Vol III Issue I Feb 2013
Impact Factor : 0.2105
ISSN No : 2230-7850

Monthly Multidisciplinary
Research Journal

Indian Streams Research Journal

Executive Editor
Ashok Yakkaldevi

Editor-in-chief
H.N.Jagtap

IMPACT FACTOR : 0.2105

Welcome to ISRJ

RNI MAHMUL/2011/38595 **ISSN No.2230-7850**

Indian Streams Research Journal is a multidisciplinary research journal, published monthly in English, Hindi & Marathi Language. All research papers submitted to the journal will be double - blind peer reviewed referred by members of the editorial Board readers will include investigator in universities, research institutes government and industry with research interest in the general subjects.

International Advisory Board

Editorial Board

Address:-Ashok Yakkaldevi 258/34, Raviwar Peth, Solapur - 413 005 Maharashtra, India
Cell : 9595 359 435, Ph No: 02172372010 Email: ayisrj@yahoo.in Website: www.isrj.net

Indian Streams Research Journal
Volume 3, Issue.1,Feb. 2013
ISSN:-2230-7850
Available online at www.isrj.net
ORIGINAL ARTICLE

ECOTOXICITY OF HEAVY METAL ON HYDROLYTIC ENZYMES IN FRESHWATER GASTROPOD, BELLAMYA BENGALENSIS FROM HOTGI TANK, SOLAPUR

K. R. RAO[1], P. V. KALE[2], S. R. ANKARAM[3], A. R. BIDKAR[4]

[1,2,3]Department of Zoology, Walchand College of Arts and Science, Solapur,Maharashtra.
[4]Department of Zoology, Yashwantrao Chavan College, Tuljapur.

Abstract:

The aim of our present investigation is to increase our understanding of the impact of toxicant on aquatic organism and ecosystem. In the present investigation, variations in acid and alkaline phosphatase activity of Bellamya bengalensis subjected to acute toxicity of copper (predetermined 96 hrs LC50 during winter= 2.5 ppm) over a period of 96 hours during winter season was used to assess ecotoxicity. Samples of digestive glands, haemolymph, kidney and gills were collected and subjected for biochemical analysis of enzyme activity at an interval of 24, 48, 72 and 96 hrs after exposure to 2.5 ppm of copper sulphate. There was generally a gradual increase in the levels of acid and alkaline phosphatase activity in digestive gland, haemolymph, kidney and gills when compared to respective control groups. Significant differences were observed in acid and alkaline phosphatase levels in the copper treated gastropods with an increase in exposure of time to the copper during winter season. Our results demonstrate that, a) the activity levels of acid and alkaline phosphatase from different organs of gastropods exposed to copper depends on type of tissue involved and time of exposure of metal ions used, b) rapid changes in the activity level of acid and alkaline phosphatase might be associated with the destabilisation of lysosomal and cell membranes by metal ions, c) activity levels of hydrolytic enzymes are significantly higher in digestive glands and haemolymph which indicates that these are the major organ systems involved in the detoxification of metals.

KEYWORDS:

Heavy metal pollution, acute toxicity, copper, hydrolytic enzymes, Bellamya bengalensis

INTRODUCTION:

Ecotoxicity deals with the study of toxicity and responses to the toxic agents at community, species, tissue, cellular and molecular levels. Heavy metal pollution in aquatic ecosystem has been recognized as a serious environmental problem due to the non-biodegradability and the tendency of the heavy metals to accumulate in animal tissues (Soegianto et al., 2008). These heavy metals accumulate in food chain and cause severe threat to aquatic organisms. In this study copper is used as heavy metal I the form of copper sulphate. Copper is considered as a 'grey listed metal' (Mason, 1996) which naturally occurs in water, soil, sediments, rocks etc. Salts of copper are used as fungicide, algaecide in agriculture. They are also used in veterinary and industrial applications (Lodhi et al., 2006). Copper is an essential micronutrient but extensive use of copper may results into the pollution of aquatic ecosystem. Molluscs are considered as good indicators of heavy metal pollution. Some authors have reported their importance as the indicators for

Title : ECOTOXICITY OF HEAVY METAL ON HYDROLYTIC ENZYMES IN FRESHWATER GASTROPOD, BELLAMYA BENGALENSIS FROM HOTGI TANK, SOLAPUR Source:Indian Streams Research Journal [2230-7850] K. R. RAO[1], P. V. KALE[2], S. R. ANKARAM[3], A. R. BIDKAR[4] yr:2013 vol:3 iss:1

monitoring heavy metals (Nurnberg et al., 1984).

Acid and alkaline phosphatases (hydrolytic enzymes) are the marker enzymes both differ in the distribution (Rahman and Siddiqui, 2004). Acid phosphatase is associated with lysosomes whereas alkaline phosphatase is a plasma membrane enzyme found on the membranes of almost all animal cells. The activities of these enzymes are involved in a variety of metabolic processes such as protein synthesis, growth and differentiation of cell, absorption and transport of molecules, steroidogenesis, etc. (Ram and Sathyanesan, 1985). Alkaline phosphatase has been reported to be sensitive to heavy metal pollution (Regoli and Principato, 1995). Copper and mercury at varying degree of concentration have shown to influence the activity of acid phosphatase in freshwater mussels (Rajalakshmi and Mohandas, 2005). Therefore, enzymes can be used as the bioindicators of metal contamination with respect to type and concentration of metal (Atli and Canli, 2007).

Bellamya bengalensis is a freshwater gastropod collected from Hotgi Tank, Solapur -Maharashtra (India) during winter season (Nov-Dec 2012). These gastropods can be used as a bioindicator of heavy metal pollution. Hotgi Tank is situated in Southern direction 10 km away from Solapur city (latitude 170-20'-20" and longitude 750-68'- 00"). It has a catchment area of 59.57 square km. This freshwater ecosystem is at high risk of pollution due to anthropogenic activities, industrialisation and agricultural use which cause severe harmful toxic effect to flora and fauna of this ecosystem due to effluent discharges. In the present investigation, heavy metal induced ecotoxicity of copper was studied during winter season with respect to acid and alkaline phosphatase hydrolytic enzymes from Bellamya bengalensis.

MATERIALS AND METHODS:

Test organism and their maintenance:

The freshwater gastropods, Bellamya bengalensis were collected during winter season (Nov-Dec 2012) from Hotgi Tank, Solapur. They were brought to the laboratory and acclimated to the laboratory conditions in a well aerated aquarium. The water was changed for every 24 hrs.

Experimental design:

Before actual experiment, 96 hrs LC50 value of copper was determined with the method described by Finney (1971). The 96 hrs LC50 was found to be 2.5 ppm during winter season. Animals were distributed into four groups of 20 animals each. They were exposed to predetermined 96 hrs LC50 of Copper (2.5 ppm) for duration of 96 hrs. A control group without toxicant was also run simultaneously. The experiments were conducted for 96 hrs with an interval of 24, 48, 72 and 96 hrs. Organs like digestive glands, kidney, gills and haemolymph were collected for further estimation from both control and experimental groups.

Preparation of sample for Enzyme assay:

At each interval 5 animals were sacrificed separately to collect various soft tissues such as digestive glands, gills, and kidney. Haemolymph (about 1 ml) was also collected for the enzyme assay. The soft organs were homogenised separately in glass homogenizers by using respective buffers. Glycine/NaOH buffer at pH-9 for alkaline phosphatase and citrate buffer at pH-4 for acid phosphatase were used. Tissue homogenate were centrifuged and the supernatants were collected which were used for the enzyme assay.

Enzyme analysis:

Acid and Alkaline phosphatase were determined spectrophotometrically with the method described by Anon (1963). The specific activity of enzymes is expressed as µg PNP released/ml.

Statistical Analysis:

The data obtained was analysed with the level of significance by using Student's't' test (Bailey, 1965). Values were taken in triplicate and expressed as Mean±SD. Graphs were prepared by using Graph Pad prism (version 5.00).

RESULTS:

The variations in acid and alkaline phosphatase activity from various organs of freshwater gastropods, Bellamya bengalensis after exposing them to acute toxicity of CuSO4 is given in the fig. 1-8.

In the present study, changes in acid and alkaline phosphatase activity from various organs due to acute toxicity showed significant variations in experimental groups when compared with the respective control groups(Table no.1 and 2). The activity of acid phosphatase in digestive glands from control group was in range between 7.800±0.780 to 8.310±0.185 µgPNP/ml. After 96 hrs of exposure to LC50 of CuSO4, there was an overall significant increase from all experimental groups as compared to respective control groups ($p<0.05$, $p<0.01$). However, the activity was more increased after 72 hrs of treatment (28.48%) and increase was little bit less after 96 hrs of treatment (16.53%). In haemolymph the activity of acid phosphatase from control group was in the range from 5.60±0.560 to 6.303±0.625 µgPNP/ml. Activity of acid phosphatase was more after 72 hrs (39.40%) and was increased less after 24 hrs (19.45%) of treatment to copper ($p<0.01$, $p<0.05$). Acid phosphatase activity in kidney from control group was in between 3.40±0.264 to 3.917±0.410 µgPNP/ml. Activity was increased more (34.63%) after 72 hrs ($p<0.01$) and was increased less (20.16%) after 96 hrs ($p<0.05$) from experimental groups. In gills, acid phosphatase activity from control group was in between 1.787±0.250 to2.100±0.210 µgPNP/ml. Activity was significantly increased and highest activity was found after 96hrs of treatment (62.45%) ($p<0.01$) and was increased less (40.37%) after 24 hrs of treatment ($p<0.01$).

Activity of alkaline phosphatase in digestive gland from control group was in the range of 11.22±0.200 to 12.13±0.289 µgPNP/ml. Maximum increase (39.76%) was observed after 72 hrs of treatment ($p<0.001$) whereas minimum increase (9.00%) was observed after 24 hrs of treatment($p<0.05$).Alkaline phosphatase activity in haemolymph from control groups was ranging in between 9.950±0.990 to 10.77±0.249 µgPNP/ml. Activity was found to be increased more significantly ($p<0.001$) after 72 hrs (51.95%) and less significantly ($p<0.01$) after 96 hrs (11.01%) from experimental groups. In kidney, the activity of alkaline phosphatase from control group was in the range of 7.910±0.487 to 9.783±0.385 µgPNP/ml. Activity of alkaline phosphatase was increased more (21.01%) after 48 hrs ($p<0.01$) and increased less (13.46%) after 72 hrs ($p<0.05$) of treatment to copper. In gills, alkaline phosphatase activity from control group was in between 7.190±0.400 to 7.580±0.540 µgPNP/ml. An increase in the activity was higher (39.90%) after 96hrs of treatment ($p<0.01$) and increase was less (17.75%) after 24 hrs of treatment ($p<0.05$).

Table No.1: Variations in the acid phosphatase activities from Digestive glands, Haemolymph, Kidney and Gills of *Bellamya bengalensis* exposed to 96hrs LC_{50}

Tissue sample	Parameter	24hrs	48hrs	72hrs	96hrs
Digestive glands	**Control**	8.00±0.800	8.310±0.185	7.800±0.780	7.903±0.795
	LC_{50}	9.837±0.212*	10.370±0.53**	10.10±1.010*	9.237±0.237*
Haemolymph	**Control**	6.00±0.600	5.60±0.560	6.303±0.625	6.300±0.630
	LC_{50}	7.167±0325*	7.303±0.725*	8.787±0.106**	7.537±0.362*
Kidney	**Control**	3.40±0.264	3.840±0.431	3.647±0.168	3.917±0.410
	LC_{50}	4.507±0.440*	4.917±0.500*	4.910±0.495*	4.707±0.227*
Gills	**Control**	1.833±0.299	1.967±0.251	2.100±0.210	1.787±0.250
	LC_{50}	2.573±0.153*	2.830±0.271*	3.103±0.305**	2.903±0.295**

(Enzyme activity is expressed in µg PNP released/ml)

Note: Based on Student's't' test, values are significant at * = p < 0.05, ** = p < 0.01, *** = p < 0.001

Table No.2: Variations in the alkaline phosphatase activities from Digestive glands, Haemolymph, Kidney and Gills of *Bellamya bengalensis* exposed to 96hrs LC50

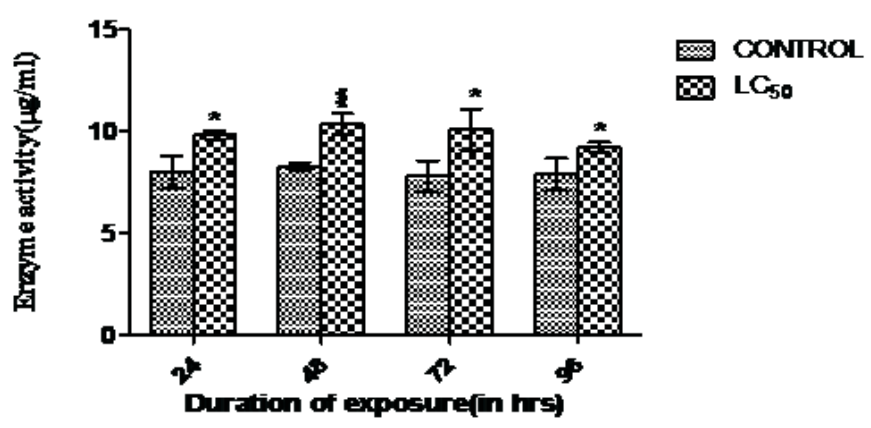
Tissue sample	Parameter	24hrs	48hrs	72hrs	96hrs
Digestive glands	**Control**	12.13±0.289	11.83±0.293	11.87±0.289	11.22±0.200
	LC_{50}	14.27±0.294***	15.97±0.783**	16.59±0.317***	12.23±0.376*
Haemolymph	**Control**	10.70±0.320	10.77±0.485	9.950±0.990	10.71±0.249
	LC_{50}	11.96±0.275**	14.06±0.310***	15.12±0.312***	11.89±0.290**
Kidney	**Control**	7.910±0.487	8.010±0.325	8.963±0.316	9.783±0.385
	LC_{50}	9.167±0.434*	9.693±0.456**	10.17±0.521*	8.303±0.825*
Gills	**Control**	7.267±0.602	7.517±0.395	7.580±0.540	7.190±0.400
	LC_{50}	8.557±0.414*	9.653±0.442**	10.18±0.478**	9.700±0.851**

(Enzyme activity is expressed in µg PNP released/ml)

Note: Based on Student's 't' test, values are significant at * = p < 0.05, ** = p < 0.01, *** = p < 0.001

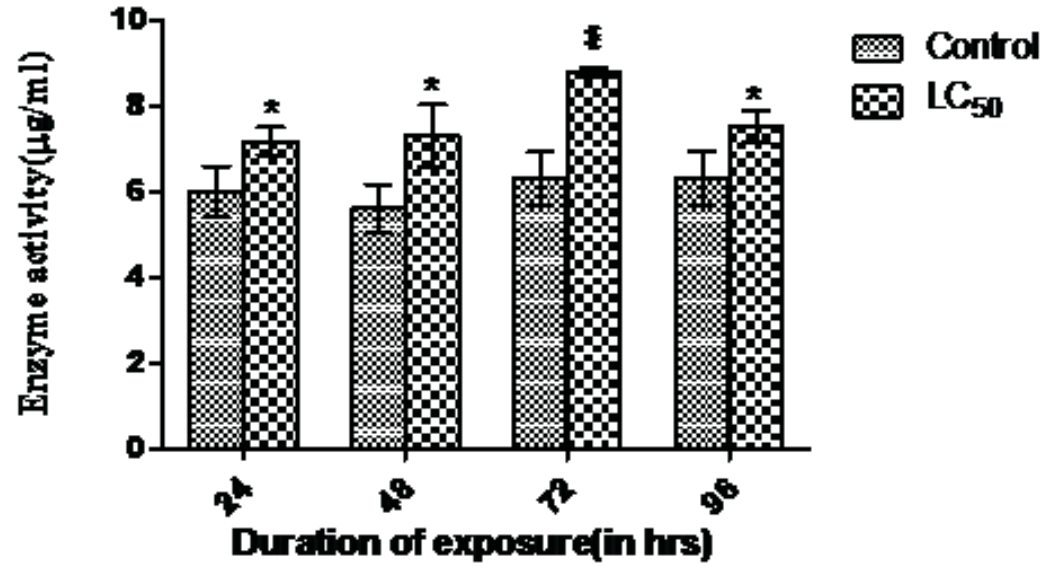
In the present study, the major activity of acid and alkaline phosphatase after exposure to copper was found in digestive gland followed by haemolymph, gills and kidney (fig.1-8) whereas according to the percent variations major increase in the activity of acid phosphatase was found in gills and alkaline phosphatase was found in haemolymph. This may be due to that gills are the primary organs to be exposed to metal and therefore an increase in the activity of acid phosphatase is more in gills whereas an increase in the activity of alkaline phosphatase was more in haemolymph because severe tissue damage results into the release of enzyme into haemolymph as gastropods have open circulatory system. Further the activity of both enzymes depends on the type of tissue involved in detoxification and the duration of exposure of metal. Activity of both hydrolytic enzymes was increased with an increase in the duration of exposure to metal. Both acid and alkaline phosphatase activities from different organs elevated significantly in experimental groups when compared with their respective control groups which indicate metabolic changes due to heavy metal stress.

Fig 1 : Variations in the Acid phosphatase level in Digestive glands from *Bellamya bengalensis* exposed to 96hrs LC_{50} of copper



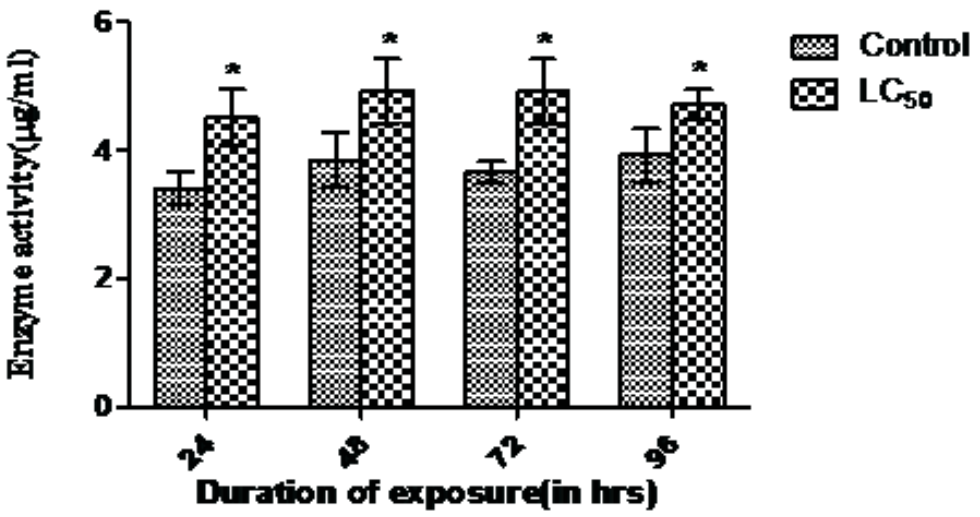
(Enzyme activity is expressed in µg PNP released/ml)
Note: Based on Student's't' test, values are significant at * = p < 0.05, ** = p < 0.01, *** = p < 0.001

Fig 2 : Variations in the Acid phosphatase level in Haemolymph from *Bellamya bengalensis* exposed to 96hrs LC_{50} of copper



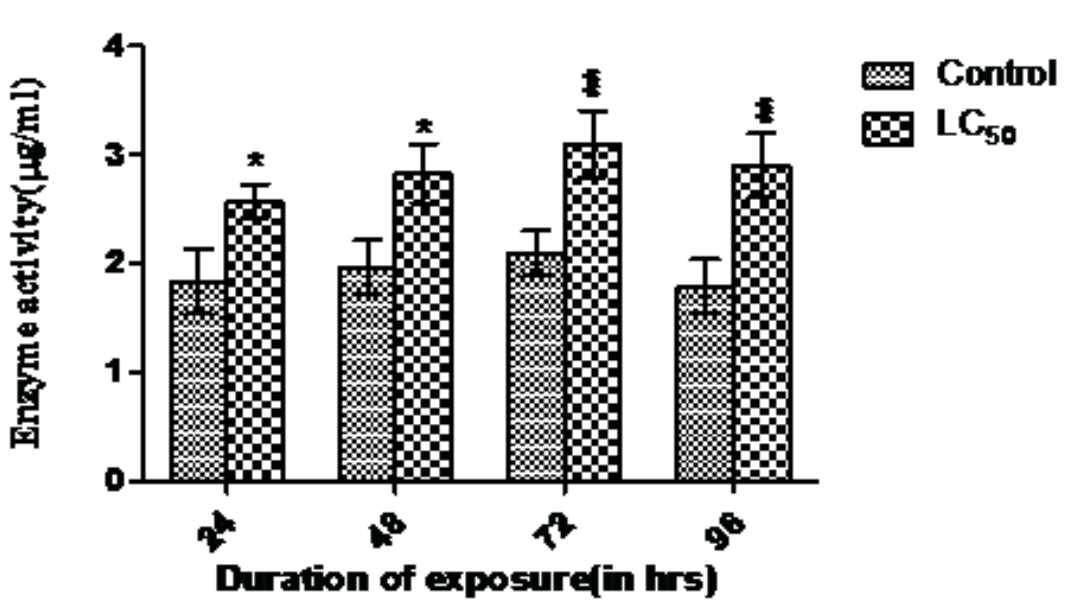
(Enzyme activity is expressed in µg PNP released/ml)
Note: Based on Student's't' test, values are significant at * = p < 0.05, ** = p < 0.01, *** = p < 0.001

Fig 3 : Variations in the Acid phosphatase level in Kidney from *Bellamya bengalensis* exposed to 96hrs LC_{50} of copper



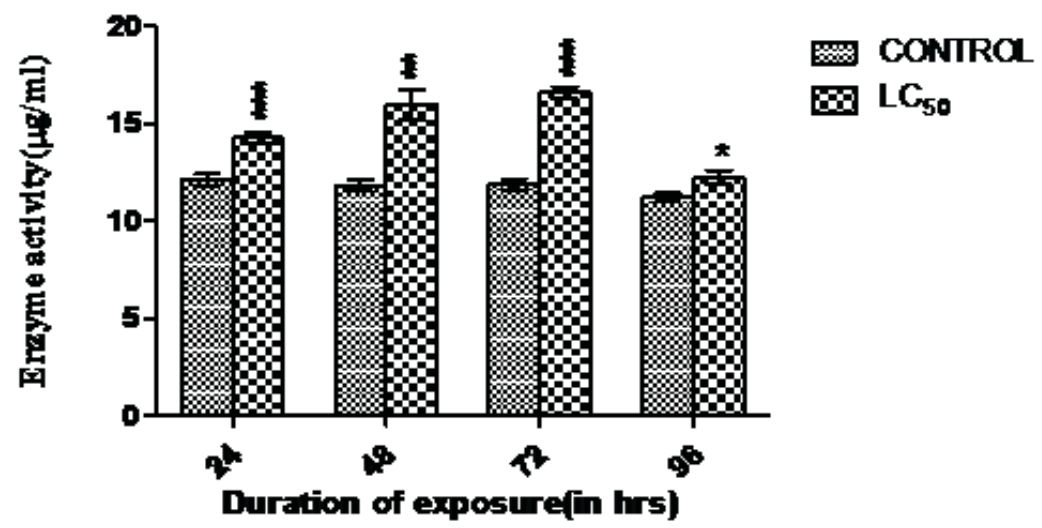
(Enzyme activity is expressed in µg PNP released/ml)
Note: Based on Student's't' test, values are significant at * = p < 0.05, ** = p < 0.01, *** = p < 0.001

Fig 4 : Variations in the Acid phosphatase level in Gills from *Bellamya bengalensis* exposed to 96hrs LC_{50} of copper



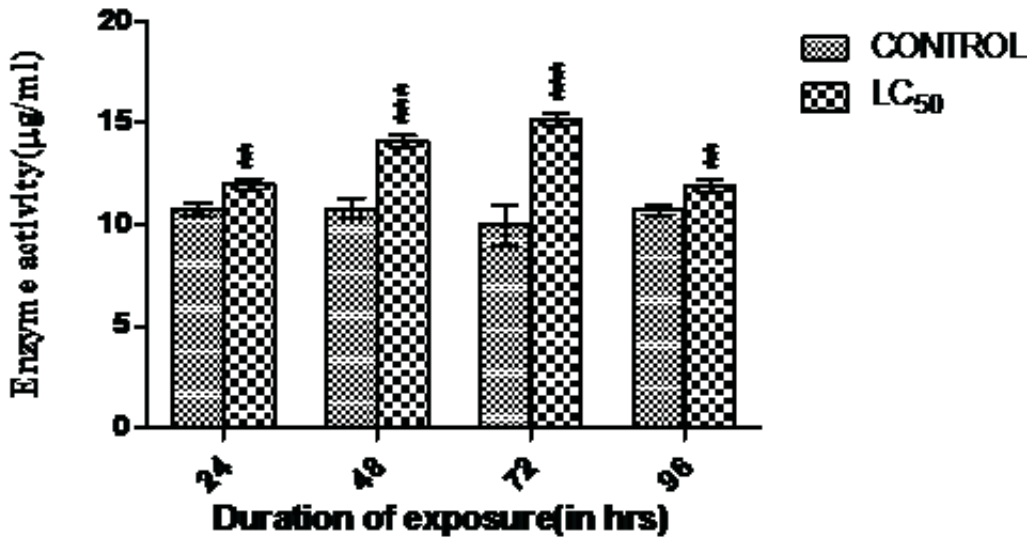
(Enzyme activity is expressed in µg PNP released/ml)
Note: Based on Student's't' test, values are significant at * = p < 0.05, ** = p < 0.01, *** = p < 0.001

Fig 5 : Variations in the Alkaline phosphatase level in Digestive glands from *Bellamya bengalensis* exposed to 96hrs LC_{50} of copper



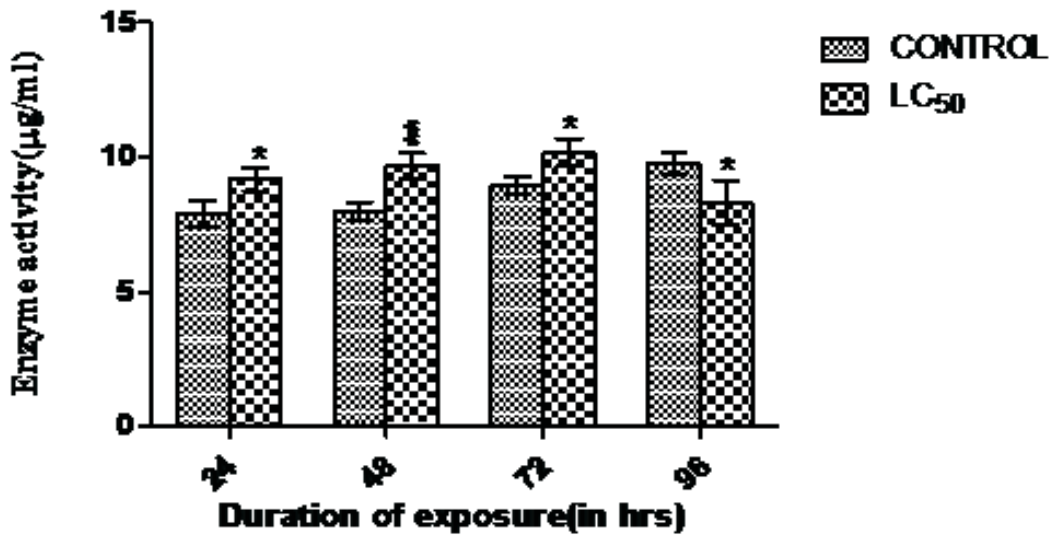
(Enzyme activity is expressed in µg PNP released/ml)
Note: Based on Student's't' test, values are significant at * = p < 0.05, ** = p < 0.01, *** = p < 0.001

Fig 6 : Variations in the Alkaline phosphatase level in Haemolymph from *Bellamya bengalensis* exposed to 96hrs LC_{50} of copper



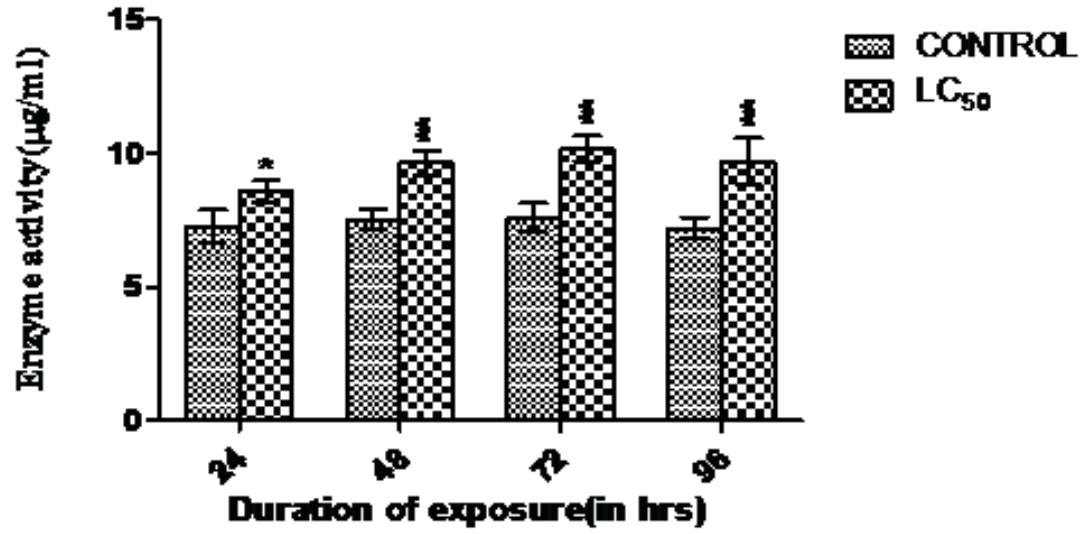
(Enzyme activity is expressed in µg PNP released/ml)
Note: Based on Student's't' test, values are significant at * = p < 0.05, ** = p < 0.01, *** = p < 0.001

Fig 7 : Variations in the Alkaline phosphatase level in Kidney from *Bellamya bengalensis* exposed to 96hrs LC_{50} of copper



(Enzyme activity is expressed in µg PNP released/ml)
Note: Based on Student's't' test, values are significant at * = p < 0.05, ** = p < 0.01, *** = p < 0.001

Fig 8 : Variations in the Alkaline phosphatase level in Gills from *Bellamya bengalensis* exposed to 96hrs LC_{50} of copper



(Enzyme activity is expressed in µg PNP released/ml)
Note: Based on Student's't' test, values are significant at * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

DISCUSSION:

In the present study an increase in the activity of acid and alkaline phosphatase suggest that the animals were under heavy metal stress. Any change in the activity of these enzymes can impair various metabolic processes.

Acid and alkaline phosphatases are involved in a variety of metabolic processes such as detoxification, biosynthesis of macromolecules and meatabolism (Rahman and Siddiqui, 2004). In the present investigation, at high metal (copper) concentration severe damage to tissues might have altered the structure, permeability and integrity of lysosomal membranes which could have resulted into diffusion of lysosomal enzyme (acid phosphatase) into cell cytoplasm. Severe damage of tissue might have resulted into the leakage of enzyme from cells, tissues to haemolymph. As a result of which the activity of acid phosphatase might be increased in gills, digestive glands, haemolymph and kidney.

Similar observations were noticed by various workers. An increase in acid phosphatase activity was observed in gills and serum of carp (Cyprinus carpio L.) exposed to the highest concentration of copper. After a recovery period decrease in enzyme activity was observed in gills and serum of Carp (Karan et al., 1998). Hypersynthesis of acid phosphatase upon metal uptake can also play a protective role in defence (Cheng, 1983). Sharma et al. (2006) speculated the hypersynthesis of lysozyme by Hg and acid phosphatase by Cu.

Alkaline phosphatase is membrane bound enzyme found almost on all animal cell membranes where active transport occurs. In the present study, an increase in alkaline phosphatase activity may be taken as an indicator of severe tissue damage. Activity of alkaline phosphatase was found to be increased significantly from haemolymph, digestive glands, gills and kidney. A significant increase in alkaline phosphatase activity from serum, kidney, lungs and liver of albino wistar rats was observed during subchronic exposure to neem based pesticide which might be due to elevated permeability of plasma membrane (Rahman and Siddiqui, 2004). Both enzymes are sensitive to heavy metals. Variations in the enzyme activity based on the concentration and length of exposure of metal is of great diagnostic value (Rajalakshmi and Mohandas, 2005). It was earlier reported that the effect of heavy metal in lysosomal enzyme acid phophatase depends on three factors viz, toxicological nature of toxicant, duration of exposure and morphophysiological status of concerned organ or tissue (Jayakumar et al., 2008). In the present study,

activity of both hydrolytic enzymes in digestive glands, haemolymph, gills and kidney from Bellamya bengalensis was increased with an increase in the duration of exposure to copper.

CONCLUSION:

In the present investigation after exposing gastropods, Bellamya bengalensis to copper for acute toxicity during winter season enzyme activity was found to be higher in digestive glands and haemolymph as compared to kidney and gills. This may suggest that digestive gland is the major organ involved in detoxification of metals. Further both enzymes are sensitive to metal which depends on the type of tissue involved in detoxification and duration of exposure of metal used. Therefore, these enzymes can be used as a biomarker in the heavy metal pollution monitoring. Further investigations are required to understand histochemical manipulations due to metal toxicity in freshwater gastropods, Bellamya bengalensis.

ACKNOWLEDGEMENT:

Authors wish thanks to the Principal Dr. A.H.Manikshete, Walchand College of Arts and Science, Solapur for providing necessary facilities.

REFERENCES:

1) Agoes Soegianto, Babang Irawan and Hamami, (2008). Bioaccumulation of Heavy metals in Aquatic animals collected from Coastal Waters of Gresik, Indonesia. Asian Journal of Water, Environment and Pollution, 6(2): 95-100.
2)Anon, (1963). The colorimetric determination of phosphatase. Sigma Tech. Bull.104: Sigma Chemicals Co. St. Louis.
3) Atli, G. and Canli, M. (2007). Enzymatic responses to metal exposures in a freshwater fish Oreochromis niloticus. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology, 145(2): 282-287.
4) Bailey, N. T. J. (1965). Statistical methods in biology. ELBS, English University press, London.
5) Cheng, T. C., (1983). Triggering of immunologic defense mechanisms of molluscan shellfish by biotic and abiotic challenge and its application. Mar. Technol. Soc. J., 17:18-25.
6) Finney, D. J. (1971). Probit Analysis (3rd Edition). Cambridge University Press.
7) Jayakumar, P., Jothivel, N. and Paul, V. I. (2008). Heavy metals induced alterations in the acid phosphatase activity in the edible freshwater mussel Lamellidens marginalis (Lamarck). The Internet Journal of Toxicology, 5(2).
8) Karan, V., Vitorovic, S., Tutundzic, V. and Poleksic, V., (1998). Functional enzymes activity and gill histology of carp after copper sulfate exposure and recovery. Ecotoxicology and Environmental Safety, 40(1-2): 49-55.
9) Lodhi, H. S., Khan, M. A., Verma, R. S. and Sharma, U. D. (2006). Acute toxicity of copper sulphate to fresh water prawns. Journal of Environmental Biology, 27(3): 585-588.
10) Mason, C. F., (1996). Biology of fresh water pollution (IIIrdEd.). Department of Biology, university of Essex. Paletisher – Longman, Singapore, Paletishers (1996).
11)Nurnberg, H. W., (1984). Bio-accumulation of heavy metals by bivalves from Lim Fjord (North Adriatic Sea). Journal of Marine Biological Sciences 81(2): 177-188.
12)Rahman, M. F. and Siddiqui, M. K. J., (2004). Biochemical effects of vepacide (from Azadurachta indica) on Wistar rats during subchronic exposure. Ecotoxicology and Environmental Safety, 59(3): 332-339.
13)Rajakajshmi, S. and Mohandas, A., (2005). Copper- induced changes in tissue enzyme activity in fresh water mussel. Ecotoxicology and Environmental Safety, 62(1): 140-143.
14)Ram, R. N. and Sathyanesen, A. G. (1985). Mercuruc chloride, cythion and ammonium sulfate induced changes in the brain, liver and ovarian alkaline phosphatase content in the fish Channa puntactus. Environ. Ecol., 3: 263-268.
15)Regoli, F. and Principato, G., (1995). Glutathione, glutathione–dependent and antioxidant enzymes in mussel Mytilus galloprovincialis, exposed to metals under field and laboratory conditions: implications for the use of biochemical biomarkers. Aquatic toxicology, 31(2): 143-164.
16)S. R. Krupesha Sharma, P. K. Asokan and A. K Sahoo, (2006). Effect of sub-lethal level of copper and mercury on digestive gland cells and lysosomal enzyme activity in the green mussel (Perna viridis L). J. Mar. Ass. India, 48(2): 245-248.