# 1. INTRODUCTION

## 1.1.HEAVY METALS

The heightened concern for reduction of environmental pollution that has been occurring over the past 20 – 25 years has stimulated active continuing research and literature on the toxicology of heavy metals. Virtually all metals can produce toxicity when ingested in sufficient quantities, but there are several which are important because they are so pervasive, or produce toxicity at such low concentrations. Occupational exposure to heavy metals has accounted for the vast majority of poisonings throughout human history. Hippocrates described abdominal colic in a man who extracted metals, and the pernicious effects of arsenic and mercury among smelters were known even to Theophrastus of Erebus (370-287 BC). Classic examples of environmental contamination include the Minamata Bay disaster and the current epidemic of arsenic poisoning in South East Asia which have entered the lexicon of heavy metal toxicology. In the 1950s, industrial effluent was consistently dumped into Japan's Minamata Bay, and mercury bioaccumulated to exceedingly high concentrations in local fish. Although some adults did develop signs and symptoms of toxicity, the greatest impact was on the next generation, into which many were born with severe neurologic deficits. It is often contented that the Fall of the Roman Empire was hastened by chronic lead poisoning experienced by the ruling classes who had water conducted through the lead plumbing and drank wine from goblets which had lead/alloy composition. Itai-itai (ouch-ouch) disease—a syndrome of chronic renal failure and osteoporosis described in the Fuchu area of Japan—is often attributed to high levels of naturally occurring cadmium in the soil coupled with increased industrial exposures around World War II.

Metals are an intrinsic component of earth's crust. Heavy metals comprise of a heterogeneous group of elements, some of which are essential cofactors for various enzymes, while others are non-essential. The essential elements include the trace elements cobalt, copper, iron, manganese, molybdenum, selenium and zinc. The non- essential group includes metals such as arsenic, cadmium, lead, mercury, plutonium, tungsten and vanadium (Johri *et al*, 2010). The non-essential metals are potent toxins and gain access to organisms by virtue of physico-chemical properties, such as ionic charge, shared with their essential counterparts (Duce and Bush, 2010). With rapid industrial development, the evolution of metal- based industries has led to the increased exposure of men and ecosystem to toxic levels, thus presenting a potential health hazard to man as well as wild life. They may enter the body through food, water, air, or by absorption through the skin followed by inflammatory responses with accompanying organ dysfunction and disease. Heavy metal pollution in aquatic environment has become a worldwide problem during past few decades. Among environmental pollutants, metals are of particular concern, due to their potential toxic effect and ability to bioaccumulate in aquatic ecosystems (Censi *et al*., 2006). Industrial and municipal waste waters and mine run offs cause various metals and metallic compounds to be released into water. These pollutants also enter the water bodies as acid rain and their sulfate and nitrate ions make water acidic. Most inorganic compounds are toxic, particularly, those of heavy metals such as mercury, chromium, lead, silver, cadmium, etc., which come from paint and plastic production, metal and metal plating units etc. These substances get attached to the tissues of aquatic organisms, produce physiological poisoning and are therefore, capable of killing living organisms of water bodies.

Among the myriad environmental pollutants, arsenic and lead merit special reference as toxic elements. Arsenic as an environmental agent, is considered to a high priority toxic substance largely due to its carcinogenic potentials in human. The arsenic affected areas of West Bengal, India lie on a sediment of younger deltaic deposition, which also extends eastward towards Bangladesh (Chowdhury *et al*., 2000). Exposure to arsenic may be elevated in population residing in the vicinity of industrial and geological sources (Rusakov *et al*., 1998). The primary biochemical mechanism of arsenic toxicity is binding of the metals to cellular sulfydryl groups resulting in inhibition of numerous enzyme systems (Squibb and Fowler, 1983).

Studies on human and animals showed marked toxicity upon lead exposure (Rodrigney *et al*., 1998; Maddison and Hawke, 1993). There is abundant evidence to indicate that lead may accumulate in significant concentration in different organs with age. Lead exposure has been found to cause alteration of hematological parameters as well as immune functions (Patocka and Cerny, 2003).

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 of its wide availability round the year, and its adaptability to laboratory conditions. 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 lead and arsenic on the fish immune system is relatively limited.

## 1.2. ANTIOXIDANT SYSTEM AND IMMUNE DEFENSES OF FISH

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 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). 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 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 the antioxidant system, damages to lipids, proteins and DNA occur and this condition is referred to as oxidative stress (Di Guilio *et al*., 1989; Storey, 1996). The induction of the antioxidant enzymes gives an early warning on adverse health effects of certain pollutants on living organisms. Like all aerobic organisms, fish are susceptible to the attack of reactive oxygen species and have developed antioxidant defenses demonstrated by research primarily dating to the 1980s. Enzymes such as catalase (CAT), superoxide dismutase (SOD), and enzymes dependent on glutathione (glutathione peroxidase, GPX and glutathione reductase, GR) have been detected in most fish species investigated to date (Rudneva, 1997).

The innate immune system is something common to all multicellular organisms. Innate or non-specific immunity comprehends defense mechanisms that protect an organism against infection without depending upon prior exposure to any particular microorganism. The immune system consists of a complex network of cells and soluble mediators that interact in a highly regulated manner to generate immune responses of appropriate magnitude and duration. Fish are one of the most primitive vertebrates, but they do have an immune system satisfactory enough to react and protect them from attacks by various organisms. The host-pathogen relationship usually elicits a response in the host (fish) which serves to protect it against the pathogen. The immune system of fish has evolved with both non-specific (innate immunity) and acquired immune functions (humoral and cell mediated immunity) to eliminate invading foreign living and non-living agents. Fish have a unique physical barrier (mucus and skin) that acts as the first line of defense against foreign agents. The mucus and skin contain immuno-reactive molecules (i.e., lysozyme, complement and immunoglobulin). Non-specific humoral molecules in fish include lectins (for carbohydrate recognition), lytic enzymes, transferrin (iron binding protein) and components of the complement system. Non-specific cells of the fish immune system include monocytes or tissue macrophages, granulocytes (neutrophils) and cytotoxic cells. Macrophages function in phagocytosis and destruction of invading foreign agent and bacteria. Macrophage activation occurs through cytokines and immunostimulation (beta- glycan and other compounds) that increases the killing ability of these cells. Macrophages are also important to the acquired immune response. Macrophages are the cells responsible for innate immunity as well as antigen processing and presentation. Acquired immunity in fish includes both humoral and cell mediated responses. B-cells of fish produce antibody when stimulated. Many components of the innate immune system appear to be evolutionary conserved (Hoffmann *et al.*, 1999; Ulevich, 2000). Thus, sensitivity of immune system mechanisms to a particular contaminant might be similar among different species. For fish populations, a link between environmental contamination and disease has long been discussed. Metals can accumulate in aquatic organisms, including fish, and persist in water and sediments (Luoma and Rainbow, 2008). Fish are an important component of human nutrition, and those from contaminated sites present a potential risk to human health. Since fish occupy the top of the aquatic food chain, they are suitable bioindicators of metal contamination. Metals are well-known inducers of oxidative stress, and assessment of oxidative damage and antioxidant defenses in fish can reflect metal contamination of the aquatic environment (Livingstone, 2003). Moreover, understanding the impact of contaminants on fish immunity is of economic relevance for fisheries as well as aquaculture.

## 1.3. HEAVY METALS, OXIDATIVE STRESS AND ANTIOXIDANT SYSTEM

The indiscriminate dumping and release of wastes containing hazardous substances like heavy metals into rivers might lead to environmental disturbance that could be considered a potential source of stress to the biotic community (Pandey *et al.*, 2003). Recent data indicate that the pollution toxicity in aquatic organisms may be associated with increased production of “reactive oxygen species” (ROS), leading to oxidative stress. In the aquatic environment, despite the presence of constitutive or enhanced antioxidant defense systems, increased levels of oxidative damage will occur in organisms exposed to contaminants that stimulate the production of ROS (Livingstone, 2001). The increased ROS production and subsequent oxidative stress has been associated with a pollutant-mediated mechanism of toxicity in fish (Tejeda-Vera *et al.*, 2007). Defensive mechanisms to counteract the impact of ROS are found in many mammalian species including aquatic animals such as fish. These systems include various antioxidant defense enzymes such as superoxide dismutases which catalyze the dismutation of superoxide radical to hydrogen peroxide, catalase acting on hydrogen peroxide, glutathione S- transferase family possessing detoxifying activities towards lipid hydroperoxides generated by organic pollutants such as heavy metals (Tjalkens *et al.*, 1998).

## 1.4. HEAVY METALS AND INNATE IMMUNE RESPONSES

In the last few decades, a possible influence of heavy metals on the aquatic environment has gained considerable interest and fish have become a favorable subject for research in this area. Considering the fact that ectothermic animals rely on their non-specific or innate immune functions more as compared to mammals, research on related functional assays has long been of great interest in fish immunology and the subject has received the attention of reviewers (Secombes, 1994; Secombes and Fletcher, 1992).Impairment of innate immunity may be more significant in fish than in mammals, as mounting an adaptive or acquired immune response takes longer in fish (Alexander and Ingram, 1992). Innate immune response is the first line of internal defense. Key cellular components of innate immunity include phagocytes such as neutrophils and monocytes/macrophages, as well as dendritic cells, mast cells, eosinophils, basophils and natural killer cells. Innate defenses also encompass physical barriers such as the epithelial and endothelial cell linings (Theron *et al*., 2012).Aquatic environment of fish is in close contact with numerous pollutants. In fish it has been shown that innate immune cell functions can be modulated by heavy metals (Bols *et al.* 2001) thus increasing the host susceptibility to infectious pathogens. Thus, an effect on fish innate immunity can serve as a warning of potential impact on human and ecosystem health because heavy metals are often released first into aquatic environments, and then, by a variety of routes, reach humans and other terrestrial animals (Zelikoff *et al.* 1998, Adams and Greesly 1999).

## 1.5. THE FRESHWATER FISH *Channa punctatus* (Bloch.)

The innate immune system is something common to all multicellular organisms. Toxicological studies with fish species were introduced in the 1930s for the determination of toxic effects in field and laboratory toxicology investigations especially for the screening of toxic chemicals, agricultural and industrial effluents and for pollution studies in rivers, lakes and the marine environment. Fish in most aquatic environments, when are mature, occupy mostly the top niche of predators in aquatic ecosystems. Fish often have been adopted as the ‘sentinel’ organisms for the health of aquatic environment. Aquatic organisms can provide model systems for investigation of how ROS damage cellular components, how cells respond, how repair mechanisms ameliorate this damage, and how oxidative stress can lead to disease (Livingstone *et al*., 1994; Di Giulio *et al*., 1989). There has been increasing concern about the aquatic disposal of industrial and non-industrial wastes and its genetic consequences on fish populations exposed to low levels of pollution over prolonged periods of time. *Channa punctatus* is an eminent tropical freshwater fish widely used for medicinal and pharmaceutical purposes, and is also an important food source in the Asia-Pacific region. *Channa* is a genus of the Channidae family of snakehead fish. *Channa* has a wide natural distribution extending from Iran in the west, to China in the east, and parts of Siberia in the Far East. It is highly popular and has great market demand in India due to its good taste and high nutritional value. It is recommended in diet during convalescence and, therefore, is a good candidate for intensive aquaculture (Marimuthu *et al*., 2009). Yaakob and Ali (1992) also noted the importance of murrels for hastening the healing of wounds and internal injuries due to the presence of certain fatty acids such as prostaglandin and thromboxane. They are one of the most common staple food fish in Thailand, Cambodia, Vietnam and other South East Asian countries where they are extensively cultured. Therefore, the choice of appropriate immunomodulatory and antioxidant assays for the species is a prerequisite in the experimental investigation of this problem.

## 1.6. OBJECTIVE OF THE PRESENT STUDY

1. The present study was designed to envisage the effect of non-lethal doses of lead and arsenic exposure in *Channa punctatus* focusing on immunosuppression consorted with oxidative stress and alteration of enzymatic as well as non-enzymatic antioxidant profiles.
2. The immunosuppressive effects of heavy metal pollution and oxidative stress resulting in higher disease susceptibility and the ensuing cytokine cross-talk have been explored here.
3. The study further endeavors to find out the interactivity effect from combined exposure to these heavy metals and determine possible synergistic effects on immunomodulatory response as well as antioxidant activity.
4. Lastly, the motive for choosing a universal trait or an innate component was that it could act as an effective biomarker for heavy metal stress or other adverse conditions in aquaculture or nature.