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CHANGES IN PROTEIN CONTENT IN DIFFERENT BODY PARTS OF BIVALVE *LAMELLIDENS MARGINALIS* DUE TO HEAVY METALS

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ABSTRACT
Freshwater bivalves *Lamellidens marginalis* (75-80 mm shell length) were exposed to lethal levels of heavy metals for 96 hrs for metal accumulation. Amongst the different body parts in control group of animals the protein (mg/100 mg) was more in whole body (58.59) followed by foot (55.28), hepatopancreas (53.54), gills (51.52), gonad (47.17) and mantle (45.52). During acute exposure of 96 hours with zinc chloride, copper sulphate and mercury chloride the decrease in protein content during 24 to 96 hrs from 45.32 to 37.00, 45.32 to 36.02 and 45.32 to 35.30 respectively in mantle. The depletion in foot during 24 to 96 hrs was 55.28 to 42.12, 55.28 to 40.0 and 55.28 to 30.32 in zinc chloride, copper sulphate and mercury chloride respectively. Further it was observed that the decrease in protein content in gill after acute exposure during 24 to 96 hrs was from 51.52 to 40.42, 51.52 to 35.39 and 51.52 to 30.39 in zinc chloride, copper sulphate and mercury chloride respectively. The protein decreased in hepatopancreas during 24 to 96 hrs from 53.54 to 40.45, 53.54 to 40.35 and 53.54 to 35.78 in zinc chloride, copper sulphate and mercury chloride respectively. The content decreased in gonads from 47.17 to 34.35, 47.17 to 33.37 and 47.17 to 30.11 in zinc chloride, copper sulphate and mercury chloride respectively. The average protein content of the bivalve *L. marginalis* was decreased after acute exposures of zinc chloride, copper sulphate and mercury chloride. The most pronounced change was observed in mercuric chloride treated animals.

KEY WORDS: bivalves, *L. marginalis*, protein, heavy metals, accumulation.

INTRODUCTION

The freshwater mussels have come into the spotlight in recent years because of the environmental movement, a growing awareness of aquatic ecosystem health, and a public desire to protect and restore native ecosystems and wildlife. Freshwater mussels 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 mussels is a result of continent-wide degradation of aquatic ecosystems and is a symbol of the loss of our native freshwater fauna. The cumulative effect of all the pollutants can be determined by bio monitoring. The overall health of the aquatic ecosystem could also be properly assessed. Bio-monitoring is the introduction of biological variables for assessment of the structural and functional aspects of ecosystems. The trace metals are known to be non-bio-degradable and highly toxic to most organisms (Kaoud and Dahshan, 2010). Benthic biota can acquire metals through ingestion of sediment particles, food and directly from pore water and overlaying water (Griscom and Fisher, 2004). 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. Biochemical indices are often very sensitive to lethal toxicants and the magnitude of the biochemical changes is often related to the severity of the toxicants (Livingston, 1985). 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 (Suryawanshi *et al.*, 2014). 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). 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).

The study on biochemical processes is very important to understand the mechanism of metal toxicity to commercially important invertebrates. Rao *et al.* (1987), Vedpathak and Mane (1988) studied the effect of fluoride and mercuric chloride on the biochemical levels in freshwater bivalve *I. caeruleus*. Kulkarni (1993) and Patil (1993) studied cadmium chloride and mercuric chloride induced changes in the biochemical composition of the freshwater bivalve *L. marginalis* respectively. Devi (1996) studied bioaccumulation and metabolic effects of zinc and mercury on marine dreissinid bivalve, *M. sallei.* Patil and Mane (1997) studied seasonal changes of protein in different body parts of *L.*

marginalis during exposure of mercury. 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). The aim of study to focus on understanding how how bivalves *L. marginalis* from Nagapur dam metabolizes and are affected by the wide range of concentration of different heavy metals in aquatic environment.

MATERIALS AND METHODS

The bivalves *Lamellidens marginalis* were collected from Nagapur dam at Parali (V). 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 the animals are micro feeders 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 (99.02 ppm) for zinc chloride, (1.72 ppm) for copper sulphate and (0.687 ppm) for mercuric chloride up to 96 hours. 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 sacrificed to their different body parts like mantle, foot, gill and hepatopancreas were separated and whole body. The tissues 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 total protein was analyzed by using Lowry's method (Lowry *et al.* 1951). The amount of protein was calculated by regression equation and expressed in terms mg/100mg dry powder.

RESULTS AND DISCUSSION

The chemical composition of any edible organisms is extremely important since the nutritive value is reflected in its biochemical contents. Many researchers devoted to study on the biochemical composition of bivalve molluscs. The aspect of energy metabolism and reproduction has been reported for a number of species of bivalves due to their commercial importance and edibility values. But the relative influence of gonad development on the distribution and storage of biochemical constituents in different body parts has been examined by only a few cases. Ansell *et al*. (1964) determined seasonal changes in biochemical composition and adductor muscle, mantle, siphon, visceral mass (gonad), digestive gland and foot from hard clam *M. mercenaria*. In the present study **(Table-1)** the protein was more in whole body (58.59) followed by foot (55.28), hepatopancreas (53.54), gills (51.52), gonads (47.17) and mantle (45.32) in control bivalves. Further, the protein was decreased in zinc chloride when it was compared with control animals. During 24 hrs the protein decreased from foot (9.36%) followed by hepatopancreas (7.0%), whole body (5.96%), mantle (4.38%), gills (3.7%) and gonads (3.67%). In 48 hrs protein more decreased from gonads (16.60%) followed by hepatopancreas (15.12%), foot (12.85%), whole body (10.38%), mantle (9.89%) and gills (7.87%). In 72 hrs the content more decreased from gonads (20.74%) followed by hepatopancreas (17.82%), foot (17.30%), mantle (14.46%) whole body (13.64%), and gills (12.35%). During 96 hrs the protein decreased from gonads (27.18%) followed by hepatopancreas (24.45%), foot (23.81%), gills (21.55%) mantle (18.72%) and whole body (18.40%). Further, the protein content decreased during 24 hrs from gills (18.97%) followed by foot (17.21%), hepatopancreas (10.52%), whole body (10.07%), mantle (8.22%), and gonads (5.20%). During 48 hrs the protein decreased from foot (24.01%) followed by gills 21.39%), hepatopancreas (15.75%), gonads (15.37%), whole body (11.24%), and mantle (10.88%). In 72 hrs the content decreased from foot (26.00%), followed by gills (25.49 %), gonads (20.27%), mantle (19.45%), hepatopancreas (19.22%) and whole body (15.16%). During 96 hrs the protein was decreased from gills (31.31%) followed by foot (27.65%), gonads (20.02%), hepatopancreas (24.45%), mantle (20.87%) and whole body (16.58%).

On the other hand the protein was decreased in mercuric chloride during 24 hrs gills (25.88%) followed by foot (25.17%), hepatopancreas (22.57%), whole body (22.43%), gonads (21.35%) and mantle (15.47%). During 48 hrs the protein more decreased from gills (31.45%) followed by whole body (30.80%), foot (28.15%), gonads (26.08%), hepatopancreas (24.45%) and mantle (16.53%). During 72 hrs the protein more decreased from gills (36.50%) followed by whole body (33.42%), foot (32.77%), gonads (29.66%), hepatopancreas (29.03%) and mantle (20.15%). During 96 hrs the protein was decreased from foot (45.16%), followed by gills (40.24%), whole body (39.48%), gonads (36.17%), hepatopancreas (33.18%) and mantle (22.46%). 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. The increase in MT levels and concomitant decrease in the accumulation of various heavy metals (Gagnon *et al*. 2006) and labile zinc in gonad

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and gill tissues might be explained by the inflammation hypothesis.

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

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	45.52 ±0.725	43.53 ±0.178 (4.38%)***	41.02 ±0.982 (9.89%)***	38.94 ±0.578 (14.46%)	37.00 ±0.145 (18.72%)	41.78 ±0.812 (8.22%)***	40.57 ±0.931 (10.88%)***	36.67 ±0.910 (19.45%)***	36.02 ±0.188 (20.87%)***	38.48 ±0.120 (15.47%)***	38.00 ±0.778 (16.53%)***	36.35 ±0.321 (20.15%)***	35.30 ±0.791 (22.46%)***
Foot	55.28 ±0.741	50.11 ±0.572 (9.36%)***	48.18 ±0.721 (12.85%)***	45.72 ±0.770 (17.30%)***	42.12 ±0.120 (23.81%)***	45.77 ±0.666 (17.21%)***	42.01 ±0.099 (24.01%)***	40.91 ±0.987 (26.00%)***	40.00 ±0.781 (27.65%)***	41.37 ±0.120 (25.17%)***	39.72 ±0.179 (28.15%)***	37.17 ±0.158 (32.77%)***	30.32 ±0.78 (45.16%)***
Gill	51.52 ±0.631	49.62 ±0.670 (3.7%)**	47.47 ±0.221 (7.87%)****	45.16 ±0.192 (12.35%)***	40.42 ±0.299 (21.55%)	41.75 ±0.318 (18.97%)***	40.50 ±0.731 (21.39%)***	38.39 ±0.751 (25.49%)***	35.39 ±0.667 (31.31%)***	38.19 ±0.712 (25.88%)***	35.32 ±0.333 (31.45%)***	32.72 ±0.079 (36.50%)***	30.39 ±0.812 (40.24%)***
Hepato-pancreas	53.54 ±1.002	49.79 ±1.701 (7.0%)**	45.45 ±0.275 (15.12%)***	44.00 ±1.002 (17.82%)****	40.45 ±0.371 (24.45%)***	47.91 ±0.198 (10.52%)***	45.11 ±0.591 (15.75%)***	43.25 ±0.751 (19.22%)***	40.45 ±0.921 (24.45%)***	41.56 ±0.781 (22.57%)***	40.00 ±0.781 *****	38.00 ±0.791 (29.03%)***	35.78 ±0.135 (33.18%)***
Gonads	47.17 ±0.157	45.44 ±1.081 (3.67%)	39.34 ±0.718 (16.60%)***	37.39 ±0.812 (20.74%)***	34.35 ±0.182 (27.18%)***	44.72 ±0.872 (5.20%)***	39.92 ±0.823 (15.37%)***	37.61 ±0.781 (20.27%)***	35.37 ±0.751 (25.02%)***	37.10 ±0.912 (21.35%)***	34.87 ±0.181 (26.08%)***	33.18 ±0.198 (29.66%)***	30.11 ±0.215 (36.17%)***
Whole body	58.59 ±0.178	55.10 ±0.717 (5.96%)	52.51 ±0.181 (10.38%)***	50.60 ±0.919 (13.64%)***	47.81 ±0.198 (18.40%)***	52.69 ±0.81 (10.07%)***	52.01 ±0.109 (11.24%)***	49.71 ±0.189 (15.16%)***	48.88 ±0.185 (16.58%)***	45.45 ±0.781 (22.43%)***	40.55 ±0.556 (30.80%)***	39.01 ±0.556 (33.42%)***	35.46 ±0.182 (39.48%)***

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). 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. marginalis* 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. 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. 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. The decrease of protein content, suggests possible utilization for metabolic purposes enhancement of proteiolysis to meet the high-energy demand under metal pollution stress condition. The fall in the protein content during pollutant exposure may be due to increase protein catabolism and decrease anabolism of protein.

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 (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. marginalis* (Satyaparameshwar *et al*., 2006). 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 which involve in repair of damaged cells and tissue organelles

(Almamoori *et al*., 2014).

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. 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. marginalis*. 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). In the present study decrease in protein might be due to increased proteolysis activity or might be due to changes in the metabolic substrate during anaerobic condition produced in the bivalves by metal. The results obtained in the present study indicate severe disturbance in the protein metabolism of the fresh water bivalve *L. marginalis* exposed to different heavy metals.

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. It was also showed that the metabolic shifts in different body parts of mussels and synthesized of lipid. However, it appears that the same organs showed protein decreases and lipid synthesizes might be due to high concentration of metal and irrespective time of exposure period such as 24 or 96 hrs.

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