Chapter - IV

HISTOPATHOLOGICAL STUDIES
Introduction

Pollution stress due to toxic substances like pesticides in aquatic animals has attracted attention of scientists all over the world. The increasing use of pesticides in order to improve the agricultural productivity to match the explosive population growth rate is a global phenomenon (Srivastava et al., 1977). Pesticides may impair the functioning of organisms in aquatic environment even at low concentration. These sub lethal concentrations of pesticides may affect the biochemical, physiological, behavioral functioning and even life cycle of organisms.

Sprague (1971) suggested that small change in environment by pollution may have ecological importance, may be in lowering the fitness of organism in its environment and not merely with in the range of adaptation of organism.

Natural run off, during the courses of its movement, brings useful as well as toxic substances like pesticides and heavy metals with it and mixed with water, some of them are present in natural resources but the rest are added by the human beings. Excess discharge of raw and partially treated industrial effluents into aquatic ecosystem leads to destruction of the environment. Incorporation of toxic compounds or their metabolites in lower organisms and in the vital tissues of fishes, birds and humans have been recorded to cause serious morphological alterations even at very low levels (Chakrabarthy and Konar, 1974; Mathur et al., 1981). Gerardo Gold – Bouchot et al. (2007) noticed biological effects of environmental pollutants in American oysters, *Crassostrea virginica*. Anitha Kumari and Ram Kumar (1997) observed degenerative changes in the intestinal serosa, mucosa and submucosa layers of *Channa striatus* and *Heteropneustes fossilis* while studying effect of water pollution on histology of intestine of these freshwater fishes from Hussainsagar Lake.

Every organism has capacity to tolerate suboptimal stress conditions. The maximum tolerance is at the extreme stress condition which exhibit physiologically defined limitations which are reflected predominantly in the structural architecture of various tissues of the animal. Traces of toxicants introduced in the body can be neutralized by immune system, but when high amount of toxicant enters, it affects the structure and function of digestive glands in the body of animal.

Structural and functional changes which occur in the tissues of animals due to different toxicants vary from tissue to tissue. In order to understand a pattern of damage caused by particular chemical to the tissue it is essential to have an insight
into the histological analysis of the tissues. This can be helpful for better understanding of the pathological condition and/or abnormalities and damages of tissues under toxic stress of pesticides. Thus histopathology is an extremely useful tool for assessing effects of toxicants at individual level.

Even though the histopathological approach is somewhat qualitative, it is very valuable because the lesions manifest integration of cumulative effects of biochemical and physiological changes in the form of injury. This approach is also important in identification of specific cell, tissues and organs that have been affected.

Histopathology was used as a tool to investigate abnormalities in the tissues caused due to toxic stress of pesticides as they are known to cause several histopathological changes in the gonads of fish (Kling, 1981; Sukumar and Karpagaganapathy, 1992). Tilak et al. (2001) studied histopathological changes in the gill, liver and kidney of Ctenopharyngodon idella exposed to fenvalerate. Veeraiah (2002) investigated histochemical and histological changes due to cypermethrin toxicity in the Indian major carp, Labio rohita. Unlu et. al. (2005) studied histopathological effects in tissues of snail, Lymnaea stagnalis exposed to sublethal concentration of thiodan.

Histopathological study is also useful to find out the exact location of the action of pollutants in the various organs and systems of animals.

Gills:-

Respiratory organs of bivalve consist of a single pair of elongated ctenidia or gills, one on each side of the foot. They lie laterally in the mantle cavity. Each ctenidium is made up of two gill plates or demibranchs. Each gill plate is formed of two similar flaps or lamellae. They are joined to each other except dorsally forming a narrow but long bag called water tubes. They open into suprabranchial chamber of the mantle cavity.

Lamellae are formed of numerous elongated and V shaped vertically parallel gill filaments. Adjacent gill filaments are joined to form interfilamentar junctions which contain holes called ostia. Ostia connect ventral inhalant chamber of a mantle cavity with water tubes. The gill filaments are covered by different kinds of cilia and supported by two chitinous rods. The cilia are of three types, those present on outer face of filaments are called frontal cilia, those on lateral parts are lateral cilia while lying in between two are latero - frontal cilia. A space between two lamellae of a gill
plate is divided by vertical bars of vascular tissue to form interlamellar junctions which contain blood vessels.

Ridehood (1903) and Ortmann (1911) studied the anatomical and histological structure of gills. Gills are the vital organs of aquatic animals which perform the function of respiration. The external surface of the gill is folded or drawn into projecting appendages covered by a thin and permeable skin which is richly supplied with thin walled blood capillaries or sinusoids containing a fluid to transport oxygen. This arrangement increases the surface area of gills that facilitates the exchange of gases at high rate. The rate of the exchange of the gases is directly proportional to the surface area of the gills.

Anabolic and catabolic activities inside the cell need energy which is obtained from the oxidation of simple food material in mitochondria through electron transport chain. Rate of oxidation depend upon the availability of oxygen to the cell for oxidation.

Along with respiration gills perform the function of osmoregulation. Gills bath continuously in surrounding water therefore; they are most vulnerable organs to various aquatic pollutants (Roberts, 1978). It results in alterations in normal respiratory surface area and the rate of diffusion of gasses through gills (Skidmore and Yovel, 1972; Hughes and Parry, 1976).

Physiological hazards of some organic pesticides are organ specific e.g. the organophosphates are neuro inhibitors and organochlorine like DDT accumulates primarily in gonads. Effect of different pesticides and heavy metals on O₂ consumption of fishes was studied by many workers (Reddy and Gomathy, 1978; Gupta et. al., 1979; Rao et. al., 1980). Lomte and Jadhav (1982) investigated the effect of heavy metals on the gill of Lamellidens corrianus.

Rao et. al. (1983) had observed the drastic effects of malathion on the gills of Tilapia mossambica. Srivastava et. al. (1987) reported the histopathological changes in the gills of fresh water fish, Heteropneustes fossilis on exposure to thiodan. Histopathological changes in Tilapia mossambica due to endosulfan exposure were studied by Muley et. al. (1996). Victor and Sarojini, (1986) studied toxicity effects of organophosphorous insecticides on ovaries of shrimp, Caridina rajdhari. Dhanapakiam et. al. (1998) observed histopathological changes in the gills of Channa punctatus in Cauvery river water. Srivastava and Gupta (2001) reported histopathological changes in the gills of fish, Channa punctatus due to zinc toxicity.
Ishak and Mohamed (1975) and Robert (1975) studied the effect of heavy metal and toxic pesticide on the oxygen consumption. Histological studies of gills of the bivalve, *Corbicula striatella* after exposure to some selected pesticides, carbaryl, endosulfan and cypermethrin were made by Jadhav (1993). Girija and Rao (1985) noted the histopathological changes in the gills of freshwater fish, *Tilapia mossambica* on exposure to heptachlor.

Pesticides affect the oxygen consumption of fishes (Reddy and Gomathy, 1978). Gupta *et. al.* (1979) and Rao *et. al.* (1983) observed drastic effects of malathion on the gill of *Tilapia mossambica*. The acute exposure of triterpenol to milkfish, *Chana chanas* induce the changes in structure of gill. Such changes in structure of gill were also observed by Dhande (2000), Daspute (2002).

**Gonads:**

Fresh water bivalves are usually dioecious i.e. sexes are separate but there is no sexual dimorphism. The paired gonads are located just above the foot and around the coils of intestine in the visceral mass. Each gonad possesses short duct which opens into the mantle cavity near excretory pore.

Many workers have studied the effect of pesticides on histopathology of gonads in different organisms (Saxena and Mani, 1985; Bagchi *et. al.*, 1990; Sukumar *et. al.*, 1992). On chronic exposure to endrin, morbid changes were observed by Eller (1977) in histopathological structure in female gonads of trout, *Salmo clarkii*. Organophosphorous insecticide, lebycid caused total atrition of ovaries in 90% of treated specimens of *Tilapia leucostica* (Klings, 1981). Victor and Sarojini (1986) also studied toxicity effect of organophosphorous insecticides on ovaries of shrimp, *Caridina rajdhari*.


Investigation regarding the histopathological impact of pesticides on tissues of freshwater bivalve is scanty therefore, in present work efforts are taken to observe
effects of pesticides viz. quinalphos and thiodan on histopathological changes in gills, gonads and digestive glands of freshwater bivalve, *Parreysia corrugata*.

**Digestive gland:**

The digestive glands of bivalve are also known as hepatopancreas. It consists of numerous tubules with blind end. These are communicated with the stomach by partially ciliated main duct and nonciliated secondary duct. The non-ciliated cells of main ducts have high and dense microvilli. They are pinocytic in function. Ciliated and nonciliated cells have a very similar fine structure. In the digestive gland there are different types of digestive tubules namely, formative, digestive and disintegrative. Each digestive tubule encloses a large lumen and contains digestive cells at different stages. The tubules are usually called hepatic lobules. Histopathological changes in the fish hepatopancreas due to industrial pollutants and chlorinated hydrocarbons are on record (Eller, 1971; Bhattacharya *et. al.*., 1975; Dubale and Shah, 1979). Amminikutty and Rege (1977) observed the effects of acute and chronic exposure to pesticides, thiodan and agallol on the liver of widow tetra *Gymnocorymbus tetroeizti*. Annes (1978) studied hepatic pathology in a freshwater teleost *Channa punctatus* exposed to sublethal and chronic levels of three organophosphorous insecticides. Usheva *et. al.* (2006) observed histopathology of the digestive gland of bivalve mollusc, *Crenomytilus grayanus*. 
Materials and method

Freshwater bivalves, *Parreysia corrugata* were collected from Ambadi dam about 50 km. away from Aurangabad city. They were cleaned and washed in a tap water and acclimatized to laboratory conditions for 4 days. During acclimatization period water in the troughs was changed every day. After the acclimatization, healthy medium sized bivalves were selected from the troughs and used for experiments.

To study the effect of pesticides at cellular level, fresh water bivalves, *Parreysia corrugata*, were exposed to the chronic dose of quinalphos and thiodan (96 hrs LC$_{50/10}$).

The acclimatized bivalves were divided into three groups with equal numbers of animals. They were kept in separate troughs for 15 and 30 days. One of the three groups was not exposed to pesticides and maintained as a control. Out of remaining two groups, one was treated by chronic concentration (LC$_{50/10}$ value of 96 hrs.) of quinalphos (0.108 ppm) and another was treated by chronic concentration of Thiodan (0.0708 ppm).

On 15$^{th}$ and 30$^{th}$ day of exposure, bivalves in each experimental group were sacrificed and their gills, gonads and digestive glands were fixed in aqueous Bouin’s fluid for 24 hours. They were washed in running tap water for about six hours so as to remove the Bouin’s fluid from tissues. The washed tissues were dehydrated through grades of alcohol (from 30% to 100% alcohol) and were dealcolised and cleared in toluene. The cleared tissues were embedded in paraffin wax (58 to 60$^\circ$C) and blocks were prepared.

Blocks of the tissues were treated and serial sections of 6 µ thickness were cut with the help of microtome. Cut sections were spread properly on the slides and were stained with Mallory’s triple stain. The stained sections were examined under light microscope for histopathological effect of above said pesticides.
Observations and results

The freshwater bivalves, *Parreysia corrugata* were exposed to chronic dose of pesticides, quinalphos and thiodan to observe histopathological effects on their gills, gonads and digestive glands.

**Histology of gills:-**

Histologically, each gill or ctenidium consists of two gill plates or demibranchs. Each gill plate is formed of two similar flaps or lamellae. They are joined to form water tubes opening into the mantle cavity. Lamellae are formed of numerous gill filaments which contain holes called ostia. The gill filaments are covered by different kinds of cilia and supported by two chitinous rods. A space between two lamellae of a gill plate contains blood vessels. Photomicroplate 4.1 shows histology of gills of bivalve in control group.

Gills of bivalves exposed to pesticides showed histopathological changes in their architecture (photomicroplate 4.2 and 4.3).

**Effect of Quinalphos:-**

Chronic exposure to quinalphos for 15 and 30 days duration induced significant pathological changes in histology of gills (photomicroplates 4.2 A & B). The epithelial cells and connective tissue cells have lost their cellular structure. Epithelium became oedematic, necrotic and vacuolated. There was fusion of secondary lamellae and decrease in space between water tubes and interlamellar junction due to hypertrophy.

Severity of necrotic effect was found to be increased with increasing period of exposure. Therefore 30 days exposed gills had swellings at the tips and degeneration of cilia was more severe than in 15 days exposed gills.

**Effect of Thiodan:-**

Chronic exposure to thiodan created more severe changes in histology of gills (photomicroplates 4.3 A & B). Respiratory epithelium was found to be enlarged due to abnormal increase in number of cell i.e. hyperplasia. Vacuolization in connective tissue was observed due to degenerative activities. Necrosis was observed in ciliated epithelium and the connective tissue.

30 days exposure induced severe damage to cilia, chitinous rods and epithelia. The epithelial linings at the tip of gill filaments were found to be disintegrated. There was damage to normal structure of gills which resulted in atrophy of secondary gill
lamellae. Cytoplasm showed disintegration due to swelling in respiratory epithelium. Cilia became clumped and exhausted. The blood vessels were ruptured causing haemorrhage. Some cells and nuclei were observed in abnormal shape.

**Histology of gonads:**

Histologically, gonads of control mussels (photomicroplates 4.4) showed previtellogenic, vitellogenic and maturing oocytes in ovaries and well developed normal testicular follicles with all stages of spermatogenesis.

Chronic exposure of gonads to pesticides resulted in to severe abnormalities in its functioning.

**Effect of Quinalphos:**

Gonads exposed to quinalphos for 15 days (photomicroplate 4.5 A & C) showed abnormal growth of oocytes, damage and disintegration of oocytes and nuclear structures, cytolysis of oocytes, degeneration of ovarian sac, and irregular shaped oocytes with damaged nucleus. Abnormal arrangement of spermatozoa and vacuoles in spermatic follicles with some degeneration were observed in testicular follicles.

30 days exposure to quinalphos (photomicroplate 4.5 B & D) showed abnormal growth of oocytes, death of oocytes, cytolysis and irregular shaped oocytes. Testicular follicles were highly shrunken with compactly arranged, disturbed spermatogenic stages indicating beginning of necrosis of testicular tissue while the spermatogenic stages were clumped and sperms were unusual.

**Effect of Thiodan:**

Chronic exposure to thiodan for 15 days (photomicroplate 4.6 A & C) resulted in decrease in number of ovarian follicles, cessation of ovarian maturation and degeneration of ovarian cords. 30 days exposure (photomicroplate 4.6 B & D) showed disintegration and cytosis of oocytes, atrophy of ovarian follicles, disturbed spermatogenesis, abnormal shaped spermatozoa, clumping and necrosis of spermatozoa. Connective tissue was degenerated with vacuolization.

The effect of both the pesticides is almost same. Severity of the damage varies with type of pesticide. Effect of thiodan is more severe than that of quinalphos and severity also increases with period of exposure.

**Histology of digestive glands:**

Digestive glands of bivalve are also called hepatopancreas. Histologically, it consists of numerous hepatic lobules. Photomicroplate 4.7 shows histology of
hepatopancreas of bivalves in control group. Each lobule is lined by columnar cells and secretary cells resting on basement membrane. In the core of hepatic lobule there is a narrow lumen. Interlobular space is filled by thin layer of connective tissue. It is the storage house of metabolic reserve which is the source of energy during physiological stress. Its secretion also plays a vital role in digestion of food.

Hepatopancreas of bivalves exposed to pesticides showed histopathological changes in their structure. (photomicroplates 4.8 & 4.9).

Effect of Quinalphos:-

Chronic dose of quinalphos for 15 and 30 days exhibited noticeable damages in hepatopancreas (photomicroplates 4.8 A & B). 15 days exposure showed slight swelling in epithelial tissue. Spaces were appeared in interlobular connective tissues. Hepatic lobules became loosely arranged. Cells were detached from the basement membrane.

Chronic treatment of quinalphos for 30 days showed more severity in effects. Epithelial cell were separated from the basement membrane and scattered in the lobules. There was increase in the size of lumen in the core of lobule. Necrosis and degeneration of the cells was observed. Connective tissue became very thin.

Effect of Thiodan:-

Chronic treatment of thiodan for 15 and 30 days manifested pronounced effect on hepatopancreas of bivalve (photomicroplates 4.9 A & B). 15 days exposure to thiodan resulted into severe damage to hepatopancreas. The basement membrane was broken and not maintained. Cells were separated from basement membrane and spread in the lobules. Cellular death was found in epithelial tissue and connective tissue due to which enlargement was observed in the lumen.

Intensity of damage was found to be increased with increase in exposure period. Hepatopancreas exposed to 30 days showed huge gaps in the connective issues. Some lobules were ruptured and the epithelial cell were scattered in the connective tissue. The cytoplasm in the cells was lost and the nucleus was degenerated. The number and size of the secretary cells was decreased.
Plate: 4.1

C    –    Chitin
WT   –    Water tube
CE   –    Ciliated epithelium
Plate: 4.2

C  –  Chitin
WT  –  Water tube
DLCE  –  Delaminated ciliated epithelium
DCE  –  Damaged epithelium
ILJ  –  Interlamellar junction
Plate: 4.3

C – Chitin
WT – Water tube
DLCE – Delaminated ciliated epithelium
DCE – Damaged epithelium
ILJ – Interlamellar junction
Plate: 4.4

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<th>Abbreviation</th>
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<tr>
<td>CT</td>
<td>Connective tissue</td>
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<tr>
<td>MB</td>
<td>Basement membrane</td>
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<td>S</td>
<td>Sperms</td>
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<tr>
<td>OC</td>
<td>Oocyte</td>
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<tr>
<td>SA</td>
<td>Sperm aggregate</td>
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<tr>
<td>DGE</td>
<td>Damaged germinal epithelium</td>
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<tr>
<td>OC</td>
<td>Oocyte</td>
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<tr>
<td>DOC</td>
<td>Damaged oocyte</td>
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<tr>
<td>DOF</td>
<td>Damaged ovarian follicle</td>
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<tr>
<td>N</td>
<td>Nucleus</td>
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Plate: 4.6

CT  –  Connective tissue
MB  –  Basement membrane
S   –  Sperms
SA  –  Sperm aggregate
DGE –  Damaged germinal epithelium
OC  –  Oocyte
DOC –  Damaged oocyte
DOF –  Damaged ovarian follicle
N   –  Nucleus
Plate: 4.7

L – Lumen
CT – Connective tissue
HL – Hepatic lobule
HC – Hepatic cells
BM – Basement membrane
Plate: 4.8

L  –  Lumen

CT  –  Connective tissue

HL  –  Hepatic lobule

HC  –  Hepatic cells

BM  –  Basement membrane

DE  –  Damaged epithelium

DLE  –  Delaminated epithelium
Plate: 4.9

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<td>L</td>
<td>Lumen</td>
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<td>DE</td>
<td>Damaged epithelium</td>
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<td>DLE</td>
<td>Delaminated epithelium</td>
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Discussion

The severity of the pathological changes depends on many factors such as type of pesticides, concentration of dose, time of exposure, type of animal species, etc. As per the toxicological study, intensity of the damages depends mostly on concentration of pesticide and on the rate of metabolism in the target tissues. Kumar and Pant (1984) stated the importance of histopathological studies in evaluating the pollution level of pesticides since their trace amount is capable of inducing considerable damage in the tissues. Many investigators have reported toxicant induced histopathological abnormalities and degenerative changes in certain tissues of various animals (Goel and Garg, 1980; Banerjee and Bhattacharya, 1997).

Observations in the present study revealed that, pesticides viz. quinalphos and thiodan have caused damages to gills, gonads and digestive glands (hepatopancreas) of freshwater bivalve, Parreysia corrugata. Severity of these pesticides was found to be increased with the time of exposure and differ with type of tissue and the type of pesticides.

Gills are the vital organs of bivalves which are engaged in respiration and osmoregulation. Since continuous water current flows through them, they come in direct contact with pesticides. Hence they are more vulnerable to damage than any other tissue.

Histopathological changes in gills of fishes caused due to pesticide exposure were studied by many workers (Gardner and Yevich, 1970; Chakrabarthy and Konar, 1974; Moses and Jayanth Rao, 1985; Radhaih, 1988; Tamse et al., 1995).

In present work, gills of bivalve are badly damaged due to quinalphos and thiodan exposure. Since thiodan is found to be more toxic than quinalphos, its destructive value is more than that of later. The epithelial cells and connective tissue cells have lost their cellular structure. Connective tissue and epithelium became oedematous, necrotic and vacuolated. There was fusion of secondary lamellae and space between water tubes and interlamellar junction was reduced due to hypertrophy and hyperplasia.

Severity of necrotic effect was found to be increased with increasing period of exposure. Therefore effect in 30 days exposed gills like swelling at the tips and degeneration of cilia was more severe than in 15 days exposed gills.
Gill et al. (1988) studied the effect of carbaryl and dimethoate in gills of *Puntius conchonius* and found wilting of pillar cell system, separation of lamellar epithelium, extensive hypertrophy of chloride cells and hyperplasia of lamellar epithelial cells leading to complete fusion of lamellae. According to them this is a mechanism to shut the gills for escaping onslaught of pesticides.

Roy and Datta (1991) observed inflammatory alterations and hyperplasia in lamellar epithelium in the gills of Malathion exposed *Cirrhinus mrigala*. Residues of endosulfan were found to cause hyperplasia and edema with lifting of lamellar epithelium in gills of all cat fishes (Nowak and Barbara, 1992).

Mattiessen and Brafield (1973) reported necrosis of gill epithelium, vacuolization and sloughing of epithelial cells with changes in their cytoplasm in gills of sickle backs, *Gasterosteus aculeatus* due to dissolved zinc. Muley et al. (1996) noticed haemorrhage in the primary gill lamellae and shortening of secondary gill lamellae, hyperplasia, hypertrophy and pycnosis in nuclei of epithelial cells in the gills of freshwater fish, *Tilapia mossambica* due to endosulfan toxicity. Vijayalaxmi and Tilak (1996) studied histopathological changes in gills of *Labeo rohita* exposed to pesticide monocrotophos and found elongation of secondary gill lamellae with bulging and bending tips, necrosis, atrophy and degeneration in the respiratory epithelial cells. They also studied effect of fenvalerate in same species.

According to Lloyed, (1961) damage to the epithelium can be expected to depend on the quantity of pollutants reaching to gills and it also depends on the ventilation volume and the concentration of the pollutant in the water. Because of this fact, hyperventilation of the gills during struggling or swimming and during hypoxia may result in the rapid poisoning to fish (Schweiger, 1957).

Mukhopadhya and Dehdari (1980) suggested that, pesticides and heavy metals inhibit the ion transporting enzyme ATPase in gills of fishes. Gardner and Yevich (1970) studied a shift from aerobic to anaerobic pathway in tissues of estuarine teleost fish *Fundulus heteroclitus* and found impairment in the gills which might be due to degenerative changes in the respiratory epithelium. Similar results were observed by Nagaratnamma and Ramamurthy (1982) in a freshwater teleost, *Cyprinus carpio* during organophosphate pesticide intoxication.

and fusion of secondary gill lamellae, vacuolization in respiratory epithelium and connective tissue and damage of chitinous rods of gill lamellae of bivalve, *Corbicula stiatella* after exposure to carbaryl, cypermethrin and endosulfan.

Jain and Mishra (1994) while studying histopathological effect of sublethal doses of herbicide, isoproturon on gills of *Puntius ticto*, found hyperaemia, hyperplasia, hypertrophy and degeneration of epithelial lining of secondary lamellae. Sultana and Sharief (2004) observed extensive damages in the internal gill architecture of copper, lead and zinc exposed fish, *Tilapia mossambica* like, degenerative changes, swelling, fusion, atrophy etc. Also found reduction in the size of primary and secondary gill lamellae and necrosis of tissue, degeneration of secondary gill lamellae with bulging tips. Filament cell proliferation, hyperplasia, lamellar fusion, epithelial lifting and aneurysm were the observations made by Wennee et. al. (2002) in the Nile tilapia *Oreochromis niloticus* exposed to glyphosate.

Almost same pathological lesions have reported in the gills of different animals after insecticidal exposure. Therefore it has been suggested that, lesions are not specific to the class and nature of toxicant (Eller, 1971; Konar, 1981; Rao et. al., 1983; Pandey and Shukla, 1985; Gupta and Rajbanshi, 1979; Mishra, 1988; Seth, 1988; Jain, 1993).

Jha (2004) observed various types of gill lesions in *Channa punctatus* on exposure to sublethal concentration of phosalone and concluded that, respiratory distress including serious hypoxic conditions affecting oxidative metabolism and ionic regulation ultimately leading to death of the fish might be due to reduced respiratory area and increased diffusion distance there by reducing the respiratory and osmoregulatory efficiency of the fish.

Tilak et. al. (2005) have studied histopathological changes in the gills of the fish, *Channa punctatus* exposed to sublethal concentration of herbicides, butachlor and machete and found bulging tips of primary gill filaments, cutting of secondary filaments, degeneration of pillar cells, necrosis and formation of vacuoles in the secondary gill epithelium and concluded that, pesticides cause irreparable architectural changes in vital organs like gills which make the fish unfit for survival. These changes can alter the physiological activities and affect metabolism.
Hepatopancreas is one of the important organs in the body of bivalve. Disintegration or partial damage of exocrine and endocrine portions of hepatopancreas of teleost treated with a variety of pesticides is documented (Amminikutty and Rege, 1977). Many workers (Clements et. al., 1980; Axiak et. al., 1988; Vincente et. al., 1988; Bright and Fill, 1989; Mariaomez et. al., 1990; Srivastava and Maurya 1991; Pillai and Menon, 1998) studied the effect of different toxicants on hepatopancreas in various aquatic animals.

In present observations, quinalphos and thiodan exposed hepatic lobules showed swelling in epithelial tissue, spaces in interlobular connective tissues, loose arrangement of lobules, broken basement membrane, detached and scattered cells in the lobules etc. There was increase in the size of lumen in the core of lobule due to cellular death, necrosis and degeneration of the cells and thin connective tissue.

Huge gaps were also observed in the connective tissues. Some lobules were ruptured and the epithelial cell were scattered. The cytoplasm in the cells was reduced and the nucleus was degenerated. The number and size of the secretary cells was decreased.

Victor et. al. (1990a) observed histopathological changes in the hepatopancreas of P. hydroaromous in response to cythion as reduction in the height of tubular epithelium, enlargement of lumen, vacuolization and atrophy. He concluded that, the histopathological changes were due to inability of animal to digest and store food properly and hence lack of nutrients resulted in the atrophy of hepatopancreas. Bhavan and Geraldine (2000) also found extensive effect of urea and naphthalene on U. triangularis in the form of vacuolation in the cells of hepatopancreas.

Suresh (2001) noticed disorganized condition of hepatopancreas in U. annulipes in response to cadmium and mercury. Mule (1990) investigated changes in the gonads and hepatopancreas of fresh water mussel, Indonaia caeruleus exposed to fluoride and found severe damages in them. There was disfigurement and enlargement in the size of lumen of lobules, disorganization and extensive vacuolization in the cytoplasm of cells. The severity of effect was dose dependent. Anita and Tilak (2003) have observed marked pathological changes like necrosis, progressive degeneration in the gill tissue and atrophy, appearance of blood streaks among hepatocytes etc in the liver of the fish Cirrhinus mrigala exposed to fenvalerate.
In thiodan exposed mosquitofish, *Gambusia affinis*, histopathological alterations were characterised as oedema, degeneration, hypertrophy, sinusoid’s enlargement, haemorrhage, pycnosis of nuclei, vacuolization of cell cytoplasm, infiltration of mononuclear lymphocyte and congestion in liver. These alterations were time and dose dependent (Cengiz *et. al.*, 2001).

Birgul *et. al.* (2004) studied the histopathological effects of endosulfan on the great rams-horn snail, *Planorbarius corneus* and observed amoebocytes infiltration, dilatation in haemolymphatic spaces between the tubules, degeneration of cells, abnormal lumen, necrosis of cells and atrophy in the connective tissue of digestive glands. Unlu *et. al.* (2005) observed irreversible necrotic changes in digestive glands of snail, *Lymnaea stagnalis* exposed to sublethal concentration of thiodan (35% EC.).

Cengiz (2005) also studied histopathological effects of thiodan on freshwater snail, *Galba truncatula* and recorded accumulation of amoebocytes in the haemolymphatic spaces between the tubules of the digestive glands, exudation in the lumen of tubules, expansion of haemolymphatic spaces between the tubules and increase of vacuolization and necrotic changes in digestive cells. Usheva *et. al.* (2006) studied histopathology of digestive glands of the bivalve mollusc, *Crenomytilus grayanus* from south-eastern Peter, the Great Bay of Japan, subjected to the effect of polluted water. They found erosive disturbances and heavy vacuolization of digestive cells in the epithelium of the tubules and channels, lipofuscin, necrosis and lysis of cells in connective tissue. Swelling of nerve fibers in some molluscs was also observed. They concluded that, these pathological changes might be due to chronic pollution of the bay and parasitic invasion.

The gonads of the quialphos treated bivalves showed abnormal growth of oocytes, damage and disintegration of oocytes and nuclear structures, cytolysis of oocytes, degeneration of ovarian sac, and irregular shaped oocytes with damaged nucleus.

The gonads of the thiodan treated bivalves showed arrest of ovarian maturity, degeneration of ovarian follicles, atrophy of ovarian cord, cytolysis of oocytes, death of oocytes etc.

Testicular follicles were highly shrunken with compactly arranged, disturbed spermatogenic stages indicating beginning of necrosis of testicular tissue while the spermatogenic stages were clumped and sperms were unusual. Regression of
spermatic cords, vacuoles in spermatic follicles, clumping of spermatozoa and atrophic condition was also observed.

Zutshi (2003) reported depletion of the sudanophilic granules and degranulation along with weak sudanophilia, cromophobia and vacuolation in the cytoplasm of interstitial cells, after exposure of freshwater fish *G. giuris* to sublethal concentration of fenthion.

It is the known fact that, the severity of the pathological alterations in gonads depends upon the sexual maturity, breeding, route of administration of pesticides and its dose. Highest concentration of carbaryl prevented the reproduction and decreased survival in *Pimephales promelas* (Carlson, 1972). Malathion caused shrinkage and vacuolization in oocyte and yolk was denatured in mature oocyte in the ovary of *Sarotherodon mossambicus* (Shukla et. al., 1984).


Sharma (1989) found cessation of proliferation in immature ovary due to damage to germinal zone in crab, *Scylla serrata* after exposure to organophosphate pesticides methylparathion and phosalone. Chronic treatment of DDT to caridian prawn, *Caridina rajdhari* increased phagocytic cells and rate of yolk synthesis in oocytes (Victor, 1984).

Reddy *et. al.* (1983) explained that, histological alterations observed in ovary of *Scylla serrata* are due to insufficient release of endogenous gonadotrophins after acute and chronic exposure to methylparathion and phosalone. When exposed to malathion, eye stalks in *Oziotelphusa senex senex* released excess gonadal inhibiting hormone leading to decreased gonadal indices (Bhagyalakshmi, 1981).

Suresh (2001) in *U. annulipes* exposed to cadmium and mercury observed changes like swelling of oocytes, vacuolization in oocytes, degeneration of oolemma, loss of normal shape of oocyte, necrosis, fusion of adjacent pocyte, picnosis in ooplasm and nucleus, atresia, turgidity in ovary, disorganized ooplasm, hyperchromatic nuclei, necrotic oocytes, and fibrosis of ovaries. Patil and Dhande (2000) noticed excessive degeneration of seminiferous tubules, distortion of
spermatids and picnosis of spermatogenetic cells in teleost, *Channa punctutus* treated with mercuric chloride, cadmium chloride and cupric chloride

Ramchandra (2000) while studying effect of sublethal exposure of malathion on freshwater fish, *Glassobius quaris* noticed significant reduction in the ovarian weight and diameter of developing oocytes, degeneration of growing oocytes, resorption and yolk of oocytes and atresia. Jyothi and Narayan (1996) studied the effect of organophosphorous insecticide phorate on gonads of fresh water fish, *Clarias batracus* and suggested that, the toxicity of any chemical necessarily impairs the reproductive physiology adversely as ovaries perform the function of producing eggs and steroid hormones and testis in the production of sperms. Kumar and Nath (1997) reported significant decrease in the number of spermatocytes per seminiferous tubule in the testes of mice due to malathion exposure. Naik *et. al.* (1996) also found significant decrease in the ovarian index in leeches, when exposed to the sublethal concentration of the heptachlor, monocrotophos and fenvalerate for 15 days.

Pollutants cause impairment of all physiological and vital processes including gametogenesis, reproduction, osmoregulation, respiration, etc. and also cause depletion of energy stored in tissues. Lipophilic contaminants accumulated by the adult may also get deposited in yolk material during oogenesis and impair embryonic development (Rossi and Anderson, 1978). Pollutant may also directly block fertilization or some steps of embryonic or larval development (Rosenthal and Alderdice, 1976)

The effect of pollutants on gametogenesis, fertilization and larval development was also observed in *Indonesia caeruleus* (Pillai, 1984) and in *Parreysia corrugata* (Thorat, 1990). Suzuki and Matsushita (1969) and Spyker *et. al.* (1972) reported nervous disorders and accumulation of mercury in vital brain centres in human beings. Waldichuk (1974) has suggested possibility of similar effects on aquatic organisms.

Any pollutants in aquatic environment are harmful to the animals as it directly or indirectly affect the maturation and growth of individuals and reproduction. It may eliminate the population of species completely which in turn imbalances the ecosystem. Environmental contamination disrupts the endocrine function in animals and humans. Many of the endocrine disruptors act as esterogenic or antiandrogenic as they influence the sex hormone profile, the development of male and female sex organs and secondary sex characteristics and thereby create fertility problem.
Werner et al. (2004) have investigated biomarker responses and survival of *Macoma nasuta* (Bivalvia) exposed to sediments from Northern San Francisco Bay and found accumulated metals, polyaromatic hydrocarbons (PAHs) and organochlorine pesticides (aldrin and P, P’ – DDT and its metabolites P, P’ – DDT and P, P’ – DDE) and observed mortality, stress protein (hsp70) in gill and histopathologic lesions in gonads, and lysosomal membrane damage was found to be significantly correlated with tissue concentrations of DDT and / or its metabolites.

Kulkarni et al. (1987) observed the effect of pesticides like endosulfan, malathion and copper sulfate on the gametogenesis of freshwater leech, *Hirudo birmanica* and found arrest in spermatogenesis and oogenesis. They have suggested that, the impaired gametogenesis in above case represent the cumulative effects of disturbed hormonal and enzymatic activities due to the effects of different pesticides. It is quite possible that, these pesticides may interfere with the secretion and release of gonadotrophins which are essential for normal gametogenesis (Mill, 1978).
Summary

- Freshwater bivalves were exposed to chronic dose (15 and 30 days) of sub lethal concentration of quinalphos (0.108 ppm) and thiodan (0.0708 ppm) so as to study the histopathological effects of pesticides on gills, gonads and digestive glands of freshwater bivalves, Parreysia corrugata.
- Severe damages were found in gills, gonads and digestive glands of pesticide exposed bivalves.
- The gills were severely damaged showing swollen tips of filaments, damage of chitinous rods, air spaces, vacuolization and detached gill filaments in the pesticide exposed bivalves.
- In the bivalves exposed to quinalphos and thiodon, the normal structure of the hepatic follicles was lost, connective tissue between the follicles was affected, and the hepatic secretary cells and the lumen were also affected.
- Gonads showed variations with respect to the male and female follicles and the stages of the development of the gametes in pesticide exposed bivalves.
- The damages in the cytoarchitacture of gills, gonads and digestive glands were less severe in quinalphos exposed bivalves than those of thiodan exposed bivalves.
- Intensity of damage in the tissues of pesticide exposed bivalves was found to be increased with increase in exposure time.