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IMPACT OF HEAVY METALS ($ZnCl_2$, $CuSo_4$ and $HgCl_2$) ON LIPID CONTENT IN DIFFERENT BODY PARTS OF BIVALVE *LAMELLIDENS MARGINALIS.*

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ABSTRACT
Freshwater bivalves *L. 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 lipid (mg/100 mg) was more in hepatopancreas (8.24) followed by gonad (7.69), foot (7.63), gill (7.55), mantle (7.43) and whole body (6.99). During lethal exposure of 96 hours with zinc chloride, copper sulphate and mercury chloride the increase in lipid during 24-to 96 hrs was 7.43 to 8.05, 7.43 to 7.94 and 7.43 to 8.25 respectively in mantle. The increase in lipid in foot was 7.63 to 8.15, 7.63 to 8.27 and 7.63 to 8.33 in zinc chloride, copper sulphate and mercury chloride respectively. Further it is also observed that the increase in lipid content in gill after acute exposure was from 7.55 to 8.15, 7.55 to 8.17 and 7.55 to 8.34 respectively. The increase in lipid in hepatopancreas was observed from 8.24 to 8.91, 7.55 to 8.91and 7.55 to 9.17 in zinc chloride, copper sulphate and mercury chloride respectively. The decrease in gonads was observed from 7.69 to 8.45, 7.69 to 8.27 and 7.69 to 8.42 in zinc chloride, copper sulphate and mercury chloride respectively. The average lipid content of the bivalve *L. marginalis* was increased 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* lipid, heavy metals, accumulation.

INTRODUCTION
Mussels are impacted by loss of fish hosts from fish kills or dams that prevent fish migration. However, in some parts of the country, it's a non-native mussel causing the most concern. In the Marathwada region, following programmes were conducted for awareness of public regarding pollution control and environment protection. It is painfully clear that in many ways humans have had a significantly negative effect on aquatic fauna and Earth's natural environment as a whole. It is essential to realize that as rational beings; humans have the ability to not only understand the problems we have created and what needs to be done to amend them, but also the capability of accomplishing these tasks. There are two basic venues of thought as to why we should protect mussels and our natural environment, one being intrinsic reasoning, and the other being anthropocentric. 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 lipid 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 lipid content on the gastropod, *Bellamya bengalensis*. In addition, biochemical assay provide both qualitative and quantitative changes of tissue level in the bivalve. Sometime specific responses shown by, for example, fishes to certain kind of toxicants such as heavy metals pesticides are particularly useful in fishery management and resources protection (Donaldson and Dye, 1975); Franzier and Baksi, 1987 Thomos, 1989). The study on biochemical processes is very important to understand the mechanism of metal toxicity to commercially important invertebrates. Rao *et al*. (1987) and Vedpathak and Mane (1988) studied the effect of fluoride and mercuric chloride on the freshwater bivalve *I. caeruleus* respectively. 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 (1995, 1996) studied bioaccumulation and metabolic effects of zinc and mercury on marine dreissinid bivalve, *M. sallei.* 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.

MATERIALS AND METHODS

The bivalves *L. 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 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 dissected and 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 lipid was analyzed by using Vanillin reagent method (Barnes and Blackstock, 1973). The amount of lipid was calculated by regression equation and expressed in terms mg/100mg dry powder.

RESULTS AND DISCUSSION

Lipids play a nutritionally and physiologically important role in bivalves by providing an efficient source of high energy content and essential fatty acids. According to our study it was observed that the lipid concentration altered due to metals and it was increased during exposure. In present study the increase trend was more in gonad, hepatopancreas and whole body in all three metals concentrations and time period when it was compared to control group of bivalves. When comparison between heavy metals the $HgCl_2$ was more affected the body parts and $ZnCl_2$ was less affected. Further it was noticed that the mantle tissue showed that lowest amount of lipid increases in all heavy metals. Our present results are consistent with the results reported by Nandkumar and Zambare (2012) different factors like age, sex, food supply, seasonal variations etc. influence the lipid content of the organisms. 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. marginalis*. In the present study the lipid (Table-1) content in control animals was more in hepatopancreas (8.24) followed by gonads (7.69), foot (7.63), gills (7.55), mantle (7.43) and whole body (6.99). Further, the lipid was increased in zinc chloride metal exposed animals when it was compared with respective tissues of control animals. During 24 hrs the lipid more increased from foot (3.15%, $p < 0.05$) followed by hepatopancreas (2.55%), whole body (2.29%), mantle (1.62%), gill (1.06%) and gonad (0.79%). In 48 hrs lipid more increased from hepatopancreas (4.13%) followed by whole body (4.01%), foot (3.28%), gills (3.18%), mantle (2.70%, $p < 0.05$) and gonads (1.57%). In 72 hrs the content more increased from whole body (8.02%) followed by mantle (6.33%, $p < 0.05$), hepatopancreas (5.47%), gonads (5.46%), gills (5.30%) and foot (4.99%, $p < 0.05$). During 96 hrs the lipid was increased from gonads (9.89%) followed by whole body (9.88%), mantle (8.35%), hepatopancreas (8.14%), foot (8.13%) and gills (7.95%). Further, the lipid was increased in copper sulphate during 24 hrs from hepatopancreas (7.74%) followed by foot (4.46%), gills (4.38%), whole body (4.30%), mantle (2.83%), and gonads (2.34%). During 48 hrs the lipid more increased from hepatopancreas (6.68%) followed by whole body (6.30%), gills (5.83%), foot (5.25%), gonads (3.91%), and mantle (3.64%). In 72 hrs the content more increased from whole body (10.02%), followed by gills (8.22 %), hepatopancreas (7.41%), foot (6.82%), gonads (6.25%) and mantle (5.93%). During 96 hrs the lipid was increased from whole body (14.31%) followed by gills (9.54%), foot (8.39%, $p < 0.001$), hepatopancreas (8.14%), gonads (7.55%) and mantle (6.87%). On the other hand the lipid was increased in mercuric chloride during 24 hrs from whole body (13.31%) followed by hepatopancreas (6.44%), mantle (5.66%), foot (5.51%, $p < 0.01$), gills (5.44%) and gonads (3.78%). During 48 hrs the lipid more increased from whole body (16.31%) followed by mantle (8.62%), hepatopancreas (7.89%), gills (7.42%), foot (6.82%, $p < 0.001$) and gonads (6.12%).

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During 72 hrs the lipid more increased from whole body (17.89%) followed by hepatopancreas (9.96%), mantle (9.95%), foot (8.39%), gills (8.22%) and gonads (7.81%). During 96 hrs the lipid was increased from whole body (20.03%), followed by hepatopancreas (11.29%), mantle (11.04%), gills (10.47%), gonads (9.50%) and foot (9.18%). A gradual increase in lipid content was observed in mantle, foot, gill, hepatopancreas of *L. marginalis* after acute treatment. The most pronounced change was observed in mercuric chloride treated animals.

Table 1: Changes in lipid 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	7.43 ±0.292	7.55 ±0.293 (1.62%)	7.63 ±0.317 (2.70%)*	7.89 ±0.725 (6.33%)*	8.05 ±0.229 (8.35%)	7.24 ±0.285 (2.83%)	7.70 ±0.578 (3.64%)	7.87 ±0.295 (5.93%)	7.94 ±0.385 (6.87%)	7.85 ±0.351 (5.66%)	8.07 ±0.378 (8.62%)	8.17 ±0.135 (9.96%)	8.25 ±0.481 (11.04%)
Foot	7.63 ±0.266	7.87 ±0.112 (3.15%)*	7.88 ±0.155 (3.28%)	8.01 ±0.135 (4.99%)*	8.15 ±0.418 (8.13%)	7.97 ±0.237 (4.46%)	8.03 ±0.378 (5.25%)	8.20 ±0.217 (6.82%)	8.27 ±0.417 (8.39%)***	8.05 ±0.432 (5.51%)**	8.15 ±0.496 (6.82%)***	8.27 ±0.517 (8.39%)***	8.33 ±0.882 (9.18%) ***
Gill	7.55 ±0.488	7.63 ±0.516 (1.06%)	7.79 ±0.496 (3.18%)	7.99 ±0.502 (5.30%)	8.15 ±0.859 (7.95%)	7.88 ±0.278 (4.38%)	7.99 ±0.611 (5.83%)	8.17 ±0.593 (8.22%)	8.27 ±0.535 (9.54%)	7.96 ±0.178 (5.44%)	8.11 ±0.851 (7.42%)	8.17 ±0.196 (8.22%)	8.34 ±0.516 (10.47%)
Hepato Pancreas	8.24 ±0.396	8.45 ±0.282 (2.55%)	8.58 ±0.496 (4.13%)	8.69 ±0.653 (5.47%)	8.91 ±0.631 (8.14%)	8.63 ±0.203 (7.74%)	8.79 ±0.151 (6.68%)	8.85 ±0.188 (7.41%)	8.91 ±0.159 (8.14%)	8.77 ±0.183 (6.44%)	8.89 ±0.488 (7.89%)	9.06 ±0.855 (9.96%)	9.17 ±0.859 (11.29%)
Gonads	7.69 ±0.178	7.75 ±0.851 (0.79%)	7.81 ±0.342 (1.57%)	8.11 ±0.493 (5.47%)	8.45 ±0. 515 (9.89%)	7.87 ±0.5.18 (2.34%)	7.99 ±0.415 (3.91%)	8.17 ±0.513 (6.25%)	8.27 ±0.551 (7.55%)	7.98 ±0.185 (3.78%)	8.16 ±0.851 (6.12%)	8.29 ±0.617 (7.81%)	8.42 ±0.692 (9.50%)
Whole body	6.99 ±0.306	7.15 ±0.185 (2.29%)	7.27 ±0.851 (4.01%)	7.55 ±0.461 (8.02%)	7.89 ±0.623 (9.88%)	7.29 ±0.179 (4.30%)	7.43 ±0.661 (6.30%)	7.69 ±0.815 (10.02%)	7.99 ±0.321 (14.31%)	7.92 ±0.032 (13.31%)	8.13 ±0.196 (16.31%)	8.24 ±0.066 (17.89%)	8.39 ±0.859 (20.03%)

(Bracket values represent percentage differences) (*,p-<0.05, **,p<0.01 and ***,p<0.001 compared to control group of bivalves)

The results obtained in the present study are supported by several investigators who reported a increase in lipid of various organisms under influence of different metals. 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 (2009) 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 Hongwei (2009) our unpublished data showed when comparison between the metals the $ZnCl_2$ was not affected much hence the lipid was not depleted more in the body organs but $HgCl_2$ metal concentration showed more pronounced to the bivalves hence more lipid 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 lipid concentration was observed. However, increase in the lipid content in the body parts of the mussels was also seen which suggest the inhibition of lipase activity and lipid synthesis likely due to impairment in metabolism and to the inhibition of enzyme activity in lipid metabolism. Other studies have reported similar increases in lipid Arasu and Shreenivasula (1995) in the gill and muscle of marine bivalve *P. viridis* during exposure to cadmium and copper. Voogt (1983) stated that lipids in bivalves are multifunctional and in different species one of the functions during the maturation of gametes, drastic environmental conditions, starvation, population stress etc. can be more noticeable. In the present study total lipid content of various tissues of *L .marginalis* was found to be increased, after lethal exposures of three metals. 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 lipid decreases and lipid synthesizes might be due to high concentration of metal and irrespective time of exposure period such as 24 or 96 hrs. On the other hand, the lipid concentration was increased during exposure period.

The increase trend was more in gonad, hepatopancreas and whole body in all three metals concentrations and time period when it was compared to control group of bivalves. When comparison between heavy metals the $HgCl_2$ was more affected the body parts and $ZnCl_2$ was less affected. While, the detected as shown in the results, enable us to make conclusions about the idea of variation in lipid and lipid was observed and we think that differences might be due to type of heavy metals, tolerance capacity, physiological status and metabolic rate of animals. However, during 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.

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