EFFECT OF COPPER AND MERCURY ON DIFFERENT TISSUES (LIVER, KIDNEY & GILL) OF THE FISH *Macrones gulio*

Histopathological alterations in animal tissues have been identified as meaningful indicators of cellular responses to pollutant induced stress. Adverse biochemical and physiological changes in an organism finally results in histopathological alterations. The advantage of cellular kidneys over other approaches lies in the fact that it is possible to identify organells, cells or organs involved as pollution targets. Histopathology is one of the methods for assessing both short term and long term xenobiotic effects. Various cytological and cytochemical techniques have been employed in investigating pollutant induced alterations in cellular structure as well as function. As such, histopathology offers a great deal to aquatic toxicological studies. According to Hinton and Lauren (1990), histopathological approaches should be obligatory components of environmental assessments and may be used to formulate monitoring systems. However, it is essential that routine histopathological studies discriminates between toxicant induced lesions and normal variations in cellular structure.

Primary effects of pollutants may be exerted at the enzyme level or at some cell function such as permeability of membranes. These in turn affect cell integrity, ultra structure and gross function such as energy budget. When these alterations are severe enough they lead to the death of cells resulting in histological lesions. As organs constitute different types of cells and impairment of one or more of these results in change in
functions of organs, the impact may be reflected on the organism's growth, reproduction etc., and ultimately affect the population. The spectrum of levels of integration (Heath, 1987) clearly shows that more specific action are observed at lower levels.

Two ways of assessing marine pollution are by measuring the chemical contaminants in the various components of the ecosystem and by ecological monitoring of the marine communities. A more rapid and direct approach is by looking for effects of pollutants on living organisms in the field and to some extent, in the laboratory. A measure of the biological effect of a pollutant on individual organisms should generally be quantifiable and more or less proportional to the levels of pollutant. Acute cell death without somatic death leads to a series of reactions important for the recovery of a tissue or organ. These may also serve as biological indices of environmental stress effect (Hinton and Lauren, 1990).

Basically, there is a fundamental unity between the structural organization and various functions of the cellular components. An interference to the balanced situation affecting the basic structure may have far reaching effects on the stipulated functions of the organelles. This has been well illustrated by studies on different organelles of various organisms which clearly show structural and related functional alterations when exposed to xenobiotics. Viarengo et al. (1980)
and Viarengo (1985) have demonstrated that metals like copper, mercury and cadmium reduce the RNA synthesis, influence the attachment of only ribosomes to rough endoplasmic reticulum and probably damage the ribosomes themselves. Accumulation of heavy metals has been reported in lysosomes, the subcellular organelles involved in the degradation of endocytosed nutrients. Heavy metal accumulation stimulates lipid peroxidation process and formation of lipofuscin granules. The lipofuscin granules in turn may trap toxic metals in a relatively stable form and may subsequently get eliminated by exocytosis of the residual bodies (Viarengo, 1985). Among the different methods of storage and detoxification of heavy metals developed by aquatic mestebration are metal compartmentation in membrane limited vesicles and formation of inorganic endo cellular precipitates. According to Viarengo (1985) mercury is able to disrupt the ionic balance in fish by altering permeability characteristic of the cell membranes thus affecting both passive and active transport processes. Thus the impact of enobiotics at both the cellular and subcellular levels of organization.

Report on cellular and subcellular responses of marine organisms to a variety of pollutants include neoplastic lesions in fish and non-neoplastic abnormalities in crabs (Malins et al. 1984), hepatopancreatic epithelial reduction in bivalve molluscs (Lowe et al. 1981; Couch, 1984), lysosomal disruption in mussels (Pickwell and Steinert, 1984, Moore et al. 1984) etc. Pollutant induced cell injury provides a highly sensitive indication of
environmental impact on the structural-functional organization. Gills are particularly vulnerable to environmental toxicants because of their external location and close contact with water and because of their permeability which makes them the principal sights of the uptake of toxicants from the medium (Roberts, 1978). Damage to gills has, for this reason, been studied in a large variety of fish exposed to various kinds of environmental pollutants at the light microscopic and electron microscopic levels (Eller, 1975., Jagoe and Haines, 1983).

Since the liver of teleosts is important in the maintenance of internal homeostasis and the metabolism of xenobiotics (Chambers and Yarbrough, 1976) and has also be shown to accumulate foreign compounds (Stathan et al. 1978) and to be susceptible to damage by toxic agent (Racicot et al. 1975; Gingerich et al. 1978) the functional integrity of the liver in fish can be affected by enobiotics (Gingerich, 1982).

The liver is specifically affected by a large number of chemical agents. The liver of mammals act as a major organ for copper storage as also many other metals. Backstorm (1967) observed that liver is one of the most important mercury accumulating organs in animals treated with phenyl mercurials. According to Buck (1978) liver is the first line of defence against copper poisoning. Copper becomes toxic only when the high binding capacity of the liver is exceeded and copper is released into blood stream. In fish also, liver is the major storage organ for copper (Buckley et al. 1982, Shearer, 1984).
El-Domiaty (1987) found that highest concentration of copper was in the liver and kidney of *Clarias lazera* after exposure to copper and suggested that the liver and kidney are vital organs in the regulation of metals and there are detoxication centres. Kidney is second only to the gills as an affector organ in ionic regulation and played an important role in the removal of the heavy metals from the body. According to Adamson (1967) the gill of fish is a poor excretory unit whereas kidney is capable of active excretion of many biotransformed derivatives of toxicants. Hence, examination of the changes in the biochemical composition and enzymatic activity in the liver and kidney are essential to understand the detoxification mechanisms in these organs.

The present study describes the histopathological changes produced in the gill, liver and kidney of the cat fish *Macorines guilo* maintained in laboratory when exposed to various concentrations of mercury and copper.

**MATERIAL AND METHODS**

Collection and acclimatization of fishes were similar as described Chapter 2. Fishes of immature stage with size range 10-13cm in length irrespective of sex were selected for the experiment. Twenty four fishes were transferred to each experimental tank contained 50 litres of water. Fishes were exposed separately to copper and mercury. For the study filtered unpolluted water was used (salinity 15±2‰, temperature 28±1°C, pH = 7.5±0.5 and dissolved oxygen > 90% saturation). Toxicant
concentration for copper was 0.01 ppm and for mercury 0.02 ppm. One tank was kept as control without metal solution and duplicates were run for each metal concentration. The test medium was renewed every 24 hr. The physico-chemical parameters were measured every 24 hr. Fishes were fed with clam meat during the exposure period and feeding stopped 24h prior to each test experiment.

The techniques for histological study and staining procedures were mainly adopted from the methods described by Bucke (1972) and Bullock (1978). Samples for the study were collected on the 1st, 5th, 10th and 15th day. The fishes were caught from the tank and immobilized. The kidney, liver and gill tissues were dissected out and fixed in Bouins fixative for 24 hrs. After fixation the tissues were graded in ascending alcohol series and cleared in xylene. The gill tissue was decalcified in 3% nitric acid before alcohol grading. The tissue was embedded in paraffin wax after proper paraffin infiltration. The sections were cut at 5μ thickness using a rotary microtome and the sections were examined under microscope. Delafield's Haematoxylin staining methods was used.

**Delafield's haematoxylin**

Dissolve 4g of haematoxylin in 25ml absolute ethyl alcohol. Mix gradually into 400ml ammonia, alum, $\text{NH}_4\text{Al(SO}_4\text{)}_2\cdot12\text{H}_2\text{O}$, saturated aqueous (approximately 1 part alum to 11 parts distilled water). Leave exposed to light in a flask with a
cotton plug for 3 - 5 days and filter. To the filtrate add 100 ml glycocerine and 100 ml methyl alcohol.

Eosin
Eosin Y - 1 g
70% ethyl alcohol - 1000 ml
Glacial acetic acid - 5 ml

Dilute with equal volume of 70% alcohol for use and added 2-3 drops of acetic acid.

Procedure
1. Deparaffinize and run slides down to water
2. Stain in Delafield's hematoxylin, until slides are well over stained : 15-20 minutes.
3. Wash in running water : 3-5 minutes.
4. Transfer to 70% alcohol
5. Concentration in eosin : 1-2 minutes
6. Transfer to 70% alcohol : 100 more drops
7. Transfer to 95% alcohol : few drops
8. Dehydrate, clear in xylene and mount in DPX.

RESULTS

Liver Control

Liver of the control did not reveal any major alterations (Plate 1). Normal architecture of parenchyma was altered very little. The hepatocytes were polyhedral in shape having a
Plate 1. Liver hepatocytes from control fish. H&E X320

Plate 2. Liver of copper treated fish showing extensive vacuolation of hepatic cells. H&E X320
Plate 3. Liver of copper treated fish showing intravascular coagulation of blood. H&E X320

Plate 4. Liver of copper treated fish exhibiting intravascular coagulation and perivascular fibrinous exudate (arrow), note also the extensive vacuolation of hepatic cells. H&E X320
Plate 5. Liver of mercury treated fish showing of the necrotic region (arrows). H&E X320

Plate 6. and Plate 7. Liver of mercury treated fish showing accumulation of mononuclear cells in perivascular region of hepatic parenchyma (arrows). H&E X320
central vesicular nucleus. The hepatocytes formed irregular cords which were separated by sinusoids lined with endothelial cells.

 Liver exposed to Copper

The liver tissue after 5 days copper exposure revealed vacuolation of hepatocytes and condensation of nuclear chromatin. The samples collected 10th day showed intravascular coagulation of blood and focal necrosis of hepatic parenchyma in addition to vascular changes. The samples taken on 15th day, showed very extensive vacuolation of hepatic cells with several foci of coagulative necrosis and mononuclear cell accumulation and perivascular mononuclear cell infiltration and intravascular coagulation of blood were also observed. Hepatic nuclei were either swollen or pyknotic (Plates 2, 3 and 4).

 Liver exposed mercury

Liver of mercury treated fish 5th day exposure exhibits intravascular coagulation. Many of the blood vessels appeared more permeable and exhibited fibrinous exudate in perivascular region. The extensive vacuolation of hepatic cells were also noticed. After 10th day of exposure the liver of treated fish showed the focal necrosis of hepatic parenchyma. After 15th day of exposure the liver of treated fish showed accumulation of mononuclear cells in perivascular region of hepatic parenchyma (Plates 5, 6 and 7).
Plate 9. Section of kidney from control fish showing congested glomerulus (arrow), haemopoietic tissue and tubules. H&E X320

Plate 10. Kidney from copper treated fish showing increased permeability of glomerulus and accumulation of exudate in Bowman's capsule (arrows).
Plate 11. High power view of Fig. 10.  H&E X800.

Plate 12. Section of kidney from copper treated fish showing extensive thickening of capillaries (arrows).  H&E X400.
Plate 13. Copper treated fish glomerulus showing increased cellularity, note also accumulation of exudate in Bowman's capsule (arrows). H&E X400.

Plate 14. Glomerulus showing sclerotic changes and adhesion (arrows). H&E X400.
Kidney Control

The kidneys were composed of excretory, haemopoietic and reticuloendothelial tissues. The nephrons consisted of a well vascularised glomeruli which were congested. The glomeruli were surrounded by Bowman's capsule which were lined by squamous epithelial cells. The Bowman's capsule continued through a ciliated neck. The proximal segments, one with a prominent brush border and other with basal striations which were separated by a ciliated segment were seen. In addition to these tubules there were distal segments which connected second proximal segments to the collecting ducts. The Proximal ducts were more eosinophilic in staining and the proximal segment of the tubule were lined by low columnar epithelium with indistinctive borders. Interstitial space was occupied by actively dividing haemopoietic tissue and elements of adrenal tissues. Numerous melanomacrophage centres were also seen (Plate 9).

Kidney exposed to copper

The samples collected on 24 hr revealed no change in glomeruli from that of control. However, swelling of epithelial cells of proximal segments with cast in the lumen were observed. On 5th day, many of the glomeruli showed more permeability and Bowman's capsule continued proteinaceous fluid and heamogenous eosinophilic materials. Some of the glomeruli revealed increased cellularity and sclerotic changes. In addition to necrosis of cells of proximal tubules, swelling of the cells and cast in the
Plate 15. Copper treated kidney glomeruli showing the sclerotic changes, note also the thickening of Bowman’s capsule. H&E X320

Plate 16. Section of kidney from mercury treated fish showing necrosis of tubules (arrows). H&E X320
Plate 17. Section of kidney from mercury treated fish depicting appearance of cast in tubule (arrow). H&E X320

Plate 18. Section of necrosed tubule showing presence of hyaline droplets (arrows). H&E X320.
lumen were also noticed. By 10th day exposure in many glomeruli the capillaries appeared highly thickend. A few Glomeruli revealed mesenchymal proliferation and also increased permeability. On 15th day of exposure the glomerulus showed sclerotic changes and adhesion (Plates 12, 13, 14 and 15).

**Kidney exposed to mercury**

The kidney's on 5th day revealed must severe changes in tubules. There were only very mild changes in glomeruli. The tubular epithelial cells were either degenerated or had undergone severe extensive necrosis. The lumen of tubule contained hyaline cast. By 10th day, considerable changes observed in glomeruli and tubules. The glomerular changes were characterised by accumulation of proteinaceous fluid in Bowman's space, the thickening of Glomerular capillaries, mesenchymal cell proliferation and adhesion of visceral and parietal layers; and periglomerular fibrosis. In 15th day samples tubular epithelial cells were necrosed and almost all the tubules contained hyaline casts (Plates 16, 17 and 18).

**Gill Control**

The control gill had structure very similar to normal gill. The gill arch was covered by typical epidermal tissue which at the origin of primary lamellae was much thicker and endowed with mucus cells. Below the epidermis there was an array of lymphoid tissue consisting of lymphocytes and large cells containing
Plate 19. Gill of control fish  
H&EX-400

Plate 20. Gill of mercury treated fish showing hyperplasia in the tip of secondary lamellae  
H&EX-400.
Plate 21. Gill of mercury treated fish showing advanced stage of hyperplasia resulting fusion of adjacent lamellae.  H&EX400

Plate 22. Gill of mercury treated fish showing complete fusion of lamellae.  H&EX400
eosinophile granules. The primary lamellae had so many lateral projections, the secondary lamellae which were covered with epithelial cells - one layer thick which was supported and protected by the pillar cells. The pillar cells form the lining of blood sinuses or lamellar sinus which connect the afferent and efferent lamellar arteries (Plate 19).

Gill exposed to mercury

The change in the mercury treated fish after 24 hrs swelling at the tip of the secondary lamellae followed by hypertrophy and hyperplasia. As the exposure continued further for 5 days the increased hyperplasia was accompanied by fusion of adjacent lamellae, and the epithelium was lined by several layers of cells instead of a single layer. On the 10th day the entire inter-lamellar space was filled with hyperplastic epithelium and the lamellar structure of the gill was completely lost. As the exposure continued for 15 days disintegration of the hyperplastic epithelium started. There was cytoplasmic vacuolation and the nuclei were either karyorhectic or pycnotic. Hemorrhage and lymphocytic infiltration was also observed (Plates 20, 21, 22 and 23).

Gill exposed to copper

The histopathological changes in the gills of the copper treated fishes are shown in plates 24, 25, 26 and 27. After 24 hr of exposure the copper treated fish showed hypertrophy and
Plate 23. Gill of mercury treated fish showing vacuolation and desquamation in lamellae. H&EX400.

Plate 24. Gill of copper treated fish showing hypertrophy and hyperplasia in secondary lamellae. H&EX400.
Plate 25. Gill of copper treated fish showing fusion of adjacent lamellae. H&EX-400.

Plate 26. Gill of copper treated fish showing widespread necrosis and vacuolation. H&EX-400.
Plate 27. Gill of copper treated fish showing advanced stage of degeneration

H&EX 400.
hyperplasia in secondary lamellae of the gill. The fusion of the adjacent lamellae and observed in the gills of copper treated fish after 5th day of exposure. As the exposure continued further for 10 days the widespread necrosis and vacuolation was observed in the gill tissue of the fish. On the 15th day the cells showed advanced stage of degeneration (Plates 24, 25, 26 and 27).

DISCUSSION

Histopathological studies of controls did not show any major alterations. The structure of liver was very similar to that described for normal fish liver by Varichak (1988), Ferguson (1974), Ellis et al. (1976), Hinton and Pool (1976) and Ellis et al. (1978). In treated fishes the liver samples revealed vacuolation of hepatic cell. This vacuolation was present in all the treated groups throughout the experimental period and increase in severity in proportion to the time of exposure and dose. Crandall and Goodnight (1963) also reported the same type of changes in hepatic parenchyma by prolonged exposure of zinc in fishes. Kumar and Pant (1981), Sultan and Khan (1981), Wester and Canton (1986, 1987), Ansari and Kumar (1987), Jambulingam (1988) have also reported hepatic vacolation in toxic condition associated with zinc, mercury, copper, cadmium etc. This study also supports the same observations.

Liver suffers from lipid accumulation in a number of conditions like deficiency of tocopherol lipotropic factors,
excessive fat in the diet and toxic damage to the liver which interfere with transport and metabolism of fat or protein synthesis in liver. Many of the toxins like antimony, arsenic, cadmium, carbon tetrachloride etc. were reported to have caused fatty liver in higher vertebrates (Runnells et al. 1965; Meiss et al. 1982; Jones and Hunt, 1983).

The heavy metals cadmium, zinc, manganese and calcium were reported to have effect on respiratory metabolism and protein synthesis of hepatic cells in fishes (Hilt ibran, 1971; Shukla and Pandey, 1986). In the present study, due to toxicants the changes became more apparent and severe as the concentration and period of exposure increased. It is believed that the injurious effect of the toxin might have produced these changes. Severe necrotic changes were observed in the liver of fish exposed to lethal concentration of copper and zinc by Kumar and Pant (1981). Necrosis of hepatocytes was a common finding in many of the studies involving heavy metal pollution and other toxic condition in fishes (Bhattacharya et al. 1985; Cruz and Tamse, 1986; Jambulingam, 1988).

Intravascular coagulation and perivascular accumulation of fibrinous material observed in this study, have also been reported by some workers like Di Michele and Taylor (1978). Fish generally has very high count of thrombocytes in blood, hence, fish blood clot very rapidly (Ellis et al. 1978). A number of workers have reported vascular damage as well as poor development
of blood vessels in the liver of animals exposed to heavy metal
toxicity and other toxic conditions (Crandall and Goodnight,
1963; Ellis et al. 1976). Increased permiability of vascular
and intravascular clotting were observed in the present study
also. The changes probably may be due to the vascular damage
which might have initiated the clotting and exudation of fibrin.

Generally, necrosis is accompanied by inflammatory reaction
and accumulation of leucocytes in the periphery of those affected
areas (Jones and Hunt, 1983). The leucocytic infiltration which
was observed in present study may be due to an inflammatory
response against the necrotic tissue. Narain and Singh (1991)
reported the acute thiodan toxicity in liver causes extensive
degeneration of cytoplasm and pyknosis of nuclei. Focal
degeneration of liver cells due to mercury toxicity was reported
by Bano and Hasan (1990).

In the control group the structure of kidney was very
similar to the euryhaline fish which was described by Ellis et
al. (1978). Occasionally some tubules showed degenerative
changes and many glomeruli appeared, congested. Since the
changes were mild in nature they were considered not very
significant.

In toxicant exposed fishes considerable changes were
observed in glomeruli and tubules. Glomerular changes consisted
of increased permeability leading to accumulation of
proteinaceous fluid in Bowman's capsule, thickening of capillaries, mesenchymal cell proliferation and sclerotic changes in glomeruli. Initially the changes in glomeruli were characterised by increased permeability indicating vascular damage to the glomerular capillaries. These glomerular changes were morphologically very similar to changes described for glomerulonephritis, in other vertebrates (Cassey et al. 1979; Slauson and Lewis, 1979; George and Somvanshi 1984). Jones and Hunt, 1983; classified Glomerulonephritis as membranous, acute proliferative, membrano proliferative and chronic sclerosing glomerulonephritis. It is believed that glomerular injury results from immunologically mediated inflammatory reaction at glomeruli. This include deposition of circulating antigen antibody complexes, auto immune reaction and compliment activation (Heyman et al. 1959; Lewis et al. 1963, 1965; Cassey et al. 1979 Slauson and Lewis, 1973 and Jones and Hunt, 1983). Glomerulonephritis is a frequent condition observed in fishes during histopathological examinations. The changes like thickening of glomerular capillaries, hyalinisation of capillaries, dilation of glomerular capillaries, shrinkage of glomeruli and dilation of Bowman's capsule were observed in experimental toxic studies on zinc, copper, cadmium, KMNO₄, B-hexachlorocyclohexane etc, in fishes including milk fish (Kumar and Pant, 1981; Sexena, 1981; Cruz and Tamse, 1986; Wester and Canton, 1986).
In the present study tubules had undergone degenerative and necrotic changes depending on concentration of toxin and period of exposure. These changes were more severe in proximal tubules and consisted of swelling appearance of hyaline droplets and complete necrosis of epithelial cells. The lumen contained hyaline casts. A large number of chemical poisons like cadmium, copper, mercury, arsenic, bismuth, chromium, potassium, dichromatic, etc; were reported to have produced same conditions in higher animals (Runnells et al. 1965, Jones and Hunt; 1983). The hyaline droplets, which appeared in many tubular epithelial cells were reported to have occurred in kidneys where protein leakage through glomeruli occurred (Jones and Hunt, 1983). In this case also glomeruli were damaged and Bowman's capsule contained the proteinaceous fluid. Tubular degeneration and necrotic conditions were common findings in many experimental studies involving chemicals and insecticide toxin in different groups of fishes (Gardner and Yevich, 1970; Koyama and Itazawa, 1977 Kumar and Pant, 1981; Cruz and Tamse, 1986; Forlin et al. 1986).

The dilation of the lamina of the kidney tubules and necrosis of tubules as observed in the present investigation after chloropyrifos treatment have been reported from various fish exposed to pollutants (Kumar and Pant, 1981, 1984; Srivastava and Srivastava 1981; Casillas et al. 1983; Sukumar and Karpagaganapathy, 1986; Gill et al. 1988). The chloropyrifos treated fish, the glomeruli are shrunken and the blood cells in
the glomerular tuft become vacuolated (Sanjay et al. 1990). Bano and Hasan (1990) studied about the histopathological lesions in the body organs of catfish (*Heteropneustes fossilis*) following mercury intoxication. Focal degeneration of liver cells and disorganization of hepatic cords were found. Furthermore centriloculular atrophy and some compensatory hepatic cells were also observed.

Physiological, histological and ultra structural studies have shown that heavy metal ions interfere with respiration and osmoregulation by disrupting the structure of gill cells in fishes (Eisler and Gardner 1973; Jones, 1975). Hypertrophy, hyperplasia and mucus production on gills are associated with prolonged, exposure to chronic levels of pesticides (Cope, 1965; Eller, 1969), heavy metals (Skidmore and Tovell, 1972; Gardner and Yevich, 1970), un-ionized ammonia (Flis, 1968) and other water borne irritants (Eller, 1975). Severe hypertrophy, hyperplasia and necrosis were seen in the gills of fish exposed to formalin (Wedemeyer, 1971). Oversecretion of mucus cells, fusion of secondary gill lamellae from the pillar cells and occurrence of necrotic cells were seen in the gill of fish *Heteropneustes fossilis* exposed to chlorpyrifos (Srivastava et al. 1989).

The effects of toxicants in the cellular structure on different tissues of the fish *Macrones guli*o treated with copper and mercury observed in the present study were mainly the
hyperplasia and necrosis of liver cells, proliferation and degeneration and oedema of lamellar epithelial cells of the gills and dialation of glomerular capillaries and proliferation of glomeral cells of kidney. In general the structural changes increased in severity in proportion to the time of exposure and dose. Such changes were also reported earlier by Skidmore and Tovell, 1972; Kumar and Pant, 1981; Sultan and Khan, 1981; Cruz and Tamse, 1986. after exposing the fishes to heavy metals including copper and mercury.