Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI: 10.26479/2019.0503.33**

EFFECT OF COPPER ON THE PHOSPHATASES ENZYME ACTIVITY OF *LITOPENAEUS VANNAMEI*

S. Arul Prasath[1], V. Valarmathi[2], K. Muthukumaravel[3]

1. Department of Zoology, Thiru Vi Ka Government Arts College, Thiruvarur, Tamil Nadu, India.

2. Department of Zoology, A.D.M College for Women, Nagapattinam, India.

3. Department of Zoology, Khadir Mohideen College, Adirampattinam, Tamil Nadu, India.

ABSTRACT: The present investigation has been carried out to study on the physiological impact of copper on the *Litopenaeus vannamei*. The acute toxicity bioassays (96 h LC_{50}) of static renewal type were carried out. The LC_{50} value for copper with 50% confidence limits and slop functions for 24, 48, 72 and 96 hr were calculated by processing the data for probit analysis. The sub-lethal concentrations namely 1/10 and 1/30 of the 96 h LC_{50} values were chosen for the present investigation for studying their effects on enzymological aspects. The chronically exposed prawn to the copper showed an increase in acid phosphatase activity with increase in the exposure periods. The alkaline phosphatases activity of various organs was found to be decreased with increasing concentrations of copper and increase in exposure period.

KEYWORDS: Pacific White Leg Shrimp, Phosphatases activity, Toxicity and Copper.

Corresponding Author: Dr. K. Muthukumaravel* Ph.D.

Department of Zoology, Khadir Mohideen College, Adirampattinam, Tamil Nadu, India.

1.INTRODUCTION

Phosphatases are key lysosomal enzymes known to play a provital role in cytolysis and differentiation processes [27] and also in acute energy crisis [19]. They serve as markers for the evaluation of disease as under of pathological conditions in biological systems [13]. Phosphatases are concerned with carbohydrate metabolism [12], oxidative phosphorylation [5] as well as growth and differentiation [1]. [14] have shown that acid phosphatase plays an important role in the utilization of yolk during embryonic development. Acid phosphates play an important role in the autolytic degradation of tissues during metamorphosis [15]. Phosphatases help in the synthesis and

transport of metabolites across the membrane, secretory activity, and protein synthesis and glycogen metabolism. Many workers reported the alteration in the enzymatic activities at chronic exposure in different tissues due to the toxicity of pesticides [18]; [10] and [17]. The present study is aimed to find out the acid and alkaline phosphates in *Litopenaeus vannamei* exposed chronically to the sublethal concentrations of heavy metal, copper.

2. MATERIALS AND METHODS

The shrimp, *Litopenaeus vannamei* were collected from the Aqua Farm near Nagapattinam, Tamil Nadu. They were acclimatized for 15 days in the laboratory condition. The water as renewed every 24 h. The LC_{50} of copper for 96h was found out by using Probit method (Finney, 1971). For phosphatises enzyme studies *Litopenaeus vannamei* were reared in sublethal concentration (10% of 96 hours LC_{50}) for a period of 10, 20 and 30 days. Acid and alkaline phosphatases were estimated by following the procedure outlined by Tennis Wood *et al.* (1976), a modified method of Bessey *et al.* (1946).

3. RESULTS AND DISCUSSION

Acid phosphatase

The highest activity of ACP in normal shrimp was observed in the hepatopancreas (1.69-1.58 μmole p-nitrophenol formed/mg protein/hr), followed by liver, and kidney. The concentration was less in muscle and least in gill. The levels of ACP activity were found to be increased in crab exposed to sublethal concentrations of copper. The increase was directly dependent on the concentration of copper and duration of exposure (Table.1). In crab exposed to 30% sublethal concentration of copper for 30 days, the activity levels of alkaline phosphatase were found to have increased by 113.61, 154.46 and 129% respectively in the hepatopancreas, gill and muscle (Table.2). In shrimp exposed to sub lethal concentrations of copper, gill showed maximum increase in activity of ACP at 30 days of exposures followed by muscle, while the increase in activity was least in the hepatopancreas for the same exposure period. Significant changes in the acid phosphatases activity in hepatopancreas were also observed in the experiments at different time periods ($F=561.16$ and $P<0.01$). Treatment versus duration TXD showed an F value 141.89 and $P<0.01$ (Table.3). Sublethal concentrations of copper have produced significant changes in the acid phosphatases activity in gill ($F=299.21$ and $P<0.01$). Similarly the values in the exposure periods showed (TXD) significant changes ($F=90.44$ and $P<0.01$) (Table.3). Significant changes were also observed in the acid phosphatases activity of muscle during the time periods ($F=247.04$ and $P<0.01$). Treatment versus duration (TXD) gave an F value of 75.97 and $P<0.01$ (Table.3). The highest activity of alkaline phosphatases in normal shrimp was observed in the hepatopancreas (6.15-6.22 μmole p-nitrophenol formed/mg protein/hr), followed by muscle, and gill. The concentration was less muscle and least in gill. The levels of ALP activity were found to be increased in the shrimp exposed to sublethal concentrations of copper. The increase was directly dependent on the concentration of copper and duration of exposure. The levels

of alkaline phosphatases were found to decrease in various tissues of the test prawns compared to controls (Table.4). In prawns exposed to 30% sublethal concentration of copper for 30 days, the levels of ALP were found to be decreased by 64.88, 58.92 and 54.42 respectively for hepatopancreas, gill and muscle. Acid phosphatases are enzymes concerned with the biosynthesis of fibrous proteins [7] and mucopolysaccharides [9], or they may serve as regulators of intracellular phosphatase concentration [6]. They are also hydrolytic enzymes which play an active part in the dissolution of the body's dead cells; stimulation or inhibition of these enzymes will thus result in metabolic disturbances [21]. The results of the present investigation showed that the activity of acid phosphatase increased significantly in all the tissues of the shrimp, following exposure to sublethal concentrations of the copper. Similar elevated trend in acid phosphatase activity has been reported by [20] in the fish *Sarotherodon mossambicus* exposed to Sevin. They observed that the increase in acid phosphatase activity might lead to severe drop in tissue protein content and RNA content. The increase of acid phosphatases due to combined pesticide effect has also been observed by [25] in *Mystus vittatus*. The same trend has also been reported by [4] in the fish, *C. catla* exposed to carbamate pesticide, methomyl. Activation of acid phosphatase in the hepatopancreas and other tissues observed in the present study could be attributed to enzyme induction by pesticides through interaction with regulators and cofactors, as suggested by [20]. Moreover, the decreased protein content observed might due to catabolic activity of increased acid phosphatases in the tissues or by the impairment of protein synthesis through RNA depletion by acid phosphatase enzymes. The elevated trend in the acid phosphatase activity in the tissues of treated shrimp might also indicate the release of these enzymes into the cellular environment by disruption of lysosomal membrane, since biocides are reported to produce cytotoxic action and alteration in the membrane stability [28]. Like acid phosphatases, alkaline phosphatases are also enzymes concerned with the biosynthesis of fibrous proteins [7] and mucopolysaccharides [9], or they may serve regulators of intracellular phosphate concentration [6]. The results of the present study revealed a significant reduction of alkaline phosphatase activity in different tissues of the shrimp exposed to sublethal concentrations of copper at 10, 20, 30 days of exposure. The decrease in alkaline phosphatase activity in hepatopancreas, gill and muscle might be associated with the direct action of the copper on the enzyme system and impairment of lysosomal metabolism in liver and muscle [21]. [26] recorded a significant reduction of alkaline phosphatase activity in the liver and kidney of the fish, *H. fossilis* following exposure to distillery wastes. [20] have also reported severe inhibition of alkaline phosphatase activity by Sevin. They opine that the reduction of alkaline phosphatase may be due to interaction of pesticides with cofactors and regulators of the enzyme. The same trend has also been reported by [29] in various tissues of *C. carpio*, *O. mossambicus* and *C. striatus* exposed to tannery effluent. Digestive gland alkaline phosphatase is known to play a role in glycogen metabolism. Alkaline phosphatases are capable of inactivating phosphorylase enzyme necessary for glycogen

breakdown [11]. The observed decrease in alkaline phosphatase activity in the hepatopancreas probably facilitates the increased activity of phosphorylase enzyme and subsequent breakdown of glycogen for energy release during metal stress. Increased utilization of stored liver glycogen to meet the energy demand under pesticide stress has been reported earlier [16]; [22]; [8] and [23]. Hence, the reduction in alkaline phosphatase enzyme activity in hepatopancreas and muscle could be considered as an adaptive feature for the shrimp to facilitate the breakdown of glycogen (possibly by anaerobic glycolysis) resulting in tissue lactic acidosis as reported by [24]. This acidosis development might be responsible for the inhibition of alkaline phosphatase in hepatopancreas, gill and muscle of *Litopenaeus vannamei* which in turn could be of adaptive importance for the shrimp to meet the energy demand via, anaerobic breakdown of glycogen.

Table 1. Levels of acid phosphatase activities in selected tissues of *Litopenaeus vannamei* exposed to sublethal concentrations of copper

Exposure Period	10 Days			20 Days			30 Days		
Tissues	**HP**	**GL**	**MC**	**HP**	**GL**	**MC**	**HP**	**GL**	**MC**
Control	1.66±0.57	1.17±0.32	1.37±0.41	1.58±0.25	1.21±0.46	1.39±0.52	1.69±0.28	1.12±0.49	1.38±0.36
10% SLC	1.89±0.41	1.32±0.35	1.51±0.42	1.95±0.65	1.49±0.28	1.96±0.38	2.39±0.44	1.82±0.18	2.01±0.36
% Variation	+13.86	+12.82	+10.22	+23.42	+23.14	+41	+41.42	+62.5	+45.65
30% SLC	2.25±0.26	1.68±0.32	1.88±0.18	2.85±0.27	2.26±0.32	2.58±0.22	3.61±0.28	2.85±0.33	3.16±0.36
% Variation	+35.54	+43.59	+37.23	+80.38	+86.8	+85.61	+113.61	+154.46	+129.00

Values are mean ± SD of six observations. – or + indicate percent decrease or increase over control
HP- hepatopancreas; GL – gill; MC – muscle

Table 2. Levels of alkaline phosphatase activities in selected tissues of *Litopenaeus vannamei* exposed to sublethal concentrations of copper

Exposure Period	10 Days			20 Days			30 Days		
Tissues	**HP**	**GL**	**MC**	**HP**	**GL**	**MC**	**HP**	**GL**	**MC**
Control	6.18± 0.47	3.12± 0.38	4.48± 0.28	6.22± 0.34	3.16± 0.26	4.55± 0.44	6.15± 0.48	3.16± 0.55	4.41±0.31
10% SLC	5.72± 0.69	2.28± 0.38	4.05± 0.41	5.16± 0.37	2.61± 0.28	3.82± 0.46	4.13± 0.35	2.11± 0.18	3.41±0.28
% Variation	-7.44	-7.7	-9.6	-17.04	-17.41	-16.04	-32.85	-32.80	-102. 68
30% SLC	4.12± 0.36	2.11± 0.29	3.26± 0.41	3.32± 0.38	1.81± 0.45	2.98± 0.32	2.16± 0.46	1.29± 0.61	2.01±0.38
% Variation	-33.33	-32.37	-27.33	-46.62	-42.72	-34.51	-64.88	-58.92	-54.42

Values are mean ± SD of six observations. – or + indicate percent decrease or increase over control
HP- hepatopancreas; GL – gill; MC – muscle

Table 3. Two way ANOVA table showing the significance of the effect copper on the acid phosphateses of *Litopenaeus vannamei*

ANALYSIS OF VARIANCE FOR ACID PHOSPHATESES - HEPATOPANCREAS

SV	DF	SS	MS	F
REP (R)	2	0.01387407	0.00693704	2.78 ns
TREATMENT	8	11.20242963	1.40030370	561.16 **
T (T)	2	7.84938519	3.92469259	1572.79 **
FACTOR D	2	1.93680741	0.96840370	388.08 **
TxD	4	1.41623704	0.35405926	141.89 **
ERROR	16	0.03992593	0.00249537	
TOTAL	26	11.25622963		

cv = 2.3%

** = significant at 1% level; ns = not significant

ANALYSIS OF VARIANCE FOR ACID PHOSPHATESES - GILL

SV	DF	SS	MS	F
REP (R)	2	0.00320000	0.00160000	<1
TREATMENT	8	7.71966667	0.96495833	299.21 **
T (T)	2	5.18028889	2.59014444	803.15 **
FACTOR D	2	1.21706667	0.60853333	188.69 **
TxD	4	1.32231111	0.33057778	102.50 **
ERROR	16	0.05160000	0.00322500	
TOTAL	26	7.77446667		

cv = 3.4%

** = significant at 1% level

ANALYSIS OF VARIANCE FOR ACID PHOSPHATESES – MUSCLE

SV	DF	SS	MS	F
REP (R)	2	0.01006667	0.00503333	1.10 ns
TREATMENT	8	9.02506667	1.12813333	247.04 **
T (T)	2	6.04442222	3.02221111	661.80 **
FACTOR D	2	1.59295556	0.79647778	174.41 **
TxD	4	1.38768889	0.34692222	75.97 **
ERROR	16	0.07306667	0.00456667	
TOTAL	26	9.10820000		

cv = 3.6%

** = significant at 1% level; ns = not significant

Table.4. Two way ANOVA table showing the significance of the effect copper on the alkaline phosphateses of *Litopenaeus vannamei*

ANALYSIS OF VARIANCE FOR ALKALINE PHOSPHATESES - HEPATOPANCREAS

SV	DF	SS	MS	F
REP (R)	2	0.00738519	0.00369259	5.53 *
TREATMENT	8	50.29191852	6.28648981	9416.66 **
T (T)	2	40.42782963	20.21391481	30278.82 **
FACTOR D	2	6.61102963	3.30551481	4951.40 **
TxD	4	3.25305926	0.81326481	1218.21 **
ERROR	16	0.01068148	0.00066759	
TOTAL	26	50.30998519		

cv = 0.5%

** = significant at 1% level; * = significant at 5% level

ANALYSIS OF VARIANCE FOR ALKALINE PHOSPHATASES - GILL

SV	DF	SS	MS	F
REP (R)	2	0.00991852	0.00495926	3.32 ns
TREATMENT	8	10.68402963	1.33550370	894.75 **
T (T)	2	9.17336296	4.58668148	3072.96 **
FACTOR D	2	0.63814074	0.31907037	213.77 **
TxD	4	0.87252593	0.21813148	146.14 **
ERROR	16	0.02388148	0.00149259	
TOTAL	26	10.71782963		

cv = 1.6%

** = significant at 1% level; ns = not significant

ANALYSIS OF VARIANCE FOR ALKALINE PHOSPHATEASES - MUSCLE

SV	DF	SS	MS	F
REP (R)	2	0.00067407	0.00033704	<1
TREATMENT	8	16.85042963	2.10630370	1273.69 **
T (T)	2	13.88227407	6.94113704	4197.33 **
FACTOR D	2	2.00156296	1.00078148	605.18 **
TxD	4	0.96659259	0.24164815	146.13 **
ERROR	16	0.02645926	0.00165370	
TOTAL	26	16.87756296		

cv = 1.1%

** = significant at 1% level

4. CONCLUSION

Thus, on the basis of results of this investigation, it can be concluded that the heavy metal copper, even in low concentrations is capable of inducing alterations in the phosphatases enzymes in prawn which may cause severe metabolic dysfunction leading to the death.

ACKNOWLEDGEMENT

The first author (S. Arul Prasath) wish to acknowledge the Principal, A.D.M College for Women, Nagapattinam – 611 001 for making available all the necessary facilities.

CONFLICT OF INTEREST

No conflict of interest.

REFERENCES

1. Barker RJ and Alexander BH. Acid and alkaline phosphatases in house flies of different apes. Ann. Entomol. Soc. Am., 1958; 51: 251-257.
2. Bessey OS, Lowry OH and Breck MJ. A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. J. Biol. Chem., 1946; 164: 321-326.
3. Finney DJ. Probit Analysis. Cambridge University Press, London, 1971; 333.
4. Geetha N, Manavalaramanujam R, Ramesh M and Chezhian A. Influence of methonyl carbamate pesticide on biochemical components of a fresh water fish *Catla catla.* Ad. Bios., 1999; 18(11): 1-6.
5. Goodman J and Rothstein A. The active transport of phosphate into the yeast cell. J. Genet. Physiol., 1957; 40: 915-925.

6. Gutmann AB. Serum alkaline phosphatase activity in diseases of skeletal and hepatobiliary system. Am. J. Med., 1959; 27: 875.
7. Johnson IR and Mc Minn RMH. Association of alkaline phosphatase with fibrogenesis. J. Anat., 1958; 92: 544.
8. Kabeer Ahamed Saheed I, Sambasiva Rao KRS and Ramana Rao KV. Dehydrogenase system of teleost, *Tilapia mossambica* under augmented sublethal malathion stress. J. Anim. Morphol. Physiol., 1983; 301(2): 101-106.
9. Kroon DB. Phosphatases and the formation of protein-carbohydrate complex. Acta Anat., 1952; 15: 317.
10. Magare SR. Enzymatic changes caused by pesticides in a fish *Barbus ticto*. Proc. 84th Indian Sci. Cong., 1997; Part – III., 105.
11. Martin DW, Mayer PA and Rodwell VM. Harper's Review of Biochemistry. 20th Edition, Lange Medical Publication. Maruzen Co. Ltd., Tokyo, Japan. 1973; 638.
12. Miller D and Grane RK. The digestive function of the epithelium of the intestine. An intracellular locus of dissacharide and sugar phosphate ester hydrolysis. Biochem. Biophys. Acta., 1961; 52: 281-293.
13. Murti R, Omkar and Shukla GS. Mercuric chloride intoxication in fresh water prawn II. Effect on phosphatase activity. Ecotoxicol. Environ. Saf., 1984; 8: 581-586.
14. Muthukrishnan J and Senthamizhselvan M. Phosphomonoesterases of *Mesogomphus lineatus* embryo. Proc. 1st Indian Symp. Odonatol., 1985; 115-124.
15. Nath J and Butler L. Acid phosphatase during development of the black carpet beetle *Attagenus megatoma* (Feb). Can. J. Biochem., 1971; 49: 311-315.
16. Quyyam MA and Shaffi SA. Changes in tissue glycogen of a fresh water cat fish *Heteropneustes fossilis* (Block) due to mercury in toxication. Curr. Sci., 1997; 46: 652-653.
17. Radhakrishnan Nair C. Changes in the acid and alkaline phosphatase activity during sublethal exposure of *Cyprinus carpio* and *Oreochromis mossambicus* to curacron. Asian J. Microbiol. Biotech. Env. Sci., 2006; **8**(4): 817-821.
18. Ravindure K, Kumar GA and Rajana MA. Physical dysfunction in a few tissues of *Notopterus notopterus* after chronic exposure to malathion. Poll. Res., 1997; 16(13): 145-147.
19. Reddy MS and Rao KVR. In vitro sub-acute physiological stress induced by phosphomidon on carbohydrate metabolism in phasic and tonic muscles of penacid prawn *Penaeus indicus* (H. Milne Edwards) during acute and chronic exposure. Proc. Indian Acad. Sci. (Anim. Sci.), 1986; 95: 525-532.
20. Saikila BI, Thangavel P and Ramasamy M. Adaptive trends in tissue acid and alkaline phosphatases of *Sarotherodon mossambicus* (Peters) under sevin toxicity. Indian J. Environ. Hlth., 1993; 35(1): 36-39.

21. Sanisa PK, Bedi R and Sosi CI. Effects of vegetable oil factory effluent on the levels of phospatases and dehydrogenases in the liver and kidney of the fresh water teleost, *Channa punctatus* (Bloch). Environ. Pollut. Sci., 1982; 4(28): 245-253.
22. Shaffi SA. Variation in tissue glycogen content, serum lactate and glucose levels due to copper intoxication in three fresh water teleosts. Curr. Sci., 1978; 47: 955-956.
23. Vasanthi M and Ramasamy M. A shift in metabolic pathway of *Sarotherodon mossambica* (Peters) exposed to thiodan (Endosulfan). Proc. Indian Acad. Sci. (Animal Sci.)., 1987; 96(1): 56-61.
24. Venkateswaran, P and Ramasamy M. Lactic acidosis in different tissues of *Sarotherdon mossambicus* exposed to sevin. Curr. Sci., 1987; 56(7): 320-322.
25. Verma SR, Tyagi AK and Datta R. Toxicity of textile waste to some teleost fish. Water, Air, Soil, Poll., 1978; 10: 351.
26. Verma SR, Tyagi AK and Datta R. Effect of distillery waste on some fresh water teleost – Biochemical studies. Environ. Poll., 1979; 19: 225.
27. Verma SR, Rani S and Dalela RC. Effects of phenol and dinitrophenol on acid and alkaline phosphatase in tissues of a fish, *Notopterus notopterus*. Arch, Environ. Contam. Toxicol., 1980; 9: 451-459.
28. Vijayendra Babu, KVK and Vasudev T. Effect of Dimecron, Rogor and Cuman on AchE and phosphatases in Fresh water mussels *Lamellidens marginalis* (Lamarck). Curr. Sci., 1984; 53(17): 935-396.
29. Viswaranjan Somanath and Muthukrishnan J. Impact of Tannery effluent on phosphatases activity of fishes. Proc. Indian. Nat. Sci. 1989.