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Changes in Protein Metabolism of Freshwater Bivalves (L. Marginalis and L. Corrianus) Due To Short Term Exposed To Heavy Metals

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Abstract
The freshwater bivalves Lamellidens marginalis and Lamellidens corrianus were exposed to LC0 and LC50 values of 96 hrs with concentrations of 1.82 ppm for zinc chloride, 1.62 ppm for copper sulphate, 1.04 ppm for cadmium chloride and 0.687 ppm for mercuric chloride. The L. marginalis showed more amount of protein in LC0 than LC50 values of metal concentration during all seasons. In monsoon, the protein content compared with the zinc content it was more in copper than cadmium and mercury. These content compared to monsoon to respective metals the protein was increased except the cadmium, the more increased rate was from mercury followed by copper and zinc. While, the content compared with monsoon and winter of respective metals the content showed in LC0 bivalves more decreased in mercury followed by cadmium and zinc respectively. In LC50 the decrease rate was in zinc followed by copper, cadmium and mercury respectively. The L. corrianus which showed content when compared to monsoon to respective metals the protein was increased and the more increased rate was in LC0 bivalves from cadmium followed by copper and mercury and zinc. In Lc50 it was decreased in all metals the decrease rate was in cadmium than zinc, copper and mercury. Further the content compared with monsoon and winter of respective metals it shows in LC0, more decreased in zinc followed by copper, cadmium and mercury respectively. In LC50 the decrease rate was in zinc followed by copper, cadmium and mercury respectively.

Keywords: *Protein, heavy metals, L. marginalis, L. corrianus acute toxicity, seasons.*

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I. Introduction

The fresh water bivalves are one of the most endangered groups of animals on Earth, and have become a symbol of the diversity and conservation of Indian rivers and dams. The conservation crisis of bivalve is a result of continent-wide degradation of aquatic ecosystems and is a symbol of the loss of our native freshwater fauna. The study on biochemical processes is very important to understand the mechanism of metal toxicity to commercially important invertebrates. The mechanism of microorganism inhibition involves the entry of heavy metal ions to the metabolic system of an organism with consequent formation of secondary metabolites, which are toxic to the organism due to the presence of heavy metals9.Biochemical composition in bivalve has been employed as biomarker in several studies that aimed to evaluate the impact of anthropogenic activities in the environment13. Heavy metals are ubiquitous and important biochemical constituent of the earth's crust and trace amounts can be released into aquatic environments through the processes of weathering and erosion 2. Small doses of metals are essential for almost all living organisms as it has a major role in numerous biochemical and physiological processes acting as a co-factor of proteins; nucleic acids, carbohydrates and lipids15. The studies on biochemical response of a bivalve to stressors have led to the better understanding as to how bivalve cope with the stressor at the biochemical level18. The change in metabolic rate has a consequence towards the change in biochemical composition; it is an indicator of stress of nature in the environment which specifically affects protein with increased catabolism and decreased anabolism5.Exposure to environmental stressors can induce oxidative stress in cells and result in a decrease in reducing potential and metabolic transformation to reactive intermediates 17. The increase in MT levels and concomitant decrease in the accumulation of various heavy metals 3 and labile zinc in gonad and gill tissues explained by the inflammation hypothesis20and6 reported biochemical variation in different freshwater bivalve using some heavy metals during different seasons. In this context the aim of our study to focus on understanding how bivalves L. marginalis and L. corrianus from Manjara dam metabolizes and are affected by the wide range of concentration of different heavy metals viz. zinc, copper, cadmium and mercury in aquatic environment.

II. Materials And Methods

The bivalve habitats are rich in flora and fauna around Manjra dam; it is large sized dam constructed on Manjra River near village Dhanegaon, Dist. Osmanabad hence selected for study and in as there is no any industry on both sides as well as in catchment area. The locality in dam is selected as per the abundance and water qualities of dam in different geographic area. The bivalves L. marginalis and L. corrianus were collected for laboratory experiments from study area during monsoon, winter and summer seasons. They were brought to the laboratory and kept in plastic troughs containing five liters of dechlorinated tap water for three days to acclimatize to laboratory conditions. Water from the plastic trough was changed after every 12 hours. The healthy bivalves of approximately same size and weight were selected for the experiments. Since the animals are micro feeders no special food was supplied during the experiment. The acclimatized bivalves were exposed to LC0 and LC50 values of 96 hrs with concentrations of 1.82 ppm for zinc chloride, 1.62 ppm for copper sulphate, 1.04 ppm for cadmium chloride and 0.687 ppm for mercuric chloride up to 96 hours. The bivalves were divided into two groups and the first group was maintained as control and each of the remaining group was exposed to different metal concentrations. After 96 hrs exposure the control and experimental bivalves were dissected and whole body were weighed and they were then kept in hot air oven at 92 0C till constant weights were obtained. The dried product was ground to obtain fine powder. From the replicates of three samples the protein was analyzed by using the method10.The amount of protein was calculated by regression equation and expressed in terms mg/100mg dry powder.

III. Results

In present study the L. marginalis and L. corrianus were exposed to different heavy metals for 96 hrs using the values of LC0 and LC50. The control group was runs simultaneously with exposed bivalves and only the mean values expressed in the Table-1. The results were calculated and compared amongst heavy metals and season, in L. marginalis showed more amount of protein in LC0 bivalves than LC50 during all seasons and metals also. In monsoon, the protein content in LC0 bivalves showed more amount in cadmium, (52.87) followed by zinc (52.50), copper (52.13) and mercury (50.25). When these contents compared with Zinc it was more in mercury (4.29%), the copper and cadmium animals showed equal amount of protein i. e. (0.71%). In LC50 the content was more in cadmium (46.50) followed by zinc (46.13), mercury (46.10) and copper (44.99). When the content compared with the zinc content it was more in copper (2.48%) than cadmium (0.81%) and mercury (0.07%) all non-significant levels. In winter the LC0 bivalves showed more amount in mercury (55.58) followed by copper (55.50), zinc (53.25) and cadmium (50.25). When these contents compared with zinc it was more in cadmium (5.64%) than mercury (4.94%) and copper (4.23%) (all non-significant level). In LC50 bivalves the more amount in copper (47.70) followed by cadmium (46.13), mercury (44.25) and zinc (43.50). When compared with the zinc content it was more in copper (9.66%) than cadmium (6.05%) and mercury (1.73%). On the other hand when these content compared to monsoon to respective metals the protein was increased except the cadmium, (4.96), the more increased rate was from mercury (84.73%; $P<0.05$) followed by copper (6.47%) and zinc (1.43%). In LC50 it was decreased in all metals except the Copper (6.03%), the decrease rate was in zinc (5.71%) than mercury (4.02%) and cadmium (0.80%). In summer the LC0 bivalves showed more amount in zinc (42.50) followed by cadmium (40.41), copper (40.20) and mercury (38.66). When these contents compared with iznc it was more in mercury (9.04%; $P<0.05$) than copper (5.42%) and cadmium (4.92%) (all non significant level). In LC50 bivalves the more amount in zinc (41.40) followed by copper (39.99),cadmium (38.56) and mercury (36.55). When compared with the zinc content it was more in mercury (11.72%; $P<0.01$) than cadmium (6.86%; $P<0.01$) and copper (3.41%; $P<0.05$). On the other hand when the protein content compared with monsoon and winter of respective metals the content showed in LC0 bivalves more decreased in mercury (23.07%; $P<0.001$), (30.82%; $P<0.001$) followed by cadmium (23.57%; $P<0.001$), (19.59%; $P<0.001$), copper (22.89%; $P<0.001$), (27.57%; $P<0.001$) and zinc (19.05%; $P<0.01$), (20.19%; $P<0.01$) respectively. In LC50 the decrease rate was in zinc (10.26%; $P<0.05$), (4.83%) followed by copper (11.12%; $P<0.05$), (16.17%; $P<0.01$), cadmium (17.08%; $P<0.01$), (17.08%; $P<0.001$) and mercury (20.72%; $P<0.01$), (20.72%; $P<0.001$) respectively.

The L. corrianus which showed more amount of protein in LC0 bivalves than LC50. In monsoon the protein content in LC0 bivalves showed more amount in copper, (54.38) followed by zinc (52.88), cadmium (52.13) and mercury (51.75). When these contents compared with zinc it was more in copper (2.84%), the mercury (2.14%) and cadmium (1.42%). In LC50 bivalves the more amounts in cadmium (48.75) followed by zinc (47.63), copper (47.60) and mercury (47.43). When compared with the zinc metal the content was more in cadmium (2.36%) than mercury (0.42%) and copper (0.07%) all non significant levels. In winter the LC0 bivalves showed more amount in copper (59.63) followed by cadmium (59.13), zinc (52.99) and mercury (52.25). When these contents compared with zinc it was more in copper (12.54%; $p<0.01$) than cadmium (11.59%; $P<0.05$) and mercury (1.40%). In LC50 bivalves the more amounts in mercury (43.50) followed by zinc (42.38), copper (42.37) and cadmium (40.88). When compared with the zinc content it was more in cadmium (3.54%) than mercury (2.65%) and copper (0.03%). On the other hand the content when compared to monsoon to respective metals the protein was increased and the more increased rate was in LC0 bivalves from cadmium (13.43%; $P<0.05$) followed by copper (9.66%; $p<0.01$) and mercury (0.97%) and zinc (0.21%). In large sizes it was decreased in all metals the decrease rate was in cadmium (16.15%; $P<0.01$) than zinc (11.03%; $P<0.01$), copper (10.99%$P<0.01$) and mercury (8.29%$P<0.01$). In summer the LC0 bivalves showed more amounts in zinc (45.65) followed by copper (44.39), cadmium (44.01) and mercury (43.0). When these contents compared with zinc it was more in mercury (5.81%) than cadmium (3.60%) and copper (2.77%) (all non-significant level). In LC50 bivalves the more amounts in cadmium (43.85) followed by copper (42.55), zinc (41.55) and mercury (40.30). When compared with the zinc content it was more in cadmium (5.54%) than mercury (3.01%) and copper (2.41%). On the other hand when the content compared with monsoon and winter of respective metals the content showed in LC0 bivalves more decreased in zinc (13.68%; $P<0.01$), (13.86%; $P<0.\ 01$) followed by copper (18.38%; $P<0.001$), (25.56%; $P<0.001$), cadmium (15.58%; $P<0.05$), (25.58%; $P<0.001$) and mercury (16.91%; $P<0.01$), (17.71%; $P<0.001$) respectively. In large sizes the decrease rate was in zinc (12.77%; $P<0.01$), (1.96%) followed by copper (10.61%; $P<0.01$), (0.43%), cadmium (10.06%; $P<0.05$), (7.27%; $P<0.05$) and mercury (15.04%; $P<0.01$), (7.36%; $P<0.05$) respectively.

IV. Discussion

When organism expose to stress tends to shift all the metabolic processes to face the toxic effects of stress and this lead to changes in biochemical and physiological mechanism in the body of organism, both duration of exposure and heavy metal concentrations important in determination of the level of biomarker response[8]. In the present study results showed upon 96 hrs exposure of metals caused some how different trend was observed, revealing different type of substrate utilization to meet the energy demand. The mussel *L. marginalis* during exposure with different heavy metals showed that the protein levels in their body parts decreased continuously when increases the time period. When exposed bivalves at all time period in metal concentration showed that more decrease was in mercury followed by cadmium and copper. Amongst metal concentration showed the decrease trend was from mercury, cadmium, copper and zinc. Further amongst seasons in summer both were more affected due to heavy metals concentration and hence protein was more depleted from these body organs when it was compared with zinc exposed bivalves. The results obtained in the present study are supported by several investigators who reported decrease in protein of various organisms under influence of different metals. It is in the level of tissue protein may also be due to excessive proteolysis to overcome the metabolic stress, as deposited protein in the cytoplasm can easily be used to replace the loss of proteins that occur during physiological stress[14]. Overall in present study the zinc showed less amount of protein decreases in all heavy metals and time period also. Apart from this it can be interpret that the utilization of protein and synthesis of lipid of the metal irrespective to its concentration in the outside medium. In present study the *L. marginalis* and *L. corrianus* showed more amount of protein in LC_0 bivalves than LC_{50} during all seasons. In monsoon, the protein content compared with the zinc content it was more in copper than cadmium and mercury. On the other hand when these content compared to monsoon to respective metals the protein was increased except the cadmium, the more increased rate was from mercury followed by copper and zinc. While, the content compared with monsoon and winter of respective metals the content showed in LC_0 bivalves more decreased in mercury followed by cadmium and zinc respectively. In LC_{50} the decrease rate was in zinc followed by copper, cadmium and mercury respectively. [11] showed that after acute and chronic exposure to mercury, protein contents in different tissues of freshwater bivalve *Corbicula striatella* were found that highly depleted and maximum protein depletion was found in foot. However, total protein content decreased on exposure to chromium in all the three tissues like gill followed by adductor muscle and mantle of freshwater bivalve *L. corrianus*[16]. The decreasing of protein, vitamins after acute exposure 24 hr and due to the consumption of Zn and Pb for using energy generation which used for defense mechanisms against heavy metals and formation of lipoprotein which involve in repair of damaged cells and tissue organelles [1]. Our present data is compatible with many studies such as ([5]and [19]) and the fall in the protein content during pollutant and heavy metals exposure may be due to increase protein catabolism and decrease anabolism of freshwater bivalves. The results obtained in the present study indicate severe disturbance in the protein metabolism of the fresh water bivalves exposed to different heavy metals. Another possible explanation for the decrease in the protein might be due to diapedesis and mucoprotein which is eliminated in the form of mucous. During perform of preliminary experiments it was noticed that the excessive secretion of mucous and diapedesis on the water surface might be scrubbing the body by bivalves due to metals and avoiding the water into the body hence supports this possibility. Moreover, biochemical variation in different body parts of freshwater bivalve *L. marginalis* using heavy metals reported by [21]. Further, the study showed in *L. corrianus* which were more amount of protein in LC_0 bivalves than LC_{50}. On the other hand the content when compared to monsoon to respective metals the protein was increased and the more increased rate was in LC_0 bivalves from cadmium followed by copper and mercury and zinc. In Lc_{50} it was decreased in all metals the decrease rate was in cadmium than zinc, copper and mercury. On the other hand when the content compared with monsoon and winter of respective metals the content showed in LC_0 bivalves more decreased in zinc followed by copper, cadmium and mercury respectively. In LC_{50} the decrease rate was in zinc followed by copper, cadmium and mercury respectively. The results obtained in present study are in agreement of most of the above observations and showed decrease in the protein in the body parts of bivalves shows its prime utilization in gearing of the metabolism. During monsoon season, gonad show maximum amount of lipid, which is correlated with the maturation of gonadal follicle and time of spawning in razor clam, *Sinonovacula constricta* [4] further they reported that the different factors like age, sex, food supply, seasons and stress influence the lipid content of the organisms**.** In response to above statement of [4]our present study showed when comparison between the metals the zinc was not affected much hence the protein was not depleted more in the body organs but mercury metal concentration showed more pronounced to the bivalves hence more protein was depleted and this indicates that the Zn is essential and Hg is not essential metal to the body of bivalves so the variation in protein concentration was observed.

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Table 1:- Changes in protein metabolism in different species of freshwater bivalves in different seasons during acute toxicity to heavy metals from Manjara dam

Species			MONSOON				WINTER				SUMMER			
	Control group	metals values	Zinc chloride	Copper sulphate	Cadmium chloride	Mercuric chloride	Zinc chloride	Copper sulphate	Cadmium chloride	Mercuric chloride	Zinc chloride	Copper sulphate	Cadmium chloride	Mercuric chloride
Lamellidens marginalis	52.90 ±1.17	LC_0	52.50 ±2.35	52.13 ±0.65 (0.71%)	52.87 ±2.98 (0.71%)	50.25 ±1.72 (4.29%)	53.25 ±1.72 (1.43%)	55.50 ±1.72 (4.23%) (6.47%)	50.25 ±1.72 (5.64%) (4.96%)	55.58 ±1.95 (4.94%) (84.73%)□	42.50 ±1.65 (19.05%)□□ (20.19%)ΔΔ	40.20 ±0.75 (5.42%) (22.89%)□□□ (27.57%)ΔΔΔ	40.41 ±0.67 (4.92%) (23.57%)□□□ (19.59%)ΔΔΔ	38.66 ±0.79 (9.04%)* (23.07%)□□□ (30.82%)ΔΔΔ
		LC_{50}	46.13 ±2.35	44.99 ±1.95 (2.48%)	46.50 ±1.72 (0.81%)	46.10 ±1.13 (0.07%)	43.50 ±1.72 (5.71%)	47.70 ±1.72 (9.66%)* (6.03%)	46.13 ±1.13 (6.05%) (0.80%)	44.25 ±0.65 (1.73%) (4.02%)	41.40 ±0.55 (10.26%)□ (4.83%)	39.99 ±0.65 (3.41%)* (11.12%)□ (16.17%)ΔΔ	38.56 ±0.22 (6.86%)** (17.08%)□□ (17.08%)ΔΔΔ	36.55 ±1.10 (11.72%)** (20.72%)□□ (20.72%)ΔΔΔ
Lamellidens corrianus	53.15 ±1.10	LC_0	52.88 ±1.95	54.38 ±0.65 (2.84%)	52.13 ±2.84 (1.42%)	51.75 ±1.95 (2.14%)	52.99 ±1.72 (0.21%)	59.63 ±1.25 (12.54%)** (9.66%)□□	59.13 ±1.72 (11.59%)* (13.43%)□	52.25 ±1.30 (1.40%) (0.97%)	45.65 ±1.65 (13.68%)□□ (13.86%)ΔΔ	44.39 ±0.75 (2.77%) (18.38%)□□□ (25.56%)ΔΔΔ	44.01 ±2.12 (3.60%) (15.58%)□ (25.58%)ΔΔΔ	43.0 ±0.95 (5.81%) (16.91%)□□ (17.71%)ΔΔΔ
		LC_{50}	47.63 ±1.72	47.60 ±1.30 (0.07%)	48.75 ±1.72 (2.36%)	47.43 ±1.16 (0.42%)	42.38 ±2.35 (11.03%)□	42.37 ±0.66 (0.03%) (10.99%)□□	40.88 ±0.65 (3.54%) (16.15%)□□	43.50 ±0.66 (2.65%) (8.29%)□□	41.55 ±1.17 (12.77%)□□ (1.96%)	42.55 ±1.35 (2.41%) (10.61%)□□ (0.43%)	43.85 ±1.17 (5.54%) (10.06%)□ (7.27%)Δ	40.30 ±1.12 (3.01%) (15.04%)□□ (7.36%)Δ

(Bracket values represent percentage differences) (*, □, Δ- P < 0.05, **, □□, ΔΔ-P < 0.01,*, □□□, ΔΔΔ- P < 0.001, *- compared to zinc , □- compared to monsoon, Δ- compared to winter of respective metal groups)**

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