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Fect of nickel induced biochemical alterations in fresh water bivalve, lammellidens marginalis

A.V. Andhale[1] and S. P. Zambare[2]

[1]Department of Zoology, Nowrosjee Wadia college, Pune- 411001 (M.S.), India.
[2]Department of Zoology, Dr. Babasaheb Amebedkar Marthwada University, Aurangabad -431004 (M.S.), India.

Abstract
This study done with the observations of biochemical changes in bimolecular contents of, DNA, RNA, protein and collagen after chronic exposure (at 15 and 30days) to nickel chloride (0.227ppm, equivalent to the $LC_{50/10}$ of 96 hours exposure) from various tissues such as gill, foot, digestive gland and whole body of control and experimental bivalves, *Lamellidens mrginalis*. It was observed that protein, collagen, DNA and RNA were dramatically decreased in treated bivalves as compeered to controlled bivalves.

Keywords: Nickel, biochemical content, bivalve

INTRODUCTION

Nickel is a compound that occurs in the environment only at very low levels. Humans use nickel for many different applications. The most common application of nickel is the use as an ingredient of steal and other metal products. It can be found in common metal products such as Jewellery, foodstuffs naturally contain small amounts of nickel. Chocolate and fats are known to contain severely high quantities. Nickel uptake will boost when people eat large quantities of vegetables from polluted soils. Plants are known to accumulate nickel and as a result the nickel uptake from vegetables will be eminent. Smokers have a higher nickel uptake through their lungs. Finally, nickel can be found in detergents. Humans may be exposed to nickel by breathing air, drinking water, eating food or smoking cigarettes. Skin contact with nickel-contaminated soil or water may also result in nickel exposure. In small quantities nickel is essential, but when the uptake is too high it can be a danger to human health. An uptake of too large quantities of nickel has the following consequences:- Higher chances of development of lung cancer, nose cancer, larynx cancer and prostate cancer- Sickness and dizziness after exposure to nickel gas- Lung embolism- Respiratory failure- Birth defects- Asthma and chronic bronchitis- Allergic reactions such as skin rashes, mainly from jewellery - Heart disorders carcinogenicity - Nickel and certain nickel compounds have been listed by the National Toxicology Program (NTP) as being reasonably anticipated to be carcinogens.

Most of the heavy metals interfere with the enzymatic action and produce many physiological and biochemical changes in the bodies of the organisms. Freshwater animals exposed to toxicants for even a short span of time may produce considerable damage of internal organs especially their enzymatic architecture. Majority of enzymes are functional in various metabolic pathways and changed pattern of enzyme activity, induced by pesticide causes functional disorders and alterations in the biochemical contents.

The accumulated heavy metal ions interacts with bimolecular and alters the physiology of organisms. The toxic compounds exert stress to organism and organism responds to it by developing necessary potential to counteract that stress. The chemical changes occurring in the body of organism give first indication of stress [1]. There is much work on the toxic effects of pesticide on specific target and non-target aquatic animal species with respect to the physiological and biochemical changes.

Higher concentrations of toxicants in aquatic environment cause adverse effect on aquatic organisms at cellular or molecular level and ultimately lead to alterations in the biochemical composition. The pollutant affects the activity of biologically active molecules such as amino acids, co-enzyme and other proteins containing sulphur and phosphorus, and affect physiological processes in tissues. Biochemical changes induced by pesticide are disturbed metabolism, inhibition of important enzymes, retardation of growth and reduction of fecundity and longevity of organisms [2].

Pollutants comprising heavy metals may alter cellular functions, ultimately affecting physiological and biochemical mechanisms of animals [3].

DNA: - DNA is master molecule of life. Heavy metals enter into the body of organism through the respiratory organs like gill, lung, etc. and through food and drinking water. It is hazardous to aquatic ecosystem and disturbs the food chain. This disturbed phenomenon has been expressed in the biochemical contents of tissues of animals.

Nucleic acid contents are considered as an index of capacity of an organism for protein synthesis. Different hormones and stress conditions may exert control over synthesis, activity and break down of nucleic acids. The nucleic acid contents can cause alterations in genetic information and genome functioning so it is important to investigate the levels of DNA and RNA periodically in different tissues of the organisms undergoing stress conditions.

DNA (Deoxyribose nucleic acid) contents can be the index of capacity of an organism for protein synthesis in the different stress conditions affected by heavy metals or any toxic metals or pesticides. Structural changes in the DNA can be monitored using biochemical

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*Corresponding Author

A. V. Andhale
Department of Zoology, Nowrosjee Wadia college, Pune- 411001 (M.S.), India.

Email: atmabiotech@gmail.com

methods and usually low quantitative changes are observed on heavy metals exposure. When the activity was assessed as the ability to suppress the synthesis of DNA, RNA and protein, only the synthesis of DNA was suppressed.

Detmar and Andrea (1992) [4] studied that cobalt is an essential trace element for mammalian nutrition, but also is classified as carcinogenic with the fidelity of DNA synthesis. Regarding anti and co-mutagenic mechanisms, the evidence for interference of Co (II) with DNA repair processes is known. An excellent example of the work at the London St. Mary's Branch and the San Diego Branch, found that in a normal cell P-53 expression levels in response to stress and DNA damaging agents caused cell cycle arrest and apoptosis before the damage to the DNA of their nuclei causing oncogenesis.

Arsenic is known to cause DNA damage and related events, such as DNA protein cross-links, micronuclei etc. [5], DNA strand breaks [6], or alterations in DNA repair enzymes [7]. Supper oxide scavengers such as Cu, Zn - SOD suppress arsenic induced DNA damage [7,8 and 6]. Tong Lu et al. (2001) [9] studied that approximately 60 genes (10%) were differentially expressed in arsenic exposed human livers as compared with those of controls, damage was also observed due to involved arsenic in the DNA of respective cells.

RNA: - Heavy metals also interact with RNA polymerases. Severe effects are expressed as such in DNA metal binding. RNA polymerase must bind site specifically to its DNA template, binds its nucleotide and primer substrates, and form a new phosphodiester bond in elongating the growing RNA. Eukaryatic RNA polymerases I, II and III are involved in the synthesis of ribosomal, messenger and transfer RNAs, respectively.

The role of RNA is to help protein synthesis in the cytoplasm hence depletion of RNA level also resulted decreased rate of protein synthesis [10]. Similar decreased amount of RNA levels was observed by Patil and Lomte (1987) [11] in *Mythima* (*Pseudoletia seperata* and by Choudhari et al. (1993) [12] in *Thiara lineata* under different toxic stress. The cellular degradation, rapid histolysis and decreased rate of protein synthesis are the possible reasons.

Ester Saball et al. (2000) [13] observed the total tissue m-RNA of liver and kidneys of control and $HgCl_2$ treated rats. Tong Lu *et al*. (2001) [9] observed that 10% genes, mostly related to cell cycle regulation, apoptosis, DNA damage response etc. were differentially expressed in the form of RNA and such abnormal RNA are vulnerable to RNA are attack.

Rao et al. (1998) [10] studied the RNA levels in various tissues of freshwater crab, *Barytelphusa cunicularis* when exposed to Fluoride.

Protein: - Proteins are long chains of amino acids forming three dimensional structures. Proteins do play both structural and functional role of cellular level. Being an integral part of the cell membrane, intracellular and extra cellular passages are linked through it. Any sort of cellular metabolism occurring in body involves one or many different proteins. The proteins are among the most abundant biological macromolecules and are extremely versatile in their function and interaction during metabolism of proteins, amino acids, enzymes and co-enzymes [14]. Deshmukh and Lomte (1998) [15] studied the biochemical content of protein in mantle, foot, gill, digestive gland and whole body of fresh water bivalve, *Parreysia corrugata* after acute and chronic exposure to copper sulphate.

Mahajan and Zambare (2001) [16] observed the protein profiles in different tissues of fresh water bivalve, *Corbicula striatella* after exposure to chronic dose of copper sulphate and mercuric chloride. Decrease in protein content in mantle, foot, gill, gonad and hepatopancreas of bivalve, after exposure to $HgCl_2$ and $CuSO_4$ treated animals might be due to alteration of membrane permeability. Mohanty et al. (2005) [17] analyzed and compared protein profile from gill, foot, and mantle of two freshwater bivalves, *L. corrianus* and *L. marginalis* and found protein markers which help to study the molluscan taxonomy.

Collagen: - Collagen is a fibrous connective tissue protein produced by fibroblast. It is the most abundant structural proteins found in the connective tissue of the animal kingdom, *Animalia*. It appears to be an amorphous substance in the basement membranes of the certain tissues and reticular fibers in extra cellular spaces. These fibers serve as a mechanical support for the tissue and represent surfaces on which cells may glide. A large quantity of collagen is produced by connective tissues. There are at least five isotypes of collagen molecules, based on slight differences in the organization of polypeptides and association with other molecules (i.e. polysaccharides and glycoproteins).

Type -I – is present in dermis, bones, tendons, cornea and dentin. Type -II – mainly in cartilage. Type -III– in foetal skin, cardiovascular system, the uterus and intestine. Type -IV– in basal laminae or basement membranes. A mesh works of type – V collagen is found in the blood capillaries and the glomerulii of kidneys. Collagen type –I, II, III and V show typical striated fibrils. Type-IV lacks is distinct fibrilar structure and is produced along with fibronectin and laminin. Basement membranes have tight relationship between collagen, fibronectin, laminin and proteoglycans. It has been found that fibronectin binds to special binding sites of the collagen molecule while making the complex. Then the cell surface attaches to it and spreads, making the focal contacts at the ventral side [18].

The basic molecular unit of collagen is tropocollagen, an elongated molecule about 300 nm long and 1.5 nm wide. Tropocollagen consists of three polypeptides of about 95,000 Daltons that are coiled together in triple helical fashion. The tertiary structure of the collagen is maintained by the disulphide bonds. The largest portion of the molecule has a ά- helix organization, with short non- helical segments of 16 to 25 residues at both ends that are called telopeptides. The knowledge of collagen type distribution in normal and diseased cardiac tissue is essential. The changes in collagen parameters may be of great importance to understand the mechanism of cardiac hypertrophy, heart failure or cardiac pathogenesis.

Lead toxicity affected osteoblast and chondrocytes and has been related to 'osteopecrosis'. Even exposure to low lead concentrations, delay in cartilage formation and decrease in type was observed during fracture healing in experimental mice [19].

The changes in plasma copper concentrations because of traumatic bone injury, because of the role of copper in collagen formation during bone repair. It is noticed that difference in copper values for patients with osteoporosis from normal patients. The fundamental structure of collagen is maintained due to disulphide bridges. The heavy metal usually binds with –SH group of proteins. Metalloid, arsenic being highly reactive can bind –SH groups of collagen and alter its structure. Disruption of collagen structure in the basement membrane leads to the poor function of epithelial cells or poor transport of bio-molecules across the membrane so it is curious to investigate interaction of heavy metal with collagen.

Thus, proteins, collagen, DNA and RNA levels in the tissues

after exposure to heavy metals can be considered as the indices for stress. In present research study Nickel salt alteration in bio-molecules proteins, collagen, DNA and RNA were study. As gene central dogma of molecular biology depends on these molecules. So, it is essential to study the pollutant impact on molecular architecture of life.

MATERIALS AND METHODS

The selected model animals, the freshwater bivalves, *Lamellidens marginalis* were collected from the Dhondwadi dam at Borana river from Tq. Parli Vijanath, Dist. Beed (M.S.), India. After collection, the bivalves were acclimatized in the laboratory condition at room temperature for 2-3 days. The active acclimatized bivalves of approximately same size were selected for experiment.

Before starting the experiment, these bivalves were divided into two groups one group of bivalves was maintained as control (which are not exposed to nickel chloride) while the second group was exposed to the chronic dose of nickel chloride (0.227 ppm , equivalent to the LC_{50}/10) for 15 and 30 days exposure.

The different tissues such as gill, foot, digestive glands and whole body from these experimental and controlled group bivalves were used for estimation of different bio-molecules as DNA, RNA, total Proteins and Collagens. These tissues were removed after 15 days and 30 days of exposure to nickel chloride and from control animals and dried in the oven at 80 0C. The dry powders of these tissues of different parts of bivalve, the DNA contents were estimated by Diphenylamine regent; RNA contents estimated by Orchinol reagents; The total protein was estimated by Lowry's reagent and collagen was estimated by estimating the Hydroxyproline by Woosner's method (1963).

The data obtained is presented in the tables 1 to4. Necessary statistical methods as standard deviation and test of significance are applied.

OBSERVATIONS AND RESULTS

Table 1 shows the decrease in the DNA contents in all tissues of the nickel exposed bivalves as compared to those of the control bivalves. The salt of nickel interacts with the DNA and disturbs its normal double helical structure and this disturbed structure is vulnerable to the attacks of the DNAs enzymes and hence the levels of the DNA are decreased.

Table 2 shows the decrease in the RNA levels of the treated bivalves as compared to those of the control bivalves. The interactions of metals with DNA affect the transcription and hence reduce the mRNA and other RNA contents and hence the levels of RNA are found to be decreased in the nickel exposed bivalves.

Table 3 shows the variations in the protein levels of the treated and control bivalves. Protein content in tissues of Gill, foot, digestive gland and whole body were dramatically decreased in experimental bivalves (which exposed to chronic dose of 0227ppm nickel chloride) with controlled bivalves (which are not exposed to nickel chloride).

The table 4 indicates the variations in the collagen levels of experimental and treated bivalves. Collagens, most prominent molecule of extracellular matrix which has role in many fundamental developmental processes like cell adhesions, cell differentiations, cell growth, cell migrations, remodeling etc, were decreased in its level in nickel exposed animals as compare to its controlled group.

DISCUSSION

Aquatic invertebrates naturally accumulate abnormally high amount of heavy metals. The effects of these heavy metals on the normal function of cells, tissues and organs are deleterious due to accumulative toxicity. Nickel chloride is hazardous when accumulated even at trace level in the system of all living organisms.

The results of biochemical estimations of DNA, RNA, proteins and collagen, chronic exposure to nickel showed drastic changes in the physiology of freshwater bivalve, *L. marginalis*.

Changes in the DNA content

DNA content, the index of capacity of an organism for protein synthesis in the different stress conditions was affected by heavy metals or any toxic metals or pesticides. It was reported that copper ions introduced into asides tumors penetrate the nucleic acid (DNA) and damage it, causing incardinating of the chromatin structure, copper associates with DNA at higher copper concentrations. Tong Lu et al. (2001) [9] observed that approximately 60 genes (10%) were differentially expressed in arsenic exposed human livers compared to controls. The differentially expressed genes induced those involved in cell cycle regulation, apoptosis, DNA damage response, and intermediate filaments.

The observed gene alterations appear to be reflective of hepatic degenerative lesions seen in the arsenic exposed patients. This array analysis revealed important patterns of aberrant gene expression occurring with arsenic exposure in human liver. Aberrant expressions of several genes were consistent with the results of array analysis of chronic arsenic exposed mouse livers and chronic arsenic - transformed rat liver cells. They suggested that clearly a variety of gene expression changes might play an integral role in arsenic hepatotoxicity and possibly carcinogenesis.

Aurintricarboxylic acid (ATA), a polyanionic form probably interferes with the electrostatic interaction between DNA and RNA in their interaction with nuclei acid binding proteins. The synthetic deutero ATA fraction was capable of inhibiting the degradation of yeast RNA by bovine pancreatic RNase and thus molecules that interact by electrostatic interactions can affect the rate of RNA catabolism. In present work it has shown that the DNA content of gill, foot, digestive gland and whole body were decrease in their percentage due to the impact stress of nickel chloride.

Changes in the RNA contents

RNA polymerase binds the binding site especially to its DNA template, binds its nucleotide and primer substrates, forms a new phosphodiester bond and elongates the growing RNA.In the present investigation, the RNA contents were decreased due to the acute exposure of mercury and arsenic. The present investigation shows the interaction of heavy metals with proteins, DNA and RNA. The decrease in proteins, collagen, DNA and RNA levels on exposure to Nickel may be due to damage in DNA, poor rate of synthesis of enzymes necessary for transcription or increased catabolism due to their abnormalities on binding to nickel.

Changes in protein contents

Protein is a key substance to show the effects of heavy metals. Proteins respond for better survival by either increasing or decreasing their levels. So, protein assessment can be considered

as a diagnostic tool to determine the physiological responses of the cells and organs.

Protein is an important organic constituent that plays a crucial role in metabolism. Being the integral part of cell membrane, intracellular and extracellular passages are linked through to it. Interactions occurring during protein metabolism in protein, amino acids, enzymes and co- enzymes were studied by Harper et al. (1978) [20].

Present investigation clearly showed that after acute exposure to Nickel, protein levels were decreased in gills, foot, digestive glands and whole body as shown in table 3 the protein contents were significantly reduced in heavy metal exposed bivalves.

Sekeri et al. (1968) [21] studied that all enzymes are proteins in nature and they control sub cellular functions. In the metabolism of protein many enzymes, co-enzymes intermediate protein and amino acids are involved.

The decrease in protein content may be due to altered size of pores in membrane [22].The decrease in average total protein content of tissue after treatment suggests enhancement of proteolysis to meet the high energy demands under heavy metal or other stress.

Mahajan and Zambare (2005) [23] observed a significant decrease in the protein content in various tissues of experimental snails *Bellamiya bengalensis* as compared to that of control. The protein contents were more in heavy metal salt with caffeine-exposed snails as compared to those exposed to only heavy metal salts.

Impact of heavy metal exposure showed the decreased levels of protein in various animals in aquatic environment. The heavy metals denature the proteins. Mahajan and Zambare (2001) [16] showed that after acute and chronic exposure to $HgCl_2$ and $CuSO_4$, protein contents in different tissues of *Corbicula striatella* were found to be highly depleted and maximum protein depletion was found in foot of $HgCl_2$ treated animals but in $CuSO_4$ treated animal decrease was slight.

Rao et al. (1994) [24] recorded that the content of sperm protein in cauda epididymis reduced significantly on exposure to mercury for 60 days. Khan et al. (2001) [25] found that the mussels, *P. viridis* when exposed to zinc chloride at 1/10th LC_0 and LC_{50} concentrations showed variation in protein content. The decrease in the protein content can be due to anaerobic metabolism**.** Protein content of brain, liver, kidney and gills of *Heteropneustes fossilis* on exposure to sublethal concentration of mercuric chloride were significantly. Exposure of fish to mercuric chloride **+** chabazite improved the protein content in comparison to fish of group II. When fish was exposed to chabazite, only, protein contents were found to be increased in comparison to their respective control.

Sastry and Gupta (1978) [26] emphasized that overall decrease in protein content was probably due to enzyme inhibition, which plays an important role in protein synthesis. Rao et al. (1987) [27] found decrease in protein levels in the hepatopancreas of *Indonaia caerules* on exposure to fluorides.

Changes in the Collagen contents

About one quarter of all of the protein in our body is collagen. Collagen is a major structural protein, forming molecular cables that strength the tendon and vast, resilient sheets that support the skin and internal organs.

Collagen is the body's most important structural protein. It is the ground substance, or cement, that supports and holds the tissues and organs together. The substance in the bones provides the toughness and flexibility and prevents brittleness. Without it, the body would just disintegrate or dissolve away. It is the substance that strengthens the arteries and veins, supports the muscles, toughens the ligaments and bones, supplies the scar tissue for healing wounds and keeps the youthful skin tissues soft, firm, supple and wrinkle free.

The disturbance or alteration in collagen formation causes the fearful effects of scurvy, the brittle bones that fracture on the slightest impact, the weakened arteries that rupture and cause hemorrhage, the incapacitating muscle weakness, the affected joints that are too painful to move, the teeth that fall out, and the wounds and sores that never heal. Sub optimal amounts of ascorbic acid over prolonged periods during the collagen, may be the factor in later life that causes the high incidence of arthritis and joint diseases, broken hips, the heart and vascular diseases that cause sudden death, and the strokes that bring on senility. Collagen is intimately connected with the entire aging process.

Effect of heavy metal on collagen content was studied by very few investigators. The present investigation shows the decrease in collagen level in the freshwater bivalves, *L. marginalis* on exposure to acute concentration of nickel chloride as compared with control bivalves.

Clinically they pose difficulty in diagnosis when there is no clear history of penetrating injury by objects containing metallic nickel. Metallic Nickel in tissue sections appears as dark, opaque globules, usually spherical in shape and of varying sizes and number. A zone of collagen necrosis often surrounds the nickel globules.

Any imbalance in the extracellular matrix or alterations in the metabolism of collagen in a pathological condition such as glomerulosclerosis lead to significantly reduced glomerular function. Reduction of type IV collagen protein and mRNA by dexamethasone on basement membrane collagens (found in the basement membrane) are most affected. Thus, they suggested that the interstitial and basement membrane collagens have been coordinately down regulated by dexamothasene.

The dramatic decrease in the type I:III ratio, observed in their study, emphasizes that the type of collage may play an important role in my Ocardial dysfunction. The effect of hypothermia on collagens of different tissues of Garden lizard was not uniform. Presumably the differences were observed in the response of tissue reflect, the differences in the type of cross-links present in each issue and the initial status of collagen at the beginning of the experiment.

The impact of decreased collagen levels in the tissues may be due to binding of heavy metals salt of nickel to disulphide linkages that maintains the triple helical tertiary structure of collagen. The abnormal collagen thus formed may be digested by the collagenase enzyme and hence the collagen contents were decreased. Alterations in the basement membranes of epithelia due to the changes in the collagen can alter the extra cellular matrix-cell interactions and the receptor cells of the epithelia resulting into poor functioning of epithelia. The hepatopancreas, foot and gills have major epithelial structures whose physiological status can be altered due to variation in the collagen levels and its structure.

Table 1. DNA content (mg/gm of dry tissue) in selected tissues of *Lamellidens marginalis* after chronic exposure to Nickel.

Treatment	Tissue	15 days	30 days
(A) Control	Gill	13.18 ± 00.362	12.56 ± 00.298
	Foot	25.90 ± 00.789	24.30 ± 00.472
	Digestive Glands	23.8 ± 0.0487	21.23 ± 00.762
	Whole body	28.95 ± 0.092	26.91± 00.6
(B) Nickel Chloride 0.227 ppm	Gill	11.63 ± 0.0756 NS (-11.7602)	10.55 ± 00.367❖❖ (-16.003)
	Foot	16.12 ±00.426❖❖❖ (-37.7606)	11.53 ±003.82❖❖❖ (- 52.5514)
	Digestive Glands	16.18 ±00.479❖❖❖ (-32.0168)	15.58 ±00.496❖❖❖ (-26.6132)
	Whole body	25.61± 0.013 (-11.5371)	21.03± 0.07 (-21.8506)

Values in the ( ) brackets indicate percent change over control, N.S. - Non Significant
❖ - Compared with respective (A), ❖ - P < 0.005, ❖❖ - P < 0.01 and
❖❖❖ -P < 0.001

Table 2. RNA content (mg/gm of dry tissue) in selected tissues of *Lamellidens marginalis* after chronic exposure to Nickel chloride

Treatment	Tissue	15 days	30 days
(A) Control	Gill	124.18 ±1.386	121.12 ±12.39
	Foot	51.23 ±0.806	45.89 ±05.63
	Digestive Glands	81.23 ±0.921	80.24 ±06.94
	Whole body	96.32±0.91	94.21±08.1
(B) Nickel Chloride 0.227 ppm	Gill	101.94 ±1.359❖ (-17.9094)	90.79 ±12.53❖❖ (-25.0412)
	Foot	44.78 ±0.208NS (-12.5902)	39.86 ±01.87❖❖ (-13.14011)
	Digestive Glands	79.69 ±05.86❖ (-1.8958)	53.21 ±04.38❖ (-33.6864)
	Whole body	85.69±04.5 (-11.0361)	81.24±06.9 (-13.7671)

Values in the ( ) brackets indicate percent change over control
N.S. - Non Significant ❖ - Compared with respective (A)
❖ - P < 0.005 ❖❖ - P < 0.01
❖❖❖ -P < 0.001

Table 3. Profiles of protein (mg/gm of dry tissue) in different tissues of fresh water bivalves *L. marginalis* (Lamarck) after chronic exposure to Nickel chloride.

Treatment		15 days	30 days
"(A)" Control (Not Treated.)	Gill	632.45±42.15	613.05±41.35
	Foot	749.00±48.05	719.16±36.41
	Digestive gland	565.33±38.17	537.31±32.75
	Whole body	619.00±45.03	606.36±35.79
(B) Nickel Chloride (0.227 ppm)	Gill	532.35±21.75 ❖ (- 15.827)	434.39±39.75 ❖ ❖ (-29.1428)
	Foot	646.19±38.75 NS (- 13.7263)	481.78±29.89❖ ❖ ❖ (-33.0079)
	Digestive gland	468.72±30.05 NS (- 17.0891)	346.57±25.75❖ ❖ (-35.4990)
	Whole body	533.08±71.35 NS (- 13.8804)	418.85±65.85❖ ❖ (-30.9238)

Values in the ( ) brackets indicate percent change over control
N.S. - Non Significant ❖ - P < 0.005
❖ ❖ - P < 0.01 ❖ ❖ ❖ -P < 0.001

Table 4. Collagen content (mg/gm of dry tissue) in selected tissues of Lamellidens *marginalis* after chronic exposure to Nickel chloride

Treatment	15 days	30 days	Treatment
(A) Control	Gill	32.54±0.136	31.86±0.156
	Foot	36.94±0.192	35.52±0.18
	D. Gland	33.69±0.178	31.75±0.167
	W. body	41.23±0.183	39.92±0.172
(B) Nickel Chloride (0.227 ppm)	Gill	24.71±0.114 (- 34.0626)	21.30±0.111 (-33.1450)
	Foot	35.54±0.125 (-3.7899)	34.12±0.117 (-3.9414)
	D. Gland	24.58±0.156 (-27.0406)	26.91±0.132 (-15.2440)
	W. body	34.13±0.127 (-17.2204)	32.50±0.119 (-18.5871)

Values in the ( ) brackets indicate percent change over control

REFERENCES

[1] Meyer, W., Kretschmer, M., Hoffmann, A. and Harich, G.1991. Biochmical and Histochmical observation on effect of two level heavy metal load (lead, Cadmium) in different organ system of the freshwater cryfish, *Astacus astacus*. *Exotoxical Environ. Saf.* 21:137-156.

[2] Murthy A. S.1986. Toxicity of pesticide to fish. CRC Press.,Inc., Boca Raton, Floridia, USA.

[3] Radhakrishnan, K. Sivaramakrishna, B. and Sonabai, P.1991. A physiological approach to study the toxicity of Hg in the fresh water mussel Lamellidens marginallis (lamark) *Z. Angew. Zool.* 78 (3):289-294.

[4] Detmar, B. and Andrea. 1992. The genetic toxicity of Cobalt, *toxicology and pharmacology. 115*:137-145.

[5] Schaumloffel, N. and Gebel, T. 1998. Hetrogenecity of DNA damage provoked by anatimony and arsenic. *Mutagenesis* 13: 281-280.

[6] Liu, F. and Jan, K.Y. 2000. DNA damage in arsenate and cadmium treated bovine aortic endothelial cells. *Free Radic. Biol. Med.* 28: 55-63.

[7] Hartwing, A.1998. Carcinogenicity of metal Compounds: possible DNA repair inhibition *Toxicol. Lett.* 102-103:235.

[8] Lynn, S., Shiung, J. N., Gurr, J. R., and Jan, K. Y.1998. Arsenic stimulate poly (ADH-ribisylation) by generation of nitric oxide *Free Radic boil. Med*. 24:442-2444.

[9] Tong Lu Jie., Liu, Edwrd L Lecuyse You, Shu Ming Liag Cheng and Michael P Walkes. 2001. Application of DNA microarray to study of arsenic induced liver diseases in population of Guizho, Chizhou, China. *Toxicol. Sci.* 56: 185-196.

[10] Rao, T. K., Chuddary, T. R., Subhas, M. and Patil, P. N.1990. Impact of plouride toxicity on nuclic acid contents of freshwater crabs, *Barytelphusa cunicularis*. *J. Aqua. Bio.* 13:104-106.

[11] Patil, P. and Lomte, N. 1987. Effect of pesticide stress on metabolic enzymes and nuclic acids of army worm, (Mythimma) sepreta proc. *Natl. Symp. Ecotorico*. pp- 85-90.

[12] Choudhari, T. R., Rao, K. R., Subsh, S. B., Deshmukh and Patil , P. N. 1993. Changes in DNA and RNA to different kinds of pesticide in *Thiara lineate*. *Porc. Acad. Envoin Biol.* 2 (2):187-192.

[13] Ester Saball, Marcella Salvarry, Esteban Serra, Guillermo pico and Marria monicva Ilias. 2000. Potential mechanism of fibronectin deposit in acute renal failure induced by mercury chloride, Abst. Instito. *De. Biol. Med.Cell. De rasario. Argintina.*

[14] Harper H, A., Rodwell V. W. and Myeres P.A. 1987. A review of physiological chemistry, Long medical Publications, California.

[15] Deshmukh, M. and Lomte, V. S. 1998. Effect of heavy metal ($CuSo_4$) on protein activity of freshwater bivalve, Parreysea corragete in relation to pollutant stress. Ph. D. thesis Dr. B. A. M. Univ. Aurangabad (M.S.).

[16] Mahajan, A. and Zambare, S.P. 2001. Ascorbate effect on copper sulphate and mercuric chloride induced alterations of protein level in freshwater bivalve, Corbicla Striatella. Asian, J. microbial, *Biotech and Envio. Science.* 3 (1-2): 95-100.

[17] Mohanty, B. P., Gupta, S. K., Yadav, L. Y. and Panwar R. S.2005. Tissue protein profile of two closely related fresh water bivalve mollusk. Natl. *Acad. Sci. Lett.* 28 (5-6).

[18] Kleinman, H. K., Kleb, R. J. and Martin, G. R. 1981. Role of collgeious matrices in the adhesion matrices in the adhesion and growth of the cells. *J. Cell Biol.* 88-473.

[19] Carmonche, J., Puzas, J., Zhang, H. and Schwar, E. 2005. Lead exposure inhibits fracture healing and is associated with increased chondrogensis, dely in cartilage mineralization and a decrease in osteoprogenitor frequency. *Environment Health. Prespect.*113(6): 48-755.

[20] Harper, H., Rodwell, V., Mayes, W and Peter, A. 1978. Review of physiological chemistry, 16$^{th\ Edn}$. Lange Los Altos, California.

[21] Sekeri, K. G., Sekeri, C. E. and Karlason, P. 1968. Protein synthesis in subcellular fracations of the blowfly during different developmental stages. *J. Insect physiology.* 14:425-431.

[22] Abel, P. D.1974. Toxicity of Synthetic detergent to the fish aquatic vertebrates *J. Fish Biology.* 6: 279-298.

[23] Mahajan, A. 2005. Effect of caffeine 137 trimethylxamine on heavy metal induced physiological alterations in the fresh water gastropod, Bellamya (Viviparas) bengalensis (L). Ph. D. Thesis, North Maharashtra University, Jalgaon (M. S.), India.

[24] Rao, M. V., Metha, A. R. and Patil, J. S. 1994. Ascorbate effect on methyl mercury toxicity in reproductive organs of male guina pigs. *Indians J. of Environmental and toxicology.* 4(2):53-58.

[25] Khan, A. K., Shaikh, A. M. and Ansari, N T. 2001. Tissue protein level in different body parts og green muscle, *Perna viridis*, exposed to zinc chloride in summer season. *J Aqua. Biol.* 16(02): 45-47.

[26] Sastry, K. V. and Gupta, P. K. 1978. Effect of mercury chloride on the digestive system of Channa punctatus, a histopatological study, *Environ.Res.* (16): 270-278.

[27] Rao, T. K., Choudhari, T. R., Shabas, M. and Patil, P.N. 1998. Impact of fluoride toxicity on nuclic acid contents of the freshwater crabs, Barytelphusa cunicularis *J. Aqua. Bio.* 13: 104-106.