

**Chapter – VIII**  
**General Summary and**  
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### General Summary and Concluding Remarks

Pollution of surface waters with sewage, combined liquid waste discharged from all domestic, municipal and industrial wastes is now very common. Sewage present in present day civilization contains appreciable amounts of detergents. Pollution in river water change the physical, physico-chemical properties of water, destroy the biological balance of self purification and closely linked with bacteria, algae and protozoans.

Phosphorus is the primary nutrient that limits the growth of algae and plants. Excessive phosphorus in a fresh water system increases plant and algal growth, which leads to changes in number of types of plants and animals leading to increase in turbidity and losses of oxygen culminating into the problem of excessively high productivity. The phosphorus more than 2 mg/l in open waters gives the sign of organic pollution. Hence phosphate as a toxicant was selected for present investigation.

The aim of the present investigation was to evaluate the acute toxicity range and LC50 for Channa striatus against phosphate concentrations.

The brief survey of the fish species used in toxicological research showed that this fish has never been used in the toxicological studies previously. Channa striatus is abundantly available in river Krishna and used as a food by the surrounding population. Therefore, this fish species was selected for present investigation. To evaluate pathogenic conditions in fishes exposed to higher doses of phosphate for prolonged periods widely accepted histopathological methods

and simultaneously histochemical techniques were employed to understand effects of phosphate giving direct evidence through microphotograph.

The survey on the existing literature showed that first response of fish to toxicants is produce large amounts of mucoid substances all over the body and within the various organ systems. Less attention has been paid to alimentary canal and target organs of control and intoxicated fish. Therefore extensive research on stomach, liver, kidney and gill for mucosubstances had undertaken.

The reasons that lead us to undertake the present investigation are describe in introduction and review of literature of the previous work on hydrology and influence of toxic substances including phosphate, pertaining to histological, histopathological and histochemical studies on the target organs, stomach, liver, kidney and gill are incorporated in the respective chapters.

#### **Hydrological studies :**

Hydrological studies on the water of river Krishna at station Mahuli near Satara city were conducted monthly for the period May 2007 to April 2008 at stations A, B, C and D mainly to know the status of water quality of the river water. The station B where sewage mixes with river water was important in present investigation.

Chaper III includes the observations and results of the hydrological studies carried out on temperature, transparency, electrical conductance, pH, dissolved oxygen, free CO<sub>2</sub>, hardness and total alkalinity. Total solids and total dissolved solids and phosphate concentration are recorded and graphically presented.

From all above hydrological studies it was evident that especially water at Station B in the river Krishna near Satara city where sewage is dumped in is highly polluted. The other stations A, C, and D are comparatively having less pollution. The water from river Krishna at station B the phosphate content is with in a range of 5.15 mg/l to 8.40 mg/l indicating phosphate pollution. The water from river Krishna thus have detrimental effect on flora and fauna in it especially edible common fishes like Channa striatus.

Such changes in the physico-chemical characters of water might be responsible for suffocation and death in fishes and increasing the toxicity of phosphate which is responsible for causing serious injury to vital organs and causing death.

#### **Dose response relationship : LC<sub>50</sub>**

Static bioassay of determining LC<sub>50</sub> to understand dose response functions of phosphate for adult Channa striatus performed. The LC<sub>50</sub> value of phosphate for adult is 0.015 M at 72 hrs.

#### **Histology, Histopathology and Histochemistry of stomach, liver, kidney and gill of Channa striatus :**

A critical survey of the literature shows that several studies have been performed to assess histopathologica and histochemical alterations especially due to pesticides, metals and phenols. Meagre work is done especially on phosphate toxicity on the target organs such as stomach, liver, kidney and gill.

Therefore the present investigation on various organs of freshwater fish Channa striatus has been undertaken to assess effects of phosphate on mucosubstance elaboration; histology and histopathology. It provides information

on the cellular localization of various mucosubstances and their alteration during stress environment of phosphate intoxication.

### **Stomach :**

The stomach, basically an organ meant for storage and mastication of food component. Stomach is generally sac like and thick walled in carnivorous and predatory fishes like Channa striatus.

The histological studies on the control fishes in the present investigation revealed that innermost mucosa is lined by columnar epithelium raised into several primary and secondary folds forming rugae. Numerous gastric glands of simple tubular type are observed below the epithelium and open in the lumen of the stomach. Gastric glands form many layered structure. Mucosa is followed by lamina propria, submucosa, muscularis and serosa. Both lamina propria and submucosa are highly vascularized. This histological structure agrees with histological structures described in other fishes. The surface mucous cells are more closely resemble with the secretory cells in the gastric glands of amphibians.

The histopathological changes in the stomach of Channa striatus in the present study were phosphate dose dependent and at different time intervals. The histopathological changes include degenerative effect, proliferation, disintegration and necrosis of mucosa, submucosa and serosa. The rugae tend to become flattened and there is sloughing off the mucosal lining. Hypertrophy of surface epithelial cells swelling or oedema of lamina propria ultimately leads to rupture the surface of epithelial lining. Flattening of folds and a cracked clay appearance was also noticed.

In the present investigation surface mucous cells contain at least. Three types of cells M1, M2 and M3 elaborating neutral, sulfo and carboxy mucins respectively. The secretion of all these cells was found to be progressively increased with higher concentration of phosphates coupled with prolonged treatment. Induced secretion of mucosubstances might be playing a role in detoxification process.

**Liver :**

Liver is yellowish brown gland consisting to two main lobes which are subdivided into smaller lobes. Most of the studies on liver toxicity are carried out by using different pesticides. Present study covers the information on phosphate toxicity on the liver of fresh water fish Channa striatus.

Histological observations of control fish Channa striatus showed a continuous mass of hepatic cords. The hepatic cells were large, hexagonal with centrally placed nucleus and homogenous cytoplasm. A large no. of blood sinusoids appeared amongst the cords of hepatic cells. Thin bile canaliculi were observed between hepatic cells. Central venule of hepatic lobule appeared distinctly.

The histopathological alterations due to phosphate intoxication indicate that exposure to 0.006 M and 0.007 M did not much effect and the architecture of liver is partially damaged. Exposure to 0.01 M and 0.15 M phosphate for a prolonged period shows number of histopathological alterations. Diffused areas of necrosis became more prominent. Sinusoids became more distended central lobular veins severely damaged. Progressive vacuolization of the hepatic cells was predominant. Hypertrophy of the hepatic cells was also

observed. Focal necrosis and hepatic lesions progressively increased. Central lobular venules severely damaged due to swelling and degeneration. Vacuolar degeneration of cytoplasm necrosis and hypertrophy was evident. Even ruptured hepatic cells are observed in some areas.

Histochemically control fish showed intense PAS but moderate ABpH-1 and ABpH-2.5 indicating elaboration glycogen and moderate amount of mucosubstances. Progressive exposure to the higher doses of phosphate resulted in the depletion of glycogen.

The fish adjust itself to cope up with extra metabolic load by compensatory hypertrophy of the liver cells i.e. increase in cell size, nuclei and vacuolized cytoplasm and this is probable due to depletion of glycogen. The cytoplasm is migrated to periphery and the cells look empty and large is discussed.

#### **Kidney :**

Kidney is mainly associated with removal of harmful substances from the body and gets affected due to exposure to pollutants. Present study incorporates an information on phosphate toxicity on kidney of fresh water fish Channa striatus.

The histology of control fish comprised of numerous excretory units, the nephrons. Each nephron is formed of renal corpuscle, coiled uriniferous tubule and interstitial haemopoietic tissues. Bowman's capsule is formed of inner quamoidal visceral epithelial cells and outer parietal layer of emboidal cells. Proximal convolutes tubule shows columnar cell with brush border along the cuminal side. The distal convoluted tubule is lined by tall dome shaped cells. Inter

tubular space is occupied by haemopoietic tissue and these cells are parenchymatous and polygonal in shape.

The histopathological alterations due to phosphate intoxication indicate that at lower doses histological architecture is partially damaged. At higher doses for prolonged period show shrunken glomerulus the haemopoietic tissue appeared as a mass of detached cells with distinct empty spaces. Glomerulus appeared as highly vacuolized due to destruction of cytoplasm and nuclei. All the proximal convoluted tubules lost their cellular material. Tubular epithelium showed necrotic changes. Lumen of the tubules was invariably dilated. Thick Bowman's capsule, increased capsular space with swelling and sloughing off the epithelium.

Histochemical control fish showed Bowman's capsule and glomerulus contained only neutral mucosubstances. Proximal convoluted tubules contained both neutral and acidic mucins. Distal tubules indicated the simultaneous occurrence of neutral and acidic mucins. Haemopoietic tissue indicate presence of neutral mucins and absence of acidic mucins.

In phosphate toxicated fishes with higher dose and prolonged period of exposure indicated increase in the mucusubstance concentration. Severely shrunken lumen of tubules is also suggestive of hindered tubular reabsorption.

There is possible role of mucosubstances related to osmoregulatory function of the kidney. Increase in acidic mucins seem to be related with the protective function by binding with toxicant.



## **Gills :**

In fishes the gill is the primary site of osmoregulation and respiration and also the main target organ for aquatic toxicants. Gill of Channa striatus comprised of primary gill filaments attached to an arch. Large number of semilunar projections of both dorsal and ventral sides form the primary filaments. The gill epithelium is primarily made up of epithelial cells, pillar cells, mucous cells and chloride cells. Histopathological structure observed in Channa striatus is similar with other teleostean fishes.

In present investigation, after exposure to phosphate at different dose and time interval showed thickening and swelling of gill epithelium. Secondary gill lamellae and interlamellar filament showed hypertrophy resulting in massive mucus secretion. Epithelial cells enlarged with vacuolated cytoplasm.

Histochemically in control fish surface epithelial cells containing neutral mucosubstances, mucous cells M<sub>1</sub>, M<sub>2</sub> and M<sub>3</sub> containing strongly sulfated, sialomucins and sulfosialomucins respectively. Cartilage cells contain neutral and sulfomucins.

The gills exposed to phosphate intoxication showed that all the mucous producing cells increase in number and size producing voluminous amount of mucous. This is obviously protective act against the intoxication.

Fusion of lamellar epithelial lining of adjacent lamellae may be due to hyperosmotic external medium and through physiologically impaired epithelial cells.

### **Concluding remarks :**

The aim and objectives of the present investigation were, To study the water quality of river Krishna at Mahuli station near Satara city where sewage enters in the river.

- To study LC50 value of Channa striatus for phosphate.
- To study histology of control fish and observe histopathological alterations in the vital organs like stomach, liver, kidney and gill of fish exposed to high doses of phosphate at different time intervals.
- To study distribution of mucosubstances and effect on phosphate concentration on them in target organs.

The result obtained in the present investigation are compared with existing literature. It is hoped that aims and objectives of the present investigations have satisfactorily been achieved.

The present investigation opens several avenues for further research in the field of toxicology and in the field of carbohydrate histochemistry.

The author is fully aware of the shortcoming during the tenure of the present investigation.

- 1) The author had to depend mainly on light microscopic observation, electron microscopic studies might have provided better insight.
- 2) The author had to depend mainly on the histochemical techniques which give the exact location of the mucosubstances in the given histological site but this information do not provide information on the exact quantity of the various mucusubstances in mathematical terms. The approximate staining intensities by visual estimation such as trace, poor weak, moderate and intense may

indicate roughly the relative amounts of mucusubstances in the given histological site of the stomach, liver, kidney and gill.

- 3) The use of recent techniques such as autoradiogrphy, immunohistochemical techniques and electron microscopy would have provided better additional information to confirm the result obtained in the present investigation
- 4) The result obtained in the present investigation will be published in due course of time.