Aquatic resources are fundamental to livelihood of man and they are a vital component in the well being of all living organisms. Aquatic resources are the resources potentially useful to humans, either for agricultural, industrial, household, recreational, research, navigation, health and environmental activities. About 97.5% of water on the earth is salt water, leaving only 2.5% as freshwater. Water resources demand already exceed supply in the world and as world population continues to rise at an unprecedented rate, many more imbalances should be expected in the supply of aquatic resources.

Massive increase of human population has lead to an exponential enhancement in technological and industrial advancements all around the world. In modern times, one of the main threats to the health of ecosystems is the exposure to a large number of toxic substances and compounds. The natural aquatic systems may extensively be contaminated with toxic chemicals released from domestic, industrial and other man-made activities (Suneetha, 2012). The outburst of these advancements has greatly increased the consideration and concern for the quality of the environment in the last decades, mainly due to the awareness that pollution can be found everywhere. Aquatic systems are considered as suitable sites for disposal and recycling of sewage and toxic wastes and drain off the excess to the sea. Water pollution may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Begum, 2004; Susan et al., 2010).

Aquatic organisms such as fish are, in most cases, exposed to multitudes of stressors that are either natural or anthropogenically introduced into the environment. Contamination of fish with pollutants might adversely impact exploitation of aquatic
resources. Among the different habitats aquatic environment is the major target of pollution. Among the different types of pollutions, chemical pollution appears to be the major type which threatens the living systems very extensively. Fish live in a wide range of habitats all over the world. However, many of their natural habitats are being altered by anthropogenic influences, including physical structures (dams and weirs), agricultural runoff, industrial waste, and urban pollutants. Contamination of water by toxic chemicals either directly or indirectly can lead to death of fish, reduced fish productivity or elevated concentrations of undesirable chemicals in edible part of fish tissue which can affect the health of human by eating such fish (Hosetti et al., 2011).

Fish are sensitive to aquatic contamination and serious concerns remains due to their populations. 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 (Das and Mukherjee, 2003). It is believed that the fish posses the same biochemical pathways to deal with the toxic effects of endogenous and exogenous agents as do mammalian species (Al-akel et al., 2010; Ahmad, 2011). Therefore, it is important to examine the toxic effects of chemical compounds on fish since they constitute an important link in food chain and their contamination by toxicants imbalance the aquatic system. The studies carried out on various fish have shown that pollutants may alter the physiological activities and biochemical parameters both in tissues and blood (Basa and Rani, 2003).

Fish have been used as indicators for contamination of aquatic environment for several years. During environmental catastrophe, fish are unable to escape from the site
affected, thus bioaccumulate toxic substances (Andrade et al., 2004). Fish acts as the last link in the food chain in the aquatic environment, they may negatively influence the food safety when they get exposed to contaminated environment. It is very important from the environmental point of view to understand the responses of the fish species to exposure to toxic substances. Early detection of the sublethal effects of a chemical may be the basic element in deciding about biodegradation and revitalization methods at polluted site (Tiwari et al., 2012).

Cyanide is one of the most toxic chemical substances on earth and is toxic to most aquatic life and human beings, even at low concentrations. Unlike other toxic metals, cyanide is not an element but a compound composed of only carbon and nitrogen. The in-depth chemistry of cyanide and its chemical behaviour in streams and sediments is complex and its toxicity is influenced by several factors, including acidity or alkalinity (Moran et al., 1980). Toxicity of the cyanide compounds is instant or acute and, once reacted, leaves little residual trace.

Numerous forms of cyanide exist in nature; they include gaseous hydrogen cyanide, water-soluble potassium and sodium cyanide salts, and poorly water-soluble mercury, copper, gold, and silver cyanide salts. In addition to this a number of cyanide-containing compounds, known as cyanogens, may release cyanide during metabolism. Several chemical forms of cyanides are present in the environment, including free cyanide, metallocyanide complexes, and synthetic organocyanides, also known as nitriles. But only free cyanide (i.e., the sum of molecular hydrogen cyanide) and the cyanide anion, (CN⁻) is the primary toxic agent, regardless of origin (Eisler, 1991).
Cyanides are readily absorbed through inhalation, ingestion, or dermal contact and are readily distributed throughout the body via blood. Cyanide is a potent and rapid-acting asphyxiant; it induces tissue anoxia through inactivation of cytochrome oxidase, causing cytotoxic hypoxia in the presence of normal hemoglobin oxygenation. Among the most consistent changes observed during acute cyanide poisoning are inhibition of brain cytochrome oxidase activity, and changes in electrical activity in heart and brain. Cyanide is generally considered to be a rare source of poisoning; however, cyanide exposure occurs frequently in individuals with smoke inhalation from residential or industrial fires. Cyanide poisoning may also occur in industries, particularly in the metal trades, mining, electroplating, jewelry manufacturing, and x-ray film recovery. It is also encountered in fumigation of ships, warehouses, and other structures. Cyanides are also used as suicidal agents, particularly among healthcare and laboratory workers, and they can potentially be used in a terrorist attack (Bhattacharya, 2000).

All living organisms exhibits broad spectrum of reactions and sensitivities when exposed to chemicals. Some are very resistant to high doses while others are very susceptible even to low doses; most of them are somewhere between very resistant and very susceptible (Eisler, 1991). In fact, when the sensitivity of a large number of animals are graphed against increasing doses of a drug, or some other chemical, the resulting distribution curve is shaped like a bell. If a certain dose of a toxic chemical is given to a large number of animals, some show no effect, some get sick, and some die. Thus, any toxicological test, do not expect all animals to be affected, nor expect all animals affected to exhibit the same degree of severity (Vutukuru, 2005).
Fish population is generally considered to be very sensitive to all kinds of environmental changes to which it is exposed as they are exclusively aquatic. Certain stages in the life cycle of freshwater fish are more susceptible to environmental and pollution stresses (Scott and Sloman, 2004). The binding of cyanide to this cytochrome prevents transport of electrons from cytochrome c oxidase to oxygen. As a result, the electron transport chain is disrupted, meaning that the cell can no longer aerobically produce ATP for energy (Ardelt et al., 1994). Although the time, dose and manner of exposure may differ, the biochemical action of cyanide is the same upon entering the body. Once in the bloodstream, cyanide forms a stable complex with cytochrome oxidase, an enzyme that promotes the transfer of electrons in the mitochondria of cells during the synthesis of ATP. Without proper cytochrome oxidase function, cells cannot utilize the oxygen present in the bloodstream, resulting in cytotoxic hypoxia or cellular asphyxiation (David et al., 2010). Lack of available oxygen causes a shift from aerobic to anaerobic metabolism, leading to the accumulation of lactate in the blood. The combined effect of the hypoxia and lactate acidosis leads to depression of the CNS that can result in respiratory arrest and death.

The toxicity of cyanide to aquatic life is probably caused by hydrogen cyanide that has ionized, dissociated or photochemically decomposed from compounds containing cyanide (Hosetti et al., 2010). Toxic effects of the cyanide ion itself on aquatic organisms are not believed to be significant, nor are the effects of photolysis of metal cyanide complex. It is therefore the hydrogen cyanide concentration of water that is of greatest significance in determining toxicity to aquatic life rather than the total cyanide concentration (Eisler, 1991).
Aquatic toxicity tests are performed in order to evaluate the response of organisms and to detect or measure the presence or effect of one or more chemical substances to the biota, alone or in combination (Yazdandoost and Katdare, 1999). Increase in the widespread usage of toxic chemicals, ultimately pollute the aquatic environment, thereby affecting the aquatic fauna mainly fish, which constitute the major economy of the country and valuable source of protein (Munian and Veeraraghavan, 1999). In the recent decades researcher have focused on the laboratory-determined lethal concentration effects of pollutants, but in reality, the levels in their actual environments are sublethal. Nominal (sublethal) concentrations can have a detrimental effect on several aspects of the fish (Tembo, 2009).

Toxicity is defined as the inherent property of a toxicant to adversely affect biological process of an organism. The toxicity of a chemical for aquatic organism is expressed in terms of LC50 (Bhattacharya et al., 2009). This value represents the amount of a toxicant, which kills 50% of the population of the test animals within a fixed period of time (Finney, 1971). If the test animals are terrestrial, the toxicant will be administered either through the oral or intramuscular or inhalation method and the toxicity will be expressed in terms of LD50. Where as if the test animals are aquatic the toxicant will be generally mixed with the ambient aquatic medium and the toxicity will be expressed in terms of LC50. The period of exposure is considerably important in evaluating the toxicity levels of toxicants in aquatic animals. Depending upon the nature of toxicant, LC50 values will be assessed at 24 or 48 or 72 or 96 h or even more (Sanoli and Kanabur, 2011).

When fish are stressed, the stress hormones are released (primary response), the composition of blood and other tissues gets altered followed by variations in ventilation
and heart rate (Israeli and Kimmel, 1998). In the long run, stress induces a reduction in the effectiveness of the immune system, resulting in high susceptibility to diseases and parasites, decreased growth rate, reduced reproduction and increased mortality. All these stress-induced changes are associated with variations in the behavior of the fish (Adams, 1990). Stressed fish change their behavior. By ‘behavior’ we refer to the series of visible actions which are operated and controlled by the nervous, sensory and endocrine organs. Behavior is a major link between the organism and its environment. Behavior provide basis for molecular, physiological, and ecological aspects of toxicology; therefore it provides insight into various levels of biological organization (Scott and Sloman, 2004). Escape or freezing, avoidance or attraction is common behavioral responses to adverse stimuli (Shwetha and Hosetti, 2009).

Stress response consists of adjustments to the physiology and behaviour of the fish that promotes the best chance of survival when the organism is faced with the toxic substance or threatening situation. The oxygen consumption rate (OCR) of fish is an important factor in metabolism. Many researchers (Gurusamy and Ramdas, 2000; Mathivanan, 2004) have observed decreasing trend in oxygen consumption in fish exposed to pollutants or toxicants. In the aquatic environment one of the most important manifestation of the toxic action of chemical is the over stimulation or decline of respiratory activity. Changes in the respiratory activity of fish have been used by several investigators as indicators of response to environmental stress. The oxygen consumption of an animal is the important physiological parameter to assess the toxic stress, because it is a valuable indicator of energy expenditure in particular and metabolism in general (Susan et al., 2010). Like most fish, carps are oxygen regulators, i.e., they maintain their...
oxygen consumption at a constant level along a gradient of environmental oxygen concentrations, until a critical oxygen concentration, is reached, below which oxygen consumption begins to fall (Shwetha and Hosetti, 2009). Under conditions of stress, this critical oxygen is likely to increase, reflecting the decreased capacity of the fish to cope with environmental contaminations.

Biochemical alterations are considered as sensitive indicators of toxicity before hazardous effects occur in fish and are important parameters for testing water and the presence of toxicants. The biochemical markers can detect early responses and prepathological alterations before other disturbances as disease, mortality or population changes occur. Such a biochemical approach has been advocated to provide an early warning of potentially damaging changes in stressed fish. It is well known that fish are ideal test organisms for investigation of certain physiological and biochemical processes of toxicants in water. Various alterations in the fish exposed to toxic chemicals show effective endpoint for evaluation of changes of water quality by toxicants in a short period (Agrahari et al., 2007). Evaluation of intermediary metabolism of fish was used, for example, to study toxicant effects caused by heavy metal (Metwally and Fouad, 2008), aromatic compositions (Mavadati and Habibian, 2011), detergents (Barbieri, 2007), pesticides (Naveed et al., 2010; 2011) and a variety of toxins (Saravanan et al., 2011; Palanisamy et al., 2011).

Biochemical approach provides an early warning of harmful changes in stressed fish. Evaluation of biochemical response of fish was used, for example, to study cyanide induced effects (de Zwaan et al., 1993). The effect of toxicant on enzymatic activity is one of the most important biochemical parameters, which is affected under stress.
condition (Das and Mukherjee, 2003). When an organ is diseased due to the effect of a toxicant, enzymatic activity appears to be stimulated or inhibited due to the active site being either denatured or distorted. Since some enzymes catalyze some steps in the metabolism of carbohydrate and protein, they are present in most tissues. The increase or decrease in their level may be sufficient to provide information of diagnostic values (Begum, 2008).

Knowledge of sublethal effects of toxic compounds on enzymatic activity is important to delineate the health status of fish and provide a future understanding of ecological impacts (Singh and Singh, 2002). Enzymatic activities are the useful 'markers' of physiological damage (Adamu and Iloba, 2008), thus are needed to be assayed in the test fish. Cyanide inhibits the mitochondrial enzyme cytochrome oxidase in the respiratory electron transport chain of the mitochondria, impairing both oxidative metabolism and the associated process of oxidative phosphorylation (Daya et al., 2000). Additionally, a number of other enzymatic processes are also inhibited which exacerbate the cyanide toxicity (Prashanth and Neelagund, 2007). Cyanide, a metabolic inhibitor which prevents resynthesis of adenosine triphosphate (ATP) in the axon, expected to reduce the release of ions to a very low value (Unnisa and Devaraj, 2007). The reduction of ATP has been suggested to cause disturbances in cellular metabolism, leading to histotoxic hypoxia in the fish (Begum, 2011).

Adenosine triphosphatases (ATPases) are complex set of enzyme systems found in invertebrates and vertebrates (Carfagna et al., 1996). These enzymes play a central role in physiological functions of a cell as energy transducers by coupling the chemical reactions. In cells, whatever mechanisms are used to maintain viability under anoxia,
ATP-reducing and ATP-utilizing reactions must be curtailed in concert so that trans-membrane ionic gradients are maintained (Cotou et al., 2001). When this balance is broken, cells inevitably become sensitive to anoxia, a process which eventually leads to inhibition of ion transport with loss of trans-membrane ion gradients and anoxic depolarization. However, if cells adapt to the new situation, a new steady state will be achieved and this include a general reduction of metabolic processes and probably a changed allocation of energy utilization (Pablo et al., 1996). Membrane localization is the key to the physiological function of ATPases which are coupled with pumping of cations across the membranes from one intracellular component to another.

ATPases required Na\(^+\)K\(^+\), Mg\(^{2+}\) and Ca\(^{2+}\) ions for their activity and involve in the cleavage of ATP to ADP / AMP and inorganic phosphate with liberation of energy (Begum, 2011). In addition to its fundamental importance to ion-transport, ATPase activity could be used as an indicator of physiological changes. Osmotic regulations in freshwater fish are intimately bound to control ionic concentration as well as cell and body volume (Kumosani, 2004). These membrane enzymes which carry out ion transport with parallel energy production are well characterized and variation in their activity can be used to measure the toxic impact of chemicals (Cotou et al., 2001).

Histopathological studies have proven to be sensitive tool to detect direct effects of chemical compounds within the target organs of the fish in laboratory and field experiments. Histopathological biomarkers can be indicators of the effects various anthropogenic pollutants on organisms and are a reflection of the overall health of the entire population in the ecosystem. The alterations in cells and tissues in fish are used as
Chapter I

Introduction

Biomarkers in many studies, but such changes occur in all vertebrates and invertebrates inhabiting aquatic basins. Histopathological biomarkers embody tissue lesions arising as a result of a previous or current exposure of the organism to one or more toxicants. Well-documented lesions based on experimental data in liver, ovary, skeleton system and skin have been used as biomarkers (Vinodhini and Narayanan, 2009).

Histopathological biomarkers are closely related to other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular changes in the affected organism. For example, the mechanism of action of several xenobiotics could initiate the formation of a specific enzyme that causes changes in metabolism, further leading to cellular intoxication and death, at a cellular level, whereas this manifests as necrosis, i.e. histopathological biomarker on a tissue level (Velkova-Jordanoska and Kostoski, 2005).

Indian Major Carps, form important commercially exploited species and are ideal animal for studying the impairment caused by the effects of toxic chemicals that are often detected in the aquatic environment. Information on the toxic effects of cyanide to fish is limited and its effects on the widely consumed Indian major carp, Labeo rohita which forms important link in the aquatic food chain, are not known. In view of this, the present study was being undertaken to know the effects of cyanide on the survival and physiology of the fish. The reported results are useful contribution in the ecotoxicity risk assessment studies of cyanide on these fish species. Present work tends to the advancement of knowledge in the field of environmental toxicology and biochemistry and for developing proper remediation measures for cyanide containing wastes.
Objectives:

Because of the importance of cyanides as water pollutants, numerous studies have been conducted on their lethal action to fish, but only a few investigations on sublethal toxic effects have been reported. To explore toxic effects sodium cyanide on fish that may have ecological significance, experiments were performed on the freshwater fish *L. rohita* using sodium cyanide with the following objectives.

1. To evaluate the toxicity of the sodium cyanide to the fish, *Labeo rohita* and to derive the safe limits of sodium cyanide.
2. To derive more sensitive end points related to the behaviour of the fish, *Labeo rohita*.
3. To evaluate effect of sublethal concentration of the sodium cyanide on some biochemical changes in the test animal.
4. To study sodium cyanide induced histopathological alteration in the fish.

This thesis is structured in to six chapters. General introduction and review of literature are presented in chapter 1 and 2 respectively. Detailed methods employed during the experimental stages are narrated in the chapter 3. The chapter 4 explains the entire results of the present study. All the experimental data are discussed in the chapter 5. Summary and conclusion are mentioned in chapter 6. This is followed by the publication and references.