TOXICITY EFFECT OF HEAVY METAL ZINC ON ACID AND ALKALINE PHOSPHATES ENZYMES ACTIVITIES IN THE ESTUARINE MUD CRAB, *Scylla serrata*

[1] P. RAMYA, [2]D. MERIN EMERALD, [3]M. SOUNDARARAJAN,

[4]P. SENTHAMILSELVI, AND [5]S.R. MANIKANDAN

1. Department of Zoology, Tamilavel Umamaheshwaranar Karanthai Arts College, Karanthai, Thanjavur, Tamil Nadu, India - 613 002.

2, 3, 4 & 5. P.G. & Research Department of Zoology, Rajah Serfoji Government College, Thanjavur, Tamil Nadu, 613 005.

Corresponding author: ***Dr. P. Ramya****, Asst. Professor & Head, Department of Zoology Tamilavel Umamaheshwaranar Karanthai Arts College, Karanthai, Thanjavur, Tamilnadu, India 613 002.*

Abstract: Impact of heavy metal is common pollutants of estuarine ecosystems where they induce adverse effects on the aquatic biota. Estuarine mud crab, *Scylla serrata* is an important carb species in Tamil Nadu region having good nutritional values. Crabs living in close association with may accumulate heavy metals. In the present investigationLC_{50} values were determined for metals zinc toxicity in the mud crab, *Scylla serrata* when subjected to varying concentrations (0.5 to 0.80 mg/L). LC_{50} values of 96 hrs exposures was 0.43 mg/L. Increased ACP activity was noticed in the gills, abdominal muscles and hepatopancreas of estuarine mud crab, *S. serrata* treated with all three sublethal (5%, 10% and 15% of LC_{50} / 96 hr) concentrations of $ZnCl_2$ on 5, 10 and 15 days of exposures. ACP and ALP activities levels were increased during the study periods of 5, 10 and 15 days exposure.

Terms Index: *Estuarine mud crab, Scylla serrata, Zinc, ACP, ALP.*

I.INTRODUCTION

Heavy metals are persistent pollutants in aquatic ecosystems. The trace metal occurs in all compartments of aquatic environment and has a tendency to accumulate in organisms from different trophic levels of aquatic food chains and food webs. The accumulation of trace metals in aquatic organisms can pose a long term burden on biogeochemical cycling in the ecosphere. Bioaccumulation becomes an environmental problem when chemicals accumulated are toxic. Once trace metals enter food chain, they may accumulate to dangerous levels and be harmful to human health[1&2].

Enzyme Phosphatases are important and critical enzymes in the biological systems responsible for detoxification process, metabolism and biosynthesis of energetic macromolecules different functions[3]. Phosphatases being important lysosomal brush border enzymes which catalyzes the spilitting on phosphoric acid from certain phosphate esters and generally located on absorptive and secretory surfaces or cells, mediating membrane transport mechanism are thus early indicators of environmental degradation[4&5].

Hinton *et al.*[6] studied the effect of mercuric chloride intoxication in channel catfish and observed marked changes in ACP and ALP in liver. Lysosomes play an important role in the sequestration and detoxication of heavy metals and are well documented although it is not certain whether this occurs through organo–metallic complexes which are partially degraded or through direct uptake from cytoplasm followed by chelation with lipofuschins[7].

Acid and alkaline phosphatase activities in *Lamellidens marginalis* exposed to heavy metals, cadmium, copper and mercury were studied by Rajalakshmi[8]. Sridevi *et al.*[9] studied the antioxidant enzyme activity in a freshwater field crab, *Barytelphusa guerinii* exposed to chromium. Alkaline phosphatase activity in *Scylla serrata* due to toxicity of dithothreitol or 2–mercaptoethanol was investigated by Rong *et al.*[10]. Heavy metal toxicity studies in two euryhaline crabs, *Callinectes sapidus* and *Carcinus maenas* with reference to carbonic anhydrase activity were carried out by Hollie *et al.*[11].

II. MATERIALS AND METHODS

Estuarine mud crab, *Scylla serrata* were collected from Keelathottam near Agniar estuary mallippattinam. Following collection, the animals were carefully transported to the laboratory and maintained for a couple of days in natural estuarine water. Healthy, crabs having equal size (30–35 mm carapace length and 70–75 gm, weight) were used for experimentation. Stock solution of Zinc and lead were prepared by dissolving appropriate amount of salt in distilled water. Physico-chemical characteristic of test water have analyzed regularly during the test periods following the standard method describe by APHA[12]. Batches of 10 healthy crabs were exposed to different concentrations of heavy metals zinc and lead to calculate the medium lethal concentration LC_{50} value (1.5 mg/L) using probit analysis Finney method[13]. The fishes (Four groups) were exposed to the two sub lethal concentrations (5%, 10% and 15%) of heavy metal zinc for 5, 10 and 15 days respectively. Another group was maintained as control. At the end of each exposure period, fishes were sacrificed and tissues such as gill, hepatopancreas and muscle were dissected and removed. The tissues (10 mg) were homogenized in 80% methanol, centrifuged at 3500 rpm for 15 minutes and the clear supernatant was used for the analysis of ACP and ALP enzymes activitities. The acid and alkaline phosphatase activities are determined following the procedure of Tennis wood *et al*[14].

III. RESULT

Bioassay toxicity test

LC_{50} values were determined for metals zinc toxicity in the mud crab, *Scylla serrata* when subjected to varying concentrations (0.5 to 0.80 mg/L). LC_{50} values of 96 hrs exposures was 0.43 mg/L.

Effect of zinc on ACP activity in the gills

Increased ACP activity was noticed in the gills of crab, *S. serrata* treated with all three sublethal (5%, 10% and 15% of LC_{50} / 96 hr) concentrations of $ZnCl_2$ on 5, 10 and 15 days of exposures. ACP activity increased levels were expressed in units of mole phenol / mg of protein / hr. Increased ACP activity levels were 7.14, 7.82 and 8.10 due to stress of 5%, 10% and 15% sublethal doses of zinc against the control, 5.84 µ mole phenol / mg of protein after 5 days of treatment. Similar increases in ACP enzyme activity after 10 days treatment in the gills and the values were 8.62, 9.40 and 10.12 as against 6.98 µ mole / phenol / mg of protein / hr in control gill tissues. At 15 days exposure, increased ACP enzyme activity levels were 10.68, 11.12 and 12.43 while in controls it was 8.52 µ mole phenol / mg of protein / hr on 5%, 10% and 15% sublethal toxic concentrations of zinc (Table 1 & Figure 1).

Effect of zinc on ACP activity in the abdominal muscles

ACP enzyme activity levels were increased as 8.36, 9.40 and 10.64 compared to 8.14 µ mole phenol / mg of protein / hr after 5 days of exposure. Similar increases in ACP activity levels were recorded as follows. 9.14, 10.16 and 12.24 µ mole phenol / mg of protein / hr while in controls it was 8.84 µ mole phenol / mg of protein / hr. ACP enzyme activity was also increased after 15 days of exposure and increased values were being 10.24, 12.12 and 13.13 µ mole phenol / mg of protein / hr as against 9.50 in control abdominal muscle tissues of crabs, *S. serrata* exposed to 5%, 10% and 15% sublethal toxicity of zinc (Table 1 & Figure 2).

Effect of zinc on ACP activity in the hepatopancreas

All three sublethal concentrations of Zinc effected increases ACP enzyme levels in hepatopancreas tissues of crab, *S. serrata* exposed for 5 days, 10 days and 15 days. At 5 days of exposure, increased ACP enzyme levels were 9.80, 10.78 and 12.42 µ mole phenol / mg of protein / hr while in control tissue it was 8.92 µ mole phenol / mg of protein / hr. 12.70, 13.83 and 14.08 were the estimated ACP enzyme levels recorded after 10 days exposure against its control, 9.70 µ mole phenol / mg of protein / hr. Likewise increased ACP enzyme activity levels were 12.79, 14.12 and 15.69 µ mole phenol / mg of protein / hr after 15 days exposure and 10.40 µ mole phenol / mg of protein / hr being the ACP enzyme level in control (Table 1 & Figure 3).

Effect of zinc on ALP activity in the gills

Three sublethal doses of zinc effected increased activity of ALP enzyme in the gill tissues of the crab, *S. serrata*. The increased ALP enzyme levels recorded at 5 days exposure were 9.40, 10.16 and 12.10 µ mole phenol / mg of protein / hr as against 7.12 in untreated gills, at 10 days exposure of similar sublethal doses caused enhanced levels in ALP enzyme and values estimated were 10.14, 12.16 and 13.40 compared to 8.20 µ mole phenol / mg of protein / hr in control gill tissues of crab exposed to same sublethal doses of zinc. 15 days exposure resulted increases in ALP enzyme activity and such increased levels were 10.96, 13.13 and 13.80 compared to 9.36 µ mole phenol / mg of protein / hr (Table 2 & Figure 4).

Effect of zinc on ALP activity in the abdominal muscles

Abdominal muscle tissues of three sublethal treated group of the crab, *S. serrata* showed enhanced ALP enzyme activity and values recorded were 10.28, 12.42 and 13.65 compared to 8.24 µ mole phenol / mg of protein / hr at 5 days exposure; 10.42, 11.65 and 12.10 were the enhanced ALP enzyme levels compared to 9.16 µ mole phenol / mg of protein / hr at 10 days exposure and 11.80, 13.80 and 14.15 µ mole phenol / mg of protein / hr calculated values for ALP enzyme as against to 10.12 µ mole values for ALP enzyme as against to 10.12 µ mole phenol / mg of protein / hr at the expiry of 15 days (Table 2 & Figure 5).

Effect of zinc on ALP activity in hepatopancreas

Three sublethal doses of zinc effected significant increases in ALP enzyme levels in the hepatopancreas tissues of the crab, *S. serrata* exposed to 5, 10 and 15 days. Increased ALP enzyme levels were 12.45, 13.23 and 14.12 µ mole phenol / mg of protein / hr in hepatopancreas tissue at the expiry of 5 days and the control value recorded was 10.68 µ mole / mg of protein / hr. Similar increases were noticed during 10 days and 15 days exposures and values were 14.92, 16.85 and 17.35 and 19.68, 22.06 and 24.63 µmole phenol / mg of protein / hr while control values recorded were 14.92 and 17.25 µ mole phenol / mg of protein / hr in their respective controls (Table 2 & Figure 5).

Table 1. Acid phosphatase activity (µ mole phenol / mg of protein / hr) in selected body tissues of mud crab, *Scylla serrata* exposed to sub lethal concentration of zinc.

Exposure Period	Concentration of metal (mg/L)	Body tissues		
		Gill	Abdominal muscles	Hepatopancreas
5 days	**Control**	5.84 ± 0.05	8.14 ± 0.04	8.92 ± 0.04
	SLC 5 % (0.022)	7.14 ± 0.06	8.36 ± 0.06	9.80 ± 0.06
	SLC10 % (0.043)	7.82 ± 0.09	9.40 ± 0.05	10.78 ± 0.07
	SLC 15% (0.065)	8.10 ± 0.04	10.64 ± 0.04	12.42 ± 0.05

10 days	**Control**	6.98 ± 0.11	8.84 ± 0.05	9.70 ± 0.04
	SLC 5 % (0.022)	8.62 ± 0.05	9.14 ± 0.11	12.70 ± 0.05
	SLC10 % (0.043)	9.40 ± 0.07	10.16 ± 0.10	13.83 ± 0.06
	SLC 15% (0.065)	10.12 ± 0.11	12.24 ± 0.07	14.08 ± 0.05
15 days	**Control**	8.52 ± 0.10	9.50 ± 0.06	10.40 ± 0.11
	SLC 5 % (0.022)	10.68 ± 0.12	10.24 ± 0.11	12.79 ± 0.19
	SLC10 % (0.043)	11.12 ± 0.21	12.12 ± 0.06	14.12 ± 0.17
	SLC 15% (0.065)	12.43 ± 0.07	13.13 ± 0.04	15.69 ± 0.49

Values are mean ± S.D of 3 observations

Table 2. Alkaline phosphatase activity (µ mole phenol / mg of protein / hr) in selected body tissues of mud crab, *Scylla serrata* exposed to sub lethal concentration of zinc.

Exposure Period	**Concentration of metal (mg/L)**	**Body tissues**		
		Gill	**Abdominal muscles**	**Hepatopancreas**
5 days	**Control**	7.12 ± 0.14	8.24 ± 0.16	10.68 ± 0.41
	SLC 5 % (0.022)	9.40 ± 0.12	10.28 ± 0.17	12.45 ± 0.21
	SLC10 % (0.043)	10.16 ± 0.13	12.42 ± 0.14	13.23 ± 0.07
	SLC 15% (0.065)	12.10 ± 0.17	13.65 ± 0.07	14.12 ± 0.17
10 days	**Control**	8.20 ± 0.05	9.16 ± 0.34	13.01 ± 0.20
	SLC 5 % (0.022)	10.14 ± 0.10	10.42 ± 0.16	14.92 ± 0.73
	SLC10 % (0.043)	12.16 ± 0.12	11.65 ± 0.05	16.85 ± 0.16
	SLC 15% (0.065)	13.40 ± 0.16	12.10 ± 0.04	17.35 ± 0.13
15 days	**Control**	9.36 ± 0.06	10.12 ± 0.14	17.25 ± 0.13
	SLC 5 % (0.022)	10.96 ± 0.11	11.80 ± 0.11	19.68 ± 0.20
	SLC10 % (0.043)	13.13 ± 0.10	13.80 ± 0.16	22.06 ± 0.32
	SLC 15% (0.065)	13.80 ± 0.04	14.15 ± 0.13	24.63 ± 0.11

Values are mean ± S. D of 3 observations

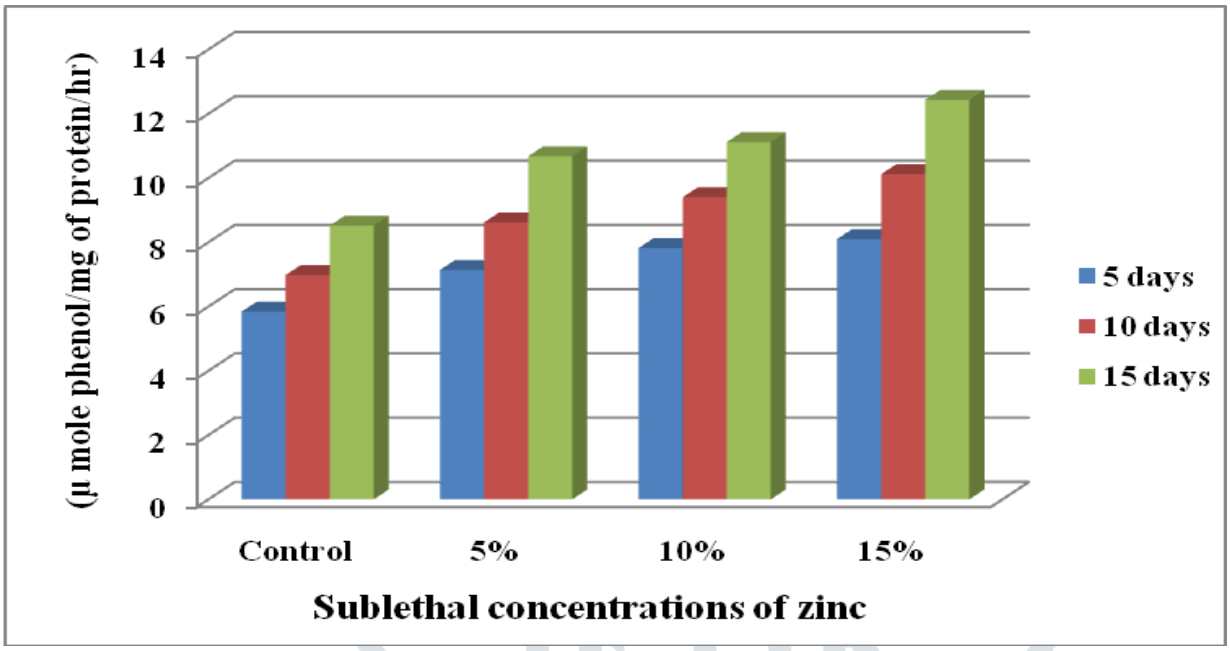

Fig. 1. Acid phosphatase activity (µ mole phenol / mg of protein / hr) of gill tissues in mud crab, *Scylla serrata* exposed to sublethal concentration of zinc.

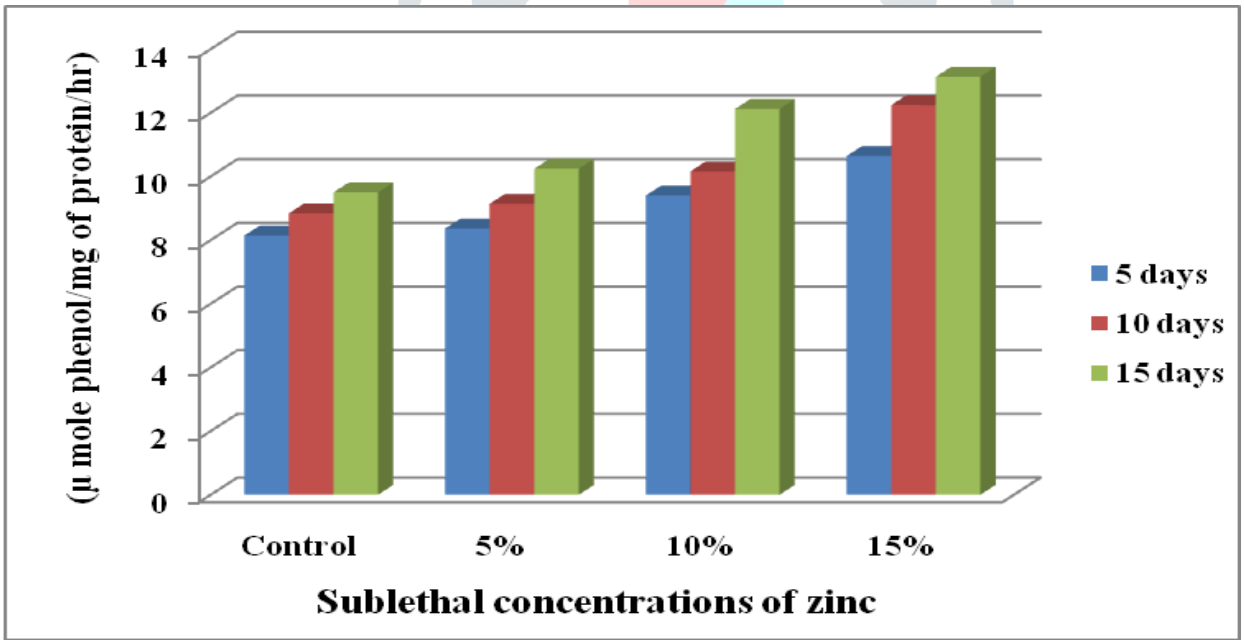

Fig. 2. Acid phosphatase activity (µ mole phenol / mg of protein / hr) of abdominal tissues in mud crab, *Scylla serrata* exposed to sublethal concentrations of zinc.

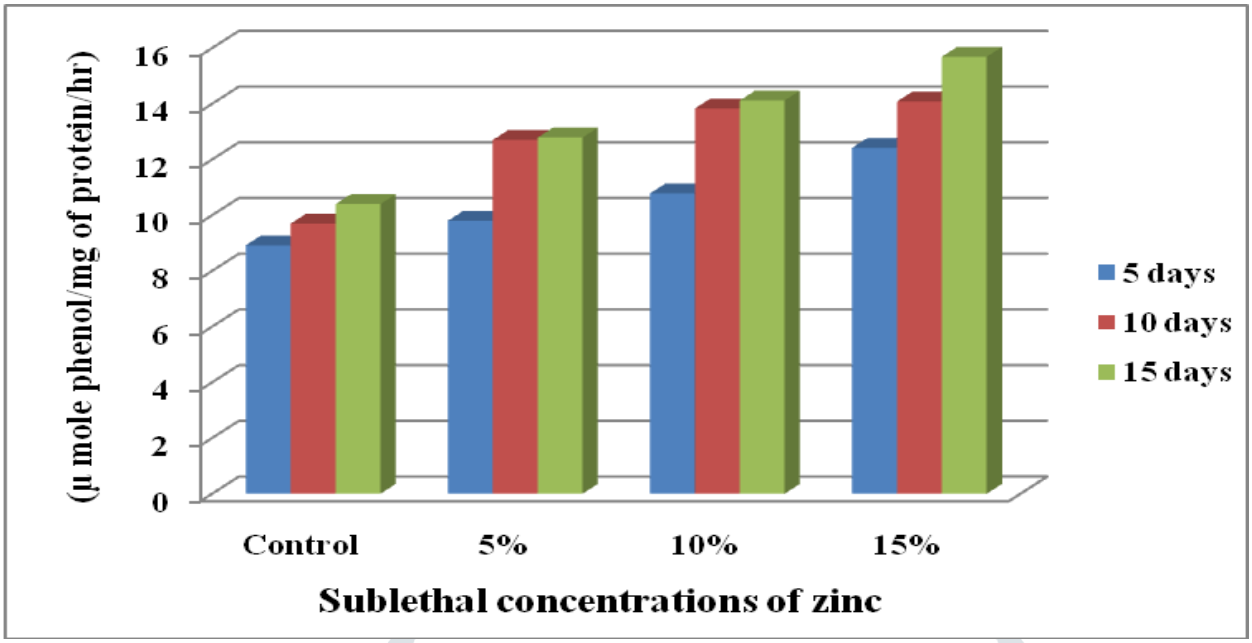

Fig. 3. Acid phosphatase activity (µ mole phenol / mg of protein / hr) of tissues hepatopancreas in mud crab, *Scylla serrata* exposed to sublethal concentrations of zinc.

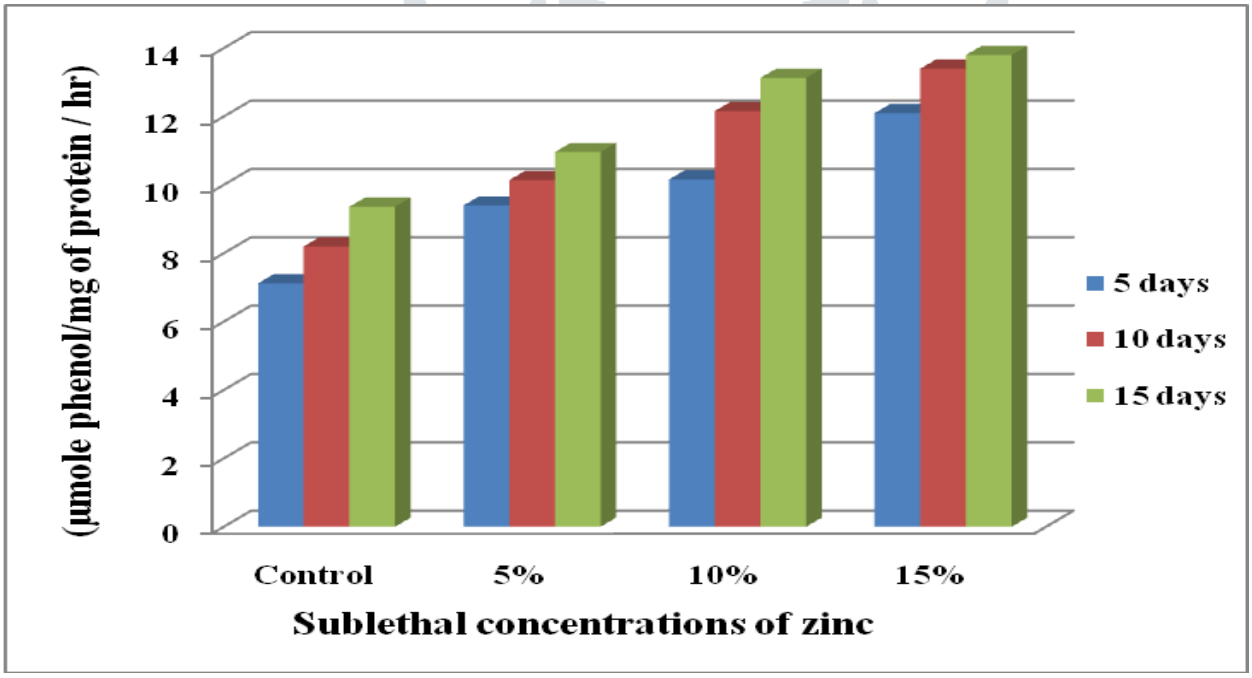

Fig. 4. Alkaline phosphatase activity (µ mole phenol / mg of protein / hr) of gill tissues in mud crab, *Scylla serrata* exposed to sub lethal concentration of zinc.

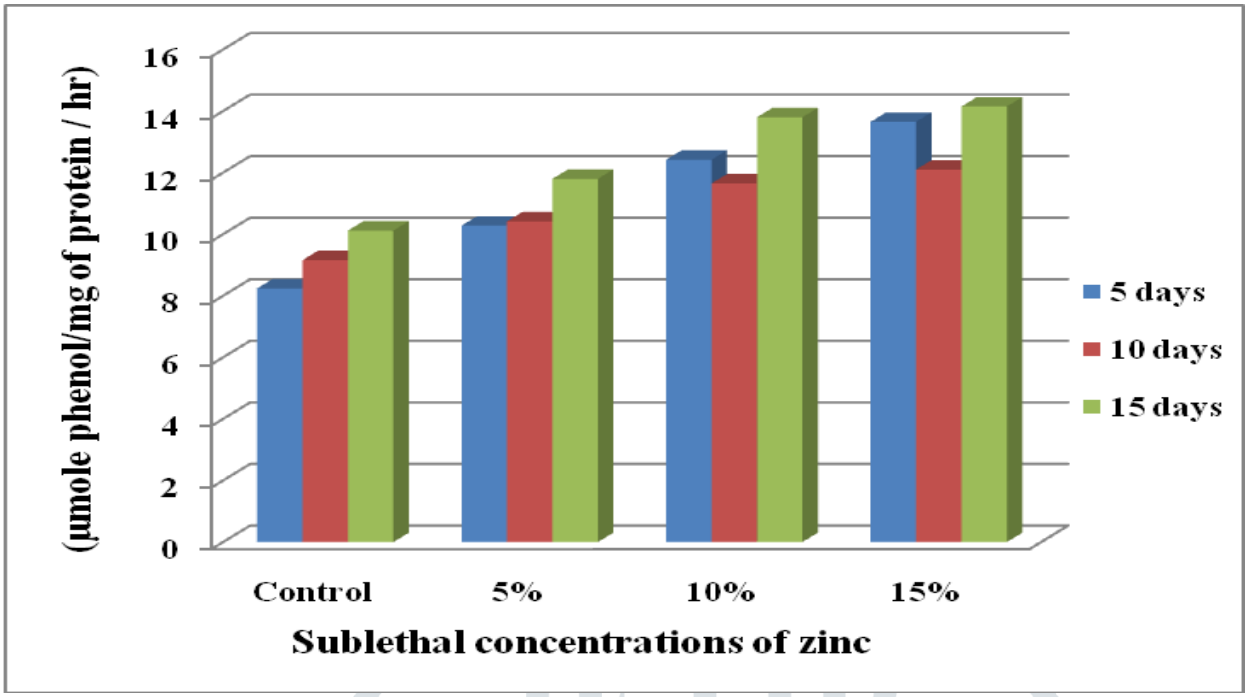

Fig. 5. Alkaline phosphatase activity (µ mole phenol / mg of protein / hr) of abdominal muscles of mud crab, *Scylla serrata* exposed to sublethal concentrations of zinc.

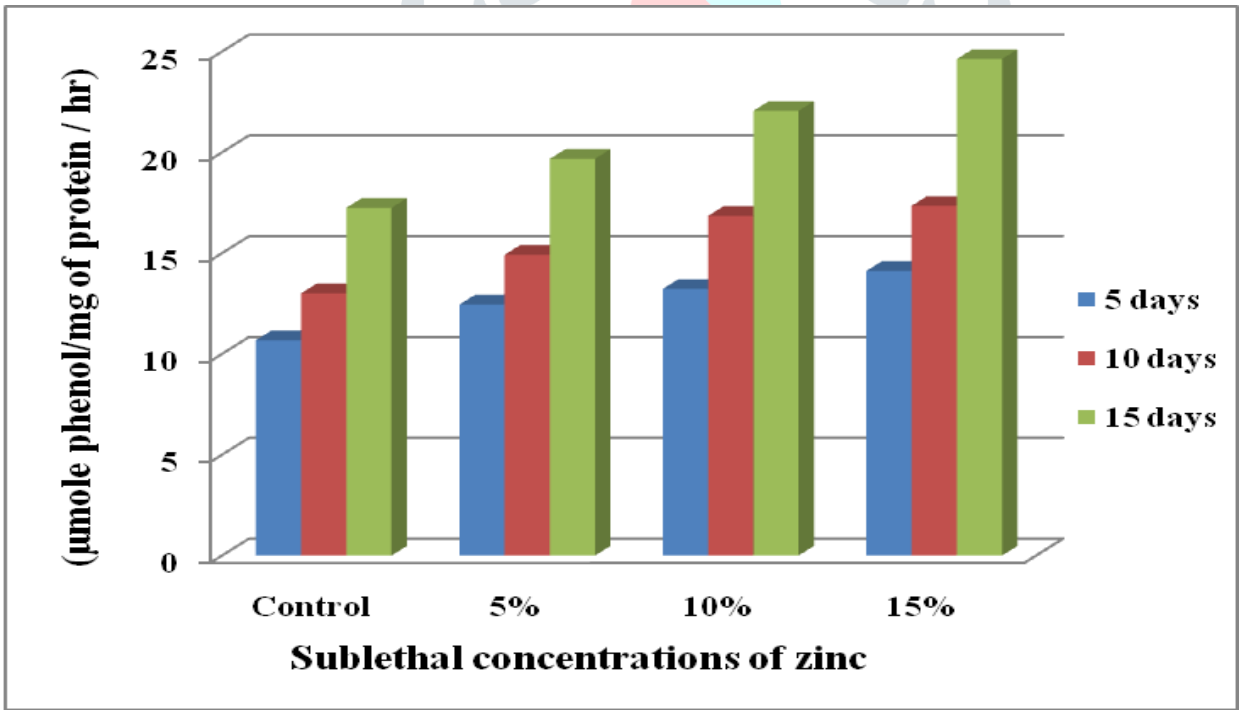

Fig.6. Alkaline phosphatase activity (µ mole phenol / mg of protein / hr) of tissues hepatopancreas in mud crab, *Scylla serrata* exposed to sublethal concentrations of zinc.

IV. DISCUSSION

In the present study LC_{50} values of heavy metal zinc of estuarine mud crab *Scylla serrata* at 96 hours LC_{50} were 0.43 mg/L. and Sub lethal concentrations namely 5%, 10% and 15% values were selected,

studying their effects on biochemical aspects. Many toxicants such as heavy metals enter the environment through the activities of man and are accumulated in different organisms. The existence and functioning of detoxification system have been considered to be of great significance recently, and changes in the levels of enzyme activities or in the total content of the enzymes are considered as specific indicators of stress[15]. It is also known that many xenobiotics cause cell injury by reacting primarily with biological membranes or membrane components[16]. Metals can combine with enzymes in many ways. Binding of metals at remote location on the enzyme molecule will influence the activity, which could range from activation to complete inhibition[17].

Hence, a study of metabolic and enzyme activities of aquatic organisms is essential to provide a tangible basis for anticipating and understanding the ecological effects of an accelerated input of heavy metals into the estuarine ecosystem King[3] reported that two phosphatases namely acid phosphatase (ACP) and alkaline phosphate (ALP) were mainly detoxifying enzymes in animals tissues to combat the toxicants such as pesticides, heavy metals etc., toxicant at each step in any metabolic process is dependent on a specific enzyme; inhibition or acceleration of one enzyme activity is bound to cause a series of metabolic disorders. The effects of toxicants on the key enzymes of metabolism have become a topic of common interest to toxicologists and biochemists. Phosphatases (ACP & ALP) were studied in the selected body tissues viz. gills, abdominal muscles and hepatopancreas of mud crab, *Scylla serrata*

In the present study, after chronic exposures (15 days) to three sublethal concentrations of metals zinc and lead, most significant increases in ACP activity were determined in the body tissues in the order of Hepatopancreas > muscles > gills. These observations agree with the findings of Reddy *et al.*[18] and they reported increases in ACP activity in the tissues of crab, *Oziotelphusa sense*. Elevated levels of ACP activity were noticed in sublethal treated *S. serrata* than in controls at all exposure periods. Similar results were obtained in the studies of Arunkumar and Hema Achyutham[19] in the liver and muscle tissues of a freshwater teleost, *Labeo rohita*; Abdul Naveed *et al.*[20] in the air breathing fish *Channa punctatus.*

As in ACP levels increased, alkaline phosphatase enzyme activity was also increased due to in all selected organs in the mud crab, *Scylla serrata*. The increase in the tissues alkaline phosphatase has also been reported by many workers Elumalai *et al.*[21] in crab, *Scylla serrata* and Jaroli and Sharma[22] in *Channa punctatus*; Kori Slakpore *et al.*[23] in catfish, *Clarias gariepinus* and Jagtap *et al.*[24] in female crab, *Spiralotelphusa hydrodomus*. Functional response of alkaline phosphatase in *Oreochromis mossambicus* has been selected to the results of the above studies.

The increases in ALP activity in selected tissues were due to the cellular damages caused by metal toxicity or a response to overcome intoxication of metals used in the present study as suggested by Barker and Alexander[25] and Navicoff[26].

V. CONCLUSION

Present study revealed that the heavy metal zinc is potent to cause toxic responses, even structural alterations, in aquatic organism like fish. The results indicate that the usage of the zinc in the agriculture fields may be a threat to aquatic fauna and flora as well as humans. Therefore, the information obtained may be useful for management and monitoring of agricultural heavy metal contamination in aquatic ecosystem. It is also recommended that before using heavy metal zinc in any aquaculture processes, the estimated safe and dischargeable concentrations should be considered important to protect living organisms as well as fish.

VI. ACKNOWLEDGEMENTS

Authors are grateful thanks to the Principal and other staff members of Zoology, Tamilavel Umamaheshvaranar Karanthai Arts College, Karanthai, Thanjavur, Tamilnadu, India for providing necessary facilities.

REFERENCES

[1] Leland, H. V., 1983. Ultrastructural changes in hepatocytes of trouts *Salmo* spp. exposed to copper and zinc. *Envt. Toxicol. Chem.* 2: 353-368.

[2] Azarbad, H., Khoi, A. J., Mirvaghefi, A., Danekar, A., and Shapoom, M., 2010. Biosorption and bioaccumulation of heavy metals by rock oyster, *Saccostrea cucullata* in the Persian Gulf. *Int. Aquat.* Res. 2: 61-69.

[3] King, J., 1965. The acid and alkaline phosphatases. In: D. Van (ed). Practical Clinical Enzymology. *Nostrand Co., London*. PP. 191-208.

[4] Mariya Dasu, P., Vivek, C. H., and Venkatarathnamma. V., 2013. Thiodicarb toxicity and its impact on biochemical changes and oxygen consumption of freshwater fish *Labeo rohita, Int. J. of Recent. Sci. Res*. 4(12): 2143-2148.

[5] Satish Chandra, 2013. Impact of pesticide rogor toxicity on serum phosphor monoesterase levels of freshwater fish, *Clarius batrachus, J. Appl. And Natural Sci.* 5(1): 20-23.

[6] Hinton, D.E., Baumann, P.C., Gardner, G.R., Hawkins, W.E., Hendricks, J.D., Murchelano, R.A., Okihiro, M.S., 1992. Histopathologic biomarkers. In: Biomarkers–biochemical, physiological and histological Markers of anthropogenic Stress. *Lewis Publishers Boca Raton, Ann. Arbor, London, Tokyo*, 155-212.

[7] George, S. G., 1983. Heavy metal detoxification in the mussel *mytilus* kidney an in vivo study of Cd and Zn binding to isolated tertiary lysosomes. *Comp. Biochem. Physiol*., 76(C): 59-65.

[8] Rajalakshmi Amma, P., 1992. Studies on the toxic effects of selected heavy metals in the freshwater mussel *Lamellidens marginalis, Ph.D., Thesis. Cochin University of Science and Technology, Cochin.* 1-150.

[9] Sri Devi, L., and Reddy, S. L. N., 1998. Effect of trivalent and hexavalent chromium on ACHE activity and oxygen consumption in freshwater field crab, *Barytelphusa guerini*. *J. Environ. & Pollut*. 5(2): 105-109.

[10] Rong – Qing Zhang, Qing – Xi Chen, Wen – Zhu Zheng, Jing – Yu Lin, Zhong – Lai Zhuang, Hai – Mng Zhou, 2000. Inhibition kinetics of green crab (*Scylla serrata*) alkaline phosphatase activity by dithiothreitol or 2 – mercaptoethanol. *Int. J. Bioche. & Cell Biol*., 32: 865-872.

[11] Hollie, S., Skaggs, Raymond Henry, P., 2002. Inhibition of carbonic anhydrase in the gills of two eurihaline crabs, *Callinectes sapidus* and *Carcinus maenas*, by heavy metals. *Comp. Biochem. Physiol*., Part C, 133: 605-612.

[12] APHA., 1998. Standard methods for the examination of water and waste water, 20th Edition, Washington, DC.

[13] Finney, D.J. 1971. Probit analysis, 3rd (Ed.), Cambridge University Press, London, 333.

[14] Tenniswood, M., Bird, C. E., and Clarkc, A. T., 1976. *Canadian J. Biochem*., 54: P. 350.

[15] Bayne, R. L., Moore, M. N., Widows, J., Livingstone, D. R., and Salked, P., 1979. Measurement of the responses of individuals to environmental stress and pollution. *Trans. R. Soc. Lond. Series* B., 286: 561-563.

[16] Moore, M. N., 1985. Cellular responses to pollutants. *Mar. Pollu. Bull*. 16: 134-150.

[17] Jackim, E., Hamlin, J. M., and Sonis, S., 1970. Effects of heavy metal poisoning on fives liver enzymes in Killi fish *Fundulus heteroclitius. J. Fish. Res. Bd. Can*. 27: 383-390.

[18] Reddy, P. S., Bhagyalakshmi, A., and Ramamurthy, R., 1986. Changes in acid phosphatase activity in tissues of crab, *Oziotelphusa senex senex*, following exposure to methyl parathion. *Bull. Environ. Contam. Toxicol.* 37: 106-112.

[19] Arunkumar, K., and Hema Achyuthan., 2007. Heavy metal accumulation in certain estuarine animals along the coast of Chennai, Tamilnadu, India. *J. of. Env. Biol*. 28(3): 637- 643.

[20] Abdul Naveed, Venkateshwarlu, P., and Janaiah, C., 2011. Biochemical alteration induced by triazophos in the blood plasma fish, *Channa punctatus* (Bloch) *Anim. Biol. Res.* 2(4): 31- 37.

[21] Elumalai, M., Balasubramanian, S. E., and Balasubramanian, M. P., 1998. Influence of naphthalene on protein, carbohydrates and phosphatases system during the vitellogenesis marine edible crab, *Scylla serrata*. *Bull. Environ. Contam. Toxicol.* 60: 25-29.

[22] Jaroli, D. P., and Sharma, B. L., 2005. Effect of organophospate insecticide on the organic constituents of *Channa punctatus. Asian J. Exp. Sci.* 19(1): 121-129.

[23] Kori – Slakpore, O., Ikomi, R. B., and Oghe, M. G., 2010. Variations in phosphatase and alkaline phosphatase in the plasma of African catfish, *Clarias gariepinus* exposed to sublethal concentrations of potassium permanganate. *Asian. J. Exp. Biol. Sci.* 1(1): 170-174.

[24] Jagtap, J. T., Sheyule, K. B., and Libarhande, S. B., 2011. Acute effect of TBTCL on protein alteration in freshwater bivalve, *Lamellidens marginalis, Int. Multidis. Res. J.* 1(8): 13-16.

[25] Barker, R. J., and Alexandar, B. H., 1958. Acid and alkaline phosphatases. *Ann. Entomol. Soc. Am.* 51: 251-257.

[26] Navicoff, A. B., 1972. Studies on acid and alkaline phosphatases activity of endosulfan exposed to fish, *Channa orientalis*. *Uttar Pradesh J. Zool.* 4(1): 154-159.