CHAPTER IV

HISTOPATHOLOGICAL STUDY
Introduction

Environmental degradation in the modern world increased considerably due to man’s excessive intellectual trespassing on delicate environmental limits. Pollution of aquatic ecosystem by chemical used in industry and agriculture is increasing day by day, heavy metals, pesticides, antifouling agents, fertilizers and agricultural drainage from water bodies adversely affects on growth and survival of aquatic animals. Today there is no brook, pond, lake, river or sea environment that is entirely free from pollution, due to the consistent rise in use of heavy toxic chemicals. These chemicals used in industries, horticulture, layer on ships, pipelines or boats reach ultimately the water bodies along with the run off. As a result, the large mortality of aquatic life has been reported due to direct attack of pollutants on aquatic animals, there is imbalance in their physiological activities with respect to respiration, reproduction, excretion, and osmoregulation etc. These chemical pollutants directly / indirectly enter into the body of aquatic animals and affect on different parts of their body and affect vital physiological mechanism.
A study pathological change in the microanatomy of tissues is known as histopathology. Since the mid nineteenth century this branch of science has been successfully employed as a diagnostic tool in medical and veterinary science. Some environmental scientists are beginning to correlate the degree of cell damage to concentrations of the toxic substance and their synergistic or antagonistic interaction, Crandall and Good night, (1963); Brown, (1968). The histopathological studies not only give an early indication of pollutants hazards but also provide useful data on nature and degree of damage to cells and tissues. It is common tool for determining the deleterious effects of toxic substances in animals. The histological techniques are promising area of research in aquatic toxicology as it gives the real picture of effects imposed and the involvement of chemical pollutants in the either disturbing or destroying the vital organs of living organisms. Histopathological studies were also useful in evaluating the pollution potential of organotins since trace amount of these chemicals which do not bring mortality over a period were capable to producing considerable organ damage, B. Indira (1989). Histological analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs, such as the gills, liver and gonads, Dutta (1996). A histological
investigation may therefore prove to be a cost effective tool to determine the health of organism's populations, hence reflecting the health of an entire aquatic ecosystem in the bio-monitoring process. Histological responses may also serve as ecotoxicologically meaningful biomarkers since they form an important link between effects at the biochemical level and those measured in whole organisms, Lowe (1988); Hinton et al., (1992). In addition, analysis of histological changes in target organs provides a valuable tool in understanding the role of specific cells and organelles in heavy metal metabolism, Hinton et al., (1992); Rubio et al., (1993). Previous attempts to use histological alterations for pollutant exposure monitoring in the aquatic environment have been focused on histopathology rather than ultrastructural changes, Couch (1984); Hinton et al., (1992); Clark et al., (2000). Many workers have reported the degenerative changes in selected tissue of animals in response to pollution by various toxicants, Bhattacharya et al., (1975); Annes (1978); Dubale and Shah (1979); Goel and Grag (1980); Ram and Sathyaneson (1987). Histological changes associated with pesticides in fish have been studied by many authors, King, (1962); Eller, (1971); Mukhopadhyay et al., (1987); Narayan and Singh, (1991); Mercy et al., (1996).
The toxicity of organotin compound varies considerably according to the number and nature of organic groups with tin and tetra dialkyltin being the most toxic form, Wong et al., (1982). TBT compounds are already being used in variety of paint formulation either alone or as an active agent or in combination with metal ion compounds Evans, (1971); Dempsey (1981). TBT compounds are extremely effective and relatively economical as biocides, contributing to rapid uptake of organotin based paints by shipping industry and small boat owners in 1970’s. These compound causes variety of effects in aquatic environment, Laughlin and Linden (1987). They interfere and interact with various physiological activities of the organism like reproduction, which is an important biological phenomenon dominating all other physiological processes, and are a need to the animal for continuity of their races, Vernberg and Vernberg (1972). The organs like gills, ovaries and hepatopancreas are known to be the sensitive indicator of physiological disturbances. His and Robert (1981, 1982) observed that copper oxide paints are less toxic to adult oyster but may have an effect on embryos and larvae’s of Crassestrea gigas and Nassaries absoletus the expression of imposex which was caused by exposure to organotin compounds reaching from antifouling paints.
Histopathological effect of pollutants on various tissues of aquatic animals was studied by many workers. Ovary is the reproductive organ when oocyte maturation takes place i.e. vitellogenesis. Histopathological alterations of gonad cells have been observed in shellfish collected from contaminated areas or exposed to contaminated sediments in the laboratory, Yevich PP (1987); Teh SJ (1993). The observed lesions ranged from dilation of reproductive follicles in mussel, *Mytilus edulis*, Sunila I. (1987), to gonadal neoplasms in soft-shell clam, *Mya arenaria*, Yevich (1983), and the *American oyster*, *Crassostrea virginica*. Peters (1997) observed neoplasms of possible germ cell origin in *M. balthica* collected from a contaminated area in the Gulf of Riga. However, the frequent occurrence of digestive cell, germ cell, and oval cell necroses and tissue inflammation may be an early indication of chronic alterations in the digestive gland and reproductive disorder caused by the sediment contaminants. Machale *et al.*, (1990) studied on the histopathological changes in the ovary of freshwater crab, *Barytelphusa guerini* exposed to copper sulphate and reported extensive damage to oocyte. Sarojini (1990) studied the effect of heavy metal pollutant, cadmium chloride on ovarian histopathology in freshwater crab, *Barytelphusa guerini*. Sarojini *et al.*, (1990) studied adverse effect of zinc sulphate on ovarian

In crustacea the hepatopancreas was considered as purely digestive gland, beside this hepatopancreas act as a center of intermediatory metabolism and an important storage depot like insect fat body and vertebrate liver and adipose tissue. The cellular diversity in hepatopancreatic tubules of crustacea has been known for some years, Momin and Rangnekar (1975). As a principle metabolic organ (detoxification organ), such digestive gland plays a major role in the uptake, accumulation, Gluth *et al.*, (1985), biotransformation, Braunbeck (1998), and excretion, Köhler (1990) of xenobiotics. Exposure to toxicants may cause histological changes in the digestive gland, which in turn could be used as a biomarker to indicate prior exposure, Hinton & Laurén (1990). Liver has the ability to degrade toxic compounds, but its regulating mechanisms can be overwhelmed by elevated concentrations
of harmful substances, and this could subsequently result in structural
damage. Similar studies on various fish species, exposed to various
toxicants, showed histopathological changes in the livers of those
effects of cadmium chloride on freshwater prawn, Macrobrachium
kistnensis showed many histopathological changes in hepatopancreas
such as vacuolization of cell, windning of tubular lumen which
ultimately resulted into syncitial mass containing large number of
vacuolated cells and phagocytes. Bhodake (1983) stated that
organopesticides affect on the hepatopancreas of freshwater crab,
Barytelphusa cunicularis. Doughtie and Rangrao (1984) studied the
histopathological changes in the hepatopancreas of grass shrimp exposed
to chromium. Gangshettiwar (1986) also found histopathological lesion
in hepatopancreas of freshwater prawn, Macrobrachium lamerrii treated
with phenol. Baker (1969) in histological and electron microscopical
study on copper poisoning in Pseudopleuronectus americanus found
degenerative and necrotic changes in liver, kidney, haemopoietic tissue
and gill of the fish exposed to copper sulphate Hanumante et al., (1981)
studied the histopathological changes in various tissues of Channa gachua
exposed to chronic bioassay of mercuric chloride and sodium penta chlorophenol.


Very few literatures were available on the impact of TBT compounds on histopathological aspects in crustacean. Hence the present investigation was under taken to find out histopathological changes in gill, ovaries and hepatopancreas in freshwater prawn, *Macrobrachium kistnensis* exposed to TBTCI.
Materials and Methods

The fresh water prawns *Macrobrachium kistnensis* were collected from Kham river near Aurangabad, Maharashtra. The prawns were maintained in plastic trough containing aerated tap water. They were acclimatized for a week in laboratory condition. The water was changed every 24 h. Prawns were fed with green algae at alternative days. 1ppm stock solution of TBTCI was prepared in acetone Laughlin *et al.*, (1983). Matured healthy female prawns were selected for experiment. For each experiment 20 animals of approximately similar size (2.5±1cm in length) were exposed to 0.26 ppm and 0.09 ppm LC$_{50}$ values of TBTCI at 24 h, 48 h, 72 h and 96 h respectively. Simultaneously group of 20 female prawns were also set up for the experimental control period in non-contaminated medium. Tissues such as ovary and hepatopancreas were dissected out from control and experimental prawns and then fixed in Bouins fluid. Respective tissues were processed for microtechnique routine procedure.
Results

Control ovary

The ovary of freshwater prawn, *Macrobrachium kistnensis* (fig. 1) was covered with an outer epithelial membrane followed by connective tissue and inner germinative epithelium. In early stage of development of germinative zone of proliferation is distinguished by the presence of compact mass of oogonial cell, which undergo meiotic division and give rise to primary oocytes. Previtellogenic oocytes were covered by a thin rim of ooplasm. The matured oocyte or vitellogenic oocytes were computably filled with yolk globule. The nutritive cells were present in close vicinity of the oocyte and supply the nutritive material to the developing oocytes. The degenerating ova are surrounded by the nutritive phagocytes.

Experimental ovary

After exposure of different concentrations of TBTCI as 0.26 ppm, and 0.09 ppm LC$_{50}$ values for 48 h and 96 h respectively of tributyltin chloride, the ovaries showed different changes in their architecture. After exposure of 48 h there was a destruction of epithelial layer, evidence of degeneration of oocyte and disorganization of nucleus (fig 2). Vacuolization at periphery, rupturing of follicular epithelium, maximum
number of degenerating oocyte with disintegrated nuclei and vacuolization and alteration in shape was observed for of 0.09 ppm (fig 3).

**Control hepatopancreas**

The tubules of hepatopancreas were enclosed by basal lamina and contained a central lumen. The hepatopancreas of *Macrobachium kistnensis* consist of innumerable oval shaped tubules bound by loose connective tissue enclosing a large central activity of lumen. The tubules were composed of collemnar epithelial cells. Two types of cells were observed in each tubule beneath the epidermal layer, the first were columnar type of cell with nucleus towards base. i.e. absorptive cells. The second type of cells was larger in size having large globular mass of cells discharge their secretion into lumen of tubules i.e. secretory cell (fig 4).

**Experimental hepatopancreas**

The hepatopancreas showed different changes in their architecture after exposure of different lethal concentrations of TBTCI for 0.26 ppm, and 0.09 ppm LC50 values at 48 h and 96 h respectively. The 0.26 ppm of TBTCI treated prawns showed increased in absorptive cells and the lumen was filled with secretary fluid and the size of lumen was reduced
Fig. 1 T. S. of the control ovary of freshwater prawn, *Macrobrachium kistnensis*

Haematoxylin-eosin X 100.

**VO**: Vitellogenic oocyte  
**OG**: Oogonia  
**PVO**: Previtellogenic oocyte  
**YV**: Yolk vesicle

Fig. 2 T. S. of the experimental ovary of the freshwater prawn, *Macrobrachium kistnensis* showing effect of 0.26 ppm of TBTCl.

Haematoxylin-eosin X 150

**DO**: Degenerating oocyte  
**DE**: Destruction of epithelial layer

Fig. 3 T. S. of the experimental ovary of the freshwater prawn, *Macrobrachium kistnensis* showing effect of 0.09ppm of TBTCl.

Haematoxylin-eosin X 100.

**VA**: Vacuolization and alteration  
**DO**: Degenerating oocyte  
**DNU**: Disintegrated Nucleus
Fig. 4 T. S. of the control hepatopancreas of the freshwater prawn, *Macrobrachium kistnensis*.

Haematoxylin-eosin X 100.

SC : Secretary Cells
L  : Lumen

Fig. 5 T. S. of the experimental hepatopancreas of the freshwater prawn, *Macrobrachium kistnensis* showing effect of 0.26 ppm of TBTCI.

Haematoxylin-eosin X 100

SC : Secretary Cells
L  : Lumen
EL : Epithelial Lining

Fig. 6 T. S. of the experimental hepatopancreas of the freshwater prawn, *Macrobrachium kistnensis* showing effect of 0.0.09 ppm of TBTCI.

Haematoxylin-eosin X 150

EL : Epithelial Lining
PN : Pycnotic Nuclei
(fig 5). Very few secretary globules were noticed in hepatopancreas of 0.09 ppm exposed prawns. Epithelial lining covering of the tubules were found ruptured and size of the lumen increased with finger like projection. The tubular absorptive cells were observed with pycnotic nuclei (fig 6).
Discussion

Chemical pollution in aquatic ecosystems, especially river systems, is a major environmental concern. Not only does this type of pollution cause a decrease in water quality, but subsequently affects all living organisms in the system Van Dyk (2003). The ecological importance of metal emanates from their general toxicity and the fact that they are nonbiodegradable and highly persistent and therefore, tend to accumulate in the environment Ursínová & Hladíková (2000); Coetzee et al., (2002). Even minute amounts of heavy metal pollutant can cause subtle or chronic biological effects that may result in irreversible long-term changes in organisms. There is a growing need to detect and assess the impact of pollution, particularly low concentrations of increasingly complex mixtures of pollutants on aquatic ecosystems. Biological and ecological responses to contaminant stressors may ranged from changes at molecular level, where genetic integrity and subcellular processes are evaluated, to population and community levels were dynamics and structure of entire food webs can be affected, Swee et al., (1996).

Histopathological characteristics of specific organs express condition and represent time integrated endogenous and exogenous
impacts on the organism stemming from alterations at lower levels of biological organization, Stebbing (1985). Therefore, histological changes occur earlier than reproductive changes and are more sensitive than growth or reproductive parameters and, as an integrative parameter, provide a better evaluation of an organism health than a single biochemical parameter, Segner & Braunbeck (1988). Histopathological changes in animal tissues are powerful indicators of prior exposure to environmental stressors and are the net result of adverse biochemical and physiological changes in an organism. Whereas biochemical and physiological techniques are also powerful in methods of pathological and histopathological changes allow the identification of specific target organs, cells, and organelles that have been infected in vivo. For field assessments, histopathology is often the easiest method of assessing both short- and long-term toxic effects, Hinton & Laurén (1990). When combined with other disciplines such as analytical chemistry or xenobiotics physiology, histopathology may also reconstruct possible general etiologies for the lesions found in the tissues, Hinton & Laurén (1990).
In the present investigation the freshwater prawn, *Macrobrachium kistnensis* was exposed to different concentration of TBTCI for 0.26 ppm and 0.09 ppm LC$_{50}$ at 48 h and 96 h respectively. The results obtained in the present study include the histopathological changes in the tissues such as ovary and hepatopancreas induced by TBTCI toxicity.

After exposure of different concentrations of TBTCI as 0.26 ppm, and 0.09 ppm LC$_{50}$ values at 48 h and 96 h respectively of TBTCI, the ovaries showed different changes in their architecture. After exposure of 48 h there was a destruction of epithelial layer, evidence of degeneration of oocyte and disorganization of nucleus (fig 2). Vacuolization at periphery of the oocytes, rupturing of follicular epithelium maximum number of degenerating oocyte with disintegrated nuclei and vacuolization and alteration in shape was observed for 0.09 ppm (fig 3). This damage observed in the ovary might be due to the direct effects of TBTCI on developing oocytes intervening the enzyme system in metabolism or destroying structure the function of hormone that controlling the ovarian growth. Similar histopathological changes were reported in different aquatic organisms exposed to different pollutants by many researchers. Machale *et al.*, (1990) observed severe changes in ovaries of crab, *Baretylphusa guerini* exposed to different concentrations
of cuprous oxide. After exposure of cuprous oxide, they reported rupture of epithelial layer of oocytes, distinct damage to proliferating zones and peripheral vacuolization of oocytes. Further degeneration proceeds from peripheral oocytes towards the center of ovary and phagocytolic cells appear. Disintegration of nucleus, nucleolus and finally the oocyte was resorbed. Damage observed in cellular destruction may be due to the direct effects on the developing oocytes through general metabolism and growth or through hormones controlling ovarian growth. B. Indira (1989) noticed shrinkage in ooplasm, vacuolization and necrosis of nucleus and nucleolus, degeneration of tissues, disintegration of nucleus and nucleolus, fragmented cytoplasm disintegration of connective tissue and reduction in cytoplasmic material in ovary of freshwater prawn, Caridina rajadhari when exposed to TBTO. R. Sarojini et al., (1990) reported the zinc sulphate adversely affects of on ovarian development of crab, Barytelphusa guerini. They investigated zinc sulphate toxicity and in their finding observed ruptured oocyte membrane and vacuolization of peripheral oocytes and disturbances to the supporting connective tissue. For longer exposure of 15 days they observed vacuolization of oocytes, striation of cytoplasm and occurrence of vacuoles. Similar results were reported by Gyananth et al., (1987);

In Macrobrachium kistnensis hepatopancreas consist of innumerable oval shaped tubules bound by loose connective tissue enclosing a large central activity of lumen. The tubules were composed of columnar epithelial cells. Two types of cells were observed in each tubule beneath the epidermal layer. Columnar types of cell with nucleus towards base were called absorptive cells. Other type cells were larger in size having large globular mass of cells discharge their secretion into lumen of tubules called secretary cells (fig 4). Hepatopancreas of crustacea is an important digestive gland like liver in fishes and plays an important role in intermediate metabolism. In the present study prawns exposed to 96 h exhibited various changes in the hepatopancreas. It was found that damage caused by TBTCI at this concentration was more pronounced when compared with that of 48 h exposure. The damage to the hepatopancreas showed, the tubules were almost in state of collapsing in most of the TBTCI treated prawns. The tubules were distinguished and lumen size was enlarged, disorganization and extensive vacuolization in the cytoplasm of cells were observed. Indira (1989) reported vacuolization of cell, windning tubular lumen and
phagocytic cell in freshwater prawn, *Caridina weberi* when exposed to TBTO and copper sulphate. Victor *et al.*, (1990) observed the histopathological changes in the hepatopancreas of *P. hydrodomus* in response to cythion resulting in a reduction in the height of tubular epithelium, enlargement of lumen, vacuolization and atrophy. The histological changes indicates that the animal were not in position to digest and store food properly because the damage of secretory and absorptive cells by TBTCI toxicity, can not produced secretory material which mainly contains digestive enzymes and hence no absorption of simple food by absorptive cell to store. The lack of nutrutents also resulted in atrophy of hepatopancreas. Suresh (2001) in *U. annipulus* observed similar disorganized condition of hepatopancreas in response to cadmium and mercury toxicity. Similar results were reported by Devi (1996); Bhavana and Geraldine (2000) and Anderson (1997).

The above histopathological changes showed that the severity of damage is dose and time dependent. Many others have reported pathological changes in different tissues of various organism induced by lethal effect of different pollutants. With the help of this, the above observation made so far show no definite conclusion regarding the
specific effect of TBTCI on ovary and hepatopancreas of

*Macrobachium kistnensis.*