CHAPTER-5

Reproduction
Introduction
Introduction:

The way for the pollutants to enter in the body of an animal is circulatory system, from where the pollutants get distributed to the whole body. Pollution of water by any pollutant can have deleterious effect on aquatic organisms. The nature of the effects varies and may cause structural and functional modifications at both, cellular and subcellular level in organisms. In order to understand the pattern of damage caused by particular chemical to the tissue is essential to have an insight into the histological analysis of the tissues. This can be helpful for better understanding of the pathological condition, abnormalities and damages of tissues under toxic stress of pollutants. Thus histology is an extremely useful tool for assessing the effect of toxicants at tissue level. Pollution by pesticide is a serious problem due to their toxicity and ability to accumulate in the biota (Islam and Tanaka 2004). There is still a general concern about the impact of pesticide in the aquatic environment (Grosell and Brix 2005). Bivalves are filter-feeders and thus uptake heavy elements not only from food and water but also from ingestion of inorganic particulate materials (Ei.Sikaily et.al.2004). Moreover this species have
been well established as bioindicators for monitoring the concentrations of pesticide.

Among the environmental contaminants pesticides have been recognized as strong biological poisons because of their persistent nature and cumulative action. These compounds have a unique property of accumulation, over a period of time, along the food chain and very high levels can be accumulate in aquatic organisms from very low concentration in water and sediment.

Increased use of various pesticides in a modern world has led to much greater emphasis on the possibilities of serious environmental contaminations arising from their use. However amongst the different pesticides the greater damage is from the use of organophosphate pesticide seems to occur when water is contaminated because aquatic organisms have the capacity to absorb the chemicals from the water and concentrate it in their fatty tissues.

From the last few decades the fresh water environment has become highly polluted throughout the biospheres with various pesticides which have become essential and virtually irreplaceable tool of the agriculturist to
increase the production of food, production of wood and controlling the
dreadful disease.

Reproduction is unable to counter balance the increased death rate
due to pollution impacts or otherwise it will cause an imbalance in the
aquatic system. (Armstrong and Nillman 1974). Survey of the literature
reveals that the effect of the pesticides on reproduction have been studied
in fishes and crustaceans by many workers, but very few workers have
focused their attention towards the impact of pesticides on the
reproduction of molluscan. Ghobale (2010) studied the effect of organotin
compounds tributyl oxide on some physiological aspects in freshwater
bivalve *Lamellidens marginalis*. Histological techniques are promising
area of research in aquatic toxicology as it gives real picture of effects
imposed and involvement of chemical pollutants either disturbing or
destroying the vital organs of the living organisms. Indira (1989) reported
that histological analysis appears to be a very sensitive parameter and is
crucial in determining cellular alteration that may occur in target organs
such as gills, liver and gonads. Dutta (1996) suggested that a histological
investigation may therefore prove to be a cost effective tool to determine
the health of animal population, hence reflecting the health of entire
aquatic ecosystem in the biomonitoring process Hinton et al. (1992) reported that histopathology has been employed to investigate the changes related to pesticide exposure in mussels. Histology can be considered as a means of providing and supporting information for measures that specifically aim to assess the historic exposure to a contaminant (Stentford et al. 2005) and Hines et al. 2007). Chagot et al. (1990) observed histological changes due to trace level of tributyltin fluoride on the oyster C. gigas. Seneewongse (2006) observed effects of TBT on tissue of various organ system in L. melenostoma. Dode (1993) reported the morbid changes in ovary and heptopancreas, in fresh water prawn Macrobrachium kistenensis due to impact of organotinmetalloids copper. Harino et al. (2005) found the distribution of organotin compounds in tissues of mussel, M edulis. Kharat (2007) observed the histological changes in ovary of fresh water prawn Macrobrachium kistenensis exposed to organotin tributylin chloride. Kamble et al. (2007) reported the effect of hexavalent chromium on the fresh water snail Lymnea from Aurangabad. Vijayavel and Balasubramaniam (2008) observed reproductive dysfunction induced by Naphthalene in an estuarine crab Scylla Serrata with reference to vitellogenesis. Huang and Chen (2004) studied the effect of chlordane and linden on testosterone and vitellogenesis levels in green neon shrimp

Like other animals, snails are not received much more attention by man from the point of their usefulness or destructive nature i.e. in the production of humus and also in the control of fungi and other aquatic weeds like algae and lichens. Unlu et.al.(2005) studied the histopathological effects in tissues of snails L. strangles exposed to sublethal concentration of Thiodan. Prigge et.al. (2007) studied the effect of reason on mussel histopathology. Patil and Mane (2001) observed histopathological changes due to mercury on the bivalve. L. marginalis. Marigomez et.al. (2006) reported the histopathological changes in the hake and anchovvy from Bay of Biscay after the oil spill.
The above literature reveals that most of work has been carried out on fishes, crustaceans and molluscan. However very few workers have focused their attention towards the effect of pollutants on the fresh water snail *Lymnea*. Therefore, present investigation was carried out on the reproduction of fresh water snail *Lymnea auricularia* exposed to folicure pesticide.
Materials and Methods
**Materials and Methods:**

The freshwater snail *Lymnea auricularia* were collected from Chankapur dam Near Kalwan, Dist.: Nashik. Immediately after the snails were brought to the laboratory mud and algal material were cleaned from the snail and acclimatized for 3-5 days to laboratory conditions in plastic troughs. Water parameters in laboratory conditions were maintained throughout the experimental period (Temperature 26±2°C, PH-7.4, Dissolved Oxygen 5.2 mg/Lit and Total Hardness of water was 190 mg/Lit).

After acclimatization, approximately same sizes of snail were used for experiments. Snails were divided into several groups for histological study of gonads. For acute study animals were exposed to different concentrations of folicure pesticide for 24, 48, 72, and 96 hrs. For chronic study 1/10th Lc 50 values of 48 hrs. was used. A batch of control snails were maintained simultaneously at laboratory conditions.

After every exposure period, i.e. 24, 48, 72, and 96 hrs. for acute study and 10, 20 and 30 days for chronic study. Sufficient number of experimental and control snails were dissected in order to collect the gonads.
The gonads were fixed in aqueous Bouin’s fluid at least for 24 hrs. After dehydration with alcohols grades, tissues were embedded in paraffin wax, mounted and stained with haemotoxylin eosin stain.

To study the histopathological lesion caused due to folicure pesticide, microphotography was done.
Results
Results:

Histological structure of gonad - control

The Hermaphrodite gonad of *Lymnea auricularia* is located in the last whorl of shell and surrounded by hepatopancreas. The gonad is made up of irregular shaped ovotesticuler follicles found embedded in loosely arranged connective tissue. At the inner part of the follicular epithelium is having zone of proliferation from which gonial cells are formed. These gonial cells after differentiation either to form or get transformed into spermatogonianal or oogonials cells.

The hermaphroditic control gonad shows primary, secondary oocytes, previtellogenic oocytes, vitellogenic ovum and degenerating ovum. The oocytes consist of distinct nucleus and nucleolus. At the bottom of each follicle amoeboid shaped vitellogenic ova are present.

The secondary spermatocytes are surrounded by sertoli cells. The secondary spermatocytes are with distinct nucleus and plum like tails. The spermatozoa sperms are arranged in the form of bundles with their heads facing towards oocytes. The phagocytes cells are present in the follicles (Fig-10).
→**Effect of 0.3548 ppm of folicure pesticide on gonads after 24 hrs.**

During acute exposure to folicure pesticide drastic changes in two gonads of Lymnea was observed after 24 hrs. exposure to 0.3548 ppm of folicure pesticide. Degeneration in vitellogenic ovum and pycnosis in nucleus was observed (Fig-11).

→**Effect of 0.253 ppm of folicure pesticide on gonads after 48 hrs.**

After 48 hrs. of exposure period, damage to ovarian layer, disruption in the proliferation process was observed after 48 hrs. exposure to folicure pesticide(Fig-12).

→**Effect of 0.2041ppm of folicure pesticide on gonads after 72 hrs.**

After 72 hrs.of exposure period, disintegration in spermatological cells, vacoulisation and arrangement of spermatogenic was found altered (Fig-13).
Fig-10: T.S. of control gonad of fresh water snail *Lymnea auricularia*.

Mallory triple×250

OR - Ova release
DO - Degenerating ovum
FE – Follicle epithelium
DF- Degenerating follicle
SG-Secretory globule
VCO- Vacuoles
VO-Vittelogenic ovum
FC-Follicle cell
SPT-Spermatocytes
TF-Transverse folliculs
O-Ovum

Fig-11: T.S. of gonad of fresh water snail *Lymnea auricularia* exposed to 0.35 ppm folicure pesticide for 24 hrs.

Mallory triple×250
Fig-12: T.S. of gonad of fresh water snail *Lymnea auricularia* exposed to 0.25ppm folicure pesticide for 48 hrs.

Mallory triple×250

Fig-13: T.S. of gonad of fresh water snail *Lymnea auricularia* exposed to 0.20ppm folicure pesticide for 72 hrs.

Mallory triple×250
→**Effect of 0.145 ppm of folicure pesticide on gonads after 96 hrs.**

After 96 hrs. of exposure to folicure pesticide, highly damaged cell in gonads were observed. Spermatogenic cells disintegrated, vacuolization, disappearance of nucleus was observed in ovum (Fig-14).

→**Effect of 0.0253ppm of sub lethal concentration of folicure pesticide on gonads after 10days.**

After 10 days exposure, drastic changes in the histological structure of gonads were observed after sublethal exposure to folicure pesticide. vacuolization and disintegration in spermatological cell were observed (Fig-15).

→**Effect of 0.0253ppm of sub lethal concentration of folicure pesticide on gonads after 20days.**

After 20 days exposure damage to follicular cells, disintegration of connective tissue were observed, very few sperm bundles were seen within the follicle (Fig-16).
Effect of 0.0253 ppm of sub lethal concentration of folicure pesticide on gonads after 30 days.

After 30 days exposure, degenerating ova in the follicles, damage to the gametogenic cells, reduction in follicular size was observed after 30 days of exposure to the follicular pesticide. (Fig-17).
Fig-14: T.S. of gonad of fresh water snail *Lymnea auricularia* exposed to 0.14ppm folicure pesticide for 96 hrs.

   Mallory triple×250

Fig-15: T.S. of gonad of fresh water snail *Lymnea auricularia* exposed to 0.025 ppm folicure (1/10LC₅₀ value of 48 hrs.) pesticides for chronic exposure to 10 days.

   Mallory triple×250
Fig-16: T.S. of gonad of fresh water snail *Lymnea auricularia* exposed chronic concentration of 0.025ppm of folicure pesticides for exposure to 20 days.

Mallory triple×250

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Fig-17: T.S. of gonad of fresh water snail *Lymnea auricularia* exposed to chronic concentration of 0.025ppm of folicure pesticides for exposure to 30 days.

Mallory triple×250
Discussion
Discussion:

The pollution of the aquatic fauna can have deleterious effects on the organisms. The nature of the effects varies and may cause structural and functional modifications at both cellular and subcellular level in organisms. These pesticides find their ways into the body of aquatic organisms by means of general body surface, gills and through circulation these pesticides reaches to the different part of the body and ultimately they impede the physiological function of the organs.

Histopathology is an undependable and powerful technique in establishing routine toxicology studies performed for the purpose of risk assessment of edible resources to human beings. The values are not only in the sensitivity in terms of toxic levels but particularly in the discloser of large organs and mechanism of action.

The present histopathological changes in the gonads of fresh water snail *Lymnea auricularia* reveals that the gonads exhibits wide spread destruction of germinal elements following acute and sublethal exposure to folicure pesticide. Most of the pesticide caused more damage in the gonad during the lethal exposure.
Sublethal exposure of pollutants can lead to their accumulation in the body tissue causing considerable physiological disturbances and reproductive failures. Prolonged exposure to the animals to pesticide can provide a clue to the resistance of animal to counteract the physiological alteration to overcome the stressed condition.

In the present investigation, when Lymnea auricularia exposed to lethal and sublethal concentration of folicure pesticide, severe damage in gonad was observed. This may be due to the high accumulation of the pesticide, generally the extent of tissue damage increased with the duration of exposure period as well as concentration of pesticides. Nimmo et.al.(1977) reported that the growth of the mysid shrimp M.bahia was reduced by chronic exposure to ketone at low concentration and altered the rate of reproduction.

General changes observed in the gonadal structure due to the pesticidal impact were disintegration in follicular cells, vacuolization, necrosis in nuclear cells, damaged connective tissue, and disappearance of nucleus from the gonadal cell were observed. Cellular deformities caused due to pesticidal impact may be because of severe dislocation of the metabolic mechanism. However disappearance of nuclei, nucleolus and
depletion in the cytoplasmic material showed decline towards reproductive activity. Similar observations were made by Deshpande (1986) on fresh water prawn *Macrobrachium kistenesis* after pesticide exposure. Martin et.al. (1990) observed the loss of ooplasmic materials and degeneration of the ovarium tissue on fresh water prawn *Caridina weberi* exposed to methyl parathion. Kamble (2007) studied the drastic histological changes in the fresh water snail *Lymnea auricularia* exposed to hexavalent chromium. Ghoble (2010) reported the impact of organotin TBTO on gonad of fresh water bivalve *Lamellidens marginalis*. Machale et al. (1990) studied the disappearance of nucleolus from the nucleus in the fresh water crab *Barytelphusa guerini* exposed to cuprous oxide. Yadav and Sarogini (1989) reported the histological deformities in the ovary of fresh water prawn *Caridina weberi* exposed to endosulfan pesticide. Mazurova et.al.(2010) reported the histological changes in ovary of crustacean *Gammarus fossarum* after exposure to chronic concentration of contaminated segments. Sonaco et.al. (2010) studied the alteration in the histology of ovary of fresh water crab *Sommaniatheplusa pax* exposed to arsenic. Manojkumar et. al. (2007) reported the histological alteration in the testes of fresh water fish *Heteropneustes fossilis* exposed to linear alkyl benzene sulphonate. Olfat et.al. (2007) studied the comparative impact of
different waste sources on reproductive parameters and histology of
gonads of fish *Siganus rivulatus*. Yadav and Patil (2012) reported the
impact of folicure pesticide on the ovary of fresh water crab *Barytelphusa
cunicularis*. Seneewangse (2006) observed effect of TBT on tissue of
various organ system in *L. melanostoma*, Unlu et.al.(2005) studied the
drastic alteration in histological structure of gonad of fresh water snail *L.
stagnalis* expose to sublethal concentration of thiodon. Deshmukh and
Kulkarni (2005) reported that fish *Channa orientalis* after exposure to
cadmium chloride showed retardation of gonadal maturation and cause
acute degeneration of testis. Various stages of spermatogenesis lose their
characteristics and greatly reduced in number .Increase in fibrous tissue
among gonadal follicles and the size and number of follicles was also
reduced. Jagtap (2010) studied the histological changes in the cells of
gonad and digestive gland of fresh water bivalve *L.marginalis* when
exposed to TBTCL similar observations were made by Jagtap et.al.(2011)
and Smolavz et.al.(2005).

The present study reveals several degenerative changes in the
histological structure of gonads expose to lethal and sublethal
concentration of folicure pesticide. It was interesting to note that
histological changes induced by folicure were more intense when compare to control group.

From the result of present investigation it can be concluded that folicure pesticide cause adverse effect on the gonad of fresh water snail *Lymnea auricularia* and due to the pesticidal impact the reproductive activity was reduced.