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Effect of Copper Sulphate on Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) Activity in Freshwater Fish *Ariopsis seemanni.*

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ABSTRACT

The heavy metals like Copper, Cadmium etc., act as environmental pollutant and they result in adverse effect on the all the life forms. Many physically and enzymatically changes occurring in the living system. Aspartate aminotransferase and Alanine aminotransferase act as bio-indicators for the environmental toxicants level and tissue damage in many living forms. Quantitative estimation of the AST and ALT activity in liver, gills and muscles tissues of catfish *Ariopsis semmani* when exposed to different concentrations of copper sulphate (2ppm, 4ppm and 6ppm) was estimated on 5^{th}, 10^{th} and 15^{th} days post exposure. The concentration of the toxicant was directly proportional to the amount of the enzyme that is produced by the fish.

Keywords: AST, ALT, *Ariopsis semmani*, bio-indicators, copper sulphate

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INTRODUCTION

Heavy metal is a major constituent of environmental toxicants that cause severe adverse effects to the health of organism. These toxicants act at cellular and molecular level which ultimately leads into physiological and biochemical disorders even sometimes leading to death of organism (More, 2012). Metal pollution has increased with the technological progress of human society. Industry, mining, advanced agriculture, household waste, and motor traffic is all among the activities considered to be major sources of metal pollution. Metals can accumulate in aquatic organisms, including fish, and persist in water and sediments (Luoma and Rainbow, 2008). Fish are an important component of human nutrition, and those from contaminated sites present a potential risk to human health. Since fish occupy the top of the aquatic food chain, they are suitable bio-indicators of metal contamination. 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, 2003).

Fishes are the simple and reliable biomarker of copper pollution in aquatic bodies (Taylor et al., 2000; Lodhi et al., 2006).The metallic ion present in water enters into the fish body and gets accumulated in various organs like liver and kidney (Al-Mohanna, 1994; Shukla et al., 2007). Copper occurs in form of several compounds. The cheapest and most commonly used form is copper sulphate (Watson and Yanong, 2006). It is a fungicide that is used to control bacterial and fungal diseases of fruits, vegetables and field crops; it is also used as an algaecide and herbicide and to kill snails and slugs in irrigation as well as municipal water treatment systems (Atamanalp et al, 2008).

Aspartate aminotransferase (AST) (L-aspartate 2-oxogluterate aminotransferase E.C. 2.6.1.1) and formerly called as serum glutamic oxaloacetic transaminase (SGOT) and Alanine aminotransferase (ALT) (L-alanine:2-oxoglutarate aminotransferase EC 2.6.1.2) and formerly called as serum glutamate-pyruvate transaminase (SGPT).

Serum aspartate aminotransferase (AST) also is a tissue enzyme that catalyzes the exchange of amino and keto groups between alpha amino acids and alpha-keto acids. AST is widely distributed in tissue principally cardiac hepatic muscle and kidney. Injury to these tissues results in the release of the AST (SGOT) enzyme to general circulation. In the present study toxicity effects of copper sulphate (from 2 ppm to 6ppm) on AST and ALT was studied in the fresh water fish *Ariopsis semmani*.

MATERIALS AND METHOD

Catfish, *Ariopsis semmani* belonging to a single population were purchased from the local market of Chennai, Tamilnadu, India. Fishes were then treated with 0.01% $KMnO_4$ solution for 15 min to avert any dermal infection before putting in aquariums. Fishes were distributed in 100 L capacity aquariums and were unfed during the first 2 days to adapt to change in environment.

Fishes were then kept for a period of fifteen days for adaptation to laboratory conditions. The experimental design consisted of a control and 3 concentrations (2, 4 and 6ppm) of copper sulphate ($CuSO_4.5H_2O$) treatment, two replicates per group with twenty fishes in each replicate. On the suggested days the fishes were sacrificed. After 5, 10 and 15 days from the treatment 2 fishes from each tank were dissected to remove gills, liver and muscle and the tissues were washed in double distilled water and preserved in 10% formalin. Before analysis, formalin was removed using filter paper from each tissue. After homogenization supernatant was used for measuring enzyme activity (Senthil Murugan et al., 2008, Adil 2013, Henry 1974, Tietz 1986, Naris 1974).

EXPERIMENTAL

Methods for the determination of AST and ALT (Bergermeyer et al., 1978)

This assay is based on the principle that AST and ALT catalyse the transfer of amino group from L-aspartate/L-alanine to α-ketoglutarate to yield oxaloacetate/pyruvate respectively. Oxaloacetate/pyruvate can oxidize NADH to NAD+ in the presence of malate dehydrogenase/lactate dehydrogenase. The decrease in

absorbance at 340nm in a spectrophotometer (Cary Varian 300) due to the oxidation of NADH is monitored kinetically and is proportional to AST/ALT activity.

RESULTS AND DISCUSSION

The fishes were treated with different sub-lethal concentration of Copper sulphate and a set of fishes were sacrificed on the apportioned days and the enzyme activity was done for gills, liver and muscles.

Enzyme Activity

Aspartate aminotransferase AST/ SGOT at 2 ppm

The maximum increase of AST was seen in the gills, followed by liver and muscles. The AST activity showed in 5 days, 10 days and 15 days was 22.8 IU/L, 31.9 IU/L and 36.5 IU/L respectively. In liver, the increase of AST level in 5 days, 10 days and 15 days was recorded as 22.1 IU/L, 55.47 IU/L and 61.1 IU/L. And finally for the muscles AST activity in 5 days, 10 days and 15 days was 0IU/L, 15.2 IU/L and 30.1IU/L were documented (Table -1).

Aspartate aminotransferase AST/ SGOT at 4 ppm

The maximum increase of AST level was seen in the gills, followed by liver and muscles was logged. The enzyme activity increase in 5 days, 10 days and 15 days in gills is 31.9 IU/L, 36.5 IU/L and 81.9 IU/L. In liver, the increase of AST in 5 days, 10 days and 15 days with respect to the control is 55.47 IU/L, 61.1 IU/L and 66.7 IU/L was noted. In the muscles the activity increase in 5 days, 10 days and 15 days with respect to the control is 15.27 IU/L, 30.1IU/L and 35 IU/L was plotted (Table-2).

Aspartate aminotransferase AST/ SGOT at 6 ppm

The maximum increase of AST was seen in the gills, followed by liver and muscles. The activity increased in 5 days, 10 days and 15 days with respect to the control is 81.9 IU/L, 100 IU/L (twice) and 127.5 IU/L. For liver, the activity increase of AST in 5 days, 10 days and 15 days with respect to the control is 55.47 IU/L, 100 IU/L (twice) and 122.1 IU/L. And finally in the muscles, the activty increase of AST in 5 days, 10 days and 15 days was 15.27 IU/L, 50 IU/L and 80 IU/L (Table-3). The comparative effects was represented in Figure -1.

Alanine aminotransferase ALT/ SGPT at 2 ppm

The maximum increase of ALT was seen in the liver followed by gills and muscles. In Gills the activity increase of ALT in 5 days, 10 days and 15 days with respect to the control is 0 IU/L 33.40 IU/L and 66.24 IU/L. For liver the activity increase of ALT in 5 days, 10 days and 15 days with respect to the control is 0 IU/L, 33.48 IU/L and 66.75 IU/L. And finally for the muscles the activity increase of ALT in 5 days, 10 days and 15 days with respect to the control is 0IU/L, 47.15 IU/L and 59.24IU/L (Table -4).

Alanine aminotransferase ALT/ SGPT at 4 ppm

The maximum increase of ALT was seen in the liver followed by gills and muscles. The percentage increase of ALT in 5 days, 10 days and 15 days was 33.4 IU/L, 102.48 IU/L, 133.74 IU/L. For liver, the activity increase of ALT in 5 days, 10 days and 15 days was 33.5 IU/L, 71.58 IU/L and 127.25 IU/L. And finally for the muscles the activity increase of ALT in 5 days, 10 days and 15 days 15.2 IU/L, 218.57 IU/L and 307.58 IU/L (Table -5).

Alanine aminotransferase ALT/ SGPT at 6 ppm

The maximum increase of ALT was seen in the liver followed by gills and muscles. The activity increase of ALT in 5 days, 10 days and 15 days with respect to the control 56.7 IU/L, 209.51 IU/L and 207.15 IU/L. For liver, the activity increase of ALT in 5 days, 10 days and 15 days with respect to the control is 66.7 IU/L, 140.75 IU/L and 278.49 IU/L. And finally for the muscles the activity increase of ALT in 5 days, 10 days and 15 days

with respect to the control is 30.1 IU/L, 397.0 IU/L and 524.84 IU/L. (Table -6) The comparative effects was represented in Figure -2.

The present study reveals the pattern shown by the enzymes and biomolecules in all the four tests above suggests that the dependence on the concentration of toxin and the duration of exposure of these toxins. When a fish was exposed at 2 ppm of copper sulphate for 10 days, the increase in the enzyme activity or the decrease in the concentration of biomolecules was almost equal to 4 ppm for 5 days. It was again similar for 4 ppm for 10 days and 6 ppm for 5 days. Here, a pattern of the augmentation enzyme activity and the decrease in biomolecules level with respect to the concentration of toxins and its exposure to the fishes was observed.

Mckim et al. (1970) found that, sublethal concentration of copper caused significant increase of pALT of *Salvelunus farttinalis* after 6 and 21 days of exposure. Then a significant decrease of pALT was observed after long term exposure (337 days). Adel Shalaby (2000) reported that changes were produced in liver and muscle of common carp*; Cyprinus carpio* L. exposed to sublethal levels of either 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 1: Curve fit values of Gills for SGOT

Days	Concentration of Copper sulphate			R^2
	2ppm	4ppm	6ppm	
5	22.8 ±0.80	31.90 ±0.25	81.90 ±0.95	0.9653
10	31.9 ±0.75	36.50 ±0.74	100.00 ±0.69	0.8184
15	36.5 ±0.47	81.90 ±0.29	127.50 ±0.27	0.9860

Table 2: Curve fit values of Liver for SGOT

Days	Concentration of Copper sulphate			R^2
	2ppm	4ppm	6ppm	
5	22.10± 0.34	55.47± 0.24	55.47± 0.54	0.8557
10	55.47± 0.68	61.10± 0.78	100.00± 0.15	1.000
15	61.10± 0.48	66.70± 0.19	122.10± 0.69	0.9636

Table 3: Curve fit values of muscles for SGOT

Days	Concentration of Copper sulphate			R^2
	2ppm	4ppm	6ppm	
5	0.0± 0.00	15.27± 0.84	15.27± 0.67	1.000
10	15.2± 0.21	30.10± 0.79	49.12± 0.82	0.9221
15	30.1± 0.73	35.00± 0.54	81.25± 0.14	0.9998

Table 4: Curve fit values of Gills for SGPT

Days	Concentration of Copper sulphate			R^2
	2ppm	4ppm	6ppm	
5	0.0± 0.00	33.40± 0.73	66.24± 0.18	0.9895
10	33.4± 0.37	102.48± 0.51	133.74± 0.42	0.9848
15	56.7± 0.45	209.51± 0.84	207.15± 0.39	0.9994

Table 5: Curve fit values of Liverfor SGPT

Days	Concentration of Copper sulphate			R^2
	2ppm	4ppm	6ppm	
5	0.00± 0.00	33.48± 0.84	66.75± 0.47	1.000
10	33.50± 0.74	71.58± 0.73	127.25± 0.15	0.9999
15	66.78± 0.97	140.75± 0.18	278.49± 0.87	0.9985

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Table 6: Curve fit values of Musclesfor SGPT

Days	Concentration of Copper sulphate			R^2
	2ppm	4ppm	6ppm	
5	0.0± 0.00	47.15± 0.77	59.24± 0.47	1.000
10	15.2± 0.17	218.57± 0.53	307.58± 0.85	0.9728
15	30.1± 0.95	397.00± 0.74	524.84± 0.14	0.9423

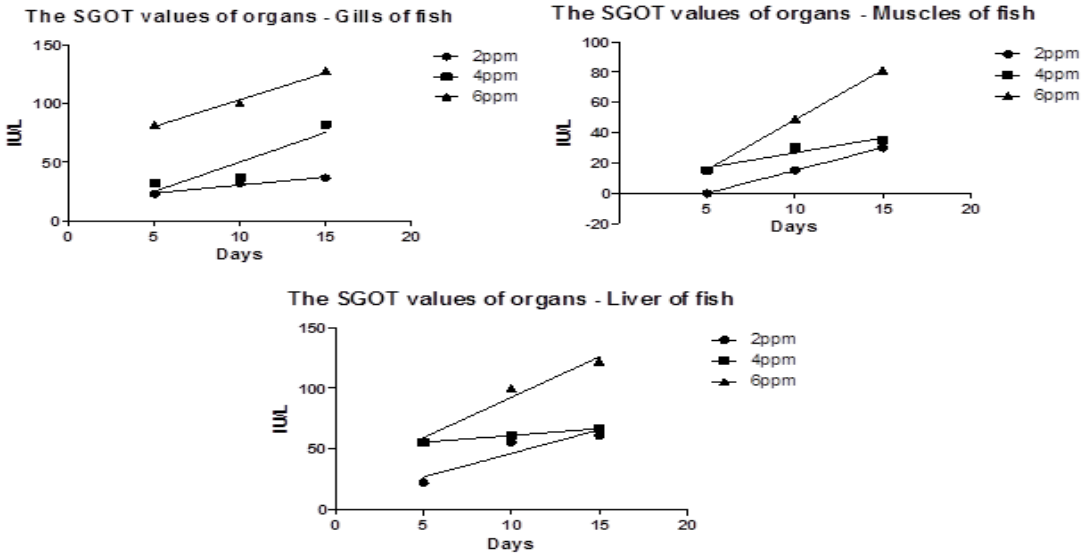

Figure 1: Comparative analysis of the AST on Gills, Liver and muscles

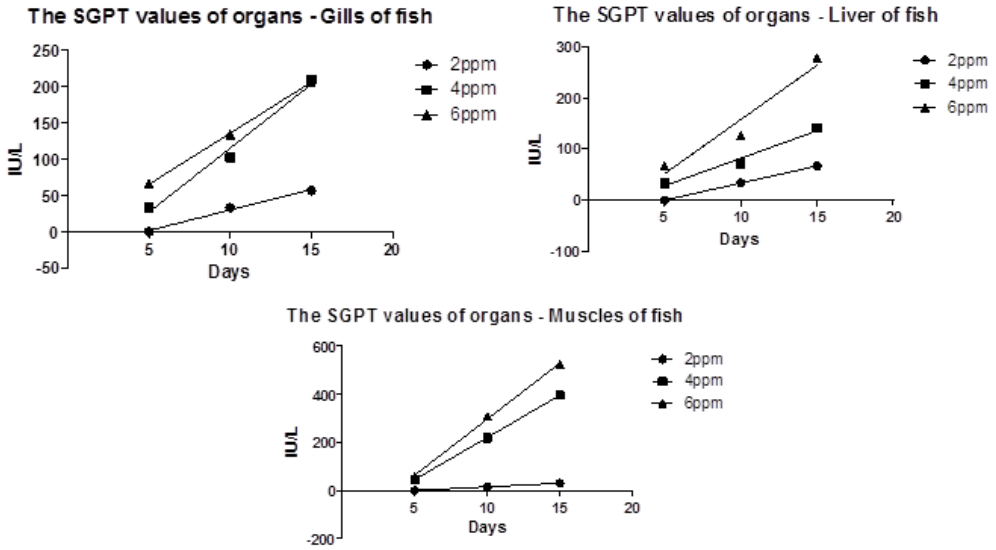

Figure 2: Comparative analysis of the ALT on Gills, Liver and muscles

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