CHAPTER : III

HISTOPATHOLOGY
INTRODUCTION

Many heavy metals are known for their strong action towards biological tissues into which they are bioaccumulated once absorbed into the body of an organism, the metal ions cause damage to cells and tissues in varying degrees depending upon their toxic phenomenon.

A study of pathological changes in the microtomy of tissues is known as histopathology. Since the midnineteenth century, this branch of science has been successfully employed as a diagnostic tool in medical and veterinary sciences. Now some environmental scientists are beginning to correlate the degree of cell damage to concentration of toxic substances and their synergistic or antagonistic interaction. The histopathological studies not only give an early indication of pollutional hazard but also provide useful data on nature and degree of damage to cells and tissues. It is a common tool for determining the deleterious effects of toxic substances in animals. Many workers have employed this tool in the study of aquatic pollution due to organic and inorganic substances (Ellis et al., 1937; Llyod 1960; Crandall and Goodnight 1963; Brown et al. 1968 and Baker, 1969). Pollutants possibly affect all the body parts of exposed organism either physiologically or by inducing histological changes.
The main way for the pollutants to enter in the body of an animal is the blood system from where the pollutant gets distributed to the whole body. It is generally agreed that the initial distribution is rapid and that the pattern of distribution roughly corresponds to the speed and pattern of blood circulation.

Pollution of water by any pollutant can have deleterious effects on aquatic organisms. The nature of the effects varies and may cause structural levels in and functional modification at both the cellular and subcellular levels in organisms. Through the gills these pollutants find their way into the body of aquatic organisms. After entering through circulation these pollutants reach different organs of the body and ultimately bring about physiological changes.

Histopathological changes in the tissue due to heavy metals poisoning, have been reported for a variety of organisms. Such as changes in the gills of fresh water prawn exposed to copper and cadmium have been reported by Couch (1977). Extensive damage has been noticed in the gills, kidney and intestine of the chosomalone, *Oncorhynchus kisutch* exposed to chromium (Strik *et al.*, 1975). In the liver, kidney and gills of winter, flounders *Pseudopleuronectes americanus* exposed to copper (Baker, 1969) Hanumante *et al.* (1981) observed histopathological changes in the
hepatopancreas of the freshwater fish, *Channa gachua* following chronic exposure to two molluscicides mercuric chloride and sodium pentachlorophenate. Vernberg and Vernberg (1972) have observed changes in the gill tissue of crab after exposure to sublethal concentration of mercury, Ghate and Mulherkar (1979) have studied the changes in the gill tissue of two fresh water prawns *Macrobranchia* and *caridina* exposed to copper, sulphate. Aken and Byared (1969) observed *Americanus* exposed to yellow phosphorus. Bodkhe (1983) reported the histopathological changes in the gills of fresh water crab, *Barytelphusa cunicularis*, when exposed to carbamate sevimol, Mary (1984) reported the structural changes in the gills following exposure to sevin in the freshwater prawn *Macrobranchium lamerei*. Establics (1979) reported structural changes in the gill, liver, pancreas, kidney, intestine and gill bladder of *Sparus aurita* exposed to mercuric chloride and methyl mercuric chloride. Similar reportes are also available for cadmium stressed marine fish Gardner and Yerich (1970). Nimmo et al. (1977) have reported structural changes in the gills of the shrimps *Penaeus vulgaris* exposed to cadmium.

Among crustaceans, hepatopancreas and gills are the important tissue. The mobilisation of lipid content and osmoregulation are mainly controlled by tissue of these two organs.
Momin and Rangnekar, (1975), Loizzi (1971) have been reported that there are different types of cell with diversified functions like absorption, storage and enzyme secretion. The evidences available suggested that the digestive enzymes are produced mainly or even solely by the hepatopancreas. The secretion of this gland however, can be passed forever into the cardiac stomach and initiate digestion. The enzymes secreted by the hepatopancreas convert the complex carbohydrate and related compounds into simple form. It is evident from the available literature that even the slight change in the cells of hepatopancreas can cause hazardous effect on the physiology of digestion. Gills are the primary site of damage due to pollutants which affect the gill functions and thereby disrupting the normal physiological activities. The gills serves as a sensitive index in toxicity studies since they occupy a strategic position between the external and internal contact with the water. It is well known that gills play an important role in the respiration and osmoregulation. Due to this even slight damage to gills can cause tremendous osmoregulatory and respiratory distress to the animals. Reproduction is a physiological process and is an essential biological need of animals for the continuity of the generation (Vernberg and Vernber, 1972). For the careful study of the strategies of an individual it is of prime importance to deleveate its reproductive
infrastructure. The aquatic environment permits to adapt the organism and to reproduce their species successfully. Sometimes aquatic environment is being threatened by a number of pollutants including pesticides and industrial effluents which would affect the biological phenomenon such as reproduction.


Due to the accumulation of the pollutants in aquatic ecosystem the reproductive process gets decelerated and on the other hand long term exposure to the pollutants causes a considerable damage to the tissues of reproductive organ, decelerating the reproductive cycle and restricting the development of eggs. Hatching of the eggs and newly hatched young ones are also affected by the exposure to pollutants and ultimately reduces
their yield. During long term exposure more pollutants gets accumulated in the tissues of the animals and thus it becomes unfit for human use.


The histological picture of tissue provides valuable information about the health of the tissue. It could be used in determining the extent of tissue damage that could be safely permitted under lethal and sublethal stress of mercury chloride and cadmium chloride. Extensive tissue damage may have a crippling effect on the physiology of the tissue in question and this may have profound impact on the vital processes of the wilds animals.

It is possible that the histopathological changes may be the manifestation of a sick tissue and these changes underline the observed pathological changes in the tissues. However, it is not possible to say whether the structural changes are really the
manifestation of sick tissue which is slowly but surely succumbing under the impact of the toxic action of the metal poison or else, constituting a protective mechanism to accumulate toxic metal ions sequestration and their eventual elimination. Only further investigation may perhaps through more light on these aspects.

On surveying the histopathological studies it is found that no attention has been paid on the effect of mercuric chloride and cadmium chloride on gonads of fresh water crab, *B. cunicularis*. Hence in the present investigation the effect of heavy metal is studied on the ovary and testes of the fresh water crab, *B. cunicularis* after lethal and sublethal exposure. The histopathological study is a mirror and an indicator to the effect of heavy metal. In this context the present investigation is carried out.
MATERIAL AND METHODS

The fresh water crab Barytelphusa cunicularis were collected from Godavari river, Paithan 50 km. away from Aurangabad. The crabs were kept acclimatized to laboratory conditions for about 2 to 3 days. In a plastic trough containing 2 litres of tap water before being used for the experiment. Selected healthy specimen were used for the experimental studies.

To study, the histopathological lesions in ovary and testes, were exposed to mercuric chloride and cadmium chloride in all the three seasons, i.e. Summer, monsoon and winter, at lethal and sublethal concentrations respectively. Simultaneously control groups of crabs were also maintained.

At the end of exposure period, the experimental and control crabs were sarificed and the tissues i.e. ovary and testes were quickly excised and fixed in Bouins fixative. After fixation for 24 hrs. the tissues were washed and dehydrated in graded series of alcohol and cleared in xylene. They were embedded in paraaffin wax and serial sections were cut at 7-8 μ and stained with mallory (1944).
RESULTS

Effect of Mercuric chloride on the ovary of the freshwater crab,  
Barytelphusa cunicularis :-

Control ovary :

Histologically the ovary of the crab, Barytelphusa  
cunicularis is covered with outer thin epithelium and inner  
germinative epithelial layer from which the oocytes proliferate. The  
mature oocyte is covered with these membranes i.e. oocyte  
membranes. Oocyte has a large rounded nucleus with one or two  
nucleoli. The nutritive cells are present in the close vacinity of  
oocytes and supply the nutritive material to the developing oocytes.  
Oocytes are covered with a layer of follicle cell. The ooplasm is  
compactly arranged with thick yolk granules ovarian follicle are  
filled with different types of maturing oocytes. (Fig. No. 1)

Effect of lethal concentration of mercuric chloride on the ovary of  
freshwater crab Barytelphusa cunicularis :- In summer

In acute ovary, after 24 hours exposure pycnosis of  
nutritive cells and nucleus of oocytes was observed due to shrinkage  
of oocytes. Destruction of epithelial layer and degeneration of  
oocytes were seen, epidermal layer was loosely arranged,  
vacuolisation was also observed in the periphery of the oocyte  
cells. (Fig. No. 2).
In ovary of 48 hours exposure brought about damage to ovarian layer, destruction of follicular epithelial layer and vacuolisation towards the periphery of oocytes, proliferation continued, but no vitellogenesis took place and increase in the number of phagocytes, as ovary enters in degenerating phase was also noticed. (Fig. No. 3).

After 72 hours exposure ovary showed irregular shape of oocytes, mixing of ooplasmic material due to disintegration of follicular epithelium maximum nature of degenerating oocytes with disintegrated nuclei was observed. Vacuoles are more in peripheral region of lobules, disappearance of nucleus in some oocytes were noticed (Fig. No. 4).

In case of 96 hours exposure, mixing of ooplasmic material due to disintegradation of follicle epithelium, fusion of three or four oocytes together and maximum number of degenerating oocytes with disintegrated nucleoli and nuclei were observed. (Fig. No. 5).

**Effect of sublethal concentration of mercuric chloride on ovary of freshwater crab Barytelphusa cunicularis:**

After 10 days of exposure oocytes were found to be scattered rupturing of epithelial layer of oocytes and vacuolisation in the oocytes were observed. (Fig. No. 6).
After 20 days of exposure the degeneration of peripheral oocytes, shrinkage of ooplasmic material and increase in number of phagocytes within the ovarian stroma were observed. (Fig. No. 7)

In case of 30 days exposure complete damage to ovarian structure was observed, such as total damage in oocyte epithelial layer and vacuolisation in the oocytes. The yolk granules and fat globules were observed in rich amount (Fig. No. 8).

**Effect of mercuric chloride on testes of the freshwater crab Barvtelphusa cunicularis control testes:**

The histological structure shows that the testicular follicles are closely packed together. Each testicular tubules had a thin membrane and a central cavity. The membrane of the tubule is made up of two layers, an outer connective tissues layer and an inner germinal epithelial layers, it grows in size and becomes spermatoblast. From the spermatoblast they form the spermatoblast. The process of spermatogenesis in crab can be divided into five phases i.e. (a) spermatogonia (b) spermatocytes (c) spermatids (d) spermatozoa and (e) spermatophore formation (Fig. No. 9).

**Effect of lethal concentration of mercuric chloride on testes of the freshwater crab Barvtelphusa cunicularis :- In summer**

After 24 hours exposure a severe damage and altered testicular activity was noticed spermatozoa were packed together, vacuolisation was also observed (Fig. No. 10).
After 48 hours exposure the testicular layer was damaged and spermatozoa were disturbed and becomes irregular (Fig. No. 11).

After 72 hours exposure irregular arrangement of testicular tubules and vacuolisation was observed. Ruptured testicular epithelium was also observed. (Fig. No. 12)

After 96 hours exposure it is noticed that irregularly arranged testicular tubules damages testicular wall and disappearance of spermatozoa was observed. Damage to testicular layer and space between testicular tubules were also noticed. (Fig. No. 13).

**Effect of sublethal concentration of mercuric chloride on testes of the freshwater crab Barystelphusa cunicularis** :-

After 10 days of exposure testicular tubules becomes irregularly arranged, some of the spermatocyte started to disappear. (Fig. No. 14)

After 20 days of exposure brought about very drastic changes like damage to tubules was completely disturbed, cell was completely degenerated. (Fig. No. 15).

In case of 30 days exposure the maximum damage to the follicular cell wall and bunches of sperm mass was noticed. Reduced spermatogenic elements and damage to the tubular cell wall occured. (Fig. No. 16)
Effect of lethal concentration of cadmium chloride on the ovary of freshwater crab, Barytelphusa cunicularis: - In summer

After 24 hours of exposure pycnosis in the nutritive cells and nucleus, shrinkage in periphery of oocytes were noticed. The destruction in epithelial layer was also seen (Fig. No. 17).

After 48 hours exposure, shrinkage and vacuolisation in oocytes were noticed. Disappearance of nucleus in some oocytes were also noticed. (Fig. No. 18).

After 72 hours exposure decrease in oocyte diameter, disappearance of nucleus from the oocytes and degeneration of ovarian wall were seen. (Fig. No. 19).

In case of 96 hours a severe histological lesions were noticed nucleus disappeared in the oocyte, increase in number of phagocytes and shrinkage in oocytes were observed. (Fig. No. 20).

Effect of sublethal concentration of cadmium chloride on the ovary of the freshwater crab Barytelphusa cunicularis: -

After 10 days exposure shrinkage in epithelial layer of oocytes, vacuolisation in oocytes were noticed. (Fig. No. 21).

After 20 days exposure, degeneration of peripheral oocytes, increase in number of phagocytes ruptured epithelial layer was observed. (Fig. No. 22).

However, 30 days exposure showed damage to epithelial layer and vacuolisation in oocytes. (Fig. No. 23).
Effect of lethal concentration of cadmium chloride on the testes of freshwater crab Barvytelphusa cunicularis :- In summer :

After 24 hours exposure, damage to the testicular cells were observed vacuolisation was also observed in testicular tubules when compared to control group. (Fig. No. 24).

After 48 hours exposure damage in testicular layer and packed spermatozoa were observed. (Fig. No. 25)

After 72 hours exposure irregular arrangement of testicular tubules and ruptured testicular epithelium was noticed (Fig. No. 26).

In case of 96 hours exposure, vacuolisation, irregular arrangement of testicular tubules and disappearance of spermatozoa were noticed (Fig. No. 27).

Effect of sublethal concentration of cadmium chloride on the testes of freshwater crab Barvytelphusa cunicularis :-

After 10 days exposure irregularly arranged testicular cells and clumping in spermatozoa was observed. (Fig. No. 28)

After 20 days exposure the testes showed damage to follicular cell wall, vacuolisation and degenerated testicular tubules were also observed (Fig. No. 29). However, after 30 days exposure, shrinkage in spermatogenic elements and damaged cell wall were noticed (Fig. No. 30).
**Effect of lethal concentration of mercuric chloride on ovary of freshwater crab Barytelphusa cunicularis : In monsoon**

After 24 hours exposure pycnosis of nutritive cells of nucleus of oocytes was observed due to shrinkage of oocytes. Destruction of epithelial layer and degeneration of oocytes was seen vacuolisation was also observed in the periphery of oocyte cells (Fig. No.31).

After 48 hours exposure shrinkage within the ooplasm, destruction as follicular membrane vacuolisation and degeneration of the peripheral ova was noticed. (Fig. No.32).

After 72 hours exposure decrease in oocyte diameter, number of phagocytes and yolk globules increased vacuolisation was also seen in peripheral region. (Fig. No. 33).

In case of 96 hours exposure the maximum damage of ovary. Nucleus was disappeared in the oocyte increase in the number of phagooytes and shrinkage in the oocytes were observed (Fig. No.34).

**Effect of sublethal concentration of mercuric chloride on ovary of freshwater crab Barytelphusa cunicularis :-**

After 10 days the follicular epithelium of the oocytes becomes ruptured. Postvitellogenic ova increased in size and vacuolization was observed (Fig. No.35).
After 20 days of exposure phagocytes follicular cells were increased in number representing slight recovery but same areas showed degenerating oocytes and shrinkage of nuclear elements (Fig. No.36).

In case of 30 days exposure complete damage to ovarian structure was observed such as total damage in oocyte epithelial layer and vacuolisation in the oocytes. The yolk granules and fat globules were observed (Fig. No.37).

**Effect of lethal concentration of mercuric chloride on testes of freshwater crab Barytelphusa cunicularis:**

- **In monsoon:**

  After 24 hours exposure damage to testicular cells were observed vacuolisation was also observed in testicular tubules when compared to control group (Fig. No.38).

  After 48 hours exposure rupture of testicular layer and vauolization of testicular lobes were noticed (Fig. No.39).

  After 72 hours exposure irregular arrangement of testicular tubules and ruptured testicular epithelium were noticed (Fig. No.40).

  After 96 hours exposure irregular arrangement of testicular tubules damaged testicular wall and disappearance of spermatzoa were observed. Damage to testicular layer noticed and space between testicular tubules were also observed. (Fig. No. 41)
Effect of sublethal concentration of mercuric chloride on testes of freshwater crab Barytelphusa cunicularis :-

After 10 days exposure caused thickening of testicular follicular layer, hypertropic changes with cytoplasmic vacuolisation in primary spermatocytes rupture of peripheres layer and accumulation thick sperm mass in lobules (Fig. No.42)

After 20 days exposure brought about very drastic changes like damage to follicular cell wall testicular material inside the tubules was completely disturbed cell wall completely degenerated. (Fig. No. 43)

In case of 30 days of exposure bundles of sperm mass was observed shrinkage in spermatogenic element and damage to the tubular cell wall occurred. (Fig. NO. 44)

Effect of lethal concentration of cadmium chloride on ovary of freshwater crab Barytelphusa cunicularis :- In monsoon :-

After 24 hours exposure pycnosis of nutritive cells and nucleus and shrinkage of oocytes was observed vacuolization in the ooplasm was noticed. (Fig. No. 45)

After 48 hours exposure the follicular layer covering the oocytes was affected. (Fig. No. 46)

After 72 hours exposure vacuolisation in the ooplasm measured and proliferating layer gets affected. (Fig. NO. 47)
After 96 hours exposure it is noticed that disappearance of nucleus and nucleolus takes place. (Fig. No. 48)

**Effect of sublethal concentration of cadmium chloride on ovary of freshwater crab Barytelphusa cunicularis:**

After 10 days exposure involves degeneration of nucleus, nuclear material and ooplasm. (Fig. No. 49)

After 20 days exposure showed impairment of the ovarian tissue and the picture is depicted in (Fig. No. 50). It indicates that there is prominent and repellent action in the form of degeneration. The cells assumed abnormal shapes.

After 30 days exposure the size of the oocyte did not show an increase in diameter but the nucleus and nucleolus have disappeared. The harmful effect of treated tissue resulted, in a decline towards reproductive activity. (Fig. No. 51)

**Effect of lethal concentration of cadmium chloride of testes of freshwater crab Barytelphusa cunicularis:** *In monsoon:*

After 24 hours exposure the arrangement of testicular tubules was disturbed and the spermatozoa become irregular. (Fig. No. 52)

After 48 hours exposure vacuolization was observed in the testicular tubules and bunches of spermatozoa were noticed. (Fig. No. 53)
After 72 hours exposure the central lobules alone containing spermatozoa while other closely associate lobules were empty. Suggesting an inhibition of spermatogenic development in the central lobules and destruction of spermatozoa. *(Fig. No. 54)*

In case of 96 hours exposure the spermatozoa disappeared in some testicular tubules and the follicular layer was damaged. *(Fig. No. 55)*

**Effect of sublethal concentration of cadmium chloride on testes of freshwater crab Barytelphusa cunicularis:**

After 10 days exposure irregular arrangement of testicular tubules and vacuolisation was observed in the testicular tubules. *(Fig. No. 56)*

After 20 days exposure tubular layer was damaged and the spermatozoa scattered throughout the lumen. *(Fig. No. 57)*

In case of 30 days exposure damage of testicular tubule was very high and spermatozoa in the tubules was destroyed. *(Fig. No. 58)*

**Effect of lethal concentration of mercuric chloride on ovary freshwater crab Barytelphusa cunicularis:** *In winter*

After 24 hours exposure caused damage to ovarian wall and vacuolization in the ooplasm *(Fig. No. 59)* shrinkage of nucleus was observed.
After 48 hours exposure the ovary showed damage to ovarian layer, increase in number of phagocytes and degeneration of ovarian cells (Fig. No.60).

After 72 hours exposure decrease in oocyte diameter disappearance of nucleus from the oocyte and degeneration of ovarian wall was seen (Fig. No.61).

After 96 hours exposure the changes include increased vacuolization in the ooplasm and rupture of oocyte membrane and degeneration of nuclei and nucleoli (Fig. No.62).

**Effect of sublethal concentration of mercuric chloride on ovary of freshwater crab Barvolphusa cunicularis:**

After 10 days exposure ruptured epithelial layer of oocytes distinct damage to proliferating zone and vacuolization in the oocyte were observed. Nucleus and nucleolus was degenerated. Damage to oocyte epithelial layer was noticed (Fig. No.63).

After 20 days of exposure degeneration of pheripheral oocytes increase in number of phagocytes, rupture in epithelial layer was observed (Fig. No.64).

The drastic damage was observed after 30 days exposure showed damage to epithelial layer and vacuolisation oocytes, phagocytes increased with vacuoles (Fig. No.65).
Effect of lethal concentration of mercuric chloride on testes of freshwater crab Barytelphusa cunicularis : - In winter

After 24 hours exposure a damage and altered testicular layer was noticed spermatozoa were packed together, was also observed (Fig. No.66).

After 48 hours exposure shows compactly arranged tubules and some abnormality in spermatophore formation as evidenced by the presence of round shpaed spermatophores instead of crescent shape as well as disruption of proliferating zone and testicular tubules. (Fig. No.67).

After 72 hours exposure caused vacuolisation in lumen tubular structure of the organ is disturbed, follicular epithelium ruptured. There is degeneration in germinal epithelium (Fig. No.68).

After 96 hours exposure caused severe damage to the testicular tissue, testicular wall was damaged testicular tubules were arranged irregularly and the disappearance of spermatozoa from the testicular tubules resulted in empty spaces suggesting an inhibition of spermatogenic development in the center lobules as well as destruction of spermatozoa. There was extensive degeneration and pycnosis of the germinal element (Fig. No. 69).
Effect of sublethal concentration of mercuric chloride on testes of freshwater crab Barytelphusa cunicularis:

After 10 days exposure the testicular tubules became irregularly arranged. Some of the spermatocytes started to disappear (Fig. No. 70).

After 20 days exposure tubular layer was damaged and the spermatozoa scattered throughout the lumen (Fig. No. 71).

However, 30 days exposure shrinkage in spermatogenic element and damaged cell wall completely lysis of lobular epithelium release of sperms from the lobules were observed (Fig. No. 72).

Effect of lethal concentration of cadmium chloride on ovary of freshwater crab Barytelphusa cunicularis: In winter

After 24 hours exposure the arrangement of oocytes was irregular and the oocytes membrane ruptured. (Fig. No. 73)

After 48 hours exposure degeneration of oocytes and the vacuolization in the ooplasm takes place. (Fig. No. 74)

After 72 hours exposure the arrangement of oocytes was irregular and shrinkage of oocytes was observed. Yolk material started disappearing. (Fig. No. 75)

In case of 96 hours exposure the oocytes degenerated and disappearance of the nucleus in some oocytes was noticed. The results are depicted in (Fig. No. 76).
**Effect of sublethal concentration of cadmium chloride on ovary of freshwater crab Barytelphusa cunicularis :-**

After 10 days exposure vacuolization was observed in the ooplasm and disappearance of nucleus in some oocyte was noticed. The result are present in (Fig. No. 77).

After 20 days exposure shrinkage of oocytes and irregular oocyte membrane was noticed. The ovarian tissue was damaged and the destruction of follicular epithelial layer around the ovary was observed. (Fig. No. 78)

After 30 days of exposure the extreme toxicity is evident from the total degeneration of oocytes and the development of oogonial cells was completely retarded complete destruction of oocytes indicates the extent of toxicity when compared with control. Results are presented in (Fig. No. 79).

**Effect of lethal concentration of cadmium chloride on testes of freshwater crab Barytelphusa cunicularis :- In winter**

After 24 hours exposure the arrangement of testicular tubules was irregular. The spermatozoa were packed together and stained dark vacuolization was observed in the testicular tubules (Fig. No. 80).

After 48 hours exposures testicular layer was damaged and destruction of spermatozoa was noticed. (Fig. No. 81)
After 72 hours exposure the central lobules alone containing spermatozoa while other closely associated lobules were empty, destruction of spermatozoa was also seen. (Fig. No. 82)

In case of 96 hours exposure irregular arrangement of testicular tubules, destruction of testicular tubular layer was more than compared with 72 hours. (Fig. No. 83)

**Effect of sublethal concentration of cadmium chloride on testes of freshwater crab Barvtelphusa cunicularis:**

After 10 days exposure the testicular tubules became irregularly arranged destruction of spermatozoa was noticed. (Fig. No. 84)

After 20 days exposure brought about vacuolization in the testicular tubules and destruction of spermatocytes. (Fig. No. 85)

In case of 30 days exposure the maximum damage to the follicular cell wall and banches of sperm mass was noticed. the lobular structure of the organ was distrubed and disorganization of spermatogenic element in many lobules was also noticed. (Fig. NO. 86)
Fig. 1. T.S. of ovary of control freshwater crab, *B. cunicularis*
Mallory Triple x 100.

FC = Follicular cell.
DO = Degenerating oocyte.
N = Nucleus.

Fig. 2. T.S. of ovary of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in summer for 24 hrs.
Mallory Triple x 100.

Fig. 3. T.S. of ovary of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in summer for 48 hrs.
Mallory Triple x 100.

Fig. 4. T.S. of ovary of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in summer for 72 hrs.
Mallory Triple x 100.

Fig. 5. T.S. of ovary of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in summer for 96 hrs.
Mallory Triple x 100.

Fig. 6. T.S. of ovary of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in summer for 10 days
Mallory Triple x 100.

Fig. 7. T.S. of ovary of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in summer for 20 days
Mallory Triple x 100.

Fig. 8. T.S. of ovary of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in summer for 30 days
Mallory Triple x 100.
Fig. 9. T.S. of testes of control fresh water crab, *B. cunicularis*
Mallory Triple x 100.

\[\begin{align*}
SG &= \text{Spermatogonia.} \\
V &= \text{Vocuole.} \\
ST &= \text{Spermatid.} \\
SC &= \text{Spermatocyte.} \\
TF &= \text{Testicular follicle}
\end{align*}\]

Fig. 10. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in summer for 24 hrs.
Mallory Triple x 100.

Fig. 11. T.S. of testes freshwater crab, *B. cunicularis*
exposed to mercuric chloride in summer for 48 hrs.
Mallory Triple x 100.

Fig. 12. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in summer for 72 hrs.
Mallory Triple x 100.

Fig. 13. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in summer for 96 hrs.
Mallory Triple x 100.

Fig. 14. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in summer for 10 days
Mallory Triple x 100.

Fig. 15. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in summer for 20 days
Mallory Triple x 100.

Fig. 16. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in summer for 30 days
Mallory Triple x 100.
Fig. 17. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to cadmium chloride in summer for 24 hrs.
Mallory Triple x 100.

Fig. 18. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to cadmium chloride in summer for 48 hrs.
Mallory Triple x 100.

Fig. 19. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to cadmium chloride in summer for 72 hrs.
Mallory Triple x 100.

Fig. 20. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to cadmium chloride in summer for 96 hrs.
Mallory Triple x 100.

Fig. 21. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to cadmium chloride in summer for 10 days
Mallory Triple x 100.

Fig. 22. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to cadmium chloride in summer for 20 days
Mallory Triple x 100.

Fig. 23. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to cadmium chloride in summer for 30 days
Mallory Triple x 100.
Fig. 24. T.S. of testes of freshwater crab, *B. cunicularis* exposed to cadmium chloride in summer for 24 hrs. Mallory Triplet x 100.

Fig. 25. T.S. of testes of freshwater crab, *B. cunicularis* exposed to cadmium chloride in summer for 48 hrs. Mallory Triplet x 100.

Fig. 26. T.S. of testes of freshwater crab, *B. cunicularis* exposed to cadmium chloride in summer for 72 hrs. Mallory Triplet x 100.

Fig. 27. T.S. of testes of freshwater crab, *B. cunicularis* exposed to cadmium chloride in summer for 96 hrs. Mallory Triplet x 100.

Fig. 28. T.S. of testes of freshwater crab, *B. cunicularis* exposed to cadmium chloride in summer for 10 days Mallory Triplet x 100.

Fig. 29. T.S. of testes of freshwater crab, *B. cunicularis* exposed to cadmium chloride in summer for 20 days Mallory Triplet x 100.

Fig. 30. T.S. of testes of freshwater crab, *B. cunicularis* exposed to cadmium chloride in summer for 30 days Mallory Triplet x 100.
Fig. 31. T.S. of ovary of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in monsoon for 24 hrs.
Mallory Triple x 100.

Fig. 32. T.S. of ovary of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in monsoon for 48 hrs.
Mallory Triple x 100.

Fig. 33. T.S. of ovary of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in monsoon for 72 hrs.
Mallory Triple x 100.

Fig. 34. T.S. of ovary of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in monsoon for 96 hrs.
Mallory Triple x 100.

Fig. 35. T.S. of ovary of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in monsoon for 10 days
Mallory Triple x 100.

Fig. 36. T.S. of ovary of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in monsoon for 20 days
Mallory Triple x 100.

Fig. 37. T.S. of ovary of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in monsoon for 30 days
Mallory Triple x 100.
Fig. 38. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in monsoon for 24 hrs.
Mallory Triple x 100.

Fig. 39. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in monsoon for 48 hrs.
Mallory Triple x 100.

Fig. 40. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in monsoon for 72 hrs.
Mallory Triple x 100.

Fig. 41. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in monsoon for 96 hrs.
Mallory Triple x 100.

Fig. 42. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in monsoon for 10 days
Mallory Triple x 100.

Fig. 43. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in monsoon for 20 days
Mallory Triple x 100.

Fig. 44. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in monsoon for 30 days
Mallory Triple x 100.
Fig. 45. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to cadmium chloride in monsoon for 24 hrs. Mallory Triple x 100.

Fig. 46. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to cadmium chloride in monsoon for 48 hrs. Mallory Triple x 100.

Fig. 47. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to cadmium chloride in monsoon for 72 hrs. Mallory Triple x 100.

Fig. 48. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to cadmium chloride in monsoon for 96 hrs. Mallory Triple x 100.

Fig. 49. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to cadmium chloride in monsoon for 10 days Mallory Triple x 100.

Fig. 50. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to cadmium chloride in monsoon for 20 days Mallory Triple x 100.

Fig. 51. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to cadmium chloride in monsoon for 30 days Mallory Triple x 100.
Fig. 52. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to cadmium chloride in monsoon for 24 hrs.
Mallory Triple x 100.

Fig. 53. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to cadmium chloride in monsoon for 48 hrs.
Mallory Triple x 100.

Fig. 54. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to cadmium chloride in monsoon for 72 hrs.
Mallory Triple x 100.

Fig. 55. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to cadmium chloride in monsoon for 96 hrs.
Mallory Triple x 100.

Fig. 56. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to cadmium chloride in monsoon for 10 days
Mallory Triple x 100.

Fig. 57. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to cadmium chloride in monsoon for 20 days
Mallory Triple x 100.

Fig. 58. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to cadmium chloride in monsoon for 30 days
Mallory Triple x 100.
Fig. 59. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to mercuric chloride in winter for 24 hrs. Mallory Triple x 100.

Fig. 60. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to mercuric chloride in winter for 48 hrs. Mallory Triple x 100.

Fig. 61. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to mercuric chloride in winter for 72 hrs. Mallory Triple x 100.

Fig. 62. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to mercuric chloride in winter for 96 hrs. Mallory Triple x 100.

Fig. 63. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to mercuric chloride in winter for 10 days Mallory Triple x 100.

Fig. 64. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to mercuric chloride in winter for 20 days Mallory Triple x 100.

Fig. 65. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to mercuric chloride in winter for 30 days Mallory Triple x 100.
Fig. 66. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in winter for 24 hrs.
Mallory Triple x 100.

Fig. 67. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in winter for 48 hrs.
Mallory Triple x 100.

Fig. 68. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in winter for 72 hrs.
Mallory Triple x 100.

Fig. 69. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in winter for 96 hrs.
Mallory Triple x 100.

Fig. 70. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in winter for 10 days
Mallory Triple x 100.

Fig. 71. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in winter for 20 days
Mallory Triple x 100.

Fig. 72. T.S. of testes of freshwater crab, *B. cunicularis*
exposed to mercuric chloride in winter for 30 days
Mallory Triple x 100.
Fig. 73. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to cadmium chloride in winter for 24 hrs. Mallory Triple x 100.

Fig. 74. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to cadmium chloride in winter for 48 hrs. Mallory Triple x 100.

Fig. 75. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to cadmium chloride in winter for 72 hrs. Mallory Triple x 100.

Fig. 76. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to cadmium chloride in winter for 96 hrs. Mallory Triple x 100.

Fig. 77. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to cadmium chloride in winter for 10 days Mallory Triple x 100.

Fig. 78. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to cadmium chloride in winter for 20 days Mallory Triple x 100.

Fig. 79. T.S. of ovary of freshwater crab, *B. cunicularis* exposed to cadmium chloride in winter for 30 days Mallory Triple x 100.
Fig. 80. T.S. of testes of freshwater crab, *B. cunicularis* exposed to cadmium chloride in winter for 24 hrs. Mallory Triple x 100.

Fig. 81. T.S. of testes of freshwater crab, *B. cunicularis* exposed to cadmium chloride in winter for 48 hrs. Mallory Triple x 100.

Fig. 82. T.S. of testes of freshwater crab, *B. cunicularis* exposed to cadmium chloride in winter for 72 hrs. Mallory Triple x 100.

Fig. 83. T.S. of testes of freshwater crab, *B. cunicularis* exposed to cadmium chloride in winter for 96 hrs. Mallory Triple x 100.

Fig. 84. T.S. of testes of freshwater crab, *B. cunicularis* exposed to cadmium chloride in winter for 10 days Mallory Triple x 100.

Fig. 85. T.S. of testes of freshwater crab, *B. cunicularis* exposed to cadmium chloride in winter for 20 days Mallory Triple x 100.

Fig. 86. T.S. of testes of freshwater crab, *B. cunicularis* exposed to cadmium chloride in winter for 30 days Mallory Triple x 100.
DISCUSSION

Pollutants possibly affect all the body parts of exposed organism either physiologically or by inducing histological changes. Once the pollutant enters the body, the organism tries to metabolise it, so that it is thrown outside the body. This is the chief mechanism of detoxification of toxic substances that find their way inside the body through one of the several pathways. Where a pollutant could not be metabolised organisms adapt in other ways of detoxification. The pollutant is distributed in the different organs in order to lessen its toxicity and again this depends on the affinity of a pollutant in connection towards certain biochemical components present in different parts of the body.

The pathological changes induced due to various pollutants which cause toxicity indicate the specific change or abnormality at tissue level. The present study on the histopathological changes in the gonads of the crabs, Barytelphusa cunicularis reveals that both the ovary as well as testes exhibit acute and chronic exposure to mercuric chloride and cadmium chloride.

Reproduction is one of the fundamental activities of the organisms. The aquatic environment permit the organism to adapt and reproductive successfully. In recent years this environment is
being threatened by a number of pollutant which may alter the
reproductive capacity of the animal. Thus the main function of the
reproductive is to replace population losses due to death and
emigration (Warren, 1971).

In the present experiments when fresh water crab, 
Barytelphusa cunicularis exposed to lethal and sublethal
concentration of heavy metals mercuric chloride and cadmium
chloride, a histological changes in testes was observed such as
destruction of spermatogenic elements, rupturing of testicular
follicles, damaged connective tissue and irregular arrangement of
spermatazoa in the testicular tubules Nagabhushanam et al (1985)
noticed changes in the gonads of the crab, Barytelphusa cunicularis
after exposure to sevimol. Mary (1984) observed testicular
abnormality and the destruction of the spermatozoa after exposure
to organophosphate in the prawn, Macrobranchium lamerrii. Rao
(1984) noticed disorganisation of lobules, fibrosis of lobules in
crab, Scylla serrata after exposure to endocel and dimecron. The
histological results are somewhat correlated with the results exposed
to pesticide. Gangashettiwar (1986) observed necrosis, inhibition
of spermatogenesis and fibrosis in the seminiferous tubules of
Macrobranchium lamerrii after exposure to phenol. Similar results
were observed by Reddy (1988) on exposure to sea anaemon crude
oil on prawn M. lamerrii.
Histopathological studies after lethal and sublethal treatment to heavy metals in the present experiment reveals that the heavy metals caused number of destructive zone disorganization of lobules disappearance of spermatozoa, etc. Jaiswal (1986) also came to similar conclusion after entoxication of hydrocarbons to the fresh water prawn, *M. lammerii*. Sambasiva Rao and Nagabhushnam (1987) noticed co related results while working on the effect of organochlorine pesticides, endocel on the testicular histology of the marine crab, *S. serrata*.

The accumulation of heavy metals within the organism body of lethal and sublethal level leads to histological lesion in the body when it is accumulated in the reproductive organ. Acute and chronic exposure of *Barytelphusa cunicularis* to heavy metal mercuric chloride and cadmium chloride causes several histological lesions in the ovary. The degeneration of oocyte was first followed by vacuolisation in the ooplasm, destruction of membrane and shrinkage of ooplasm was observed after acute and chronic exposure. The present results are in agreement with the results of Gyananath (1983) and Reddy (1982) who observed the same effect on the ovarian tissue of fresh water prawn, *Macrobranchium lammerii* and *Caridina weberi* respectively, after exposure to pesticide. Victor (1984) reported that pesticidal exposure caused,
changes in the normal structure of the ovary in prawn, *Caridina rajadhari*, Sarojini (1986) reported that fenitrothion toxicity causes damages by vacuolization in the ovarian tissue of freshwater prawn *Caridina weberi*. In the present study perinuclear space may occur due to shrinkage of chromatin material. The changes in the shape of all destructed oocytes indicate the toxic nature of heavy metals. The disappearance of nucleus results in decline of reproductive activity vacuolization probably may be due to the sudden alteration in membrane permeability leading to the active transport of heavy metals. Gyananth *et al.* (1987) working on the effect of potassium ferrocyanide on the ovary of the freshwater prawn *M. lamertii* reported that after acute treatment, the oocyte showed many abnormalities including the loss of ooplasmic material and disappearance of nucleus. Machale *et al.*, (1990) studied that cuprous oxide exposure induced significant alterations in the ovary of the crab *B. guerini*. Reddy *et al.* (1983) have reported that after exposure to insecticide sumithion to a fresh water rice field crab, *Oziotelphusa senex senex* resulted in a decrease in ovarian growth, after sumithion exposure, might be due to release of gonads inhibiting hormone which act on the brain and thorasic ganglia. Fingerman (1984) concluded that neuroendocrine tissue responsible for ovarin development exert a direct effect on ovary. Similar results
were noted by Bhagyalakshmi et al. (1982). During sublethal exposure to heavy metals ruptuering of oocyte, vacuolization, irregular arrangement of oocyte and disappearance of nucleus was observed in fresh water crab, Barytelphusa cunicularis. Similar results were obtained by Sarojini (1986), Deshpande (1985) etc. Victor (1984) observed increase in phagocyte cells in prawn, Caridina rajadhari after exposure to D.D.T. Saraf (1997) on freshwater crab, Barytelphusa cunicularis after exposure to Ekalux-25.

The result of the present studies of freshwater crab, Barytelphusa cunicularis, after exposure to mercuric chloride and cadmium chloride was supported by the above mentioned findings.