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Effects of sub-lethal dose of nickel on the biochemical parameters in the tissues of female crab *Scylla serrata*, from Mumbai coast.

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Abstract: *The effects of sub-lethal concentration of nickel on the biochemical composition such as glycogen, protein, cholesterol, lactic acid, pyruvic acid in gills, hepatopancreas, muscles and sugar contents along with haemolymph in female crab, Scylla serrata procured form local fish market in Mumbai (Sassoon Dock) were studied. The female crabs were exposed to sub-lethal dose of nickel LC_0 and LC_{50} were 200ppm and 500ppm respectively. Among the control set the glycogen contents were found to be the highest in muscles (44.616±0.225 mg/gm wt); protein contents were maximum in gills (66.5232±0.170 mf/gm wt); cholesterol contents were highest in ovary (2.009±0.006 mg/gm wt). The sub-lethal dose of nickel caused decline in the contents of glycogen (1.030±0.0141), protein (11.299±0.055), cholesterol (1.373±0.008) and pyruvic acid (0.440±0.195) in most of the tissues studied. The lactic acid contents increased in all the tissues studied in response to the exposure of nickel. The sugar and lactic acid contents in haemolymph were elevated as a result of exposure whereas the contents of protein, cholesterol and pyruvic acid in haemolymph declined due to the sub-lethal exposure in female crabs.*

Key words: Scylla serrata, Nickel, Glycogen, Protein, Cholesterol, Lactic acid, Pyruvic acid.

Introduction

Nickel compounds are among those chemicals which are much sought after as these are used in varied aspects of industry like electroplating, electroforming and for the production of nickel batteries, electronic equipments, as a component of alloy to be used for the manufacture of tools, machinery, armaments, appliances, jewelry, medical prostheses etc. Further nickel is among the listed priority pollutants and is regarded as one of the major hazardous and noxious heavy metals which are potential component to human health and the biota in metallic as well as in combined form. Paez-Osuna and Torn-Mayen (1996) have reported on the contamination of coastal waters by various forms of pollutants, all these affect the marine biota and human adversely and effectively. Jewett and Naidu (2000) found that various metal ions are distributed in the tissues such as muscles, hepatopancreas of crab, *Paralithodes camtischaticus*. Darllinger and Kaut (1985) reported that when fish living in polluted fresh water bodies with nickel in sediment and contaminated food, nickel is ingested. Gautam and Sharma (2012) have reported that when fresh water fish, *Clarius batrachus* was exposed to copper nitrate (chronic dose, 2.5mg/l) for 30 days, cadmium and iron contents showed variations in liver and kidney; it was found that copper affected calcium metabolism in liver and iron contents were not affected in liver but in kidney it got accumulated. Chou *et al.*, (2002) have reported that crustaceans accumulate heavy metals from the environment and many of them are used as bioindicators. Sokolova and Sokolova (2005) have studied the effects of Cd on oyster and Valarmathi and Azariah (2003) have evaluated the effects of Cu on the enzyme activities in

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crab, *Sesarma quadrates*. Chavez-Crooker *et al.*, (2002) and (2003) have investigated the fate of copper and zinc during intracellular localization. Considerable data on crustacean is available related to acute toxicity tests with heavy metals like Hg, Zn, (Portman, 1972; Newell and Brown, 1972); Fales (1978). Limited data related to toxicity of nickel and its effects on reproduction in crustaceans is available, in general and decapods estuarine crab in particular. Therefore it was imperative to carry out the studies related to nickel and its effects on female crab, *Scylla serrata*, a common inhabitant of marine habitat.

Materials and Methods:

Matured female crabs, *Scylla serrata*, were procured from the local fish market (Sassoon Dock). The selected specimens were devoid of ecto-parasites and external injuries. These were transported to laboratory very carefully and care was taken to avoid over crowding, injuries, stress to the animals. The specimens were maintained, acclimatized to the laboratory conditions for 15 days in a large glass aquaria containing filtered and aerated sea water in which the crabs were half immersed. For experimentation the specimens of uniform weight 125 gm to 155 gm and carapace length 10.5 cm to 12.0 cm were used.

The crabs were subjected to various doses of nickel to estimate LC_0 and LC_{50} values which were 200 ppm and 500 ppm respectively. The selected specimens were exposed to the 200 ppm and 500 ppm concentrations of nickel for 30 days.

The biochemical parameters like glucose, glycogen, protein, cholesterol, lactic acid and pyruvic acid were carried out. Glucose contents in haemolymph were estimated by adopting the method of Folin-Wu as described by Hawk (1965). 1 ml of haemolymph was deprotinized by adding 1ml of 10% Na-tungstate solution and 1ml of 2/3 N sulphuric acid followed by dilution up to 10 ml. 2ml of this filtrate was mixed with 2ml of alkaline $CuSo_4$ and boiled for 10 minutes in water bath. The resultant mixtures were cooled down to room temperature and OD of these was read at 420 nm on Spectronic 20 spectrophotometer. Along the experimental set controls and blanks were also maintained.

Glycogen contents in the tissues were estimated by Anthrone reagent method of Seifer *et al.*, (1950). Appropriate weights of the different tissues were extracted from the experimental animals and were digested in 30% of KOH. The resultant KOH digests were diluted to 100 ml with distilled water. 10 ml of freshly prepared Anthrone reagent were added to the 5 ml of respective aliquots. The mixtures were subjected to boiling for 10 minutes in water bath. The resultant mixtures were cooled to room temperature. The O. D. of these was read at 625 nm on Spectronic 20 spectrophotometer. Standard calibration curve was prepared to calculate glucose values. The glucose values obtained were converted into glycogen using Morrison's factor 1.11.

Protein contents were estimated by adopting the method of Folin-Phenol reagent as described by Lowry *et al.*, (1951). Appropriate weight of the respective tissues were homogenized (1:10 W/V). 5 ml of alkaline $CuSO_4$ were added to 1ml of tissue homogenates and subjected to incubation for 10 minutes at room temperature, 0.5 ml of Folin-Phenol reagent (diluted) were added to the respective reaction mixtures followed by through mixing and allowed to stand for 30 minutes. The color developed was read at 750 nm on Spectronic 20 spectrophotometer. Bovine serum albumin was used as standard for the estimation.

Cholesterol contents in the respective tissues were estimated by the method of Hawk (1965). Appropriate weights of the respective tissues were homogenized (1:10). Ferric chloride ($FeCl_3$) reagent was added to the homogenates followed by incubation for 30 minutes. The reaction mixtures were centrifuged. 5 ml of supernatant were mixed with 3ml of conc. H_2SO_4 and these were kept standing for 20 minutes at room temperature. The O. D. of the

final reaction mixtures were read at 560 nm on Spectronic 20 spectrophotometer.

The contents of lactic acid were estimated by the method of Barker and Summerson, (1974) as described by Hawk (1965). Appropriate weights of the respective tissues were homogenized with 10% TCA. The respective supernatants were used for the estimation of lactic acid contents. 2 ml of the aliquot from this step (protein free) were treated with 1 ml of 20% $CuSO_4$. These mixtures were diluted to10 ml and 1 mg of $Ca(OH)_2$ was added followed by vigorous shaking to disperse it uniformly and the resultant mixtures were allowed to stand for one hour. The reaction mixtures then centrifuged. One ml of clear aliquot was transferred to wide mouth test tube and to it 0.5 ml of 4% $CuSO_4$ were added followed by 6 ml of H_2SO_4. The different reaction mixtures were boiled for 5 minutes followed by cooling to room temperature. To these cooled reaction mixtures 0.1 ml of p-hydroxydiphenyl reagent were added drop-wise. These tubes were allowed to be at 30?C for 30 minutes with intermittent shaking. There after these test tubes were kept for boiling in water bath for 90 seconds. The respective O. D. was read at 560 after cooling them to room temperature.

The pyruvic acid contents were estimated by following the method of Hawk (1965). The protein free supernatants of the respective tissues which were prepared for the estimation of lactic acid were used. 2 ml of distilled water was added to 1 ml of aliquot and to these mixtures 0.5 ml of 2.4-dinitrophenylhydrazene reagent (0.1% in 2N HCl) and 3 ml of 2.5 N NaOH were added. The reaction mixtures were allowed to stand for 10 minutes and there after their OD was read at 540 on Spectronic 20 spectrophotometer.

Results and Discussion

The observations of the parameter studied are presented in tables 1 to 6. The sub-lethal exposure of nickel to female crab has resulted in the impairment of some of the basic biochemical parameters reflecting its role in the various metabolites and their metabolism. McLeay and Brown, (1975) have reported that hyperglycemic conditions caused possibly are due to increased glycogenolysis in the hepatopancreas caused by the nature of the toxicant, exposure duration and strength of stimulus. It is reported that fall in muscle glycogen suggests an increased turnover of glycogen and that the muscle glycogen dose not contributes towards hypoglycemia. Hepatic glycogen becomes the major source of hyperglycemia in blood due to metabolization of glucose molecules from liver to blood. The decrease in hepatic glycogen and corresponding increase in blood glucose level observed during the present investigation lends support to the hypothesis. The decrease in glycogen contents in tissues like hepatopancreas and muscle, suggests its metabolization to meet the energy demands warranted by toxic environment. Larson *et al.*, (1980) are of the opinion that such a response may be caused by general state of stress, but it can also be a result of hypoxic state which can be the result of strict mechanical/heavy metal action on gill function. Ranganekar *et al.*, (1971) and Pathak and Ranganekar, (1979) have suggested that Crustacean Hyperglycemic hormone (CHH) is likely to elevate blood sugar levels during hypoxia. Laul *et al.*, (1974) have suggested that the changes induced in the carbohydrate metabolism by severe muscular exercise are due to stress exerted by the heavy metals uptake in vertebrates and invertebrates. Gabbott and Bayne (1973) and Bayne (1975) investigated stress caused by the pollutants in bivalve, *Mytilus edulis* and observed that seasonal and laboratory induced stress due to a pollutant could be detected by studying parameters like blood sugar contents, disruption of energy balance represented by alterations in glycogen, protein and lipid contents of tissues.

Dange and Masurekar (1981) have proposed that changes in pyruvic acid and lactic acid levels in experimental animals in comparison

with control animals reflect the metabolic or physiological fluctuations. Huckabee (1958) explained that upward trend in the lactic acid relative to pyruvic acid in exposed animals is like to create inadequate oxygen supply to the tissues and caused disturbance in the normal metabolic functioning. When similar conditions are crated in the experimental animals may attribute to condition in which animal can not derive enough oxygen form the surrounding medium because of the damage caused to the gills possibly leading to hypoxia. In the present investigation significant increase in the lactic acid contents was observed in female crab whereas the pyruvic acid contents in hepatopancreas, gills, muscles and blood exhibit prominent decrease; there seems to be a possibility of conversion of pyruvic acid in to lactic acid causing elevation in the contents of lactic acid. Further the hypoxic conditions partly suppress the aerobic respiration. Sastry and Subhadra (1982) have shown that pollutants cause stress and this result in increase in the level of lactic acid in blood. Burton *et al*., (1972) have reported similar conditions and observations in the tissues of fish. Mayekar *et al.*, (2003) reported similar observations in *Scylla serrata* due to Cd exposure. Buddenbrock (1938) has proposed that lethal concentrations of nickel might initiate the state of “lactic acidosis” in crab, a state of elevated level of lactic acid in tissues. In the present study persistent stress might lead to stimulation of lactate due to anaerobic respiration and this condition causes CHH secretion and helps to maintain hyperglycemia. Dhananjay *et al.*, (2011) studied the residue of Cd and Hg in liver and muscle of a fresh water cat fish, *Heteropneustes fosiilis* (Bloch) and found Cd residue was higher in liver as compared to muscle but Hg was found to be more in muscles than in liver.

In the present investigation a significant decrease in the protein contents in all the tissues and in haemolymph too due to exposure to nickel. This decline may be attributed to the enhanced proteolysis resulting in the depletion of protein contents. Reddy *et al.*, (1983) have observed that when rice field crabs were exposed to sumithion it causes breaks down of protein which in turn causes damage to hepatopancreas. Reddy *et al.*, (1983) and Bhagyalakhmi *et al.*, (1983) have observed constant elevation in protein contents in the crab, *Oziotelphusa sensex sensex* when exposed to benzene hexachloride and sumithion. Further, increase is due to the stimulation of the hepatopancreas itself that encountered with destruction and necrosis by the exposure to heavy metals resulting in decrease in protein contents. Bhagyalakhmi *et al.*, (1983) reported that when glycogen breaks down in muscle and gills the proteins are used to produce more energy in order to meat the stress caused by the heavy metals. Further enhanced proteolysis results in efflux of protein in blood/haemolymph. Mohite *et al.*, (2011) have found that exposure of sub-lethal dose of Cd caused an increase GOT activity in the tissues of mantle, gills, hepatopancreas, adductor muscle, siphon, foot and gonad of the bivalve, *Perna viridis* (L) while exposure of Zn resulted an increase in ALP activity.

Adiyodi and Adiyodi (1970) have reported decline in the lipid and free sugar contents in hepatopancreas of the crab, *P. hydrodromus* and suggested that these metabolites are relatively pressed in to service as fuel to meet the metabolic demands. Gill and Pant (1983) have stated that heavy metals cause persistent stress and hyper metabolic state resulting in the utilization of stored lipid. Since lactic acid forms a precursor of lipid metabolism there is a correlation between the two. In the present investigation decline in lactic acid in haemolymph, muscle, gill and hepatopancreas may support this view and also in agreement with the findings of Prior (1978). Krzynowek *et al.*, (1982) have mentioned that cholesterol is the major sterol in the cellular membranes of crustaceans and can account for 5 and 12% of the total membrane lipid. Wodtke (1978) observed significant decline in the molar ratio of cholesterol to phospholipids with cold

acclimation in carp liver mitochondria. Desai *et al.*, (2002) have observed notable changes in the protein, ascorbic acid and cholesterol level due to the exposure of toxic effects of nickel in fresh water fish, *Channa punctaus*.

The current study reveals that sub-lethal dose of nickel has caused changes in the contents of glycogen, protein, cholesterol, lactic acid and pyruvic acid. This further indicates that sub-lethal exposure has the ability to affect the metabolism of carbohydrate, lipid and protein, thus sub-lethal dose of Ni inflicts physiological fluctuations in these biochemical parameters. Observations on glucose, lactic acid and pyruvic acid contents indicate that the physiological stress on the redoxy metabolism and adaptability of the female crab to ensure its survival in the stressed conditions. It may be concluded that female crab can act as bio-indicator with respect to nickel in the aquatic environment.

Table 1. Effects of sub-lethal exposure of nickel for 30 days on glycogen contents in the tissues of female crab, *Scylla serrata*

Sr. No.	Tissues	Control	Lc_0 200 ppm	Lc_{50} 500 ppm
1.	Gills	3.8030 ±0.0128	**3.276 ±0.009	**3.069 ±0.0318
2.	Hepatopancreas	1.4393 ±0.0410	1.372 ±0.0141	**1.030 ±0.0141
3.	Muscles	44.616 ±0.2254	**43.736 ±0.1924	**42.321 ±0.190
4.	Ovary	2.254 ±0.0281	2.234 ±0.0128	**2.175 ±0.221

All values are expressed as mg/gm wt
Values are mean ± SD of 5 estimations.
*significantly different from control,
* P?0.05 by ANOVA,
**P?0.05 by ANOVA

Table 2. Effects of sub-lethal exposure of nickel for 30 days on protein contents in the issues of female crab, *Scylla serrata*

Sr. No.	Tissues	Control	Lc_0 200 ppm	Lc_{50} 500 ppm
1.	Gills	66.523 ±0.170	**58.349 ±0.248	**49.225 ±0.0991
2.	Hepatopancreas	13.710 ±0.209	**12.222 ±0.164	**11.299 ±0.055
3.	Muscles	32.695 ±0.153	**28.219 ±0.0991	**23.737 ±0.149
4.	Ovary	24.812 ±0.110	**21.882 ±0.090	**19.303 ±0.0534

All values are expressed as mg/gm wt
Values are mean ± SD of 5 estimations.
*significantly different from control,
* P?0.05 by ANOVA,
**P?0.05 by ANOVA

Table 3. Effects of exposure of sub-lethal dose of nickel on the cholesterol contents in the tissues of female crab, *Scylla serrata*

Sr. No.	Tissues	Control	Lc_0 200 ppm	Lc_{50} 500 ppm
1.	Gills	0.810 ±0.008	**0.092 ±0.007	**0.346 ±0.008
2.	Hepatopancreas	1.307 ±0.008	**1.143 ±0.003	**0.125 ±0.0031
3.	Muscles	1.521 ±0.181	1.509 ±0.0509	**1.439 ±0.0164
4.	Ovary	2.009 ±0.006	**1.451 ±0.0334	**1.373 ±0.008

All values are expressed as mg/gm wt
Values are mean ± SD of 5 estimations.
*significantly different from control,
* P?0.05 by ANOVA,
**P?0.05 by ANOVA

Table 4. Effects of exposure of sub-lethal dose of nickel on the lactic acid contents in the tissues of female crab *Scylla serrata*

Sr. No.	Tissues	Control	Lc_0 200 ppm	Lc_{50} 500 ppm
1	Gills	0.492 ±0.005	*0.554 ±0.0231	*0.664 ±0.0306
2.	Hepatopancreas	0.179 ±0.024	*0.447 ±0.0316	*0.812 ±0.0115
3.	Muscles	0.562 ±0.047	*0.851 ±0.0467	*0.790 ±0.0198
4.	Ovary	0.252 ±0.0341	*0.686 ±0.005	*0.526 ±0.010

All values are expressed as mg/gm wt
Values are mean ± SD of 5 estimations.
*significantly different from control,
* P?0.05 by ANOVA,
**P?0.05 by ANOVA

Table 5. Effects of exposure of sub-lethal dose of nickel on pyruvic acid contents in the tissues of female crab *Scylla serrata*

Sr. No.	Tissues	Control	Lc_0 200 ppm	Lc_{50} 500 ppm
1.	Gills	0.661 ±0.0218	0.658 ±0.0268	0.651 ±0.0222
2.	Hepatopancreas	1.1434 ±0.0425	1.065 ±0.0477	1.179 ±0.0627
3.	Muscles	0.5356 ±0.0262	0.440 ±0.195	0.493 ±0.0952
4.	Ovary	I.132 ±0.0205	1.117 ±0.001	1.110 ±0.008

All values are expressed as mg/gm wt
Values are mean ± SD of 5 estimations.
*significantly different from control,
* P?0.05 by ANOVA,
**P?0.05 by ANOVA

Table 6. Effects of exposure of sub-lethal dose of nickel on the biochemical parameters of haemolymph of female crab *Scylla serrata*

Sr. No.	Biochemical parameter	Control	Lc_{50} 200 ppm	Lc_{50} 500 ppm
1.	Sugar	0.916 ±0.058	*1.240 ±0.056	*1.457 ±0.107
2.	Protein	84.655 ±3.901	**56.738 ±2.058	**63.562 ±1.440
3.	Cholesterol	0.0854 ±0.0034	**0.274 ±0.0044	**0.0206 ±0.002
4.	Lactic acid	12.792 ±0.401	*16.099 ±0.778	*18.021 ±1.646
5.	Pyruvic acid	1.113 ±0.0021	1.106 ±0.005	1.111 ±0.0078

All values are expressed as mg/gm wt
Values are mean ± SD of 5 estimations.
*significantly different from control,
* P?0.05 by ANOVA,
**P?0.05 by ANOVA

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