# Toxicological Effects in Fresh Water Teleost Fish *Heteropneustes fossilis* (Bloch) Induced by Cadmium

## A Thesis

**Submitted for the award of the degree of**

Doctor of Philosophy

in

**Zoology**

to


SACHIN RASTOGI

**M.Sc. (Zoology)**


Under the supervision

of


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**Maharishi University of Information Technology**

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# *Contents*

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# CERTIFICATE

This is to certify that the thesis entitled **"Toxicological Effects in Fresh Water Teleost Fish *Hetropneustes fossilis* (Bloch) Induced by Cadmium"** submitted to Department of Zoology, Maharishi University of Information Technology, Lucknow, Uttar Pradesh, in the fulfillment of the requirement for the award of degree of 'Doctor of Philosophy' in Zoology, embodies the original research work carried out by **Mr. Sachin Rastogi**, under my supervision and has not been submitted in part or full for any degree or diploma of this or any other University. It is further certified that scholar fulfills all the requirements as per the ordinance of the University for the purpose of submission of Ph.D. thesis.

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# DECLARATION

I, hereby, declare that the work presented in this thesis entitled **"Toxicological Effects in Fresh Water Teleost Fish *Hetropneustes fossilis* (Bloch) Induced by Cadmium"** in fulfillment of the requirements for the award of Degree of **'Doctor of Philosophy'** of Maharishi University of Information Technology, Lucknow, Uttar Pradesh, is an authentic record of my own research work carried out under the supervision of **Prof. Madhulika Singh**, Department of Zoology, Maharishi University of Information Technology, Lucknow.

I also declare that the work embodied in the present thesis is my original work and has not been submitted by me for any other Degree or Diploma of any university or institution.

**April 2021**

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# CHAPTER-1

# *INRODUCTION*

The universal presence of metals in universe has considerable possibilities for reacting with a wide range of systems; therefore, the identification of specific molecular targets for metals is one of the major difficulties. However, metals and their ions play a critical role by acting as catalysts or structural components of large molecules with specific functions and are indispensable for life. The biologically active metals such as Sodium (Na), Potassium (K), Magnesium (Mg), and Calcium (Ca) are present in large amounts and needed to maintain for the osmolarity of biological fluids, the structural and functionality of cell membrane, the synthesis of protein, and the conduction of nerve impulse and contraction of muscles. Ca plays the important part in forming the structures flexible or rigid but it (and also some time Mg) can also trigger many of reaction possibly by effecting a structural change. Na and K because of their abundance function many as charge carries.

Large varieties to inorganic and organic compounds are released into the environment during industrial and agricultural activities. Many of their chemicals which are foreign to living systems (xenobiotics) can cause harm to organisms. These chemical Pollutes air, water and food, and thus finally affecting the health of human beings, plants and animals. Metals are ubiquitous in our environment and have a versatile role in living systems as essential nutrients; as active entities of catalytic and

transport process; as component of structural origination; as beneficial drug and unfortunately some act as harmful toxicants, therefore the study of biological role of metals is fascinating aspect of environmental toxicology. In fact the increasing concern about the adverse effect of metals, polluting occupational and community environment, on human live stock and agricultural ecology is among the major problems of these days. Mechanistic understanding of how the metal parses, there ionic species or organic combinations to reach the molecular targets at specific tissue loci in biological systems and how they interred with the specific protein in manifestation of toxicity is an important aspect of chemical toxicology.

Various natural and anthropogenic activities led to release of heavy metals in aquatic ecosystem creating serious problems to its flora and fauna. Heavy metal pollution is among five major pollution problems of aquatic bodies. Heavy metals have long been recognized as serious pollutant of the aquatic life. Pollution by heavy metals has becomes a serious environmental as well as public health hazard concern because the construction related into the environment from industrial processes often exceed permissible levels. Due to their bio-accumulative and non biodegradable properties heavy metals constitute a scare group of aquatic pollutants also. These chemical accumulate in the tissues of aquatic organisms at concentrations many times higher than concentrations is water may biomagnified in the food chain to levels the course physiological impairment of higher tropic levels in human consumes. It is reported that reproduction rate of aquatic organisms may be affected due to exposure

to heavy metals and can lead to a gradual extinction of their generations in polluted waters.

In the list of heavy metals such as lead (Pb), mercury (Hg) and cadmium (Cd) are considered to cause public health hazards. Cd is a naturally occurring non-essential heavy metal present at higher concentrations in association with Cd-rich soils, including shales, oceanic and lacustrine sediments, and phosphorites. However, more than 90% of Cd in the surface environment is the result of industrial and agricultural processes (Pan et al., 2010). Burning of fossil fuels and mineral oil, smelting, mining, alloy processing and industries that use Cd as a dye (CdS: yellow; CdSeO3: red) in their manufacturing processes (Swarup et al., 2007) are all potential sources of Cd for farmed ruminants, with exposure decreasing with distance from the pollution source (Vos et al., 1990). Cd may also enter into the atmosphere from zinc, lead or copper smelter. It can enter water through disposal of wastes from households or industries. Further usually, air concentrations of Cd of between 0.01 and 0.35 $\mu g/m^3$ have been reported (US Department of Health, Education and Welfare, 1966), with the highest concentrations in industrialized cities. Fertilizers often contain some Cd. Cd is also a pollutant in phosphate fertilizers (Järup, 2003), leading to Cd being added to land through normal farming practice (Roberts et al., 2014). The Cd content in phosphate fertilizers varies considerably, depending on source, ranging from 3.6 to 527 mg/kg (Satarug et al., 2003). Sewage sludge is also recognized as an important source of Cd contamination (Patrick, 2003).

Cd is primarily stored in the liver and kidneys, which account for half of the body's total stores of Cd, rest in bone, pancreas, adrenals and placenta (Pope and Rall, 1995). The rate of Cd excretion, primarily in urine, is slower than that of uptake, highlighting the need for animals detoxify and store excessive Cd (George and Coombs, 1977; Wilkinson et al., 2003; Smiejan et al., 2003; Klaassen et al., 2009). Cd damages the kidney and cause signs of chronic toxicity, including impaired kidney function, poor reproductive capacity, hypertension, tumors and hepatic dysfunction (Pope and Rall, 1995).

Fishes construct one of the major sources of protein for human beings. Fishes are major part of the human diet and it is therefore unsurprisingly numerous studies have been carried out on metal pollution in different species of edible fish. The dietary worth of fish depends on their biochemical contents (protein, amino acids, vitamins, mineral contents, etc.) in different tissues. Cd is non-biodegradable element with still not known biological functions and reported to be a major containment of aquatic ecosystems causing adverse effects on aquatic organisms (Hollis et al., 1999, McRae et al., 2018). It is enters into aquatic ecosystem through diverse sources including both natural and anthropogenic activities. The major sources of Cd contamination include electroplating paper PVC plastic pigment & ceramic industries battery, mining and moldering units and many other modern industries (Gupta et al., 2003). It also enters into aquatic bodies through sewage sludge and with the run also enters off from agricultural lands as it’s is one of the major components of the phosphate fertilizers (Cherian and Goyer, 1989). Afterward, it produces deleterious

effects on aquatic flora and fauna via affecting their various physiological, biochemical and cellular processes (Gill et al., 1988; Venugopal & Reddy 1992; Faverney et al., 2001; Drastichova et al., 2004; Patro, 2006; Mai et al., 2006).

Nowadays, the aquatic life of water resources/bodies is in danger due to continuous addition of industrial pollutants, domestic and agricultural wastes, heavy metals etc. Numerous studies confirmed that pollutants are adversely affecting the aquaculture (Hollis et al., 1999; Opaluwa et al., 2012; Haloi et at., 2013; McRae et al., 2018). The presence of pesticides and various toxic metal accumulations were observed and reported in the freshwater and marine fish organs (Agrahari & Gopal, 2007). It is well known that Cd is a highly toxic and essential heavy metal. In the modern age its application is increasing in chemical and agricultural industries. It gets accumulated and damages the fish organs. The higher concentrations of Cd damage the organs and affect the fish behavior. Any changes in fish behavior are considered as one of the sensitive biomarker to evaluate the exposure to the toxicant (Conrad et al., 2011). It had been observed that the fish behavior alters due to the toxicants like heavy metals, pesticides, etc. (Ghanbohadur, et al., 2015; Deshmukh, 2016). Any change in fish behavior can be measure by some common parameters like erratic swimming, escaping from toxic water mucus secretion, convulsions and food intake etc. (Ghanbohadur, et al., 2015 and Deshmukh, 2016). Further, metal accumulation causes an increase in generation of highly reactive oxygen species (ROS), i.e. hydrogen peroxide, super oxide radical, hydroxyl/ radical leading to oxidative stress in fish. Heavy metals are suggested to promote oxidative damage in living system by

directly increasing the cellular concentration of ROS. Antioxidant enzymes contribute to the maintenance of a relatively low level of the reactive and harmful hydrogen/radical generals through the Haber–Weiss Reaction between superoxide radical and hydrogen peroxide in the presence of Cd. CAT primary antioxidant defense component eliminates hydrogen peroxide.

The fresh water air breathing sting catfish *Heteropneustes fossilis* (*H. fossilis*; The Asian stinging catfish or fossil cat, is a species of airsac catfish widely found in India, Bangladesh, Pakistan, Nepal, Sri Lanka, Thailand, and Myanmar) is a cherished table fish in India is distributed throughout the India sub-continent in various fresh water ecosystems including muddy marshy & derelicts ponds having law levels of water and dissolved oxygen. They are seen ever contaminated water bodies also. Hence, *H. fossilis* is suggested as a fish having nutrition value as well as a better bio indicator of fresh water stream, lakes and ponds to observe the behavioral alterations proposed by any toxicants/pollutants (Kamal et al., 2007; Farah et al., 2004).

Toxicity studies of Cd in fishes have stimulated considerable interest in recent years. Studies have shown that metals, vitamins, chelating agents and protein diets can alter the physiological, biochemical and behavioral aspects of fish. Micronutrient and essential metal as zinc have also been well known their role in animal physiology. Respiratory parameters of living organism are important for assessing the toxic stress as they are valuable indicators of the functions of all vital life.

Cd is reported to be toxic to all tissues of animals. Skeletal deformities making fishes unable to swim, food capture, mating and guarding its territory have been reported due to various heavy metals in fish. Cd is heavy metal commonly use in electrological studies because is concentration rises in the environment due to some industrial wastes and domestic sewage waste streams (Wright and Wellborn 1994; Groening et al., 1995). It is an important xenobiotic in aquatic ecosystems. Cd is regarded as one of most toxic heavy metals and potential for fish exposure has increased with increasing industrial use of metal. As non-degradable and cumulative pollutant Cd is regarded as ecologically significant problem due to its ability to be accumulated in tissues of living organisms (Jensen & Bro Rasiriurren 1992; Alazemi et al., 1996). A large amount of Cd maybe accumulated rapidly but last very slowly. Fish in contaminated areas are often expired to higher concentrations of Cd than there in unpolluted environments. In fishes Cd has been shown to alter the structure and causes morphopathological changes (Lemaire- Gany and Lemaire, 1992; Thophon et al., 2004). Heavy metals have long been recognized as serious pollutant of the aquatic environment and cause damage to aquatic life. A large part of their elements exert their toxic effect by generating reactive oxygen species (ROS), causing oxidative stress. Most of the heavy metal ions are toxic and carcinogenic in nature; pose a threat to human health and environment. Cd is a nonessential heavy metal; however it is considered as one of the most toxic water contaminants and could causes toxicity at each level in organism, from populations and communities to cell elements. Even at sublethal concentration Cd has a cumulative polluting effect and could course serious

disturbance in fish metabolism such as abnormal behavior, locomotion anomalies or anorexia. Cd may also affect the blood cells.

The physiological effects of water borne Cd exposure on freshwater fish are well documented. Chromium is also a compound of biological interest, probably having a role in glucose and lipid metabolism as an essential nutrient (Langard et al., 1979). Chromium (VI) compounds have been found to be mutagenic and carcinogenic (Forstner and Wittman, 1979; Vengo, 1985). Among the heavy metal, chromium is an important pollutant from industrial effluents and causes deleterious effects on non-target aquatic organism resulting imbalance of an ecosystem (Singh et al., 2004).

However, fish develops an increases tolerance to acute Cd toxicity during acclimatization to chronic water borne Cd exposure (Hollis et al., 1999), and probably due to the induction of Cd sequestering proteins (e.g. metallothionin) which bind to Cd and keep it in a metabolically inactive from (Hollis et al., 2000, Chowdhury et al., 2004).

Dietary Cd explore also contributes significantly to Cd accumulation in fresh water fishes (Szebedinszsky et al., 2001; Chowdhary et al., 2004; Ng and Wood 2008). Yet, its toxicological implications remain largely unknown. The current information on the effects of dietary Cd exposure on ion homeostasis in fish is contradictory because both no effects and modest disturbances have been reported (Pratap et al., 1989; Chowdhary et al., 2004). Dietary Cd exposure may affect iron metabolism and homeostasis in fishes. Given that Cd can interact with intestinal iron

transport as well as systemic iron handling. Biochemical changes in fish tissues such as ovary, testes and liver have drawn the attention of several researchers, because tissues are the major source of protein, carbohydrate and lipid and have a high calorific value (Joshi et al., 1979). Researchers interpreted that these changes may occur due to the various physiological factors such as maturation, and spawning. Freshwater fish have high food value and are economically important too. Biochemical composition of the fish is subjected to variations depending on the season were reported in *Sillago sihama* (Shamsan and Ansari, 2010). On global scale, fish and fish products are the most important source of protein in the human diet. The fish proteins are of relatively high digestibility when compared to other animal protein source. The protein content in the body of the fish changes depending on the stages of maturity of the gonads were reported in *Onchorhynchus nerka* (Brett et al., 1969) and *Salmo gairdneri Richardson* (Weatherley and Gill, 1983). Additionally, protein content in a fish population is relatively constant. The positive correlation between fish condition and percentage of protein in the gonads shows that healthy fish are likely to be more successful in breeding. Total protein content was reported by (Minimary, 1996) in tissues such as gonads and liver of fishes *Gotvla gotvla*, *G.annandali* and *G. lissorhynchus*. Seasonal variation in total protein and glycogen content of ovaries were reported in the fish, *Garra mullya* by (Khan and Mehrothra, 1991).The inter-relationship between the liver and gonads were also reported in the fish, *Cyprinus carpio* (Sivakami et al., 1986) during different stages of maturity. Seasonal changes and annual variation in the protein, glycogen and cholesterol content

in the ovary and testes were studied in *H. fossilis* by (Hunge and Baile, 2003). Though a lot of work has been carried out on relation to biochemical aspects of ovary, testes and liver, in fishes this study suggests the effects of salinity and correlates the biochemical composition with reproductive cycle.

The work done in this thesis **"Toxicological Effects in Fresh Water *Teleost* Fish *Hetropneustis fossilis* (Bloch) Induced by Cadmium''** is conceptualized with the basic thought to found the applicability of fresh water fish *H. fossilis* as a biological model to decipher the mechanism underlying aquatic environmental pollutants induced morphological, biochemical, histopathological, and behavioral alterations. **Thus, the current investigation was carried out with the objectives to determine the effects of $CdCl_2$ doses on morphology, behavior, biochemistry and histopathological alterations in the largest organs (liver and kidney) of fresh water fish *H. fossilis.***

# CHAPTER-2

# *LITERATURE REVIEW*

Aquaculture is totally centered on aquatic ecosystem and accounts for substantial contribution to Indian economy and also responsible for source of revenue to millions of people. Outstanding achievements have been made in the field of aquaculture by adapting contemporary techniques of fish culture and hence there is substantial growth in the production of culture fishes. Globally, India rank 3$^{rd}$ in Fisheries and 2$^{nd}$ in aquaculture. Combining the production of all types of fisheries (capture and culture), the total fish production in the country reached about 11.41 million tonnes in 2016-17 (National Fisheries Development Board, Govt. of India). Fishes are highly nutritious and consumed as a delicacy food throughout country (Prasad et al., 1992, 2007). But, addition of pollutants in water has ill impact on the aquatic ecosystem and poses adverse effect on the fish health. As a result, fish production is generally overloaded and fish farmers countenance an enormous fiscal loss. Thus monitoring and understanding haematological and biochemical parameters of various toxicants would be helpful in minimizing losses and providing safeguard to public health.

A variety of contaminants including toxic heavy metals (Cd, Cu, Hg and Zn) are reported to be ubiquitously present in rivers, reservoirs and are disadvantageous for

aquatic organisms (Olsson, 1998). In general, they are not biodegraded, and therefore, their bio accumulation in fishes, oysters, mussels, sediments and other components of aquatic ecosystems have been reported from all over the world. Changes in stream temperature and bottom characteristics lead to habitat destruction and alteration of fish species diversity. Further the addition of toxic substances to such water can have either acute or chronic effects on aquatic organisms (Gaufin, 1973; Sanders, 1997).

The heavy metal toxicity is widely present in our environment. They find their way into the water bodies during monsoon, thus causing pollution hazards to aquatic life. If these are allowed to drain and accumulate into water bodies continuously, the commercial fisheries will get a serious setback in future. Several experimental studies in which fishes have been exposed to sublethal concentration of heavy metal and other pollutents have provided unquestionable evidence of toxicity as evident by histopathological changes due to such exposure (Dutta et al., 1993, 1996; Deore et al., 2012; Ghanbahadur et al., 2015). A lot of literature on the morphology, histology and haematological parameters on the various organs of fishes is available but their study on histopathology is scanty and controversial. We investigated the acute toxicity parameters and biochemical effects of heavy metal (Cd) pollution on exposed fish specimens. The various sections in this chapter, therefore, discuss each of these aspects with special reference to this study.

Drinking-water contamination, high ambient air concentrations near emission sources, or intake via the food chain may cause heavy metal poisoning. Heavy metals

can also be accumulated by some organisms either directly or through the food chain, eventually propounding a serious health risk to inhabitants of an ecosystem, including humans (Galloway et al., 1982, Angelone et al., 1992). The bioaccumulation of toxicants including heavy metals, by living organisms is frequently a superior integrative marker of exposure and has been extensively used to assess contamination levels of heavy metals in polluted ecosystems (Philips et al., 1994, Ullah et al., 2016). These metals are accumulated at various tropic levels through food chain. Arsenic causes hyperkeratosis, a disease related with cancer (Chen et al., 1962) and Cd leads to a disease known as Itai-Itai (Kobayashi, 1970). Considerable attentiveness has been focused on the providence of heavy metals and their effects in the aquatic environment during the past decades. The gravity of heavy metals contamination was fingered for the first time during the epidemic of "Minamata disease" (Monk, 1983); a direct result of eating mercury contaminated shell fish. Several diseases caused by the excessive accumulation of heavy metals were reported following this. The excessive accumulation of Pb in human system produces nephritis, a stage of severe contamination of kidney. Numerous studies demonstrate that some species accumulate high level of heavy metals in the whole animals and in the tissues (Kalmaz et al., 1981). Furthermore, a recent study suggested that the Cd level in fish muscle was above the threshold level and these fishes are not safe for human consumption (Dalzochio et al., 2018).

Heavy metal contaminants have been proved to be associated with numerous toxic effects in aquatic organisms via waterborne exposure. In recent years, due to the

importance of the impacts of these contaminants on biota which may include human resources of food; would best serves as test organisms in toxicity bioassays, toxicity studies have become an integral part of pollution assessment. Bioassays have become basic tools for detecting, evaluation and moderation of water pollution, during the past decades. The values of bioassays for detecting and evaluating the toxicity from industry in connection with its treatment and safe disposal are being realized increasingly. Therefore, the manipulation of bioassays has increased greatly. In addition, there has been a great increase in varieties of organisms used for bioassays (APHA, 1981).

As a tool to identify suitable organisms as a bio-indicator and to derive water quality standards for chemicals, toxicity testing has been extensively used. Toxicity testing is an indispensable tool for assessing the consequence and providence of toxicants in aquatic ecosystems. The ability of various pollutants and their derivatives/metabolites, to mutually affect their toxic actions lay down hurdles for the risk assessment based exclusively on environmental levels (Calabrese, 1991). Researchers primarily concerned with establishing the potential relationship between a pollutant source and an ecological effect caused by exposure of organisms to the pollutant (Suter, 1993).

## *Sources of Cadmium pollution:*

Cd is an element that occurs naturally in the earth's crust and obtained rank 7 of ASTDR's "Top 20 list" (ASTDR, 1999). It is an environmental contaminant that

disguises brutal threats to aquatic organisms and their associated ecosystem. Since, it is found in range of agrochemical (insecticides, fungicides, sludge, and commercial fertilizers) which are consistently used in agriculture; its percentage in the upper soil has been increasing. Other known sources of Cd are dental alloys, electroplating, motor oil and exhaust. It is also used for the following: batteries (83%), pigments (8%), coating and plating (7%), stabilizers for plastic (1.2%), nonferrous alloys, photovoltaic devices, and other uses (0.8%) (Thornton, 1992). For this reason, anthropogenic activities have augmented Cd intensification in the environment. Overall 10% of total Cd in the environment is consequential from natural sources, whereas remaining 90% is derived from anthropogenic activities (Okada et al., 1997). Volcanic activities contribute about 62% of natural emissions and other natural causes include decaying of vegetation (25%) airborne soil particles (12%) and forest fire (2%). Its non-corrosive and accumulative nature has made it very important to its applications in electroplating or galvanizing. Anthropogenic undertakings like; smelting operations, use of phosphate fertilizers, pigment, cigarettes' smokes, automobiles etc. have contributed to the admittance of cadmium into human and animal food chain (WHO, 1992; Okada et al., 1997; Kumar et al., 2007). Higher concentration of Cd than the maximum acceptable limits in water, vegetation and food is well reported.

As a source of Cd, higher level of Cd has also been detected in sewage sludge (rich in almost all nutrients and hence generally used as plant fertilizer), various vegetables (Roblenbeck et al., 1999), animals feed and their tissues (Kumar et al.,

2007). Topsoil enrich in sludge contributes Cd accumulation in the blood, milk, hair, liver and kidney of sheep, goat, cow, buffalo (Allen 1995, Swarup et al., 2007; Patra et al., 2005, 2007). In India, various levels of Cd concentration have been reported to be present in aquatic ecosystem which is more than 5ng/ml in the Yamuna river water at Agra, Delhi, Etawah and Mathura (Ajmal et al., 1985) and 0.50-114.8 mg/kg in the Yamuna river sediments at Agra and Delhi. However, the water of Yamuna River around the industrial areas has been found to contain higher levels of cadmium (Singh, 2001; Kaushik et al., 2003). Similarly, Hindon River (Uttar Pradesh) has also been contaminated with heavy metals including Cd (Jain and Sharma, 2001; Sharma, 2003). Moreover, high concentration of Cd (70-100 ng/ml) has been detected in Bombay city (Agrawal and Raj, 1978), Baroda city (Kannan, 1997) and fish and chicken meat in western UP markets (Kumar et al., 2006 and 2007; Burger, 2008).

As metals Cd is both; persistent and toxic; metal contamination in aquatic systems is a particular apprehension (Clark 1992). Animals that accumulate Cd in their bodies ("body burden") can be eaten by others, and so on, such that Cd will both accumulate and bio-magnify in the food chain (Environmental Planning and Assessment Regulation 2000; Dalzochio et al., 2018). Cd and other metals released from mining sites can contaminate drinking and other water sources (Younger et al., 2002; Peplow et al., 2004).

## *Absorption and accumulation of Cadmium by aquatic organisms:*

In aquatic systems, Cd is most readily absorbed by organisms directly from water in its free ionic form Cd (II). The toxicity of Cd to aquatic organisms is variable, even between closely related species, and is related to the free ionic concentration of the metal. Cd interacts with the calcium metabolism of animals. In fish, it causes lack of calcium, probably by inhibiting calcium uptake from the water. However, high calcium concentrations in the water protect fish from Cd uptake by competing with the uptake medium. Effects of long term exposure can include larval mortality and temporary reduction in growth rate. The toxicity is variable in fish species, salmonoids being particularly susceptible to Cd. Sub-lethal effects in fish, notably malformation of the spine, have been reported (AMAP 1998).

When, where and how an animal consumes, Cd can play a role in behavior of its effect. Fish can accumulate Cd from both the water and by eating foods contaminated with Cd (contaminated food chain). It is important to note that bioaccumulation as well as bio magnification occur when a substance cannot be easily metabolized or excreted. Cd exhibits this persistence (ATSDR Medical Fact-Sheet, 2008) in fish body and tissue. It accumulates in considerable concentrations in various organs of fish (Sindayigaya et al., 1994; Kumar et al., 2008; Dalzochio et al., 2018). De Smet et al., (2001) reported that Cd accumulates in tissues of carp *Cyprinus carpio* in following order: kidney> Liver> Gills. Kidney is the prime target structure for Cd. Liver also stores a considerable quantity of the accumulated Cd. It is redistributed to these organs

directly following uptake through the gills and intestine, but there may also be redistribution of Cd from other organs (Olsson et al., 1987). A study revealed that some insects can also accumulate high levels of Cd without showing any adverse effects (Jamil et al., 1992). Additionally an increased bioaccumulation of chromium, Cd and Pb mixture in muscle, gills, liver, kidney and intestine tissue of *Cyprinus carpio L.* exposed for 30 days was observed (Rajeshkumar et al., 2017).

At molecular level there is variety of approach of Cd uptake in aquatic organism, where it is most readily absorbed by organisms directly from the water in its free ionic form Cd (II) (AMAP 1998). In broad metal ions are usually absorbed through passive diffusion or carrier mediated transport over the gills while metals associated with organic materials are ingested and absorbed by endocytosis through intestine (Kumar et al., 2008). Olsson suggested that Cd ions enter the chloride cells in the gills through calcium channels (Olsson, 1998). Once enter in the cells the metal is made available for the interaction with cytoplasmatic components such as enzymes and metallothioneine. Although metallothoneine is induced in the gills it does not appear to be as capable of sequestering the vast majority of accumulated Cd2+, as it is in the liver (Olsson and Hogstrand, 1987). The reason for this is believed to be due to the high affinity of Cd2+ for Ca 2+ binding sites in the gills (Flick et al., 1987), and it is also believed that Cd2+ binds to the active sites on the basolateral Ca2+ -pump in chloride cells. It thus seems that Cd2+ enters the gills through Ca-channel on the apical side and is further translocated to the circulation interactions with Ca2+ -ATPases on the basolateral side.

## *Toxicity of Cadmium:*

Cd toxicity may be manifested by a variety of syndromes and effects including renal dysfunction, hypertension, and hepatic injury, lung damage after inhalation exposure, reproductive toxicity, teratogenic effects and bone defects (Friberg et al., 1974; Nriagu, 1989). In a recent mechanistic study adult female zebrafish were exposed to Cd (1mg/L) for for 24h and 96h, and the oxidative stress and inflammatory responses induced by Cd were evaluated in the brain, liver and ovary. Cd has been reported to exert deleterious effects in terms of nephrotoxic, cytotoxic, genotoxic, immunotoxic and carcinogenic material (ATSDR, 1999; Lippmann, 2000). Study demonstrated that water born Cd can induced oxidative stress and immunotoxicity in fish, possibly through transcriptional regulation of Nrf2 and NF-κB and gene modifications at transcriptional, translational, post-translational levels (Zheng et al., 2016 a.b).

Studies have revealed that the fish exposed to Cd exhibited significant reduction in RBCs, haemoglobin and haematocrit. The reduction of these parameters in *Nile tilapia*, and *Oreochromis niloticus* at sublethal levels of Cd might be due to the destruction of mature RBCs and the inhibition of erythrocyte production due to reduction of hemesynthesis that is affected by pollutants (Wintrobe, 1978). Also, the decrease in RBCs count may be attributed to haemato-pathology or acute haemolytic crisis that results in severe anemia in the majority of vertebrates including fish species exposed to different environmental pollutants (Khangarot et al., 1991) or may be the decrease in the RBCs may be attributed to reduction of growth and other food

utilization parameters which can cause severe aneamia (James et al., 1991 and 1999). Also was found a significant reduction in the RBCs, Hb and Hct in American eel (*Anguilla rostrata*) after exposure to 50 g/L Cd. Karuppasamy et al., (2005) found in his study a significant decline in total erythrocyte count, haemoglobin content, haematocrit value and mean corpuscular haemoglobin concentration in air breathing fish, *Channa punctatus* after exposure to sublethal dose of Cd. Accumulation of Cd in ex-vivo exposed trout (*Oncorhynchus mykiss*) erythrocytes and Cd dose-dependent effect in terms of RBC viability, cytosolic and mitochondrial ROS levels as well as its effects on mitochondrial membrane depolarization, hemoglobin stability and precipitation was evaluated by Orlando et al., (2017). Study revealed that metal accumulation was associated with an increase in oxidative indexes, (except decreased mitochondrial superoxide anion production) which was probably Cd-dependent mitochondrial membrane depolarization. $C_d$ $C_{l2}$ induced genotoxicity and cytotoxicity has been assessed in the peripheral blood erythrocytes of freshwater *fish Labeo rohita* exposed to 0.37 and 0.62mg/L of $C_dC_{l2}$ in water for 100 days (Jindal and Verma 2015). The metal exposed groups showed significant variation in the frequency of cellular abnormalities as well as the extent of DNA damage in comparison to controls (Jindal and Verma 2015).

From the available data, it is clear that Cd exposure upraises the stress level in fish as the elevation in blood glucose level is generally observed. Cd resistivity was greater in case of air-breathing (*H. fossils*) as compared to non air-breathing (*Labeo rohita*) fishes with regards to their duration of survival and growth (Dass and

Banerjee, 1980). Sub-acute levels of Cd exposure show a definite stress condition on both species as they respond differently towards carbohydrate metabolism as observed from liver and muscle glycogen content, liver microsomal glucose-6-phosphatase activity and serum glucose level. Cd induced hyperglycemia with decrease in liver glycogen in catfish, *H. fossilis* (Sastry and Subhadra, 1985). Blood glucose is a sensitive and reliable indicator of environmental stress in fishes. Soengas et al., (1996) suggested that hyperglycemia occurred in Atlantic salmon (*Salmo salar*) after toxic exposure with cadmium, possibly be due to changes in liver carbohydrate metabolism (activation of liver glycogenolysis and glycolysis) as well as increased levels of plasma glucose and lactate. Protein level is a frequently measured parameter for metal poisoning in fish. James et al., (1999) suggested that protein was significantly decreased in *H. fossilis* exposed to Cd. The activity of AST and ALT enzymes in blood may also be used as a stress indicator. The significant changes in activities of these enzymes in blood plasma indicates, tissue impairment caused by stress (James et al., 1991). Sastry et al., (1985) recorded a significant reduction in ALP in liver and kidney of catfish, *H. fossilis* after toxic exposure to Cd. Poornima et al (2007) evaluated the toxicity of Cd and its impact on biochemical constituents like glucose, glycogen, total proteins, lipid and free amino acids in the fresh water edible carp *Catla catla*. According to Poornima et al., (2007) elevated levels of glucose indicated the organism's response to the toxicant stress and thus the fish cultured in the aquatic systems closer to the industrial locations would not have the expected nutritive value.

## *Oxidative stress induced by Cadmium:*

Cd is a bivalent cation and is unable to generate free radicals directly; nevertheless there is increased production of Reactive Oxygen Species (ROS) after Cd exposure. Cd alters antioxidant defense systems and increase production of cellular ROS, such as singlet oxygen, hydrogen peroxide, and hydroxyl radicals (Pathak and Khandelwal 2006, Ognjanovića et al., 2010). ROS can lead to oxidative stress within cells by reacting with macromolecules causing damages, such as mutation, destruction of protein function and structure, and peroxidation of lipids as well as alterations in gene expression and apoptosis (Valko et al., 2006). The effects of Cd induced oxidative stress in the tissues and cells of animals and plants have been reported by Venod (2009).

## *Organ toxicity in fishes induced by Cadmium*

The uptake of Cd through the food chain in aquatic organisms may lead to morphological alterations and pathological disorders (George and Coombs, 1977; Gill and Pant 1985; Giari et al., 2007; Gill and Raine 2014). The pathological changes caused by Cd poisoning in the digestive tract, kidney and gill of *Findulus heterocities* have been observed by (Gardner and Yevich ,1970). Cd in sub-lethal doses induces structural and functional alterations in various vital organs including liver, kidney of fishes. Histopathological study thus gives us useful data about the concerning tissue change, prior to external expression. It has been shown to alter the structural morphology of fish and cause histopathological changes to varying digress in various

fish tissues. Accumulated heavy metals may lead to alterations in the tissues of fish (Olsson et al., 1998). Cd can trigger liver and kidney cell apoptosis through the activation of caspase-3A. Caspase-3A may play an essential role in Cd-induced apoptosis (Gao 2013a and b).

***In liver tissue:***

Cd accumulates in the liver tissues of fishes in sub-lethal concentrations (De Smet and Blust, 2001; Rangsayatorn et al., 2004). It also induces a variety of pathological alterations in the liver tissues including engorgement of blood vessels, congestion, vacuolar degeneration of hepatocytes, necrosis of pancreatic cells and fatty changes in the hepatocytes (Rani and Ramamurthi 1989, Dangre et al., 2010). Bouraoui et al., (2008) reported acute effects of Cd on liver phase I and phase II enzymes and accumulation metallothionein in *Sparus aurata*. Cd (dose 30µg/L) exposure also induced severe pyknotic nuclei, evident ultrastructure damage, and considerable lipid inclusions in the hepatocytes (Zheng et al., 2016 b). Further, authors explained that the negative effects caused by Cd doses may be through an increase in hepatic oxidative damage, as reflected by the enhanced levels of lipid peroxidation and protein carbonylation and reduced activity of Cu/Zn-superoxide dismutase and catalase. On contrary a study suggests that exposure of Cd induced no alterations on spleen and liver organo-somatic indexes whilst produced progressive deleterious morphological alterations in liver and exocrine pancreas that correlated with the hepatic Cd-accumulation (Guardiola et al., 2013).

***In kidney:***

Cd accumulates in the kidney tissues of fishes in high concentrations and has been reported to possess nephrotoxic action in various animals including fishes (Avallone et al., 2017). Kidney is the principle target organ for Cd toxicity and its chronic exposure in almost all animal species is characterized by varying degree of renal damage such as glomerulus, tubules and Bowman's capsule (Shukla and Gautam 2004; Kumar et al., 2006, Vesey 2010).

***In Gills:***

Gills are also reported to act as storeroom of Cd (Allen, 1995; Ramesh and Nagaranjan, 2007). Morphological and biochemical changes in the gills of Tilapia (*Oreochromis mossambicus*) after Cd exposure was noted by Wong and Wong (2000). Researchers noted the microbridges in pavement cells and an increase in the apical membrane of chloride cells of kidney. They further reported chloride cells as a prime target of Cd toxicity, resulting into fish hypocalcemia (Miandare et al., 2016) also noted histological alterations in gills of *Acipenser persicus* following the doses of Cd.

***Miscellaneous:*** Organs like intestine and gonads of fishes also emerge susceptible for toxic effects of Cd (Taylor, 1983; Kumari and Ramkumar, 1997; Singh et al., 2007; Kumar, 2007).

## *Effects of Cadmium on fish behavior:*

Recently behavioural toxicology has come out as a capable discipline to link the laboratory-to-field divide. behaviour of living things integrates the physiology of the creature and their external environment. Toxicant induced behavioural impairments frequently summit to underlying physiological deficits that can be used effectively to evaluate ecological risk: especially if the affected behaviour relates directly to survival, growth, or reproduction. Behavioral changes in animals are indicative of such internal disturbances of the body functions. There are many other studies confirming behavioural changes in fish due to various toxic chemicals (Rani and Kumaraguru, 2014, Sabullah et al., 2015, Green and Planchart, 2017). The elimination of aquatic animals by small dangerous physiological or behavioural changes has been reported to be more serious than a massive fish kill, since it is less likely to be observed and corrected (Larsson et al., 1976). Behavioral abnormalities in various fish species on exposure to heavy metal have been reported by several researchers. Hyperactivity, erratic swimming and loss of equilibrium in Brook trout, *Salvalinus fontinallis*, in response to lead was noted Holcombe et al., (1970). Similarly, loss of equilibrium, frequent surfacing, sinking, burst of erratic swimming, and gradual onset of inactivity in Rainbow trout, *Salmo gairdneri*, on Hg exposure (MacLeod and Pessah, 1973). According to Ghatak and Konar (1990) when *Tilapia mossambica* exposed to Cd showed frequent surfacing with irregular opercular movements and loss of equilibrium. Several chemicals have shown divergent alteration in behaviour of fishes such as interpretation fish sluggish

and alter their swimming ability making them more susceptible to be preyed, reduce their ability to feed, maintain their position and defend their territories (Prashanth et al., 2005; 2011). These chemicals have also shown interrupting the schooling behaviour (Gill and Raine, 2014) of fish due to dangling, erratic and irregular movements and disturbed swimming (Nagaraju et al., 2011). Erratic swimming, jerky body movements, rolling the body, convulsions, mucous secretion , loss of equilibrium, rapid opercular movements, difficulty in respiration, and lethargy was noted by the doses of Cu (Siddiqui and Arifa 2011)

Distraction of schooling behaviour also makes the fish extra susceptible and easily preyed. On account of stress due to chemicals, fish became stressed and immune compromised, which make them more susceptible and vulnerable to diseases, secondary infections and pathogens (Satyavardhan, 2013). Methyl parathion resulted in increased movements of opercula, rapid jerk movement, equilibrium loss, body colour alterations, frequent surfacing, and elevated mucus secretion in *Catla catla* (Ilavazhahan et al., 2010). Cypermethrin caused darting, erratic and irregular swimming movements, equilibrium loss, hyper-excitability and sinking to bottom in *Labeo rohita* (Marigoudar et al., 2009). Pesticides also alter the migratory behaviour of migratory salmonid fish (Nagaraju et al., 2011). Yet studies have shown that adult salmon use to circumvent pollutants and contaminated areas during their migration, altering their migratory pattern, which result in postponement of spawning (Satyavardhan, 2013). Sodium cyanide induced certain behavioural changes such as hypexcitability, darting and erratic movements, and imbalance swimming, in

*Oreochromis mossambicus*, *Catla catla*, *Cirrhinus mrigala*, *Labeo rohita*, and *Cyprinus carpio* (David et al., 2012). Cypermethrin resulted in jumping, increased surface activity, balance loss, increased air gulping, equilibrium loss, abrupt swimming, sluggishness, motionlessness, adopting vertical positions and internal haemorrhage in *Tor putitora* (Ullah et al., 2014).

After going through extensive literature search we arrived at the conclusion that in the last couple of decades, there have been extensive efforts to unravel the mechanistic understanding of impaired morphological, biochemical, and behavioral disarrays due to exposure to environmental chemicals such as heavy metals including Cd that has met with limited success. The toxic responses of Cd, a widely present heavy metal and an established response as toxicant of fresh water fishes, has been subjected to intense investigation however its underlying mechanisms at pathological and biochemical level still remain not clear. Furthermore seeing as animal's behavioral response to any environmental changes has received considerable attention we thus directed our work towards an understanding of how Cd causes neurotoxicity by assessing the dose dependent responses of fish behavior.

# CHAPTER-3

# *MATERIALS AND METHODS*

The present study is intended to investigate the toxicity of Cadmium to a freshwater, air-breathing, stinging catfish, *Heteropneustes fossilis* Bloch (Order: Siluriformes; Family: Heteropneustidae). It includes Morphological, Behaviour, Biochemical and Histopathological aspects of fish to toxicants.

## FIELD WORK

### COLLECTION OF FISH SPECIMENS

Fishes were collected from river Gomti and water reservoirs in and around Lucknow, U.P. (India) with the help of local fisherman, brought to laboratory (N- 26º51’59’’, E- 80º56’17’’) and acclimatized to laboratory conditions for 15days before the experiments. Stock solution of Cadmium chloride (CdCl2.21/2 H2O, M.W. = 228.35AR Grade, Manufactured by Thomas baker chemicals ltd. Mumbai, India) was prepared by dissolving weighed amount of salt in double distilled water.

## LABORATORY WORK

For toxicity test six aquaria of 50 liter capacity were taken having 30 liters of dechlorinated tap water (Physico-chemical properties, pH = 7.6±0.2; Temp. = 26±20C; Alkalinity = 65±4.5 mg/L; Total Hardness =265±2.5 mg/L; D. O. = 7.0±0.2 mg/L).

Series of three concentrations of Cadmium chloride *viz*. 25, 50 and 75 mg/l (Toxic range was predetermined by exploratory tests) was prepared by adding calculated amount of stock solution.

One aquarium having diluent water without Cadmium chloride served as control. Adult 10 fishes of similar size and weight (average length 15± 1.5cm and weight 26.5± 2.0gm) were introduced to each test as well as control aquaria from stocking tank. Feeding was suspended 24 hour before start and throughout the experiment to avoid dissolved Cadmium losses due to particulate adsorption. Proper aeration was maintained in test as well as control aquaria by air pumps and stone diffusers throughout the experiments.

## PARAMETERS STUDIED

### 1. MORPHOLOGY AND BEHAVIOUR

Experiment was carried out according to guidelines of APHA *et al*. (1998) and replicated thrice. The LC50 values of various intervals were calculated according to Trimmed Spearman Karber's Method (Hamilton et al., 1977) on PC. Behavioural

characteristics were also recorded with respect to activity, movement, mucous secretion, skin colouration and opercular beats. The data obtained for opercular beats were statistically analyzed for student t-test and ANOVA using MINITAB software on PC.

## 2. BIOCHEMISTRY

The biochemical constituents viz., Glucose, Glycogen, Total proteins, Total Lipids and Choloestrol were estimated by standard procedures in 5 tissues viz., Muscle, Gill, Liver, Heart and Kidney of the healthy fish (Control) and of those from the fish exposed to sub-lethal and lethal concentrations of Cadmium chloride (Merck).

One-tenth of the lethal concentration was taken as sub-lethal dose and the fish were exposed to sub-lethal dose for a period of 30 and 60 days before sacrifice for the biochemical analysis.

### Estimation of the Glucose and Glycogen

The Glucose and Glycogen in tissues were determined by the method of Kemp et al., (1954)

**Reagents:**

KOH 30%

Anthrone Reagent

Con: Sulphuric Acid

Ethyl Alcohol

Standard glucose (40mg/ml)

**Estimation of Total Protein** (Lowry's method – Folin Ciocalteu method)

The total protein of tissue samples were assayed by the method of Lowry et al., (1951).

**Reagents:**

Sodium Hydroxide Solution 0.1N

Sodium Carbonate Solution 2%

Copper Sulphate Solution 0.04m (1%)

Sodium Potassium tartarate Solution 1%

Alkaline Copper Reagent:- A mixture of 50ml of Sodium carbonate solution and 0.5 ml of each of copper sulphate solution and sodium potassium tartarate solution.

Folin's phenol reagent (1:1 dilution with distilled water) Standard protein solution- 100mg in 0.1N NaOH

**Estimation of Total lipid**

Total Lipids were estimated by methods of Pande et al (1963).

Reagents:

Chloroform – Methanol Solution

Calcium Chloride Solution

Sodium Chloride Solution

Lipid Standard Solution

Stock acid dichromate reagent

Palmitic Acid Lecithin

**Estimation of Cholesterol**

Estimation of cholesterol was carried out by Zlatkis et al., (1953) method.

**Reagents:**

Glacial acetic acid

0.05 % Ferric chloride

Concentrated sulphuric acid

0.9 % Sodium chloride solution

Cholesterol standard

Working standard (0.04 mg/ml)

They were acclimatized to laboratory conditions in Fiberglass Reinforced Plastic (FRP) Tanks of 500 L capacity for one month prior to exposure to Cadmium. The water was changed every day. Fishes were fed with chicken liver *ad libitum* daily.

## 3. HISTOPATHOLOGY

The experimental design was based on Static Renewal Test (SRT), Range Finding and Definitive Test (Acute Toxicity Test) described by Sprague22 and USEPA23. For each bioassay test, a series of three test concentrations of Cadmium and a control were used.

The tissue samples were taken from the fishes exposed to the first three concentrations only. At the end of the experiment (60 days), live fish samples were collected from the above-mentioned three concentrations, sacrificed and their liver and kidney were excised out, they were subsequently washed in distilled water and

processed through graded series of alcohol, cleared in xyline and embedded in paraffin wax. Sections of 10 micron thickness were cut; stained with Harris haematoxylin and eosin and mounted in DPX. Stained sections were examined with light microscope for histopathological changes and also, light photomicrographs were taken. The morphological changes of the liver and kidney sections noted in the experimental fish were compared with those of control fish.

**Reagents:**

Physiological saline (0.75%NaCl sol.)

Neutral buffered formalin

Graded series of alcohol

Xyline

Harris haematoxylin

Eosin

DPX mountant

# CHAPTER-4

# *RESULTS*

## 1. MORPHOLOGICAL MANIFESTATION

The stining catfish, *H. fossilis* is classified into Subclass Teleostomi; Family: Heteropneustidae commonly known as the stining catfish or Singhi. It is considered one of the most highly demanded freshwater air breathing fish species in the Indian sub-continent and Southeast Asia.

The *H. fossilis* has an elongate body, sub-cylindrical up to the pelvic fin base, compressed behind. Head depressed with the top and the sides covered with osseous peats. Mouth is small and terminal; teeth in villiform bands on the jaws. The rounded caudal fin is separated from both dorsal and anal fins. Barbals are in 4 pair and well developed. Dorsal fins are short, inserted usually above the tip of the pectoral fins. Pectoral fin with a strong spine, serrated along its inner edge and with a few serrated by a distinct notch from the caudal fin, the latter rounded. Outstanding anatomical feature is a pair of long hollow air sacs on either side of the vertebral column. The pectoral spine is stout, sharp, smooth or sometimes a little roughened and covered with skin except near its tip. These vicious spines are capable in inflicting painful wounds. Usually are dark purplish-brown above and lighter below; and with two yellowish lateral bands. Young ones are reddish, mature specimen almost black.

During this study, we documented the specific site of Cd compounds action on *H. fossilis*. Among morphological changes, discoloration of skin, chemical deposition on skin and aquarium, lesions were recorded and effects were concentration dependent **(Table-1)**. In is reported that among Cd compounds, $CdCl_2$ caused maximum morphological changes in comparatively low concentration than the others. The schooling is the characteristic of this fish was found weakened by the toxicities of $CdCl_2$ during the present study. At higher concentration of $CdCl_2$ (75 mg/l), scales depletion started, skin lesion observed from dorsal to lateral side of the body of fish and these were deepens. Furthermore, copious mucous secretion and clumping of gills increased with the increasing concentration of Cd. The skin lesions around the head region, base of caudal fins and pectoral fins were prominent in the 90% of the fish in higher concentrations. The fishes lost their natural coloration and become almost pale yellow in color.

## 2. BEHAVIORAL MANIFESTATIONS

After 30 days of exposure fish showed more surfacing, air gulping, restlessness, escaping movement, erratic swimming and loose schooling. Sudden irregular swimming, increased mucus secretion and high rate of opercular beat rate was also noticed, fish became lethargic, less active, irresponsive which resided at the bottom of aquaria. Schooling was found completely disturbed and fish were scattered. Fish showed very less response for food and became very weak. Haemorrhage, colour fading, peeling of skin and rashes were also observed (**Table 2**). Responsiveness to

stimuli and food consumption became less the control fish. Ulcerative tubercles, haemorrhages and skin rashes were also start to appear on caudal and abdominal region.

After 60 days of exposure increased surfacing, fast swimming, jerky movement, restlessness, loss of balance, loose schooling and erratic swimming was observed in exposed fishes (**Table 2**). Fish became less active and less responsiveness to external stimuli and food. The skin was found peeling off more with increased haemorrhages and skin rashes on body surface particular in opercular and caudal region along skin colour fading in exposed animals.

After 30 day of exposure CdCl2 induced marked effects on opercular beats/minute of *H. fossilis* in the test fishes which were found significantly higher throughout the experiment (**Table-3**). Though a decline was noticed from 30 to 60 days exposure but the values were still significantly higher than the controls. The differences between means of opercular beats of test animals and controls animals were highly significant at 30 days ($t=12.50$; $P<0.0001$) and 60 days exposure ($t=11.55$, $P<0.0001$). The overall fluctuations in means of opercular beats from 30 days to 60 days were found significant in test animals ($F=15.29$; $P<0.001$) as well as in control animals ($F=12.75$; $P<0.001$) using One Way ANOVA.

## 3. HISTOPATHOLOGICAL CHANGES

In the present work liver and kidney of *H. fossilis* have been obtained for their histopathological investigations. For above observation, fishes were kept in 25 mg/l,

50 mg/l and 75mg/l $CdCl_2$ mixture for durations of 30 and 60 days, with every aquarium containing experimental fishes, parallel control were set.

**LIVER:**

Liver of *H. fossilis* is bilobed consisting of two subequal lateral lobes disposed longitudinally, small anterior lobe and larger posterior lobe with the gallbladder between the two. The liver consists of polygonal glandular hepatic cells arranged in groups. The hepatic cells are provided with a centrally placed darkly stained spherical and granular cytoplasm.

In our study liver of control fish *H. fossilis* is a dark brownish red coloured, bilobed gland composed of hepatocyte cells which are the parts of mass and forms a typical normal architecture. The hepatocytes are arranged in a radial manner around hepatic veins to form hepatic cords. The cords are however formed of liver parenchyma which encloses the blood sinusoids. There are lymphatic glands which contain bile pigments and lymphocytes. The liver cells are polygonal in shape and contain a prominent nucleus which possesses densely stained nucleoli. The binding together of liver cells is brought about by the connective tissue (Plate 1).

After acute and chronic exposure to $CdCl_2$, liver of *H. fossilis* becomes more fragile and darker in colour but no tumour like out growth is seen anywhere in the liver. Histologically, following the exposure to $CdCl_2$, hypertrophy of hepatic cells has been observed. Polygonal shape of the hepatic cells is completely lost at various places. Hepatocytes are found scattered in the hepatic tissue, vacuolization and

pycnotic changes have been observed. Dilation of extra cellular spaces, bile canaticuli has also been noticed.

Histopathological changes were much more remarkable after chronic exposure to $CdCl_2$ as higher degree of atrophy has been noticed. Principal histological changes are necrosis hypertrophy, shrinkage in various hepatic cells and complete deformation of polygonal shape of the hepatic cells. Progressive splitting in tissues and clumping of cells were important pathological lessions. Nuclei have also been noticed acentric.

In the liver of the fish exposed to $CdCl_2$ at 25 mg/l, histological changes included ruptured nucleus, increased kupffer cell, ruptured hepatic tissue, cellular necrosis and increased pycnotic nucleus. Cd at 50 mg/l concentration induced highly distinct changes such as cellular necrosis, ruptured hepatic tissue, ruptured nucleus and focal necrosis in the liver of fish. Changes became more pronounced when the concentration of Cd was 75 mg/l. The changes included focal necrosis, increased pycnotic nucleus, cellular necrosis and ruptured hepatic tissue (PLATE 1).

**KIDNEY**

Like other fishes practically no clear cut distinction of cortical and medullary regions exists in the kidney of *H. fossilis*. The kidney is divided in two portions, the head kidney and trunk kidney. Externally the kidney is lined by peritoneal layer and internally it consists of glomeruli, uriniferous tubules, Bowmen's capsule and psedolymphoidal tissues. The malpigion body is composed of capillary network. The

head kidney is made-up of lymphoid, haemopoietic inter renal tissue. The uriniferous tubules are lined with nucleated epithelial cells.

Histological studies revealed that the kidney section of control group showed normal histoarchiteceture.

In 30 days $CdCl_2$ treated group fishes kidney exhibited the degeneration of tubules and necrotic condition. The cells of renal tubules were fused and cytoplasm condensed in the central region. Renal tubules lost original appearance and degeneration of cytoplasm leading to wide spaces. The hypertrophy of glomerulus exhibited. The glomerulus in the Bowman's capsule exhibited shrunken or clumped condition and leading to wide space in the capsule. The deshaped glomerulus were also exhibited .The pyknotic nuclei in haemopoietic tissue were visible. The cytoplasm is not evenly distributed and it was shrunkened from periphery of the cells to load of $CdCl_2$. Eccentric and pyknotic condition of nuclei exhibiting in almost all cells (PLATE 2).

In 60 days duration $CdCl_2$ treatment the renal tubules undergo degeneration. The cellular structure becomes hypertrophied. The cells of renal tubules exhibited vacuolated condition, due to clumped cytoplasm in the central region. It gives blurred appearance. The cell boundaries were disturbed and become indistinct. Glomeruli in the Bowman's capsule were had only thick mass. Due to this empty Bowman's capsule were visible. The haemopoietic cells were in vacuolated stage (PLATE 2). Duration sever condition were visible. The renal tubules show wide spaces due to

fusion of cells and clumped cytoplasm. The cytoplasm appears atrophied and granular texture. At places the oedema was visible in the renal tubules. The glomerulus exhibited atrophied condition. The atrophied and hemorrhagic condition was remarkable tissues. All the cellular structure including haemopoietic tissues were present in necrotic configuration due to loss of cytoplasm. Severe degeneration was seen in haemopoietic tissue.

## 4. BIOCHEMICAL CHANGES

The variation in different organic reserves in different tissues of *H. fossilis* after the exposure of CdCl2 during 30 days and 60 days at sub lethal concentration were observed. These changes are as follows.

### GLUCOSE

The glucose content level in muscle, gill, liver, heart and kidney were significantly ($P<0.05$, $P<0.001$) increased after the 30 days and 60 days exposure to $CdCl_2$. The glucose level varied from 9.16 mg/gm to 12.32mg/gm in muscle in gills and liver it changed from 2.84mg/gm to 5.26 mg/gm and 11.34mg/gm to 13.64mg/gm. In heart and kidney glucose level ranges from 5.97mg/gm to 8.25mg/gm and 7.51mg/gm to 9.42mg/gm, respectively, after the of different sub lethal concentrations of $CdCl_2$ for 30 days (Table 4 and Fig 1).

The glucose level increased from 9.16mg/gm to 14.34mg/gm in muscle, 2.84mg/gm to 7.21mg/gm, in gills, 11.34mg/gm 15.04mg/gm in liver 5.97mg/gm to

9.11mg/gm in heart and from 7.50mg/gm to 10.36mg/gm in kidney after 60 days exposure at 75mg/l of CdCl2 (Table 5 and Fig 2).The maximum 13.64mg/gm and 15.04mg/gm glucose level found in liver at 75mg/l of 30 days and 60 days exposure.

**GLYCOGEN**

The glycogen level in muscle, gill, liver, heart & kidney were significantly ($P<0.05$, $P<0.001$) decreased after 30days and 60 days exposure to CdCl2. The glycogen level decreased from 7.81mg/gm to 7.12mg/gm in muscle, 5.43mg/gm to 4.26mg/gm in gill, 16.82mg/gm to 11.79mg/gm in liver , 3.51mg/gm to 2.68 mg/gm in heart and 6.87mg gm to 5.87mg/gm in kidney after 30 days exposure of at 75mg/gm CdCl2(Table 6 and Fig 3).

After 60 days exposure at 75mg/l CdCl2 the glycogen level decreased from 7.81mg/gm to 6.87mg/gm in muscles, 5.43mg/gm to 3.52mg/gm in gill, 16.82mg/gm to 10.93mg/gm in liver , 3.51mg/gm to 2.07mg/gm in heart and 6.87mg/gm to 5.42mg/gm in kidney (Table 7 and Fig 4).The minimum glycogen content were observed in heart after the 30 and 60 days exposure of 75mg/l CdCl2.

**TOTAL PROTEIN**

Fish are the important component in our diet and rich source of protein. Fishes contains good quality, balanced and digestible protein. Protein content in fish meat varied between 16 and 21%. In fish's two types of muscles are present i.e. dark muscles (red) and white muscles. Dark muscles contain low level of moisture and

protein than the white muscle. Proteins are classified on the basis of shape, solubility and chemical structure. On the basis of solubility in salt solution, Proteins are of three groups' Sarcoplasmic protein, Myofibrillar protein and Stroma. Sarcoplasmic protein (Albumin and Globulin) constitutes 25-30% of protein. Myofibrillar protein (myosin, actin, tropomyosin, troponin) are structural protein constitute 65-70 % of total protein. Stroma proteins are also known as connective tissue protein. It constitutes 3% of the total protein. Proteins are complex organic compounds and made up of Prolong chain of amino acid bounded together by peptide bonds. Fresh fish meat provides good source of protein for human diet, about 90-95% of fish protein is assimilated by human. In this experiment, we need to know how much protein is present in our sample or to measure the concentration or amount of protein in fish muscle samples.

The total protein level in muscles gill, lever, heart and kidney were significantly ($P<0.05$, $P<0.001$) decreased after 30 and 60 days exposure to CdCl2.

The total protein level were observed in decreasing manner from 172.6mg/gm to 132.9mg/gm in muscles, 58.1mg/gm to 37.9mg/gm 91.2mg/gm to 47.7mg/gm in heart and 126.2mg/gm to 107.2mg in kidney after 30 days exposure of 75mg/l CdCl2 (Table 8 and Fig 5).

After the 60days exposure of 75mg/l CdCl2 the protein level were observed from 172.6mg/gm 121.6mg/gm in muscles, 58.1mg/gm to 31.2mg/gm in gill, 91.2mg/gm to 41.6mg/gm in liver, 16.1mg/gm to 8.9mg/gm in heart and 126.2mg/gm to 101.8mg/gm in kidney (Table 9 and Fig 6).

The maximum decrease 10.7mg/gm to 8.9mg/gm of total protein level was found in heart after 30 days and 60 days exposure and the minimum decrease of total protein 132.9mg/l and 121.6mg/l was found in muscles.

**TOTAL LIPID**

The total lipid level in muscle, gill liver, heart and kidney were significantly ($P<0.05$, $P<0.001$) decreased after 30 days and 60 days exposure to CdCl2.

The value of total lipid were decreased from 8.62mg/gm to 5.41mg/gm in muscle, 9.51mg/gm to 6.40mg/gm in gills,71.28mg/gm to 39.32mg/gm in liver, 48.80mg/gm to 30.18mg/gm in heart and 8.72mg/gm to 3.98mg/gm in kidney after the 30days exposure at 75mg/l $CdCl_2$ (Table 10 and Fig 7).

The value of total lipid were decreased after the 60days exposure at 25mg/l from 8.62mg/gm to 4.62mg/gm in muscle, 9.5mg/gm to 6.19mg/gm in gill 71.28mg/gm to 33.68mg/gm in liver, 48.80mg/gm to 28.18mg/gm in heart and 8.72mg/gm 4.82mg/gm in kidney(Table 11and Fig 8).The maximum decrease 3.98mg/gm was observed in kidney after 60days exposure at 75mg/l and the minimum decrease.

**CHOLESTEROL**

The cholesterol level in muscle, liver, and heart were significantly increased ($P<0.05$ and $P<0.001$) after 30 and 60 days exposure to CdCl2.

The value of cholesterol increases in muscle, liver and heart tissues. It found from 6.61mg/gm to 8.08mg/gm in muscle, 21.12mg/gm to 25.85mg/gm in liver and 3.40 mg/gm to 4.82 mg/gm in heart after the 30days exposure at 75mg/l $CdCl_2$ (Table 12 and Fig 9) .Whereas after 60 days exposure of $CdCl_2$ the values increases from 6.61mg/gm to 8.22 mg/gm in muscle, 21.12mg/gm to 26.07mg/gm in liver and 3.40 mg/gm to 5.04 mg/gm in heart. (Table 13 and Fig 10)

The value of cholesterol decreases in gill and kidney tissues. It observed from 1.82 mg/gm to 0.98 mg/gm in gills and 33.36 mg/gm to 28.64 mg/gm in kidney after the 30days exposure at 75mg/l $CdCl_2$. After 60 days exposure this values decreases from 1.82 mg/gm to 0.86 mg/gm in gills and 33.36 mg/gm to 27.24 mg/gm in kidney tissues.

**TABLE 1: Morphological changes of freshwater fish *Heteropneustes fossilis* of Cadmium Chloride exposure.** .

| S.No. | Morphological changes | Control | $CdCl_2$ |
|---|---|---|---|
| 1. | Discoloration of skin | - | ++++ |
| 2. | Lesions on skin | - | ++++ |
| 3. | Shedding of scale | - | ++++ |
| 4. | Mucus secretion | - | ++++ |
| 5. | Sedimentation of chemical on body | - | ++++ |
| 6. | Muscular bleeding | - | ++++ |
| 7. | Clumping of gills | - | ++++ |
| 8. | All fins damage | - | ++++ |

**C-Control, E-Exposed (Nil); + (Less); ++ (Moderate); +++ (Prominent)**

**TABLE 2: Effects of Cadmium Chloride exposure on behavioral pattern of *Heteropneustes fossilis***

| Duration of exposure | Loss of balance | | Surfacing | | Circling | | Mucous secretion | | Hyperactivity | | Schooloing | | Tail wagging | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | E | C | E | C | E | C | E | C | E | C | E | C | E |
| 30 days | - | ++ | + | +++ | - | + | - | ++ | - | +++ | +++ | + | + | ++ |
| 60 days | - | +++ | + | ++ | - | ++ | - | +++ | - | +++ | +++ | + | + | +++ |

**C-Control, E-Exposed (Nil); + (Less); ++ (Moderate); +++ (Prominent)**

**TABLE 3: Opercular beats/minute of *Heteropneustes fossilis* after acute exposure of Cadmium chloride**

| Exposure Duration (days) | Control | Exposed |
|---|---|---|
| 30 | 62 ± 3.78 | 87 ± 3.64** |
| 60 | 75 ± 3.87 | 102 ± 4.19*** |

** denotes differences in means to be significant at P<0.001.
*** denotes differences in means to be significant at P<0.0001.

**TABLE 4: Effect of Cadmium Chloride on glucose (mg/gm wet tissue) of *Heteropneustes fossilis* after 30 days exposure.**

| ORGAN | CONTROL | 25mg/l | 50mg/l | 75mg/l |
|---|---|---|---|---|
| **MUSCLES** | 9.16 ± 0.54 | 9.30± 0.52 | 10.10 ±0.31* | 12.32 ± 0.77** |
| **GILLS** | 2.84 ± 0.54 | 3.21 ± 0.51 | 4.19 ± 0.14* | 5.26 ±0.12** |
| **LIVER** | 11.34 ± 0.44 | 12.04 ± 0.43 | 12.41 ±0.58* | 13.64 ± 0.51** |
| **HEART** | 5.97 ±0.38 | 6.45 ± 0.30 | 7.64 ± 0.72* | 8.25 ±0.35** |
| **KIDNEY** | 7.51 ± 0.32 | 7.92 ± 0.31 | 8.13 ±0.18* | 9.42 ±0.31** |

*Significant at P<0.05level, ** Significant at P<0.001 level

**TABLE 5: Effect of Cadmium Chloride on glucose(mg/gm wet tissue) of *Heteropneustes fossilis* after 60 days exposure.**

| ORGAN | CONTROL | 25mg/l | 50mg/l | 75mg/l |
|---|---|---|---|---|
| **MUSCLES** | 9.16 ± 0.54 | 9.83± 0.54 | 11.44 ±1.84* | 14.34± 1.17** |
| **GILLS** | 2.84 ± 0.54 | 3.67 ± 0.31 | 4.55 ± 0.81* | 7.21 ±0.88** |
| **LIVER** | 11.34 ± 0.44 | 12.62 ± 0.64 | 13.51 ±0.62* | 15.04 ± 0.86** |
| **HEART** | 5.97 ±0.38 | 7.05 ± 0.32 | 8.09 ± 0.82* | 9.11 ±0.35** |
| **KIDNEY** | 7.51 ±0.32 | 8.53±0.42 | 9.12 ±0.91* | 10.36 ±0.36** |

**TABLE 6: Effect of Cadmium Chloride on glycogen (mg/gm wet tissue) of *Heteropneustes fossilis* after 30 days exposure.**

| ORGAN | CONTROL | 25mg/l | 50mg/l | 75mg/l |
|---|---|---|---|---|
| **MUSCLES** | 7.81 ± 0.13 | 7.72 ± 0.12 | 7.48 ± 0.33 | 7.12 ± 0.32* |
| **GILLS** | 5.43 ± 0.44 | 5.18 ± 0.95 | 4.83 ± 0.31 | 4.26 ± 0.33* |
| **LIVER** | 16.82± 1.13 | 15.91 ± 0.14 | 13.84 ± 0.14 | 11.79 ± 0.21* |
| **HEART** | 3.51 ± 0.42 | 3.26 ± 0.11 | 3.03 ± 0.37* | 2.68 ± 0.30* |
| **KIDNEY** | 6.87 ± 0.32 | 6.69 ± 0.13 | 6.01 ± 1.11 | 5.87 ± 0.12* |

*Significant at P<0.05level, ** Significant at P<0.001 level

**TABLE 7: Effect of Cadmium Chloride on glycogen (mg/gm wet tissue) of *Heteropneustes fossilis* after 60 days exposure**

| ORGAN | CONTROL | 25mg/l | 50mg/l | 75mg/l |
|---|---|---|---|---|
| **MUSCLES** | 7.81 ± 0.13 | 7.68 ± 0.31 | 7.23 ± 0.27 * | 6.87 ± 0.32** |
| **GILLS** | 5.43 ± 0.44 | 5.01± 0.51* | 3.97± 0.41** | 3.52± 0.30** |
| **LIVER** | 16.82± 1.13 | 14.93 ± 0.72 | 12.13 ± 0.84* | 10.93 ± 0.31** |
| **HEART** | 3.51 ± 0.42 | 3.18 ± 0.33 | 2.56 ± 0.33* | 2.07 ± 0.37** |
| **KIDNEY** | 6.87 ± 0.32 | 6.57 ± 0.61 | 5.93 ± 0.81* | 5.42 ± 0.12* |

*Significant at P<0.05level, ** Significant at P<0.001 level

**TABLE 8: Effect of Cadmium Chloride on Total Protein (mg/gm wet tissue) of *Heteropneustes fossilis* after 30 days exposure.**

| ORGAN | CONTROL | 25mg/l | 50mg/l | 75mg/l |
|---|---|---|---|---|
| **MUSCLES** | 171.6 ± 1.30 | 165.4 ± 1.0 | 151.2 ± 1.11 | 132.9 ± 1.08* |
| **GILLS** | 58.1 ±2.29 | 56.7 ± 1.12 | 51.4 ± 1.15* | 37.9 ± 1.12** |
| **LIVER** | 91.2 ± 1.03 | 82.3 ± 1.02 | 71.4 ± 0.54 | 47.7 ± 0.51** |
| **HEART** | 16.1 ± 1.03 | 12.8 ± 0.59 | 11.2 ± 0.19 | 10.7 ± 0.21* |
| **KIDNEY** | 126.2 ± 1.38 | 120.9 ± 0.21 | 112.7 ±0.17 | 107.2 ± 0.27* |

*Significant at P<0.05level, ** Significant at P<0.001 level

**TABLE 9: Effect of Cadmium Chloride on Total Protein (mg/gm wet tissue) of *Heteropneustes fossilis* after 60 days exposure.**

| ORGAN | CONTROL | 25mg/l | 50mg/l | 75mg/l |
|---|---|---|---|---|
| **MUSCLES** | 171.6 ± 1.30 | 159.3 ± 1.14 | 159.8 ± 5.86* | 121.6 ± 1.11** |
| **GILLS** | 58.1 ±2.29 | 52.3 ± 1.16* | 49.5 ± 1.81* | 31.2 ±0.50** |
| **LIVER** | 91.2 ± 1.03 | 72.8 ± 1.08 | 57.9 ±0.97* | 41.6 ±1.13* |
| **HEART** | 16.1 ± 1.03 | 11.9 ± 0.37 | 10.2 ±0.66 | 8.9 ±1.14** |
| **KIDNEY** | 126.2 ± 1.38 | 114.9 ± 2.45 | 107.3 ±2.97 | 101.8 ± 3.68 |

*Significant at P<0.05level, ** Significant at P<0.001 level

**TABLE 10: Effect of Cadmium Chloride on Total Lipid (mg/gm wet tissue) of *Heteropneustes fossilis* after 30 days exposure**

| ORGAN | CONTROL | 25mg/l | 50mg/l | 75mg/l |
|---|---|---|---|---|
| **MUSCLES** | 8.6 2± 0.32 | 7.81 ± 0.26 | 6.43 ± 0.19 | 5.41 ± 0.31***** |
| **GILLS** | 9.51 ± 0.47 | 8.12 ± 0.30 | 7.23 ± 0.16 | 6.40 ± 0.38***** |
| **LIVER** | 71.28 ± 1.32 | 64.97 ± 0.72 | 51.14 ±1.14 | 39.32 ± 0.13***** |
| **HEART** | 48.80 ± 5.28 | 45.24 ± 0.33 | 41.18 ±1.18 | 31.18 ± 0.97***** |
| **KIDNEY** | 8.72 ± 0.31 | 7.65 ± 0.36 | 5.42 ± 1.17***** | 3.98 ± 0.32***** |

*****Significant at P<0.05level, ****** Significant at P<0.001 level

**TABLE 11: Effect of Cadmium Chloride on Total Lipid (mg/gm wet tissue) of *Heteropneustes fossilis* after 60 days exposure.**

| ORGAN | CONTROL | 25mg/l | 50mg/l | 75mg/l |
|---|---|---|---|---|
| **MUSCLES** | 8.62 ± 0.32 | 6.92 ± 0.31 | 5.23 ± 1.13* | 4.62 ± 0.31** |
| **GILLS** | 9.51 ± 0.47 | 7.92 ± 0.33 | 7.12 ± 1.10* | 6.19 ± 1.02** |
| **LIVER** | 71.28 ± 1.32 | 59.23 ± 1.29 | 43.21 ± 1.31** | 33.68 ± 1.17** |
| **HEART** | 48.80 ± 5.28 | 41.62 ± 4.10 | 34.81 ± 4.27* | 28.18 ± 5.99** |
| **KIDNEY** | 8.72 ± 0.31 | 6.23 ± 0.48 | 5.42 ± 1.12* | 4.82 ± 0.32** |

*Significant at P<0.05level, ** Significant at P<0.001 level

**TABLE 12: Effect of Cadmium Chloride on Cholesterol (mg/gm wet tissue) of *Heteropneustes fossilis* after 30 days exposure.**

| ORGAN | CONTROL | 25mg/l | 50mg/l | 75mg/l |
|---|---|---|---|---|
| **MUSCLES** | 6.61± 0.31 | 7.12± 0.13 | 7.83± 0.18 | 8.08± 0.14***** |
| **GILLS** | 1.82± 0.18 | 1.71± 0.16 | 1.54± 0.17 | 0.98± 0.15***** |
| **LIVER** | 21.12± 1.30 | 22.03± 0.41 | 24.13± 0.32 | 25.85± 0.98***** |
| **HEART** | 3.40± 0.31 | 3.81± 0.32 | 4.08 ± 0.17 | 4.82± 1.18***** |
| **KIDNEY** | 33.36± 1.32 | 32.16± 0.26 | 30.64± 0.15 | 28.64 ± 1.30***** |

*****Significant at P<0.05level, ****** Significant at P<0.001 level

**TABLE 13: Effect of Cadmium Chloride on Cholesterol (mg/gm wet tissue) of *Heteropneustes fossilis* after 60 days exposure.**

| ORGAN | CONTROL | 25mg/l | 50mg/l | 75mg/l |
|---|---|---|---|---|
| **MUSCLES** | 6.61± 0.31 | 7.52± 0.18* | 7.92± 0.31* | 8.22± 0.12** |
| **GILLS** | 1.82± 0.18 | 1.61± 0.04 | 1.03± 0.08* | 0.86± 0.06* |
| **LIVER** | 21.12± 1.30 | 22.83± 1.31 | 24.97± 1.18* | 26.07± 1.02* |
| **HEART** | 3.40± 0.31 | 3.98± 0.28 | 4.26 ± 0.28 | 5.04± 0.41* |
| **KIDNEY** | 33.36± 1.32 | 31.07± 1.38 | 29.12± 1.48 | 27.24 ± 1.38* |

*Significant at P<0.05level, ** Significant at P<0.001 level

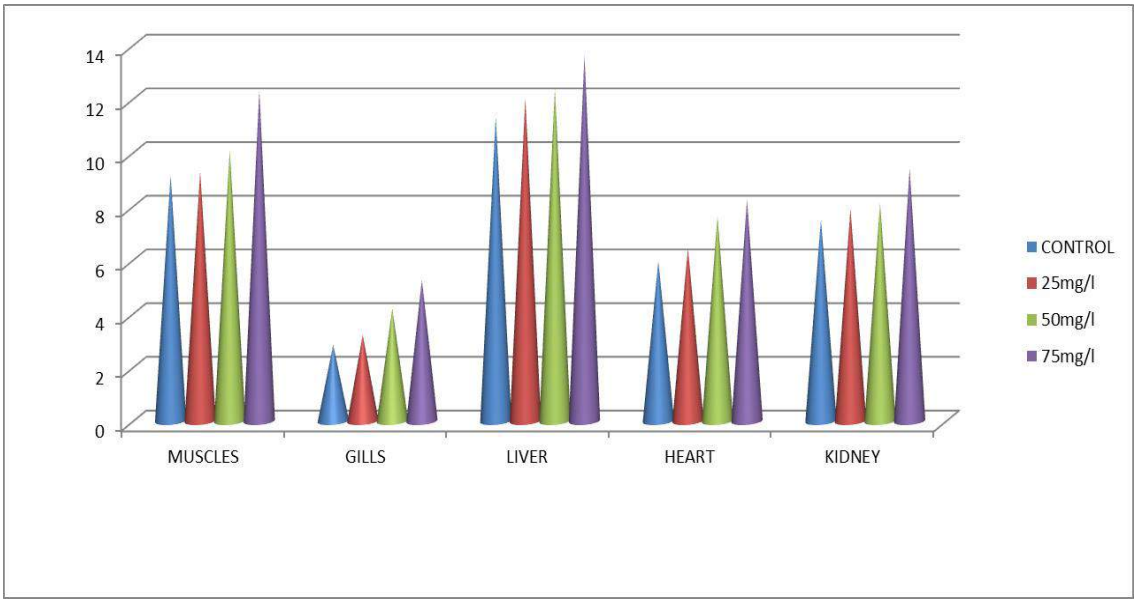



**FIG: 1- Effect of Cadmium Chloride on glucose (mg/gm wet tissue) of *Heteropneustes fossilis* after 30 days exposure.**

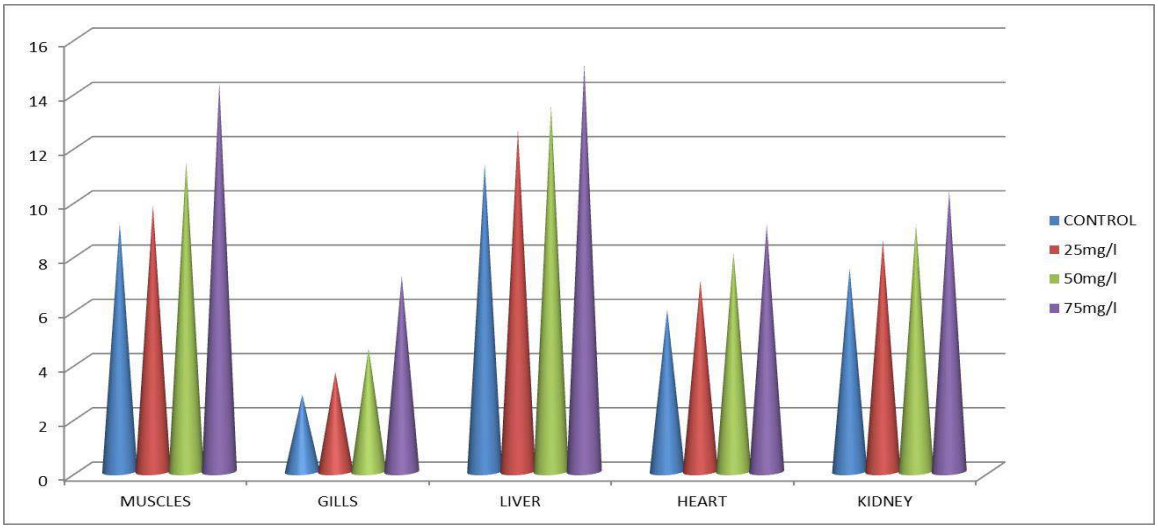



**FIG: 2- Effect of Cadmium Chloride on glucose (mg/gm wet tissue) of *Heteropneustes fossilis* after 60 days exposure.**

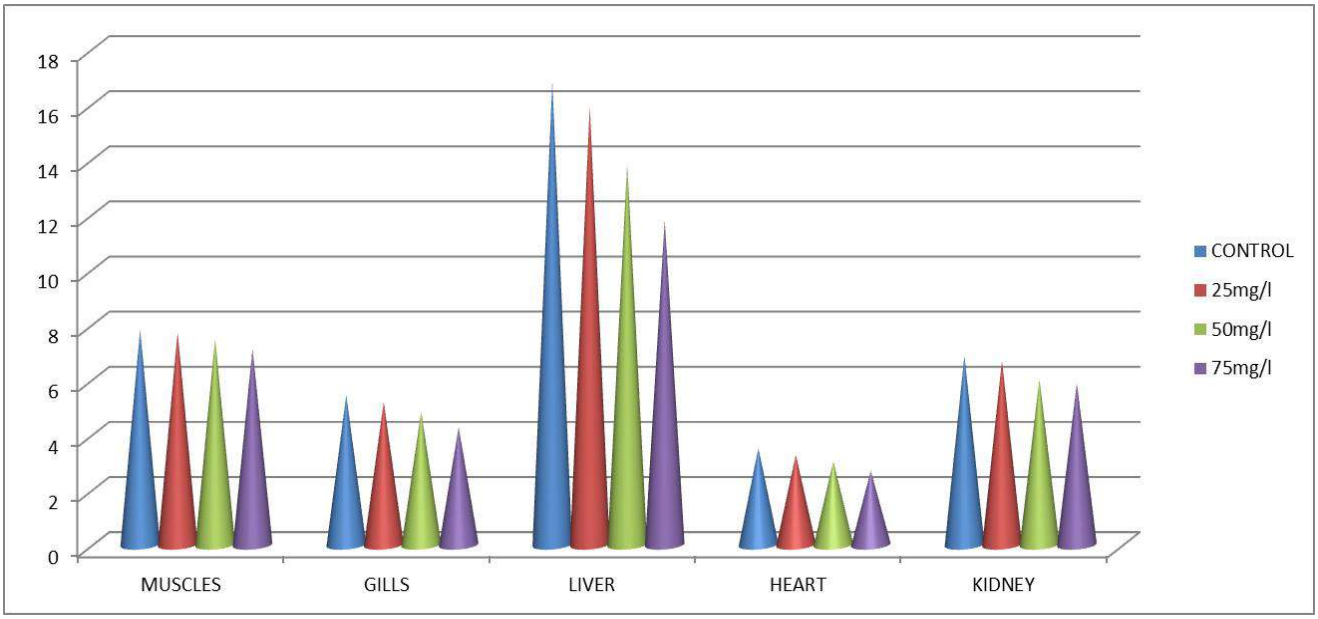



**Fig: 3- Effect of Cadmium Chloride on glycogen (mg/gm wet tissue) of *Heteropneustes fossilis* after 30 days exposure.**

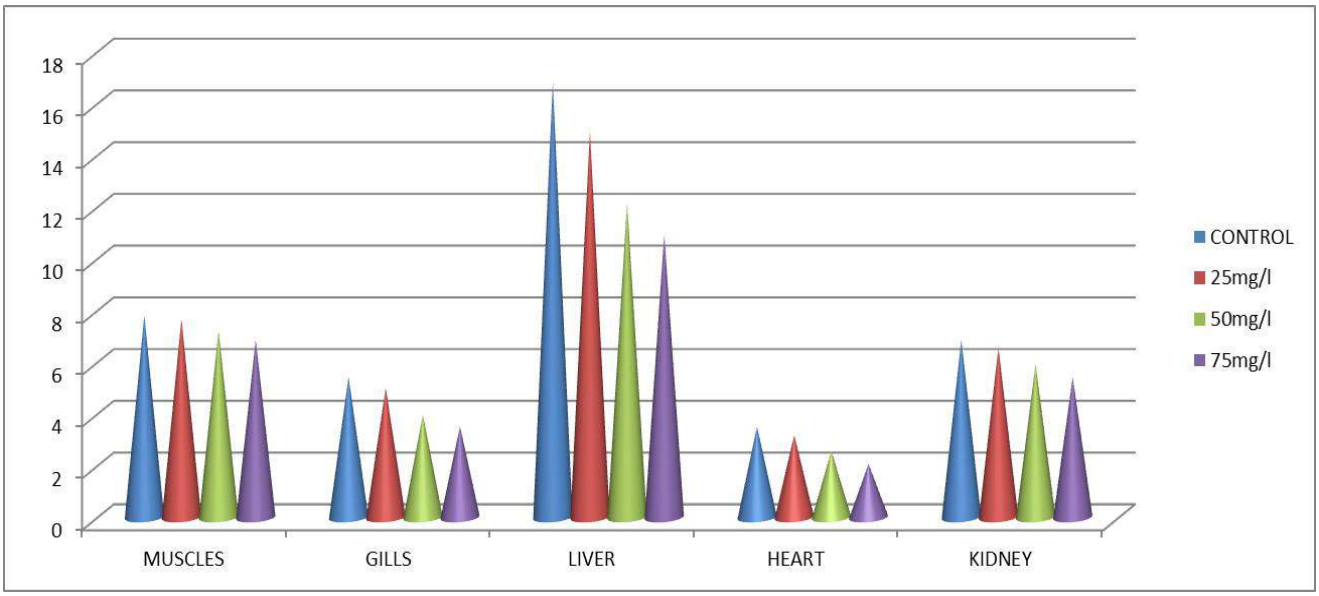



**FIG: 4- Effect of Cadmium Chloride on glycogen (mg/gm wet tissue) of *Heteropneustes fossilis* after 60 days exposure.**

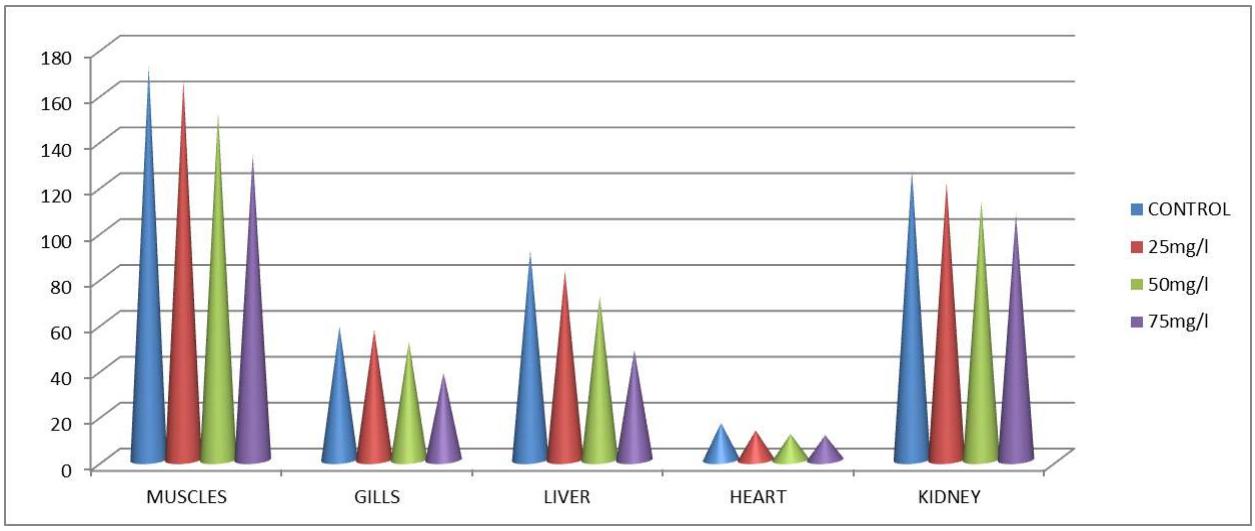



**FIG: 5- Effect of Cadmium Chloride on Total Protein (mg/gm wet tissue) of *Heteropneustes fossilis* after 30 days exposure.**

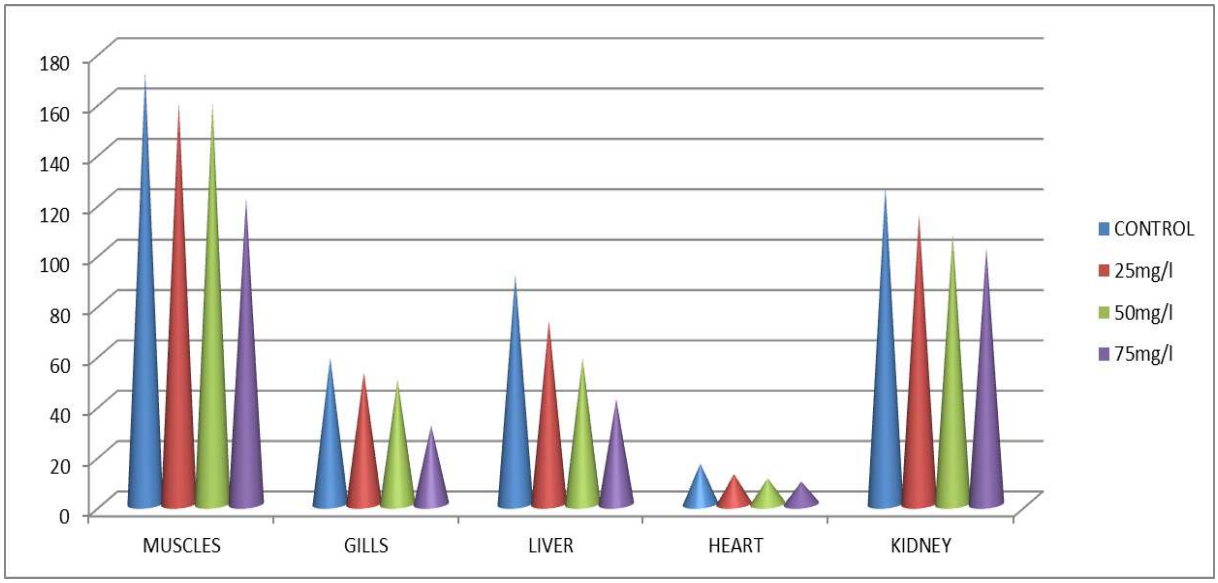



**FIG: 6- Effect of Cadmium Chloride on Total Protein (mg/gm wet tissue) of *Heteropneustes fossilis* after 60 days exposure**

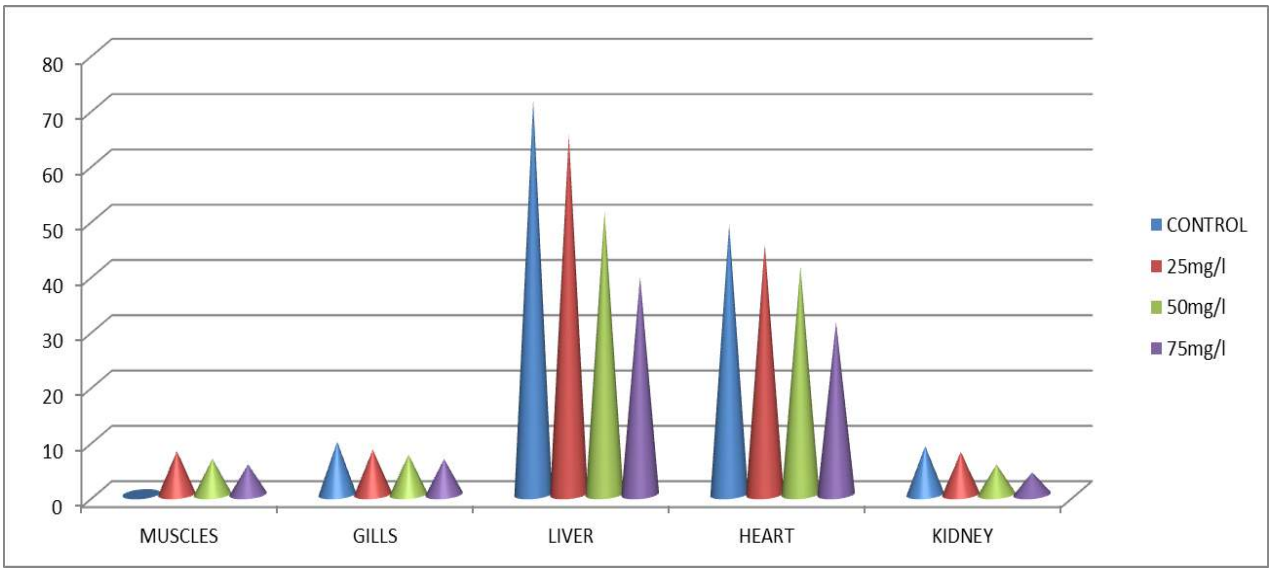



**FIG: 7- Effect of Cadmium Chloride on Total Lipid (mg/gm wet tissue) of *Heteropneustes fossilis* after 30 days exposure.**

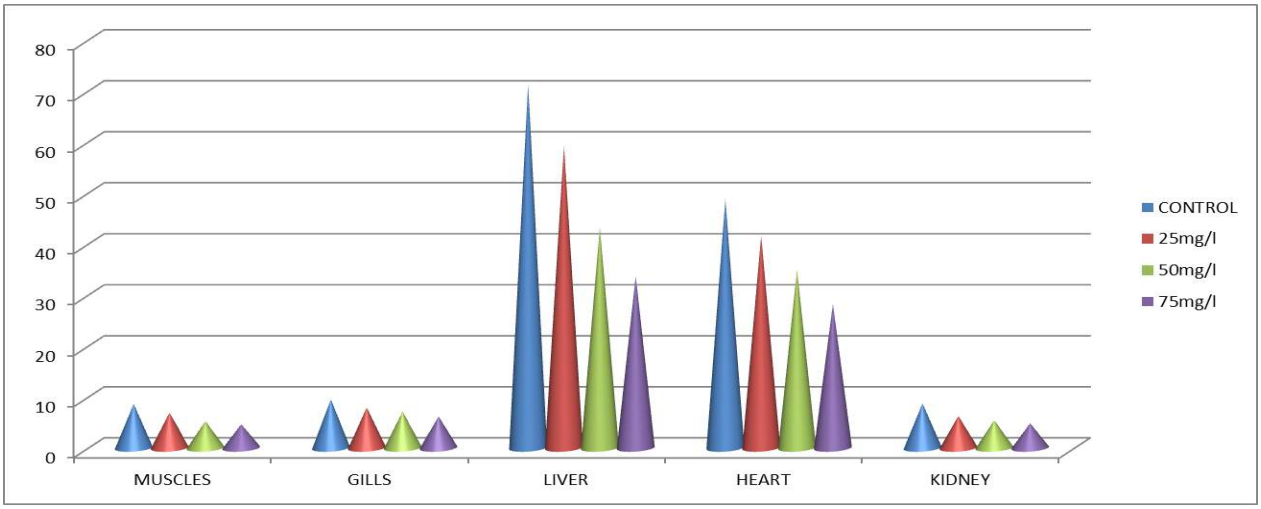



**FIG: 8- Effect of Cadmium Chloride on Total Lipid (mg/gm wet tissue) of *Heteropneustes fossilis* after 60 days exposure.**

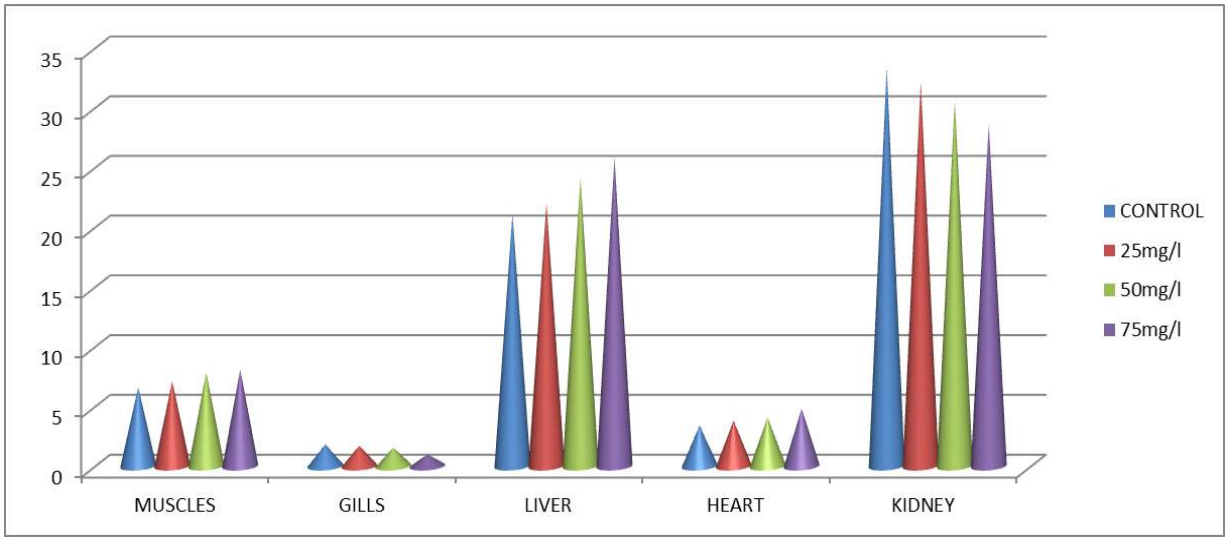



**FIG: 9- Effect of Cadmium Chloride on Cholesterol (mg/gm wet tissue) of *Heteropneustes fossilis* after 30 days exposure.**

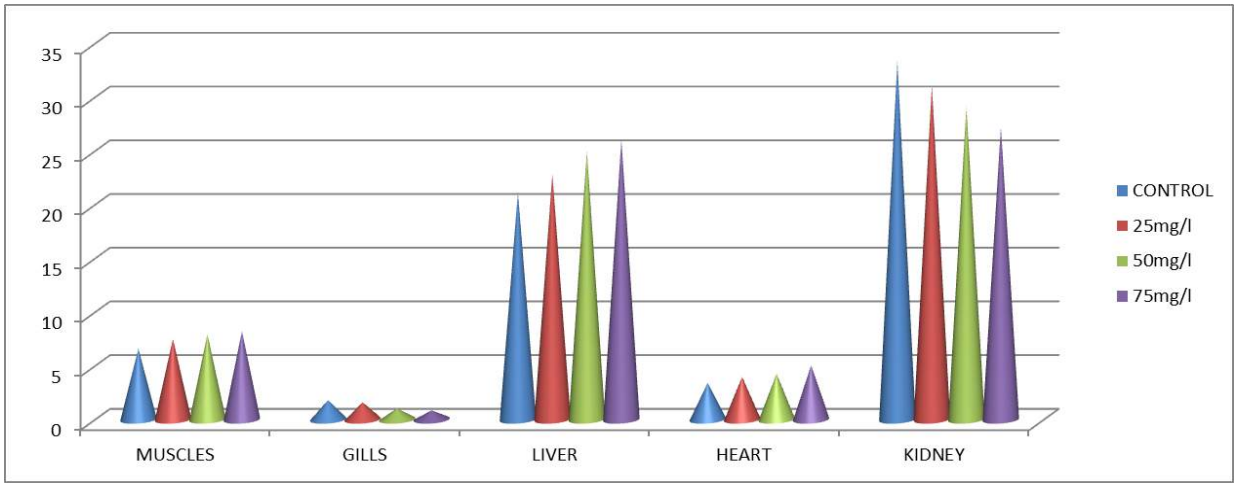
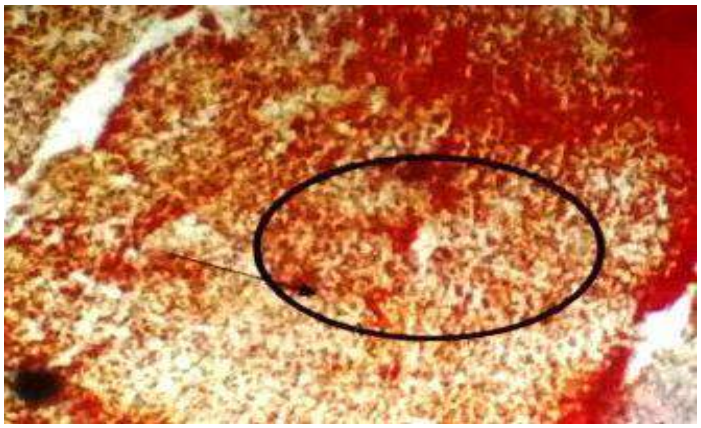
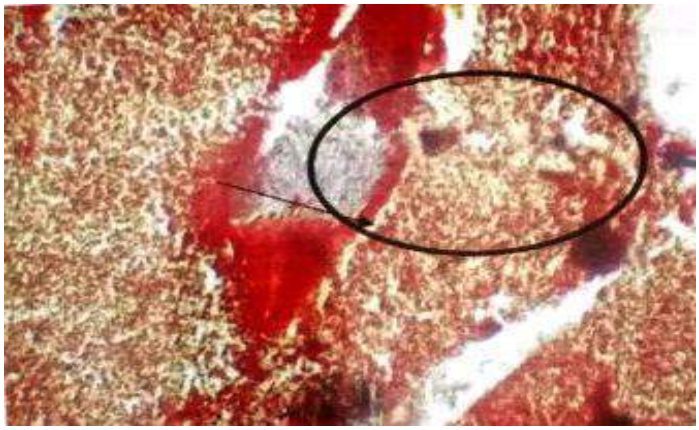


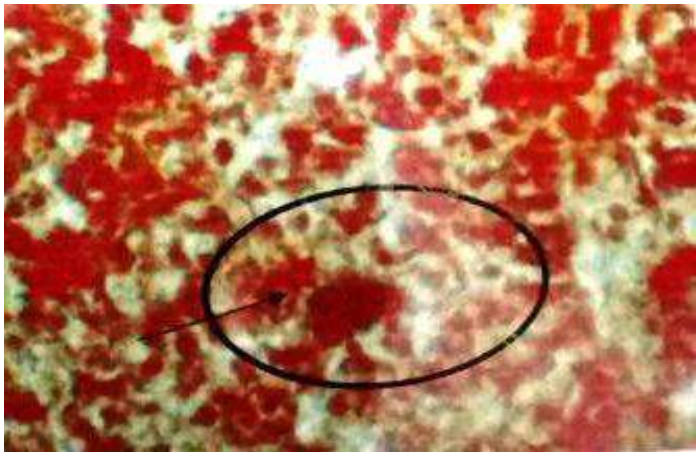

**FIG: 10- Effect of Cadmium Chloride on Cholesterol (mg/gm wet tissue) of *Heteropneustes fossilis* after 60 days exposure**



**Section of Liver of *H. fossilis* control**

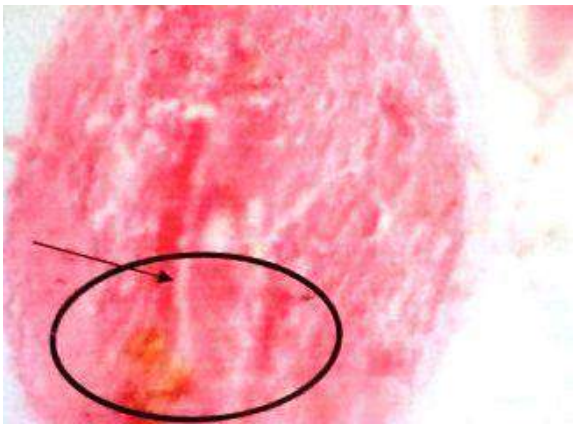


**Section of Liver of *H. fossilis following the* 30 days exposure of $CdCl_2$**



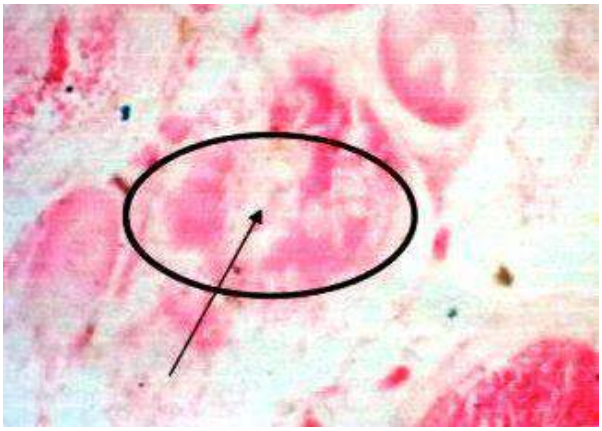
**Section of Liver of *H. fossilis following the* 60 days exposure of $CdCl_2$**

**Plate-1**



**Photomicrograph of section of kidney (control) of *H. fossilis***

**Section of Kidney of H. fossilis following the 60 days exposure of CdCl2**



**Section of Kidney of *H. fossilis following the* 60 days exposure of $CdCl_2$**

# CHAPTER-5

# *DISCUSSION*

## MORPHOLOGY AND BEHAVIOUR

Cd compounds pose toxic effects on the fresh water fish *H. fossilis.* In our study documented fish mortality was due to manifestation of Cd compounds toxicity through absorption, bio-accumulation or greater activity of chemical in animal's body. The differences in the value between 30 to 60 days at 25 mg/l -75mg/l dose of $CdCl_2$ on *H. fossilis* in present study and other earlier reports may be attributed to the fact that metal induced changes fluctuate from metal to metal, species to species and from one experimental stipulation to other (Maruthayanagam et al., 2002, Kasherwani et al., 2009). Similarly the higher concentration of the toxicant may because of adoptive competence of fish to heavy metals polluted aquatic environment (Vinodini and Narayanan, 2008). The difference in toxicity level among Cd compounds might be related to change in sensitivity of chemo receptors (Suterlin, 1974). The exact causes of death due to heavy metal poisoning are multiple and depend mainly on time-concentration combination.

In present study various behavioral alterations were observed. Behavioral abnormalities in various fish species on exposure to heavy metal have been well documented by a number of scientists. After acute exposure of $CdCl_2$, initial

hyperactivity, restlessness, fast opercular beats, increased surfacing, loss of balance, loose schooling, colour fading, skin peeling off, jerky movement, erratic swimming, formation of ulcerative tubercles, haemorrhages on caudal and pectoral fins with heavy deposition of mucus on gill region and whole body surface were noticed. In sub acute exposure abdominal swelling, loss of food detection capacity, feeble response on gentle paddling, high rate of gulping air were noticed in addition to acute toxicity. Almost similar alterations in fish behavior after exposure of various metal compounds were observed by several workers (Masud et al. 2005; Borah and Yadav, 1995; Kalavathy et al., 2001; Sindal et al., 2004). According to Ghatak and Konar (1990) *Tilapia mossambica* when exposed to Cd showed frequent surfacing with irregular opercular movements and loss of equilibrium. Similarly Holcombe et al (1970) reported hyperactivity, erratic swimming and loss of equilibrium in Brook trout, *Salvalinus fontinallis*, in response to lead treatment. The loss of equilibrium, frequent surfacing, sinking, burst of erratic swimming, and gradual onset of inactivity in Rainbow trout, *Salmo gairdneri*, on mercury exposure, have also been determined (MacLeod and Pessah, 1973).

The initial hyperactivity and restlessness of the fish invariably in the lethal and sub lethal exposure may be probably due to hindrance in the functioning of enzyme AChE in relation to nervous system as suggested by many authors (Agarwal and Nair, 1989; Murthy et al., 1986). It leads to accumulation of acetyl choline, which is likely to cause prolonged excited postsynaptic potential. These may first leads to stimulation and later cause a block in the cholinergic system (Prashanth et al., 2005.). The erratic

swimming in Cd exposed fish in the present study indicates loss of equilibrium. It is likely that the area in the brain associated with the maintenance of equilibrium is affected by Cd (Prashanth et al., 2005; Rao and Rao, 1987). Loss of balance is also due to change in blood cortisol and glucose level (Svecevicus, 2005). According to Lewis and Lewis, (1971) *Notiemigonus crysoleucus*, when exposed to Cu (5 ppm) piped at the surface, became restless and finally lost equilibrium. Similar results achieved when *Lepomis macrochiurs* treated with diverse concentration of Cu (Ellgaard and Guillot, 1988). Lethargic response and frequent surfacing along with gulping of air in exposure to Cu (0.25 ppm) were observed in *H. fossilis* (Singh and Reddy, 1990). *Eutroplus maculatus* on exposure to Cu, Hg and selenium showed irregular erratic swimming, frequent surfacing, gulping of air, revolving, and convulsions and faster ventilation with rapid arrhythmic opercular and mouth movements (Veena et al., 1997). Additionally increased opercular movement and decreased locomotary activities as observed in present studies are probably associated to compensate for loss of efficiency in the oxygen uptake by decreasing the physiological oxygen demand and increasing the amount of oxygen passing over the bronchial tissue per unit time (Panigrahi and Mishra, 1978; Menezes and Qasim, 1984). Further, the increased opercular movements and corresponding increase in frequency of surfacing of H. *fossils* in the present study clearly indicate that fish must adaptively be shifting towards aerial respiration from aquatic one and trying to avoid contact with the toxicant through gill chamber (Siddiqui and Noori, 2011). Similar finding were also recorded by many workers (Menezes and Qasim, 1984.). The

reduced oxygen consumption may be due to the injury of red blood cells (Ganapati and Alikunhi, 1950). It may be also due to the coagulation of mucus on gills and inhibition of enzyme system of mitochondria and due to severe hypoxia in fish after metal induced stress. *H. fossilis* schooling behavior is based on the senses of their barbels (Jain and Sahai, 1990). Vision is the primary sense involve in the schooling behavior of fish. The disruption of schooling behaviour of exposed fish indicates the loss of the group hydrodynamic effect due to stress and respiratory problem (Devi and Fingerman 1995; Devi, 2003).

Behavioral abnormalities have been attributed to nervous impairment due to blockage of nervous transmission between the nervous system and various effectors sites (Nriagu, 1979, 1981, 1989 and 1996). According to Cearley (1971) the dysfunctions of enzyme may cause paralysis of respiratory muscles and depression of respiratory centre in the medulla oblongata. Disturbances in energy pathways which results in depletion of energy may a cause of lethargy in fish due to toxicity (Ellgaard and Guillot, 1988). Cd having toxic effects on CNS and skin epidermal cells may results in the reduced olfaction and touch response due to degenerative change in barbels which might be a cause for disrupt schooling behaviour of exposed fishes as well as affecting vision, olfactory organs and lateral line system. The toxicants interfere with the functions of the nervous system resulting in loss of coordination and locomotion, instability followed by hyper excitability, tremors and convulsions (Wouters and Van den, 1978). The hyper excitability of fish exposed to Cu (0.75 ppm) probably due to disturbances in acetylcholine esterase activity in the nervous system

(Reddy, et al., 1990) which leads to accumulation of acetylcholine causing prolonged postsynaptic excitability.

Excessive mucus secretion on gill region and general body surface of *H. fossilis* exposed to Cd toxicity may be an adaptive response perhaps providing additional protection against corrosive nature of the toxicant and to avoid the absorption of toxicant by the general body surface. This is in agreement with the earlier findings (Das and Mukherjee, 2003; Yilmaz, 2004; Prashanth et al., 2005). Behavioural changes induced by many toxicants resulted in a series of adverse symptoms likely hyperactivity, tremors, ataxia, convulsions, eventually paralysis and death observed by several workers (Muniyan and Veeraraghavan, 1999, Mushigeri and David, 2004). Basically the toxicant in natural environment mostly trapped or sticked with mucus coating fish body, made up of by glycoprotein barrier (Coombs et al., 1972; Lock and Van Overbeeke, 1981). The skin ulceration, colour fading and haemorrhages in present studies were observed after Cd exposure. It is suggested that a vascular damage in the dermis possibly affected the blood supply and cause ischemia, leading to degenerative changes in the epidermis. The ulceration and haemorrhages developed in experimentally stressed fish are suggestive that a very severe perturbation of homeostasis is needed for this response. It may believe that the cytoplasm of necrotic cells at the inability cells to control the influx of water and from outflow of cellular protein (Frenkel et al., 1999). While both necrosis and apoptosis appeared to play a role in epidermal loss with ulceration.

Present study indicates that various behavioral parameters are highly sensitive to heavy metals like Cd and provide information of toxicity. After proper standardization behavioral parameters can serve as better biomarkers than other physical and biochemical parameters.

## HISTOPATHOLOGY

The toxicity effect of heavy metals on liver bas been studied by several workers. The effects of acute Cd on the liver of *H. fossilis* is in conformity to other similar kind of studies (Naigaga, 2002; Nasiruddin et al., 2009; Bais and Lokhande, 2012; Sharma et al., 2013; Selvanathan et al., 2013). Histological alterations like degeneration of hepatocytes, vacuolization, congestion of hepatic tissues, subcapsular vacuolization, necrosis, indistinct cell boundaries and pyknotic nuclei were observed in the liver of the catfish, *Clarias batrachus* exposed to Cd (Selvanathan et al, 2013). Degenerative changes like hepatocellualar dissociation, necrosis and hypertrophy were observed in the freshwater fish, *Ophiocephalus striatus* exposed to $CdCl_2$ (Bais and Lokhande, 2012). Mathur (1962) has been reported the several degenerative changes in the liver of *Ophiocephalus punctatus, H. fossilis, Trichociaster fasciatus* and *Barbus Stigma* due to chemicals treatment. Chemicals like DDT can induce notable histological changes in liver, and intestine also described. Elezaby et al., (2001) studied hemorrhage, necrosis and lipidosis in the liver *Oreochromis niloticus* and *Clarias gariepinus due* to malathion and organophosphorus insecticide (Hostathion) toxicity. The fresh water fish *Anabas testudines* when exposed to paper mill effluents similar

histopathological changes were reported by Nanda and Panigrahi (2004). Gardner and La Roche (1973) have reported a significant damage due to Cu in hepatic cells. Sastry (1977) observed toxic effects of methyl parathion in the liver of *H. fossilis*. He had reported anomalies like cytoplasmic ascuolation necrosis, hypeilrophy of hepatic cells. They found liver necrosis and pycnosis of hepatocytes of liver due to chronic exposure to thiodon. Also in this study, the sequential manifestation of lesion in the order of hepatic vacuolar degeneration, fatty degeneration and necrosis indicates a steady raise in liver damage with duration and dose Cd concentration. This suggests the liver hyperfunction to initial liver lesion formed due to vacuolar degeneration and attributed liver hypofunction to fatty degeneration and early stages of necrosis which could be related to the damage to cellular organelles like mitochondria (Metcalfe, 1998). The presence of macrophage aggregates in the liver is a generalized non-specific marker of environmental stress (Metcalfe, 1998). In addition initial lesion in the liver during the present study might be due to physiological changes that took place in the liver tissue in the process of trying to homeostatistically regulating and detoxifying the metal during continuous exposure as suggested by Naigaga (2002). Our present observation on histological alterations on liver is in conformity with observations made in similar work carried out with different toxicants on various fish species (Ikram and Malik, 2009; Pantung et al., 2008; Thophon, 2003).

Histopathological alterations result depending upon the type of metal and concentrations, length of exposure, species of fish, and additional physico-chemical factors. Degeneration in the epithelial cells of renal tubules, pycnotic nuclei in the

hematopietictissue, dilation of glomerular caplillaries, degeneration of glomerulus, intra cytoplasmic vacuoles in the epithelial cells with tubular lumen were observed in the kidney tissues of fish exposed to diltamethrin (Cengiz,2006).Valmurugan, et al., (2007) reported pycnotic nuclei in tubular epithelium, hypertrophied epithelial cells of renal tubules, contraction of glomerulus and expension of space inside the Bowman's capsule in the kidney of *Cirrhinus mrigala* exposed to monocrotophos.

The acidic pH of water magnifies the toxic effect of certain environmental pollutants including heavy metals (Dey et al., 2001). The alterations of the liver parenchyma, such as vacuolation and necrosis are often associated with acid water (Myers et al., 1997). The findings of the present study are in accordance with above studies since we have also noted the same changes in liver of fish exposed to $CdCl_2$. Vacuoles in the cytoplasm of the hepatocytes can contain lipids and glycogen, which are related to the normal metabolic function of the liver (Camargo and Martinez, 2007; Pacheco and Santos, 2002)**.** Further vacuolation of the hepatocytes is as a signal of degenerative process that bring about metabolic damage on exposure to contaminated water (Camargo and Martinez, 2007; Pacheco and Santos, 2002). Similar histopathological alterations with both Cu and Cd were observed in liver of many teleostean fishes **(**Ibrahim and Mahmoud, 2005; Tayel et al., 2008).

In the present study, it was observed that the severity of the lesion was metal specific and pH dependent. The liver of fish exposed to Cd for 60 days at different pH exhibited several histological alterations like degradation of hepatocytes, distended

sinusoids with pyknotic nuclei, development of vacuoles in cell cytoplasm and necrosis of hepatic tissue. The magnitude of changes differed in proportion to the concentrations of two different metals and variation in water pH, probably due to the synergistic effect of metal and pH. Similar results have been reported in *Clarias batrachus, Cyprinus carpio, Oreochromis mossambicus* and *Oreochromis niloticus* exposed to copper sulfate and $CdCl_2$ (Rani and Ramamurthi, 1889; Vanranka et al. 2001; Figueiredo-Fernandes, 2007; Bilal , 2011). Numerous organic compounds induce toxicopathic lesions in the liver of fishes. The acute toxic injury usually includes cloudy swelling or hydropic degenerations and pyknosis, karyorrhexis and karyolysis of nuclei (Hawkes 1980**;** Hinton and Lauren 1990; Visoottviseth et al., 1999; Jiraungkoorskul et al., 2003).

Several studies used histological characteristic of kidney as an indicator of pollution. Histology of kidney of control fishes showed normal type of cells (Plate 2). Histological studies revealed that the kidney sections from control fishes showed normal histoarchitecture. Kidney is characterized by well-built haemopoietic tissue, uriniferous tubule and glomerulus with clear Bowman's capsule. In the present work, histological changes in the kidney after exposure to Cd at 25 mg/l (30 days) were vacuolation, increased periglomerular space, shrunken glomerulus . Some distinct changes like melanomacrophages, increased periglomerular space, vacuolation, increased peritubular space and shrunken glomerulus were observed in the kidney of the fishes treated with Cd at 50 mg/l. The changes like melanomacrophages, increased peritubular space and periglomerular space and loss of cytoplasm were observed in

fishes exposed to 75 mg/l Cd. Almost similar kind of observations was made in *Channa punctatus (*Arulvasu et al., 2010), *Cirrhinus mrigala* (Prabhahar et al. 2012), *Labeo rohita* (Ikram and Malik 2009), Hybrid walking catfish (*Clarias macrocephalus and C. gariepinus*) (Pantung et al., 2008) and *Lates calcarifer* (Thophon, 2003). Chronic Cd exposure in the freshwater fish *Colosoma macropomum* produced an anomalous head kidney structure and induced an inflammatory process in this organ, affecting haematopoietic cell differentiation, especially with regard to granulocytes and perhaps affecting its functions (Salazar-Lugo et al., 2013).

In this study histological section of liver of control fish (acclimatized under laboratory condition) showed the normal hepatocytes and exhibits a homogenous cytoplasm around the spherical nucleus. The histological alterations in the liver of *H. fossilis* induced by Cd revealed vacuolated hepatocytes in diffused manner, probably due to glycogen infiltration, and the necrosis as common characteristic.

**BIOCHEMISTRY**

Freshwater fish have high food value and also are economically important. Biochemical studies on fish tissues such as muscles, gills, liver, heart and kidney have drawn the attention of several researchers, because tissues are the major source of glucose, glycogen, protein, lipid and cholesterol, have a high calorific value (Joshi et al., 1979). Changes in biochemical parameters may occur due to the various physiological factors such as season, food availability, maturation, spawning etc.

Biochemical composition of the fish is subjected to disparities depending on the season were reported in *Sillago sihama* (Shamsan and Ansari, 2010).

In present study we have noted the increased glucose levels in muscles, gills, liver, heart and kidney tissues by the doses of $CdCl_2$. Decreased and increased glucose levels on Cd exposure have been reported in *H. fossilis* and *Labeo rohita*, respectively (Das and Banerjee, 1980). The varying levels of blood glucose are indicative of abnormal carbohydrate metabolism and possibly the result of impaired hormonal control (Andersson et al., 1988). Heavy metal exposure alter carbohydrate metabolism in fish (*Cyprinion watsoni)* and thus resulted in the depletion of energy. This depletion of energy might be the cause of behavioural impairments as we noted in our experimental fish, *H. fossilis*. Unquestionably the generally results of the present study are in conformity with the findings of the above researchers.

On global scale, fish and fish products are the most important source of protein in the human diet. The fish proteins are of relatively high digestibility when compared to other animal protein source. The protein content in the body of the fish changes depending on the stages of maturity of the gonads were reported in *Onchorhynchus nerka* (Brett et al., 1969) and *Salmo gairdneri Richardson* (Weatherly and Gill, 1983). The protein content in the fish population is relatively constant. The positive correlation between fish condition and percentage of protein in the gonads shows that healthy fish are likely to be more successful in breeding. Total protein content was reported by Minimary (1996) in tissues such as gonads and liver of fishes. Seasonal

variation in total protein and glycogen content of ovaries were reported in the fish, *Garra mullya* (Khan and Mehrothra, 1991).The inter-relationship between the liver and gonads were also reported in the fish, *Cyprinus carpio* during different stages of maturity (Sivakami et al., 1986). Seasonal changes and annual variation in the protein, glycogen and cholesterol content in the ovary and testes were studied in *H. fossilis* (Hunge and Baile, 2003); *Puntius amphibious* and *Mystus kelatius* (Minimary, 1996). Studies on the biochemical constituents in relation to the reproductive cycle were reported in fishes like *Mugil cephalus*; *Channa striatus*; *Clarias batrachus etc.* (Das, 1978; Jyotsna, 1995; Sinha and Pal, 1990).

The biochemical composition in the different fish species has been reported in relation to their age, sex, habitat and seasons (Stansby, 1962; Nazrul Islam and Abdul Razaaq Joadder, 2005). Seasonal variations in the biochemical composition of fish were reported in *Mugil cephalus* (Das, 1978; Hoar, 1957) and in *H. fossilis* (Joshi, Gupta and Chaturvedi, 1979). Annual correlative changes were reported in some biochemical contents of testes in the catfish *Clarias batrachus* (Singh and Joy, 1999). Investigations on biochemical composition of different fishes were reported in *Macrognathus aculeatus* (Nabi, 1989) and *Cirrhinus mrigala* (Muslemuddin, 1991). The main source of energy reserves in fish are protein and lipid in three-spined-stickle Back, *Gasterosteus aculeatus* L. (Chellappa, 1988). In rainbow trout, *Onchorhynchus mykiss*, an increased use of hepatic carbohydrates is reported to take place during gonadal maturation (Washburn et al., 1990). Several changes in liver and gonadal

metabolism were observed during the onset of testicular recrudescence in *Oreochromis mossambicus* (Soengas et al., 1993).

Seasonal variation in the biochemical composition of Koi fish *Anabas testudineus* (Bloch) were reported by Nargis (2006). In present study, there was decrease in glycogen content of all the tissues at sublethal and lethal concentrations of Cd in dose-dependent manner. The findings are accordance with several researchers those have shown similar effect due to different pollutants (Maruthi and Rao 2000). This decrease in tissue glycogen may be due to glycolysis for production of energy to overcome toxic effect (Maruthi and Rao 2000). Decrease in glycogen has also been suggested by Shaffi (1978), to explain depletion in glycogen. Similar depletion in glycogen content in this study may be attributed to its utilization to meet high energy demand created by stress of Cd. This might have occurred by speedy glycogenolysis and inhibition of glycogenesis through activation of glycogen phosphorylase and depression of transferase (Jha and Pandey, 1989; Jha and Jha, 1995 a, b). Depletion of the glycogen content in the liver and muscle was also observed by other workers in fish *Mystus cavasius* exposed to electroplating industrial effluent (Palanisamy et al., 2011), and *C. punctatus* exposed to distillery effluent (Maruthi and Subba Rao, 2000). Srivastava and Srivastava (2008) reported that glycogen reserves consistently decreased from in *C. punctatus* when exposed to sublethal concentrations of ZnSO4. Since carbohydrates serve as the instant energy source during stress so during acute condition blood glucose level increases due to glycogenolysis but reduction can be correlated to utilization of stored glycogen to meet up the energy require or chronic

exposure. In liver, glycogen mobilized to glucose whereas in muscle glycogen/glucose served as readily available source of energy.

In present study, there was decrease in protein content of all organs at tested doses. Significant decrease in total protein content of fishes indicates that, stress due to pollutant induces proteolysis. Stress has been reported to accelerate protein metabolism in man and animals (Nichol and Rosen, 1963). Protein decrease may be due to stress in fish as protein is likely to undergo hydrolysis and oxidation through TCA cycle to full fill the augmented requirement for energy caused by the toxicant induced stress (Somnath, 1991). Increase in liver protein may be due to increase in synthesis of detoxification enzymes as suggested by Chitra (1983). The alteration in the tissue protein, in the present study suggests disturbance in the physiological activity of fishes due to Cd toxicity. Electroplating effluent at sublethal and lethal concentration there was decrease in protein content in gill, liver and muscle except kidney (Muley et al., 2007). Additionally textile mill effluent exposure causes a decrease in protein content at both sublethal and lethal concentrations (Muley et al., 2007).

Additionally, in present study, we noted that there was decrease in lipid content of all organs at tested doses. The depletion in the total lipid in liver tissues after the Cd doses could be due to their active mobilization towards the blood and/or tissue metabolism (Murthy et al., 1994). According to Rao and coworkers (1985) this decrease might be due to the utilization of lipid content to meet the additional energy

requirement under stress situation. Toxic substances might have accumulated in the brain of fish, causing disintegration of nerve cells, clotting of blood and reduction in transport of oxygen to brain (Panigrahi and Misra, 1978). Loss of lipids noticed in this study may be due to inhibited lipid synthesis and mobilizing the stored lipid, either through oxidation or through a gradual unsaturation of lipid molecules as suggested by Jha (1991).The observations from the present study showed that, these Cd at sublethal and lethal concentrations altered the biochemical composition (glycogen, protein and lipid) of the various organs of test fish, due to utilization of biochemical energy to counteract the Cd induced stress.

The cholesterol variation in certain fishes was reported by researchers in H. *fossilis* (Singh and Singh, 1979) *Channa punctatus* (Siddiqui, 1966). Seasonal variations in the biochemical composition of ovary and testes were reported in catfish *H. fossilis* (Shreni Kalpana, 1980). The cholesterol being the precursors for the synthesis of steroids which in turn influence the maturation phenomenon were reported in the female pearl spot *Etroplus suratensis* during different maturity stages (Diwan and Krishnan, 1996). The cholesterol dynamics in relation to egg maturation were reported in Garra mullya (Ehsan et al., 1991). Change in tissue cholesterol levels during different reproductive phases were reported in the female freshwater fish *Notopterus notopterus* (Pallas) (Shankar and Kulkarni, 2007). Cholesterol concentration in the serum of Cd exposed fish also showed a different pattern. A study showed a reduction in serum cholesterol within 15-days, possibly due to tissue damage in the kidney (Heydarnejad et al., 2013). On the contrary, in *Oreochromis niloticus*, an

increase in cholesterol was seen during a 21 day period due to Cd (Oner et al 2008). This alteration in cholesterol concentration could be due to hazardous effects of metals on cell membrane. Thus, decrease in tissue cholesterol levels are good indicators of Cd stress in tested fishes. In conclusions, present findings of the present study demonstrate that Cd is highly toxic for the aquatic fauna specially fishes. Cd influences all the aspects of exposed fish *H. fossilis,* in both concentration and time dependent manner. Cd induced toxic effects on *H. fossilis* are reflected in morphological, behavior, histopathology and biochemical parameters. Cd changes the morphological characters, behavior pattern, biochemical parameters in fish as compare to control. The liver and kidney showed the structural changes at cellular level. Our study recommended that Cd is very lethal for aquatic organism so it is necessary to treat the water body and keep free from heavy metals like Cd.

# CAPTER-6

# *SUMMARY*

Among several elements of the periodic table, there are 35 metals are associated with community and occupational exposure. Out of these, 23 are described as heavy metals. These elements are generally released in small amounts into the environment by processes like weathering of rocks, volcanic eruptions etc. and their intake/exposure is necessary in trace amounts for good health. But, presently, there is a steady increase in their concentration in all habitats owing to mining, electroplating, paints and dye, battery making industries etc. The release is rapid with the rapidly growing technology and heavy metal application in these industries. The two most important factors that contribute to the deleterious effects of heavy metals as pollutants are their indestructible nature through bioremediation unlike organic pollutants and their tendency to accumulate in environment especially in the bottom sediments of aquatic habitats in association with organic and inorganic matter. Cadmium (Cd), one of the heavy metal toxicants, is widely used in Ni-Cd batteries manufacture, metal and mining industry, dentistry etc. because of its non-corrosive nature. Cd is released in considerable amounts through industrial effluents into soil, surface and ground water systems. These excess amounts in addition to naturally occurring levels gradually build up to toxic levels causing damage to the biota of the aquatic ecosystem. It shows biomagnifications and has greater half-life periods. Cd

was found to interfere with many protein and carbohydrate metabolisms by inhibiting the enzymes involved in the processes. Toxicity of Cdto fishes has stimulated considerable interest in recent years. Studies have shown that other heavy metals, vitamins, chelating agents and protein diets which also can alter the physiological, biochemical and behavioral aspects is fish. In the present study the effect of Cd on the morphology, behavior, histopathology and biochemical parameters of different tissues of the fresh water fish *Heteropneustes fossilis* were investigated.

The work in the thesis entitled '**Toxicological Effects in Fresh Water Teleost Fish *Hetropneustis fossilis (Bloch)* Induced by Cadmium'** is briefly summarized below and divided into chapters:

## *Chapter 1: 'Introduction'*

The first chapter introduces the thesis in the form of "Introduction" and focuses on the work taken up in the research plan and correlates it with the utility in regard to cadmium toxicity of fish.

## *Chapter 2: 'Literature Review'*

In this chapter the past work on aquatic environment toxicity and cadmium contamination is reviewed. The toxicological impacts of the contaminates including Cd on morphology, behavior, histopathology and biochemical parameters (of different tissues) of the fresh water *H. fossilis* have been defined.

## *Chapter 3: 'Material and Methods'*

This section of thesis includes experimental Techniques and Methods of Analysis along with a brief account of the methods used in the study of morphological, histopathological, behavioral and biochemical parameters. Experimental Techniques and Methods of Analysis", comprises of methodology undertaken for the study incorporating the various techniques adopted for the execution of the research plan.

## *Chapter 4: 'Results'*

Deals with the details of results of morphological, histopathological, behavioral and biochemical changes of *H. fossilis* following the different doses of Cd. Regular observations in fresh water teleost *H. fossilis* for the Cd tocxicity were recorded.

This chapter is fully illustrated, supported with tables, diagrams and photographs which have been appended in the text at appropriate places.

1. **Morphological manifestation**: The schooling is the characteristic of this fish was found weakened in the Cd during study. At higher chemical concentration (75 mg/l), scale depletion start, skin lesion observed from dorsal to lateral side of the body of fish and these were deepens, copious mucous, clumping of gills increases with the increasing of concentration of toxicant.
2. **Behavioral manifestations:** After the exposure of Cd doses 25, 50 and 75 mg/l, fish showed more surfacing, air gulping, rest lessness, escaping movement, erratic swimming and loose schooling. Sudden irregular swimming, increased mucus secretion and high rate of opercular beat rate were also noticed. Fishes became lethargic, less active, irresponsive which resided at the bottom of aquaria.

Schooling was found completely disturbed and fish were scattered. Fishes showed very less response for food and became very weak. Haemorrhage, colour fading, peeling of skin and rashes were also observed.

**3. Histopathological changes:**

**(i) Liver**

**Liver** of *H. fossilis* becomes more fragile and darker in colour after both acute and chronic exposure to $CdCl_2$. But no tumour like out growth is seen anywhere in the liver. Microscopically changes hepatic cells as hypertrophy has been observed. Furthermore, polygonal shape of the hepatic cells is completely lost at various places.

Fishes exposed to $CdCl_2$ for a period of 30days, liver did not show any marked pathological changes.

**(ii) Kidney**

**Kidney** in 30 days $CdCl_2$ treated group exhibited the degeneration of tubules and necrotic condition. The cells of renal tubules were fused and cytoplasm condensed in the central region. Renal tubules lost original appearance and degeneration of cytoplasm leading to wide spaces. The hypertrophy of glomerulus exhibited. The glomerulus in the Bowman's capsule exhibited shrunken or clumped condition and leading to wide space in the capsule. The deshaped glomerulus was also exhibited.

In 60 days duration the renal tubules undergo degeneration. The cellular structure becomes hypertrophied. The cells of renal tubules exhibited vacuolated condition, due to clumped cytoplasm in the central region. It gives blurred appearance. The cell boundaries were disturbed and become indistinct. Glomeruli in the Bowman's capsule were had only thick mass. Due to this empty Bowman's capsule were visible. The haemopoietic cells were in vacuolated stage.

**4- Biochemical changes:**

The variation in biochemical content of tissues of *H. fossilis* after the exposure of $CdCl_2$during 30 days and 60 days at sub lethal concentration were observed. These changes are as follows.

**Glucose-** The maximum 13.64mg/gm and 15.04mg/gm glucose level found in liver at dose of 75mg/l of $CdCl_2$ for 30 days and 60 days exposure, respectively.

**Glycogen-** The minimum 2.68mg/gm and 2.07mg/gm glycogen content were observed in heart after the 30 and 60 days exposure of 75mg/l $CdCl_2$, respectively.

**Total Protein-** The maximum 10.7mg/gm to 8.9mg/gm of total protein level was found in heart after 30days and 60days exposure of 75 mg/l $CdCl_2$, respectively. Total protein 132.9mg/gm and 121.6mg/gm was found in muscles.

**Total Lipid-** The maximum decrease 3.98mg/gm was observed in kidney after 60days exposure at 75mg/l $CdCl_2$.

**Cholesterol-** The values decreases in gills and kidney from 1.82mg/gm to 0.98 mg/gm and 0.86 mg/gm following the doses of $CdCl_2$.

## *Chapter 5: 'Discussion'*

The possible mode of action of has been discussed with the help of the literature available in this field.

## *Chapter 6: 'Summary'*

The summary of the thesis is an anthology of the entire research work executed in the research plan and summarizes the thesis in nutshell.

The present findings show the Cadmium is very toxic for the aquatic fauna specially fishes. The cadmium toxicity affect all the aspects of *Heteropnuestus fossilis*. After inducing the Cadmium compound at different level of doses, it showed the toxic effect in the Morphological, Behaviour, Biochemical and Histopathology.

The cadmium toxicity changes the morphological characters as well as the behaviour pattern of fish shown in the previous chapter.

There are several variations found in the biochemical parameters after giving the different doses of cadmium compound as compare to control. The liver and kidney showes the structural changes at cellular leval. Cadmium compound is very lethal for aquatic organism so it is neccessory to treat the water body and keep free from heavy metals.

## *Chapter 7: Bibliography*

It is a compilation of references which have been consulted in connection with the implementation of the research work wherever required at various places of text.

# CAPTER-7

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## Published Research Paper

1. **Rastogi S,** Yadav P and Singh M. Cadmium Toxicity and its Effect on the histolopathology of liver and kidney of the fresh water teleost *Heteropneustes fossilis* (Bloch). International J of Scientific Research. 2018; 7: 377-379 ISSN No.: 2277-8179 IF:4.758

2. **Rastogi S,** Yadav P and Singh M. Toxicity of cadmium and its effect on the morphology of the fresh water teleost, *Heteropneustes fossilis* (bloch). International Journal on Biological Sciences 2018; 9 (1): 60-62, ISSN No.: 0976-4518; NAAS Rating 3.14; UGC List of Journal No. 7775

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# TOXICITY OF CADMIUM AND ITS EFFECT ON THE MORPHOLOGY OF THE FRESH WATER TELEOST, *HETEROPNEUSTES FOSSILIS*. (BLOCH)

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## ABSTRACT

Cadmium(Cd) compounds are serious pollutants of aquatic environment because of their environmental persistence and ability to be accumulated by aquatic organisms. In the present study, the acute toxicity of cadmium chloride and their toxicological effects on morphology of widely consumed Indian snakehead fish *H. fossillis* was observed for 60 days. In this study percentage mortality was recorded in freshwater fish via treatment with various concentrations of cadmium compounds. Freshwater fish *H. fossillis* was exposed to various concentration of CdCl, (25mg/l, 50mg/l, 75mg/l).

**Keywords:** Cadmium, toxicity,morphology, *Heteropnuestes fossilis*.

## INTRODUCTION

Cadmium, which is the most venomous and non-essential heavy metal has wide distribution in the earth's Cadmium, crust and aquatic environments. In the list of heavy metals such as lead, mercury and cadmium are considered to cause public health hazards. Burning of fossil fuels or incineration of municipal waste materials are known to be largest sources of cadmium release to the general environment (such as coal or oil). Cadmium may also enter into the atmosphere from zinc, lead or copper smelter. It can enter water through disposal of wastes from households or industries. Fertilizers often contain some cadmium, Reproduction rate of aquatic organisms may also be effected due to Exposure to heavy metals and can lead to a gradual extinction of their generations in polluted waters. For example, cadmium and mercury damage the kidney and cause signs of chronic toxicity, including impaired kidney function, poor reproductive capacity, hypertension, tumors and hepatic dysfunction. Any changes in fish behavior are considered as one of the sensitive biomarker to evaluate the exposure to the toxicant (Reddy *et. al*; 2011). It had been observed that the fish behavior alters due to the toxicants the heavy metals pesticides, etc. (Ghanbohadur, *et. al*; 2015 and Deshmukh, 2016). Erratic swimming, escaping from toxic water mucus secretion, convulsions and food intake etc. can be considered as some common parameters to measure the changes in fish behavior.

## MATERIAL METHODS

Experiment was carried out according to guidelines of APHA *et al.* (1998) and replicated thrice. The LC50 values of various intervals were calculated according to Trimmed Spearman Karber's Method (Hamilton *et al.*, 1977) on PC. Behavioural characteristics were also recorded with respect to activity, movement, mucous secretion, skin colouration and opercular beats. The data obtained for

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opercular beats were statistically analyzed for student t-rest and ANOVA using MINITAB software on PC.

## RESAULT

During this study, we documented the specific site of cadmium compounds action. Among morphological changes, discoloration of skin, chemical deposition on skin and aquarium, lesions were recorded and effects were concentration dependent (Table-1). Among cadmium compounds, cadmium chloride caused maximum morphological changes in comparatively low concentration than the cadmium sulphate and cadmium nitrate. The schooling is the characteristic of this fish was found weakened in the cadmium chloride during study. At higher chemical concentration75 mg/l, scale depletion start, skin lesion observed from dorsal to lateral side of the body of fish and these were deepens, copious mucous, clumping of gills increases with the increasing of concentration of toxicant. The skin lesions around the head region, base of caudal fins and pectoral fins were prominent in the 90 % of the fish in higher concentrations. The fishes lost their natural coloration and become almost pale yellow in color.

**Table 1: Morphological changes of freshwater fish *Heteropneustes fossilis* of Cadmium. Chloride exposure.**

| Sl. No. | Morphological changes | Control | CdCl2 |
|---|---|---|---|
| 1. | Discoloration of skin | - | ++++ |
| 2. | Lesions on skin | - | ++++ |
| 3. | Shedding of scale | - | ++++ |
| 4. | Mucus secretion | - | ++++ |
| 5. | Sedimentation of chemical on body | - | ++++ |
| 6. | Muscular bleeding | - | ++++ |
| 7. | Clumping of gills | - | ++++ |
| 8. | All fins damage | - | ++++ |

**C-Control, E-Exposed (Nil); + (Less); ++ (Moderate); +++ (Prominent)**

## DISCUSSION

Cadmium compounds poses toxic effects on the freash water fish Heteropnuestus fossilis. The fish mortality may have resulted by absorption, bio-accumulation of cadmium compounds or greater activity of chemical in body of fish. Increased opercular movement and decreased locomotary activities as observed in present studies are probably associated to compensate for loss of efficiency in the oxygen uptake by decreasing the physiological oxygen demand and increasing the amount of oxygen passing over the bronchial tissue per unit time. Similar finding were also recorded by many workers ( Panigrahi, A. K. and Mishra, B.N.,1978 and Menezes, M. R. and Qasim, S. Z., 1984.). Excessive mucus secretion on gill region and general body surface in present study is a general response of protection against toxicant. Basically the toxicant in natural environment mostly trapped or sticked with mucus coating fish body, made up of by glycoprotein barrier ( Coombs, T.L.,etal.,1972, Lock, R. A. C. and Van Overbeeke A. P.,1981 ). The skin ulceration, colour fading and haemorrhages in present studies were observed after cadmium exposure.

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ERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

# IUM TOXICITY AND ITS EFFECT ON THE HISTOLOPATHOLOGY OF LIVER D KIDNEY OF THE FRESH WATER TELEOST, *HETEROPNEUSTES FOSSILIS* (BLOCH)

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## ABSTRACT

ic cadmium chloride ($CdCl_2$) toxicity was studied in an experimental aquatic exposure. *Heteropneustes fossilis* (an air-breathing catfish) ically exposed to different concentration of $CdCl_2$ (25, 50 and 75 mg/L) for 30 and 60 days. Morphological and histological changes in g following the doses of $CdCl_2$ were studied. Histopathological investigations revealed greater changes in liver and kidney tissues after posure. Necrosis of epithelial cells of renal tubules, Glomerular contraction and reduction of Bowman's space were observed in the ssue of exposed fish. Changes observed in the liver tissue post exposure included necrosis, degradation of hepatocytes, degeneration of oesels, distended sinusoids with pyknotic nuclei and vacuolation of cells. The degree of damage to the liver and kidney tissue was ed with the concentrations and duration of the $CdCl_2$ used.

## KEYWORDS

Cadmium Chloride, histology, liver, kidney, *Heteropneustes fossilis*.

## UCTION

etals have long been recognized as serious pollutant of the life. Pollution by heavy metals has becomes a serious ental and public health hazard because the construction m the environment from industrial processes often exceed ble levels. Due to their bio accumulative and non adable properties heavy metals constitute a care group of ollutants.Cadmium, which is the most venomous and non-heavy metal has wide distribution in the earth's Cadmium, aquatic environments. In the list of heavy metals such as lead, ird cadmium are considered to cause public health hazards ,2003). Burning of fossil fuels or incineration of municipal metals are known to be largest sources of cadmium release to al environment (such as coal or oil). Exposure of Cd can ly influence living organisms and leads to pathological s (Cope et al., 1994; Annabi et al., 2011). As a persistent vironment pollutant, freshwater fish are particularly to cadmium exposure (Sorensen, 1991).

al methods represent a practical tool to evaluate the effects m on living organisms (Cengiz and Unlu, 2005). It has also sively used in the evaluation of cadmium toxicity to fish population (Thophon et al.,2003; Au,2004).In fish tions of cadmium in the liver, gills, kidney and inal tract is reported (Norey et al., 1990).While kidney is l as one of the main targets for cadmium accumulation al2011).Thus, in this paper we aim to investigate cadmium istopathological changes in liver and kidney of *H. fossilis*.

## RIAL AND METHODS

**ence of fishes:** Air-breathing fish *H. fossiles* were kept in arium containing cadmium chloride doses ($CdCl_2$) at room ure (28-30°C). The control groups were maintained in condition without $CdCl_2$. During the experimental period and 60 days) the fishes were fed *ad libitum* with a complete (Silver and Coates, 1957). For each bioassay test, a series of concentrations (25 mg/l, 50mg/l and 75mg/l) of $CdCl_2$ and a ereased.

samples were taken from the fishes exposed to the first three ations (25 mg/l, 50mg/l and 75mg/l). At the end of the od (30 and 60 days), live fish samples were collected from the ntained three concentrations, sacrificed and their liver and ere excised out and fixed in Bouins fixative (for 24 hrs.) and for histological analysis according to standard procedures ed in successive grades of ethanol series and embedded in erial longitudinal sections (thickness 4–5 µm) were stained matoxylin and eosin (H/E) for histological examination under roscope Also, light photomicrographs were taken. The gical changes of the liver and kidney sections noted in the alfish were compared with those of control group fish.

## RESULT

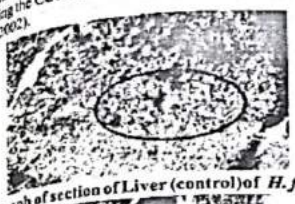
The liver of control *H. fossilis* appears dark brownish red coloured, bilobed gland composed of hepatocytes which are the parts of mass and forms a typical architecture (plate 1). The hepatocytes are arranged in a radial manner around hepatic veins to form hepatic cords. The liver cells are polygonal in shape and contain a prominent nucleus which possesses densely stained nucleoli. Following the exposure of $CdCl_2$, liver of fish *H. fossilis* becomes more fragile and darker in color but no tumour like out growth was seen anywhere in the liver at all tested doses. Histopathologically hypertrophy of hepatic cells has been observed (plate 1). Polygonal shape of the hepatic cells was completely lost at various places. Hepatocytes are found scattered in the hepatic tissue, vacuolization and pycnotic changes have been observed. Dilation of extra cellular spaces, bile canaticuli has also been noticed. In fishes exposed to 25 mg/l. $CdCl_2$ changes included ruptured nucleus, increased kupffer cell, ruptured hepatic tissue, cellular necrosis and increased pycnotic nucleus. Very distinct marked changes such as cellular necrosis, ruptured hepatic tissue, ruptured nucleus and focal necrosis were observed in the liver of fishes exposed to 50 mg/l $CdCl_2$. Further 75 mg/l dose of $CdCl_2$ pronounced the changes including focal necrosis, increased pycnotic nucleus, cellular necrosis and ruptured hepatic tissue (plate 1). All these effects were more pronounced in 60 days groups.

Kidney of control group showed normal histoarchitecture however in $CdCl_2$ exposed fishes concentration and duration dependant changes were noted. In 30 days $CdCl_2$ (75mg/l) treated group kidney exhibited the degeneration of tubules and necrotic condition. The cells of renal tubules in the central region were fused and cytoplasm of these cells was condensed. Loss of original appearance and degeneration of cytoplasm renal tubules was also noted. The hypertrophy of glomerulus demonstrated. Glomeruli in Bowman's capsule revealed shrunken condition thereby wide space in the capsule was noted. The pyknotic nuclei in haemopoletic tissue were visible and cytoplasm was unevenly distributed. Eccentric and pyknotic condition of nuclei exhibiting in almost all cells (plate 2). In 60 days duration $CdCl_2$ (75mg/l) treatment the renal tubules undergo further degeneration and the changes were more pronounced than 30 days exposure. The cellular structure becomes more hypertrophied, the cells of renal tubules exhibited vacuolated condition and the cell membrane becomes indistinct. Glomeruli in the Bowman's capsule were had thick mass like appearance. The haemopoletic cells were highly vacuolated and severe degenerative changes was seen in haemopoletic tissue (plate 2).

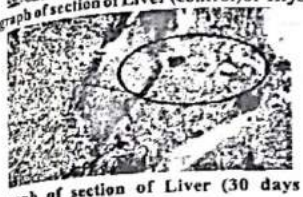
## DISCUSSION

The toxicity effect of heavy metals on fishes pathology been studied by several workers. In this study toxic effect of $CdCl_2$ on the external morphology, and histopathology of liver and kidney of *H. fossilis* is in conformity to other similar kind of studies.

al alterations in liver tissues like degeneration of hepa-
vacuolization, congestion of hepatic tissues, subcapsular
ation, necrosis, indistinct cell boundaries and pyknotic nuclei
served in the liver of the catfish, *Clarias batrachus* exposed to
priyanshan et al (2013). Initial lesion in the liver during the
study might be due to physiological changes that took place in
tissue in the process of trying to homeostatistically regulating
ntifying the Cd metal during continuous exposure as suggested
ga (2002).



ticrograph of section of Liver (control)of *H. fossilis*



ticrograph of section of Liver (30 days exposure of
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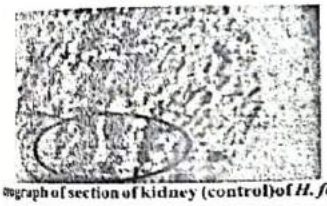
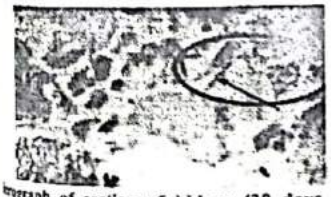
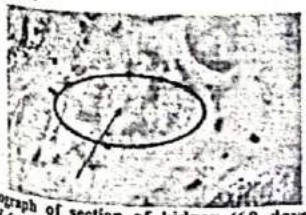


ograph of section of kidney (control)of *H. fossilis*



rograph of section of kidney (30 days exposure of
*H. fossilis*



rograph of section of kidney (60 days exposure of
*H. fossilis* Plate-2

Our present observation on histological alterations on liver is in conformity with observations made in similar work carried out with different toxicants on various fish species too (Ikram and Malik 2009; Pantung et al., 2008) In hepatic tissue, the histological alterations noted during the chronic exposure (30 days and 60 days), focal necrosis, increased pycnotic nucleus, cellular necrosis and ruptured hepatic tissue in Cd concentration dependant manner. These findings are consistent with cadmium inducing greater hepatic alteration in fish after chronic exposure ( Van Dyk et al., 2007). Further, in this study identified alterations of liver cells may be the result of diverse biochemical alteration in liver following the Cd toxicity and act as a signal of degenerative processes that suggests metabolic damage also (Pacheco and Santos, 2002). In addition to above chances vacuolation of hepatocytes is also noted which is suggested to be associated with the inhibition of protein synthesis, energy depletion or a shift in substrate utilization (Hinton and Lauren, 1990).

The fish kidney is one of the first organs to be affected by water pollutants (Thophon *et al.*, 2003) and is suggested as preferential site for Cd toxicity in fish (Brown *et al.*, 1984; Allen, 1995). In this study alterations of kidney tissue during the $CdCl_2$ (25, 50 and 75 mg/l) exposure were rigorous and was both concentration and time dependant manner. Following the chronic exposure, severe glomerular alteration was noted in kidney tissue of fishes. These findings are in confirmation of several previous studies on fishes (Olsson et al 1996; Thophon et al., 2003). Upon acute exposure of CdCl2 renal tubule necrosis and degeneration in fish (*Leiostomus xanthorus)* was noted (Hawkins et al., 1980). Hypertrophy of Bowman's capsule cells in fishes exposed to heavy metals was noted (Handy and Penrice, 1993). We also noted presence of pyknotic and fragmented nuclei in kidney epithelial cells is suggestive of apoptotic and necrotic cell death (Weber et al., 2003). Moreover, the presence of dilated tubules is to be a result of dead and dying epithelial cells, while a thickening of Bowman's capsule is due fibrosis (Weber et al., 2003).

## CONCLUSION

In conclusion histological alterations in the catfish, *H. fossilis* under the influence of $CdCl_2$ toxicity can be used a sensitive method to monitor the aquatic pollution. The present result suggested that exposure to $CdCl_2$ concentrations leads to alterations in morphology and damages in the tissues of liver and kidney of fresh water fishes.

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## and Increasing Plant Growth

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The concept of sustainability also involve use of sustainable agriculture and to achieve this al, there is a increase need to adopt environmental-friendly approach in providing plant nutrition d sustaining soil fertility with increase productivity.

The use of beneficial organisms to combat pests on economic crops is being aggressively sued worldwide in an attempt to eliminate or reduce the application of pesticides, which can lute the soil and adversely affect commodity quality.

The *Trichoderma* has gained highest attention among biocontrol agents because of its some que features viz., mycoparasitism, antibiosis, competition and moreover present in nearly ." ricultural important soil. *Trichoderma* species acclaimed as effective, ecofriendly and chea lifying the ill effects of chemicals. Application of *Trichoderma* in agriculture has four beneficial acts for plants First, it can colonize plant root and its rhizosphere, second, *Trichoderma* fungi ntrol plant pathogens thought parasitism, and antibiosis production, and promote systemic resis- nce. Third, it improve plant health through increasing plant growth. Ultimately, *Trichoderma* fungi mulate root growth and improve plant growth.

Soil is the repertoire of both beneficial and pathogenic microbes Delivering of *Trichoderma* o to soil will increase the population dynamics of augmented fungal antagonists and thereby uld suppress the establishment of pathogenic microbes onto the infection court *Trichoderma* is pable of colonizing farm yard manure (FYM) and therefore application of colonized FYM to the soil more appropriate and beneficial This is the most effective method of application of *Trichoderma* rticularly for the management of soil-borne diseases.

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## Environmental Policy of India: A View

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A view of environmental policy of India is related to that Legislation for the protected areas and e of other natural resources of future generations. India's judicial activism on environmental issues s, some suggest, delivered positive effects to the Indian experience. India's Supreme Court has livered a new normative regime of rights and insisted that the Indian state cannot act arbitrarily but ust act reasonably and in public interest on pain of its action being invalidated by judicial interven- n *Legislation in India:* Indian Forest Act, 1927, British India (*Developed procedures for setting and protection of reserved forests, protected forests, and village forests*) Use of other natural sources in regulation of movement (*Special focus on timber*).1951 1st, 1956 2nd and 1961 3rd Five ear Plan, (*Almost the same but with extra deer saving acts*), 1969 4th Five Year Plan and1972 ildlife Protection Act (India except J&K) *Formalisation of national parks, wildlife sanctuaries, con- ervation reserves and community reserves Development of National Board for Wildlife and State oards for Wildlife for identification of future protected areas Penal codes for animal poaching, and ade in products derived from protected animals* 1973 National Wildlife Action Plan, 1974 5th and 378 6th Five Year Plan, 1980 environment protection act 1986 *it is a legislation which signifies the entral government determination to take effective steps to protect the environment. (Forest (Con- ervation) Act*, 1980 (with Amendments Made in 1988) 7th Five Year Plan (No State Government or ther authority shall make any order directing- that any reserved forest shall cease to be reserved



any portion thereof may be assigned by way of lease or otherwise to any private person or to any authority, corporation, agency or any other organisation not owned, managed or controlled by Government and any forest land or any portion thereof may be cleared of trees which have grown naturally in that land or portion, for the purpose of using it for reafforestation). 1986 Environment (Protection) Act, 1988 National Forest Policy, 1992 Foreign Trade Act, 1992 8th, 1997 9th and 2002 10th Five Year Plan, India signed and ratified the Protocol (KYOTO) in August 2002. 2006 National Environmental Policy, 2007 11th Five Year Plan, which contains mandatory and voluntary measures to *increase efficiency in power generation and distribution and increased use of nuclear power and renewable energy.* The Indian government tried to stop Greenpeace freedom of expression in 2015. *(See also Energy policy of India; Indian Council of Forestry Research and Education; Environmental policy of India)*

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## Behavioral Changes of the Freshwater Teleost *Heteropneustes Fossilis (Bloch)* Exposed to Malathion

Nisha Yadav[1], Ramakant Maurya[1], Pushpa Yadav[2] and Madhulika Singh[1]
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These days organophosphate (OP) chemicals have been chosen as the most widely preferential insecticide to make the crop free from pest intended for larger production. Excessive use of these chemicals has health risk for human as well as threatens non-target organisms. Malathion (C, H, O PS ) one of the earliest OP developed in1950 being extensively used to control the pest. Very little information is available regarding the concentration of Malathion which produces deleterious effects on growth and behavior of aquatic organisms. In this study toxicity of individual treatment of malathion has been evaluated in a freshwater teleost, *Heteropneustes fossilis* (Bloch) by determining the values of lethal concentration ($LC_{50}$) and observing the behavioral changes at 96 hours duration. Bioassay methods have been use to determine the concentration of the Malathion exposure. Results of the study revealed that treatment of fishes by deferent doses of Malathion (25.00, 31.25, 39.06 and 48.80 ppm) for 96h duration induced behavioral changes as exhibited by irregular and spiral swimming, loss of equilibrium and sinking to the bottom. In conclusion doses of Malathion are inducing behavioral changes in fishes in dose dependant manner.

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## A Study on Biochemical Changes on Fresh Water Teleost, *Heteropneustes Fossilis* (Bloch) Exposed to the Heavy Metal Toxicant Cadmium

Sachin Rastogi, Pushpa Yadav[2] Ramakant Maurya1and Madhulika Singh[1]
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Cadmium (Cd) one of heavy metal environmental toxicants, is widely used in batteries manufacture, metal and mining industry, dentistry etc. Cd is released in extensive amounts through industrial effluents and reported to cause versatile biotic changes into soil surface and ground water systems as well as damage to the living organisms of the aquatic ecosystem. It shows biomagnification and has greater half-life periods. Cd was found to interfere with many protein and carbohydrate

...effects of Cd toxicity in fresh water teleost fish at biochemical level. Following the acute ...oses of Cd (96h) significant fall in levels of glycogen, total protein and lipids in tissues of liver, ...idney, muscles and gills was noted ($p<0.05$). However, the glucose level was elevated in all tissues ...n conclusion study suggests organs toxicity impact of Cd in fishes and such fishes when consumed ...s food leads to the deposition of heavy metals in consumers.

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## Ecological Degradation and Its Impact on Socio-Economic Development

**Rama Nishad**
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Environmental degradation is the deterioration of the environmen: through depletion of re-urces such as air, water and soil; the destruction of ecosystems, habitat destruction the extinc-n of wildlife: and pollution It is defined as any change or disturbance to the environment perceived be deleterious or undesirable There are many problems, challenges and opportunities associated th living in the environment today. The earth and its treasure base, is experiencing a siege from all ...spects of human endeavors ranging from misuse abuse and degradation of the environment that ...eve become so easily spotted and there are disruptions every day and almost everywhere The ...stainable management of the environment and natural resources is vital for economic growth and ...man wellbeing When managed well, renewable natural resources, watersheds productive land-...capes and seascapes can provide the foundation for sustained inclusive growth food security and ...overty reduction The primary causes of environmental degradation in India are attributed to the ...pid growth of population in combination with economic development and overuse of natural re-...ources. Major environmental calamities in India include land degradation, deforestation, soil ero-...on, habitat destruction and loss of biodiversity. Economic growth and changing consumption pat-...rns have led to a rising demand for energy and increasing transport activities Air, water and noise ...ollution together with water scarcity dominate the environmental issues in India

**Key Words:** Environment, Degradation, Ecosystem Economic growth Biodiversity

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## Floristic Diversity Around Orai of Jalaun District In Bundelkhand Region, Uttar Pradesh

***R.K. Gupta, Praveen Kumar and Ghan Shyam**
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Dayanand Vedic College, Orai (Jalaun)
(Affiliated to Bundelkhand University, Jhansi)
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Floristic diversity of Orai, district Jalaun of Bundelkhand region U P has been analyzed in the ...esent study The area has unique phytogeographical position for floristic survey The vegetation ...ound Orai mainly represents tropical dry deciduous forest, thorn forest and scrub forest

The floristic analysis shows 133 species belongingto107genera and 48 families of ...giosperm. Dominant family is Fabaceae (21sp) followed by Apocynaceae (08sp) Euphorbiaceae



...the medicinal purpose. The paper comprises a brief account of ... type Some species are also known for their timber, fodder and ...

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## National Water Policy

**Manju Yadav**
Assistant Professor, Department of Chemistry
M.P. Govt. P.G. College Hardoi

The water is a prime natural resource, a basic human need and a precious national asset. Planning and development of water recourses need to be governed by national perspectives. Na-tional water policy is formulated by ministry of water recourses of the government of India to govern the planning and development of water recourses and their optimum utilization. The first national water policy was adopted in 1987. It was reviewed and updated in 2002 and later in 2012. India ...ccount for 15 percent of the world population and about 4 percent of world's water resources. One ... the solution to solve the country's water woes is to link the rivers. It has been successful in ...eating live water storage capacity of about 253 billion cubic meter so far. In a first the ecological ...eeds of rivers have also been taken into consideration.

The main emphasis of national water policy 2012 is to treat water as economic good which ...e ministry claims to promote its conservation and effective use. The policy also does away with the ...iorities for water allocation mentioned in 1987 and 2002 version of the policy.

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## Impact of Invasive Fishes on the Native Fish Fauna of River Yamuna at Mathura District, U.P.

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In terms of the status of invasive fish species significance presence of *Oreochromis niloticus*, *Cyprinus carpio*, *Hypoptnalmicthys molitrix and Clanas ganpineus* is evident in majority of the river stretches Abundance of these species is recorded due to less stressed condition which reflects the cominance in terms of biomass than any other species. A preliminary record shows that 48-fish species belonging to 13-families were recorded in Mathura waters so far. Species of the family Cyprinidae were most dominant followed by Bagaridae, Schilbeidae, Clupeidae, Ophiocephalidae As far as concern with the trophic ublization of fishes, carnivorous fishes were dominant followed by herbivorous and omnivorous. Now present condition has changed in terms of native fish fauna are gradually decreasing.

It has been recorded that presence of invasive fishes gradually establishing themselves as a breeding population replacing the Indian Native Fish Fauna. Study reveals that the use of Yamuna river water for the purpose of hydal projects, irrigation and drinking purpose and water pollution are the main threats affecting the habitat of native species and has provide a favorable environment for the alien fishes So, need of the hour is to check the entry of alien fishes in river Yamuna and monitoring the river water in terms of water pollution can be a mile stone in conservation of life and environment as well.

**Key Words:** Yamuna River, Native fish fauna, Invasive fishes, Conservation.

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## Toxicity of Cadmium and its effect on the Morphology of the fresh water teleost, Heteropneustes fossilis. (Bloch)

**Sachin Rastogi1, * Madhulika Singh1 and Pushpa Yadav2**
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2 Deptt. Of Zoology ,NSCB,Govt. College,Lucknow(India)22601

**Abstract:**
Cadmium(Cd) compounds are serious pollutants of aquatic environment because of their environmental persistence and ability to be accumulated by aquatic organisms. In the present study, the acute toxicity of cadmium chloride and their toxicological effects on morphology of widely consumed Indian snakehead fish H.fossillis was observed for 60 days. In this study percentage mortality was recorded in freshwater fish via treatment with various concentrations of cadmium compounds. Freshwater fish H.fossillis was exposed to various concentration of CdCl2 (25mg/l ,50mg/l, 75mg/l)

**Keywords:** Cadmium, toxicity,morphology,Heteropnuestesfossilis

## A Preliminary survey of the invasive alien angiospermic Plant species of Pilibhit tiger reserve, Pilibhit, U.P. (India)

**Deepak Singh[1] and A.K. Khare[2]**
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**Abstract:**
The district of Pilibhit is the north-eastern most district of Rohilkhand division which is situated in the Sub Himalayan belt on the boundary of Nepal. Pilibhit wild life sanctuary under Pilibhit forest division & some area of Shahjhanpur has been notified as Pilibhit Tiger Reserve by the State government in the year 2014. The Tiger Reserve is located in terai region which is known for its complex of sal forest, tall grasslands and swamps maintained periodic flooding, is one of the most threatened ecosystems in India. In a Preliminary survey of invasive alien angiospermic plant species of the Tiger Reserve, a total 37 species belonging to 17 Families were recorded. Among these Species Dicots represented by 31 Species whereas monocots with 6 Species. Among all Alien Species, the maximum number of species (7) was from the family Asteraceae, followed by Amaranthaceae (4), Papilionaceae (3) Euphorbiaceae (2) and Caesalpiniaceae (1) Pontederiaceae (1) Verbenaceae (1).
The data revealed that herbs accounted for 18 Species, undershrubs 4 species, shrubs 5 species, climbers 3 species and trees 3 species grasses and sedges represented with 2 species of each. Alien species are a major threat to local biodiversity and an important cause of homogenization of ecosystems. These invasive alien species not only compete for nutrients, moisture and light but for space too. Some important examples of invasive alien species are *Ageratum conyzoides, Ageratum houstonianum, Anagallis arvensis, Argemone mexicana, Calotropis gigantea, Calotropis procera, Cuscuta reflexa, Eclipta prostrata, Eicchornia Spp., Euphorbia hirta, Lantana indica, Mimosa pudica, Parthenium hysterophorus.*

**Keywords:** Invasive alien species, Pilibhit Tiger Reserve.

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*We wish his/her best for future endeavours.*

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