CHAPTER II

REVIEW OF LITERATURE

Fishes are highly nutritious and consumed as a delicacy food throughout India. But addition of pollutants in water has impact on the aquatic ecosystem and poses adverse effect on the fish health. As a result fish production is generally encumbered and fish farmers face a great economic loss. Thus monitoring and understanding pathophysiology of various toxicants would be helpful in minimizing losses and providing safeguard to public health. Among the various toxic pollutants, heavy metals are particularly severe in their action due to tendency of bio-magnification in the food chain. The global heavy metal pollution of water is a major environmental problem. With the advent of agricultural and industrial revolution, most of the water sources are becoming contaminated (Khare and Singh, 2002). Industrial discharges containing toxic and hazardous substances, including heavy metals (Gbem et al., 2001; Woodling et al., 2001) contribute tremendously to the pollution of aquatic ecosystem. A variety of contaminants including toxic heavy metals (lead and zinc) are reported to be ubiquitously present in rivers, reservoirs and are disadvantageous for aquatic organisms. In general, they are not biodegraded and therefore, their bioaccumulation in fish, oyster, mussels, sediments and other components of aquatic ecosystems have been reported from all over the world. It appears that problem of heavy metals accumulation in aquatic organisms including fish needs continuous monitoring and surveillance owing to biomagnifying potential of toxic metals in human food chain (Das and Kaviraj 2000;
Laxi, 2005; Jayakumar and Paul 2006; Kumar et al., 2007; Kumar et al., 2008). Zinc and lead smelters, together with refineries, are large contributors to anthropogenic Zn and Pb emissions (Dolgopolova et al., 2006). The toxic effects of heavy metals have been reviewed, including bioaccumulation in fish (Waqar, 2006; Adami et al., 2002; Rasmussen and Anderson, 2000; Usharani, 2000; Aucoin et al., 1999). The potential effects of metal on fish can be assessed examining their accumulation in target tissues (Rajotte et al., 2003; Mendil and Uluözlö, 2007). Several studies have demonstrated various concentrations of metals that can affect wild fish species (Romeo et al., 1999).

Svobodova et al., (1995) and Kolacz et al., (1996) showed the extent of metal accumulation in fish muscles to be species-specific. Windom et al., (1987) suggested that those differences were affected by the toxicity of a chemical compound, in addition to fish size and age.

Fish living in lakes and rivers near mines, smelters and metal-finishing industries are often chronically exposed to elevated levels of metals such as lead (Pb) and zinc (Zn). Accumulation of these metals by fish increases the risk of toxicity such as physiological impairment (Laflamme et al., 2000; Rajotte and Couture, 2002; Couture and Kumar, 2003) and lipid peroxidation (Farag et al., 1995). Metal accumulation by fish in natural systems depends on many factors including water chemistry, temperature (Kock et al., 1996; Greenfield et al., 2001) and the relative importance of food and water as uptake routes. In addition to these extrinsic environmental factors that control bioavailability, factors intrinsic to the fish also play an important role in determining how the metal is handled once assimilated. The association of metals with different cellular ligands could influence their bioavailability within the cell. For example, metals sequestered by cytosolic proteins such as metallothionein (MT) (Olsson and Haux, 1986; Hogstrand et
or metals stored within lysosomes or metal-rich granules (Weis et al., 1986; Bunton et al., 1987; Lanno et al., 1987; Wallace et al., 2003) are considered detoxified, and thus are not bioavailable to more sensitive cellular fractions.

Fish can accumulate heavy metals including Pb and Zn, primarily through dietary exposure (U.S.EPA, 2000). The gill is an important site for the entry of heavy metals that provokes lesions and gill damage (Bols et al., 2001; Lock and Overbeeke, 1981). Liver is another organ where high accumulation of metal is observed. The higher accumulation in liver may alter the levels of various biochemical parameters in liver. This may also cause severe liver damage (Ferguson, 1989; Mayers and Hendricks, 1984). Kidney is the gateway for heavy metal detoxification in body. Accumulation pattern of heavy metals in kidney was studied in *Cyprinus carpio* (Vinodhini and Narayanan, 2008).

**Lead** (Pb) is a naturally occurring, ubiquitous compound that can be found in rocks, soils, water, plants, animals and air. Lead is cheap and there is a long tradition of its use. But the toxic effects of Pb have also been recognized for centuries. As a result, western societies have greatly reduced many traditional uses of Pb, including many paints, gasoline and solders because of threats to the health of humans and the environment. Legislation in several countries has eliminated the use of lead shot for hunting waterfowl. Despite these advances, a great many Pb products continue to be readily available. Conservationists recognize that hunting, angling and shooting sports deposit thousands of tons of Pb into the environment each year. Even today, our knowledge of the long-term sublethal effects of Pb on human health continues to grow dramatically. Our knowledge about lead poisoning in domestic animals is significantly
less. For wild animals, our understanding of lead poisoning is roughly where our knowledge about humans was in the mid-1800s when Tanquerel Des Planches made his famous medical observations (Tanquerel Des Planches 1850). Lead is an element and a metal (atomic number 82). It is soft, has a low melting point (327.5 °C), a high density (11.34 g/cm³) and is found naturally in a variety of minerals including galena, cerussite and anglesite. Unlike many natural elements, lead is not known to be required by any living organism. Acute effects of Pb can cause sudden death, severe abdominal cramps, anemia, ataxia, strange headaches, and behavioural changes, such as irritability and appetite loss. Chronic effects are most often the result of smaller amounts of lead being taken in over longer times – months to years. These effects can be quite subtle and nonspecific, but include all body systems. A brief list of effects documented in people includes such effects as lowered sex drive, decreased fertility (in males and females), miscarriages and premature births, learning problems, hypertension, cardiovascular disease, increased aggression and kidney problems (Pokras and Greenfield, 2009). In the body, lead can bind important enzymes (primarily at their sulfhydryl groups) and inactivate them. Pb can also displace biologically important metals, such as calcium, zinc and magnesium, interfering with a variety of the body’s chemical reactions. Lead toxicity affects all organ systems, but the most profound effects are seen in the nervous, digestive, and circulatory systems. Every time nerves transmit messages around the body, calcium is required. Thus lead can interfere with functions dependent on nerve conduction such as learning, blood pressure, reaction time and muscle contraction. The Mechanisms of Pb toxicity (Needleman 1991, Casas and Sordo 2006, Ahameda and Siddiqui 2007) are as follows:

- It substitutes for and competes with Ca++
• It disrupts Ca++ homeostasis
• It binds with sulfhydryl groups
• It stimulates release of Ca++ from mitochondria
• It damages mitochondria and mitochondrial membranes
• It substitutes for Zn in zinc mediated processes
• Increases oxidative stress
• Inhibits anti-oxidative enzymes
• Alters lipid metabolism

Pb can induce neurologic effects by following means (Needleman 1991, Casas and Sordo 2006, Ahameda and Siddiqui 2007):
• increase in affinity for Ca++ binding sites
• disrupts Ca++ metabolism
• substitutes for Ca++ in Ca/Na ATP pump
• blocks uptake of Ca++ into mitochondria and endoplasmic reticula
• interference with neural cell adhesion
• impairment of cell to cell connections
• alters some neurotransmitter function
• activates protein kinase C
• alters Ca++ mediated apoptosis

The situation seems to be similar in fish and mammals. Adult fish of some species appear to be relatively insensitive to acute toxicity, but their eggs and larvae can show dramatic effects at low levels of exposure, sometimes resulting in population level effects and ecosystem alteration (Carpenter 1924a, b, Dilling et al., 1926, Jones 1964, Srivastava and Mishra 1979, Birge et al., 1979, Johansson-Sjöbeck and Larsson 1979,
Newsome and Piron 1982, Hodson et al., 1984, Sorensen 1991, Weber et al., 1997, Kasthuri and Chandran 1997, Chaurasia et al., 1996, Chaurasia and Kar 1999, Shafiq-ur-Rehman 2003, Martinez et al., 2004, Shah 2006). It seems that as more studies explore the sub-lethal effects of lead exposure in non-human species, there will be increased emphasis on integrating our thinking so that threats to human health are understood in the context of an overall environmental well-being. Some of this regards direct toxicity, but there is also literature on the ability of some invertebrates to accumulate lead (and other heavy metals) and to cause indirect toxicity to vertebrates that eat them (Grosell et al. 2006, Ma 1982, 1987, 1989, Scheuhammer 2003).

The current Water Quality Standards establish hardness dependent lead criteria. At a water hardness of 100 mg/L CaCO$_3$, the dissolved Pb criteria are 65 μg/L and 2.5 μg/L for short-term and long-term exposures, respectively. Corresponding total recoverable lead criteria are 62 μg/L and 3.2 μg/L for short-term and long-term exposures, respectively. Concentrations of lead associated with background fresh water systems are estimated to be <3.0 μg/L (Moore and Ramamoorthy, 1984). Aquatic organisms are sensitive to lead and are affected more strongly by dissolved rather than total lead. Likewise, the toxicity of lead is increased when it forms organo-lead compounds and when environmental conditions consist of high temperature and low pH. Fishes are also more sensitive at younger life stages and when exposure durations are greater. Many studies have determined LC$_{50}$ values for various life stages of rainbow trout. The 32-week LC$_{50}$ value for embryo/larval stages was 220 μg/L (hardness=101). Two month old fry had an LC$_{50}$ of 8,000 μg/L (hardness=82-132 mg/L). For juvenile rainbow trout, the 21-day LC$_{50}$ value was calculated as 2,400 μg/L (hardness=135). Finally, in adults,
LC<sub>50</sub> values ranged from 1,170 µg/L to 471,000 µg/L to 542,000 µg/L depending upon the hardness values: 28, 353, 290, respectively (Eisler, 1988).

Lead has been shown to bioconcentrate (an increase in concentration in relation to the ambient concentration) in aquatic species. Invertebrates tend to have higher bioconcentration factors (BCF) than vertebrates. For example, the BCF for the freshwater snail, *Lymnaea palustris*, is 1,700 and the BCF for the blue mussel, *Mytilus edulis*, is 2,570. In the freshwater snail, *Physa integra*, tissue concentration changes were correlated with changes in dissolved lead in the water column, but not with changes in the amount of lead found in substrate. Similarly, *Campeloma decisum* (sub-tropical freshwater snail) had lower tissue concentrations than the substrate even though the organism was associated closely with contaminated sediments. Lead was found to accumulate in the ganglia of freshwater snails (*Lymnaea stagnalis*). In vertebrates, such as brook trout embryos, the BCF is 42 (Eisler, 1988). Although Pb is concentrated by biota from water, there is no convincing evidence that it is transferred through food chains (Branica and Konrad, 1980; Settle and Patterson, 1980). Lead concentrations tended to decrease markedly with increasing trophic level in both detritus-based and grazing aquatic food chains (Wong et al., 1978). In the marine food chain of seawater (<0.08 ug Pb/l), to a brown alga (*Egregia laevigata*), to the red abalone (*Haliotis rufescens*), Pb concentrations in the alga and abalone were both <0.04 mg Pb/kg fresh weight after 6 months, indicating negligible biomagnification (Stewart and Schulz-Baldes, 1976). When seawater contained 1,000 ug Pb/l, young abalones that fed on *Egregia* for 6 months contained up to 21 mg Pb/kg fresh weight, but neither growth nor activity was affected; Pb selectively accumulated in the digestive gland (38 mg/kg), and was lowest in muscle (<1 mg/kg)—the part normally consumed by humans (Stewart and
Schulz-Baldes, 1976). In the freshwater food chain of an alga (*Selenastrum capricornutum*), to a daphnid (*Daphnia magna*), to the guppy (*Poecilia reticulata*), Pb accumulation progressively decreased from the alga to the guppy. Thus, in organisms held for 28 days in solutions containing 5 ug Pb/l, Pb content was 460 mg/kg dry weight in the alga, 23 mg/kg in the grazing daphnids, and 4 to 16 mg/kg in the guppies that fed on the daphnids (Vighi, 1981). Concentrations of Pb in the freshwater snail, *Lymnaea peregra*, collected near an abandoned Pb mine were positively correlated with the Pb content in its diet; the digestive glands contained up to 5,600 mg/kg dry weight (Everard and Denny, 1984). The gut contents of eels (*Anguilla anguilla*) grazing on contaminated snails contained up to 4,350 mg Pb/kg, but the Pb was rapidly released; feces from both snails and eels return the Pb to the ecosystem as particulates and detritus (Everard and Denny 1984).

**Zinc** (Zn) belongs to a class of microelements which are essential for proper functioning of the body (Hilmy *et al.*, 1987; Kotze *et al.*, 1999). Elevated levels of zinc in aquatic systems can be due to liquid effluent discharge, atmosphere deposition, the leaching of domestic sewage and metal bearing minerals insecticides and galvanizing processes (DWAF, 1996; Nussey, 1998). Zinc has an extensive industrial use in alloys, galvanizing, pigments and electrical equipments. On a relative basis, surface drainage and atmospheric fallout are the most important inputs of zinc to aquatic environments (Spear, 1981). Though zinc is an essential trace element for organisms and plays a vital role in the physiology of living systems, higher concentrations can be toxic to organisms (Williams and Mount, 1965; Ambrose *et al.*, 1994). The toxicity of zinc to aquatic life was intensively investigated during the previous decades and a considerable amount of experimental data was compiled and reviewed (US EPA, 1980; Tuurala and
Soivio, 1982; Somasundaram et al., 1984; Larson and Hyland, 1987; Bagdonas and Vosylien, 2006). Zn is essential for normal growth and development. It is the constituent of many enzymes and proteins. However excessive amounts can lead to toxic symptoms. Zinc is an essential trace element for all living organisms. As a constituent of more than 200 metalloenzymes and other metabolic compounds, zinc assures stability of biological molecules such as DNA and biological structures such as membranes and ribosomes (Vallee 1959; Casey and Hambidge 1980). Clinical manifestations of zinc deficiency in animals include growth retardation, testicular atrophy, skin changes, and poor appetite. In all these, zinc exerts adverse effect in fish accruing structural damage, which affects the growth, development and survival of fish. Sub-lethal levels of zinc have been known to adversely affect hatchability, survival and haematological parameters of fish (Cardeihac et al., 1981; Clarkson, 1998; Dickman and Leung, 1998). For instance, exposure to sub-lethal concentrations (300 mg/l) of zinc sulphate for 20 days resulted in drastic changes in the male testis. Metals that accumulate in adult fish were reported being possibly transferred to the eggs during oögenesis, which in turn induced harmful effects onto the embryonic development (Sehgal and Saxena, 1986). Aquatic populations are frequently decimated in zinc-polluted waters (Solbe and Flook 1975; Everall et al. 1989b). Zinc in the aquatic environment is of particular importance because the gills of fish are physically damaged by high concentrations of zinc (NAS 1979). Zinc toxicosis in humans is not a common medical problem, although it may appear in some metal workers and others under special conditions (NAS 1979). Zinc composes 0.004% of the earth's crust and is 25th in order of abundance of the elements (Vallee, 1959). Uses of zinc include the production of noncorrosive alloys, galvanizing steel and iron products, and the
therapeutic treatment of zinc deficiency (Elinder, 1986). Zn is an essential micronutrient that acts as cofactor for the activity and folding of protein (Sekler et al., 2007). Zn is largely present in aquatic system due to effluent discharge, atmosphere deposition, domestic sewage, insecticides and galvanizing processes (Nussey, 1998). However excess of Zn can be harmful in fish causing structural damage, which affects the growth, development and survival of fish. Acutely toxic concentrations of Zn can kill fish by destroying gill tissue and impairing branchial calcium influx leading to hypocalcemia (Hoogstrand et al., 1994). Excessive Zn exposure has also been associated with behavioural anomalies in fish (Ololade and Ogini, 2009). Zn effects on catfish range from altered enzyme levels, bioaccumulation, and haematological to histopathological anomalies. In acute studies, high zinc concentrations (close to LC$_{50}$) decreased the concentration of haemoglobin and the per cent of haematocrit in the blood of carp (*Cyprinus carpio*). In long-term studies, while exposing fish to 30 mg/l of zinc, no changes in blood indices were found (Svobodova et al., 1994). However, according to other authors, after 1-day exposure of carp to sublethal concentrations of zinc, the erythrocyte count and haemoglobin concentration in the blood increased but the total leucocyte count did not change (Tishinova and Ilieva, 1994). After 96-hour exposure to 22 mg/l and 32 mg/l of zinc, the concentrations of haemoglobin and haematocrit increased in such freshwater fish as catfish (*Clarias lazera*) and tilapia (*Tilapia zilli*) (Hilmy et al., 1987). During experiments on acute toxicity (96 hours), 140 mg/l of zinc caused a significant decrease in leucocytes in the blood of carp (*Cyprinus carpio*). It was due to a significant decrease in the count of small lymphocytes while the amount of neutrophiles increased (Svobodova et al., 1994). However, in the studies of acute toxicity, it was determined that exposure to zinc causes an increase in the leucocyte
count in the blood of dogfish (*Scyliorhinus canicula*) (Torres *et al.*, 1984). High concentrations of zinc (up to 1 g/l) decreased phagocytic activity of macrophages in carp (*Cyprinus carpio*) while low concentrations, on the contrary, stimulated it (Dunier and Siwicki, 1992). Glucose. 96-hour exposure to LC50 of zinc increased the concentration of glucose by 80% in the blood of salmonid fish (McLeay, 1977). An increase in glucose concentration was also observed in rainbow trout after 7-day exposure of this fish to 214 mkg/l of zinc (Watson and McKeown, 1976). Zinc exerts adverse effect in fish accruing structural damage, which affects the growth, development and survival of fish. Sub-lethal levels of zinc have been known to adversely affect hatchability, survival and haematological parameters of fish (Cardeihac *et al.*, 1981; Clarkson, 1998; Dickman and Leung, 1998). For instance, exposure to sub-lethal concentrations (300mg/l) of zinc sulphate for 20 days resulted in drastic changes in the male testis. Metals that accumulate in adult fish were reported being possibly transferred to the eggs during oögenesis, which in turn induced harmful effects onto the embryonic development (Sehgal and Saxena, 1986). The studies carried out on various fishes have shown that heavy metals including zinc may alter the physiological activities and the biochemical parameters both in tissues and in blood (Tort and Torres, 1988; Canli *et al.*, 1998; Svoboda, 2001; Basa and Rani, 2003; Witeska, 2003). Acute lethality of zinc salts to teleosts, have been reported (Crespo and Balasch, 1980). In addition, Zn can also cause adverse chronic effects at concentrations less than acutely lethal levels with continuous Zn exposure (Chapman, 1978). Although the mode of toxic action of zinc is uncertain, acutely toxic concentrations can kill fish by destroying gill tissue (Skidmore, 1964). Acute exposures can also cause an impaired brachial calcium influx leading to hypocalcemia (Hoogstrand *et al.*, 1994). Zinc is essential
element acting as structural component and having specific properties indispensable for life (Bengari and Patil, 1986). The danger of zinc is aggravated by its almost indefinite persistence in the environment because it cannot be destroyed biologically but are only transformed from oxidation state or organic complex to another. Zn is a potential toxicant to fish (Everall et al., 1989), which causes disturbances of acid-base and ionoregulation, disruption of gill tissue and hypoxia (Hogstrand et al., 1994). Because zinc combines with biomolecules in target species and most of these species accumulate more than they need for normal metabolism, data showing bioconcentration factors for target receptors may be misleading. Little to no evidence exists indicating the successive biomagnification of zinc in tissues of fish. This assumption is based on several factors. First, existing BCF data (EPA, 1987) shows that the greatest BCF was seen in mayflies while the least was found in Atlantic salmon. Bioaccumulation of metals reflects the amount ingested by the organism, the way in which the metals are distributed among the different tissues and the extent to which the metal is retained in each tissue type. Accumulation of zinc has attained a serious dimension causing a pathogenic stage like Alzheimer’s disease. Zn in certain concentration is desirable for the growth of freshwater animals but its over accumulation is hazardous to exposed organisms as well as to those who consume them directly or indirectly through food chain. The pattern of metal accumulation in fish tissue can be utilized as effective indicator of environmental contamination (Sultana and Rao, 1998). Fish exposed to high concentrations of trace metals in water may take up substantial quantities of these metals. Hogstrand et al. (1994) suggested an adaptation to water borne Zn by a change of $K_m$ of a mutual $\text{Ca}^{2+}/\text{Zn}^{2+}$ carrier which may have reduced Zn influx. When exposure to high Zn level occurs and the liver’s capacity may be removed by the exceeded level
of Zn and the more toxic type of Zn (Zn$^{2+}$) may be transported through blood stream to other organs. Zinc can be accumulated via the gills and/or the digestive track, however the role of water as source of Zn uptake is not fully elucidated (Spry et al., 1988).

On reviewing existing literature, an wealth of information on various works in the field of Pb and Zn toxicities in fish were found. However, the mechanism of uptake, regulation and their possible sequestration patterns in fish system was not properly understood. As metals bioaccumulation through aquatic food webs to fish, humans and other piscivorous animals are of environmental and human health concern, therefore, it has become imperative to observe toxicities Pb and Zn along with their uptake, regulation and sequestration patterns in a teleost, *Clarias batrachus*. 