General
Introduction
Water is a unique solvent essential for our survival and its pollution is a major global problem. Our rivers, lakes and seas have limits for absorbing pollutants with an increasing world population. There is a subsequent increase in the discharge of sewage, industrial effluents and garbage. The contamination of natural waters with man made chemicals has caused serious problems to the aquatic biota (Brezonik et al., 1991).

Increased industrialization, urbanization, population growth and overall man's greed to overexploit Mother Nature has created a serious threat to all kinds of life in the form of pollution which has now become a global problem. Among all types of pollution, aquatic pollution is of greater concern as each and every kind of the life depends on water. Among all types of aquatic pollutants, heavy metals are of greatest concern. Heavy metals when reach the aquatic bodies deteriorate the life sustaining quality of water and cause damages to both flora and fauna. Being intrinsic components of the earth crust, nature has not provided effective control mechanisms for these metals. The problem increases many folds due to their long half-life period and properties of non-biodegradability, bioaccumulation and biomagnifications (Mukherjee and Kumar, 2005; Woodling et al., 2001).

The tremendous increase in the use of heavy metals over the past few decades has inevitably resulted in an increased flux of metallic substances in the aquatic environment. The metals are of special concern because of their diversified effect and the range of concentration stimulated toxic ill effect to the aquatic life forms. Industrial wastes constitute the major source of metal
pollution in natural water. Aquatic systems are exposed to a number of pollutants that are mainly released from effluents discharged from industries, sewage treatment plants and drainage from urban and agricultural areas. These pollutants cause serious damage to aquatic life. Most of the heavy metal ions are toxic or carcinogenic in nature and pose a threat to human health and the environment. The river systems may be excessively contaminated with heavy metals released from domestic, industrial, mining, and agricultural effluents. Due to natural, geochemical and anthropogenic factors, the infiltration of toxic heavy metals into aquatic ecosystems is on the increase. These toxic heavy metals in aquatic ecosystems are carried via the food chain to the upper trophic levels and create important ecological problems.

Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms. Among animal species, fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants. Fish are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic systems. The studies carried out on various fishes have shown that heavy metals may alter the physiological activities and biochemical parameters both in tissues and in blood. The toxic effects of heavy metals have been reviewed, including bioaccumulation. The organisms developed a protective defense against the deleterious effects of essential and non-essential heavy metals and other xenobiotics that produce degenerative changes like oxidative stress in the body (Vinodhini and Narayanan, 2008).
India which ranks 9th among the fish producing nations of the world, is endowed with vast and varied fishery resources comprising fresh as well as brackish water. These resources constitute the freshwater zones of extensive river system, ponds, large tanks, lakes and reservoirs. Millions of people are dependent for their protein and other nutritional requirements on the fisheries sector. It is now realized that natural ocean and freshwater fisheries are being harvested to their near limits and the increased demand can only be met through diversification and application of modern tools. In India freshwater fish farming have been successfully developed to enhance fish production, but on the other hand toxicants originated from industrial, agricultural and municipal sources are directly or indirectly dumped into the aquatic ecosystem, which ultimately affects non target organisms like fish. The mass mortality of fishes encountered in India are witnessing the pollution of freshwater lakes, alarming the water quality.

Aquatic ecosystem is the traditional recipient of many industrial pollutants of which heavy metals are predominant. Even though they are the marvelous gifts of the nature playing a key role in the industrial development, the injudicious and unprogrammed discharge of them into the natural waters in the form of effluents from a number of industries is endangering the productivity and survival of freshwater organisms, leading to harmful effect even on human beings due to the consumption of affected organisms.

In recent past increased inputs of anthropogenic contaminants and other stresses such as the destruction of habitats have brought changes in aquatic life, correspondingly increased concerns has centered on:
a. The accumulation and catastrophic effect of contaminants on the survival of aquatic organisms.

b. The uptake and accumulation of pathogenic organisms and chemical contaminants in aquatic resources destined for human consumption.

c. The release of biodegradable organic matter and nutrients which under quiescent condition may result in localized eutrophication, organic enrichment and oxygen depletion.

In response to these events, aquatic toxicology has undergone significant changes in the past decade. The science that initially focused on the burdens of toxic environment and its acute bioassay which had lethality as the principal end point, has expanded into studies involving distinct biochemical process.

Chemicals derived from agricultural operations (pesticides and herbicides) and industrial effluents, such as metals, ultimately find their way into a variety of different water bodies and can produce a range of toxic effects in aquatic organisms, ranging from alterations to a single cell, up to changes in whole populations. The accumulation of toxic metals to hazardous levels in aquatic biota has become a problem of increasing concern. Excessive pollution of surface waters could lead to health hazards in man, either through drinking of water and/or consumption of fish. The increasing importance of fish as a source of protein and the interest in understanding the accumulation of heavy metals at the trophic levels of the food chain, extend the focus towards finfish. Pollutants enter fish through five main routes: Via food or non-food particles, gills, oral consumption of water and the skin. On absorption, the pollutants
carried in blood stream to either a storage point or to the liver for transformation and/or storage. Pollutants transformed in the liver may be stored there or excreted in bile or transported to other excretory organs such as gills or kidneys for elimination or stored in fat, which is an extra hepatic tissue. The concentration of any pollutant in any given tissue therefore depends on its rate of absorption and the dynamic processes associated with its elimination by the fish (Mohammed and Al-Kahtan, 2009).

Heavy metals include transition metals like Arsenic, Antimony, Bismuth, Cadmium, Cobalt, Copper, Lead, Mercury, Nickel, Zinc Etc. having atomic number 22 to 92 in all the groups from III-VII in the periodic table. The common features of these metals are that they are relatively toxic to aquatic organisms even at fairly low concentrations and readily accumulate in their tissues. The presence of heavy metals in the aquatic media is further compounded by the fact that they are water soluble non-degradable and having the ability of binding to many biochemical’s, especially polypeptides and proteins (Clements, 1991). All the heavy metals are toxic to aquatic organisms at high concentrations, but some are highly toxic even at lower concentrations.

Heavy metals are the important source of pollution for aquatic habitats. In spite of their natural occurrence in the aquatic ecosystem, heavy metals represent a major problematic environmental issue of increasing concern (Gill et al., 1990; Hunaiti and Soud, 2000) and their monitoring has received worldwide significant attention in the field and under laboratory conditions (Christensen, 1975; Gupta and Sastry, 1981; Adham et al., 1999; Gilli et al.,
Industrial effluents and agricultural runoff containing toxic and hazardous substances including heavy metals discharge into water and tremendously contaminate the aquatic environment (Munger et al., 1999, Gbem et al., 2001 and Woodling et al., 2001). The presence of heavy metals in different kinds of food constitutes serious health hazards, depending on their relative levels. Lead for example causes renal failure and liver damage (Emmerson, 1973). Some other metals cause poor reproductive capacity, hypertension and tumours and hepatic dysfunction (Mansour and Sidky, 2002).

Cadmium (Cd) belongs to the group of highly toxic heavy metals. Naturally, it occurs in water only in trace amounts, but recently its levels have increased due to anthropogenic activities (Papina, 2001). Most cadmium contamination comes from metal foundries, the dye industry, production of plastics and of accumulators. Cadmium is primarily used in zinc smelting and electroplating. In addition cadmium is widely used in the metal industry as protective covering for iron, copper, and steel. Cadmium electroplated parts are used in televisions and radio sets. Cadmium oxide is used as the negative electrode in cadmium-nickel and cadmium-silver rechargeable batteries and is an important component in low melting alloys in bearings, solders, nuclear reactor control rods and cadmium copper telephone wires. It is also used in PVC heat stabilizers and to improve high temperature properties of rubber and
plastics as well as in semiconductors and in ceramic glazes. Cadmium interacts with several other essential elements such as iron, copper, zinc, calcium, selenium and could play an important regulatory role with these elements.

It may enter the aquatic and ambient environment as a toxic pollutant from various anthropogenic sources such as zinc, copper and lead mining, various industries, iron & steel, non-ferrous metals cement production etc, electroplating, phosphate fertilizers, nickel-cadmium batteries, coal utilization and tobacco smoking. In high concentrations, cadmium may affect human health. Cadmium contamination of the fishes was the main cause for the episodal pollution and endemic borne disease "itai-itai" reported from Japan, during which several hundreds of peoples were affected.

Cadmium was discovered by German scientist as a by-product of the zinc refining process during the year 1817. Its name has been derived from the Latin word cadmia and the Greek word kadmeia. Natural cadmium levels in the atmosphere and earth's crust are 0.1 to 5 ng/m^3 and 0.1 to 0.5 µg/g respectively. It is estimated that for every 10 million parts of earth's crust containing about 2 parts of cadmium. Long-term occupational exposure can cause adverse health effects on the lungs and kidney. Following this increasing scientific interest has been devoted to this element as an environmental contaminant. Initially studies were concentrated on the effects of cadmium on man, while the studies related to the behavior of cadmium in the aquatic environment and its effects on biota were of recent origin. Like mercury, cadmium has been shown to pose a real health threat to human populations,
which receive high level of exposure. Over the years cadmium accumulates in body particularly in bones and culminates in specific disease. There are increasing awareness and regulatory regimes to protect the environment as well as human population from the undesirable effects of environmental hazards of cadmium.

It accumulates in liver and kidneys of Cd exposed animals and humans. Cadmium half-life is about 10 years, so the symptoms of cadmium intoxication may occur several years after the exposure (Farhat and Abdul, 2010).

The exposure of cadmium results in pathological changes in water ecosystems, mostly demonstrated in fishes, which are affected by heavy metals through the respiratory and digestive systems and through the skin. In general, toxic effects of all heavy metals are similar, including pathological changes in parenchymatous organs and the nervous system. Indeed, long-term exposure of cadmium has some specific effects like impairment of reproductive function and endocrine disruption. Current accepted opinion of cadmium action as well as other metals is related mainly to their influence on protein molecules, particularly enzymes. They have a strong affinity to bond with the aminoacid moieties of proteins and may cause changes in enzyme structures. The most obvious consequences of these changes are the inhibition of enzymes (Drastichova, 2004).

Sediments absorb heavy metals such as lead, zinc, copper, nickel, cadmium, chromium etc. from water and accumulate in its different tiers. The view is emerging in certain quarters that cadmium from phosphorus fertilizers
poses a potentially serious threat to soil quality and, through the food chain, to human health (Oosterhuis et al., 2000). Aquatic organisms take up heavy metals, concentrate them to amounts considerably higher than those found in the environment (Ferard et al., 1983) and exhibit toxicity effects (Witeska et al., 1995 and Pelgrom et al., 1994). Benthic communities take up heavy metals from environment and are generally characterized by reduced abundance, lower species diversity, and shifts in community composition from sensitive to tolerant taxa (Bedii and Kenan, 2005; Deepak et al., 2001; Clements, 1991). Jana and Das (1997) put forwarded that *Lamellidens* might be used as a biofilter for the reclamation of cadmium contaminated aquatic environment. Cadmium is also an important xenobiotic and cumulative pollutant in aquatic ecosystems and fish are particularly vulnerable to cadmium exposure (Sorensen, 1991). In fish, cadmium uptake is took place mainly through three routes namely, gills, skin and also from food via the intestinal wall and accumulated (OECD, 1996; Edgren and Notter, 1989 and Kumada et al., 1980). On the other hand, the metal retention capacity of fish is dependent on the metal assimilation and excretion capacities of the fish concerned (Rao and Patnaik, 1999).

Cadmium is a ‘priority pollutant’, not only from the human health perspective, but also from a broader ecosystem viewpoint (Campbell, 2006). In general, it is a biologically non-essential, non-biodegradable, persistent type of heavy metal and its compounds are known to have high toxic potentials. There is increasing concern regarding the likely impact of cadmium in the
environment, because it is a carcinogenic element posing risks to ecosystems and humans, and is hazardous in excessive amounts (SCOPE, 2000). Another important adverse property of cadmium is their ability of amply accumulating in the sediments and in the aquatic flora and fauna (by bioaccumulation and biomagnification) that causing a gross biological impact.

Detoxification systems for metals in plants, invertebrates, fishes, birds and mammals are derived from the specific feature of stress induced molecules to bind heavy metals. Particularly it was discovered that organisms protect themselves against the toxic effect of metals by synthesis of extra proteins called metallothioneins (MTs), which are abundant throughout the whole animal kingdom (Fabrik, et al., 2008). These proteins are rich in cysteine and are able to bind metal ions (Ryan and Hightower 1994). MTs are a group of low molecular mass (6,000–7,000 Da) single-chain proteins, containing about 25–35% cysteine, due to which they have high binding capacity for metals. All SH-groups may bind a metal ion, however, about 50% of metal-binding sites are always saturated with Zn. One MT molecule can sequester 6-7 cadmium molecules (Hamer, 2009). In fish, MTs occur mainly in the liver, kidneys, gills and digestive tract (Kito, et al., 1982). Among fish species negligible differences in amino-acid composition, spectral characteristics and stoichiometric properties of MTs were found. Two MT isoforms corresponding to classes MT-I and MT-II (6,227 Da and 6,435 Da) were isolated from the hepatopancreas of the carp (Cyprinus carpio). These isoforms occur in trace amounts in cells, because their primary function is to maintain homeostasis of
copper and zinc and the protection of cells against oxidative damage (Adam, et al., 2008). Nevertheless, sublethal concentrations of metals such as Cu, Cd, Ag or Hg induce their synthesis. Based on the data published by other authors (Hamer, 2009; Kagi, et al 1988; Bremner, 1994), factors such as environmental stress, starvation, or bacterial infection may cause an increase in MT levels in animals, including fish. The molecular level of MTs is induced by interleukin-1, glucocorticoids, interferon and heavy metals as a matter of course (Kille, et al, 1992). For detection of metallothioneins, various analytical methods including spectrometry, liquid chromatography, capillary electrophoresis, and electrochemistry can be employed. In this study, a very sensitive technique, the differential pulse voltammetry Brdicka reaction was utilized for detection of this protein (Petrlova, et al., 2006). In our biological experiments, the influence of cadmium chloride in water on levels of cadmium in muscles, liver and kidneys and to levels of metallothioneins in these tissues of fish was studied.

Acute toxicity may result from the ingestion of cadmium through contaminated foods and beverages. Mammalian organisms are exposed to cadmium by its technological utilization and the epidemiological studies shows that cadmium is one of the most toxic of the heavy metals to humans. Exposure to sublethal concentration of cadmium to the fishes leads to haemolysis. Cadmium produces toxic lesions in various tissues; the largest amount of this metal is deposited in the liver and kidney tissues. It causes morphological and functional changes in the liver. Cadmium ingestion causes testicular abnormalities in early sperm development in mice. Cadmium inhibits the
activity of enzymes by binding to their sulfhydryl groups (-SH), consequently causing the peroxidative destruction of cell membranes. Cadmium is also known to alter several physiological activities such as alteration in carbohydrate metabolism in rat, mice and rabbit (Karthikeyan and Bavani 2009).

Cadmium causes significant metabolic alterations and injuries in biological system at different levels. In recent times enormous quantities of cadmium is being discharged into the aquatic environment due to the activities of highly industrial societies. Studies on the toxicity of the non essential element reported its harmful nature for the productivity of freshwater fishes, which serve as staple food for human beings. One among the Indian major carp, *Labeo rohita* inhabiting the freshwater sources is widely cultured in ponds, tanks and lakes of this region. This fish is largely preferred for table purpose as low cost protein and vitamin source. Hence this fish has a good commercial value and also forms an important component of the freshwater ecosystem. This fish is considered best suitable for composite fish culture. Owing to its high productivity, easy availability, adaptability to laboratory conditions and wide consumption by the natives. Hence the species *Labeo rohita* was considered as suitable experimental studies.

The studies on the effect of cadmium on different physiological, biochemical, and histopathological aspects of fish exposed to median lethal and sub lethal concentrations are very limited. There is wide lacuna in this field of cadmium toxicity on freshwater fishes prompted to take up this investigation.
The survey of literature reflects that, the heavy metal cadmium affects a wide range of non target organisms such as fishes and other aquatic organisms.

In view of the foregoing account the present investigation was projected to understand the impact of median lethal concentration and sub lethal concentration of cadmium on the freshwater fish, *Labeo rohita* with the following objectives.

**Chapter 1: Toxicity Evaluation**

The LC$_{50}$/96 hr value for the fish, *Labeo rohita* was determined after conducting static renewal bioassay test. The LC$_{50}$ studies believed to provide information on the relative lethality of a toxicant to an organism. One fifth of the LC$_{50}$ was selected as sub lethal concentration for sub acute studies (1, 5, 10, 15 days) to find out cadmium induced metabolic derangements.

**Chapter 2: Behavioral Toxicology**

Heavy metal cadmium brings tremendous changes in the organism. Changes in regular behavioural pattern of the organism link it with the disturbed physiology. In the laboratory fish behaviour can be a sensitive marker of toxicant induced stress. Hence an attempt has been made to study physical, morphological and behavioural changes in cadmium exposed fish.

**Chapter 3: Whole animal oxygen consumption**

Respiration is highly influenced by the stress inducted by the heavy metal cadmium. The whole animal oxygen consumption was determined in the control as well as cadmium exposed fish.
Chapter 4: Accumulation of cadmium (residue analysis)

Fish tend to accumulate the metal from the surrounding medium into different tissues through various modes. The quantity of cadmium accumulated in the tissues (gill, kidney and liver) was quantified by employing (AAS) Atomic Absorption Spectroscopy technique.

Chapter 5: Ions and associated ATPases

Studies on the concentrations of ions (Na\(^+\), K\(^+\) and Ca\(^{2+}\)) and activities of associated ATPases (Na\(^+\)- K\(^+\), Ca\(^{2+}\) and Mg\(^{2+}\)) have been initiated to look at deleterious effects on ion transport and osmoregulatory functions of tissue (gill, kidney and liver) of control and cadmium exposed fish tissues.

Chapter 6: Carbohydrate metabolism

Studies on changes in carbohydrate metabolism include estimation of blood glucose, glycogen, lactate, pyruvate and activity measurement of enzymes such as succinate dehydrogenases (SDH), Lactate dehydrogenase (LDH), Glycogen phosphorylase, Glucose-6-Phosphatase in the control and experimental tissues (gill, kidney and liver).

Chapter 7: Haematology

Haematological studies play an important role in understanding variation of blood characteristics in relation to stress factors. The blood composition of a fish results to some extent to metabolic and other
physiologic process. Effect of cadmium on some haematological parameters like RBC's, WBC's, haemoglobin and on certain hematocrit indices like packed cell volume (PCV), mean corpuscular haemoglobin volume (MCHV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) were in blood of control and experimental fish.

Chapter 8: Histopathological studies

Biochemical changes in the tissues are compared with the histopathological changes in gill, kidney and liver of fish, L. rohita exposed to lethal and sub lethal concentrations of cadmium.