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Alteration of Lipid in Different Tissues of Freshwater Bivalve Lamellidens Marginalis After Exposed to Tributyltin Chloride

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ABSTRACT:

The freshwater bivalve, *Lamellidensmarginalis* exposed to 4.65 ppm, 3.40 ppm, 2.65 ppm and 1.72 ppm concentration of tri-butyltin chloride on lipid content in the tissue of gonad gills and digestive gland up to 96 hours exposure. Compare to control group there was significant change in lipid contents from gonad, gills and digestive gland in tri-butyltin chloride exposed groups. The percent decrease of total lipid content was in the order of gills, digestive gland and gonad. The depletion was maximum ingills.The result showed that depletion of Lipid in the gonad, Gills and Digestive gland of the test animals *Lamellidensmarginalis* due to the increase period of acute concentration of tributyltin chloride and also utilization of the products of their degradation for metabolic purpose.

KEYWORDS: Toxicity, Lamellidensmarginalis, Tri-butyltin chloride, Lipid alteration.

INTRODUCTION

Organotins compounds are responsible for water pollution and hence non target organisms in contaminated water bodies come in continuous and direct contact with organotin compound. After penetrating in the body, these organotin compound attacks on the biochemical composition of tissues in the organisms due to pollution stress. Organotin compounds have many applications, which include use as stabilizers in PVC (polyvinyl chloride) catalysts in chemical reactions, glass coatings, agricultural pesticides, biocide in marine antifouling paints and wood treatment and preservatives (Batt, 2006). The most concerned organotins compound is triorganotin such as tributyltin and triphenyltin compounds, which are used as industrial biocides, agricultural chemicals, wood preservatives, and antifouling agents.

In freshwater ecosystems along main shipping routes, the occurrence of concentrations that might endanger certain species is probable. TBT compounds exhibit the highest toxicity of all organotins (acute toxicity and immunotoxicity) and have been characterized as one of the most toxic groups of xenobiotics ever produced and deliberately introduced into the environment. This compound is known to be harmful to man and "non-target" aquatic organisms, particularly molluscs (Horiguchi*et al*., 1997). The freshwater mussels are an ecologically important fauna because they are used as sensitive biomarkers of aquatic ecosystem pollution. Bivalves are stationary filter-feeding organisms able to bioaccumulate and concentrate most pollutants even if they are present fairly low concentrations (Niyogi*et al*., 2001).

The biochemical changes occurring in the body gives the important indication of stress. Different tissues and organs have different activities and metabolic rates and therefore their responses to the same toxicant may be different. Much work carried on toxic effect of heavy metals on specific target and non target aquatic invertebrate and vertebrates with respect to the biochemical changes. Lipid is one of the important biochemical constitute in animal tissues which plays an important role in energy metabolism and provides energy in metabolic process, Shigmastus and Takeshita, (1959). Lipids are also important in the cellular and subcellular membranes. Lipids are used as energy resources and these are stored and transported in the form of glycerol and esters. Since *Lamellidensmarginalis* is an economically important freshwater bivalve, an attempt has been made to study the changes in lipid composition of different tissues like gonads, gills, digestive glands, which have vital and metabolic importance.KewalJaiswal*et al.,* (1989) reported changes in biochemical constituent such lipid when exposed to naphthalene to freshwater prawn, *M. kistnensis*. Higher concentrations of toxicant in aquatic environment cause adverse effect on aquatic organism at cellular or molecular level and ultimately it leads to disorder in biochemical composition (Waykar and Lomte, 2001).

Some toxicologist focused certain attention on the impact of pollutants on the lipid reserves of aquatic animal. Nagabhushanam*et al.,* (1972) reported decrease in lipid level in hepatopancreas of the freshwater prawn, *Macrobrachiumkistnensis*in response to pesticidal toxicity. Villalan*et al.,* (1990) observed the reduction in lipid content in muscle due to chromium stress in *Macrobrachiumidella.*Lomte and Muley, (1993) reported the decrease in lipid level in the freshwater snail and bivalve, *Thairatuberculata*and*Parresia corrugate* exposed to copper toxicity.Sarvana and Geraldine, (1997) reported the reduction in lipid content in freshwater prawn, *Macrobrachiummalcomsonii*when the prawn exposed to endosulfan. Sarojini*et al.,* (1989) found significant decreased in lipid in crab, *Barytelphusaguerini*exposed to zinc sulphate and copper sulphate. Romeo and Mauricette, (1997) studied the effect of heavy metals on lipid peroxidation in the mediterranean clam, *Ruditapesdecussatus.*Zambre, (1991) studied the effect of heavy metals on lipid content of freshwater bivalve, *Corbiculastriatella* due to heavy metal exposure. Deshmukh and Lomte, (1998) studied the effect of copper sulphate on lipid metabolism of the freshwater bivalve, *Parreysiacorrugata*. Many workers studied the lipid alterations in various animals after exposure to toxicants (Bhagyalakshmi, 1981; Verma and Tonk, 1983; Patil, 1986; Ribeiro*et al*., 2001).

Very few literatures are available on biochemical constituent under the stress of TBT in freshwater mussels. Hence, the present investigation was undertaken for the study of variation in lipid constituent in gonad, digestive gland and gill in freshwater bivalve, in *L. marginalis* under stress of tributyltin chloride.

MATERIAL AND METHODS

The freshwater bivalves, *L. marginalis* were collected from Godavari river at Paithan, 45 Km. away from Aurangabad city of Maharashtra state. They were collected and transported to the laboratory. Animals were brought and kept in plastic troughs containing aged water and acclimatize to them in laboratory conditions for 3 to 4 days. The water in the troughs was changed regularly. Before the experiment carried out the physiochemical characteristic of tap water were recorded.

1ppm stock solution of tributyltin chloride was prepared in acetone Laughlin *et al.,* (1983). After the acclimatization, healthy and medium sized bivalves were selected for experiments. The biochemical

analyses from different body parts were made of the bivalve, *L. marginalis* belonging to the control, and experimental.

For each experiment 10 animals of approximately similar size were exposed to acute concentrations as 4.6 ppm, 3.4 ppm, 2.6 ppm and 1.8 ppm for 24, 48, 72 and 96 hours of tributyltin chloride in monsoon season.

Along with experimental group a control group of 10 bivalves were also set up for the experimental period in non-contaminated freshwater medium to compare the results to study the effect of tributyltin chloride at different concentrations on the biochemical constituents of gonads, digestive glands, and gills. After experimental period the gonads, digestive glands, and gills were dissected out often control and experiments wet tissues were weighed and further processes for the analysis of lipid and of freshwater bivalve, *L. marginalis.*

Estimation of Lipid

Vanillin reagent method of Barnes and Blackstoch (1973) was used to estimate the amount of total lipids in the tissue.100 mg tissue was homogenized by adding 10 ml of chloroform Methanol (2:1) mixture. The homogenate was filtered and 1 ml of this (filtrate) was kept at room temperature in laboratory at 37^{o}C for 2 days to dry. 1ml of concentrated sulphuric acid was added to the dry mixture and kept in boiling water bath for exactly 10 minutes, following by cooling rapidly under tap water. 0.2 ml of this solution was taken; 5 ml Vanillin reagent was added and kept aside 30 minutes at room temperature (37^{o}C) before calorimetric estimator. Lipid content expressed as mg lipid/100mg wet tissue. The intensity of the colour developed was measured in colorimeter at 530mμ filter. The cholesterol was used as a standard.

The obtained data were analyzed by using various statistical analysis. The level of significance was calculated by student‘t’ test, R.A. Fisher and Yates (1963).

OBSERVATIONS AND RESULTS

The changes in biochemical composition of gonad, gills and digestive gland of freshwater bivalve, *Lamellidensmarginalis* exposed to acute concentrations of organotin tri-butyltin chloride were studied along with control animals. The data was supported by various stasticalanalysis and the standard deviation and standard error of the mean were calculated. Student‘t’ test was used to find out significance. The level of significance was used in the present study ($P<0.1$, $P<0.05$, $P<0.01$, $P<0.001$), R.A. Fisher and Yates, (1963).

During monsoon season the gonad of control bivalve, the lipid level was observed (15.7104 ± 0.2811). The bivalves exposed to 4.6 ppm, 3.4 ppm, 2.6 ppm and 1.8 ppm concentration of tributyltin chloride induced depletion in lipid content (15.0612 ± 0.4868, 4.1320%; $P<0.1$), (14.0875 ± 0.4868, 10.3301%; $P<0.05$), (13.6006 ± 0.4868, 13.4292%; $P<0.05$) and (13.1137 ± 0.4868, 16.5283%; $P<0.05$)% mg at 24, 48, 72 and 96 hours respectively, the results showed in (Table-01). In the gill of control bivalve, observed lipid content found to be (11.8966 ± 0.4216). In the gills of experimental bivalves the lipid depletion were recorded and the observed values were (10.5171 ± 0.2811, 11.5956%; $P<0.1$), (9.5433 ± 0.2811, 19.7807%; $P<0.05$), (8.9753 ± 0.2434, 24.5554%; $P<0.05$) and (8.0827 ± 0.1405, 32.0585%; $P<0.01$), % mg at 24, 48, 72 and 96 hours respectively, the results showed in (Fig-01). The digestive gland of control bivalve, the observed lipid content was (16.5219 ± 0.4868). In the digestive glands of experimental bivalves the lipid decreases (15.2235 ± 0.2811, 7.8582 %; $P<0.1$),

(14.3309 ± 0.6440, 13.2608%; P<0.1), (13.0326 ± 0.3718, 21.1191%; P<0.05) and (12.7080 ± 0.6126, 23.0837%; P<0.05), % mg at 24, 48, 72 and 96 hours respectively, the results showed in (Table-01).

In the present study results clearly indicate that biochemical constituent of Lipid in the gonad, Gill and Digestive gland of the test animals *L. marginalis* decreases significantly as the period of acute concentration of tributyltin chloride increases.

Table -01 (Monsoon)
Lipid content from different body parts of freshwater bivalve, *amellidensmarginalis*, exposed to acute concentration of TBTCl.

Sr. No	Tissue	Control	24h. Exp.	48h. Exp	72h. Exp	96h. Exp
1	Gonad	15.7104 ± 0.2811	15.0612 ± 0.4868 4.1320%	14.0875 ± 0.4868*** 10.3301%	13.6006 ± 0.4868*** 13.4292%	13.1137 ± 0.4868*** 16.5283 %
2	Gill	11.8966 ± 0.4216	10.5171 ± 0.2811 11.5956%	9.5433 ± 0.2811 *** 19.7807%	8.9753 ± 0.2434 *** 24.5554%	8.0827 ± 0.1405 ** 32.0585 %
3	Digestive gland	16.5219 ± 0.4868	15.2235 ± 0.2811 7.8582%	14.3309 ± 0.6440 $^{N.S}$ 13.2608%	13.0326 ± 0.3718*** 21.1191%	12.708 ± 0.6126 *** 23.0837 %

1. Values expressed as mg/100mg of wet wt. of tissue.
2. ± indicate S.D. of three observations.
3. P<0.1^{NS}- Non significant, P<0.05***, P<0.01**, P<0.001*.

DISCUSSION AND CONCLUSION

Although the general awareness of the hazard of environmental pollution had resulted in an upsurge of interest in toxicological studies pertaining to a freshwater bivalves. The actual metabolic effects of tributyltin chloride have received very little, if any inquiry. This motivated the design of laboratory oriented acute chemotoxicity tests to probe into the changes undergone at the biochemical level i.e., variations in lipid. Consequently, the freshwater bivalve, *L. marginalis* was exposed to the acute stress of tributyltin chloride to study such changes.The present findings indicated decrease of lipid in gonad, digestive gland and gills analysed for biochemical composition in *L. marginalis* exposed to

acute concentration 4.65 ppm, 3.40 ppm, 2.65 ppm and 1.72 ppm for 24, 48, 72 and 96 hours of tributyltin chloride in monsoon season respectively.In the present study, total lipid significantly decreases as period of exposure increases. (Table- 01).

The reasons for the decrease in lipid content in the bivalve, *L. marginalis*after tributyltin chloride stress may be due to reduced synthesis of lipid or increased activity of lipase involved in oxidation of lipids (Hollands, 1978). The overall decrease in the lipid content of tissues indicates the pronounced lipolysis and its utilization during tributyltin exposure. The effect of mercury on oxygen consumption and body biochemical composition of a marine bivalve *M. salleiwas* investigated by Uma Devi *et al.,* (1985) and results were significant decrease in oxygen consumption with increasing concentration of the toxicant. The effect of exposure time and concentration of Hg on body biochemical composition was also studied in *M. sallei* a decrease in the glycogen, protein and lipid was observed in time-dependent experiments. In concentration-dependent experiments, there was also a decrease in glycogen lipid and protein at all exposure concentrations.

The results of the present investigation supported and indicated that *L. marginalis* exhibited a differential preference in their utilization of biochemical constituent during time- and concentration-dependent stress of tributyltin chloride. The decrease in oxygen consumption together with the utilization of lipid and protein during tributyltin chloride exposure suggests that these bivalves might shift to anaerobic metabolism in order to encounter the heavy metal stress in the environment. Sujatha*et al.,* (1996) reported effect of tributyltin oxide induced biochemical changes in estuarine clam. They suggested tributyltin compounds are generally lipophilic and may reduces the lipid level in gemal muscle and digestive gland under stress condition of TBTO in clams, similar results were reported by Lundebye*et al*; (1997); Suguruinoue*et al*; (2006). Shiva Prasad Rao and RamanaRao (1979) stated that, considerable decrease in the total lipid in muscle might be due to drastic decrease in glycogen content in the same tissue which is an immediate source of energy during toxic stress conditions and after glycogen lipid content may be used for energy production to overcome the toxic stress. Romeo and Mauricette, (1997) in vitro experiments indicated that cadmium, which does not undergo redox cycling, was found unable to stimulate the lipid peroxidation process, where as copper and mercury may exist under different oxidation states and have detrimental effects on the antioxidant defence system of the Mediterranean clam, *Ruditapes decussates*. Deshmukh and Lomte, (1998) observed significant depletion in the lipid content in all the tissues tested after acute treatment of copper sulphate.Mahajan P.R,(2014) Showed the alteration of lipid in foot, digestive gland and whole body parts of freshwater bivalve*L. marginalis* due to increase the exposure period of heavymetal (Lead nitrate) and also due to chronic treatment of heavy metal.SwapnajaKamble and NitinKamble, (2015) showed in the experimental freshwater snail *Bellamyabegalensis* the lipid content was significantly decrease in different tissues up to the 96hrs of exposure.

Possibility of another factors may influences environmental parameters (temperature, salinity and available food) on the condition reproductive activity and biochemical composition of a native population of gametogenesis proceeded slowly and spawning took place in May- June, although the predicted time of ripening was early March. Available food appeared to be a very important factor in controlling gonad growth, once gametogenesis initiated.

Although during present investigation lipids level decreases in gill. The depletion in these metabolites in monsoon might be due to, over burden of tributyltin chloride stress on the cells of gills as the gills are the first organ which directly comes in contact with toxicant, ultimately affect the

physiology of gill function via, respiration, due to variety of effects on mitochondrial gill cells, which correlates with increase in O_2 consumption and at initial stress of toxicant the metabolites may examted to liberate the tremendous energy under stress of acute concentrations. (Wulf and Byington, 1975; Aldrich, 1976). Another possibility of decrease in organic constituent showed in gill, might be due to unfavorable conditions generally occurred during monsoon, unavailability of food, and no bloom of phytoplankton.

In conclusion depletion of lipid in the tissues in freshwater bivalve, *L. marginalis* mainly due to stress condition of tributyltin chloride exposure. Many factors Such as biological and environmental may also cause depletion in lipid level, particularly in gonad and digestive gland. It is need of further investigation to evaluate the extent of toxic effect of tributyltin chloride to focus the degree of bioaccumulation and bio assessment of tributyltin chloride in tissues of freshwater bivalve, *L. marginalis.*

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