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# Depletion Of Glycogen In Different Tissues Of Freshwater Bivalve, *Lamellidens Marginalis* Exposed To Tributyltin Chloride

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**Abstract**

The freshwater bivalve, *Lamellidens marginalis* exposed to 3.5 ppm, 2.5 ppm, 1.8 ppm and 1.0 ppm concentration of tri-butyltin chloride on glycogen content in the tissue of gonad gills and digestive gland up to 96 hours exposure. Compare to control group there was significant change in glycogen contents from gonad, gills and digestive gland in tri-butyltin chloride exposed groups. The percent decrease of total glycogen content was in the order of gills, digestive gland and gonad. The depletion was maximum in gills. The result showed that depletion of glycogen in the gonad, Gills and Digestive gland of the test animals *Lamellidens marginalis* due to the increase period of acute concentration of tributyltin chloride and hypoxic condition might have been prevailed in the bivalve to provide excess energy by its utilization.

Keywords: *Toxicity, Lamellidensmarginalis,Tri-butyltin chloride, Lipid alteration.*

## 1. Introduction

The heavy metal pollutant gives rise to alteration in the metabolic and
physiological activity on both short and long term exposures. The biochemical changes in the organs of animal exposed to heavy metals have no definite pattern and the physiological state of metabolic activity of an organism reflects in the utilization of their biochemical energy to counteract toxic stress. To investigate the physiological changes after heavy metal treatment, point is to study the changes in the biochemical constituents like carbohydrate, protein and lipid as they are important metabolites to provide energy for different vital processes.

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.

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).

## 2. Review of Literature

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. Since *Lamellidens marginalis* is an economically important freshwater bivalve, an attempt has been made to study the changes in biochemical composition of different tissues like gonads, gills, digestive glands, which have vital and metabolic importance. Carbohydrates are the major biochemical components, which act as source of energy for various physiological functions including reproduction (Giese, 1969).

Carbohydrate plays not only a structural role in every cell but also serve as a reservoir of the chemical energy to be increased or decreased according to the organismal needs. Animals store polysaccharides in tissues as glycogen, and is

considered to be the major source of energy and hence all metabolic events depend upon the supply of glycogen. Glycogen in tissues is also considered to be the immediate source of energy for animals adapted to the changing environmental conditions (Latner, 1975).

Several workers have reported the impact of various aquatic pollutants on glycogen metabolism of different species. Nagabhushanam*et al*., (1988) reported altered carbohydrate metabolism in freshwater prawn, *Macrobrachiumkistnensis* exposed to sublethal concentration of naphthalene. Reddy and Rao, (1991) observed considerable decreased in total glycogen level exposed to phosphomidan toxicity to marine penaeid prawn, *Metapenaeusmonoceros*. Sreenivasulu Reddy and Bhagyalakshmi, (1994) studied the alteration in carbohydrate metabolism in selected tissues of freshwater crab *Oziotelphusasensex*when exposed to pyrethroid insecticide. Dhanapakiam and Ramasami, (2001) reported toxic effects of copper and zinc mixture on some hematological and biochemical parameter in common carp, *Cyprinuscarpio*.Ramalingam and Indra, (2002) showed decrease in glycogen level when exposed to copper sulphate toxicity on tissue phosphatases and carbohydrate turned over in *Achatinafulcia*. K. Satyaparameshwar*et al.,* (2006) reported decreased carbohydrate metabolism in freshwater bivalve, *Lamelidensmarginalis* exposed to copper sulphate and observed decrease in carbohydrate content level in labial palp, gill and mantle. Chaudhari*et al.,* (2002) observed the effect of heavy metal, on the biochemical component like glycogen of various tissues of freshwater bivalve, *Parreysia cylindrical*. The significant decreases in total glycogen content of gill, digestive gland were observed due to pollution stress caused by nickel chloride.

Shah *et al.,* (1998) investigated changes in glycogen contents in estuarine *Anadararhombea*exposed to TBTO. Rao *et al*., (1983) observed significant decrease in total carbohydrates and glycogen in hepatopancreas, mantle and foot in *Pila globosa*to sub lethal concentration of methyl parathion and also decreased phosphorylase activity. Mandal and Ghose (1970) observed the glycogen depletion in digestive gland of the snail, *Achatinafulica* exposed to calcium arsenate. Reddy *et al*, (1986) and Shah *et al*., (1998) also studied the glycogenolytic phenomenon during stress.The glycogen contents in mollusks after exposure to pollutant were estimated by Lomte and Alam (1982).

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

## 3. Materials 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 3.5 ppm, 2.5 ppm, 1.8 ppm and 1.0 ppm for 24, 48, 72 and 96 hours of tributyltin chloride in summer 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 glycogen of freshwater bivalve, *L. marginalis.*

**Estimation of Glycogen**

Glycogen was estimated by Anthrone reagent method (Dezwaan and Zandee, 1972). 100 mg tissue was prepared in 30% KOH and the homogenate mixture was kept in boiling water bath for 5 to 10 minutes to dissolve the tissue and then cooled. Before centrifugation 2 ml of 96% ethyl alcohol was added and the mixture was kept overnight in refrigerator. After 24 h this mixture was centrifuged at 3000 rpm for 15 minutes. The glycogen cake settled down to the bottom. 2 ml of distilled water was added to cake and mixed well. This mixture was kept at 70□ C for 5 minutes in hot water bath. 0.1 ml from this solution was mixed with 0.9 ml distilled water and 5 ml of anthrone reagent was added. This mixture was kept in hot water bath for 10 minutes. Glycogen content is expressed in terms of mg glucose/100mg wet weight tissue. The intensity of the colour developed was measured in colorimeter at 620mμ filter.

The obtained data for summer season were analyzed by using various statistical analysis. The

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level of significance was calculated by student't' test, R.A. Fisher and Yates (1963).

## 4. Results and Discussion

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 stastical analysis 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 summer season the gonad of control bivalve, the glycogen level was observed (9.3771 ± 0.17012). The bivalves exposed to 3.5 ppm, 2.5 ppm, 1.8 ppm and 1.0 ppm concentration of tributyltin chloride induced significantly depletion in glycogen content (8.4698 ± 0.0982, 9.6759%; P<0.05), (7.9027 ± 0.0982, 15.7234%; P<0.01), (7.2222 ± 0.0982, 22.9803%; P<0.01) and (6.5417± 0.0982, 30.2373%; P<0.001)% mg at 24, 48, 72 and 96 hours respectively, the results showed in (Table-1). In the gill of control bivalve, glycogen content found to be (7.6759 ± 0.1701). In the gills of experimental bivalves the glycogen content depletion were recorded, and the observed values were (6.9386 ± 0.0982, 9.6041%; P<0.05), (6.3716 ± 0.0982, 16.9919%; P<0.01), (5.6911 ± 0.0982, 25.8572%; P<0.01) and (5.2374 ± 0.0982, 31.7675%; P<0.001), % mg at 24, 48, 72 and 96 hours respectively, the results showed in (Table-1). The digestive gland of control bivalve, the observed glycogen content was (8.6966 ± 0.1701). In the digestive glands of experimental bivalves the glycogen content significantly decreases to (8.2429 ± 0.0982, 5.2165%; P<0.1), (7.4490 ± 0.0982, 14.3454%; P<0.01), (6.7685 ± 0.0982, 22.1702%; P<0.01) and (5.9746 ± 0.1701, 31.2992%; P<0.001), % mg at 24, 48, 72 and 96 hours respectively, the results showed in (Table-1 & Fig-1).

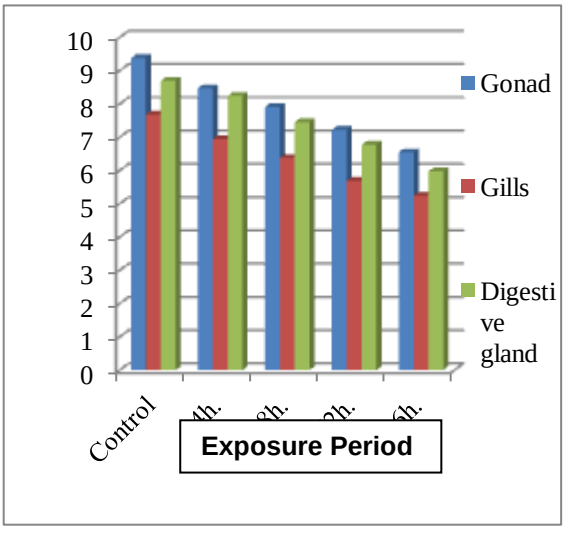
In the present study results clearly indicate that biochemical constituent Glycogen in the gonad, Gill and Digestive gland of the test animals *L. marginalis* decreases significantly as the period of acute concentration of tributyltin chloride increases.

Table 1: Glycogen content from different body parts of freshwater bivalve, *Lamellidens marginalis*, exposed to acute concentration of TBTCl.

| Sr. No | Tissue | Control | 24h. Exp. | 48h. Exp | 72h. Exp | 96h. Exp |
|---|---|---|---|---|---|---|
| 1 | **Gonad** | 9.3771 ± 0.17012 | 8.4698 ± 0.0982 *** 9.6759 % | 7.9027 ± 0.0982 ** 15.7234% | 7.2222 ± 0.0982 ** 22.9803% | 6.5417 ± 0.0982* 30.2373% |
| 2 | **Gills** | 7.6759 ± 0.1701 | 6.9386 ± 0.0982 *** 9.6041 % | 6.3716 ± 0.0982 ** 16.9919% | 5.6911 ± 0.0982 ** 25.8572% | 5.2374 ± 0.0982* 31.7675% |
| 3 | **Digestive gland** | 8.6966 ± 0.1701 | 8.2429 ± 0.0982 5.2165 % | 7.4490 ± 0.0982 ** 14.3454% | 6.7685 ± 0.0982 ** 22.1702% | 5.9746 ± 0.1701* 31.2992% |

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*.

Fig. 1 Showing the depletion of glycogen in different tissues after exposure to Tributyltin chloride.




The last decades revealed that there is growing evidence of the increased ecological risk to aquatic organisms inhabiting contaminated sites. These organisms may accumulate a wide variety of pollutants in their tissues and known that the effects, induced by the environmental contaminants, are most frequently initially displayed at the molecular and biochemical level and, as a result of such

disturbances, various biochemical, cytological, morphological, and physiological and other changes occur. At the later stages, changes may occur at higher biological levels and they affect the ability of organisms to grow, to reproduce or to survive. Anderson *et al.,* (1994).

The mode of action of pollutant may be responsible for cellular disorganization offering the storage and metabolism of the organic constituents. The pollutant give the heavy physical irritated stress causing rapid movement and increased respiration rate thus increased the utilization of reserve constituents like lipids and glycogen to meet the high energy demand of body causing decrease in organic constituents content Bhagylakshmi, (1981).

Glycogen is considered to be the major source of energy in animal tissues and maintenance of glycogen reserves is an essential feature of the normal organismal metabolism. It also plays an important role in the structural part of the cell membranes De Zwaan and Zandee, (1972). The decrease in glycogen content form the whole body of the bivalve, *L. marginalis*suggeststhe possibility of the glycogenolysis which in turn produce energy to cope up the adverse stress conditions. It has been described that in molluscs the glycogen is the chief source to liberate energy and its increase or decrease will alter the metabolism (Chaudhari and Lomte, 1992). Any stressful condition alters the biochemical composition. The change in metabolic rate leads towards the change in biochemical composition hence, the change in biochemical composition is an indicator of stress of chemical or physical nature in the surrounding which mainly affects glycogen contents.

Significant depletion in glycogen level suggests possibility of its rapid utilization to provide excess energy for cellular biochemical process through glycolysis. Hypoxic condition might have been prevailed in the bivalve to provide excess energy by its utilization. Glycogen level is generally high in bivalves (Dezwaan 1983).

The present findings, total glycogen percentage decreases gradually in gonads, digestive gland and gills in the summer significantly as period of tributyltin chloride exposure increases. (Table -1& Fig-2).

Depletion in glycogen levels in the present study might be attributed to hypoxic conditions under tributyltin chloride, our results are in agreement with Sujatha *et al.,* (1996) and Kharat, (2007).Many workers evaluated the glycogen content under heavy metal stress conditions (George *et al.*, 1986; Langstone, 1986; Reddy *et al.*, 1986; Bourgion and Risk, 1987; Langstone and Burt, 1991; Satyaparameshwar*et al.,* 2006). (Almeida *et al.*, 1999; Osada and Mori 2000) studied the biochemical composition of *Crossostreagigas*and *Ostrea edulis* with respect to seasonal variations under stress condition and reported decrease of glycogen in different tissues. According to Clarke, (1975) Glycogen is the immediate source of energy; under stressed conditions the glycogen depot is exhausted. The decrease in glycogen might be due to great breakdown of glycogen in the digestive gland subjected to glycogenolytic activity (Koundinya and Ramamurthi, 1979) as that of the liver of vertebrates (Kabeer*et al.*, 1977). Shah *et al.,* (1998) also found a reduced level of glycogen in the digestive gland due to glycogenolysis in blood clam, *A. rhombea*.

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. Seasonal changes in the main biochemical components of this bivalve were determined for a standard individual. When food was abundant, energy reserves were built up. Spawning produced a decrease in biochemical constituent levels, and recovery coincided with the phytoplankton bloom. The stored reserves, mainly lipids, were used to overcome a state of energy imbalance in winter associated with low food availability. Results show this bivalve to be an opportunist organism which concentrates its reproductive effort during a short period of favorable conditions and which is directly dependent on nutritive availability in the environment. Gabbott, and Bayne, (1973), have shown that seasonal variations in biochemical composition of molluscs depend on environmental parameters such as temperature and available phytoplankton and factors such as timing of the reproductive cycle and the rate of turnover of stored energy.

The glycogen depletion in summer 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 constituents showed in gill, might be due to unfavorable conditions generally occurred during summer, such as increase in water and atmospheric temperature, low dissolved oxygen, unavailability of food, and no bloom of phytoplankton.

## 5. Conclusions

Very few literatures are available on the biochemical constituent under the stress of TBTCL in freshwater mussels. Hence, the present

investigation was undertaken. 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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