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Acute Toxicity Study of Tributyltin Chloride on the Freshwater Bivalve, *Lamellidens marginalis*

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Abstract: The active substance TBT is highly toxic and showed damage to a multitude of non target species. The bivalves have been used for many years to determine the pollution status of water. In the present study static bioassays were performed on bivalve, *Lamellidens marginalis* to evaluate the median lethal concentrations of tributyltin chloride (TBTCl) for 24, 48, 72 and 96 hrs. The LC_{50} values were 5.33, 4.02, 3.05 and 2.12 ppm, after 24, 48, 72 and 96 hrs respectively. The results showed that the LC_{50} values decreased with increase in exposure period.

Key words: Toxicity % Godavari river % Tributyltin chloride % *Lamellidens marginalis* % $LC_{50,}$ ppm

INTRODUCTION

The toxicological studies of pollutants are gaining more significance in recent time and worldwide attempts has been made to identify a "hazard" from toxic chemical present or released in aquatic environment. Polluted state of the water resources has led to steady decline in aquatic flora and fauna.

Freshwater mussels are in serious global decline and in urgent need of protection and conservation. The declines have been attributed to a wide array of human activities resulting in pollution and water-quality degradation. 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 [1]. Godavari river is considered one of the most important water bodies in Maharashtra state, large, shallow and exposed to high levels of pollutants from industrial, domestic and agricultural resources.

Organotin tributyltin compounds are among the most hazardous pollutants known so far in aquatic ecosystems [2] 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 mollusks [3]. Tributyltin chloride is of particular importance because of its widespread use as biocide, namely in antifouling paints on ships and in wood protection. Since the late 1970's considerable quantities of Tributyltin chloride were introduced into the aquatic environment and as a result, widespread pollution of marine and freshwater harbours and adjacent areas resulted. Due to the extreme toxicity and the ecotoxicological hazards associated with TBTCl in antifouling paints, biocide and in wood protection, restrictions on its use have been implemented in many countries in the mid to end 1980's. In spite of regulation and prevention act the release of organotin Tributyltin chloride in aquatic and terrestrial environments has decrease recently, but inputs still occur and previously contaminated sites continue to act as source [4].

Since last three decades acute toxicity bioassays in general are useful in measuring the toxicity of different pollutants to aquatic organisms. Mane and Muley [5] observed the toxicity of cythion-malathion to two freshwater bivalve molluscs, *L. marginalis* and *Lamellidens corrianus* [6] Studied the cadmium toxicity to the marine edible gastropod, *Babylonia spirata*. [7] Showed the toxicity bioassay of the Juvenile freshwater snail, *M. martensi* exposed to mercury and cadmium.

Many authors have been extensively studied the effect of organotin compounds on experimental animals Alzieu *et al*. [8] found mortality in pacific oyster,

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Crassostrea gigas exposed to TBT. Reproductive abnormalities have been observed by toxic effect of TBT in the European flat oyster, *Ostrea edulis* [9]. Morri and Roberts [10] studied acute toxicity of tributyltin chloride to embryos and larvae of two bivalve mollusks, *Crassostrea virginica* and *Mercenaria mercenaria*. Verslyce *et al*. [11] Revealed that the cellular energy allocation in the esturine mysid shrimp *Neomysis integer* to different TBT exposure. Rabbito *et al*. [12] have been studied the effect of TBT on Neotropical fish, *Hoplias malabaricus.* Sousa *et al*. [13] observed the acute toxicity of tributyltin to veliger larvae of *Nassarius reticulates*.

The present study has been planned to evaluate the impact of organotin tributyltin chloride on toxicity of the freshwater bivalve, *L. marginalis*.

MATERIALS AND METHODS

The freshwater bivalves, *Lamellidens marginalis* were collected from the Godavari river at Paithan, 45 km away from Aurangabad city. The bivalves were brought to the laboratory and cleaned to remove the fouling algal biomass and mud. The bivalves kept in plastic troughs containing dechlorinated tap water for 3 to 4 days to acclimatize to the laboratory conditions.

The bivalves were exposed to diffused day light during the daytime, where the daily photoperiod was about 10-12 hrs. Pilot experiments were conducted to find out the range of the toxicity of the toxicant used tributyltin chloride. The chosen range of concentration was such that it resulted in 0 to 100% mortality.

1-ppm stock solution was prepared in acetone [14]. The series of statistic bioassay were conducted under laboratory condition as described by Finney [15].

Acute toxicity tests were conducted over 96 hrs. The experimental troughs containing 5 liters dechlorinated water were used to keep the animals. For each experiment ten bivalves, *L. marginalis* of approximately similar size (50-55mm in shell length) were exposed to different concentrations of tributyltin chloride. After every 12 hours the polluted water was changed by the fresh solution of the same concentration. The resulting mortality was noted in the range of 10 to 90% for each concentration for the duration of 24, 48, 72 and 96 hrs. Each experiment was repeated thrice to obtain constant results.

The data collected was analyzed statically by means of probit method on transforming toxicity curve (% mortality vs. concentration), which allows the average median lethal concentration of LC_{50} to be calculated for 24, 48, 72 and 96 hrs. Dead bivalves were counted individually.

RESULTS

The LC_{50} values were calculated for 24, 48, 72 and 96 hours by Finney's method [15]. The LC_{50} values obtained for tributyltin chloride exposed for 24, 48, 72 and 96 hours exposure were 5.33, 4.02, 3.05 and 2.12 ppm respectively. The results showed that LC_{50} values decreases with increasing periods of exposure of tributyltin chloride.

The LC_{50} values, regression results, Chi square, variance and 95% fiducial limits, lethal concentration and safe concentration were calculated and are shown in Table 1. From the above results it appears that the freshwater bivalve, *Lamellidens marginalis* is highly sensitive to organotin Tributyltin chloride.

DISCUSSION

The determination of the LC_{50} value is of immense importance since it provides fundamental data for the design of more complex disposal model. The values obtained are highly useful in the evaluation of safe level or tolerance level of a pollutant [16].

In the present study the *L. marginalis* exposed to tributyltin chloride, the acute toxicity level was expressed in terms of LC_{50} values. The LC_{50} values were found to be 5.33, 4.02, 3.05 and 2.12 ppm at 24, 48, 72 and 96 hours respectively. The 96 hours LC_{50} value was the low,

Table 1: Relative toxicity of TBTCL to the freshwater bivalve, *Lamellidens marginalis*

Time of exposure	Regression equation	LC50			Fiducial limits		Lethal	Safe
(Hrs.)	Y=yG+(X-xG)	Values in ppm.	Variance V	Chi-square	m1	m2	dose 111.7368	conc. (ppm)
24	Y=19.3152X-9.0402	5.3327	0.000091753	0.15276105	0.69658486	0.73413369	127.9848	0.4588
48	Y=14.6742X-3.8753	4.0258	0.000153900	0.17244250	0.57665780	0.62528150	193.2384	
72	Y=12.0731X-0.8517	3.0532	0.000238150	0.09207855	0.44492966	0.50542295	219.8304	
96	Y=7.3390X+2.5989	2.1244	0.000644580	0.21162092	0.24684964	0.34637292	203.9424	

however the mortality scored was high. The LC_{50} values decreased with increase in exposure period. Therefore the LC_{50} values and exposure period showed a direct relationship. The similar result was found by [17] they reported that the LC_{50} values depend on the concentrations of pesticides and also with the time of exposure. Reddy *et al*. [18] reported that the LC_{50} values and the exposure period showed inverse relation. The results showed that the LC_{50} values decreased with increase in exposure period and vice-versa. The reaction and survival of aquatic organism, under toxic conditions depend upon several factors, such as kind, toxicity and concentration of the toxicant and the temperature, salinity, dissolved oxygen, pH and physiological factors such as reproductive cycle and seasons, in addition to the type and time of exposure to the toxicant [19, 20].

The toxic effect on common oyster larvae exposed to 0.02-100 g/L tributyltin acetate were studied by [21], as a result, in the group of larvae exposed to tributyltin acetate at 0.05 g/L (0.05 g/L in terms of tributyltin chloride) or over, growth was inhibited and deaths were observed within 10 days. Beaumont and Budd, [22] Exposed veliger larvae of the mussel (*Mytilus edulis*) to TBTO for 15 days. No larvae survived longer than 5 days in 10 µg/L TBTO, or longer than 10 days in 1 µg/L TBTO. About half the larvae exposed to 0.1 µg/L TBTO were dead on Day 15 (i.e., 15-d LC_{50} approximately 0.1 µg/L TBTO) and most surviving larvae were moribund and had grown significantly more slowly than controls.

Holwerda and Herring, [23] found that the freshwater clam *Anodonta anatine* could not survive exposure to tributyltin oxide in a concentration equivalent to 5ìg Sn/L for longer than 6 weeks. Dode, [24] Reported that LC_{50} values of all the five size groups of fresh water prawn, *Macrobrachium kistnensis* exposed to different concentrations of cuprous oxide for 24, 48, 72 and 96 hours, they show that relative toxicity increases with increasing exposure time since LC_{50} values decreased as the exposure period increased. Kungolos *et al*. [25] studied toxicity tests were performed in order to determine the toxic properties of four organotin compounds to freshwater crustacean; *Daphnia magna* tributyltin chloride was found to be the most toxic substances on test organisms.

Shejule *et al*. [26] reported that, LC_{50} values of the organotin tributyltin chloride exposed to freshwater prawn, *Macrobrachium kistnensis*; up to 96 hours and decreased with increase in exposure period. Kharat, [27] shows the same results of LC_{50} values of the organotin tributyltin chloride exposed to freshwater prawn, *Macrobrachium kistnensis.*

From the above discussion and all the available literature, we can conclude that the TBTCl is very toxic to the freshwater bivalve, *Lamellidens marginalis*, therefore the release of organotin compounds in aquatic environment especially in freshwater ecosystem might be controlled.

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