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# Effect of Sub-lethal Concentration of Tributyltin Oxide on Enzyme Activity of Fresh Water Crab *Barytelphusa cunicularis*

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**Abstract:** The present study was conducted to determine the effect of organotin compound on alteration of enzyme activity in fresh water crab *Barytelphusa cunicularis* exposed to sub-lethal concentrations of tributyltin oxide (TBTO) (0.244 ppm which is 1/10th of 96 h $LC_{50}$). The $LC_{50}$ value of TBTO were found as 5.29 ppm for 24 h and 2.44 ppm for 96 h. Crabs (*Barytelphusa cunicularis*) were exposed to sub-lethal concentrations (0.244 ppm) of TBTO for 7, 14, 21 and 28 days. The activity of enzymes Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) in the hemolymph of crabs showed significant increase after exposure to 0.244 ppm TBTO for 28 days. The increase metabolic enzymes in hemolymph of treated animals compared with control indicated heaptopancreatic damage caused by TBTO. These changes are an outcome of metabolic as well as physiological stress.

**Keywords:** Tributyltin oxide, *Barytelphusa cunicularis*, Aspartate aminotransferase, Alanine aminotransferase, Alkaline phosphatase, Toxicity, $LC_{50}$

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## Introduction

Environment managers in various nations have asked whether the organotin compounds pose risks to human health or environmental quality that might justify further restricting their use (Champ, 2000**).** Tributyltin oxide (TBTO) is antifouling agent used as biocides in various industries to control aquatic organism (Fent, 1996; Hoch, 2001). Tributyltin (TBT) and triphenyltin (TPT) compounds are used extensively for several purposes owing to their strong biocidal activity toward a variety of aquatic organisms such as crustaceans, fishes, mollusca, fungi and algae. They have been used as antifouling agent in paints for boats and cooling towers to prevent adhesion of aquatic organisms, and also as wood preservatives, in industrial processes, as slimicides and molluscicides to prevent schistosomiasis. TPT is also utilized for its fungicidal property. However, the finding that TBT was damaging oyster production in France in the 1970s, made people recognize the negative facets about consumption of these compounds in the aquatic environment (Alzieu, 1981). Human exposure to these compounds occurs through consumption of aquatic food such as crab, fish etc. through which they enters in food chain and shows accumulation (Kannan *et al*., 1995, 1996; Iwata *et al*., 1997; Kannan *et al*.,1998,1999; Hoch, 2001). It causes immune toxicity such as thymus atrophy and thereby interferes with T-lymphocyte-mediated immune responses (Vos *et al*., 1984; Snoeij *et al*., 1987). In living organisms, metabolic pathways carried out with biocatalyst in control manner. Whenever animals were exposed to stress conditions, alteration in physiological conditions were seen in response to toxicant and showed hyper- or hypo- level of enzyme secretions. The trace metal concentrations in Queensland Estuarine crabs, *Australoplax tridentate* have been observed (Mortimer, 2000). Various chemicals entering the aquatic ecosystem through human activities, either accidentally or by design may cause adverse effects on the aquatic biota, including deleterious changes which disrupt metabolic activity at the biochemical levels (Hirth, 1964). Aquatic ecosystems are more intense to release of industrial wastewater (Páleaz-Cid *et al*., 2013). Stress exerted by exposure of freshwater crabs to organotin compound drained into water bodies had altered activity of enzyme components, which indicated major impact of toxic component to crab as a significant species of aquatic ecosystem used as biomarker (Patil *et al*., 2014). Clinically Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) are measured as a part of a diagnostic assessment of hepatocellular damage, to determine liver health (Wang *et al*., 2012). AST and ALT may serve as strategic links between protein and carbohydrate metabolisms, which is known to alter under several physiological and pathological conditions (Shivakumar, 2005). AST and Alkaline phosphatase (ALP) are stress indicators related with detoxification. Succinate dehydrogenase bound to the inner mitochondrial membrane of cells. It is the only enzyme that participates in both the citric acid cycle and the electron transport chain (Oyedotun and Lemire, 2004). If any alteration is observed in the enzymatic activity (biomarkers), it indicates drastic physiological and biochemical changes along with considerable marked index of hepatotoxicity (Reuben, 2004), and it can serve as an index of liver biosynthetic capacity (Johansson *et al*., 2008; Ingawale *et al*., 2014; Contreras-Zentella *et al*., 2016). Jiaxiang Luo *et al*. (2020) investigated effects of dietary Zn level on growth performance, Zn bioaccumulation, antioxidant capacity and innate immunity in juvenile mud crabs *Scylla paramamosain.* The present study was conducted to determine the effect of organotin compound on alteration of enzyme activity (ALP, ALT, AST) in hemolymph of fresh water crab *Barytelphusa cunicularis* exposed to sub-lethal concentrations of tributyltin oxide (TBTO) (0.244 ppm which is $1/10^{th}$ of 96 h $LC_{50}$).

## Materials and Methods

Fresh water crabs, *Barytelphusa cunicularis* were caught from paddy field area of Aurangabad, India and transported to the laboratory in aerated plastic troughs. They wereacclimated for seven days in an aquarium under laboratory condition. The $LC_{50}$ values of the TBTO for 24, 48, 72 and 96 h were determined by exposing crabs to range of concentrations of TBTO diluted with tap water. It was calculated by using Finney (1964). $LC_{50}$ value for 96 h was found to be 2.44 ppm. Chronic toxicity bioassay was carried out by exposing crabs to 0.244 ppm i.e. $1/10^{th}$ concentration of 96 h $LC_{50}$. Animals were exposed to this concentration for 7, 14, 21, 28 days. Simultaneously control set of animals was also maintained. Hemolymph of *Barytelphusa cunicularis* was collected aseptically from the base of one of the second walking legs using a sterile syringe. Hemolymph was centrifuged at 3000 rpm for 15min at 4°C. Supernatant was used for enzyme assay as follow:

*Alkaline phosphatase* (*ALP*): Alkaline phosphatase activity was estimated by Bergmeyer and Hagen

(1972) method at 405 nm. ALP was determined by concentration of p-nitro phenyl phosphate.

*Alanine aminotransferase* (*ALT*): The activity of alanine aminotransferase (ALT) were determined by Reitman and Frankel (1957) method in which ALT catalase the reaction between α-oxoglutarate and L-alanine to give L-glutamate and pyruvate. The concentration of ALT was measured by monitoring the concentration of pyruvate hydrazine formed with 2,4-dinitrophenyl-hydrazine at 545 nm.

*Aspartate aminotransferase* (*AST*): The activity of alanine aminotransferase (AST) were determined by Reitman and Frankel (1957) method in which AST catalase the reaction between α-oxoglutarate and L-aspartate to give L-glutamate and oxaloacetate. The concentration of AST was measured by monitoring the concentration of oxaloacetate hydrazine formed with 2, 4-dinitrophenylhydrazine at 545 nm.

## Results and Discussion

In all the concentrations of the TBTO, the $LC_{50}$ values decreased with increasing exposure period. In the mature crabs, the $LC_{50}$ values of TBTO were found to be 5.29, 4.26, 3.17 and 2.44 ppm after 24, 48, 72, and 96 h, respectively. From the above results it was observed that the $LC_{50}$ values of 96 h were found lowest among all the exposure periods (Table 1).

The extremely high body residues of TBT in the past and our extrapolations on heightened intracellular partitioning allow suggesting that the TBT toxicity in the marine environment has been underestimated compared to the water exposure pathway toxicity (Koen *et al*., 2019). In the present study the percentage mortality of crabs increased progressively up to 96 h AST, a key enzyme of nitrogen metabolism and energy mobilization in invertebrates, is frequently used as a biochemical index of stress (Calabrese *et al*., 1977). The Aminotransferases serve as a strategic link between carbohydrate and protein metabolisms under environmental stress (Knox and Greengard, 1963). Increased activities of AST and ALT in the present study indicate that there is an active transamination of amino acids, possibly to provide Keto acids in the TCA Cycle. Elevation in the activities of AST, ALT and ALP provides evidence for amino acid utilization, as observed in the present study, and such utilization indicates the channeling of ketoacidosis to the Krebs Cycle, in the event of blockage of glycolytic metabolism, as reported by Reddy *et al*. (1989). ALP is responsible for phosphorylation, biosynthesis, secretory activity, mediation of membrane transport, involvement in active transport and good indicator for stress condition of biological systems. Hemolymph is more susceptible to oxidative stress originated by AgNPs and temperature stress, whereas the gills constitutes the main storage organ (Walters *et al*., 2017). The enzymes play an important role in the innate immune response and are involved in the defensive system of mud crabs. There were higher hemolymph protein and UN levels in the normal mud crabs than the red sternum mud crabs indicating that the red sternum syndrome affected the nitrogen metabolism of mud crabs (Chantana and Jintana, 2018). TBT has a toxic effect on the female reproductive system of the hermit crab *Clibanarius vittatus*, which showed ovarian disorganization and atrophy. This impact may result reductions of fertility and embryo success, and even population decreases (Bruno *et al*., 2012). The activity of aspartate transaminase (AST) enzyme in the hemolymph of freshwater crab *Barytelphusa cunicularis* exposed to sub-lethal concentrations (0.244 ppm) of TBTO after 7, 14, 21 and 28 days exposure period showed significant difference as compared to control crab. Hemolymph AST activity in control crab varied between 21.20±1.30 and 23.90±2.18 (IU/L) during 7 to 28 days. The activity of AST in the hemolymph showed significant increase after exposure to 0.244 ppm TBTO revealing 24.90±2.33, 25.70±1.26, 27.10±1.89 and 28.40±1.96 (IU/L) at 7, 14, 21 and 28 days, respectively as compared to control. Alanine transaminase (ALT) activity was significantly increased in tributyltin oxide exposed group of

Table 1: $LC_{50}$ values of fresh water crab, *Barytelphusa cuniclularis* exposed to TBTO for 96 h

| Concentration of TBTO in ppm | No. of crab used | Corrected % Mortality | Log of conc. to base 10 | Probit analysis |
|---|---|---|---|---|
| 2.40 | 10 | 30 | 0.3802 | 4.8107 |
| 2.42 | 10 | 40 | 0.3838 | 4.9104 |
| 2.44 | 10 | 50 | 0.3873 | 5.0073 |
| 2.46 | 10 | 60 | 0.3909 | 5.1070 |
| 2.48 | 10 | 60 | 0.3944 | 5.2039 |
| 2.50 | 10 | 70 | 0.3979 | 5.3008 |

Table 2: Effect of sub-lethal concentration (0.244 ppm) of TBTO on hemolymph enzyme activity of fresh water crab *Barytelphusa cunicularis*

| Exposure period (days) | AST ( IU/L) | | ALT( IU/L ) | | ALP ( IU/L) | |
|---|---|---|---|---|---|---|
| | Control | TBTO | Control | TBTO | Control | TBTO |
| 7 | 21.20 ±1.30 | 24.90 ± 2.33* | 12.10 ±1.53 | 15.20 0.98** | 13.90 ±2.62 | 15.74 ± 1.23* |
| 14 | 22.70 ± 0.52 | 25.70± 1.26** | 13.85 ±2.56 | 16.33±1.75* | 14.09 ± 2.39 | 17.51 ± 3.71* |
| 21 | 23.03 ± 2.30 | 27.10 ± 1.89* | 14.25 ± 2.46 | 17.50±3.27* | 14.69 ± 0.98 | 18.63 ±2.47** |
| 28 | 23.90±2.18 | 28.40 ± 1.96** | 14.87 ± 2.04 | 18.64±1.45** | 15.37 ± 1.33 | 19.24 ± 2.04* |

Values are the mean ±S.D. (n=3); * and ** indicate significance $P<0.05$ and $P<0.01$, respectively

crab in present study. The control values of ALT activity in hemolymph were measured as 12.10±1.53, 13.85±2.56, 14.25±2.46 and 14.87±2.04 IU/L at 7, 14, 21 and 28 days, respectively. In hemolymph ALT level increased significantly ($P<0.05$, $P<0.01$) as compared to control crab. Observed ALT values are 15.20±0.98, 16.33±1.75, 17.50±3.27 and 18.64±1.45 (IU/L) at 7, 14, 21 and 28 days, respectively (Table 2). The alterations in the levels of activity of transaminases induced by the tributyltin oxide showed the increased energy demands under stress. The level of alkaline phosphatase (ALP) was found to increase in hemolymph of the test crab compared to controls (Table 2). In crab exposed to 0.244ppm of TBTO for 7, 14, 21 and 28 days, the levels of ALP in hemolymph were found to be more significantly ($P < 0.01$) increased which are 15.74 ± 1.23, 17.51 ± 3.71, 18.63 ± 2.47 and 19.24 ± 2.04IU/L, respectively (Table 2). The present results indicated elevation of ALP enzymes due to the test toxicant stress which lead to alterations in the protein and carbohydrate metabolism.

## Conclusion

In the present investigation, the increase in aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) activity in the hemolymph of experimental crab was noticed after 0.244 ppm TBTO treatment. This may be due to efflux of enzymes into circulation from damaged organs and dominance of anaerobic nature of carbohydrate metabolism to meet the increased energy demands under sustained and prolonged stress. Therefore, alterations in the metabolism of the crab reveals the differential effects of stress and can be considered as a bio-monitoring tool in the assessment of environmental pollution by TBTO on non-target organism.

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