



Variation in Biochemical Contents in Hepatopancreas of Bivalve *Lamellidens Marginalis* Induced by Tributyltin Oxide

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ABSTRACT

In present biochemical study, bivalve *Lamellidens marginalis* exposed to acute concentrations of TBTO for 24hr, 48hr, 72hr, 96hr in three seasons and protein, lipid, glycogen contents of hepatopancreas were analyzed during year June2014-May2015. Animals were exposed in acute concentrations in ppm were 2.261, 1.679, 1.272, 0.885 in monsoon; 2.634, 1.921, 1.458, 1.094 in winter and 1.618, 1.111, 0.857, 0.398 in summer season, values are 24, 48, 72 and 96 hrs respectively for each season. In control group and experimental group of bivalves found biochemical contents high in summer season and under the stress of TBTO biochemical contents decreased significantly up to 96hr in all seasons. Biochemical contents level present in hepatopancreas was found as Glycogen>protein>Lipid.

KEYWORDS : *Lamellidens marginalis*, hepatopancreas, biochemical contents, Seasonal, LC50, TBTO

Introduction

Seasonal changes in physicochemical factors and contamination of pollutants in aquatic bodies there is assumed stress conditions consequently there may effect on physiology of aquatic organisms. Protein, lipid and glycogen are most important biochemical constituents which are energy source for metabolism as well as among them mainly protein take part in structural and functional purpose of cellular level. The main carbohydrate reserve in the tissue is glycogen; Lipids play vital role in energy metabolism after glycogen lipids are used as energy source¹⁻³. Hepatopancreas in bivalve works as biochemical reserve organ containing digestive cells, the digestive cells of the bivalve are initial target of endocytic absorption and intracellular digestion, the digestive gland is the key site of heavy metal storage. Animals have a natural or innate protective mechanism to oppose and struggle against toxicants and their effect. The toxic substance are once entered in the body, they start to damage target organs at cellular, molecular level hence animal have required more demand of energy. If contamination occurring in body, biochemical processes give alarming indication that may be stress condition⁴, so biochemical processes might be helpful to monitor the pollutants' toxicity⁵. Biochemical study could be essential to analyze the health of organism and intensity of toxicity of pollutants present in aquatic animals and aquatic ecosystems⁶.

Heavy metals are the most common anthropogenic pollutants in water bodies. The heavy metals are known as non bio-degradable while highly toxic to almost all aquatic organisms⁷. TBTs are widely utilized organometallic compounds and they have been using worldwide⁸⁻⁹. Once the organometals are deposited in the environment create a serious threat to global ecosystems¹⁰. Organometallic compounds are increased risk due to their potential reactivity, bio concentration, bioaccumulation and biomagnifications¹¹. *L. marginalis* is regarded as biomarkers of metals in aquatic ecosystem. Biochemical contents in hepatopancreas seasonally exposed to TBTO have been reported less figure of workers thus in present study protein, lipid and glycogen in hepatopancreas of *Lamellidens marginalis* exposed with TBTO was investigated seasonally.

Material and methods

The freshwater bivalves, *L. marginalis* were collected from the Godavari river at Kaigaon village, 45 km away from Aurangabad city in Maharashtra state. During June2014-May2015 a period, one year was selected for experiments. The bivalves were cleaned to remove the algal biomass and mud then put 3 to 4 days for acclimation in laboratory condition. 1ppm stock solution of tributyltin oxide was prepared in acetone¹². For each experiment 10 animals of approximately similar size (60-65mm) were exposed to acute concentrations (LC₅₀) as 2.261 ppm, 1.679 ppm, 1.272 ppm, and 0.885 ppm in monsoon (August-September); 2.634 ppm, 1.921 ppm, 1.458 ppm and 1.094 ppm in winter season (December-January); 1.618 ppm, 1.111 ppm, 0.857 ppm and 0.398 ppm in summer season (April-May) for each season 24, 48, 72, and 96 hrs respectively, A control group of 10 bivalves were maintained simultaneously, animals were dissected and 100mg of each wet tissues were taken for biochemical analysis. Protein, lipid and glycogen level in hepatopancreas of controlled and experimental bivalves were determined. Protein estimation was made by Lowry's method¹³, lipid by Vanillin reagent method¹⁴ and glycogen by Anthrone reagent method¹⁵. The biochemical contents of hepatopancreas were calculated and expressed in terms of mg/100mg wet weight of the tissue. In the present study, Standard solutions were used, O.D. taken from spectrophotometer and the level of significance was used (P<0.001, P<0.01, P<0.05)¹⁶.

Observation and results

Biochemical contents in hepatopancreas of freshwater bivalve, *Lamellidens marginalis* exposed to acute concentrations of organotin tri-butyltin oxide (TBTO) observed along with control animals were showed in tables (1-3). In the present study the biochemical contents present in hepatopancreas decreased considerably up to 96hr when exposed in acute TBTO concentrations and they observed maximum in the summer and minimum in monsoon season. Biochemical contents level present in hepatopancreas was found as Glycogen>protein>Lipid.

Table-1 (summer season)

Tissue	Control Group	24hr	48hr	72hr	96hr
Glycogen	37.82 ±3.43	34.43** ±1.58 8.96%	29.71 ±2.63 21.44%	23.84** ±0.83 36.96%	20.27*** ±3.19 46.40%
Protein	28.73 ±1.82	21.83 ±1.64 24.01%	18.87* ±1.30 34.31%	15.04*** ±0.83 47.65%	11.69* ±1.26 59.31%
Lipid	21.42 ±1.43	19.40* ±1.89 9.43%	16.24 ±1.19 24.18%	14.31* ±2.01 33.19%	10.93** ±0.86 48.97%

Biochemical contents in hepatopancreas of freshwater bivalve, *Lamellidens marginalis*, exposed to acute concentration of TBTO. Values are mg/100mg of wet weight of tissue.

Significant difference from control value (P<0.05=*, P<0.01=**, P<0.001=***)

Table-2 (winter season)

Biochemical contents in hepatopancreas of freshwater bivalve, *Lamellidens marginalis*, exposed to acute concentration of TBTO. Values are mg/100mg of wet weight of tissue.

Tissue	Control Group	24hr	48hr	72hr	96hr
Glycogen	32.13 ±3.14	28.73** ±2.17 10.58%	26.28* ±0.79 18.20%	23.93* ±1.09 25.42%	19.57 ±3.20 39.09%
Protein	24.53 ±2.83	21.34* ±1.28 14.81%	18.42 ±1.69 24.90%	14.76** ±2.61 39.82%	12.89*** ±0.78 47.45%
Lipid	18.94 ±1.40	16.04** ±1.36 15.31%	14.31 ±1.24 24.44%	12.82*** ±1.22 32.31%	9.46** ±0.73 50.05%

Significant difference from control value (P<0.05=*, P<0.01=**, P<0.001=***)

Table-3 (monsoon)

Biochemical contents in hepatopancreas of freshwater bivalve, *Lamellidens marginalis*, exposed to acute concentration of TBTO. Values are mg/100mg of wet weight of tissue.

Tissue	Control Group	24hr	48hr	72hr	96hr
Glycogen	29.76 ±1.31	25.81* ±2.36 13.27%	22.09 ±1.15 25.77%	18.37** ±1.36 38.27%	15.52*** ±0.92 47.84%
Protein	23.19 ±1.23	21.35* ±1.22 7.93%	17.79* ±2.37 23.28%	15.49** ±1.84 33.20%	12.74* ±0.96 45.06%
Lipid	16.37 ±1.40	13.96* ±1.23 14.72%	10.94*** ±1.37 33.17%	7.26 ±2.36 55.65%	6.98** ±0.96 57.36%

Significant difference from control value (P<0.05=*, P<0.01=**, P<0.001=***)

DISCUSSION AND CONCLUSION

Acute effect of TBTO on hepatopancreas of bivalve *L. marginalis* resulted as reduction in protein, lipid and glycogen content in all over three seasons as compared with control animals they were also changed biochemical contents as per seasonal stress. In response to changes in variable seasonal physicochemical factors animal may change metabolic activities. Food availability, health of organism, reproductive cycle may influence on biochemical constitute of organism. Animal increases membrane protein for changed temperature, the biochemistry of cell membrane made as more fluid in cold temperatures and less fluid in warm temperatures¹⁷. During summer season there may gametogenesis¹⁸; on the contrary reproduction may take place in monsoon¹⁹ that may causes decreased biochemical constitute in control animal group.

In the stress condition of TBTO hepatopancreas may be the initial sites for action of pollutant in the body of bivalve where animal re-

acts against toxicant with making some physiological changes within body or body parts like excess mucus secretion, antioxidant defense mechanisms²⁰ resulted more energy demand and that got from stored biochemical in tissue. It give the impression to be the main site of degradation and detoxification of toxicants resulting into increasing utilization of major energy sources like protein, lipid and glycogen hence there have the more demand of energy for the metabolic processes and start to decrease biochemical contents²¹⁻²². It was observed, glutathione dependent antioxidant enzymes in mussel, *Mytilus galloprovincialis* exposed to metals²⁰. Changes in hepatopancreas of the bivalve *Lamellidens marginalis* were observed in summer when exposed to acute toxicity of Cadmium²³. *Lamellidens marginalis* after exposed to TBTO found to be significant depletion of biochemical contents during different seasons when increased exposure period up to 96hr²⁴. Similar results were found, *L. marginalis* exposed to sub chronic period of colour pigments which used for idols resulted as biochemical contents decreased with increase concentration of toxicant²⁵. Protein content of *L. marginalis* deceased when exposed to nickel²⁶ and nickel chloride without ascorbic acid²⁷ as compare to bivalves exposed in Nickel with ascorbic acid. Recently observed oysters *Crassostrea cattuckensis* exposed to sub lethal lower and higher concentrations of mercury for 15 days resulted decrease in protein contents in all body parts during the detoxification process performed by animal²⁸. *L. marginalis* exposed to tannery wastewater for 10 and 20 day observed same results significantly decrease in biochemical contents with increased exposure period²⁹. Seasonal changes in biochemical content in *L. marginalis* exposed to different pollutants were observed³⁰⁻³³ and when exposed to heavy metal³⁴. Seasonally variations in difference physicochemical parameters in the aquatic system such as, temperature, light, pH, total suspended solids, dissolved organic carbon³⁰⁻³² and sometimes difference in water flow like monsoon, which may cause variations in the physiology. Glycogen is the immediate source of energy; under stressed conditions the glycogen store is exhausted³⁵. Seasonal variation in biochemical contents level in *Donax cuneatus* was reported³⁶. Affect of trace metals to mussel *perna perna* observed concentrations affects on sex, weight and seasonal cycle³⁷. Thus present investigation concluded that, TBTO contamination make adversely effects on health of freshwater bivalve *L. marginalis*, Decreased biochemical contents level in hepatopancreas of *L. marginalis* may indicates stress condition so study of biochemical can useful in monitoring the toxic effects of pollutants on freshwater animals.

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