Study on the Effects of Exposure of Sub-Lethal Dose of Nickel on the Enzyme Activities in the Tissues of Female Crab *Scylla serrata* from the Coast of Mumbai

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Abstract

Enzyme activities such as acid phosphatase (ACP), alkaline phosphatase (ALP), glutamate-oxaloacetate transaminase, (GOT), glutamate-pyruvate transaminase (GPT), succinate dehydrogenase (SDH), lactate dehydrogenase (LDH) and adenosine tri-phosphatase (ATPase) and amount of nickel accumulated in tissues like gills, hepatopancreas, muscle and ovary of female crab, *Scylla serrata*, have been carried out. The female crabs were exposed to sub-lethal dose of nickel, LCo and LCso values were 200 ppm and 500 ppm respectively. The maximum quantity of nickel was found in gills, while hepatopancreas and muscle showed relatively less amount of nickel, very less amount of nickel was accumulated in ovary at LC_0 and LC_{50} levels. Acid phosphatase (ACP) and alkaline phosphatase (ALP) activities have been increased in gills, hepatopancreas and muscle at LC_0 and LC_{50} levels but in case of ovary the ACP and ALP activities increased at LC_0 but at LC_{50} level these activities showed slight rise. GOT and GPT activities were found to be elevated in all the tissues studied; GOT was found to be increased at LC_0 and LC_{50} levels in hepatopancreas, gills, muscle and in ovary this activity was found to be slightly higher. Relatively low SDH activity and high LDH activity were found in the tissues studied. ATPase activity at LC_0 level was found to be decreased in hepatopancreas, muscle and ovary but not in gills while at LC_{50} level ATPase activity declined in all the tissues.

Key words : *Scylla serrata*, acid phosphatase, alkaline phosphatase, glutamate-oxaloacetate transaminase, glutamate-pyruvate transaminase, succinate dehydrogenase, lactate dehydrogenase, adenosine tri-phosphatase.

Introduction :

Nickel is amongst those metals which are listed under priority pollutants; it is accepted as hazardous and noxious heavy metal having potential danger to human health and the biota in combine and metallic form. Darllinger and Kautzky (1985) reported the presence of nickel in the body of fish living in polluted water body having nickel in its sediment. Paez-Osuna and Ton-Mayen (1996) have observed that contamination of coastal water due to different pollutants affect adversely the marine biota. Harris and Santos (2000) have studied the contamination and physiological variability in Brazilian mangrove crab. Chou *et al* (2002) have suggested that crustaceans can be used as bio-indicators because heavy metals get accumulated in their body. Chavez-Crooker *et al* (2002) and (2003) have studied the fate of copper and zinc during intracellular localization. Sokolova and Sokolova (2005) investigated the effects of cadmium on oyster. Gautam and Sharma (2012) reported that cadmium and iron contents exhibited variations in the liver and kidney of *Clarius batrachus* when exposed to chronic dose (2.5 mg/l) 30 days. They reported that copper affected the iron contents in kidney. Mayekar *et al* (2012) have reported on the effects of sub-lethal exposure of nickel on biochemical parameters in the issues of female crab, *Scylla serrata* obtained from the coast of Mumbai; the observations on the biochemical parameters obtained have envisaged to conduct study of those enzymes which reflect on the health of the tissues which may be adversely affected by the above mentioned exposure of nickel.

Materials and Methods :

Healthy specimens of female crab, *Scylla serrata* with no external injuries and ectoparasites were procured form the Sassoon Docks, Mumbai and were transported to laboratory without causing stress, over crowding and discomfort to the experimental animals. The selected specimens were of uniform sizes and body weight. In laboratory, these were acclimated at room temperature in glass aquaria containing filtered and aerated sea water for 15 day. During this period sea water was changed twice every day and the animals were fed on prawn muscles. The experimental specimens were exposed to nickel as nickel sulphate and LC_0 of 200 ppm and LC_{50} of 500 ppm for 30 days. The control sets were also maintained along with the experimental sets. In the current study nickel was estimated in the tissues like gills, hepatopancreas, muscles and ovary and the enzyme

activity of acid phosphatase (ACP), alkaline phosphatase (ALP), glutamate-oxaloacetate transaminase, (GOT), glutamate-pyruvate transaminase (GPT), succinate dehydrogenase (SDH), lactate dehydrogenase (LDH) and adenosine tri-phosphatase (ATPase) were studied.

For the study of accumulation of nickel in the tissues of the experimental animal for 30 days the respective tissues were extracted and dried for 2 days in oven; 500 mg of each tissue was gently digested at room temperature with 4:1 nitric acid : perchloric acid. The residue was dissolved in 0.1 N HCl and volume was made up to 25 ml with double distilled water. The samples were stored in refrigerator and nickel was studied by atomic absorption spectrophotometric method. The data is expressed as ìg/ gm of tissue of crab. The acid phosphatase activity (ACP) and alkaline phosphate activity (ALP) were estimated by following the method of Andersch and Szcypinsk (1947). To study acid phosphatase activity 1.0 ml of citrate buffer and 0.2 ml of p-nitrophenol phosphate were mixed. To this reaction mixture 0.2 ml of respective homogenates were added and the final reaction mixtures were subjected incubation at 37p C for 30 minutes. This was followed by addition of 4.0 ml of 0.2 N NaOH to stop the reaction. The final reaction mixtures were shaken thoroughly and respective O D were read at 410 nm. The specific activity of acid phosphatase activity (ACP) was the measure as n-mole of p-nitrophenol released/30 minutes/mg protein. To estimate the alkaline phosphatase activity 1.0 ml of glycine-NaOH buffer and 0.2 ml of p-nitrophenol phosphate were mixed with 0.2 ml of respective tissue homogenates and there reaction mixtures were incubated at 37p C for 30 minutes. At the end of incubation 4.0 ml of 0.2 N NaOH was added to the reaction mixture, this step prevented the further reaction. The reaction mixtures thus obtained were thoroughly shaken. The respective O D of the final reaction mixtures was read at 410 nm. One phosphate unit is the amount of enzyme which releases an n-mole of p-nitrophenol under the experimental conditions. The specific activity is expressed as n-mole of p-nitrophenol released/30 minutes/mg of protein. Method of Lowery *et al* (1951) was followed to estimate the protein. To evaluate the alkaline phosphatase activity (ALP) method of Andersch and Szcypinsk (1947) was adopted. 1.1 ml of glycine buffer and 0.2 ml of p-nitrophenol reagent were added to 0.2 ml of tissue homogenates, the respective reaction mixtures were incubated at 37p C for 30 minutes. Thereafter, 4.0 ml of 0.2 N NaOH were added to stop the enzymatic reaction. The final reaction mixtures were shaken thoroughly; this was followed by taking OD at 410 nm. One phosphate unit is the amount of enzyme which liberates an n-mole of p-nitrophenol under assay conditions and specific activity is represented as n-mole of p-nitrophenol liberated /30 minutes/mg protein.

The method of Bergmeyer and Bernt (1965a) was followed with slight modifications Mohite (2002) to evaluate glutamic oxaloacetic transaminase activity (GOT). 1.0 ml of substrate buffer solution (0.1N phosphate buffer pH7.4), 1ml of 0.1 N 1-aspartate oxoglutarate and 0.2 ml of the homogenates of respective tissues were mixed and incubated for 1 hour at 30p C. After this step 1.0 ml of ketone reagent (which consisted of 10N 2-4 Di-nitrophenyl hydrazine) was added. These reaction mixtures were kept at room temperature for 20 minutes. 10.0 ml of 0.4 N NaOH were added. There after OD of the final respective reaction mixtures were taken at 540 nm. The specific activity of GOT is expressed as unit/g protein. The method of Bergmeyer and Bernt (1965b) with slight modifications was adopted to estimate glutamic pyruvate transaminase activity (GPT). The reaction mixtures were consisted of 1.0 ml of substrate buffer (7.4 pH, 0.2 M DL alanine and $2X10^3$M oxoglutarate) and 0.2 ml of homogenates. These reaction mixtures were incubated for 30 minutes at 37p C. Thereafter 1.0 ml of ketone reagent (10N 2-4 Di-nitrophenyl hydrazine) was added to these. The resultant mixtures were kept at room temperature for 20 minutes. 10 ml of 0.4N NaOH were added. The OD was read after five minutes at 540 nm. The specific activity of GPT is expressed as unit/g protein.

Adenosine tri-phosphatase enzyme activity was studied by following the method of Terri *et al* (1973) with slight modification. The reaction mixtures consisted of 0.5 ml of respective tissue homogenates, 0.2 ml of tris-HCl buffer, 20 ì moles of NaCl, 25 ì moles of KCl, 8ì moles of disodium salt of ATP. These mixtures were incubated at 37p C for 15 minutes. 2.0 ml of ice-cold 10% TCA were added to stop the reaction of the enzyme. The reaction mixtures were centrifuged. The organic phosphorous was estimated in 1.0 ml of supernatant obtained using Fisk and Subbarao (1925). Units of ATPase activity is expressed as ì moles of Pi liberated/mg protein/ hr.

Succinate dehydrogenase enzyme activity was assayed by using the method of Slatter and Bonner (1952). The respective reaction mixtures consisted of 0.3 ml of neutralized KCN (0.01M), 0.3 ml of 0.01 M K*f* $Fe(CN)_6$, 0.2 ml of sodium succinate, 2.0 ml of phosphate buffer (0.15 M, 7.5 pH), 0.5 ml of respective tissue homogenates. The reaction was carried out at room temperature. The reaction mixtures were thoroughly mixed and immediately the optical density of each was read at 430 nm at the interval of one minute for 6 minutes. The enzyme activity was expressed as decrease in OD after addition of the enzyme which is denoted by the reaction of potassium ferrricyanide. Specific activity of SDH was calculated as/ mg of protein. Lactate dehydrogenase enzyme activity (LDH) was estimated by following the

method of Bergmeyer and Brent (1965a). The reaction mixtures contained 2.85 ml of phosphate-pyruvate solution and 0.05 ml 0f NADH solution and 0.1 ml of respective homogenates, these were mixed rapidly. The optical density (OD) of the respective final mixtures was read on Spectronic 20 at 340 nm at one minute interval, it continued for five minutes. Specific activity of LDH was calculated as unit/mg protein.

Observations and Discussion:

Current study has shown that in case of control sets of experimental specimens nickel was found to be present in the tissues such as gills, hepatopancreas, muscle and 0.040 ± 0.020, 0.020 ± 0.010, 0.0133 ± 0.0057 respectively, however, nickel was not found in ovary. Sub-lethal exposure has elevated the amount of nickel in all the tissues studied even ovary showed increased amount of nickel at LC_0 and LC_{50} levels (Table 1). The activities of enzymes studied indicate the general health of normal cells, the elevated amount of nickel at LC_2 and LC_{50} levels reflects on the fluctuations in the enzyme activities and the stress caused. Mohite *et al* (2011) have also supported this view while working on the exposure of sub-lethal dose of cadmium and zinc on the tissues of green mussel, *Perna viridis* (L). Current study on acid phosphatase (ACP) and alkaline phosphatase (ALP) activities have been found to be increased in gills, hepatopancreas and muscle at LC_0 and LC_{50} levels but in case of ovary the ACP and ALP activities increased at LC_0 while at LC_{50} level these activities showed gentle increase (Tables 1, 2, 3). These observations reflect on the probable stress due to the accumulation of nickel. Mayekar *et al* (2002) have also supported the view that metals like cadmium have the ability to cause histo-pathological stress in the tissues of crab. The present investigation support the view expressed. Morton (1965), Mayekar and Raut (2003) opined that ACP and ALP enzymes activities are basically co-related with hydrolysis of phosphoester to release and maintenance of orthophosphate pool, transfer of phosphoryl group, hydrolysis and esterification of metabolites moving cross membrane within the cell and between cells and extracellular space.

Varley (1969), has suggested that GOT and GPT enzymes are key enzymes of nitrogen and energy metabolism. Further, they opined that aminotransferase enzymes function as link between protein and carbohydrate metabolism because these enzymes bring about inter-conversion of metabolites like á-ketoglutaric acid, pyruvic acid, oxalic acid along with alanine and aspartic acid. Thus, the activities of these enzymes have been investigated to evaluate physiological fluctuations in an organism in ecological and environmental stress. Mokim *et al* (1970), Sastry and Subhadra (1982) have reported that GOT and GPT activities increase due to lesions formed in liver. Harris and Santos (2000) have observed physiological variations in the tissues of Brazilian mangrove crabs, *Ucides cordiatus* and *Callinectus danoe* due to contamination of water due to heavy metals. Chon (2002) and Agwuocha *et al* (2011) have suggested that the activities of GPT and GOT increases because of damage caused to the tissues either due to pollution of heavy metals like cadmium, nickel and petroleum hydrocarbons like benzene and xylene. The present investigation has shown elevated GOT and GPT activities in all the tissues studied; GOT was found to be increased at LC_0 and LC_{50} levels in hepatopancreas (0.0455±0.0002. 0.0484±0.0003), gills (0.2223±0.0150, 0.0306±0.0015), in muscle (0.0125±0.005, 0.0242±0.0004) in ovary this activity was found to be least at LC_0 (0.0108±0.0004) and LC_{50} slightly higher and reaching close to control values (0.0131±0.0004). The amount of nickel was found to be the minimum in ovary at LC_{50} level. The GPT activity was also found to be more in gills and decreased in hepatopancreas, ovary and muscle (Table 1, 4, 5). The current observations are in agreement with those of Mohite *et al* (2011), Agwuocha *et al* (2011). Mayekar *et al* (2012) have reported decline in protein contents as the accumulated amount of nickel increased in the tissues at LC_0 and LC_{50} levels.

Varley (1969) has suggested that lactate dehydrogenase (LDH) enzyme is widely distributed in all types of cells, its activity is related to aspartate transaminase and higher LDH activity has been found in muscular dystrophy, liver hepatitis. Devi *et al* (1993) have reported that LDH activity in the hepatopancreas and abdominal muscles of fiddler crab, *Uca pugilator* as a result of exposure of $CdCl_2$; Reddy and Bhagyalaxmi (1994) have observed LDH activity in crabs after exposing them to $CdCl_2$; their finding suggested that decrease in LDH activity is likely to be related to mobilization of the pyruvate into citric acid cycle. Fox and Rao (1977), Chance and William (1956) have reported inhibition of succinate dehydrogenase (SDH) activity in blue crab, *Callinactes sapidus* due to exposure to nickel and sodium pentachlorophenate (PCP) and 2-4-dinitrophenol. Bell (1968) suggested that damaged tissue allows leakage of such enzymes into the ambient medium. Dalela *et al* (1980) and Verma *et al* (1980) have also observed inhibition of SDH activity in the liver of *Notopterus notopterus*. The present observations have revealed that relatively low SDH activity and high LDH activity in the tissues studied, further, nickel accumulation was found to be quite high in gills, hepatopancreas and muscle in decreasing order but SDH activity was found to be more in hepatopancreas followed by that in gills and muscles. The LDH activity was observed to be the maximum in gills then in declined in hepatopancreas, muscle and ovary (Table 1, 6, 7). SDH and LDH activities appeared to be the indicative of stress in the tissues studied. Mayekar *et al* (2012) have reported higher values of lactic acid and pyruvic acid in the tissues of hymolymph of female crab *Scylla*

serrata as a result of sub-lethal exposure of cadmium and nickel. The higher values of nickel found appeared to be the probable cause of cellular damage and physiological stress.

Adenosine tri-phosphatase (ATPase) enzyme is related with energy metabolism, any impairment in this activity directly affects availability of cellular energy. Schmidt and Nielson (1974) have observed that inhibition of Naz /Kz ATPase activity in gills and liver of rain bow trout as a response to exposure of cadmium and mercury. Verma *et al* (1983) reported that ATPase activity is closely associated with cellular membrane and when ATPase activity is inhibited its action is blocked across the cell membrane. With current study it has been observed that sub-lethal exposure of nickel has decreased the ATPase activity at LC_0 level in hepatopancreas, muscle and ovary but not in gills while at LC_{50} level ATPase activity declined in all the tissues in response to the accumulation of nickel there by causing physiological stress in the tissues of female crab (Table 1and 8). These observations are in agreement with those of Schmidt and Nielson (1974), Verma *et al* (1983). Mayekar *et al* (2012) have reported that since biochemical parameters are adversely affected due to sub-lethal exposure of nickel and female crabs tend to survive in stressed conditions both physiological and environmental, the current observation on the activities of various enzymes are in congruency and support that nickel plays its role in causing the said stress.

References:

Andersch M A and Szcypinsk (1947) 'Estimation of phosphatase activity' *Am J Clin Pathol,* **9**:37-42

Agwuocha S, Kulkarni B G, Mohite and Lahir YK (2011) 'Study on the effects of xylene exposure on the enzymatic activities in *Gafrarium divaricatum* (Gemelin),'*Biochem Cell Arch,* **11(1)**: 153-159

Bell G R (1968) 'Distribution of transaminase (aminotransferase) in the tissue of Pacific Salmon, Onchorhynchus,' *J Fish Res Bd Can,* **25**:1247-1268

Bergmeyer H U and Bernt E., (1965a), Glutamate-oxaloacetate transaminases, In: Methods of enzymatic analysis, (Ed Bergmeyer H U) Academic Press, New York, 837-847

Bergmeyer H U and Bernt E., (1965b) , Lactate dehydrogenase, In: Methods of Enzymatic analysis, (Ed Bergmeyer H U) Academic Press, New York, 736-743

Chance B and Williams C R., (1956) , The respiratory chain of oxiphosphorylation, Adv Enzymol, Vol. 17,65-134.

Chavez-Crooker P., Garrido N and Ahearn G A., (2002), Copper transport by lobster, *Homarus americanus* hepatopancreatic mitochondria, *J Exp Biol,* Vol. 205, 405-413.

Chavez-Crooker P., Pozo P., Castro H., Dice M S., Boutet I., Tanguy A., Moragac D., Ahearn G A., (2003) ,Cellular localization of calcium, heavy metals and metallothionein in lobster *Homarus americanus* hepatopancreas, Comp Biochem Physiol Part C, Vol. 136, 213-224

Chou C L., Paon L A and Moffatt J D., (2002), Cadmium, copper, manganese, silver and zinc in Rock crab, Cancer irroratus from highly copper contaminated sites in the inner bayof Fundi, Atlantica, Canada, Bull Environ Contam Toxicol, Vol.68, 885-892.

Dalela R C., Soraj Rani and Verma S R., (1980), Physiological stress induced by sub-lethal Concentration of phenol and pentachlorophenol in *Notopterus notopterus*: hepatic acid and alkaline phosphatase and succinic dehydrogenase, Env Poll (series-A), Vol.21, 3-8

Darllinger R and Kautzky H., (1985), The importance of contaminated food for the uptake of heavy metals by rainbow trout *Salmo gairdneri*; A field study', *Oecologia*, **62**: 82-89

Devi M, Reddy P S, and Fingerman M (1993) 'Effect of cadmium exposure on lactate-Dehydrogenase activity in hepatopancreas and abdominal muscle in fiddler crab, *Uca pugilator,' Comp Biochem Physiol,* **106-C**: 739-742

Fiske C H and Subbarao Y (1925) 'The colorimetric determination of phosphorus' *J Biol Chem*, **226**: 497-509

Fox F R and Rao K R (1977) 'Effects of sodium pentachlorophenate and 2-4-dinitrophenol and hepatopancreatic enzymes in blue crab, *Callinectes sepides*,' *Proc Symp, pentachlorophenol abstract.* Univ West Florida, 17-18

Gautam R K and Sharma S (2012) 'Copper nitrate alter inorganic compounds in liver and kidney of fresh water fish, *Clarius batrachus*', *J Exp Zool India,* **15:**209-211

Harris R R and Santos M C F (2000) 'Heavy metals contamination and physiological variability in the Brazilian mangrove crab *Ucides cordiatus* and *Callinectus danoe*', Marine Biol., 137:691-703.

Lowry O H, Rosenbrough N J, Farr A L and Randall R J (1951) 'Protein measurement with Folin-phenol reagent,' *J Biol Chem,* **193:** 265-275

Paez-Osuna F and Ton-Mayen I (1996) 'Concentration and distribution of heavy metals in tissues of wild and framed

shrimp, *Penaeus vannamei*, from the northwest coast of Mexico,' *Environ Int*, **22:** 443-450

Mayekar V G, Wasnik N L, Pawar, S S, Raut P D (2002) 'Ultrastructutral studies in histopathological changes in hepatopancreas of crab, Scylla serrata, exposed to heavy metal cadmium, *National symposium on Physiology and Biochemistry of cultivable crustaceans,'Dept of Zoology, Univ of Madras,* Chennai

Mayekar V G and Raut P D (2003) 'Some aspects of endocrine physiology of the crab, *Scylla serrata* (Forskal) exposed to cadmium' M Sc Thesis, Univ of Mumbai

Mayekar V, Pathare M, Afonso G, Mohite V T, Lahir Y K and Raut P D (2012) ' Effects of sub- lethal dose of nickel on the biochemical parameters in the tissues of female crab *Scylla serrata,* from Mumbai coast', *J Ecophysiol Occup Helth,* **12:** 69-76

Mohite V T (2002) 'Base level of heavy metals and detoxification mechanism in mussel from the Coast of Maharashtra state', Ph D Thesis, Univ of Mumbai

Mohite V T, Lahir Y K, Pathare M, Agwuocha S, and Mane U H (2011) 'Study on the effects on the exposure of sub-lethal dose of Cd and Zn on enzymatic activity in the tissues of green mussel, *Perna viridis* (L) from Ratnagiri coast, MS', *J Ecophysiol Occup Helth,* **11:**131-140

Mokim J M, Christensen G M and Hunt E P (1970) 'Changes in the blood of brook trout *Salvenalis fontinalis*, after short and long term exposure to copper', *J Fish Res Bd Can,* **27**:1883-1889

Morton R K (1965) 'Phosphatase; In Comparative Biochemistry, Vol-16, (Ed) Florkin M and Stots E H, Elsevier, New York

Reddy S P, and Bhagyalaxmi A (1994) 'Changes in oxidative metabolism in selected tissues of crab, *Scylla serrata* in response to cadmium toxicity, 'Ecotox *Environ Saf,* **29:**255-264

Terri R, Lagerspetz K YH and Kohen J (1973) 'Temperature dependence of ATPase activity in Brain homogenates during postnatal development of rat',*Comp Biochem Physiol,* **44:**473-480

Sastry K V and Subhadra (1982) Effects of cadmium on some aspects of carbohydrate metabolism in a fresh water catfish, *Heteropneustes fossilis*,' *Toxicol Lett,* **14**:45-55

Schmidt Neilson (1974) 'Osmoregulation: effect of mercuric chloride on salinity and heavy metals', *Fed Proc,* **33:**21-37

Slatter E C and Bonner W D (1952) 'Effect of fluoride on the succinate oxidase system,' *Bio Chem,* 52: 185-196

Sokolova I M and Sokolova E P (2005) 'Evolution of mitochondrial uncoupling proteins: novel invertebrate UCP homologues suggest early evolutionary divergence of the UPC family,' *FEBS lett* **579:**313-317

Varley H (1969) 'Practical clinical biochemistry, '4th Ed, The ELBS and William Heinemann Book Ltd, London

Verma S R, Jain M and Tonk I P (1983) "in vivo effect of mercuric chloride on tissue ATPase of *Notopterus notopterus*', *Toxicol Lett,* **16:**305-309

Sr. No	Tissues	Control	LC_0	LC_{50}
1.	Gills	0.040 ±0.020	0.760* ±0.020	1.520*±0.10
2.	Hepatopancreas	0.0.20±0.010	0.410* ± 0.020	0.651*±0.040
3.	Muscle	0.0133±0.0057	0.080 ± 0.040	0.030±0.020
4.	Ovary	0.000 ±0.000	0.030± 0.002	0.01330±0.0057

Table 1 Study of nickel accumulation in the tissues of female *Scylla* serrate based on atomic absorption spectrophotometric method at LC_0 and LC_{50} levels

Activity expressed as μg gm^{-1} dry weight of tissue. Values are mean ± SD of 5 estimates. * Significantly different from control; *P>0.05 by ANOVA; ** P<0.05 by ANOVA

Sr. No.	Tissues	Control	LC_0	LC_{50}
1.	Gills	3.1440 ± 0.0752	4.0256* ± 0.0.263	5.9280* ± 0.0296
2.	Hepato-pancreas	6.5304 ± 0.1100	8.8330* ± 0.0811	9.5520* ± 0.0986
3.	Muscle	0.5600 ± 0.0235	0.7720* ± 0.0413	2.3230* ± 0.0314
4.	Ovary	5.8180 ± 0.1040	7.8360* ± 0.1017	7.840* ± 0.0370

Table 2 Effects of sub-lethal exposure of nickel for 30 days on acid phosphatase activity in the tissues of female crab, *Scylla serrata* at LC_0 and LC_{50} levels

Values are mean ± SD of 5 estimates. * Significantly different from control; *P>0.05 by ANOVA; ** P<0.05 by ANOVA

Sr. No.	Tissues	Control	LC_0	LC_{50}
1	Gills	1.2950 ± 0.0080	3.1080* ± 0.0175	4.8270* ± 0.0290
2	Hepato-pancreas	5.2620 ± 0.0488	7.6110* ± 0.0890	6.2860* ± 0.0350
3	Muscle	1.3000 ± 0.0172	2.9290* ± 0.0285	3.8190* ± 0.0335
4	Ovary	0.6750 ± 0.1660	4.1680* ± 0.08387	2.3050* ± o.1280

Table 3 Effects of sub-lethal exposure of nickel for 30 days on alkaline phosphatase activity in the tissues of female crab *Scylla serrata* at LC_0 and LC_{50} levels

Values are mean ± SD of 5 estimates. * Significantly different from control; *P>0.05 by ANOVA; ** P<0.05 by ANOVA

Sr. No.	Tissues	Control	LC_0	LC_{50}
1.	Gills	0.0173 ± 0.0020	0.0223* ± 0.0150	0.0306* ± 0.0015
2.	Hepato-pancreas	0.0253 ± 0.0002	0.0455* ± 0.0002	0.0484* ± 0.0003
3.	Muscle	0.0108 ± 0.0006	0.0125* ± 0.0005	0.0242* ± 0.0004
4.	Ovary	0.0132 ± 0.0016	0.0180* ± 0.0004	0.0131* ± 0.0004

Table 4 Effects of sub-lethal exposure of nickel foe 30 days on glutamate oxaloacetate transaminase activity in the tissues of female crab, *Scylla serrata* at LC_0 and LC_{50} levels

Values are mean ± SD of 5 estimates. * Significantly different from control; *P>0.05 by ANOVA; ** P<0.05 by ANOVA

Sr. No.	Tissues	Control	LC_0	LC_{50}
1.	Gills	0.0150 ± 0.0004	0.0155 ± 0.0004	0.0217* ± 0.0005
2.	Hepato-pancreas	0.0051 ± 0.0006	0.0059 ± 0.0003	0.0065 ± 0.0028
3.	Muscle	0.00173 ± 0.0005	0.00296* ± 0.0003	0.0233* ± 0.0020
4.	Ovary	0.00146 ± 0.0015	0.00366 ± 0.0026	0.0023* ± 0.00025

Table5 Effects of sub-lethal exposure of nickel for 30 days on glutamate pyruvate transaminase activity in the tissues of female crab, *Scylla serrata* at LC_0 and LC_{50} levels

Values are mean ± SD of 5 estimates. * Significantly different from control; *P>0.05 by ANOVA; ** P<0.05 by ANOVA

Sr. No.	Tissues	Control	LC_0	LC_{50}
1.	Gills	0.0583 ± 0.0065	0.0482 ± 0.0036	0.0480 ± 0.0055
2.	Hepato-pancreas	0.0732 ± 0.0028	0.0691 ± 0.0050	0.0706 ± 0.0040
3.	Muscle	0.0.423 ± 0.0027	0.0355 ± 0.0043	0.0368 ± 0.0051
4.	Ovary	0.0331 ± 0.0025	0.0266 ± 0.0047	0.0294 ± 0. 0037

Table 6 Effects of sub-lethal exposure of nickel for 30 days on succinate dehydrogenase activity in the tissues of female crab, *Scylla serrata* at LC_0 and LC_{50} levels

Values are mean ± SD of 5 estimates. * Significantly different from control; * P>0.05 by ANOVA; ** P<0.05 by ANOVA

Sr. No.	Tissues	Control	LC_0	LC_{50}
1.	Gills	1.937 ±1.140	2.1880 ±0.0135	1.596 ±0.007
2.	Hepato-pancreas	0.1910 ±0.0089	0.1870 ± 0.0050	0.1820 ±0.0010
3.	Muscle	0.0547 ±0.0011	0.0540 ± 0.0009	0.0538 ± 0.0011
4.	Ovary	0.0817 ± 0.0008	0.0810 ± 0.00026	0.0808 ±0.0027

Table 8 Effects of sub-lethal exposure of nickel for 30 days on adenosine tri phosphatase activity in the tissues of female crab, *Scylla serrata* at LC_0 and LC_{50} levels

Values are mean ± SD of 5 estimates. * Significantly different from control; *P>0.05 by ANOVA; ** P<0.05 by ANOVA