STUDIES ON THE TOXIC EFFECTS OF SELECTED HEAVY METALS IN THE FRESHWATER MUSSEL *LAMELLIDENS CORRIANUS* (LEA)

THESIS

SUBMITTED TO

COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

UNDER THE FACULTY OF ENVIRONMENTAL STUDIES

By

RAJALEKSHMI AMMA P.

SCHOOL OF ENVIRONMENTAL STUDIES

COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY

COCHIN - 682 016

1992

C E R T I F I C A T E

This is to certify that this thesis is an authentic record of the research work carried out by Smt. P. Rajalekshmi Amma., under my scientific supervision and guidance in the School of Environmental Studies, Cochin University of Science and Technology, in partial fulfilment of the requirements for the degree of Doctor of Philosophy of the Cochin University of Science and Technology under the Faculty of Environmental Studies, and no part thereof has been presented for the award of any other degree, diploma, or associateship in any University.

Dr. A. MOHANDAS
Head
School of Environmental Studies
Cochin University of Science and Technology
Cochin-682 016.

D E C L A R A T I O N

I, Rajalekshmi Amma, P., do hereby declare that this thesis entitled "STUDIES ON THE TOXIC EFFECTS OF SELECTED HEAVY METALS IN THE FRESHWATER MUSSEL, *LAMELLIDENS CORRIANUS* (LEA)" is a genuine record of the research work done by me under the scientific supervision of Dr. A. Mohandas, Head, School of Environmental Studies, Cochin University of Science and Technology, and has not previously formed the basis for the award of any degree, diploma, or associateship in any University.

Cochin-682 016.

RAJALEKSHMI AMMA, P.

A C K N O W L E D G E M E N T

I am greately indebted to Dr. A. Mohandas, Head, School of Environmental Studies, my supervising teacher, for his guidance, encouragement, and critical evaluation of the thesis.

I wish to express my sense of gratitude to Prof. Dr. K.P. Balakrishnan, Dean, Faculty of Environmental Studies, for his help, and guidance.

I thank the authorities of Cochin University of Science and Technology for granting me permission and facilities to carry out my research work.

I thank Dr. M.V. Subba Rao, Scientist, Zoological Survey of India for the identification of the bivalve species studied.

I would like to express my profound thanks to Prof. Dr. N.R. Menon, Director, School of Marine Sciences, for his encouragement and help.

I am very much obliged to Dr. M.V. Harindranathan Nair, Lecturer, School of Environmental Studies, for the invaluable help and encouragemennt rendered by him during the preparation of the thesis.

I would like to express my sincere thanks to Dr. Suguna Yesodharan, Lecturer, School of Environmental Studies, for her help and encouragement.

I am very much thankful to my colleagues, the members of the non-teaching staff, School of Environmental Studies, for their co-operation and support.

I gratefully acknowledge the invaluable help and support rendered by my friends, Dr. Suresh, P.G., and Dr. Reju, M.K.

My sincere thanks are due to Mrs. Manju, M.R., Mr. Bennichan Thomas, Dr. K.S. Gopalakrishnan, Dr. K. Suresh, Mr. Babu Jose, P., and Mrs. Mini Shankar who helped me in one way or the other.

I would like to express my sincere thanks to Dr. S. Ashok Kumar and Dr. Pauly, K.V., Dept. of Industrial Fisheries for all the invaluable help rendered by them during the preparation of the thesis.

C O N T E N T S

C H A P T E R - I

INTRODUCTION

Industrialisation affects air, water, and soil. Industrial effluents which enter the aquatic environment either by direct disposal or through run off, affect living organisms at morphological and physiological levels. In any living tissue toxic materials exert their effects first at molecular and biochemical levels (Robbins and Angell, 1976). Most of the industrial effluents contain elevated concentrations of organic and inorganic chemicals capable of eliciting stimulatory or inhibitory effects on the metabolism of aquatic organisms.

Heavy metals form an important group of environmental pollutants. Effects of pollution on the aquatic environment by heavy metals have received considerable attention in recent years due to their toxicity even at very low levels, persistence in the environment, and chances of getting biomagnified. A pollutant that does not affect a particular process under normal unstressed condition may affect the ability of the animal to adjust to changing environmental conditions which ultimately decrease its chances of survival (Thurberg et al., 1973). Because of the ecological hazards, heavy metals have become of increasing concern as environmental pollutants. The well documented cases

of cadmium contamination as well as mercury poisoning of Minamata Bay have revealed the devastating effects of these metals on human beings. The chief sources of mercury contamination are chloralkali plants and agricultural application. Mercury is widely used as a fungicide, seed dresser, and also in the manufacture of scientific equipments, and electrical appliances. Copper is widely used as molluscicides and algicides. Since copper is an essential metal, the toxic effect of copper gets manifested only when the toxic limit is exceeded. Cadmium and its compounds are being increasingly used in industries. It is widely used in electroplating. Cadmium compounds are used as colour pigments in plastics and various types of paints.

Metals may alter the enzyme activity or function in many ways. They may bind to enzyme active sites or may interfere with substrate binding and cause alterations in enzyme activity (Ulmer, 1970). Metal ions may also alter concentrations of cofactors and membrane permeability (Passow, 1970). Presence of heavy metals leads to toxic effects mainly because of direct metal interaction on enzyme systems (Tallendini et al., 1986). Mercury is also found to disrupt ionic balance, altering cell membrane permeability (Larry et al., 1974). Physiological damage brought about by a toxicant is as important as mortality. Cadmium and

lead are found to react with phosphate groups of lipid bilayer before being complexed by intracellular ligands (George and Viarengo, 1984). In vivo studies on the effect of cadmium and copper on mussel digestive gland have demonstrated that the first alteration detected at lysosomal level is loss of membrane stability (Viarengo et al., 1981 Moore et al., 1984). Involvement of heavy metal in the stimulation of mitochondrial lipid peroxidation could bring about alteration in the organism's physiology (Viarengo, 1985). Metallothioneins play a very significant role in metal metabolism. Studies concerning the metal detoxification in the digestive gland of metal exposed mussels have shown that like other cytosolic proteins, thioneins are also taken up by lysosomes (George, 1983 b Viarengo et al., 1984). The elimination of heavy metals is closely linked with the biochemical characteristics of lysosomes which may vary in different cells of the same organism (Viarengo et al., 1985). The induction of hepatic metallothionein has been considered as the most satisfactory explanation of acquired resistance of the animal to metal toxicity (Brown and Parsons, 1978; Dixon and Sprague, 1981; Cousins, 1985).

Heavy metals are the most active polluting substances as they cause serious metabolic, physiological and structural

impairment in animals. Exposure to heavy metals can cause tissue hypoxia and induce metabolic alterations (Tort et al., 1984). Metal induced hypoxia was also suggested by Burton et al. (1972). Marine organisms are capable of accumulating very high levels of metals in their tissues with no apparent biological effects (Goldberg et al., 1978). This tolerance may be attributed to the presence of metallothioneins (Noel- Lambot et al., 1978; Olafson et al., 1979 a; 1979 b; Overnell and Trewhella, 1979).

Molluscs are known for their ability to accumulate metals from media, and tolerate high tissue concentrations. Higher tissue tolerance is related to the presence of chelating proteins (see Dillon and Neff, 1978), and lysosomes (Desnoyers and Chang, 1975; Fowler et al., 1975). Cadmium has been found to be directly toxic to macrophages (Loose et al., 1978 a), and depress phagocytic capacity (Loose et al., 1978 b). Gill is intimately involved in osmoregulation and respiration. High metal uptake by gill tissue during initial period is reported (Eisler et al., 1972; Hutcheson, 1974). Cadmium induced changes in gill respiration are reported in crabs (Collier et. al. 1973). Improved gas transfer efficiency and reduction in energetic requirements are found to be compensatory mechanisms. The feature and intensity of damage depend on the nature and level of the metal.

Morphological changes in gill epithelial cells after exposure to mercury were reported in the crab *Uca pugilator* by Vernberg et al.(1974). Vernberg and Vernberg (1972) found that hepatopancreas, gills, and antenal glands are targets of mercury deposition. Cadmium accumulation in hepatopancreas of invertebrates is reported by Ray et al. (1980, 1981) and Delthlefsen, (1977). It has been reported that metallothioneins increased with time in mussels on exposure to metals and that the thioneins accumulate in the lysosomes before being eliminated by exocytosis (Viarengo et al., 1984). Lysosomes are able to include metal chelating compounds in order to eliminate them (see Cassini et al., 1986) and thus play an important role in metal metabolism. Many marine bivalves are able to accumulate high metal concentrations in tissues without being affected because of the presence of metallothioneins (Cherian and Nordberg, 1983).

Pollutants are having profound influence on the filtration rates of bivalves and the system. Effects of pollutants on the filtering rate of *Mytilus edulis* have been studied by Abel (1976). Watling and Watling (1982) conducted experiments on the comparative effects of metals on the filtering rate of *Perna perna* and reported that while mercury decreased the rate of filtration, selenium dramatically increased it. Valee and Ulmer

(1972) have postulated that mercury can bind with carrier protein molecule as the metal shows strong affinity for ligands such as phosphate, cycteinyl, and histidyl side chains of proteins resulting in inhibition of glucose and fructose transport. Mercury interference with sodium pump during sugar transport is also reported (Sastry and Rao, 1983). Cadmium uptake by mussel may involve prior complexation with a carrier ligand before translocation of the complex takes place across the gill membrane (George and Coombs, 1977).

Freshwater mussels have been widely used in toxicity evaluation and water quality management programme in view of their role as bioindicator of toxicity levels and as water purifiers in maintaining environmental quality (Reddy, 1984). The high tolerance of bivalves to metals may be due to their ability to withdraw into the shells and thereby reduce the penetration of the toxicant into the soft parts (Calabrese et al., 1973). If contaminants affect the homoeostatic mechanisms that control concentration of biochemicals in organisms, measurment of affected metabolites in normal and stressed organisms provides a means to evaluate the stress condition. Mussels are attractive indicators of pollution in the freshwater ecosystem as they do not migrate extensively from their native

streams. As they are filter feeders, they will also absorb and concentrate toxic material in their tissues. In a study on the metal toxicity of freshwater mussels, Cassini et al. (1986) observed that cadmium concentration in the gill tissue was maximum while hepatopancreas recorded the lowest. Tallendini et al. (1986) reported that the metal concentration in freshwater bivalves was concentration as well as duration dependent. They also opined that gill responded more quickly to metal exposure than hepatopancreas. Presence of large amounts of calcium concretions in the gills of freshwater mussel *Anodonta* exposed to cadmium chloride was observed by Pynnoenen et al. (1987) who suggested the possible role of these concretions in metal detoxication. Everard and Denny (1984), in a study on the uptake of lead by freshwater mussel, found that the digestive gland is the major site of metal regulation although other tissues may also act as long term immobilisation reservoirs. They also observed that shell acts as a "sink" for the metal ions as the metal ions are found irreversibly bound to shell structure. Dermott and Lum (1986) also have reported of the greater affinity of lead to the shell in freshwater clams. However, Anderson (1977) found an equal distribution of lead in both shell and soft tissues.

Bivalves, being sessile in habit, passively experience the varying conditions in the overlying water coloumn. As they pump large quantity of water for feeding and respiration, they accumulate contaminants and are found to be responsive to many environmental pollutants without showing a prolonged stress (Widdows et al., 1980). Benthic macroinvertebrates are, in general, very sensitive to changes in the environment as they can integrate the combined effect of many stressers through changes in their ecology, morphology and pathology (Bostwick, 1973). Amongst the multitude of benthic organisms, bivalve molluscs such as clams, mussels, and oysters have widely been used as sentinel organisms for assessing and monitoring the chemical and biological effects of pollution (Hung et al., 1975). The capacity of bivalves to concentrate metals makes them useful indicators of pollution (Frazier, 1976; Phillips, 1977; Cunnigham 1979; Zaroogian et al., 1979). Since they have the capacity to concentrate metals, they also have the capacity to sequester the toxicant (Engel and Brouwer, 1982). The ability of lamellibranch molluscs to concentrate heavy metals to levels higher than that of their medium is well documented (See Manly and George, 1977).

A number of abnormal cellular conditions have been reported in mussels and bivalves exposed to pollutants. Presence of

proliferating neoplasmic cells, pollutant-induced alteration in epithelial cells causing epithelial thinning, formation of giant lysosomes associated with membrane destabilisation or lysosomal labilisataion etc. are reported in mussels (Lowe and Moore, 1978).

Reports on the occurrence and accumulation of heavy metals in pelycepods have been limited to marine forms. Reports of heavy metal toxicity studies on the freshwater bivalves are very scanty. Freshwater bivalves are found to be very tolerant to aquatic pollutants by virtue of their inherent metabolic adaptive capabilities, conferring greater survival chances for them when compared to other aquatic forms. Talbot and Magee (1978) found that cadmium, present in the gill and viscera of mussels from cadmium polluted area, is found in high molecular weight metallothionein proteins and low molecular weight aminoacids. Presence of cadmium bound to protein smaller than metallothioneins is also reported (Luten et al., 1986). Seasonal variation in the response of mussels to toxicants is well documented. Akarte and Mane (1988) observed that *Lamellidens marginalis* and *Lamellidens corrianus* are most sensitive to pesticides during summer than in monsoon and winter. Mane and Akarte (1987) also reported that the three species of freshwater

mussels studied, required low pesticide concentration during summer than in winter and monsoon to induce mortality and alteration in behaviour pattern. Stebbing (1976) opined that freshwater organisms are more sensitive to cadmium than marine forms.

Various chemicals that enter the aquatic ecosystem through anthropogenic activities may adversely affect the aquatic fauna and flora, inflicting deleterious changes which disrupt the metabolic activity at the biochemical level. Various studies have been conducted on the effects of heavy metals on the physiological as well as the biochemical aspect of fishes. But only sparse information is available on bivalves, especially freshwater bivalves, regarding the above aspects. Particular information is lacking regarding the effects of heavy metals on enzyme activity pattern. Transaminases are widely distributed enzymes which play an important role in metabolic pathways. The overall number of aminoacids transaminated in freshwater bivalves appears to be lower than that of gastropods and marine bivalves (Falany and Friedl, 1981). Since alanine and aspartic acids are the major components of aminoacids as well as active substances of transamination, catabolism of these aminoacids could be chanelised through them (Falany and Friedl, 1981).

Physiological and biochemical measurements are useful in setting priorities for determining chemicals for which more comprehensive hazard measurement is needed. They are also useful in field investigation (Mehrle and Mayer, 1978). Perturbations at different levels of functional complexity are brought about by pollutants. Xenobiotics are found to inhibit or stimulate enzyme synthesis or cause membrane disturbances by causing damage to lysosomes, mitochondria and enzyme reaction (Moore, 1985). Injury, resulting in destabilisation of lysosomal membrane bears quantitative relationship of the magnitude of response (Bayne et al., 1979, 1982). Displacement of essential metals from metalloenzymes by non-essential metals causes changes in the confirmational shape of the enzyme resulting in the loss of enzyme activity (Friedberg, 1974).

Since effluents from industrial complexes entering the water bodies contain an array of chemicals, it is impractical to monitor water quality by chemical analysis alone. Therefore, it is highly essential to study the biological availability of metals as well as their impact on biological systems. A chemical renders the animal more susceptible to infectious agents limiting its defence mechanism. It has been well established that many of the metals compromise the immune mechanism of the experimental

animals (Koller, 1980). Metals could alter the enzyme activity not only by inhibiting, but also by stimulating the catalytic function of the enzymes (Eichhorn et al., 1969). Metals bind to low molecular weight cytosolic compounds,most of which are substrates of enzymatic reaction (Scoppa, 1975; Zaba and Harris, 1978). Exposure to metals in some way alters the enzyme and changesits response to co-factors, temperature and pH (Jackim, 1974). Exposure to cadmium causes a drain of metabolic energy due to increased enzyme biosynthesis and loss of metabolic flexibility by way of reduced sensitivity to magnesium modulation (Gould, 1976). But metal exerts its toxic effects only when the binding capacity of metallothioneins has been exceeded resulting in an interaction of toxic trace metals with the enzyme pool (Bayne et al., 1983).

Freshwater mussels form an integral part of the ecosystem. They are bottom dwelling and are good markers of aquatic pollution as they absorb all soluble chemicals. Eventhough derth of literature is available regarding metal toxicity in animals, there is a paucity of literature on the influence of toxicants on freshwater mussels. Importance of mussels and clams as a source of protein is widely recognised and freshwater mussel is considered as poor man's food.

Lamellidens corrianus (Lea) is an edible freshwater bivalve common in ponds, pools, and rivers all over India. They live partialy burried in mud or sand and form the cheapest form of nutrient. *Lamellidens* is also of high academic interest. Besides, they are also being used for producing pearl, and their shell used in lime industry. Since large quantites of industrial effluents are being discharged into the rivers, the freshwater mussels are subjected to intense pollution. As heavy metals form a major component of the industrial effluents, it was thought worth while to undertake a study on the effect of these metals on this freshwater mussel. Mercury, copper, and cadmium are metals toxic to many organisms even at very low concentrations. Barring copper, these metals are not at all beneficial to living beings. Copper, mercury, and cadmium were chosen as the challenge metals because of their widespread contamination of the aquatic system as well as their high toxicity to aquatic life. Cadmium pollution is of particular interest because of their cumulative toxic effect. The metal toxicity was assessed by studying the effect of the above metals on two tissues, gill and hepatopancreas. Gill is intimately involved in osmoregulation as well as respiration and hence the study on the effect of the metal on gill tissue is of paramount importance. Gill is found to be the initial target of metals. Hepatopancreas has also been

proved to be the target organ for the deposition of heavy metals like mercury (Vernberg and Vernberg, 1972). Hepatopancreas acts as a "sink" for a variety of toxic substances (Gibson and Barker, 1979). Hepatopancreas also plays the pivotal role in metal detoxication, as it is rich in lysosomes (Summer, 1969; Owen, 1972; Moore and Halton, 1973, 1977; Moore, 1976). The study of enzyme offers potential for the development of both general and specific sublethal indices of stress (Bayne et al., 1985).

If contaminants or abnormal condition affect the homoeostatic mechanism that controls concentration of biochemicals in organism, measurement of affected metabolites in normal and stressed organism may provide a means of evaluating degraded aquatic habitats (Gardner et al., 1981). More over, a better understanding of the mode of action of toxicants in the aquatic organism is necessary to predict the potential harm of various pollutants discharged into the environment. The present study is intended :(i) to gain more information regarding the internal disturbances in mussels on exposure to heavy metals, and (ii) also to enhance our understanding of their mode of action.

C H A P T E R - II

EFFECT OF HEAVY METALS ON THE RATE OF OXYGEN UPTAKE

2.1 INTRODUCTION

Industries built adjacent to rivers and other bodies pollute the very aquatic environment by easy disposal of their waste. Pollutants seriously alter the environmental conditions of the affected area causing serious damage to the aquatic life. Bivalves are known to concentrate heavy metals in their tissues resulting in a shift in the physiological activities (Phillips, 1977; George and Coombs 1977; Lomte and Jadhav, 1982). As pollutants gain entry into the body of the bivalve through the gills, the first physiological function to be affected is oxygen uptake. Anderson et al. (1974) remarked that respiratory response of an animal may be merely indicative of its ability to regulate in some way to an irritating environment.

Several studies have stressed the possible deleterious effects of mercury and cadmium on aquatic organisms (Eisler, 1971; Calabrese and Nelson, 1974; Eknath and Menon, 1979 a; George and Frazier, 1982). Changes in oxygen consumption serve as a good indicator of stress, and are used in evaluating changes in the metabolism due to alteration in the environment (Hawkins et al., 1986). Metal induced variation in oxygen consumption is amply recorded in invertebrates (MacInnes and

Thurberg, 1973; Saliba and Vella, 1977; Waldichuk, 1974; Torreblanca et al., 1987). Metal induced changes alter from metal to metal and species to species (Thurberg et al., 1974; Bryan, 1976). Sublethal concentrations of metals are found to either depress or elevate functions, especially respiration.

Mercury, cadmium, and zinc are found to depress respiration in *Perna indica* (Baby and Menon, 1986 a). Variability in oxygen uptake due to copper stress is reported by Brown and Newell (1972). Brown and Newell (1972), reported that inhibition in respiration in *Mytilus edulis* by heavy metals was due to suspension of ciliary activity rather than direct inhibition of respiratory activity. Mohan et al. (1986 b) found mercury and cadmium acting as respiratory depressants in *Perna viridis*. Salanki (1965, 1968) found that bivalves close their valves under unfavorable conditions resulting in decreased oxygen uptake. In *Meretrix casta*, and *Mytilus edulis*, copper acted as respiratory depressant (Scott and Major, 1972) while copper had no effect on respiration in *Carcinus maenus* (Thurberg et al., 1973). Anaerobic respiration following metal exposure is documented in *Scorbicularia* (Akberali and Black, 1980).

Bivalves show great variability in respiratory rate. Even under constant external condition, oxygen consumption of a given

species is variable (Wilbur and Yonge, 1966). Fluctuations in respiratory levels are demonstrated in *Ostrea* (Galtsoff and Whipple, 1930) and *Anadora* species (Weinland, 1919). Patil and Kaliwal (1983) found decrease in oxygen consumption followed by increase on exposure to metals. Disruption in oxygen uptake and osmoregulatory function due to metal toxicity is reported by Sullivan and Cheng (1975) in gastropods.

The variation in oxygen consumption may not be essentially due to the direct effect of metal on the respiration rate. It can be due to the effect on filtration rate or ciliary activity (Mathew and Menon, 1983). Morphological changes in gill epithelial cells after exposure to mercury are reported (Vernberg et al., 1974; Bubel, 1976). Experimental studies with zinc have been found to impair gill respiration (Lloyd, 1960; Skidmore, 1970). Gill damage resulting from zinc toxicity modifies gas exchange and create hypoxia at tissue level (Skidmore, 1970).Anderson (1978) reported decrease in oxygen uptake in animals exposed to lead. Disruption of gill structure following heavy metal exposure, resulting in variation in respiration and osmoregulation is well documented in crustaceans and fishes (Baker, 1969; Eisler and Gardner, 1973; Jones, 1975).

Shaffi (1978 a) stated that coagulation of mucus on gills

might have reduced the oxygen transfer to various internal organs when the animal is subjected to pollutants. Activity of cilia of inner and outer gills is differentially modulated by pesticides in freshwater mussel (Basha and Swami,1987). Increase in oxygen consumption under pesticide stress is reported in *Lamellidens corrianus* (Muley and Mane, 1987). Closure of valves in response to stress and thereby decrease in oxygen uptake is reported in bivalves. (Gilles, 1972; Bayne, 1973). Variation in the rate of respiration in relation to concentration and exposure period is reported (Chinnaya, 1971; Collier et al. 1973). Severe pathological changes were observed by Couch (1977) in gills of crustaceans exposed to mercury, cadmium, copper, and chromium.

Respiratory chain is of vital importance for the supply of energy to the living cells. It is an important physiological index reflecting the animal's overall functional well being. As respiration is sensitive to an array of environmental and biological variables, respiration may be used as a tool for the assessment of the toxic effects of a pollutant. The nature of the respiratory response varies between species as well as toxic substances and is often a function of concentration of toxicant (Verriopoulos et al., 1986). Oxygen consumption rate in mud snail *Nassarius obsoletus* had been found to be lowered by copper,

arsenic, silver and zinc but elevated by cadmium (MacInnes and Thurburg, 1973). Extensive alteration in the ultra structure of gill tissue filaments due to mercury exposure is reported in crabs (Vernberg et al. 1974). Lobster was shown to elevate gill tissue oxygen consumption on exposure to cadmium (Thurberg et al., 1977) in contrast to depression in oxygen consumption in gill tissue of green crab *Carcinus maenas* (Thurberg et al., 1973). Jones (1942) found stimulation of respiratory rate in *Gammarus pulex* and *Polycellis nigra* when exposed to copper and mercury. Similar observation with reference to copper was reported by Hunter (1949). But Corner and Sparrow (1956) found copper and mercury as respiratory depressants. Eisler et al. (1972) reported that the level of cadmium doubled in the gills of lobster after exposure to the metal, resulting in changes in respiration. Elevation of gill tissue oxygen consumption under stress is reported by Thurberg et al.(1977).

Decline in oxygen consumption on exposure to chromium suggests that chromium interferes with energy supplying metabolic processes, resulting in inhibition of ciliary activity and vice-versa (Capuzzo and Sasner, 1977). Cadmium is also reported to stimulate oxygen consumption in zoo plankton (Kettle et al., 1980). Exposure to silver resulted in increased oxygen

Oxygen consumption is a useful measure of sublethal effects because energy processes serve as an indicator of overall physiological state (Sigmon, 1979). This chapter deals with the study on the effect of heavy metals on the rate of oxygen uptake by *L. corrianus*.

2.2 MATERIAL AND METHODS

2.2.1 Test animal

The animal selected for the study is the common freshwater mussel, *Lamellidens corrianus* (Lea). *Lamellidens corrianus* is a freshwater mussel common in the ponds and rivers. Specimens were collected from Desam, near Aluva, Ernakulam district, Kerala.

2.2.2 Laboratory conditioning of the animal

After collection, the clams were immediately taken to the laboratory, with minimum disturbance, in polythene bags filled with water. In the laboratory they were maintained in large fibre glass tanks, containing well aerated, chlorine free water of pH 7-7.5 and temperature 30 $\pm$ 1^{o}C. The clams were acclimated to laboratory conditions for 48 hrs and during the period of acclimation they were fed with green algae, *Scenedesmus*. The medium was changed every 24 hrs. All animals taken for the study

were of the same population.

2.2.3 Selection of animals

The animals selected for the study belonged to the size group of 5.5 cm ± 1 cm. The above size group, being the common size group, was selected for the study. After acclimatising the animals for 48-96 hrs they were transferred to the test medium for the required period of time.

2.2.4 Toxicity studies

Lethal toxicity of individual toxicants

Lethal toxicity studies provide information regarding the relative lethality of a toxicant. This test is designed to determine the highest concentration of a pollutant which will cause 50% mortality of a limited number of organisms. The static renewal test technique was employed for the study as described in APHA (1975). Exploratory tests were conducted prior to acute toxicity studies.

For lethal toxicity studies, laboratory conditioned mussels of uniform size (5.5± 1cm) were exposed to the test medium, containing a series of concentrations of the toxicants. The toxicants selected were copper, mercury, and cadmium. Standard

solutions of the metals were prepared by dissolving the salts of the selected metals in glass distilled water. Standard solutions of copper and cadmium were prepared using CuSo4 5 H_2o (Qualigens, AR grade) and 3 CdSo4 8 H_2o (Sarabhai chemicals, AR grade) respectively. Standard solutions of mercury was prepared by dissolving $Hgcl_2$ (Qualigens, AR grade) in glass distilled water. Fibre glass tanks were used for the experiment. Ten animals were selected for each concentration. The experimental tanks were kept covered to minimize external disturbances. The tests were carried out at room temperature (30 $\pm$ 1.5^{o}C). The animals were not fed during the experiment. Appropriate replicates and controls were invariably maintained for all the experiments. The test media were replenished totally every 24 hr. The animals were inspected at regular intervals of 12 hrs and the cumulative mortality was recorded. Valve gaping beyond 5mm and/or inability of the animal to close the valves under mechanical stimulation were the indices of death. The 96 hr LC50 value was calculated by Probit Analysis (Finney, 1971). The LC50 values were 2.66 ppm, 2.278 ppm, and 2.994 ppm for copper, mercury, and cadmium respectively.

2.2.5 Sublethal toxicity studies

The objective of these studies was to find out changes in

the activities of the animals under the stress of sublethal concentrations of metals. Three sublethal concentrations were selected for each metal. For copper, the concentrations selected were 100, 200, and 400 ppb and for mercury, the selected concentrations were 75 ppb, 150 ppb, and 300 ppb. The sublethal concentrations for cadmium were 150 ppb, 300 ppb, and 600 ppb. Appropriate quantities of the above solutions were added to the test media to get the respective metal concentrations. Assessment of the different parameters was carried out at 24, 72, 120 and 168 hrs. The test medium was replenished every 24 hr. Controls were invariably run along with the experimentals. Standard solutions of the different metals were prepared by dissolving the corresponding salts in glass distilled water. Appropriate quantities of the above solutions were added to the test media to get the respective metal concentrations.

2.2.6. Rate of oxygen uptake

For each concentration as well as control the total number of animals used was six. Animals pre- exposed to the respective toxicant concentration for different periods of 24, 72, 120 and 168 hrs were taken for oxygen uptake studies. The animals were kept in conical flasks of 2 l capacity containing 2 l of the test medium, along with a control group. To avoid gaseous exchange

with the atmosphere, the water column in the experimental flasks was sealed with inert liquid paraffin. After one hour a definite volume of the water sample was drawn from the flask and the oxygen content was determined by the Winkler method. After the experiment, the animals were dissected, the soft tissues removed, cleaned in distilled water and dried at 70-80 ^{o}C for 48 hrs and the dry weight recorded to constancy. For each concentration the total number of animals used was six. Oxygen consumption was expressed as mg O_2 $^{h-1}$ gm^{-1} (dry wt).

The data were statistically analysed by student's `t' test (Croxton et al., 1975) to manifest the variation in comparison with the controls. The variations were represented at three significance levels, viz. $P<0.05$, 0.01, 0.001. All the computations were carried out using a personal computer (Casio fx - 730P).

2.3 RESULTS

2.3.1 Copper (Table 1, Figure, 1)

When 100 ppb copper pre-exposed animals were compared with the control, significant reduction in oxygen consumption was noticed at 24 and 72 hr ($P<0.001$). But at 120 and 168 hr time period the change in oxygen consumption was not significant. In 200 ppb pre-exposed mussels, reduction in oxygen uptake was

Table. 1 Rate of Oxygen uptake in Lamellidens corrianus exposed to three sublethal concentrations of copper (mg $O_2 h^{-1} gm^{-1}$ dry wt)

	Hours	24	72	120	168
Control	N	6	6	6	6
	Mean value	0.819	0.808	0.771	0.761
	± SD	0.040	0.034	0.035	0.041
	Range	0.774-0.845	0.753-0.852	0.711-0.820	0.698-0.768
100 ppb of copper exposed	N	6	6	6	6
	Mean value	0.336 ***	0.233 ***	0.658	0.676
	± SD	0.172	0.053	0.143	0.100
	Range	0.175-0.570	0.145-0.291	0.480-0.864	0.205-0.821
200 ppb of copper exposed	N	6	6	6	6
	Mean value	0.432 ***	0.376 ***	0.475 ***	0.457 **
	± SD	0.109	0.099	0.077	0.152
	Range	0.313-0.552	0.250-0.541	0.362-0.600	0.294-0.663
00 ppb of opper xposed	N	6	6	6	6
	Mean value	0.444 ***	0.516 ***	0.586 *	0.521 ***
	± SD	0.137	0.069	0.138	0.070
	Range	0.223-0.657	0.462-0.621	0.442-0.804	0.449-0.520

ignificant levels *$P<0.05$ **$P<0.01$ ***$P<0.001$

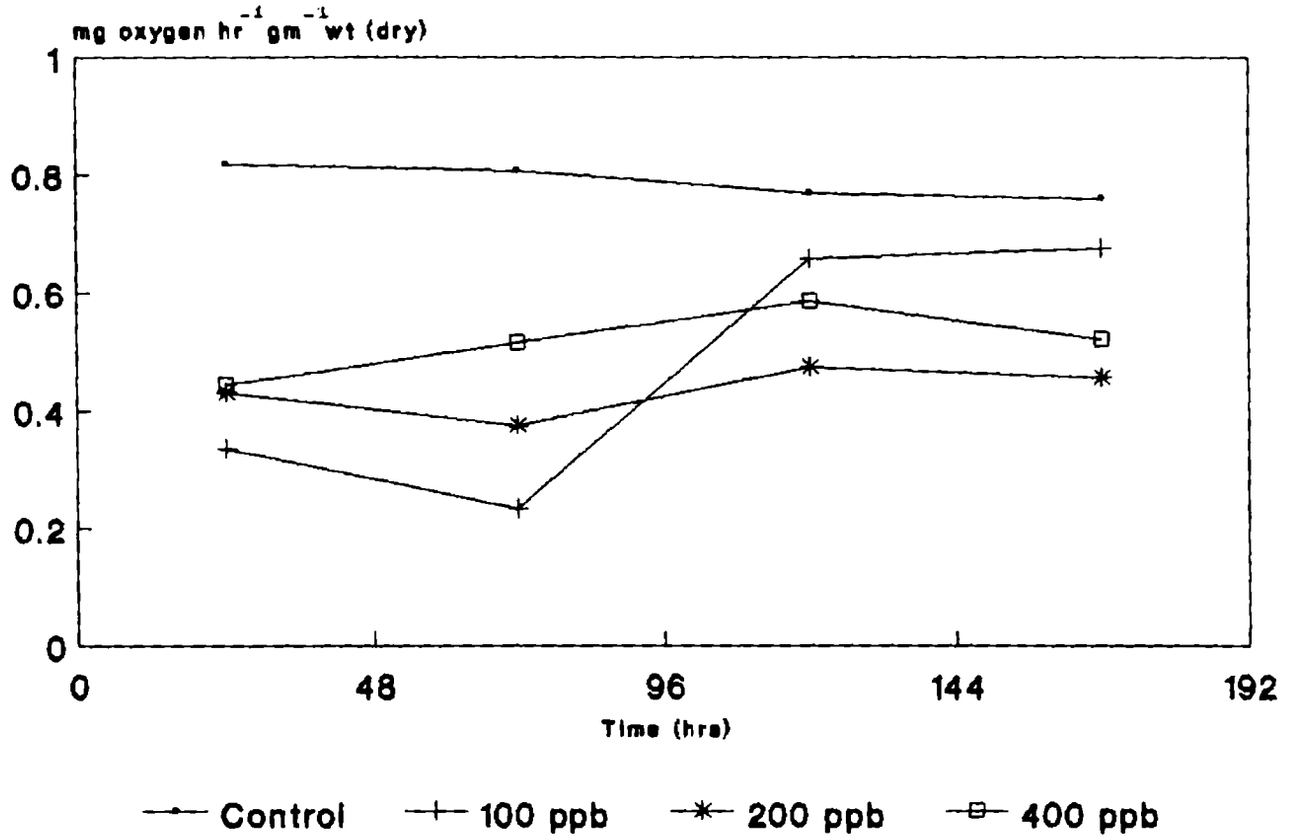


Figure 1. Rate of oxygen uptake in *Lamellidens corrianus* exposed to three sublethal concentrations of copper.

observed at all time periods ($P<0.001$, 0.001, 0.001 and 0.01) respectively. Mussels pre-exposed to 400 ppb also exhibited significant reduction in oxygen consumption at all time periods ($P<0.001$, 0.001, 0.05, 0.001).

When the rate of oxygen uptake in 100 and 200 ppb pre-exposed animals, was compared, significantly higher rate of oxygen consumption was observed in 200 ppb pre-exposed animals at 72 hr ($P<0.05$) while at 120 and 168 hr, the 100 ppb pre-exposed animals had higher rate of oxygen consumption ($P<0.05$). When 100 and 400 ppb pre-exposed animals were compared, significantly higher rate of oxygen consumption was observed at 72 hr in 400 ppb pre-exposed animals ($P<0.001$). When 200 and 400 ppb pre-exposed mussels were compared, higher value was found at 72 hr in 400 ppb pre-exposed animals ($P<0.05$).

2.3.2 Mercury (Table 2, Figure 2)

When mussels pre-exposed to mercury were compared with that of control, lower rate was observed in all the three concentrations at all time periods ($P<0.001$).

Comparison of mussels pre-exposed to 75 and 150 ppb showed significant variation at 24 hr pre-exposure. At 24 hr, 75 ppb

Table. 2 Rate of Oxygen uptake in *Lamellidens corrianus* exposed to three sublethal concentrations of mercury (mg O_2 h^{-1} gm^{-1} dry wt)

	Hours	24	72	120	168
Control	N	6	6	6	6
	Mean value	0.819	0.808	0.771	0.761
	± SD	0.040	0.034	0.035	0.041
	Range	0.774-0.845	0.753-0.852	0.711-0.820	0.698-0.768
75 ppb of mercury exposed	N	6	6	6	6
	Mean value	0.592 ***	0.499 ***	0.547 ***	0.228 ***
	± SD	0.070	0.082	0.095	0.085
	Range	0.498-0.688	0.390-0.576	0.467-0.679	0.133-0.328
150 ppb of mercury exposed	N	6	6	6	6
	Mean value	0.278 ***	0.535 ***	0.508 ***	0.305 ***
	± SD	0.056	0.110	0.063	0.021
	Range	0.184-0.326	0.418-0.681	0.405-0.564	0.284-0.335
300 ppb of mercury exposed	N	6	6	6	6
	Mean value	0.199 ***	0.398 ***	0.340 ***	0.309 ***
	± SD	0.050	0.070	0.033	0.066
	Range	0.133-0.259	0.315-0.478	0.301-0.378	0.242-0.395

Significant levels *** $P<0.001$

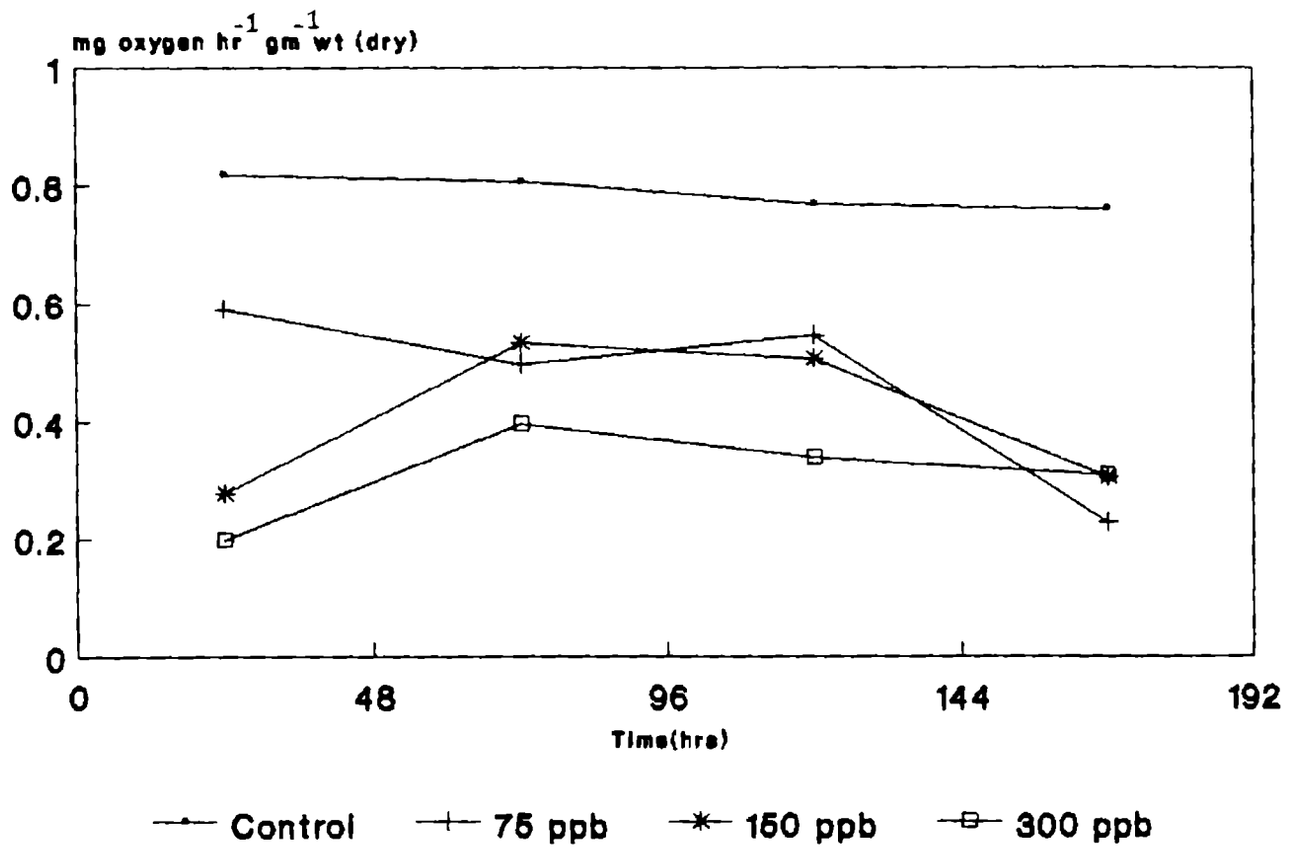


Figure 2. Rate of oxygen uptake in *Lamellidens corrianus* exposed to three sublethal concentrations of mercury.

Table. 3 Rate of Oxygen uptake in Lamellidens corrianus exposed to three sublethal concentrations of cadmium (mg O_2 h^{-1} gm^{-1} dry wt)

	Hours	24	72	120	168
Control	N	6	6	6	6
	Mean value	0.819	0.808	0.771	0.761
	± SD	0.040	0.034	0.035	0.041
	Range	0.774-0.845	0.753-0.852	0.711-0.820	0.698-0.768
150 ppb of cadmium exposed	N	6	6	6	6
	Mean value	0.349 ***	0.147 ***	0.170 ***	0.185 ***
	± SD	0.069	0.024	0.040	0.012
	Range	0.245-0.407	0.113-0.167	0.120-0.214	0.168-0.200
300 ppb of cadmium exposed	N	6	6	6	6
	Mean value	0.110 ***	0.134 ***	0.170 ***	0.207 ***
	± SD	0.028	0.040	0.040	0.011
	Range	0.084-0.155	0.097-0.208	0.120-0.214	0.185-0.215
500 ppb of cadmium exposed	N	6	6	6	6
	Mean value	0.102 ***	0.103 ***	0.116 ***	0.183 ***
	± SD	0.015	0.004	0.006	0.015
	Range	0.084-0.118	0.098-0.109	0.108-0.129	0.158-0.200

Significant levels ***$P<0.001$

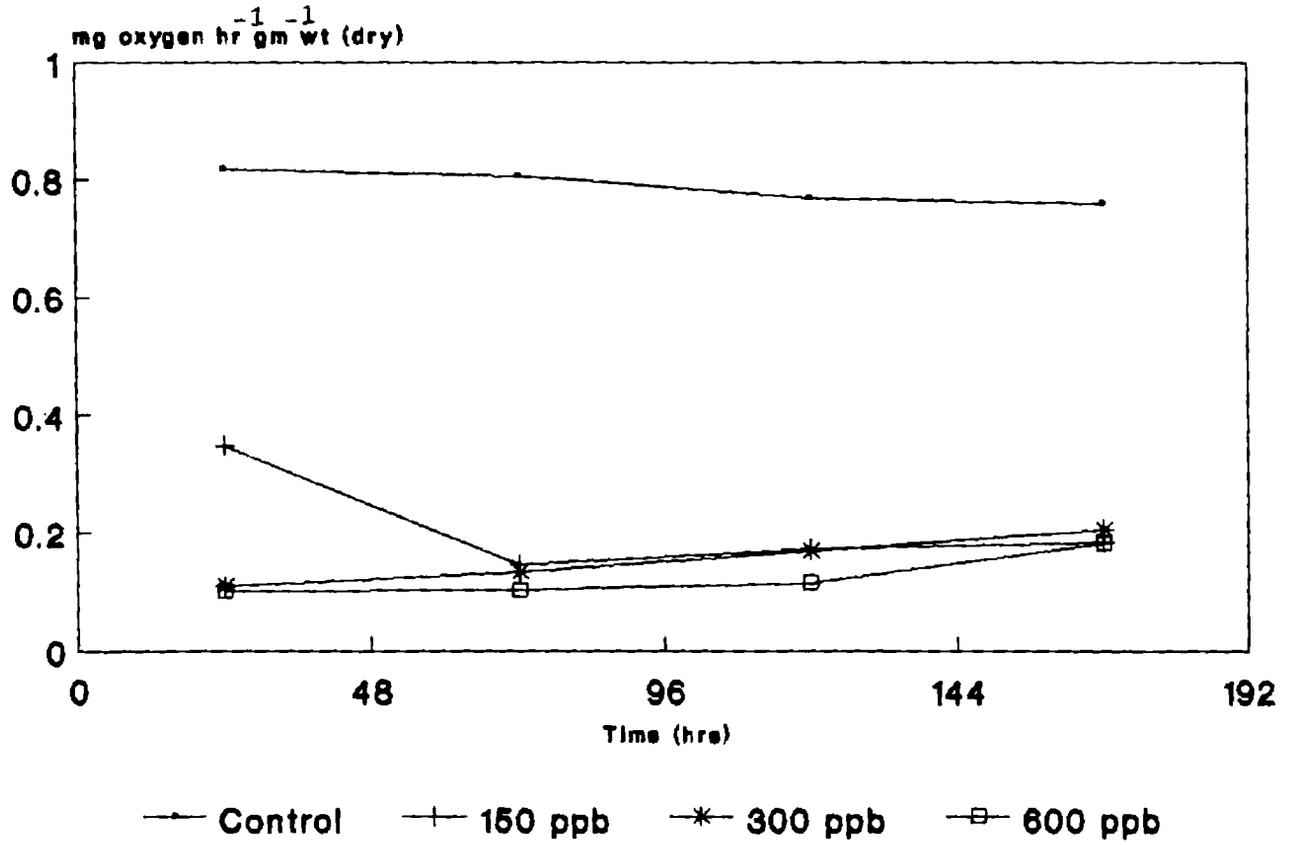


Figure 3. Rate of oxygen uptake in *Lamellidens corrianus* exposed to three sublethal concentrations of cadmium.

pre-exposed animals showed higher rate of oxygen consumption ($P<0.001$). When 75 and 300 ppb pre-exposed animals were compared, 75 ppb pre-exposed ones showed higher rate of oxygen uptake at 24 and 120 hr ($P<0.001$). On comparison between 150 and 300 ppb pre-exposed animals, 150 ppb pre-exposed ones showed significantly higher values at 24, 72 and 120 hr ($P<0.05$, 0.05 0.001)

2.3.3. Cadmium (Table 3, Figure 3)

When animals pre-exposed to cadmium werecompared with the control, lower oxygen uptake rate was shown by all the experimentals ($P<0.001$).

When 150 and 300 ppb cadmium pre-exposed mussels were compared, 150 ppb pre-exposed ones showed higher value at 24 hr butlower value at 168 hr ($P<0.001$, 0.01). In a comparison between 150 and 600 ppb pre-exposed mussels, animals pre-exposed to 150 ppb showed higher rate at 24, 72 and 120 hr ($P<0.001$,0.01, 0.05). 300 ppb pre-exposed animals showed higher rate of oxygen consumption at 120 and 168 hr ($P<0.05$) when compared with 600 ppb pre-exposed ones.

2.4 DISCUSSION

Copper exposed animals showed significant reduction in

oxygen consumption during 24 and 72 hr time period in all the experimentals. Comparatively higher reduction in value was found at early time periods in 100 ppb exposed animals. However, towards the later stage of the experiment those exposed to 100 ppb copper reverted to normal level of oxygen uptake. The oxygen uptake values at higher concentrations were lower at all time periods. Sublethal concentrations of metals are reported to either depress or elevate rate of respiration. Copper, zinc, and silver were found to depress oxygen consumption in *Perna viridis* and *Meretrix casta* (Baby, 1987). Reduction in oxygen consumption at low concentrations of copper and temporary elevation at medium concentrations were reported by Mathew and Menon (1983). But such a feature was not observed in the present study. Reduction in oxygen uptake under copper stress is amply recorded in bivalves (Brown and Newell, 1972; Scott and Major, 1972; Prabhudeva and Menon, 1986). In the case of copper stressed animals it can be seen that the reduction in oxygen uptake during 24 hr time period was an immediate response to the toxicant. The retrieval to control level may be related to the detoxification process taken place in the lower concentration of the toxicant. It can also be well assumed that damage caused to the gill tissue was minimum, and hence the retrieval. Increasing copper concentrations causing increase and decrease in the rate of

respiration were also reported in barnacles (Bernard and Lane, 1961). Marked changes in oxygen uptake in lower concentrations of toxicants were reported by Anderson et al. (1974), and Verriopoulos et al. (1986). The present study has clearly indicated that the rate of oxygen consumption is seriously affected in all the concentration tested, and in the lower concentration as the time period increased, the animal is able to adjust to the normal conditions, but in higher concentrations, retrival is never attained.

In mercury exposed animals, rate of oxygen uptake was drastically reduced at the initial and final periods of the experiment. The immediate response of the bivalve to a toxicant is shell closure. Shell closure and resultant reduction in oxygen uptake are responsible for the drastic decline in oxygen uptake during the initial period particularly at higher concentrations. Mercury is found to depress oxygen uptake in *Perna viridis* (Baby and Menon, 1986 b; Mohan et al., 1986 b). The reduction in oxygen uptake on prolonged exposure may be due to gill tissue impairment or due to binding of mercury to mucus secretion resulting in a faulty gaseous exchange. Depression in oxygen uptake by mercury is reported in crustaceans (Vernberg and Vernberg, 1972; Vernberg et al. 1974; Depledge, 1984). Mercury

is also found to act as a respiratory depressant in *Lamellidens marginalis* (Venkatsubbaia et al., 1984).

As in the case of copper and mercury exposed animals, oxygen uptake rate in cadmium exposed animals was also drastically reduced throughout the exposure period. Cadmium has been reported to be extremely toxic to aquatic forms and that its toxicity is cumulative (Ray and Coffin, 1977). Mohan et al. (1986 a) have found that mercury and cadmium depress the rate of filtration to a considerable extent. The consistantly low uptake of oxygen in cadmium exposed animals may be attributed to the impairment of filtration causing less water to be irrigated over the gills. The present result is in agreement with the observation of Radhakrishnaiah (1988) who reported reduction in oxygen consumption in cadmium stressed mussels. Radhakrishnaiah (1988) also reported reduction in ciliary beat in the cadmium exposed freshwater mussels and indicated irrevocable suppression of oxygen uptake in lethal cadmium concentrations and reversible suppression in sublethal concentrations. The present observation is in disagreement with the above report, as no revival in oxygen uptake was observed. Reduced efficiency of gill tissue exposed to heavy metals was reported by Jones (1947). Gill tissue damage by cadmium is also reported by Eisler (1971) and Nimmo et al. (1977).

Respiratory depression may be due to either valve closure or direct metabolic effect. A more or less uniform irrevocable depression in oxygen consumption in cadmium exposed animals indicates cellular damage of gills and cilia bearing cells. Similar observations were made by Prabhudeva and Menon (1986) in *Perna viridis* subjected to copper stress. Depression in gill tissue oxygen consumption is reported in crabs subjected to cadmium and copper toxicity (Thurberg et al., 1973). Oxygen uptake is very much dependent on the rate of filtration. Reduction in filtration rate by heavy metal is widely reported (Reading and Buikema, 1980; Prabhudeva and Menon, 1985; Abraham et al., 1986). Inhibitory effect of copper, silver, and zinc on filtration ability of mussels has also been reported (Abel, 1976; Murthy, 1982; Mathew and Menon, 1984). Rate of filtration and oxygen consumption are also directly related to valve closure mechanism. Phillips (1977) suggested that the pollutant itself might elicit valve closure, thereby decreasing the rate of filtration.

Reduction in oxygen consumption with increasing concentration of mercury and cadmium is documented in *Perna viridis* (Mohan et al., 1986 b). Oxygen consumption in bivalves is the product of two factors, ventilation rate, and efficiency

of gas exchange. Filtration rate, in turn, can be affected by the frequency of ciliary activity and changes in the level of gill irrigation (Reddy and Menon, 1979). Reduced filtration rate under pollutant stress was reported by Epifanio and Srna (1975). Effect of heavy metals on clearance rate of mussels was worked out by Abel (1976), Eknath and Menon (1979 b), Mathew and Menon (1984) and Mohan et al. (1986 b)

Behavioural responses such as frequency of shell closure, siphonal activity, and rate of gill irrigation influence rate of oxygen uptake. The toxicant stress that results in the alteration or modification of any of the above processes can affect the rate of oxygen consumption (Mathew and Menon, 1983). Dunning and Major (1974) also opined that oxygen uptake depends on the rate of movement of water over the gill surface. The comparatively greater reduction in oxygen consumption in cadmium exposed animals may be due to the fact that cadmium is depurated slowly from the body of the animal as suggested by (Viarengo, 1985).

In general all these metals were found to adversely affect the rate of oxygen uptake.

C H A P T E R - III

EFFECT OF HEAVY METALS ON TISSUE GLYCOGEN CONTENT

3.1 INTRODUCTION

In lamellibranch molluscs glycogen is stored in considerable quantity in certain tissues, while in other tissues it is insignificant. Mane et al.(1986) conducted biochemical studies on some freshwater mussels and found that mantle was the most prominent glycogen storage site. Hepatopancreas and gonad were also found to have considerable glycogen reserve while gill contained the least. The glycogen level in mussel tissues is subjected to seasonal as well as age dependent variation (Madar and Pora, 1981). Variation in glycogen level also occurs in aestivating mussels (Sahib et al., 1983). Coasta and Indrasena (1984) have studied the proximate composition of the freshwater mussel *Lamellidens lamellatus*. Heavy metals are potential metabolic inhibitors of aquatic as well as terrestrial organisms as they induce several functional alterations in the animal's body. Heavy metals, like many other pollutants, cause hypoxia or anoxia. Hypoxia causes reduction in oxidative metabolism at mitochondrial level and anoxia or hypoxia causes increase in carbohydrate consumption (DeZwaan and Zandee, 1972). Moorthy et al.(1985), in a study on the glucose metabolism in hepatopancreas and gill of *Lamellidens marginalis* exposed to pesticide, found

increase in lactate content indicating reduced mobilisation of pyruvate into TCA cycle. Occurrence and significance of various anaerobic pathways other than glycolysis have been reported in invertebrates (Hochachka et al., 1973). Alanine and succinate are found to be the end products of glucose degradation in the mussel *Rangia* (Stokes and Awapara, 1968). Badman and Chin (1973) have worked on the metabolic responses of freshwater mussel *Pleurobema coccineum* under anaerobic conditions. Engel et al.(1972) suggested that toxicants can cause metabolic alterations in bivalve molluscs leading to an increase in glucose catabolism concomitantly with a decrease of gluco-neogenesis flux. In the change from aerobic to anaerobic metabolism in bivalves, regulation of pyruvate kinase is important (De Vooys, 1980). Wernstedt (1944) studied the metabolism of gill epithelium of *Druissena* and found that glycogen disappears from the gill three times faster under anaerobic condition than under aerobic. Rao and Chari (1981) reported that bivalve molluscs, in general, possess high glycogen content, and depend more on glycogen metabolism. Generally, glycogen and protein are the energy source in molluscs, but fat may also act as additional source (Shulman, 1974). High succinic dehydrogenase activity, regarded as an index of overall glycolytic metabolism by Hammen, (1975), is reported in *Lamellidens corrianus* (Rao and Chari, 1981), and

overall metabolic depression is reported in *Lamellidens corrianus* on toxicant exposure (Kulkarni et al., 1984). Metabolic substrate utilised for catabolism varies in molluscs. Some have lipid oriented metabolism (Emerson and Duerr, 1967) while others rely on carbohydrates for energy (Emerson, 1967). Lowering of tissue glycogen due to heavy metal toxicity is reported in invertebrates (Garanina, 1984)

Heavy metals have disruptive influence on structural organisation of gill tissue (Skidmore and Tovell, 1972; Sunila and Lindstorm, 1985). Most research on effects of cadmium on carbohydrate metabolism has been performed in vertebrates (Sporn et al., 1970; Ithakissios et al., 1974; Singhal et al., 1974). Shore et al. (1975) in a study on the effect of cadmium in Limpet, found progressive reduction in glycolytic rate as tissue cadmium concentration increased. Pollution by heavy metals has been shown to profoundly affect respiration and carbohydrate metabolism in molluscs (Mac Innes and Thurberg, 1973; Shore et al., 1975). The activity of glycogen synthesising enzymes is inhibited to different extends, depending on the toxicant used and duration of exposure (Gormosova, 1979). He also observed that glycogen is intensively utilised and excessive oligosaccharides are accumulated in mussels when they switch over

to anaerobic respiration during hypoxic and toxic conditions.

In this chapter results of the study on glycogen content in tissues of the mussel exposed to heavy metals are reported.

3.2. MATERIAL AND METHODS

Methods of collection of specimens, acclimation and exposure to toxicants were the same as described in Chapter 2. For each concentration of each metal as well as control, eight animals were used.

The supernatant of both the tissue extract (gill and hepatopancreas) in TCA was taken for the estimation of glycogen. The tissue glycogen was determined following the method of Montgomery (1957). To 1ml of the supernatant, 1.2 ml of 95% ethyl alcohol was added and kept undisturbed overnight in a refrigerator and then centrifuged at 2500 rpm for 15 minutes. The supernatant was gently decanted. The precipitate was dissolved in 2.0 ml distilled water and 0.1 ml of 80% phenol was added and mixed well. To this 5 ml of conc.sulphuric acid was added forcefully with a blowout pipette for thorough mixing and kept for 30 minutes at room temperature. After cooling, the optical density was read at 490 nm in HITACHI U 2000 UV-VIS

spectrophotometer and the glycogen present in the tissue extract was measured from a standard graph using glucose as the standard. The glycogen present in the extract was expressed as mg/gm wet wt(glucose equivalents)

3. 3 RESULTS

3.3.1 Copper

3.3.1.1 Gill tissue (Table 4, Figure 4)

Significantly lower values were exhibited by mussels exposed to varying concentrations of copper at all time periods when compared with the control values. In 100 ppb exposed animals significantly lower values were obtained at all sampling periods ($P<0.001, 0.001, 0.05$, and 0.001). At 200 ppb also reduction in values was highly significant at all time periods, when compared with the control values ($P<0.001$), while at 400 ppb, reduction in glycogen values was significant at all time periods ($P<0.01$, 0.001, 0.001) except at 72 hrs

Mussels exposed to 100 ppb of copper had higher values than that of mussels exposed to 200 ppb at 120 hr ($P<0.001$) but lower values at 72 hr ($P<0.01$) than that of those exposed to 400 ppb. 100 ppb exposed mussels had higher value at 120 and 168 hr also

able 4. Gill Glycogen (mg glucose/gm wet wt) in Lamellidens corrianus exposed to three sublethal concentrations of copper

	Hours	24	72	120	168
ontrol	N	8	8	8	8
	Mean value	2.528	2.501	2.487	2.297
	± SD	0.632	0.407	0.503	0.669
	Range	1.625-3.430	2.094-3.126	1.925-3.015	1.378-3.249
00 ppb of opper xposed	N	8	8	8	8
	Mean value	1.083 ***	1.041 ***	1.982 *	0.907 ***
	± SD	0.238	0.272	0.176	0.152
	Range	0.844-1.512	0.839-1.666	1.747-2.300	0.627-1.128
00 ppb of opper xposed	N	8	8	8	8
	Mean value	1.190 ***	1.020 ***	1.489 ***	1.188 ***
	± SD	0.201	0.382	0.257	0.383
	Range	0.956-1.540	0.541-1.512	1.135-1.723	0.741-1.775
00 ppb of opper xposed	N	8	8	8	8
	Mean value	1.292 ***	2.134	1.495 ***	0.708 ***
	± SD	0.517	0.652	0.350	0.102
	Range	0.702-2.140	1.206-2.832	0.990-1.952	0.584-0.791

ignificance level : *P<0.05 **P<0.01 ***P<0.001

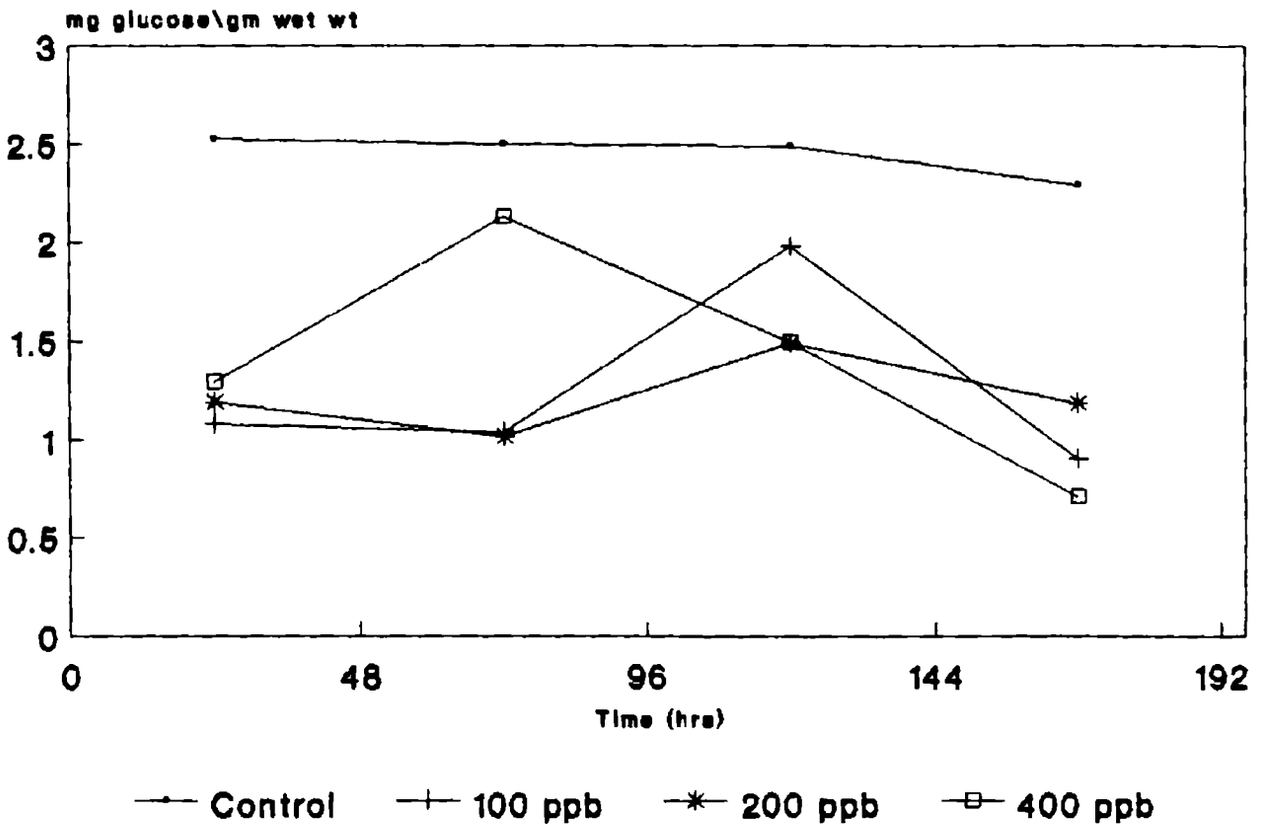


Figure. 4. Gill Glycogen (mg glucose/gm wet wt) in *Lamellidens corrianus* exposed to three sublethal concentrations of copper.

when conpared with 400 ppb exposure ($P<0.01$, 0.05). Those exposed to 200 ppb had lower value at 72 hr ($P<0.01$) but higher at 168 hr ($P<0.01$) than those exposed to 400 ppb.

3.3.1.2 Hepatopancreas tissue (Table 5, Figure 5)

Mussels exposed to 100 and 200 ppb copper showed significant reduction in glycogen content in the hepatic tissues when compared with controls ($P<0.001$) at all time periods. At 400 ppb, also there was significant reduction in values at all time periods ($P<0.001$, 0.01, 0.001, 0.001)

When values of 100 ppb exposed mussels were compared with those of 200 and 400 ppb exposed ones no significant variation was found between the values of 100 and 200 ppb exposed ones at any exposure period, while a consistant reduction in values was observed in 100 ppb exposed ones at all time periods except at 168 hr, when compared with those exposed to 400 ppb ($P<0.001$, 0.001, 0.001). Those exposed to 400 ppb registered significant elevation at all time periods, except at 168 hr ($P<0.001$,0.001,0.01) when the values were compared with those exposed to 200 ppb.

Table 5. Hepatopancreas Glycogen (mg glucose/gm wet wt) in Lamellidens corrianus exposed to three sublethal concentrations of copper

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	10.572	10.229	11.396	9.951
	± SD	1.552	2.134	2.660	2.337
	Range	9.060-13.252	6.789-13.423	9.253-17.216	7.895-14.895
100 ppb of copper exposed	N	8	8	8	8
	Mean value	4.423 ***	2.222 ***	2.120 ***	2.139 ***
	± SD	0.648	0.405	0.218	0.400
	Range	3.677-5.092	1.788-2.854	1.889-2.535	1.493-2.762
200 ppb of copper exposed	N	8	8	8	8
	Mean value	4.611 ***	2.700 ***	2.299 ***	1.942 ***
	± SD	0.775	1.027	0.799	0.634
	Range	3.565-5.997	1.170-3.093	1.170-3.226	1.309-3.309
400 ppb of copper exposed	N	8	8	8	8
	Mean value	7.399 ***	6.793 **	4.419 ***	2.570 ***
	± SD	1.097	1.622	1.391	0.835
	Range	5.409-8.890	4.366-8.140	2.365-6.720	1.596-4.016

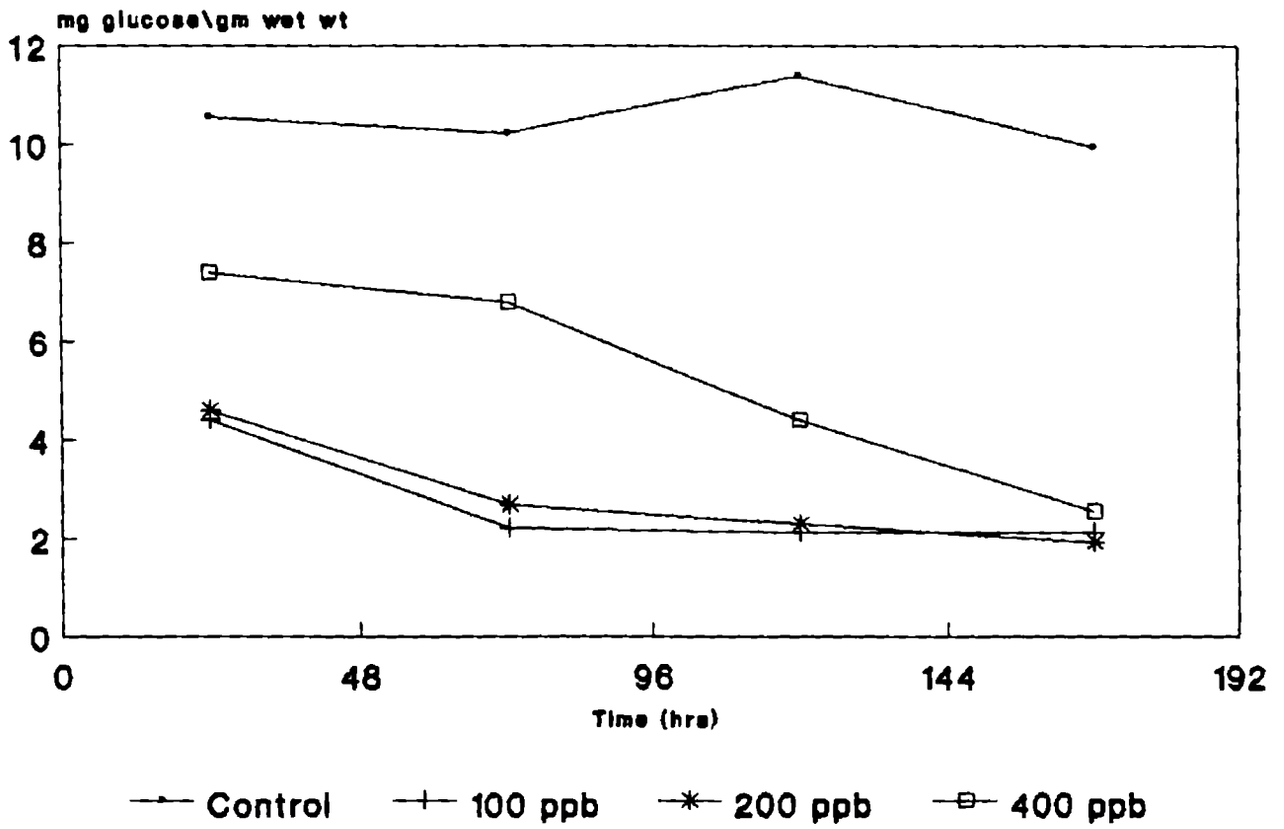


Figure. 5. Hepatopancreas Glycogen (mg glucose/gm wet wt) in *Lamellidens corrianus* exposed to three sublethal concentrations of copper.

3.3.2 Mercury

3.3.2.1 Gill tissue (Table 6, Figure 6)

Values of mussels exposed to 75 ppb when compared with those of the control, significantly higher values were found at 24 and 72 hr ($P<0.01$), but significantly lower value at 168 hr ($P<0.001$). At 300 ppb, significant reduction was noticed at 120 and 168 hr ($P<0.001$, 0.01) while in the remaining exposure periods no significant variation in glycogen level was observed. At 150 ppb, significant reduction in glycogen content was observed at 120 and 168 hr ($P<0.001$).

Significant elevation in glycogen level was shown by 75 ppb at 24, 72 and 120 hr ($P<0.001$, 0.01, 0.001), but reduction at 168 hr ($P<0.001$), when compared with the values of mussels exposed to 150 ppb. On comparing the values of 75 and 300 ppb exposed ones, those exposed to 75 ppb had significantly higher values at 24, 72 and 120 hr ($P<0.01$, 0.001, 0.01) but significantly lower value at 168 hr ($P<0.001$). Significantly lower values were shown by 150 ppb exposed ones at 120 and 168 hr ($P<0.001$) while significantly higher value was registered at 72 hr ($P<0.05$) when compared with the values of those exposed to 300 ppb.

ble 6. Gill Glycogen (mg glucose/gm wet wt) in Lamellidens corrianus exposed to three sublethal concentrations of mercury

	Hours	24	72	120	168
	N	8	8	8	8
	Mean value	2.631	2.685	2.607	2.444
ntrol	± SD	0.550	0.631	0.557	0.621
	Range	1.794-3.286	1.809-3.533	1.869-3.319	1.667-3.429
	N	8	8	8	8
ppb of	Mean value	3.939 **	3.939 **	2.867	0.184 ***
rcury	± SD	0.868	0.585	0.751	0.037
posed	Range	2.594-5.118	3.118-4.778	2.324-4.480	0.139-0.234
	N	8	8	8	8
0 ppb of	Mean value	2.214	2.817	1.033 ***	1.013 ***
rcury	± SD	0.245	0.482	0.103	0.141
posed	Range	1.900-2.772	2.117-3.489	0.892-1.196	0.790-1.196
	N	8	8	8	8
0 ppb of	Mean value	2.365	2.181	1.661 ***	1.661 **
rcury	± SD	0.786	0.409	0.178	0.126
posed	Range	1.445-3.960	1.570-2.732	1.425-1.938	1.480-1.886

gnificance level : **$P<0.01$ ***$P<0.001$

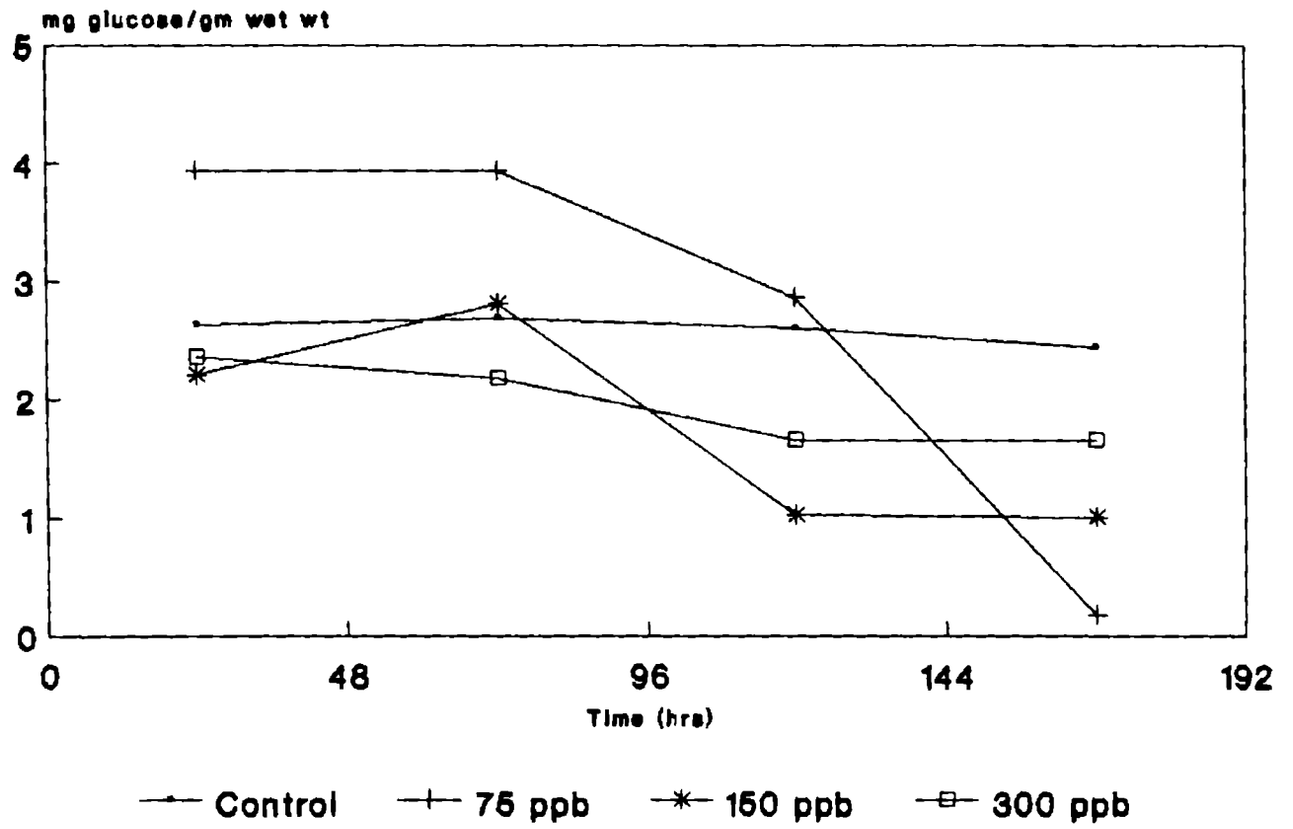


Figure. 6. Gill Glycogen (mg glucose/gm wet wt) in *Lamellidens corrianus* exposed to three sublethal concentrations of mercury.

3.3.2.2 Hepatopancreas tissue (Table 7, Figure 7)

Significant decrease in value was found in 75 ppb exposed ones at all time periods ($P<0.01$, 0.05, 0.05) except at 72 hr when compared with the control values. But at 150 and 300 ppb, reduction in glycogen value was significant at all time periods. At 150 ppb exposure, the levels of significance were $P<0.01$, 0.001, 0.01,0.001 and at 300 ppb exposure those were $P<0.05$, 0.05, 0.05, 0.01.

Comparison of values among mussels exposed to different concentrations showed significant changes in glycogen level at certain periods only. When values of 75 ppb were compared with those of 150 and 300 ppb, significant elevation was found in 75 ppb exposed ones at 72 and 168 hr in both cases ($P<0.001$, 0.001, 0.05). On comparing the values of 150 and 300 ppb exposed ones, significant change was recorded only at 168 hr ($P<0.05$).

3.3.3 Cadmium

3.3.3.1 Gill tissue (Table 8, Figure 8)

When the values of cadmium exposed animals were compared with those of the control, the following pattern emerged.

Table 7. Hepatopancreas Glycogen (mg glucose/gm wet wt) in *Lamellidens corrianus* exposed to three sublethal concentrations of mercury

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	11.419	10.582	10.284	9.984
	± SD	3.315	2.070	2.700	3.025
	Range	7.843-18.496	8.363-14.008	7.424-14.286	7.260-16.028
75 ppb of mercury exposed	N	8	8	8	8
	Mean value	7.060 **	11.794	6.979 *	6.928 *
	± SD	1.211	1.635	2.039	1.215
	Range	5.398-8.525	9.136-14.514	3.513-9.374	5.121-8.375
150 ppb of mercury exposed	N	8	8	8	8
	Mean value	7.274 **	6.456 ***	6.049 **	4.167 ***
	± SD	1.040	0.877	0.889	1.070
	Range	5.912-8.618	5.012-7.702	5.345-7.541	2.412-5.531
300 ppb of mercury exposed	N	8	8	8	8
	Mean value	7.554 *	7.852 *	6.758 *	5.454 **
	± SD	1.081	1.563	1.657	1.073
	Range	6.025-8.960	5.260-9.952	4.552-9.450	3.921-6.952

Significance level : *$P<0.05$ **$P<0.01$ ***$P<0.001$

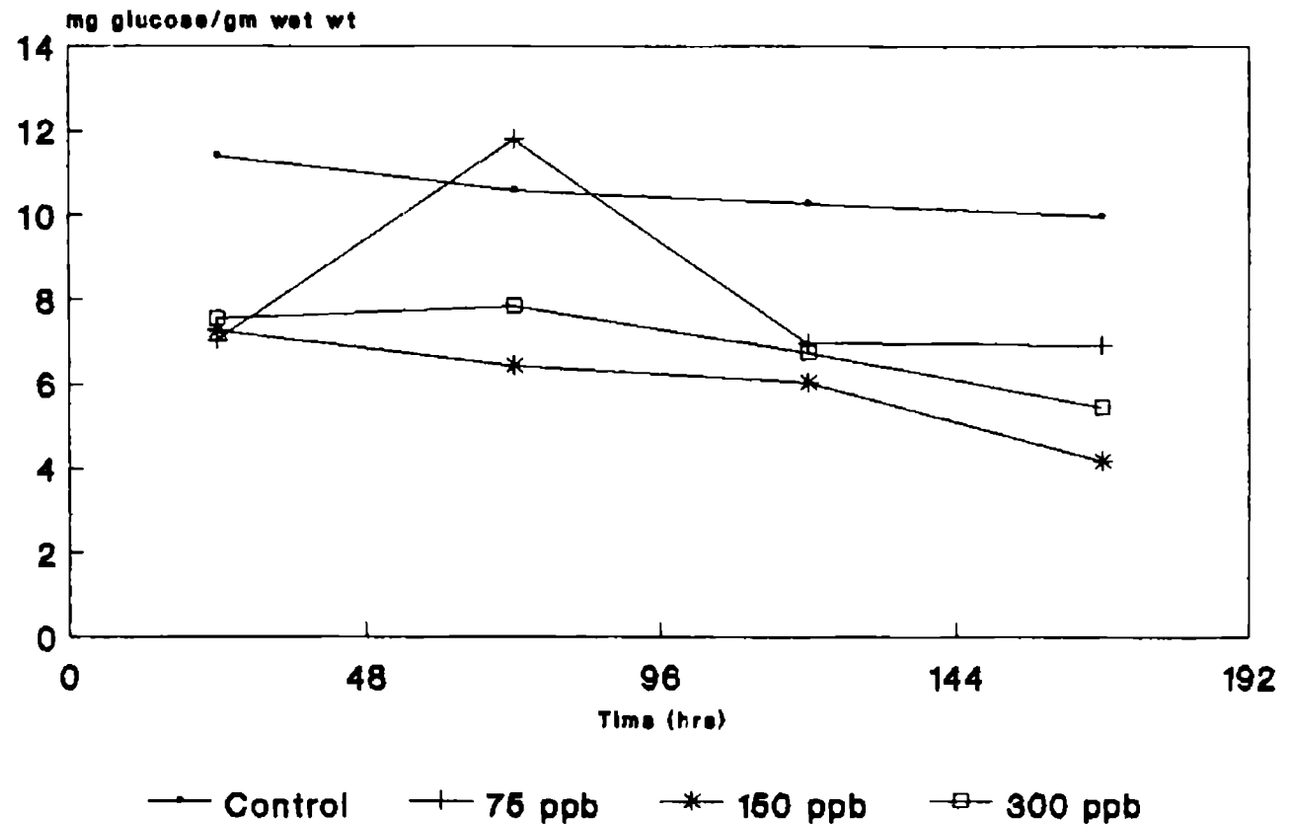


Figure. 7. Hepatopancreas Glycogen (mg glucose/gm wet wt) in *Lamellidens corrianus* exposed to three sublethal concentrations of mercury.

Table 8. Gill glycogen (mg glucose/gm wet wt) in Lamellidens corrianus exposed to three sublethal concentrations of cadmium

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	2.482	2.643	2.614	2.339
	± SD	0.515	0.551	0.504	0.641
	Range	1.798-3.129	1.908-3.545	1.899-3.126	1.550-3.256
150 ppb of cadmium exposed	N	8	8	8	8
	Mean value	2.931	2.559	1.813 **	0.772 ***
	± SD	0.400	0.554	0.414	0.115
	Range	2.286-3.311	2.077-3.590	1.463-2.728	0.638-0.983
300 ppb of cadmium exposed	N	8	8	8	8
	Mean value	1.287 ***	2.630	1.923	1.546 *
	± SD	0.185	0.635	0.860	0.550
	Range	1.065-1.574	1.966-3.936	1.026-3.848	0.968-2.240
600 ppb of cadmium exposed	N	8	8	8	8
	Mean value	1.038 ***	0.959 ***	1.058 ***	0.545 ***
	± SD	0.425	0.367	0.350	0.065
	Range	0.616-1.685	0.512-1.665	0.775-1.879	0.431-0.638

Significance level : *$P<0.05$ **$P<0.01$ ***$P<0.001$

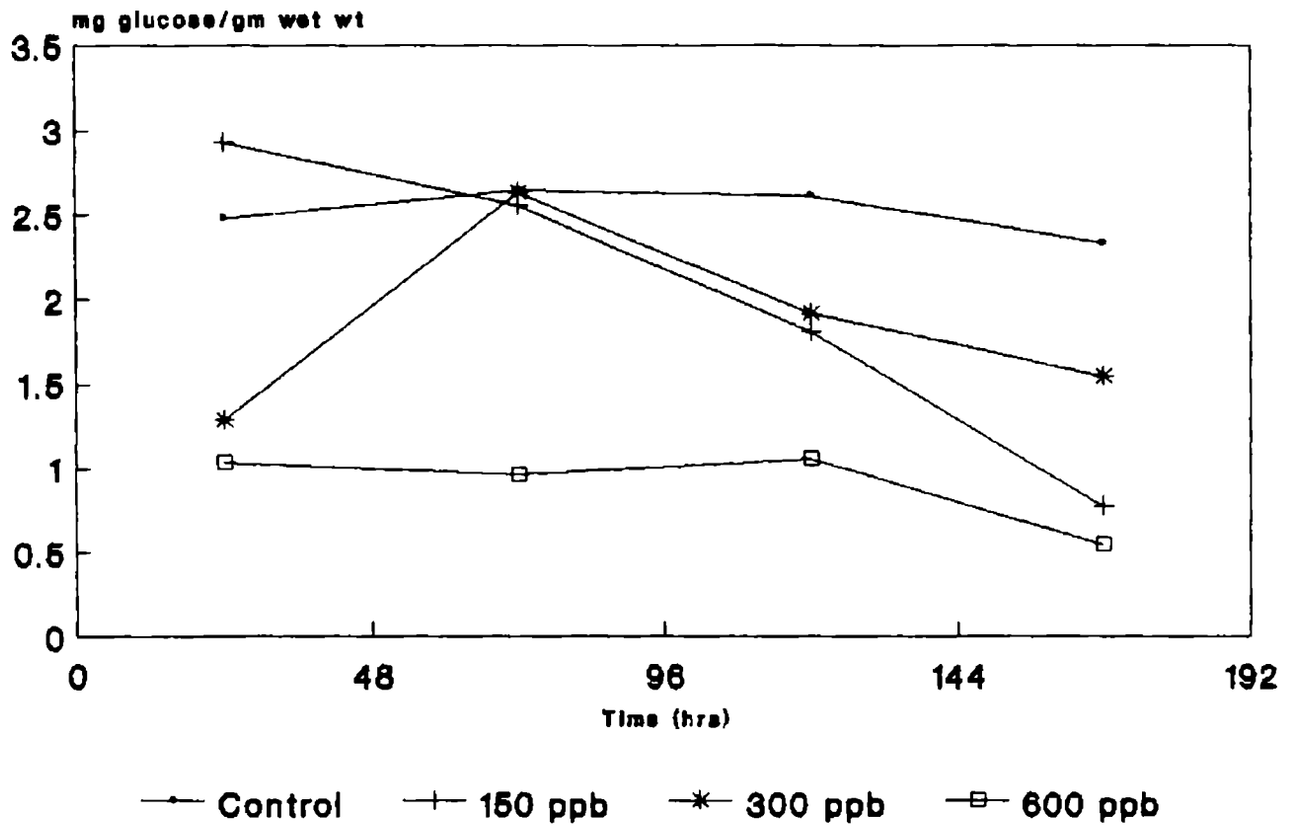


Figure. 8. Gill Glycogen (mg glucose/gm wet wt) in *Lamellidens corrianus* exposed to three sublethal concentrations of cadmium.

Values of 150 ppb cadmium exposed animals when compared with those of control, significant decrease was observed at 120 and 168 hr ($P<0.01$, 0.001). Animals exposed to 300 ppb showed significantly lower values at 24 and 168 hr ($P<0.001$, 0.05). Significant reduction in 600 ppb exposed ones was observed at all time periods when compared with the control values ($P<0.001$).

On comparing the values of 150 and 300 ppb exposed ones, significantly higher value was shown by 150 ppb exposed ones at 24 hr ($P<0.001$) and lower value at 168 hr ($P<0.01$). On comparing values of 150 and 600 ppb exposed ones, 150 ppb exposed ones registered elevated activity at all time periods ($P<0.001$, 0.001, 0.01,0.001). Those exposed to 300 ppb exhibited significant elevation at all exposure periods, except at 24 hr, when compared with the values of 600 ppb exposed ones. The levels of significance were $P<0.001$, 0.01, 0.001

3.3.3.2 Hepatopancreas tissue (Table 9, Figure 9)

When glycogen content in hepatopancreas of cadmium exposed animals was compared with that of the control, the following changes were observed. -At 150 ppb ,significant reduction was recorded at all time periods ($P<0.01$, 0.001 ,0.01, 0.001) and at

able 9. Hepatopancreas Glycogen (mg glucose/gm wet wt) in Lamellidens corrianus exposed to three sublethal concentrations of cadmium

	Hours	24	72	120	168
ontrol	N	8	8	8	8
	Mean value	10.742	10.840	10.284	10.476
	± SD	2.134	2.345	2.700	2.655
	Range	7.989-14.121	7.925-14.124	7.424-14.286	8.495-16.128
50 ppb of admium xposed	N	8	8	8	8
	Mean value	8.001 **	6.549 ***	6.113 **	6.001 ***
	± SD	1.042	0.870	1.600	0.845
	Range	6.017-9.126	5.439-8.267	4.136-8.662	4.964-7.427
00 ppb of admium xposed	N	8	8	8	8
	Mean value	10.023	8.098 **	6.368 **	2.763 ***
	± SD	1.068	0.612	0.907	0.535
	Range	8.595-11.495	7.168-9.135	5.178-7.671	1.936-3.562
00 ppb of admium xposed	N	8	8	8	8
	Mean value	10.004	7.756 **	5.481 ***	3.924 ***
	± SD	0.588	1.409	0.946	0.454
	Range	8.988-11.136	6.090-9.488	4.526-7.007	3.275-4.427

ignificance level : *$P<0.05$ **$P<0.01$ ***$P<0.001$

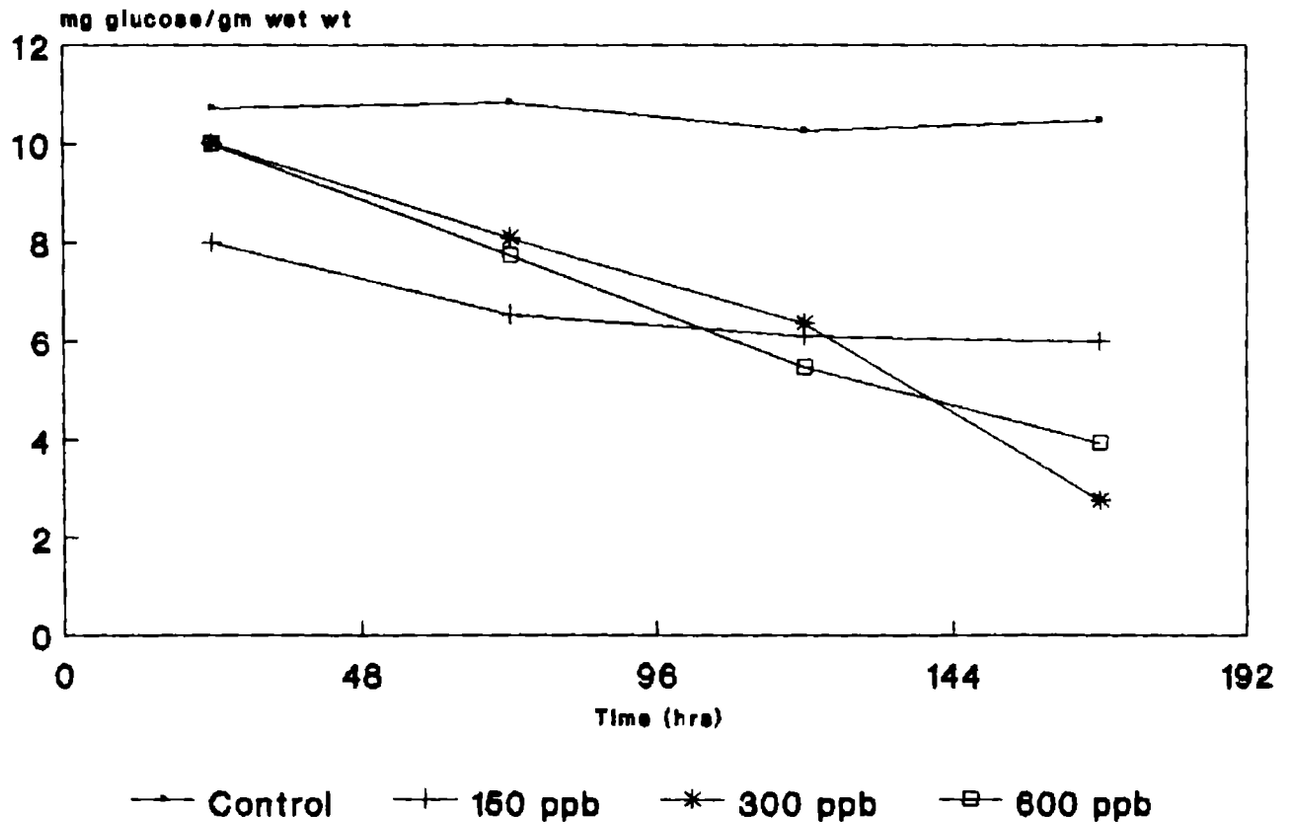


Figure. 9. Hepatopancreas Glycogen (mg glucose/gm wet wt) in *Lamellidens corrianus* exposed to three sublethal concentrations of cadmium.

300 and 600 ppb also significant reduction was observed at all time periods, except 24 hr. At 300 ppb the levels of significance were $P<0.01$, 0.01, 0.001 and at 600 ppb they were $P<0.01$, 0.001, 0.001

When the values of 150 ppb exposed ones were compared with those of 300 ppb ,significant reduction in glycogen value was shown by 150 ppb exposed ones at 24 and 72 hr ($P<0.01$), but at 168 hr, the value was higher ($P<0.001$). On comparing the values of 150 and 600 ppb exposed ones significant reduction in 150 ppb at 24 hr ($P<0.001$) was found to elevate at 168 hr ($P<0.001$). In the other combination ,significant change was noticed only at 168 hr where the 300 ppb exposed ones registered a lower value ($P<0.001$) than that of 600 ppb exposed ones.

3.4 DISCUSSION

Glycogen content in the gill tissue of copper exposed animals showed a general trend of reduction when compared with the controls. The reduction in glycogen values was found to be significant at almost all exposure periods.

In the hepatopancreas, glycogen content in the 400 ppb exposed animals declined slowly and steadily in a time dependent

manner while in the lower two concentrations, the glycogen content remained more or less steady during the later exposure periods, although significantly lower than the control at all time periods. A drop in glycogen was observed during the initial period.

Breakdown of glycogen during stress is reported in bivalve molluscs (Sahib et al., 1983; Moorthy et al., 1985; Mane et al., 1986; Mohan et al., 1987.). The breakdown of glycogen in both the tissues may be considered in terms of hypoxia caused by the metal. Glycogen breakdown during hypoxic condition is widely reported (De Zwaan et al., 1973; Shaffi, 1978 a; Babu et al., 1985 Lakshmanan and Nambisan, 1985; Sathyanathan et al., 1988). Shaffi (1978 b) observed increased glycogen breakdown in tissues of fishes exposed to copper sulphate as a result of hypoxia. Collip (1920), Dugal (1939), and Beyers and Warwick (1968) have reported that in the absence of oxygen there is a rapid decrease in glycogen content, and an increased acidity in the cells. Glycogen is reported to be the immediate energy source utilised during stress (Ahamed et al., 1978 Rao and Rao, 1983; Reddy et al., 1986 a).

The depletion of glycogen in gill and hepatopancreas can be attributed to the prevailing hypoxic condition. But the

breakdown of glycogen seems to show an inverse relation with the concentration of the toxicant. Such an inverse relationship between concentration of copper and tissue glycogen is reported by Shaffi (1978 b). The breakdown of glycogen may be due to suppressed gluconeogenesis or inhibition of glucose 6-phosphatase.

Mohan et al. (1987) observed high lactic acid content in tissues of *Lamellidens marginalis* exposed to toxicants and suggested the emphasis laid on glycolysis on conditions of stress. The steady level of glycogen during later periods in the lower concentrations may be due to gluconeogenesis or shift to aerobic metabolism. A shift towards normal respiratory activity can be envisaged during the later periods, from the oxygen uptake study. However, there was no revival in the level of glycogen even though respiration rate was found approaching control levels. This may be due to the high energy demand for the restoration of deranged aerobic metabolic activities. The sharp decline in glycogen at 168 hour may be attributed to damage caused to gill tissue resulting in hypoxia at tissue level

Gill glycogen content in the lowest concentration of mercury exposed animals was significantly higher during 24 and 72 hr time

periods, but lower at 168 hr, while in the other two concentrations it showed a general trend of lower values specifically at later time periods. In hepatopancreas of almost all the experimentals, there was a consistant reduction in glycogen level. Reddy et al. (1986 b) studied the effect of mercury on freshwater mussel, *Parreysia rugosa* tissues and reported that due to inhibition of dehydrogenase activity energy supply through oxidative pathway decreased. In the present study also such a condition can be envisaged and a stepped up breakdown of glycogen to meet the stress is found to be the reason for decreased glycogen concentration. In the animals exposed to lowest concentration, increased glycogen level in the gill at early time periods may be due to glycogenesis. Since damage caused is less, the glycogen synthesis may not have been affected. Since hepatopancreas act as a `sink' for metal ions, impairment caused to metabolic activities is more severe and hence increased glycogen breakdown occurs to meet the impending stress. Verma and Tonk (1983) also observed depletion in tissue glycogen on exposure to mercury. Lakshmanan and Nambisan (1985), and Satyanathan et al.(1988) also have reported glycogen depletion in bivalves due to mercury toxicity. Lakshmanan and Nambisan (1985) have reported depletion in glycogen to non detectable level in *Perna viridis* following copper and mercury

exposure. The maintenance of a near steady level of glycogen in all most all the experimentals though lower, towards the later periods is suggestive of an alternate pathway of energy metabolism. Replenishment through gluconeogenesis is also a possibility. Sparing of glycogen after an initial breakdown under hypoxia is reported by Badman and Chin (1973). Maintenance of a steady level of glycogen under hypoxic condition is also reported (Babu et al., 1985). In lamellibranch molluscs lipids are considered to be a source of energy other than carbohydrates. On prolonged exposure, the animal utilises free fatty acids as energy source (Lal et al., 1986). This may also lead to a steady glycogen concentration (Mane et al., 1986). The significantly higher level of glycogen in 75 ppb exposed animal's gill tissue at early time periods may be due to inhibition of glycogenolysis or that gluconeogenesis and glycogenesis increased to some extent. It can be assumed that low mercury concentrations stimulate certain physiological processes while high doses inhibit the process. Similar observations were made by Grant and Mehrle (1973).

In general, glycogen levels in both the tissues were significantly lower in those animals exposed to higher cadmium concentrations. The decline in glycogen may be due to its

immediate utilisation to withstand stress. In the animals exposed to lower concentrations, hepatopancreas glycogen concentration was consistantly lower. Husain et al. (1980) observed increase in blood glucose due to increased breakdown of hepatic glycogen and activation of glycolytic enzymes following the administration of manganese. Husain et al. (1980) suggested that alterations in the level of insulin, cyclic AMP, and bivalent cations can be responsible for the metal induced changes. Since cadmium is physiochemically similar to manganese (Nath and Kumar, 1987), increased glycogen breakdown observed in the present study can be attributed to the above mentioned alterations. Nath and Kumar (1987) also observed glycogen breakdown following cadmium treatment. The stepped up glycolysis in all the cadmium exposures may also be due to the prevailing hypoxic condition. During the initial period, in hepatopancreas of the experimentals at higher concentrations and in gill of those exposed to lower concentrations, gluconeogenesis or a shift towards lipid metabolism may be responsible for the almost steady glycogen level. Pollution by cadmium has been shown to profoundly affect respiration and carbohydrate metabolism in molluscs (Mac Innes and Thurberg, 1973 Shore et al., 1975). Increased blood glucose levels on exposure to cadmium are reported in fishes (Larsson, 1975). Engel et al. (1972)

suggested that sublethal doses of toxicants can cause metabolic alterations with an increase in glucose catabolism concomitantly with a decrease of gluconeogenic flux in bivalves. Moorthy et al.(1985) has reported impaired energy system as a result of reduced aerobic oxidation of glucose in *Lamellidens marginalis* exposed to toxicants.

Since succinate dehydrogenase is an important enzyme in TCA cycle, with the inhibition of SDH activity, metabolic pathway might have shifted towards anaerobic side to meet the increased energy demands due to stress (Radhaiah et al., 1987).

From the results it appears that the animal was geared to a lowered oxidative and increased glycolytic enzyme activities. There is a close topographical relationship between endoplasmic reticulam membrane and glycogen depot and that inhibition of glucose-6-phosphatase altered endoplasmic reticulam accompanied by depletion of cellular glycogen (see verma and Tonk, 1983). Decrease in glycogen may also be due to inactivation of enzymes involved in carbohydrate synthesis as suggested by Nagabhushanam and Kulkarni (1981).

C H A P T E R - IV

EFFECT OF HEAVY METALS ON TISSUE TOTAL PROTEIN CONTENT

4.1 INTRODUCTION

When metals cross the cell membrane, they react with the cellular components in different ways. Metals can combine with different functional groups. Most of the heavy metals can cause serious impairment in the metabolic as well as physiological activities of the animals.

The general response of an organism to stress is the utilisation of nutrient reserves to meet the metabolic requirements, which gets enhanced above the normal values. This response to stress can be measured in terms of alteration in the balance between catabolism of carbohydrates, protein, and lipid substrates (Bayne et al., 1985). The potentially toxic heavy metals can be detoxified intra-cellularly by partitioning into lysosomes (Moore, 1980) or by binding to protein metallothionein (Bayne et al., 1985). Metallothioneins are a group of low molecular weight proteins involved in the binding of metals such as cadmium, copper, mercury, zinc etc. Protein induction, defined as an adaptive increase in the number of molecules of a specific protein or decreased rate of its degradation is a

possible selective means for regulating levels of specific protein (Rana and Kumar, 1980). Protein system undergoes regulation as a result of changes in environmental parameters (Rana and Kumar, 1980).

High levels of metallothionein are reported in gill tissue of bivalves (Roesijadi, 1979). The levels of metallothioneins can be greatly increased, even up to 40 times, on exposure to heavy metals such as copper, cadmium, mercury, and tin (Winge et al., 1975; Sabbioni and Marafante, 1975). Cadmium induced metallothionein is reported in *Mercenaria, Mytilus, Cancer, Carcinus and Littorina* species (George, 1982). Viarengo et al.(1981) reported that when mussels were exposed to lower copper concentrations for 48 hrs, biosynthesis of copper binding proteins was stimulated in the gills, digestive gland and mantle.

A number of cadmium binding protein molecules, totally unlike metallothionein, is reported from invertebrates (Stone and Overnell, 1985). Harrison et al.(1983) have reported changes in protein content in *Mytilus edulis* subjected to copper toxicity, that are dependent on exposure period and concentration of the toxicant.

Reduction in protein content as a result of exposure to

toxicant or stress is widely reported (McLeay and Brown, 1974; Sakaguchi and Hamaguchi, 1975 Baneerjee and Choudhuri, 1985). Decrease in protein content in freshwater mussels during heavy metal as well as pesticide exposure is also reported (Rao et al., 1980 Reddy and Chari, 1985 Moorthy et al., 1986). Ahamed et al.(1978) observed reduction in total protein in various tissues of *Lamellidens marginalis* under pesticide stress. Metal ions are known to inactivate protein molecules through non-specific binding or cross linking of essential side chains and by promoting irreversible denaturation (Ulmer, 1970)

The development of tolerance to metal ions may be due to the induction of synthesis of metallothioneins which by preferential binding, may reduce the extent to which essential metabolic activities would otherwise be inhibited (Chaterjee and Bhattacharya, 1986). The induction of hepatic metallothionein is well documented (Brown and Parsons, 1978; Dixon and Sprague, 1981; Cousins, 1985). Cadmium and copper exposure induced synthesis of metallothionein in the gills of mussels (Sunila, 1986). Synthesis of protein induced by stress and heavy metals is also reported by Oh et al.(1978).

This chapter deals with the study of protein content in tissues of heavy metal exposed mussels.

4.2 MATERIAL AND METHODS

Methods of collection of specimens, acclimatisation and exposure to toxicants were the same as described in Chapter. 2.

For each concentration of each metal as well as control, eight animals were used.

Residue of the tissue (gill and hepatopancreas) extract in TCA was taken for the estimation of protein. Protein estimation was carried out following the method of Lowry et al.(1951). The precipitated protein was dissolved in 1ml of 0.1N NaoH. From this 0.5 ml of the sample was pipetted into another test tube and made up to 1 ml with distilled water. After thorough mixing, 5 ml. of alkaline copper reagent was added and mixed well. After 10 minutes, 0.5 ml of Folin's phenol reagent was added and mixed well. The optical density of the sample was determined spectrophotometrically at 500 nm, after 45 minutes. Bovine serum albumin (Sigma) was used as the standard. The concentration of protein is expressed as mg protein/gm. wet weight of tissue.

4.3 RESULTS

4.3.1 Copper

4.3.1.1 Gill tissue (Table 10, Figure 10)

When the gill protein content of 100 ppb copper exposed animals was compared with that of the control, significantly lower value was obtained in 100 ppb exposed animals at 24 hr ($P<0.05$). But at 120 and 168 hr post-exposure, the protein values were significantly higher than those of the controls ($P<0.001$). In 200 ppb exposed animals also significantly lower value was obtained at 24 hr ($P<0.01$), but significantly higher values at 120 and 168 hrs ($P<0.001$). In 400 ppb exposed mussels at 72 and 120 hr, increase in protein values was noted when the values were compared with the controls ($P<$ 0.01, 0.001)

When the total protein values in animals exposed to varying concentrations of copper were compared the following picture emerged. Comparison of protein content between 100 ppb and 200 ppb exposed animals showed lower value in 100 ppb exposed animals at 72 hr ($P<0.01$). At 120 hr 100 ppb exposed animals showed higher protein value than that of 200 ppb exposed ones ($P<0.001$). When values of 100 ppb and 400 ppb exposed animals were compared, initial periods of exposure brought about significant reduction in protein content ($P<0.05$, 0.001) while later periods of exposure brought about elevation in 100 ppb exposed animals

able 10. Gill Total Protein (mg protein/gm wet wt) in Lamellidens corrianus exposed to three sublethal concentrations of copper

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	46.416	45.445	46.086	45.309
	± SD	9.534	9.947	7.890	7.181
	Range	38.278-60.484	29.875-58.659	32.863-54.735	37.230-60.168
00 ppb of copper exposed	N	8	8	8	8
	Mean value	37.314 *	37.894	87.593 ***	64.133 ***
	± SD	4.856	4.431	5.889	8.776
	Range	34.00-45.160	29.642-45.408	80.698-99.851	56.739-82.219
00 ppb of copper exposed	N	8	8	8	8
	Mean value	33.677 **	48.503	70.909 ***	63.870 ***
	± SD	5.433	5.972	4.683	8.029
	Range	25.569-38.582	42.786-59.304	64.028-78.032	53.016-78.714
400 ppb copper exposed	N	8	8	8	8
	Mean value	48.475	61.112 **	72.002 ***	50.078
	± SD	11.923	5.650	13.704	12.532
	Range	33.923-65.550	51.693-69.753	52.997-88.541	38.300-73.600

ignificance levels * $P<0.05$ ** $P<0.01$ *** $P<0.001$

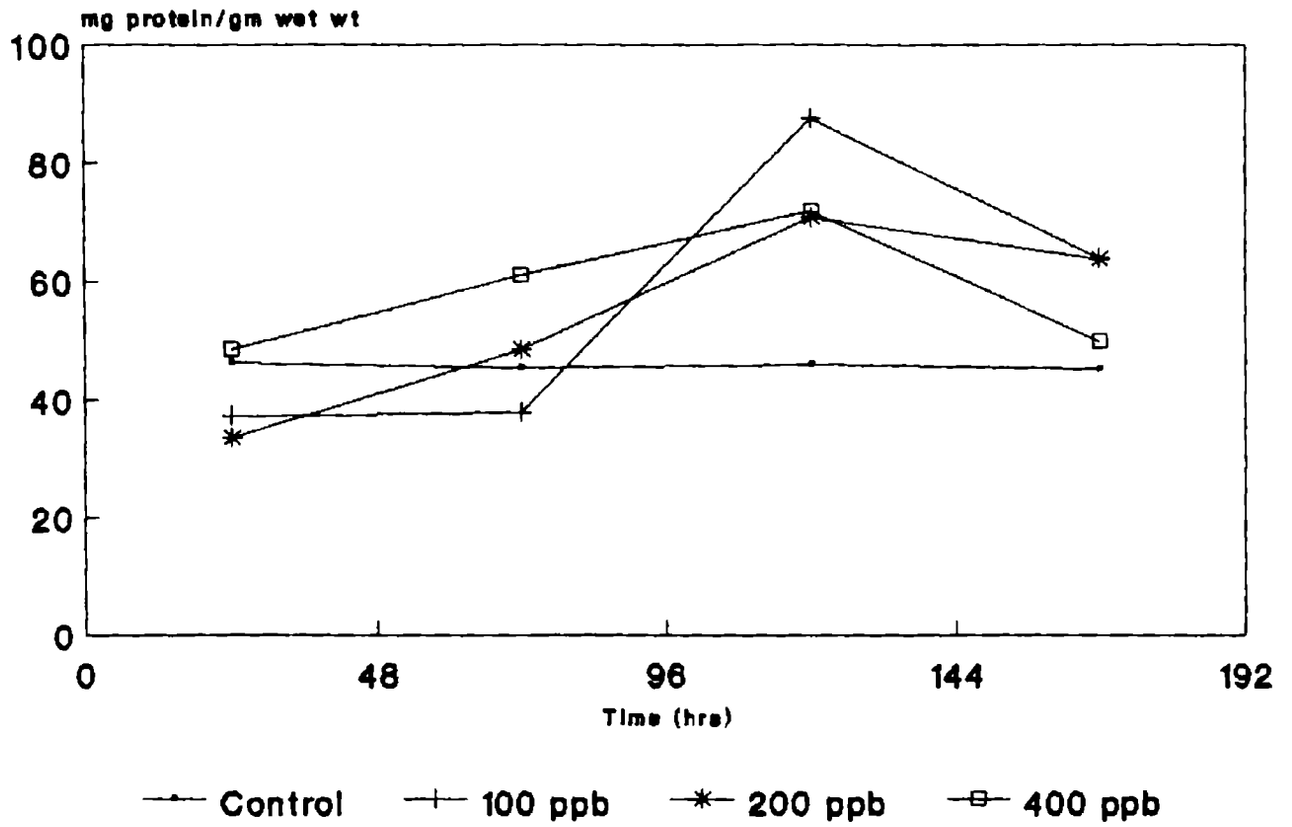


Figure. 10. Gill Total Protein (mg protein/gm wet wt) in *Lamellidens corrianus* exposed to three sublethal concentrations of copper.

($P<0.05$). On comparing values in 200 ppb and 400 ppb exposed animals, lower values were shown by 200 ppb exposed mussels at 24 and 72 hr ($P<0.01$) but higher at 168 hr ($P<0.05$).

4.3.1.2 Hepatopancreas tissue (Table 11, Figure 11)

The 100 ppb copper-exposed animals had significantly lower protein value at 24 hr ($P<0.01$), but at 120 hr,the protein value wassignificantly higher than those of the controls ($P<0.001$). In 200 ppb exposed animals, significantly lower values were observed at 24 and 72 hr time periods ($P<0.001$, 0.01) while significantly higher value was found at 120 hr ($P<0.001$). Significantly lower values at 24 and 168 hr ($P<0.05$, 0.01), but significantly higher value at 72 hr ($P<0.001$) were found in 400 ppb exposed animals when compared with the control values.

When values in 100 ppb exposed animals were compared with those in 200 ppb exposed ones, significantly higher value was found at 168 hr ($P<0.05$) in 100 ppb exposed animals. When values in 100 and 400 ppb exposed animals were compared, significantly lower value was shown at 72 hr ($P<0.001$) but higher values at 120 and 168 hr post exposure in 100 ppb exposed animals ($P<0.001$, 0.01). When values in animals exposed to 200 and 400 ppb were compared, significantly lower values were found at 24 and 72 hr

able 11. Hepatopancreas Total Protein (mg protein/gm wet wt) in *Lamellidens corrianus* exposed to three sublethal concentrations of copper

	Hours	24	72	120	168
ontrol	N	8	8	8	8
	Mean value	49.611	49.462	47.698	48.654
	± SD	6.212	7.206	6.354	5.942
	Range	42.134-60.756	31.341-60.812	38.832-57.648	40.366-52.695
00 ppb of copper exposed	N	8	8	8	8
	Mean value	34.944 **	43.509	79.325 ***	57.438
	± SD	8.684	11.118	8.357	10.547
	Range	19.227-47.220	26.093-62.933	66.505-90.036	39.552-66.459
00 ppb of copper exposed	N	8	8	8	8
	Mean value	30.973 ***	36.171 **	79.956 ***	42.093
	± SD	3.905	8.595	8.172	10.983
	Range	29.060-36.556	26.024-50.766	67.869-93.828	21.920-54.103
00 ppb of exposed	N	8	8	8	8
	Mean value	40.050 *	69.455***	49.068	41.275 **
	± SD	6.241	7.705	10.610	2.380
	Range	32.853-49.900	59.285-80.872	39.760-65.864	38.200-44.354

ignificance level: *$P<0.05$, **$P<0.01$, ***$P<0.001$

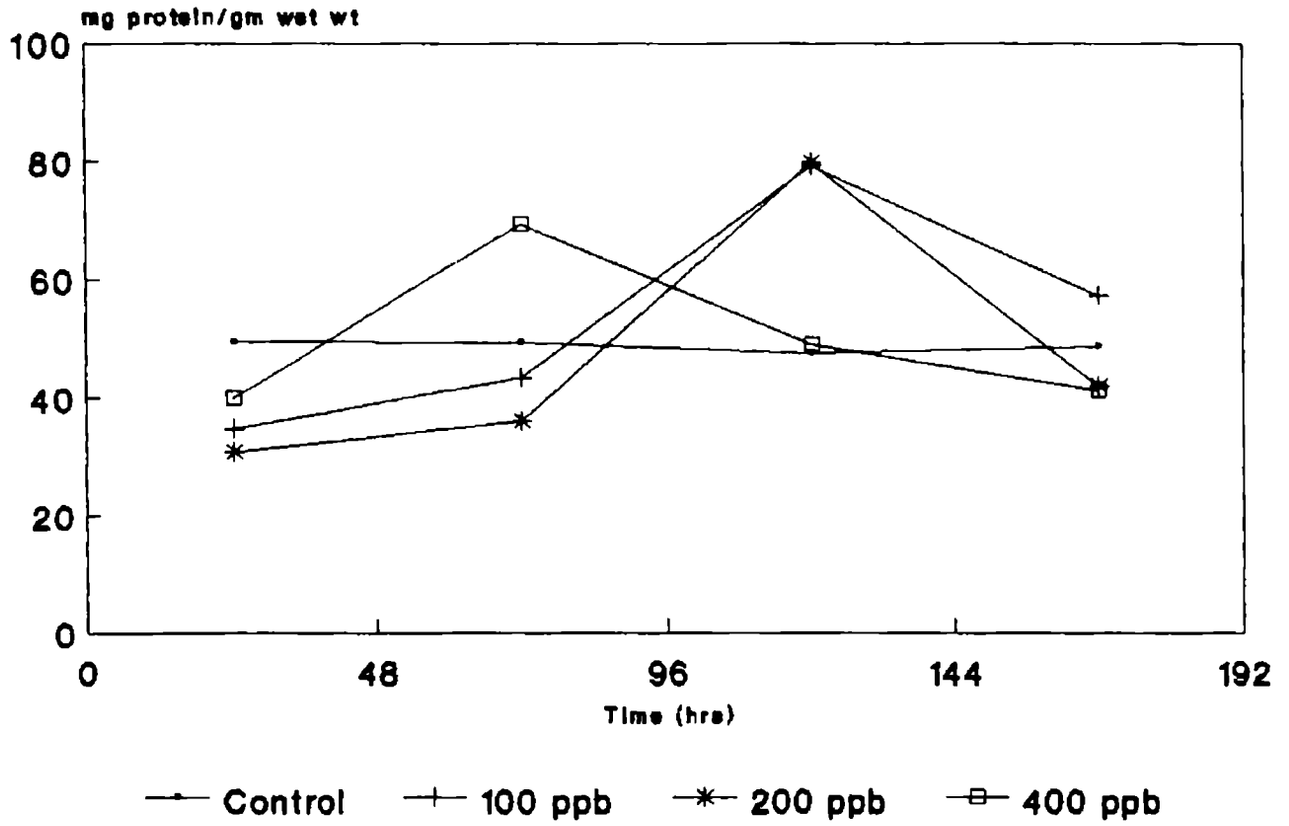


Figure. 11. Hepatopancreas Total Protein (mg protein/gm wet wt) in *Lamellidens corrianus* exposed to three sublethal concentrations of copper

($P<0.01$, 0.001) but significantly higher value at 120 hr ($P<0.001$) in 200 ppb exposed mussels.

4.3.2 Mercury

4.3.2.1 Gill tissue (Table 12, Figure 12)

When values in 75 ppb mercury exposed animals were compared with those of the controls, significant increase was observed only at 168 hr ($P<0.05$). But in 150 ppb exposed animals significantly higher values were obtained at 24, 120 and 168 hr when compared with the controls ($P<0.01$, 0.01, 0.001). In 300 ppb mercury exposed animals significantly lower protein value at 168 hr ($P<0.05$), but significantly higher value at 24 hr ($P<0.01$) were observed.

On comparing protein content in 75 and 150 ppb exposed animals, significantly lower values were found in 75 ppb exposed ones at 24 and 168 hr ($P<0.01$, 0.05). During the remaining exposure periods, no significant change was noticed. On comparison of values in 75 and 300 ppb exposed mussels, significantly lower value at 24 hr ($P<0.01$) but significantly higher values at 72 and 168 hr ($P<0.05$) were found in 75 ppb exposed ones. Those exposed to 150 ppb had significantly higher value at 168 hr ($P<0.001$), when compared to the values in 300 ppb

Table 12. Gill Total Protein (mg protein/gm wet wt) in Lamellidens corrianus exposed to three sublethal concentrations of mercury

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	42.395	42.782	41.959	40.804
	± SD	6.720	9.950	6.059	6.299
	Range	30.540-49.793	30.250-56.889	36.042-51.231	32.259-47.600
75 ppb of mercury exposed	N	8	8	8	8
	Mean value	34.780	58.852	54.062	52.619 *
	± SD	16.523	18.749	15.857	9.953
	Range	13.240-56.840	34.800-89.429	29.842-75.476	34.043-65.205
150 ppb of mercury exposed	N	8	8	8	8
	Mean value	60.350 **	49.339	55.432 **	68.792 ***
	±SD	13.746	9.457	7.106	11.106
	Range	38.800-77.147	36.984-63.800	45.160-63.800	51.736-85.920
300 ppb of mercury exposed	N	8	8	8	8
	Mean value	56.618 **	37.331	47.200	30.546 *
	±SD	6.818	15.445	8.830	9.566
	Range	48.000-66.485	16.791-64.300	33.365-58.906	16.520-45.079

Significance levels: *$P<0.05$, **$P<0.01$, ***$P<0.001$

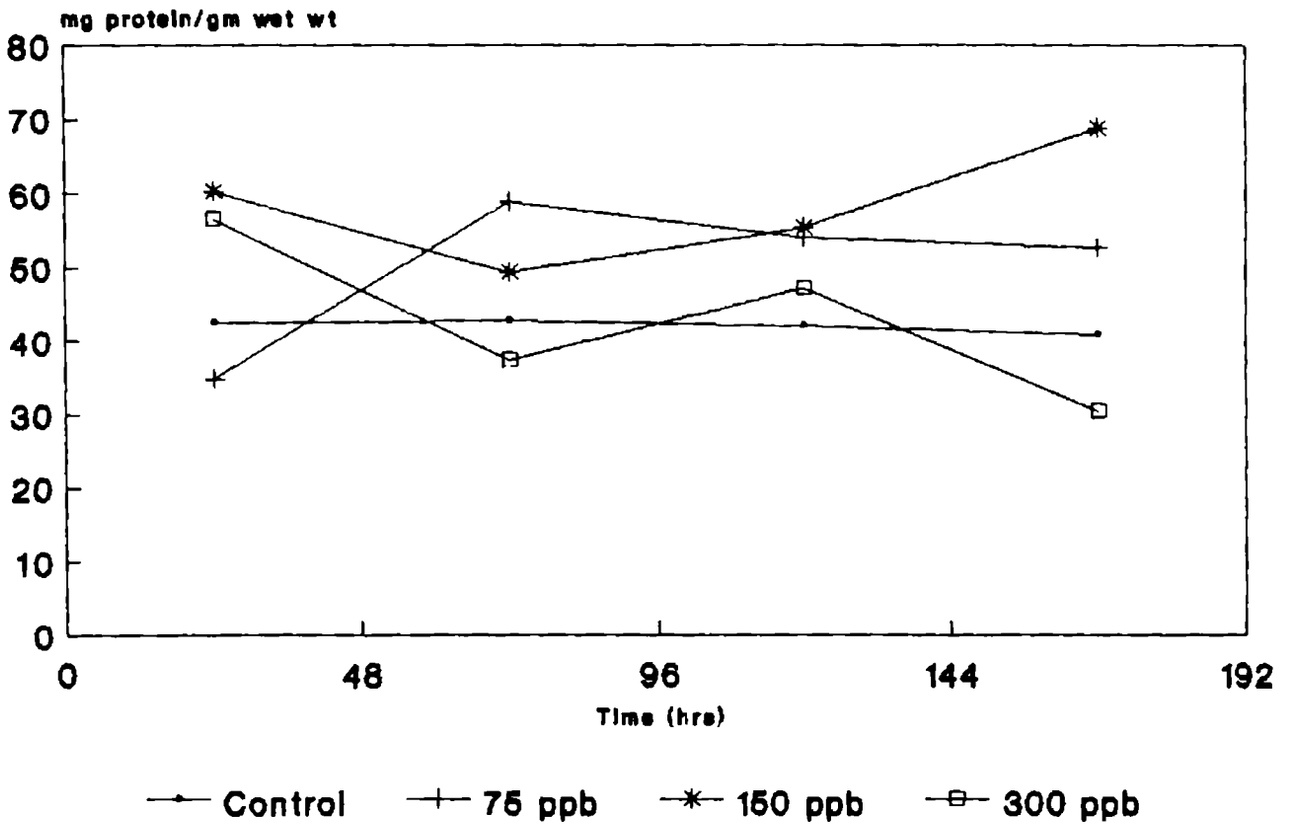


Figure. 12. Gill Total Protein (mg protein/gm wet wt) in *Lamellidens corrianus* exposed to three sublethal concentrations of mercury

exposed animals.

4.3.2.2 Hepatopancreas tissue (Table 13, Figure 13)

When values in mercury exposed animals were compared with those of the controls, significant variation was observed at 24 hr in 75 ppb exposed animals and at 168 hr in 150 ppb exposed ones. In 75 ppb exposed animals lower value was found at 24 hr ($P<0.05$) and in 150 ppb exposed ones at 168 hr ($P<0.01$). But in those exposed to 300 ppb, significantly lower values were observed at 72 and 168 hr ($P<0.01$, 0.001). Values in the rest of the exposure periods did not show any significant change.

On comparison of values in 75 and 150 ppb exposed mussels, no significant change was observed during any of the exposure periods. On comparison of values in 75 and 300 ppb, and between 150-300 ppb exposed mussels, significantly lower value was shown by 300 ppb exposed animals at 72 and 168 hr ($P<0.001$). Values at other exposure periods did not show any significant variation.

4.3.3 Cadmium

4.3.3.1 Gill tissue (Table 14, Figure 14)

When values in 150 ppb cadmium exposed animals were compared with those of controls, significantly lower value was found in

Table 13. Hepatopancreas Total Protein (mg protein/gm wet wt) in *Lamellidens corrianus* exposed to three sublethal concentrations of mercury

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	48.564	47.342	46.265	47.268
	± SD	7.631	8.028	5.086	5.811
	Range	40.436-61.670	35.714-63.812	36.328-53.864	36.366-54.568
75 ppb of mercury exposed	N	8	8	8	8
	Mean value	37.718 *	51.386	55.808	36.914
	± SD	6.238	10.724	11.693	12.152
	Range	30.352-46.105	38.960-62.520	39.258-75.800	20.554-57.811
150 ppb of mercury exposed	N	8	8	8	8
	Mean value	41.072	59.111	50.080	33.485 **
	± SD	12.307	14.386	7.890	8.740
	Range	25.372-55.400	31.979-71.680	38.965-60.789	19.695-45.183
300 ppb of mercury exposed	N	8	8	8	8
	Mean value	42.842	31.420 **	45.078	14.833 ***
	± SD	9.986	6.284	10.331	2.728
	Range	26.642-57.962	21.160-39.247	29.770-59.504	10.440-18.187

Significance levels: *$P<0.05$, **$P<0.01$, ***$P<0.001$

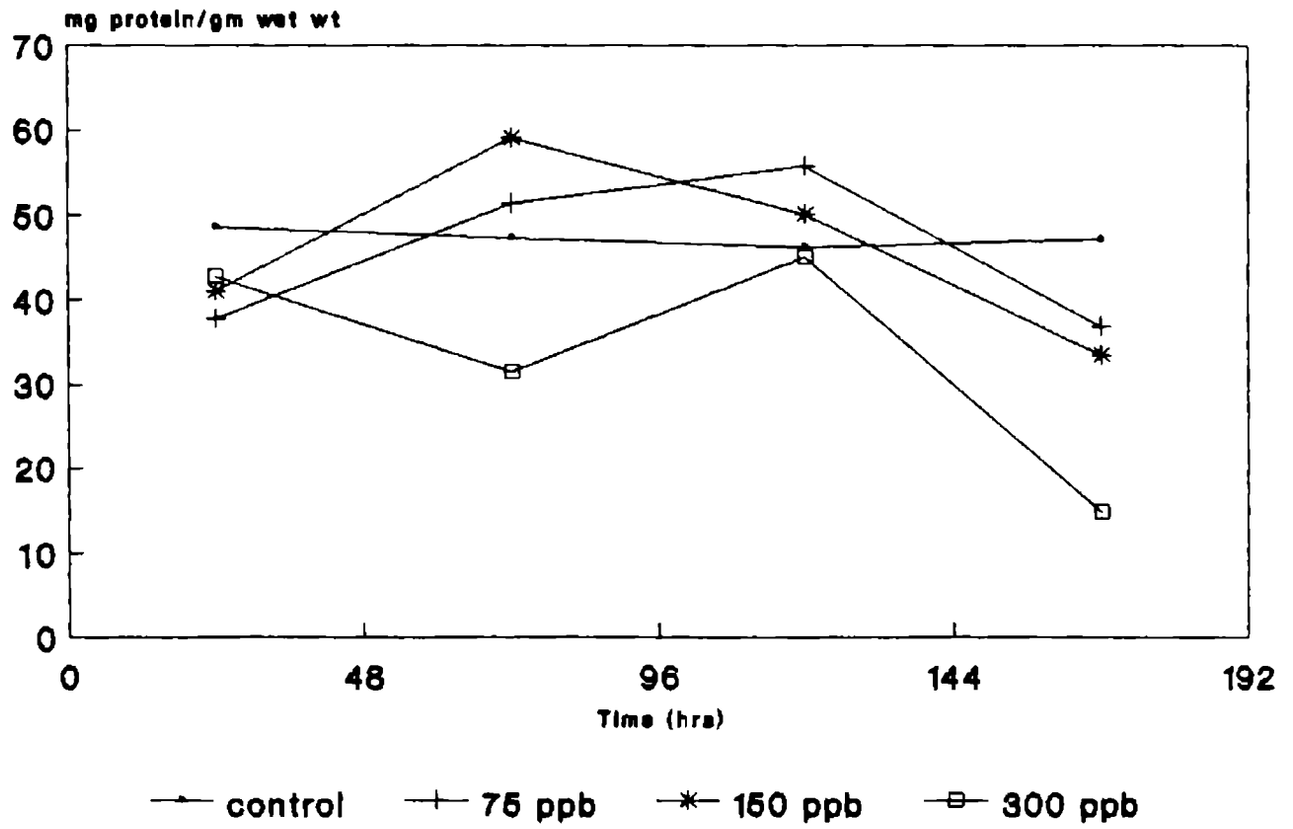


Figure. 13. Hepatopancreas Total Protein (mg protein/gm wet wt) in *Lamellidens corrianus* exposed to three sublethal concentrations of mercury

Table 14. Gill Total Protein (mg protein/gm wet wt) in *Lamellidens corrianus* exposed to three sublethal concentrations of cadmium

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	46.416	46.298	45.982	48.278
	± SD	9.534	5.849	6.761	8.858
	Range	38.278-60.484	34.738-52.774	31.869-52.576	38.283-55.928
150 ppb of cadmium exposed	N	8	8	8	8
	Mean value	35.253 *	58.463 *	39.084	56.395
	± SD	6.837	9.162	7.813	13.887
	Range	23.680-43.887	41.086-70.078	26.317-47.011	37.880-78.357
300 ppb of cadmium exposed	N	8	8	8	8
	Mean value	40.441	52.177	59.116 **	41.567
	± SD	6.112	6.432	6.434	8.770
	Range	31.445-47.102	46.440-63.480	50.669-69.480	29.702-53.669
600 ppb of cadmium exposed	N	8	8	8	8
	Mean value	54.887	37.092 *	56.615 **	30.365 ***
	± SD	18.295	8.023	6.328	3.434
	Range	33.037-82.786	27.261-49.693	48.447-65.327	24.548-34.338

Significance levels: *$P<0.05$, **$P<0.01$, ***$P<0.001$

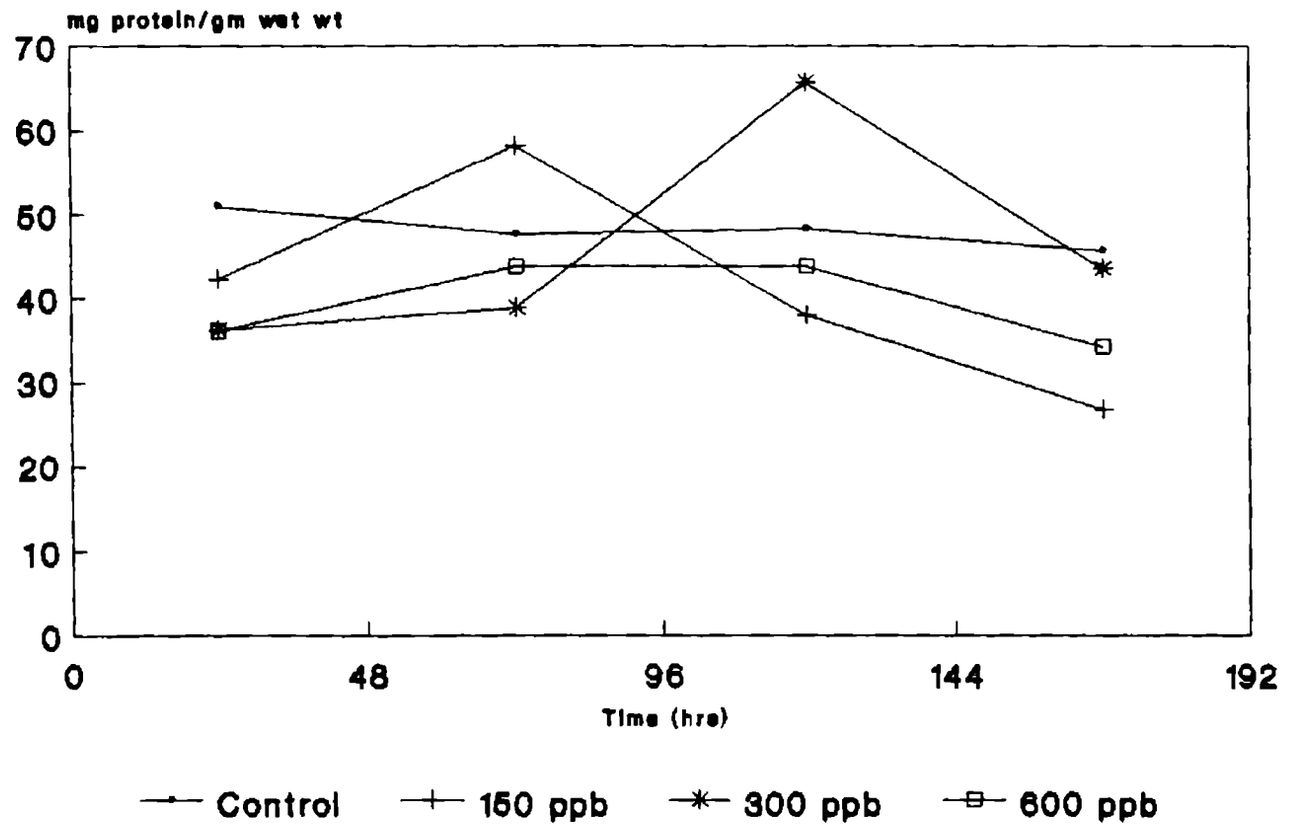


Figure. 14. Gill Total Prtotein (mg protein/gm wet wt) in *Lamellidens corrianus* exposed to three sublethal concentrations of cadmium.

150 ppb exposed ones at 24 hr ($P<0.05$) but significantly higher value at 72 hr ($P<0.05$). In 300 ppb cadmium exposed animals, total protein in gill tissue was significantly higher only at 120 hr when compared with the controls ($P<0.01$). In 600 ppb exposed mussels, the significantly higher value at 120 hr ($P<0.01$) got reduced significantly by 168 hr ($P<0.001$). Significant reduction was found at 72 hr time period also ($P<0.05$). But the initial exposure period did not produce any significant change.

On comparison of values in 150-300 ppb exposed animals, significantly lower value was found at 120 hr ($P<0.001$) and significantly higher value at 168 hr ($P<0.05$) in 150 ppb. When values in 150 ppb exposed animals were compared with those in 600 ppb exposed ones, significantly lower values at 24 and 120 hr ($P<0.05$ and 0.001), but higher at 72 and 168 hr were found in 150 ppb ($P<0.001$) exposed ones. When values in animals exposed to 300 ppb and 600 ppb were compared, significantly higher values were found in 300 ppb exposed ones at 72 and 168 hr ($P<0.01$). During the remaining time periods no significant change was observed.

4.3.3.2 Hepatopancreas tissue (Table 15, Figure 15)

Table 15. Hepatopancreas Total Protein (mg protein/gm wet wt) in Lamellidens corrianus exposed to three sublethal concentrations of cadmium

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	51.075	47.705	48.416	45.753
	± SD	5.246	8.035	7.970	5.762
	Range	45.986-60.756	40.714-63.812	35.823-58.126	37.856-54.127
150 ppb of cadmium exposed	N	8	8	8	8
	Mean value	42.273	58.260 *	37.988 **	26.792 ***
	± SD	10.590	9.263	3.389	3.100
	Range	29.360-59.906	41.874-68.674	34.450-44.185	21.309-30.693
300 ppb of cadmium exposed	N	8	8	8	8
	Mean value	36.283 **	38.883	65.802 ***	43.540
	± SD	8.461	7.540	8.643	3.407
	Range	25.472-47.936	26.835-49.101	56.520-78.960	39.533-48.000
600 ppb of cadmium exposed	N	8	8	8	8
	Mean value	36.183 **	43.880	43.800	34.201 ***
	± SD	8.259	9.165	9.783	3.008
	Range	25.472-47.936	29.224-54.069	29.769-60.320	30.926-40.253

Significance levels: *$P<0.05$, **$P<0.01$, ***$P<0.001$

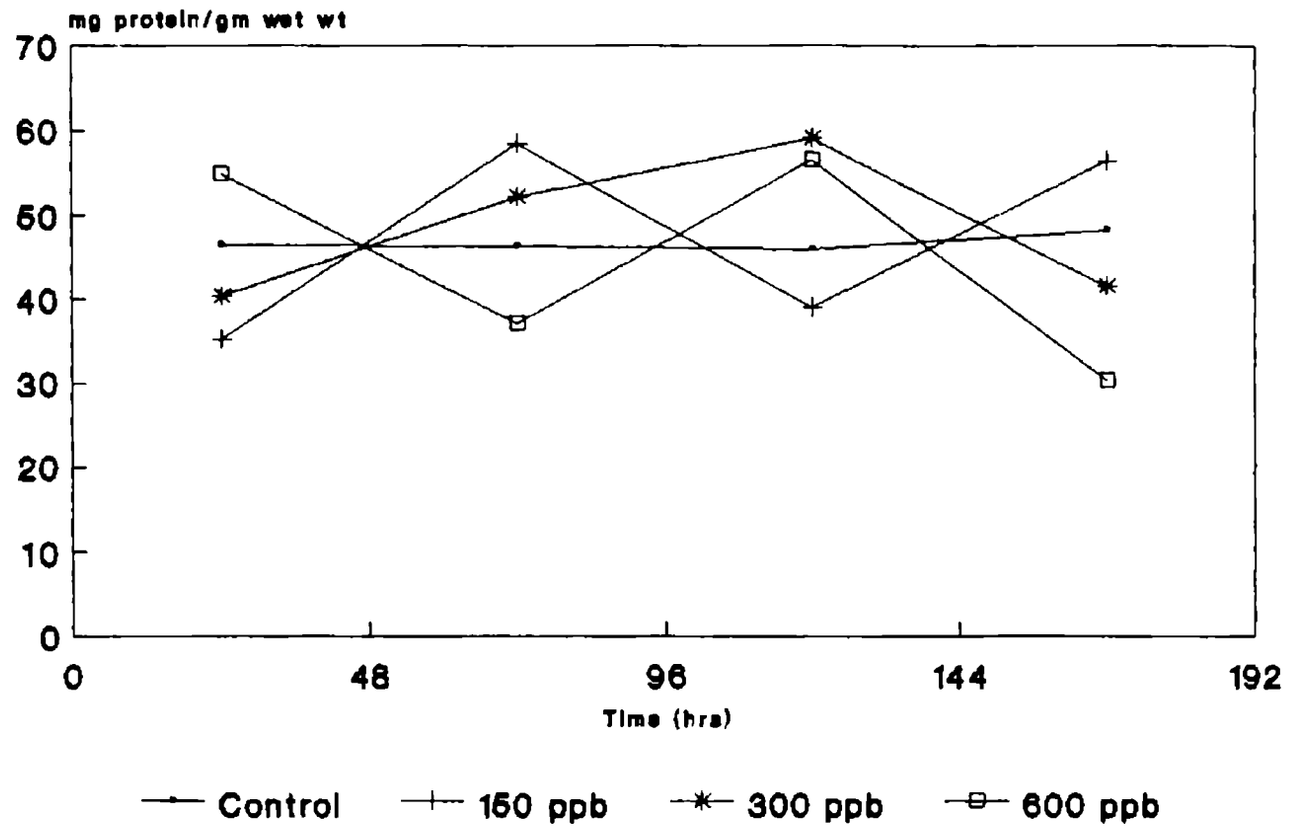


Figure 15. Hepatopancreas Total Protein (mg protein/gm wet wt) in *Lamellidens corrianus* exposed to three sublethal concentrations of cadmium.

When values in cadmium exposed animals were compared with those of the controls, significantly lower values were found at 120 and 168 hr ($P<0.01$, 0.001) while higher value was found at 72 hr ($P<0.05$) in 150 ppb exposed animals. In 300 ppb exposed animals, significantly lower value was found at 24 hr ($P<0.01$) but higher value at 120 hr ($P<0.001$). In animals exposed to 600 ppb, significantly lower values were found at 24 and 168 hr ($P<0.01$, 0.001)

On comparison between values in animals exposed to 150 and 300 ppb, significantly higher value at 72 hr ($P<0.001$) but lower values at 120 and 168 hr exposure periods ($P<0.001$) were observed in 150 ppb exposed animals. When values in 150 ppb exposed animals were compared with those of 600 ppb exposed ones, significantly higher value was found in 150 ppb exposed ones ($P<0.05$) at 72 hr but lower at 168 hr ($P<0.001$). On comparison between values in 300 and 600 ppb exposed mussels, 300 ppb exposed ones showed significantly higher values at 120 and 168 hr ($P<0.001$). No significant change was observed in values at 24 and 72 hr exposure periods.

4.4 DISCUSSION

Total protein content in gills of animals exposed to the

lower two concentrations of copper was significantly low at 24 hr time period, and high at 120 and 168 hrs. Towards later periods (ie: 120 and 168 hr) there was a general trend of increase in protein level from the level seen at early time periods.

The initial decrease in protein content can be attributed to the increased proteolytic activity. The elevation in transaminase activity observed during the same period suggests the replenishment of amino acid pool. Decrease in total protein is also reported by Ahamed et al.(1978) in *Lamellidens marginalis* subjected to pesticide toxicity. Decline in total protein content during stress is reported by Bhaskar et al.(1982) in gill of fish. Bhaskar et al.(1982) also observed differences in soluble and structural fractions of protein. After the initial decline, the protein level was found to get elevated and reached a peak at 120 hr in all the concentrations. This increase may be due to enhanced protein biosynthesis in the tissue. Ammonia formed during stress may be converted to glutamine and stored in the tissue to be utilised for aminoacid and protein syntheses (Rao et al., 1981). Since glutamate based ammonia production is suggested in tissues of *Lamellidens* species during stress (Mohan et al., 1987) the increased protein content in the present study

may possibly be due to the above reason. The low protein content in the early phase of exposure may again be due to the utilisation of the protein synthesised during the earlier hours, ie. before 24 hrs. Proteolysis at structural level of organisation of gill is reported in freshwater fish *Tilapia mossambica* under stress (Bhaskar et al., 1982).

In the hepatopancreas, the pattern of protein concentration is more or less similar to that of gill. Here also during the initial period protein content wasat a low profile. Unlike gill, in hepatopancreas, the decline in protein value may be due to the mobilisation of protein to other parts of the body as indicated by Viarengo et al.(1982). Hepatopancreas also showed a general trend of increase in protein level in a time dependent manner up to 120 hr. The maximum protein concentration was observed at 120 hr post exposure in 100 and 200 ppb, while in 400 ppb post exposure, the maximum value was found at 72 hr. The significant elevation in protein level may be due to augmented protein biosynthesis early at higher concentration and a little later in the other two. Heavy metal induction of metallothionein is widely reported inmolluscs (Roesijadi, 1980; Viarengo et al., 1980, 1981). Since hepatopancreas plays an important role in detoxication, the increased protein content may be indicative of

the synthesis of metallothionein. Moreover, the pattern of increase, ie. earlier in highest concentration and later in low concentration, is also suggestive of the induction of metallothionein in relation to metal concentration. Hepatic protein induction in copper exposed mussels is reported by Katticaran (1988). After the detoxication of metals, the protein synthesis declines, and the declining trend observed during 168 hr may be indicative of completion or near completion of detoxication. Chaterjee and Bhattacharya, (1986) observed increase in hepatic protein during early period and decline towards the later period of exposure. However, they also found that the protein content of the experimentals did not reach the control level.

Gill protein profile of mercury exposed animal tissue is very much different from that of copper exposed mussels. The initial decrease in protein (though insignificant) in low concentration suggests their possible degradation due to increased proteolytic activity. Decrease in protein content during stress is reported in freshwater mussel (Ahamed et al., 1978). A relatively high GPT activity during the same period further substantiates the possibility of increased proteolysis which in turn could contribute to the increase in amino acids to

be fed into the TCA cycle as keto acids. The increase in protein contentmay be due to decreased proteolytic activity or increased protein synthesis. The decreased and increased levels of protein suggest the possibility that in low concentration the degradation of protein exceeds protein synthesis where as in higher concentration, the synthesis exceeds degradation as indicated by Rao et al.(1987). Similar inverse relation between concentration and protein level was reported by Baktavalhsalam and Reddy (1984). Baktavalhsalam and Reddy (1984) suggested that the energy demand was high in lower concentration than in higher concentration of the toxicant. During other periods, protein concentration in the animals exposed to lower two concentrations remained more or less steady, possibly due to the activation of synthetic phase of protein metabolism. But in the highest concentration, protein synthetic mechanism seemed to get affected by prolonged exposure, as evident from the value at 168 hr,probably due to structural damage inflicted on the tissue. Damage to gill tissue on toxicant exposure is reported (Daughtie and Rao, 1984 Sunila and Lindstorm, 1985) The trend of elevation of GPT and ACP activity in gill in 300 ppb exposure during the same period, further strengthen the possibility of tissue damage.

Protein concentration in the hepatopancreas showed lower value early in low concentration but late in higher concentrations. The variation in protein level may be due to the alteration in general metabolism (Gilbertson et al., 1967). The general trend of decline in protein level in 150 and 300 ppb exposed ones at later time period, as well as during the early period in lower concentration may be attributed to impairment of protein synthetic mechanisms. Cellular degradation by mercury with increase in exposure duration is documented (Hilmy et al., 1981). The reduction in protein content may also be due to inhibition of uptake of amino acids into polypeptide chain or by decreasing the rate of degradation of protein in the tissue (Baneerjee and Choudhuri, 1985), or due to the possible utilisation of these compounds for metabolic purposes. A significant increase in hepatic protein and free amino acids was observed by Rao et al.(1980) in *L. marginalis* on exposure to toxicants. Rao et al.(1980) suggested that the increase may be due to the possible diversion of the system towards the synthetic phase to face the augmented stress condition. Due to increased stress in higher concentration as well as prolonged exposure, the protein synthetic mechanism is further affected and deranged. Viarengo et al.(1980) reported that cadmium, mercury, and copper reduce protein synthesis not only by reducing RNA synthesis, but

by influencing attachment to rough endoplasmic reticulum or by damaging the ribosomes.

In cadmium exposed mussels, protein content in gill tissue showed a peculiar pattern. During the initial period, protein content in 150 ppb exposed animals was significantly lower than that of control, while in those exposed to higher concentrations, no significant change was observed at this period. But from 72 hr post exposure onwards, protein concentration in 150 and 600 ppb exposed mussels showed an inverse relation. At 72 hr post exposure, when significantly high protein level was found at 150 ppb, the protein in 300 ppb exposed animals was low. Similarly, during 120 hr, protein content in 600 ppb exposed mussels was significantly high while in those exposed to 150 ppb was low. In 300 ppb exposed animals significant change in protein content occurred only at 120 hr after which the protein concentration reached the control level. The significantly low protein content during early time period in 150 ppb exposed animals may be due to increased proteolysis for gluconeogenesis in order to combat stress. Decrease in protein content during stress is reported (McLeay and Brown, 1970). But the protein concentration was found to be recouped during the subsequent exposure period. In 600 ppb exposed animals, the significantly high value during 120

hr may be due to increased synthesis of metallothionein. The decline in value during later period suggest detoxification. The nonsynchronised pattern of protein concentration may be due to the overall impairment of metabolic activity. The variation may also be due to alteration in RNA precursor uptake (Gilbertson and Michelson, 1969).

The decrease in total protein content in the hepatopancreas during the initial period may be attributed to increased proteolytic activity as in the case of copper and mercury exposed mussels. After the non significant initial decline, protein content rose in 150 ppb exposed mussels at 72 hr probably due to the induction of metallothionein. According to De Bruin (1976) the defect in protein synthesis is due to altered relationship between the ribosomes and membranes of endoplasmic reticulum. A biphasic effect of cadmium chloride on hepatic metallothionein synthesis is reported in fish (Chaterjee and Bhattacharya, 1986). Augmented protein level in 300 ppb exposed animals at 120 hr also suggests the probable involvement of metallothionein. Metallothionein synthesis is well documented for cadmium and copper in mussels (Noel-Lambot, 1976; Phillips,1976 a; 1976 b Viarengo et al., 1980, 1981, 1985; Langston and Zhou, 1987). At 600 ppb, it appears, the synthesis was also affected.

The inductable nature of metallothionein may exert a protective function against the cytotoxic effects of heavy metal (Piscator, 1964). Binding of metals to such protein may account for the apparent tolerance of the organism to a high metal concentration, although there may be a threshold level beyond which the animal cannot metabolically contain the excess metal.

C H A P T E R - V

EFFECT OF HEAVY METALS ON THE ACTIVITY PATTERN OF TISSUE PHOSPHATASES

5.1. INTRODUCTION

Many xenobiotics enter the environment through the activity of man, and are accumulated in different organisms. Molluscs, especially bivalves, are found to rapidly accumulate various organic and inorganic toxicants in their tissues. 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 (Bayne et al., 1979; Lee et al., 1980; Moore, 1985). It is also known that many xenobiotics cause cell injury by reacting primarily with biological membranes or membrane components (Moore, 1985)

Changes in enzyme activity levels are of diagnostic value in various pathological conditions particularly in mammals. But in bivalves very little information is available in this respect. Although studies on physiological and biochemical responses to toxicants are extensively carried out in mammals, such studies have not been an integral part of aquatic toxicology (Mehrle and

Mayer, 1978) 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 (Jackim et al., 1970). Toxicant concentration, exposure periods, temperature, salinity etc. are factors which greatly influence enzyme activity.

Copper is found to affect respiratory enzymes, and enzymes related to the excretion of metals (Hubschmann, 1967). The principal reaction of mercury involves binding of thiols and formation of stable mercaptides (Hughes, 1957). Alteration in cell membranes brought about by binding of mercury to sulfydryl groups could stimulate membrane turn over and production of hydrolytic enzyme (Arstila and Trump, 1968). Mercury is also shown to inhibit enzymes of Kreb's cycle (Yoshino et al., 1966), reducing levels of ATP which could decrease extrusion of hydrolytic enzymes from cells and there by causing increased activity of acid phosphatase (ACP) in lysosomal mitochondrial fraction (Hinton and Koenig, 1975). Cadmium is also found to inhibit enzymatic and metabolic activities (Jackim et al., 1970).

Lysosomal membrane is often the target of injury by xenobiotics, resulting in destabilisation. The destabilised membrane allows release of lysosomal hydrolytic enzymes into the

cytoplasm and extra cellular environment (Moore and Stebbing, 1976).

Lysosomes, a store house of about three dozen hydrolytic enzymes are found to sequester many anthropogenic substances and play an important role in their bio-accumulation (Dingle and Fell, 1969). Many of the molluscan cell types are rich in lysosomes. They are involved in intracellular digestion, storage, excretion, resorption, cell proliferation, and immune mechanisms (Summer, 1969; Owen, 1972; Moore et al., 1978 a; 1978 b; Cheng, 1983). A variety of environmental stressers and pollutants, including heavy metals are found to affect the lysosomal structure resulting in labilisation and subsequent release of hydrolytic enzyme into the cytoplasm. Many heavy metals such as cadmium, zinc, iron etc. have been demonstrated to be lysosomal inclusions in many bivalve cells (Lowe and Moore, 1979; George, 1983 a).

Lysosomes and cell membrane are the first target of xenobiotics as lysosomes are concerned with the disintegration of foreign bodies, and cell membrane is the first barrier to a pollutant. If the lysosomal membrane is made unstable, the enzymes will be released into the cytoplasm. Thus, monitoring of

alteration of lysosomal latency and acid hydrolase activity is found to be an index of pollution (Moore and Stebbing, 1976). Phosphatases are metal requiring enzymes whose activity may get modified by added cations.

Acid and Alkaline phosphatases (ACP and ALP) are groups of enzymes that hydrolase phosphomonoesters in a relatively non-specific manner with optimum activity in the acidic and alkaline regions, respectively. Acid phosphatase is a lysosomal marker enzyme and is reported to be a good stress indicator in biological systems. Xenobiotics can cause injury to lysosomes which later release the hydrolytic enzyme leading to cell atrophy. This type of injury resulting in destabilisation of lysosomal membrane bears a quantitative relationship to the magnitude of stress response (Bayne et al., 1979, 1982). Alkaline phosphatase is a plasma membrane bound enzyme involved in membrane transport and other intra cellular functions. It is a purported zinc metalloenzyme (Reynolds and Schlesinger, 1969). A variety of metals including copper and zinc are involved structurally in the formation of metallo-enzymes in molluscs. Alkaline phosphatase is one among these enzymes (George and Coombs, 1975). Liver ALP exists at least in two forms in rats and humans (Unnakami et al., 1987), which are different in heat

stability, chemical inhibition etc. Kominami et al. (1984) showed that rat liver ALP exists as confirmationally different forms in the serum and liver membrane. Polymeric forms of ALP are also found to exist in molluscs (Pricipato et al., 1982)

ACP and ALP are enzymes concerned with the bio-synthesis of fibrous protein (Johnson and McMinn, 1958) and mucopolysaccharides (Kroon, 1952), or they may serve as regulator of intracellular phosphatase concentration (Gutman, 1959). They play an active role in the dissolution of dead cells. Stimulation or inhibition of these enzymes will result in the disturbance of metabolism.

Martin and Field (1934), Monod(1944), Allen and Price (1950), and Gupta et al. (1975) have observed the diverse effects of various toxicants on the activity of various hydrolytic and oxidative enzymes of different organisms. Hinton et al. (1973) studied the effect of mercuric chloride intoxication in channel cat fish and observed marked changes in ACP and ALP in liver. According to Hinton et al. (1973), mercuric chloride inhibits ACP and ALP activity. Lysosomal membranes are adversely affected by mercury, resulting in the release of acid hydrolytic enzymes into the cytoplasm (Verity and Reith, 1967). Inhibitory effect of mercury is also quoted by Chavapil et al. (1972). Increased acid

and alkaline phosphatase activity is reported in *Lamellidens marginalis* on post exposure to pesticides (Babu and Vasudev, 1984). The accumulation of a wide range of metals in lysosomes and their effect on enzymes have been reviewed by Sternlieb and Goldfischer (1976). The marine mussel *Mytilus edulis* is reported to accumulate very high levels of metals in the tertiary lysosomes of kidney (George et al., 1976; Schulz-Baldes, 1978). Tertiary lysosomes can accumulate large amounts of metals in hepatopancreas and excretory tissues (George, 1982). Invertebrates are able to excrete these bodies by exocytosis. Such observations are reported in gills of *Mytilus edulis* (George et al., 1976), hepatopancreas of *Carcinus maenus* (Hopkins and Noh, 1980), and kidney of *Mytilus edulis* (George and Pirie, 1980).

Many molluscan cells are rich in lysosomes. The cytoplasmic granules in ameboid cells of *Mytilus edulis* are found to accumulate iron and lead (Moore and Lowe, 1977). The secretory cells of kidney in *Mytilus edulis* are shown to contain zinc and iron in lysosomes (George et al., 1976). In a study on the effect of mercury and selenium on blood clam, *Anadara granosa* Chandy and Patel (1985) observed an increase in the activity of acid phosphatase and aryl sulphatase. Mercury has been demonstrated as a lysosomal inclusion in rat liver, leading to an

increase in the quantity of lysosomes (Koenig, 1963). This increase in the availability of hydrolytic enzymes enables the system to metabolise and sequester the metal ion in a non-toxic form.

Increase in acid hydrolases due to toxicant intoxication is characteristic of tissue damage and is used for measuring hepatic toxicity (Tietz, 1970). In a study on the effect of Sumithion on a freshwater crab, Reddy et al. (1984) observed increase in ACP activity in all the exposed animals after 24 hrs. Lysosomal hydrolases are thought to contribute to the degradation of damaged cells and thus facilitate their replacement by normal tissue (De Duve, 1963). ACP and ALP are stated to show an increase in activity under various pathological conditions (Lutner, 1975). Vijayakumari et al. (1978) working on the effect of scorpion venom on guinea pig found that the maximum enzyme activity was in blood serum. Phosphatases are quickly transported to the serum from the liver, where they are synthesised. Suresh and Mohandas (1990) observed a high level of ACP in the hemolymph of *Sunetta scripta* exposed to 1 ppm copper, and a lower value at higher concentration. Many xenobiotics are known to cause lysosomal destabilisation (Moore and Lowe, 1985) leading to the release of hydrolases into the cytosol. Cadmium,

a common aquatic pollutant, is found to be accumulated in large amounts in the gills (Carpene and George, 1980), and digestive diverticula (George and Coombs, 1977) of bivalves.

Hilmy et al. (1985) suggested that sensitivity of enzyme to cadmium varied in different tissues without obeying any rule and suggested the difference in tissue enzyme sensitivity may be related to the ability of the compound to alter the cellular membrane configuration by binding with lipid protein of membrane and thus blocking the movement of substances by active transport. Loomis and Lippman (1948), and Kelly and Syrett (1964) have pointed out that dinitrophenol prevents the orderly formation of energy-rich compounds prior to the synthesis of ATP, thus acting as uncoupling agents that hinder phosphorylation that normally accompanies oxidation. Excess toxicant is also found to injure the mitochondrial system, thus blocking the enzyme action (Simon, 1953). In the hydroid, *Campanularia flexuosa*, release of lysosomal hydrolases is involved in cytotoxic response of copper, cadmium and mercury (Moore, 1977). Such mechanism may be involved in the kidney secretory cells and digestive cells of *Mytilus edulis* (Lowe and Moore, 1979).

At the sub cellular levels, lysosomes are found to play an

important role in the detoxification of many trace metals through binding and sequestration (Moore, 1980). The monitoring of alteration in lysosomal latency and acid hydrolase activity has been suggested as an index of pollution (Moore and Stebbing, 1976; Lowe and Moore, 1979). Yager and Harry (1964) reported that the permeability of surface epithelial membrane was disrupted and as a result copper uptake was lowered in snails. The lysosomes play a fundamental role in homoeostasis as well as in the detoxification of metals in the digestive gland of copper exposed mussels (Viarengo et al., 1985). In *Mytilus edulis* concentration dependent labilisation of lysosomes was observed on exposure to copper (Harrison and Berger, 1982). Suresh and Mohandas (1990) have reported increased ACP activity in copper stressed bivalves during early time periods and indicated that it may be due to destabilisation of the lysosomal membrane resulting in the release of enzyme into the haemolymph.

Sastry and Gupta (1987) reported the adverse effect of mercury on aerobic and anaerobic metabolism by inhibiting the activities of hexokinase glucose-6-phosphatase, lactate de hydrogenase and succinic dehydrogenase in fishes. The inhibitory effect of enzyme activity may be due to direct binding of mercury and other heavy metals with the enzyme protein (Passow et al.,

1961), or due to decrease in enzyme synthesis (Blackwood et al., 1965). Dalela et al. (1980) studied the effect of sublethal concentration of phenol and phenolic compounds on hepatic ACP and ALP and found significant inhibition. Saxena et al. (1982), in a study on the effect of vegetable oil factory effluent on *Channa punctatus*, reported significant decline in enzyme activity in liver and kidney.

Within a single species the cellular enzyme content may vary with physiological conditions. For example, humans with bacterial infection have elevated ALP level, while those with viral infection have normal or below normal enzyme activity (Kaplaw, 1973). ALP are a group of membrane associated enzymes of low substrate specificity which hydrolases a variety of organic monophosphate esters. The enzymes are present in many tissues, and isoenzymes of bone, liver, and intestine are thought to give rise to ALP normally present in circulation (Kaplan, 1972).

The activity of the enzyme and the lysosomal latency are dependent on the physiochemical nature of habitat which in turn is influenced by pollution (Patel and Patel, 1985). Patel and Patel (1985) also observed that change in ACP activity may be

caused by some alteration in the properties of enzymes or their inactivtion due to a drop in the bio-availability of metallic enzyme activators. Onikieno (1963), and Bhatia et al. (1972) observed an increase in serum ACP and ALP in rats due to liver damage when exposed to pesticides. Increased levels of phosphatases in serum of *Mystus vittatus* were reported to be due to degeneration of live cells by the toxic action of pesticides which induced the production of hepatic microsomal enzymes that subsequently came into the blood (Verma et al., 1984). ALP is reported to be involved in protein synthesis (Pilo et al., 1972), synthesis of certain enzymes (Summer, 1965), secretory activity (Ibrahim et al., 1974), and glycogen metabolism (Gupta and Rao, 1974). The activation or inhibition of ALP could be indicative of disturbance in the above processes. Naurigal and Singh (1985) observed in *Puntius chilinoides* that ALP activity increased in ovaries during the maturing phase indicating that during this period, synthesis of new protein takes place as ALP is involved in protein synthesis.. Undoubtedly, degradative damage to functional physiological modules within a cell will eventually have wide ranging effects on the integration of cellular, tissue, and ultimately whole organism's physiological processes (Bayne et al., 1985).

Alkaline phosphatase was selected for the present study because they are metal requring enzymes, traces of added cations might displace or affect the metal containing moiety and modify enzyme activity. Acid phosphatase, being a lysosomal enzyme, plays an important role in mineral metabolism and metal detoxification, and hence it was selected. In this chapter are included results of the study on the effects of heavy metals on the activity pattern of these two phosphatases in gill and hepatopancreas of *L. corrianus*.

5.2 MATERIAL AND METHODS

Methods of collection of specimens, acclimatisation and exposure to toxicants were the same as described in Chapter 2. For each concentration of each metal as well as the controls, eight animals were used.

The control and the experimental animals were sacrificed at the end of the test period and the gill and hepatopancreas were removed immediately. After rinsing in chilled glass double distilled water, tissues were accurately weighed and homogenised separately, using a Potter-Eivehfen type homogeniser. A 5% homogenate of each of the two tissue samples wasprepared for the present study. The homogenates were then centrifuged at 15,000

rpm at 4°C for 15 minutes. The supernatant thus obtained was the source for the estimation of activities of selected enzymes and protein concentration. The supernatant was kept frozen and analysed for enzyme activity within 2-3 hours after preparation. The tissue extract was kept frozen till incubation.

5.2.1 Assay of Acid phosphatase activity

Acid phosphatase activity was determined by employing the method described in Sigma Technical Bulletin No. 104 with slight modification (Annon, 1963) To 1 ml of (0.1 M) frozen citrate buffer of pH 4.2, 0.1 ml of enzyme extract was added. To this buffer enzyme mixture, 0.1 ml of substrate containing 2.0 mg of *P* - Nitrophenyl phosphate sodium salt (Merck) in 0.1 ml of distilled water was added and incubated for 60 minutes at 37 $\pm$ 0.05°C. After the incubation, the reaction was stopped by adding 2ml of o.25 N NaoH. *P*- Nitro-phenyl phosphate was hydrolysed to *P* - Nitrophenol by the enzyme during the incubation period. The yellow colour developed in the alkaline medium was read spectrophotometrically at 410 nm. The concentration of *P*-Nitrophenol formed was calculated from the standard graph. Simultaneously, the protein content of the extract was also estimated by Lowry's method, (1951). From this μ moles of

P-Nitrophenol liberated /mg. protein / hour was calculated.

5.2.2 Assay of Alkaline phosphatase activity

Alkaline phosphatase activity was determined according to the method described in Sigma Technical Bulletin No.104 with slight modifications (Annon, 1963). To study the enzyme activity, 0.05 M glycine-NaoH buffer of pH 9.0 and 9.4 was used for gill and hepatopancreas extracts, respectively. To 1ml of frozen buffer, 0.1 ml of extract was added. To this buffer enzyme mixture, 0.1 ml of substrate (2.0mg of *P*-Nitrophenyl phosphate sodium salt (Merck) in 0.1 ml distilled water) was added and incubated in a water bath for 60 minutes, at 37 $\pm$ 0.05^{o}C. After incubation the reaction was stopped by adding 2 ml of 0.25 N NaoH. The yellow colour developed was read at 410 nm. For alkaline phosphatase also protein content of the extract was estimated by Lowry's method (1951). From this μ moles of *P*-Nitrophenol liberated was calculated, and the enzyme activity is expressed as μ moles *P*-Nitrophenol liberated/mg protein/hour.

5.3 RESULTS

5.3.1. Acid phosphatase activity (ACP activity)

5.3.1.1 Copper

5.3.1.1.1 Gill tissue (Table 16, Figure 16)

Table 16. Gill Acid Phosphatase Activity (μ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of copper

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	0.2686	0.2689	0.2874	0.2180
	± SD	0.0568	0.0607	0.0659	0.0498
	Range	0.1998-0.3550	0.1998-0.3549	0.1417-0.3299	0.1535-3129
100 ppb of copper exposed	N	8	8	8	8
	Mean value	0.8304 ***	0.2858	0.4039 **	0.2031
	± SD	0.2413	0.0822	0.0559	0.0398
	Range	0.4389-1.1463	0.2014-0.4183	0.3189-0.4802	0.1486-0.2769
200 ppb of copper exposed	N	8	8	8	8
	Mean value	0.5202 ***	0.5711 ***	0.5510 ***	0.2285
	± SD	0.1114	0.1033	0.0807	0.0558
	Range	0.3514-0.7093	0.4617-0.7653	0.4401-0.7253	0.1257-0.2881
400 ppb of copper exposed	N	8	8	8	8
	Mean value	0.5952 ***	0.5441 ***	0.3992*	0.3458 ***
	± SD	0.0952	0.0384	0.1282	0.0450
	Range	0.4595-0.7448	0.4682-0.5842	0.2504-0.6183	0.3084-0.4201

Significance level : *$P<0.05$, **$P<0.01$, ***$P<0.001$

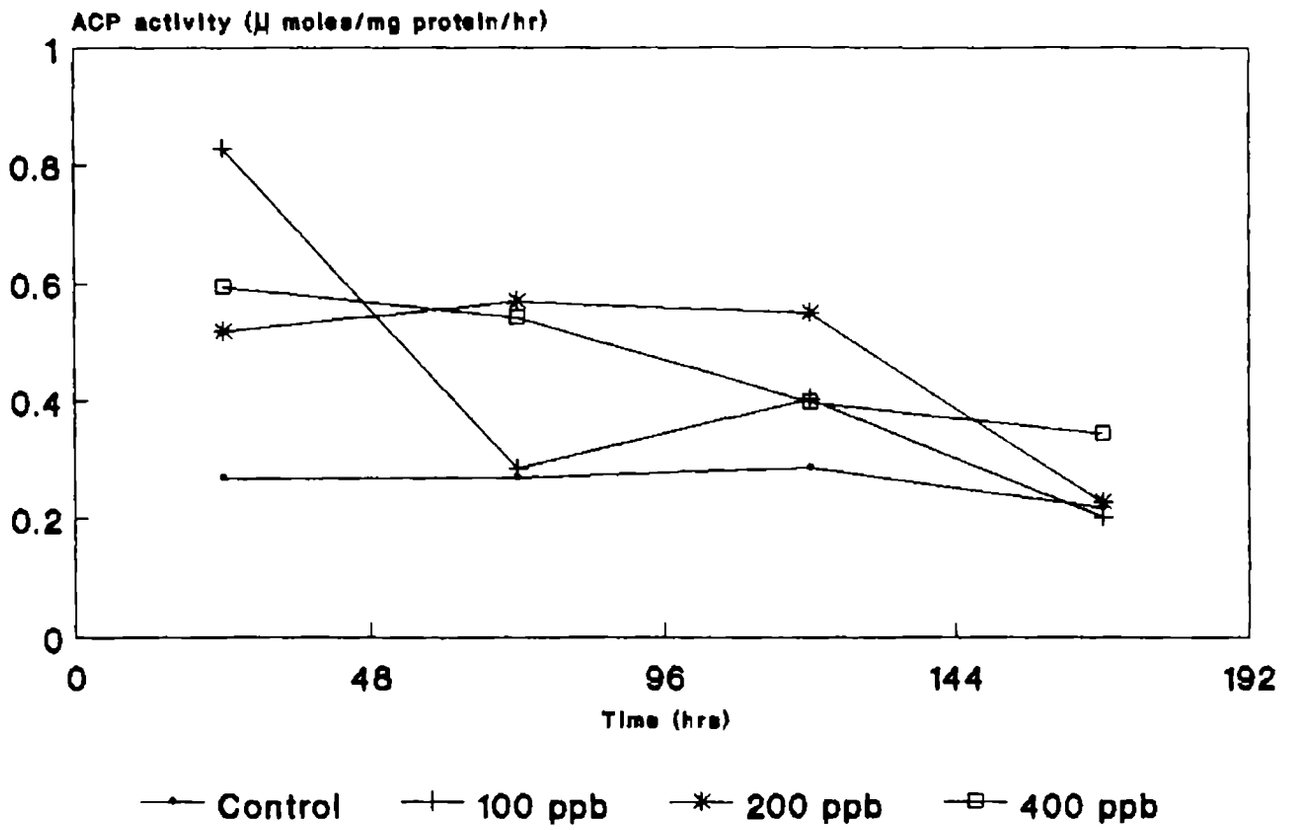


Figure 16. Gill Acid Phosphatase activity (µ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of copper.

Significant increase in gill ACP activity was found in clams exposed to 100 ppb copper at 24 hr post-exposure, when compared with the activity in the controls ($P<0.001$). Animals exposed to 100 ppb showed an increase in activity also at 120 hr ($P<0.01$). But in the remaining exposure periods, no significant change in activity was observed.In 200 ppb copper exposed animals, enzyme activity was significantly higher at all time periods ($P<0.001$) except at 168 hr. Significant elevation in activity was found at 24, 72, and 168 hr post-exposure in 400 ppb exposed animals ($P<0.001$) when compared with the controls. At 120 hr post exposure also significant increase in activity ($P<0.05$) was observed in 400 ppb exposed animals when compared with control. A decrease in activity depending upon the period of exposure was obvious in 400 ppb exposed animals.

At 72 and 120 hr post-exposure, reduction in activity was observed in 100 ppb exposed animals when compared with the activity in 200 ppb exposed ones($P<0.001,0.01$). The reduction in activity at 168 hr post-exposure in 100 ppb exposed animals was not significant. At 24 hr post-exposure, elevation in activity in 100 ppb exposed animals was significant ($P<0.01$) when compared with the activity in those exposed to 200 ppb. Significant decrease in activity at 72 and 168 hr post-exposure

($P<0.001$) but significant elevation in activity at 24 hr time period ($P<0.05$) were observed in 100 ppb exposed animals when compared with the values in those exposed to 400 ppb. When the activity levels in mussels exposed to 200 and 400 ppb were compared, significantly higher activity at 168 hr ($P<0.001$), but significantly lower activity at 120 hr ($P<0.05$) were observed in 400 ppb exposed mussels.

5.3.1.1.2. Hepatopancreas tissue (Table 17, Figure 17)

Comparison of enzyme activity in the hepatopancreas of copper exposed animals with that of the controls, exhibited the following pattern. Enzyme activity was significantly lower in 100 ppb exposed animals at all time periods ($P<0.01, 0.001, 0.001$) except at 120 hr when compared with the control values. In 200 ppb exposed animals, reduction in enzyme activity was significant at all time periods except at 24 hr post-exposure ($P<0.001$, 0.05, 0.001). In 400 ppb exposed mussels, enzyme activity showed no significant change when compared with the control values.

At 24, 72, and 168 hr post-exposure, 100 ppb exposed animals showed significant reduction in activity ($P<0.01$, 0.05, 0.05) when compared with the activities in 200 ppb exposed animals. The reduction in activity in 100 ppb exposed animals at the above time periods was also significant when compared with the activity

Table 17. Hepatopancreas Acid Phosphatase Activity (µ moles/mg protein/hour) in Lamellidens corrianus exposed to three sublethal concentrations of copper

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	0.8969	0.9649	0.9185	0.8637
	± SD	0.1669	0.1063	0.1865	0.1935
	Range	0.6976-1.2374	0.7896-1.1700	0.7005-1.1965	0.6859-1.2328
100 ppb of copper exposed	N	8	8	8	8
	Mean value	0.6447 **	0.6040 ***	0.8097	0.1810 ***
	± SD	0.0977	0.1067	0.0533	0.0528
	Range	0.4903-0.7391	0.4297-0.7218	0.6947-0.8659	0.1321-0.2927
200 ppb of copper exposed	N	8	8	8	8
	Mean value	0.9390	0.7244 ***	0.7432 *	0.3761 ***
	± SD	0.2061	0.1053	0.0987	0.1975
	Range	0.6225-1.2772	0.5933-0.8628	0.6051-0.9472	0.1553-0.7632
400 ppb of copper exposed	N	8	8	8	8
	Mean value	1.0400	0.9618	0.7342	0.9219
	± SD	0.3476	0.1814	0.1946	0.3040
	Range	0.7035-1.6187	0.7175-1.2640	0.4873-1.1414	0.5410-1.3656

Significance levels * $P<0.05$ ** $P<0.01$ *** $P<0.001$

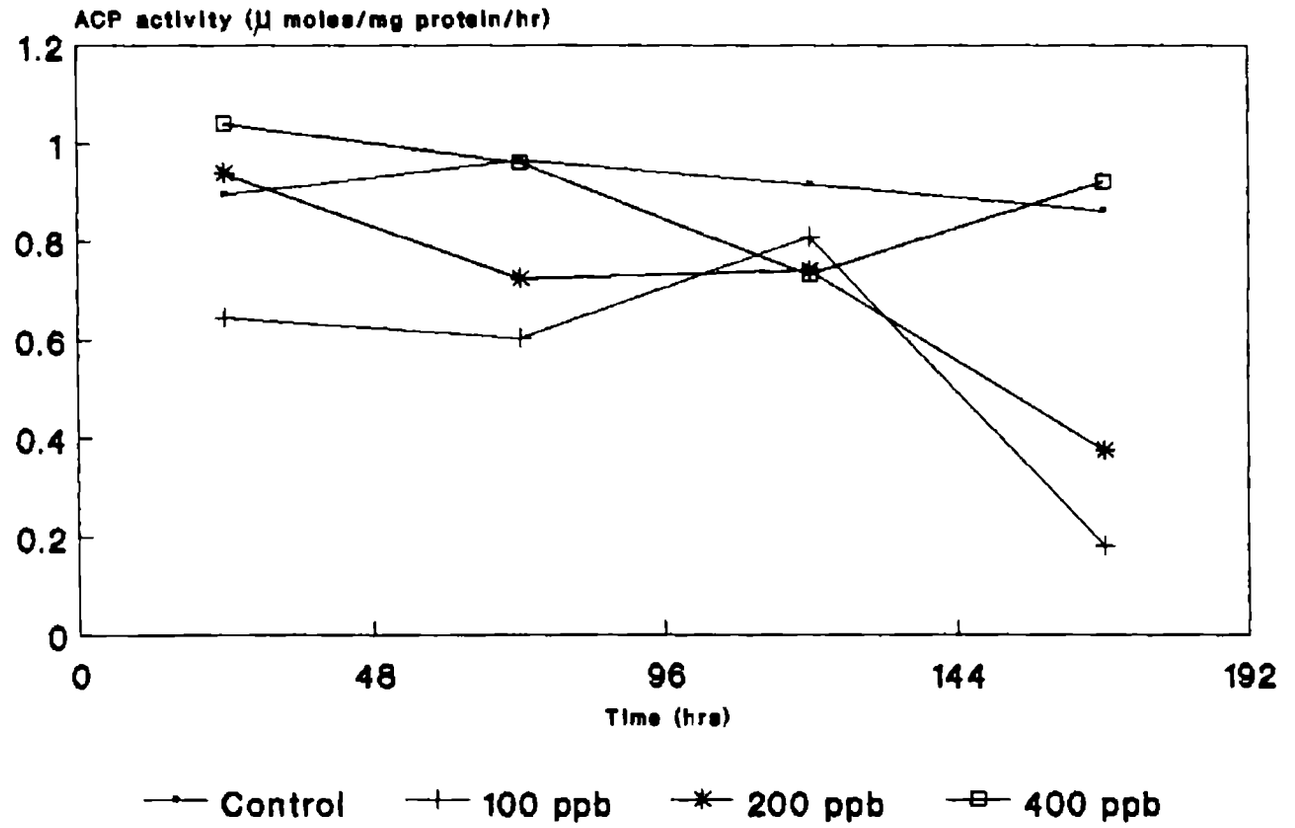


Figure 17. Hepatopancreas Acid Phosphatase activity (µ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of copper.

levels in 400 ppb exposed animals ($P<0.05$, 0.001,0.001). At 72 and 168 hr post exposure, significantly higher values were observed in 400 ppb exposed mussels ($P<0.01$) when compared with those of 200 ppb exposed animals.

5.3.1.2 Mercury

5.3.1.2.1 Gill tissue (Table 18, Figure 18)

Mussels exposed to 75 ppb mercury showed elevation in activity at 24 and 72 hr post-exposure ($P<0.001$, 0.01) but reduction in activity at 120 hr ($P<0.05$) when compared with the control values. When the activity levels in 150 ppb exposed animals were compared with those in the controls, enzyme activity was significantly higher at 72 and 168 hr post-exposure ($P<0.001$). Enzyme activity in 300 ppb exposed animals was significantly higher at 72 and 168 hr post exposure ($P<0.05$, 0.01) when compared with the controls.

Comparison of values among mussels exposed to different concentrations revealed a dose dependent decrease in activity at 24 hr post-exposure. Animals exposed to 75 ppb registered marked reduction in activity at 72, 120 and 168 hr post exposure ($P<0.01$, 0.001, 0.001) when compared with the activity in 150 ppb exposed animals, but showed elevation in activity at 24 hr post-exposure ($P<0.001$). Comparison of values in mussels exposed

Table 18. Gill Acid Phosphatase Activity (μ mole/mg protein/hour) in Lamellidens corrianus exposed to three sublethal concentrations of mercury

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	0.2801	0.2873	0.2754	0.2337
	± SD	0.0545	0.0758	0.0630	0.0524
	Range	0.2017-0.3550	0.1996-0.3945	0.1519-0.3495	0.1780-0.3222
75 ppb of mercury exposed	N	8	8	8	8
	Mean value	0.5235 ***	0.4260 **	0.2141 *	0.2337
	± SD	0.0652	0.0620	0.0238	0.0274
	Range	0.4425-0.6291	0.3425-0.5291	0.1733-0.2606	0.1952-0.2713
150 ppb of mercury exposed	N	8	8	8	8
	Mean value	0.3053	0.7716 ***	0.3870	0.5796 ***
	± SD	0.0300	0.2146	0.0889	0.1723
	Range	0.2589-0.3571	0.3979-1.0616	0.2813-0.5117	0.3563-0.8790
300 ppb of mercury exposed	N	8	8	8	8
	Mean value	0.2545	0.3960 *	0.2612	0.3343 **
	± SD	0.0345	0.0739	0.0816	0.0691
	Range	0.2147-0.3285	0.2992-0.5261	0.1915-0.3869	0.2458-0.4245

Significance level: *$P<0.05$, **$P<0.01$, ***$P<0.001$

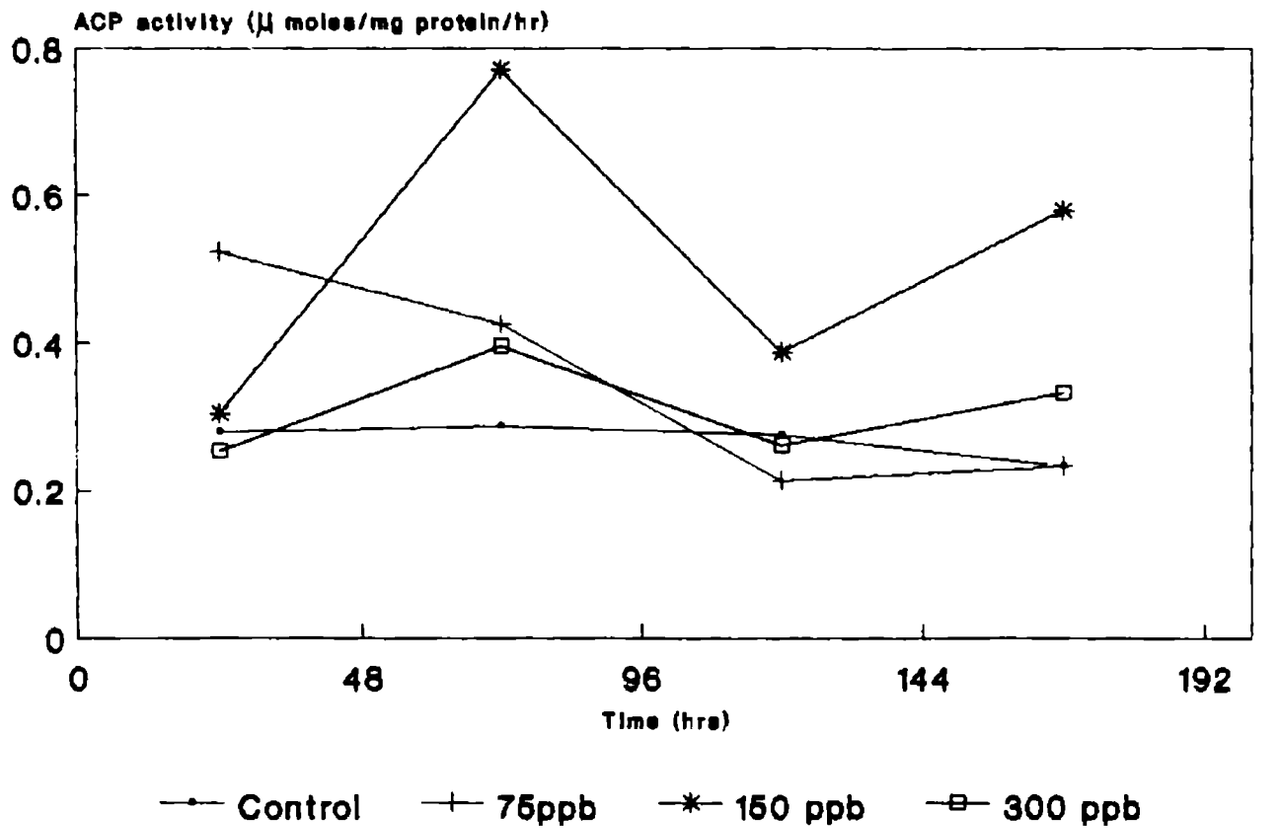


Figure 18. Gill Acid Phosphatase activity (µ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of mercury.

to 75 and 300 ppb mercury showed significantly higher activity at 24 hr ($P<0.001$) but lower activity at 168 hr ($P< 0.01$) in 75 ppb exposed animals. Consistantly higher values were found in 150 ppb exposed animals when compared with the values in those exposed to 300 ppb ($P<0.05$, 0.001 0.05, 0.01).

5.3.1.2.2 Hepatopancreas tissue (Table 19, Figure 19)

Significantly higher hepatic ACP activity at 24 hr post-exposure ($P<0.001$) but lower activity at 120 hr post-exposure($P<0.001$) were noticed in 75 ppb exposed animals when compared with the controls. But in 150 ppb exposed animals, significant reduction in enzyme activity was registered at 72 hr post-exposure ($P<0.001$) when compared with the control activity. At 300 ppb, significantly higher value was noticed at 72 hr ($P<0.01$) when compared with control.

On comparing the values of 75 and 150 ppb exposed animals, significantly higher value in 75 ppb exposed ones at 24 hr and 72 hr post-exposure ($P<0.001$), but significantly lower value at 120hr post-exposure ($P<0.001$) were observed. Significant reduction in activity was observed at all exposure periods except at 24 hr in 75 ppb exposed animals when compared with the activity in 300 ppb exposed mussels ($P< 0.001$, 0.001, 0.01). At 24 hr post-exposure, the enzyme activity at 75 ppb was higher

Table 19. Hepatopancreas Acid Phosphatase Activity (μ mole/mg protein/hour) in Lamellidens corrianus exposed to three sublethal concentrations of mercury

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	0.8050	0.9649	0.9448	0.8689
	± SD	0.2634	0.1063	0.1915	0.2888
	Range	0.5571-1.3647	0.7896-1.1700	0.7283-1.2478	0.5320-1.3528
75 ppb of mercury exposed	N	8	8	8	8
	Mean value	1.7928 ***	1.1840	0.4574 ***	0.7076
	± SD	0.2039	0.3076	0.0507	0.1071
	Range	1.600-2.2710	0.8864-1.8504	0.3934-0.5556	0.5399-0.9160
150 ppb of mercury exposed	N	8	8	8	8
	Mean value	0.7087	0.3542 ***	0.8546	0.9248
	± SD	0.0392	0.0913	0.1826	0.2603
	Range	0.6349-0.7609	0.2460-0.5109	0.6223-1.1974	0.6778-1.5093
300 ppb of mercury exposed	N	8	8	8	8
	Mean value	0.7473	1.6533 **	0.9210	0.9836
	± SD	0.0833	0.4594	0.2117	0.1658
	Range	0.5854-0.8374	1.0274-2.1831	0.6782-1.2742	0.7854-1.2978

Significance level: **$P<0.01$ ***$P<0.001$

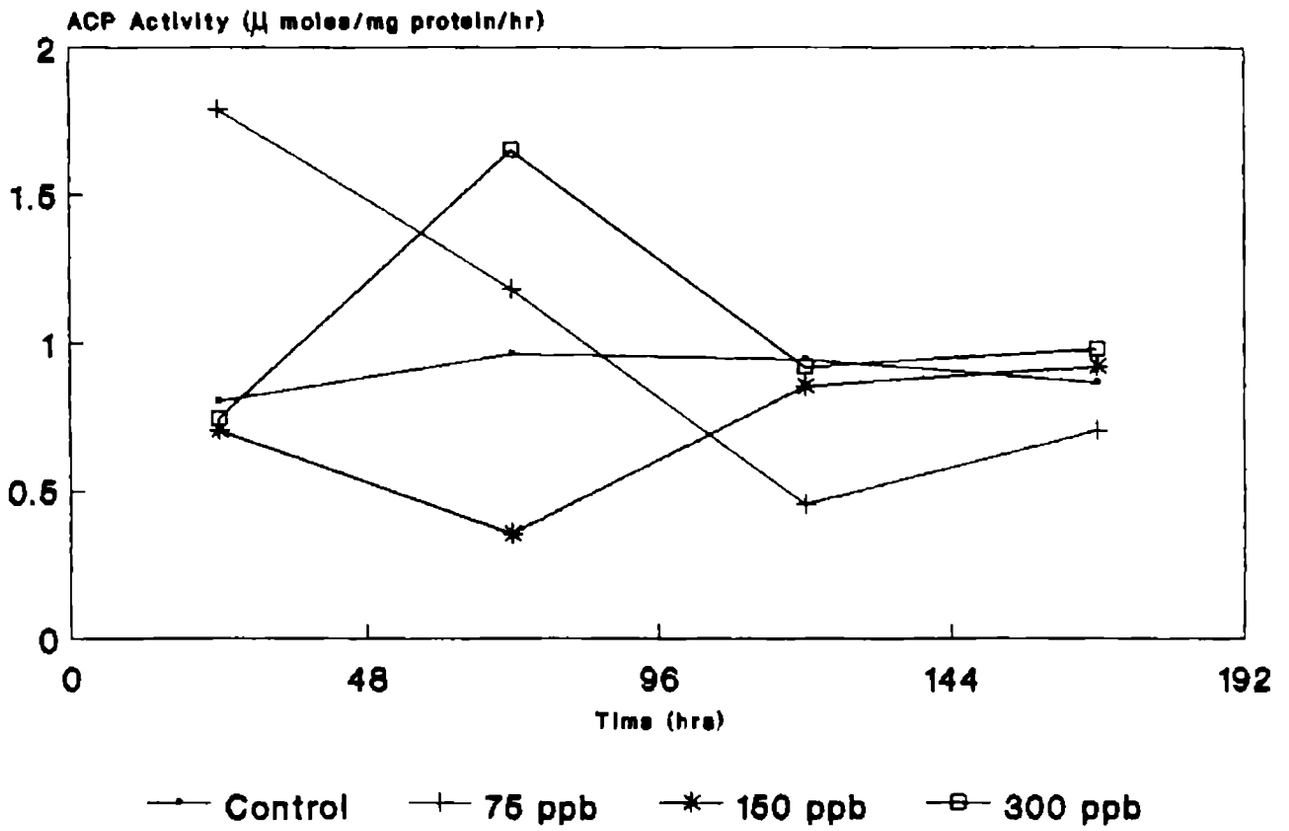


Figure 19. Hepatopancreas Acid Phosphatase activity (µ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of mercury.

($P<0.001$). Significantly lower activity was observed in 150 ppb exposed animals at 72 hr when compared with the activity in 300 ppb exposed ones ($P<0.001$). No significant change was observed in the remaining exposure periods.

5.3.1.3 Cadmium

5.3.1.3.1 Gill tissue (Table 20, Figure 20)

In 150 ppb exposed mussels, no significant change in activity was observed at any exposure period when compared with the controls. When 300 ppb exposed animals were compared with the controls, significantly higher values were found in the experimentals at all exposure periods ($P<0.05$, 0.01, 0.001, 0.001). Animals exposed to 600 ppb showed higher values at 24 and 72 hr post-exposure ($P<0.001$) when compared with the controls.

On comparison between 150 and 300 ppb exposed animals, enzyme activity in 150 ppb was consistantly low at all time periods ($P<0.01$, 0.001, 0.001, 0.001). Comparison between 150 and 600 ppb exposed animals also showed a similar pattern except at 168 hr ($P<0.001$, 0.001, 0.05). At 168 hr post-exposure, change in activity was not significant. When values in 300 ppb exposed animals were compared with those of 600 ppb exposed ones, higher values were found at 24 and 72 hr ($P<0.001$, 0.05) but

Table 20. Gill Acid Phosphatase Activity (μ moles/mg protein/hour) in Lamellidens corrianus exposed to three sublethal concentrations of cadmium

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	0.2810	0.2680	0.2584	0.2172
	± SD	0.0590	0.0750	0.0447	0.0472
	Range	0.1998-0.3550	0.1896-0.3945	0.2070-0.3195	0.1535-0.3000
150 ppb of cadmium exposed	N	8	8	8	8
	Mean value	0.2219	0.2883	0.2085	0.2448
	± SD	0.0713	0.0322	0.0229	0.0373
	Range	0.1258-0.3614	0.2351-0.3485	0.1758-0.2563	0.1744-0.2915
300 ppb of cadmium exposed	N	8	8	8	
	Mean value	0.3642 *	0.4607 **	0.4855 ***	0.3283 ***
	± SD	0.0830	0.0814	0.0818	0.0204
	Range	0.2234-0.4930	0.3468-0.5914	0.3663-0.6037	0.3087-0.3596
600 ppb of cadmium exposed	N	8	8	8	
	Mean value	0.8381 ***	0.5995 ***	0.4224	0.2266
	± SD	0.1295	0.1501	0.2139	0.0679
	Range	0.6094-0.9770	0.3716-0.7805	0.2318-0.7960	0.1380-0.3159

Significance level : *$P<0.05$, **$P<0.01$, ***$P<0.001$

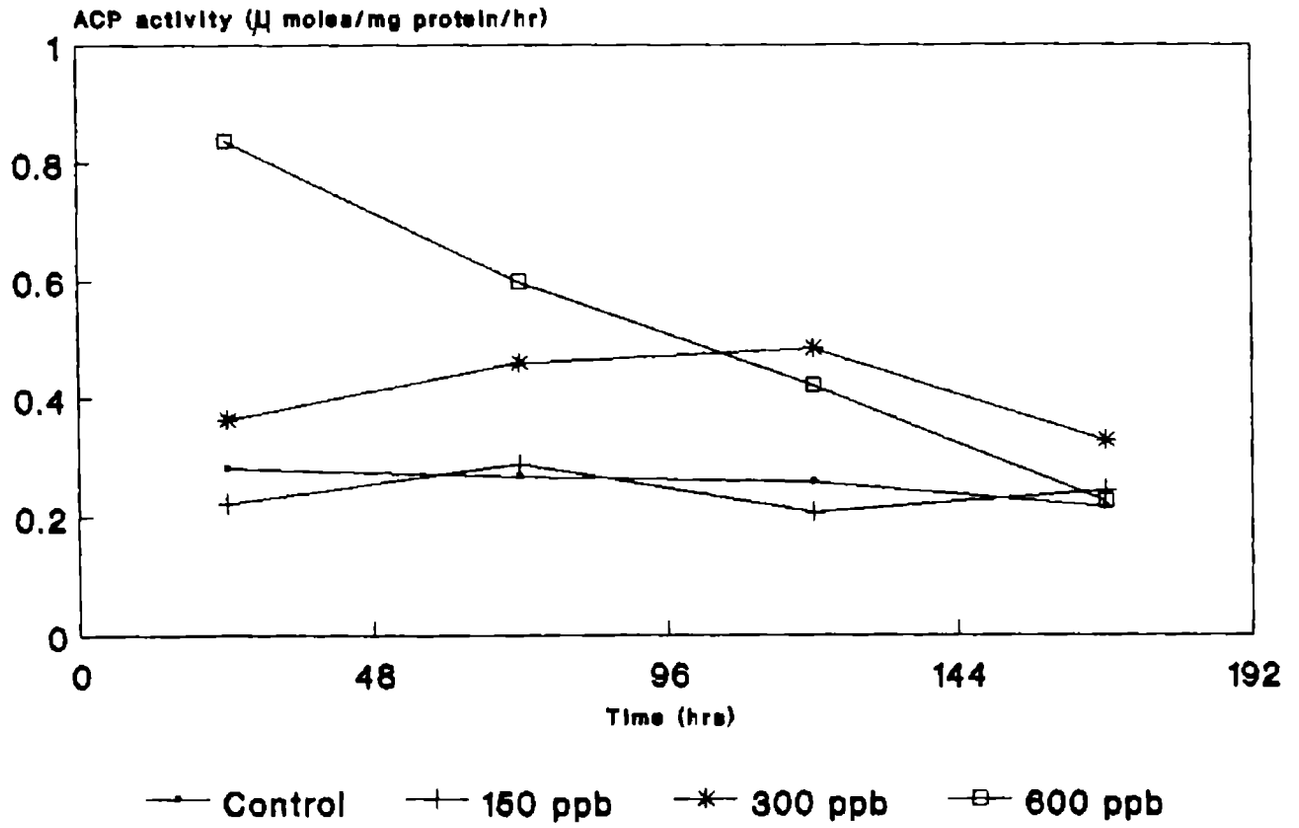


Figure 20. Gill Acid Phosphatase activity (µ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of cadmium.

lower at 168 hr ($P<0.01$) in 600 ppb exposed animals.

5.3.1.3.2 Hepatopancreas tissue (Table 21, Figure 21)

When the enzyme activity in hepatopancreas of 150 ppb exposed animals were compared with those of the controls, significant decrease was observed at 24, 120 and 168 hr post-exposure ($P<0.05$). Significantly higher enzyme activity was observed at 120 hr in 300 ppb exposed animals ($P<0.001$) and at 24 and 120 hr in 600 ppb exposed ones ($P<0.001$, 0.05) when compared with controls. At 168 hr post-exposure in 600 ppb, the enzyme activity was lower than that of the control ($P<0.001$). A dose dependant increase in activity was observed at 24 and 72 hr post-exposure in all the exposed animals.

Animals exposed to 150 ppb on comparison with those exposed to 300 ppb showed significantly lower values at 24 and 120 hr ($P<0.001$). Significant reduction in activity was also observed in 150 ppb exposed animals at 24 and 120 hr ($P<0.001$), but elevation at 168 hr ($P<0.001$) when compared with the activities in 600 ppb exposed animals. When values of 300 ppb exposed animals were compared with those of 600 ppb exposed ones, significantly lower value was found at 24 hr ($P<0.001$). However, at 120 and 168 hr post- exposure, significant elevation in activity was observed

Table 21. Hepatopancreas Acid Phosphatase Activity (μmoles/mg protein/hour) in Lamellidens corrianus exposed to three sublethal concentrations of cadmium

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	0.7924	0.7896	0.9287	1.0032
	± SD	0.2967	0.3106	0.2015	0.1291
	Range	0.4898-1.4367	0.5163-1.3285	0.7305-1.2370	0.8976-1.2215
150 ppb of cadmium exposed	N	8	8	8	8
	Mean value	0.5848 *	0.8727	0.7397 *	0.7553 *
	± SD	0.0493	0.0890	0.0750	0.2172
	Range	0.5128-0.6457	0.7314-0.9733	0.6061-0.8424	0.4964-1.1066
300 ppb of cadmium exposed	N	8	8	8	8
	Mean value	0.8103	0.9382	1.8179 ***	0.8591
	± SD	0.0239	0.1608	0.3372	0.1440
	Range	0.7797-0.8489	0.6833-1.1210	1.4037-2.3953	0.8000-1.2152
600 ppb of cadmium exposed	N	8	8	8	8
	Mean value	2.0153 ***	0.9871	1.2761 *	0.3817 ***
	± SD	0.4418	0.2158	0.2710	0.0696
	Range	1.3399-2.7090	0.5384-1.1864	1.7446-1.0719	0.2772-0.4383

Significance level : *P<0.05, **P<0.01, ***P<0.001

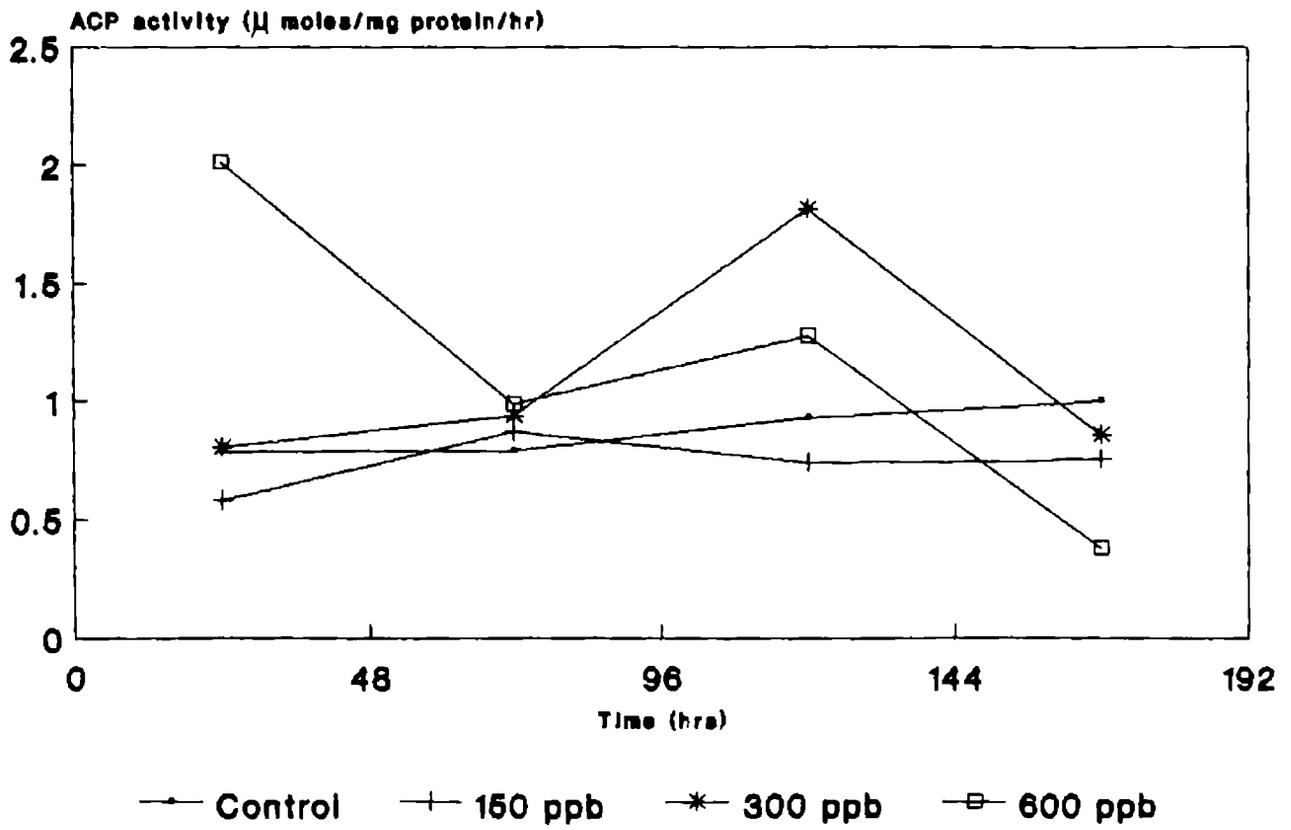


Figure 21. Hepatopancreas Acid Phosphatase activity (µ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of cadmium.

in 300 ppb exposed animals ($P<0.01$, 0.001).

5.3.2 Alkaline phosphatase activity (ALP activity)

5.3.2.1 Copper

5.3.2.1.1 Gill tissue (Table 22, Figure 22)

Significantly lower enzyme activity was found in 100 ppb exposed animals at 72 and 168 hr post-exposure ($P<0.01$, 0.001) when compared with the control values and in 200 ppb exposed animals, at 168 hr post-exposure ($P<0.001$). Comparison of values of 400 ppb exposed mussels with those of the controls showed significant reduction at 120 and 168 hr post-exposure ($P<0.001$, 0. 001).

Mussels exposed to 100 ppb copper had significantly lower values than those of mussels exposed to 200 ppb at 72 and 168 hr ($P<0.001$, 0.05), and again at 72 and 168 hr post-exposure ($P<0.001$) when compared with those of mussels exposed to 400 ppb. But at 120 hr post-exposure 100 ppb exposed animals showed higher enzyme activity ($P<0.001$) than those of mussels exposed to 400 ppb. Those animals exposed to 200 ppb copper had higher value at 120 hr ($P<0.001$) but lower value at 168 hr ($P<0.05$) when compared with the values of 400 ppb exposed animals.

5.3.2.1.2 Hepatopancreas tissue (Table 23, Figure 23)

Table 22. Gill Alkaline Phosphatase Activity (μ moles/mg protein/hour) in Lamellidens corrianus exposed to three sublethal concentrations of copper

Hours		24	72	120	168
Control	N	8	8	8	8
	Mean value	0.8404	0.8218	0.7943	0.7987
	± SD	0.2495	0.2399	0.1817	0.1440
	Range	0.6189-1.2385	0.5517-1.2127	0.5194-0.9985	0.6059-0.9985
100 ppb of copper exposed	N	8	8	8	8
	Mean value	1.0291	0.5077 **	0.6897	0.2183 ***
	± SD	0.1735	0.0644	0.0481	0.0180
	Range	0.9153-1.4158	0.3895-0.5523	0.5866-0.7294	0.2033-0.2537
200 ppb of copper exposed	N	8	8	8	8
	Mean value	1.0720	0.7327	0.7214	0.3240 ***
	± SD	0.1841	0.0644	0.0767	0.1104
	Range	0.9257-1.4953	0.6142-0.8141	0.6584-0.8917	0.2025-0.4899
400 ppb of copper exposed	N	8	8	8	8
	Mean value	1.0935	0.8673	0.4736 ***	0.4559 ***
	± SD	0.2063	0.1975	0.0579	0.0806
	Range	0.7151-1.2457	0.5634-1.2480	0.4099-0.5380	0.3222-0.5652

Significance level: **$P<0.01$ ***$P<0.001$

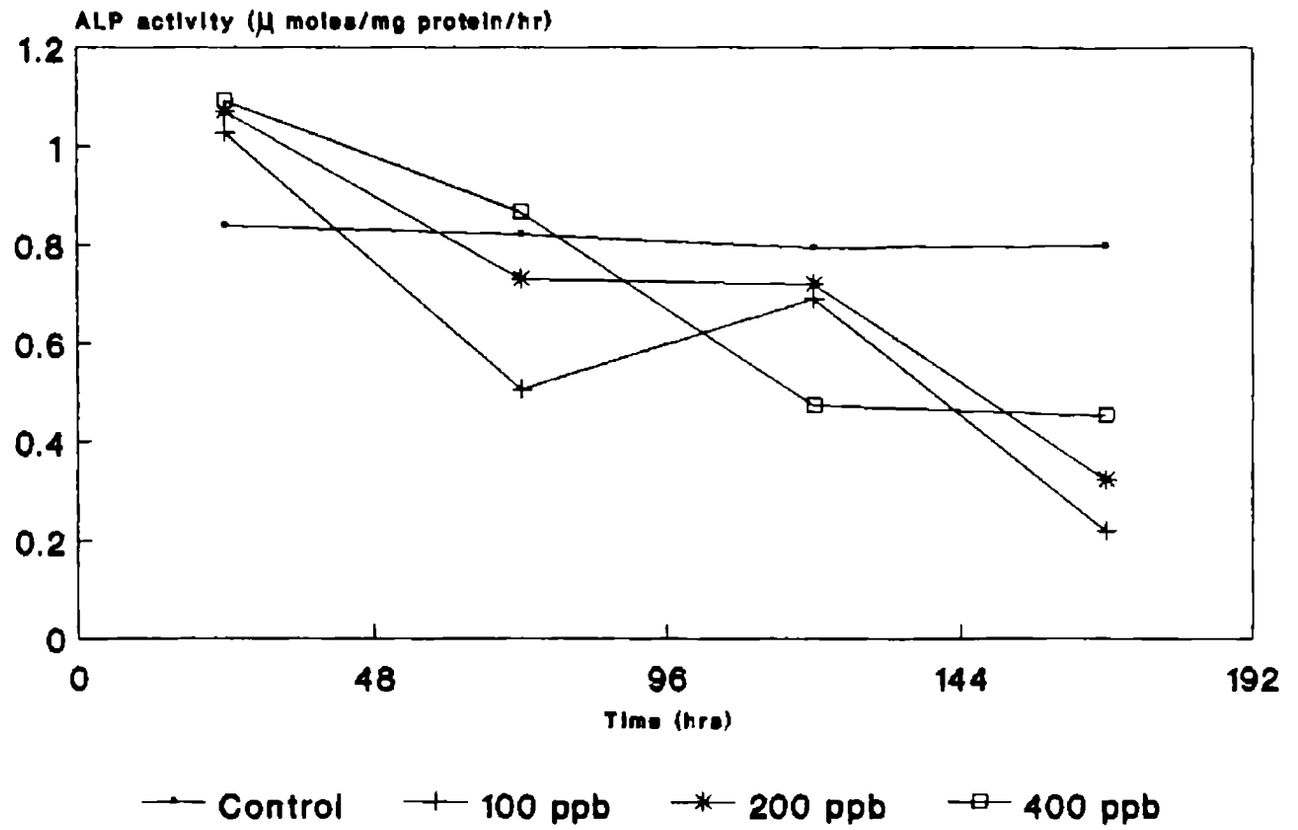


Figure 22. Gill Alkaline Phosphatase activity (µ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of copper.

able 23. Hepatopancreas Alkaline Phosphatase Activity (μ moles/mg protein/hour) in Lamellidens corrianus exposed to three sublethal concentrations of copper.

ours		24	72	120	168
ontrol	N	8	8	8	8
	Mean value	0.1835	0.1709	0.1568	0.1549
	± SD	0.0583	0.0268	0.0228	0.0280
	Range	0.1221-0.2656	0.1371-0.2065	0.1235-0.1850	0.1285-0.2063
00 ppb of opper xposed	N	8	8	8	8
	Mean value	0.1065 **	0.1117 *	0.1755	0.0257 ***
	± SD	0.0117	0.0497	0.0182	0.0050
	Range	0.0986-0.1305	0.0512-0.2011	0.1443-0.2094	0.0215-0.0374
00 ppb of opper xposed	N	8	8	8	8
	Mean value	0.1127 *	0.0879 **	0.1055 *	0.0558 ***
	± SD	0.0251	0.0454	0.0218	0.0203
	Range	0.0678-0.1309	0.0336-0.1666	0.0557-0.1274	0.0306-0.0954
00 ppb of opper xposed	N	8	8	8	8
	Mean value	0.0777	0.1524	0.1004 *	0.2259 *
	± SD	0.0169	0.0573	0.0208	0.0563
	Range	0.0530-0956	0.0669-0.2469	0.0705-0.1386	0.1489-0.3268

ignificance level : *$P<0.05$ **$P<0.01$ ***$P<0.001$

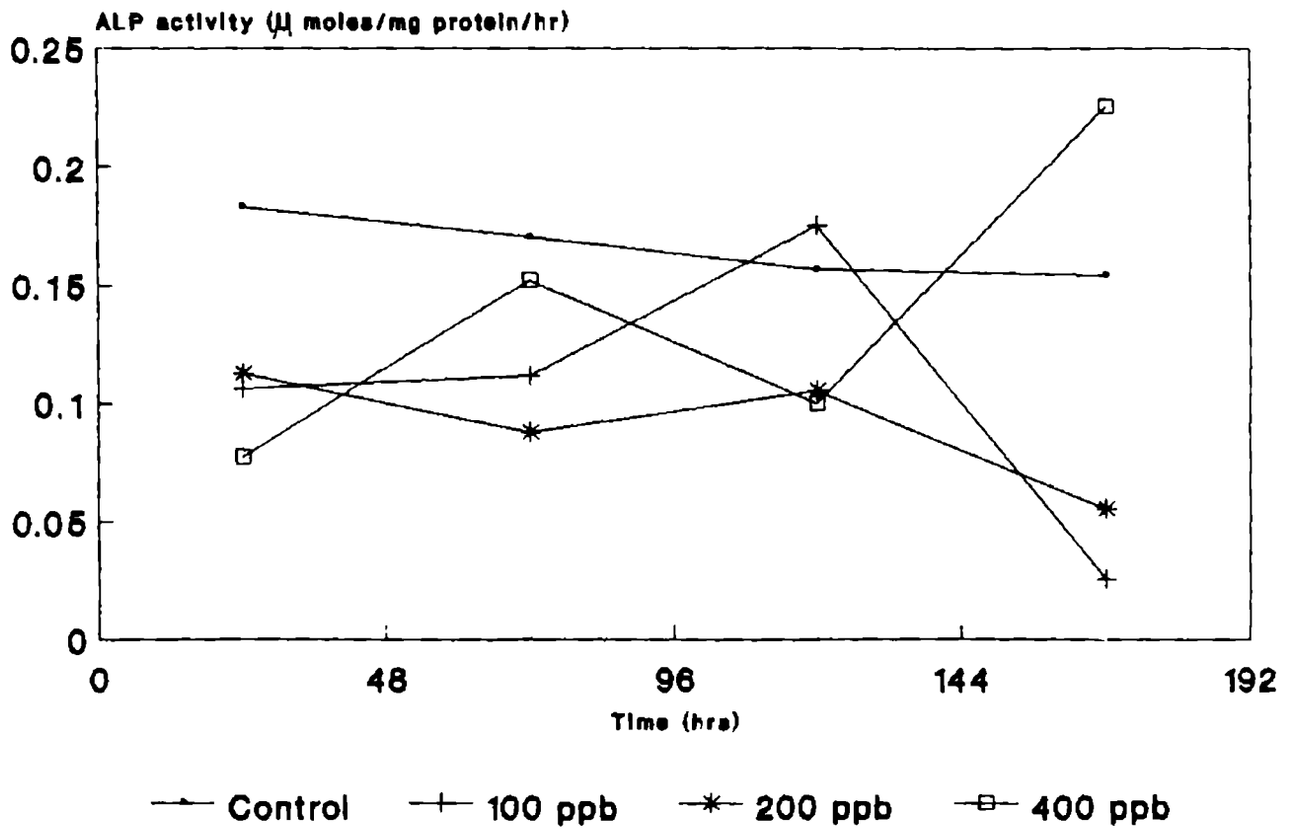


Figure 23. Hepatopancreas Alkaline Phosphatase activity (µ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of copper.

ALP activity in the hepatopancreas of 100 ppb exposed mussels was significantly lower at 24, 72, and 168 hr (P <0.01, 0.05, 0.001) when compared with the controls. Animals exposed to 200 ppb showed consistantly lower activity at all exposure periods (P<0.05 ,0.01,0.05 0.001) when compared with the controls. Values of 400 ppb exposed animals also were significantly lower than those of the controls at 24 and 120 hr (P<0.01,0.05) but significantly higher at 168 hr post-exposure (P<0.05) when compared with the controls.

Mussels exposed to 100 ppb had higher value at 120 hr (P<0.01) but lower value at 168 hr (P<0.01) than those of mussels exposed to 200 ppb. They also had lower value at 168 hr (P<0.01) but higher values at 24 and 120 hrs (P<0.01) than those exposed to 400 ppb. Comparison of values between 200 and 400 ppb exposed animals showed higher value in 400 ppb at 72 and 168 hr (P<0.05, 0.001) but lower value at 24 hr post-exposure (P<0.001).

5.3.2.2 Mercury

5.3.2.2.1 Gill tissue (Table 24, Figure 24)

Significantly higher values were exhibited by mussels exposed to 75 ppb at 24 hr (P<0.001) but lower value at 120 hr

Table 24. Gill Alkaline Phosphatase Activity (μ moles\mg protein\hour) in Lamellidens corrianus exposed to three sublethal concentrations of mercury

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	0.8466	0.8277	0.8302	0.8236
	± SD	0.1406	0.2620	0.1780	0.2407
	Range	0.6747-1.0360	0.5517-1.3294	0.5466-1.0593	0.4748-1.0851
75 ppb of mercury exposed	N	8	8	8	8
	Mean value	1.7225 ***	0.9071	0.5779 **	0.7316
	± SD	0.1198	0.0713	0.0523	0.1934
	Range	1.6114-1.9967	0.7995-1.0178	0.4845-0.6789	0.4748-1.0851
150 ppb of mercury exposed	N	8	8	8	8
	Mean value	1.1789 ***	2.2483 ***	0.9756	0.7925
	± SD	0.1541	0.1959	0.1462	0.1160
	Range	1.0101-1.4550	1.9068-2.4763	0.8528-1.3092	0.7207-1.0562
300 ppb of mercury exposed	N	8	8	8	8
	Mean value	0.7504	0.9051	0.4211 ***	0.9376
	± SD	0.1090	0.1035	0.0876	0.1328
	Range	0.5835-0.9033	0.7480-1.0313	0.3130-0.5427	0.7789-1.1619

Significance level : **P<0.01 ***P<0.001

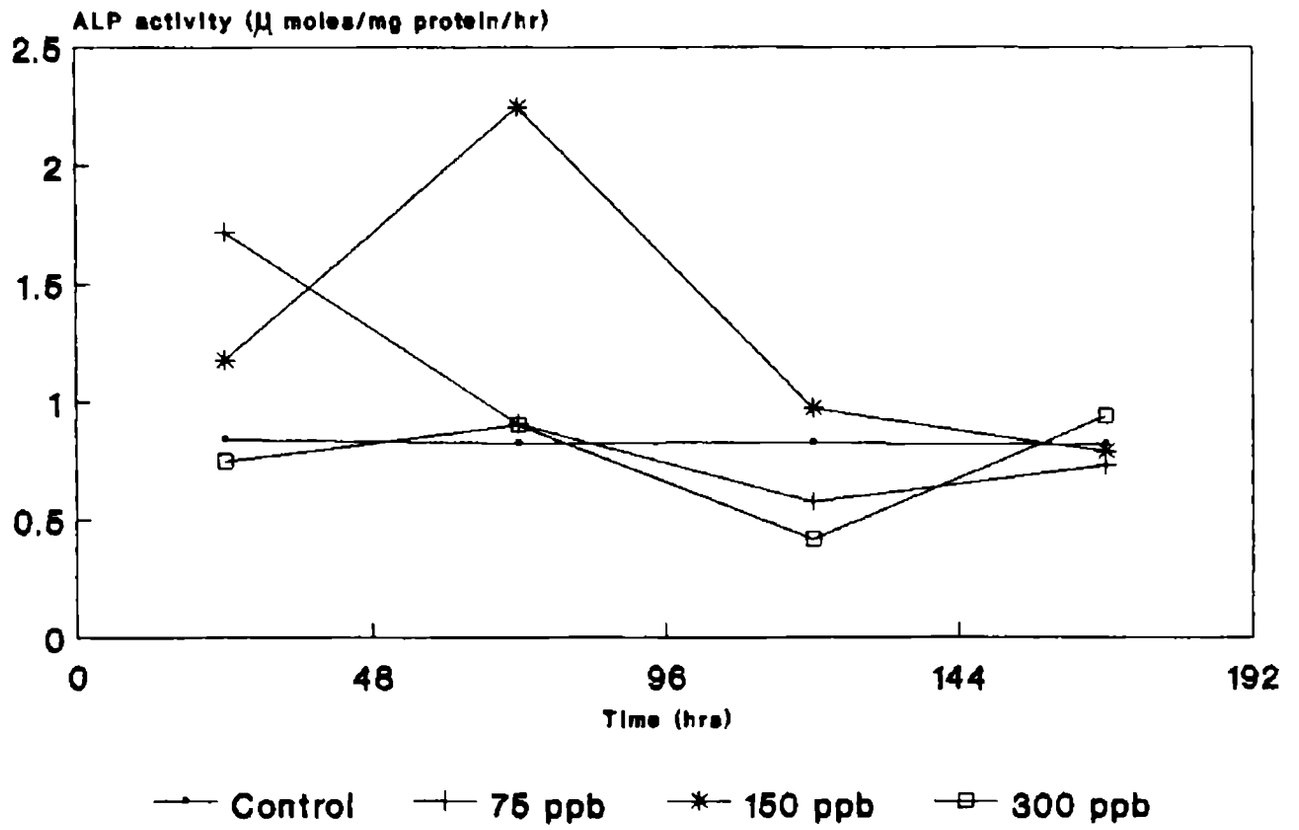


Figure 24. Gill Alkaline Phosphatase activity (µ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of mercury.

post-exposure ($P<0.01$) when compared with the controls. In 150 ppb exposed animals, significantly higher enzyme activity was observed at 24 and 72 hr time periods ($P<0.001$) when compared with the controls. Animals exposed to 300 ppb showed significant variation in activity only at 120 hr when compared with the controls. They showed lower value ($P<0.001$) at 120 hr post-exposure.

When values of 75 ppb exposed mussels were compared with those of 150 ppb exposed ones, higher values were noticed at 72 and 120 hr ($P<0.001$) but lower value at 24 hr ($P<0.001$) in 150 ppb exposed mussels. On comparison between values in mussels exposed to 75 ppb and 300 ppb copper, significantly higher value was shown by 75 ppb at 24 and 120 hr post-exposure ($P<0.001$, 0.01) but significantly lower value at 168 hr post-exposure ($P<0.05$). On comparing values of 150 ppb and 300 ppb exposed mussels, significantly higher values were found at 24, 72 and 120 hrs ($P<0.001$) but significantly lower value at 168 hr in 150 ppb exposed ones ($P<0.05$).

5.3.2.2.2 Hepatopancreas tissue (Table 25, Figure 25)

Comparison of enzyme activity in the hepatopancreas of the mussels exposed to different concentrations with that of the

Table 25. Hepatopancreas Alkaline Phosphatase Activity (μ moles\mg protein\hour) in Lamellidens corrianus exposed to three sublethal concentrations of mercury

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	0.1909	0.1745	0.1342	0.1571
	± SD	0.0767	0.0354	0.0234	0.0271
	Range	0.1339-0.2999	0.1439-0.2474	0.1060-0.1684	0.1307-0.2064
75 ppb of mercury exposed	N	8	8	8	8
	Mean value	0.3676 **	0.3359 *	0.1702	0.2824 ***
	± SD	0.0830	0.1481	0.0427	0.0121
	Range	0.2341-0.5128	0.0983-0.5496	0.1096-0.2556	0.2318-0.4105
150 ppb of mercury exposed	N	8	8	8	8
	Mean value	0.4551 ***	0.1220 **	0.3318 ***	0.3030 ***
	± SD	0.0931	0.0145	0.0765	0.0578
	Range	0.3241-0.6084	0.1016-0.1443	0.2222-0.4515	0.1962-0.3872
300 ppb of mercury exposed	N	8	8	8	8
	Mean value	0.3768 ***	0.3733 **	0.2847	0.1579
	± SD	0.0889	0.1660	0.2010	0.0441
	Range	0.2482-0.4956	0.1798-0.6354	0.0699-0.1274	0.0957-0.2163

Significance level : *$P<0.05$ **$P<0.01$ ***$P<0.001$

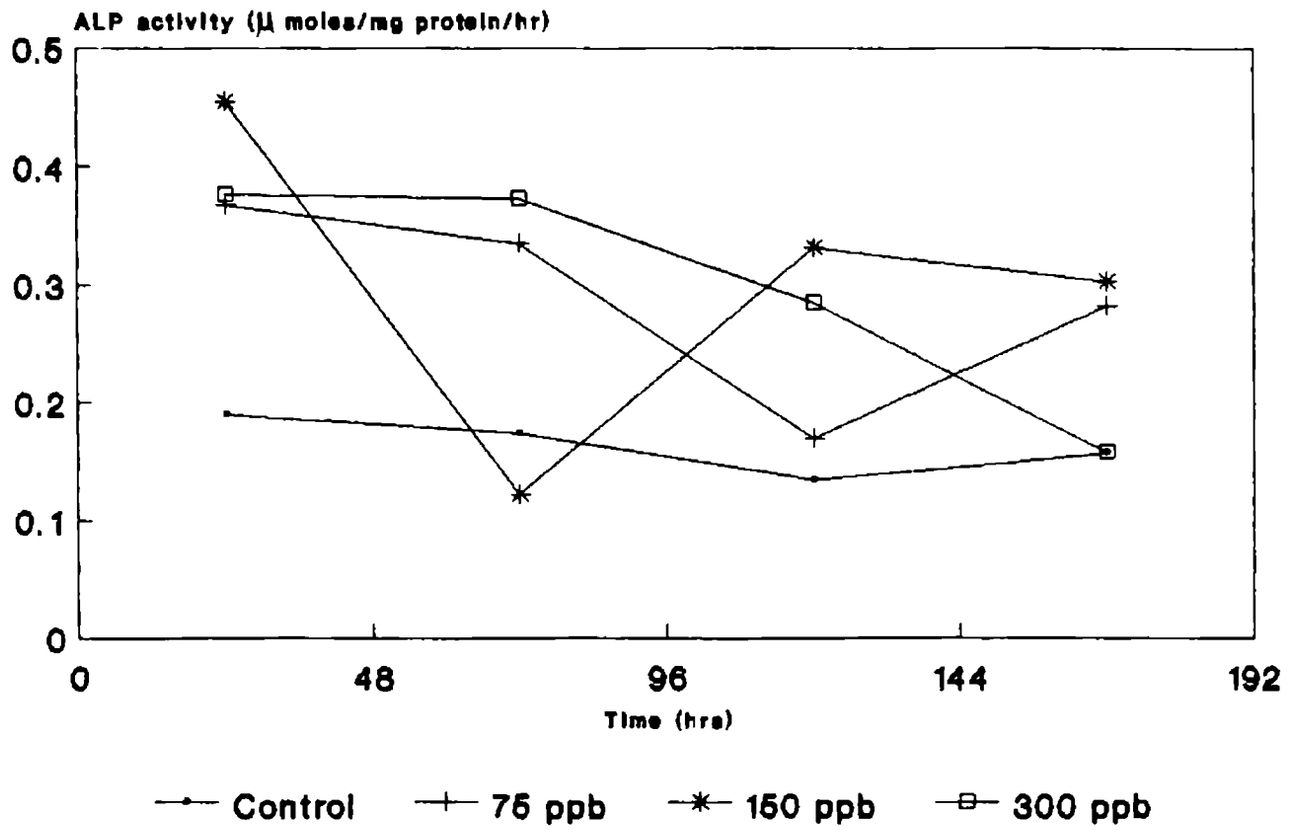


Figure 25. Hepatopancreas Alkaline Phosphatase activity (µ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of mercury.

controls revealed the following pattern. Animals exposed to 75 ppb showed higher enzyme activity at 24, 72 and 168 hr ($P<0.01$, 0.05, 0.001) when compared with the controls. In 150 ppb exposed mussels, significantly higher values were obtained at 24, 120 and 168 hr ($P<0.001$) but significantly lower value at 72 hr time period ($P<0.01$). When 300 ppb exposed animals were compared with the controls, significant elevation in activity was observed at 24 and 72 hr ($P<0.001$, 0.01).

Animals exposed to 75 ppb mercury had higher value at 72 hr ($P<0.01$) but lower value at 120 hr ($P<0.001$) than the values in those exposed to 150 ppb. But 75 ppb exposed animals had higher value also at 168 hr ($P<0.001$) when compared with that of mussels exposed to 300 ppb. On comparison between 150 and 300 ppb exposed animals, 150 ppb showed significantly lower value at 72 hr ($P<0.01$) but significantly higher value at 168 hr ($P<0.001$).

5.3.2.3 Cadmium

5.3.2.3.1 Gill tissue (Table 26, Figure 26)

When the enzyme activity in the gill tissue of cadmium exposed animals was compared with that of the controls, the following pattern was observed.Significantly lower values were shown by 150 ppb exposed mussels at all exposure periods ($P<0.001$, 0.05, 0.001, 0.01). Enzyme activity in 300 ppb

Table 26. Gill Alkaline Phosphatase Activity (μ mole/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of cadmium

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	0.8435	0.8559	0.7968	0.8470
	± SD	0.1593	0.2178	0.2052	0.2074
	Range	0.6712-1.1192	0.6217-1.2349	0.5001-1.1193	0.6698-1.0735
150 ppb of cadmium exposed	N	8	8	8	8
	Mean value	0.5082 ***	0.6057 *	0.3588 ***	0.5060 **
	± SD	0.0721	0.0229	0.0644	0.0589
	Range	0.3772-0.6053	0.5909-0.6429	0.2512-0.4395	0.4059-0.5668
300 ppb of cadmium exposed	N	8	8	8	8
	Mean value	0.9178	0.8994	0.8005	0.6228
	± SD	0.1571	0.3600	0.2693	0.0693
	Range	0.6665-1.2234	0.4493-1.4327	0.3643-1.1392	0.5143-0.6678
600 ppb of cadmium exposed	N	8	8	8	8
	Mean value	2.3950 ***	1.4948 ***	0.4885 *	0.4472 **
	± SD	0.8903	0.1055	0.1843	0.1506
	Range	0.8916-3.7735	1.2520-1.5782	0.1815-0.7120	0.2469-0.6210

Significance level : *$P<0.05$ **$P<0.01$ ***$P<0.001$

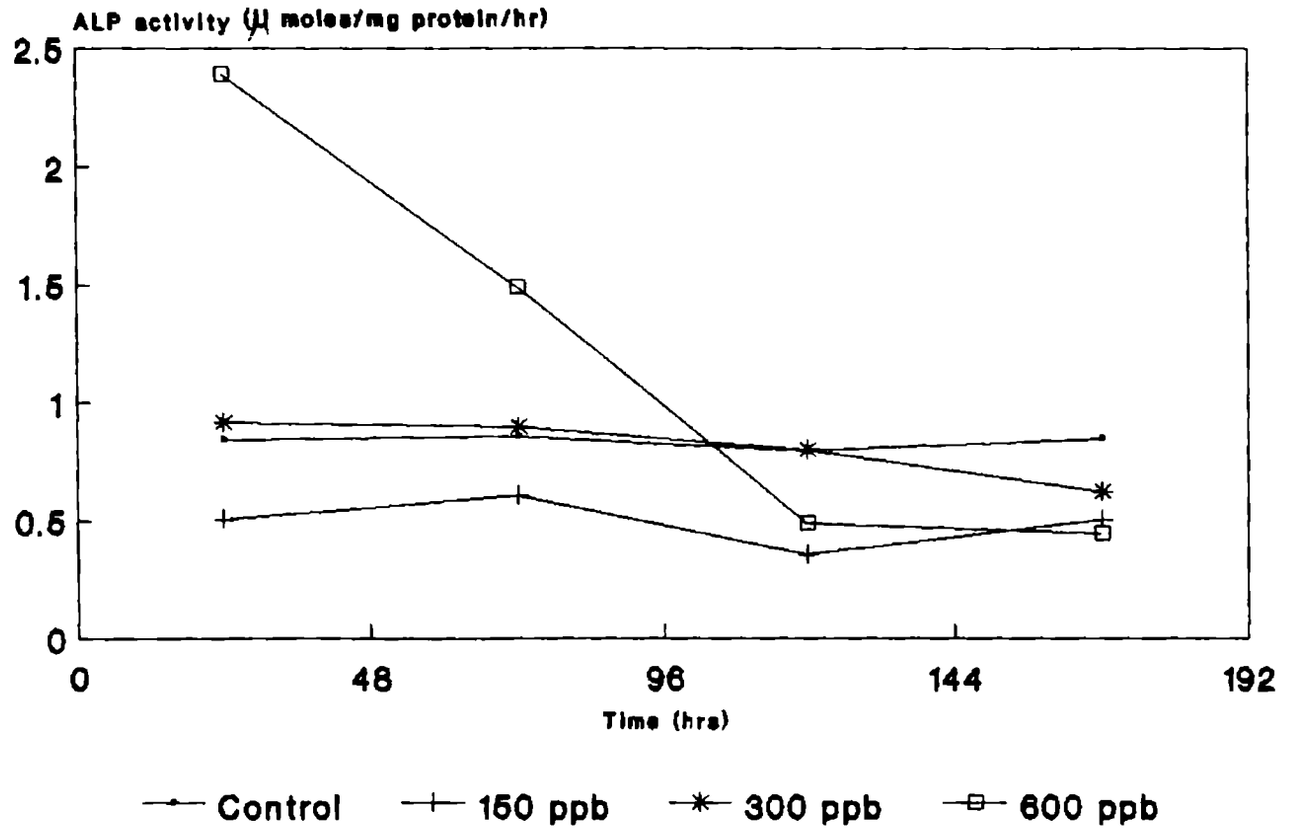


Figure 26. Gill Alkaline Phosphatase activity (μ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of cadmium.

exposed mussels did not show any statistically significant variation at any of the exposure periods. In 600 ppb exposed animals, significantly higher values were obtained at 24 and 72 hr time periods ($P<0.001$) but significantly lower values at 120 and 168 hr ($P<0.05$, 0.01)

Mussels exposed to 150 ppb cadmium showed lower values at all time periods ($P<0.001$, 0.05, 0.001,0.01) when compared with the values in 300 ppb exposed animals. When values in 150 ppb exposed animals were compared with those exposed to 600 ppb, significantly higher value was noticed in the latter at 24 and 72 hr ($P<0.001$). Animals exposed to 300 ppb showed higher values at 120 and 168 hrs ($P<0.05$) butlower values at 24 and 72 hrs ($P<0.001$) when compared with the values in 600 ppb exposed animals.

5.3.2.3.2 Hepatopancreas tissue (Table 27, Figure 27)

150 ppb exposed animals exhibited significantly higher value at 24 hr post-exposure ($P<0.05$), but significantly lower value at 72 and 120 hr ($P<0.01$, 0.001) when compared with controls. Enzyme activity in 300 ppb exposed animals was higher at 24 and 120 hr ($P<0.001$) but lower at 72 hr ($P<0.001$) than those of the controls. But in 600 ppb exposed animals, significantly higher

Table 27. Hepatopancreas Alkaline Phosphatase Activity (μ mole/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of cadmium

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	0.1857	0.1800	0.1495	0.1561
	± SD	0.0657	0.0258	0.0150	0.0312
	Range	0.1256-0.2777	0.1535-0.2276	0.1345-0.1800	0.1273-0.2123
150 ppb of cadmium exposed	N	8	8	8	8
	Mean value	0.2783 *	0.1293 **	0.0861 ***	0.1455
	± SD	0.0542	0.0212	0.0165	0.0377
	Range	0.1992-0.3554	0.0961-0.1622	0.0569-0.1007	0.0903-0.1989
300 ppb of cadmium exposed	N	8	8	8	8
	Mean value	0.3083 ***	0.1113 ***	0.2026 ***	0.1722
	± SD	0.0342	0.0122	0.0193	0.0021
	Range	0.2592-0.3388	0.0905-0.1296	0.1842-0.2370	0.1695-0.1752
600 ppb of cadmium exposed	N	8	8	8	8
	Mean value	0.3752 **	0.1575	0.3783 ***	0.0475 ***
	± SD	0.1267	0.0262	0.1051	0.0063
	Range	0.2543-0.6354	0.1043-0.1872	0.2543-0.6037	0.0372-0.0518

Significance level : *$P<0.05$ **$P<0.01$ ***$P<0.001$

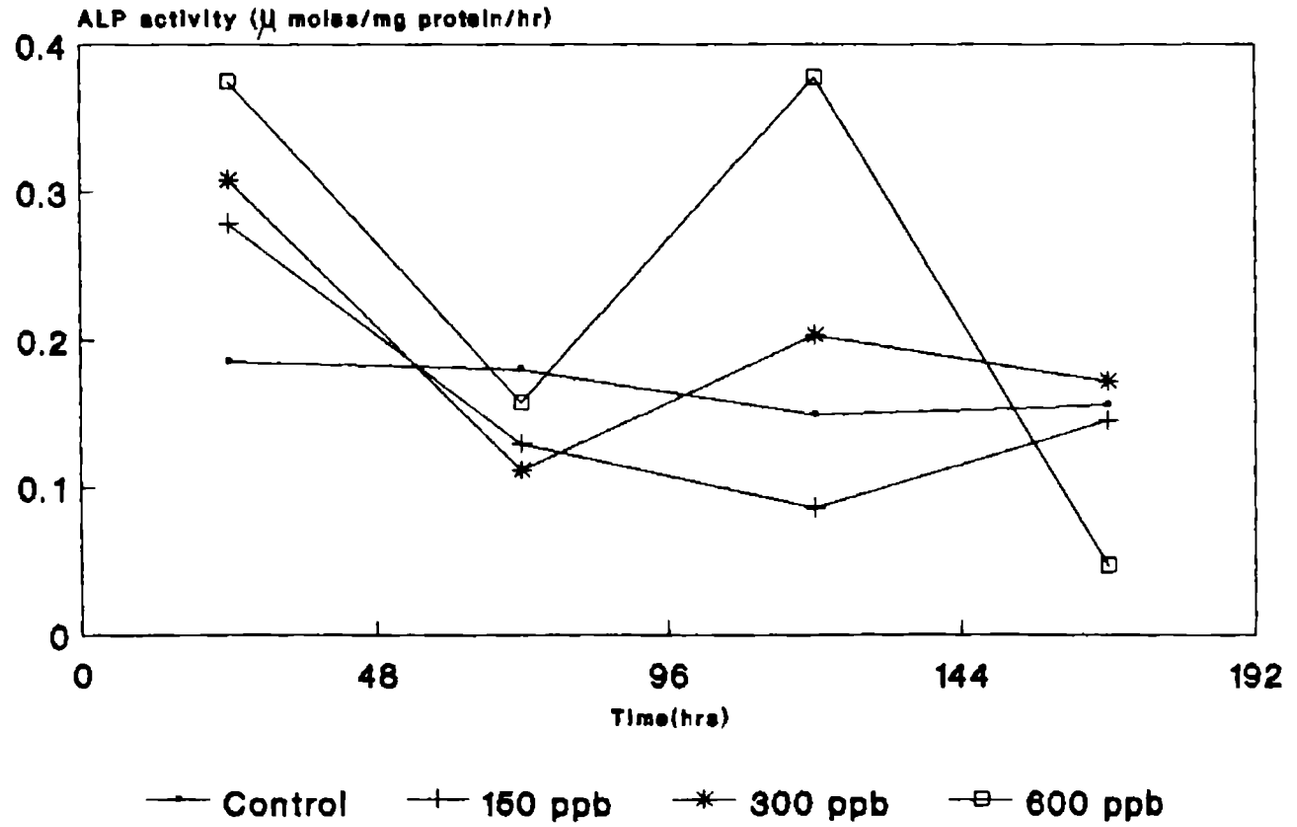


Figure 27. Hepatopancreas Alkaline Phosphatase activity (µ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of cadmium.

values were noticed at 24 and 120 hr ($P< 0.01, 0.001$) but lower value at 168 hr post-exposure ($P< 0.001$)

On comparison between values of 150 ppb and 300 ppb exposed animals, lower values were found in mussels exposed to 150 ppb at 24 and 120 hrs ($P<0.001$). When values in 150 ppb exposed animals were compared with those in 600 ppb exposed ones, lower values were found in 150 ppb exposed animals at 72 and 120 hr ($P<0.05, 0.001$) but higher at 168 hr post-exposure ($P< 0.001$). Values in animals exposed to 300 ppb on comparison with those exposed to 600 ppb showed lower activity at 24, 72, and 120 hr ($P<0.001$) but higher activity at 168hr ($P<0.001$).

5.4 DISCUSSION

ACP activity in the gill tissue of copper exposed animals showed significant elevation in all the three concentrations at 24 hr time period. In 100 ppb exposed animals, ACP activity exhibited further elevation at 120 hr which subsequently got normalised. In 200 ppb and 400 ppb exposed ones, though enzyme activity showed time dependent reduction, the activity was higher than the normal.

Acid phosphatase is a hydrolytic enzyme which can be

activated by a number of stress conditions. The enhanced activity in all the experimentals can be attributed to hypersynthesis of the enzyme. Increase in ACP activity is reported in *Lamellidens marginalis* following pesticide exposure (Babu and Vasudev, 1984), in the gills of the clam *Protothaca staminia* following copper exposure (Roesijadi, 1980), and in the haemolymph of the clams *Sunetta scripta* and *Villorita cyprinoides* var.*cochinensis* on copper exposure (Suresh and Mohandas, 1990). The elevation in activity in 100 ppb exposed animals during 120 hr may be due to the fact that the enzyme released during the early period is insufficient to detoxify the metal ions, and therefore there is subsequent hypersynthesis of the enzyme. The consistantly higher enzyme activity observed in the two higher concentrations may also be attributed to the same phenomenon. Higher ACP activity persisting for longer period is observed by Reddy (1986a)in crab tissue. Presence of metal ion causes increased availability of lysosomal acid hydrolases to metabolise and sequester the metals in a non-toxic form (Chandy and Patel, 1985). With the beginning of recovery, the enzyme activity started declining and approached control level. The higher enzyme activity in 100 ppb exposed mussels at 24 hr, when compared with the other two concentration exposures, may be due to the fact that the hypersynthesized enzyme is not immediately

made use of and is reflected as increased enzyme activity at the early period as suggested by Suresh (1988). In 400 ppb exposed mussels, the recovery was much more delayed when compared to the other exposures as is evident from the elevated enzyme level even at 168 hr post-exposure as a result of hypersynthesis. A general trend of enhanced enzyme activity in gill tissue has been attributed also to extensive tissue damage as it is in direct contact with the toxicant (Couch, 1977; Dalela et al., 1979; Sunila, 1986).

In the hepatopancreas, the enzyme activity in 200 ppb exposed animals did not show significant variation from the control level during the early time period. But in mussels exposed to 400 ppb, non significant change was observed. However, during the later periods there was significant drop in enzyme activity in the two lower concentrations. Significant inhibition in hepatic ACP activity is reported (Saleem and Alikhan, 1974; Dalela et al., 1980; Saxena et al., 1982). In the hepatopancreas, the copper ion might have inhibited enzyme synthesis by binding with the enzyme protein. The general trend of increased inhibition of enzyme activity can be attributed to the failure of protein synthesis. A generally low ALP activity also supports this argument. Inhibition of protein synthesis on exposure to toxicants is widely reported (Murthy and Devi, 1982;

Ramalingam and Ramalingam, 1982). The overall depression in phosphatase activity can also be attributed to the injury elicited to the mitochondrial system. Copper toxicity due to the reactivity of the metal with mitochondria is reported in snails (Babu and Rao, 1982). Hwang et al.(1972) has shown that copper binds to the protein binding sites of the mitochondrial membrane resulting in changes in permeability and activation of energy linked ion movements. In the present study in 400 ppb exposed mussels, the enzyme activity in general was found to get elevated during the exposure period though not statistically significant. This elevation in activity may be due to the labilisation of lysosomes by the toxicant which gets accumulated with prolonged exposure resulting in increased enzyme release into the cytosol.

Among mercury exposed animals, significant elevation in gill ACP activity was observed in 75 ppb exposed ones during the initial period, while in those exposed to higher concentrations, the enzyme activity at this period was found to be normal. In 150 ppb exposed animals, elevation in enzyme activity occurred at 72 hr post-exposure which remained significantly elevated at 168 hr. From the pattern of enzyme activity, it can be assumed that peak enzyme activity in these mussels may be a slightly delayed response to the toxicant. Xenobiotics are known to induce

alteration in the bounding membrane of the lysosomes leading to destabilisation (Moore and Lowe, 1985), and subsequent release of hydrolases into the cytosol (Moore, 1976). When the metal ion concentration is low, hypersynthesised enzyme is not immediately inactivated by the metal ion and this was reflected in the increased enzyme activity in 75 ppb exposed mussels at early time periods while in higher concentrations not only hypersynthesised enzyme is not inactivated by the higher metal ion concentration, the activity is extended. Suresh and Mohandas (1990) have reported similar observations. Since the hypersynthesised enzyme was sufficient enough to detoxify the metal at early time periods, it was not sufficient by 120 hr. Later, it is presumed, the animal could stabilize, and hence the activity became normal. As the concentrations of metal increased, there appear to be lysosomal destabilisation also resulting in increased release of the enzyme. The higher activity of the enzyme during the later periods might also be indicative of the increased damage elicited on the lysosomal system. Alterations in cell membrane brought about by binding of mercury to sulphydril groups could stimulate membrane turn over and also hydrolytic enzyme production (Arstila and Trump, 1961).

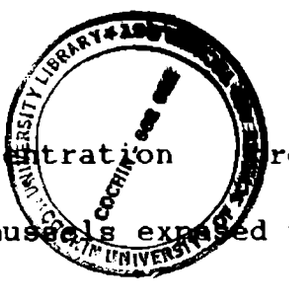
In hepatopancreas, the enzyme activity during the initial

period was more or less similar to that of the gill tissue. In this tissue also, significant elevation in activity was observed in 75 ppb exposed mussels, while in those exposed to other two concentrations, the activity was found to be normal during the initial period. As in the case of gill tissue, in hepatopancreas also hypersynthesis of enzyme might have occurred at an early period (before 24 hrs) in the animals exposed to the higher concentrations and hence not detected early. Since hypersynthesized enzyme in lower concentration exposure is not immediately inactivated by the metal ion, it was reflected as increased enzyme activity in 75 ppb exposed animals. Similar observation was documented by Suresh and Mohandas (1990). Although the hypersynthesized enzyme during the early time period was enough to detoxify the metal for some time there was time dependent decline in activity in 75 ppb exposed animals which ultimately, however, was found to attain the control level. In 300 ppb exposed animals, there was a subsequent elevation in activity at 72 hr after which the enzyme activity normalised. This enhancement in activity may probably be to detoxify the excess metal ion that gets accumulated by prolonged exposure. The increased enzyme activity may also be due to increased synthesis, decreased removal, or both. The decline in activity in 150 ppb exposed animals at 72 hr can not be explained in this

context. However, it is possible that the hypersynthesized enzyme of early time periods is not sufficient to detoxify the metal at 72 hr post - exposure and there was no further synthesis but the animal, however, stabilises at later time periods. Yoshino and Cheng (1976) have suggested that lysosomes represent a chemically heterogenous population or that there is a non-synchronised chemical cycle occurring with in the granules. Thus, the discrepancy observed in enzyme activity in the present study can be attributed to the chemically heterogenous population of lysosomes. Similar observations are reported by Suresh and Mohandas (1990) in copper exposed clams.

ACP activity in the gill tissue of mussels exposed to the lower concentrations of cadmium was not much affected by the metal. This may be due to the detoxication and consequent removal of the metal ion before they could damage the lysosomes. In mussels exposed to 300 ppb there was increase in enzyme activity at all time periods which can be attributed to hypersynthesis of the enzyme at early time periods, which was more than sufficient to detoxify the metal even by 168 hrs. But in mussels exposed to 600 ppb of cadmium, the hypersynthesized enzyme was just sufficient to detoxify the metal by 168 hrs and hence no significant fluctuation in activity was observed beyond

72 hrs. Since the release of ACP is associated with tissue damage and lysosomal destabilisation, the increased activity observed in the present case may also be due to tissue damage caused by the higher concentration.



In hepatopancreas lower cadmium concentration produced significant reduction in ACP activity. In mussels exposed to 600 ppb, there was a significant elevation at 24 hr. This elevation in activity can again be considered as an indication of hepatic injury. Many environmental factors can alter the properties of the lysosomal membrane, resulting in the release of its contents (Lowe and Moore, 1979). This has been demonstrated in *Mytilus* (Moore, 1977). Increase in lysosomal activity in injured cell occurs as part of pre-necrotic change (Nevicoffe, 1961). Elevation in ACP activity has also been attributed to increased hystocytic reaction resulting from pollution (Sastry and Gupta, 1978). As is evident from the graph, the peak in activity is found to be dose dependent as well as time dependent. Elevation in activity in 300 ppb exposed mussels could be expected at a much later period also. In animals exposed to the lower concentration, the significant reduction in activity of the enzyme may be due to inhibition of activity by the low metal concentration. In animals exposed to higher metal concentration,

the metal ion concentration in the lysosomes get elevated and thereby crosses the threshold level, liberating the hydrolytic enzymes into the cytoplasm, resulting in enhanced activity. The significant drop in activity in 600 ppb exposed animals towards the later period is attributed to the leakage of the enzyme into the haemolymph following tissue damage, or inhibition of enzyme synthesis.

Gill alkaline phosphatase activity in copper exposed mussels was found not to be affected during the initial period. Alkaline phosphatase is believed to be associated with the plasma membrane and endoplasmic reticulum (Davison and Gregson, 1965; Hart and Fouts, 1965). ALP is involved in membrane transport, carbohydrate metabolism etc. Jackim et al. (1970) has observed stimulation of both ACP and ALP in liver of animals exposed to lower copper concentrations. Increase in ALP is reported by Babu and Vasudev (1984) in *Lamellidens marginalis*. The time dependent decrease in activity observed in mussels exposed to all the three concentrations may be due to increased inhibition of enzyme activity. According to Hilmy et al. (1981) diminished enzyme activity occurs when the metal conjugates are formed with the enzyme. Sharp decline in activity towards the later periods can be attributed to the leakage of enzyme into the haemolymph

following tissue damage.

The generally low enzyme activity in hepatopancreas can be due to the inhibition of protein synthesis. The binding affinity of heavy metal ion and protein is generally intense (Hilmy et al., 1981). The metal ion may also cause injury to mitochondrial system which markedly blocks the action of enzyme. Since the ACP activity was also found to be at a low profile, inhibition of protein synthesis is a strong possibility. Byczkowski and Boryszwicz (1976) reported that copper affects the mitochondrial membrane permeability. Copper also causes changes in mitochondrial membrane affecting passive permeability of anions, activation of energy linked ion movements, uncoupling, and changes in rate of respiration (Hwang et al., 1972). The significantly low enzyme activity in gill tissue when compared with hepatopancreas could be attributed to heavy localised tissue damage. Damage to gill tissue following toxicant exposure is reported (Sunila, 1986). Moreover, the acidic condition prevailing in gill tissue as a result of shift in metabolism also explains the above finding.

The ALP activity in the gill tissue of 75 and 150 ppb mercury exposed mussels was significantly high during the 24 hr

time period. The peak in enzyme activity in animals exposed to the lower concentrations was found to be time dependent. Mercury is reported to stimulate phosphatase activity at lower concentration and inhibit it at higher concentration exposure (Jackim et al.,1970). In the present study also, higher concentration of mercury was found to inhibit enzyme activity, while lower concentration was found to stimulate it. However, enzyme activity in the lower concentration exposure was found to get inhibited by prolonged exposure, probably due to the formation of metal protein conjugants. Inhibition of ALP activity in gill tissue is reported (Hilmy et al., 1987, Plaveux 1977). Decrease in mercury toxicity due to replacement of Mg^{++} by mercury and subsequent release of nontoxic Mg^{++} is reported by Hilmy et al.(1981). The normalisation of enzyme activity in the present study may also be associated with the above finding. The variation in enzyme activity in 150 ppb exposed animals may be attributed to the existence of polymorphic forms of ALP (Carpene and Wyne, 1986). In the hepatopancreas, the enzyme activity in all the exposed animals was significantly high during the initial period. Increase in ALP activity following the release of the rich supply of the enzyme in the digestive gland as a result of tissue damage is reported (Muller, 1965). The generally high activity of both phosphatases suggest

tissue damage. The time dependent decrease in activity observed in animals exposed to the higher concentration may be attributed to the slow but steady inhibition of ALP synthesis by mercury. Such slow inhibition of enzyme activity is reported (Hilmy et al., 1981). Alternatively, the decline in enzyme activity may also be due to inactivation of the metal ion and recovery of the tissue. Alteration brought about by different environmental factors resulting in the release of enzyme is demonstrated in *Mytilus* (Moore et al., 1978 a). This type of alteration could possibly result in the mobilisation and retoxication of previously immobilised metal ions (Lowe and Moore, 1979). This phenomenon explains the sudden elevation in enzyme activity observed at certain time periods.

Cadmium is reported to cause inhibition of ALP activity in gill tissue of fishes (Plaveux, 1977; Hilmy et al., 1981). The low enzyme activity in 150 ppb exposed mussels may be due to the inhibitory nature of cadmium. Cadmium inhibition suggests the substitution of active centre Zn by cadmium and producing a less active enzyme (Coleman and Gettings, 1983). The time dependent decline in activity in the higher concentration may also be attributed to the inhibition of enzyme activity.

In the hepatopancreas, the enzyme activity in all the three

exposures was significantly elevated at early time period suggesting the release of the enzyme into the cytoplasm. Stimulation of ALP activity on exposure to pesticides is reported by Babu and Vasudev (1976) and suggested that increase in ALP is due to increased uptake of certain metabolites. But Jackim et al. (1970), and Hilmy et al. (1985) reported that cadmium inhibits enzyme synthesis in fish tissue. The relative decrease in enzyme activity in the lower concentration exposure may perhaps be due to the inhibition of enzyme protein as metal conjugates with the protein molecule. It can be presumed that in lower concentration exposure the metal only brings derangement of metabolic activities by binding to the protein molecule and that the activity of the enzyme is restored at a later period as a result of detoxification. But in 600 ppb exposed mussels, very high peak in activity at 120 hr suggests tissue damage and liberation of the enzyme into the cytosol. The sharp decline in activity at 168 hr indicates inhibition of the enzyme synthesis. Rise in serum ALP following cadmium treatment is reported (Hilmy et al., 1985).

Results of the present study show differential tissue enzyme sensitivity to metals. Similar observations are made by Wells et al.(1974), Verma et al. (1978), and Hilmy et al. (1985). This

may be associated with the ability of the metal to alter the cellular membrane configuration by binding with lipid protein of membrane and thus block movement of substances by active transport (Hilmy et al., 1985). The different effects of metal ion on tissue could also be related to the different role that the same metal plays in the biochemistry of lipid peroxidation, a process that represents central point in the alteration of lysosomal physiology (Viarengo et al., 1985). The inhibition of acid and alkaline phosphatase activity may also be caused by uncoupling of oxidative phosphorylation as reported by Loomis and Lipman (1948), Simon (1953), and Gallagher and Rees (1960), or due to oxidation accompanied by phosphorylation. The observed inconsistancy in phosphatase activity may be associated with the heterogenous population of lysosomes as well as polymeric forms of alkaline phosphatase. Interaction with regulators or cofactors could also be accounted for the activation or inhibition of enzyme activity. The biphasic response of phosphatase observed during certain time periods could be due to initial induction followed by direct inhibition, as the toxic cation increases in tissue. The normalisation of enzyme activity during prolonged exposure in general, shows that in spite of the early derangement and impairment of metabolic activity, the animal seems to have the necessary compensating mechanism to combat the adverse effect of the toxicant.

C H A P T E R - VI

EFFECT OF HEAVY METALS ON THE ACTIVITY PATTERN OF TISSUE TRANSAMINASES

6.1 INTRODUCTION

Although the study of changes in enzyme activity is an important aspect of mammalian toxicology, very little information is available regarding the effect of toxicants in molluscs. Study of enzymatic activities is an important tool to get an insight into the mechanism of metal poisoning.

Aminotransferases are of great significance in protein metabolism by virtue of their ability to synthesise and degrade amino acids. Transamination serves as a pathway of conversion of alpha-ketoacids to L-aminoacids and as an alternate means of replenishing pyruvate pool. Glutamate Oxaloacetate Tranaminase (GOT) or Aspartate Aminotransferase (AAT, E.C.2.6.1.1), and Glutamate Pyruvate Transaminase (GPT) or Alanine Aminotransferase (ALAT, E.C. 2.6.1.2.) are two widely investigated transaminases. Alanine and aspartate aminotransferases serve as link between carbohydrate and protein metabolism, thus favouring gluconeogenesis (Lehninger, 1975). Chaplin et al. (1967) reported that alanine aminotransferase activity can be considered as an index of adaptability. These two enzymes are active under

stress, starvation, or any altered physiological condition (Nichol and Rosen, 1963). It is found that toxic agents which lead to chronic impairment of animal metabolism will cause changes in the activities of some enzymes (see Bell, 1968; Mckim et al., 1970; Lockhart et al., 1972).

The liver parenchyma is a rich source of GOT and has a very high concentration of GPT. The absolute amount of GPT is always found to be less than that of GOT. Although hepatic tissue damage leads to elevated serum concentration of both transaminases, increase in GPT level than GOT is more pronounced in liver damage (Sherlock, 1968). It is reported that the level of aspartate activity in any tissue including serum is five to twenty times higher than that of alanie activity (Das et al., 1986). Similar observations were also made by Wilson (1973), and Nemesock et al.(1981). Racicot et al. (1975) have reported seventeen times less activity for ALAT in a study on the effect of carbon tetra-chloride on rainbow trout

Environmental pollution appears to be one of the factors that affects aminotransferase activities in animal tissue (Lane and Scura, 1970). Elevated levels of hepatic enzymes are indicative of increased metabolism and tissue damage following hepatotoxicity (Bell, 1968). Hepatotoxicity and

hepatopathological damage are the most common responses of various xenobiotics in the case of fishes (Couch, 1975). Levels of alanine and aspartate aminotransferases are found to elevate in response to hepatotoxicity. Lower levels of hepatic GOT is indicative of physiological adaptation of the animal to the toxicant (Rice and Mills,1987). According to Malhotra et al. (1986), GPT will be higher when alanine transamination is more than aspartate utilisation. Malhotra et al. (1986) also reported that alanine generated through degradation of branched chain aminoacids may be converted to aspartate during the degradation phase of muscle repair. Elevation in aminotransferase activity during stress is well documented (Knox and Greenguard, 1965; Kulkarni and Mehrotra, 1973; Ahamed et al., 1978; Altland and Ratner, 1979; Goel et al., 1984; Baneerjee and Choudhuri, 1985; Murthy et al., 1985).

The reciprocal relationship between alanine aminotransferase and glutamic dehydrogenase activity has been reported in bovine liver tissue (Tomkins et al., 1961). Alanine aminotransferase and glutamic dehydrogenase form enzyme complexes and both glutamate and aspartate as substrates, enhance the binding of these enzymes (Fahien and Kmiotek, 1979). Glutamic dehydrogenase is also found to regulate the levels of transaminases by

controlling the level of glutamate (Hochachka and Somero, 1973). Low transaminase activity due to low glutamic dehydrogenase activity and vice-versa has been reported in certain amphibian species (Lakshmipathi and Reddy, 1984).

Hilmy et al. (1981) observed a relatively slow but constant inhibition of serum GOT in mercury exposed fish. This constant inhibition in enzyme activity is considered to be due to cellular degradation by mercury. Bell et al. (1968) opined that as in the case of mammals, transaminases are indicative of liver, heart, and other organ dysfunction in salmonides. Stimulation of alanine and aspartate aminotransferases activity in lower concentration, and inhibition in higher concentration of toxicants have been reported by Cheng (1965), and Fox and Rao (1978). Time dependent inhibition and enhancement of AAT and ALAT by cadmium has been reported by Hilmy et al.(1985). The biphasic response of AAT to cadmium toxicity, induction followed by inhibition, is also reported by Jackim et al.(1970), and Christensen (1972) after heavy metal exposure. Racicot et al. (1975) observed cellular vaccuolisation a short while after the animals were exposed to carbon tetra chloride and were of opinion that vaccuolisation may be related to disorganisation at cellular level and the variation of the plasma enzyme level.

Contradicting the above finding, Pfeifer et al.(1980) reported that prominent histochemical vaccuolisation in control animals or treated ones was due to glycogen deposit and not due to lipid accumulation.

GOT and GPT activities have been reported in all molluscs. GOT level tends to be a bit higher than the GPT levels in the hepatopancreas of some molluscs (Reddy and Naidu, 1978; Sollock et al., 1979). Aminotransferases are also involved in shell formation (Hammen and Wilbur, 1959). Various factors like species difference, season, type of food, size of the animal etc., are found to influence the activity of GOT and GPT. The tissue levels of GOT and GPT were higher in active snail than in aestivating snail (Swami and Reddy, 1978). ALAT and AAT activities are found to be much higher in brachiuran species. Alanine and aspartate aminotransferases activities of muscle were found to be higher than those of gill and mid gut gland (Rao and Dayakar, 1987). They also reported that extra hepatic tissue has higher aminotransferase activity than hepatic tissue indicating the more emphasis given to physiological function than to metabolic function. Aspartate and alanine aminotransferases are known to play a strategic role in metabolising L-aminoacids gluconeogenesis, and function as link between carbohydrate and

protein metabolism under altered physiological, pathological and environmental stress condition (Nichol and Rosen, 1963; Knox and Greenguard, 1965; Harper et al., 1979). When the organism is under stress, AAT and ALAT activities enhance, whereby gluconeogenesis occurs to mitigate the effect of toxic stress. Transaminases are reported to be present in a wide variety of molluscan tissues such as body fluid of *Biomphalaria glabrata* (Rodrick and Cheng, 1974), body fluid of *Viviparous bengalensis* (Prashad et al., 1983), haemolymph of *Lymnea leuteola* (Manohar et al., 1972), and *Pila globosa* (Swami and Reddy, 1978). Kulkarni and Kulkarni (1987) reported elevated levels of transaminase in the gill and mantle of the clam *Katelisia opima* when exposed to mercury. Similar enhancement in GOT and GPT activity is also reported by Ahamed et al. (1978) in *Lamellidens marginalis* after exposing to certain pesticides. Increase in serum GOT and GPT is reported by Vatal and Aiyar (1988) in rats following lithium administration. Kulkarni (1983) observed enhanced GOT and GPT in serum of *Sylla serrata* exposed to naphthalene and pointed out that the change was brought about by damage to hepatopancreas. Histopathological damage of liver tissue is also reported by many investigators (Dalela et al., 1979; Koundinya and Ramamurthy, 1980; Mandal and Kulshrestha, 1980; Shareef et al., 1983; Bhatnagar et al., 1987).

Transamination is of particular importance under conditions that impose heavy drain on the animal's store of metabolites (Goddard and Martin, 1966). The feeding of aminoacids into carbohydrate and lipid metabolism is mobilised by aminotransferase. Alanine, Aspartate and Tyrosine aminotransferases are reported to be present in molluscan tissues (Read, 1962; Hammen, 1968; Rao, 1974). Free aminoacids are said to play a significant role in osmotic regulation in freshwater mussels (Little, 1965). Glutamate and aspartate are related to neuronal metabolism in many ways by transamination. They may control oxidative metabolism through Kreb's cycle (West et al., 1967).

Transaminases are widely distributed enzymes which play an important role in metabolic pathways. Falany and Friedl (1981) observed that the overall number of aminoacids transaminated by the two freshwater mussels *Anodonta couperiana* and *Popenais buckleyi* was lower than that of gastropods and marine bivalves. AAT, as well as ALAT have been detected in the cytosol and mitochondria of bivalve molluscs (Bishop et al., 1983; Burcham et al., 1983). Increased aminotransferase activity in response to varying salinity is reported in marine bivalves (Dapaul and Webb, 1974). Aspartate can be utilised along with carbohydrate as an

immediate response to oxygen depletion (Collicut and Hochachka, 1977; Meinardus and Gade, 1981). When aspartate is involved, glycolysis and TCA cycle are connected indirectly by aminotransferase reaction. Aminotransferases are widely acknowledged for their significance in protein metabolism by virtue of their ability to regulate synthesis and degradation of aminoacids. Changes in activities of aminotransferases brought about by any internal or external factors are associated with changes in many other metabolic function. The altered metabolic activities will, in turn, affect the organism's physiology.

In this chapter the sublethal effect of copper, cadmium and mercury on the activity pattern of GOT and GPT is discussed

6.2 MATERIAL AND METHODS

6.2.1 Assay of Glutamate Oxaloacetate Transaminase (Aspartate Aminotransferase)

Methods of collection of specimens, acclimation, and exposure to toxicants were the same as described in Chapter 2. Tissue extraction procedure was carried out as described in Chapter 5.

Glutamate oxaloacetate transaminase activity was determined according to Reitman and Frankel (1957) method. Buffer substrate mixture (containing 0.1 M phosphate buffer, 0.1 M aspartate and 2mM 2-oxoglutarate) of pH 7.4 was used for hepatopancreas. For gill tissue the pH of buffer substrate mixture was 7.8.

To 1 ml of the frozen buffer/substrate mixture, 0.1 ml of tissue extract was added and incubated for exactly 60 minutes at 37 $\pm$ 0.05^{o}C. At the end of incubation period, the enzymatic reaction was stopped by adding 1 ml of chromogen solution (2,4-dinitrophenyl hydrazine) and mixed well and kept for 20 minutes at room temperature. Afterwards 10 ml of 0.4 N NaoH was added and the colour developed was determined spectrophotometrically at 546 nm. Sodium pyruvate (Merck) was used as the standard. The estimation of protein was done by Lowry's method (1951). The activity is expressed as U moles pyruvate liberated/mg protein/hr.

6.2.2 Assay of Glutamate Pyruvate Transaminase (Alanine aminotransferase)

Glutamate pyruvate transaminase activity was determined by the method of Reitman and Frankel (1957). The pH of the buffer substrate solution was 7.2 for both the tissues.

To 1ml of buffer substrate solution (containing 0.1 M phosphate buffer of pH 7.2, 0.2 M DL-alanine and 2mM 2-oxoglutarate) 0.1 ml of tissue extract was added and incubated for 60 minutes at 37 $\pm$ 0.05 ^{o}C. The reaction was stopped by adding 1ml of 0.45 mM chromogen solution and allowed to stand for 20 minutes at room temperature. Later, 10 ml of 0.4 N NaoH was added and the colour developed was measured at 546 nm. Sodium pyruvate was used as the standard. The protein content was determined by Lowry's method (1951). The enzyme activity is expressed as μ moles pyruvate liberated/mg protein/hour.

6.3 RESULTS

6.3.1 Glutamate Oxaloacetate Transaminase Activity

6.3.1.1 Copper

6.3.1.1.1 Gill tissue (Table 28, Figure 28)

The GOT activity in the gill tissue of copper exposed animals showed the following pattern when compared with control. Higher value at 24 hr post exposure ($P<0.001$), but lower values at 72 ($P<0.001$), 120 hr ($P<0.05$), and at 168 hr ($P<0.001$) were observed in 100 ppb exposed animals. In 200 ppb exposed animals,

Table. 28. Gill Glutamate Oxaloacetate Transaminase Activity (μ moles/mg protein/hour) in Lamellidens corrianus exposed to three sublethal concentrations of copper

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	1.0087	1.1613	1.0413	1.1003
	± SD	0.1144	0.3754	0.2808	0.2925
	Range	0.8684-1.2210	0.7658-1.2698	0.5505-1.3746	0.7631-1.6839
100 ppb of copper exposed	N	8	8	8	8
	Mean value	1.6647 ***	0.5518 ***	0.7396 *	0.4365 ***
	± SD	0.1311	0.0743	0.1218	0.0697
	Range	1.3670-1.7566	0.5094-0.7348	0.5490-0.8627	0.3507-0.5107
200 ppb of copper exposed	N	8	8	8	8
	Mean value	1.8994 ***	1.4017	0.6069 **	0.6458 *
	± SD	0.3490	0.1715	0.0483	0.3048
	Range	1.3905-2.4216	1.2233-1.7349	0.5474-0.6861	0.2896-1.2360
400 ppb of copper exposed	N	8	8	8	8
	Mean value	1.6794 ***	0.9728	0.9932	0.8351
	± SD	0.0870	0.2095	0.2367	0.1451
	Range	1.5685-1.8516	0.7756-1.2841	0.6480-1.3137	0.5454-1.0634

Significance levels * $p<0.05$ ** $p<0.01$ *** $p<0.001$

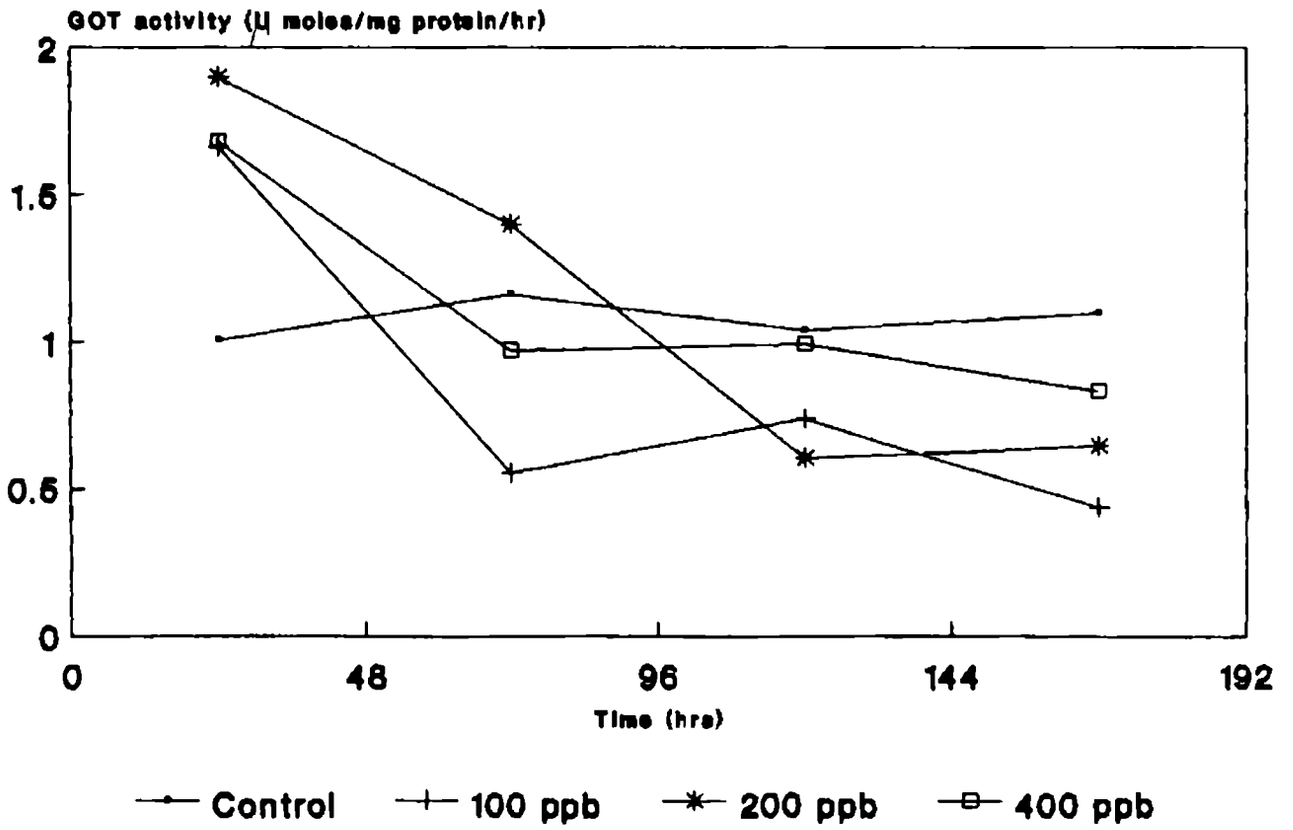


Figure 28. Gill Glutamate Oxaloactate Transaminase activity (µ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of copper.

higher value was noticed at 24 hr ($P<0.001$), but lower values at 120 and 168 hr ($P<0.01$, 0.05) post-exposure. 400 ppb exposed animals showed significantly higher value at 24 hr post-exposure ($P<0.001$).

Among experimentals, it was found that 100 ppb exposed mussels had lower value at 72 hr ($P<0.001$) but higher value at 120 hr ($P<0.05$) post-exposure when compared with the values in 200 ppb exposed animals and lower value at all time periods except 24 hrwhen compared with those in 400 ppb exposed ones ($P<0.001$, 0.05, 0.001). Values in 200 ppb exposed animals when compared with those in 400 ppb exposed ones were higher at 72 hr ($P<0.001$) but lower at 120 hr post-exposure ($P<0.001$).

6.3.1.1.2 Hepatopancreas tissue (Table 29, Figure 29)

The following results were obtained on comparison between experimentals and controls. 100 ppb exposed animals showed lower values at 24 hr ($P<0.001$), 72 hr ($P<0.01$) and 168 hr ($P<0.001$) post exposure. In mussels exposed to 200 ppb, lower values were obtained at 72 hr ($P<0.001$) and 168 hr ($P<0.01$). At 400 ppb, lower value was noticed at 72 hr post-exposure ($P<0.05$).

In a comparison between the varying concentrations, it was

Table. 29. Hepatopancreas Glutamate Oxaloacetate Transaminase Activi ty (μ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of copper

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	1.3308	1.4812	1.1694	1.2057
	± SD	0.3487	0.2245	0.3354	0.5002
	Range	0.7585-1.5658	1.1567-1.7307	0.6578-1.7895	0.7370-2.1056
100 ppb of copper exposed	N	8	8	8	8
	Mean value	0.1455 ***	1.0654 **	1.1076	0.2100 ***
	± SD	0.0348	0.1958	0.1435	0.0189
	Range	0.1156-0.2039	0.7378-1.4076	0.8132-1.2467	0.1813-0.2448
200 ppb of copper exposed	N	8	8	8	8
	Mean value	1.6005	0.9866 ***	1.1331	0.4889 **
	± SD	0.2695	0.1523	0.1469	0.2680
	Range	1.2059-2.0974	0.8439-1.2550	1.0222-1.4817	0.2183-0.9280
400 ppb of copper exposed	N	8	8	8	8
	Mean value	1.5953	1.2080 *	1.0076	1.5660
	± SD	0.3966	0.2290	0.1744	0.2324
	Range	1.1145-2.3759	0.7609-1.5293	0.7718-1.3317	1.4082-2.1198

Significance level: * $P<0.05$, ** $P<0.001$, ***$P<0.01$)

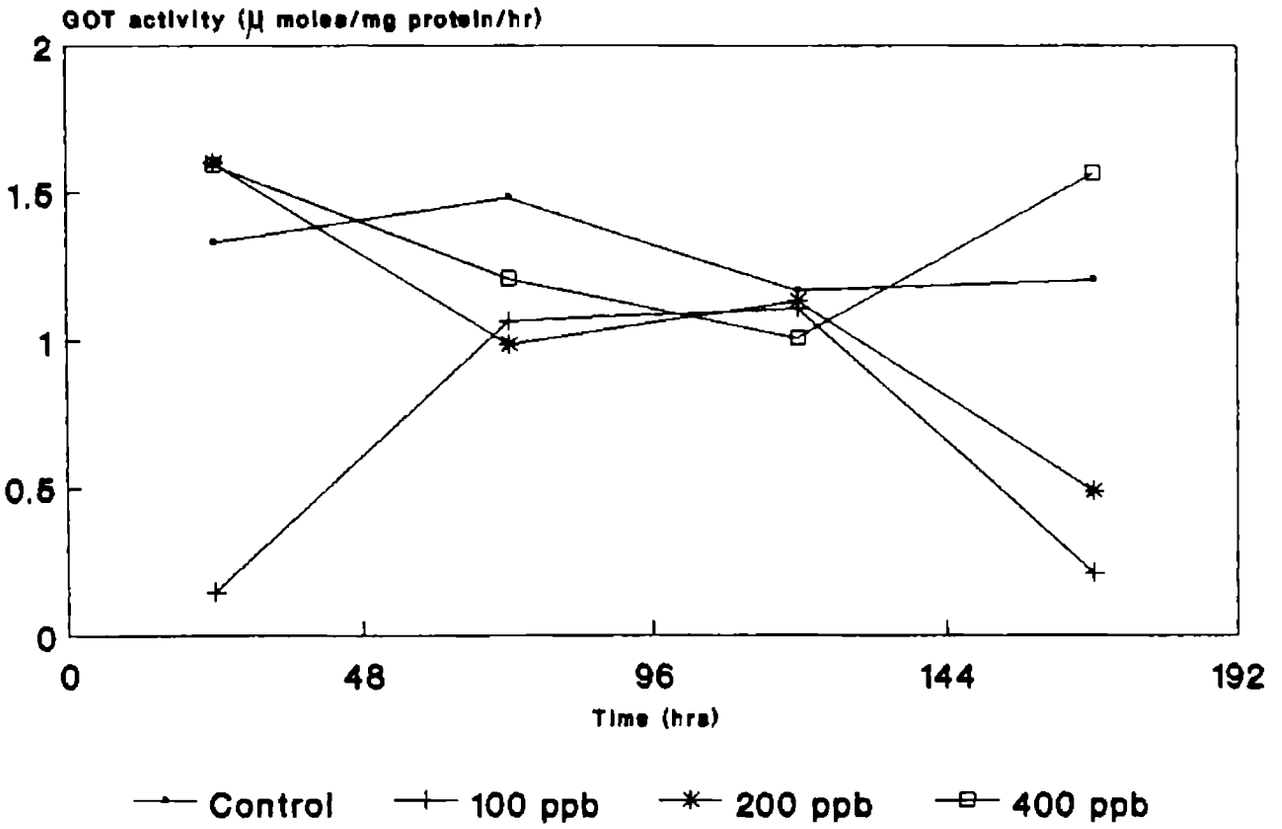


Figure 29. Hepatopancreas Glutamate Oxaloacetate Transaminase activity (μ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of copper.

noticed that 100 ppb exposed animals had lower enzyme activity at 24 hr ($P<0.001$) and 168hr ($P<0.05$) when compared with 200 ppb exposed mussels. 100 ppb exposed animals when compared with 400 ppb exposed ones, had lower value at 24 hr ($P<0.001$) and 168 hr ($P<0.001$). Animals exposed to 200 ppb showed lower value at 168 hr ($P<0.001$) when compared with 400 ppb exposed ones.

6.3.1.2 Mercury

6.3.1.2.1 Gill tissue (Table 30, Figure 30)

Significantly lower value was obtained at 24 hr and 120 hr ($P<0.001$) in 75 ppb exposed animals. In mussels exposed to 150 ppb, lower value was found at 24 hr ($P<0.01$). 300 ppb exposed mussels did not show any variation at any of the exposure periods.

Comparison of values in 75 ppb exposed animals with thoseof 150 ppb exposed ones showed lower values at 72 hr ($P<0.05$), 120 hr ($P<0.001$) and 168 hr ($P<0.001$) post-exposure. When compared with 300 ppb exposed mussels, 75 ppb exposed ones had lower enzyme activity at 24 hr ($P<0.05$) and 120 hr ($P<0.001$) post-exposure. When 150 and 300 ppb exposed animals were compared, higher value was found in 150 ppb at 72 hr ($P<0.01$), 120 hr ($P<0.001$) and 168 hr ($P<0.01$).

Table. 30. Gill Glutamate Oxaloacetate Transaminase activity (μ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of mercury

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	1.0635	1.1490	1.0477	1.1563
	± SD	0.1639	0.3646	0.3172	0.3971
	Range	0.8684-1.4051	0.6586-1.3316	0.5001-1.4677	0.6331-1.8639
75 ppb mercury exposed	N	8	8	8	8
	Mean value	0.6605 ***	1.1687	0.3284 ***	0.9123
	± SD	0.1780	0.2134	0.0456	0.0931
	Range	0.4342-1.0502	0.8959-1.6079	0.2606-0.4081	0.8196-1.1005
150 ppb mercury exposed	N	8	8	8	8
	Mean value	0.7591 **	1.4371	1.0743	1.2507
	± SD	0.1377	0.2145	0.0169	0.1135
	Range	0.6388-1.0633	1.1283-1.7321	1.0479-1.0957	1.0528-1.4739
300 ppb mercury exposed	N	8	8	8	8
	Mean value	0.9130	1.0321	0.7729	0.9378
	± SD	0.1879	0.1879	0.1525	0.1940
	Range	0.7596-1.2361	0.7596-1.2361	0.5299-1.0040	0.6759-1.2076

Significance level: *$P<0.05$, **$P<0.01$, ***$P<0.001$

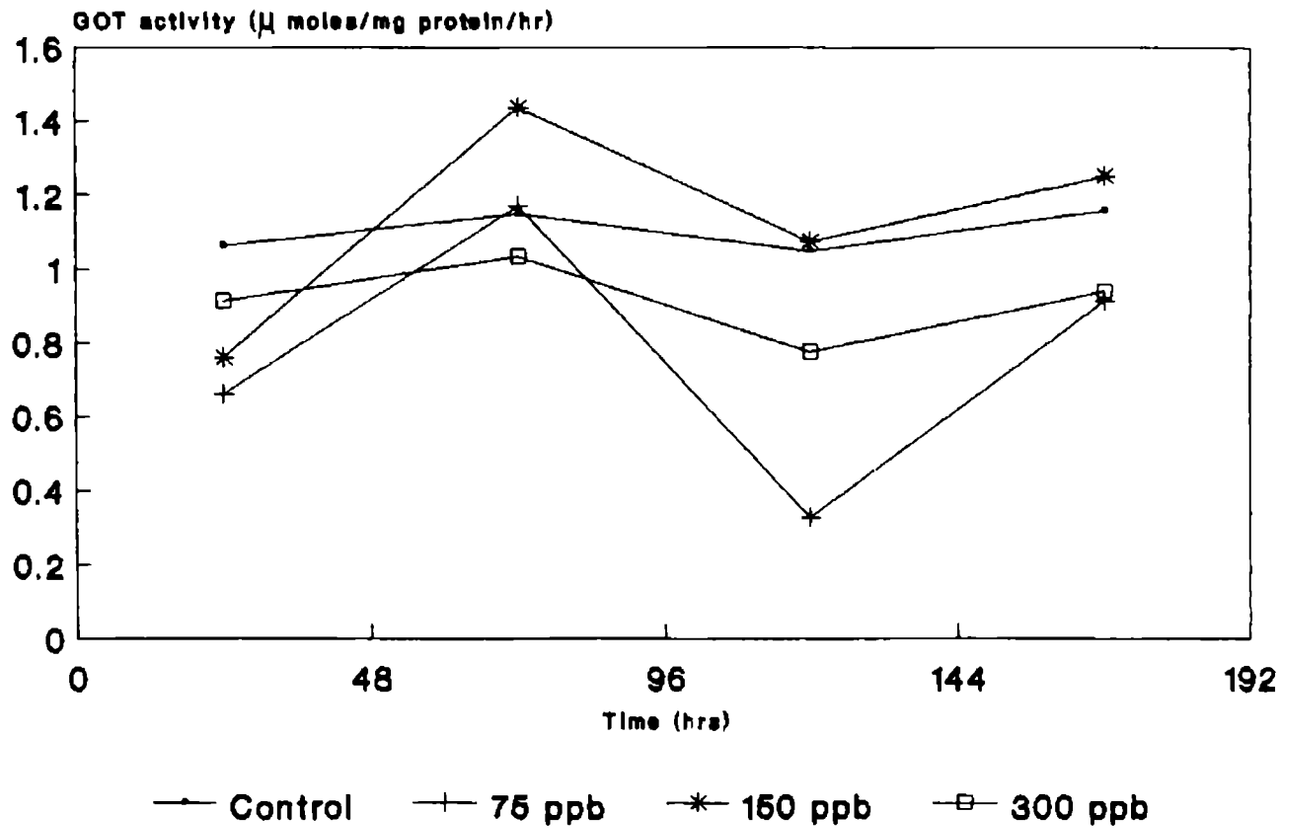


Figure 30. Gill Glutamate Oxaloacetate Transaminase activty (μ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of mercury.

6.3.1.2.2 Hepatopancreas tissue (Table 31, Figure 31)

Mussels exposed to 75 ppb had higher value at 72 hr ($P<0.001$) but lower values at 120 hr ($P<0.001$) and 168 hr ($P<0.05$) when compared with control. 150 ppb exposed animals showed lower value at 24 hr ($P<0.01$) and 72 hr ($P<0.001$) post-exposure, when compared with control. Animals exposed to 300 ppb did not show any significant variation.

75 ppb exposed mussels when compared with 150 ppb and 300 ppb exposed ones, had higher value at 72 hr ($P<0.001$) butlower values at 120 and 168 hr ($P<0.001$). 150 ppb exposed animals when compared with 300 ppb exposed ones showed lower value at 24 hr ($P<0.05$), 72 hr ($P<0.001$) and 168 hr ($P<0.05$) post-exposure.

6.3.1.3 Cadmium

6.3.1.3.1 Gill tissue (Table 32, Figure 32)

When the cadmium exposed animals were compared with the controls, the following pattern of GOT activity was observed. 150 ppb exposed mussels showed lower values at 24 hr ($P<0.05$), 72 hr ($P<0.05$) and 120 hr ($P<0.001$) post-exposure. 300 ppb exposed animals did not show any significant variation in activity. In 600 ppb exposed animals elevation in activity at 24 hr ($P<0.001$)

Table. 31. Hepatopancreas Glutamate Oxaloacetate Transaminase Activity (μ moles/mg protein/hour) in *Lamellidens corrrianus* exposed to three sublethal concentrations of mercury

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	1.3813	1.4188	1.2180	1.3060
	± SD	0.3645	0.2493	0.3767	0.5785
	Range	0.8583-2.009	1.0529-1.7001	0.5687-1.8837	0.8370-2.4566
75 ppb of mercury exposed	N	8	8	8	8
	Mean value	1.2242	2.1313 ***	0.4414 ***	0.7844 *
	± SD	0.4338	0.3070	0.1441	0.1900
	Range	0.6187-1.9047	1.3932-2.3795	0.3052-0.7372	0.4570-1.0513
150 ppb of mercury exposed	N	8	8	8	8
	Mean value	0.9143 **	0.5714 ***	1.0385	1.3714
	± SD	0.1322	0.1019	0.1537	0.2971
	Range	0.6672-1.0957	0.4341-0.7775	0.8001-1.2171	0.9045-1.9745
300 ppb of mercury exposed	N	8	8	8	8
	Mean value	1.2314	1.2383	1.1506	1.6943
	± SD	0.3255	0.3661	0.1468	0.2564
	Range	0.8611-1.7873	0.7488-1.8440	0.8742-1.1990	1.3241-2.0086

Significance level: *$P<0.05$, **$P<0.01$, ***$P<0.001$

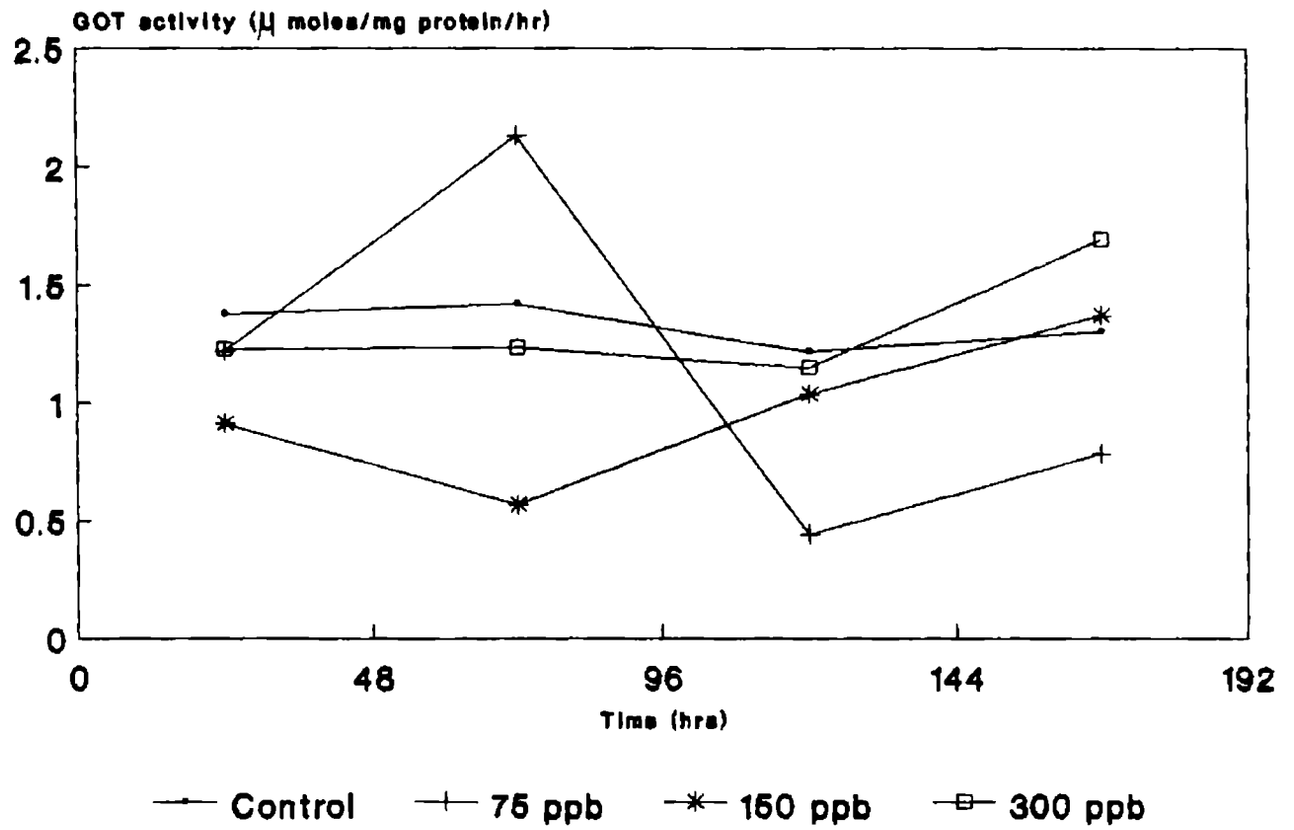


Figure 31. Hepatopancreas Glutamate Oxaloacetate Transaminase activty (μ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of mercury.

Table 32. Gill Glutamate Oxaloacetate Transaminase Activity (μ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of cadmium

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	1.0610	1.1465	1.0180	1.1116
	± SD	0.1775	0.3426	0.2496	0.3343
	Range	0.8898-1.2246	0.6085-1.7840	0.6018-1.3480	0.7331-1.7385
150 ppb of cadmium exposed	N	8	8	8	8
	Mean value	0.7425 *	0.7516 *	0.4505 ***	0.9407
	± SD	0.2495	0.0892	0.0752	0.0902
	Range	0.3884-1.0905	0.6195-0.9044	0.3250-0.5493	0.8699-1.0845
300 ppb of cadmium exposed	N	8	8	8	8
	Mean value	0.8543	1.5473	1.2750	1.3408
	± SD	0.2173	0.4811	0.1180	0.4138
	Range	0.6952-1.3217	0.8907-2.2395	1.1050-1.5329	1.0946-1.7373
600 ppb of cadmium exposed	N	8	8	8	8
	Mean value	2.4050 ***	1.3450	1.4359 *	0.8175 *
	± SD	0.3012	0.1462	0.2016	0.1076
	Range	1.9880-2.7561	1.0890-1.4610	1.2569-1.8772	0.6951-0.9514

Significance level: *$P<0.05$, **$P<0.01$, ***$P<0.001$

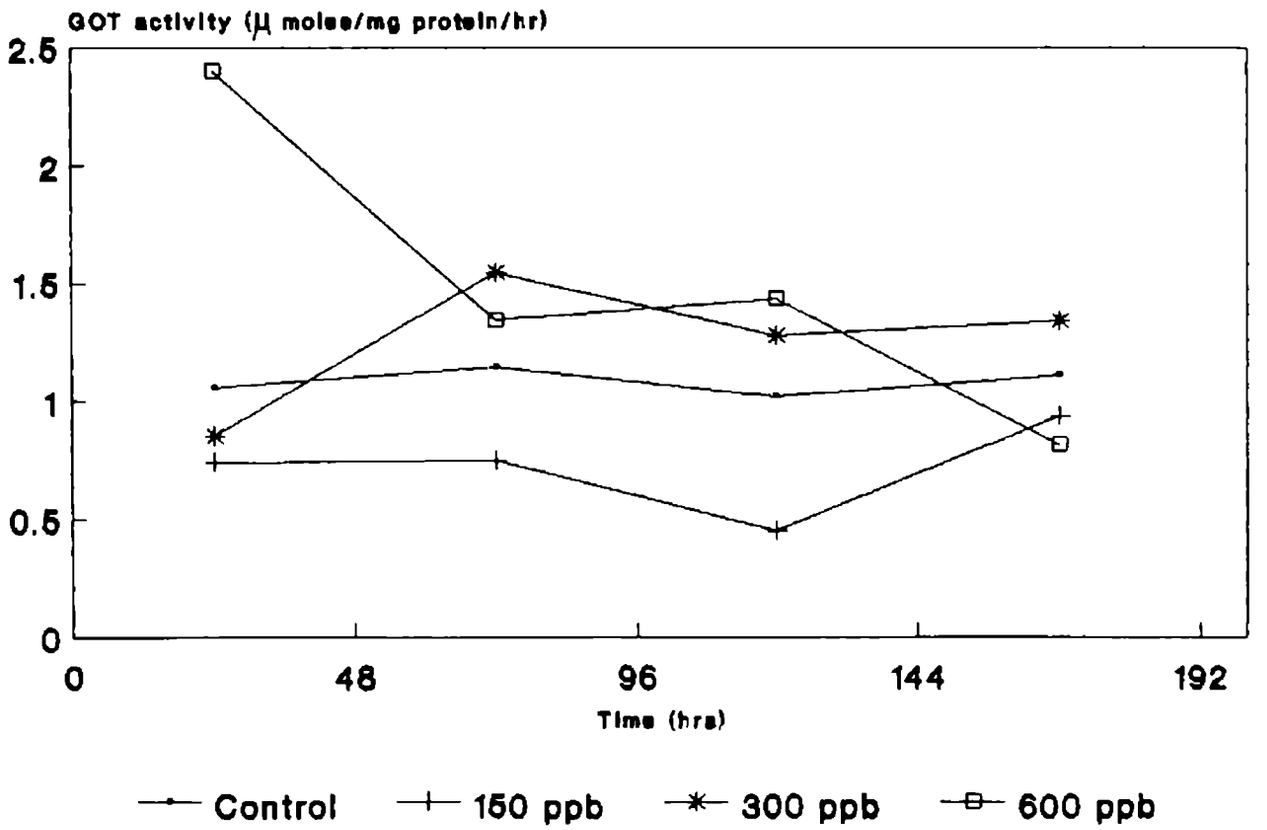


Figure 32. Gill Glutamate Oxaloacetate Transaminase activity (µ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of cadmium.

and 120 hr ($P<0.05$), while reduction at 168 hr ($P<0.05$) were observed.

150 ppb exposed mussels when compared with 300 ppb exposed ones had lower values at 72 hr ($P<0.001$), 120 hr ($P<0.001$) and 168 hr ($P<0.05$) post-exposure. Mussels exposed to 150 ppb showed lower values at 24, 72, and 120 hr ($P<0.001$) but higher value at 168 hr ($P<0.05$), when compared with 600 ppb exposed animals. 300 ppb exposed mussel had lower value at 24 hr ($P<0.001$) buthigher value at 168 hr ($P<0.01$) post-exposure when compared with the values of 600 ppb exposed ones.

6.3.1.3.2 Hepatopancreas tissue (Table 33, Figure 33)

When 150 ppb exposed animals were compared with control, lower values were found at 72 hr ($P<0.001$) and 120 hr ($P<0.01$) time period. 300 ppb exposed animals on comparison with control showed lower value at 24 hr ($P<0.001$) but higher value at 120 hr ($P<0.001$). In 600 ppb exposed animals higher value at 24 hr ($P<0.001$) but lower value at 168 hr ($P<0.05$) were observed.

150 ppb exposed animals when compared with those exposed to 300 ppb showed higher valuesat 24 hr ($P<0.001$) and 168 hr ($P<0.05$) while lower values at 72 hr ($P<0.001$) and 120 hr

Table. 33. Hepatopancreas Glutamate Oxaloacetate Transaminase Activity (µ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of cadmium

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	1.3861	1.4590	1.2279	1.2433
	± SD	0.3651	0.1645	0.3578	0.4901
	Range	0.9107-2.1015	1.2506-1.7106	0.7370-1.9258	0.6853-2.2015
150 ppb of cadmium exposed	N	8	8	8	8
	Mean value	1.2499	0.7722 ***	0.6818 **	1.0562
	± SD	0.1172	0.1457	0.0400	0.1741
	Range	1.1622-1.5078	0.5491-1.0136	0.5964-0.7272	0.7928-1.2310
300 ppb of cadmium exposed	N	8	8	8	8
	Mean value	0.7103 ***	1.2281	2.3312 ***	0.8453
	± SD	0.2248	0.1716	0.3867	0.0913
	Range	0.5332-1.1595	0.9530-1.5760	1.7922-3.1768	0.6339-0.9449
600 ppb of cadmium exposed	N	8	8	8	8
	Mean value	3.1761 ***	1.5389	1.1313	0.7156 *
	± SD	0.3252	0.2611	0.2161	0.1378
	Range	2.6461-3.5785	1.0873-1.9488	0.8304-1.4538	0.5812-0.9271

Significance level: *$P<0.05$, **$P<0.01$, ***$P<0.001$

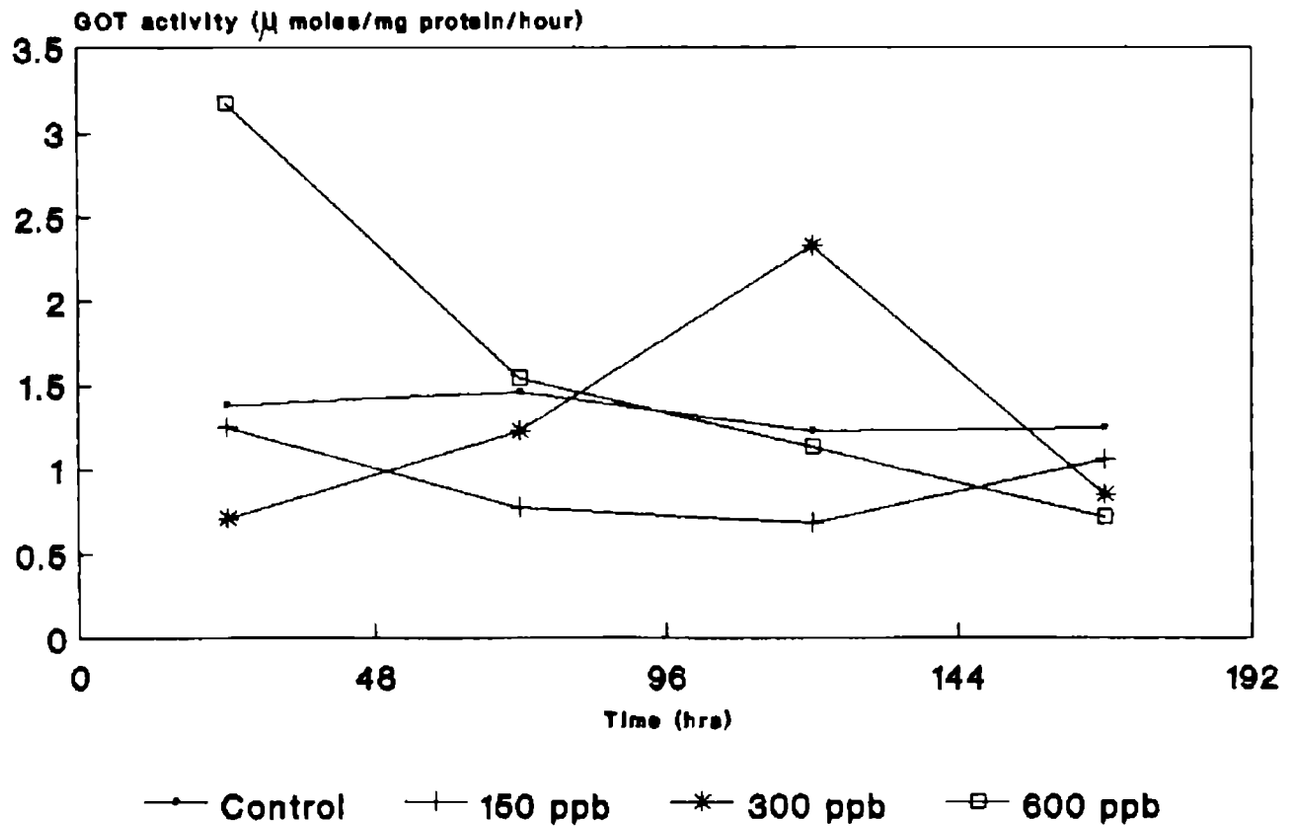


Figure 33. Hepatopancreas Glutamate Oxaloacetate Transaminase activity (μ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of cadmium.

($P<0.001$) post-exposure. Mussels exposed to 150 ppb showed lower values at 24, 72, and 120 hr ($P<0.001$) post-exposure buthigher value at 168 hr ($P<0.01$) when compared with 600 ppb exposed ones. 300 ppb exposed mussels had lower values at 24 hr ($P<0.001$) and 72 hr ($P<0.05$) while higher value at 120 hr ($P<0.001$) post-exposure, when compared with those exposed to 600 ppb cadmium.

6.3.2 Glutamate Pyruvate Transaminase Activity

6.3.2.1 Copper

6.3.2.1.1 Gill tissue (Table 34, Figure 34)

The following observations were made when the GPT activity of copper exposed mussels was compared with that of control. 100 ppb exposed mussels showed higher value at 72 hr ($P<0.001$) but lower value at 168 hr ($P<0.001$) post-exposure. Those exposed to 200 ppb showed higher activity at 24 hr ($P<0.01$) and 72 hr ($P<0.001$) post-exposure. 400 ppb exposed mussels had higher values at 24 hr ($P<0.001$) and 72 hr ($P<0.01$) time periods.

When GPT activity was compared among the experimentals, significant variation was observed at certain time periods only. When 100 and 200 ppb exposed animals were compared, lower values were found at 24 hr ($P<0.01$) and 72 hr ($P<0.001$) time period in

Table 34. Gill Glutamate Pyruvate Transaminase Activty (μ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of copper

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	2.2496	2.4381	2.3557	2.5581
	± SD	0.3157	0.4139	0.4808	0.4986
	Range	1.7896-2.6138	2.1123-3.0985	1.6365-3.0010	2.020-3.2896
100 ppb of copper exposed	N	8	8	8	8
	Mean value	2.2940	5.1146 ***	2.8952	1.3164 ***
	± SD	0.1592	0.6602	0.5397	0.1443
	Range	2.1500-2.6129	4.0863-6.5181	2.3496-3.7946	1.1086-1.5548
200 ppb of copper exposed	N	8	8	8	8
	Mean value	2.9546 **	8.3290 ***	2.6159	1.9150
	± SD	0.4516	0.9897	0.4499	1.0454
	Range	2.1968-3.3382	7.3505-9.9639	1.7840-3.2550	0.8216-3.7533
400 ppb of copper exposed	N	8	8	8	8
	Mean value	3.4953 ***	3.4676 **	2.3993	3.0287
	± SD	0.5833	0.6709	0.6983	0.4378
	Range	2.6319-4.5582	2.5829-4.5065	1.3794-3.5991	2.2962-3.6406

Significance levels: *$P<0.05$, **$P<0.01$, ***$P<0.001$

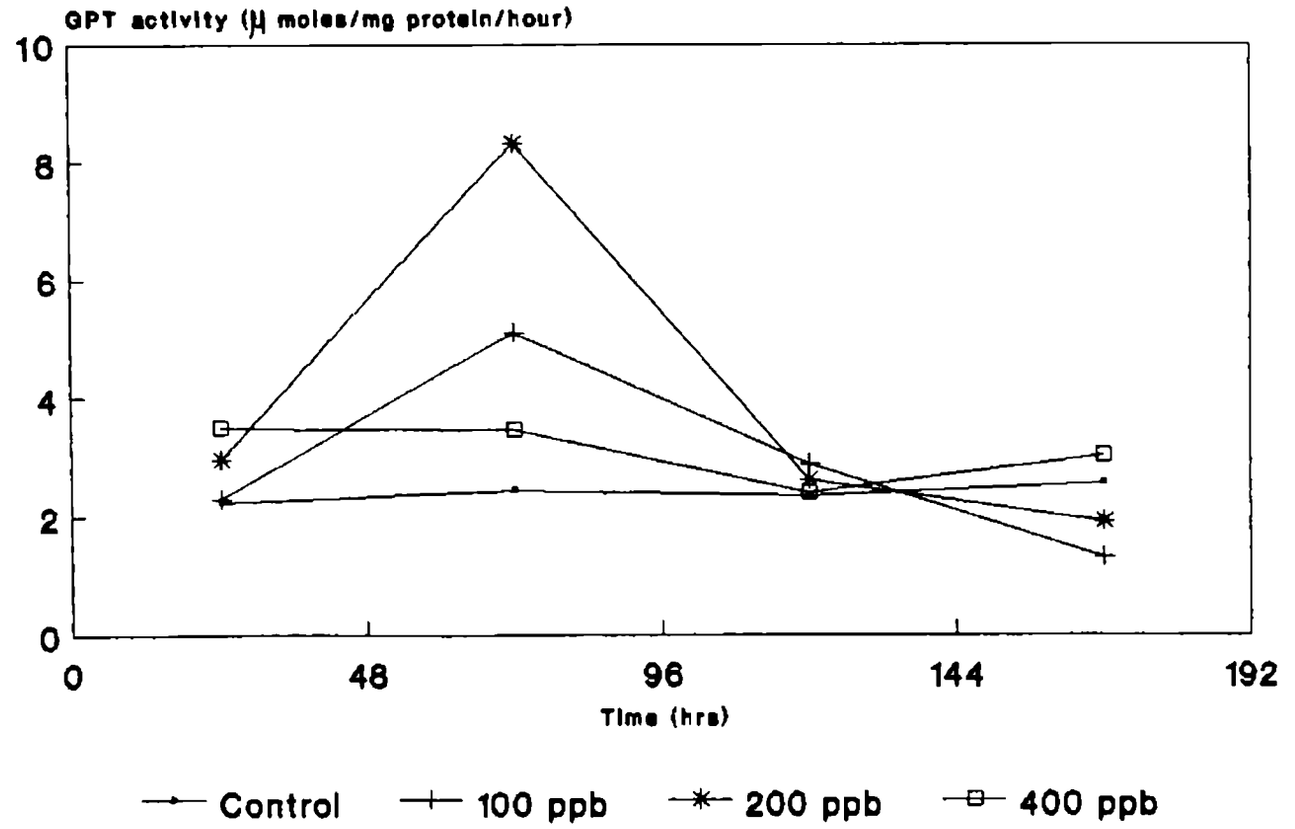


Figure 34. Gill Glutamate Pyruvate Transaminase activity (μ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of copper.

100 ppb exposed animals. 100 ppb exposed animals also showed lower values at 24 hr ($P<0.001$) and 168 hr ($P<0.001$) but higher value at 72 hr ($P<0.001$) when compared with 400 ppb exposed ones. Comparison between 200 and 400 ppb exposed animals showed higher value at 72 hr ($P<0.001$) but lower value at 168 hr ($P<0.05$) in 200 ppb exposed animals.

6.3.2.1.2 Hepatopancreas tissue (Table 35, Figure 35)

The following were the values obtained when 100, 200, and 400 ppb copper exposed animals were compared with thoseof control. 100 ppb exposed mussels had higher values at 72 hr ($P<0.001$) and 120 hr ($P<0.01$) but lower value at 168 hr ($P<0.001$). 200 ppb exposed animals had higher value at 72 hr ($P<0.001$) but lower value at 168 hr ($P<0.05$) post-exposure. Mussels exposed to 400 ppb showed higher values at 24 hr ($P<0.05$), 72 hr ($P<0.001$), 120 hr ($P<0.05$) and 168 hr ($P<.001$) post-exposure.

100 ppb exposed animals when compared with 200 ppb exposed ones, had higher value at 120 hr ($P<0.05$) but lower value at 168 hr ($P<0.01$) post exposure. When values in 100 and 400 ppb exposed animals were compared, the former showed lower values at 24 hr ($P<0.001$) and 168 hr ($P<0.001$) but higher value at 72 hr

Table 35. Hepatopancreas Glutamate Pyruvate Transaminase Activity (μ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of copper

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	2.0242	2.0192	1.8859	1.7431
	± SD	0.8407	0.3933	0.5817	0.1417
	Range	1.1128-3.2082	1.3935-2.450	1.1276-2.644	1.5485-1.9057
100 ppb of copper exposed	N	8	8	8	8
	Mean value	1.5756	6.3509 ***	2.9955 **	0.5304 ***
	± SD	0.1984	1.4266	0.7079	0.0815
	Range	1.3373-1.9171	3.7020-7.4825	2.0744-4.2068	0.4155-0.6899
200 ppb of copper exposed	N	8	8	8	8
	Mean value	1.5358	5.0139 ***	2.2359	1.2542 *
	± SD	0.3320	1.6739	0.4380	0.5557
	Range	1.3340-2.2030	2.8675-8.0158	1.6801-2.9791	0.6671-2.1590
400 ppb of copper exposed	N	8	8	8	8
	Mean value	3.6548 *	3.6443 ***	2.6224 *	4.6423 ***
	± SD	1.3829	0.4655	0.4168	1.2805
	Range	2.0151-6.1357	2.8995-4.3108	2.0947-3.2342	3.0615-6.2984

Significance levels: *$P<0.05$, **$P<0.01$, ***$P<0.001$

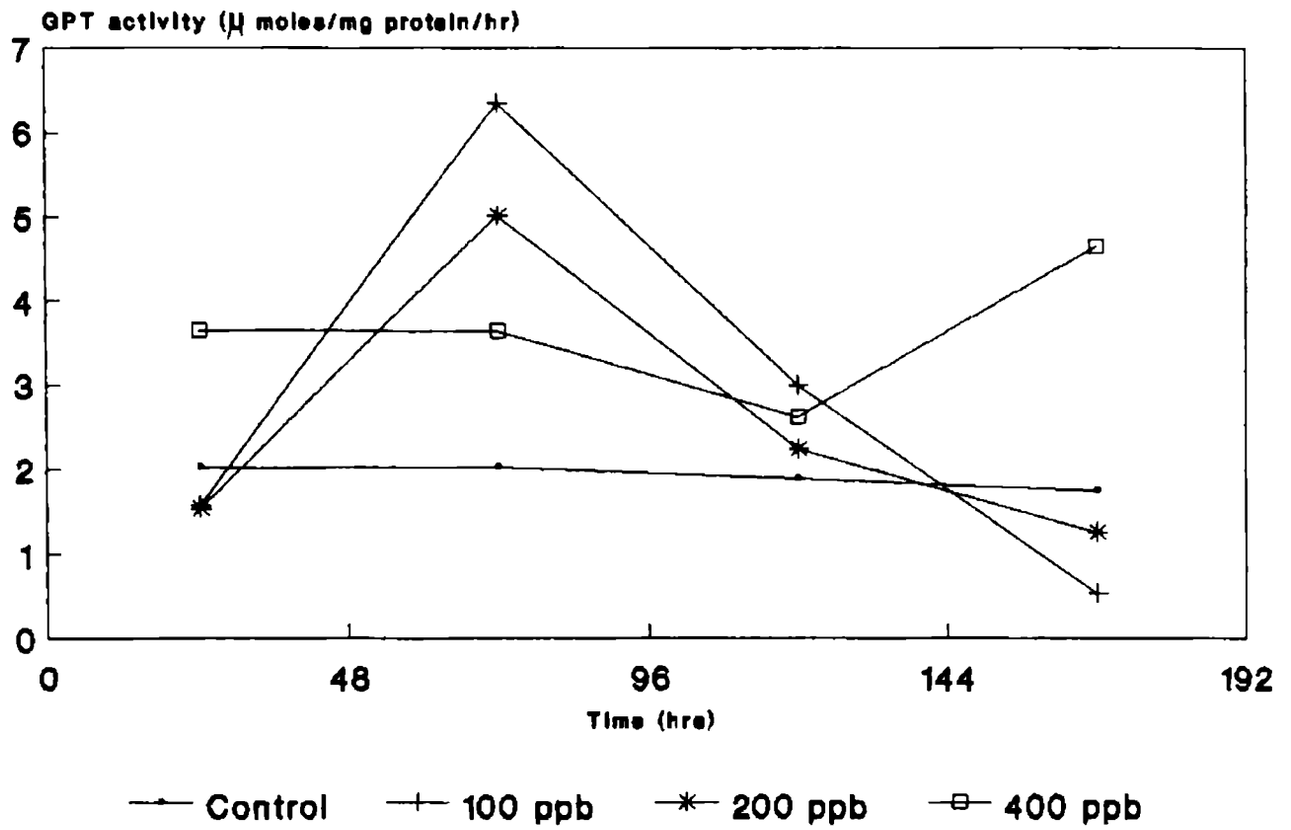


Figure 35. Hepatopancreas Glutamate Pyruvate Transaminase activity (μ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of copper.

($P<0.001$). When values in 200 and 400 ppb exposed animals were compared, the former showed lower values at 24 hr ($P<0.01$) and 168 hr ($P<0.001$) post-exposure.

6.3.2.2 Mercury

6.3.2.2.1 Gill tissue (Table 36, Figure 36)

75 ppb exposed animals when compared with control had higher values at 24 hr ($P<0.01$) and 72 hr ($P<0.05$) while lower value at 120 hr ($P<0.001$) time periods. Animals exposed to 150 ppb had higher values at 72 and 120 hr ($P<0.001$) post-exposure when compared with control. 300 ppb exposed mussels showed higher activity at 24 hr ($P<0.001$), 72 hr ($P<0.05$) and 168 hr ($P<0.01$) while lower activity at 120 hr ($P<0.05$) post-exposure.

75 ppb exposed mussels showed higher value at 24 hr ($P<0.01$) but lower values at 72 and 120 hr ($P<0.001$) post-exposure when compared with 150 ppb exposed mussels. 75 ppb exposed animals when compared with 300 ppb showed lower values at 24 hr ($P<0.05$), 120 hr ($P<0.01$) and 168 hr ($P<0.05$) post-exposure. 150 ppb exposed animals showed lower value at 24 hr ($P<0.001$) buthigher values at 72 hr ($P<0.01$), and 120 hr ($P<0.001$) when compared with 300 ppb exposed ones.

6.3.2.2.2 Hepatopancreas tissue (Table 37, Figure 37)

Table 36. Gill Glutamate Pyruvate Transaminase Activity (μ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of mercury

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	2.2622	2.4390	2.4444	2.6142
	± SD	0.2616	0.4850	0.4425	0.5267
	Range	1.9478-2.5944	1.9822-3.2871	1.7852-3.1031	2.0385-3.3644
75 ppb of mercury exposed	N	8	8	8	8
	Mean value	2.8584 **	2.9956 *	0.8847 ***	2.8738
	± SD	0.3684	0.3795	0.1578	0.4775
	Range	2.1721-3.4044	2.5139-3.7632	0.7414-1.2196	1.9897-3.5420
150 ppb of mercury exposed	N	8	8	8	8
	Mean value	2.0486	6.2117 ***	3.3995 ***	3.1427
	± SD	0.3755	1.1631	0.2788	0.4910
	Range	1.6641-2.8861	4.2209-7.2931	3.0283-3.7829	2.1541-3.7550
300 ppb of mercury exposed	N	8	8	8	8
	Mean value	3.4382 ***	3.7763 *	1.7551 *	3.7204 **
	± SD	0.5488	1.2903	0.6120	0.8290
	Range	2.7416-4.4294	2.2242-5.7901	0.8300-2.5275	2.6433-5.1803

Significance levels: *$P<0.05$, **$P<0.01$, ***$P<0.001$

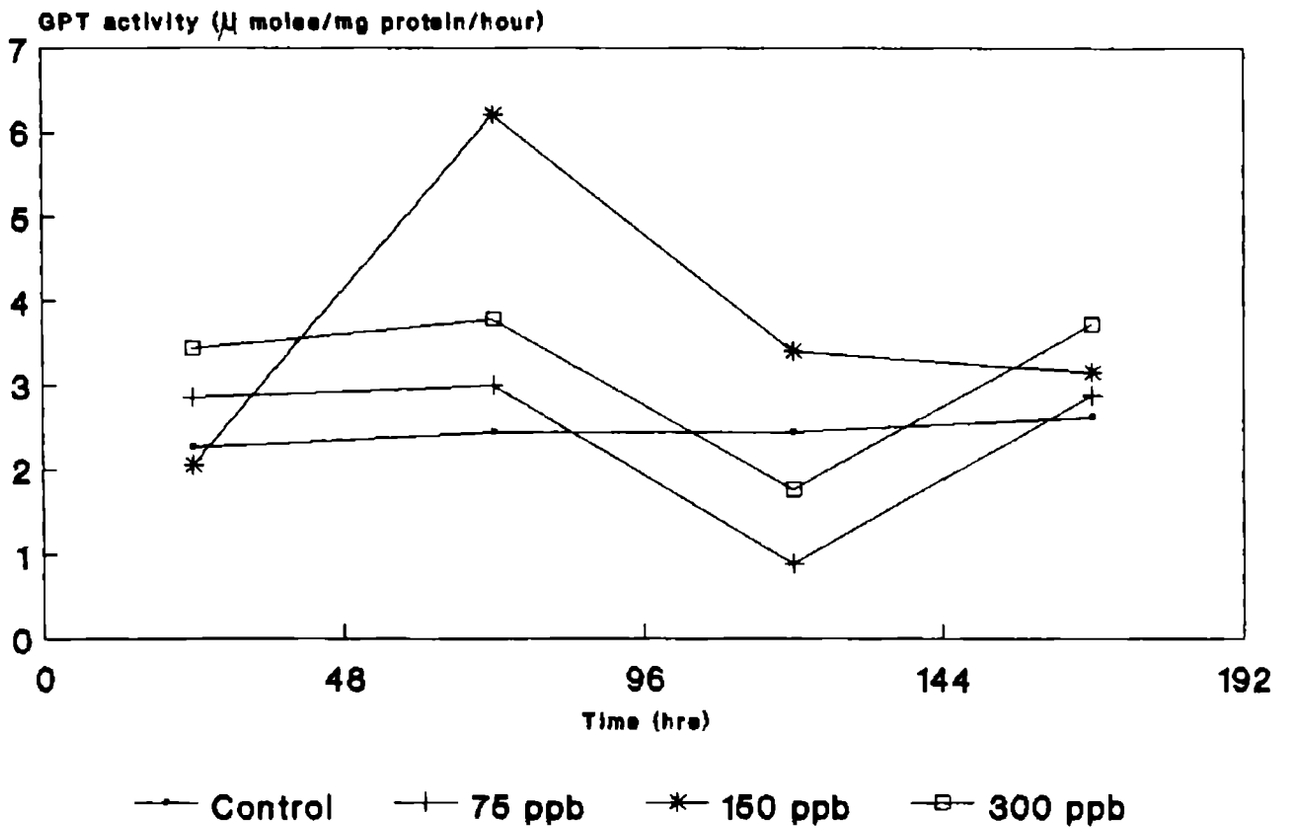


Figure 36. Gill Glutamate Pyruvate Transaminase activity (µ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of mercury

Table 37. Hepatopancreas Glutamate Pyruvate Transaminase Activity (μ mole/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of mercury

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	2.1131	2.0827	1.9366	1.7816
	± SD	0.8314	0.3614	0.5765	0.1452
	Range	1.1974-3.2628	1.4793-2.54	1.1450-2.6445	1.5663-1.9372
75 ppb of mercury exposed	N	8	8	8	8
	Mean value	3.9762 **	4.6855 ***	0.8509 ***	2.6120 ***
	± SD	1.1023	0.3557	0.0531	0.3356
	Range	2.2575-5.6776	4.1725-5.0153	0.7560-0.9158	2.0421-3.1496
150 ppb of mercury exposed	N	8	8	8	8
	Mean value	1.9922	0.9885 ***	2.6439 *	3.3800 ***
	± SD	0.2404	0.2654	0.2816	0.6735
	Range	1.6729-2.3045	0.6651-1.3884	2.1671-2.9815	2.0446-4.2896
300 ppb of mercury exposed	N	8	8	8	8
	Mean value	3.5919 **	1.8753	2.0037	1.2294 ***
	± SD	0.5496	0.7281	0.3514	0.0616
	Range	2.8316-4.2106	0.6227-2.9978	1.3488-2.4337	1.1494-1.3556

Significance levels: *$P<0.05$, **$P<0.01$, ***$P<0.001$

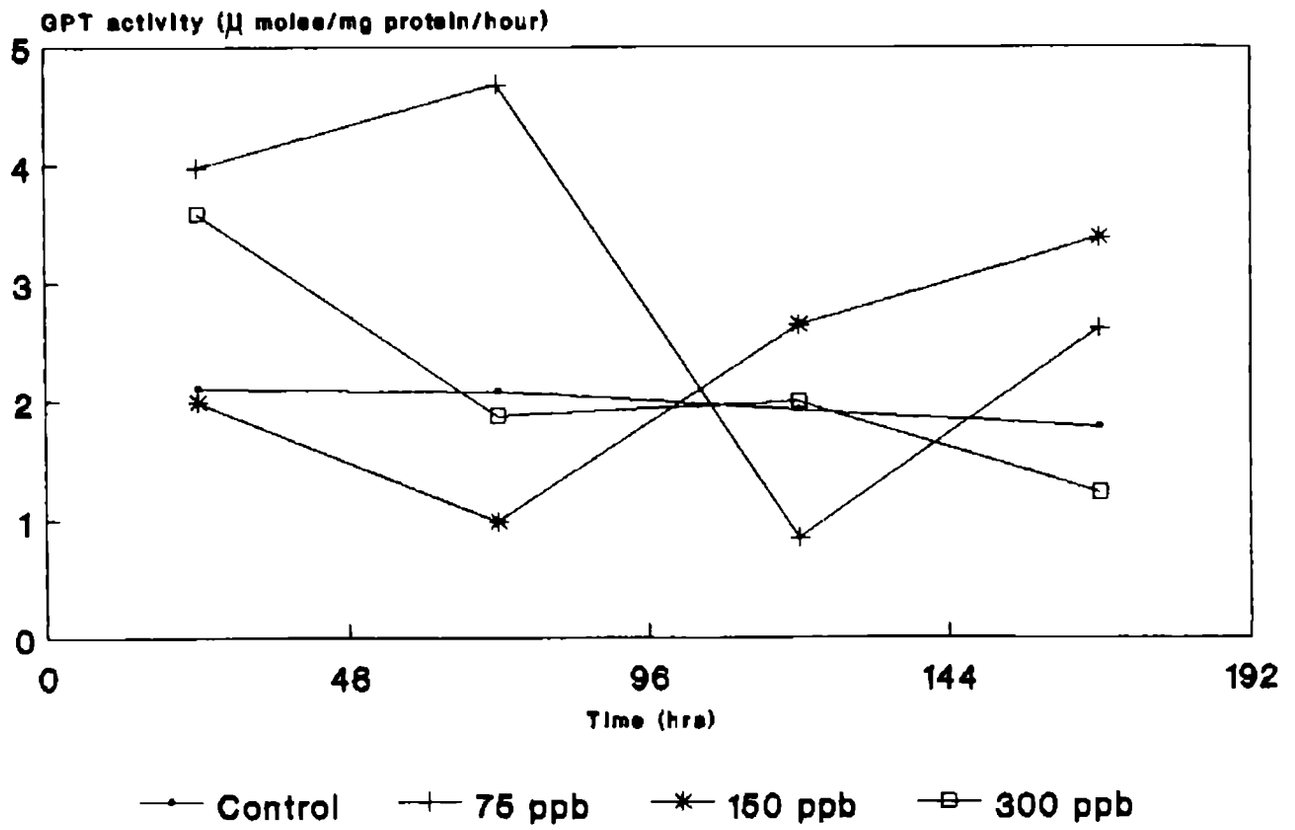


Figure 37. Hepatopancreas Glutamate Pyruvate transaminase activity (μ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of mercury.

When 75 ppb exposed animals were compared with control, values were higher at 24 hr ($P<0.01$), 72 hr ($P<0.001$) and 168 hr ($P<0.001$) while lower at 120 hr ($P<0.001$) post-exposure. Mussels exposed to 150 ppb showed lower value at 72 hr ($P<0.001$) but higher values at 120 hr ($P<0.05$), and 168 hr ($P<0.001$) post-exposure. 300 ppb exposed animals had higher value at 24 hr ($P<0.01$) but lower value at 168 hr ($P<0.001$) post exposure.

75 ppb exposed animals had higher values at 24 hr ($P<0.001$), and72 hr ($P<0.001$), but lower value at 120 hr ($P<0.001$) and 168 hr ($P<0.05$) when compared with 150 ppb exposed ones. 75 ppb exposed mussels had higher value at 72 hr ($P<0.001$) and 168 hr ($P<0.001$) while lower value at 120 hr ($P<0.001$) post-exposure when compared with 300 ppb exposed ones. 150 ppb exposed animals had lower values at 24 hr ($P<0.001$), and 72 hr ($P<0.01$) while higher values at 120 hr ($P<0.01$) and 168 hr ($P<0.001$) post-exposure.

6.3.2.3 Cadmium

6.3.2.3.1 Gill tissue (Table 38, Figure 38)

Mussels exposed to 150 ppb had lower values at 24 hr ($P<0.01$) and 120 hr ($P<0.01$) but higher value at 168 hr ($P<0.001$)

Table 38. Gill Glutamate Pyruvate Transaminase Activity (μ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of cadmium

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	2.1753	2.3570	2.4735	2.5549
	± SD	0.3156	0.4221	0.3755	0.4339
	Range	1.7489-2.6001	1.8575-3.0010	1.8589-3.0130	2.0515-3.1285
150 ppb of cadmium exposed	N	8	8	8	8
	Mean value	1.6020 **	2.2348	1.6690 **	3.8427 ***
	± SD	0.3352	0.4095	0.3058	0.2077
	Range	1.0755-2.0766	1.8076-3.1467	1.0522-2.0460	3.6371-4.2476
300 ppb of cadmium exposed	N	8	8	8	8
	Mean value	2.0946	4.4531 ***	3.3632 ***	4.6237 ***
	± SD	0.4070	0.9338	0.3104	0.2390
	Range	1.4214-2.6943	2.9325-5.5446	2.6705-3.7694	4.5097-5.2119
600 ppb of cadmium exposed	N	8	8	8	8
	Mean value	6.9204 ***	3.9509 ***	3.2094 *	1.7597 ***
	± SD	0.7915	0.7274	0.7883	0.1257
	Range	5.7342-7.6839	2.7894-4.7287	1.8435-4.6737	1.6484-1.9148

Significance levels : *$P<0.05$, **$P<0.01$, ***$P<0.001$

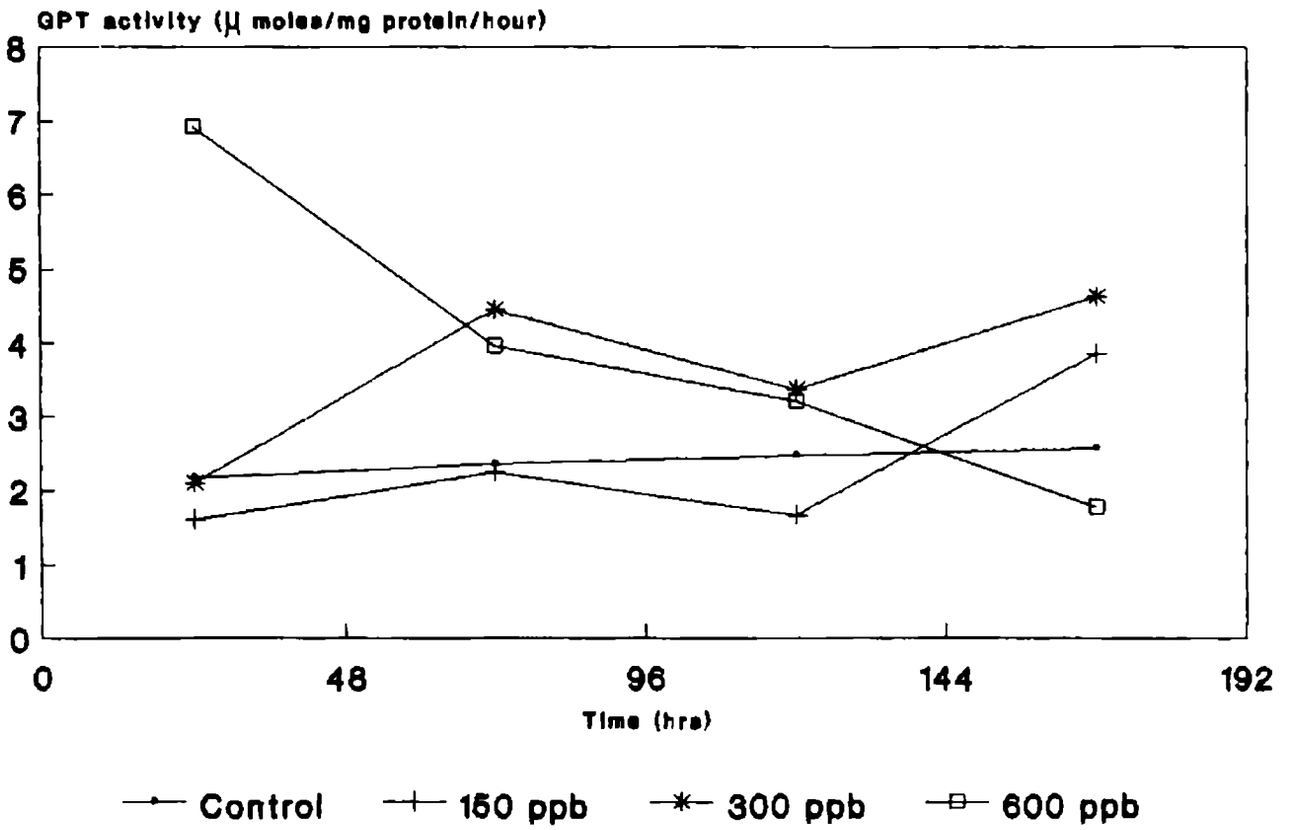


Figure 38 Gill Glutamate Pyruvate Trannsaminase activity (μ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of cadmium.

when compared with control. 300 ppb exposed animals on comparison with control showed higher values at 72, 120, and 168 hr ($P<0.001$) exposure periods. In 600 ppb exposed animals, higher values were obtained at 24 hr ($P<0.001$), 72 hr ($P<0.001$) and 120 hr ($P<0.05$) while lower value at 168 hr ($P<0.001$) when compared with control.

150 ppb exposed animals when compared with 300 ppb exposed ones had lower values at 24 hr ($P<0.05$), 72 hr ($P<0.001$), 120 hr ($P<0.001$), and 168 hr ($P<0.001$). 150 ppb exposed animals on comparison with 600 ppb exposed ones had lower values at 24, 72, and 120 hrs ($P<0.001$) but higher value at 168 hr ($P<0.001$) post-exposure. Comparing values of 300 ppb exposed mussels with those of 600 ppb exposed ones, showed lower value at 24 hr ($P<0.001$) but higher value at 168 hr ($P<0.001$) post-exposure.

6.3.2.3.2 Hepatopancreas tissue (Table 39, Figure 39)

150 ppb exposed mussels had higher activity at 120 hr ($P<0.05$) when compared with control. Mussels exposed to 300 ppb had lower value at 24 hr ($P<0.01$) but higher values at 120 hr ($P<0.001$) and 168 hr ($P<0.001$). 600 ppb exposed animals had higher value at 24 hr ($P<0.001$) and120 hr ($P<.05$) butlower value at 168 hr ($P<0.001$).

Table 39. Hepatopancreas Glutamate Pyruvate Transaminase Activity (μ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of cadmium

	Hours	24	72	120	168
Control	N	8	8	8	8
	Mean value	2.1706	2.0063	1.9402	1.7730
	± SD	0.7045	0.3353	0.5778	0.1017
	Range	1.2845-3.1718	1.5103-2.3998	1.1178-2.6212	1.6258-1.9273
150 ppb of cadmium exposed	N	8	8	8	8
	Mean value	1.6793	1.6269	2.5860 *	1.9067
	± SD	0.3649	0.3333	0.1695	0.6099
	Range	1.3775-2.3315	1.0910-2.0509	2.2609-2.8218	1.0170-2.6734
300 ppb of cadmium exposed	N	8	8	8	8
	Mean value	1.0853 **	2.3818	5.0390 ***	2.0747 ***
	± SD	0.2011	0.3908	0.8269	0.0150
	Range	0.6657-1.2049	1.7454-2.7658	3.9430-6.0692	2.0565-2.0859
600 ppb of cadmium exposed	N	8	8	8	8
	Mean value	4.9823 ***	2.3032	2.8497 *	1.1257 ***
	± SD	0.9166	0.5970	0.6952	0.2343
	Range	4.2183-6.7558	1.3668-3.1240	2.1739-4.3616	0.8147-1.1965

Significance levels : *$P<0.05$, **$P<0.01$, ***$P<0.001$

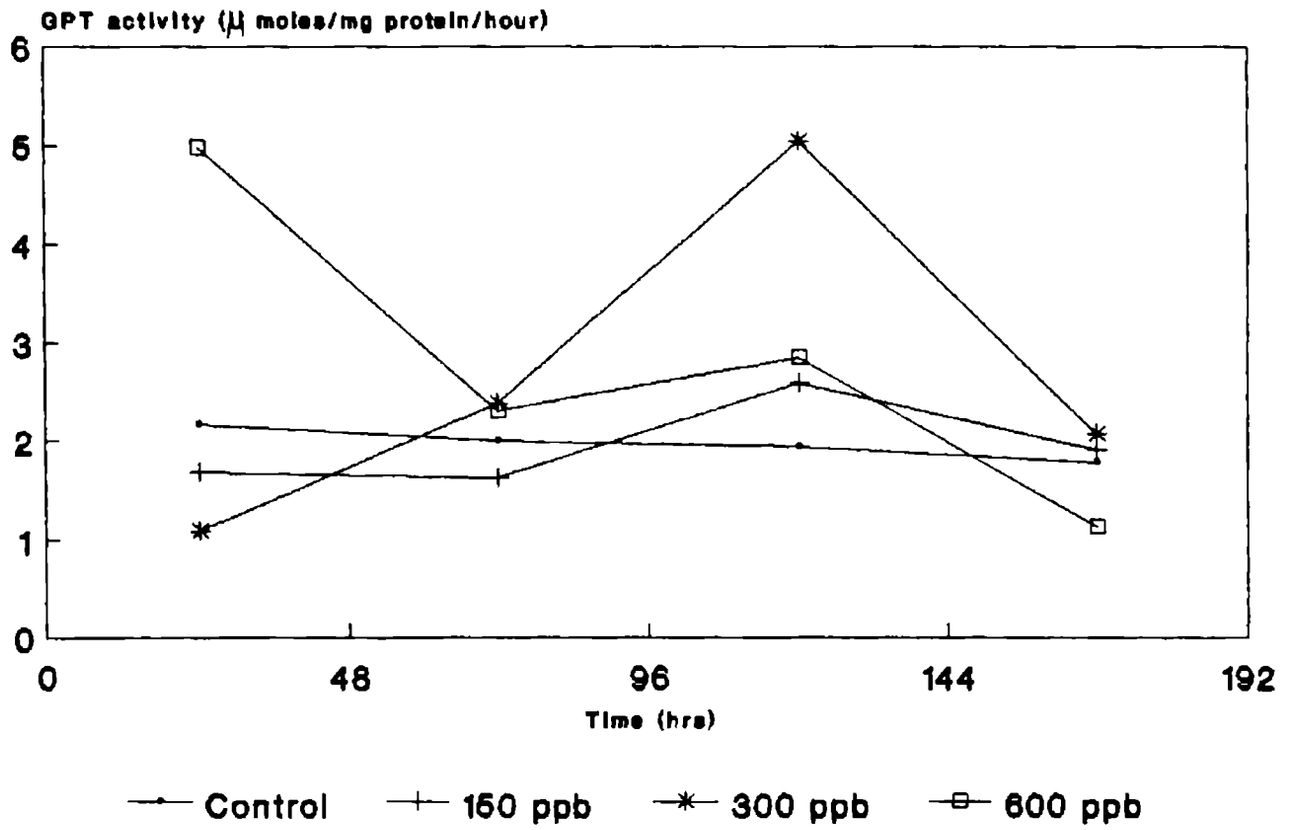


Figure 39. Hepatopancreas Glutamate Pyruvate Transaminase activity (μ moles/mg protein/hour) in *Lamellidens corrianus* exposed to three sublethal concentrations of cadmium.

Comparison of enzyme activity among the experimentals showed the following pattern. 150 ppb exposed animals when compared with 300 ppb exposed ones showed higher activity at 24 hr ($P<0.01$) but lower activity at 72 hr ($P<0.01$) and 120 hr ($P<.001$) exposure periods. When 150 and 600 ppb exposed mussels were compared, the former had lower values at 24 hr ($P<0.001$) and 72 hr ($P<0.05$) while higher value at 168 hr ($P<0.01$). When 300 ppb and 600 ppb exposed mussels were compared, 300 ppb exposed ones showed lower value at 24 hr ($P<0.001$) but higher values at 120 hr and 168 hr ($P<0.001$) post-exposure.

6.4 DISCUSSION

In the gill tissue of all the copper exposed animals GOT activity was high, whileGPT activity was significantly high only in higher concentrations during the initial period.

Increase in GOT and GPT activity is reported to be an indication of stress (Knox and Greenguard, 1965; Kulkarni and Mehrotra, 1973). In the present study, elevation in activity of both the transaminases may be an immediate response to the toxicant stress. Since glycogen level has depleted drastically during the same period, a shift in metabolism from aerobic to

anaerobic phase is a strong possibility. The decrease in oxygen uptake rate also is suggestive of a shift in metabolism. Therefore, gluconeogenesis might have become operative to meet the excess energy demand.

Elevation in the activity of GOT and GPT during stress is reported in the freshwater mussels, *Lamellidens marginalis* (Ahamed et al., 1978), and in *Parreysia rugosa* (Reddy and Chari, 1985). By 72 hr time period, the GPT activity was found to attain a peak in all the exposures while GOT activity showed a general trend of decline. Therefore, it can be presumed that during this time period, alanine transamination was more than aspartate utilisation as indicated by Malhotra et al.(1986). High ALAT level is reported in amphibian species (Laksmipathi and Reddy, 1984). Elevation in GPT activity can also be considered as a sign of adaptability to the toxicant (Moorthy et al., 1985; Vijayalakshmi and Rao, 1985). Since there was a tendency for increased oxygen uptake during the later periods, adaptability to the toxicant on prolonged exposure is a possibility. During the later periods, GOT activity declined steadily, more so in the lower concentrations. This decrease in GOT activity further proves a shift in alanine transamination in preference to aspartate.

Decrease in GOT and increase in GPT activity is reported by Chetty et al.(1980) in *Tilapia mossambica* during stress. Damage caused to the mitochondrial membrane also leads to decline in GOT activity (Chow and Pond, 1972). But in the present study, since decrease in activity was more in lower concentrations, possibility of mitochondrial damage can be ruled out. The low activity may be attributed to the decreased availability of oxalo-acetate. The peak activity in turn may be to compensate for the loss of GOT activity and also due to increased availability of pyruvate formed due to lactate dehydrogenase activity (Chetty et al., 1980). ALAT activity enhancement could be attributed to the generation of pyruvate under hypoxic condition, and not to the formation of pyruvic acid from protein break down as observed from the upward trend in total protein content.

In the case of hepatopancreas, there was significant fluctuation in both the transaminase activities, indicating stress. In 100 ppb exposed animals, the GPT activity was normal while GOT activity showed a sharp decline at 24 hr. GPT activity in all the three concentration exposures exhibited a peak activity at 72 hr while GOT activity was generally low. Alanine transamination was found to be more than that of aspartate

utilisation. Increased ALAT activity suggests the mobility of aminoacid towards the formation of pyruvic acid (Reddy et al., 1985). Decreased total protein during the initial period further suggests pyruvic acid formation from aminoacids. In bivalves under anoxic or hypoxic condition, malic enzyme catalases in the direction of pyruvate production (Hochachka et al., 1973). Also, a shift towards alanine transamination is an adaptive response (Moorthy et al., 1985). However, at all time periods, there occurred an increase in the activity of both the transaminases in 400 ppb exposure. This elevation indicates enhanced hepatic injury on prolonged exposure at higher concentration. Hepatotoxicity and tissue damage are strong possibilities as the ALP also showed a trend of elevation in activity during the same period. The lower glycogen level in spite of the recovery in oxygen uptake is suggestive of a heavy drain of metabolites during the stress condition.

GOT and GPT activities exhibit a biphasic response in 400 ppb exposure. Similar biphasic response is reported in other animals (Koeferal, 1972; Racicot et al., 1975; Statham et al., 1978 Pfeifer, 1979; Dalich et al., 1982). Copper ions are reported to inhibit both GOT and GPT activity in mussels (Gormosova and Tomozhnyaya, 1979)

In mercury exposed mussels, GOT activity in the gill tissue remained significantly lower than that of the control in both 75 and 150 exposure during early exposure period. In the case of mercury exposed animals alanine transamination occurred at a higher rate than aspartate transamination during the initial period as against the same phenomenon which occurred during the later periods in the gill of copper exposed animals. During the 72 hr time period, significantly high GPT activity was observed which again indicated the increased formation of pyruvate. Catabolism of alanine in most of the molluscs involves transamination to pyruvate (Livingstone and De Zwaan, 1983). Feng et al.(1970) has also suggested the possibility of oxaloacetate resulting from transamination being converted to pyruvate. The decrease in activity of transaminases during certain time periods may alsobe attributed to the competition of glutamate dehydrogenase in the presence of ammonia for NADH and L-ketoglutarate (D'Appollonia and Anderson, 1980). The decrease may also be due to inhibition of proteolytic enzymes by the metal as it leads to depletion of precursor for transamination. This inhibition reaction being reversible, (Hilmy et al., 1981) there was further restoration of normal activity during later periods.

In the case of hepatopancreas of mercury exposed mussels, GOT activity in 150 ppb alone was significantly below the control value during the initial periods. During 72 hr time period both enzymes exhibited a peak in activity in 75 ppb exposed animal tissue, elevation being caused by the metal stress. Mercury is reported to elevate transaminase activity in bivalves (Reddy and Chari, 1985 Kulkarni and Kulkarni, 1987). Hilmy et al. (1981) observed an increase in serum GOT and GPT as a result of hepatotoxicity following mercury poisoning in fish. At 168 hr GOT activity remained normal but GPT activity got reduced in 300 ppb exposed animals, and a shift in metabolism could be envisaged. It could be assumed that during this time period, oxaloacetate oriented metabolism occurred. Elevated GDH activity and high aspartate aminotransferase activity are reported in *Lamellidens marginalis* during conditions of stress (Mohan et al., 1987). Increase in GOT activity is also documented in fishes (Christensen, 1975) and in crabs (Gould, 1976) following heavy metal toxicity. In 150 ppb mercury exposure, GPT activity was found to show a trend of elevation during the later periods. This elevation probably indicates hepatotoxicity following prolonged exposure. The general trend of enhanced activity may be due to cellular degradation following prolonged exposure (Hilmy et al., 1981). The enzyme activity in

mercury exposed animals showed an erratic pattern almost throughout the experimental period. Bitensky et al.(1965) reported that factors influencing the state of aggregation of molecules affect changes in the activities of enzyme. The stimulation of activity of enzyme may in turn disturb the metabolic processes (Gupta and Dalela, 1985). The discrepancy observed in the enzyme activity in these animals may be attributed to the interaction of cofactors or regulators thereby altering membrane permeability (Passow, 1970).

In 600 ppb cadmium exposed mussels, high gill GOT and GPT activity was observed almost throughout the experimental period except in the last phase. In the case of hepatopancreas, both GOT and GPT were very high during the 24 hr time period in the above concentration exposure while in the other two exposures the enzyme activity was rather at a low profile. In all the exposures, both the transaminases followed an almost similar pattern. In 600 ppb exposed animals there was a time dependent lowering of enzyme activity.

Elevation in the activity of GOT and GPT is considered to be stress mediated (Chaplin et al., 1967;Ahamed et al., 1978;Rao and Rao,1984).Hilmy et al. (1985) reported an elevation in the activity of both GOT and GPT in all tissues except liver, on exposure to

cadmium. In the liver, activity of the enzyme was reported to be inhibited. Initial induction followed by inhibition of enzyme activity by heavy metals is documented (Jackim et al., 1970). The generally high enzyme activity, particularly GPT in both the tissues in the present study indicate large scale gluconeogenic activity. Since the carbohydrate reserves might have depleted due to the prevailing hypoxic condition, energy demand should be met through gluconeogenesis. The slow and steady decline in transferses in hepatopancreas on prolonged exposure could be due to the inhibition of enzyme activity by the metal. Cadmium compounds are known to inhibit enzymatic as well as metabolic processes (Webb, 1966). In the lower concentration exposures, towards the later periods, near normal level or an elevation in enzyme activity was observed. This may be due to the increased concentration of the metal in the tissues, on prolonged exposure. In the hepatopancreas, peak enzyme activity was more or less dose dependent. Since damage caused by the highest concentration was more severe, there was an early rise in activity in 600 ppb exposed animals. In 300 ppb exposed animals, the peak in activity occurred at 120 hr. Therefore, a similar peak in 150 ppb exposed animals might be a much delayed response. High hepatic transaminase activity indicates cellular damage or tissue injury (Tietz, 1970). The comparatively low activity of the

enzyme in 150 ppb in both the tissues may be due to the binding of cadmium with protein and there by inhibiting enzyme synthesis. The apparent lack of inhibition in high concentrations may be due to the precipitation of cadmium at higher concentration (Hilmy et al., 1985). The consistant decrease in enzyme activity in the highest concentration may be attributed to the constant inhibition of the enzyme by the metal. The sharp decline in activity at certain time periods is also suggestive of the leakage of the enzyme into the haemolymph (Hilmy et al., 1985).

Toxin induced changes in enzyme activity could represent initial disorders or they can be simply be symptoms of metabolic impairments (Jackim et al., 1970). Differential tissue enzyme sensitivity to pollutants could be attributed to the ability of the compounds to alter the cellular membrane configuration by binding with lipid protein of membrane and thus blocking the movement of substances by active transport (Hilmy et al., 1985). Metals may alter the enzyme activity in several ways. Binding at or near active sites may cause confirmational changes leading to alterations in enzyme activity (Ulmer, 1970). Replacement of normal co-factor by a different metal ion may also alter enzyme activity (Tucker and Matte, 1980).

C H A P T E R - VII

SUMMARY

In the present study, an attempt has been made to analyse the variations brought about in the systems of the freshwater mussel *Lamellidens corrianus* (Lea) on exposure to heavy metal toxicants in terms of oxygen uptake, organic constituents, and activity patterns of selected phosphatases and transaminases. Activity patterns of alkaline and acid phosphatases, alanine and aspartate transaminases were studied to understand the changes caused by metal pollution.

The thesis consists of seven chapters followed by a list of references. The first chapter is a general introduction in which the current status of heavy metal pollution studies and the significance of the present work are discussed.

The second chapter is on the sublethal effect of heavy metals, copper, mercury and cadmium, on the rate of oxygen uptake. Mussels pre- exposed to sublethal concentrations of the metals for a period of 24, 72, 120 and 168 hr were subjected to oxygen uptake study. Metals were found to drastically reduce the rate of oxygen uptake in the freshwater mussel. In 100 ppb copper pre-exposed animals, oxygen uptake, which was very much

reduced during the early period of exposure, was found to revert to near normal level during the later periods. In both mercury and cadmium pre-exposed animals oxygen uptake rate was very much reduced. The present study has clearly indicated that the rate of uptake of oxygen in the freshwater mussel *Lamellidens corrianus* is invariably affected by the metals, as in other bivalves. The reason for this reduction is discussed in terms of possible gill tissue damage or low filtration rate.

In the third chapter, sublethal effects of the metals on glycogen content in gill and hepatopancreas are discussed. The introductory part gives an updated review of literature in related field. Glycogen in the gill tissue of animals exposed to all the three metals showed a generally low level. Increased glycogen breakdown was also observed in the hepatopancreas of all the exposed mussels. Based on the present investigation, it can be said that there is enhanced glycogen break down in the tissues to meet the impending stress. The prevailing hypoxic condition might have resulted in glycogen breakdown.

Variations in protein content in metal exposed mussels form the subject matter of the fourth chapter. In copper exposed mussels, protein concentration in gill and hepatopancreas was found to increase during the course of the experiment. In

mercury exposed animals protein concentration in gill tissue increased in the lower concentrations while in the higher concentration, the protein value more or less fluctuated near the control level. In the hepatopancreas eventhough protein concentration in the low concentration exposure decreased early and stabilised later, in 150 and 300 ppb exposure protein was low at later time period. The gill tissue of cadmium exposed mussels showed a rather non synchronised pattern of protein concentration. In the hepatopancreas also a definite pattern was lacking.

The fifth chapter consists of the study on the effects of metals on the activity pattern of acid and alkaline phosphatases. ACP activity in the gill tissue of copper exposed animals was significantly high in all the experimentals almost at all time periods. In hepatopancreas, however the activity in lower two concentrations was generally significantly low, but in higher concentration no significant change was observed. In copper exposed mussels, gill ALP activity was generally low, particularly at later time-periods and in hepatopancreas also the activity was low at almost all time periods. In general, except perhaps gill ACP activity, the activity of both ACP and ALP was low in gill and hepatopancreas. In mercury exposed mussels higher

ACP activity was found in the lower concentration during the initial period while enzyme activity was not much affected in the higher concentration at this period. However, at later time periods, the activity was elevated. The hepatopancreas also showed a more or less similar pattern. In mercury exposed animals, gill ALP activity in the lower concentrations were high during the initial periods. However, the activity was found to get normalised during the course of the experiment. In 300 ppb, the enzyme activity fluctuated near the control level. In hepatopancreas, the activity in general was high. In cadmium exposed mussels, the reduction and elevation in enzyme activity is discussed in terms of possible enzyme inhibition and tissue damage. Gill ACP activity in general, was high at higher concentrations in cadmium exposed animals, and in hepatopancreas lower concentration produced decreased activity and higher concentration increased activity at most of the time periods. Gill ALP activity was generally low in lower concentration and in higher concentration the values were significantly high during the initial periods and low towards the later period. In hepatopancreas, at early time period, in all concentrations, the activity was high, but later at various time periods, activity was low in all the concentrations. The variation in enzyme activity is discussed in terms of possible hypersynthesis of the

enzyme stimulated by the metals. The discrepancy found is related to the heterogenous population of lysosomes or the nonsynchronised chemical cycles occurring within the lysosomes. Results of the study show differential tissue enzyme sensitivity to metals. A rather biphasic response of enzyme activity was produced by the metal at times.

Effects of sublethal concentrations of metals on the glutamate oxaloacetate and glutamate pyruvate transaminases are reported in the sixth chapter. In copper exposed animals gill GOT activity was significantly high during the initial period. But in lower two concentrations, the activity was low at later time periods. In hepatopancreas a generally low enzyme activity was observed. GPT activity of copper exposed mussels showed a more or less similar pattern in both gill and hepatopancreas. In both tissues, the activity was at its peak during the 72 hr time period.In mercury exposed mussels, GOT in gill tissue was generally low while GPT exhibited a peak at 72 hr. In the hepatopacreas, GOT activity in 75 ppb exposed mussels had a steep decline towards the later periods and in 150 ppb exposed mussels values were lower at early time periods. Those exposed to 300 ppb did not show any variation. GPT activity in the hepatopancreas was slightly erratic. In 75 ppb exposed mussels,

GPT activity was significantly high at most of the time periods while in 300 ppb, the activity was generally low. In 150 ppb exposed mussels, activity was highest at 168 hr. In gill tissue of cadmium exposed mussels, GOT activity was significantly low in the lowest concentration almost through out the experimental period, while in the middle concentration enzyme activity did not show any significant variation. In 600 ppb exposed animals, enzyme activity showed a gradual decline during the course of the experiment. GPT activity in 150 ppb exposed mussels was generally low during most of the exposure period. However, during 168 hr enzyme activity was significantly high. In 300 ppb exposed animals, enzyme activity was generally high while in 600 ppb exposed ones, enzyme activity was high at almost all time periods. GOT activity in hepatopancreas was significantly low during 72 and 120 hr in 150 ppb exposed animals. In 300 ppb, the enzyme activity was generally low during most of the exposure periods. However, during 120 hr, the enzyme activity was high. In 600 ppb, the activity declined during the course of the experiment GPT activity in the lowest concentration generally did not show much variation while in the highest concentration, activity was high during the initial period and low towards the end of exposure period. In the middle concentration the enzyme activity was generally high during later time periods.

Differences in the extent of variations caused by the different metals were indicated in the present study. Heavy metals do affect the animal seriously causing variations in metabolic activities. Serious metabolic impairments occur as a result of hypoxia caused by the metal.

REFERENCES

REFERENCES

Abel, P.D. 1976. Effect of some pollutants on the filtration rate of *Mytilus*. Mar. Pollut. Bull. 7, 228-231.

Abraham, T.J., Salih, K.Y.M., and Chacko 1986. Effect of heavy metals on the filtration rate of Bivalve *Villorita cyprinoides* (Hanley) var. *cochenensis*. Ind. J. Mar. Sci. 15, 195-196.

Ahamed, J.K., Begum, Md.R., Sivaiah, S., and Rao, K.V.R. 1978. Effect of malathion on free aminoacids, total proteins, and some enzymes of pelycepod *Lamellidens marginalis* (Lamarck). Ind. Acad. Sci. 87 (Animal Sciences) B 12, 377-380.

Akarte, S.R., and Mane, U.H. 1988. Toxicity of Folithion to three species of freshwater bivalve molluscs in different seasons. J. Environ. Biol. 9 (1), 27 - 38.

Akberali, H.B., and Black, J.E., 1980. Behavioural responses of the bivalve, *Scrobicularia plana* (de Coasta) subjected to short term copper (Cu 11) concentrations. Mar. Environ. Res. 4, 97- 107.

Allen, P.J., and Price, W.H. 1950. The relationship between respiration and protoplasmic flow in the slime mold, *Physarum polycephalum*. Am. J. Bot. 37, 393-399

Altland, P.D., and Rattner, B.A. 1979. Effects of nicotine

and carbon monoxide on tissue and systemic changes in rats. Environ. Res.19, 202.

Anderson, J.W., Neff, J.M., Cox, B.A., Tatem, H.E., and Hightower, G.M. 1974. Charactaristics of dispersions and water soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. Mar. Biol. 27, 75 - 88.

Anderson, R.V. 1977. Concentration of cd, cu, Pb, and zn in 35 genera of freshwater macro invertibrates from the Fox River, Illinois. Bull. Environ. Contam. Toxicol. 18, 345-349.

Anderson, R.V. 1978. The effects of lead on oxygen uptake in the cray fish, *Orconectes virilis* (Hager). Bull. Environ. Contam. Toxicol. 20, 394-400.

Annon, 1963. The colorimetric determination of phosphatase. Sigma Tech. Bull. No.104, Sigma Chemicals C0., St.Louis.

APHA. 1975. Standard Methods for the Examination of Water and Waste Water. Rand, M.C., Greenberg, A.E., and Taras, M.J. (eds). American Public Health Assos. Washington. 14th Edition. P 834-869.

Arstila, A.V., and Trump, B.F. 1968. Studies on cellular autophagocytosis. The formation of autophagic vacuoles in liver after glucagon administration. Am. J. Path. 53,

687-734.

Babu, G.R., Jyothirmayi, G.N., and Rao, P.V. 1985. Effect of hypoxia on tissue metabolism of midgut gland of the scorpion *Heterometrus fulvipes*. Proc. Indian. Acad. Sci.(Animal Sci). 94 (2), 139-143.

Babu, G.R., and Rao, P.V. 1982. Heavy metal ion toxicity in the freshwater gastropod snail host, *Lymnea leuteola*. Ind. J. Physiol. Pharmac. 26 (2), 142-146.

Babu, K.V.R.V., and Vasudev, T., 1984. Effect of Dimecron, Rogor and Cumen on AchE and Phosphatase activity in freshwater mussel, *Lamellidens marginalis*. Curr. Sci. 53. 935-936.

Baby, K.V. 1987. Combined toxicity of heavy metals and petroleum hydrocarbons on selected marine organisms. Ph.D thesis. Cochin. Univ. Sci. Technol. Cochin. Kerala.

Baby, K.V., and Menon, N.R. 1986. a. Oxygen uptake in the brown mussel *Perna indica* (Kuriakose and Nair) under sublethal stress of Hg, Cd, and Zn. Ind. J. Mar. Sci. 15, 127-128.

Baby, K.V., and Menon, N.R., 1986. b. Oxygen consumption under copper and zinc stress in *Perna viridis*. Fish. Tech. 23, 24-26.

Badman, D.G., and Chin, S.L. 1973. Metabolic responses of the

freshwater bivalve *Pleurobem coccineum* (Conrad) to anaerobic conditions. Comp. Biochem. Physiol. 44 (B), 27-32.

Baker, J.T.P. 1969. Histological and electron microscopical observations on copper poisoning in the winter flounder, *Pseudopleuronectes americanus*. J. Fish. Res. Bd. Can. 26, 2785 - 2793.

Baktavalhsalam, R., and Reddy, T.S. 1984. Importance of protein metabolism during acute exposure of *Anabas testudineus* to Lindane. Environ. Ecol. 2 (3), 194 - 198.

Baneerjee, U., and Choudhuri, D.K. 1985. Effect of nicotine on the biochemical components in the central nervous system of sand burrowing carnivorous insect *Schizodactyles monstrosus olary* (Insecta). Proc. Ind. Nat. Sci. Acad. B 51 (2), 194-201.

Basha, S.M., and Swami, K.S. 1987. Ciliary activity in freshwater mussel *Lamellidens marginalis* exposed to pesticide stress at various temperature. Environ. Ecol. 5 (3), 514-516.

Bayne, B. 1973. The responses of three species of bivalve molluscs to declining oxygen tension at reduced salinity. Comp. Biochem. Physiol. 45 A, 793-806.

Bayne, B.L., Moore, M.N., Widdows, J., Livingstone, D.R., and Salked, P. 1979. Measurements of the responses of

individuals to environmental stress and pollution. Trans. R. Soc. Lon. Series B 286, 561-563.

Bayne, B.L., Widdows, J., Moore, M.N., Salked, P., Worral, C.M., and Donkin, P. 1982. Some ecological consequences of the physiological and biochemical effects of petroleum compounds on marine molluscs. Phil. Trans. R. Soc. Lond. B.297, 219-239.

Bayne, B.L., Salked, P.N., and Worrall, C.M. 1983. Reproductive effort and value in different populations of the marine mussel, *Mytilus edulis* L. Oecologia. 59, 18 - 26.

Bayne, B.L., Brown, D.A., Burns, K., Dixon, D.R., Ivanovici, A., Livingstone, D.R., Lowe, D.M., Moore, M.N., Stebbing, A.R.D., and Widdows, J. 1985. The effects of stress and pollution on marine animals. Praeger Scientific, New York. P 383.

Bell, G.R. 1968. Distribution of transaminases in the tissues of Pacific Salmon, with emphasis on the properties and diagnostic use of GOT. J.Fish. Res. Bd. Canada. 25, 1247-1268.

*Bell, G.H., Davidson, J.N., Scarborough, H., and Garry, R.S. 1968. Text book of physiology and biochemistry. 7th ed. Livingston. Edinburgh.

Bernard, F.S., and Lane, C.E. 1961. Absorption and excretion of Cu ions during settlement and metamorphosis of the barnacle

* Not referred to the original

Balanus amphitrite. Biol. Bull. 121, 438 - 448.

Beyers, R.J., and Warwick, R.W., 1968. The production of carbondioxide by *Rangia cuneata*. Contrib. Mar. Sci. 13, 43-50.

Bhaskar, M., Reddy, G.V., Murthy, V.K., Reddama, P., and Govindappa, S. 1982. Branchial protein metabolism of freshwater fish, *Tilapia mossambica* (Peters) during acute exposure and acclimation to sublethal alkaline water. Proc. Ind. Acad. Sci. (Animal Sciences). 91 (3), 235-241.

* Bhatia, S.C., Sharma, S.C., and Subramaniam, T.A.V. 1972. Quoted from " Effects of pesticides and their combinations on three serum phosphatases of *Mystus vittatus* (Verma, S.R., Rani, S., and Dalela, R.C.). Water. Air. Soil. Pollut.(1984). 21, 9-14.

Bhatnagar, M. C., Bana, A.K., and Dalela, R.C. 1987. Histopathological alterations in liver of *Channa gachua* (Ham) exposed to endosulfan. Environ. Pest. Toxicity. 205-209.

Bishop, S.H., Ellis, L.L., and Burcham, J.M. 1983. "Aminoacid metabolism". In Mollusca. Vol. 1. Metabolic biochemistry and molecular biomechanics. Hochachka, P.W., and Wilbur, K.M. (eds). Academic Press, New York. P 243 - 327.

Bitensky, M.W., Yielding, K.L., and Tomkins, G.M. 1965. Reciprocal changes in Alanine and glutamate dehydrogenase

activities after exposure of crystalline bovine-L-glutamate dehydrogenase to organic mercury. J. Biol. Chem. 240, 663-667.

*Blackwood, W., Mc menemcy, W.H., Mayer, A., Norman, R.M., and Russel, D.S. 1965. Greenfield neuropathology. Edward. Arnol. Ltd. London.

Bostwick, H.K. 1973. Ecological effects of marine pollution. In Special publication dedicated to Dr. N.K. Panikkar. (A Marine Biological Association of India Publication). 7-28.

Brown, B.E., and Newell, R.C. 1972. The effect of copper and Zinc on the metabolism of *Mytilus edulis*. Mar. Biol. 16, 108-118.

Brown, D.A., and Parsons, T.R. 1978. Relationship between cytoplasmic distribution of mercury and toxic effects to zooplankton and chum salmon (*Oncorhynchus keta*) exposed to mercury in a controlled ecosystem. J. Fish. Res. Board. Can. 35, 880-884.

Bryan, G.W. 1976. Heavy metal contamination in the sea. In Marine Pollution. Johnston, R. (ed). Academic Press. New York. 185-302.

Bubel, A. 1976. Histological and electron microscopical observations on the effects of different salinities and heavy metal ions on the gills of *Jaera nordmani*, Crustacea,

Isopoda. Cell. Tiss. Res. 167, 65-95.

Burcham, J.M., Paynter, K.T., and Bishop, B.H. 1983. Coupled mitochondria from oyster gill tissue. Mar. Biol. Lett. 4, 349-356.

Burton, D.T., Jones, A.H., and Cairns, J. Jr. 1972. Acute zinc toxicity to rainbow trout *Salmo gairdneri* confirmation that the hypothesis that death is related to tissue hypoxia. J. Fish. Res. Board. Can. 29, 1463-1466.

Byczkowski, J.J., and Boryszwicz. 1976. Action of some neurotropic drugs and Cu^{++} cations on mitochondrial membrane permiability. Gen. Pharmac. 7, 364-369.

Calabrese, A., and Nelson, D.A. 1974. Inhibition of embryonic development of the hard clam *Mercenaria mercenaria* by heavy metals. Bull. Environ. Contam. Toxicol. 11, 92 - 97.

Calabrese, A., Collier, R.S., Nelson, D.A., and MacInnes, J.R. 1973. The toxicity of heavy metals to embryos of the American oyster *Crassostrea virginica*. Mar. Biol. 18, 162-166.

Capuzzo, J.M., and Sasner Jr, J.J. 1977. The effect of chromium on filtration rates and metabolic activity of *Mytilus edulis* L. and *Mya arenaria* L. In Physiological responses of marine biota to pollutants. Vernberg, F.J., Calabrese, A., Thurberg, F.P., and Vernberg, W.S. (eds). Academic Press. New

York. 225-237.

Carpene, E., and George, S.G. 1980. Absorption of cadmium by gills of *Mytilus edulis* (L). Mol. Physiol. 1, 23-24.

Carpene, E., and Wynne, D. 1986. Properties of an alkaline phosphatase from the dinoflagellate *Peridinium cinctum*. Comp. Biochem. Physiol 8 8B (1), 163-167.

Cassini, A., Tallandini, L., Favero, N., and Albergoni, V. 1986. Cadmium bioaccumulation studies in the freshwater molluscs, *Anodonta cygnea* and *Unio elongatulus*. Comp. Biochem. Physiol. 84. C (1), 35-41.

Chandy, J.P., and Patel, B. 1985. Do selenium and glutathione (GSH) de toxify mercury in marine invertebrates? Effects on lysosomal response in the tropical blood clam, *Anadara granosa*. Dis. Aquat. Org. 1, 39-47.

*Chaplin, A.E., Huggins, A.K., and Munday, K.A. 1967. Comp. Biochem. Physiol. 20, 195

Chaterjee, S., and Bhattacharya, S. 1986. Inductive changes in hepatic metallothionein profile in the climbing Perch *Anabas testudineus* (Bloch) by industrial pollutants. Ind. J. Exp. Biol. 24, 455-457.

Chavapil, M., Ryan, J.N., and Brada, Z. 1972. Effects of selected chelating agents and metals on the stability of liver lysosomes. Biochem. Pharmacol. 21, 1097-1105.

*Cheng, J.T. 1965. The effects of potassium pentachlorophenate on selected enzymes in fish. Master's thesis. Oregon state Univ. Corvallis, U.S.A.

Cheng, T.C. 1983. The role of lysosomes in molluscan inflamation. Amer. Zool. 23, 129-144.

Cherian, M.G., and Nordberg, M. 1983. Cellular adaptation in metal toxicicology and metallothionein. Toxicology. 28, 1-15.

Chetty, C.S., Naidu, R.C., Reddy, Y.S., Aruna, P., and Swami, K.S. 1980. Tolerence limits and detoxication mechanisms in the fish *Tilapia mossambica* subjected to ammonia toxicity. Ind. J. Fish. 27, 177-182.

Chinnaya, B. 1971. Effect of heavy metals on the oxygen consumption by the shrimp, *Caridna rajadhari* (Bourier). Ind. J. Exp. Biol. 9, 277-278.

Chow, K.V., and Pond, W.G. 1972. Biochemical and morphological swelling of mitochondria in ammonia toxicity. Proc. Soc. Exptl. Biol. Med. 139, 150-156.

Christensen, G. 1972. Effects of metal cations and other

chemicals upon the invitro activity of two enzymes in the blood plasma of the white sucker *Catostomus commersomi*. Chem. Biol. Interact. 4, 351-361.

* Christensen, G.M., 1975. Biochemical effects of methyl mercuric chloride, cadmium chloride and lead nitrate on embryos and levins of the brook trout *Salvelinus fontinalis*. Toxicol. Appl. Pharmacol. 32, 191-197.

Coasta, H.H., and Indrasena, W.M. 1984. The morphometrics and the proximate composition of the edible freshwater mussel, *Lamellidens lamellatus* (Lea) from Bathalagoda Tank, a man-made reservoir in SriLanka. J. Natl. Aquat. Resour. Agency. (Srilanka) 31, 97-108.

Coleman, J.E., and Gettings, P. 1983. Alkaline phosphatase, solution structure and mechanism. Adv. Enzymol. 55, 381-451.

Collicut, J.M., and Hochachka, P.W. 1977. The anaerobic oyster heart coupling of glucose and aspartate fermentation. J. Comp. Physiol. 115, 147-157.

Collier, R.S., Miller, J.E., Dawson, M.A., and Thurberg, F.P. 1973. Physiological response of the mud crab, *Eurypanopeus*

depressus to cadmium. Bull. Environ. Contam. Toxicol. 10, 378-382.

*Collip, J.B. 1920. Studies on molluscan coelomic fluid. Effect of change in environment on the carbon dioxide content of the coelomic fluid. Anaerobic respiration in *Mya arenaria*. J. Biol. Chem. 45, 23-49.

Corner, E.D.S., and Sparrow, B.W. 1956. The mode of action of toxic agents - 1. Observations on the poisoning of certain crustaceans by Cu & Hg. J. Mar. Biol. Ass. U.K. 35. 531-548.

Couch, J.A. 1975. Histopathological effects of pesticides and related chemicals on the liver of fish. In The pathology of fishes. Ribelin, W.E., and Migaki, G. (eds), University of Wisconsin press, Madison Wis. 559-584.

Couch, J.A. 1977. Ultrastructural study of lesions in gills of a marine shrimp exposed to cadmium. J. Invertebr. Pathol. 29, 267-288.

Cousins, R.J. 1985. Absorption, transport, and hepatic metabolism of copper and zinc Special reference to metallothionein and ceruloplasmin. Physiol. Rev. 65, 238-309.

Croxton, F.E., Cowden, D.J., and Klein, S. 1975. "Applied general statistics. 3rd Edn. Printice - Hall of India Pvt. Ltd. New Delhi. PP 754.

Cunnigham, P.A. 1979. The use of bivalve molluscs in heavy metal pollution research, In Marine pollution Functional responses, Vernberg, W.B., Thurberg, F.P., Calabrese, A., and Vernberg, F.J. (eds), Academic Press, New York. 183 -222.

D' Appollonia, S., and Anderson, D. 1980. Optimal assay conditions for serum and liver GOT, and sorbitol dehydrogenase from the rainbow trout, *Salmo gairdneri*. Can. J. Fish. Aquat. Sci. 37, 163-169.

Dalela, R.C., Bhatnagar, M.C., Tyagi, A.K., and Verma, S.R. 1979. Histological damage of gills in *Channa gachua* after acute and subacute exposure to Endosulfan and Rogor. Microskopie 35, 301-307.

Dalela, R.C., Rani, S., and Verma, S.R. 1980. Physiological stress induced by sublethal concentrations of Phenol and Pentachloro Phenol in *Notopterus notopterus* Hepatic acid and alkaline phosphatases and succinic dehydrogenase. Environ. Pollut. A. 21 (1), 3-8.

Dalich, G.M., Larson, R.E., and Gingerich, W.H. 1982. Acute and chronic toxicity studies with Monochlorobenzene in rainbow

trout. Aquat. Toxicol. 2, 127 -142.

Dapual, W.D., and Webb, K.L. 1974. Salinity induced changes in the alanine and aspartate aminotransferase activity in three marine bivalve molluscs. Archs. Int. Physiol. Biochem. 82, 817-822.

Das, S., Samantaray, K., and Das, A.B. 1986. Tissue distribution of alanine and aspartate aminotransferases in an air breathing fish, *Channa punctatus* (Bloch). Proc. Ind. Nat. Sci. Acad. B. 52. (5), 637-641.

Davison, A.N., and Gregson, N. 1965. Cytochemistry of the nervous system. In Neurohistochemistry. Adams, C.W.A. (ed). Elsevier. New York. 189 - 235.

De Bruin, A. 1976. Biochemical toxicology of environmental agents. Elsievier. Amsterdam. 902 -935.

*De Duve, C. 1963. Lysosomes. De Reuck, A.V.S., and Gameron, M.P. (eds), Churchil LTD. London.

*Delthlefsen, V. 1977. Uptake, retention, and loss of cadmium by brown shrimp (*Crangon crangon*). Meeresforch. 26, 137-152.

Depledge, M.H. 1984. Disruption of circulatory and respiratory activity in shore crabs (*Carcinus maenas)* exposed to heavy metal pollution. Comp. Biochem. Physiol. 78 C, 445 - 459.

Dermott, R.M., and Lum, K.R. 1986. Metal concentrations in the annual shell layers of the bivalve, *Ellptio complanata*.

Environ. Pollut. (Sereies B). 12, 131-143.

Desnoyers, P.A., and Chang, L.W. 1975. Ultrastructural changes in the liver after chronic exposure to methyl mercury. Environ. Res. 10, 59 - 75.

De Vooys, C.G.N. 1980. Anaerobic metabolism in sublittoral living *Mytilus galloprovinsialis* Lam. in the Mediterranian-11. Partial adaptation of pyruvate kinase and phosphoenol pyruvate carboxikinase. Comp.Biochem. Physiol.65 B, 513-518.

De Zwaan, A., and Zandee, D. 1972. The utilisation of glycogen and accumulation of some intermediates during anaerobiosis in *Mytilus edulis*. L. Comp. Biochem. Physiol. 43 B, 47-54.

De Zwaan, A., Marrewijk, W.J.A.V., and Holwerda, D.A. 1973. Anaerobic carbohydrate metabolism in the sea mussel, *Mytilus edulis*, L. Neth. J. Zool. 23 (2), 225-228.

Dillon, F.M., and Neff, J.M. 1978. Mercury and the estuarine marsh clam, *Rangia cuneata* Gray. 11. Uptake, tissue distribution and depuration. Mar. Environ. Res.1. 67-77.

Dingle, J.T., and Fell, H.B. 1969. Lysosomes in biology and pathology. North Holland Publ. Co. Amsterdam. PP 451.

Dixon, D.G., and Sprague, J.B. 1981. Copper bioaccumulation and hepatoprotein synthesis during acclimation to copper by juvenile rainbow trout. Aquat. Toxicol. 1, 69-81.

Doughtie, D., and Rao, K.R. 1984. Histopathological and ultrastructural changes in the antenal gland, midgut, hepatopancreas, and gill of grass shrimp, *Palaemonectes puglo*, following exposure to hexavalent chromium. J. Invert. Pathol. 43, 83-108.

Dugal, L.P. 1939. The use of calcarious shell to buffer the products of anaerobic glycolysis in *Venus mercenaria*. J. Cell. Comp. Physiol. 13, 235-251.

Dunning, A., and Major, C.W. 1974. The effects of cold sea water extracts of oil fractions upon the blue mussel, *Mytilus edulis*. In :Pollution and physiology of marine organisms. Vernberg, F.J., and Vernberg, W.B. (eds). Academic Press. New York. 349 - 366.

Eichhorn, G.L, Clark, P., and Tarien, E. 1969. The interaction of metal ions with polynucleotides and related compounds. J. Biol. Chem. 244, 937-942.

Eisler, R. 1971. Cadmium poisoning in *Fundulus hetroclitus* and other marine organisms. J. Fish. Res. Bd.Canada.28, 1225 - 1234.

Eisler, R., and Gardner, G.R. 1973. Acute toxicity to an estuarine teleost of mixtures of cadmium, copper and zinc. J. Fish. Biol. 5, 131-142.

Eisler, R., Zaroogian, G.E., and Hennekey, R.J. 1972. Cadmium

uptake by marine organisms. J. Fish. Res. Bd. Can. 29 (9), 1367-1369.

Eknath, A.E., and Menon, N.R. 1979.a. Mercury toxicity in two intertidal tropical marine molluscs. Fish. Tech. 20, 84-89.

Eknath, A.E., and Menon, N.R. 1979. b. Effect of organochlorines on the life and activities of non-target marine animals. In Symposium on pesticides residues in the environment in India. Edwards, C.A., Veerush, G.K., Krueger, H.R. (eds). Allied Publishers. Bangalore. 513 - 519.

Emerson, D. 1967. Carbohydrate oriented metabolism of *Planorbis comeus* (Mollusca, planorbidae) during starvation. Comp. Biochem. Physiol. 22, 571-579.

Emerson, D., and Duerr, F. 1967. Some physiological effects of starvation in the intertidal prosobranch, *Littorina planaxis* (Phillippi, 1847). Comp. Biochem. Physiol. 20, 45 - 53.

Engel, D.W., and Brouwer, M. 1982. Detoxification of accumulated trace metals by the american oyster, *Crassostrea virginica* Laboratory VS Environment. In Physiological mechanisms of marine pollutant toxicity. Vernberg, W.B., Calabrese, A., Thurberg, F.P., and Vernberg, F.J. (eds). Academic Press. New York. 89 - 109.

Engel, R.H., Neat, M.J., and Hillman, R.E. 1972. Sublethal, chronic effects of DDT and Lindane on glycolytic and

gluconeogenic enzymes of the quahog, *Mercenaria mercinaria*. In Marine Pollution and Sea Life. Fishing News (Books), London. 257 - 259.

Epifanio, C.E., and Srna, R.F, 1975. Toxicity of ammonia, nitrite ion, nitrate ion and orthophcsphate to *Mercenaria mercenaria* and *Crassostrea virginica*. Mar. Biol. 33, 241-246.

Everard, M., and Denny, P. 1984. The transfer of lead by freshwater snails in Ulls water, Cumbria. Environ. Pollut. (Series A). 35, 299-314.

Fahien, L.A., and Kmiotek, E. 1979. Precipitation complexes between glutamate dehydrogenase and mitochondrial enzymes. J. Biol. Chem. 254, 5983-5990.

Falany, C.N., and Friedl, F.E. 1981. Aminoacid transamination in the freshwater clams, *Anodonta couperiana* and *Popenaias buckley*. Comp. Biochem. Physiol. B. 68 (1), 119-123.

Feng, S.Y., Khairallah, E.A., Canzonier, N.S. 1970. Haemolymph free aminoacids and related nitrogenous compounds of *Crassostrea virginica* injected with *Bucephalus* sp. and *Minchinia nelsoni*. Comp. Biochem. Physiol. 34, 547-557.

Finney, D.J. 1971. Probit analysis. Cambridge Univ. Press. Cambridge, P 333.

Fowler, B.A., Wolfe, D.A., and Kettler, W.F. 1975. Mercury and iron uptake by cytosomes in mantle epithelial cells of quahog clams (*Mercenaria mercenaria*) exposed to mercury. J. Fish. Res. Bd. Canada. 32, 1767 - 1775.

*Fox, F.R., and Rao, K.R. 1978. Effects of Sodium pentachlorophenate and 2-4 dinitrophenol on hepatopancreatic enzymes in the blue crab *Callinectes sapidus*. Pentachlorophenol. 12, 265-276.

Frazier, J.M. 1976. The dynamics of metals in the American oyster *Crassostrea virginica*. 11. Environmental effects. Chesapeake Sci. 17, 188-197.

*Friedberg, F. 1974. Effects of metal binding on protein structure. Q. Rev. Biophyics. 7, 1 - 33.

Galtsoff, P.S., and Whipple, D.V. 1930. Oxygen consumption of normal and green oysters. Bull. U.S. Bur. Fisheries. 46, 489 - 508.

Gallagher, C.H., and Rees, K.R. 1960. Levels of pyridine nucleotides in liver poisoning. Nature. London. 187, 217-243.

Garanina, S.N. 1984. Effect of heavy metals on some aspects of carbohydrate metabolism in Caspian Sea gammarids. Hydrobiol. J. 20 (4), 69-71.

Gardner, W.S., Miller 111, W.H., and Imlay, M.J. 1981. Free aminoacids in mantle tissues of the bivalve *Amblema plicata* Possible relation to environmental stress. Bull. Environ. Contam. Toxicol. 26, 157-162.

George, S.G. 1982. Subcellular accumulation and detoxication of metals in aquatic animals. In Physiological mechanisms of marine pollutant toxicity. Vernberg, W.B., Callabrese, A., Thurberg, F.P., and Vernberg, F.J. (eds). Academic press, New York. 3 - 52.

George, S.G. 1983. a. Heavy metal detoxication in *Mytilus* kidney - an in vitro study of Cd and Zn binding to isolated tertiary lysosomes. Comp. Biochem. Physiol. 76 (C), 59-65.

George, S.G. 1983. b. Heavy metal detoxication in *Mytilus edulis*. L. Composition of Cd containing kidney granules (tertiary lysosomes). Comp. Biochem. Physiol. 76. C, 49-58.

* George, S.G., and Coombs, T.L. 1975. A comparison of trace metal and metallo enzyme profiles in different molluscs and during development of the oyster. Proc. Eur. Symp. Mar.

Biol. 9th Aberdneen Scot, 433-449.

George, S.G., and Coombs, T.L. 1977. The effects of chelating agents on the uptake and accumulation of cadmium by *Mytilus edulis.* Mar. Biol (Berlin). 39, 261-268.

George, S.G., and Frazier, J.M. 1982. Some acpects of the relationship between tolerence of heavy metal pollution and metabolism of Cd, Cu, and Zn in oysters. Thalassia Jugosl. 18, 203 - 219.

George, S.G., and Pirie, B.J.S. 1980. Metabolism of zinc in *Mytilus edulis*. J. Mar. Biol. Assoc. U.K. 60, 575-590.

*George, S.G., and Viarengo, A., 1984. An integration of current knowledge of the uptake, metabolism and intra cellular control of heavy metals in mussels. Sixth symposium on Pollution and physiology of marine organisms. 1-3, Nov. 1984. Mystic, Connecticut, U.S.A.

George, S.G., Pierie, B.J.S., and Coombs, T.L. 1976. The kinetics of accumulation of ferric hydroxide in *Mytilus edulis* and its distribution in tissues. J. Exp. Mar. Biol. Ecol. 23, 71-84.

Gibson, R., and Barker, D.L. 1979. The decapod hepatopancreas. Oceanogr. Mar. Biol. A. Rev. 17, 285-346.

Gilbertson, D.E., Etges, F.G., and Ogle, J.D. 1967. Free aminoacids of *Australorbis glabratus* hemolymph Comparison of four geographic strains and effect of injection by *Schistosoma mansoni*. J. Parasitol. 53, 565-568.

Gilbertson, D.E., and Michelson, E.H. 1969. Invitro incorporation of precursors of RNA into the digestive gland of normal and of *Schistosoma*-infected *Biomphalaria glabrata*. J. Parasitol. 55, 276-278.

Gilles, R. 1972. Osmoregulation in three molluscs *Acnanthochitona discrepaus* (Brown), *Glycymeris glycymeris* (L), and *Mytilus edulis* (L). Biol. Bull. Mar. Biol. Lab., Woods Hole. 142, 25-35.

Goddard, C.K., and Martin, A.W. 1966. Carbohydrate metabolism. In Physiology of Mollusca 11. Wilbur, K.M., and Yonge, E.M.(eds) Academic Press, New York. P 275-308.

Goel, K.A., Kalpana, Sandhya, and Agarwal, V.P. 1984. Alachlor toxicity to a freshwater teleost, *Clarius batrachus*. Curr. Sci. 53 (19), 1051-1052.

Goldberg, E.D., Bowen, V.T., Farrington, J.W., Harvey, G., Martin, J.H., Parker, P.L., Risebrough, R.W., Robertson, W.,

Schneider, E., and Gamble, E. 1978. The mussel Watch. Environ. Cons. 5. 101-125.

Gormosova, S.A. 1979. The effect of hypoxia and some poisons on the rate of degradation and synthesis of carbohydrates in mussel tissues. Biol. Morya 48, 66-69.

Gormosova, S.A., and Tamozhnyaya, V.A. 1979. The levels of transaminase activity in mussel tissues under normal conditions and during hypoxia. Biol. Morya 48, 70-75.

Gould, E. 1976. Alteration of enzymes in winter flounder *Pseudopleuronectes americanus*, exposed to sublethal amounts of cadmium chloride. In Physiological responses of marine biota to pollutants. Verberg, F.J., Calabrese, A., Thurberg, F.P., and Vernberg, W.B.(eds). Academic Press, New York. 209 - 224.

Grant, B.F., and Mehrle, P.M. 1973. Endrin toxicosis in rainbow trout (*Salmo gairdneri*). J. Fish. Res. Bd. Canada. 30, 31-40.

Gupta, P.K., Dhar, U., and Bawa, S.R. 1975. Effect of malathion and radiation seperately and jointly upon rat enzymes in vivo. Environ. Physiol. & Biochem. 5, 49-53.

Gupta, S., and Dalela, R.C. 1985. Effect of phenol on invivo activity of tissue transaminases in a freshwater fish *Notopterus notopterus*. J. Environ. Biol. 6, 151-156.

Gupta, V., and Rao, G. 1974. Histological studies on the choroid plexes of the goat embryos. 111. Histological distribution of acid and alkaline phosphatases. Acta. Histochem. 49, 60-63.

Gutman, B. 1959. Serum alkaline phosphatase activity in disease of skeletal and hepatobilary system. Amer. J. Med. 27, 875.

Hammen, C.S. 1968. Aminotransferase activities and aminoacid excretion of bivalve molluscs and brachiopods. Comp. Biochem. Physiol. 26, 697-705.

Hammen, C.S. 1975. Succinate and lactate oxidoreductases in bivalve molluscs. Comp. Biochem. Physiol. 50. B. 407-412.

Hammen, C.S., and Wilbur, K.M. 1959. Carbondioxide fixation in marine invertebrates. 1. The main pathway in the oyster. J. Biol. Chem.234, 1268-1271.

*Harper, H.A., Rodwell, V.M., and Mayes, P.A. 1979. In Review of physiological chemistry. Large Medical Publications, Maruzan Asia (PVT) LTD. P 282.

Harrison, F.L., and Berger, 1982. Effects of copper on the latency of lysosomal hexosaminidase in the digestive cells of *Mytilus edulis*. Mar. Biol. 68, 109-116.

Harrison, F.L., Lam, J.R., Berger, R. 1983. Sublethal responses of *Mytilus edulis* to increased dissolved copper. In

Biological availability of trace metals, chemical estimation, ecological and health implications. Jenne, E.A., and Wildung, R.E. (eds), 141-158.

Hart, C.G., and Fouts, J.R. 1965. Studies of possible mechanisms by which chloridine stimulate hepatic microsomal drug metabolism in the rat. Biochem. Pharmacol. 14, 263-270.

Hawkins, A.J.S., Bayne, B.L., Menon, N.R., and Damoderan, R. 1986. The mussels *Perna viridis* and *P.indica*as transplantable indicators of pollution Comparison of their metabolic response to reduction of both oxygen tension and salinity. In Proceedings National Seminar on Mussel Watch. Univ. Cochin. Publ. Cochin. 51-65.

Hilmy, A.M., Shabana, M.B., and Daabees, A.Y. 1985. Effects of cadmium toxicity upon the invivo and invitro activity of proteins and five enzymes in blood serum and tissue homogenates of *Mugil cephalus.* Comp.Biochem. Physiol. 81 (C), 145 -153.

Hilmy, A.M., Shabana, M.B., and Said, M.M. 1981. The role of serum transaminases (SGO-T and SGP-T) and alkaline phosphatase in relation to inorganic phosphorus with respect to mercury poisoning in *Aphanius dispar* Rup. (Teleostei) of the Red Sea. Comp. Biochem. Physiol. 68 C, 69-74.

Hilmy, A.M.,EL Domiaty, N.A., Daabees, A.Y., and Latife, H.A.A. 1987. Some physiological and biochemical indices of Zn toxicity in two freshwater fishes, *Clarius lazera* and *Tilapia zeith*. Comp. Biochem. Physiol. 87 C (2), 297-301.

*Hinton, D.E., Kendall, M.W., and Silver, B.B. 1973. Use of histological and histochemical assesments in the prognosis of the effect of aquatic pollutants. Amer. Soc. Test. Mat. Spec. Tech. Publs. 528. 194-208.

Hinton, E.D., and Koenig, C.J. 1975. Acid phosphatase activity in subcellular fractions of fish liver exposed to methyl mercuric chloride. Comp. Biochem. Physiol. 50 B. 621-625.

Hochachka, P.W., and Somero,G.N. 1973. Strategies of biochemical adaptations. In Pollution and physiology of marine organisms. Vernberg, F.J., and Verberg, W.B. (eds). Academic Press. New York.PP 358.

Hochachka, P.W., Fields, J., and Mustafa, T. 1973. Enzymes in facultative anaerobiosis of mollusc. 1 Malic enzyme in oyster adductor muscle. Comp. Biochem. Physiol. 45 B. 625-637.

Hopkins, S.P., and Noh, J.A. 1980. Studies on the digestive cycle of the shore crab, *Carcinus maenus* (L) with the special reference to the B cells in the hepatopancreas. J. Mar. Biol. Assoc. U.K. 60, 891-907.

Howell, R., Grant, A.M., and Maccoy, E.J.N. 1984. The effect of treatment with reserpine on the change in filtration rate of *Mytilus edulis* subjected to dissolved copper. Mar. Pollu. Bull. 15, 436-437.

Hubschmann, J.H. 1967. Effect of copper on the cray fish *O. rusticus*. 11. Mode of toxic action - Crustacea. 12, 141-151.

Hughes, W.L. 1957. A physico-chemical rationale for the biological activity of mercury and its compounds. Ann. N.Y. Acad. Sci. 65, 454-460.

*Hung, T.C., Chen, J.C., Lin, L.P., and Liang, N.K. 1975. Pollution studies in shell fish cultivating area of Taiwan western coast. Spl. Pub. (Inst. Oceanogr. National Taiwan Univ.). 6, 60.

* Hutcheson, M.S., 1974. The effect of temperature and salinity on cadmium uptake by the blue crab, *Callinectes sapidus*. Chesapeake Sci. 15, 237-241.

Hunter, W.R. 1949. The poisoning of *Marinogammarus marinus* by cypric sulphate and mercuric chloride. J. Exp. Biol. 26(2), 124-126.

Husain, R., Mushtaq, M., and Seth, P.K. 1980. Effect of manganese on some aspects of carbohydrate metabolism in rats. Bull. Environm. Contam. Toxicol. 25 (4), 646-650.

Hwang, K.M., Scott, K.M., and Brierley, G.P. 1972. Ion transport by heart mitochondrea. The effects of Cu^{++} ion on membrane permiability Arch. Biochem. Biophys. 150, 746-756.

Ibrahim, A.M., Higazi, M.G., and Demian, E.S. 1974. Histological localization of alkaline phosphatase activity in the alimentary tract of the snail, *Marisa caenuarietus* (L). Zool. Soc. Egypt. Bull. 26, 94-105.

Ithakissios, D.S., Kessler, W.V., Arvesen, J.V., and Born, G.S. 1974. Effects of multiple doses of cadmium on glucose metabolism in the rat. J. Pharm. Sci. 63, 146-151.

Jackim, E. 1974. Enzyme responses to metals in fish. In Pollution and physiology of marine organisms". Vernberg, F.G., and Verberg, W.B. (eds). Academic Press, New York, 59-65.

Jackim, E., Hamlin, J.M., and Sonis, S. 1970. Effects of heavy metal poisoning on five liver enzymes in killi fish (*Fundulus heteroclitus*). J. Fish. Res. Bd. Can. 27, 383-390.

Johnson, F.R., and Mc Minn, R.M.H. 1958. Association of alkaline phosphatase with fibrogenesis. J. Anat. 92, 544

Jones, J.R.E. 1942. The effects of ionic Cu on the O2 consumption of *Gammarus pulex* and *Polycellis nigra*. J. Exp. Biol. 18. 153-161.

Jones, J.R.F. 1947. The oxygen consumption of *Gasterosteus aculeatus* in toxic solutions. J. Exp. Biol. 23, 191-311.

Jones, M.B. 1975. Synergistic effects of salinity, temperature and heavy metals on mortality and osmoregulation in marine and estuarine isopods (Crustacea). Mar. Biol. 30, 13-20.

Kaplan, M. 1972. Alkaline phosphatase. New. Eng. J. Med. 286, 200-202.

Kaplaw, L.S. 1973. Clinical significance of leucocyte alkaline phosphatase determination. Rev. Roum. Med. Int. 10, 365-372.

Katticaran, C.M. 1988. Studies on the biology of the clam *Sunetta scripta* (Linn) from the subtidal waters of Cochin. Ph.D thesis, Cochin Univ. Sci. Tech. Cochin.

Kelly, D.P., and Syrett, P.J. 1964. Inhibition of formation of ATP in *Thiobacillus thioparus*, by 2-4 dinitrophenol. Nature 202, 597-598.

Kettle, W.D., de Noyellus, F.Jr., and Lei, C.H. 1980. Oxygen consumption of zooplankton as affected by laboratory and field cadmium exposures. Bull. Environ. Contam. Toxicol. 25 (4), 547-553.

Knox, W.E., and Greenguard, O. 1965. An introduction to enzyme physiology. Weber, G. (ed). Pergamon Press. New York. 3, 247 - 248.

*Koeferl., M.T., 1972. Potentiation of acute carbon tetrachloride hepatotoxicity by dietary exposure to chlorophenolthane(DDT) in rats. Ph.D thesis. Oregon State Univ. Corvallis. PP 182.

Koenig, H. 1963. Intravital staining of lysosomes by basic dyes and metallic ions. J. Histochem. Cytochem. 11, 120-121.

Koller, L.D. 1980. Immunotoxicology of heavy metals. Int. J. Immunopharmac. 2, 269-279.

Kominami, T., Oda, K., and Ikehara, Y. 1984. Induction of rat hepatic alkaline phosphatase and its appearence in serum Electrophoretic charactarisation of liver membranous and serum soluble forms. J. Biochem. 96, 901-911.

Koundinya, P.R., and Ramamurthi, R. 1980. Toxicity of Sumithion and Sevin to the freshwater fish, *Sarotherodon mossambica* (Peters). Curr. Sci. 49, 875.

Kroon, D.B. 1952. Phosphatases and the formation of protein carbohydrate complex. Acta. Anat. 15, 317.

Kulkarni, A.N., Kamble, S.M., and Keshavan. R. 1984. Metabolic depression in the freshwater bivalve, *Lamellidens corrianus* exposed to an insecticide, Hildan. Proc. Symp. Assesment. Environm. Pollution, Aurangabad. 20-22 Dec. 225-227.

Kulkarni, A.P. and Mehrotra, K.N. 1973. Effect of Dieldrin and Sumithion on the aminoacid nitrogen and protein in the

haemolymph of desert locust *Shistocerca gregaria*. Forsk. Pestic. Biochem. Physiol. 3, 420-434.

*Kulkarni, B.G. 1983. Toxicity studies, effects of some petroleum hydrocarbons on some marine animals. Ph.D thesis. Bombay Univ.

Kulkarni, B.G., and Kulkarni, R.G. 1987. Effect of mercury exposure on enzymes in the clam *Katelysia opima* (Gmelin). Ind. J. Mar. Sci. 16, 265-266.

Lakshmanan, P.T., and Nambisan, P.N.K. 1985. Tissue lactic acid and glycogen level of molluscs exposed to Cu and Hg. Curr. Sci. 54 (10), 478-479.

Lakshmipathi, V., and Reddy, P.V. 1984. Activity of certain enzymes of aminoacid metabolism in the liver and kidney of three amphibian species. Proc. Ind. Sci. Academy, 93 (1), 65-70.

Lal, B., Singh, A., Kumari, A., and Sinha, N. 1986. Biochemical and haematological changes following Malathion treatment in the freshwater cat fish *Heteropneustes fossilis* (Blotch). Environ. Pollut. (Series A). 42 (2), 151-156.

Lane, C.E., and Scura, E.D. 1970. Effects of dieldrin on glutamic oxaloacetic transaminase in *Poecilia lalirinna*. J. Fish. Bd. Can. 27, 1869-1871.

Langston, W.J., and Zhou, M. 1987. Cadmium accumulation,

distribution, and elimination in bivalve *Macoma balthica*. Neither metallothionein nor metallothionein like protein are involved. Mar. Environ. Res. 21, 225-237.

Larry, R.J., Nielsen, B.D., Miller, D., Benos, D., and Allen, J. 1974. Methyl mercury and inorganic mercury Uptake, distribution, and effect on osmoregulatary mechanism in fishes. In Pollution and physiology of marine organisms. Vernberg, F.J., and Vernberg, W.B. (eds), New York, Academic Press. 101-122.

*Larsson, A. 1975. In Sublethal effects of toxic chemicals on aquatic animals. 111. Koeman, J.H., and Strik, J.J.T.W.A. (eds), Elsevier Scientific Publishing Company, Amsterdam. P. 3.

Lee, R.F., Davies, J.M., Freeman, H.C., Ivanovici, A., Moore, M.N., Stegman, J., and Uthe, J.F. 1980. Biochemical techniques for monitoring biological effects of pollution in the sea. In Biological effects of marine pollution and the problems of monitoring. Rapp. P.V. Reum. Cons. Int. Explor. Mer. 179, 48-55.

Lehninger, A.L. 1975. Biochemistry. Worth Publishers Inc. New York. PP 562.

Little, C. 1965. The formation of urine by the prosobranch gastropod mollusc *Viviparous viviparous* Linn. J. Exp. Biol.

43, 39-54.

Livingstone, D.R., and De Zwaan, A. 1983. Carbohydrate metabolism of gastropods. In "The Mollusca". 1. Wilbur, K.M. (ed-in-chief), Academic Press, New York. 177-242.

Lloyd, R. 1960. Toxicity of zinc sulphate to rainbow trout. Ann. Appl. Biol. 48, 84.

Lockhart, W.L., Uthe, J.F., Kenney, A.R., and Mehrle, P.M. 1972. Methyl mercury in northern pike (*Esox lucius*). Distribution, elimination, and some biochemical charactaristics of contaminated fish. J. Fish. Res. Bd. Can. 29, 1519-1523.

Lomte, V.S., and Jadhav, M.L. 1982. Effects of toxic compounds on oxygen consumption in the freshwater bivalve, *Corbicula regularis* (Prime). Comp. Physiol. Ecol. 7, 31-33.

Loomis, W.F., and Lipman, F. 1948. Reversible inhibition of the coupling between phosphorylation and oxidation. J. Biol. Chem. 173, 807-814.

Loose, L.D., Silkworth, J.B., and Simpson, D.W. 1978. a. Influence of cadmium on the phagocytic and microbial activity of marine peritoneal macrophages, pulmonary alveolar macrophages and polymorphonuclear neutrophils. Infec. Immun. 22, 378-381.

Loose, L.D., Silkworth, J.B., and Warrington, D. 1978. b. Cadmium induced phagocyte cytotoxicity. Bull. Environm.

Contam. Toxicol. 20, 582.

Lowe, D.M., and Moore, M.N. 1978. Cytology and quantitative cytochemistry of a proliferative atypical hemocytic condition in *Mytilus edulis* (Bivalvia, Mollusca). J. Natn. Cancer Inst. 60, 1455-1459.

Lowe, D.M., and Moore, M.N. 1979. The cytochemical distribution of zinc (Zn 11) and iron (Fe 111) in the common mussel, *Mytilus edulis*, and their relationship with lysosomes. J. Mar. Biol. Ass. U.K. 59, 851-858.

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.

Luten, J.B., Bouquet, W., Burggraaf, M.M., and Rus, J. 1986. Accumulation, elimination, and speciation of cadmium and zinc in mussels, *Mytilus edulis*, in the natural environment. Bull. Environm. Contam. Toxicol. 37, 579-586.

Lutner, A.L. 1975. Clinical Biochemistry. W.B. Saunders Comp. London. pp 566

Mac Innes, J.R., and Thurberg, F.P. 1973. Effects of metals on the behaviour and oxygen consumption of the mud snail. Mar. Pollut. Bull. 12, 320-323.

Madar, N.S., and Pora, E.A. 1981. Age dependant changes in the glycogen content in some tissues of *Mytilus galloprovincialis*

(L) of the black sea. Riv. Roum. Biol. Ser. Biol. Anim. 26 (2), 143-148.

Malhotra, R.K., Kaul, R., and Malhotra, N. 1986. Transaminase activity in normal and irradiated minced muscle autografts on frog gastronemins. Ind. J. Exp. Biol. 24, 414-415.

Mandal, P.K., and Kulshrestha, S.K. 1980. Histopathological changes induced by the sublethal Sumithion in *Clarius batrachus*. Ind. J. Exp. Biol. 18 (5), 547-552.

Mane, U.H., and Akarte, S.R. 1987. Toxicity of Lebaycid to three species of freshwater lamellibranch molluscs in different seasons. Environ. Ecol. 5 (2).

Mane, U.H., Akarte, S.R., and Kulkarni, D.A. 1986. Acute toxicity of Fenithion to freshwater lamellibranch mollusc, *Indonaia caeruleus* (Prashad 1918), from Godavari river at Paithan- a biochemical approach. Bull. Environm. Contam. Toxicol. 37 (4), 622-628.

Manly, R., and George, W.O. 1977. The occurrence of some heavy metals in populations of the freshwater mussel, *Anodonta anatina* (L) from river Thames. Env. Pollut. 14, 139-154.

Manohar, L., Rao, P.V., and Swami, K.S. 1972. Variations in aminotransferase activity and total free aminoacid level in the body fluid of the snail, *Lymnea luteola* during different larval trematode infections. J. Invertbr. Pathol. 19, 36-41.

*Martin, A.W., and Field, J. 1934. Quoted from "Physiological stress indicated by sublethal concentrations of phenol and pentachlorophenol on *Notopterus notopterus* Hepatic acid and alkaline phosphatases and succinic dehydrogenase (Dalela, R.C., Rani, S., and Verma, S.R.). Environ. Pollut.(1980). A. 21 (1), 3-8.

Mathew, R., and Menon, N.R. 1983. Oxygen consumption in tropical bivalves, *Perna viridis* (Linn) and *Meretrix casta* (Chem) exposed to heavy metals. Ind. J. Mar. Sci. 12, 57 - 59.

Mathew, R., and Menon, N.R. 1984. Filtration in *Perna viridis* and *Meretrix casta* under heavy metal stress. Mahasagar. 17, 183-186.

Mc Kim, J.M., Christensen, G.M., and Hunt, E.P. 1970. Changes in the blood of brook trout *(Salvelinus fontinalis)* after short-term and long term exposure to copper. J. Fish. Res. Bd. Canada. 27, 1883-1889.

Mc Leay, D.J., and Brown, D.A. 1970. Stress and chronic effects of untrated and treated bleached kraft pulpmill effluent on the biochemistry and stamina of juvenile coho salmon *(Oncorhynchus kisutch)*. J. Fish. Res. Bd. Canada. 36, 1049-1059.

Mc Leay, D.J., and Brown, D.A. 1974. Growth stimulation and

biochemical changes in juvenile coho salmon (*Oncorhynchus kisutch*) exposed to bleached kraft pulp mill effluent to 200 days. J. Fish. Res. Bd. Can. 31, 1043-1049.

Mehrle, P.M., and Mayer, F.L. 1978. Biochemistry and physiology. In Estimating the hazard of chemical substances to aquatic life. Cairns, J., Dickson, K.L., Maki, A.W. (eds). ASTM, Philadelphia. 264 - 282.

Meinardus, G., and Gade, G. 1981. Anaerobic metabolism of the cockle, *Cardium edule*. 1V. Time dependant changes of metabolites in the foot and gill tissue induced by anoxia and electrical stimulation. Comp. Biochem. Physiol. 70 B, 271 - 277.

Mohan, C.V., Gupta, T.R.C., Shetty, H.P.C., and Menon, N.R. 1986. a. Combined toxicity of mercury and cadmium to the tropical green mussel, *Perna viridis*. Des. Aquat. Org. 2, 65-72.

Mohan, C.V., Menon, N.R., and Gupta, T.R.C. 1986. b. Filtration in some tropical intertidal bivalves exposed to mercury and cadmium mixtures. Fishery. Tech. 23, 204-210.

Mohan, P.K., Reddy, L.V., Shankara, C.R., and Indira, K. 1987. Metabolic consequences of methyl parathion exposure in the bivalve, *Lamellidens marginalis* (Lamarck) Bull. Contam. Toxicol. 38 (3), 509-514.

* Monod, J. 1944. Inhibition de'l adaptation enzymatique chez B. Colien presence de 2-4 dinitrophenol. Anns. Inst. Pasteur. Paris. 70, 381-384.

Montgomery, R. 1957. Determination of glycogen. Arch. Biochem. Biophys. 67, 378-386.

Moore, M.N. 1976. Cytochemical demonstration of latency of lysosomal hydrolases in digestive cells of the common mussel, *Mytilus edulis*, and changes induced by thermal stress. Cell. Tissue. Res. 175, 279-287.

Moore, M.N. 1977. Lysosomal responses of environmental chemicals in some marine invertibrates. In pollution effects on marine organisms. Giam, C.S. (eds), Heath, Tronto. P 143-154.

Moore, M.N. 1980. Cytochemical determination of cellular responses to environmental stressors in marine organisms. In Biological effects of marine pollution and the problems of monitoring organisms. Mc Intyre, A.D., and Pearce, J.B. (eds). Rapp. P.V. Retten. Cons. Int. Explor. Mer, 179, 7-15.

Moore, M.N. 1985. Cellular responses to pollutants. Mar. Pollut. Bull. 16 (4), 134 - 139.

Moore, M.N., and Halton, D.W. 1973. Histochemical changes in the digestive gland of *Lymnea truncatula* injected with ***Fasciola hepatica*** • Z. Parasitenk. 43, 11 - 16.

Moore, M.N., and Halton, D.W. 1977. The cytochemical localisation of lysosomal hydrolases in the digestive cells of littorinids and changes induced by larval trematode infection. Z. Parasitenk. 53, 115 - 122.

Moore, M.N., and Lowe, D.M. 1977. The cytology and cytochemistry of the hemocytes of *Mytilus edulis* and their responses to experimentally injected carbon particles. J. Invertebr. Pathol. 29, 18-20.

Moore, M.N., and Lowe, D.M. 1985. Cytological and cytochemical measurements. In The effects of stress and pollution on marine animals. Bayne, B.L.(ed). Prager Scientific, New York. 46-74.

Moore, M.N., and Stebbing, A.R.D. 1976. The quantitative cytochemical effects of three metal ions on a lysosomal hydrolase of a hydroid. J. Mar. Biol. Assoc. U.K. 56, 995-1005.

Moore, M.M., Lowe, D.M., Feith, P.E.M. 1978 a Lysosomal responses to experimentally injected anthracene in the digestive cells of *Mytilus edulis*. Mar. Biol. 48, 297-302.

Moore, M.M., Lowe, D.M., and Feith, P.E.M. 1978 b. Responses of lysosomes in the digestive cells of common mussel, *Mytilus edulis* to sex steriods and cortisol. Cell. Tiss. Res. 188, 1-9.

Moore, M.N., Widdows, J., Cleary, J.J., Pipe, R.K., Salked, P.N., Donkin, P., Farrar, S.V., Evans, S.V., and Thomson, P.E. 1984. Responses of the mussel, *Mytilus edulis* to copper and phenanthrene. Mar. Environm. Res. 14, 167-183.

Moorthy, K.S., Reddy, B.K., Swami, K.S., and Chetty, C.S. 1985. Glucose metabolism in hepatopancreas and gill of *Lamellidens marginalis* during methyl parathion toxicity. Pestic. Biochem. Physiol. 24 (1), 40-44.

Moorthy, K.S., Reddy, B.K.,Swami, K.S., Chetty, C.S., and Sreeramula, K. 1986. Dichlorvos induced metabolic changes in tissues of freshwater mussel, *Lamellidens marginalis*. J. Environm. Biol. 7 (2), 101-106.

Muley, D.V., and Mane, U.H. 1987. Malathion induced changes in oxygen consumption in two species of freshwater lamellibranch mollusc from Godavari river, Maharastra state, India. J. Environm. Biol. 8 (3), 267-275.

Muller, G. 1965. The histochemical localisation of phosphatases and esterases in *Austalorbis glabratus*. Proc. Zool. Soc. London. 144, 229-237.

*Murthy, C.K. 1982. Combined toxicity of silver and copper on a few marine invertebrates and ecological monitoring of the inshore waters of the Arabian sea off Mangalore. M.F.Sc thesis, Univ. Agricul. Sci. Bangalore.pp 274.

Murthy, A.S., and Devi, A.P. 1982. Effect of Endosulfan and its isomers on tissue protein, glycogen, and lipids in the fish, *Channa punctatus*. Pesticide. Biochem. Physiol. 17, 280-286.

Murthy, B.N., Reddy, M.S., and Rao, K.V.R. 1985. Significance of aminotransferase activity of the freshwater teleost, *Oreochromis mossambicus* (Trewavas) under Lindane toxicity. Curr. Sci. 54 (20), 1077.

Nagabhushanam, R., and Kulkarni, G.K. 1981. Freshwater palaemonid prawn, *Machrobrachium kistens* (Tiwari)-effect of heavy metal pollutants. Proc. Ind. Nat. Sci. Academy. B. 47 (3), 380-386.

Nath, K., and Kumar, N. 1987. Toxicity of manganese and its impact on some aspects of carbohydrate metabolism of a freshwater teleost, *Cotisa fasciatus*. Sci. Total. Environm. 67 (2), 257-262.

Naurigal, B.P., and Singh, H.R. 1985. Some biochemical changes in the reproductive cycle of a hill stream teleost, *Puntius chilinoides* (Mc clelland). Proc. Indian. Acad. Sci. 94 (1), 67-72.

Nemesok, J., Benedecyky, I., Boross, L., Asztabos, B., and Orban, L. 1981. Subcellular localisation of transaminase enzymes in fishes and their significance in the detection of water pollution. Acta Biologica. Szeged. 27, 9-15.

Nevicoff, A.B. 1961. In The cell. Vol. 11. Mirsky (ed). Academic Press. New York. P 423.

Nichol, C.A., and Rosen, F. 1963. Advances in enzyme regulation.Weber, B. (ed). Pergamon Press, New York. 341.

Nimmo, D.W.R., Lightner, D.V., and Bahner, L.H. 1977. Effects of cadmium on the shrimps, *Penaeus duorarum, Palaemonetes pugio* and *Palaemonetes vulgaris*. In Physiological responses of marine biota to pollutants. Vernberg, F.J., Calabrese, A., Thurberg, F.P., and Vernberg, W.B. Academic Press, New York. P 131-183.

Noel-Lambot, F. 1976. Distribution of Cd, Zn, and Cu in the mussel, *Mytilus edulis*. Existence of Cd binding protein similar to metallothioneins. Experimentia. 32, 324-325.

Noel-Lambot, E., Gerday, C.N., and Disteche, A. 1978. Distribution of Cd, Zn, and Cu in liver and gills of the eel *Anguilla anguilla* with special reference to metallothioneins. Comp. Biochem. Physiol. 61 C, 177-187.

Oh, S., Deagen, J., Whanger, P., and Weswig, P. 1978. Biological functions of metallothionein. V. Its induction in rats by various stressess. Amer. J. Physiol. 234. E, 282.

Olafson, R.W., Sim, R.G., and Boto, K.G. 1979. a. Isolation and chemical charactarization of the heavy metal-binding protein metallothionein from marine invertebrates. Comp. Biochem.

Physiol. 62 B, 407-416.

Olafson, R.W., Sim, R.G., and Kearns, A. 1979.b. Physiological and chemical charactarisation of invertebrate metallothionein like protein. In "Metallothionein" Proceedings of the first international meet on metallothionein and other low molecular weight metal-binding proteins. Kagi, J.H.R., and Nordberg, M. (eds). Birhauzer Verlag, Boston. 177-204.

*Onikieno, F.A. 1963. Enzymic changes from early stages of intoxication with small doses of chlorogenic insecticides. Gig. Primen. Toxicol. Pestits. Khn. Otrcylennii P Zabolivanni. 77, 137.

Overnell, J., and Trewhella, E. 1979. Evidence for the national occurence of (cadmium, copper) metallothionein in the crab, *Cancer paguru*. Comp. Biochem. Physiol. 64 C, 69-76.

Owen, G. 1972. Lysosomes, peroxisomes and bivalves. Sci. Prog. Oxf. 60, 299-318.

Passow, H. 1970. The red blood cell Penetration, distribution, and toxic actions of heavy metals. In Effects of metals on cells, subcellular elements and macromolecules. Maniloff, J., Coleman, J.R., and Miller, M.W. (eds). Sprinfield, 111. Charles. C. Thomas. P 291.

Passow, H., Rothstein, A., and Clarksow, T.W. 1961. The general pharmacology of heavy metals. Pharmacol. Rev. 13, 185-190.

Patel, S., and Patel, B. 1985. Effects of environmental parameters on lysosomal marker enzymes in the tropical blood clam, *Anadara granosa*. Mar. Biol. 85, 245-252.

Patil, H.S., and Kaliwal, M.B. 1983. Influence of cadmium, copper, and zinc on oxygen consumption rate of freshwater prawn, *Macrobrachium hencles sodyanum*. Environ. Ecol. 1, 175-177.

*Pfeifer, K.F.. 1979. Biochemical and physiological aspects of carbontetra chloride toxicity in the rainbow trout, *Salmo gairdneri*. Ph. D thesis. Oregon State Univ. Corvallis. PP 184.

Pfeifer, K.F., Weber, L.J., Larson, R.E. 1980. Carbontetrachloride induced hepatotoxic response in rainbow trout, *Salmon gairdneri* as influenced by two commercial fish diets. Comp. Biochem. Physiol. 67 C, 91-96.

Phillips, D.J.H. 1976 a. The common mussel, *Mytilus edulis* as an indicator of pollution by zinc, cadmium, lead, and copper. 1. Effects of environmental variables on uptake of metals. Mar. Biol. 38, 59-69.

Phillips, D.J.H. 1976 b. The common mussel *Mytilus edulis* as an indicator of pollution by zinc, cadmium, lead, and copper. 11. Relation of metal in the mussel to those discharged by the industry. Mar. Biol. 38, 71-80.

Phillips, D.J.H. 1977. The use of biological indicator

organisms to monitor trace metal pollution in marine and estuarine environments - A review. Environm. Pollut. 13, 281-318.

Pilo, B., Asnani, M.V., and Shah, R.V. 1972. Studies on the wound healing and repair in pigeon liver. 111. Histochemical studies on acid and alkaline phosphatase during the process. J. Anm. Morphol. Physiol. 19, 205-212.

*Piscator, M. 1964. On cadmium in normal human kidneys together with a report on the isolation of metallothionein from livers of cadmium exposed rabbits. Nard. Hyg. Tidskr. 45, 76-82.

*Plaveux, A. 1977. L' influence du cadmium sur la activite due la phosphatase alkaline dans diverstissues de *Lepomis gibbosus*. 1. (Pisces, Centrarchidae). Hydrobiologia. 55, 251-256.

Poulsen, E., Riisgard, H.V., and Mohlenberg, F. 1982. Accumulation of cadmium and bioenergetics in the mussel *Mytilus edulis*. Mar. Biol. 68, 25-31.

Prabhudeva, K.N., and Menon, N.R. 1985. Rate of filtration by *Perna viridis* pre-exposed to heavy metals. Fish. Tech. 22, 102-104.

Prabhudeva, K.V., and Menon, N.R. 1986. Oxygen consumption under copper and zinc stress in *Perna viridis*. Fish. Technol. 23, 24-26.

Prashad, B.S.V., Reddy, B.R., Damodaran, C., and Rao, P.V. 1983. Effect of different larval trematode infections on enzyme activities of body fluid of the banded pond snail, *Viviparous bengalensis*. Ind. J. Comp. Anim. Physiol. 1 (2), 61-65.

Principato, B.G., Aisa, M.C., Biagioni, M., and Giovannini, E. 1982. Partial purification and charactarisation of an alkaline phosphatase in *Helix nemoralis* and *Octopus vulgaris*. Comp. Biochem. Physiol. 72 B, 325-328.

Pynnoenen, K., Holwerda, D.A., and Zandee, D.I. 1987. Occurrence of calcium concentration in various tissues of freshwater mussels and their capacity for cadmium sequestration. Aquatic. Toxicol. 10, 2-3.

Racicot, J.G., Gaudet, M., and Leray, C. 1975. Blood and liver enzymes in rainbow trout (*Salmo gairdneri* Rich) with emphasis on their diagnostic use.: Study of CCl4 toxicity and a case of aeromonas injection. J. Fish. Biol. 7, 825-835.

Radhaiah, V., Girija, M., and Rao, K.J. 1987. changes in selected biochemical parameters inthe kidney and blood of the fish, *Tilapia mossambica* (Peters) exposed to Heptachlor. Bull. Environ. Contam. Toxicol. 39 (6), 1006-1011.

Radhakrishnaiah, K. 1988. Effect of cadmium on the freshwater mussel, *Lamellidens marginalis* (Lamarck) - A physiological approach. J. Environ. Biol. 9 (1), 73-78.

Ramalingam, K., and Ramalingam, K. 1982. Effect of sublethal level of DDT, Malathion, and mercury on tissue protein of *Sarotherodan mossambicus* (Pters). Proc. Ind. Acad. Sci. 91 (6), 501-506.

Rana, S.V.S., and Kumar, A. 1980. Proteins, lipids and carbohydrates in the liver and kidney of rats after molybdenum and copper treatment. Bull. Environ. contam. Toxicol. 25 (1), 146-152.

Rao, G.M., and Chari, N. 1981. Metabolic differentiation of white and pink components of catch and heart muscles of a freshwater mussel, *Lamellidens corrianus* (Lea). Indian. J. Exp. Biol. 19, 225-227.

Rao, K.S., and Rao, R.K.V. 1983. Regulation of phosphorylases and aldolases in tissues of the teleost (*Tilapia mossambica*) under methyl parathion impact. Bull. Environ. Contam. Toxicol. 31, 474-478.

Rao, K.S., Moorthy, K.S., Reddy, B.K., Swami, K.S., and Chetty, C.R. 1987. Effect of Benthiocarb on protein metabolism of freshwater teleost, *Sarotherodan mossambicus*. Ind. J. Environ. Health. 29 (91), 45-51.

Rao, K.S.P., Ahamed, I.K., and Rao, K.V.R. 1981. Impact of methyl parathion on the tissue NH_3- changes in the fish *Tilapia mossambica* (Pters). Proc. Ind. Nat. Sci. Acad. B. 47

(3), 394-397.

Rao, K.S.P., Prasad, K.S., and Rao, K.V.R. 1980. Sublethal effect of methyl parathion on tissue proteolysis in the freshwater mussel, *Lamellidens marginalis* (Lamarck). Proc. Indian. Nat. Sci. Acad. B. 46 (2), 164-167.

Rao, K.S.P., and Rao, K.V. 1984. Tissue specific alteration of aminotransferases and total ATP as in the fish (*Tilapia mossambica*) under methyl parathion impact. Toxicol. Lett. 20 (1), 53-57.

*Rao, K.V.R. 1974. Studies on some aspects of selected enzyme system of gastropod, *Pila globosa* (Swanson) with special reference to aestivation. Ph.D thesis. S.V. Univ. Tirupathi.

Rao, K.V.R., and Dayakar, Y. 1987. Significance of tissue aminotransferases in brachyuran species of diverse habitats. Ind. J. Mar. Sci. 16, 267-268.

Ray, S., and Coffin, J. 1977. Ecological effects of cadmium pollution in the aquatic environment- A review. Fish. Mar. Serv. Tech. Rep. 734, P 24.

Ray, S., Mc Leese, D.W., Waiwood, B.A., and Pezzack, D. 1980. The disposition of cadmium and zinc in *Pandalus montagui*. Arch. Environ. Contam. Toxicol. 9, 675-681.

Ray, S., Mc Leese, D.W., and Bueridge, L.E. 1981. Cadmium in tissues of lobsters captured near a lead smelter. Mar.

Pollut. Bull. 12 (11), 383 - 386.

Read, K.R.H. 1962. Transamination in certain tissue homogenates of the bivalve molluscs *Mytilus edulis* (L) and *Modiolus modiolus* (L). Comp. Bioch. Physiol. 1, 15-22.

Reading, J.T., and Buikema Jr. A.L. 1980. Effects of sublethal concentrations of selenium on metabolism and filtering rate of *Daphnia pulex*. Bull. Environ. Contam. Toxicol. 24 (6), 929 - 935.

*Reddy, B.K. 1984. The possible metabolic diversions in the nitrogen metabolism adapted by freshwater mussel, *Lamellidens marginalis* (Lamarck) under short term methyl parathion stress. Ph.D thesis, S.V. Univ. Tirupathi.

Reddy, M.S., Venketeswarlu, Y., Surendranath, P., and Rao, K.V.R. 1985. Changes in nitrogen metabolism in the selected tissues of a penaeid prawn, *Penaeus indicus* exposed to Phosphamidon. Environ. Ecol. 3, 500-503.

Reddy, M.V., and Naidu, B.P. 1978. Role of hepatopancreas in the metabolism of copper in the indian amphibious snail, *Pila globosa* (Swainson) with special reference to aestivation and starvation. Curr. Sci. 47, 145-147.

Reddy, N.A., and Menon, N.R. 1979. Effects of ammonia and ammonium on tolerence and byssogenesis in *Perna viridis*. Mar. Ecol. Prog. Ser. 1, 315-321.

Reddy, P.S., Bhagyalakshmi, A., and Ramamurthi, R. 1984. Invivo subacute physiological stress induced by sumithion on the hepatopancreatic acid phosphatase activity in the freshwater crab, (*Oziotelphusa senex senex*). Water. Air. Soil. Pollut. 22 (3), 299-302.

Reddy, P.S., Bhagyalakshmi, A., and Ramamurthi, R. 1986 a. Chronic Malathion toxicity Effect on carbohydrate metabolism of *Oziotelphusa senex senex* the Indian rice field crab. Bull. Environ. contam. Toxicol. 37 (6), 816-822.

Reddy, R., Vijayakumar, N., and Chari, N. 1986 b. Effect of mercuric chloride on carbohydrate metabolism of mantle, foot, and gill of freshwater mussel, *Parreysia rugosa* (Gmelin). J. Environ. Biol. 7 (4), 225-230.

Reddy, T.R., and Chari, N. 1985. Effect of sublethal concentration of mercury and copper on alanine aminotransferase, aspartate aminotransferase and glutamate dehydrogenase of freshwater mussel, *Parreysia rugosa* (G). J. Environ. Biol. 6(2), 67-70.

Reitman, S., and Frankel, S. 1957. Colorimetric method for the determination of serum transaminase activity. Amer. J. Clin. Pathol. 28, 56.

Reynolds, J.A., and Schlesinger, M.J. 1969. Alterations in the structure and function of *Escheriachia coli* alkaline

phosphatase due to Zn binding. Biochemistry. 8, 588-593.

Rice, C.D., and Mills, R.R. 1987. Effect of Kepone tolerence on liver alkaline phosphomonoestarase and glutamic oxaloacetate aminotransferase from *Fundulus hetroclites*. Bull. Environ. Cont. Toxicol. 39, 655-662.

*Robbins, S.L., and Angell, M. 1976. In Basic pathology, 2nd edition, W.B. Saunders co. Philadelphia. 3-30.

Rodrick, E., and Cheng, T.C. 1974. Activities of selected haemolymph enzymes in *Biomphalaria glabrata* (Mollusca). J. Invertbr. Pathol. 24, 374-375.

Roesijadi, G. 1979. Taurine and Glycine in the gills of the clam, *Protothaca staminea* exposed to chlorinated sea water. Bull. Environm. Contam. Toxicol. 22, 543 - 547.

Roesijadi, G. 1980. Influence of copper on the clam, *Protothaca staminea* Effects on gills and occurence of copper-binding proteins. Biol. Bull. 158, 233-247.

Sabbioni, E., and Marafante, E. 1975. Heavy metals in rat liver cadmium binding protein. Environ. Physiol. Biochem. 5, 132 - 141.

Sahib, I.K.a., Narasimhamurthy, B., Begum, M.R.S.O., Prasad, K.S., and Rao, K.V.R. 1983. Orientation in the glycolytic potentials of carbohydrate metabolism in the selected tissues of the aestivating freshwater pelecypod, *Lamellidens*

marginalis (Lamark). Comp. Physiol. Ecol. 8 (3), 180-184.

Sakaguchi, H., and Hamaguchi, A. 1975. Physiological changes in the serum and hepatopancreas of yellow tail injected with carbon tetra chloride. Bull. Jpn. soc. Scient. Fish. 41, 283-290.

Salanki, J. 1965. Oxygen levels as a specific regulator of the rythmic activity of freshwater mussel, *Anodonta cygnea*. Acta. Biol. Acad. Sci. Hung. 15, 299-310.

*Salanki, J. 1968. Role of cerebral ganglia in the regulation of activity in freshwater mussel, *Anodonta cygnea*. In neurobiology of invertebrates". Salanki, J. (ed). Akade 'miai kiado'. Budapest. 493-501.

Saleem, M., and Alikhan, M.A. 1974. Charactaristics of acid phosphatase in *Porcellio laevis latreille* (Porcellionidae, Isopoda). Comp. Biochem. Physiol. 49 B, 129-137.

Saliba, L.J., and Vella, R. 1977 Effects of mercury on the behaviour and oxygen consumption of *Monodonta articulata*. Mar. Biol. 43, 277-282.

Sastry, K.V., and Gupta, A.D. 1987. Enzymological profile in some tissues of a teleost fish, *Channa punctatus* chronically exposed to mercury, nickel, and chromium. Environ.& Pesticide. Toxicity. 211-217.

Sastry, K. V., and Gupta, P.K. 1978. Histopathological and

enzymological studies on the effects of chronic lead nitrate intoxication in the digestive system of a freshwater teleost, *Channa punctatus*. Environ. Res. 17, 472-479.

Sastry, K.V., and Rao, D.R. 1983. Mercuric chloride induced alterations in the intestinal transport of glucose and frectose in the freshwater mussel, *Channa punctatus*. Water. Air. Soil. Pollut. 19 (2), 143-147.

Sathyanathan, B., Nair, S.M., Chacko, J., and Nambisan, P.N.K. 1988. Sublethal effect of copper and mercury on some biochemical constituents of the estuarine clam, *Villoritta cyprinoides* var *Cochinensis* (Hanly). Bull. Environ. Contam. Toxicol. 40, 510-516.

Saxena, P.K., Bedi, R., and Soni, G.L. 1982. Effects of vegetable oil factory effluent on the levels of phosphatases and dehydrogenases in the liver and kidney of the freshwater teleost *Channa punctatus* (BL). Environ. Pollut. 28 (4), 245-253.

*Schulz-Baldes, M. 1978. Lead transport in the common mussel, *Mytilus edulis*. In "Proceedings of the 12th European symposium on marine biology. Mc Lusky, D.S., and Berry, A.J. (eds). Pergamon Press. Oxford and New York. 211-219.

*Scoppa, P. 1975. Cadmium isocitrate complex Its stability as a function of ionic strength. Z. Naturrforch. 30, 555-561.

Scott, D.M., and Major, C.W. 1972. The effect of copper (11) on

survival, respiration, and heart rate in the common blue mussel, *Mytilus edulis*. Biol. Bull. 143, 679-688.

Shaffi, S.A. 1978. a. Cadmium intoxication of tissue glycogen content in three freshwater teleosts. Curr. Sci. 47, 868-870.

Shaffi, S.A. 1978. b. Variations in tissue glycogen content, serum lactate and glucose levels due to copper intoxication inthree freshwater teleosts. Curr. Sci. 47, 954-956.

Shareef, K., Shareef, S., and Wagh, S.B. 1983. Effect of sublethal dose of Saquin (R) (Quinolphos 25% w/w) on liver and kidney of two freshwater cyprinid fishes, *Barbus ticto* and *Rasbara daniconius* (Ham). In: Environmental biology Coastal ecosystem.Dalela, R.C., Madhyastha, H.N., and Joseph, M.M. (eds). The academy of environmental biology, Muzaffarnagar, India.

*Sherlock, S. 1968. Diseases of the liver and bilary system. 4th edn. Blackwell. Oxford.

Shore, R., Carney, G., and Stygall, T. 1975. Cadmium levels and carbohydrate metabolism in limpets. Mar. Pollut. Bull. 6, 187-189.

*Shulman, G.E. 1974. Life cycles of fish physiology and biochemistry. Keter publ. House. Jeruselam.

Sigmon, C. 1979. Oxygen consumption in *Daphnia pulex* exposed to

2, 4-D or 2, 4,5-T. Bull. Environ. contam. Toxicol. 21 (6), 822-825.

Simon, E.W. 1953. Mechanism of dinitrophenol toxicity. Biol. Rev. 28, 453-479.

Singhal, R.L., Merali, Z., Kacew, S., and Sutherland, D.J.B. 1974. persistence of cadmium induced metabolic changes in liver and kidney. Science. 183, 1094-1096.

Skidmore, J. 1970. Respiration and osmoregulation in rainbow trout with gill damage by zinc sulphate. J. Exp. Biol. 52, 481.

Skidmore, J.F., and Tovell, P.W.A. 1972. Toxic effects of zinc sulphate on the gills of rainbow trout. Water. Res. 6, 217-230.

Sollock, R.L., Vornhaben, T.G., and Campbell, J.W. 1979. Transaminase reactions and glutamate dehydrogenase in gastropod hepatopancreas. J. Comp. Physiol. B. 129, 497-526.

Sporn, A., Dinu, I., and Stoenescu, L. 1970. Influence of cadmium administration on carbohydrate and cellular energetic metabolism with rat liver. Rev. Roum. Biochem. 7, 299-305.

Statham, C.N., Croft, W.A., and Lech, J.J. 1978. Uptake, distribution and effects of carbon tetrachloride in rainbow trout (*Salmogairdneri*). Toxicol. Appl. Pharmacol. 45, 131-140.

Stebbing, A.R.D. 1976. The effects of low metal levels on clonal hydroid. J. Mar. Biol. Ass. U.K. 56, 977-994.

Sternlieb, I., and Goldfischer, S. 1976. Heavy metals and lysosomes. In Lysosomes in biology and pathology, Dingle, J.T., and Dean, R.T. (eds). American Elseiver Publishing Company, New York. 185-200.

Stokes, T.M., and Awapara, J. 1968. Alanine and succinate as end products of glucose degradation in the clam *Rangia cuneata*. Comp. Biochem. Physiol. 25, 883-892.

Stone, H., and Overnell, J. 1985. Non-metallothionein cadmium binding proteins. Comp. Biochem. Physiol. 80 C (1), 9-14.

Sullivan, J.T., and Cheng, T.C. 1975. Heavy metal toxicity to *Biomphalaria glabrata* (Mollusca, Pulmonate). Ann. New York. Acad. Sci. 266, 437-444.

Summer, A.T. 1965. The cytology and histochemistry of the digestive gland cells of *Helix*. Q.J. Microse. Sci. 106, 173-193.

Sumner, A.T.1969. The distribution of some hydrolytic enzymes in the cells of the digestive gland of certain lamellibranchs and gastropods. J. Zool. (London). 158, 277 - 291.

Sunila, I. 1986. Chronic histopathological effects of short term Cu and Cd exposure on the gill of mussel, *Mytilus edulis*. J.Invertebr. Pathol 47, 125-142.

Sunila, I., and Lindstorm, R. 1985. The structure of the intrafilamentar junction of the mussel (*Mytilus edulis* L) gill and its uncoupling by copper and cadmium exposures. Comp. Biochem. Physiol. 81 C, 267 - 272.

Suresh, K. 1988. Haematology of some marine and estuarine molluscs of commercial importance. Ph.D thesis. Cochin. Univ. Sci. Tech. Cochin, Kerala.

Suresh, K., and Mohandas, A. 1990. Haemolymph acid phosphatase activity pattern in copper-stressed bivalves. J. Invertbr. Pathol. 55, 118-125.

Swami, K.S., and Reddy, Y.S. 1978. Adaptive changes to survival during aestivation in the gastropod snail, *Pila globosa*. J. Sci. Ind. Res. 37, 144-157.

Talbot, V., and Magee, R.J. 1978. Naturaly -occuring heavy metal binding proteins in invertebrates. Arch. Environ. Contam. Toxicol. 7, 73-78.

Tallendini, L., Cassini, A., Favero, N., and Albergoni, V. 1986. Regulation and subcellular distribution of copperr in the freshwater molluscs *Anodonta cygnea* (L) and *Unio elongatulus* (Pf). Comp. Biochem. Physiol. 84 C (1), 43-49.

Thurberg, F.P., Cable, W.D., Dawson, M.A., Mac Innes, J.R., and Wenzloff, D.R. 1975. Respiratory response of larval, juvenile and adult surf clams, *Spisula solidissima* to silver.

In Respiration of marine organisms. Cech, J.J. Jr., Bridges, D.W., and Halton, D.B. (eds). Trigom publ. Portland Mc. 41-52.

Thurberg, F.P., Calabrese, A., and Dawson, M.A. 1974. Effect of silver on oxygen consumption of bivalves at various salinities. In Pollution and Physiology of marine organisms". Vernberg, F.G., and Vernberg, W. (eds). Academic Press, New York. 67 - 78.

Thurberg, F.P., Calabrese, A., Gould, E., Greig, R.A., Dawson, M.A., and Tucker, R.K. 1977. Response of the lobster, *Homarus americanus* to sublethal levels of cadmium and mercury. In "Physiological responses of marine biota to pollutants". Vernberrg, F.J., Calabrese, A., Thurberg, F.P., and Vernberg, W.B. (eds). Academic Press. New York. 185-197.

Thurberg, F.P., Dawson, M A., and Collier, R.S. 1973. Effect of copper and cadmium on osmoregulation and oxygen consumption in two species of estuarine crabs. Mar. Biol. 23, 171-175.

*Tietz, N.W. 1970. Fundamentals of clinical biochemistry. Saunders, Philadelphia.

Tomkins, G.M., Yielding, K.L., and Curran, J. 1961. Steroid hormone activation of L-alanine oxidation catalysed by a sub unit of crystalline glutamic dehydrogenase. Proc. Natl.

Acad. Sci. U.S.A. 47, 270-278.

Torreblanca, A., Diaz-Majans, J., Ramo, J.D., and Nunez, A. 1987. Oxygen uptake and gill morphological alterations in *Procambarus clarkii* (Girard) after sublethal exposure to lead. Comp. Biochem. Physiol. 86 C(1), 219-224.

Tort, L., Torres, P., and Hidalgo, J. 1984. Short-term cadmium effects on gill tissue metabolism. Mar. Pollut. Bull. 448-450.

Tucker, R.K., and Matte, a. 1980. Invivo effects of cadmium and lead on ATPases in the gill of the rock crab, *Cancer ieroratus*. Bull. Environ. Contam. Toxicol. 24, 847-852.

Ulmer, D.D. 1970. Effects of metals on protein structure. In Effects of metals on cells, subcellular elements and macromolecules. Maniloff, J., Coleman, J.R., and Miller, M.W. (eds). Charles C. Thomas Publishers, Springfield, 11-35.

Unnakami, S., Komoda, T., Watanabe, M., Tanimoto, Y., Sakagishi, Y., and Ikezawa, H. 1987. Molecular nature of three liver alkaline phosphatases detected by drug administration invivo: Differences between soluble and membranous enzymes. Comp. Biochem. Physiol. 88 B (1), 111-118.

Valee, B.L., and Ulmer, D.D. 1972. Biochemical effects of mercury, cadmium, and lead. Annual. Review. Biochemistry. 41, 91-128.

Vatal, M., and Aiyar, A.S. 1988. Effect of lithium carbohydrate on serum glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and lactate dehydrogenase of rats. Ind. J. Exp. Biol. 26, 801-803.

Venkatsubbaia, M.B., Phillip, G., Purushottam, K.R., Ramamurthi, R. 1984. Effect of mercury on oxygen consumption of the freshwater mussel, *Lamellidens marginalis*. Geobios, 11(2), 87-89.

Verity, M.A., and Reith, A. 1967. Effect of mercurial compounds on structure linked latency of lysosomal hydrolases. Biochem. J. 105, 685-690.

Verma, S.R., Gupta, A.K., Bansal, S.K., and Dalela, R.C. 1978. The invitro distribution of ATP dependant active transport following treatment with aldrin and its epoxy dieldrin in *Labeo rohita*. Toxicol. 11, 193-201.

Verma, S.R., Rani, S., and Dalela, R.C. 1984. Effects of pesticides and their combinations on three serum phosphatases of *Mystus vittatus*. Water. Air. Soil. Pollut. 21. 9-14.

Verma, S.R., and Tonk, I.P. 1983. Effect of sublethal concentrations of mercury on the composition of liver, muscles, and ovary of *Notoptrus notopterus*. Water, Air, Soil Pollut. 20 (3), 287-292.

Vernberg, W.B., De coursey, P.J., and O' Hara, J. 1974.

Multiple environmental factor effects on physiology and behaviour of the fiddler crab, *Uca pugilator* In Pollution and physiology of marine organisms. Vernberg, F.J., and Vernberg, W.B. (eds), Academic Press. New York. 381 - 425.

Vernberg, W.B. and Vernberg, J. 1972. The synergistic effects of temperature, salinity and mercury on survival and metabolism of the adult fiddler crab, *Uca pugilator*. Fish. Bull. 70, 415 - 420.

Verriopoulos, G., Moraitou-Apostolopoulou, M., and Xatzispirou. 1986. Evaluation of metabolic responses of *Artimia salina* to oil dispersant as a potential indicator of toxicant stress. Bull. Environ. Contam. Toxicol. 36 (3), 444-451.

Viarengo, A. 1985. Biochemical effects of trace metals. Mar. Pollut. Bull. 16 (4), 153-158.

Viarengo, A., Moore, M.V., Pertica, M., Mancinelli, G., Zanicchi, G., and Pipe, R.K. 1985. Detoxification of copper in the cells of the digestive gland of mussel The role of lysosomes and thioneins. The Sci. Total. Environ. 44, 135-145.

Viarengo, A., Pertica, M., Mancinelli, G., Palmero, S., and Orunesu, M. 1982. Effects of Cu^{2+} on nuclear RNA polymerase activities in the mussel digestive gland. Mar.

Biol. Lett. 3, 345-352.

Viarengo, A., Pertica, M., Mancinelli, G., Orunesu, M., Zanicchi, G., Moore, M.N., Pipe, R.K. 1984. Possible role of lysosomes in the detoxication of copper in the digestive gland cells of metal exposed mussels. Mar. Environ. Res. 14, 469-470.

Viarengo, A., Pertica, M., Mancinelli, G., Palmero, S., Zanicchi, G., and Orunesu, M. 1981 Synthesis of cu-binding proteins in different tissues of mussels exposed to the metal. Mar. Pollut. Bull. 12 (10), 347-350.

Viarengo, A., Pertica, M., Mancinelli, G., Zanicchi, G., and Orunesu, M. 1980. Rapid induction of copper-binding proteins in the gills of metal exposed mussels. Comp. Biochem. Physiol. 67 C (2), 215-218.

Vijayakumari, K., Mohan, P.M., and Sasirababu, K. 1978. Effect of scorpion (*Heterometrus fulvipes*) venom on phosphatase activities in guinea pig. Curr. Sci. 47 (17), 650-652.

Vijayalakshmi, P., and Rao, K.V.R. 1985. Effect of Phosphamidon on tissue aminotransferase activities in *Metapenaeus monoceros* (Fabricius). Ind. J. Mar. Sci. 14 (3), 165-166.

Waldichuk, M. 1974. Some biological concerns in heavy metal pollution. In Pollution and physiology of marine organisms. Vernberg, F.J., and Vernberg, W.B. (eds). Academic Press. New

York. 1 - 57.

Watling, H.R., and Watling, R.J. 1982. Comparitive effects of metals on the filtering rate of the brown mussel (*Perna perna*). Bull. Environ. Contam. Toxicol. 29, 651-657.

Webb, J.L. 1966. Enzymes and metabolic inhibitors. Academic Press. New York.

*Wells, M. R., Phillips, J.B., and Murphy, G.G. 1974. Quoted from "Effects of cadmium toxicity upon the invivo and invitro activity of proteins and five enzymes in blood serum and tissue homogenates of *Mugil cephalus*".(Hilmy, A.M., Shabana, M B., and Dabees, A.Y. 1985) Comp. Biochem. Physiol. 81 (C), 145-153.

*Weinland, E.1919. Beobachtungen Uler den gaswechsel von *Anodonta cygnea*. Z. Biol. 69. 1-86.

West, E.S., Todd, W.R., Mason, H.S., and Van Brugger, J.J. 1967. Text book of biochemistry. Mac millan Pub. Company. London.

Wernstedt, C. 1944. Metabolism of gill epithilium of a freshwater mussel. Nature. 154, 463.

Widdows, Y., Salked, P.N., Moore, M.N., and Livingstone, D. 1980. The ecological effects of sublethal stress and pollution in estuarine invertebrates. In Environment and quality of life. Second environmental research programme, 1976-80. (Brussels Commission of the European Communities).

Wilbur, K.M., and Yonge, C.M. 1966. Physiology of mollusca. Academic Press. New York. 175-208.

Wilson, R.P. 1973. Nitrogen metabolism in channel fish *Ictatarus punctatus* L. Tissue distribution of aspartate and alanine aminotransferases and glutamic dehydrogenase. Comp. Biochem. Physiol. B. 46, 617-624.

Winge, D.R., Premakumar, R., and Rajagopalan, K.V. 1975. Metal-induced formation of metallothionein in rat liver. Arch. Biochem. Biophys. 170, 242 - 252.

Yager, C.M., and Harry, H.W. 1964. The uptake of radioactive zinc, cadmium and copper by the freshwater snail, *Taphius glabratus*. Malacologia. 1, 339-353.

Yoshino, T.P., and Cheng, T.C. 1976. Fine structural localisation of acid phosphatase on granulocytes of the pelycepod *Mercenaria mercinaria*. Trans. Amer. Microsc. Soc. 95, 215-220.

Yoshino, Y., Mozai, T., and Nakao, K. 1966. Biochemical changes in the brain in rats poisoned with an alkyl mercury compound, with special reference to the inhibition of protein synthesis in brain cortex slices. J. Neurochem. 13, 1223-1230.

Zaba, B.N., and Harris, E.J. 1978. Accumulation and effects of trace metal ions in fish liver mitochondria. Comp. Biochem.

Physiol. 61 B, 89-93.

Zaroogian, G.E., Morrison, G., and Heltshe, J.F. 1979. *Crassostrea virginica* as an indicator of lead pollution. Mar. Biol. 52, 182 - 196.

* Not referred to the original