CHAPTER 1

INTRODUCTION

1.1. Heavy metals

Heavy metals are members of an ill-defined subset of elements that exhibit metallic properties. These include the transition metals, some metalloids, lanthanides, and actinides. The term “heavy metal” assumes a variety of different meanings throughout the different branches of science. Although “heavy metal” lacks a consistent definition in medical and scientific literature, the term is commonly used to describe the group of dense metals or their related compounds, usually associated with environmental pollution or toxicity (Duffus 2002). These metals are a cause of environmental pollution from sources such as leaded petrol, industrial effluents, and leaching of metal ions from the soil into lakes and rivers by acid rain. Any toxic metal may be called heavy metal, irrespective of their atomic mass or density (Singh *et al.,* 2011).

Any metal (or metalloid) species may be considered a “contaminant” if it occurs where it is unwanted, or in a form or concentration that causes a detrimental human or environmental effect. Metals/metalloids include lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), chromium (Cr), copper (Cu), selenium (Se), nickel (Ni), silver (Ag), and zinc (Zn). Other less common metallic contaminants include aluminium (Al), cesium (Cs), cobalt (Co), manganese (Mn), molybdenum (Mo), strontium (Sr), and uranium (U). (Mcintyre 2003)

Living organisms require varying amounts of "heavy metals". Iron, cobalt, copper, manganese, molybdenum, and zinc are required by humans. Excessive levels can be damaging to the organism. Other heavy metals such as mercury, cadmium, plutonium, and lead are toxic metals and their accumulation over time in the bodies of animals can cause serious illness. Certain elements that are normally toxic are, for certain organisms or under certain conditions, beneficial. Examples include vanadium, tungsten, and even cadmium (Singh *et al*., 2011).

Acute heavy metal intoxications may damage central nervous function, the cardiovascular and gastrointestinal (GI) systems, lungs, kidneys, liver, endocrine glands, and bones (Jang 2011; Adal 2013). Chronic heavy metal exposure has been implicated in several degenerative diseases of these same systems and may increase the risk of some cancers (Galanis 2009; Wu 2012).

While a specific toxic metal has the potential to exert detrimental effects by select mechanisms, there are several common features among toxic heavy metals. One of the most widely studied mechanisms of action for toxic metals is oxidative damage due to direct generation of free radical species and depletion of antioxidant reserves (Ercal 2001). Mercury, cadmium, and lead, for example, can effectively inhibit cellular glutathione peroxidase, reducing the effectiveness of this antioxidant defense system for detoxification (Reddy 1981). Many toxic heavy metals act as molecular “mimics” of nutritionally essential trace elements; as a result, they may compete with essential metallic cofactors for entry into cells and incorporation into enzymes (Jang 2011).

1.2. Riverine accumulation of pollutants including heavy metals

Surface water is usually rain water that collects in surface water bodies, like oceans, lakes, or rivers. Another source of surface water is groundwater that discharges to the surface from springs. Surface water pollution occurs when hazardous substances come into contact and either dissolve or physically mix with the water. Because of the close relationship between sediments and surface water, contaminated sediments are often considered part of surface water contamination. Sediments include the sand and soils on the bottom of an ocean, lake, or rivers.

Surface water can become contaminated in many ways. Surface water can be contaminated when hazardous substances are discharged directly from an outfall pipe or channel or when they receive contaminated storm water runoff. Direct discharges can come from industrial sources or from certain older sewer systems that overflow during wet weather. Storm water runoff becomes polluted when rain water comes into contact with contaminated soil and either dissolves the contamination or carries contaminated soil particles. Surface water can also be contaminated when contaminated groundwater reaches the surface through a spring, or when contaminants in the air are deposited on the surface water.

Contaminated surface water can also affect the health of animals and humans when they drink or bathe in contaminated water or, for aquatic organisms, when they ingest contaminated sediments. One of the major concerns associated with contaminated surface water is the ability of aquatic organisms, like fish, to accumulate and concentrate contaminants in their bodies. When other animals or humans ingest these organisms, they receive a much higher dose of contamination than they would have if they had been directly exposed to the original source of the contamination.

Surface waters are most exposable to pollution due to their easy accessibility for disposal of waste waters. Both the anthropogenic influences such as urban, industrial, and agricultural activities increasing exploitation of water resources as well as natural processes such as precipitation inputs, erosion, and weathering of crustal materials degrade surface waters and damage their use for drinking, industrial, agricultural, reaction, or other purposes (Jarvie *et al*., 1998; Simeonov *et al*., 2003).

In recent years the accumulation of heavy metals in aquatic systems has become a problem of great concern throughout the world. These metals may accumulate to a very high toxic levels and cause severe impact on the aquatic organisms without any visible sign. A river close to an urban center has both naturally occurring and anthropogenically originated heavy metals in its environment. Natural sources come from physical and chemical weathering of rocks of the catchment area of rivers. Anthropogenic sources include industrial, domestic wastes and sewage effluents originating from nearby urban center and draining into rivers.

The geographic location and regional topography of the area (Fig. 1.1) where this work has been carried out (Assam University, Silchar) merits special mention as they make the present study relevant as a global phenomenon with major local implications. Barak valley of southern Assam, India is bordered by the Barail, Khasi and the Jaintia hills (Fig. 1.2). The Barak riverine system has its tributaries from the neighboring states of Meghalaya, Manipur, Mizoram, Nagaland and Tripura. Thus, this river valley acts as a catchment area receiving run-off water from feeder-rivers descending from the hills of neighboring states. As the river enters Surma valley of Bangladesh, it meanders widely, leading to heavy silt deposition and a slow rate of flow. Thus, any heavy metal load that drains into this riverine system tends to stagnate. Frequent floods occur due to shallowness of the river bed, thus, further contaminating the river.

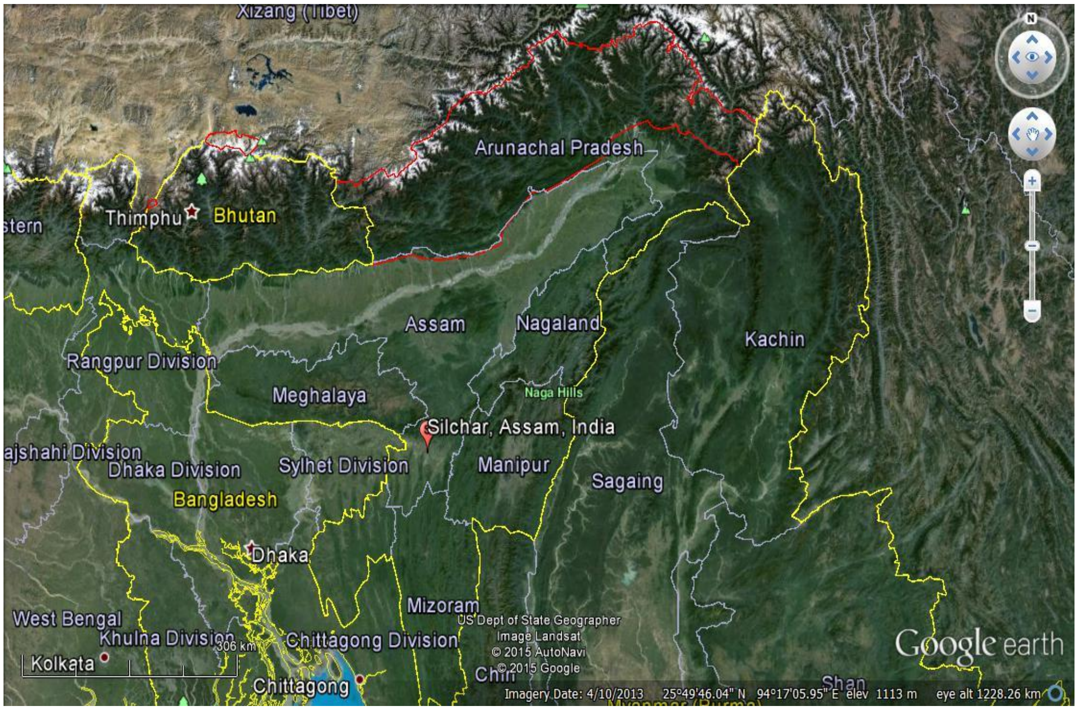

Fig. 1.1: Physical map of North East India with its neighboring states and international political borders. The map highlights the proximity to Bangladesh (Source: Google earth)

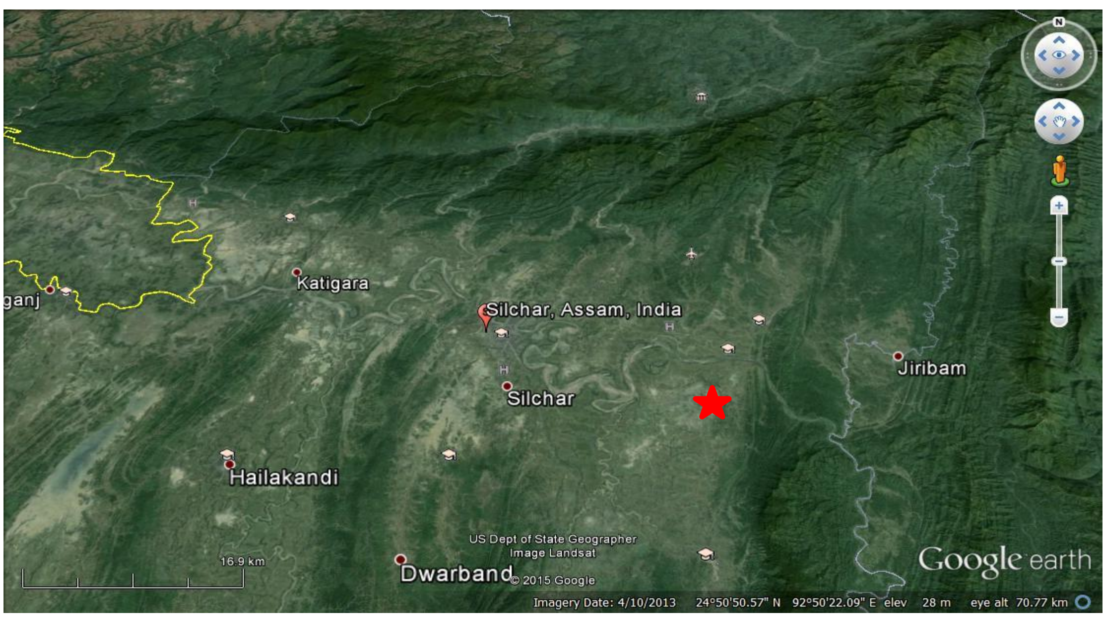

Fig. 1.2: The map of Silchar Assam, India showing the Barak riverine system (red star) (Source: Google earth).

1.3. Heavy Metals in Aquatic Communities

Heavy metals derived from agricultural operations (pesticides and herbicides), industrial effluents and other man made activities 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 (Bernet *et al*., 1999). 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 (Mathis *et al*., 1973)

Another reason for the interest in heavy metals and behavior in aquatic communities is that heavy metals may have behavioral effects at concentrations much less than at which they have lethal effects (Scott and Sloman 2004), suggesting that regulatory pollution limits based upon standard toxicological studies may be too high to prevent damage to aquatic communities through these sub-lethal behavioral effects. This realization has led to calls for increased integration of behavioral studies into eco-toxicological investigations (e.g., Clotfelter *et al*. 2004; Klaschka 2008).

Unlike organic pollutants, heavy metals do not decay and thus pose a different kind of challenge for remediation. Plants, mushrooms, or microorganisms are occasionally successfully used to remove some heavy metals such as mercury. One of the largest problems associated with the persistence of heavy metals is the potential for bioaccumulation and bio-magnification causing heavier exposure for some organisms than is present in the environment alone. Coastal fish (such as the smooth toadfish) and seabirds (such as the Atlantic Puffin) are often monitored for the presence of such contaminants.

The fungus *Aspergillus niger* plays a role in the solubilization of heavy metal sulfides (Singh 2006).

Heavy metals are natural constituents of the earth's crust, but indiscriminate human activities have drastically altered their geochemical cycles and biochemical balance. This results in accumulation of metals in plant and many aquatic organisms including fishes. Prolonged exposure to heavy metals such as mercury, cadmium, copper, lead, nickel, and zinc can cause deleterious health effects in humans.

Intense activity in industrial and agricultural sectors has inexorably increased the levels of toxic heavy metals in aquatic environment. Heavy metals are serious pollutant of the aquatic environment because of their environmental persistence and ability to be accumulated by aquatic organisms (Veena *et al.,* 1997). Marine or fresh water animals such as fish are able to readily absorb metals and their bodies regulate to accommodate their presence. They are easily stored in fatty tissue and will bio-accumulate if the fish is exposed to further contamination. Among heavy metals, mercury and cadmium are the wide spread metal pollutant of high toxicity not only to warm blooded vertebrates, but also to aquatic animals including fishes. There is currently triple the amount of mercury falling from the sky than at the time of the Industrial Revolution. It is also contaminating many areas as a result of unregulated gold mining. The metal is absorbed by fish and other aquatic animals, and passed up the food chain to any other fish-eating species. Scientists today are concerned that people who eat fish contaminated with mercury and cadmium will be put at risk – particularly pregnant mothers and children as mercury poisoning has been shown to adversely affect brain development. However it is also being linked to a higher risk of heart disease and stroke in men as well as memory loss, and central nervous system damage. Cadmium is very toxic to humans and has been linked to kidney disease, lung problems, and prostate cancer.

A neurological disease, known as Minamata disease is caused by severe mercury poisoning. Minamata disease was first discovered in Minamata city in Kumamoto prefecture, Japan in 1956. It was caused by the release of methyl mercury in the industrial wastewater from the Chisso Corporation's chemical factory, which continued from 1932 to 1968. This highly toxic chemical bio-accumulated in shellfish and fish in Minamata Bay and the Shiranui Sea, which when eaten by the local populace resulted in mercury poisoning. While cat, dog, pig and human deaths continued over more than 30 years, the government and company did little to prevent the pollution.

Fish are excellent subject for the study of various effects of contaminants present in the aquatic environment since they can metabolize, concentrate and store water borne pollutants. The presence of metal pollutant both in fresh and marine water has been found to disturb the delicate balance of aquatic ecosystem (Asaolu *et al.*, 2005). Fish are disreputable for their ability to concentrate metals in their body tissues and since they play important role in human nutrition, they need to screened to ensure that unnecessarily high level of some toxic metals are not being transferred to man through fishes (Kakulu *et al*., 1988). Like all vertebrates, fish possess a wide array of defense systems to protect themselves against heavy metals. Teleost fish have proved to be good models to evaluate the toxicity and effects of contaminants on animals, since their biochemical responses are similar to those of mammals and of other vertebrates (Sancho *et al*., 2000). *Channa punctatus* is a freshwater teleost fish and was selected as an experimental model because it has well documented general biology, short developmental time easy culturing and year reproduction. Because of these characteristics *Channa punctatus* was considered suitable for toxicity test. Fish offer a number of advantages over the mammalian models of immunotoxicological study because they are not only amenable to laboratory and field studies but also provide a large repository of immune cells. Moreover, knowledge on the effect of cadmium and mercury on the fish immune system is relatively limited.

1.4. Oxidative stress

A disturbance in the balance in between the pro-oxidants and antioxidants leading to detrimental biochemical and physiological effects is known as oxidative stress. This is a harmful condition in which increases in free radical production, and/or decrease in antioxidant levels can lead to potential damage. Indicators of oxidative stress include changes in the antioxidant enzyme activity, damaged DNA bases, protein oxidation products and lipid peroxidation products. Oxidative stress is known to play a critical role in the pathology of several human diseases, including pulmonary fibrosis, cancer, neurodegenerative diseases such as Parkinson's, Alzheimer's etc. (Thannical and Fanburg 2000).

All forms of life maintain a reducing environment within their cells. This reducing environment is preserved by enzymes that maintain the reduced state through a constant input of metabolic energy. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including protein, lipid, and DNA. Mercury, cadmium, and other heavy metals have a high affinity for sulfhydryl (-SH) groups, inactivating numerous enzymatic reactions, amino acids, and sulfur-containing antioxidants (NAC, ALA, GSH), with subsequent decreased oxidant defense and increased oxidative stress. Both bind to metallothionein and substitute for zinc, copper, and other trace metals reducing the effectiveness of metalloenzymes. Mercury induces mitochondrial dysfunction with reduction in ATP, depletion of glutathione, and increased lipid peroxidation; increased oxidative stress is common. Selenium antagonizes mercury toxicity. The overall vascular effects of mercury include oxidative stress, inflammation, thrombosis, vascular smooth muscle dysfunction, endothelial dysfunction, dyslipidemia, immune dysfunction, and mitochondrial dysfunction including proteins, lipids, and DNA (Houston, 2007).

Oxidative stress as stated above is an imbalance between the production of reactive oxygen species the cells ability to reduce ROS, detoxify reactive intermediates, and repair damage that may occur in the cellular molecules. This imbalance may occur as a result of increased ROS production, a decrease in defense mechanisms, or both. ROS are endogenously produced from a wide range of sources within the cell.

A large number of heavy metals and xenobiotics that are present in the environment as a direct result of human activity can cause an increase in ROS production in the cells of the exposed individual. As the amounts and diversity of chemicals entering in to the aquatic environment increase, biological loads in fishes have also increased with deleterious effects. Many pollutants mediate their toxicity through oxidative stress resulting in changes in antioxidant defenses as well as damage to proteins, membrane lipids and DNA molecules.

The result of such exposure leading to oxidative stress can impair cellular or biological function which can lead to disease (Almroth 2008).

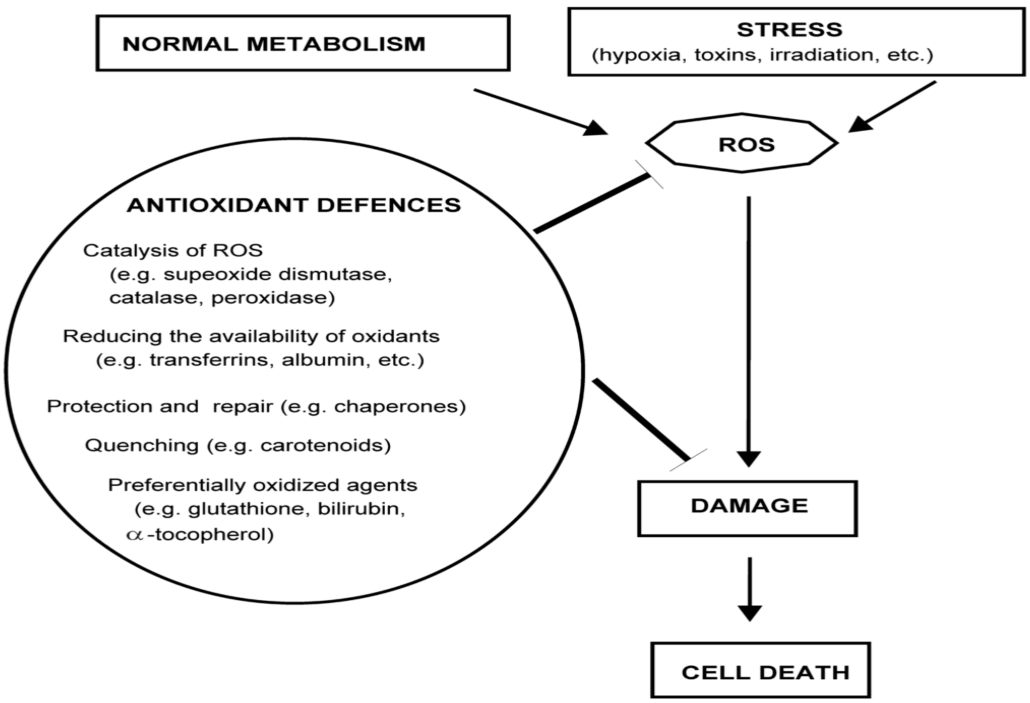

Fig.1.3: Effects of oxidative stress on living organism

1.5. Biomarkers

A biomarker, according to Peakall and walker (1996), is any biological response to an environmental chemical at the individual level or below demonstrating a departure from the normal status. Oxidative stress as a result of environmental pollution has been documented in numerous fish species over the past decades (van der Oost *et al*., 2003, Valavanidis 2006).

Measuring heavy metals in aquatic organisms may be a bioindicator of their impact on organism and ecosystem health (Krishnakumar *et al*. 1994), but a true evaluation of the damage inflicted by heavy metals should come from comprehensive biomarker studies. Biomarkers are more telling than bio-indicators as measurement of heavy metal contamination because they deal with chemical and physiological changes on the organism level and assess contamination based on a direct measure of change in the organism (de Lafontaine *et al*., 2000, Allen & Moore 2004). Research over time has focused on various species and various biomarkers to determine the amount of heavy metal toxicity in aquatic environments. Albaugh (2002) used sea anemones, Aspholm and Hylland (1998) used sea urchins, Downs *et al.,* (2001) used grass shrimp, and Regoli *et al*., (2002) used a benthic fish called the red mullet. Biomarkers in mussels such as glutathione (GSH) and metallothionein are often used to evaluate heavy metal contamination (Rainbow *et al*., 2000).

Several studies have shown that antioxidants that are affected by reactive oxygen species show adaptive responses to xenobiotics that produce oxyradicals (Di Giulio *et al*., 1995) and are potential biomarkers for oxidative stress in fish (van der Oost *et al*. 2003). Biomarkers of oxidative stress, such as changes in antioxidant enzyme activity or in degree of accumulation of damaged molecules, can offer an early warning sign for exposure to redox-active xenobiotics. These oxidative stress parameters have been associated with various disease pathologies and organism longevity in a number of species, thereby establishing ecological relevance in these cases. A large number of biomarkers of oxidative stress have been used in fish studies and these include both the antioxidant defense mechanisms possessed by the cell, both enzymatic and molecular, as well as oxidative damage products.

Increasing attention has also been paid to the immune system of fish as a bioindicator of exposure to environmental pollutants. Cells isolated from the anterior kidney (pronephros) of the sea bass *Dicentrarchus labrax* were used to investigate the non-specific immune activity (Bennani *et al*., 1995), this organ being the main hematopoietic tissue in fish (Stave *et al.*, 1983).

1.6. Antioxidant defenses in fish

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. All living creatures have an antioxidant defense system which can neutralize the harmful effects of reactive oxygen species (ROS) including hydroxyl radicals, superoxide radical anion, and hydrogen peroxide. The antioxidant systems in living organisms may be divided in to two types. One is represented by enzymes, such as superoxide dismutase, catalase and the peroxidases, which remove reactive oxygen species. The other group of antioxidative compounds scavenges free radicals; they are generally of low molecular weight and may be water or lipid soluble. These antioxidants reduce free radicals and are themselves oxidized. After that they may be reduced to their active forms by other reducing systems. The antioxidant defense system includes antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and other lower molecular weight substances such as glutathione (GSH), vitamins and proteins located in different tissues (Frei, 1999).

Antioxidants can act at different stages in the oxidation process and some may have more than one mechanism of action. Their mode of action can be classified into the following categories (Symons 1998):

- Removing oxygen or decreasing local O_2 concentrations.
- Removing catalytic metal ions.

- Removing reactive oxygen species such as $O_2^{\bullet-}$ and H_2O_2.
- Scavenging initiating radicals such as •OH, LO• and LOO•.
- Breaking the chain of an initiated sequence.
- Quenching or scavenging singlet oxygen.

Thus antioxidants may directly or indirectly inhibit the initiation and propagation steps of lipid oxidation. They sometimes have multiple effects and their mechanisms of action are therefore often difficult to interpret. According to their mode of action, antioxidants can be categorized into preventive inhibitors and true antioxidants. There are several natural compounds that participate in the antioxidative defense mechanism of fish (Hultin 1992, 1994; Undeland 1997). These include enzymes (catalase, peroxidase, glutathione and superoxide dismutase), carotenoids, peptides, amino acids and phenolic compounds (tocopherols, ubiquinones). These compounds are found in the cell plasma, mitochondria of cell membranes.

The antioxidant enzymes are found in almost all tissues of vertebrates, and their activities are especially high in the liver, a major organ responsible for the transformation of ROS. The ROS are generated during normal metabolism and the amounts are well-controlled under normal physiological conditions. When pollutants such as heavy metals, xenobiotic molecules enter the body, they undergo redox cycling and generate ROS. The body produces more antioxidant enzymes to get rid of the undesired ROS. This response is also called induction of antioxidant enzymes. When the generation of ROS overwhelms or insufficient levels of antioxidants or inhibition of the antioxidant enzymes occurs it may cause oxidative stress and may damage or kill cells. Oxidative stress is an unavoidable aspect of aerobic life. It is the result of an imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses in living organisms (Nishida, 2011). Mechanisms of antioxidant defenses in fish include the enzyme system and low molecular weight antioxidants, similar to those in mammals, although the specific isoforms of enzymes in various fish species have not been well identified (Di Giulio and Meyer, 2008). Superoxide dismutase (SOD), catalase (C AT), glutathione peroxidase (GPx), and glutathione-s-transferase (GST) are the main antioxidant enzymes and important indicators of oxidative stress. Reduced glutathione (GSH) and oxidized glutathione disulphide (GSSG) play a key role in non-enzymatic antioxidant defense. Metal binding proteins such as ferritin, ceruloplasmin, and metallothioneins (MTs) have special functions in the detoxification of toxic metals, and also play a role in the metabolism and homeostasis of essential metals (Kelly *et al.*, 1998).

1.7. Immune system of teleost fish

Fish are heterogeneous group of different organisms which include the agnathans (hagfishes and lampreys), condryctians (sharks and rays) and teleosteans (bony fish). Like all vertebrates, fish have cellular and humoral immune responses, and central organs whose the main function is involved in immune defence.(Cabezas, 2006; Nelson, 1994; Tort *et al*., 2003; Zapata *et al*., 1996).

The immune system serves to protect the host from infectious diseases and developing neoplastic cells and is highly conserved across all vertebrate species, with remnants also existing in invertebrates (Roitt *et al*., 1998). The teleost immune system shares many structural and functional similarities with the mammalian immune system and humoral, cell-mediated and non-specific immune responses have all been described. Fish are able to reject allografts, exhibit hypersensitivity responses, produce specific antibodies following antigenic stimulation, respond to mitogens and elicit a mixed lymphocyte reaction (van Muiswinkel *et al*., 1985).

The teleost defense system is basically similar to that described in mammals. For cellular defense systems in fish, teleosts have phagocytic cells similar to macrophages, neutrophils, and natural killer (NK) cells, as well as T and B lymphocytes. Teleosts also have various humoral defense components such as complement (classical and alternative pathways), lysozyme, natural hemolysin, transferrin and C-reactive protein (CRP). The existence of cytokines (such as interferon, interleukin 2 (IL-2), macrophage activating factors (MAF)) has also been reported (Secombes *et al*., 1996, Sakai, 1999). But the morphology of the immune system is quite different between fish and mammals. One of the important facts of fish immune system is that, fish lack bone marrow and lymph nodes. Instead, the head kidney serves as a major lymphoid organ, in addition to the thymus and spleen (Press & Evensen, 1999). Gut associated lymphoid tissues are also known lymphoid organs, and have been shown to function in eliciting immune responses in carp (Joosten *et al.*, 1996).

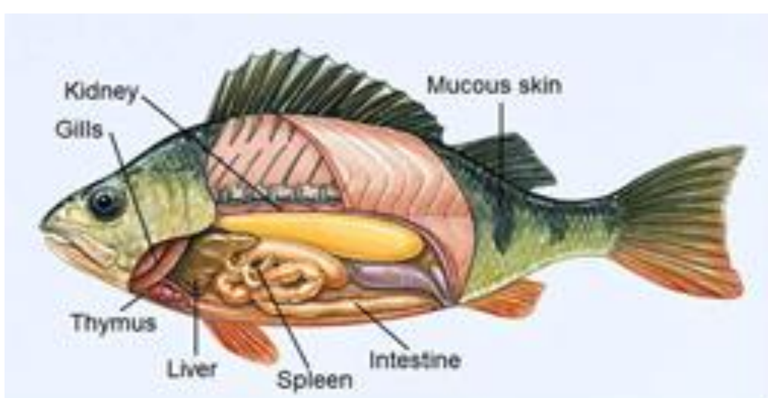

Fig. 1.4: Immune structures in teleost fish

1.8. Innate (Non- specific) immune system

The classical division of the immune system is into the innate and the adaptive systems. Despite the fact that dividing immune system into the innate and the acquired immunity is a common practice, recent studies in both fish and mammalian immunology demonstrate that these are combined systems rather than independent systems. Thus, the innate immune response is also important in activating the acquired immune response (Fearon & Locksley, 1996; Jimeno, 2008; Medzhitov, 2007; Shoemarker *et al*., 2001).

Macrophages are multifunctional cells of the immune system with extended functional roles including clearance of microorganisms, xenobiotic material, and apoptotic cells to the regulation of both innate and acquired immune responses. They are involved in phagocytosis, antigen presentation and release of antimicrobial and antitumour agents, as well as in the inflammatory reaction by secreting different types of cytokines and chemokines (Mackenzie *et al.,* 2006). The innate immune system is the only defense weapon of invertebrates and a fundamental defense mechanism of fish. The innate system also plays an instructive role in the acquired immune response and homeostasis and is therefore equally important in higher vertebrates. It is commonly divided into 3 compartments:

- Physiochemical barriers and/or the epithelial and/or mucosal barrier such as scales, epithelial surface (on gills, skin and gut) with secreted mucus,
- The humoral parameters such as cell secretions of complement, CRP, IFN, lysozyme, transferrin, lectins, antimicrobial peptides, and
- The cellular components such as non-specific cytotoxic cells (or NK cells), monocytes/macrophages, thrombocytes, granulocytes (or neutrophils), lymphocytes

(Buonocore & Scapigliati, 2009; Jansson, 2002; Magnadóttir, 2010; Rodriguez-Tovar *et al*., 2011).

The general terminology for these innate parameters is pattern recognition proteins or receptors. These parameters recognize pathogen associated molecular patterns (PAMPs) associated with microbes and also inhered danger signals from malignant tissue or apoptotic cells. under normal conditions the fish maintains a healthy state by defending itself against the potential invaders by a complex system of innate defense mechanisms.

1.9. The fresh water teleost *Channa punctatus* (Bloch.)

Channa punctatus (Bloch 1793) is a freshwater teleost. It is commonly known as spotted snake head or green snakehead, belonging to the family Channidae of the order Channiformes and has accessory respiratory organs that help the fish to survive in inhospitable situations. These fishes are elongated, more or less cylindrical with long, entirely soft-rayed dorsal and anal fins, large mouth with toothed jaws and palate, and head depressed with large shield-scale above. They have two accessory air-breathing pharyngeal air cavities. Snakeheads live in the freshwater bodies like ponds, swampy areas, flood plains, reservoirs, lakes, and rivers. All of them are carnivorous and mainly feed on different aquatic organisms with special preferences for fishes. It has a natural distribution in India and the other neighboring countries. *Channa punctatus* can very well be termed as a ‘lean' fish because of its very low lipid contents throughout the year. Moreover these fishes have very little or no adipose tissues this may be an important reason for the easy digestibility of this species, according to Ghosh (2006).

1.10. Classification of *Channa punctatus* (Marcus Elieser Bloch. 1973)

Kingdom:	Animalia
Phylum:	Chordata
Class:	Actinopterygii
Order:	Perciformes
Family:	Channidae
Genus:	*Channa*
Species:	*punctata*

Fig. 1.5: *Channa punctatus* (Bloch. 1973)

1.11. Overview of the intestine of *Channa punctatus* (Bloch.)

The gastrointestinal system of teleost fishes varies considerably due to factors such as adaptation to food type and phylogeny (evolutionary history of species). Since the present research project used a freshwater teleost species, *Channa punctatus* (Bloch.), as the experimental model, the overview of the gastrointestinal function is focused on this species.

As opposed to herbivorous or omnivorous fish which typically have no stomach, *Channa punctatus* (Bloch.) is a carnivorous fish and has a defined stomach for initial physical and enzymatic breakdown of food. The intestinal tract can be broadly divided into three sections morphologically: the anterior intestine which contains a number of blind-sacs known as pyloric caecae, the mid intestine which is narrower and free of caeca, and the posterior intestine which has a larger diameter, visible external annular folds and internal mucosal folds. In general, cellular uptake of nutrients across the intestine involves three steps: (1) absorption of nutrients through the apical membrane into the epithelial cells, (2) intracellular trafficking of nutrients to the basolateral membrane, and (3) extrusion of nutrients from the cells into the bloodstream.