CHAPTER-1

INTRODUCTION
Due to industrialization and population explosion, indiscriminate exploitation of natural resources have increased. Massive amount of domestic waste and effluents are discharged into the water bodies including rivers. Industrial waste containing soup of both inorganic compound (including heavy metal) and organic waste, contaminate the fresh water bodies with a wide range of pollutants. These pollutants not only threatened public health and water supplies, but also damage the aquatic life. Contamination of a river with heavy metals has devastating effect on the ecological balance of the aquatic environment. The diversity of aquatic organisms becomes limited with the extent of contamination (Suziki et. al. 1988). Metals are an integral part of our biosphere, made available naturally, often in minor quantities through weathering of rocks. Anthropogenic activity such as burning of fossil fuels, mining, smelting and discharge of industrial, agricultural and domestic wastes, however have accelerated their accumulation in environment. Studies have shown that fish are able to accumulate and retain heavy metals from their environment and it has been shown that accumulation of metal in tissues of fishes is dependent upon concentration and duration as well as other factors such as salinity, temperature, hardness and metabolism of animals (Pagenkopt 1983; Heath 1987; and Allen 1995).
Once Heavy metals are accumulated by aquatic organism they can be transferred to upper class of food chain. Heavy metals generally do not degrade and tend to biomagnify in man, through food chain. Thus human health eventually is threatened by the consumption of such food. For example Minamata disease, which occurred in Kumamoto, Japan in 1953, was the result of consumption of fish and shrimps, contaminated by methyl mercury from waste water discharge by alkaline factories. According to Tinsli (1982) there are two ways for penetration of heavy metal into the organisms either by direct water absorption or by consuming fish as food. Komarrovskii and Polishtuk (1981) reported larger metal load in the tissue of predatory fish species. The ecological specificity of this pollutant is that there are practically no self-cleaning mechanism known for them when present in water, they pass through the trophic chain of aquatic communities. Heavy metal level in the tissues of aquatic animal is occasionally monitored, because the heavy metal concentration in the tissue reflects, past exposure via environment and food. It can demonstrate the current grave situation of aquatic animals, before the toxicity of metals affect the ecological balance of population in aquatic environment (Canli and Kalay 1998).
Heavy metal can enter the water naturally due to erosion and wash out processes, by discharge of the effluent inflow from industrial waste water, rain water and flow through the urban water way. The amount of heavy metals in water steadily increased. They are present in water in dissolved form only at low levels, since heavy metal compounds have low solubility. Mineral suspension and precipitation substances are able to store heavy metal ions on their outer surface. Heavy metals are neither removed nor detoxified readily by metabolic activity. As a result they accumulate and cause deterioration in quality of environment. Microbial transformation of these metals in sediment may however lead there way in food chain as reported for Mercury, Arsenic, Tin, and Selenium (Khan 2001). Several adverse, reports on metal exposure and toxicity have made human being more conscious all over the world. I.T.R.C. (Industrial Toxicology Research Centre 2000), has prescribed permissible limits of heavy metals for drinking water, are summerised in table -1.

Toxic pollutants such as heavy metals are particularly harmful to animal life. Pollutant cause disease, behaviour abnormalities, cancer, gene mutation, physiological malformation or physical deformation of organism that eventually
ingest to absorb them. Toxicity of metals can contaminate their flesh, decrease their value as food or even kill them. Toxicity of ingested heavy metal has been important to human health issues for decades. Many studies have shown that fish are capable of accumulating high level of metal from contaminated water. This is an important exposure pathway for people, who consume fish grown in contaminated water. The population, most affected by heavy metal toxicity are pregnant woman or very young child (Boon and Soltanpour 1992).

Neurological disorders, central nervous systems and cancer of various body organism in some reports is the effect of heavy metal poisoning (ATSDR 1994; ATSDR 1999a; ATSDR 1999b and ATSDR 2000). Mahaffey et. al. (1981) reported lower birth rate and mental retardation of new born children in some cases where pregnant mother ingested toxic amount of heavy metal. According to Dianne et. al. (1999) metals have been recognized as powerful toxins in many studies. Metal with atomic radii often have important cellular function which depend on the formation of their preferred co-ordination complex with oxygen or nitrogen, the ligands of comparable size for example, to control the quaternary arrangement in structure or catalytic domains of protein (eg. Zinc and Copper in superoxide dismutase)
and in the transport of amino acid with Sodium and electrons by Iron and Copper in cytochrome c oxidase. Metallothionein is low molecular weight protein with about 30% sulphur containing amino acid. Metallothionein is known to be important in the regulation of copper and zinc metabolism and in the detoxification of heavy metals, particularly Calcium (Dianne et. al. 1999).

Some metals are naturally found in the body and are essential to health, Iron for example prevent anemia, Zinc is a cofactor in over 100 enzyme reaction. Some metals such as Mercury, Aluminium, Arsenic, Cadmium, Nickel etc. that act as poison, interfere with the enzyme system and hence effect the metabolic activities of the body. Heavy metal are stable elements (they can not be metabolized by the body), they bioaccumulate and are passed up through food chain to human beings. Heavy metals are taken into body via inhalation, ingestion and skin absorption. Some heavy metals are extremely toxic, even in most minute doses, where as others have low toxicity, even in high doses. Iron can cause several oxidative damage, while Copper may compromise with the liver function. Most metal serve a functional role in the body, for example, selenium is needed in the enzyme activity that restores oxidized glutathione.
Important function of selenium is, its role as a powerful antioxidant in preventing cancer. Some metal have no physiological function, Mercury, Lead, Aluminium are in this group. Even the smallest amount have negative physiological effects. If heavy metal enter and accumulate in body tissue faster than the body detoxification pathway, can deposit in them and gradual built up of these toxins will occur. Heavy metal overload in the adrenal gland, reduce the production of hormones, which cause early aging, process. Heavy metal lead to neurological disease such as depression and loss of thinking power. Human exposure to heavy metal has risen dramatically in last 50 years. This has been due to an exponential increase in the use of heavy metal in industrial processes and product. In modern industrial society there is no escaping exposure to toxic chemical and metals. In general, heavy metal are systemic toxins with specific neurotoxic, nephrotoxic, teratogenic effects. Neurotoxins are substances attracted to nervous system. They are absorbed by nerve endings and travel inside the neuron to the cell body, on their way they disrupt vital functions of cells, such as axonal transport of nutrients, mitochondrial respiration and proper DNA transcription. Heavy metal can directly influence behaviour by impairing mental and neurological function, influencing
neurotransmitter production utilization and altering numerous metabolic body processes.

Heavy metals effect on fish in different ways such as alteration of behaviour. Bioaccumulation of metal in the body of organism effect histological and biochemical alteration in fish. Metal also effect early life stage of fish. Behaviour abnormality in various fish species on the exposure of heavy metal have been reported by several studies. Syed Lal and Shah (2002) reported little behaviour change in low concentration and lethargy and loss of equilibrium in high concentration of Copper exposed fish.

The effect of environmental factors, on accumulation of Copper, has been investigated by Cogun and Kargia (2004) in fish. Geeth et.al. (1996) observed that the exposure concentration and duration effect the accumulation of Copper on Lepidocephalacathys thermalis. Heavy metals disturb the homeostasis of the fish due to which metal exposed fish show stress. The stress reaction involve various physiological changes which include alteration in blood composition and immune response. Christensen et. al. (1972) have reported that change in blood of bull head fish occur when exposed to Copper solution.
The 96h LC-50 values for most metals are known, to us. It is very important to determine the concentration of specific metal present in the medium. It may be very small concentration in the aquatic medium. Study has to be made in detail as it causes initial occurrence of cellular damage.

Heavy metals effect on specific vital organs such as liver, gill and kidney. Liver contain highest metal concentration because it is an organ of storage and detoxification of metal (Avenant and Marx 2000). Changes in histological structure of specific vital organs due to exposure of sub lethal concentration of metal in various fishes has been reported by many workers. The body constantly tries to eliminate heavy metals via the available exit routes: the liver, kidney and skin. Detoxification mechanism include acetylation, sulfonation, oxidation etc. The liver is most important in these processes. Here most elimination products are expelled though the bile in to the small intestine and should leave the body via the digestive tract. Alteration of epithelium surface of gill in Copper exposed fish Catla catla and Labeo rohita has been reported by Ahmad and Munshi (1987). Histological analysis appears to be a very sensitive parameter and is crucial in determining cellular change that may occur in target organs, such as gill and Liver (Dutta 1996). The liver is
detoxification organ and essential for both the metabolism and excretion of toxic substances. Liver has ability to degrade toxic compounds but its regulating mechanism can be overwhelmed by elevated concentration of these compounds and could subsequently result in its structural damage. Heavy metals have the ability to bioaccumulate in the liver and kidney, the target organ of heavy metal pollution, and also the body's detoxification organs.

Fish are an important source of human nutrition. Aquatic ecosystem polluted with heavy metal, may therefore threaten human nutrition and health directly. Toxicity of heavy metals can kill fish, contaminate their flesh and decrease their value as food.

Heavy metals can cause genetic mutation. Heavy metal disrupt metabolic processes. Heavy metal alter prooxidant/antioxidant balance and bind to free sulphydryl groups resulting in inhibition of glutathione metabolism, numerous enzyme and hormone function. According to Rik et. al. (2004) chemically reactive pollutant such as electrophiles, react with different nucleophilic biological molecules. Depending on its electrophilicity, an electrophilic pollutant react with soft
nucleophilies, such as thiol groups in protein and peptides or with harder nucleophiles, such as nucleotides in DNA. They also pointed out that reaction with peptides and proteins interfere with the cellular-reducing capacity through conjugation with glutathione or interfere with enzyme activity, while DNA damage leads to mutation.

Nutritionally, heavy metals action is directly antagonistic to essential trace elements and compete with nutrient elements binding site on transport of strong protein, metaloenzyme, enzyme and receptor which result in disturbance of the metabolism and in balance of nutrient element viz carbohydrate protein / aminoacid, lipids, neurotransmitters and hormone. Ranjanna et.al.(1981) reported enhancement of protein contents due to heavy metal. Cadmium, Lead, Copper, Arsenic, Mercury, & Chromium exposed fish reported decrease in R.N.A. and protein content (Jana and Bandyopadhaya 1987).

Fish are relatively sensitive to changes in their surrounding environment including an increase in pollution. Fish health may thus reflect, and give a good indication of the health status of specific aquatic ecosystem. Early toxic effect of pollution is evident on cellular or tissue level before significant
changes can be identified in fish behaviour or external appearance.

Heavy metal pollution in Indian aquatic ecosystems, especially river systems, is a major environmental concern. Recent studies on Gandhi Sagar reservoir in Chambal river near Nagada and Kota, Khan river near Indore, Kshipra river near Ujjain and lower lake of Bhopal have shown accumulation of metal such as Zinc, Manganese, Copper Nickel, Mercury, and Lead in water. Lead concentration in submerged plant and fish from various sites of river Ganga were observed only in downstream site and in fishes collected at Kanpur (NEERI 1987). Fish population will either be adapted to environmental changes, or may die a slow death. Environmental pollution in the aquatic ecosystem is usually at low level but chronic in nature. Physiological and histological studies with more traditional acute toxicity test, may help one to gain insight into the mode and site of toxic action.

Base-line laboratory studies will provide an essential foundation starting necessary research in this field, where external condition can be controlled and fish can be experimentally exposed to one or more metal known to be present.
in natural water. The result of these laboratory studies will be useful in predicting the effects of metal exposure in natural aquatic systems and eventually determine species and metal specific histological lesions and changes. Certain heavy metals are necessary for specific body functions due to their nutritional value. High concentration of these metals can however cause toxic effect within an organism.

1.2 **Aim of study**: -

The aim of present study has been to evaluate the effect of toxic stress caused by acute and chronic heavy metal exposure on selected parameter such as bioaccumulation and histopathological abnormality in selected tissue (Gill, Liver and Kidney), growth and behavioral changes in *Cyprinus carpio* and *Heteropneustes fossilis* due to different concentration of Cadmium, Mercury and Copper.

**Objective I**: -

Exposure of fish to different concentration of heavy metals.

**Objective II**: -

Exposing the fish over various time periods including both short and long term exposure.
1.3 Motivation for work :-

This study can accordingly be summarized in the following statements :-

1. Heavy metal pollution is of great environmental concern and its harmful effect must be investigated.

2. Controlled laboratory studies are necessary to determine species and metal specific toxicity.

3. It is necessary to determine the concentration of heavy metal, however minute that concentration may be, that will cause initial structural damage at the histological level.

4. Short and long term exposure periods may significantly influence the histological damage, bioaccumulation of metal in vital organs, growth and behavioral alteration caused by exposure to heavy metal.