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# Changes in Protein Content in Different Body Parts of *Lamellidens Marginalis* When Exposed To Heavy Metals

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***ABSTRACT***
*The bivalves Lamellidens marginalis were collected from Nagapur dam and the bivalves of approximately same sizes (75-80 mm shell length) were selected for the experiments and no special food was supplied during the experiment. The acclimatized bivalve were exposed to $LC_0$ and $LC_{50}$ values of 96 hrs with concentrations of 88.02 ppm for $ZnCl_2$, 1.72 ppm for $ZnCl_2$, $CuSo_4$ and 0.687 ppm for $HgCl_2$ metal salts. The protein was more in whole body followed by hepatopancreas gills, foot, gonad and mantle in control animals. Further, the protein was decreased in zinc chloride when it was compared with control animals. During 24 hrs the protein decreased from hepatopancreas followed by gonads, gill, whole body, mantle and foot. In 48 hrs it was more decreased from hepatopancreas followed by gonads, gill, whole body, mantle and foot. In 62 hrs protein more decreased from hepatopancreas followed by gonads, gills, mantle, whole body and foot. During 96 hrs protein more decreased from followed by gonads hepatopancreas, gill, foot, mantle and whole body. Further, the protein decreased during 24 hrs when the bivalves exposed to copper sulphate and it was more in gonads (14.08%) followed by hepatopancreas gill, foot, whole body and mantle. In 48 hrs protein more decreased from gonads followed by hepatopancreas, foot, gill, whole body and mantle. In 62 hrs it was more decreased from gonads followed by hepatopancreas, foot, gill, mantle and whole body. During 96 hrs it was more decreased from gonads followed by hepatopancreas, foot, gill, mantle and whole body.*

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## I. INTRODUCTION

Invertebrate's changes in the biochemical constituents are pronounced which are cyclic in reproduction, since a great amount of energy must be channelized to the gonad during reproduction. The trace metals are known to be non-bio-degradable and highly toxic to most organisms (Kaoud and Dahshan, 2010). 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 level. Small doses of zinc 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; metabolism of proteins, nucleic acids, carbohydrates and lipids (Rosabal *et al.,* 2012). Zinc is a 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 (Batty *et al.,* 2010). The inorganic constituents of water have effect on the diversity of the bivalves, the texture of the sediment and the quantity of organic matter seemed to have played a role in their distribution and bivalves are able to survive even in the presence of sandy soil and lesser organic matter Shafakatullah Nannu, (2012). The study is intended to compare the response of exotic and native freshwater bivalves to mercury discharges coming from a chloralkali industry located at the lower course of the Ebro River by (Melissa *et al.,* 2010). The mechanism of microorganism inhibition involves the entry of heavy metal ions (Zn2+, Cu2+, Cd2+, Ag+, etc.) 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 metals (Lim *et al.,* 2013). Biochemical composition in bivalve has been employed as biomarker in several studies that aimed to evaluate the impact of anthropogenic activities in the environment (Nahrgang *et al.,* 2013). 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 anabolism (Jagtap *et al*., 2011). Mahajan (2005) studied the biochemical changes induced by heavy metals, lead, mercury and arsenic in the protein content on the gastropod, *Bellamya bengalensis*. Exposure to environmental stressors can induce oxidative stress in cells and result in a decrease in reducing potential and metabolic transformation to reactive intermediates (Simmons *et al.,* 2011). The effects of chronic exposure of color pigments which is using in paintings on changes in the biochemical constituents like protein, glycogen and lactic acid, in different body parts of fresh water mussels *L. corrianus* for 10 and 20 days (Phadnis *et al.,* 2013). The study to evaluated the toxic effects of monocrotophos, using organophosphorus pesticide, on *L. corrianus* with a wide battery of biomarkers consisting of inhibition, lipid peroxidation, the

levels of antioxidant enzymes, and histopathological changes,(Mundhe and Pandit, 2014). In addition, biochemical assay provide both qualitative and quantitative changes of tissue level in the bivalve. Sometimes specific responses shown by bivalves to certain kind of toxicants such as heavy metals pesticides are particularly useful in fishery management and resources protection (Shafakatullah and Krishnamoorthy, 2014, Jadhav *et al.*, 2012; Rane and Zambare, 2014, Goncalo Vale at al., 2014.). The aim of study to focus on understanding how bivalves *L. marginalis* from Nagapur dam metabolizes and are affected by the wide range of concentration of different heavy metals in aquatic environment.

## II. MATERIALS AND METHODS

The bivalves *Lamellidens marginalis* were collected from Nagapur dam. Soon after the fishing 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 bivalves of approximately same sizes (75-80 mm shell length) were selected for the experiments and no special food was supplied during the experiment. The acclimatized bivalve *L. marginalis* were exposed to $LC_0$ and $LC_{50}$ values of 96 hrs with concentrations of 88.02 ppm for $ZnCl_2$, 1.72 ppm for $ZnCl_2$, $CuSo_4$ and 0.687 ppm for $HgCl_2$ metal salts. The bivalves were divided into four groups and the first group was maintained as control and each of the remaining three groups was exposed to different metal concentrations. After 24, 48, 72 and 96 hrs exposure the control and experimental the bivalves were dissected for their different body parts like mantle, foot, gill, gonad and hepatopancreas and whole body were separated. The tissues were weighed and they were then kept in hot air oven at $82^0$C till constant weights were obtained. The dried product was ground to obtain fine powder. From the replicates of three samples the total protein was analyzed by methods (Lowry *et al.*, 1951). The amount of protein were calculated by regression equation and expressed in terms mg/100mg dry powder.

## III. RESULTS

The protein **(Table 1)** was more in whole body (48.48) followed by hepatopancreas (46.44), gills (44.28), foot (41.42), gonad (40.42) and mantle (44.42) in control animals. Further, the protein was decreased in zinc chloride when it was compared with control animals. During 24 hrs the protein decreased from hepatopancreas (11.84%) followed by gonads (10.06%), gill (8.26%), whole body (4.96%), mantle (4.28%) and foot (2.6%). In 48 hrs protein more decreased from hepatopancreas (18.62%) followed by gonads (12.11%), gill (12.84%), whole body (10.28%), mantle (8.88%) and foot (6.96%). In 62 hrs protein more decreased from hepatopancreas (22.18%) followed by gonads (18.08%), gills (116.20%), mantle (14.46%), whole body (12.64%) and foot (12.24%). During 96 hrs protein more decreased from followed by gonads (24.08%), hepatopancreas gill (22.81%), foot (21.44%), mantle (18.62%) and whole body (18.40%). Further, the protein decreased during 24 hrs when the bivalves exposed to copper sulphate and it was more in gonads (14.08%) followed by hepatopancreas (12.40%) gill (12.20%), foot (12.64%), whole body (10.06%) and mantle (8.22%). In 48 hrs protein more decreased from gonads (20.88%) followed by hepatopancreas (18.44%), foot (18.68%), gill (16.66%), whole body (11.24%) and mantle (10.88%). In 62 hrs protein more decreased from gonads (24.46%) followed by hepatopancreas (22.41%), foot (21.61%), gill (20.46%), mantle (18.44%) and whole body.

During 96 hrs protein more decreased from gonads (28.88%) followed by hepatopancreas, foot (22.44%), gill (22.18%), mantle (20.96%) and whole body (16.48%). In mercuric chloride during 24 hrs gonads (26.46%) followed by hepatopancreas (24.62%), gill (22.24%), whole body (22.42%), foot (18.64%) and mantle (14.46%). During 48 hrs the protein more decreased from hepatopancreas (22.80%) followed by gonads (20.88%), gills (26.24%) foot (24.64%), whole body (24.66%), and mantle (16.42%). During 62 hrs the protein more decreased from gonads (40.26%) followed by hepatopancreas (26.62%), gill (22.84%), foot (28.62%), whole body (26.60%), and mantle (20.14%). During 96 hrs the protein was decreased from hepatopancreas (40.26%) followed by gonads (48.21%), gills (28.62%) foot (22.28%), whole body (22.64%), and mantle (22.46%). These all values were significant at $p<0.001$ compared to control group of bivalves.

## IV. DISCUSSION

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 level. In present study the 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. corrianus* during exposure with different heavy metals and time period showed that the protein levels in their body parts decreased continuously when increases the time period. When exposed mussels at all time period in $ZnCl_2$ metal concentration showed that more decrease was in hepatopancreas followed by gonad and gill. In CuSo4 metal concentration showed the decrease trend was from gonad followed by hepatopancreas and foot. 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 (Lehtonen *et al*., 2003). The present study results obtained are supported by several investigators who reported decrease in protein of various organisms under influence of different metals. In $HgCl_2$ metal concentration the protein was more decreases in gonad and hepatopancreas alternate time period followed by gill and foot. Further amongst body parts the hepatopancreas, gonad and gill was more affected due to heavy metals concentration and hence protein was more depleted from these body organs when it was compared with control group of bivalves. 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 (Patil, 2011). Mahajan and Zambare (2001) showed that after acute and chronic exposure to $HgCl_2$, 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* (Satyaparameshwar *et al*., 2006). Overall in study the mantle showed less amount of protein decreases in all heavy metals and time period also. It is evident that decrease in the protein from gonad, hepatopancreas and gills in the mussels in all the metal concentration probably caused metabolism restricted to lipogenesis and maintenance by utilizing protein substrate. 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. Our present data is compatible with many studies such as (Jagtap *et al*., 2011) the fall in the protein content during pollutant exposure may be due to increase protein catabolism and decrease anabolism of bivalves *L. corrianus*. Further in study it was showed the decreasing of protein, vitamins after acute exposure 24hr & due to the consumption of Zn and Pb for using energy generation which used for defense mechanisms against heavy metals and formation of lipoprotein. The present study showed decrease in protein, suggests possible utilization for metabolic purposes enhancement of proteolysis to meet the high-energy demand under stress condition. Whereas, the protein decreases in organism due to largest need of energy for the metabolic process which leads to increases utilization of protein to meet energy and increase the proteolysis to reach the high energy demands under heavy metal stress in fresh water bivalves (Patil, 2011). The results obtained in the present study indicate severe disturbance in the protein metabolism of the fresh water bivalve *L. corrianus* 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. ). On the other hand the effect of exposure time and concentration of mercury on body biochemical composition of *M. sallei* studied by Devi (1896) and noted that the time dependent experiments, protein and carbohydrates were consumed in concentration dependent exposure. Andhale and Zambare (2011) studied the nickel induced biochemical alterations in freshwater bivalve, *L. corrianus* and reported that the protein contents were decreased in treated animals than the control. Nagpure and Zambare (2005) observed that on acute and chronic exposure to tetracycline and chloramphenicol, *L. corrianus* showed decrease in protein levels, in proportion with the period of exposure.

It was observed that the great increase in total lipid in different tissues of *L. corrianus* and *P. cylindrica* when bivalves came across the stressed conditions. Moreover, Shaikh (2011) reported that the lipid molecules deposited in large amount of body tissues and biochemical changes seasonally in *L. corrianus*. 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* Hongwei (2008) further they reported that the different factors like age, sex, food supply, seasons and stress influence the lipid content of the organisms. 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. On the other hand, upon 96 hrs exposure to metals the protein decreased from all body parts but the more decrease was in hepatopancreas, gonad and gills of mussels this showed greater demand of energy over the utilization of body reserves in this organs, where in protein metabolites decreased. In response to above statement of Hongwei (2008) our present study showed when comparison between the metals the $ZnCl_2$ was not affected much hence the protein was not depleted more in the body organs but $HgCl_2$ 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 mussels so the variation in protein concentration was observed.

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**Table 1:** Changes in protein content from different body parts of *L. marginalis* after acute exposure to different heavy metals in Nagapur dam

Parali (V)

| Body parts | Control | Zinc chloride | | | | Copper sulphate | | | | Mercuric chloride | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 24 hrs. | 48hrs. | 72 hrs. | 96 hrs. | 24 hrs. | 48hrs. | 72hrs. | 96 hrs. | 24 hrs. | 48hrs. | 72 hrs. | 96hrs. |
| Mantle | 44.42 ±0.624 | 42.42 ±0.168 (4.28%)*** | 41.02 ±0.882 (8.88%)** | 28.84 ±0.468 (14.46%) | 26.00 ±0.144 (18.62%) | 41.68 ±0.812 (8.22%)*** | 40.46 ±0.821 (10.88%)*** | 26.66 ±0.810 (18.44%)** | 26.02 ±0.188 (20.86%)** | 28.48 ±0.120 (14.46%)*** | 28.00 ±0.668 (16.42%)** | 26.24 ±0.221 (20.14%)** | 24.20 ±0.681 (22.46%)** |
| Foot | 41.42 ±0.621 | 48.62 ±0.660 (2.6%)* | 46.46 ±0.221 (6.86%)** | 44.16 ±0.182 (12.24%)** | 40.42 ±0.288 (21.44%) | 42.84 ±0.218 (16.64%)** | 41.88 ±0.621 (18.68%)** | 40.28 ±0.641 (21.61%)** | 24.28 ±0.666 (22.44%)** | 41.81 ±0.612 (18.64%)** | 28.24 ±0.222 (24.64%)** | 26.62 ±0.068 (28.62%)** | 24.28 ±0.812 (22.28%)** |
| Gill | 44.28 ±0.641 | 40.11 ±0.462 (8.26%)** | 48.18 ±0.621 (12.84%)*** | 44.62 ±0.660 (16.20%)** | 42.12 ±0.120 (22.81%)** | 46.88 ±0.666 (14.01%)** | 46.01 ±0.088 (16.66%)** | 42.81 ±0.886 (20.46%)** | 40.00 ±0.681 (22.18%)** | 42.26 ±0.120 (22.24%)** | 40.62 ±0.168 (26.24%)** | 28.16 ±0.148 (22.84%)** | 20.22 ±0.68 (28.62%)** |
| Hepato-pancreas | 46.44 ±1.002 | 48.68 ±1.601 (11.84%)* | 44.44 ±0.264 (18.62%)*** | 44.00 ±1.002 (22.18%)* | 40.44 ±0.261 (28.46%)** | 48.81 ±0.188 (12.40%)** | 46.11 ±0.481 (18.44%)** | 42.24 ±0.641 (22.41%)** | 40.44 ±0.821 (28.46%)** | 42.46 ±0.681 (24.62%)** | 28.00 ±0.681 (22.80%)*** | 24.68 ±0.681 (26.62%)** | 28.12 ±0.124 (40.26%)** |
| Gonads | 40.42 ±0.146 | 44.44 ±1.081 (10.06%) | 42.8 ±0.618 (12.11%)*** | 41.28 ±0.812 (18.08%)** | 28.24 ±0.182 (24.08%)** | 42.80 ±0.862 (14.08%)*** | 28.82 ±0.822 (20.88%)** | 26.61 ±0.681 (24.46%)** | 24.26 ±0.641 (28.88%)** | 26.10 ±0.812 (26.46%)** | 24.86 ±0.181 (20.88%)** | 22.18 ±0.188 (40.26%)** | 20.11 ±0.214 (48.21%)** |
| Whole body | 48.48 ±0.168 | 44.10 ±0.616 (4.86%) | 42.41 ±0.181 (10.28%)*** | 40.60 ±0.818 (12.64%)** | 46.81 ±0.188 (18.40%)** | 42.68 ±0.81 (10.06%)** | 42.01 ±0.108 (11.24%)** | 48.61 ±0.188 (14.16%)** | 48.88 ±0.184 (16.48%)** | 44.44 ±0.681 (22.42%)** | 42.44 ±0.446 (24.66%)** | 42.01 ±0.446 (26.60%)** | 28.46 ±0.182 (22.64%)** |

(Bracket values represent percentage differences and all are significant at ***, $p<0.001$ compared to control group of bivalves)