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METAL INDUCED OXYGEN TENSION AND BEHAVIOURAL CHANGES IN FRESHWATER SNAIL *BELLAMYA BENGALENSIS:* COMPARATIVE STUDY

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ABSTRACT

Heavy metals are major pollutants released into the environment. Comparative study of these metals against physiological alterations provides information about toxicity nature and biomonitoring mechanism. Present investigation deals with intoxication of zinc sulphate and lead acetate against respiratory mechanism and oxygen tension occurred in snail for pre-determined mean LC_{50} concentration 1.06 ppm for zinc sulphate and 0.77 ppm for lead acetate respectively, for different exposure period. Histological damage in to gills and rate of gas transportation, resulted to problem of oxygen scarcity. As a impact, snails under experiment changed normal behavior and tried to escape from toxic environment for their survivality. Lead acetate found to be more hazardous than zinc sulphate, as rate of oxygen consumption was drastically reduced with increased oxygen tension. Present investigation provides comparative metal toxicity and respiratory dysfunction and altered behavioral pattern in aquatic snail *Bellamya bengalensis* (L).

KEYWORDS: Comparative toxicity, heavy metals, behavior change, oxygen tension, *Bellamya bengalensis.*

INTRODUCTION

Respiration proved to be vital phenomenon of life and acts as important physiological index of any organism (Rajalekshmi and Mohandas, 1998). When any toxicant accumulate in to body, as first toxic mechanism, immediately it affects respiration i.e. rate of oxygen consumption of animal. Generally it has been found that, metal get mixed in large part of river systems, coastal and marine environments due to agricultural runoff, industrial waste and mining activities Fu and Wang, (2011). Machado et. al., (2014) documented for responses of biomarkers in wild freshwater mussels chronically exposed to complex contaminant mixtures, they concluded cellular pathology and drastic behavioral changes. Ellis, (1940), recorded that atmospheric pollutants and contact with mining wastes including heavy metals, could impact aquatic fauna.

Chaudhari et. al., (1988) documented that, change in oxygen consumption can be considered as a tool in evaluation of toxicity in different aquatic animals. Sing and Saxena (2001) found that the prosobranch snails were more sensitive to low temperature, while pulmonate snails to high temperature. Nagabhushnam and Chintawar, (1976) studied oxygen consumption of *Indoplanorbis exustus* exposed to atmospheric air, in relation to body size, salinity, temperature, pH, oxygen tension and starvation. Other parameters as, alkalinity can have a similar effect in that at pH 7.5 *Physa acuta* snails respond to moving into safer habitats for the survivality (Turner and Chislock, 2010). David and Ray, (1966) and Venkatraman, (1966) reported that, mixing of poisons in the body can reduce concentration of oxygen, scarcity of oxygen level in the body increase the rate of mortality of animals.

Mikkelsen and Weber ,(1992) observed that the oxygen capacity of respiratory pigment, hemocyanin (HC) and isolated alpha-HC component of snail *Helix pomatia* was changed due to induced toxicity. Toxicity assessment of different contaminants on gill pathology and biochemical changes has been analyzed in several studies as observed by Wu and Chen, (2004). Jezierska and Sarnowski, (2002) noticed adverse effects of various heavy metals such as Cd, Cu, Cr, Hg and Zn on respiratory capabilities of different fish species. Das and Gupta, (2012) carried out study about, effect of cadmium chloride on oxygen consumption gill morphology of Indian flying barb, *Esomus danricus* and documented several major deformities in the gill architecture and respiratory functions.

Mahajan, (2007) documented effect of certain heavy metal induced physiological alteration in the freshwater bivalve, *Lamellidins marginalis.* Witeska et. al., (2006) recorded that, some of the physiological effects of chronic exposure to cadmium at sub lethal levels were manifested in the form of disturbance in respiration. Similarly , Lacroix and Hontela, (2004) also verified disruption in whole-body or plasma ion regulation, reduction in growth, changes enzyme activity and other blood parameters, including content of glucose, total protein, triglyceride and cortisol which has revealed major stress response in experimental animal. Lefcort et. al., (2000), observed that, snails alter their behavior when exposed to kairomones released from crushed snail cells which where acts as indicator. They also mentioned that, snails reduce their movements and due to metals stress and finaly impaired their total response to became slugish. Nanaware *et al.* (2004) studied effect of Thimet 10-G and NaPCP on *Viviparus bengalensis* and observed decrease in oxygen consumption and lost the mobility in the experimental animals.

Earlier research and available literature shows that, still more affords are required towards the analysis of toxicity impact against rate of respiration and oxygen consumption in aquatic animals. Very few investigators have attended work related to comparative effect of heavy metals on oxygen consumption and behavioral change in invertebrates. Therefore, in the present investigation, we have carried out study in relation to effect of heavy metals on respiratory physiology of freshwater snail *Bellamya bengalensis* (L).

MATERIAL AND METHODS

In the present investigation water miscible heavy metals as Zinc sulphate ($ZnSO_4.7H_2O$) and Lead acetate $(CH_3COO)_2$ $Pb.3H_2O$ were used to study their effects on the oxygen consumption and behavior of freshwater snail *Bellamya bengalensis* (L).

Experimental design

The freshwater snail *Bellamya bengalensis* (L) were collected from 'Rajaram tank', freshwater lake, near the campus of Shivaji University, Kolhapur, Dist. Kolhapur, Maharashtra State, India. Animals were acclimatized under laboratory conditions for a week. The snails having same size and same weight (26-28 mm shell height and 2.8 to 3.5 gm weight) were selected for the experiments. For the study, two sets having 5 troughs in each set were prepared. In each set one trough was used for control group and four troughs were used as experimental trough, in which snails were intoxicated with pre-determined mean LC_{50} concentration of heavy metal 1.06 ppm for zinc sulphate and 0.77 ppm for lead acetate respectively. The snails in all experimental troughs were exposed to 24 hrs., 48 hrs., 72 hrs. and 96 hrs.

After completion of exposure four snails were selected from each trough and placed in specially designed air tight respiratory glass jar upto an hour, for the measurement of oxygen consumption. After one hour snails were removed from jar. The water from jar was chemically analyzed for dissolved oxygen content, as per the standard method of Trivedi and Goel, (1998) before and after the completion of toxicity period. All experiments were repeated thrice in order to avoid the errors. The rate of oxygen consumption was measured in turns of ml of O_2/lit/hr/gm body wt.

Calculations

Formula to calculate dissolved oxygen:

$$\text{Dissolved oxygen mg/lit} = \frac{(Ml \times N) \times 8 \times 1000}{V_2 \left\{ \dfrac{V_1 - V}{V_1} \right\}}$$

Where:

V = Volume of $MnSO_4$ and KI solution. (2 ml)

V_1 = Volume of sample bottle after placing stopper (328 ml).

V_2 = Volume of part of content titrated.

Normality of sodium thiosulphate = 0.025 N.

$$\therefore \text{Dissolved oxygen, mg/lit} = \frac{(Ml \times 0.025) \times 8 \times 1000}{100 \left\{ \dfrac{328 - V}{328} \right\}}$$

Formula for oxygen consumption:

$$\text{Oxygen consumption mg/lit/hr/gm body wt.} = \frac{\text{Initial} - \text{Final}}{\text{Average wt. of animal}}$$

RESULT AND DISCUSSION

While studying effects of intoxication of two heavy against freshwater snail *Bellamya bengalensis*, we found that during experiment, control snails were moved together and formed pairing or groups. They remained attached to each other for longer period. The normal courtship behavior was observed in the trough. They raised their heads, pressed against each other. After some time they detached from each other, snails were found more sencitive and active all the while in the experimental troughs to mechanical pin touch as protective response.

Effect of Zinc Sulphate on the Oxygen Consumption of Snail *B. bengalensis*:

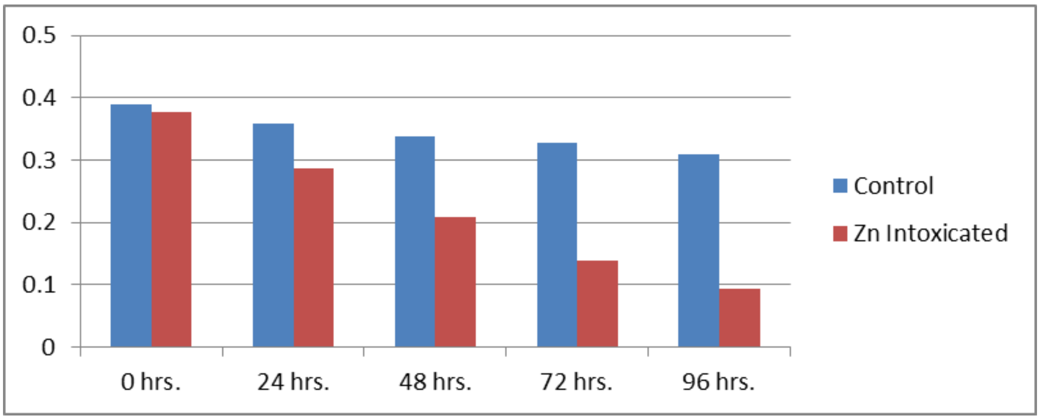
The intoxicated snails after exposure for 96 hrs to zinc sulphate were found attached at one place, protective response to mechanical stimuli was lacking. The tentacular movements were slowed and seen less active in their regular movements. Foot part was dragged inside the shell, without creeping movement. Milky whitish - yellow gelatinous mucus was seen. The opercular movement was reduced. The courtship behavior was not observed among the snails. The snails remained isolated and inactive. Similar type of behavior pattern was documented in *Solea senegalensis* after copper induction by (Arellano et. al., 1999).

After chemical analysis, oxygen consumption in snails of controlled group was 0.390, 0.358, 0.339, 0.327 and 0.309 mg/lit/hr/gm body wt. at 0 hr, 24 hrs, 48 hrs, 72 hrs and 96 hrs respectively. Compare to this, rate of oxygen consumption in experimental snails was initially decreased up to 0.378 mg/lit/hr/gm body wt. at 0 hr. but later on, with increasing the intoxication period in zinc sulphate solution. But after that, there was significant decrease in oxygen consumption. It was 0.286 after 24 hrs., 0.209 after 48 hrs., 0.138 after 72 hrs and 0.094 after 96 hrs of exposure, showing 24.32%, 42.52%, 64.30% and 75.93% decreased, oxygen consumption respectively against zinc toxicity. The results of the oxygen consumption have been recorded in **Table No.1** and have been graphically represented in **Graph No.1.**

Table 1: The oxygen consumption of freshwater snail, *Bellamya bengalensis* exposed to heavy metal Zinc sulphate.

Experimental Condition	Oxygen consumption in mg/lit/hr/gm body wt.				
	Intoxication Period				
	0 hrs.	**24 hrs.**	**48 hrs.**	**72 hrs.**	**96 hrs.**
Control	0.390 ± 0.045	0.358 ± 0.038	0.339 ± 0.044	0.327 ±0.040	0.309 ± 0.028
Intoxicated	0.378 ± 0.038 **(-3.08%)**	0.286 ± 0.024 **(- 20.11%)**	0.209 ± 0.018 **(-38.35%)**	0.138 ±0.040 **(-57.80%)**	0.094 ± 0.010 **(-69.58%)**

* = p < 0.001, ** = p < 0.01, ***= Mean ± SD (n=3)



Graph 1: The oxygen consumption of freshwater snail, *Bellamya bengalensis* exposed to heavy metal Zinc sulphate.

B. Effect of Lead Acetate on Oxygen Consumption of Snail *B. bengalensis*

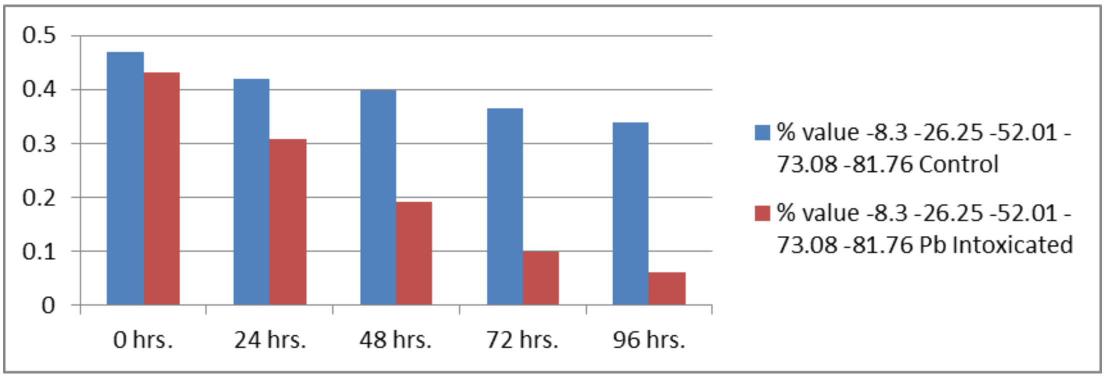
After 96 hrs. of exposure to lead acetate, intoxicated snails does not showed any protective behavior due to the toxic chemical stress. They remained motionless. Since the snails had closed their shell opening by operculum, no tentacular movements and the foot movements were observed. The snail does not showed any type of response to the external mechanical stimuli. Only milky white thick gelatinous more concentrated mucus was observed in to the trough. The courtship behavior was not observed. Mule and Lomate, (1994) have studied the effects of CuSO4 and HgCl2, on the behavior and respiratory physiology in relation to oxygen consumption in the snail *Thiara tuberculata* and have recorded significant reduction in the oxygen consumption with lack of mobility due to heavy metal toxicity.

The rate of oxygen consumption in a control group of snails during all the exposure periods remained more or less 0.470, 0.419, 0.398, 0.364 and 0.340 mg/lit/hr/gm body wt. for 0 hr, 24 hrs, 48 hrs, 72 hrs and 96 hrs respectively. In lead intoxicated snails, rate of oxygen consumption was notably decreased up to 26.25% after 24 hrs., 52.01% after 48 hrs., 73.08% after 72 hrs. and 81.76% after 96 hrs of intoxication period. The observations were recorded in **Graph No.** 3.

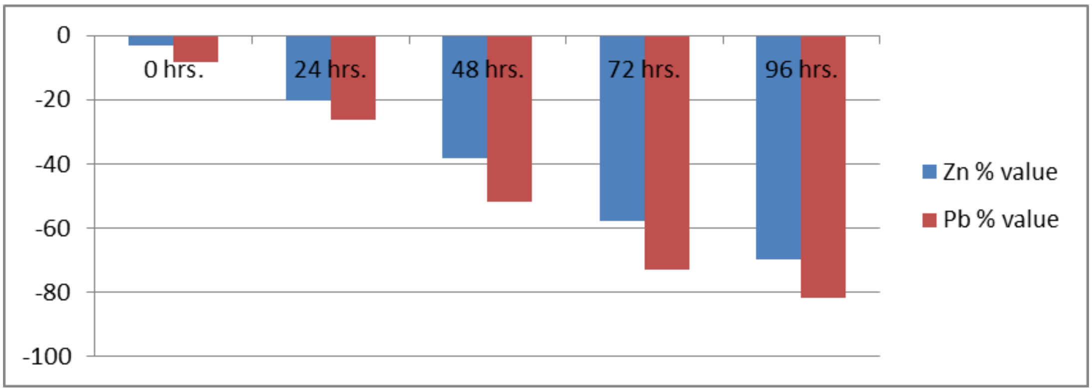
Table 2: The oxygen consumption of freshwater snail, *Bellamya bengalensis* exposed to heavy metal Lead acetate.

Experimental Condition	Oxygen consumption in mg/lit/hr/gm body wt.				
	Intoxication Period				
	0 hrs.	**24 hrs.**	**48 hrs.**	**72 hrs.**	**96 hrs.**
Control	0.470 ± 0.058	0.419 ±0.030	0.398 ±0.029	0.364 ±0.052	0.340 ±0.042
Intoxicated	0.431 ±0.060 **(-8.30%)**	0.309 ±0.052 **(-26.25%)**	0.191 ±0.029(- **52.01%)**	0.098 ±0.010 **(-73.08%)**	0.062 ±0.007 **(-81.76%)**

*= $p < 0.001$, ** = $p < 0.01$, *** = Mean ± SD (n=3)



Graph No. 2 The oxygen consumption of freshwater snail, *Bellamya bengalensis* exposed to heavy metal Lead acetate.



Graph No. 3. Comparative % value difference of oxygen consumption in freshwater snail, *Bellamya bengalensis* exposed to heavy Zinc sulphate and Lead acetate.

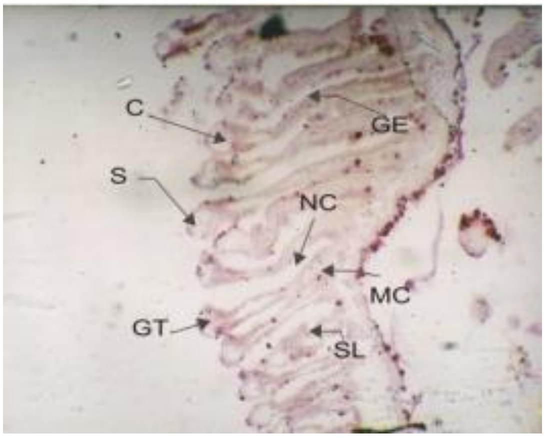


Figure 1: Light micrograph of gill lamellae in control snail snail *B. bengalensis* Stained with HE 20 x 4.

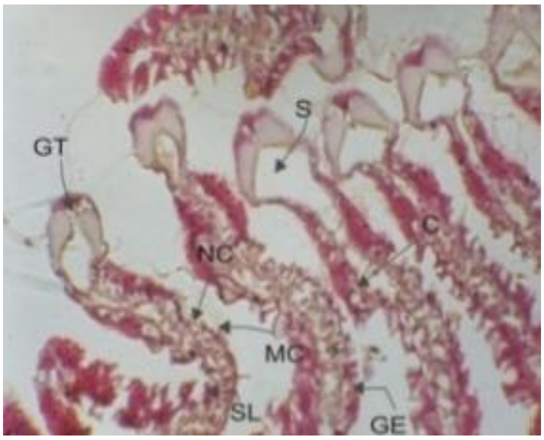
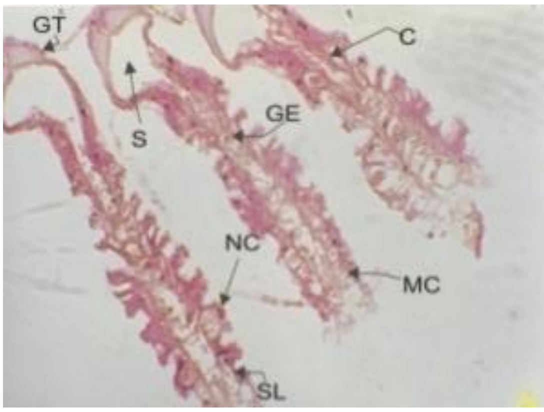


Figure 2: Magnified micrograph of gill lamellae in *B. bengalensis* Stained with HE 20 x 4.



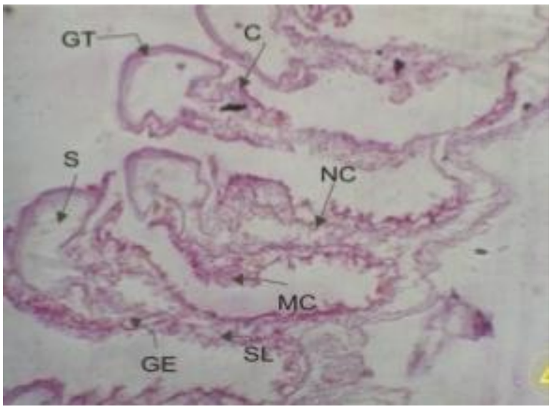


Figure 1: Zinc sulphate induced changes in gill lamellae of lamellae of snail *B. bengalensis* Stained with HE 20 x. **Figure 1: Lead acetate induced changes in gill 4 snail *B. bengalensis* Stained with HE 20 x 4.**
Key to Lettering: GT – Gill Tip, S – space ,C – cilia, GE- gill epithelium NC- nucleated cells.

DISCUSSION

In developing countries like India, most of the industries discharge their effluents into the aquatic and soil media, which can contaminate agriculture, aquaculture and other domestic regions, (Peebua et. al., 2006). Sediments act as a reservoir for all contaminants and dead organic matter in the ecosystem as documented by (Hamed and Emara 2006). Molluscs have capacity to bioconcetrate heavy metals in their soft tissues, (Jara-Marini et. al., 2009 and Tapia et. al., 2010). It was well noticed that, gill tissue of *Mytillus galloprovincialis* found responsible for the uptake of metal ions from water. (Znidaric et. al., 2005). Gulbhile, (2006) documented toxicity of mercuric chloride exposure on *Lamellidens corrianus*, where lamellae of gill showed cytological changes such as rupturing of the ciliated epithelium, increased size of lamellae, increased inter lamellar junction.

Panigrahi and Misra , (1980) documented, blindness, swelling of eyes, decreased oxygen consumption, reduction in blood proteins, blood hemoglobin and RBC in *Tilapia mossambica* during an exposure of 5 days at 0.005 mg L-1 mercury. Jawale (2006-4) also recorded that, after chronic exposure to lead and zinc, it showed marked histopathological changes and exhibited an initial reaction of epithelial damage, together with necrotic changes at the basement membrane and intertubular connective tissue of bivalve, *Lamellidens corrianus*.

Engel and Fowler (1979) found cytological deformities in gill tissue with reduced rate of body oxygenation due to effect of copper and cadmium induction. We found similar thickening of the basal lamina in gill filaments, vacuolization and necrosis of lamellar epithelial cells, congestion of central lamellar vein and hyperplasia of lamellar epithelial cells when subjected to toxic exposure of Zn and Pb respectively. Fig. No. 1, 2, 3 and 4. The percent decrease in body oxygen after 96 hrs. of exposure in zinc sulphate intoxicated snails was 69.58%, and 81.76% in lead acetate intoxicated group of snails. These snails were found more disturbed and under physiological stress. We found some of them were more sluggish and died because of lack of oxygen. Regarding some behavioral change, it was reported that, marine abalone when exposed to acidic waters showed impaired behavior and impaired feeding mechanism, Vargas et al. (2013).

Investigators working on invertebrate and vertebrate animals noticed that, the decrease in the oxygen consumption was due to cytological deformities and reduced membrane transportation in gill tissue, (Engel and Fowler, 1979). Histopathological changes and deformities in biomechanics can be responsible for stressed behavior of animal, (Holden, 1973). The loss of cilia may be caused by the interaction of heavy metals with the cell membrane, which induces changes in composition, fluidity and stability of the membrane (Axiak et. al., 1988). Lomate and Jadhav, (1982) observed a decrease in the oxygen consumption of *Corbicula regularis* due to $CuSO_4$, NaCN, KCN, potassium ferrocyanide, potassium oxalate, ammonium ferrocyanide, $ZnSO_4$ and $HgCl_2$. Scientifically it was proved that, Pb probably had limited availability since it was strongly bound to humic acids in the sediment and can be more harmful to cell metabolism (Campbell and Evans, 1987). Bhavan and Geraldine (2000) studied the histopathology of gill of the prawn *Macrobrachium malcolmsonii* exposed to Endosulfan and recorded reduced oxygen consumption. Our observations also coincide with the observations of Reichert *et al.* (1979) were they documented toxicity of heavy metals to aquatic organisms and reported scarcity of oxygen level and death of animals. Similar type of pathology and loss of function was documented by Rao et. al., (1988) against mercuric chloride induced alterations freshwater bivalve molluscs, *Indonaia caeruleus*.

CONCLUSION

Present investigation is more attentive about comparative effect of heavy metals zinc sulphate and lead acetate and its effects on rate of oxygen consumption with behavioral changes in freshwater snail *B. bengalensis*. We found that even at low dose, snais were more disturbed and stressed due to lead acetate toxicity compare to zinc sulphate. Results also supported by behavioral changes such as protective response, tentacular movements, foot movements, responses to external stimuli and courtship behavior were reduced/suppressed in very early exposure to lead acetate intoxication as compared to zinc sulphate indicating lead has higher toxicity to the experimental snail *Bellamya bengalensis.* Zinc might be utilized as essential metal up to certain limit, but has shown list toxic effect.

CONFLICTS OF INTEREST

The author declares that no conflicts of interest.

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