CHAPTER – IX
HISTOLOGICAL STUDIES

INTRODUCTION:

The most commonly used tools for determining the deleterious effects of pesticides/ pollution in fishes are histology and histopathology. In order to review the intensity of damage of the organs, histological study plays a significant role in toxicology. The gills of fishes are vital respiratory structures which are important in the homeostasis of the milieu interior.


Effects of Chromium on the respiratory and histopathological response of Catla catla were studied by Christy (1995), and histological impairment of gills were noticed. Influence of dyes on the histopathology of intestine of the fish Cyprinus carpio, reveals extensively damaged intestinal tissues, elongated and degenerated nucleus.

Histopathological abnormality and variations in the biochemical levels have been studied in the fish Puntius stigma exposing pesticides metasystox and endosulfan (Khillaere, 1992) and observed cellular damage and tissue abnormality treated fish than in metasystox.

Histopathological changes in the intestine, kidney and liver tissue were studied in the cat fish Mystus seengla exposed to ammonium sulphate and NPK fertilizers (Subramanium, et al, 2004)
Signs of toxicity are always preceded by the biochemical, physiological responses to the toxicants in any animal. Measuring these changes qualitatively and quantitatively provide valuable insights into the mechanism of toxicity. Hence a study was made to know the histological changes of gills, intestine and muscle under phenol and super phosphate stress.

The histology is associated with physiology of organism. However, the histological changes induced by the pollutants reveal the consequences to be faced by the fish. As there is no histological studies are available on the effect of phenol and super phosphate in an air-breathing fish, the present work is planned and carried out.

MATERIALS AND METHODS:

Healthy fishes were collected from nearby ponds and acclimatized for a week in the laboratory by feeding liver. 25 fishes were isolated and separated into 5 groups. Two sets were given 1ppm and 2ppm doses of phenol and the other 2were given 1ppm and 2ppm doses of super phosphate. One group was kept as control. The experiment was continued for 60 days. After 60 days, the gills and gut were separated and preserved in 5% formalin and then processed for histological studies.

The gills, and gut were carefully removed and immediately transferred to Bouin’s fluid and kept overnight. Then they were washed in running water. Then the specimens were dehydrated through grades of alcohol and then cleared in acetone and benzene. Then the specimens were mounted in paraffin wax. Then serial sections were made at 6 /u thickness. Then they were stained with routine staining process in hematoxylin and eosine. Then they were made permanent mount in DPX mountant and observed under microscope. The selected views were photographed by using Nickon coolpix digital microphotographic unit and the variations in micro-architecture of cells were described.
RESULTS AND DISCUSSION:

Toxicity of phenol and super phosphate on the cells in the gills and intestine were studied and the histological observations were recorded.

Gills:

The micrograph of gills in control (fig:9.1), fishes treated with phenol (fig:9.2) and superphosphate (fig:9.3) are presented. This reveals that phenol has adversely influenced the micro-architecture of gills. While superphosphate did not induce such changes. The impact of phenol caused damage and rupture in the epithelial wall of secondary lamellae. The pillar cells were displaced with vacuolization in some of them. Separation of respiratory epithelium from the pillar cells, at few places observed. Degenerative changes were also observed in blood capillaries and epithelial cells. Similar observations were recorded in Barbus ticto (Jadhavi, Joshi, and Kulkarni, 2004).

Wrinkling of cell membrane, vaculization, thickening of cell membrane, disappearance of cell membrane etc were noticed (Kapila manoj and Ragothaman, 1999). Large amount of mucous exudation, necrosis of the secondary gill lamellae, decrease the respiratory surface area, oedaema at the tip of secondary lamellae under pollution stess (Narayan Chandra Rooj, 1993).

Studies in Channa gachua on exposure to industrial effluent reveals swollen pillar and epithelial cells and pillar cells showed hyperplasia but no nuclear psychosis and hemorrhage. In 50% Conc. significant charges were recorded as the epithelial hyperplasia and shrinkage in inter-lamellar space (Sonawane, et al., 2004).
The fish exposed to 0.13 ppm of endosulfan for 24 hours showed that the epithelial walls of secondary lamella were damaged, the secondary lamella was ruptured, and the pillar cells were displaced with vacuolization in some of them. The inter-lamellar distance was decreased. The separation of respiratory epithelium from the pillar cells at certain places was observed. Marked degenerative charges were noticed in pillar cells, blood capillaries, epithelial cells and blood cells in different regions of gill filament, debris of damaged blood cells were observed (Jadhavi, Joshi and Kulkarni, 2004).

Large amount of mucous exudation, necrosis of the secondary gill lamellae, decrease the respiratory surface area, oedema at the tip of secondary lamellae under pollution stess (Narayan Chandra Rooj, 1993) were studied. In the present study also phenol treated *Channa punctatus* showed mucous exudation. The gills have shown that the epithelial walls of secondary lamellae were damaged and ruptured. The pillar cells were displaced with vacuolization in some of them. Separation of respiratory epithelium from the pillar cells, at few places were observed. Marked degenerative changes were observed in pillar cells, blood capillaries, epithelial cells and blood cells in treated fishes of *Barbus ticto* (Jadhavi, Joshi, and Kulkarni , 2004).

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The results of the present study reveals that the action of phenol is similar to pesticides/ insecticide toxicity. Destruction of respiratory epithelium was observed in many fishes which were clearly observed in treated samples. When compared with these, the phenol treated gills shown distractions of epithelial layers. However the fishes treated with superphosphate do not show such degenerative changes. Hence it
reveals that the toxic nature of phenol and the non-toxic nature of lower concentrations of superphosphate.

**Intestine:**

In the intestinal wall, damaged tissues were observed in phenol treated tissues. The muscle fibres showed some alterations or damages in the treated tissues. In the fishes treated with superphosphate, no such adverse changes were found.

Heavy metals exposure cause meaningful results and the intensity of changes are hyalinization, hepatocyte vacuolation, cellular swelling and congestion of blood vessels (Van Dyke *et al*, 2006). Bhakthavatsalam *et al.* (1983) studied histology of intestine of the fish *Anabas testudineus* exposed to furadon and observed vacuolization and dilation of sub-mucosal cells, necrosis of mucosal folds and severe damage to sub mucosal region.

In the present investigation similar changes in the sub-mucosal cells and mucosal folds were found in fishes treated with phenol (Fig:9:5). Shrinkage of cells were also observed in phenol treated fishes. On the other hand, the fishes treated with superphosphate does not show any degenerative changes (Fig:9.6) which is similar to control (Fig:9.4). This reveals the harmless or non-toxic nature of superphosphate and toxic nature of phenol on *C. punctatus*.