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ACUTE TOXICITY OF HEAVY METALS (CADMIUM CHLORIDE ($CDCL_2$) AND MERCURIC CHLORIDE ($HGCL_2$) ON FRESH WATER BIVALVE *LAMELLIDENS MARGINALIS* FROM PRAVARASANGAM OF GODAVARI RIVER, MS. INDIA

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ABSTRACT

The Bio-filter potential of the freshwater bivalve, *Lamellidens marginalis* was examined in experiments, collected from Pravara-Sangam of Godavari River. Bivalves are known for their low susceptibility to heavy metal toxicity. Since toxicity is based on the effect and toxicant produces at a target site in the body of organism. Establishment of relationship between the concentration of substance at target site of animal and the subsequent toxic effect which may can provide tools for predicting toxicity. Cadmium and Mercury are the major contaminants found in the river. The behaviour of single toxicant could not be fully understood without the knowing the fact of physical and biochemical properties of the substance that can change. To understand this, the acute toxicity of Cadmium chloride ($CdCl_2$) and Mercuric chloride ($HgCl_2$) on *L. marginalis* was determined. LC_{50} of $CdCl_2$ and $HgCl_2$ at 96 hr were noticed at 12 and 6 ppm respectively. More protein depletion observed in the digestive gland than gills and foot. The present research work served as an experimental tool and bio-indicators for the first line evaluation of environmental pollution.

KEYWORDS: Acute, Toxicant, *Lamellidens marginalis*, $CdCl_2$, $HgCl_2$.

INTRODUCTION

Aquatic ecosystems are progressively coming under permanent pressure of anthropogenic activity, Pollution might be directly or indirectly toxic to the aquatic life and thus pollution in aquatic environments has become a global issue.[1] Rapidly expanding industries and agriculture has significantly resulted aquatic pollution, are biggest menace to the aquatic animal and rising concern to the *Human being*.[2] The excessive use of chemical fertilizers and pesticides has resulted in creating various environmental threats and becomes leading pollutant agents.[3] Chemical industries, Agricultural waste and urban or rural sewages contamination has increased the potential pollution of the Godavari River. Aquatic animals accumulate large quantities of xenobiotics, the accumulation depends upon the intake and the elimination from the body. Freshwater clams, oysters, and mussels accumulate large quantities of heavy metals.[4] Mercury and Cadmium are recognized as toxic contaminants of aquatic environment.[5] These are highly toxic heavy metals enter into the body of living organism including man through the food chain and accumulate in the tissues and exert their effects at molecular and biochemical levels of organism.[6] The heavy metals bind to the cell membrane; therefore, they are very difficult to remove from cell membrane, the great challenge is to remove heavy metal from water and animals body. Mercury chief sources are fungicide, seed dresser, in manufacturing of scientific equipments, and electrical appliances and several industries of paints, electroplating etc. and colour pigments in plastics and various types of paints are the cadmium sources.

Among the macro-invertebrates, molluscs are an integral component of aquatic ecosystem and are very sensitive to changes in water quality, making them an excellent indicator *Lamellidens marginalis* used to assess the tropic status of freshwater systems.[7] Therefore native species of fresh water bivalves, *Lamellidens marginalis* have been selected to investigate lethal concentration and protein depletion. Study would able to provide all aspect of heavy metal accumulation and its impact on protein depletion in invertebrate group.

MATERIALS AND METHODS

Bivalves *L. marginalis* about 65-70 mm in length from Pravarasangan, Godavari River were collected and transferred to the laboratory. Water samples were analyzed for different physico-chemical parameters.[8] Animals were acclimatized in well maintained aquarium

for a week, water changed after an interval of 11-12 hrs every day. Five clean aquariums were filled with 20 litter water and kept10 animals in each, experiments were conducted for 96 hrs. Cadmium chloride and Mercuric chloride were used as toxicants. Test solutions were renewed once after 24hrs replacing the test solution[9] First aquariums were kept as a control and remaining four were experimental; $CdCl_2$ and $HgCl_2$ doses were given to the selected bivalve in 10, 11, 12, 13 and 5, 6, 7, 8 ppm respectively. Live bivalves from LC_{50} group were anesthetized with chloroform[10] gills, foot and digestive glands were dissected. Samples were dried for 3-4 days to remove water content, transferred to the hot air oven maintained at 90-100 ^{0}C. 20 mg of dried tissue of gills, foot and digestive gland were weighed separately. Sample were homogenized in 1N NaOH and centrifuged at 3000 rpm for 15 min, supernatant were discarded, 5ml of alkaline copper Sulphate reagent were added and allowed to stand at room temp for about 10 min. After that 0.5ml of Folic-phenol reagent (1:1 folic-phenol: distilled water) added, solution were mixed thoroughly and incubated at room temp for about 30 min and the color developed measured at 650 nm. Values were calculated against BSA used as standard and were represented as mg protein/gm dry weight of the tissue, whole proteins were estimated.[11]

Study area: Pravara-Sangam of Godavari River of Ahmednagar District, M.S.India.

Figure: Map of Maharashtra state, India (Blue colour indicates sample station).

RESULTS AND DISCUSSION

In the first experiment, Bivalve exposed to Cadmium chloride ($CdCl_2$) concentration. In the 10 ppm was 30%, in 11 ppm was 40%, in 12 ppm was 50% and in 13 ppm was 60% mortality observed within 96 hrs and shown in the table 3 and figure 3.

Table 3.1: *L. marginalis* Percent and Probate Mortality of Cadmium chloride ($CdCl_2$)

Cadmium chloride ($CdCl_2$) (ppm)	Dead animal	Live animal	Percentage mortality	Probate mortality
Control	0	10	0.0	0.0
10	3	7	30	4.48
11	4	6	40	4.75
12	5	5	50	5.00
13	6	4	60	5.25

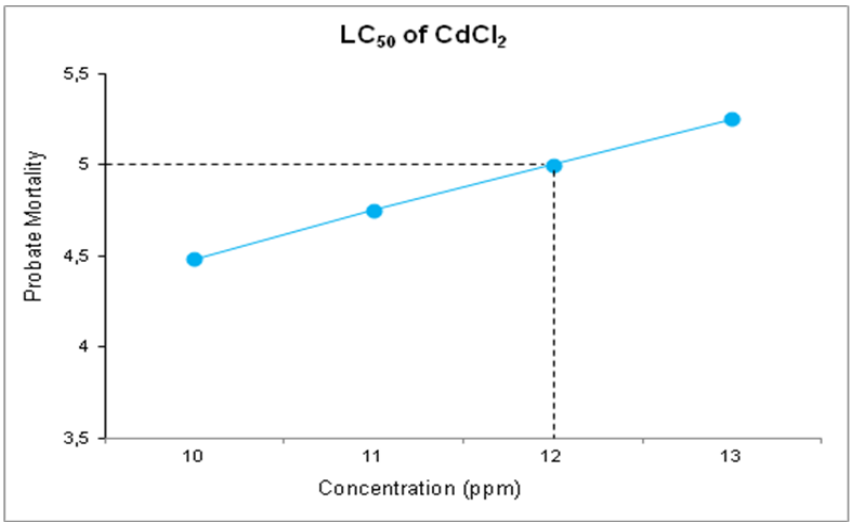

Fig. 3.1: *L. marginalis* LC_{50} of Cadmium chloride ($CdCl_2$)

In the second experiment, Bivalve exposed to the Mercury chloride ($HgCl_2$) concentration. In the 5 ppm was 40%, in 6 ppm was 50%, in 7 ppm was 60% and in 8 ppm was 70% mortality observed within 96 hrs and shown in the table 4 and figure 4.

Table 3.2: *L. marginalis* Percent and Probate Mortality of Mercury chloride ($HgCl_2$).

Mercury Chloride ($HgCl_2$) (ppm)	Dead animal	Live animal	Percent mortality %	Probate mortality
Control	0	10	0.0	0.0
5	4	6	40	4.75
6	5	5	50	5.00
7	6	4	60	5.25
8	7	3	70	5.52

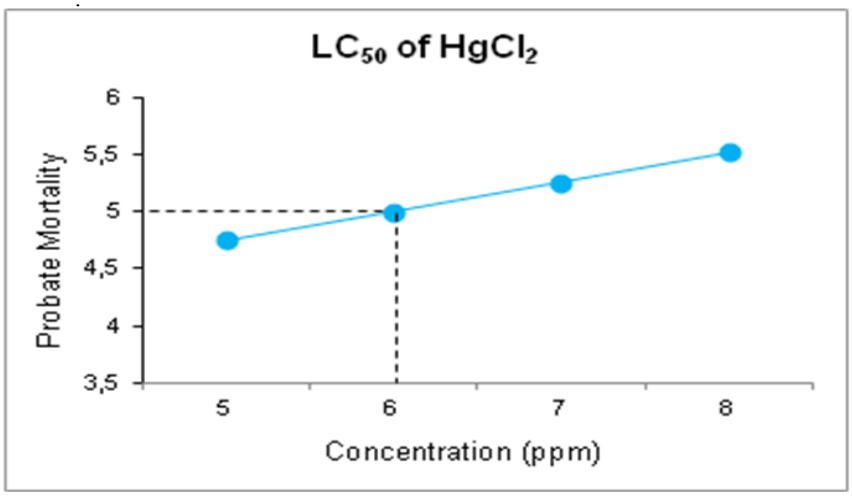

Fig. 3.2: *L. marginalis* LC_{50} of Mercury chloride ($HgCl_2$)

The determination of the LC_{50} values has an immense importance since it provides fundamental data for the design of more complex disposal model. Heavy metal affects behaviour and get accumulate in the test animals and reduce survival rate. Extensive studies have been carried out all over the world for the effects of heavy metals on aquatic animals.[12] Many investigations have reported the toxicity of pesticides and heavy metals to different aquatic invertebrate species. In the present investigation, the freshwater bivalve *L. marginalis* established its LC_{50} values of Cadmium chloride ($CdCl_2$) and Mercury chloride ($HgCl_2$) were 12 ppm and 6 ppm respectively (shown in Fig. 3&4). The rate of mortality has increased with increasing concentration and the time of exposure to Cadmium chloride ($CdCl_2$) and Mercury chloride ($HgCl_2$). Hence, mortality rate is directly proportional to the time of exposure and concentration of the heavy metal. Since the LC_{50} value of Cadmium chloride ($CdCl_2$) within 96 hrs was less (12ppm) than Mercury chloride ($HgCl_2$)$HgCl_2$ (6 ppm). Study clearly

indicated that any pollutant present in the aquatic environment is toxic. The toxic substance once get entered in the body they certainly damage and weaken the mechanisms concern, such damage may be at cellular and molecular level.

The changes in biochemical composition in the Gill, Foot and Digestive gland of freshwater bivalve, *L. marginalis* exposed to acute concentrations of cadmium chloride and mercuric chloride for 96 hrs were studied along with control group. The data was supported by various statistical analysis and the standard deviation of the mean were calculated.[13] Table 3 and figure 3 show depletion in the amount of total protein. The protein depletion occurred due to cadmium chloride toxicity has been compared with control group of bivalve, significant decrease in protein level was observed in all the three organs. The total amount of protein in the Gill of control bivalve was observed 11.4351 mg/100mg but it was significantly reduced after 96 hrs as 7.9064 mg/100mg (-30.8584%), Foot showed 9.3099 mg/100 mg of protein in control group, but it was decreased up to 6.6870 mg/100 mg (-28.1732%), Digestive gland of control bivalve showed 13.6249 mg/100mg of protein in it, but it was depleted after 96 hrs as 8.7363mg/100 (-35.8798%) exposed to lethal concentration (12 ppm) of cadmium chloride.

Table 3: Total Protein content of Gill, Foot and Digestive gland of *Lamellidens marginalis* exposed to Cadmium chloride ($CdCl_2$) (Mean ± S.D; n = 3)

Tissue	96 hr control	96 hr (12ppm)	%Variation
Gill	11.4351 ±0.8086	7.9064 ±0.2701	30.8584
Foot	9.3099 ±0.6862	6.6870 ±0.7532	28.1732
Digestive Gland	13.6249 ±0.5908	8.7363 ±0.5414	35.8798

(Values expressed as mg/100mg of Dry wt. of tissue.± indicates S.D. of 3 observations.)

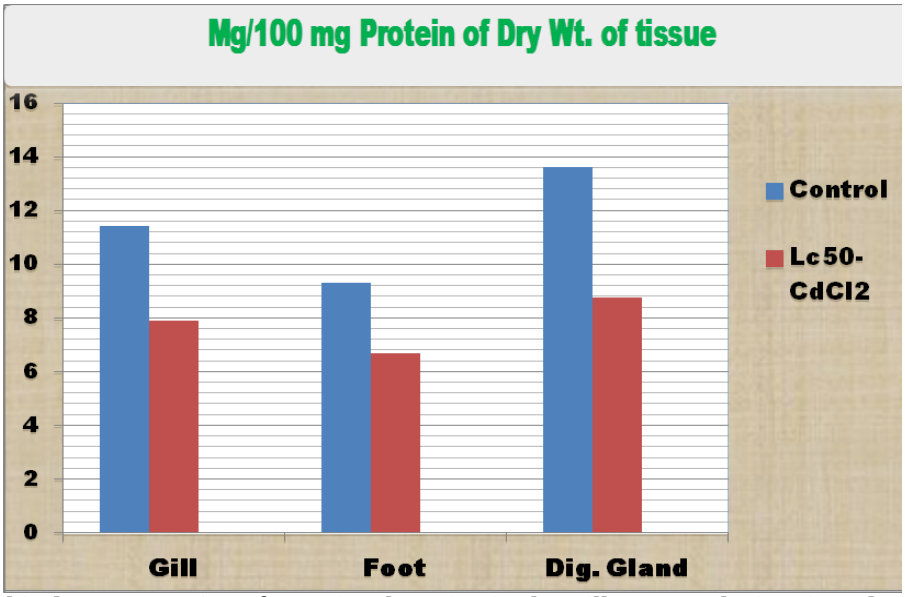

Figure 3.3: Graphical representation of Protein Alteration in the Gill, Foot and Digestive Gland of *Lamellidens marginalis* exposed to LC_{50} $CdCl_2$ (Mean ± S.D; n = 3)

Table 4 and figure 4 show depletion in the amount of total protein. The protein depletion occurred due to mercuric chloride toxicity has been compared with control group of bivalve. Significant decrease in protein level was observed in all the three organs. The total amount of protein in the Gill of control bivalve was observed 11.4351 mg/100mg but it was significantly reduced after 96 hrs as 7.110 mg/100mg (-37.8195%), Foot showed 9.3099 mg/100 mg of protein in control group, but it was decreased up to 6.7167 mg/100 mg (-27.8542%), Digestive gland of controlled bivalve showed 13.6249 mg/100mg of protein in it, but it was depleted after 96 hrs as 8.2458 mg/100 (-39.4799%) exposed to lethal concentration (6 ppm) of mercuric chloride.

Table 3.4: Total Protein content in the Gill, Foot and Digestive gland of *Lamellidens marginalis* exposed to $HgCl_2$ (Mean ± S.D; n = 3)

Tissue	96 hr control	96 hr (6ppm)	% Variation
Gill	11.4351 ±0.8086	7.1104 ±0.5540	37.8195
Foot	9.3099 ±0.6862	6.7167 ±0.6319	27.8542
Digestive Gland	13.6249 ±0.5908	8.2458 ± 0.8729	39.4799

(Values expressed as mg/100mg of Dry wt. of tissue. ± indicates S.D. of 3 observations.)

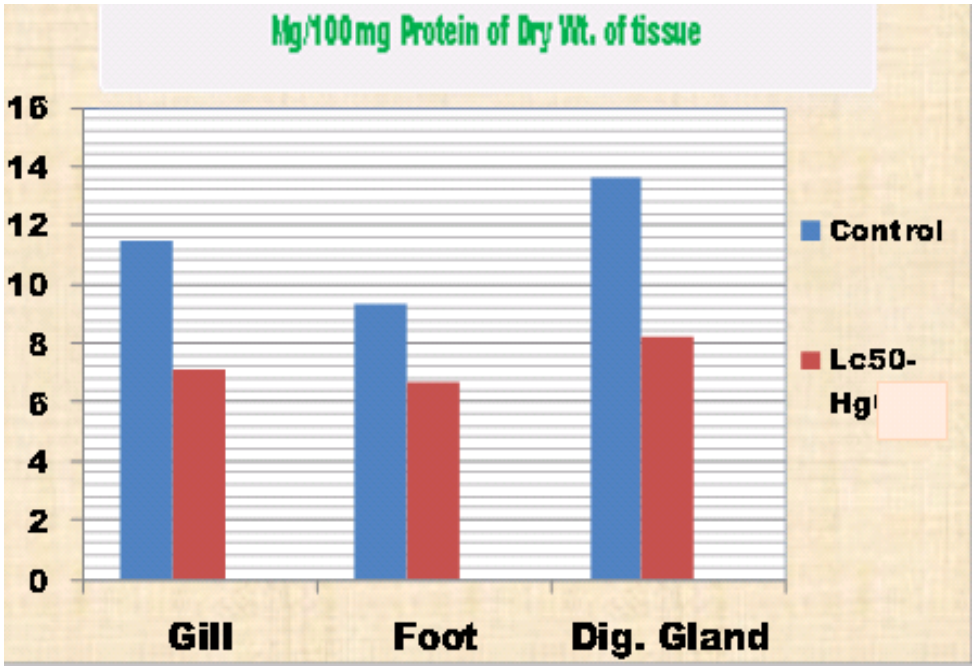

Figure 3.4: Graphical representation of Protein Alteration in the Gill, Foot and Digestive Gland of *Lamellidens marginalis* exposed to LC_{50} $HgCl_2$ (Mean ± S.D; n = 3)

During any stressful condition alters the biochemical composition in aquatic animals. The protein depletion during pollutant exposure may be due to increased catabolism and decreased anabolism of proteins. Depletion in protein content in animal tissue after exposure to organism[14] pollutant and other heavy metals were reported by few researchers. Kharat et al.[15] reported that the reduction in total protein content in the gill, foot, hepatopancreas and muscles of the *Macrobrachium kistnensis* exposed to different conc. of tributyltin chloride, and it was possibly due to stress condition caused by toxicity on protein metabolism similar results were reported by Sole et al.[16] Significant decrease in protein level was observed in all the three organs. Bivalve exposed to the lethal concentration of cadmium chloride (12ppm), amount of total protein in Gill (-30.8584%), Foot (-28.1732% and Digestive gland (-35.8798%) and while exposed to mercuric chloride (6ppm) in Gill (-37.8195%), Foot (-27.8542% and digestive gland (-39.4799%) were found significantly lower compared to control. Higher protein depletion in digestive glands followed by gills and foots, depletion might be due to more utilization of these constituents under stress of Cadmium chloride ($CdCl_2$) and Mercury chloride ($HgCl_2$) contamination. The study results clearly indicated that digestive gland was the most affected organ followed by gill and foot. The higher depletion might be due to high metabolic potency and efficiency of the gland under pollutant stress and are provided better indication of the extent of toxicity. The digestive gland seems to be the main site of degradation and detoxification of toxicants and hence has the largest demand of energy for the metabolic processes resulting in increasing utilization of protein to meet energy demand. More protein depletion observed in digestive gland exposed to Mercury chloride ($HgCl_2$) than the Cadmium chloride ($CdCl_2$), Hence the investigations also confirm that the species has found higher sensitivity to Mercury chloride ($HgCl_2$) compared to Cadmium chloride ($CdCl_2$) Singaraju et al.[17], reported the most alteration of protein in digestive gland under stress. Swami, et al.[18] recorded significant decline in protein content in fresh water bivalve, *L. marginalis* exposed to flodit and metacid. Similar observations were made by number of workers in molluscs.[19, 20, 21] Hence the above discussion and all the available literature, The Mercuric chloride is very toxic to the freshwater bivalve, *L. marginalis.* The release of Cadmium and Mercury

compounds in aquatic environment especially in freshwater ecosystem might be controlled.

CONCLUSION

The present study revealed that the toxicity of Cadmium chloride and Mercuric chloride on *L. marginalis* resulted into death within 96 hrs of exposure. Amount of protein in Gills, Foot and Digestive gland were found significantly lower compared to control, higher protein depletion in digestive glands followed by gills and foots, and depletion might be due to more utilization of these constituents under stress of $CdCl_2$ and $HgCl_2$ contamination. The investigations also confirm that the species has found higher sensitivity to $HgCl_2$ compared to $CdCl_2$, which says that species has the potential to monitor aquatic pollution and can be widely used in water quality monitoring program. The present research served as an experimental tools and bio-indicators for the first line evaluation of aquatic pollution.

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