops like mildew, leaf spots, blights and apple scab. It is also used as an algaecide, a herbicide in irrigation and municipal water treatment system and for controlling phytoplankton in fish ponds and lakes as well as a herbicide used in aquatic weed control Carbonell and Tarazona [3]. Qiu et al. [4] showed that copper has toxic effects on the larval development of the barnacle *Balanus Amphitrite* and that molting was a more sensitive end-point than survival.

Studies on different biochemical parameters have proved useful in determining the adaptive and protective mechanisms of the body to resist the toxic effects of the toxic substances. Any alteration in biochemical parameters can result in serious outcomes in the form of various diseases in both the fish and its consumer. In the tissue protein, carbohydrate and lipids play a major role as energy precursors for aquatic organisms exposed to stress conditions [5]. An alteration in biochemical and physiological changes in the crab, *Portunus pelagicus* due to copper and zinc have been reported by Hilmy et al. [6]. Similarly [7] reported that the variation in carbohydrate and protein contents in the clam, *Sunetta scripta* during its exposure to copper. Villalan et al. [8] observed that heavy metals altered protein, lipid and carbohydrate levels in the crab, *Thalamita crenata.* Baden et al. [9] also reported similar changes in the distribution of glycogen in the tissues of Norway lobster, *Nephrops norvegicus* exposed to copper. Maharajan et al. [10,11] observed the biochemical changes of various tissues and haemolymph of spiny lobster, *Panulirus homarus* and the fresh water crab*, Paratelphusa jacquemontii* when exposed to sublethal doses of copper. The development and growth of the fishes depend upon the DNA and RNA which serve as biochemical indices Buckley et al. [12]. Cellular enlargement and active protein synthesis are dependent on DNA and RNA content.

The protein content in the tissues of animals plays a significant role in the metabolism. Palanivelu et al. [13] stated that the protein content of the cell may be considered as an important tool for evaluation of physiological standards. The soluble protein fraction represents the activity level of enzymes in general. The structural protein fraction forms the structural moiety of a cell Lehninger, [14]. Begam and Vijayaraghavan [15] observed that the protein decrease in the fish tissues indicates the physiological strategy in order to meet the energy demand and to adopt itself to the changed metabolic system which may lead to the stimulation of degradative processes like proteolysis and utilization of degraded products for increased energy metabolism. When any aquatic animal is exposed to polluted medium, a sudden stress is developed for which the animals should meet more energy demand to overcome the toxic stress Maharajan et al. (2012a, 2014); Paruruckumani et al. [16,17]. Verma et al. [18] reported on the toxic effects of sublethal concentration of copper sulphate on certain biologically important enzymes in *Saccobranchus fossilis.*

The purpose of present investigation is to determine the effect of copper sulphate on protein content, free amino acids, ASAT and ALAT levels in certain tissues, i.e. muscle and liver of *Mozambique tilapia.*

2. MATERIALS AND METHODS

The fish weighing about 12- 14 g used in the present study were procured from State fisheries culture tanks. They were transported to the laboratory in oxygenated containers and treated with $KMnO_4$ to avoid dermal infection and acclimatized to laboratory conditions for 10 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 containers was replaced by fresh water every 24 hours. Biochemical parameters (total proteins, amino acids, ASAT and ALAT) were estimated in muscle and liver by exposing to four sub lethal 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 3 mg/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). ASAT and ALAT levels were estimated by Bergmeyer method (1965).

3. RESULTS

After exposing the fish to different sublethal cconcentration 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 on protein content, Free amino acids, AST and ALT levels in Muscle and Liver of *Oreochromis mossambicus* fish were studied and the results were statically analyzed. The variations in levels of protein, Free amino acids, AST and ALT levels in different tissues given in figures (Fig. 1 to 8) in terms of mean with Standard error values over control . Proteins gradually decreased with increased exposure period of sublethal concentrations. 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.

The observation was made in both control and $CuSO_4$ exposed fish, muscle contains high levels of protein in comparison to liver tissue. At the end of the experiment the protein levels were decreased in both tissues. The order of protein levels decrease in two tissues when exposed to sublethal concentrations was observed as muscle > liver of fish. Decrease of protein levels was more at higher concentrations of $CuSO_4$ (12 mg/L, 6 mg/L, 4 mg/L and 3 mg/L) and higher durations (40, 30, 20, and 10 days).

In the present study, free amino acids levels in different tissues like muscle and Liver were increased under sublethal exposure of $CuSO_4$. The free amino acid levels along with mean and Standard error over the control were given in Fig. 3 and 4. There was a significant increase in Free Amino acid levels in all tissues at all durations and in all sublethal doses were observed. The order of increase in different tissues when exposed to sublethal concentrations was observed as Liver > Muscle of fish.

The Aspartate aminotransferase (AST) levels in different tissues like muscle and Liver were observed under sublethal exposure of $CuSO_4$. The mean values of AST with Standard error over the control were given in Figs. 5 and 6. There was a significant increase in AST levels in both the tissues at all durations and in all sublethal doses were observed. The order of increase in different tissues when exposed to sublethal concentrations was observed as Liver > Muscle of fish.

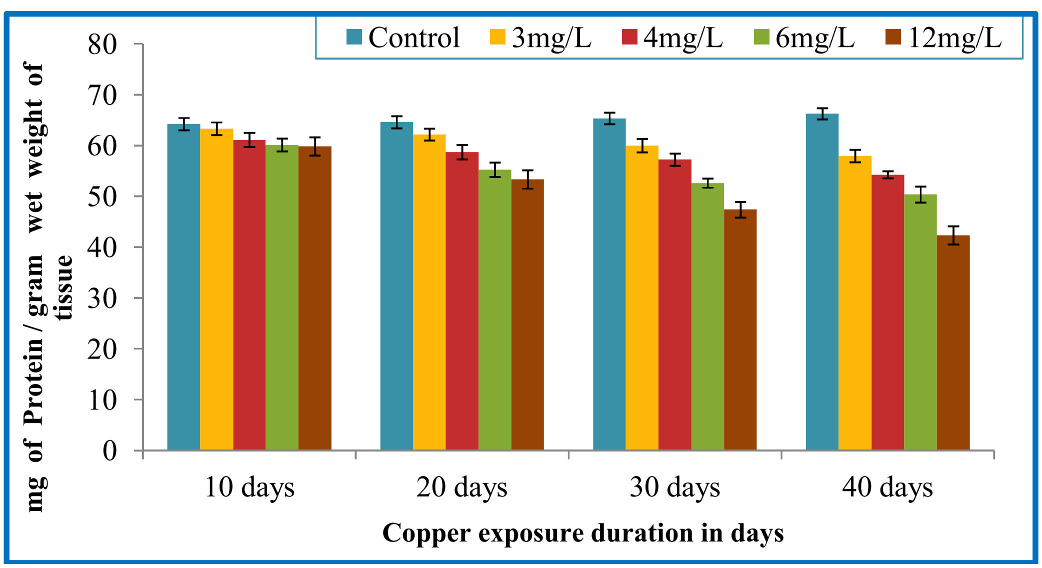

Fig. 1. Protein content in fish Muscle after exposure to sublethal concentrations of Copper Compared to control (Mean ± SE)

Table 1. Protein content in fish muscle after exposure to sublethal concentrations of Copper compared to control

$CuSO_4$ mg/ L		Days of exposure			
		10 days	20 days	30 days	40 days
Control	Mean	64.240	64.590	65.340	66.270
	SE	±1.200	±1.211	±1.144	±1.090
3mg/L	Mean	63.320^{NS}	62.170^{NS}	59.980^{NS}	57.940*
	SE	±1.239	±1.171	±1.311	±1.236
	%V	-1.43	-3.73	-8.20	-12.57
4mg/L	Mean	61.120^{NS}	58.720*	57.230**	54.260***
	SE	±1.386	±1.403	±1.176	±0.691
	%V	-4.85	-9.09	-12.41	-18.13
6mg/L	Mean	60.100^{NS}	55.280**	52.600***	50.370***
	SE	±1.260	±1.414	±0.890	±1.565
	%V	-6.45	-14.49	-19.49	-23.99
12mg/L	Mean	59.840^{NS}	53.340**	47.310***	42.320***
	SE	±1.772	±1.811	±1.544	±1.808
	%V	-6.86	-17.41	-27.46	-36.13

Each value is the Mean ± SE of six individual observations
Values are expressed as mg of protein / gram wet weight of tissue SE - Standard Error,
*%V- Percent variation. NS : Not Significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$*

Table 2. Protein content in fish Liver after exposure to sublethal concentrations of Copper Compared to control

$CuSO_4$ mg/ L		Days of exposure			
		10 days	20 days	30 days	40 days
Control	Mean	50.300	50.490	51.480	52.250
	SE	±1.282	±1.274	±1.253	±1.189
3mg/L	Mean	50.370^{NS}	49.140^{NS}	48.220^{*}	47.190**
	SE	±1.108	±1.187	±1.183	±1.141
	%V	0.12	-2.66	-6.34	-9.68
4mg/L	Mean	49.270^{NS}	48.260^{NS}	46.280*	44.220**
	SE	±1.067	±1.107	±1.186	±1.091
	%V	-2.06	-4.40	-10.10	-15.36
6mg/L	Mean	48.280^{NS}	45.290**	43.410**	41.280***
	SE	±1.123	±1.105	±1.201	±1.059
	%V	-4.03	-10.29	-15.68	-20.99
12mg/L	Mean	47.220*	43.280***	40.180***	37.260***
	SE	±1.124	±1.169	±1.141	±1.099
	%V	-6.14	-14.27	-21.94	-28.68

Each value is the Mean ± SE of six individual observations
Values are expressed as mg of protein / gram wet weight of tissue SE - Standard Error,
*%V- Percent Variation. NS : Not Significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$*

The Alanine aminotransferase (ALT) levels in different tissues like muscle and Liver were observed under sublethal exposure of $CuSO_4$. The mean values of ALT with Standard error over the control were given in Figs. 7 and 8. There is a significant increase in ALT levels in both the tissues at all durations and in all sublethal doses were observed. The order of increase in different tissues when exposed to sublethal concentrations was observed as Liver > Muscle of fish.

4. DISCUSSION

During experiment of the treating of the fish, *Oreochromis mossambicus* to different sublethal concentration of copper sulphate ($CuSO_4$), proteins gradually decreased with increased exposure period of sublethal concentrations. Amino acids, AST and ALT levels increased with increased exposure period and the increase was observed to be directly proportional to increased sublethal concentrations.

The observation was made in both control and $CuSO_4$ exposed fish, muscle contains high levels of protein in comparison to liver tissue. At the end of the experiment the protein levels were decreased in both the tissues. The decrease in protein levels may be due to metabolic stress and proteolysis under toxic exposure of fish. Similar decrement in protein content was also observed when fish were exposed to Copper sulphate by Mikhaylova et al. [19].

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 [20]. Amino acids are essential intermediate substances in the process of protein synthesis and its degradation

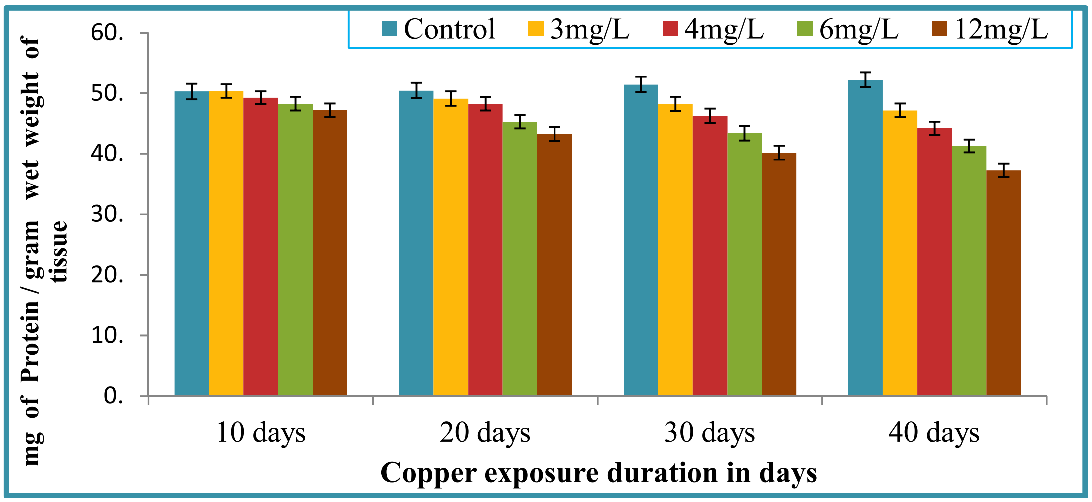

Fig. 2. Protein content in fish Liver after exposure to sublethal concentrations of Copper Compared to control (Mean ± SE)

Table 3. Free Amino acid content in fish muscle after exposure to sublethal concentrations of Copper compared to control

$CuSO_4$ mg/ L		Days of exposure			
		10 days	**20 days**	**30 days**	**40 days**
Control	**Mean**	20.540	20.740	21.070	20.970
	SE	±0.343	±0.362	±0.557	±0.516
3mg/L	**Mean**	20.900^{NS}	21.120^{NS}	21.810^{NS}	22.820^{NS}
	SE	±0.338	±0.426	±0.629	±0.508
	%V	1.73	1.83	3.49	8.79
4mg/L	**Mean**	21.100^{NS}	21.720^{NS}	22.340^{NS}	22.920*
	SE	±0.466	±0.704	±0.510	±0.366
	%V	2.30	4.75	6.01	9.29
6mg/L	**Mean**	21.130^{NS}	22.010^{NS}	22.690*	23.340*
	SE	±0.566	±0.549	0.000	±0.285
	%V	2.84	6.12	7.67	11.30
12mg/L	**Mean**	21.380^{NS}	22.810**	25.080**	26.880***
	SE	±0.446	±0.066	±0.322	±0.301
	%V	4.05	10.01	19.03	28.14

Each value is the Mean ± SE of six individual observations
Values are expressed as mg of free amino acids/gram wet weight of tissue, SE-Standard Error
*%V- Percent variation. NS : Not Significant, * P < 0.05, ** P < 0.01, *** P < 0.001*

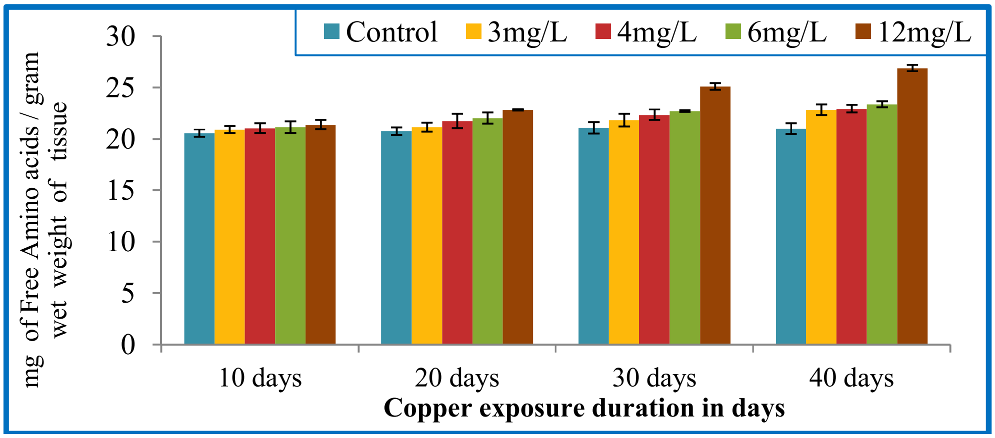

Fig. 3. Free Amino acid content in fish muscle after exposure to sublethal concentrations of Copper compared to control (Mean ± SE)

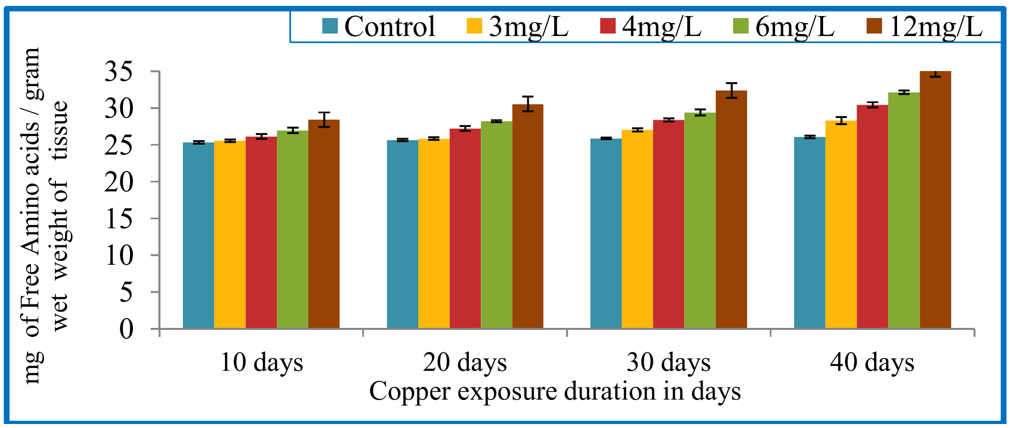

Fig. 4. Free Amino acid content in fish Liver after exposure to sublethal concentrations of Copper compared to control (Mean ± SE)

Table 4. Free Amino acid content in fish Liver after exposure to sublethal concentrations of Copper compared to control

$CuSO_4$ mg/ L		Days of exposure			
		10 days	**20 days**	**30 days**	**40 days**
Control	**Mean**	25.320	25.650	25.840	26.060
	SE	±0.165	±0.156	±0.112	±0.179
3mg/L	**Mean**	25.530^{NS}	25.840^{NS}	27.000**	28.280**
	SE	±0.178	±0.166	±0.217	±0.481
	%V	0.84	0.72	4.47	8.52
4mg/L	**Mean**	26.120^{NS}	27.210*	28.360**	30.420***
	SE	±0.314	±0.315	±0.222	±0.357
	%V	3.14	6.08	9.72	16.74
6mg/L	**Mean**	26.930*	28.190**	29.390**	32.130***
	SE	±0.374	±0.150	±0.412	±0.244
	%V	6.37	9.90	13.72	23.28
12mg/L	**Mean**	28.390**	30.530***	32.370***	35.260***
	SE	±0.329	±0.318	±0.414	±0.457
	%V	12.11	19.02	25.26	35.29

Each value is the Mean ± SE of six individual observations
Values are expressed as mg of free amino acids /gram wet weight of tissue SE-Standard Error
*%V- Percent variation. NS : Not Significant, * P < 0.05, ** P < 0.01, *** P < 0.001*

Table 5. AST level in fish Muscle after exposure to sublethal concentrations of Copper compared to control

$CuSO_4$ mg/ L		Days of exposure			
		10 days	**20 days**	**30 days**	**40 days**
Control	**Mean**	53.040	54.220	53.040	54.220
	SE	±1.021	±0.589	±1.021	±0.589
3mg/L	**Mean**	54.810^{NS}	56.580^{NS}	58.930**	60.120**
	SE	±1.021	±1.021	±0.5890	±1.021
	%V	3.33	4.34	11.11	10.87
4mg/L	**Mean**	56.580^{NS}	58.930**	60.110**	61.880**
	SE	±1.021	±0.589	±1.021	±1.021
	%V	6.66	8.69	13.33	14.13
6mg/L	**Mean**	57.760*	60.110**	63.060**	65.420***
	SE	±0.589	±1.021	±1.179	±1.021
	%V	8.89	10.87	15.88	20.65
12mg/L	**Mean**	61.290***	62.470***	64.830***	68.750***
	SE	±0.589	±0.589	±0.589	±0.853
	%V	15.55	15.21	18.18	26.80

Each value is the Mean ± SE of six individual observations
Values are expressed as µ moles of Pyruvate /mg / hour SE - Standard Error,
*%V- Percent variation. NS : Not Significant, * P < 0.05, ** P < 0.01, *** P < 0.001*

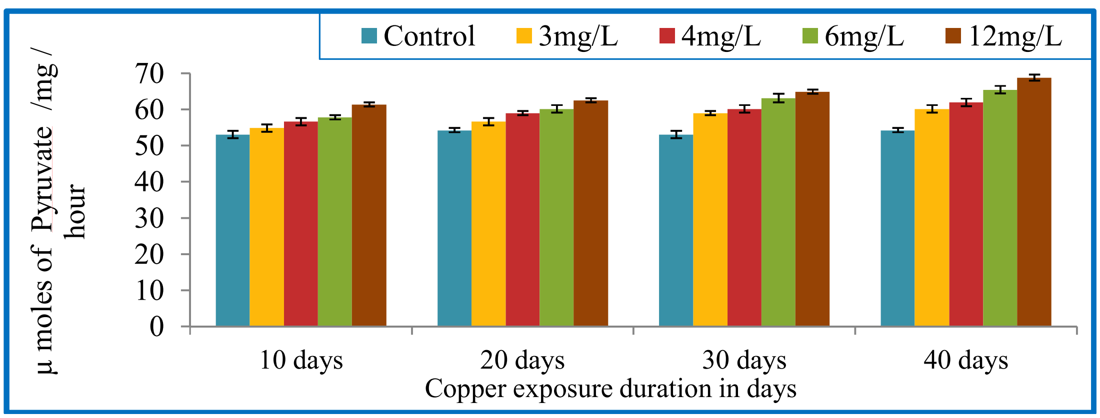

Fig. 5. AST level in fish Muscle after exposure to sublethal concentrations of Copper compared to control (Mean ± SE)

products appear in the form of various nitrogenous compound [21]. The present study revealed that, free amino acids levels in different tissues like muscle and Liver were increased under sublethal exposure of $CuSO_4$. The increase in the free amino acid content is supported increased proteolytic action caused by the stress due to Copper sulphate. From the results, 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 different tissues like muscle and Liver were observed under sublethal exposure of $CuSO_4$. The Alanine aminotransferase (ALT) levels in different tissues like muscle and Liver were observed under sublethal exposure of $CuSO_4$.

Mckim et al. [22] found that, sublethal concentration of copper caused significant increase of pALT of *Salvelunus farttinalis* after 6 and 21 days of exposure. Shalaby [23] reported that changes were produced in liver and muscle of common carp; *Cyprinus carpio* L. exposed to sublethal levels of copper, cadmium or zinc alone or a combination of them for 7 to 30 days. The hepatic Aspartate aminotransferase (AST) in liver was increased. Also, hepatic Alanine aminotransferase (ALT) showed significant increase in fish.

Table 6. AST level in fish liver after exposure to sublethal concentrations of Copper compared to control

$CuSO_4$ mg/ L		Days of exposure			
		10 days	20 days	30 days	40 days
Control	Mean	71.310	71.310	71.900	72.490
	SE	±0.589	±0.589	±0.589	±1.021
3mg/L	Mean	73.670^{NS}	76.030^{NS}	77.800*	80.740**
	SE	±0.589	±1.022	±1.021	±0.589
	%V	3.30	6.61	8.20	11.38
4mg/L	Mean	79.560*	81.330**	85.450***	91.350***
	SE	±1.019	±1.021	±0.589	±0.589
	%V	11.57	14.05	18.85	26.01
6mg/L	Mean	83.690**	86.630***	92.530***	100.190***
	SE	±0.589	±1.021	±0.589	±1.559
	%V	17.35	21.48	28.69	38.21
12mg/L	Mean	88.400***	96.070***	104.900***	117.280***
	SE	±1.021	±1.559	±1.559	±2.569
	%V	23.96	34.71	45.90	61.79

Each value is the Mean ± SE of six individual observations
Values are expressed as µ moles of Pyruvate /mg / hour SE - Standard Error,
*%V- Percent variation. NS : Not Significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$*

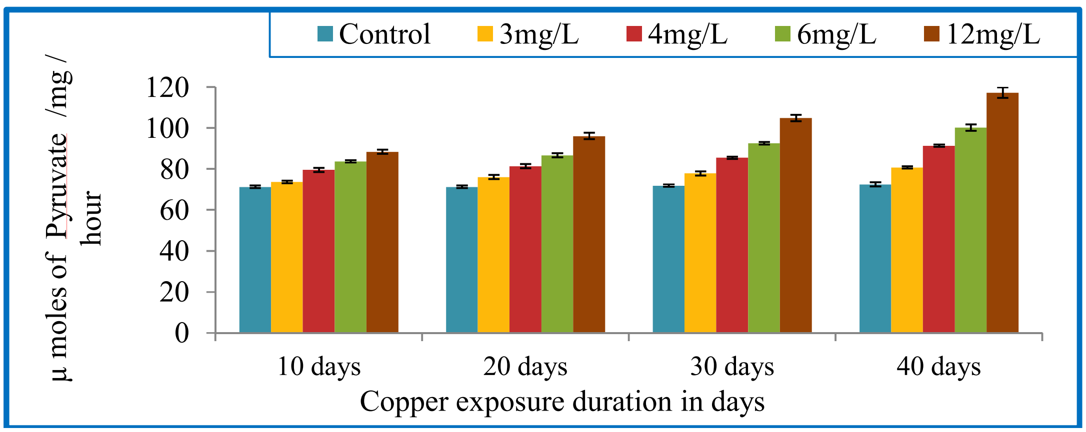

Fig. 6. AST level in fish Liver after exposure to sublethal concentrations of copper compared to control (Mean ± SE)

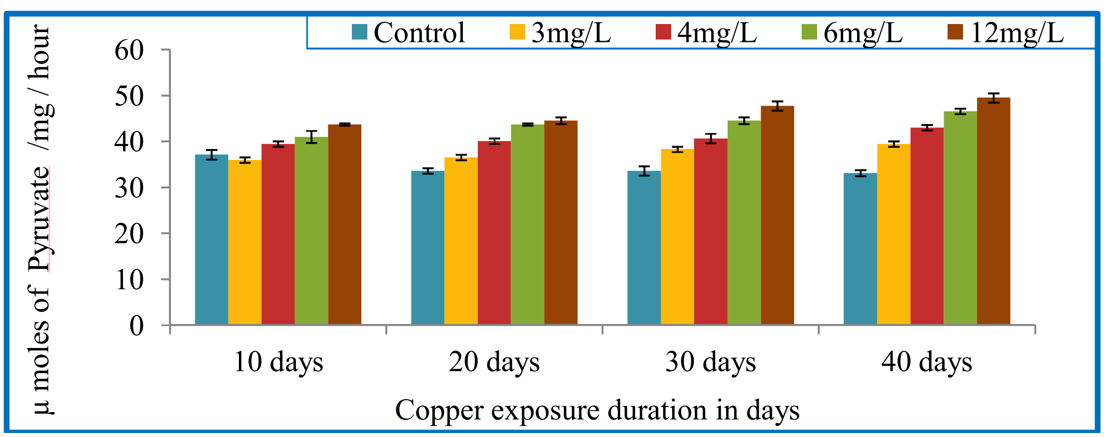

Fig. 7. ALT level in fish Muscle after exposure to sublethal concentrations of copper compared to control (Mean ± SE)

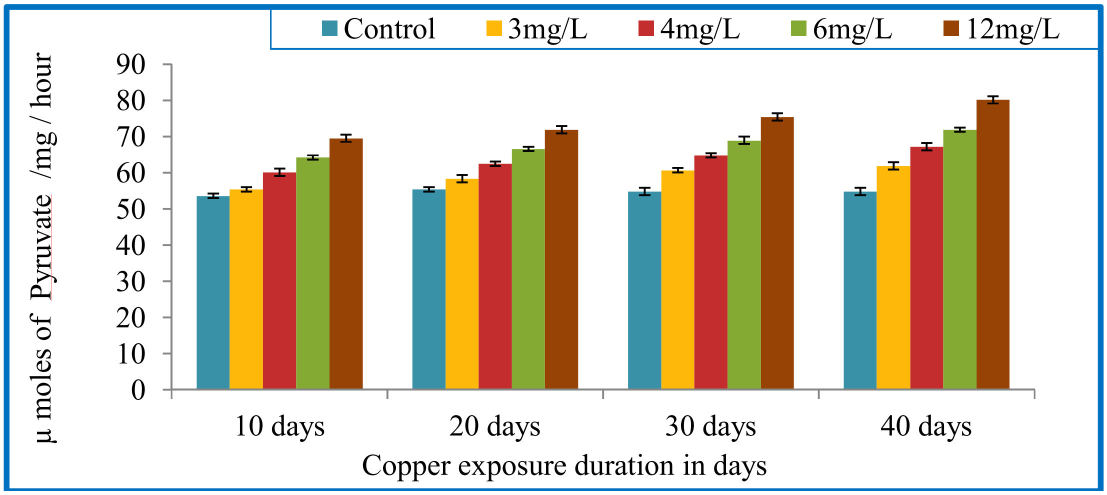

Fig. 8. ALT level in fish Liver after exposure to sublethal concentrations of Copper compared to control (Mean ± SE)

Table 7. ALT level in fish Muscle after exposure to sublethal concentrations of Copper compared to control

$CuSO_4$ mg/ L		**Days of exposure**			
		10 days	**20 days**	**30 days**	**40 days**
Control	**Mean**	37.128	33.592	33.592	33.128
	SE	±1.020	±0.590	±1.020	±0.680
3mg/L	**Mean**	35.950^{NS}	36.540^{NS}	38.310*	39.480**
	SE	±0.590	±0.590	±0.590	±0.590
	%V	-3.17	8.77	14.03	19.19
4mg/L	**Mean**	39.480*	40.070**	40.660*	43.020***
	SE	±0.590	±0.590	±1.020	±0.590
	%V	6.35	19.29	21.05	29.86
6mg/L	**Mean**	40.910*	43.690***	44.530***	46.560***
	SE	±1.320	±0.260	±0.750	±0.590
	%V	10.42	30.05	32.57	40.53
12mg/L	**Mean**	43.690***	44.530***	47.740***	49.500***
	SE	±0.260	±0.750	±1.020	±1.020
	%V	17.67	32.57	42.10	49.43

Each value is the Mean ± SE of six individual observations
Values are expressed as µ moles of Pyruvate /mg / hour SE - Standard Error,
*%V- Percent variation. NS : Not Significant, * P < 0.05, ** P < 0.01, *** P < 0.001*

Table 8. ALT level in fish Liver after exposure to sublethal concentrations of Copper compared to control

$CuSO_4$ mg/ L		**Days of exposure**			
		10 days	**20 days**	**30 days**	**40 days**
Control	**Mean**	53.630	55.400	54.810	54.810
	SE	±0.590	±0.590	±1.020	±1.020
3mg/L	**Mean**	55.400^{NS}	58.340^{NS}	60.700*	61.880*
	SE	±0.590	±1.020	±0.590	±1.020
	%V	3.29	5.32	10.75	12.90
4mg/L	**Mean**	60.110**	62.470***	64.830***	67.180***
	SE	±1.020	±0.590	±0.590	±1.020
	%V	12.08	12.76	18.28	22.58

$CuSO_4$ mg/ L		Days of exposure			
		10 days	20 days	30 days	40 days
6mg/L	Mean	64.230***	66.590***	68.950***	71.900***
	SE	±0.590	±0.590	±1.020	±0.590
	%V	19.77	20.21	25.80	31.18
12mg/L	Mean	69.540***	71.900***	75.430***	80.150***
	SE	±0.590	±0.590	±1.560	±0.590
	%V	29.67	29.78	37.63	46.23

Each value is the Mean ± SE of six individual observations
Values are expressed as μ moles of Pyruvate /mg / hour SE - Standard Error,
*%V- Percent variation. NS : Not Significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$*

5. CONCLUSION

The present investigation revealed that $CuSO_4$ caused alterations in biochemical parameters and enzymes of *Oerochromis mossambicus* might be caused by intoxication of heavy metal. It is concluded that the utilization of Copper sulphate should be minimized and awareness should create awareness among the people about the toxicity of Copper Sulphate on animals as well as on human. Since majority of heavy metals are released cumulatively and regularly, through the industrial and human activities, their residues are known to bioaccumulate in the tissues of fish and other animals, and transfer via food chain to the human bodies, they create risk to the health of the people who consume these fish seems to be considerable. The need to protect the people from undue exposure to the heavy metals through the food chain cannot be over emphasized.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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