REVIEW OF LITERATURE

Heavy metals occur naturally in the environment and are found in varying levels in all ground and surface waters (Martin and Coughtrey, 1982). Anthropogenic activities, such as agriculture, mining, and industry, have exponentially increased the amount of metals in many ecosystems, particularly during the last century (Guimaraes-Soares et al., 2006). Increased discharge of both essential and non-essential metals into natural aquatic ecosystems can expose aquatic organisms to unnaturally high levels of these metals where they pose a serious threat because of toxicity, long persistence, bioaccumulation, and biomagnification in the food chain (Kucykbay and Orun 2003; Van Dyk et al., 2007). Al-Attar (2005) reported that contamination of freshwater with heavy metals causes devastating effects on ecological balance of the aquatic environments. Contamination of aquatic environments by heavy metals, whether as a consequence of acute or chronic events, constitutes an additional source of stress for aquatic organisms (Kori-Siakpere and Ubogu, 2008). Among all kinds of aquatic organisms, fish have been widely used in the evaluation of the quality of aquatic environments as bioindicators for environmental pollutants. It is also important to examine the toxic effects of metals on fish since they constitute an important link in food chain and their contamination by metal causes imbalances in the aquatic system (Firat and Kargin, 2010).

During the last two decades, the interest in using toxicological endpoints so called biomarkers or bioindicators (endpoints at levels of higher biological organization) - as monitoring tools to assess environmental pollution has steadily increased (Adams, 2002). A biomarker is defined as a change in a biological response (ranging from molecular through cellular and physiological responses to behavioral changes) which can be related to exposure to or toxic effects of environmental chemicals (Peakall, 1994). Zhang et al. (2004) has pointed out that the potential utility of biomarkers for monitoring both environmental quality and the health of the organisms inhabiting contaminated ecosystems has aroused increasing attention during the last few years.
In environmental risk assessment, fish bioaccumulation markers and biological biomarkers are used to demonstrate exposure to and effects of environmental contaminants. Fish bioaccumulation markers may be used to elucidate the aquatic behavior of environmental contaminants, to identify certain substances present at low concentrations, and to assess exposure of aquatic organisms (Van der Oost et al., 2003). The monitoring of metal bioaccumulation is necessary, because this will give an indication of the temporal and spatial extent of the process, as well as an assessment of the potential impact on organism health (Has-Schön et al., 2007).

A number of bioaccumulation of metals in fish exposed to acute and sublethal concentrations of metals are reported in the literature (Perevoznikov and Bogdanov, 1999; Uysal et al., 2008; Kamunde and MacPhail, 2011). Biochemical and physiological biomarkers are frequently used for detecting or diagnosing sublethal effects in fish exposed to different toxic substances (Theodorakis et al., 1992; Lavado et al., 2006). Prominent among these biomarkers are physiologic variables, such as serum levels of metabolites (DiGiulio et al., 1995) and ions (Martinez and Souza, 2002); levels of hormones (Benguira and Hontela, 2000); and biochemical variables, such as antioxidants (Orbea et al., 2002) and enzyme activities (De la Tore et al., 2000) are used as stress indicators.

Heavy metal exposure has been linked to endocrine disruption in several aquatic species (Depledge and Billinghurst, 1999; Thangavel et al., 2005) and hormone regulation may be impaired due to exposure to environmental pollutants like heavy metals (Folmar, 1993; Ernst et al., 2007). Thyroid hormones (TH) have been implicated in a variety of critical fitness-related processes such as growth, reproduction, development, and mediation of other hormone activities and physiological functions. Environmentally induced thyroid dysfunction has been an increasing concern for some time. Indeed, a variety of organic and inorganic chemicals have been found to be thyroid disruptors (Brown et al., 2004). Numerous studies have demonstrated that environmental contaminants affect plasma TH levels and cause thyroid dysfunction in fish (Waring and Brown, 1997; Thangavel et al., 2005; Thangavel et al., 2010). Development of sensitive assays for thyroid hormones is therefore essential for investigation of the thyroid status in fish species and its suspected disruption by environmental toxicants (Zhou et al., 2000; Colborn, 2002).
The antioxidant mechanisms related with oxidative stress have gained considerable interest in the field of ecotoxicology. Therefore, antioxidant enzymes are considered as sensitive biomarkers in environmental stress before hazardous effects occur in fish, and are important parameters for testing water for the presence of toxicants (Geoffroy et al., 2004). It is well known that both organic and inorganic contaminants can induce oxidative stress in animals by producing reactive oxygen species (ROS) such as hydrogen peroxide ($H_2O_2$) and superoxide anion ($O_2^-$). ROS may initiate a sequence of reactions which produce free lipid radicals and hydroperoxides that are extremely toxic to cells (Livingstone, 2001). Defensive systems to counteract the impact of ROS are found in many aquatic animals. These systems include various antioxidant defense enzymes such as superoxide dismutases (SOD), which catalyze the dismutation of superoxide radical to hydrogen peroxide, and the glutathione S-transferase (GST) family, which possesses detoxifying activities towards the lipid hydroperoxides generated by pollutants such as heavy metals (Livingstone et al., 1990).

GSH serves in a multitude of critical cellular defensive functions including, detoxication of electrophiles and maintenance of thiol-disulfide status and signal transduction (Meister and Anderson, 1983; Sen and Packer, 1996). Moreover, glutathione is the cofactor of many enzymes catalyzing the detoxification and excretion of several toxic compounds. Glutathione-S-transferase (GST, EC. 2.5.1.18) form a family of multifunctional phase II biotransformation enzymes, it present in the cytosol of most cells catalyzing the conjugation of the tri-peptide glutathione to a variety of compounds with an electrophilic group (George and Buchanan, 1990), which play an important role in the biotransformation and detoxification of a number of electrophilic compounds, by conjugation to glutathione (Venkateswara Rao, 2006). GPx constitutes a family of enzymes, which are capable of reducing a variety of organic and inorganic hydroperoxides to the corresponding hydroxy compounds, utilizing GSH and/or other reducing equivalents (Uner et al., 2005).

GPx serves as the most important peroxidase for hydroperoxide detoxification (Hermes-Lima, 2004), and CAT eliminates hydrogen peroxide, which can penetrate through all biological membranes and inactive several enzymes (Vutukuru et al.,
Lipid peroxidation can be a major contributor to the loss of cell function through cellular membrane disruption and activation of calcium dependent proteases and lipases (Hermes-Lima et al., 1995). The most widely used assay for lipid peroxidation is the malondialdehyde (MDA) formation, which represents the secondary lipid peroxidation product with the thiobarbituric acid reactive substances test (Draper et al., 1993). The concentration of MDA is the direct evidence of toxic processes caused by free radicals (Sieja and Talerczyk, 2004). The activation of antioxidant (AD) systems in response to exposure to pollutants has been reported in various fish species (Marcon and Wilhelm Fillho, 1999; Amado et al., 2006; Yonar and Sakin, 2011).

The impact of contaminants on aquatic ecosystems can be assessed by the measurement of biochemical parameters in fish that respond specifically to the degree and type of contamination (Petrivalsky et al., 1997). Biochemical biomarkers are particularly responsive to low doses of environmental stimulation and can provide advance warning of impending environmental changes. Biochemical biomarkers can also indicate if organisms are, or have been, under environmental stress and are useful models for simulating the effects of pollutants on organisms (Woo et al., 2009). Stress response could be attributed to the mobilization of glucose (glycogenolysis) linked to changes in carbohydrate metabolism of fish and ultimately increasing energy availability and utilization to improve the response to toxic stress (Cazenave et al., 2006b). Plasma glucose, liver and muscle glycogen responses appear particularly suitable for measuring stressful levels of pollutants and have been used as indicators of stress in fish (Pickering et al., 1982).

Nemsock and Boross (1982) reported that blood glucose appeared to be a sensitive indicator of environmental stress in fish. Many investigators have reported plasma glucose levels under various toxicant exposure conditions (Teles et al., 2003; Sepici-Dincel et al., 2009). The protein is the major intake of energy source (Agrahari and Gopal, 2009). Blood serum protein is a fairly labile biochemical system, precisely reflecting the condition of the organism and the changes happening to it under influence of internal and external factors (Hadi et al., 2009). Thus, the influence of toxicants on the total protein concentration of fish has been taken into consideration in evaluating the response to stressors and consequently the increasing demand for
energy (Osman et al., 2010). Significant alterations in plasma/organ protein level were noted in many fish species exposed to metals (Kori-Siakpere and Ubogu, 2008; Kopp et al., 2011). Bilirubin concentrations are attributable to liver and/or biliary tract disease. Serum bilirubin is often used for the evaluations of the liver condition (Jung et al., 2003). Several investigators reviewed the bilirubin concentrations in fish exposed to various toxicants (Jyothi and Narayan, 1999; Okonkwo and Ejike, 2011).

In recent years, there has been a rapid development of enzymatic biomarkers. This is not only due to advances in biochemistry but also to modern methods of measurement. The measurement of fish cellular enzymes is an indicator of health condition and has been used as diagnostic tool in monitoring programs of aquatic pollution (Fernandes et al., 2008). Phosphatases are good indicators of stress condition in the biological systems (Verma et al., 1980). Acid and alkaline phosphatases are general enzymes present in almost all the tissues. They are hydrolytic enzymes concerned with the process of transphosphorylation and have an important role in the general energetics of an organism. They are associated with the transport of metabolites, with metabolism of phospholipids, phosphoproteins, nucleotides and carbohydrate, and with synthesis of proteins (Srivastava et al., 1995). Acid phosphatase is a lysosomal enzyme that hydrolyses the ester linkage of phosphate esters and helps in autolysis of cell after its death. Alkaline phosphatase (ALP) is a membrane-bound enzyme related to the transport of various metabolites (Lin et al., 1976). It has been also proposed as a good biomarker in ecotoxicology because of its sensitivity to metallic salts (Boge et al., 1992). Alterations in acid phosphatase (ACP) and alkaline phosphatase (ALP) activities in tissues and serum have been reported in fish species (Jyothi and Narayan, 2000; Rogers et al., 2003; Atencio et al., 2008).

Histopathological investigations have long been recognized to be reliable biomarkers of stress in fish for several reasons (van der Oost et al., 2003). Results from histopathological studies are useful in establishing water quality criteria (USEPA, 1992). The overall toxic impact on organs like gill, kidney and liver may seriously affect the metabolic as well as physiologic activities and could impair the growth and behavior of fish (Mishra and Mohanty, 2009). Histopathological characteristics of specific organs can express condition and represent time-integrated
impacts of both exogenous and endogenous origin (Teh et al., 1997). Gills, liver and kidney have been frequently used in the assessment of impact of aquatic pollutants in marine as well as freshwater habitats. Several studies have revealed histological alterations in gills, liver and kidney of fish exposed to toxic compounds (Jimenez-Tenorio et al., 2007; Liu et al., 2011).

Among the various metals cadmium is most toxic to many aquatic organisms (Ruparelia et al., 1990). Cd is a non-essential metal whose dispersion in the environment has increased over the past decades due to its widespread industrial use as a color pigment in paints, in electroplating and galvanizing, in batteries, etc. (Guinee et al., 1999). There are estimates that 30,000 tons of Cd is released into the environment each year, with an estimated 4000–13,000 tons coming from human activities (ATSDR, 2003b). Cd causes impairment of reproductive activity and disrupts endocrine function in fish population exposed to environmentally relevant concentrations of Cd. It has been shown to modulate endocrine pathways in teleosts in vivo and in vitro (Hontela, 1998). In fish, Cd can exert a wide range of pathological effects like skeletal deformities (Muramoto, 1981), damage in gill structure (Thophon et al., 2003), disturbances in respiration (Chowdhury et al., 2004), changes in hematology (Zikic et al., 2001) and other blood parameters, such as cortisol and glucose, which reveal the stress response in fish (Fu et al., 1990; Lacroix and Hontela, 2004) and disruption in whole-body or plasma ion regulation (Chowdhury et al., 2004). To our knowledge the effect of cadmium on bioaccumulation markers and biological biomarkers in Indian major carps are scanty.

The objective of the present study was to assess the toxic impact of the cadmium in an Indian major carp Cirrhinus mrigala using bioaccumulation markers and biological biomarkers. The presence of heavy metals such as cadmium has gained wide interest in the scientific community in recent years due to its potential human health hazards. Fish bioaccumulation markers may be useful in order to elucidate the aquatic behavior of environmental contaminants as bioconcentrators to categorize certain substances with low water levels and to assess exposure of aquatic organisms. Further in order to assess exposure to or effects of environmental pollutants on aquatic ecosystems, the biological biomarkers may be useful for environmental monitoring programme.
The main goals of the present work were to:

➢ To derive the median lethal concentration (LC 50) of Cd for 24 and 96 h in *Cirrhinus mrigala*.

➢ To document cadmium accumulation patterns in gill liver and kidney of fish in relation to acute and sublethal concentrations of Cd and to assess the tissue metal burdens and differential accumulation patterns among tissues.

➢ To study hormonal changes (T4 and T3) in the plasma of fish after treated with acute and sublethal concentrations of Cd and to use these endpoints as stress biomarkers.

➢ To analyze antioxidant defenses (GSH, GST, GPx and LPO) in the blood of fish exposed to acute and sublethal concentrations of Cd and to use these endpoints as an indication of early detection.

➢ To find out biochemical changes (glucose, protein, total bilirubin, ACP and ALP) in the plasma of fish related to acute and sublethal concentrations of Cd, which generally indicates the health status of fish.

➢ Finally to obtain information about histopathological changes in gill, liver and kidney of fish treated with acute and sublethal concentrations of Cd, which provides important of qualitative and quantitative information of toxicants.