INTRODUCTION

Introduction

Water and life are closely linked. This has been recognized throughout history by civilizations and religions and is still the case with scientists today. Liquid water is required for life to start and for life to continue. No enzymes work in the absence of water molecules. No other liquid can replace water. We are very fortunate, therefore, that our planet is so well endowed. Water is a common material in the Universe, being found as widely dispersed gaseous molecules and as amorphous ice in tiny grains and much larger asteroids, comets and planets, but water needs particularly precise conditions to exist as a liquid as it does on Earth. It is most likely that this water arrived from multiple sources, such as comets and asteroids, somewhat after solid planet Earth was formed.

Water possesses particular properties that cannot be found in other materials and that are required for life-giving processes. These properties are brought about by the hydrogen-bonded environment particularly evident in liquid water. The earliest life forms appeared in water; nearly all fish live exclusively in water, and there are many types of marine mammals, such as dolphins and whales that also live in the water. Some kinds of animals, such as amphibians and some mollusc spend portions of their lives in water and portions on land. Plants such as algae grow in the water and are the basis for some underwater ecosystems. Plankton is generally the foundation of the ocean food chain.

Water is necessary for all living animals and human being are not exception. They can survive for several weeks without food, but for only a few days without water. From this one can understand the neccessity of water in our life, but nowadays this water is getting polluted by various pollutants. Water pollution is todays major problem infront of the world beacause there are so many water pollutants and they can not be easily removed from water. Water pollution is the contamination of water bodies such as lakes, rivers, oceans, and groundwater caused by human activities, which can be harmful to organisms and plants, which live in these water bodies.

Although natural phenomena such as volcanoes, algal blooms, storms, and earthquakes also cause major changes in water quality and the ecological status of water, water is typically referred to as polluted when impaired by anthropogenic contaminants and either does not support a human use (like serving as drinking water) or undergoes a marked shift in its ability to support its constituent biotic communities. Water pollution has many causes and characteristics. The primary sources of water pollution are generally grouped into two categories based on their point of origin. Point-source pollution refers to contaminants that enter a waterway through a discrete "point source". Examples of this category include discharges from a wastewater treatment plant, outfalls from a factory, leaking underground tanks, etc. The second primary category, non-point source pollution, refers to contamination that, as its name suggests, does not originate from a single discrete source. Non-point source pollution is often a cumulative effect of small amounts of contaminants gathered from a large area. Nutrient runoff in stormwater from sheet flow over an agricultural field, or metals and hydrocarbons from an area with high impervious surfaces and vehicular traffic are examples of non-point source pollution. The primary focus of legislation and efforts to curb water pollution for the past several decades was first aimed at point sources. As point sources have been effectively regulated, greater attention has come to be placed on non-point source contributions, especially in rapidly urbanizing / suburbanizing or developing areas.

The specific contaminants leading to pollution in water include a wide spectrum of chemicals, pathogens, and physical or sensory changes. While many of the chemicals and substances that are regulated may be naturally occurring (iron, manganese, etc) the concentration is often the key in determining what is a natural component of water, and what is a contaminant. Many of the chemical substances are toxic. Pathogens can produce waterborne diseases in either human or animal hosts. Alteration of water's physical chemistry includes acidity, electrical conductivity, temperature, and eutrophication. Eutrophication is the fertilization of surface water by nutrients that were previously scarce. Water pollution is a major problem in the global context. It has been suggested that it is the leading worldwide cause of deaths and diseases and that it accounts for the deaths of more than 14,000 people daily.

In the developing world, 90% of all wastewater still goes untreated into local rivers and streams (UNEP International Environment; 2002). Some 50 countries, with roughly a third of the world's population, also suffer from medium or high water stress, and 17 of these extract more water annually than is recharged through their natural water cycles (Ravindranath *et al.*, 2002). The strain not only affects surface freshwater bodies like rivers anc lakes, but also degrades groundwater resources.

In India, up to 70% water spread area has gone polluted (Basu, 1986). Many times water pollution is due to toxic heavy metal which has received worldwide attention as the heavy metals play important role in determining drinking water quality (Ponangi *et al.*, 2000). Metals may occur in the aquatic environment as hydrated ions or they may form a number of soluble and insoluble complexes with different organic and inorganic ligands (Vanloon, 1977). The term 'heavy metal' although often not rigidly defined is commonly held for those metals, which have specific weights more than $59/cm^3$ (Hollemman and Wiberg, 1995).

There are about 40 elements that fall into heavy metal category. Heavy metals constitute a very heterogeneous group of elements widely varied in their chemical properties and biological functions. These metals are largely found in disperse form in rock formations, Industrial processes involving the use of water fossil fuel combustion, discharge of waste waters or effluents from various industries, primarily electroplating, metal finishing, textiles, painting and dyeing, is one of the main cause of water pollution by toxic heavy metal (Ponangi *et al.*, 2000, Nair; 1984). Heavy metals are largely found in disperse form in rock formations. These metals have largest availability in soil and aquatic ecosystems and to a relatively smaller proportion in atmosphere as particulate / vapours.

Heavy metal toxicity to plants vary with plant species, specific metal, concentration, chemical form and soil composition and pH, as many heavy metals are considered to be essential for plant growth. Similarly, many heavy metals are essential trace nutrients of animals and human body (Wintz *et al.*, 2002). Some of these heavy metals like Cu and Zn serve either as cofactor and activators of enzyme reaction e.g. informing enzymes or substrate metal complex or exert a catalytic property such as prosthetic group in metalloproteinase (Mildvan, 1970).

An alternative classification of metals based on their co-ordination chemistry, categorizes heavy metals as class B metals that come under non-essential trace elements, which are highly toxic elements such as Hg, Ag, Pb, Ni (Nieboerand, 1980). These heavy metals are persistent and bioaccumulative, and do not readily break down in the environment or not easily metabolized. Such metals accumulate in ecological food chain through uptake at primary producer level and then through consumption at consumer levels (Doelman *et al.*, 1984). Heavy metals enter the human body either through inhalation / ingestion, which is the main route of entry for general population. However, urbanization, industrial, agricultural activities, waste incineration and mining have significantly contributed to the heavy metals into food chain (Sharma and Agrawal, 2005).

Heavy metal contamination is a general term given to describe a condition in animals that have abnormally high levels of toxic metals in the body. Common examples are mercury, lead, cadmium and arsenic. This contamination can be very real, detrimental to health and deadly. Unfortunately, many scam artists are propagating myths, distortions and lies about heavy metal contamination as scare tactics to sell fraudulent or partially effective products for treating the supposed contamination. Heavy metals are subtle, silent, stalking killers. They enter the body with our food, water, air we breathe and by skin contact. They slowly accumulate in the kidneys, liver, pancreas, bones, central nervous system and brain where they degrade health without being noticed or diagnosed. Heavy metals can and do cause cancer without ever being implicated in the diagnosis. Heavy metals cause sodium retention leading to high blood pressure. Heavy metals can and do cause heart disease and mental retardation. Everyone is contaminated with heavy metals, some seriously, without ever knowing it.

Lead is one such heavy metal with specific toxicity and cumulative effects (Ponangi *et al.*, 2000). 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). Ancient people used lead for ornaments, dishes, trays as a core for bronze status; as sinkers for fishing nets etc. Greeks and Romans used lead extensively for sweetening the bitter taste of the food cooked in bronze pots, storing olive oil, preparing wine etc (Muley, 1985).

Lead is considered as a general protoplasmic poison which is cumulative, slow acting and subtle. It produces a variety of symtoms similar to other heavy metals, it has affinity for sulfur. It interacts with carboxyl and phosphoryl groups. The major biochemical effect of lead (Pb) is its interference with heme synthesis leading to hematological damage. Pb inhibits several important enzymes involved in the synthesis of hemoglobin and other respiratory pigments such as cytochromes, which require heme. Higher levels of Pb in the blood can cause kidney dysfunction, and brain damage because it is toxic to the central and peripheral nervous system (Dara *et al.*,1995) and its permeable blood brain barrier leading to neurotoxic effect even at low concentration (Jarup, 2003). Chronic lead poisoning may cause three general disease syndromes:

1. Gastrointestinal disorder
2. Neuromuscular effects (lead lapsy) weakness, fatigue and muscular atrophy.
3. Central nervous system effects or CNS Syndromes that may result to coma and death.

Also causes constipation, abdominal pain, etc. (Manahan *et al.*, 1984). A relatively common variant of the second enzyme in heme biosynthesis, of δ aminolevulinic acid dehydratase (ALAD) has been reported to be associated with susceptibility to higher blood lead concentrations among children and workers (Astrin *et al.*,1987; Wetmur *et al.*,1991; Ziemsen *et al.*,1986). One of the most insidious effect of inorganic lead is its ability to replace Ca in bones and accumulate there as a reservoir of long term release. This lead is subsequently remobilized along with phosphate from the bones and exerts toxic effects when transported to soft tissue (Dara, 1995).

The effect of heavy metals on aquatic organisms is currently attracting widespread attention, particularly in studies related to pollution. Heavy metals are being introduced into aquatic environments through various sources; Lead is also not exception for this. Lead composition in typical uncontaminated soils range in 2 - 200 ppm d. wt. while in agricultural crops it is about 0.1 - 10 ppm d.wt. (Allaway, 1968). The actual toxic limit of Pb is › 500 µg/l toxic concentration in blood and 250 - 550 μg / l (for children) while the recommended intake of Pb for human health is 20 - 282 μg / day for adults and 9 - 278 μg / day for children. Pb concentrations in agricultural amendments are, in

Sewage sludge	⇒ 2 - 7000 μg / g.
Compost refuses	⇒ 1.3 – 2240 μg / g.
Farmyard manure	⇒ 0.4 – 27 μg / g.
Phosphate fertilizers	⇒ 4 – 1000 μg / g.
Nitrate fertilizers	⇒ 2 – 120 μg / g.
Lime	⇒ 20 – 1250 μg / g.
Pesticides	⇒ 11 – 26 μg / g.

From all these sources Lead (Pb) enters into aquatic ecosystems (Ross, 1994).

Indian standards for Pb in soil 250 - 500 mg/kg, in food 2.5 mg/kg and in drinking water 0.1 mg/l (Awasthi, 2000). Although Schwarz (1974) reported some evidence for possible essential function for lead, under certain unusual conditions lead is stimulatory and enhances protein synthesis, increases erythropoiesis, respiration, DNA synthesis, Cell replication and reproduction (Luckey, 1975b). Small doses of intraperitonealy injected lead stimulate DNA synthesis in rat kidney (Choie and Richter, 1974).

Most of the lead was found in the lysosomes. Reductions in number of lysosomes and vacuolar dilation of the intercellular space were noted on lead acetate administration (Schooeer *et al.*, 1982). Heavy metals like Pb, Cd, Zn etc. can be burried in land fills / washed into sediments. After getting entry into aquatic bodies, these metals may precipitate, absorb in water or may be taken up by fauna and flora (Leland;1977, Oehme; 1978, Reimbers *et al.*,1975). In India use of Hg, Cd, Al, Zn, Ni, Pb etc. in various compound forms pesticides, Insecticides, fungicides and subsequent waste discharge on leaching to water bodies is common (Kumaranguru *et al.,*1979; Panda, 1981).

This lead enters in animal body by various sources which are referred earlier. From which industrial, source is the major source of lead, by which the soil, water and atmosphere becomes contaminated (Sharma and Agarwal, 2005). Lead and other heavy metals are incorporated in the body by aquatic organisms and their concentration gradually increases during the life span of an organism called bioaccumulation (Chaudhari and Hazra; 2001).

The studies on metal concentration have been carried on many invertebrates. Regulation of excess metal concentration in animal tissues is accomplished through several means such as temporary absorption, storage and release by hepatopancreas, losses across the gill (Narayanan *et al.,* 1988). The accumulation of heavy metals form the water columns by bivalve molluscs have been found to be relatively rapid and to reflect ambient exposure levels closely (Boyden and Phillips, 1981). So bivalve molluscs are used for study of bioaccumulation of lead and other heavy metals in India and all over the world.

The critical evaluation of the literature on the water pollution, mainly on the river system, indicates that the test animal used for such studies is mainly fish from vertebrate and for invertebrate study the other major component of fresh water animal that is freshwater mussels is selected. A river pollution study and its effects on fish in India is worked out by a number of scientists (Bhilave, 2001).

Though the fishes are used as the test organisms, fresh water mussels also have been a popular research material for experimental studies. Mussels have been a subject of considered amount of research effort, reflecting both their ecological and economical importance. The mussels are worldwide in their distribution. They have therefore aroused the interest not only in the factors controlling their own population ecology, but also in their role in structure and function of the communities of which they are characteristics and an important component. The mussles constitute a significant part of the molluscan fauna today and have gained tremendous importance since prehistoric times. They are extensively used as human food. In recent years, mussles have become essential food items of human. Recent developments in shell fish resource management and cultivation shows a great practice in the production of proteins which has already been exploited by a number of countries. The mussles act as a food chain component of the ecosystem. They also act as a biological control by removing a number of bacterial population and toxic substances. Their shells are used for the production of ornamental articles, production of pearls and medicine. Therefore, in development and developing countries like USA, Japan, England, France, USSR, India, Australia, and Canada. A good amount of work has been carried out in connection with the cultivation of commercial species of mussles (Halstead, 1965; Manson, 1972; Milne, 1972; Mostert, 1972).

Mollusca are the 2nd largest phyla of invertebrates. The mollusks in India are playing a living or vital role yet shedding many of their past association and reported miraculous properties but; assuming newer and vastly more utilitarian roles. The trade in shells as raw material for the traditional handicraft products appears still to exist. The ancient time infants drinking spot fashioned out of chank lingers among some of the poorer classes, while the richer strata are supplied with carved shell ash trays of nautilus, reading lamps or window pane, oyster lamp shades.

Apart from the well established fisheries along various coastal area may clams, oysters, mussels, squids and other minor shell fishes constitute smaller fisheries of local importance in many other regions. Oysters, mussels and clams from regular fisheries of considerable local importance along the east and west coast. The meat is widely eaten and considered delicious by those, who develop a taste for it. As mentioned earlier, the resources of mollusks that can sustain regular and very productive fisheries as well as of scientific interest are abundant in our water. Great as the industrial use of mollusks, is perhaps the significance of molluscs in future would be greater as a potential source for human consumption and also in scientific researchers (Muley,1985).

The freshwater molluscs are abundant in Indian inland waters. Among these, lamellibranch and gastropod molluscs are widely distributed. The freshwater lamellibranch embrace the genera like *Lamellidens, Parreysig, Indonaia, Corbicula, Pseudodon, Balawantia, Physunia* and *Unio,* of the freshwater gastropods, those belonging to Prosobranchiata are common.

It has generally been found that concentrations of heavy metals in bivalve molluscs change with those in the environment. Therefore, the study on accumulation of Pb and other heavy metals by different molluscs facilitates the assessment of water quality as well as the selection of suitable bio-indicator species. As a consequence the use of bivalves as biomonitors of heavy metal pollution has been well established (Chaudhuri and Hazra, 2001). These bivalves are filter feeders. They permit feeding while buried in the sediments, and siphons permit access to fresh food and oxygen-bearing water without leaving the sediment.

The shells of bivalves are facilitated for burrowing in soft sediments. In these sediments, heavy metals concentrations are more than that on the surface waters (Kataria, 1994). So the use of bivalve molluscs to monitor the low level pollution in the marine habitat was initially proposed as, "Mussel Watch Concept" by Goldberg (1975). However, studies on monitoring of metal pollution in freshwater systems using fresh water molluscs are inadequate (Havlikand Marking, 1987).

The systematic studies of various mussels has been completed in 18th and 19th century and also different scientist has given the characteristics features of shell, gill and foot of Bivalvia, Lamellibranchiata or Pelecypoda under phylum Mollusca. Satyamurti (1960), Patil (1965, 1968) have taken a systematic survey of freshwater mussels in India. Apart from systematic studies morphological and anatomical investigations also appeared from time to time. The anatomical work, especially on soft part of many species, have thrown much light on the exact systematic position of bivalve species where true relationship could either to be correctly inferred from the study of their shell and external characters alone.

Ghosh (1918) has studied the anatomy of *Lamellidens marginalis*, while Patil (1965) has studied the morphology and anatomy of various organs of a freshwater mussel, *Lamellidens corrianus*. Ghosh and Ghosh (1918) have studied the reproductive organs of freshwater mussels. In 1967 Dinamani has studied variation in the structural pattern of stomach and he also correlated it with the interaction between walls of stomach and digestive diverticula.

Patil (1965) have studied the gills with reference to the formation of marsupium during the breeding season. Alello (1960, 1970), Alello and Guideri (1965), Paparo and Alello (1975) and Paparo (1972) have indicated that the activity of the epithelial cells of gills is under nervous control. Sleigh (1969, 1974) has provided upto date comprehensive review of the literature on bivalve gill. Histochemical investigations on the gill are given by Eble Albert (1968) demonstrated presence of glycogen, glycogen phosphorylase and branching enzymes in the gills of Oyster. Pasteels (1971) has demonstrated the acid phosphatase activity and Golgian polarity in the gills of bivalve. With the help of alcian blue technique the presence of acid mucosubsatance secreting cells has been reported by Reid and Reid (1979). Kale (1981) extended the histochemical studies on the gill of freshwater mussels and pointed out the presence of different types of mucous cells, secreting sulphated and nonsulphated acid mucoplysaccharides.

Biomarker and bioaccumulation responses of Asian clams exposed to aqueous cadmium and combining these data with observations of mortality in higher cadmium treatments suggests that impairment of DNA structural integrity and reduced digestive enzyme activity may indicate metal-induced stress in clams (Barfield *et al.*,2001) and the influence of suspended particles on the acute toxicity of 2-chloro-4-nitro-aniline, cadmium, and pentachlorophenol on the valve movement response of the zebra mussel, *Dreissena polymorpha* was observed by Borcherding *et al.,* 2001. Lares and Orians (2001) also reported the differences in Cd elimination from *Mytilus californianus* and *Mytilus trossulus* soft tissues. The most disturbing pollutants tested seem to be Pb, Zn and Cr which caused highly decreased overall compositions, namely with respect to protein, and glucosamine, in comparison to the control group. This suggests that this group contributes to a decrease of the metabolic activity, and thus mineralization, in the exposed animals (Moura *et al.,* 2000). The further study was designed to assess and validate potential sublethal toxicity of hexavalent chromium (Cr-VI) in clams under controlled laboratory exposure (The *et al.,* 2000). Also the Characterization of the acute toxicity of photoactivated fluoranthene to glochidia of the freshwater mussel, *Utterbackia imbecillis* was carried out (Weinstein, 2001). The literature exists showed that the primary studies on the effect of pollution on mollusca deals with bivalve population, O_2 consumption, reproductive behaviour and bioaccumulation of certain heavy metals.

Imabayashi (1983) noticed depletion of ambient dissolved O_2 leads to decline in the number of small bivalve *T. lubrica*. Hydrocarbon pollution causes significant ecological damage to bivalve population by affecting fecundity and residual reproductive value of the individual (Bayne *et.al.*, 1981), whereas Shaw *et al.,* (1986) noticed selective retention of different hydrocarbons by *M. edulis* and *M. balthica* in the area of discharge of petroleum hydrocarbons. Pollution effects due to the heavy metals in young mollusks have been reported by Nilov (1986). His studies showed that the young mollusks have great power of bioaccumulation of strontium 90. Sloft *et.al.,* (1983) carried out an acute toxicity tests with respect to these chemicals and showed that the susceptibility of the species was highly pollutant specific. Further, Reddy and Rao (1983) studied the molluscicidal effect of $CuSO_4$ treatment alters the lipid components of foot, mantle and digestive gland.

The bioaccumulation of metals varies from metal to metal and differs among various organisms. Some of the metals are bioaccumulated through the food chain, so that predators have highest concentration, but the highest concentrations of metals appear to occur in the invertebrates for which bivalves are not exception (Vernberg and Vernberg, 1974). Durfort (1982) has reported the presence of Cu, Si, Fe, Mg, Zn, Cd and Na in the digestive gland of Trachydesma whereas Pfeitter *et al.,* (1985) have reported the presence of Cu, Cr, Cd, Zn, Mn and Pb in body tissue of filter feeding mollusks. Bargogli and Leonzia (1985) studied the industrial pollutants such as Pb, Ni, Zn, and Fe which contaminated the coastal water of Bay of follonica, which increases the trace element deposists in mussels and limplets. Molluscicidal activity of Cu mainly affects the weight of the organs and gametogenic activity (Martincic *et al.,* 1987) and also interferes with the TCA cycle at the L - Ketoglutarate level (Babu *et al.*, 1957). Bioaccumulation of Zn in mantle tissue of *M. galloprovincials* and *Strea edulis* from the ambient water has been reported by Martincic *et al.*, (1987) and showed that Zn has simulative effect on filtration rate.

An organophosphate pesticides affected behaviour and respiration in a number of molluscan species (Rao *et al.,* 1978; Mane *et al.*, 1979, Kabeer *et al.*, 1979; Vijayandra *et al.,* 1982; Mane *et al.*, 1984). Lethal concentration for 50% and 0% mortality have been studied for various heavy metal toxicity for example, LC_{50} at 96 hours for Cd accumulation in clams have been reported by Henery *et al.,*(1984) while malathion induced changes in oxygen consumption in LC_0 and LC_{50} groups of *L.marginalis* and *L. corrianus* during all seasons has been reported by Muley and Mane (1987) and showed significant decrease in O_2 consumption of LC_{50} group at 96 hours during summer. Also the effect of Folithion and Lebaycid on the gill ciliary activities of three freshwater lamellibranch have been reported by Mane and Akarte (1988) and reported a significant increase in the ciliary activities in LC_{50} groups of freshwater bivalves during different seasons.

Accumulation of Cd, Cu, Pb and Zn in the tissue of *Mytilus edulis* and *M. planulatus* was examined under cyclic conditions of exposure in order to establish whether the rate of accumulation of heavy metal is proportional to time exposed to the elevated concentration, when exposed to a single metal, the accumulation of Pb, Zn was directly proportional to the time. Accumulation of both Pb and Cd are directly proportional to the exposure time (Eliott *et al.,* 1985).

The accumulation of Cd, Cu and Pb has their effect on aspartate and alanine aminotransferase in digestive gland, gills, foot and soft body in the clam *Ruditapes philippinarum* were examined. They were exposed at various concentrations and the highest concentration of Pb is in gills and the lowest values were observed in the foot (Blasco and Puppo, 1999). Gill and mantle exhibited higher concentration of metals than adductor muscles (Mitra and Choudhary, 1994).

Accumulation of heavy metals i.e. Cu and Pb were studied in the molluscs *Bellamya bengalensis* and *Lamellidens marginalis*. Lead concentration varied from 134.80 to 133 µg / g in the bivalve species, it accumulated a high amount of Pb in the post monsoon period (Gupta and Banerjee, 1998). Extensive work has been carried out by scientists in the field of heavy metal toxicity to demonstrate bioaccumulation of Lead (Pb) and other heavy metals in tissues. The work related to the removal of these Pb and other heavy metals by the chelating therapy is scanty.

Chelation Therapy :

Chelation is the primary method used to remove toxic heavy metals from the body. Chelation is a term commonly seen in the discussion of heavy metal contamination. Chelation is the binding or complexation of a bi- or multidentate ligand. Chelating compounds, agents or molecules have the affinity for bonding metal ions with two or more of the atoms of the chelating agent. The resulting bond is stronger than the existing bond of the metal ion in the body. The chelator pulls the metal ion away from the body molecules and carries it away. Another important feature of a chelating agent is the ability to hold the metal ion while being discharged by the liver into the digestive tract or the kidneys into the urine. The metal ion must not be allowed to recycle from the intestinal tract back into the blood stream. Toxic heavy metal ions can become captured within the body.

Other ions are carried in the blood where they are captured or discharged by the liver or kidneys. Those ions discharged by the liver into the intestinal tract are often picked up again and recycled back into the blood. Some of the ions can remain in the urine and feces where they can be measured in the laboratory. Toxic heavy metal ions can be discharged from the body in feces, urine, hair, sweat and discarded skin cells. Chelation of toxic heavy metals from the body can be accomplished by the use of intravenous chelators, oral chelators or by chelating vitamins, minerals and supplements (Kotlikoff, 2006).

The role of nutritional status in altering susceptibility to lead toxicity has been recognized through most of the studies in this century. A variety of methods e.g. experimental studies, clinical assessments, epidemiological investigations have been used to establish the association between nutritional status and susceptibility to lead toxicity (Kathryn, 1995). Age, dietary calcium, vitamin D. influences absorption of lead (Taylor *et al.,* 1962, Forbes and Reina, 1972). Vitamin B complex fortified with Vitamin C decreased the accumulation and toxic effects of lead in animals. Ascorbic acid and thiamine have been shown to enhance the efficiency of calcium disodium ethylenediamine tetraacetate (EDTA) to counteract Pb intoxication. Thiamine, ascorbic acid and their combination were investigated for their ability to prevent or treat Pb toxicity in rats (Swaran *et al.*, 1986). In 1972, Mahaffey-Six and Goyer first reported that rodents fed on iron-deficient diet experienced increased susceptibility to lead toxicity that means iron is also helpful to decrease the toxicity of lead (Pb).

Injected 'zinc' appeared to have little protective effect on lead haematotoxicity. There is limited evidence to suggest that low-protein diets may be associated with increased retention of lead (Mahaffey-Six and Goyer, 1972, Barltrop and Khoo, 1975). Similarly, high-fat diets may enhance alimentary absorption of lead (Barltrop and Khoo, 1975; Quaterman *et al.*, 1977).

There may be a complex interactive effect between different nutritional components; such as calcium, phosphorus and vitamin D. The highest blood lead levels in children were associated with both low calcium and low vitamin-D intake (Soreell *et al.,* 1977). A diet low in protein, minerals, (particularly calcium, phosphorus and iron) and certain vitamins may not only enhance the circulating dose of lead but may also reduce the general health status of the growing child (Rat cliffe,1984). Moreover, in few recent studies we observed a beneficial role of essential trace metals (zinc and copper) supplementation during the course of chelating treatment of lead (Flora and Tandon, 1991).

Along these all, Selenium supplementation alone could also reduce the lead concentration of blood, liver and kidney (Tandon *et al.*, 1992). Among these all, the uptake and metabolism of lead may be modified by calcium status (Sorrell *et al.*, 1977).

Bioaccumulation of lead is influenced by divalent ions like Calcium, Zinc, Iron etc. among which calcium is naturally present and it is involved in cell signaling (Alberts, 2002). A number of animal studies have suggested that low dietary calcium, increase the absorption of lead (Mahaffey *et al.*, 1973; Kostial *et al.*, 1971; Six and Goyer, 1970). Calcium intake was found to be inversely related to both absorption and retention of lead in infants (Ziegler *et al.*, 1978).

Mostly calcium disodium ethylenediamine tetracetic acid ($CaNa_2$ EDTA) has been used for prevention or therapy of lead intoxication (Tandon and Flora, 1992). Extensive data indicate that dietary calcium intake and nutritional status of calcium influence susceptibility to lead toxicity (Mahaffey *et al.,* 1981, 1985 and 1990).

As understanding of calcium mediated cell functions has expanded, the hypothesis that fundamental calcium against role might be central to lead toxicity has been postulated (Pounds *et al.,* 1984, 1991). The particular, lead induced changes in skeletal development and regulation of skeletal mass assume particular significance because of the role of bone as the major internal source of lead and calcium (Pounds *et al.,* 1991).

The lead stream follows the calcium stream (Aub *et al.*, 1926). Early studies with experimental animals indicated that there were some aspects of physiological responses to calcium that altered biological response to lead. However, nutritional investigation of trace elements was not well developed during this period. Many of the early studies used diets that were deficient or imbalanced in more than one nutrient, making clear identification of the role of calcium uncertain. In the early 1970s, Mahaffey and colleagues identified a clear role of nutritional status for calcium on susceptibility to lead toxicity. These investigators observed that rats ingesting a low-calcium diet had blood-lead concentrations about four times higher than rats on a normal calcium diet, although the quantities of lead ingested were equal. Bone-lead concentration also increased on the low-calcium diet. However, at the lowest level of dietary calcium intake, renal and blood-lead concentrations also increased sharply. Lead induced impairment of hematopoiesis and changes in renal histology were much greater on the low calcium diets.

The initial observations with rodents were confirmed in several species including humans (Blake and Mann, 1983; Heard *et al.,* 1982; Ziegler *et al.,* 1978). Lower dietary calcium intake among children with higher blood-lead concentrations occurred (Mahaffey *et al.* 1976; Johnson *et al.*, 1979) and general population groups e.g. Second National Health and Nutrition Examination Survey (NHANES) II data reported by Mahaffey *et al.*, 1986. A major difficulty in these surveys of calcium status is the assessment techniques. Dietary recall or dietary history provides only a very approximate indicator of nutritional status of Ca^{++}.

The interaction between lead and calcium has been described at the cellular level. Many of the physiological processes that exercise metabolic control over calcium are affected by the presence of lead. Lead is known to mimic Ca^{2+} in various biological systems or to alter Ca^{2+} mediated cellular processes (Dave *et al.*, 1993). Lead will compete with calcium in enzyme systems, alter regulation of calcium metabolism e.g. synthesis of 1-$\propto$ - 25 hydroxyl – cholecalciferol (Edelstein *et al.*,1985) or inhibit 1, 25-dihydroxy-vitamin D-3 regulation of calcium second messenger system e.g. Long *et al.*,(1992) impair normal modulation of intracellular calcium homeostasis ; modify induction of calcium regulating proteins e.g. lead inhibition of renal 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).

Broadly speaking the research and public health experiences provide a substantial basis to identify an increased susceptibility to lead toxicity resulting from inadequate to marginal dietary calcium intakes; and a fundamental, lead-induced alteration, in calcium mediated cellular processes and in physiological mechanisms that are Ca^{2+} dependent or control Ca^{2+} homeostasis.

Therefore, present investigation deals with removal of lead toxicity by calcium chelation therapy in the freshwater mussel, *Lamellidens marginalis* most of the freshwater bivalves are used as food by human beings. There is need to try the elimination of lead entering food chain from the important carrier organisms. The freshwater bivalves are one of the most important carriers of Pb towards higher vertebrates. In the present investigation an attempt has been made to remove lead from the bivalve mussels by calcium supplimantation.

Alteration in protein contents were studied by number of workers to prove the toxic effect of corresponding toxicant (Jadhav and Lomate, 1982; Chaudhari *et al.,* 1989; Somnath, 1991; Subramanian *et al.*, 1993; Chandravarthy, 1994; Ambrose *et al.*, 1994; Karanjkar, 2001; Satyaparameshwar, 2006). Therefore total protein study from various organs was undertaken to understand impact of pollutants on the health of the organisms. The role of biological significance of phosphatases has attracted considerable attention, 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. Therefore enzymes like alkaline phosphatase and acid phosphatase were studied in various tissues.

Light microscopical study was used as a biomarker for studying changes at cellular level after exposure of the animal to the lead acetate and remedial effect of calcium acetate. For present investigation, Hematoxylin and Eosin staining were used. This staining helps to demonstrate the effect of toxicant on histoarchitecture of tissue and also can give information about physiochemical alterations within the tissue.

Collagen structure study is one of the important studies to understand extent of lead toxicity. The collagens are a family of fibrous proteins found in all multicellular animals. They are secreted by connective tissue cells, as well as by a variety of other cell types. As a major component of skin, bone and body wall connective tissue of most of invertebrates like mollusk. They are the most abundant proteins in invertebrate and vertebrate, constituting 25% of the total protein mass in these animals. Connective tissue and epithelial tissue represent two extremes of organization. In connective tissue, the extracellular matrix is plentiful, and cells are sparsely distributed within it. The matrix is rich in fibrous polymers, especially collagen and it is the matrix rather than the cells that bear most of the mechanical stress to which the tissue is subjected (Albert, 2002). In gill and mantle of freshwater bivalve connective tissue is the major part and collagen is the main constituent of it and therefore histological architecture of gill and mantle is totally depending upon collagen fibers. The toxic exposure of animal breaks down collagen, which may leads into the disturbance of histological architecture of gills and mantle. To understand these alterations collagen is a very important parameter taken for study.

The present study is presented in five chapters. The first chapter gives a detailed introduction of the topic. The second chapter gives detailed material and methods used in the investigation. The third chapter deals with detailed observation of all parameters studied. The fourth chapter deals with discussion of all parameters studied. The fifth chapter deals with the summary and precise conclusions. The dissertation also gives a detailed bibliography of the references which are directly and indirectly referred for the co-relation of the present investigation.