International Journal of Engineering Science Invention
ISSN (Online): 2319 – 6734, ISSN (Print): 2319 – 6726
www.ijesi.org ||Volume 6 Issue 8|| August 2017 || PP. 65-70

Effect of Heavy Metals on Lysosomal Enzyme Acid Phosphatase Activity of Bivalve L. marginalis…..

Sandhya M. Sonawane.
Department of Zoology, JDMVPS ASC College, Jalgaon, Dist. Jalgaon. (M.S.) Mobile No. 9422977824
E-mail I.D. : sandhyamahendra11@gmail.com,
Corresponding Author: Sandhya M. Sonawane.

ABSTRACT: *Enzymes are referred as biological, catalysts which obey certain general rules. The literature shows investigative lacunae with regard to effects of heavy metals on Acid phosphatase activity of aquatic animals especially where fresh water molluscs are concerned. Hence an attempt has been made to study the effect of heavy metals $CuSO_4$, $HgCl_2$ and $CdCl_2$ on fresh water bivalve, Lamellidens Marginalis with respect to change in the level of acid phosphatase enzymes. The level of acid phosphatase was elevated due to heavy metal stress. The elevation was more prominent in $HgCl_2$ stress than $CuSO_4$ and $CdCl_2$.*
Keywords : Heavy metals $CuSO_4$, $HgCl_2$ and $CdCl_2$, L. marginalis, Acid phosphatase enzyme, acute, chronic.

I. INTRODUCTION

Enzymes are referred as biological, catalysts which obey certain general rules. The enzyme catalyzed reactions take place at physiologically low temperatures (37^0C) and require extremely small amounts of enzymes. Chemically, enzymes are complex protein molecules synthesized in the cells where they act as biocatalysts in carrying out various physic-chemical reactions. These proteins have their own specificity and kinetics. The enzymes help in attaining a reaction in a state of equilibrium. An enzyme recognizes its specific substrate and reacts with it to form products and gets regenerated at the end of the reaction.

The usual measure applied for the assessment of any environmental effect of a pollutant on animal is mortality. However, other effects which are gradual and are indicative of physiological change can be as detrimental as mortality to the animal's survival.

Although instantaneous effects of heavy metal poisoning may be physical such as retraction of animal in the shell, loss of locomotory activity, changes is normal behaviour, reproductive disorders, suffocation, coating of respiratory surface with mucus, long term effect would be exclusively due to physiological alterations.

The most fundamental effects would be the study of changes in enzyme levels, since these organic cellular catalysts control the formation of biochemical intermediates which are indispensable to all normal physiological processes. Interactive effects of metal pollution and ocean acidification on physiology of marine organisms Anna V. Ivanina, Inna M. Sokolova, (2015)

The lysosomes which contain several acid hydrolases are believed to constitute an intracellular digestive pattern (Berhet, 1965; de Durve and Wattiaux 1966) and have been implicated in the digestion and ingested substances in various types of mammalian cells (Cohn and Hirsch, 1960; Straus, 1964 a, b). However, there is little evidence that intracellular digestion in mollusks is due to lysosomes. Acid phosphatase are lysosomal enzyme. Summer (1969) demonstrated the presence of these enzymes in lysosomes and food vacuoles of the digestive gland of Mytilus edulis and Helix aspersa.

The acid phosphatase, a non specific monosterase and alkaline phosphatase, a nonspecific hydrolase are the two considerably important enzymes widely spread in the animal kingdom (Standtonan 1961). Acid phosphatase, is a lysosomal enzyme which hydrolyses the ester linkage of phosphate esters and helps in autolysis of the cell. Acid Phosphatase concerned with the presence of transphosphorylation and have an important role to play in the general energetic of an organism.

This enzyme have been studied by many workers – Bhattacharya et al. (1975) in the fish Clarius Batrachus exposed to endrin and Anees (1976) in the fish Channa punctatus noticed the activity of these enzymes in effect of heavy metals like Cu, Hg and Zn on these hydrolases in Viviparus bengalensisFor going survey of literature showed that only a few workers have studied metabolic enzymes of lamellibranch mollusks. The literature shows investigative lacunae with regard to effects of heavy metals on metabolic processes of aquatic animals especially where fresh water molluscs are concerned. Hence an attempt has been made to study the effect of heavy metals on fresh water bivalve, Lamellidens Marginalis with respect to change in the level of lysosomal enzymes.

II. MATERIAL AND METHODS

The bivalves Lamellidens Marginalis were collected from the Godavari river at Paithan. After bringing the bivalves to the laboratory, they were cleaned thoroughly and placed in plastic troughs. They were acclimatized to the laboratory conditions for 5 to 6 days prior to subjecting them to experiments. The water in the troughs was changed every day. Only active and healthy animals were chosen for experiments. During chronic treatment the animals were fed on crushed fresh water algae and Hydrilla.

The bivalve were exposed to median lethal concentration and sublethal concentration of popputant as acute and chronic treatment respectively. The acclimatized bivalves were divided into four groups, of ten each. The first groups of bivalves was kept as control. The remaining groups were exposed to 1.6 ppm $Cuso_4$, 0.6 ppm $HgCl_2$ and 3.9 ppm cadmium chloride for 72 hours, for acute treatment. The concentrations used for chronic exposure were 0.82 ppm copper sulphate, 0.32 ppm mercuric chloride and 1.95 ppm cadmium chloride. The chronic treatment was given upto 20 days. The control and treated bivalves were fed on freshwater crushed algae and Hydrilla during exposure period.

The digestive glands from five to ten mussels were separated and washed in distilled water. These digestive glands were then dried between the folds of muslin cloth, dehydrated and defatted by treatment with ice cold acetone (Summer and Summer, 1947). The material was
ground in a clean ice chilled glass morter and repeatedly washed in acetone and filtered till and filtrate was colourless. The powder thus obtained was dried under fan and stored in a clean bottle in freezer, at 3 to 5^0C. In all the experiments, 1% homogenate of digestive gland prepared in glass distilled water was used. Half of this extract was boiled for half an hour to destroy the enzyme activity and this boiled extract was used as control for all the experiments.

Estimation of acid Phosphatase :

Acid phosphatase activity was measured by the method of Gutman and Gutman (1940). In a medium of 2.0 ml of citrate buffer (pH 4.8), 2.0 ml of 0.1 ml. disodium phenyl phosphate, 2.0 ml of 20% tissue homogenate were added and incubated at 37^0C for 30 minutes. After stopping the reaction by the addition of 1.8 ml of Folin ciocalteu phenol reagent, the reaction mixture was centrifused at 3000 rpm for 5 minutes. To 4.0 ml of the supernatant, 2.0 ml of 15% sodium carbonate were added. After further incubation of 10 minutes at 37^0C. The blue colour developed was read at 660 um in a colourimeter. A simultaneous blank was run with distilled water. Phenol was used for determining the standard graph value for both acid and alkaline phosphatase. For the calculation of acid phosphatase activity, the same formula as that for alkaline phosphatase was used. The values were expressed as Ka/ml/hr/at 37^0C.

III. OBSERVATIONS AND RESULTS

The activity of acid phosphatase was studied in the normally fed and pollutant (heavy metals) treated freshwater bivalve, Lamellidens marginalis. The experimental findings obtained are summarized in Tables 1 to 2.

The activity of the enzyme acid phosphatase was greatly enhanced in all the tissues of the bivalve L. Marginalis though it was time dependent. The acid phosphatase activity in control bivalves was found to be 2.005, 1.565, 0.990 um at Pi/mg protein/hr in digestive gland, foot and mantle respectively. All the types of heavy metal exposures caused an increase in acid phosphatase activity.

$CuSO_4$ acute exposure showed increased activity and it was 2.103, 1.630 and 1.290 ug of Pi/mg protein/hr after 72 hours in digestive gland, foot and mantle respectively. $HgCl_2$ acute exposure showed increased activity and it was 2.130, 1.43 and 1.136 ug of Pi/mg protein/hr after 72 hours in digestive gland, foot and mantle respectively. The values after acute exposure of $CdCl_2$ were 1.938, 1.572 and 1.200 ug of Pi/mg of protein/hr after 72 hours.

Similar trend was also observed in chronic exposure to all the heavy metals. The acid phosphatase activity in control bivalves was 2.430, 0.994 and 0.734 after 20 days in digestive gland, foot and mantle respectively. The increase was highest at 5 days of $CuSO_4$, $HgCl_2$ and $CdCl_2$ stress and lowest at 20 days of the three heavy metals. In $CuSO_4$ the values were 2.520, 1.289 and 0.830 ug of Pi/mg protein/hr, after 20 days of exposure. The values after $HgCl_2$ stress were 2.869, 1.486 and 0.963 ug of Pi/mg protein/hr after 20 days of exposure, while at the end of 20 days exposure of $CdCl_2$ the value were 1.199, 1.175 and 0.802 ug of Pi/mg protein/hr in digestive gland foot and mantle respectively.

The maximum percentage increase was upto 9.224 in digestive gland, 65.80% in foot and 36.60% in mantle after 24 hrs. $HgCl_2$ stress. In chronic exposure maximum percentage increase was upto 50.16% (5 days $HgCl_2$ stress in foot) the increase in activity of acid phosphatase in acute exposure of $CdCl_2$ of 48 hrs is not significant statistically whereas all other values are statistically significant at $P<0.05$ and $P<0.001$ levels. The increase in acid phosphatase after chronic exposure to $CdCl_2$ at 10 days and decrease at 20 days are not significant, whereas all other values are statistically significant at $P<0.01$, $P<0.001$ and $P<0.05$ levels.

Table - 1

Level of acid phosphatase activity in the bivalve, L.marginalis after acute exposure of Copper sulphate, Mercuric chloride and Cadmium chloride

Pollutant	**Exposure Period (in hrs)**	**Digestive gland**		**Foot**		**Mantle**	
1	**2**	**3**		**4**		**5**	
Control		2.005±0.0050		1.565±0.0034		0.990±0.0032	
$CuSO_4$	24 hrs	2.138±**0.005** **P<0.05**	±15.71	1.780±**0.0035** **P<0.001**	+70.11	1.330±**0.0020** **P<0.001**	+58.01
	48 hrs	2.122±**0.0043** **P<0.04**	+12.11	1.750±**0.0042** **P<0.05**	+61.13	1.301±**0.0028** **P<0.001**	+48.20
	72 hrs	2.103±**0.0035** **P<0.001**	+10.01	1.630±**0.0023** **P<0.05**	+57.18	1.290±**0.0022** **P<0.001**	+45.80
$HgCl_2$	24 hrs	2.152±**0.004** **P<0.05**	+19.22	1.780±**0.0025** **P<0.001**	+65.80	1.545±**0.0037** **P<0.05**	+36.6
	48 hrs	2.145±**0.0034** **P<0.05**	+17.67	1.722±**0.0024** **P<0.05**	-60.71	1.433±**0.0029** **P<0.05**	+33.13
	72 hrs	2.130±**0.0038** **P<0.001**	+10.14	1.433±**0.0022** **P<0.001**	-60.50	1.136±**0.0024** **P<0.05**	-32.14
$CdCl_2$	24 hrs	2.109±**0.0034** **P<0.05**	+7.34	1.594±**0.0028** **P<0.05**	+19.70	1.290±**0.0025** **P<0.05**	+25.24
	48 hrs	2.000±**0.0040** **N.S.**	+3.10	1.579±**0.0020** **P<0.05**	+18.80	1.230±**0.0022** **P<0.05**	+20.10
	72 hrs	1.938±**0.6062** **P<0.05**	+2.02	1.572±**0.0022** **P<0.05**	+15.30	1.200±**0.0020** **P<0.05**	+12.81

Acid phosphatase activity is expressed as ug of Pi/mg protein/hr
Each value is the mean of five observation ± S.D.
Values are significant at P<0.001 and 0.05 level or NS = Not significant
+ve sign indicates percentage increased .

Table - 2

Level of Acid Phosphatase activity in the tissue of L.marginalis after chronic exposure of heavy metals, copper sulphate, Mercuric chloride and Cadmium chloride

Pollutant	**Exposure Period (in Days)**	**Digestive gland**		**Foot**		**Mantle**	
1	**2**	**3**		**4**		**5**	
Control	5	2.239±0.0020		0.998±0.0018		0.789±0.0042	
	10	2.237±0.0023		0.995±0.0019		0.734±0.0015	
	15	2.232±0.0032		0.992±0.0010		0.735±0.0017	
	20	2.430±0.0018		0.994±0.0015		0.734±0.0015	
$CuSO_4$	5	2.635±**0.0026** **P<0.05**	+8.352	1.324±**0.0018** **P<0.05**	+32.40	0.883±**0.0032** **P<0.05**	+20.23
	10	2.630±**0.0022** **P<0.05**	+3.433	1.320±**0.0024** **P<0.05**	+31.43	0.880±**0.0024** **P<0.001**	+19.16

	15	2.559±**0.0019** **P<0.05**	+2.225	1.290±**0.0020** **P<0.001**	+30.81	0.875±**0.0023** **P<0.001**	+18.19
	20	2.520±**0.0020** **NS**	+0.9785	1.289±**0.0019** **P<0.001**	+27.72	0.830±**0.0030** **P<0.001**	+11.72
$HgCl_2$	5	2.889±**0.0002** **P<0.05**	+14.05	1.537±**0.0015** **P<0.05**	+54.16	0.992±**0.0022** **P<0.05**	+31.32
	10	2.880±**0.0012** **P<0.001**	+5.579	1.522±**0.0012** **P<0.05**	+52.86	0.986±**0.005** **P<0.001**	+30.83
	15	2.872±**0.0028** **P<0.001**	+5.043	1.498±**0.0015** **P<0.05**	+50.70	0.972±**0.0016** **P<0.001**	+22.54
	20	2.869±**0.003** **P<0.001**	+3.448	1.486±**0.0015** **P<0.05**	+49.19	0.963±**0.0019** **P<0.001**	+17.80
$CdCl_2$	5	2.240±**0.0034** **P<0.001**	-4.530	1.235±**0.0013** **P<0.001**	+23.87	0.822±**0.0020** **P<0.001**	+11.70
	10	2.222±**0.0031** **P<0.001**	+2.352	1.212±**0.0023** **P<0.001**	-22.80	0.818±**0.0017** **P<0.001**	+4.583
	15	2.129±**0.0018** **NS**	+1.0362	1.180±**0.0016** **P<0.001**	+19.51	0.812±**0.0016** **NS**	+1.80
	20	1.199±**0.0012** **NS**	+1.0054	1.175±**0.0027** **P<0.001**	+18.40	0.802±**0.0027** **NS**	-1.272

Acid phosphatase activity is expressed as ug of Pi/mg. protein/hr.
Each value is a mean of five observations.
Values are significant at P<0.001, P<0.05 level or NS = Not Significant
-ve sign indicates % inhibition and
-ve sign indicates % stimulation.

IV. DISCUSSION

Digestion is a sequential process which renders food absorbable through the gut wall by breaking the food into simple molecular components through enzymatic action, such enzymes are secreted by specialized cells at digestive tract. Digestion although dependent on enzymatic action is determined by the functional organization and the structure of digestive system. Out of the many devices which can be applied to investigate the physiological alterations made by the pesticidal and heavy metal treatment, the most fundamental one would be the study of changes in the enzyme activities, since these organic cellular catalysts control the formation of biochemical intermediates which are indispensible to all the normal physiological processes.

Recently it was considered that digestion in molluscs was wholly intracellular, by way of phagocytosis or pinocytosis, proceeding specially in the digestive gland. The presence of digestive enzymes in the extract of digestive diverticula of mollusks was first shown by Fredrucq (1878) who found protease in Mya and Mytilus. A number of digestive enzymes were found to occur in the digestive gland particularly, amylase, maltase, lactase, esterase, lipase, protease (Mansourbek, 1954; Vonk, 1960; Vanwell, 1961; Arvy, 1969 and Vanwell, 1970).

There is no doubt that the effect of certain metals is profound on the enzyme activity in aquatic organisms though the mode of action of these heavy metals has not been clearly outlined with regard to their mechanism of action in certain key enzymes which are responsible for the general energetic of animals. There is a considerable amount of literature devoted to the study of organic pesticides concerning the enzyme system of various animals (Yap et al., 1975; Koundinya and Ramamurthi, 1978; Natrajan, 1981; Shastry and Malik, 1981 and Dalela et al., 1982). However, information regarding the effect of heavy metals on the enzyme action is restricted (Hewitt and Nicholas 1963 and Jackim, 1974).

The high elevation in acid phosphatase activity observed in all the concentrations and at all the exposures might be due to the necrotic changes occurring in different parts of the body. Rees and Sinha (1969) have suggested that damaged organs produce augmented quantity of enzyme. Damaged tissue of L. Marginalis might have secreted large quantities of acid phosphatase especially in mantle and foot where the activity of the enzyme was greatly enhanced. Similar results have been also reported by Alam (1984) after heavy metal treatment to Viviparous bengalensis.

Novikoff (1961) and De Duve (1968) have also suggested that increased lysosomal activity occurs as a part of the prenecrotic changes. Increase in lysosomal activity ultimately results in elevated acid phosphatase activity. These observations support the present findings. During all acute and chronic exposures the activity of acid phosphatase was enhanced indicating constant cell necrosis which was highest in $HgCl_2$ followed by $CuSO_4$ and $CdCl_2$. Similar findings were also reported by Alam (1984) in Viviparous bengalensis when exposed to salts of heavy metals. Gupta and Paul (1978) have observed an elevation in the activities of acid phosphatase in Bubalus Bubalus after dermal subacute malathion treatment.

Elevation of acid phosphatase activity in the present investigation may be due to the increased phosphorylation or disintegration of the cells which normally elevate the lysosomal activity. Sastry and Sharma (1978) also obtained similar results.

Simon (1953) reported that the concentrations higher than those needed to prevent oxidative phosphorylation damage the mitochondrial network to a degree where the action enzyme involved in oxidative metabolism is blocked. According to pressman (1963) uncouplers promote conductivity of protons within the mitochondrial membrane and subsequently present the formation of gradient across the membrane. It is generally supposed that alterations in mitochondrial mechanisms are reflected in their morphological changes and that normal metabolic profiles are dependent on the continuous supply of energy rich interference of uncouplers. These pathways are blocked finally affecting the activity of phosphatases. In the present investigation the mode of action of heavy metals might be the same as that of pesticides.

V. SUMMARY

The influence of different heavy metals, copper sulphate, mercuric chloride and cadmium chloride on the enzymatic activity of the bivalve, Lamellidens Marginalis was observed. The important lysosomal enzymes acid phosphatase was studied in relation to heavy metal stress. The level of acid phosphatase was elevated due to heavy metal stress. The elevation was more prominent in $HgCl_2$ stress than $CuSO_4$ and $CdCl_2$.

REFERENCES

[1.] Alam, S.M. (1984) : Some aspects of physiology of Viviparus bengalensis, Ph.D. Thesis, Marathwada University, Aurangabad, M.S.

[2.] Anees, M.A. (1976) : Intestinal pathology in fresh water teleost Channa punctatus (Bloch) exposed sublethal and chronic levels of three organophosphorus insecticides. Acta. Physiol. Latino – Amer. 26(1) : 63-67.

[3.] Anna V. Ivanina , Inna M. Sokolova (2015): Interactive effects of metal pollution and ocean acidification on physiology of marine organisms, Current Zoology 61 (4): 653–668.

[4.] Arvy, L. (1969) : Le's enzymes chez les crustacean. Ann. Biol. 8 : 505-580.

[5.] Berhet, J. (1965) : La digestion intracellulaire et les lysosomes. Archs. Biol. (Liege) 76 : 387-388.

[6.] Bhattacharya, S., Mukherjee, S. and Bhattacharya S. (1975) : Toxic effects of endrin on hepatopancreas of the fish Clarius bactrachus (Lin.) Ind. J. Exp. Biol., 13 : 185-186.

[7.] Cohn, Z.A. and Hirsch, J.G. (1960) : The isolation and properties of the specific cytoplasmic granules of rabbit J. Exp. Med. 112 : 483-1004.

[8.] Dalela, R.C., Rani S. and Verma, S.R. (1980) : Physiological stress induced by Sublethal concentrations of Phenol and Pentachlorophenol in Notopterus notopterus; Hepatic acid and alkaline phosphate axes and a succinic dehydrogenase. Environ. Pollut., 213-218.

[9.] Dalela, R.C., Rani S. and Verma, S.R. (1982) : Acid phosphatase activity in tissues of Notopterus notopterus chronically exposed to phenolic compound,. Proc. Indian Acad. Sci. 1 : 7-12.

[10.] De Duve, C. and Wattiaux, R. (1966) : Functions of Lysosomes. A Rev. Physiol., 28 : 435-492.

[11.] De Duve, C. (1968) : In lysosomes a new group of cytoplasmic particles. Subcellular particles edited by T. Hayashi, Renald Press, New Yo4k, 128.

[12.] Feredericq, L. (1978) : La digestion des matieres albuminoides chez quclques invertebrates, Arch. Zool. Exper. 7 : 391.

[13.] Jackim F. (1974) : Enzyme responses to metals in fish. In : Pollution and physiology of marine organisms. Eds. Vernberg F.J. and Vernberg, W.B., Academic Press, New York and London.

[14.] Koundinya, P.R. and Ramamurthi, R. (1978) : Effect of sumithion (fenitrothion) on some selected enzyme systems in the fish Tilapia mossambica (Peters), Ind. J. Exp. Biol., 16(7) : 808-811.

[15.] Mansour-bek, J.J. (1954) : The digestive enzymes in invertebrate and protochordate, Tab. Biol. 21 : 76-367.

[16.] Natarajan, G.M. (1981) : Effect of lethal (LC50/48 hrs) concentration of metasstox on selected oxidative enzyme tissue respiration and histology of gills of the freshwater air breathing fish, Channa striatus. Curr. Sci. 50 (220) : 985-989.

[17.] Novikoff, A.B. (1961) : The cells (Eds. J. Brachent and A.P. Mirsky) New York, Academic Press, 423.

[18.] Pressma, B.C. (1963) : In : Energy linked functions of mitochondria Ed. B. Chance New York, Academic Press, New York, 188-191.
[19.] Prosser, C.L. (1973) : Comparative Animal Physiology edited by C.L.Prosser, W.B.Sounders, Co., Philadelphia, London/ Toronto.
[20.] Rees, K.R. and Sinha, K.P. (1960) : Blood enzymes in liver injury J. Pathol. Bacterial. 80 : 297-307.
[21.] Shastry, K.V. and S.K. Sharma (1978) : Bull. Environ. Contam. Toxicol.. 20 : 456.
[22.] Shastry, K.V. and Malik, P.V. (1981) : Acute and chronic effects of diazinon on some enzymes in certain tissues of a fresh water teleost fish, Channa punctatus (Bloch), J.Environ.Biol., 2 (3) : 19-28.
[23.] Summer, J.B. and Sommers, C.F. (1947) : Chemistry and Methods of enzymes, Academic Press, New York.
[24.] Summer, A.T. (1969) : The distribution of some hydrolytic enzymes in the cells of the digestive gland of certain jour.zool. 158 (20) : 277-291.
[25.] Vonk, H.J. (1960) : Digestion and metabolism. In T.H.Waterman (Ed) " The Physiology of crustacean", Vol. 1, Academic Press, New York.
[26.] Yap, H.M., Desaiah, D., Cutkamp, L.K.and Koch, R.B. (1975) : In vitro inhibition of fish brain ATPase toxicity by cyclo ooliene insecticide and related compounds. Bull. Environ. Contam. Toxicol. 14 : 163-167.

International Journal of Engineering Science Invention (IJESI) is UGC approved Journal with Sl. No. 3822, Journal no. 43302.

* Sandhya M. Sonawane." Effect of Heavy Metals on Lysosomal Enzyme Acid Phosphatase Activity of Bivalve L. marginalis ." International Journal of Engineering Science Invention (IJESI) 6.8 (2017): 65-70.