Histopathology
INTRODUCTION:

Histology, the study of the microanatomy of specific tissues has been successfully employed as a diagnostic tool in medical and veterinary science since the first cellular investigations were carried out in the mid 19th century (Veichow, 1858).

The histological studies on a fish is noteworthy and promising field to understand the extent to what changes in the structural organization occur in the organs due to pollutants in the environment. Most of the information about the environmental pollution on aquatic animals has been obtained from mortality studies (Singh et al., 1990). These structural changes of the organ at microscopic, cellular and organelle level lead to alterations in functional systems (Jagadeesan, 1999). The histopathological changes are irreversible while altered functional systems are considered as a reversible effect. The severity of histological damages in any particular aquatic organism is directly proportional to the concentration of a pollutant in the medium. Moreover, the histopathological picture of the organs can corroborate with a biochemical changes accounting for the functional disruption in the activity of the organs due to cellular damage. Vijaymadhavan and Iwai, (1975) have reported that the extent of damage varies with organs, nature of pollutant, medium and duration of test.

Toxicological histopathology gives useful data concerning the change induced by the chemicals at cellular and tissue level. All the organs in the body of a fish may be potential targets for the effects of pollutants. Hence, the
pathology of exposed animals is an important confirming factor in the assessment of toxicity of pollutants. Histopathological assessment through light on the nature of tissue alteration and the extent of tissue damage. This intern helps to evaluate the nature of toxic compound. Therefore histology can give useful insight on the deleterious effects on different pollutants on fishes. Histological investigations so may be considered as biomarker i.e., an indicator of the impact of xenobiotics compound on different levels of biological organizations (Cells, organs, individuals, populations; Paolini et. al., 2005).

The extent of severity of tissue damage of a particular compound depends on the toxic potentiality of the organisms (Tilak et al., 2001). It is even greater in different animal groups. However, the location of the major damage may be determined by the mode of action of the chemical. The mode of action of each poison and the pattern of tissue vulnerability has been well defined and the toxic level of each agent at which a fairly standard distinctive pattern of tissue damage has been studied.

Histopathology is mainly directed to study the effect of chemicals, pesticides and other pathogens on the structural components of the living system and the ways in which cells and tissues respond to injury. A chemical or a derivative acting directly on the cell most frequently causes chemical cytotoxicity by altering its environment. The cells in turn respond histopathologically by degeneration, proliferation, inflammation and repair. The chemical affects the cell by altering the external environment, oxygen and nutrient transport system or the endocrine and immune system.
Degeneration is the most common symptom seen within the cell or population of cells. This may be produced by reduced blood and nutrient supply or endocrine deficiency. Intracellular accumulation like water, fat and proteins is seen very often, ultimately killing the cells, which are dead and still form a part of the living body. This leads to necrosis. Histologically necrosis is characterized by a sequence of morphological changes, which takes several hours to develop after actual death of cells. Gross necrotic changes are of three types.

1. **Liguetactine necrosis**: Resulting from rapid enzymatic digestion of cells.

2. **Coagulative necrosis**: The result of ischemia or loss of blood supply to an area.

3. **Fat necrosis**: The area has soapy fat consistency. In section, the stages of necrosis are best assessed in terms of the nucleus. They are,

   I Pyknosis: Nucleus is shrunken and very dark. Rupture of the nuclear membrane and fragmentation of nuclear chromatin

   II Karyolysis: characterized by fading and dissolving of nucleus leaving a ghost outline.

Anthropogenic contamination of the aquatic environmental by cadmium has increased substantially in this last several decades and resulted in the elevation of cadmium in the tissue of aquatic organisms at all trophic levels. Although acute toxicity tests are used to safe concentrations of toxicants in the
environment, they provide little information on the mode of toxic action or environmental situations were accessory factors affect toxicity. By combining physiological, hematological, and histological studies leads one can gain insight into the mode and site of toxic action, as well as determine environmentally safe concentration of toxicant. Cadmium is highly able to accumulated in the living organisms. In fish, the gills, kidney, liver are the primary target organs for the cadmium (Giles, 1988). Due to its ability to accumulate in organs several pathobiochemical and histological alterations appeared (Mohamed and Mikotaj, 1990).

A few reports are available on the damage caused to different internal organs of freshwater animals exposed to various heavy metals. Gardiner and Yevich (1970) reported that cadmium concentrates in gill tissues and causes impairment of respiratory and osmoregulatory functions due to hypertrophy and hyperplasia of interlamellar epithelium and separation of lamellar epithelial layer in the estuarine teleost, Fundulus heteroclitus exposed to cadmium.

Jagadeesan 1999 reported the metallic salts are capable of producing severe damage and changes in its cellular levels in gills with remarkable changes in primary and secondary lamellae and leads to death of fish on exposure of L. rohita in different concentrations of mercury. Lindahl and Hell 1970 reported a decrease in oxygen consumption of gill filaments in Leucisus rutilus due to damage to the secondary gill lamellae on exposure to phenyl mercuric hydroxide.
Tafenelli 1972 studied the effect of cadmium chloride on gold fish and observed kidney lesions. Bilinski and Jonas (1973) in their studies on rainbow trout *Salmo gairdneri* exposed to copper and cadmium salts found separation of epithelial layer hyperplasia and hypertrophy of interlamellar cells in the gill tissue. Palaniappan *et al.*, (2003) observed the changes in the histology of gills which lead to disturbance in the basement membrane, degeneration of gill lamellae, swelling of base, interlamellar space, pyknotic and necrotic chloride cells, hyperplasia and fusion of secondary lamellae, when fish *Cirrhinus mrigala* exposed to heavy metal Nickel. Strick *et al.*, 1975 observed damage to gill lamellae in Coho salmon *Onchorhynchus kisutich* following exposed to cadmium. Structural impairment in liver leading to the destruction of hepatocytes was observed in *Cyprinus carpio* after Zinc and copper poisoning (Wong et, 1977). Copper sulphate treatment resulted in hemorrhage in the gill filaments of *Heteropneustes fossilis* (Rajbanshi and Gupta 1979). Kapilamanoj and Ragothaman (1999) reported the severe changes in the histology of gills lead to the disturbance in basement membrane, degeneration of gill lamellae, cyst formation, swelling of base increased intercellular space etc. These changes finally caused the failure of the respiratory mechanism which resulted into the death of fish on exposed to sublethal concentration of cadmium. Ghate and Masurekar, (1979) observed distention of gill plates, vacoulation and necrosis of the gill tissues in two species of freshwater prawns *Machrobrachium kistnensis* and *M. Cardina* exposed to copper sulphate. Kumar and pant (1981) reported that the severity of damage caused by
cadmium was greater in liver and kidney of *Puntius conchonius* compare to the t in the gills of the same fish. The toxic effects of cadmium on liver of freshwater teleost, *Garra mulya* (Sykes) were studied by Wani and Latey (1983) and reported that the damage in the cadmium treated liver was in the form of extensive vacoulation, liver chord disarray and nuclear pyknosis indicating necrosis.

Many histological alterations observed in gill lamellae, such as buldging at basal and distal parts of the lamellae, hypertrophy and hyperplasia of lamellar and interlamellar cells, separation of respiratory epithelial layer atrophy and necrosis of gill lamellae in *Sarthorodon mossambicus* exposed to both the lethal and sublethal concentration of mercury (Akhilendar Naidu *et al.*, 1983a). Heavy metal cadmium is known to cause injury to most of the internal organs of higher vertebrates (Phillopotts, 1986). Cadmium induced renal toxicity and histopathological changes in the kidney of fish *Mylio macrocephalus* (Ooli and Law, 1989). Ghosh and Chakrabarti, 1993 reported that, cadmium chloride caused histopathological and histochemical changes were observed in liver, Pancreas and Kidney. The important observations include cytoplasmic vacoulation, eocentric nucleas, rupture of cell membrane of hapatocytes in liver, rupture of tubular epithelium, degeneration of glomeruli in kidney on exposure to sublethal concentration of freshwater fish, *Heteropneustes fossilis* and similar types of observations were made by Ramesh and Singh, (1997) in *Clarius batrachus* on exposure to lead nitrate. Sushithra *et al.*, (2007) investigated the histopathological manifestation of
cadmium toxicity includes bulging of the hyperplasmic secondary lamellae of gill, necrosis, hemorrhage, fusion of secondary lamellae of *Heteropneustes fossilis* on exposure to sublethal concentration of cadmium chloride. Thophon et. Al., (2003) were also reported the same symptoms in *Lates calcarifer* in acute and subacute cadmium exposure. Cadmium and lead induced histopathological alterations in liver of Cat fish, *Clarius gariepenes* (Tawari Fufeyin, 2008; Pedro et., al 2009).

Several authors reported that, untreated effluent discharged from pulp and paper mills into receiving waters is known to be toxic to some aquatic organisms. Manifestation of toxicity in fish include fin necrosis, increase of parasites, change in physiology, kidney tumors, neoplastic lesions in the liver and skin tumors (Khan *et al.*, 1992; Lindejoo and Thulin, 1990; Muckittrick *et al.*, 1991; Bucher *et al.*, 1992; Lindstrom and Oikari, 1990; Myers *et al.*, 1987; Hawkins *et al.*, 1990; Moore, 1991; Muralidharan *et al.*, 2000 and Vardhani and Gowri, 2002).

Mandal and Kulshrestha, (1983) and Vinod Ghanathay, (1989) studied histopathological changes in *Clarias batrachus* and *Channa punctatus* exposed to sumithion and BHC respectively. Similar histopathological studies were carried out by Sowbhagya, (1991) and Vijayander Reddy, (1993) in fishes exposed to paper mill effluents and chromium respectively. Thorat, (2001) reported histopathological changes in the intestine of the fish, *Catla catla* exposed to endosulfan. Anitha and Ramkumar, (1997 a & b) revealed degenerative changes in the serosa, mucosa and submucosa layers, focal
necrosis, proliferation and desquamation of the superficial part of villi in the fishes *Channa punctatus* and *Heteropneustes fossilis* collected from polluted Hussainsagar Lake. Effect of many dyes was studied by Saraswathi and Padmavathi, (1994), and reported extensive damage to the intestinal tissue and the spherical nucleas became elongated and seen in degenerated conditions.

Subhadra Banerjee and Bhattacharya (1994), reported the drastic histopathological changes i.e., degeneration and dispersion of chromoffin tissue, kidney lesions, karyolysis, dilation and shrinkage of Bowman’s capsule and glomerulus in *Channa punctatus* on exposure to mercury and ammonia. Cadmium effect on the histology of kidney and gill was studied by Ooi and Law, (1989) and Kapila and Ragothaman, (1999) respectively reported necrosis, damage of the renal tissue, disturbance in basement membrane, degeneration of gill lamella, cyst formation, swelling of base and increased interlamellar space in gill.

Toxicants impair the metabolic and physiological activities of the organisms but such studies alone do not satisfy the complete understanding of pathological condition of tissues under toxic stress. Hence, it is useful to have an insight into histological analysis regarding the extent damage of the tissues like gill, liver, and kidney when cadmium enters the body *Labeo rohita*. The foregoing literature clearly documents that the pesticides cause structural changes in different organs of freshwater animals. The histological studies of the organs of freshwater fishes subjected to heavy metal cadmium in relation to the concentration of the cadmium and period of exposure. As the line of
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structural changes in different organs of freshwater animals. The histological
studies on the organs of freshwater fishes subjected to cadmium in relation to
the concentration of the metal and period of exposure however are not reported.
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hematological alterations in the organs of the *Labeo rohita* exposed to
cadmium stress.

RESULTS:

Histology of Gill (Control)

Gills are the vital organs for respiration in fish which establish a
direct contact with the medium through which a pollutant largely enters into the
body. The teleosts have four pairs of gill arches. The normal structure of the
gill lamellae are flat leaf like structure laterally compressed and situated
alternately on either side of the inter brachial septum. The primary gill lamellae
consist of centrally placed rod like supporting axis with a row of secondary gill
lamellae present on each side of it. The secondary gill lamellae also turned as
respiratory lamellae are highly vascularized and covered with a thin layer of
simple squamous epithelial cells separated by mucus cells. Blood vessels can
be seen extended into each secondary gill lamellae. The blood cell has single
nucleus, which is flat in appearance. The region between two adjacent respiratory lamellae is turned as inter lamellar region (Plate 1 Fig. 1 & 2).

**Histological changes in the Gill of exposed fish**

Fish on exposure to the lethal concentration of cadmium, on day 1, the enlargement of the base of primary gill lamellae was observed (Plate, 1 and Fig 3 & 4). On day 2 the secondary gill lamellae showed buldging at the distal and basal portions (telangiectatic tissue) (Plate 1 fig 5 & 6). Fusion of these lamellae was noticed all along their length. The cells seemed to have undergone a clear increased hyperplasia. On further exposure, on Day 3 the gills exhibited cytoplasmic vacuolization of superficial epithelial cells, hollow spheres due to greater loss of mucous from mucous cells followed by atrophy of gill lamellae, (Plate 2, Fig. 1 & 2) and at day 4 (Plate 2, Fig. 3 & 4). The thinning down of secondary lamellae with desquamation of the hyperplastic epithelium may appearance pillar cells and capillaries, loss of the lamellar structure of the gill with cytoplasmic vacuolation and pyknotic nuclei and more number of telangiectatic secondary lamellae were seen all the gill filaments (Plate, 2 and Fig. 5 & 6).

In the sublethal concentration of cadmium the gills of the fish exhibited a mild degree of degenerative changes on day 1 of exposure, the slight damage to the base of secondary lamellae and inter lamellar tissue (Plate, 3 and Fig. 3 & 4). On day 5 the changes were more marked when compared to day 1 (Plate, 3 and Fig. 5 & 6). The degeneration of epithelial cells encapsulating primary and secondary gill lamellae with necrosis. Fusion of secondary lamellar,
bubbling of primary gill lamellae, atrophy is also observed. The excess secretion of mucus and necrosis of basal filament was observed. But on further exposures, for 10 and 15 days the gill structure was just similar to that of control fish except mild degree of precipitation of mucus over the gill lamellae (Plate, 4 and Fig. 1, 2, 3, 4 & 5).

Histology of Liver (Control)

The liver of fish comprises a continuous mass of large hexagonal hepatic cells (Hepatic parenchyma). Hepatic cells are of roundish or polygonal shape containing clear spherical nucleus. They are located among sinusoids forming cord like structures known as hepatic cell cords. In fish these structures are generally obscure. Bile canaliculus is centrally located in each cord. There is no clear division of hepatic cells into lobules. These cells contained granular cytoplasm and with distinct nuclei either exocentric or slightly centrally placed. Hepatic cells have many vital functions other than the secretion of bile. They play an important role in protein, lipid and carbohydrate metabolism. They serve as storage site for some nutrients. Detoxification is another important function. A large number of blood sinusoids and lipid glycogen granules are found in the hepatic mass (Plate 7 Fig 5).

Histological changes in the Liver of exposed fish

On day 1 of exposure to the lethal concentration of cadmium, the liver of fish exhibited enlarged nuclei and vacoulation in hepatic cells. Liver cords were seen disarrayed (Plate 5 Fig 1& 2). On day 2 of exposure the paranchymatous nature of the liver was greatly disrupted with congested blood
vessels. The hepatocytes cell membranes were ruptured and granular degeneration was evident in most of the hepatocytes. Nuclei became slightly hypertrophic (Plate 5 Fig 3 & 3). On further exposure, day 3 severe degrees of atrophic changes were noticed in the liver cords. Hemorrhagic condition was prominent with heavy vacuolization in the liver tissue. At some regions exfoliation and congregation of hepatocytic nuclei and focal necrosis were seen (Plate 5 Fig 5 & 6). This was followed by the severe degree of vacoulation, shrinkage of hepatocytes, atrophy, granular degeneration, rupture of blood vessels, necrosis dissolution of laminar structure and cytoplasmic disintegration in hepatocytes on day 4 of exposure (Plate 6 Fig 1, 2, 3, 4, 5 & 6).

Compared to the structure of the liver of control fish, exposed to sublethal concentration of cadmium initially exhibited few changes like slight disarray of liver lobes, mild degree of degeneration of cytoplasm, occasional blood clots and congregation of nuclei at day 1 (Plate 7 Fig 1 & 2) and cloudy swelling of hepatocytes, granulization of cytoplasm, hypertrophic and pyknotic nuclei on day 5 (Plate 7 Fig 3 & 4). However, on further exposure to day 10 certain degree of reorganization in the structure of liver cords was observed. The nuclei appeared normal, with a very little degree of cytoplasmic vacuolization (Plate 8 Fig 1 & 2). At 15 days of exposure, no significant changes were seen different from controls, except a slight degree of hyperchromatic condition of the nuclei (Plate 8 Fig 3 & 4).
Histology of Kidney (Control)

The basic unit of kidney in fish consists of a renal corpuscle, Bowman’s capsule and glomerulus and various segment of the renal tubules, namely proximal tubule, intermediate segment, distal tubule and collecting duct. Proximal tubules have prominent brush borders (Microrilli) bathed in the vascular bed in the interstitial tissues. Distal tubules and collecting ducts, both devoid of brush borders, and are sparsely distributed. The intermediate segments between proximal and distal tubules are rarely seen. The renal corpuscles are located in close vicinity of renal tubules and blood vessels in the interstitial tissue. Pigments and leucocytes are very common in the interstitial tissue (Plate 9 Fig 1, 2 & Plate 11 Fig 1 & 2).

Histological changes in the Kidney of exposed fish

In lethal concentration of cadmium, the kidney showed reduction in renal cell number in the proximal and distal collecting tubules which have resulted in narrowness of lumen. The tubular cells have undergone hypertrophy and some of the renal tubules have lost their normal shape. Vacuolation due to degeneration of cytoplasm is quite obvious. The nuclei of epithelial cells have become quite dominant and are found infiltrating into the surrounding tissue. The perforation of kidney tubules is commonly observed. The kidney demonstrated hyperplasia, vacuolation, degeneration and necrosis leading to the complete necrosis. Cuboidal epithelial cells lining the tubules showed complete vacuolation with degenerating cytoplasm and more nuclear division and their disorderly scattering nature. The haemopoitic tissue was fully studded
with lymphatic cells at the highest rate of nuclear division. The lumen of the tubules was found to be dilated. Kidney tubules were also found to be perforated (Plate 9 Fig 3, 4, 5, Plate 10 Fig 1, 2, 3, 4, 5 and 6).

In sub lethal concentration of cadmium the kidney of the fish exhibited a mild degree of changes. On 1st, 5th and 10th day shows more changes (Plate 11 & 12 Fig 3, 4, 5, 6 & 1 & 2). i.e., epithelial cells of the tubules which showed desquamation, irregular orientation of the nuclei in the cells, lumen of the tubules became wider as a result of flattening of epithelial cells. Ruptures of the tubules were quite prominent, cell fragments could be seen inside the lumen of some tubules. Vacuolar degeneration was seen in the few tubules. Haemopoitic tissue was degenerated. But on 15th day kidney showed recovery tendency (Plate 12 Fig 3 & 4). Glomerular cells attained normalcy in structure. Cytoplasm appeared clear and vacuolization and Karyolysis of cell was completely reduced. Necrotic changes in uriniferous tubules were reduced. Clumping of damaged blood cells was seen.

**DISCUSSION:**

The most common route of the entry of the water soluble toxicants in fishes is through gills. Fish have direct contact with pollutant medium containing pollutants (Holden, 1973). The morphological appearance of an organ or organism is the documentary evidence on the adverse effects of metals in an animal. Thus histopathological responses of an animal at lethal and sublethal exposure to metals can bring a relationship between
the level of accumulation of the metal and to the animals various physiological and biochemical activities (Paulose, 1989). In the present study, compared to the controls, the progressive degeneration changes in the gills of the fishes exposed to the lethal concentration of cadmium. The changes include swellings of the base of the secondary gill lamellae, fusion of primary and secondary gill lamellae all over their length, erosion of superficial cells, hypertrophy and hyperplasia, nuclear pyknosis, from 1 to 4 days reveal severe toxic effects of acute concentrations of cadmium on the respiratory organ. Thus the structural changes in the gill filaments, particularly secondary gill lamellae offer a favorable material for the studies on the effects of toxic substances because they have key positions in the body of the fish due to their role in the transport of oxygen.

The progressive degenerative changes in the gills of the fish over time of exposure to the lethal concentrations of cadmium suggest that, the major route of entry of metal ions is through the respiratory structure. The changes in the secondary gill lamellae indicates that, the deaths of fish exposed to the lethal concentration of cadmium might have occurred due to the failure of gaseous exchange across the respiratory epithelium. This is clearly evidenced from the present study by the drastic decrease in the rate of oxygen consumption of the exposed fish. Skidmore and Tovell
(1972) and Khangarot and Somani (1980) also reported that with action of high concentrations of zinc and mercury pollutants, the epithelial covering of secondary lamellae were lifted away in the form of continuous sheets from the pillar cells system, thus increased the diffusion distance from the water and blood and finally fish died of tissue hypoxia. Similar histopathological changes have been reported in the gills of *Lepomis macrochirus* exposed to cadmium (Donald and John, 1986); *Salmo gairdneri* to lead (Sippel and Hodson, 1983); *Channa punctatus* to methoxy ethyl mercuric chloride (Sastry and Rao, 1983); *Rasbora daniconius* to mercury (Gupta and Vinod, 1995). However, the damage in the pillar cells was well marked at higher concentrations, which was also reported in *Cyprinus carpio* exposed to cadmium (Suresh, 1992). Rainbow trout exposed to zinc sulphate (Skidmore and Tovell, 1972); *Labeo rohita* exposed to mercury (Jagadeesan, and Mathivanan 1999).

Suresh (1992) reported enlargement of secondary gill lamellae and inflammation of interlamellar epithelium in fingerlings of *Cyprinus carpio* on exposure to cadmium. He also observed increased numbers of mucous cells in the secondary lamellae of the experimental fish as compared to control fish, indicating the defensive response of the fish to the toxicant. The pillar cell system also appeared to be collapsed pilaster columns were seen curled, pools of congested blood have also seen with
the subepithelial space. Collapse of the pillar cells system is believed to occur when a fall in the hydrostatic pressure causes this system to fail as vascular endoskeleton (Bijya, 2002). Due to the formation of subepithelial space, the blood supply between the pilaster cells and epithelial lining as well as water balance is affected (Prasanth, 2002).

Degeneration of epithelial cells indicates the damage in the gill lamellae which reduces the activity. This consequence is also likely to limit the respiratory capacity of the gills. The destruction in the arrangement of the pillar cells and red blood cells observed in the present observation has substantiated the findings of Natarajan (1979) in Barbus stigma exposed to lead and of Jagadeesan and Mathivanan, (1999) in Labeo rohita exposed to sub lethal concentrations of mercury. Marja, (1986) observed that the secondary lamellae of the gills were shortened, fused and deformed and the epithelial cells were disassociated. He also observed microfilament bundles in its pillar cell. Cytoplasm had disintegrated. The fusion of secondary lamellar in gill may take place as an ultimate result of massive lamellar hyperplasia, which results in a solid fusion of many or all of the lamellar capillaries within a mass of hyperplasic epithelium (Ronald J Roberts, 1989). The chloride cells were swollen and alterations had occurred in their mitochondria and nuclei. He had proposed that his observations were probably specific to DDT, but this histopathological features noted in the present study were similar. Probably the metals in general might cause similar pathological manifestations. These histological changes shown in gills, caused improvement in oxygen
consumption of fish. Shrinkage of gill lamellae resulted in the restriction of the flow of water through the gill seine, for respiration. Thickening of lamellae due to inflammation of epithelial cells results in the lifting and dissociation of epithelium. This reduces the availability of water space and constricts the blood capillaries (Kapilamanoj and Ragothaman, 1999).

Telangiectatic secondary lamellae were common in almost all the exposures. It was in short exposure times, (Plate 1 Fig 5 & 6) when exposure days was prolonged telangiectatic tissue also increased in number (Plate 3 Fig 1, 2, 3, 4 & 5). The dominance of telangiectatic tissue is observed at 96 hrs of exposure. Histologically it is obvious that the lesion has its genesis in the rupture of the retaining pillar, or pilaster cells, which normally join the dorsal surface of secondary lamellae to the ventral. The result is dilation of the lamellar capillary and pooling of the blood, which thromboses and eventually fibroses fuses with adjacent lamellae or resorbs. If there are many telangiectatic lamellae, respiratory function will be impaired especially at higher temperatures when dissolved oxygen levels are lower and metabolic oxygen demand is high, but also, in such case further traumatized, rupture and fatal hemorrhage may supervene. Extensive telangiectasis takes considerably longer to resorb than hyperplastic lesions of the gill (Ronald, 1989). Bulging of secondary lamellae were observed in may species, Brachydanio rerio (Karlsson et al., 1985); (Channa marulius by Bijya, 2002); Anabas testudineus, (Santhakumar et al., 2001); Boleophthalmus dussumeri, (Kapilamanoj and Ragothaman, 1999); Labeo Rohita, (Jagadeesan and Mathivanan 1999) and Punitus stigma, (Khilleare and Davane, 1998).
Hyperplasia and fusion of gill filaments due to separation of epithelium reduces the surface area available for gaseous and other exchanges (Skidmore and Tovel, 1972). In fish, the respiratory epithelium is the barrier between the blood and the surrounding water through which respiratory exchanges take place (Narain et al., 1990). Hyperplasia of lamellar epithelium is generally due to an increase in numbers and migration of the malapighion cells of the primary lamella. Hyperplasia is a long term response of the malapighion cells, often to lower levels of irritations. Cells are principally derived from the primary lamellae. They migrate distally, often in the early stages resulting in an accumulation of cells at the leading edge of the secondary lamella, known colloquially as ‘clubbing’ of the lamellae. There may be an increase in numbers of mucous cells at the base of the lamellae. Eventually the intercellular space may be filled with new cells and the respiratory area greatly reduced (Ronald, 1989). Any damage to this epithelium affects not only ventilatory process but also other vital process like ion-exchange, secretary and excretory function of the gills (Narain et al., 1990; Bijay, 2002 and Sarita and Sudha, 2002).

Metal stress induced in the gill epithelium leads to events like increased influx of hydrogen ions which reduces the pH of the blood and thus decreases the oxygen carrying capacity of the hemoglobin (Suresh, 1992). On the other hand the ionoregulatory and excretory functions of the gills were hampered. Epithelial damage disturbs the exchange of ammonium and bicarbonate ions of the blood with sodium and chloride ions of the medium, which normally occurs across the gill epithelium of fish (Love, 1980).
Since gills are not only the respiratory but also the osmoregulatory organs of the fish, the cellular damage induced by cadmium might also impair the osmoregulatory function of fish as evidenced from the decreased oxidative metabolism uptake of vital ions and the associated ATPase activates which also could be one of the possible reasons for the death of the fish. Similar reason also suggested by Sushithra et al., 2007 for the death of the Heteropneustes fossilis on exposure to Cadmium chloride. Similar observations made in Rainbow trout, (Hollis, 1999), lates calcarifer (Thophon et. al., 2003), Cyprinus carpio and Oreochromis mossambicus (Coutinho and Gokhale, 2000), neotrophical fish species prochilodus lineatus (Marina and Claudia, 2007). The progressive dissolution of gill structure in the fish exposed to lethal concentration provides a good support for the progressive decrease in the soluble and structural protein levels in this organ.

In the sub lethal treatment of cadmium the changes in gills and the trend was totally different. On 1st and 5th day hyperplasia, fusion of gill epithelium due to separation of epithelium, necrosis of gill epithelium, degeneration of pilaster cells and telangiectatic in secondary lamellae were observed (Plate 3 Fig 3, 4, 5 & 6). Followed by slow recovery on 10th day of exposure (Plate 4 Fig 1, & 2) attributing to the decrease in concentration of cadmium and also longer days of exposure when the fish acclimatized to the surrounding water media. On 15th day of exposure gill structure showed maximum recovery (Plate 4 Fig 3, 4, & 5). Recoveries from virtually all proliferate or an oedematous lamellar lesion appears to be possible provided that adequate time
and water quality are maintained. The time scale required is often remarkably short. Fukuda (1983) and Suresh, (1992) have both shown complete recovery from severe rective hyperplasia in less than a month when the stimulus was removed. The progressive recovery in gill of *Labeo rohita* exposed to mercury has also been noticed (Jagadeesan and Mathivanan, 1999).

The changes appeared in the organs of fish, at initial periods of exposure to the lethal concentration of cadmium might be a part of defense mechanism, but on prolonged exposure the further accumulation of cadmium caused the condensation of the tissue nuclear material. Dubale and Shah, (1979) and Ooi and Law, (1989) reported that the appearance of atrophic or pyknotic changes in the nuclei of kidney tubules of *Mylio macrocephalus* increased over time of exposure to cadmium concentration. The degree of destruction appeared to be linearly proportional to the period of exposure, similar reports were made by Ramesh Mishra and Singh, (1997) in *Clarius batrachus* exposed to lead nitrate and dichromate.

During sub lethal treatment of cadmium, the extent of damage in all the organs of fish is considerably low compared to the damage observed in the lethal concentration. The slight damage at the tips of secondary gill lamellae with a mild degree of precipitation of mucus observed in the fish on day 1 indicate that the sub lethal concentration also affected the organ systems during initial period of exposure. However, the changes may be a part of defense mechanism. The maximum structural reorganization of gills of fish observed at day 10th and 15th of exposures supports the ability of the fish to resist the sub
lethal stress, and could repair whatever the damage caused to the vital organs, by enhancing the protein synthetic potentials and other associated activities of the cell.

The fish also have mild to moderate damages caused to the gills, kidney and liver at day 1 and 5 on exposure to the sub lethal concentration, as evident by the hypertrophy, necrosis, nuclear proliferation of gills, slight disarray of liver lobes, swellings of hepatocytes, hypertrophy and pyknotic nuclei of liver of the vital organs of the fish, Labeo rohita. But the fish slowly developed good resistance to the influx of lower doses of cadmium as in organs at day 10th and 15th. It appears that these animals vigor in order to detoxify or eliminate the accumulated cadmium. The recovery from the suppression of oxidative metabolism and the domination of protein synthesis might have facilitated them to activate the structural reorganization.

The liver of the fish does not show the diversity of pathology seen in higher animals probably as a result of the lack of kuffer cells in the liver sinusoids. However, it is susceptible to a number of toxic and metabolic differences. Acute and extensive necrosis of liver cells may occur in toxic condition (Ronald, 1978).

Liver is involved in the metabolism of most toxicants which can usually be detoxified, but many of them can be bioactivated and in turn becomes more toxic. The toxicology of liver is complicated by the variety of liver injuries caused. The liver has a high concentration of xenobiotic metabolizing enzymes, some of which activate the toxicants to induce lesions locally (Sastry and Rao,
Toxicants induced changes in the liver of fishes can be regarded as an index for the identification of pollution stress on fishes (Jayantha Rao et al., 1985). In the present investigation the appearance of degenerative changes in the liver of fish exposed to the lethal concentration of cadmium support the metabolic disorders observed in it. The disarrayed liver cords, vacoulation in hepatic cells, dilated sinusoids, coagulation of blood cells, serves degree of nuclear atrophy followed by the shrinkage of hepatocytes and dissociation of laminar structure suggest that the depletion in its glycogen reserves (Plate: 5 Fig 1 to 6; Plate: 6 Fig 1 to 6; Plate: 8 Fig 1 to 4).

The pathological changes in liver due to metals have been reported by a number of workers (Naidu and Ramamurthi, 1983; Natarajan 1982; Sastry and Gupta, 1978; Kapilamanoj and Ragothaman, 1999). These pathological changes may be associated with the accumulation of the metals (Camargo and Matinez, 2007). Giari et. Al., 2007 reported histopathology of liver exposed to cadmium. The changes in the liver was characterized by necrosis, hepatic cells lost their original shape, cell boundaries begin to rupture and disintegrate which lead to the formation of multinucleated giant cells. Similar responses were also observed in the fish subjected to anthropogenic stress (Hinton et. al, 1992). The necrosis of hepatocytes vacuolization and swelling of liver cords were noticed by some workers in different fishes treated with various toxicants. The liver of blue gills treated with methoxychlor showed cell vacuolization and swelling of liver cords. These changes were also noticed by other investigators in different fishes treated with various toxicants. The liver of blue gills treated with
methoxychlor showed cell vacuolization (Khan et al., 1994). The metoxyethyl mercuric chloride treated Channa punctatus showed vacuolization of hepatocytes, necrosis, and rupture of cell membrane (Sastry and Rao, 1983). Olojo et. al., 2005 reported vacoulation of connective tissue and grouping of hepatocytes culminating in focal necrosis, etc in Clarius gariepinus on exposure to lead,. Basanta Kumar Das and Subhas Chandra Mukherjee, 2000 observed dilation of sinusoids, deformation of hepatic cells and necrosis in Labeo rohita on exposure to hexachlorocyclohexane. Battacharya et al., (1975) reported swollen liver cells with irregular surface in Clarius batrachus exposed to various concentration of indexin. The cells were either binucleated or the nucleus was enlarged. Degenerative changes were shown by rupture and vacoulation of hepatic cells; some times with the appearance of inter cellular spaces indicating a severe necrotic condition. The damage to liver was more in the fishes at higher concentrations of cadmium but the damage at lower concentration was not significant.

Suresh (1992) stated that the concentration of cadmium is more important in bringing the histological changes in liver of fish; hence these changes could be used as a tool for assessing the toxic effects of the cadmium in aquatic environment. The differences in the degree of liver damages noticed in the concentrations of the pesticide in the present study may be due to its mode of action, accumulation, persistence and concentration.

Cadmium exposure induced marked abnormalities in the kidney initiated with disruption of tubular organization. Thereafter degeneration of tubular
epithelial cells and lymphocytic infiltration was evident. Most of these pathological changes persisted with vacoulation, clotting of blood in some sinusoids and glomerular degeneration.

Cadmium accumulates preferentially in the kidney tissues when the body burden of Cadmium increases, new proteins such as metallothionein are synthesized in the liver and kidney (Ooi and Law, 1989). The membranous organelles, such as mitochondria, endoplasmic reticulum and nuclear envelope, are most easily affected by cadmium in which disorganization, rearrangement and malfunction may occur. Thus, the proximal tubules which posses numerous mitochondria rather than the distal tubules are easily damaged by cadmium. The collecting ducts are usually more resistant to Cadmium exposure. The injuries to collecting ducts are only obvious in the fish exposed to higher concentration of cadmium.

The appearance of atrophic or pyknotic nuclei in fish kidney increases with the increase of time course. The phenomenon of nuclear changes in fish is probably similar to that found in other animals. It has been suggested that a nuclear and nucleolar changes are induced preceding a trophy and necrosis of cells in other animals. At the beginning, the change may probably form part of a defuse mechanism, leading to defuse an activation of synthetic or other activities in the cell, such as synthesis of metallothionein. However, during prolonged treatment, further accumulation of cadmium causes a condensation of nuclear material to form rarely stained pyknotic nuclei.
Leucocytes are common in the interstitial space of control fish, but they are rarely aggregated so densely and abundantly as in cadmium treated renal tissue. The increase of leucocytes may have been an inflammatory response to cadmium. Leucocytes may either remove or engulf injured and non functional cells. The dilation of the lumen of the kidney tubules, degeneration in the haemopoitic tissue rupture in the collecting tubules and necrosis as observed in the present investigation. Gupta and Dalela (1987) reported degeneration and dissolution of epithelial cells of renal tubules and hypertrophy and necrosis of renal cells of the kidney of *Notopterus notopterus* exposed to sub lethal concentrations of phenolic compounds. Similar observations were made by Konar (1977) in *Heteropeustes fossilis* and *Labeo rohita* chronically exposed to DDVP, Phosphamidon. The deformation of renal tubules was observed (Bakthavathsalam et al., 1984) in *Anabas testudineus* chronically exposed to furadon. Rashtwar and Ilyas (1984) reported the histolopathological changes in kidney to lead to cloudy swelling of renal tubules in *Nemachellus denisoni* acutely exposed to phosphamidon. In the present study also the swelling of renal tubules in acute exposure was evident. Changes like vacoulation of epithelial cells of renal tubules and pronounced enlargement of the tubules were observed at higher sub lethal concentration and prolonged exposure to cadmium.

Necrosis and vacoulation were observed by Dhanapakiam and Premalatha (1994) in *Cyprinus carpio* exposed to malathion. Sastry and Sharma, (1979) observed a number of striking changes in the histological
structure of the kidney of *Channa punctatus* exposed to sub lethal concentration of endrin. Konar, (1979) observed shrinkage and degeneration of glomerulus and vacoulation of tubules in carp chronically treated with hepatochlor. Vinod Ghanathay, (1989) studied histopathological changes in the kidney of *Channa punctatus*, exposed to BHC. He noticed that, the glomeruli were shrunken, slightly vacuolated, cloudy swelling and hydropic degeneration of interstitial tissues. The tubular epithelium was fibrosed.

The changes appeared in the organs of the fish at the initial period of exposure to the lethal concentrations of cadmium might be a part of defense mechanism, but on prolonged exposure the further accumulation of cadmium causes the condensation of the tissue nuclear material there by the synthetic ability of the metallothionein is repressed and the free cadmium ions destroyed the organ structure. The degree of destruction appeared to be linearly proportional to the period of exposure which is also reported by Dubale and Shah (1979, 1981b) in *Channa punctatus* exposed to cadmium concentrations. Ooi and Law (1989) reported that the appearance of atrophic or pyknotic changes in the nuclei of kidney tubules of *Mylio macrocephalus* increased over time of exposure to cadmium concentrations. Paulose (1985) also suggested that the accumulation of mercury and morphological changes in the gills of *Labeo rohita* were time dependent.
In the sublethal concentrations of cadmium in the fishes show a mild to moderate damage caused to the gills, kidney, liver at 1 and 5 days of exposure as evident by the hypertrophy, necrosis, nuclear proliferation of gills, tubular necrosis, vacuolization in parenchymatous cells of haemopoietic tissue and dilation of glomeruli of kidney, slight disarray of liver lobes, swelling of hepatocytes, glomerulization of cytoplasm, hypertrophic and Pyknotic nuclei of liver and fibrillation and thinning down of muscle fibers indicate moderate structural disorganization of the total organs of the fish Labeo rohita. But even the fish slowly developed good resistance to the influx of lower doses of cadmium as observed from the histological sections of their organs at 10th and 15th days. It appears that, these animals rendered their structures to gain the original vigor in order to detoxify or eliminate the accumulated cadmium. The recovery from the suppression of oxidative metabolism and the domination of protein synthesis might have facilitated them to activate the structural reorganization. For the synthesis of cadmium binding proteins might have prevented the metal ions to intervene with structural dissolution. On the whole the lethal concentration of cadmium caused irreversible damage to the organs of the Labeo rohita. This is considerably worse in the organs of the fish. Whereas the sublethal concentration though caused initially a mild damage to the organs of the fish but on prolonged exposure these animals could develop enough resistance and replenish the loss by
activating the protein synthetic machinery and energy cycles. The replenishment however seem to be slow in the organs and not to the full extent. Thus the changes induced by cadmium in the structure of the organs of the *Labeo rohita* are only dependent on the concentration of metal.

In view of the literature cited above, it is apparent that in the present investigation, cadmium at both the lethal and sub lethal concentration caused considerable histological damages to the organs studied and extend support to the earlier mentioned alterations in hematological aspects, ions and carbohydrate metabolism however, intensive studies in these aspects are required to arrive at definite and meaningful conclusion.
Plate-1

Fig 1 and 2: Section of gill of control, *Labeo rohita* showing normal structure. H & E X 400.

PGL = Primary gill lamellae
SGL = Secondary gill lamellae
ILS = Inter lamellar space

Fig 3 and 4: Section of gill of fish, *Labeo rohita* exposed to cadmium for 1 day, showing hyperplasia (HP) and formation of telangietatic tissues (T), lamellar fusion and lamellar clotting. H & E X 400.

Fig 5 and 6: Section of gill of fish, *Labeo rohita* exposed to cadmium for 72 h, showing more telangietatic secondary lamellae (TSL). H & E X 400.
Plate 2

Fig 1 and 2: Section of gill of fish, *Labeo rohita* exposed to cadmium (15 mg/L) for 96 h, showing more telangiectatic secondary lamellae (TSL), Hyperplasia (HP) and Atrophy (A). H & E X 400.

Fig 3 and 4: Section of gill of fish, *Labeo rohita* exposed to cadmium (15 mg/L) for 96 h showing talaengiectic secondary lamellae (TSL) and lamellar fusion (TF). H & E X 400.

Fig 5: Section of gill of fish, *Labeo rohita* exposed to cadmium (15 mg/L) for 96 h showing single filament with talaengiectic secondary lamellae (TSL). H & E X 400.

Fig 6: Section of gill of fish, *Labeo rohita* exposed to cadmium (15 mg/L) for 96 h showing all gill filaments with talaengiectic secondary lamellae (TSL). H & E X 400.
Plate 3

Fig 1and 2: Section of gill of control fish, *Labeo rohita* showing normal structure. H & E X 400.
PGL= primary gill lamellae
SGL= Secondary gill lamellae
ILS= Inter lamellar space

Fig 3and 4: Section of gill of fish, *Labeo rohita* exposed to cadmium (3 mg/ L) for 1 day showing loss of gill lamellae and damage of basal gill filaments (BGF). H & E X 400.

Fig 5and 6: Section of gill of fish, *Labeo rohita* exposed to cadmium (3 mg/ L) for 5 day showing bulging of secondary lamellae tips, hypertrophy (HT), lamellar fusion (LF) and atrophy (A). H & E X 400.
Plate 4

Fig 1 and 2: Section of gill of fish, *Labeo rohita* exposed to cadmium (3 mg/L) for 10 day showing lamellar fusion (LF), Hyperplasia (HP) and shortening of secondary lamellae. H & E X 400.

Fig 3 and 4: Section of gill of fish, *Labeo rohita* exposed to cadmium (3 mg/L) for 15 day showing recovery in structure of gill filament. H & E X 400.

Fig 5: Section of gill of fish, *Labeo rohita* exposed to cadmium (3 mg/L) for 15 day showing view of recovery tendency in the entire gill filament. H & E X 100.
Plate 5

Fig 1 and 2: Section of liver of fish, *Labeo rohita* exposed to cadmium (15 mg/L) for 48 h showing Hyperplasia (HP) and damaged blood vessels (BV). H & E X 400.

Fig 3 and 4: Section of liver of fish, *Labeo rohita* exposed to cadmium (15 mg/L) for 72 h showing hyperplasia (HP) and nucleus (N). H & E X 400.

Fig 5 and 6: Section of liver of fish, *Labeo rohita* exposed to cadmium (15 mg/L) for 96 h showing formation of vacuolization (VZ), damaged blood vessels and hyperplasia (HP). H & E X 400.
Plate 6

Fig 1 and 2: Section of liver of fish, *Labeo rohita* exposed to cadmium (15 mg/L) for 96 h showing vacuolization of hepatocytes (VZ) hypertrophy (HT). H & E: X 400.

Fig 3 and 4: Section of liver of fish, *Labeo rohita* exposed to cadmium (15 mg/L) for 96 h showing vacuolization (VZ), atrophy cells (A), degenerated blood vessels (BV) and diffused necrosis (NC). H & E: X 400.

Fig 5 and 6: Section of liver of fish, *Labeo rohita* exposed to cadmium (15 mg/L) for 96 h showing Severe necrosis (NC), lymphatic infiltration and vacuolization of hepatic cells (VZ). H & E: X 400.
Plate 7

Fig 1 and 2: Section of liver of fish, *Labeo rohita* exposed to cadmium (3 mg/L) for 1 day showing slight necrosis (NC), damage of blood vessels (BV) and vacuolization of hepatic cells (VZ). H & E: X 400.

Fig 3 and 4: Section of liver of fish, *Labeo rohita* exposed to cadmium (3 mg/L) for 5 day showing diffused necrosis (NC), Cytoplasmic degeneration, severe damage of blood vessels (BV) and vacuolization of hepatic cells (VZ). H & E: X 400.

Fig 5: Section of liver of control fish, *Labeo rohita* showing normal structure.

Plate 8

Fig 1 and 2: Section of liver of fish, *Labeo rohita* exposed to cadmium (3 mg/L) for 10 day showing less damage of Hepatocytes, hepatic cord and blood vessels. H & E: X 400.

Fig 3 and 4: Section of liver of fish, *Labeo rohita* exposed to cadmium (3 mg/L) for 15 day showing a recovery liver structure. H & E: X 400.
Plate 9

Fig 1 and 2: Section of kidney of control fish, *Labeo rohita* showing. H & E: X 400.
P= proximal tubule
G= Glomerulus
BV= Blood vessel

Fig 3 and 4: Section of kidney of control fish, *Labeo rohita* showing enlarged proximal tubule (P) with Blood vessel (BV) and interstitial tissue (IT). H & E: X 1000.

Fig 4 and 5: Section of kidney of fish, *Labeo rohita* exposed to cadmium (15 mg/L) for 24 h showing enlargement of tubular lumen, vacuolization (VZ), necrotic material (N), and damage of proximal and distal tubule (P and DT). H & E: X 400.
Plate 10

Fig 1 and 2: Section of kidney of fish, *Labeo rohita* exposed to cadmium (15 mg/L) for 28 h showing necrosis of proximal tubule (N), Glomerulus shrinkage (G), Vacuolization (VZ), tubular degeneration. H & E: X 400.

Fig 3 and 4: Section of kidney of fish, *Labeo rohita* exposed to cadmium (15 mg/L) for 72 h showing maximum damages like necrosis of proximal tubule (N), vacuolization (VZ) and tubular degeneration. H & E: X 400.

Fig 5 and 6: Section of kidney of fish, *Labeo rohita* exposed to cadmium (15 mg/L) for 96 h showing degeneration of interstitial tissue (IT), tubular degeneration, necrosis of proximal tubule (N), and vacuolization (VZ). H & E: X 400.
Plate 11

Fig 1 and 2: Section of kidney of control fish, *Labeo rohita* showing normal structure.

P = Proximal tubule
G = Glomerulus
IT = Interstitial tissue
BV = Blood vessel
DT = Distal tubule.

H & E: X 400.

Fig 3 and 4: Section of kidney of fish, *Labeo rohita* exposed to cadmium (3 mg/L), for 1 day showing desquamation and degeneration of tubules, necrosis (N), vacuolization of tubules (VZ), damage to the proximal tubule (P), Interstitial tissue (IT). H & E: X 400.

Fig 5 and 6: Section of kidney of fish, *Labeo rohita* exposed to cadmium (3 mg/L), for 5 day showing degeneration of tubular epithelial cells, infiltration in interstitial space, tubular degeneration, necrosis of proximal tubule (N), vacuolization (VZ). H & E: X 400.
Plate 12

Fig 1 and 2: Section of kidney of fish, *Labeo rohita* exposed to cadmium (3 mg/L), for 10 day showing enlargement of tubular lumen, damage of proximal tubules and vacuolization of tubules (VZ). H & E: X 400.

Fig 3 and 4: Section of kidney of fish, *Labeo rohita* exposed to cadmium (3 mg/L), for 15 day showing recovery structure, cellular damages were reduced. H & E: X 400.