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Oxygen Consumption of Freshwater Crab, *Barytelphusa Cunicularis*, in Relation to Organotin Compound and Nanoparticles Stress

Somvanshi Varsha[1*], Salve Pooja[2] and Shejule K.B.[1]

[1]Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad 431004, India

[2]Department of Biophysics, Government Institute of Science, Aurangabad 431004, India

**Corresponding Author*

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Abstract: The present investigation explore the respiratory response and behavioural changes of the freshwater crab *Barytelphusa cunicularis* after exposure to lethal concentration (1.00 ppm) of silver nanoparticles (AgNPs) and Tributyltin oxide (TBTO) (2.44 ppm). The animals after acclimatization to the laboratory conditions were exposed for 0, 2, 4, 8, 12 and 24 h to the toxicants. Total oxygen consumption was estimated by Winkler's method (1960) and the behaviour changes wereassessed by visually. In the present study, total oxygen consumption showed a gradual decline from 0 to 24 h in the experimental animals exposed to silver nanoparticles and TBTO.

Keywords: *Barytelphusa cunicularis*, Tributyltin oxide, Silver nanoparticles, Wrinkler's method, Oxygen consumption, Behavioural response

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Introduction

The effectiveness of Tributyltin oxide (TBTO) is a result of the fact that it gradually leaches from the hull killing the fouling organisms in the surrounding area. Organotin compounds are effective antifouling agents and have saved the shipping industry billions of dollars by reducing fuel consumption and reducing dry dock time. In 1980's it was recognized that TBTO and other organotin antifouling agnts have relatively long half-lives in the environment. TBTO is organotin compound which has been released in large quantities from industrial, agricultural and urban activity and thereby discharged into freshwater. TBTO used as antifouling agent (Ozturk *et al*., 2008). It causes damage to the reproductive system and influence on the growth of aquatic organisms. The body activity of animals can be measured in terms of oxygen uptake which represents growth and development of aquatic animals. The aquatic organisms have to pass large quantities of water over their respiratory surface and are subjected to relatively high risk of exposure to the toxic substances (Shelke and Wani, 2005). AgNPs is used in coating and paint preparations to avoid bio-deterioration (Zeilecka *et al*., 2011). AgNPs developed a more effective antimicrobial vegetable oil-based paint with embedded AgNPs but with a higher silver concentration (34 mg of silver benzoate/4.8 g of alkyd paint). AgNPs were produced in a single step at ambient conditions without using external reagents (Kumar *et al*., 2008). Holtz *et al*. (2012) demonstrated that 1% (m/v) AgNPs supported on silver vanadate nanowires dispersed in a commercial water-based paint showed effective antibacterial activity.

Respiration is the most significant energetic process shown by living organisms to obtained energy in the form of ATP as energy currency to perform different biological and physiological functions likes reproduction, locomotion, muscular contraction. Metabolic processes are most sensitive parameters as indicator of stress as all enzymatic reactions on the substances and physiological responses are incorporated and regulated in a unique manner (Dillon *et al*., 1981). It is well known that respiration is a vital phenomenon of life and rate of oxygen consumption in turn control the metabolic activities and act as a measure of the intensity of metabolism along with change in behaviour pattern of an organism.The Metabolic response of an organism to a changing or stressful environment is an overall outcome of its adaptive ability. Different species of crustaceans vary in their ability to reduce metabolism. Therefore any change in the respiratory activities can be rightfully used as an indicator of stress in general and toxicant induced changes in exposed animal in particular stress behaviour (Fry, 1957). The present investigation explore the respiratory response and behavioural changes of the freshwater crab *Barytelphusa cunicularis* after exposure to lethal concentration (1.00 ppm) of silver nanoparticles (AgNPs) and Tributyltin oxide (TBTO) (2.44 ppm).

Materials and Methods

Fresh water crab *Barytelphusa cunicularis* (40-45 g) were collected from Aurangabad district (Maharashtra), Kaigaon tok and were brought to the laboratory. They were acclimatized in the laboratory for three to four days before they were used for experiments. Only healthy crabs were carefully chosen for experimentation to avoid problems of sex and size. The animals were fed with earthworms. For each experiment 10 animals were exposed to 2.44 ppm and 1.00 ppm lethal concentration of 96 h LC_{50} of tributyltin oxide and silver nanoparticles (Finny, 1971). The oxygen consumption for control group crab and toxicant exposed crabs was measured for 0, 2, 4, 8, 12 and 24 h. Estimation of oxygen consumption was carried by Wrinkler's method as modified by Strickland and Parsons.

The water from the respiratory jar was carefully siphoned out in the stopper bottle and oxygen content was determined according to Golterman and Clymo (1969). The crab was then taken out from the jar and carefully blotted to remove excess of water, then weighed to obtain weight of animal. The oxygen consumed by the animal is expressed as mg of O_2/h/l /g body weight. Simultaneously control group was also maintained in tap water with experimental animal group. The wet body weight of the individuals used in each set of experimental animals. Each experiment was repeated thrice and mean was taken for calculations. The data are expressed as mean±SE and statistically analysed for Student 't' test (Mungikar, 1997).

Results and Discussion

There was normal feeding and all the crabs looked healthy. Crawling movements were normal and showed no signs of stress and eye movements and body posture were normal at 0 h in control and toxicant treated (AgNPs and TBTO) crabs.

The animals looked quite restless at 2 h in response to exposure to AgNPs and TBTO. Movements increased initially then became dull gradually with normal eye movements at 4 to 8 h. Most of the animals showed aggressive behaviour at 12 and 24 h in AgNPs concentration compared with the TBTO. Rapid movement of chelate legs were observed with crawling upon each other and trying to attack each other. Amount of fecal matter was determined after exposure of crabs to TBTO and AgNPs and control crabs for 24 h. It was found that the amount of fecal matter is more and sticky in toxicants exposed crabs than control crab.

The freshwater crab *Barytelphusa cunicularis* are exposed to 2.44 ppm of TBTO and 1 ppm of AgNPs concentrations at 0, 2, 4, 8, 12 and 24 h. The first sets of observations were made using from the stock aquarium containing tap water. This served as control. Table 1 illustrates the changes in oxygen consumption of crabs exposed to TBTO and AgNPs. It is evident from the result that oxygen consumption slightly changed at 0 - 2 h which was 0.77 and 0.79 with concentrations of 2.44 ppm TBTO and 1 ppm AgNPs, respectively and gradually decreased at 8 to 24 h in TBTO and AgNPs treated crabs (Fig. 1).

The use of measures of animal behaviour as indicators and provide sensitive information about a stress condition exposed to population. For example, toxicologists are currently using behavioural measures e.g. activity, feeding, and anti-predator behaviour as bio indicators (Clotfelter *et al*., 2004). It has been reported that increased concentration of chloride, nitrate and phosphate can be used as bio-indicator of pollution as the plankton population was found to be increased (Trivedi and Goel, 1986). Under stress conditions the ability to locate food can also be affected (Breithaupt *et al*., 1999). To overcome the stress induced by TBTO and AgNPs concentration, experimental animals showed increase in body activity with aggressive behavioural changes due to increased rate of metabolic reactions. It was reported for some brachyuran crabs that individuals reach adulthood at smaller sizes compared to the time before populations were affected by intense fishing pressure (Abbe, 2002). Rapid tail flipping and escape behaviour was recorded in lobsters (Butterworth *et al*., 2004). Respiration and heartbeat rates are two important physiological parameters in ectothermic organisms, such as crabs, bivalves etc. For this reason RR and HBR have been used to predict physical and chemical stress effects for a long time. Several researchers have reported a dose-dependent decrease of HBR for the chronic effects of heavy metal exposure, such as copper (Curtis *et al*., 2000). Few researchers have also reported depletion in protein content in the different tissue exposed to acute and chronic concentrations of different pollutants. The decrease in protein content was reported in freshwater prawn *Macrobrachium kistnensis* exposed to pesticides (Nagbhushnam *et al*., 1972). It should be pointed out that, in the present study, silver was continuously available in a nonreactive form, that is, the elemental state. Preliminary data obtained by experiment suggest a sudden precipitation of AgNPs in seawater and a relatively low dissolution rate of silver to Ag^{+}. Dominguez-Wong *et al*. (2014) prepared acrylic paints with TiO_2 de-corated with AgNPs (15%v/v of a water dispersion of the particles) and evaluated their antibacterial biofilm activity at different humidity conditions, finding that at lower relative humidity the antibacterial activity dropped significantly. Main classes of emerging contaminant in water industry, urban runoff, Mining, agricultural and port activities can release heavy trace metals such as cadmium (Cd), aluminium (Al), cobalt (Co), chromium (Cr), gallium (Ga), lead (Pb), copper (Cu), iron (Fe), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), tin (Sn), silver (Ag), vanadium (V) and zinc (Zn) and metalloids like arsenic (As), antimony (Sb) and selenium (Se) of environmental concern to coastal waters (Angel BM, 2010). Some of these are termed lithophile A-type metals like Sn, Mn, Co, V, Cr and Ni (Schlenk, 1999). Whereas atmophile B-type metal As, Hg, Se, Pb and Sn are those with greater atmospheric mass transport than that in streams/rivers (Abraham and Susan, 2017). Atmophile can be methylated and released into the atmosphere as vapours. As, Hg and probably Se can also be free as inorganic vapour from burning of coal (Ayandiran *et al*., 2014).

It is well-known that the increased level of dissolved oxygen can provide a higher possibility of existence of life in aquatic habitat. The dissolved

Table 1: Oxygen Consumption of crabs after TBTO and AgNPs exposure

Hours	Control	TBTO (2.44 ppm)	AgNPs (1.00 ppm)
0	0.73±0.016	0.77±0.015**	0.79± 0.021*
2	0.69±0.032	0.73±0.028*	0.76±0.018**
4	0.57±0.028	0.68±0.012**	0.68±0.012**
8	0.53±0.017	0.59±0.017*	0.61±0.032*
12	0.49±0.029	0.47±0.020*	0.49±0.016**
24	0.43±0.028	0.39±0.016**	0.40±0.027*

Values are mean±SE (n=3) for oxygen consumption, * and ** indicate significance value $P<0.05$ and $P<0.01$, respectively

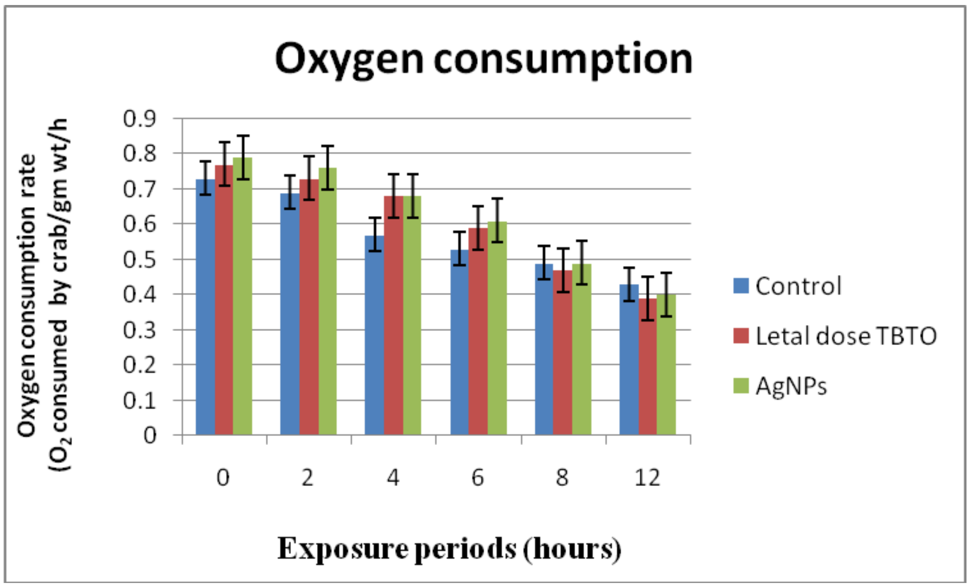

Fig. 1: Oxygen Consumption of crabs after TBTO and AgNPs exposure.

oxygen is highly beneficial for the survival of all aquatic organisms, not only for fish but also for invertebrates such as crabs, clams, and zooplankton. Moreover, it was observed that the pollutant such as heavy metals, in the rivers were lower than those from STPs. It was established that a high pollutant concentration can cause an excess algal growth in the rivers and progress as eutrophication. Temporal variability in AgNPs concentrations can be principally explained by temporal variability in the water discharge. It has been reported that a higher water discharge from the sewage effluent causes a higher AgNPs dilution (Dumont *et al.*, 2015).

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