ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2020; 6(6): 37-40
www.allresearchjournal.com
Received: 02-04-2020
Accepted: 05-05-2020

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Acute toxicity studies of tributyltin chloride on fresh water bivalve, lamellidens marginalis in summer season

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Abstract
Toxicity study is essential to provide the safe level of concentration and determine the sensitivity of animals towards the toxicant. The active substance TBT is highly toxic and also damaging to a multitude of non-target species. Static bioassays were performed on bivalve, *Lamellidens marginalis* to evaluate the median lethal concentrations of TBTCl (tributyltin chloride) for 24, 48, 72 and 96 hrs. The LC_{50} values were 3.55 ppm, 2.42ppm, 1.79 ppm and 0.98ppm respectively. The study revealed that the bivalves were more sensitive to TBTCL stress and the result LC_{50} values decreased with increase in exposure period.

Keywords: Lamellidens marginalis, tributyltin chloride, acute toxicity, LC_{50}, summer season

Introduction
Organotin compounds have developed into important industrial commodities in the past 50 years because of the wide variety of their uses. About 70% of the organic application (Hoch M. 2001) [17] of the world annual production of organotin compounds estimated at 50,000 tons (Fent, K., 1996) [11] is the use of mono/dialkyltin derivatives as heat and light stabilizer additives in PVC processing. Major Organotin compounds released to the environment are triphenyltin (TPT), tricyclohexyltin (TCT), di-n-octyltin (DOT), di-n-butyltin (DBT), dimethyltin (DMT), and tri-n-butyltin (TBT). TBT compounds are used as biocides for colling systems and as antifouling agents for boat and fishnet paints in maritime activities since the early 1960s, Omae, I. (2003) [33]. TBT-based antifouling paints preventing the attachment of algae and invertebrates on ship hulls have been the main source of organotins (Batley, G. 1996; Alzieu, C., 2000) [7, 1]. Biocidal uses, such as in antifoulants, agrochemical and wood preservatives make up about 20% of the total annual production of organotin compounds, Omae, I. (2003) [33]. TBT compounds exhibit the highest toxicity of all organotins, Lijiao *et al*., (2014) and have even been characterized as one of the most toxic groups of xenobiotics ever produced and deliberately introduced into the environment. Ayanda *et al*., (2012) [5] reported reported that high concentration of organotin compounds have also been found in the tissues of marine mammals and its presence has been linked to mass mortalities of marine mammals.

Tributyltin chloride is known to be harmful to many, “non-target” aquatic organisms, particularly molluscs, Horiguchi *et al.*, (1997) [18]. Considerable work has been carried on effect of TBT on marine organisms, Alzieu *et al*., (1980) [2] found 100% mortality in pacific oyster, *Crassostrea gigas* exposed to TBT. Newton *et al*., (1985) [5] observed significantly enhanced growth and hatching success in *California grunion*, *Leuresthes tenuis* after effect of TBTO in the duration of 10 days. Reproductive abnormalities have been observed by toxic effect of TBT in the European flat oyster, *Ostrea edulis,* Thain, (1986) [43]. Salazar and Salazar, (1996) [37] observed accumulation of TBT in blue mussel, *Mytilus* species. Meador, (1997) [28] reported that tributyltin chloride strongly effect on amphipod, *Rhepoxynius abronius.* Rabbito, (2005) [36] have been studied the effect of TBT on Neotropical fish, *Hoplias malabaricus.* The effects of organotin compounds have been extensively studied on experimental animals (Wada *et al.*, 1982; Merkord and Henninghausen 1989; Takagi *et al.*, 1992) [44, 29, 42].

The evaluation of acute toxicity is essential for determination of sensitivity of animals to the toxicants and also useful for evaluating the degree of damage to the target organs and the consequent physiological and behavioral disorders, Arome and Chinedu, (2018) [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, (1987) [25] studied the acute toxicity in summer of Cythion-Malathion to two freshwater bivalve molluscs, *L. marginalis* and *Lamellidens corrianus*. Andhale *et al*., (2011) [3] studied toxicity evaluation of Nickel on Lamellidens marginalis and they observed what types of role play the toxicant due to increasing period. Shuhaimi *et al*., (2012) [40] calculated acute toxicity of eight heavy metals and showed that freshwater molluscs Melanoides tuberculata was sensitive to metals. Piansiri and Pachanee, (2008) [35] detected the toxicity bioassay of the Juvenile freshwater snail, *M. martensi* exposed to mercury and cadmium. Since many workers directed the studies towards the toxicity evaluation (Hickey *et al*., 1997; Bhavani and Dawood, 2003;, Kumar *et al*., 2015, Sharma M, 2019) [16, 8, 45, 38].

Molluscs have been used extensively as bioindicators of heavy metal pollution in aquatic system, Aziz *et al*., (2018) [6]. They are more sensitive than the fish species from the middle and the inferior parts of the rivers. When the mussels disappear, it means that the river is seriously affected, Fuller, (1974) [13], leading to a decreasing in the life support capacity of the ecosystem. Compare to marine very little work about TBT had to be focus on freshwater animals some of the workers, (Humbe *et al*. 2016; Mohate 2013) [19, 30] studied acute toxicity effect of TBT on fish, prawns, bivalves etc. Hence the present study has been focused to evaluate the acute toxic effects of tributyltin chloride to freshwater bivalves, *L. marginalis* as bioindicator, of local importance.

Material and Method

The freshwater bivalves, *Lamellidens marginalis* were collected from the Godavari River at Paithan, 45km away from Aurangabad city. The bivalves were brought to the laboratory and acclimatize to the laboratory conditions. 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, Laughlin *et al*., (1983) [23]. The Series of statistic bioassay were conducted under laboratory condition as described by Finney (1971) [12].

Acute toxicity tests were conducted over 96 hrs. The experimental troughs containing 5 litres 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. Stock solution of the tributyltin chloride was prepared. After every 12 hours the polluted water was changed, 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.

Results

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

Time of exposure (Hrs.)	Regression equation $Y=\bar{y}+(X-\bar{x})$	LC50 Values in ppm.	Variance V	Chi-square	Fiducial limits		Lethal dose 111.7368	Safe conc.(ppm)
					m1	m2		
24	Y=13.9590X-2.6867	3.5532	0.00017093	0.09511119	0.51701658	0.5682674	85.2768	0.2245
48	Y=8.7563X+1.6394	2.42	0.00042949	0.14310022	0.33653968	0.4177784	116.16	
72	Y=4.8998X+3.7610	1.7902	0.0014463	0.00430609	0.12260716	0.2716855	128.8944	
96	Y=9.1523+2.2790	0.9829	0.0004779	0.2026317	-0.206948	0.2926412	94.3584	

The LC_{50} values were calculated for 24, 48, 72 and 96 hours by Finney's method (1971). The results of toxicity evaluation are summarized in table No.1.

The LC_{50}values obtained in summer season for tributyltin chloride exposed to 24, 48, 72, and 96hours were 3.5535 ppm, 2.42ppm, 1.7902 ppm and 0.9829ppm respectively.

The variance 'V' values of LC_{50} for 24, 48, 72 and 96 hours were recorded 0.00017093, 0.00042949, 0.0014463 and 0.0004779 respectively. The calculated minimum and maximum fiducial limits of organotin tributyltin chloride for 24, 48, 72 and 96 hours were 0.5170 to 0.5682, 0.3365 to 0.4177, 0.1226 to 0.2716, and -0.2069 to 0.2926 respectively. The Chi-square values were 0.09511119, 0.14310022, 0.00430609 and 0.2026317 calculated. These values were used to test homogeneity of data. Lethal dose of tributyltin chloride were 85.2768 ppm, 116.16 ppm, 128.8944 ppm and 94.3584 ppm respectively. The safe concentration of tributyltin chloride in summer season is 0.2245 was calculated.

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. Mary, (1984) [27] has reported that the LC_{50} values depend on the concentrations of pesticides and also with the time of exposure. The 96 hours LC_{50} value was the low, however the mortality scored was high. In the present study the results obtained for toxicity evaluation of tributyltin chloride on *L. marginalis* indicated that tributyltin chloride found to be more toxic in 96hr. the results shows that LC_{50} values decreases with increasing periods of exposure. In the same way Srinivasulu reddy *et al*., (1985a) [41] reported that the LC_{50} values and the exposure period showed inverse relation. Patil S.S, (1993) [34]. Examined, the LC_{50} value observed was less in summer compare to monsoon and winter, the less values indicated high toxicity so it was concluded that in summer season the bivalve, *L. marginalis* was more sensitive and winter it was less due to mercury stress. Gokhale A.A, (1994) [15] showed acute toxicity tests performed on *L. marginalis* in different seasons for 96 hours, and reported test species revealed comparatively more sensitive to fluoride in summer than in monsoon and winter. Dode, (1993) [9] 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. Martina *et al*., (2003) [26] studied the toxicity of Tributyltin to the freshwater Mudsnail, Potamopyrgus antipodarum in a new sediment biotest. Their results indicated that P. antipodarum is highly sensitive to endocrine disruptors TBT at environmentally relevant concentrations. Kungolos *et al*., (2001) [22] studied the toxicity of four organotin compounds towards freshwater crustacean, *Daphnia magna*. Tributyltin chloride proved to be the most toxic among all four organotin compounds. Shejule *et al*., (2006) [39] reported LC_{50} values of the organotin tributyltin chloride exposed to freshwater prawn, *Macrobrachium kistnensis*; to 24, 48, 72 and 96 hours, LC_{50} values were found to be 0.33 ppm, 0.26 ppm, 0.17 ppm and 0.09 ppm respectively. They showed the LC_{50} values decreased with increase in exposure period. Nikam and Shejule, (2015) [32] they reported similar results when Bis (tributyltin) oxide (TBTO) exposed on freshwater fish Nemacheilus botia. Kamble and Kamble (2014) [20]: observed physiological and morphological changes induced due to LC 50 concentration of copper sulphate (0.56) and reported that the toxicity of copper sulphate is responsible for behavioral changes in freshwater snail, Bellamya bengalensis. Since many workers directed the studies towards the toxicity evaluation and put forth the same result (Hickey *et al*., 1997; Bhavani and Dawood, 2003;, Kumar *et al*., 2015, Sharma M, 2019) [16, 8, 45, 38].

The physiological factors also influence the toxicity of the aquatic pollutants. Eisler, (1977) [10] found that in static bioassay, temperature influenced the toxicity of pesticide. Moreover, he had also found that different salinity's have little effect on the toxicity of agrochemicals, temperature and pH had greatest effect on toxicity of pollutants. In the present study the rate of mortality of freshwater bivalve *Lamellidens marginalis* has increased with increasing concentration and the time of exposure. The rise in temperature, low oxygen content and low food availability in the water body inhabiting the animals in the summer are mainly accounting for the physiological demand to the survival of the species. Addition of tributyltin chloride stress increased the demand and thereby the animal becomes sensitive to the tributyltin chloride stress. The effect of tributyltin chloride on freshwater organisms is quite insufficient compare to marine organisms, so in the present work it is attempted to study the effect of tributyltin chloride on survival of freshwater bivalve, *L. marginalis*.

Acknowledgement

Authors are thankful to Head Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (M.S) India and The Principal Dr. B.D. Khandare Swami Vivekanand Sr. College Mantha for giving support and provided necessary facilities during experimentations.

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