

Effect of Heavy Metals on Gills of Fresh Water Bivalve *Lamellidens marginalis*.

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Abstract: *The effects of heavy metals on the histopathology gills of the fresh water bivalve *L.marginalis* was investigated. Samples were collected from the Godavari river at paithan. The bivalves were exposed to 1.6 ppm CuSO_4 , 0.6 HgCl_2 and 3.9 ppm CdCl_2 for 72 hours acute treatment. For chronic exposure were exposed to 0.82 ppm CuSO_4 , 0.32 ppm HgCl_2 and 1.95 ppm CdCl_2 . Histopathological changes observed in the gills were edema and lifting of lamellar epithelia, lamellar degeneration, necrotic changes in intercellular epithelial cells. This research simulate a reliable indicator of the aquatic ecosystem contamination and the possible negative impact of the surrounding environment*

Keywords: *Lamellidens marginalis, gills, histopathology heavy metals*

I. Introduction

Godavari river is an important river in India and it flows from western to southern India. The river is 1,465 km long and ranks as the second longest river in the country. Paithan is located 56 kms south of present day Aurangabad on the Godavari river. In last few decades increase in population density, heavy industrialization and agricultural activities have resulted in more and more waste entering in fresh water resources.

There are over 6500 species of marine bivalves in the phylum Mollusca (Robertson, L.J. 2007). Marine bivalves are known to be natural unique accumulators of contaminants (Madkour, H.A. 2005). There is an increased body of evidence implicating heavy metals as a potential threat to aquatic organism by way of studies on their physiology, behavior, biochemistry and ecology (V.R.Chavan and D.V.Muley, 2014). Marine organisms are characterized by a greater spatial ability to accumulate some metals when compared with bottom sediments (Kaladharan P, Prema D. 2005). The shellfish represents an important source of protein for coastal communities. It has been predictable, for instance, that over 90% of human health exposure to several contaminants occurs through diet primarily seafood and meat (Kim, M., Wolt J.D. 2011, Smith A.G. 2002). Contamination of fresh water with a wide range of pollutants has become a matter of concern over last few decades (Vutukuru, 2005). Heavy metals have devastating effects on ecological balance of the recent environment and a diversity of aquatic organisms (Farombi et.al, 2007). In order to evaluate the adverse effect of the pollutants on aquatic organisms, there is a world wide trends to complement physical and chemical parameter with bio markers in aquatic pollution monitoring (Abdel et.al, 2012). Bivalves are used in monitoring programmes in the marine environment due to their ability to concentrate pollutants to several orders of magnitude above ambient levels in seawater. O Connor, T.P. (2002) and Jung, K, Zauke G.P. (2008) reported that bivalves had been used as successful biomonitors of aquatic metallic pollutants levels internationally prominent examples including the United States National Oceanic and Atmospheric Administration Mussel Watch Programmes and the Australian Oyster Watch Programmes.

Effects of heavy metals on gills showed lamellar degeneration, epithelial lifting and necrotic changes in intercellular epithelial cells (V.R.Chavan and D.V.Muley, 2014). Necrosis of the epithelial cells on secondary lamellae, hyperplasia of the epithelial lining of gills filament, abnormal dilation of capillary lumen and destruction of blood cell in fish *Carassius carassius* (N.senol, S.Tekin Ozan, 2013). The gills of heavy metal expose group show some epithelial lesions when exposed to heavy metals. The filament regions constitute edema with intense lamellar vasso dilation. The gills exhibit a stratified fusion of stratified pigments in numerous spaces are found in the gill of heavy metal exposed common carp *Cyprinus carpio* (Vinodini, R and Narayanan, 2008). The present study was aimed to evaluate the histopathological effects of heavy metals copper, mercury and cadmium on gills of *L.marginalis*.

II. Material and methods

The bivalves, *L.marginalis* were collected from the Godavari River at paithan. The bivalves were acclimatized to laboratory conditions upto 2-3 days, before subjecting them to experiments. healthy active animals of approximately the same size and weight were chosen the acclimatized active bivalves were divided into two groups. one group of bivalves was exposed to lethal concentration (LC_{50} values of 96 hours) concentrations 1.9 ppm, 0.6 ppm and 3.9 ppm of CuSO_4 , HgCl_2 and CdCl_2 respectively upto 96 hours of

treatment. Another group of acclimatized group of was exposed to sub lethal concentration of heavy metals, pollutants upto 20 days for chronic treatment. After acute and chronic exposure the animals were dissected and the gills were removed and fixed in Bouin's fluid. After dehydration they were embedded in paraffin wax and were sectioned at 5 microns. The sections were stained by the hematoxylene –eosin method and observed under a light microscope. All the precautions recommended by (ATSDR,2003) to minimize risks of sample contamination were followed during collection and treatment of sample.

III. Result And Discussion

Histopathological changes after CuSO₄, Hgcl₂ and Cdcl₂ exposes are anticipatory in nature. These abnormalities which occur in the tissues and cellular level are the results of complex physiological dis function since gills are the major respiratory organ . Gill surfaces are constantly exposed to water currents in aquatic bivalves; they have to face of all the toxic and harmful substances. The observations made in the present study indicate that the damage in the tissue architecture in the gill is more severe in higher concentrations. This concentration dependent toxic intensity has also been reported by Ghate and Mulherkar (1979) in the gills of two species of fresh water prawns exposed to CuSO₄. Histopathological alteration of gills of bivalve tissues have been shown to be responsive and sensitive to wide range of contaminants because they play an important role in respiration and food collection.(Au,D.W.2004,Abdel Nabi,I.M,Taha I.A. & Moawad,T.I.2007).The histological changes of gill of bivalve in the present study are in agreement with (EI Shenawy,N.S., Moawad,T.I.2009) as they reported irregularity of gill lamellae of the cells ,swelling of gill filament and haemocytes infiltration of bivalve.epithelial cells of gills play a crucial role (Choi H.J.,Ahn,I.Y.2003) the damage in the epithelium results in serious dysfunction of tissue consequently leading to deleterious effect on the organization levels(Madkour,H.A.2005) Bivalves possess different measures of defense against environmental hazards e.g. particle rejection and formation of pseudo faces, reduce filtration rate and valve closure (Waterman,B.T.,Herlyn,M...2008) Bonneris ,E,Fiala,Medoni(2005) reported that metal concentration recorded in soft tissue and muscle increased without a source of extra metals in water.

Manduzio,H.& Rocher,B.(2004) concluded that gills food preferentially be used in bio monitoring studies in blue muscles .The gill tissue of *Mytillus galloprovincialis* is responsible for the uptake of metal ions from water.(Znidaric,M.T.,Falnoga,I,and Turk,V.2005)Cd is not an essential element for animals (Madkour,H.A.2005)

Gills from copper sulphate (plate II.a)

The first noticeable effect of copper sulphate in general on the gills of bivalve was the enlargement of respiratory epithelial cells. Nuclear size was greatly increased and cytoplasm shows granular structure, nuclei showed irregular shape and they also showed the granular appearance. Cytoplasm also showed disintegration, because of swelling of respiratory epithelium .the interlamellar space was reduce . in the central core there was conjection of blood cells. At certain places connective tissue core also showed the changes like enlargement of capillaries, vacuolization and disintegration of normal structure.

The mercury treated gills (plate II.b)

Exhibited severe alternate in the normal architecture of gill lamellae. The epithelial cells showed totally altered structure. The shape was lost, the nuclei show pycnotic conditions, moderate necrotic changes in interlamellar epithelial cells.,crysting of gill filament tips,infiltration of cells in primary axis. The shrinkage was prominent and because of shrinkage cavities were form between epithelial cells.at certain places mercury treated lamellae showed fusion and loss of interlamellar spaces.

Cadmium treated gills (plate II.c)

Showed enlargement of nuclei in the epithelial cells. Cytoplasm showed the degenerative changes and therefore mass of nuclei was observed in the lamellae .The central core of lamellae showed dialation.In certain regions the interlamellar space was filled with coagulated mucous and it was reduce due to enlargement of respiratory epithelium.

Gills from chronic exposure (plate III a)

Chronic exposure of copper sulphate showed drastic changes in the normal architecture of gill lamellae, cytoplasm showed disintegration because of swelling of respiratory epithelium. The gills of lead exposed fish showed dilation and congestion in blood vessel of primary gill filament (V.R.Chavan and D.V.Muley, 2014).due to copper sulphate there was overall enlargement of the lamellae which reduce the interlamellar space. Also the border of epithelial cells at some places showed degeneration. Some studies revealed that interstitial edema is one of the more frequent lesions observed in gill epithelium of fish exposed to heavy metals.(Mallatt,1985) edema with lifting of lamellar epithelium would be serve as a mechanism of defense (Arellano et.al.,1999)

Mercury treated gill lamellae (plate III.b)

Exhibited highly abnormal architecture, necrosis was evident in gill epithelium from the appearance of pycnotic nuclei .towards proximal and disintegration of nuclear material was more prominent than distal region. The blood capillaries also showed congestion of blood cells. At certain places blood cell show shrinkage and vacuolization.hyperplacia of epithelial cells between secondary lamellae led to fusion and separated from pillar system.vacuolisation and necrosis of lamellar epithelial cells ,congestion of central lamellar vein and hyperplasia of lamellar epithelial cells was evident in gill of fish exposed to lead (V.R.Chavan and D.V.Muley, 2014)

The chronic treatment of cadmium chloride altered (plate III.c)

the nuclei showed granular nature , the blood vessels in the connective tissue showed presence of leucocytes which also shows shrinkage. Some lamellae were fused at the proximal end and degenerative changes were observed in distal ends of lamellae. some studies reveals that histopathology evaluation of the toxic effect of heavy metals in the nucleus changes in the cell, lamellae structure ,necrosis inflammation hyperplacia,hypertrophy,athrophyad amount of mucous in epithelial cells were examined (N.Senol,S.Tekin Ozan,2013).the main histopathological changes observed in the gills were edema and lifting of lamellar epithelia(Vinodini,R and Narayanan M,2009).

Similarly Shanmugam A and Kesavan K(2007) reported that bioaccumulation of heavy metals such as magnesium, iron, zinc and copper concentration in different body parts and shell of C.melo from Cuddalore coast. Huang J.Y.(2007) reported that, level of accumulation of heavy metals such as mercury, cadmium, lead, zinc, copper and arsenic in soft tissues of 15 species of benthic invertebrates from Zhejiang costal waters, East China. The concentration of iron in different soft tissues and byssus and also studied the potential role of the byssus as an excretion route for iron in P. viridis(Yap CK, Tan SG 2007). Cadmium is widely distributed at low level in the environment and most foods have an inherently low level of Cd which has been shown to bind to the protein and accumulate significantly in higher level(FDA 2011).

Plate 1

Fig 1- L.S. of gill of Lamellidens marginalis showing normal architecture of gill, lamellae, respiratory epithelium, cilia,nuclei and interlamellar space x100.

- GL- Gill Lamellae
- RE- Respiratory Epithelium
- C- Cilia
- N- Nuclei
- ILS- Interlamellar Space

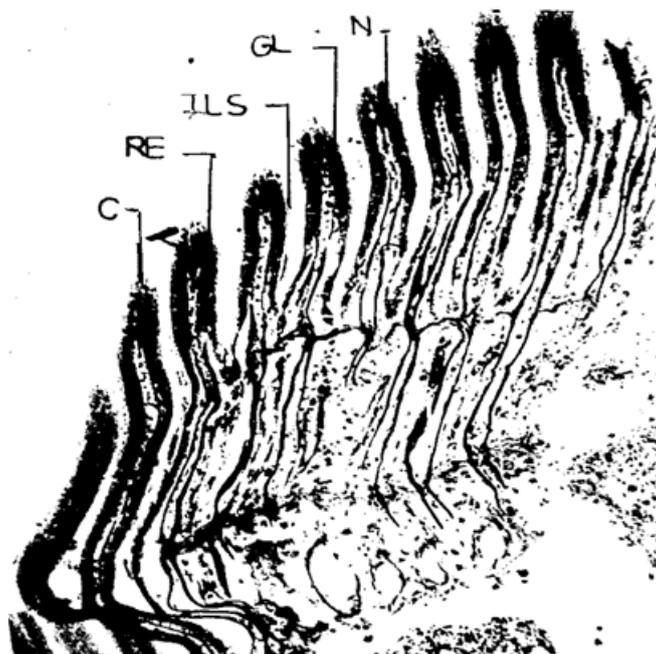
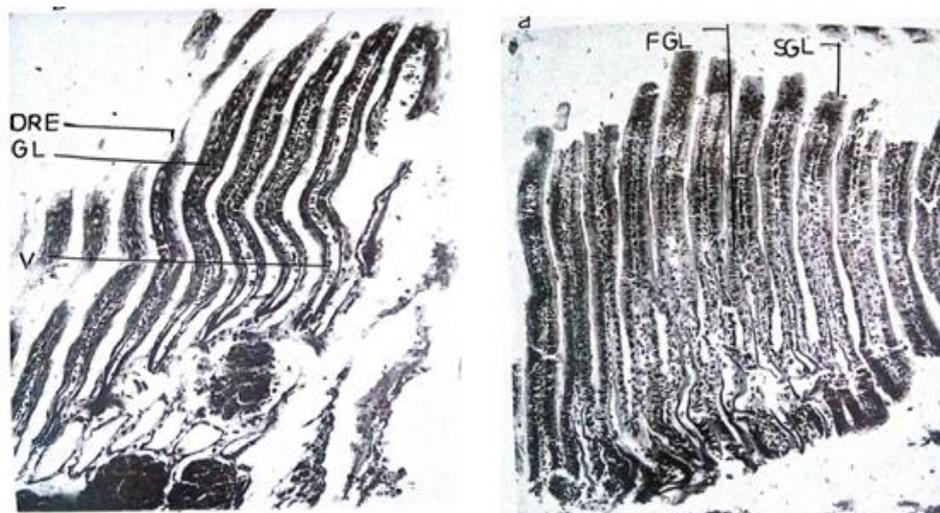


Plate 2



GL- Gill Lamellae
SGL- Swollen Gill Lamellae
DRE- Degenerated Respiratory Epithelium
FGL- Fused Gill Lamellae
CR- Chitinous Rod

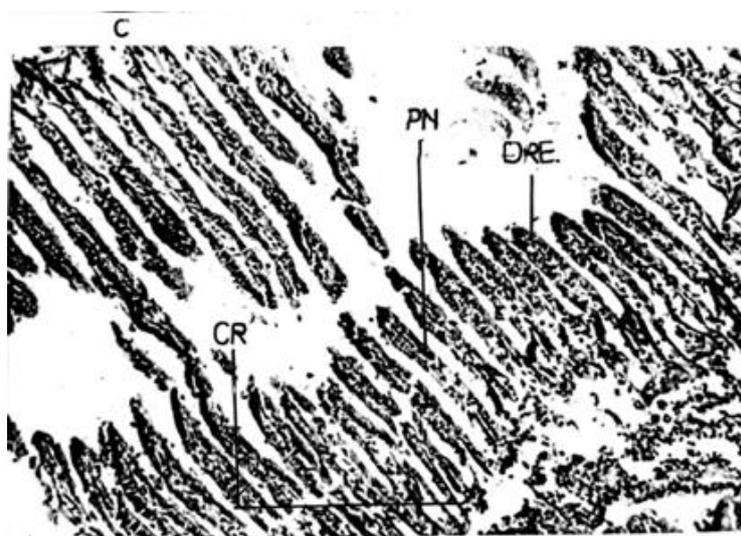


PLATE 2:

L.S. of gill of *L. marginalis* exposed to acute treatment of (96 hrs) of copper sulphate, mercury chloride and cadmium chloride.

Fig:A Acute Copper Sulphate(96 hrs) x 100

Gill lamellae showing increased nuclear size, granular cytoplasm and nuclei, reduced interlamellar space, swollen gill lamellae and lamellar fusion.

Fig:B Acute Mercury Chloride (96 hrs) x 100

Gill lamellae showing vacuolization, degeneration of nuclei, necrosis and dissolution of respiratory epithelium.

Fig:C Acute Cadmium chloride (96 hrs) x100

Gill lamellae showing shrinkage of cytoplasm, vacuolization and degeneration of respiratory epithelium.

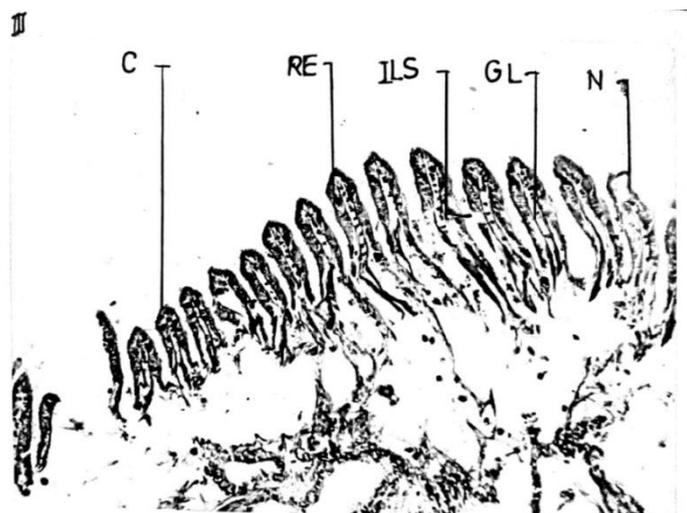


PLATE 3:
L.S. of gill of *L. marginalis* exposed to chronic treatment (20 days) CONTROL

PLATE 4:

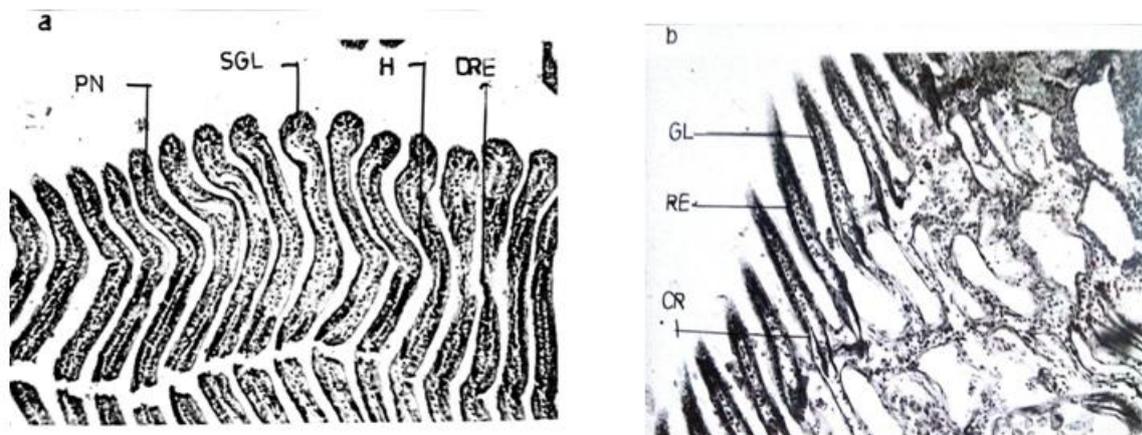


PLATE 4:
L.S. of gill of *L. marginalis* exposed to chronic treatment (20 days) of heavy metals, CuSO_4 , Hgcl_2 and Cdcl_2 .

Fig a; Chronic CuSO_4 x100

Gill lamellae showing swelling at their tips, pycnotic nuclei, vacuolization, congestion of haemocytes and degeneration of respiratory epithelium.

Fig b: Chronic Hgcl_2 x100

Gill lamellae showing degenerating nuclei, necrosis and dissolution of respiratory epithelium, vacuolization.

Figc: Chronic Cdcl_2 x100

Gill lamellae showing vacuolization, shrinkage of cytoplasm, degeneration of respiratory epithelium.

GL Gill Lamellae

SGL Swollen Gill Lamellae

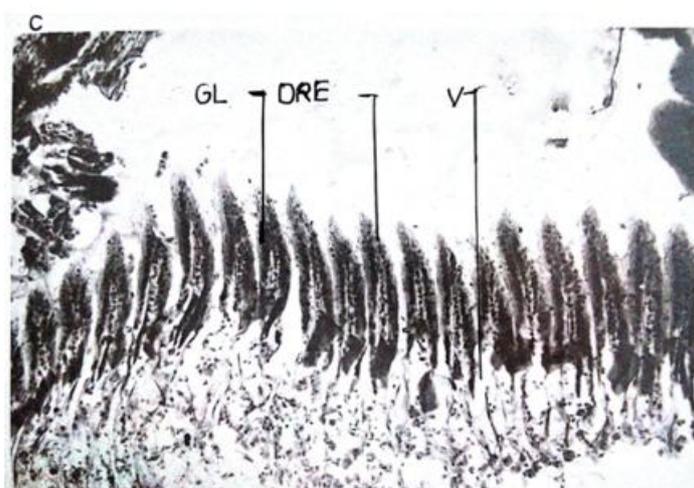
H Haemocytes

PN Pycnotic Nuclei

DRE Degenerative Respiratory Epithelium

CR Chitinous Rod

V Vacuoles.



IV. Conclusion

Histopathological changes in bivalve *L.marginalis* due to effects of heavy metals can be used as a sensitive model to monitor the aquatic pollution and are widely used in water quality monitoring programmes in many countries. The current results indicate that heavy metal contamination definitely affects the gills by necrosis, edema, proliferation, hyperplasia, hypertrophy. It is recommended to treat the effluent (detoxification) before discharging to the resources to avoid negative impact on aquatic biota. The present research work served as an experimental tool and bio indicators for the first line evaluation of environmental pollution.

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