CHAPTER-X

HISTOPATHOLOGY

In this the present study of microanatomy of specific tissues/organs has been successfully exerting as an investigative tool in medical as well as in veterinary science since the first cellular investigations were carried out in the mid-19th century. The histological studies on a fish species is remarkable and auspicious 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. These structural changes of the organ/tissues at microscopic, cellular and organelle level lead to variations in functional systems (Jagadeesan, 1999). The histopathological changes are irreversible while altered functional systems are considered as a reversible effect. In any particular aquatic organism the nastiness of the histological damages is directly comparative to the absorption of a pollutant in the aquatic medium condution. 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 Tiwai, (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.

The effect of chemicals, pesticides and other pathogens on the structural
mechanisms of the living system and the ways in which cells and organs/tissues
retort to injury due to the mainly directed to Histopathology. A chemical or a
derivative acting directly on the cell most commonly 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.

In this way of study the degeneration is the most common symptom seen
within the cell or population of the cells and this may be produced by concentrated
blood and nutrient supply or endocrine deficiency. Intracellular accrual like proteins,
water and fat 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. In this way the gross necrotic changes are of three
types.

- **Liguentactine necrosis:** Resulting from rapid enzymatic digestion of cells.

- **Coagulative necrosis:** The loss of blood supply was the result of ischemia in this
  area.

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

  I Pyknosis: Nucleus is emaciated and also is very dark in nature. Rupture of the
  nuclear membrane and fragmentation of nuclear chromatin taking place.

  II Karyolysis: The categorized by diminishing and dissolving of nucleus leaving a
  ghost out line.
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. In the environment although the acute toxicity tests are used to safe absorptions of toxicants and 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 accumulate in the living organisms. In fish, the gills, kidney, liver are the primary target organs for the Cadmium (Giles, 1984). Due to its ability to accrue 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 in 1999 reported the metallic salts are talented of producing severe harm 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 harm 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 al., 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, and These changes lastly triggered the failure of the respiratory mechanism which caused into the death of fish on exposed to sub-lethal concentration of Cadmium compound. 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 in the gills of the same fish. The lethal effects of Cadmium on liver of freshwater teleost, Garra mullya (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.

In this study many histological modifications was 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 sub-lethal absorption of mercury (Akhilendar Naidu et al., 1983a). Most of the internal organs of higher vertebrate’s the heavy metal Cadmium is known to cause injury (Phillopotts, 1986) and the 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, kidney and pancreas and the important observations include cytoplasmic vacoulation, eocentric nucleas, rupture of cell membrane of hepatocytes in liver, rupture of tubular epithelium, degeneration of glomeruli in kidney on exposure to sub-lethal 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. Susitra 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 sub-lethal absorption of Cadmium chloride. Thophon et al., (2003) were also reported the same symptoms in Lates calcarifer in acute and sub-acute 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, crude effluent discharged from paper mills into receiving waters and pulp is known to be toxic to some aquatic organisms. Manifestation of toxicity in fish contain increase of parasites, fin necrosis, kidney tumors, change in physiology, neoplastic lesions in the liver and skin tumors (Khan et al., 1992; Lindesjoo 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 technique 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 technique changes in the intestine of the fish, Catla catla exposed to endosulfan. Anitha and Ramkumar, (1997 a & b) exposed to deteriorating 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 Ooli 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 physiological activities and metabolic process of the animals but such studies alone do not fulfill the complete understanding of pathological condition of tissues/organs under toxic stress. Therefore, it is useful to have a vision into histological analysis regarding the extent damage of the tissues like liver and kidney when Cadmium chloride enters the body *Cyprinus carpio*. The foregoing literature clearly documents that, heavy metals cause structural changes in different organs of freshwater animals. The histological studies on the organs of freshwater fishes subjected to Cadmium chloride in relation to the absorption of the heavy metal and the time period of exposure though these are not reported. As this line of research provides support for the physiological, biochemical and hematological alterations in the tissues/organs of the fish *Cyprinus carpio* exposed to Cadmium chloride stress.
10.1 RESULTS

10.1.1 Histology of Liver (Control)

The liver of fish comprises a continuous mass of large hexagonal hepatic cells (Hepatic parenchyma). Hepatic cells are of polygonal shape or roundish containing clear sphere-shaped nucleus and they are also located among sinusoids forming string like structures known as hepatic cell cords. In this type of fish species these structures are generally unclear in nature and the 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. The secretions of bile have many vital functions other than Hepatic cells and also they play an important role in carbohydrate metabolism, protein and lipid. They serve as storage site for some nutrients and the detoxification is another important role. A large number of blood sinusoids and lipid glycogen granules are found in the hepatic mass (Plate 3 Fig 5).

10.1.2 Histological Changes in the Liver of Exposed Fish

In this investigation the day 1 of exposure to the lethal absorption of Cadmium chloride, the liver of the fish showed vacoulation and enlarged nuclei in hepatic cells and finally in the liver strings were seen disarrayed (Plate 1 Fig 1 & 2). And day 2 of exposure the paranchymatous nature of the liver was greatly interrupted with blocked the blood vessels. The hepatocytes cell membranes were cracked and granular weakening was evident in most of the hepatocytes and Nuclei became slightly hypertrophic (Plate 1 Fig 3 & 4). On further exposure, day 3 Spartan degrees of atrophic changes were noticed in the liver strings and Hemorrhagic condition was protuberant with heavy vacuolization in the liver organs/tissue. At some regions exfoliation and congregation of hepatocytic nuclei and focal necrosis were seen (Plate 1 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 cytoplasmatic disintegration in hepatocytes on day 4 of exposure (Plate 2 Fig 1, 2, 3, 4, 5 & 6) and Fig 1 shows Photomicrographs of the liver of Cyprinus carpio.

And compared to the structure of the liver of control fish which exposed to sub-lethal absorption of Cadmium chloride firstly displayed few changes like slight
confusion of liver lobes, mild degree of degeneration of cytoplasm, occasional blood clots and congregation of nuclei at day 1 (Plate 3 Fig 1 & 2) and cloudy swelling of hepatocytes, granulization of cytoplasm, hypertrophic and pyknotic nuclei on day 5 (Plate 3 Fig 3 & 4 & 5). Though, 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 4 Fig 1 & 2). At 15 days of exposure, no substantial changes were seen different from controls, except a slight degree of hyperchromatic condition of the nuclei (Plate 4 Fig 3 & 4).

10.1.3 Histology of Kidney (Control)

In this experiment the basic unit of kidney in fish contains of a renal corpuscle, glomerulus, Bowman’s capsule and various segment of the renal tubules, namely proximal tubule, distal tubule, intermediate segment and collecting duct. Proximal tubules have projecting brush borders (Microvilli) covered in the vascular bed in the interstitial tissues. Distal tubules and collecting ducts, both devoid of brush borders and are sparsely distributed. The in-between and the segments between proximal and distal tubules are rarely seen and the renal corpuscles are located in close locality of renal tubules and blood vessels in the interstitial tissue and the pigments and leucocytes are very common in the interstitial tissue (Plate 5 Fig 1, 2, 3, 4 & 5).

10.1.4 Histological Changes in the Kidney of Exposed Fish

In this study shows that the lethal absorption of Cadmium chloride and the kidney exhibited the lessening in renal cell number in the proximal and the 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. The vacoulation due to degeneration of cytoplasm is quite obvious and the nuclei of epithelial cells have become quite dominant and are found insightful into the surrounding tissue. The puncture of kidney tubules is commonly observed and this kidney demonstrated hyperplasia, vacoulation, degeneration and necrosis leading to the complete necrosis. Cubiodal epithelial cells coating the tubules showed comprehensive vacoulation with degenerating cytoplasm and more nuclear division and their confused sprinkling in nature. The haemopoitic tissue was fully studded with lymphatic cells at the highest rate of nuclear division and the lumen of
the tubules was found to be expanded. The kidney tubules were also found to be perforated (Plate 6 Fig 1, 2, 3, 4, 5 and 6) and Fig 2 shows Photomicrographs of the kidney of *Cyprinus carpio*.

In sub-lethal concentration of Cadmium chloride the kidney of the fish displayed a mild degree of changes. On 1\textsuperscript{st}, 5\textsuperscript{th} and 10\textsuperscript{th} day shows more changes (Plate 7 Fig 1, 2, 3, 4, 5 & 6). 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 destruction of epithelial cells and the separations of the tubules were quite protuberant in nature, and the cell fragments could be seen inside the lumen of some tubules. The vacuolar degeneration was seen in the few tubules. Haemopoitic tissue was degenerated. But on 15\textsuperscript{th} day kidney showed recovery propensity (Plate 8 Fig 1, 2, 3 & 4). Glomerular cells reached normalcy in structure and the cytoplasm seemed clear and vacuolization and karyolysis of cell was completely reduced. Necrotic changes in uriniferous tubules were reduced and clumping of damaged blood cells was seen.
Fig. 1: Photomicrographs of the liver of Cyprinus carpio. [A] normal hepatic tissue, showing hepatocytes (H) blood sinusoid (BS) and central vein (CV); [B] hepatocytes hypertrophy (HH); [C] nuclear hypertrophy (NH); [D] cellular degeneration (CD) and blood congestion (BC); [E] nuclear pyknosis (NP) nuclear degeneration (ND) and bile stagnation (BS); [F] cytoplasmic vacuolation (CV) and blood congestion in sinusoids (BC); [G] cellular necrosis (CN) and blood congestion (BC); [H] melanomacrophages aggregate (M) close to a bile duct (BD).
Fig. 2: Photomicrographs of the kidney of *Cyprinus carpio*. [A] normal renal corpuscle showing the glomerulus and the Bowman’s space well defined (RC) renal tubules (RT); [B] glomerular expansion (GE) and dilation of Bowman’s space (BC); [C] increasing in the diameter of renal tubules (RT); [D] deterioration of glomerulus (G) and dilation of Bowman’s space (BC); [E] cellular degeneration (CD), melanomacrophages aggregate (M) with hemorrhage (H); [F] edematous fluid (ED), shrinkage of renal corpuscle (RC) and cellular degeneration in tubules (CD); [G] hyaline droplet degeneration and deformation in renal tubules architecture (HDD); [H] acute cellular degeneration and occlusion of the tubular lumen (CD).
Plate-4

Fig 1 and 2: Section of liver of fish, *Cyprinus carpio* exposed to Cadmium chloride (14 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, *Cyprinus carpio* exposed to Cadmium chloride (14 mg/L) for 72 h showing hyperplasia (HP) and nucleus (N). H & E X 400.

Fig 5 and 6: Section of liver of fish, *Cyprinus carpio* exposed to Cadmium chloride (14 mg/L) for 96 h showing formation of vacuolization (VZ), damaged blood vessels and hyperplasia (HP). H & E X 400.
PLATE 5

[1] VZ

[2] HT

[3] VZ

[4] BV

[5] VZ

[6] VZ
Plate-5

Fig 1 and 2: Section of liver of fish, *Cyprinus carpio* exposed to Cadmium chloride (14 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, *Cyprinus carpio* exposed to Cadmium chloride (14 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, *Cyprinus carpio* exposed to Cadmium chloride (14 mg/ L) for 96 h showing severe necrosis (NC), lymphatic infiltration and vacuolization of hepatic cells (VZ). H & E: X 400.
Plate-6

Fig 1 and 2: Section of liver of fish, *Cyprinus carpio* exposed to Cadmium chloride (2.8 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, *Cyprinus carpio* exposed to Cadmium chloride (2.8 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, *Cyprinus carpio* showing normal structure. H: Hepatocytes, N: nucleus, BV: Blood vessels. H & E: X 400.
PLATE-7

[1] H BV N

[2] BV

[3] H BV N

[4] N BV
Plate-7

Fig 1 and 2: Section of liver of fish, *Cyprinus carpio* exposed to Cadmium chloride (2.8 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, *Cyprinus carpio* exposed to Cadmium chloride (2.8 mg/ L) for 15 day showing a recovery liver structure. H & E: X 400.
Plate-8

Fig 1 and 2: Section of kidney of control fish, *Cyprinus carpio* showing. H & E: X 400.

P= proximal tubule
G= Glomerulus
BV= Blood vessel

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

Fig 5: Section of kidney of fish, *Cyprinus carpio* exposed to Cadmium chloride (14 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-9**

Fig 1 and 2: Section of kidney of fish, *Cyprinus carpio* exposed to Cadmium chloride (14 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, *Cyprinus carpio* exposed to Cadmium chloride (14 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, *Cyprinus carpio* exposed to Cadmium chloride (14 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-10

[1] IT G P BV

[2] IT DT

[3] BV P N

[4] VZ N IT

[5] R N VZ

Plate-10

Fig 1 and 2: Section of kidney of control fish, *Cyprinus carpio* 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, *Cyprinus carpio* exposed to Cadmium chloride (2.8 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, *Cyprinus carpio* exposed to Cadmium chloride (2.8 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-11

Fig 1 and 2: Section of kidney of fish, *Cyprinus carpio* exposed to Cadmium chloride (2.8 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, *Cyprinus carpio* exposed to Cadmium chloride (2.8 mg/L), for 15 day showing recovery structure, cellular damages were reduced. H & E: X 400.
10.2 DISCUSSION

In this present investigation 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 sub-lethal exposure to metals can carry a relationship between the level of accrual of the metal and to the animals various physiological and biochemical activities (Paulose, 1989).

In the present study, associated 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 aimed at 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 kidney and liver of the fish species and in this above the time of exposure to the toxic absorptions of Cadmium chloride 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 potency have occurred due to the failure of gaseous exchange across the respiratory epithelium. This is clearly evidenced from the present study by the drastic changes and the oxygen consumption was decrease in the rate 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). Rainbow trout exposed to zinc sulphate (Skidmore and Tovell, 1972); *Labeo rohita* exposed to mercury (Jagadeesan, and Mathivanan 1999). He also observed increased numbers of mucous cells in the secondary lamellae of the experimental fish species as compared to control fish, demonstrating the defensive response of the fish to the toxicant in this the pillar cell system also seemed to be collapsed pilaster and the columns were seen curled and the pools of overfilled blood have also seen with the sub-epithelial space. The collapse of the pillar cells system is supposed to occur when a fall in the hydrostatic pressure causes this system to fail as vascular endoskeleton (Bijya, 2002). Due to the formation of sub-epithelial space, the blood supply between the pilaster cells and epithelial lining as well as water balance is affected.

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 absorptions of mercury. (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. The lamellae was Solidifying due to irritation 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). And widespread telangiectasia takes significantly longer to resorb than hyperplastic lesions of the liver and kidney (Ronald, 1989). Bulging of secondary lamellae was observed in many 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 *Puntius stigma*, (Khillare and Davane, 1998).

Hyperplasia and fusion of gill filaments due to separation of epithelium reduces and the surface area available for gaseous and other exchanges (Skidmore
and Tovel, 1972). In fish, the respiratory epithelium is the wall between the blood and the surrounding water media and 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 and also 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). Epithelium cell damage in any of the organs this 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). On the other hand the ion regulatory and excretory functions of the gills were hindered and the epithelial damage disturbs the exchange of ammonium and bicarbonate ions of the blood with sodium ions 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 tissues of the fish species and the cellular injury and it has the tempted 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 Susitra 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). Fukuda (1983) have shown whole recovery from severe rective hyperplasia in less than a month when the stimulus was removed and the progressive recovery in gill of Labeo rohita exposed to mercury has also been noticed (Jagadeesan and Mathivanan, 1999).

The changes seemed in the tissues/organs of fish, at initial periods of exposure to the toxic concentration of cadmium might be a part of defense
mechanism, but on prolonged exposure the further accrual of Cadmium caused the concentration of the tissue nuclear material. Dubale and Shah, (1979) and Ooli and Law, (1989) reported that the presence 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 seemed to be linearly comparative to the time 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 chloride, 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 tissues/organ systems throughout early time period of exposure. However, the changes may be a part of defense mechanism. The maximum structural reorganization of liver 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 in the exposure period.

The fish also have mild to moderate damages caused to the liver and kidney at 1 day and 5 day on exposure to the sub-lethal absorption, 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 variety of pathology seen in higher animals perhaps 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. Extensive necrosis of liver cells may occur in acute toxic condition (Ronald, 1978).
Liver is involved in the metabolism of most toxicants which can usually be detoxified, but many of them can be bio-activated 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, 1983). 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 study the appearance of degenerative changes in the liver of fish exposed to the lethal absorption 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 tracked by the decline of hepatocytes and dissociation of laminar structure propose that the depletion in its glycogen assets (Plate: 1 Fig 1 to 6; Plate: 2 Fig 1 to 6; Plate: 3 Fig 1 to 5 and Plate: 4 Fig 1 to 4) of the liver tissue.

The pathological changes in liver due to heavy metals must have been stated by a number of workers (Naidu and Ramamurthi, 1983; Natarajan 1982; Sastry and Gupta, 1979; Kapilamanoj and Ragothaman, 1999). These pathological changes may be associated with the accumulation of the metals (Camargo and Matinez, 2007). 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., 1992). 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. Bhattacharya *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; sometimes 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. The concentration of Cadmium chloride 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 which induced noticeable anomalies in the kidney initiated with trouble of tubular organization. Thereafter deterioration of tubular epithelial cells and lymphocytic penetration was evident. Most of these pathological changes persisted with vacoulation, clotting of blood in some sinusoids and glomerular degeneration.

Cadmium chloride 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 (Ooli and Law, 1989). The membranous organelles, such as nuclear envelope, mitochondria, endoplasmic reticulum and are most easily affected by Cadmium in which inefficiency, reorganization and malfunction may occur. Consequently, the proximal tubules which possess numerous mitochondria rather than the distal tubules are easily damaged by Cadmium and finally 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 chloride.

In this present investigation the appearance of pyknotic or atrophic nuclei in fish kidney increases with the increase of time course. The phenomenon of nuclear variations in fish is probably similar to that found in other animals. It has been suggested that a nuclear and nucleolar variations are induced foregoing 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. (Plate: 5 Fig 1 to 5) which shows the control day 1 to 4 and (Plate: 6 Fig 1 to 6, Plate: 7 Fig 1 to 6 and Plate: 8 Fig 1 to 4) which shows in lethal and sub-lethal groups of the kidney. However, during prolonged treatment, further accumulation of Cadmium causes a condensation of nuclear material to form rarely stained pyknotic nuclei.

The leucocytes are common in the interstitial space of control fish, but they are rarely combined so thickly and plentifully as in Cadmium treated renal tissue and it also increase of leucocytes may have been an inflammatory response to Cadmium and the leucocytes may either remove or engulf injured and non-functional cells. The dilation of the lumen of the kidney tubules, deterioration in the haemopoietic tissue break in the collecting tubules and necrosis as observed in the present investigation. Gupta and Dalela (1987) stated that the dissolution and degeneration 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 Heteropheustes 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 histopathological 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 chloride.

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 early time period of exposure to the lethal concentrations of Cadmium chloride strength and be a part of resistance appliance, but on prolonged exposure the further accrual of Cadmium causes the strengthening 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 obliteration seemed to be linearly comparative to the period of exposure which is also reported by Dubale and Shah (1979, 1981b) in Channa punctatus exposed to Cadmium concentrations. Ooli 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 (1989) also suggested that the accumulation of mercury and morphological changes in the gills of Labeo rohita were time dependent.

In the sub-lethal absorptions of Cadmium chloride in the fishes show a mild to moderate damage caused to the liver, kidney at 1 and 5 days of exposure as evident by the hypertrophy, necrosis, nuclear proliferation of gills, tubular necrosis, vacuolization in parenchymatous cells of haemopoitic tissue and dilation of glomeruli of kidney, minor disorder of liver lobes, swelling of hepatocytes, glomerulization of cytoplasm, hypertrophic and Pyknotic nuclei of liver and fribillation and thinning down of muscle fibers indicate moderate structural disorganization of the total organs of the fish Cyprinus carpio. But even the fish slowly developed good resistance to the influx of lower doses of Cadmium chloride 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 chloride. 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 chloride binding proteins might have prevented the metal ions to intervene with structural dissolution. On the whole the lethal concentration of Cadmium chloride triggered unalterable injury to the organs of the Cyprinus carpio. This is considerably worse in the organs of the fish. Whereas the sub-lethal 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 chloride in the structure of the organs of the *Cyprinus carpio* are only dependent on the concentration of metal.

In view of the literature cited above, it is apparent that in the present investigation, Cadmium chloride 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.