STUDIES ON TOXICITY EFFECT OF HEAVY METAL ZINC ON HISTOLOGICAL ALTERATIONS IN THE ESTUARINE MUD CRAB, *Scylla serrata*

[1] P. RAMYA, [2]M. SOUNDARARAJAN, [3]D. MERIN EMERALD,

[4]P. SENTHAMILSELVI, AND [5]S.R. MANIKANDAN

1. Department of Zoology, Tamilavel Umamaheshwaranar Karanthai Arts College, Karanthai, Thanjavur, Tamilnadu, India - 613 002.

2, 3, 4 & 5. P.G. & Research Department of Zoology, Rajah Serfoji Government College, Thanjavur, Tamilnadu, 613 005.

Corresponding author: ***Dr. P. Ramya***, *Asst. Professor & Head, Department of Zoology Tamilavel Umamaheshwaranar Karanthai Arts College, Karanthai, Thanjavur, Tamilnadu, India 613 002.*

Abstract: Impact of heavy metal is common pollutants of estuarine ecosystems where they induce adverse effects on the aquatic biota. Estuarine mud crab, *Scylla serrata* is an important carb species in Tamil Nadu region having good nutritional values. Crabs living in close association with may accumulate heavy metals. In the present investigationLC_{50} values were determined for metals zinc toxicity in the mud crab, *Scylla serrata* when subjected to varying concentrations (0.5 to 0.80 mg/L). LC_{50} values of 96 hrs exposures was 0.43 mg/L. Increased ACP activity was noticed in the gills, abdominal muscles and hepatopancreas of estuarine mud crab, *S. serrata* treated with sublethal (15%) concentration of $ZnCl_2$ on 5, 10 and 15 days of exposures. The present work on histological observation was carried out to know lesions in gill, abdominal muscle and hepatopancreas that had resulted from 15% sublethal concentration of heavy metal zinc exposed to estuarine mud crab, *Scylla serrata.*

Terms Index: *Estuarine mud crab, Scylla serrata, Zinc, Gill, Abdominal muscle, Hepatopancreas.*

I.INTRODUCTION

Heavy metals are potential pollutants in aquatic bodies. The presence of heavy metals in water and sediments of rivers and estuaries can lead to greater environmental problems when the contaminated water and sediment loaded metals are uptaken by molluscs and crustaceans which are purely filter feeding invertebrates[1&2].

Heavy metals such as zinc and lead are soluble in water, tending to adsorb onto the suspended particulate matter in the aquatic bodies and affect aquatic organisms. Toxic effects do not normally manifest themselves after the toxin enters the environment and organisms they usually become apparent only after a few years[3]. The duration of an organism's exposure to heavy metals has a significant effect on their bioaccumulation; sometimes, despite a relatively short exposure period, the amount of metal deposited in an organism may be considerable[4].

The processes by which metals accumulate in the tissues and organs of crustaceans are species dependent and are related to the mechanism of detoxification and metabolism. The accumulation of heavy metals at different levels of trophic structure causes serious problems as far as the health of aquatic environment is concerned. In these circumstances, the selection of suitable crustacean species such as prawns, crabs etc., could be used as biomonitors[5&6].

Studying the effect of heavy metals on the mud crab *Scylla serrata* many reports have pointed out pathological manifestations[7&8]. Sarojini *et al.*[9] conducted histopathological studies to find out the adverse effects of metal exposure to ovarian development in the fresh water crab *Barytelphusa guerini*. Lawson *et al.*[10] studied the ultrastructural changes in the gill epithelium of *Carcinus maenas* after exposure to copper. Kalita *et al.*[11] studied the behavioral and hispathological changes in the gill tissue of the freshwater crab *Paratelphusa spinigera*.

II. MATERIALS AND METHODS

Estuarine mud crab, *Scylla serrata* were collected from Keelathottam near Agniar estuary mallippattinam. Following collection, the animals were carefully transported to the laboratory and maintained for a couple of days in natural estuarine water. Healthy, crabs having equal size (30–35 mm carapace length and 70–75 gm, weight) were used for experimentation. Stock solution of Zinc and lead were prepared by dissolving appropriate amount of salt in distilled water. Physico-chemical characteristic of test water have analyzed regularly during the test periods following the standard method describe by APHA[12]. Batches of 10 healthy crabs were exposed to different concentrations of heavy metals zinc and lead to calculate the medium lethal concentration LC_{50} value (0.43 mg/L) using probit analysis Finney method[13]. The fishes (Four groups) were exposed to 15% sublethal concentration of heavy metal zinc for 5, 10 and 15 days respectively. Another group was maintained as control. At the end of each exposure period, fishes were sacrificed and tissues such as gill, hepatopancreas and muscle were dissected and removed. The major steps involved in histopathological analysis are fixation, tissue processing, decalcification, section cutting[14] and staining[15]. For histological analysis section were cut at 5- 6μm thickness and stained with Haematoxylin and Eosin. After stained the slides were examined under light microscope and photographed (Labomed)

III. RESULT

Bioassay toxicity test

LC_{50} values were determined for metals zinc toxicity in the mud crab, *Scylla serrata* when subjected to varying concentrations (0.5 to 0.80 mg/L). LC_{50} values of 96 hrs exposures was 0.43 mg/L.

Histological studies in selected body tissues of mud crab, *Scylla Serrata* due to sublethal toxicity of metal, zinc

Normal gill histology of mud crab, *Scylla serrata*

The gills of mud crab, *Scylla serrata* are constituted by a series of broad flattened plates called lamellae. These are arranged serially in pairs along a central stem or bar. The gill at its outer surface is enclosed by a thin layer of chitin. A basement membrane lies at the border of gills. Basement membrane together with serially arranged lamellae form the architect of the gills (Plate 1).

Gill histology under acute exposure

In the crabs treated with acute sublethal toxicity of zinc, the gills exhibited degeneration of gill lamellae, shrinkage of gill lamellae, abnormal gill tips, accumulation of haemocytes, disintegration of epithelium, necrosis and vacuolation in the cytoplasm at 15% sublethal concentrations for 5, 10 1nd 15 days exposure respectively (Plate 1)

Normal abdominal muscle of mud crab, *Scylla serrata*

In control crabs, the abdominal muscle was formed of myocytes and myofibrils. Myocytes contained several fibers called myofibrils myocytes and multinucleate myofibrils assemble on a basement membrane (Plate 2).

Abdominal muscles histology under acute exposure

Abdominal muscles of mud crab, *S. serrata* treated with sublethal doses of zinc for 96 hours exhibited structural changes. Myofibrils or muscle filaments become degenerated while myocytes, atrophy, edema, splitting of muscle fibers, degeneration of muscle bundle were noted at 15% sublethal concentration of zinc treatments for 5, 10 and 15 days respectively (Plate 2).

Normal hepatopancreas of mud crab, *Scylla serrata*

In control crabs, the hepatopancreas is found in the cephalothoric cavity and yellowish brown in colour. It is composed of tubules covered with epithelial cells, B–cells, E–cells, R–cells and F–cells. They were concentrated in the distal tips of tubules. All these cell types are secretory in function, though they are highly differentiated in structure. Among the four cell types, R–cell are most abundant and storage in function (Plate 3).

Hepatopancreas histology under acute exposure

In 15% sublethal concentration of zinc treated crab were observed degeneration of hepatocytes of the crab, *S. serrata* was noticed while clumping of cells was evident in medium and atrophy of hepatopancreas tubular filaments, tissue debris, necrotic cells were also observed by the higher a sublethal concentration (15% of zinc) for 5, 10 and 15 days exposure period respectively (Plate 3).

Plate 1. Normal and 15% (SLC) zinc treated gill in the mud crab, *Scylla serrata* exposed to 5, 10 and 15 days exposure.

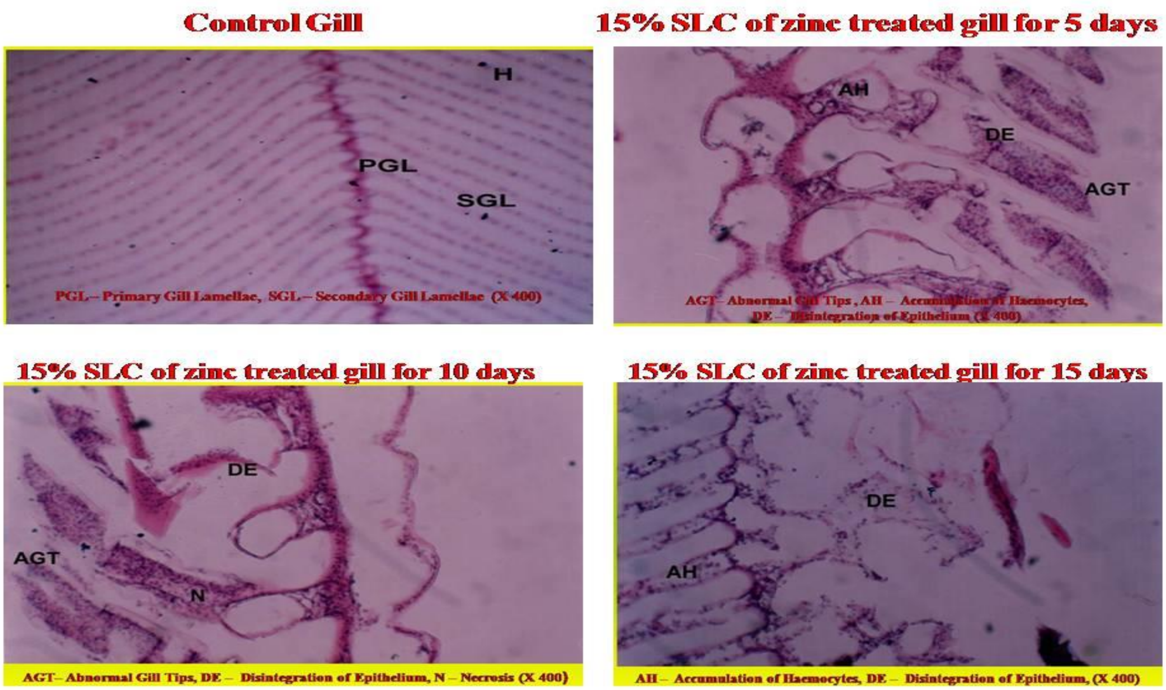


Plate 2. Normal and 15% (SLC) zinc treated abdominal muscle in the mud crab, *Scylla serrata* exposed to 5, 10 and 15 days exposure.

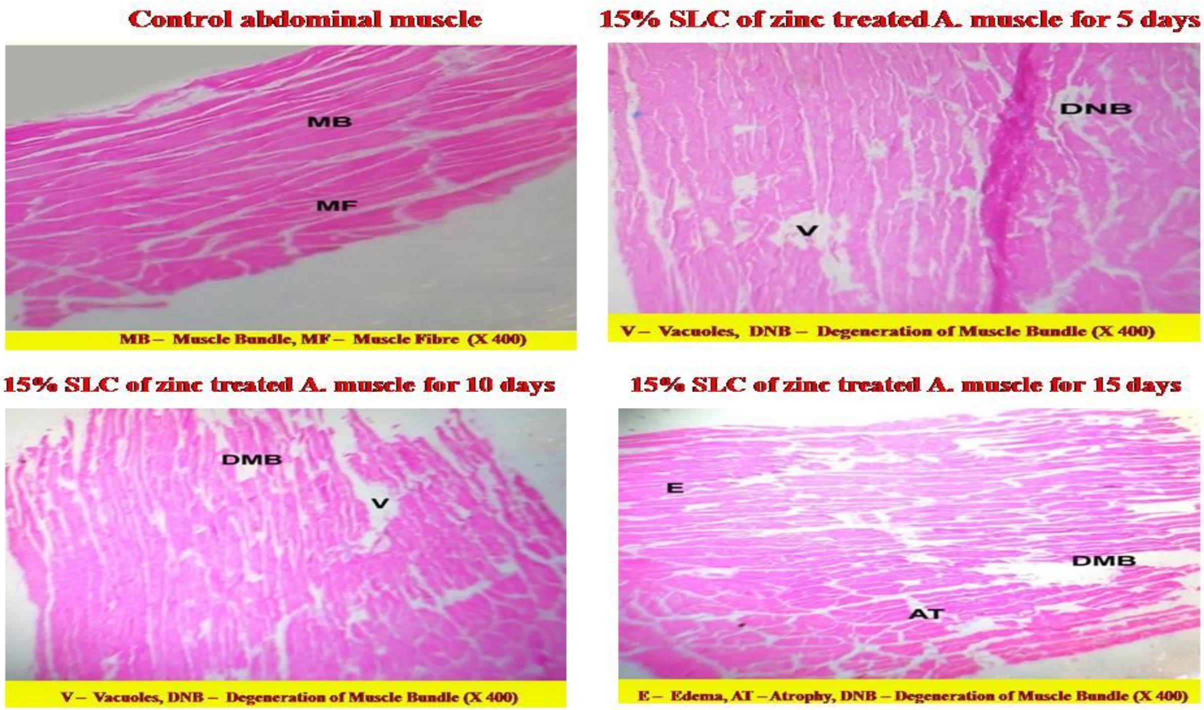
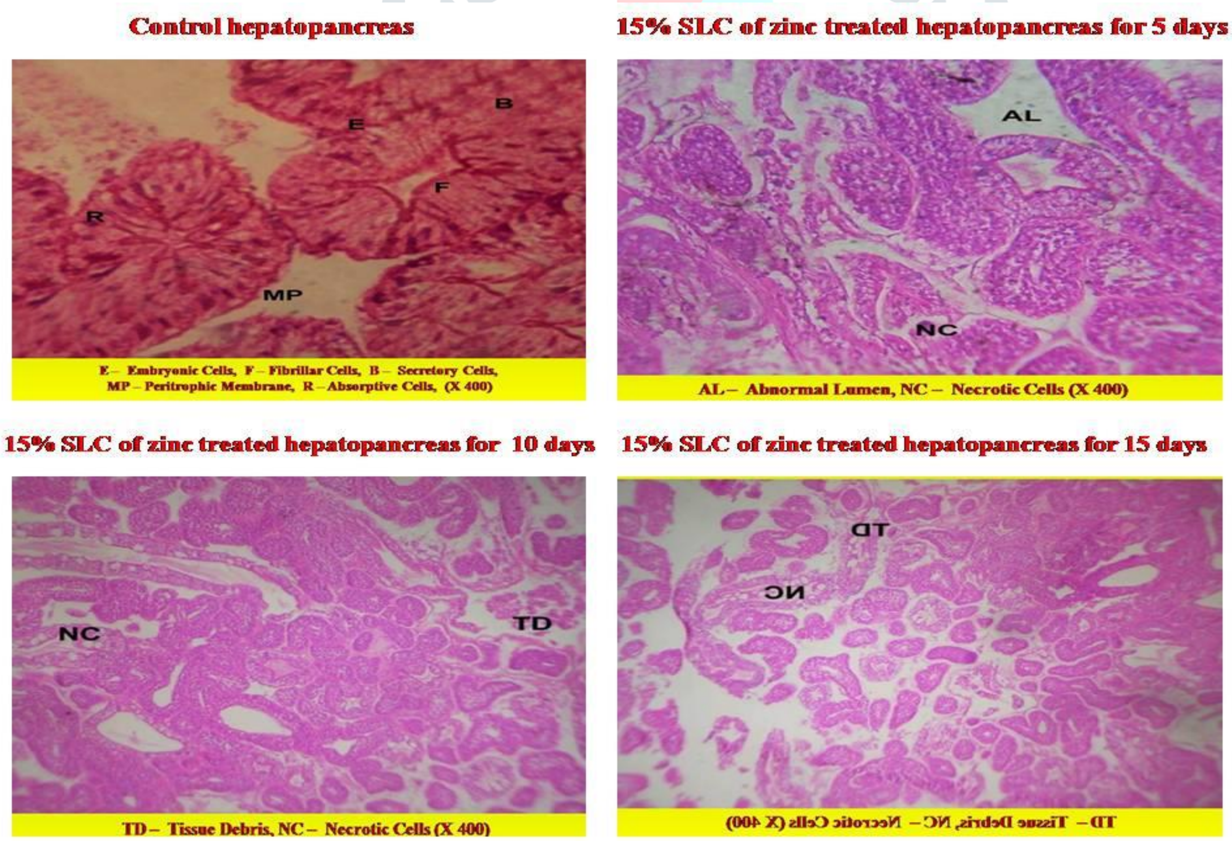


Plate 3. Normal and 15% (SLC) zinc treated hepatopancreas in the mud crab, *Scylla serrata* exposed to 5, 10 and 15 days exposure.



IV. DISCUSSION

In the present study LC_{50} values of heavy metal zinc of estuarine mud crab *Scylla serrata* at 96 hours LC_{50} were 0.43 mg/L. and sublethal concentration 15% value was selected, studying their effects on biochemical aspects. Histological biomarkers of toxicity in animal tissues are a useful indicator or environmental pollution[16&17]. Several histopathological investigations have been reported in the gills, liver, muscles, kidneys, etc. of aquatic animals. In the present study, a wide spectrum of histopathologies was revealed in the gills, abdominal muscles and hepatopancreas of mud crab, *Scylla serrata* after exposures to 15% sublethal concentration of heavy metal zinc chloride.

The gills, having large surface area and being directly exposed to environmental changes, are the prime target of pollution. The accumulation of zinc and lead by the gills results in morphological changes within the cells, which suggest effects on the functions of the organelles. The gills have been reported to be the main sites of absorption of heavy metals present in the medium[18] were the other indications of metal toxicity.

Specific groups of toxicants have been linked to a number of histological alterations in gills. Several investigations have reported histopathological changes in the gills of different fish species exposed to heavy metals[19] in European are *Anguilla Anguilla.* Histopathological changes in the gills were observed in the fish *Fundulus heteroclitus* exposed to Cadmium Gardner *et al.*[20] in *Tilapia nilotica* exposed to lead acetate, mercuric chloride and cadmium chloride[21] and in turbot, *Scophthalmus maximus* exposed to Cu, Cd and Zn[22]. Similar observations were made by Usharani[23] in *Scylla serrata* exposed to zinc and lead and Shagnas Banu[24] in *Scylla serrata* exposed to mercury.

As crab's gills are critical organs for their respiratory and osmoregulatory functions, the injuries in gill tissues of the crab, observed as a result of sublethal exposures of metals, zinc and lead reduced the oxygen consumption and resulted in the disruption of the osmoregulatory function of the crab. As the gills are the major sites of osmotic and ionic regulation in crab, any change in gill histology may result in perturbet osmotic and ionitic status which was observed as decreased ATPase activity in the present study. Also, the histopathological alterations could be attributed to increased peroxidative damage to gill membrane in crabs exposed to a variety of pollutants such as pesticides and metals[25].

Significant deformations in the structure of the abdominal muscles of the mud crab, *Scylla serrata* were noticed, when the crabs subjected to lethal (acute) and sublethal (chronic) exposures of both metal zinc. Acute toxicity included rupturing of basement membrane, disintegration of myocytes and vacuolation in myofibrils, while chronic metal toxicity resulted in dissolution of myocytes, necrosis and atrophy of myofibrils and cytoplasm of myocytes. Similar observations were reported by Palaniappan *et al.*[26] in muscle tissues of freshwater fish *Catla catla*; Agah *et al.*[27] in five fishes of Persian Gulf and Khan *et al.*[28] in the muscles and gills of African catfish *Clarias batrachus*. Rupturing of the basal membrane of muscular tissues, disintegration of muscle cells, atrophy, necrosis and vacuolation were the significant changes studied by there authors and such muscular histological changes fall in line with the observations made in the abdominal muscles of the crab, *S. serrata* of the present study.

As Sublethal doses caused destructive effect in the gills, abdominal muscles and hepatopancreas tissues of *S. serrata*. These results are in agreement with earlier studies by Sujatha *et al.*[29] in *Liza parsia*; Dhanapakkiam and Premalatha[30] in *Cyprinus carpio* and Deore and Wagh[31] in *Channa gachue*. The observed respiratory behaviour and altered histopathology of vital organs demonstrate the severe adverse effects to exposure of metal toxicity in *S. serrata*.

Hepatopancreas being the main organ of various key metabolic pathways, toxic effects of chemicals usually appear primarily in hepatopancreas[24]. In the present study, the hepatopancreas showed changes in their constituent cells (B, E, F and R cells) in sublethal concentrations of zinc and lead and cells were found clumped and intercellular spaces invisible in the metal sublethal concentrations and a general degeneration, loss of tubules structures, vacuolation and necrosis of cells in the high concentrations of both zinc and lead exposed *Scylla serrata*. Similar observations were observed in the *Scylla serrata* exposed to zinc and cadmium, lead, arsenic and selenium as reported by Krishnaraja *et al.*[32] and Krishnamoorthy *et al.*[33] also reported changes such as elongation of hepatopancreatic cells and shrunken cells in *Macrobrachium lamarrei* exposed to low (0.0065 ppm) and high (0.0215 ppm) concentrations of copper. Stressor – associated alterations clumping of cells, or loss of cells or both may be found in the internal structure of hepatopancreas of *S. serrata* exposed to sublethal toxicity of metal zinc.

V. CONCLUSION

Present study revealed that the heavy metal zinc is potent to cause toxic responses, even structural alterations, in aquatic organism like fish. The results indicate that the usage of the zinc in the agriculture fields may be a threat to aquatic fauna and flora as well as humans. This findings of the present histological investigations demonstrate a direct correlation between exposure to zinc compounds and histopathological observations indicated that exposure to sublethal concentration of Zn. It is also recommended that before using heavy metal zinc in any aquaculture processes, the estimated safe and dischargeable concentrations should be considered important to protect living organisms as well as fish.

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