EFFECT OF HEAVY METAL COPPER ON GLYCOGEN AND PROTEIN ALTERATIONS IN THE EDIBLE CLAMS, *MERETRIX MERETRIX*

[1]T. SIVA*, [2]M. SUKUMARAN, AND [3]R. RENGARAJAN,

1&2. P.G. & Research Department of Zoology, Rajah Serfoji Govt. College (Autonomous), Thanjavur, Tamil Nadu, India - 613 005.

3. Department of Zoology, Government Arts College, Ariyalur, Tamil Nadu, India.

Abstract: Heavy metal copper sulphate is the most commonly used fungicides worldwide in the fungal diseases control of crops. Edible clams, *Meretrix meretrix* is an important species in Tamil Nadu region having good nutritional values. Clams living in close association with may accumulate heavy metal. To analyze glycogen and protein content in edible clams, *Meretrix meretrix* exposed under heavy metal copper sulphate. Present study, toxic effects of the heavy metal copper sulphate LC_{50} 10.4µg was observed. The 10% and 30% sublethal concentrations of glycogen and protein contents in the tissue of gill, digestive gland and foot muscle of edible clams *Meretrix meretrix* were estimated. Toxicity effect of heavy metal copper sulphate resulted in decrease of glycogen and protein contents in all concentration of time intervals. There is decreased in all tissues of glycogen and protein contents increasing exposure periods compared with control edible clams *Meretrix meretrix*. Present results indicated the toxic nature of the heavy metal copper sulphate. Therefore it is advised to heavy metal workers to take all precautions regarding protection from heavy metal exposure and proper use of prophylactic supplementation for their healthy life.

***Index Terms*:** Heavy metal copper sulphate, *Edible clams*, *Meretrix meretrix, Glycogen, Protein.*

I.INTRODUCTION

Estuarine invertebrates such as crustaceans and bivalves differ in their update and accumulation of heavy metals[1,2&3]. An estuarine bivalve namely clams and mussels have been used as very useful experimental organisms to assess the toxic effects of heavy metals. These animals are capable of tolerating variable conditions provided in the laboratories. The capability of bivalves to function normally under laboratory conditions have made them potential experimental animals to assess the toxic metal pollution which varies over space and time in and between aquatic habitats[4].

Any information on the biochemical composition is helpful in assessing the nutritive value of organisms. In the changed scenario of increased environmental pollution, changes in biochemical constituents of biota can be used as a convenient index or tool to assess the degree of impairment caused by the pollutants

on the organisms. No doubt, certain heavy metals are quite essential in trace quantities, but when the concentration exceeds the required level, they disrupt the metabolic processes, and thus altering physiological stat of the organisms. Under metal contaminated environmental conditions, the concentrations of heavy metal in organisms can increase considerably to which the aquatic organisms have not previously been exp0osed. Under such unfavorable conditions the organisms develop certain adaptive methods such as mobilization of energy from reserves to tide over the crisis and to protect themselves[5].

Investigations on toxic effects of heavy metal to estuarine invertebrates are a field of ecotoxicology which has gained momentum in recent years[6,7&8]. Among estuarine invertebrates bivalve molluscs have assumed a major role in assessing levels of toxicants worldwide. This is a result of strategic advantages in terms of case of collection, cosmopolitan distribution, relatively sedentary habits, suitable size and often, ecological and economic importance. On the basis of heavy metals, bivalves have involved a number of subcellular systems for accumulation and storage of the excess of essential and non essential metals[9].

Heavy metals like zinc (Zn), Nickel (Ni) and Chromium (Cr) are potentially harmful not only to prawns, shrimps, clams and mussels but also to human beings who ultimately consume these food items[10&11]. Among estuarine biota, bivalves are desirable organisms for Biomonitoring purposes. A biomonitor is a biological response (for example a bio–chemical, cellular, physiological or behavioural variation) that can be measured in tissue or body fluid samples or at the level of whole organism, that provide a measure of exposure to and for effects of a toxicant[12&13].

The use of biomonitors in environmental toxicology is becoming increasingly important. The need to detect and assess the impact of pollutants at low sub lethal concentrations, on environmental quality has led to the development of a range of biomarkers measured in a number of different species living in estuarine and coastal waters. Prior to death or over sickness, organisms may respond to stress by changing molecular physiological or behavioural responses. The ability to recognize and measure these changes, defined here as biomarkers may provide an early warning of later, much more serious consequences[14].

Biochemical alternations in the content of biomolecules (Glycogen, Proteins and Lipids) found in the body tissues of animals may be due to the exposure of heavy metals and also to the stressful conditions followed thereafter[15&16]. In the present investigation envisaged some biochemical studies of two important tissues namely the gills which are copiously bashed by external medium resulting in considerable water tissue contact and the digestive diverticular an internal organ which gets the impact of heavy metal toxicity via water or food. In this chapter, biochemical analysis has been conducted, since stress caused by metal toxicity induces alterations in biochemical composition. The change in biochemical composition is an indicator of stress of chemical nature in the surrounding which mainly effects biomolecular contents.

Present study, an experiment was designed to ascertains the biochemical alterations to tissue carbohydrates and protein in the edible clams, *Meretrix meretrix* to two sublethal ($1/10^{th}$ and $1/30^{th}$ of LC_{50}/96) contraptions of heavy metal $CuSo_4$ at three tissues 5, 10, and 15 days.

II. MATERIALS AND METHODS

Edible clams, *Meretrix meretrix* with uniform body length (70–80 mm) were collected from the "clam bed" (60x60 cm) which consested of specimens *M. meretrix* sampled from stations I and II of Muthupet estuary during 2012 – 2013. Clam samples were brought to the laboratory in plastic buckets with estuarine water. clams were maintained in glass circular pneumatic troughs (40 x 30 x 30 cms), each containing 15 clams and 10L of freshly collected estuarine water for the days of acclimation to the laboratory conductions (Temp: 26 ± 2ºC; pH: 7.8 ± 0.3). The medium water was renewed daily to avoid faecal matter clams were fed with algal food ad *libitum*.

Long term (Chronic) accumulation Study

Based on the 96 hrs LC_{50} values (10.4µg for Cu) two sublethal concentrations *viz* $1/10^{th}$ and $1/30^{th}$ were chosen for the metals Cu. Experimental media were prepared with faltered estuarine water. Test media were renewed once every 24h. Water was well aerated and the media were prepared a fresh daily. A group of 10 clams was exposed to the 2 sublethal concentrations (5 each metal) of Cu individually for a period of 15 days and an equal number of animals of the same size served as controls and were maintained in circular glass pneumatic trough felled well faltered estuarine water. All the few clams after the expiry of 5, 10, and 15 days of exposure from each concentration and an equal number of control were sacrificed. Selected body tissues *viz*. Gills, digestive gland and food. Were dissected out from the specimens, washed properly in double distilled water, kept in an oven at 110^0C up to 24 hrs and then digested as per the methodology described by Kamaru Zzaman *et al*.[17]. Copper contents were estimated by Atomic Absorbtion Spectrophotomrter (Peren Elmer). The values of heavy metal concentrations were calculated based on day weights and expressed as mg/gm dry wt. Quantitative estimation of glycogen determined by following methods[18]. Protein content in the selected body tissues was determined following the method[19].

III. RESULTS

In the present study, effect of copper on the biochemical constituents of gill, digestive gland and foot muscle of *M. meretrix* were presented in the Tables 1 and 2. Glycogen content was found to be decreased in the tissues of gill, digestive gland and foot muscle exposed to sublethal concentrations of heavy metal copper sulphate for the period of 5, 10 and 15 days exposure (Table 1 and Fig. 1-3). The amount of protein content were decreased with increasing sublethal concentrations of heavy metal copper sulphate for the period of 5, 10 and 15 days exposure, when compared to control clams (Table 2 and Fig. 4-6).

Table 1. Glycogen content in selected body tissues of *Meretrix meretrix* exposed to two sublethal concentration of Cu So_4

Body Tissue	Days	Control	Sublethal Concentration	
			1/10 (1.04µg)	1/30 (3.12µg)
GILLS	5 days	4.80±0.33 (100.0)	3.40±0.21 (70.83)	2.15±0.10 (44.80)
	10 days	4.00±0.15 (100.0)	2.70±0.06 (67.50)	1.24±0.32 (31.00)
	15 days	3.16±0.12 (100.0)	1.75±0.26 (55.38)	0.92±0.05 (29.12)
DIGESTIVE GLAND	5 days	4.83±1.04 (100.0)	3.86±0.10 (79.12)	3.06±0.16 (63.36)
	10 days	4.79±0.33 (100.0)	3.56±0.13 (74.32)	2.06±0.11 (54.28)
	15 days	3.96±0.16 (100.0)	3.14±0.25 (79.29)	1.84±0.08 (46.46)
FOOT MUSCLE	5 days	4.20±0.19 (100.0)	3.4±0.21 (81.42)	2.60±0.14 (61.90)
	10 days	3.46±0.12 (100.0)	2.75±0.06 (79.48)	1.90±0.13 (54.91)
	15 days	2.16±0.14 (100.0)	1.77±0.26 (81.94)	1.06±0.36 (49.07)

Values are the means of five observations values expressed as mg/100 gm dry weight of the time values in parentheses are % of change over control

Table 2. Protein content in selected body tissues of *Meretrix meretrix* exposed to two sublethal concentration of Cu So_4.

Body Tissue	Days	Control	Sublethal Concentration	
			1/10 (1.04µg)	1/30 (3.12µg)
GILLS	5 days	68.60±2.84 (100.0)	58.13±2.12 (84.74)	49.95±2.05 (72.82)
	10 days	65.14±2.16 (100.0)	62.36±2.04 (95.74)	46.50±1.92 (71.38)
	15 days	63.05±1.69 (100.0)	60.98±1.49 (96.72)	45.28±1.43 (71.87)
DIGESTIVE GLAND	5 days	56.90±1.59 (100.0)	50.84±1.50 (89.35)	44.28±1.43 (77.82)
	10 days	52.10±1.23 (100.0)	49.28±1.48 (94.58)	42.99±1.78 (82.52)
	15 days	51.13±1.53 (100.0)	46.27±1.46 (90.49)	40.33±1.52 (78.87)
FOOT MUSCLE	5 days	18.78±1.24 (100.0)	15.48±1.20 (82.43)	13.08±1.20 (69.64)
	10 days	15.70±1.16 (100.0)	13.80±1.13 (87.89)	11.50±1.21 (53.48)
	15 days	14.70±1.20 (100.0)	11.00±1.12 (74.82)	9.44±1.44 (64.21)

Values are the means of five observations values expressed as mg/100 gm dry weight of the time values in parentheses are % of change over control.

Fig. 1. Glycogen content in the gill tissues of *Meretrix meretrix* exposed to the two sublethal concentrations of Cu So_4 (N = 5).

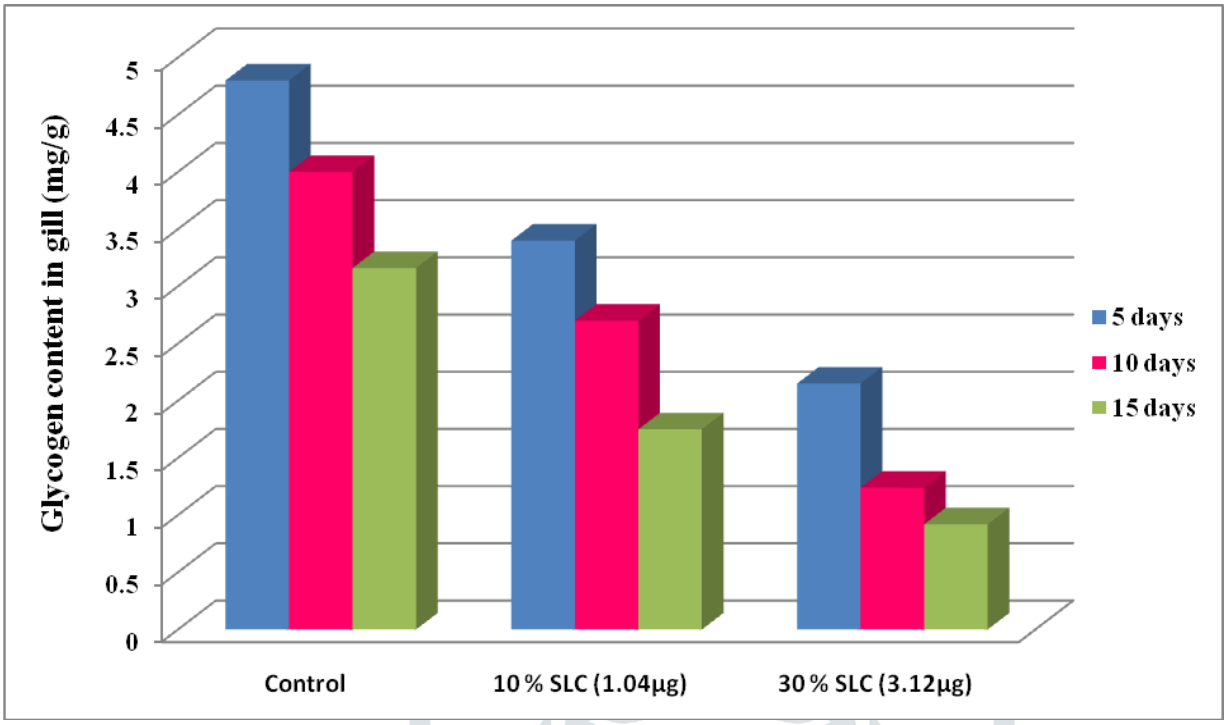


Fig. 2. Glycogen content in the digestive gland tissues of *Meretrix meretrix* exposed to the two sublethal concentrations of Cu So_4 (N = 5).

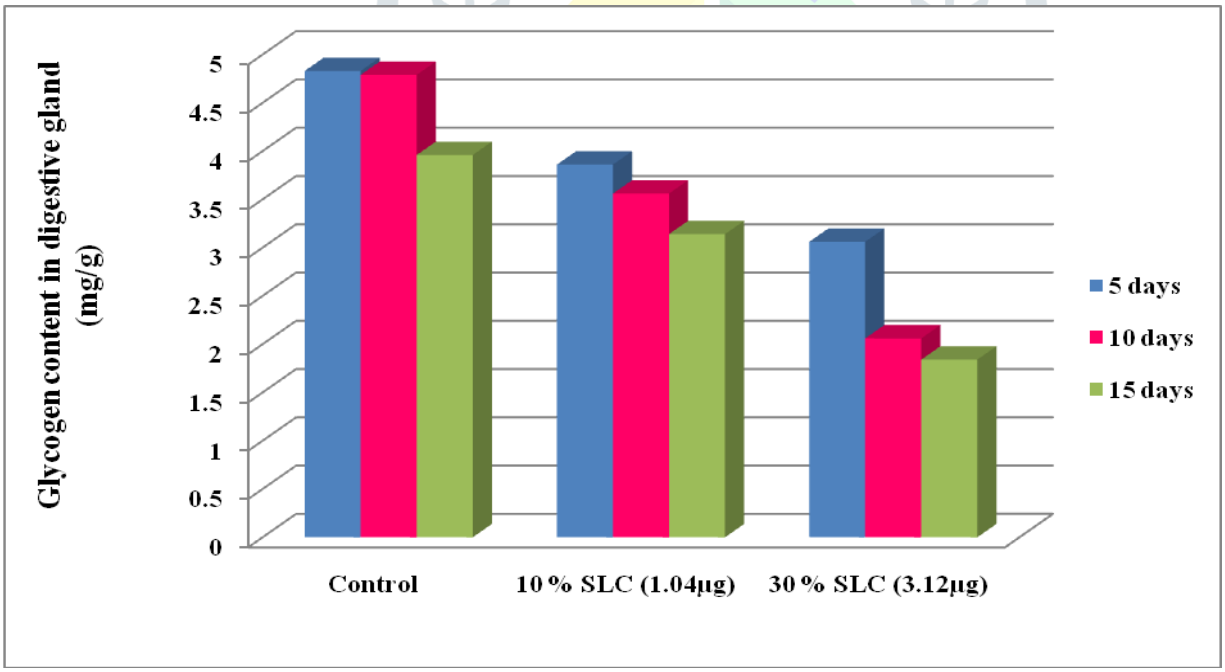


Fig. 3. Glycogen content in the foot tissues of *Meretrix meretrix* exposed to the two sublethal concentrations of Cu So_4 (N = 5).

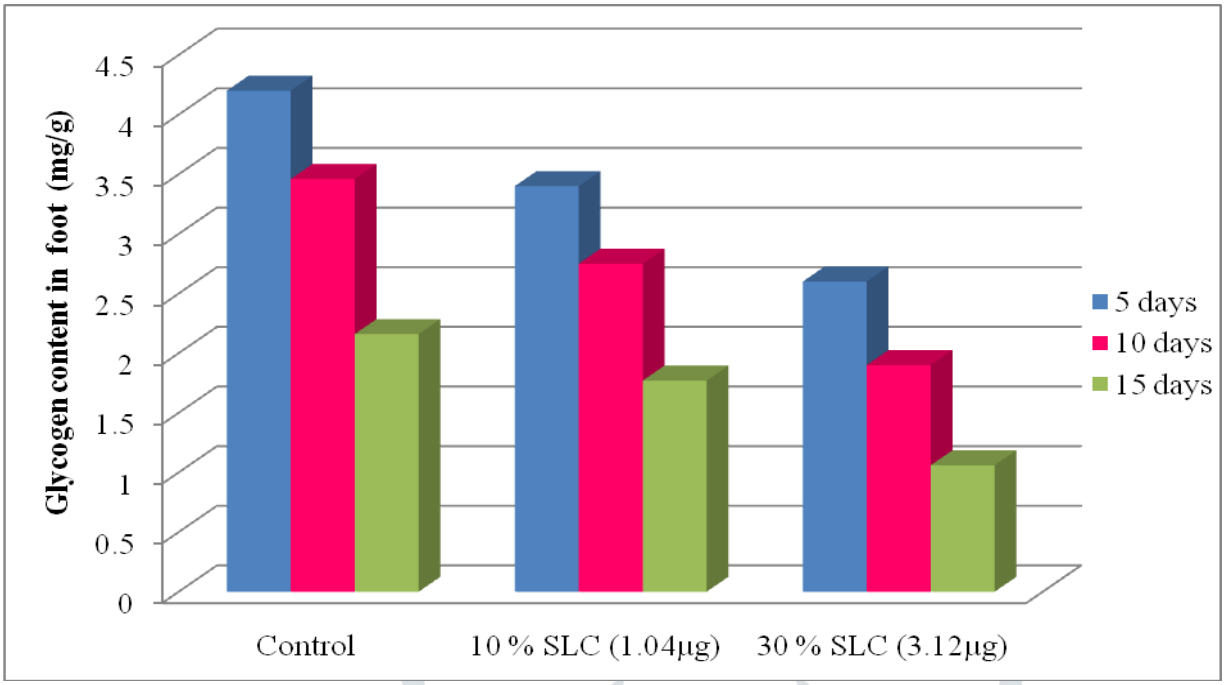


Fig.4. Protein content in the gill tissues of *Meretrix meretrix* exposed to the two sublethal concentrations of Cu So_4 (N = 5).

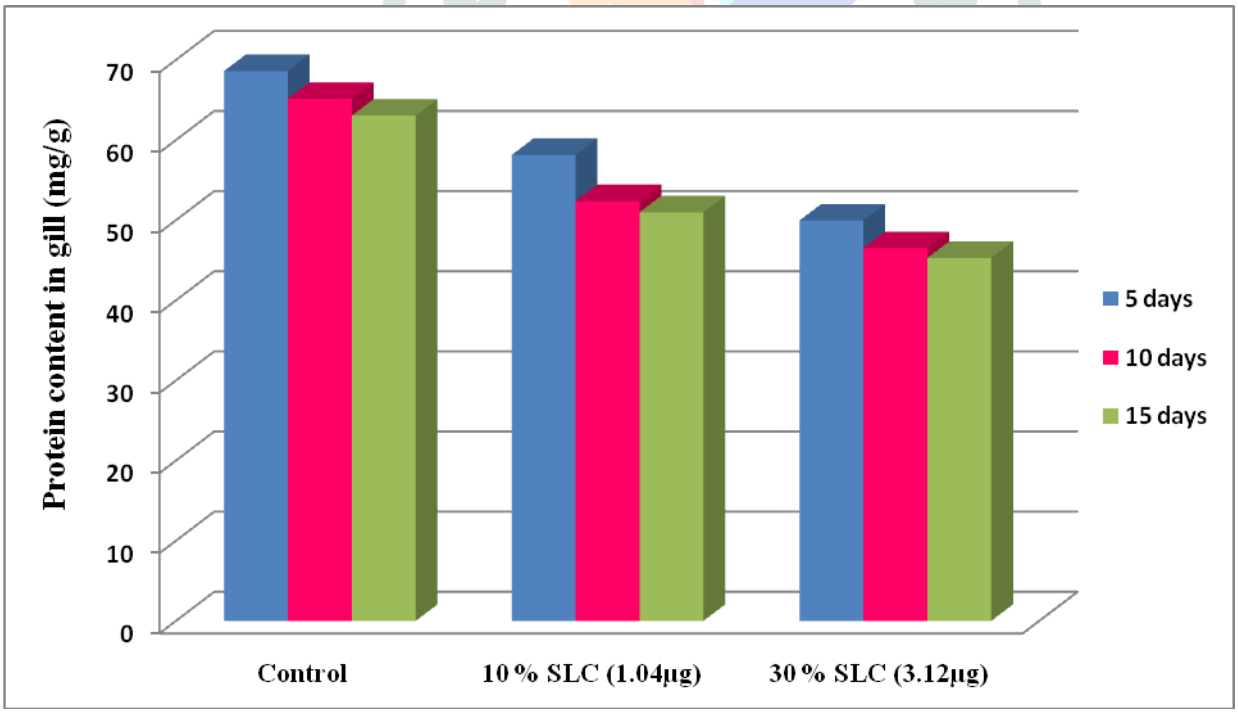


Fig.5. Protein content in the digestive gland tissues of *Meretrix meretrix* exposed to the two sublethal concentrations of Cu So_4 (N = 5).

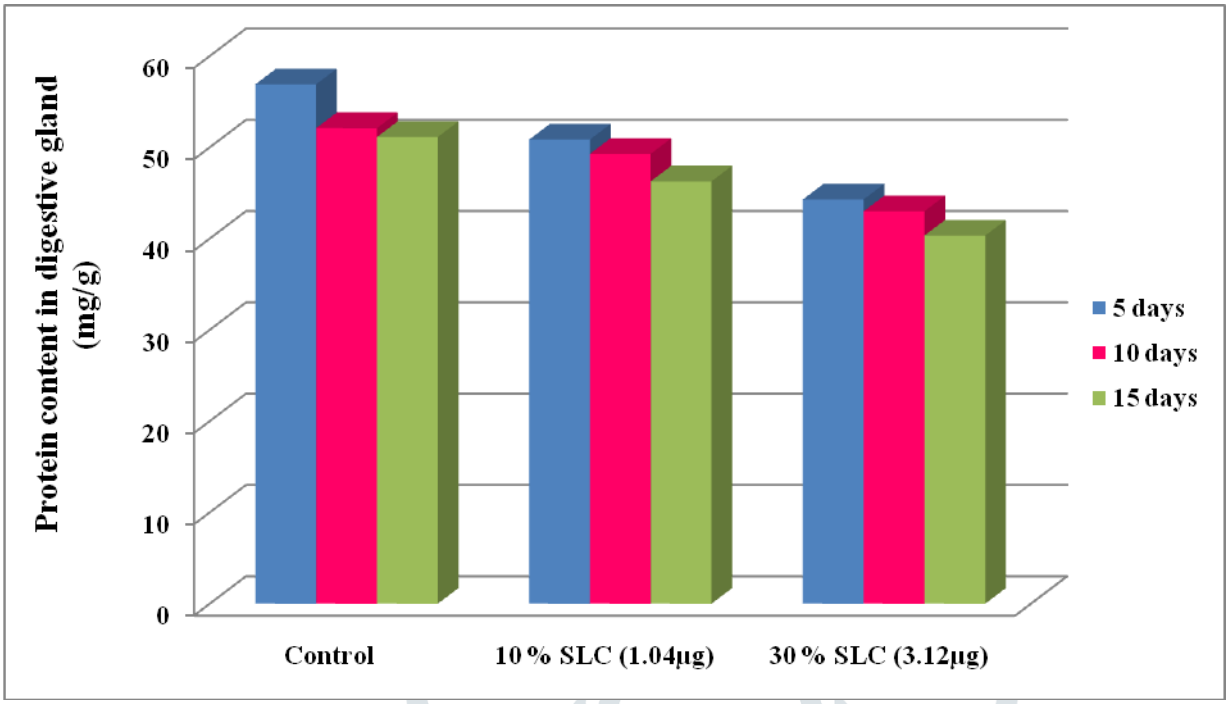
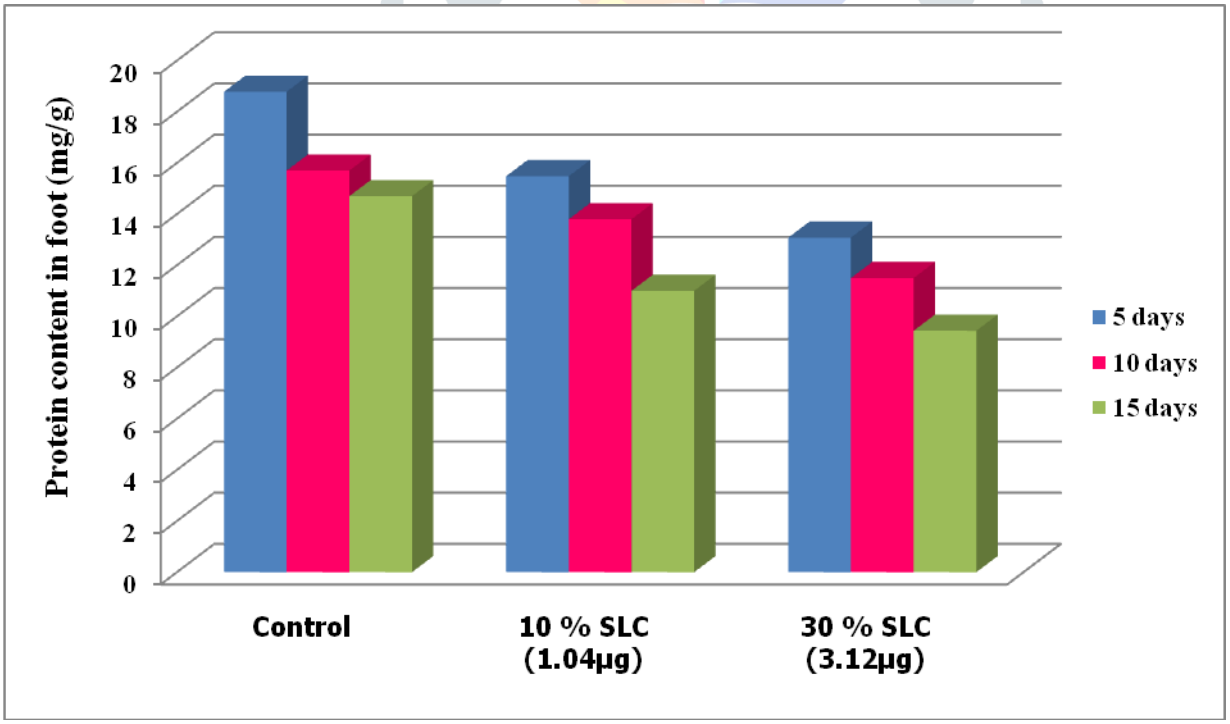


Fig.6. Protein content in the foot tissues of *Meretrix meretrix* exposed to the two sublethal concentrations of Cu So_4 (N = 5).



IV. DISCUSSION

In the present investigation the impact of heavy metals copper sulphate on the biochemical constituents like glycogen and proteins were studied. The amount of glycogen content in the edible clams *Meretrix meretrix* different tissues off gill, digestive gland and foot muscle were found to be decreased exposed to sublethal concentration of heavy metal copper sulphate for the period of 5, 10 and 15 days exposure. In the earlier worker documented that decrease in the glycogen content may be due to enhanced breakdown of glycogen to glucose through glycogenolysis in the fish tissues to withstand the existing stress condition, mediated by catecolamine and adenocortical harmones[20]. Alterations may be due to rapid utilization of glycogen to meet the energy demands under stress condition and supply energy demand in the form of glucose which undergoes breakdown to produce energy rich compound ATP through glycolytic pathway as suggested by Omkar *et al*.[21].

Sandhya and Mayur[22] noted glycogen content in freshwater bivalve, *L. marginalis* was altered indicating the effects of heavy metals. The average glycogen content in acute and chronic treatment by heavy metal copper sulphate was decreased in the whole body. The depletion of glycogen content was greater in the digestive gland as compared to the foot and mantle of the bivalve, when exposed to pollutants. The greater breakdown of glycogen may suggest the need of high energy to animal in stress conditions caused due to pollutants.

In the present study, the protein content was observed from edible clams *Meretrix meretrix* treated sublethal concentrations of copper sulphale (10% & 30%) for 5, 10 and 15 days showed a decreasing trend in the protein content in the tissues of gill, digestive gland and foot muscle when compared to control. In the similar reporter estimated protein concentration in the tissues of liver, gill, kidney, ovary and testis were found to be reduced sublethal concentrations of zinc during the exposure periods[23]. Reduced protein level was observed in the pesticide exposed fish liver[24].

Hameed and Muthu [25] studied two sublethal concentrations (10% and 30% of LC_{50}) of Cd for a period of 96 hrs on the fish *Oreochromis mossambicus*. The protein content of fish decreased with time due to increased Cd concentrations. A decline in the protein content was 24.3 to 25.5 percent. James *et al*.[26] reported toxic effects of Cu, Zn and Cd in *Oreochromis mossombicus*. Cu was most toxic followed by Zn and Cd. A significant decrease in protein was observed in muscles, liver, gill and the whole body of *O. mossambicus* exposed to metals individually and in combinations except when exposed to Cu alone.

V. CONCLUSION

Present study indicates that presence of low concentration of heavy metal copper sulphate in the water is toxic to fishes and alters the glycogen and protein of the edible clam tissues. This result indicates that the

usage of the heavy metal copper sulphate fungicide 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 heavy metal contamination in aquatic ecosystem.

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