Chaper 8

HISTOPATHOLOGY

8.1. INTRODUCTION

The increasing input of xenobiotic into the environment has elicited growing concern regarding their possible ecological impact. A particular problem with heavy metals is their persistence. They cannot be eliminated from aquatic ecosystem, but persist in sediments, from where they are slowly released into the overlying water. The suspension feeding bivalve molluscs accumulated and concentrate most of the pollutants within their tissues to the concentration significantly above ambient levels in the environment, thus facilitating accurate chemical analysis and assessment. The use of bivalve molluscs in particular mussels, as sentinel organisms for indicating levels of pollutants in coastal marine water has been established through various mussel watch programmes, (Bryan,1973, Philips,1976).

The term “pollution” implies a biological effect and if the levels of pollution are to be determined, biological techniques must be deployed preferably in concert with chemical measurements (Bayne,1989).

Pollution of aquatic ecosystem by chemicals used in industry and agriculture is increasing day by day. The histopathological studies on aquatic organisms is a noteworthy and promising field to understand the extent to which changes in the structural organization occurring in the organs due to environmental pollutants
(Palaniappan et al., 2002). Singh et al., (1990) studied about the environmental pollution and its effects on aquatic animals.

The histopathology is a promising field of research in aquatic toxicology (Anees, 1976) as it provides the real picture of the toxic effects of xenobiotics in the vital functions of living organism. The extent of histopathological damages induced in the test animals and the amount of cell damage in relation to concentration of toxicants are utilized in assessing the toxicity of pesticides and heavy metals. It is generally evident that structural changes are more serious than functional abnormalities. Moreover structural alteration is irreversible while altered function is considered as a reversible effect. The changes in the structure at cellular, tissue and organ level can be corrected with the functional alterations. Organs like gill, intestine, mantle, digestive gland and foot are the best suited ones for histopathological studies. (Couch, 1975).

The histological methods have been in use for assessing the effects of pollution on aquatic organisms, since such studies bear a direct testimony to the deleterious effects of toxicants (Hinton, et al., 1973). The gross pathological alterations in tissues, and mainly aid in determining the route of entry of a toxicant into organism by showing where pathological lesions have occurred. Further, histopathological examinations of tissues may also be used for the assessment of water quality in the environment.

Studies on the histology and histopathology have proved to be a very useful tool in assessing the pollutant induced injury to whole animal. Pathological changes caused by
various pollutants in the gills of some species of bivalves have been reported by Ansell, (1961), Stainken, (1975) and Barnes, (1980).

Bivalve molluscs especially mussels have been used internationally as a sentinel organisms for pollution monitoring programme because they concentrate pollutants of many kinds in their vital tissues (Tripp et al., 1984; Langston, 1986; Livingstone et al., 1988). Due to bio accumulative property, the bivalves have gained great importance as indicator organisms though numerous studies have shown the impact of heavy metals on the function morphology and biochemical aspects of selected tissues in a number of bivalve species (Lee et al., 1974; George et al., 1986; Bright, 1988; Langston and Burt, 1991; Regoli et al., 1991; Shah et al., 1988; Amanulla Hameed, 2005).

Srikala Pillai and Menon(1998) observed structural alterations in the hepatopancreas of *Perna viridis* exposed to histopathological changes in the hepatopancreas of *Penaeus duoraran*. Narayanan (1989) observed chromium induced changes in the hepatopancreas of *Scylla serrata*.

Although toxicant impairs the metabolic and physiological activities of the organisms, physiological studies alone do not satisfy the complete understanding of pathological conditions of tissues under toxic stress. Hence, it is useful to have an insight into histological analysis. The extent of severity of tissue damage is a consequence of the concentrative of toxicant and time dependence. Also the severity of damage depends on the toxic potentiality of particular compound or heavy metals accumulation in the tissues.
Hence, an attempt is made to study the histopathological changes of tissues of *L. marginalis* exposed to heavy metals.

**8.2. MATERIALS AND METHODS**

Specimens of *L. marginalis* with shell length 57± 5 mm were exposed to 30% sublethal concentrations (derived from acute toxicity tests) of copper, cadmium and synergistic metals for 30 days. On 30th day, fresh water mussel was taken out, sacrificed and the tissues of gill, foot, mantle and digestive gland were excised out. The complete autopsy was completed in less than 4 mts. The tissues were fixed in bouin’s fluid and then they were processed adopting the usual procedure (Gurr, 1959) and embedded in paraffin wax (58-60°C). Serial sections of 6 to 8 μm thickness were cut and de-parafined sections were stained with haematoxylin and counterstained with aqueous eosin for microscopic observation.

**8.3. RESULTS**

**8.3.1. Histology of normal fresh water mussel**

The histological studies of organs like digestive gland, gill, mantle and foot were done after exposing the mussel to sub lethal concentration for a period of 30 days. The histology of the selected organs of the mussel in control experiment and mussels exposed to heavy metals were observed. In addition the synergistic effects of heavy metals were observed.
Digestive gland

The digestive gland of *L. marginalis* consists, as in other bivalves, of numerous blind ending tubules which communicate with the stomach by partially ciliated main ducts and non-ciliated secondary ducts. The non-ciliated cells of main ducts are characterized by high and dense microvilli and a strong pinocytotic activity. Ciliated and non-ciliated cells have a very similar fine structure. The digestive tubules have a large lumen and contain digestive cells at different phases of absorption, digestion and excretion (Plate-3, fig 12).

Gill

The gills of bivalves are plate-like structures, formed by the fusion of successive branchial or gill filaments. The normal gill filaments of *L. marginalis* showed ciliated epithelium surrounding water tube and blood sinus. The gill filaments are joined to one another by horizontal bars called inter- filamentar junction. Two lamellae of a lamina are joined together by inter - lamellar junction. The inter - lamellar junctions between two lamellar divide the space into distinct compartments called water tuber (Plate-5, fig-16).

Mantle

In control mussel, the mantle tissue comprised of muscular and connective tissue network. The columnar epithelium covered the mantle, an inner ciliated epithelium containing mucus-secreting cells and nacre secreting cells (Plate-7, fig 20).
Foot

The foot of *L. marginalis* is mottled brown due to pigments in the outermost epithelium. The epithelium contain subepithelial mucus cells, centrally bundle of muscles was observed. In between the muscle bundles the elastin cells were noted. The byssal thread had elastic properties resulting from the secretions (Structural proteins) from two types of glands, elastin and basal. Most of the secretions are from the centrally placed, conspicuous, compact elastin gland formed of pyriform cells that secrete the elastin type of structural protein. The basal glands are responsible for the synthesis of a keratin – type protein, which is intermixed with phenolic protein (Plate 9, Fig. 24).

8.3.2. Histological alterations in Cadmium treated fresh water mussel:

Digestive gland

Several pathological changes were noticed in the digestive gland of cadmium treated mussel. The cytoplasm of the cells was disintegrated and due to its absence, the cells became empty and looked like vacuoles. The cell membranes in most of the cells were ruptured. The cells were found to be dislodged and partial or complete disintegration (Plate-3, fig-13).

Gill

In sublethal exposure of cadmium, the gill of *L. marginalis* showed marked histological changes such as sloughing of basal epithelium, damaged epithelial cell and swollen of lamellae with haemocytes. Vacuolization of epithelial cells were found in the 30 days of 30% SLC treated mussel (Plate.5, fig.17).
Mantle

Marked toxic effects were observed at the structural and cellular level in the mantle. The surface epithelium of mantle in the treated mussels revealed focal areas of necrosis, extensive desquamation of epithelial cells and in some areas signs of tissue regeneration (Plate-7, fig 21).

Foot

Cadmium induced severe damages in foot of *L. marginalis*, shrinkage of outer epithelium, damaged muscle bundles and decreased number of elastin cells were observed. The cells were found to be dislodged and partial or complete disintegration. (Plate 9, Fig. 25)

8.3.3. Histological alterations in Copper treated fresh water mussel

Digestive gland

Mussels which were exposed to 30% SLC of copper for 30days, showed tubular and cellular damage. Heavy vacuolization of cells was noticed. Gastric tubules contained enlarged vacuolated cells to the extent of obliterating the lumen. The digestive cells of the digestive gland were significantly reduced in height (atrophy) and exhibited reduced lysosomal membrane stability. (Plate-4, fig-14).

Gill

Copper induced severe damages in gills. Clumping and enlargement of filament, sloughing of basal epithelium, damaged epithelial cells and swollen lamellae with
haemocytes were observed. Higher degree of hypertrophy of gill lamellae and loss of inter lamellar junctions were also noticed (Plate-6, fig-18).

Mantle

Appreciable changes were noticed within 30 days of 30% sub lethal exposure of copper in mantle. In some areas, focal proliferation of the epithelial cells and appearance of increased number of mucus secreting cells were noted. The shrinkage of outer columnar epithelium was also observed (Plate-8, fig-22).

Foot

In sublethal exposure of copper, the foot of *L. marginalis* showed marked histological changes. Several necrotic lesions in the epithelial layer, disintegrated muscle bundles and loss of subepithelial mucus cells were observed (Plate 10, Fig. 26).

8.3.4. Histological alteration in synergistic treated fresh water mussel

Digestive gland

The external lining mainly of collagenous layer showed shrinkage. Cells of digestive tubules were sporadically sloughed off. Increases in cell volume and damages to the epithelial lining were observed. Gastric tubules contained enlarged vacuolated cells to the extent of obliterating the lumen were also noticed in 30 days exposure for 30% SLC of copper and cadmium (synergistic) (Plate-4, fig-15).
Gill

The mussels exposed to 30% SLC (synergistic) for a period of 30 days showed, the vacuolization of epithelial cells, enlargement of gill filament, sloughing of basal epithelium and swollen of lamellae with haemocytes. (Plate-6, fig-19).

Mantle

Several pathological changes were noticed in the mantle of synergistically treated mussel. The surface epithelium of mantle revealed focal areas of necrosis and appearance of increased number of mucus secreting cells. In some areas, focal proliferation of the epithelial cells was also noticed. The extensive desquamation of epithelial cells and in some areas signs of tissue regeneration were also seen (Plate – 8, fig 23).

Foot

In the mussel, *L. marginalis*, a marked ruptured epithelium, erosion of plastin cells and degeneration of muscle bundles and loss of sub epithelial cells were noticed. (Plate 10, Fig.27).

8.4. DISCUSSION

Toxic effects are often due to physical changes in the tissue at the cellular or ultra-structural levels and can only be speculated upon, unless they are visualized. Histopathological studies with light microscopy and electron microscopy are necessary for the description and evolution of potential lesions in aquatic animals exposed to various toxicants (Meyers and Hendricks, 1985).
8.4.1. Histopathological lesions in the digestive gland

In mussels the digestive gland performs several function viz, intracellular digestion, secretion of enzymes, storage of glycogen, lipid and calcium (Barnes, 1982). Phillips, (1976) said that the digestive gland is the organ of accumulation of some heavy metals.

In the present study the digestive glands of the mussels exposed to 30% sublethal concentrations of cadmium, copper and synergistic organizations. The results of the present study indicate one such damage resulted in the digestive gland which is the chief metabolic organ (Kabeer *et al* 1977, Pirett *et al*., 1988, Turner & Miller 1989).

The normal structure of the tubule showed a sheath of collagen fibres covered with smooth muscle fibres forming a mesh work. This is the normal structure of digestive tubules of majority of bivalves (Sreekala pillai & Menon 1998, Shah *et al*., 2003). The digestive cells which occupy the internal lining of the tubules are large and are characterized by the presence of irregularly shaped empty vesicles. The lumen of the tubules is provided with internal dentations.

In the present study, collagenous layer had disintegrated exposing the muscular layer. The cells were found to be dislodged and partial or complete disintegration of cells (Plate 3 & 4, fig 13,14 &15). These findings were in agreement with the observations made by Sreekala pillai and Menon, (1998) in the hepatopancreas of green mussel *Perna viridis* and Shah *et al.*, (2003) in the digestive glands edible clam, *Mactra violacea*. The digestive cells of the digestive gland of *L. marginalis* were significantly reduced in height as reported in *Venus verrucosa* after 20 days exposure to PHC by Axuak *et al.*, (1988) and in *M. violacea* by Shah *et al.*, (2003) exposed to 21 days in TBTO. Further more several cellular alternations were recorded in the digestive gland of some heavy metals exposed *L. marginalis* including as an increase in cell volume and damages to the epithelial lining of the digestive cells (Bright & Ellis 1989, Vincente *et al* 1998, Mariaomez *et al.*, 1990).

In present study, heavy vacuolization of cells in the external lining mainly of collagenous layer showed shrinkage and gastric tubules contained enlarged vacuolated cells to the extent of obliterating the lumen (Plate 4, fig 15). These observations were similar in accordance with the observation made by Sreekala pillai and Menon (1998). The histological alterations observed in *L. marginalis* are related to the time of exposure and the dose of the contaminant (Clements *et al.*, 1980)

On the basis of the above findings, it is suggested that the histopathology of the digestive gland can be used as a reliable tool to assess the pollutant induced toxicity.
8.4.2. Histopathological Lesions in the Gill


Injury of gill epithelium in common response to extreme levels of environmental pollutants and other variables were discussed by Dalela et al., (1979). According of Venkatesan and Subramanian (2007) active secretion of mucous in gills depending on the concentration of pollution and exposure period.

Sloughing of gill epithelium, swollen of lamellae with haemcytes, loss of inter lamellar junction and hypertrophy (Plate 5 & 6, fig 17,18 & 19) were observed in the present study are conformity with report of Donde (2006) studied the gills of clam Gafrarium diverticatum exposed to WSF- Crude oil.

In the present study indicates the vacuolization, enlargement of gill filament and loss of inter lamellar junctions in the gills of L. marginalis exposed to cadmium, copper
and synergistic, which is conformity with Sukumaran (2006) in case of *Meretrix casta* exposed to heavy metals.

Necrosis and sloughing of the gill epithelium were noted in the *Crassostrea madrasensis* (Gijo Ittop *et al.*, 2006) whelk *Busycon canaliculatum* (Betzer and Yevich, 1975), Sunila (1986, 1988) has also reported the above changes in *M. edulis*. Impaired feed filtration by the gills can be suggested as a reason for the reduced feed intake observed in the heavy metals exposed animals. Since gills are involved in the respiration and gas exchange of the animals, the damage caused is likely to influence the respiration also.

### 8.4.3. Histopathological Lesions in the Mantle

The mantle structure of the normal animal resembled that of *T. gigas* (Norton and Jones, 1992). In the present study, the mantle revealed areas of necrosis in the epithelium and increased in the number of goblet cells. Focal proliferation of the epithelial cells was also noted (Plate 7 & 8, fig 21, 22 & 23). The present findings are in good agreement with the observation of Gijo Ittoop *et al.*, (2006) in *Crassostrea madrasensis*.

In the higher dose of heavy metals, extensive necrosis was observed and signs of regeneration were also noticed. These changes indicate that heavy metals produces mild to moderate irritation, which causes proliferative and secretory responses. The increased response of mucus secreting cells is reported in response to metal pollution (Viant *et al.*, 2002).
The physiological functions such as opening and closure of the shell valves and feed intake were found to be affected in metal exposed animals. The damages to the tissues will definitely have serious repercussions in other physiological functions also thus lowering their general health and defence capacity.

**8.4.4 Histopathological lesions in foot**

The foot is chiefly a locomotory organ and is adapted for burrowing. Mucous on the surface of the foot forms the mechanism by which the attachment disc adheres radially. Subepidermal mucus glands are one type of mucus gland functionally associated with the main byssal threat formed along the median groove, and a third in the large compact mucous glands, which have a functional relationship with the stub or anchoring device. In present study, loss of heavy metals exposed freshwater mussel. These findings were in agreement with the observations made by Anisa Banu *et al.*, (1976b) in the foot of green mussel *Perna viridis*.

The histology of the mussel foot in relation to the byssus and associated glands. Since there are three groups of mucus glands, each associated with a particular function, it could be argued that the subepidermal glands serve to adhere the distal disc to the substratum. The other two groups of mucus glands are associated with some mechanism in the final polymerization and compacting of structural protein. Tamarin *et al.*, (1974) speculated on the role of mucus secretions in adhesion and Waite (1983) commented that it is too early to appreciate fully how the attachment disc is adhesive to the substratum under water. Two types of glands were recognized in *M. edulis* by Brown (1956).
In the present study indicates the several necrotic lesions in the epithelial layer, disintegrated muscle bundles and Erosion of elastin cells in the foot of *L. marginalis* exposed to cadmium, copper and synergistic metal which is conformity with Bharathi and Ramalingam (1983) study of the foot of *P. viridis*, Waite (1985) reported in *M. edulis* Koha Shyamasundari and Hanumantha Rao (1988) observed in the *Perna viridis* and *Mytilopsis sallei*.