DISCUSSION

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The results on the toxicity of lead and detoxification of it by using calcium in the freshwater bivalve, *Lamelliidens marginalis* shows that there are marked differences in the sensitivity to lead. The lead toxicity was studied by number of workers. This heavy metal Pb, enter in aquatic animals through various sources and it disturbs the metabolism. Kamble also confirmed this 2008; in freshwater gastropod mollusk, *Bellamea bengalensis*. This accumulated lead was entering in to higher vertebrate animals through food chain. The study on removal of this lead was carried in human beings by using various chelating agent like calcium, iron and other nutrients. Also the detoxification process was studied in several species of mussels, through the production of Calcium phosphate granules (Jeffree *et al.,* 1992; Byrne, 2000). As the information is very scarce and fragmentary with invertebrate models the present investigation is carried out to understand the toxic effects of Pb and chelating effect of calcium on lead accumulation. Bivalve molluscs such as mussels and oysters are commonly used in environmental monitoring programs because of their ability to concentrate relatively high concentrations of anthropogenically derived chemicals. Of the various molluscan species, metallothionein (MT) induction has been studied in greatest detail in the oyster, *Crassostrea virginica*, the first invertebrate species reported to possess metallothionein (MT) (Hamer, 1985).

The toxicity of heavy metal altered by physico - chemical parameters of water like pH, COD, BOD, Nitrate, Hardness, Calcium and heavy metals like Pb etc. discussion on the results obtained during the investigation, analysis of river water and experimental water, general toxicity, biochemical changes, enzyme activities, metal accumulation and histopathological changes observed in gills and mantle.

4.1 Physico - chemical parameters of experimental and collection site water:

Metal availability in aquatic organisms is influenced by many external factors such as season, pH, hardness of water, concentration and composition of particulate matter (Phillips, 1977; Luoma and Bryan, 1979). The physico chemical properties of experimental and collection site water used in this investigation were in accordance with the studies made by earlier investigator (Fadanis, 2008). A physico - chemical characteristic of water plays an important role in effects of aquatic organisms.

Temperature which is one of the major abiotic factor affecting the growth and survival of aquatic organisms is defined as, the measure of the hotness of any material. This measure of temperature of water is important basically for its effect on the chemistry and biochemical reaction in the organisms.

In the present study temperature of both water samples were similar with each other. Therefore there was no effect of temperature observed on Pb toxicity. In the case of Pb or any metal toxicity termpreture can alter the rate of toxicity. The toxicity of lead to *Gammarus pulex pulex* was decreased by decreasing temperature. Similar effects of temperature were shown by Bryant *et al.,* (1885) on the toxicity of chromium, arsenic, nickel and zinc to a variety of marine and estuary invertebrates. These environmental variables temperature can influence the solubility and toxicity of a toxicant (Jones, 1975). Denton and Burden-Jones (1982) measured the influence of temperature on the acute toxicity of heavy metals, including nickel, to the tropical banana prawn, *Penaeus merguiensis..* They found that the toxicity of all metals increased at higher temperatures, and that the toxicity of all metals tested. For nickel, toxicity was greater at high temperature, although the authors stated that the differences were not significant. Babich and Stotsky (1983) found that nickel toxicity to microbes in marine systems was reduced by decreasing temperature and incorporating simulated sediment.

There have been several reports on the effect of temperature on zinc toxicity. Jones (1975) and Fernandez (1983) both reported that zinc toxicity increased at higher temperature. McKenney and Neff (1979), in a study on the effects of temperature and zinc on the larval development of the grass shrimp *Palaernonetes pugio,* found viability reduced outside the optimal ranges of 17 to 27 gm and 20° to 27°C and that survival through complete larval development was progressively reduced in the presence of zinc. The study was also undertaken to establish the effect of temperature on nickel and zinc toxicity to 2 species of invertebrates which are of ecolôgical importance in European estuaries, the amphipod *Corophiurn volutator* Pallas and the bivalve *Macoma balthica* (L.). Bryant *et al.*, (1985) concluded that the metals are having their maximal effect in reducing the median survival times of the 2 species (*Corophium volutator, Macoma balthica)* when the concentrations of heavy metals are present in their optimal temperature conditions.

In the present investigation pH of both water samples detected were not so much differ from each other. The pH is one of the important parameter controlling the uptake of lead from wastewater and aqueous solution. The percentage adsorption increases with pH to attain a maximum at pH 5 – 6 there after it decreases with further increase in pH (Meena *et al.,* 2002). pH of the water bodies showed significant rise in values after the immersion of idols during festival. Increase in pH is due to the addition of plaster of paris which mainly composed of calcium sulphate. This pH carried various changes in freshwater bivalve, *Lamellidens marginalis* (Fadanis, 2008). Altretion in glycogen, protein, lipid and ascorbic acid of various organ of *L. marginalis* when exposed to subnormal or supranormal pH were studied by Muley *et al.*, (1995). They also stated that, during anaerobiosis, the fate of fermentatively generated ATP is hydrolysis for the support of various cell functions. Such demand might have occurred in gills and hapatopancreas exposed to abnormal pH in combination with endosulfan. The catalytic and regulatory properties of Phosphofructokinase (PFK) are also notably sensitive to pH (Ebberink, 1980). However, pH can

influence the distribution patterns of *C. fasciatus*, in coincidence with the conclusions of Courtney and Clements (2000) who found that sensitive Mayflies were eliminated from streams with lower pH and metal pollution conditions in experimental studies. According to Nussey *et al.* (2000), the toxicity of Pb is dependent upon the life stage of the fish, pH and hardness of the water, in addition to the presence of organic materials. Seymore *et al.* (1995) stated that as the pH of the water decreases, the ionic state of the metal becomes more prevalent and toxicity increases acutely. Therefore, to understand whether there is change in pH of water used for experiment, the pH study was carried out.

Dissolved Oxygen the most important chemical factor in freshwater for existence of life. The dissolved oxygen is responsible for the biological and biochemical procsses.The freshwater bodies receive oxygen from atmospheric absorption at the surface and as by-product of photosynthesis from the aquatic plants. In present study. In present investigation the DO of both water samples were within standard limits. The river water quality has deteriorated with respect to some of the major water quality parameters like Dissolved Oxygen (DO), Biological Oxygen Demand (BOD) and phosphates levels. *M. bruchina* and *M. dydima* were correlated with the concentration of DO and this association is closely related during winter for all sites except Buñirigo downstream studied by C. S. Ocon *et al.*, (2004). The reduced concentration of dissolved oxygen could be attributed to the presence of organic matter in high proportions (Paka and Rao, 1997). Effect of tannery wastewater on dissolved oxygen level of water is studied by (Kambale, 2008). The dissolved oxygen of the water bodies decreased significantly after the immersion of idols during festival (Fadanis, 2008). Dissolved oxygen affects the solubility and availability of many nutrients and therefore controls productivity of aquatic system. DO is very important to maintain animals in the experimental water.

In the present study, total hardness of the water bodies showed increase in values of experimental water as compared to values of collection site water.

Total hardness is the property of water which prevents the formation of lather with soap, caused mainly by the multivalent metallic cations (Guruprasad and Narayana, 2004). Major contribution of hardness comes from calcium and magnesium. Fadanis, (2008) observed significant increase in hardness in the river water after immersion of idols. This increase in hardness always affect on the Pb toxicity.

In the present study, a rise in BOD levels of experimental and collection site water was within standard limits. But BOD level of the collection site was observed to be somewhat higher than the water used for experiment purpose. The water contents high amount of pollutants showed low amount of BOD, it means water from the collection site was more polluted. Thus, temperature also affects the pH level of the water. Due to increase in temperature there is decrease in solubility of oxygen and intensification of BOD. Many substances are more toxic when dissolved oxygen in water is reduced (Carins *et al.,* 1975). Oxygen concentration in water is affected by temperature.

In the present study Chemical Oxygen Demand was found within a limit. Even water having more industrial effluent discharge show increased COD which may be harmful to living organisms. In tannery wastewater COD is also high due to use of chemicals in it and results in reduction of Dissolved Oxygen contents of water (Kambale, 2008). Aquatic organisms can not survive in water bodies contaminated with chemicals through and having poor Dissolved Oxygen. The micro - invertebrate Juan Blanco stream showed the highest values of BOD (16 mg/l), COD (122mg/l) and nutrient concentration due to industrial pollution (C. S. Ocon *et al.*, 2004).

In an aquatic ecosystem nitrates are formed on biological oxidation of organic nitrogenous matter received from domestic sewage, agricultural run off and industrial effluents. In addition to this metabolic waste, excretory product and dead organisms add organic nitrogen. In the present study, nitrates at experimental water showed 10.37 mg/l and collection site water showed 0.68 mg/l. The recorded nitrate was within standard limits. Nitrogen occurring in the freshwater is in the form of element or nitrogenous composition. Nitrogen is mostly derived from atmosphere and denitrifying bacteria giving nitrites and ammonia. Increased organic load in the water bodies alters the nitrate content in the water.

Calcium is an important nutrient essential for metabolism of organisms. It is mostly used as a chelating agent of lead. Lead inhibition of renal tissue increases in the vitamin – D dependent, calcium binding protein calbindin (Bogden *et.al.*, 1992) and interfere with energy metabolism e.g., reduced rate of glucose metabolism in brain capillaries of calves (Ahrens,1993). Calcium occurs 0.820 mg/gm and 0.996 mg/gm in entire male and female clams respectively while mantle showed 2.08 mg/gm and gill showed 0.594 mg/gm (Kale, 1997). The given amount of calcium from shell of *L. marginalis* was 316.875 mg/gm it means calcium is the major mineral in bivalve mollusc. In the present study calcium of collection site water was very high than experimental water.

Divalent lead ions cause significant public health hazards when present in drinking water at parts per million concentrations. The chief sources of lead in water are the effluents of lead and lead processing industries. In some plastic water pipes, lead is used as a stabilizer, which may lead to contamination of the water. Lead is also used in storage batteries, insecticides, food, beverages, ointments and medicinal concoctions for flavoring and sweetening (Sharma, 2001). The impact of sublethal concentration of Pb on the biochemical and physiological aspects of fish. This Pb accumulation in catfish, *Mystus gulio* was

studied by Kasthuri *et al.,* in 1997. In the present investigation, Pb level in both water samples was showed little fluctuation.

4.2 Accumulation study:

Pollutants, introduced into the environment, are distributed in water, sediment, and biota. In order to determine the concentration of a contaminant surveys in one of those media can be chosen. However, all the surveys have negative and positive sides. Pollutants in water exist in solution, suspension or when they are absorbed. Mixing or settling of sediment also affects the amount in water. Both water and sediment analysis does not show anything about the bioavailable part of a pollutant and therefore also about toxic effects (Phillips, 1977).

Bivalve molluscs are one of the most suitable bioindicators because they are sedentary, widespread and have a long life span (Farrington *et al.,* 1983). Despite many studies on the uptake of stable metals in bivalves, variables affecting the results of bioindication surveys remain partly unknown. Some data is available on the effects of season, salinity, water temperature, and coexistence of several metals, organisms age, weight, size, and sex on the uptake of metals (Phillips, 1977). The average concentration of Cu reached 5.5 µg/g in specimens of *A. cygnea* inhabited the Vilhelmo Channel near the waterworks. The amounts of Cu, Ni, and Zn were highly variable in the tissues of *A. cygnea* inhabited the channel either near the waterworks. The concentration of Cu in the tissues of *U. tumidus* was 7.6 µg/g. Although the high individual variability of Ni concentrations was determined in the tissues of Unionidae specimens, *U. tumidus* have accumulated from both sites on average exactly the same amount of Ni 0.9 µg/g (Dalia, 1999).

Positive relationships between metal concentrations in whole body tissues and body size have been reported occasionally from a variety of bivalves and gastropods (Boyden 1974, 1977, Cossa, 1989, Odzak *et al.* 1994). It is likely that

when tissues grow more quickly than the metal can be absorbed; there will be a reduction in metal concentrations in soft tissue. Since in nearly all species, smaller (younger) individuals grow faster than the older ones, dilution of metal concentrations by tissue growth should have a greater effect in smaller individuals than in larger ones, causing a positive slope in the metal concentration body size relationship (Strong and Luoma 1981).

The different accumulation patterns between Zn and Cu on the one hand and Cd on the other may be related to the fact that Zn and Cu are essential for metabolic activities. For example, carbonic anhydrase, carboxy pepeptidase A and B and several dehydrogenases contain zinc, while haemocyanin contain copper. Conversely, Cd is considered a non-essential metal because it has no known biological function (Bryan, 1984). Accumulation patterns for essential metals include regulation of body metal concentration, accumulation without excretion, and accumulation with some excretion either from the metabolically active pool or from the detoxified store (Depledge and Rainbow, 1990; Rainbow, 2002). In the littoral crustacean, *Palaemon elegans*, the body concentration of Zn does not show any change over an increasing range of dissolved Zn exposures until a threshold external dissolved availability is reached. Non-essential metals may be accumulated without excretion or with some excretion (Rainbow, 2002). Much of the Cd accumulated by aquatic invertebrates is bound to metallothionein in the cytosol of the organ predominantly used for accumulated Cd storage (Langston and Zhou, 1987; Rainbow, 2002). The interactions obtained in the accumulation of Cd in mixed solutions suggested synergistic effects at the low.

High amount of heavy metals in mantle may be due to more surface area of the organ and in gills as it circulates more water for respiration (Satyaparameshwar *et al.,* 2006). It is a well known fact that the gills of aquatic animals have the ability to excrete invading heavy metals (Nakatani, 1966). Absorption of heavy metals by crustaceans could be a process of diffusion through gills is due to absorption of metals onto the cuticle of the gills (Bryan,

1964; Engel, 1983). The metal thus entering the gills get bound to the soluble ligands like protein and is then transported through blood to organs like hepatopancreas etc (Engel, 1983).

Pb is also known to accumulate in the tissues of fish, including skeletal bones, gills, kidneys, liver and scales (Nussey *et al.,* 2000). Seymore *et al.* (1995) stated that the uptake of aqueous Pb^{++} across the gills into the blood stream is the primary mode of uptake in freshwater fish. According to Nussey *et al.* (2000), the toxicity of Pb is dependent upon the life stage of the fish, pH and hardness of the water, in addition to the presence of organic materials. Increasing concentrations of the metals Pb cause significant increases in the mortality in both brood and larvae of *C. kraussi.* Increasing Pb concentrations in permutation with varying salinities exhibit a significant negative influence on brood and larval development of *C. kraussi.* The synergistic effect of Pb result in significantly higher mortalities on brood and larvae of *C. kraussi* than these metals on their own. According to Kamble (2008). Pb is more toxic and having with high accumulating power than Cd and Zn in freshwater gastropod, *Bellamea bengalensis.*

In present investigation accumulation of Pb was higher in 16 days treatment of 28 ppm Pb. But this accumulation was affected by calcium chelating. Therefore, calcium can be used for remove Pb stress from freshwater mussel, *Lamellidens marginalis.*

4.3 Behavioral study:

The toxicity effects of heavy metal Pb were observed on behavior of animal also. Due to high Pb concentration the animal showed stress behavior. The movement of bivalve, *L. marginali* was affected first due to 28 ppm dose of Pb at 8 and 16 days exposure periods. The large exposure leads into death. The similar results were observed by Zupo, (2000) in *H. inermis* expoed to high water temperature, reacts very sensitively to artificial environmental conditions. Wolf *et*

al., (1998) studied effects of Cd on *Daphnia magna.* The survivability decreased with increasing Cd concentration. During the adaptation reaction, a part of the metabolic energy is used to restore this imbalance. The protection reaction is characterized by decreased motility. During this phase, spontaneous muscular activity becomes depressed due to higher maintenance costs resulting in higher metabolic rates in certain non-muscular tissues (Heath, 1995, Knops *et al.* 2001). The 1st order escape reaction is characterized by increased ventilation and motility, whereas the 2nd order escape reaction is characterized by very greatly increased ventilation activity and motility due to powerful beats of the pleon. This energy mobilization during the 2nd order escape reaction is the last chance for the animal to escape unfavorable environmental conditions, or otherwise it will die (irreversible toxicological effects). A protection reaction was shown by the test animals exposed to lower Cd concentrations were observed. In this phase, they utilized their metabolic energy for increased muscle activity. Similar stress response mechanisms have been described for other crustaceans, like daphnids (Wolf *et al.* 1998, Untersteiner *et al.* 2003). In the amphipod *Gammarus pulex* (L.), Gerhardt (1999) observed a general behavioral response pattern to toxicants, the so-called stepwise stress model. The model describes how reaction to a toxic compound, an organism shows a sequence of behavioral stress responses above their respective threshold of resistance. Within a species-specific tolerance range, organisms can regulate their behavior (e.g. ventilation behavior) in order to keep their body functions unaffected. The model further states that beyond an organisms resilience range, toxic effects appear, such as hyperactivity, resulting in exhaustion, decreased activity, and ultimately lethality. To avoid such toxic effects, organisms are able to switch to another stress-response behavior. This behavioral plasticity of organisms as a strategy against toxic stress as it is explained by the stepwise stress model has also been observed in other crustaceans, like the freshwater prawn *Macrobrachium nipponense* (Crustacea: Palaemonidae) (Gerhardt *et al.* 2002). Heavy metal stress behavior were also studied by Kamble, (2008) in freshwater gastropod

mollusk, *Bellamea bengalensis* exposed to Pb, Cd and Zn. Ranade, (1977) also studied behavior of mussle, *Mytilus virides* for different salinity changes.

4.4 Biochemistry and metabolism:

Bivalves being a source of rich protein as compared to other non vegetarian diets. Mostly it is important diet of people who lives near sea region and fresh water reservoirs. Variation in protein constituents of bivalve helps in understanding their correlation with ability of bivalve to overcome toxic effects and also the changes induced by heavy metal toxicity.

Proteins play vital role in building up material of bodily tissues and it is regarded with various functions of body tissues of not only human but also bivalves. Number of workers has worked on biochemical study pertaining to protein aspects. Becker *et. al.*, (1975) studied the effect of starvation on total protein and hemoglobin concentration on haemolymph of *Biomphalaria glabrata* and reported that the protein content of the haemolymph of *B. glabrata* was clearly decreased by 7 days of starvation, also in starvation proteins were the main substrate (Brand *et.al.,* 1957). It was also reported by Brand *et.al,* 1957 that the total protein in various organs of *Samperula maculate* were decreased significantly, after starvation.

The protein is affected by not only the starvation but also by various toxic environments. The protein content in all the tissues of fish, *Labio rohita* decreased considerably upon acute exposure to 30% electroplating effluent (Karanjkar, 2001). He also worked out that the decrease in protein content was different for different tissues. Also the tannery effluent badly affect the protein level in fresh water prawn, *Macrobranchium idella* and this protein level was decreased with increasing effluent concentration(Subramanian *et al.*,1993).

Wardle, (1972) found decrease in protein during biochemical and physiological stress of animals. This protein content in pulmonate snails, *Cassidula nuaeus* and *Melampus ceylonics* was changed due to stress environment.

Now, it has been realized that heavy metal pollution is a vary serious problem all over the world. These heavy metals through food chain enters in animal body and affects protein content of animal tissue. Heavy metals from the sediments easily enter in the body of aquatic animals like bivalve mussels. Many scientists had taken keen interest to study from the various angles the toxicological effects of heavy metals in the bivalve mollusc.

Depletion in the protein content has been observed due to heavy metal toxicity in other bivalves and gastropod mollusks. Protein content in the mantle, hepatopancreas and adductor muscle of bivalve *L. marginalis* was decreased due to mercury toxicity (Patil, 1998) also Mule and Lomate (1994) observed decrease in protein concentration in mantle, foot and whole body due to mercury toxicity in gastropod, *Thiara tuberculata.* Effect of tannery effluent on the biochemical constituents like total free amino acids, total protein, glycogen and lipids in different tissues like gills. mantle and foot of *Pila globossa* were studied by Varadraj *et al.*, (1994). The work suggested that protein and other constituents were decreased on the dose of the effluent and duration of exposure period.

In snail, *Bellamea bergalensis*, the glycogen, protein and lipids were significantly decreased due to intoxication of Zn, Cd and Pb (Kamble,2007). Also this decrease was directly proportional to the concentration and time of exposure period. Particularly in Pb toxication the decrease in protein and other constituents was very fast, acute and highest.

In gastropods, protein content was decreased due to the herbicide toxicity (Chaudhary *et al.*, 1998) during this type of stress condition the protein synthesis and interconversion of amino acids, glucose and fatty acids to liberate energy get affected (Mane *et al.*,1986). Molluscicide copper sulphate induced toxicity in a fresh water pulmonate, *L. luteola* was studied by Mathur, (1994) in mantle, foot and HP. Ahmed *et al.,* (1977) and Ramana Rao and Ramamurtny (1981) studied the effect of heavy metals on terrestrial snail, *Zootecus insularis.*

This decrease in protein synthesis is due to binding of lead to phenylalanyl and lysyl t- RNA. Hence, these two components fails to form complex with ribosome in rat (Dhar and Banerjee, 1979). Another reason for depletion in protein is by toxic dose of lead acetate and lead nitrate induced DNA breaks determined by nick translation (Royetal, 1992). Further, it gives support to above work and stated that, in Chinese hamster cells, lead acetate enhance the mutations induced by N-methyl-N-nitro-N-nitrosoguanidine (MNNG) by lead. Once DNA damaged, its repair was inhibited by lead ions interaction (Hartwig,1994). Also due to toxic effects or heavy metal stress condition, membrane permeability becomes altered (Albel, 1974) and the amount of RNA decreased because of this, protein level gets depleted. Bivalve mollusks contain large amount of free amino acids in their extra cellular fluids which play an important role in the osmoregulation and ion regulation (Schoffenits *et al.*, 1972). Mantle tissue of *L. marginalis* exposed to abnormal pH might have an additional demand of proteins. This protein undergoes catabolism to overcome from stress condition. The use of protein as a substrate is indicated by a quantitative increase in ammonia excretion in case of bivalves (Muley and Mane, 1995).

The amounts of protein content are lowered in the animals collected from the heavy metal polluted environment. Thus, it appears that the proteins and phospholipids in these organs are essential for synthesis of mucoproteins and glycoproteins. Therefore they are gradually decreased in the polluted animals. These macromolecules might be utilized for the compensation of loss of epithelial cells, tissues deformation, tubular dilation, atrophy, macrophage aggregates, tubular cell necrosis, cell vacuolation and tissue inflammation of the organs like mantle and gill induced by the heavy metal deposition in these organs (Bhoite, 2006).

In present investigation, a significant decrease in protein content in all the tissues observed in animals exposed to lead, this decrease mainly due to severe stress exerted by lead resulting in increased proteolysis caused depletion in the

protein content. The decrease was also observed in 28 ppm and 56 ppm dose of calcium for 8 days, but this decrease was at very minor level. The protein levels were increased due to increase in calcium exposure period. At Pb exposure significant decrease was observed. The animals treated to lead and calcium showed much improvement in protein content. The calcium showed chelating effect and therefore, forms a complex with lead and increases its absorption. Thus, very little alteration in protein were observed.

Enzymes are biochemical catalysts for the chemical reaction that occur in the biological systems, which are necessary for the metabolic processes within the tissues studied on the enzyme activities help a great deal in giving deeper insight into the vital phase of metabolic pathways.

Acid and alkaline phosphatases are collective terms. They denote group of several hydrolytic enzymes, which occur in higher concentrations in the location specially related with secretion, absorption and excretion. The role of biological significance of phosphatases has attracted considerable attention (Von Brand, 1952, Erasmus, 1957), but it still remains to be undecided and controversial issue. Presence of phosphatases at the transport site of substrates has been noticed in several studies. Changes in the phosphatase activity in bivalve and other animals due to heavy metal toxicity have been studied. Knowledge of the distribution of a toxic metal within the cells is important for understanding the clinical picture of intoxication. It is a phosphate monoester, which catalyzes the hydrolysis of a wide variety of phosphate monoesters and phosphoproteins. Yamagen, (1963) suggested that ACP play important role in the breakdown of phospholipids. The pH optimum is 4.8 to 5.

Acid phosphatase is a phosphomonoesterase, which catalyzes the hydrolysis of wide variety of phosphate monoesters and phosphoproteins. The phosphodiesters and phospholipids are not hydrolyzed by acid phosphatase. The enzymes attacks pyrophosphate from several sources. In addition to the work on

chemistry of acid phosphatase. Variations in the ACP activity in the degenerating tissue and cells have been reported by various workers and furthermore, association of ACP along with other lysosomal enzymes in the lysosomes definitely indicated a lytic role for this enzyme in the functioning of cells and tissues. A role in intracellular digestion has been postulated by Duve and Wattiaux, (1966) and Gahan, (1967), Allison, (1967) believed that lysosomes might function as trigger for cell division by hydrolyzing certain chemical inhibitors of cell division. Das and Banergiee, (1980) demonstrated the changes in activity of lysosomal enzymes.

Acid phosphatase has been studied in various tissues in relation to secretary process. Novikoff, (1962) suggested that acid phoaphatase may be involved in secretary granules condensation or it may be 'carried over' as membrane bounded sites of activity on the membrane of the secretary granules (Osinchak, 1964). Smith and Farquhar, (1966) suggested that ACP plays an important role in regulating the secretary process by incorporating and degrading undischarged secretary granules especially in endocrine glands. Helminer and Ericsson, (1970) have also suggested a role for prostatic acid phosphatase in regulation and modification of secretary process.

Lysosomes are surrounded by unit membrane (Novikof, 1993) and can easily be ruptured by the accumulation of mercury compounds to release ACP (Taylor, 1965). On account of this, enzyme may diffuse into the cell and is utilized for the digestion of other cellular organelle. Much work has been done on functional role of ACP and toxic effects on the enzyme activity in liver and kidney. Comparatively little is known on the ACP activity in intestine of fish.

Mahendra and Agrawal, (1983) reported increased ACP in hepatopancreas, mantle, intestine and foot of *Lymnaea acuminate* exposed to phorate and mexacarbate. In present study ACP activity was increased with increasing toxicity of Pb while this level of ACP was depleted by Calcium supplementation and finally came near to the control one.

On exposure of *Lamellidens marginalis* to lead acetate resulted into increase in acid phosphatase in high amount acid secretion due to lysosomal rupture caused by heavy metal stress. This activity was comparatively high on 16 days exposure which might be due to long exposure period result into more harm to tissue. But when bivalve were exposed to calcium very small alteration were observed in acid phosphatase in both gill and mantle. In remedial group different concentrations of calcium and lead showed good results. The ACP activity was very close to that of normal, which is the indication of less lysosomal rupture which proves the chelation effect cause by calcium.

Phosphatases exhibiting optimal activity at high pH values are known as alkaline phosphatases (Aruna and Muley, 1987,88). ALP (Orthophshoric monoester phosphohydrolase) is known to be widely distributed in animal tissues. Besides their vital role in the metabolism of carbohydrates, nucleotide and phospholipids (Summer *et al.*, 1955), they are involved in the liberation of newly synthesized protein from the nucleoprotein complex (Bradfield, 1951). There were much literature available on alkaline phosphatase activities in variety of fishes. Rana (1970) studied the alkaline phosphatase activity in the respiratory

organs. Alkaline and β-glucoronidase in gills of two fishes *Heteropheustus fossils* and *Labeo roita* in relation with cadmium toxicity.

Rana *et al.*,(1982) has shown that there is dose response relationship between the amount of mercury retained in the liver and inhibition of enzyme (i.e. alkaline phosphatase, glucose-6-phosphatase, amylase, maltose, lactose, lipase and dehydrogenases).

Kargarot, (1984) showed an increased acid phosphatase activity in the secondary gill lamellae and pillar cells system of *Channa punctatus* exposed to zinc. According to them, the acid phosphatase release from ruptured lysosomes. Satry *et al.*, (1985) has shown that acid phosphatase activity was inhibited in liver, ovary and gills but the enzyme activity increased in kidney and intestine, when freshwater catfish, *Heteropheustes fossils* exposed to cadmium (0.26mg/l) of 15, 30 and 60 days. The activity of alkaline phosphatase decreased in liver, kidney and intestine but elevation was recorded in ovary and muscle in above exposure period and concentration. Katoley and Katoley, (1988) demonstrated the alkaline and acid phosphatase activity in gill of mosquito fish, *G. affinis* exposed to low doses of cadmium chloride. They further reported that the acid phosphatase activity was more in comparison with alkaline phosphatase.

Alkaline phosphatases from other sources are fairly heterogenous, and it is uncertain whether they represent separate genetic groups or whether the differences result from carbohydrate prosthetic groups. Alkaline phosphatase from an osteosarcoma has been shown to be completely different from liver, intestinal, kidney and placental alkaline phosphatase on immunological grounds (Singh and Tsng, 1975). Alkaline phosphatase contains zinc as an integral part of the molecule and is therefore considered a metalloenzyme. Its active centre contains a serine residue, and the mechanism of action involves the formation of phosphoryl serine residue. The enzymes contain a number of carbohydrate

prosthetic groups including sialic acid, but the amount varies from one type to another.

High concentration of alkaline phosphatase is often associated with absorptive cells, especially in the brush borders of the intestinal mucosa and the proximal tubules of the kidney. Alkaline phosphatase is always present wherever calcification occurs, particularly in osteoblasts and in chondrocytes of cartilage that is about to give way to endochondral bone formation.

In addition to being able to hydrolyze a wide variety of monophosphates, alkaline phosphate is also quite capable of hydrolyzing pyrophosphate (Cox and Griffith, 1965). It has also been reported to hydrolyze ATP (Moss and Walli, 1969). It has long been known that most types of alkaline phosphatases are activated by magnesium ions, but Moss (1969) showed that only the orthophosphatase activity of alkaline phosphatase is activated, magnesium ions actually inhibit pyrophosphatase activity. Zinc ions inhibit alkaline phosphates of bone and cartilage (Takada *et al.*, 1968). Highly specific inhibitors of alkaline phosphatase, L- tetramisole, and related compounds were introduced by Borgers (1973). These inhibitors make it possible to distinguish clearly between alkaline phosphatsase and other phosphatase whose specificities overlap with alkaline phosphatase (Borgers and Thone, 1975 and 1976).

Lead exposure caused a significant inhibition of hepatic and renal activities of glutamic pyruvic transaminase (GTP), glutamic oxaloacetic transaminase (GOT) and alkaline phosphatase (ALP). Hepatic ALP activity increased following $CaNO_3$ DTPA treatment (Tandon, 1992). High alkaline phosphatase activity, high type 1 collagen synthesis. Cyclic adenosine monophosphate (cAMP) and intracellular calcium responses to parathyroid hormone and an osteocalcin response to 1, 25 dihydroxy-vit. D3. ALP activity and the synthesis of an anti apoptotic protein, encoded by the proto-oncogene Bcl-2 are both characteristic of bone cells (Liebeherr *et al.,* 1993).

They are typically membrane bound enzymes that are often associated with brush borders. Increase in membrane permeability does not cause their release into plasma (Stockham, 2002; Bain, 2003). Only increases in serum ALP measurements are helpful clinically. There are no significant causes of decrease in ALP (Willard *et al.,* 1999). Measurement of ALP concentration in urine has been used as an indicator of early toxic tubular injury (Wisloff *et al.*, 2003). ALP is most helpful in detecting hepatobilliary disease in small amimals if an increase in serum ALP activity is seen, it is important to consider young age, drug therapy and hyperadrenocorticium as causes of increased enzyme activity before proceeding with a hepatic biopsy (Willard *et al.,* 1999). Here the ALP activity was inhibited by lead stress and thus administration of calcium removes the lead and results into alkaline phosphate close to normal.

4.5 Histological study:

In the present investigation bivalve, *Lamellidens marginalis* exposed at different concentrations of Pb and Ca, for different exposure periods showed changes in the structure of the gills followed by the disintegration and vacuolization of connective tissue. Similar kinds of observations were reported in gills of bivalve, *Parreysia cylindrica* exposed to pesticides (Lomate and Wayekar, 1998). In aquatic mollusk the main respiratory organs are gill and mantle. In bivalves the gills have taxanomic importance as the class bivalvia is further classified into different orders on the basis of their structural pecularities. In mussels, as in some gastropods, apart from their respiratory activity, the gills are involved in mucocilary mode of feeding. The cilia present over the surface of the gill create water current, which enters into the mantle cavity through the inhalant aperture and passes out through exhalent aperture. The particles which enter into the mantle cavity along with the water current by the action of cilia get spread over the surface of gill and then get directed to the mouth on the basis of which White, (1937) has suggested that the primary function of bivalve gills is to collect food rather than respiration. Apart from respiration and mucociliary mode

of feeding the gills in the freshwater mussel, also act as a marsupium in which the fertilized eggs develop into the larvae (Yokley, 1972).

Earlier contributions on the bivalve gills were mainly with reference to their, anatomical and histological structure. The gills have been also studied with reference to feeding mechanism (Patil, 1974). Gilmer (1974) have attributed the role of mucus secretion to the gills and in the transport of food particles. They have also described the mucociliary mechanism of gill epithelium which performs major defence activity of clearing foreign bodies from surface of air exposed cavities and direct the mucus coated material towards the mouth. Motokawa *et al.*, (1975) has pointed out an importance of the calcium in the control of ciliary movement. Wright *et al.*, (1975) has observed transport of amino acids by isolated gill of marine mussel, *Mytilus californianus.* Wright and Stephens, (1978) have pointed out that the amino acids are removed during a single passage of water across the gill of marine mussel. Eble Albert, (1968) has demonstrated the presence of glycogen, glycogen-phosporylase and branching enzymes, histochemically in the gill of American Oyster. Seah and Hobbden (1969) have reported the presence of Mn++ in the gill of freshwater bivalves. Pastells (1971) has noticed acid phosphatase activity in gills. Paparo and Caleb, (1972) have noticed catecholamine in the gill of bivalve. Lagerspetz and Senius, (1979) have pointed out that the stimulation of ATPase activity is done by Na+/K+ in gills of freshwater mussel, *Anodonta.*

Reid and Reid, (1969) have demonstrated the presence of acid mucosubstance secreting cells in the gills of marine bivalves by employing alcian blue staining technique. Kale and Patil, (1977) have analyzed chemical nature of gill- mucin histochemically and pointed out that the gills secrete both neutral and acid mucopolysaccharide. In the gills, the ciliated margine was badly affected due to Pb toxicity. The epithelial cells, mucocytes and columnar cells were swollen and showed atropy. These Pb induced changes were very acute, severe and permanent (Kamble, 2008).

Bivalve gills are unique organs that show continuous terminal growth by the addition of new elements in correlation with the lifelong increase in shell size (Neumann and Kappes, 2003). In aquatic vertebrates and invertebrates gills are the organs directly exposed and the most susceptible to environmental variations hence they are target tissues for different pollutants (Rodriguez *et al.*, 2005).

In the present investigation gill was damaged due to Pb toxicity and this damage was increased with exposure time period. After calcium supplementation some curative effects were observed and these positive altraretions were dose dependent.

Each mantle lobe, at its free margin is usually divided into three folds. The outer fold in close contact with the shell is primarily secretary in function, the middle fold is considered to be sensory and the inner fold a muscular flap controlling the flow of the water into the mantle cavity (Hyman, 1967; Hillman, 1968). The literature available on the bivalve mantle mainly deals with its morphology, feeding process (Bernard, 1974; Petit *et al.*, 1978) and shell formation (Kale and Patil, 1976, 1977).

Mucus secretion is also one of the important functional activities of the mantle and it is suspected that mucopolysaccharides secreted by the mantle get involved in many, hitherto unsuspected, physiological roles such as feeding and shell formation (Preznt, 1979). In the epithelium of mantle neutral mucopolysachharides, glycogen and protein have been reported by Mane and Patil, (1980). Hillman, (1968) has pointed out two types of mucous cells, on secreting acid mucopolysachharides and the other neutral mucopolysachharides. Further, he has pointed out that the acid mucopolysaccharides are both non sulphated and sulphated in nature. Kale and Patil, (1976, 1977) have pointed out three types of mucous cells in the ventral mantle edge of *Parreysia corrugate Var nagporensis* containing neutral mucopolysachharides, sulphated and non sulphated acid muucopolysachharides. According to Harris, (1969) and Wilbur,

(1972) the mucopolysaccharides secreted by the mantle along with the protein involved in the transport of calcium ions which are essential for the formation of shell.

In the present investigation Pb toxicity also create similar effects on mantle tissue and this damage was increased with exposure period. After calcium supplementation some curative effects were observed and these positive altraretions were increased with increase in calcium dose.

The histological picture of collagens provides valuable information about the extent of tissue damage due to heavy metal toxicity. Histological alteration in collagen of freshwater bivalve, *L. marginalis* may cause the histological disorder, which lead to severe and permanent disorder in animal. Collagens are proteins that constitutes the major structural element in extracellular matrix. It is principle protein of metazoan connective tissue (Adams, 1978). It is also principle structural protein of connective tissue of most of the invertebrates (Gosline, 1983). He also stated that mollusk bivalve collagen is similar with vertebrate collagen in their structural properties. There is limited but growing body of evidence indicating that invertebrates tissue collagens are similar to, and evolutionarily related to, vertebrate collagens (Adams, 1978).

With heating, collagen fibers shrink to one third of their original length and become rubbery (Gosline, 1983). The effect of the presence of various electrostatic and hydrophobic surfactants in the environment on collagen fibrilogenesis has been studied by Honya *et al.*, (1974). They also concluded that, the rate of collagen fibril formation and fibril – fibril interactions were influenced by environmental factors. In mollusk gills and mantle are mainly consist of connective tissue and the connective tissue is the main site of collagen synthesis. The net of collagen and elastin fibers crosslink with each other to form compact structure of connective tissue. in freshwater bivalve collagens were

distributed all over the tissue. The main role of collagen is to provide rigid structure.

In present study, collagen structure was affected by Pb toxicity because of this damages occured in gill and mantle tissue. These damages were cured by calcium doses.

4.6 Chelating effect of Calcium :

The potential importance of metallothionein (MT) in toxicologic responses to metals was recognized at the time of its initial discovery (Kagi,1960). These low molecular weight, metal-binding proteins and polypeptides (Fowler,1987) are inducible by metals and are believed to participate in functions associated with the metabolism and detoxification of metals (Kagi,1987). Although the precise cellular function of MT has remained elusive to investigators, there is considerable evidence to support a purported role in regulating or controlling the intracellular availability of essential metals such as Cu and Zn and the nonessential metal Cd. MTs are capable of donating Cu and Zn to appropriate receptor molecules such as metalloenzymes (Udom 1980;Brouwer, 1991) and transcriptional Metallothionein are strong chelating agents of heavy metals.

The sharp increases in Ca levels observed in bivalves exposed to heavy metals, but not in the controls, indicate an alteration of Ca homeostasis as a result of heavy metals accumulation. A significant increase of total Ca content was also found in the gills of mussels exposed to Cu (Viarengo *et al.*, 1988b) and in the digestive gland after exposure to Cu in presence of hydrocarbons (Viarengo *et al.*, 1988a). The pattern of Ca variations in the digestive gland was rather similar to those of the heavy metals. The increase in Ca concentrations in whole organisms exposed to Cu or to Cd, much larger than those observed in some organs, probably reflect the high content of Ca in the kidney, where numerous granules containing Ca phosphate were found to accumulate in renal cells during exposure to metals. The gills of *Donacilla cornea* are used for

monitoring heavy metals in seawater; its digestive gland is particularly studied on the effect of heavy metals on Ca homeostasis (Regolil, 1991).

Ca, vitamin C, thiamine and iron etc. are used as Pb chelating agents. Ascorbic acid (Goyer and Cherian ,1978) or thiamine (Flora *et al.*, 1986) greatly enhances the efficacy of calcium versenate in Pb intoxification including mobilization of Pb from central nervous system. The administration of ascorbic acid plus Zn (Papaioannou and Sohler, 1978) or ascorbic acid plus Fe (Suzuki and Yoshina, 1979) helped in amelioration of Pb intoxification. Thiamine prevents the toxic effect and clinical symptoms of Pb poisoning (Bratton *et al*, 1981). There may be a complex interactive effect between different nutritional components; in this case, calcium, phosphorus and vit.D. The highest blood lead levels in children were associated with both low calcium and low vitamin-D intake (Soreell et.al;1977). As understanding of calcium mediated cell functions has expanded, the hypothesis that a fundamental calcium against role might be central to lead toxicity has been postulated (Pounds J.G. *et al.*, 1984, 1991).

The foregoing discussion suggests that the exposure of the fresh water bivalve, *Lamellidens marginalis* to the heavy metal lead at $1/10^{th}$ of LC_{50} concentrations as 28 ppm and also its chelating agent Ca^{++} at three different concentrations, 28 ppm, 56 ppm and 84 ppm leads to the accumulation of Pb and its detoxification by Ca^{++} in the organs like gills and mantle. It also alters biochemical composition as well as metabolism, clearly shown by enzyme studies. It also had its impact on the collagen synthesis of the animal. These all alterations were shown by light microscopic study. In present investigation, Ca was used as chelating agent of Pb. Due to this chelating therapy Pb stress was successfully decreases with increasing Ca concentration.