CHAPTER – 5

HISTOPHTHOLOGICAL STUDIES
5.1 **INTRODUCTION:**

Heavy metals are more hazardous pollutants of ecosystems with deleterious effects on aquatic biosystems. Rapid industrialization, urbanization and other developmental activities have lead to deterioration of the environment (Salunke *et al.* 1982). Heavy metal toxicants have been shown to enter and accumulate in aquatic fauna and flora causing heavy modification within biota (Berman and Lal, 1994). Earlier reports reveal that they may disturb cellular functions, altering vital physiological and biochemical mechanisms of animals (Larsen *et. al*; 1976; Shastry and Sharma, 1978; Muthu-krishnan *et al*; 1986 and Radhakrishniah *et al.* 1991).

The deleterious effects caused by the toxicity of chromium, have been reported and reviewed (Merts, 1969; Binishi and Lewis, 1986 and Anna and Barlow, 1987). Extensive information is available on effects of chromium to Ichthyofauna (Adelman and Smith, 1976; Benoit, 1976; Buhler *et al*, 1977; Arillo *et al*, 1982; Srivastava *et al*, 1982 a; Doughtie *et al*, 1984; sastry and Sunitha, 1984; Taylor *et al*, 1985 Srivastava and Maurya, 1991; Venugopal and Reddy, 1992; Ambrose and Vincent, 1994; Abbasi *et al*,1995; Mahipal Singh, 1995; Anusuya *et al*, 2005 and Recently by Khedkar *et al*, 2007. Scant attention has been paid on chromium toxicity to the invertebrate taxa

There is a paucity of information on such effects in freshwater snail, except few reports by Ravera, (1977); American Public Health Association (APHA), (1980) and Khangarot, et al, (1981). Further it is also essential to evaluate toxic effects of chromium or any heavy metal on snails, since, like other animals, snails are not received much more attention by man from the point of their usefulness or destructive nature. Snails also play a role in the nature i.e. in the production of humus, in the control of fungi and other aquatic weeds like algae and lichens.

The present snail also act as a bioindicator of pollution in the freshwater environment. Therefore, the present investigation aims to evaluate the histopathological lesions caused by hexavalent chromium toxicity to the freshwater snail, *Lymnaea*. 
5.2 MATERIAL AND METHODS

Normal sized specimens of *Lymnaea acuminata* collected from Kham River near Aurangabad. Immediately after the snails were brought to the laboratory, mud and algal material was removed from the shell and acclimatized for 3 days to laboratory conditions in plastic troughs, water parameters in laboratory conditions maintained constant throughout the experimental period. (Temperature 28 ± 2°C, pH 7-8, dissolved oxygen 5.2 ± 0.5 mg/lit and total hardness of water was 175-180 mg/lit).

After acclimation, normal sized (with an average shell length, 1.8 - 2.0 cm) active healthy snails were selected for histopathological studies.

The snails were exposed to 1/10th concentration of LC$_{50}$ value of 24 hours of hexavalent chromium i.e. 12.690 ppm upt0 10 days. A batch of control snails were maintained simultaneously at laboratory conditions.

After every exposure period viz, 24, 48, 78 and 96 hours and 5, 7, and 10 days sufficient number of experimental and control snails were sacrificed in order to collect different body components i.e. foot,
mantle, hepatopancreas and gonad separately for histopathological study.

The foot, mantle, hepatopancreas gonad and cerebral ganglion, were fixed in aqueous Bouin’s fluid at least for 24 hrs. After usual dehydration with alcohol grades, tissues were embedded in paraffin (melting point at 56 -58°C) and serial sections were cut at 4-5µ for cerebral ganglion and 7-9 µ for rest of tissues. The serial section were mounted and stained with hematoxylin-eosin staining method and alterations in the histological structure of foot, mantle, hepatopancreas and gonad were observed after exposure for 24,48,72 and 96 hours and 5,7, and 10 days.

For the histomorphological study of neurosecretory cells, the snails were exposed to same concentration of chromium as general histopathological study, but after every exposure period viz, 1, 2, 4, 8, 12, 24, 48, 72 and 96 hours and 5, 7, and 10 days the experimental and control snails were sacrificed in order to collect cerebral ganglion from nerve ring separately.

The sections of the cerebral ganglion were stained with Aldehyde-Fuchsin (Even, 1962) method. Light microscopical observations were made to study the normal histology of cerebral neurosecretory cells. Cell and nuclear diameters of neurosecretory cells
in the cerebral ganglion were measured with the help of ocular micrometer. The neurosecretory material intensity (NSM) of neurosecretory cells was measured by visual arbitrary scale method as described by Nagabhushanam and Hanumante (1977).

1 - No N.S.M.
2 - Less N.S.M.
3 - Moderate N.S.M.
4 - Considerable amount
5 - Fully loaded.

In order to check the histopathological lesions caused due to chromium exposure were noted down light microscopically. Various types of cell groups, cell number, staining material intensity and their cell and nuclear diameters were measured and compared with normal control snails.
5.3 OBSERVATION AND RESULTS

1) Histomorphology of control foot:

The foot region of the snail, *L. accuminata* forms major part of the body organ which is sole like and is used for creeping when present on hard substratum, it is also used to float on the water surface at the time of swimming. The transverse sections show a cuticular lining from the outer side, these layers protect the foot region inner to the cuticular lining, there is a columnar epithelial layer, the cells of this epithelium are tall with basal nuclei. Intermitantly these columnar cells get modified into a sac like unicellular glands, which open through cuticular layer exterior to foot surface. The unicellular glands are covered by a thin cell with distinct nucleus at the bottom, these unicellular glands are involved in the secretory process of mucous secretion. These glands remain embedded transversely running muscle fibers. These muscles are also called as longitudinal muscles, major part of the foot region is made up to thickely arranged oblique muscle fibers at its inner side. (See Plate-II and Fig-A).
24 - Hours Exposed Foot:

After 24 hrs of exposure period to hexavalent chromium, the outer most cuticular layer is prone to get affected, there is increased secretion of mucous within the mucous glands indicated by distinct appearance of these glands, there is slight change in the cells of tall columnar epithelial layer. (See Plate - 2 and Fig-B).

48 - Hours Exposed foot:

After 48 hrs. of exposure to hexavalent chromium, there is damage to the cuticular lining layer, its surface layer becomes irregular (See Plate - 2 and Fig-C). The columnar layer gets also severely affected, there is derangement within longitudinal muscle layer.

72 - Hours Exposed foot:

After 72 hrs. of exposure to hexavalent chromium, the cuticular layer shows damages throughout its covering, also there is damage to the columnar epithelium. Regular arrangement of basal nuclei got affected (See Plate – 2 and Fig-D).

96 - Hours Exposed foot:

After 96 hrs. of exposure to hexavalent chromium, the cuticular lining which was thick in normal animal but in 96 hrs exposure this
layer gets thin, may due to removal of outer layer. The unicellular glands gets severely affected from the point of their mucus secretion, there size becomes smaller when compared to that of normal animals foot (See Plate - 3 and Fig – A). Due to damage to the oblique muscles there is formation of spaces within this layer may be shifting of oblique muscle fibers.

5 – Days Exposed foot:

After 5 days exposure period, the mucous secretory activity stopped, which was evidenced from observation. Cuticular lining starts getting broken here and there. (See Plate – 3 and Fig- B).

7 - Days Exposed foot:

After 7 days of exposure period, there is shrinkage within the cuticular layer, the unicellular glands gets extended their secretory pouches in to inner muscular layer. The oblique muscle layer too severaly damaged. (See Plate – 3 and Fig. C).

10 - Days Exposed foot:

After exposure to 10 days the cuticular lining layer shows finger like projections above the surface of foot, may be due to further shrinkage of this layer together with columnar epithelium. The number
of empty spaces within the muscular layer increased (See Plate – 3 and Fig – D).

2) **Histomorphology of Control Mantle:**

The mantle is the anterior most body wall which form the dorsal covering for the enclosed body visceral organs. It is thin in the mid-dorsal region and thick at the sides. A transverse section of the mantle when observed histologically shows that it is formed of various types of tissues, such as commencing from the outermost are the epidermis, which is having made up of columnar epithelial cells, then is connecting tissue, within which are sac like glands, nerve fibres and branches of blood capillaries.

The unicellular glands situated in this region, opens to the outside through small necklike apertures (See Plate – 4 and Fig – A). Inner to connective tissue is the innermost part of the mantle wall is formed by the muscular bands running in to each other in a network like fasion. The muscular bands are consisting of longitudinal and transverse muscle bands.

**24 - Hours Exposed Mantle:**

After exposure to the hexavalent chromium for 24 hrs, the outermost covering layer, epidermis shows changes in its cell structure.
The secretory activity of unicellular glands get affected, may be due to this effect, the outermost covering layer becomes turgid. (See Plate – 4 and Fig – B).

48 - Hours Exposed Mantle:

After exposure to hexavalent chromium for 48 hrs, columnar cells become compactely arranged with damage to the unicellular glands, transverse and longitudinal muscles starts showing changes in their arrangement. (See Plate – 4 and Fig – C).

72 - Hours Exposed Mantle:

After exposure to 72 hrs, columnar cells epithelium got severaly affected, unicellular glands become emptied, also thre is damage to the connective tissue layer which is evident by formation of spaces in this region. (See Plate – 4 and Fig – D).

96 - Hours Exposed Mantle:

After exposure for 96 hrs, the outermost epidermal covering layer shows damage here and there. This location of unicellular gland sacs occured may be due to destruction to the connective tissue layer. There is damage to the transverse and longitudinal epithelial cell layer. (See Plate – 5 and Fig-A).
5 - Days Exposed Mantle:

After 5 days of treatment with hexavalent chromium there starts disintegration of outer most mucoidal layer. The unicellular gland sac gets shrunken, connective region becomes thin, degeneration of connective tissue cells, may be the cause to become this region thin. (See Plate – 5 and Fig – B)

7 - Days Exposed Mantle:

After 7 days of exposure the outermost epidermal layer starts showing folds in it, because of this fact the unicellular glands are severly damaged. There is derangement in the arrangement of inner muscular region (See Plate – 5 and Fig – C).

10 - Days Exposed Mantle:

After 10 days of exposure, number of folds within the epidermal layer increased. The connective tissue layer becomes more compactely arranged cells. Transverse muscle band show change in their regular network like arrangement, these bands gets intermingelled with each other (See Plate - 5 and Fig – D)
3) **Histomorphology of Control Hepatopancreas**:

Hepatopancreas or midgut gland of *L. acuminata*, is the large sized major digestive gland, remain associated with 2/3 posterior part of the digestive system. Also it remains covered with peripherally to the hermaphrodite gonad. Normally it is red brown in colour and remains enclosed by a thin covering membrane, “Tunica propria.” It is made up of irregular shaped, some times rounded hepatopancreatic or digestive tubules. Each tubule is made up of single cellular epithelium with centrally enclosed lumen. After getting stained with various conventional staining methods, at least three or four types of epithelial cells can be identified. There are large number of digestive cells which are columnar having secretory globules at the apex region facing towards lumen. The other types of cells are calcium cells and basophilic cells. The epithelial layer rests on the basement membrane forms the outer most covering layer. The lumen of these tubules is of irregular shape and goes on changing with respect to season of the year and type of food material consumed by the snail. There is a connective tissue in between these tubules. The secreatory globules are released in the lumen of tubules. All these tubules are joined by the ductules and at last forming the hepatopancreatic duct which opens in the midgut or stomach region of the digestive system (See Plate – 6 and Fig – A).
24 - Hours Exposed Hepatopancreas:

After exposure to the hexavalent chromium for 24 hrs the “Tunica propria” got affected. There is increase in intertubular space. The secretory globules from the digestive cells discharged within the lumen, and simultaneously released outside the tubule i.e. within digestive tract. (See Plate – 6 and Fig – B)

48 - Hours Exposed Hepatopancreas:

After exposure for 48 hrs to the hexavalent chromium, the epithelial lining looks like vacuolar may be because of secretion of material in the form of globules. There is a rupture through the basement membrane, derangement of epithelial cells starts from this time period of exposure to chromium. (See Plate – 6 and Fig – C).

72 - Hours Exposed Hepatopancreas:

After exposure for 72 hrs, there is increase in intertubular space. Digestive cells get severely affected, also there is damage to calcium secretory cells. Secretory columnar cells show changes in their cytomorphological picture, such as shifting of nucleus from basal region towards lumen. (See Plate – 6 and Fig- D).
96 - Hours Exposed Hepatopancreas:

After exposure for 96 hrs. there is change in normal shape of the hepatopancreatic tubules to irregular manner. Vacuolization within lumen of the follicle is more evident. Also there is formation of large sized vacuoles within enter tubular space (See Plate - 7 and Fig- A).

5 - Days Exposed Hepatopancreas:

After exposure to chromium for 5 days period, there is enhanced tendency of vacuolization, degeneration of follicular epithelium is observed. From the day onward there is significant damage to the tubules occurred with termination of secretory process within the digestive cells. (See Plate – 7 and Fig – B).

7 - Days Exposed Hepatopancreas:

After exposure for 7 days, there starts changes in the normal picture of the hepatopancreatic tubule. Irregular arrangement of tubules with destruction to the epithelial layer is observed. The number of hepatopancreatic tubules decreased their by increase in the inter tubular space. (See Plate - 7 and Fig – C)
10 - Days Exposed Hepatopancreas:

After exposure for 10 days, the histomorphological picture of hepatopancreas shows some sort of abnormalities in their tubular structure, arrangement and in secretory processes. Inter tubular connective tissue cells are severely damaged forming fragmentary remains within the inter tubular space. (See Plate – 7 and Fig – D).

4) Histomorphology of Gonads:

The hermaphrodite gonad of *L.acuminata* is located in the last whorl of the shell and gets surrounded by hepatopancreas from outer side. The gonad is made up of irregular shaped various sized some times lobulated hermaphroditic or ovotesticular follicles. These follicles remain embedded within loosely arranged connective tissue. Each follicle is having single cellular epithelial wall. At inner part of the follicular epithelium is having zone of proliferation, from which gonial cells are formed. These gonial cells after attaining sufficient number of population through meiotic 1 or 2 divisions undergo differentiation, either to form or get transformed into spermatoggnial cell or oogonial cell. Accordingly further process of development and maturation of gamates occur.
Control Gonad:

Histological picture of hermaphroditic control gonad shows, various stages of developing oocytes, such as primary, secondary oocytes, previtellogenic oocytes, vitellogenic ova and very few degenerating ova which are present at the bottom of the hermaphrodite follicle. The primary and secondary oocytes are rounded with distinct nucleus and nucleolus. The ooplasmic material is neutral it’s staining with haematoxylin eosin. These oocytes are near by to the neck region of the follicle. The previtellogenic ova are pyriform in shape and are present at the bottom of the follicle with strong basophilic ooplasm. On further development these oocytes get surrounded by two thin follicle cells. At the end of maturation process, previtellogenic oocytes gets converted to vitellogenic ova. At the bottom of each follicle oval to amoeboided shaped vitellogenic ova are present. The ooplasmic material of these ova is eosinophilic in nature, which is evident by pink colour.

Within these follicles also there are various stages of sperm development. The secondary spermatocytes get arranged themselves surrounded to Sertoli cell. The function of Sertoli cell is to provide nutritive substances, specially carbohydrate to the developing sperm. On the further maturation secondary spermatocytes are with distinct nuclei and plume like tails. Through the process of spermiation, the
spermatids get metamorphosed to sperm. The spermatozoa these sperms are arranged in the form of bundles with their heads facing towards oocytes and their tails free within the lumen of the follicle. The other types of the cells which are present within the hermaphrodite follicles are nutritive phagocytes. The population of nutritive phagocytes increases within the degenerating follicles. (See Plate – 8 and Fig – A and B).

24 - Hours Exposed Gonads:

After 24 hrs. exposure to hexavalent chromium, the gonial follicles shows following changes.

The vitellogenic ova or matured ova are released which is evident from having scars within the follicles, there is disruption within the proliferation process. (See Plate – 8 and Fig –C).

48 - Hours Exposed Gonads:

After 48 hrs of exposure periods, there is no any further release of gonial cells within the follicle. The vitellogenic ova show symptoms of degeneration, such as nuclear picnosis occur in vitellogenic ova. Number of sperm bundles few when compared with control gonad (See Plate - 8 Fig – D).
72 - Hours Exposed Gonads:

After 72 hrs of exposure period, there is complete disappearance of the nucleus with vacuolization within the ooplasm. There is a distinct vacuolar ring observed within the peripheral parts of the ovum (See Plate – 9 and Fig – A). The number of degenerating ova increased per follicle. The spermatogenic process gets affected.

96 - Hours Exposed Gonads:

After 96 hrs of exposure to hexavalent chromium, the hermaphrodite follicles becomes compactely arranged. There is further progress in degenerative process of matured ova, the spermatogenic mass start getting disintegrated. Most of the peripheral hermaphrodite follicles are observed empty. (See Plate – 9 and Fig – B).

5 - Days Exposed Gonads:

After 5 days of exposure period, the hermaphrodite follicular epithelium gets affected. The hermaphrodite follicles are having very few vitellogenic ova, there is reduction in follicle size. The normal picture of arrangement of spermatogenic cells start getting altered. (See Plate - 9 and Fig –C).
7 - Days Exposed Gonads:

After 7 days of exposure period, there is presence of numerous degenerating ova within the follicles. The connective tissue mass gets affected within which the hermaphrodite follicles are present. Proliferation of gonial cells totally stopped, hence there are no any earlier stages of spermatogenic cells at the neck region of follicle (See Plate – 9 and Fig – D). There is damage to the follicular epithelium, very few sperm bundles are present within the lumen of follicle.

10 - Days Exposed Gonads:

After 10 days of exposure periods, the effects of hexavalent chromium are very drastic. The normal picture of the gonad gets changed, the follicular epithelium gets damaged at several sides. The process of degeneration is at its peak. The follicles are seen empty may be due to degeneration of both types of gametogenic cells. The cytoarchitecture of hepatopancreatic tubules surrounding the follicles gets changed. (See Plate – 9 and Fig-E).
A) Histomorphology of Control Cerebral Neurosecretory cells:

The nervous system of *L. acuminata* is in the form of circumoesophageal ring. It is composed of two parts i) Superoesophageal ring – It is made up of paired buccal and cerebral ganglia. ii) The sub oesophageal ring is having paired, pedal, pleural and single visceral ganglion.

For histomorphological study, after exposure to hexavalent chromium, attention has been paid on the changes in the cerebral ganglionic neurosecretory cells, which forms the major neuroendocrine center of this animal. There are two cerebral ganglia united by cerebral commissure.

Topographically, there are three groups of neurosecretory cells such as mediodorsal cells (MDC), Laterodorsal cells (LDC) and caudodorsal cells (CDC). From cytomorphological characteristics features, each group is having two types of neurosecretory cells.

a) Types ‘A’ neurosecretory cells. These are large sized neurons with oblong cell body and elongated axonal pathways. These neurosecretory cells are less in number in each group and having large sized polymorphic nucleus with nucleolus, when stained with
paraldehyde fuchsin method, the cytoplasmic material stains deep violet in colour and the nuclear material orange in colour.

These cells measures $35.44 \pm 2.64 \ \mu m$ cell diameter and $28.32 \pm 2.11 \ \mu m$ nuclear diameter. In normal animals these cells show neurosecretory material intensity 5.

b) The other neurosecretory cell type in each group is ‘B’ type of neurosecretory cells. These cells are smaller in size when compared with type ‘A’ cells. These cells are more in number in each group the cell body is rounded to oval in shape there is centrally located nucleus, the staining characteristic features are similar to those of type ‘A’ cells. The neurosecretory axons traverses towards neuropile area of each ganglion. These cells measures $13.64 \pm 1.16 \ \mu m$ cell diameter and $8.83 \pm 0.98 \ \mu m$ nuclear diameter and their neurosecretory material intensity is 4. (See Plate – 10 and Fig – A) and (Table 5.1)

B) Effect of hexavalent chromium (12.690 ppm) on cerebral neurosecretory cells of *Lymnea*:

After exposure to hexavalent chromium for 1 hour time period, there is increase in the cell diameter $(36.17 \pm 2.18 \ \mu m)$ of ‘A’ cells. The number of cells counted are $14 \pm 2$ compared with $12 \pm 1$ in control animals. There is slight increase in the nuclear diameter
28.87 ± 2.64 µm). The neurosecretory material intensity in their cell perikarya is 4.

The number of ‘B’ cell types with staining material increased drastically (36 ± 4) with slight increase in their cell diameter (13.86 ± 1.93 µm). There is also increase in nuclear diameter (91.61 ± 0.88 µm) and the neurosecretory material intensity remains same as control. (See Table 5.1).

After exposure for 2 hrs time period there is further increase in number of ‘A’ cell type (15 ±1) with increase in cell and nuclear diameter compared normal animals. However there is an increase in neurosecretory material intensity compared with animal exposed for 1 hour time period (See Table 5.1).

Cerebral ‘B’ types of cells shows increase in both cells as well as nuclear diameters, with decrease in number of cells compared with earlier group (See Table 5.1).

After exposure for 4 hrs. time period, the neurosecretory cell number (17 ± 2) cell diameter (39.93 ± 2.86 µm), nuclear diameter (29.99 ± 2.35 µm) of ‘A’ types indicates an increase with neurosecretory material intensity same as control (See Table 5.1).
The other cell type ‘B’ shows increase in cell as well as nuclear diameter with staining material intensity as 5. However the neurosecretory cell number decreased compared with 2 hrs exposure period (See Table 5.1).

After exposure for 8 and 12 hrs time period to chromium, the number of neurosecretory cell type ‘A’, cell and nuclear diameter as well as their neurosecretory material intensity deceased further (See Table 5.1), However the neurosecretory cell type ‘B’ shows significant increase in their number (38±3), after exposure for 8 hrs. But their cell and nuclear diameters along with staining material intensity decreases further exposure for 8 and 12 hrs, only decrease in their number (20±2) after 12 hrs exposure. (See Table 5.1).

After exposure for 24 hrs there is increase in both type of cell number, but there is decrease in cell as well as nuclear diameters of ‘A’ and ‘B’ types. The cell and nuclear diameters of ‘A’ and ‘B’ neurosecretory cells are 25.64 ± 2.89, 20.26 ± 1.95 and 10.17 ± 1.53, 8.60 ± 1.42 respectively. There is increase in neurosecretory material intensity of ‘B’ types compared with 12 hrs exposure period (See Table 5.1).

After exposure for 48 and 72 hrs both cell types show decrease in cell and nuclear diameters along with cell number upto 48 hrs
exposure period. However the number of cell types increases further exposure for 72 hrs. From 4th day onwards exposure up to 10 days, the cell number, cell diameter and nuclear diameter along with neurosecretory material intensity decreases. Along with these changes, there is nuclear picnosis and vacuolization within the cell perikaryon is noticed during exposure for 5, 7 and 10 days. Also there is damage to the perineurium wall layer which covers the cerebral ganglion (See Plate – 12 Fig A, B, C and D).
5.4 DISCUSSION

Histopathological lesions caused due to hexavalent chromium exposure to freshwater snail, *L. acuminata* are severe and deleterious effects have been noticed in the normal histomorphology of foot, mantle, hepatopancreas, gonad and cerebral neurosecretory cells. The mantle tissue forms one of the vital organs of the snail *Lymnaea*, since it is involved in the process of respiration. Hence, it is the prime organ to get affected, after treatment of the hexavalent chromium.

Heavy metal toxicants have been shown to enter and accumulate in aquatic fauna and flora causing heavy modifications (Berman and Lal, 1994) and they may disturb cellular functions (Larson *et al*, 1976; Muthukirishnan *et al*, 1986 and Radhakrishnaiah *et al*, 1991). Histological approach is the most valuable tool for assessing the action of toxicant at tissue level provide data concerning tissue damage (Sprague, 1971). Visible histopathological abnormalities caused due to toxicity of heavy metals in animals restricted to fish species (Chiquoine, 1963; Clegg and Carr, 1966; Gardner and Yevich, 1970; Newman and Mclean, 1974; Kuman and Pant, 1981; Srivastava *et al*, 1982; Rameshkumar *et al*, 1988 and Srivastava and Maurya, 1991).
Practically, very few scattered reports are available about histopathological alterations caused due to metal pollution to invertebrates (Ferene et al, 1977; Engle and Fowler; 1979; Hanumante et al, 1979; Rondelund and Dreyfuss, 1996; Utkar and Kulkarni, 2000; and Mathur and Saini, 2003).

The mantle and foot region of *L. acuminata* are having unicellular mucous secreting glands just below the cuticular lining. These are the modified form of epithelial cells involved in the secretion of mucous may help in creeping movement of the snail to slide over the surface of different type of substrata. After exposure to chromium there is damage to the surface epithelial layer of foot and mantle initially, further exposure causes loss of epithelial layer, showing erosion of epithelial cells with necrosis of tissue. Similar types of changes have been reported in the gill tissue epithelium of the frog tadpoles after treatment with chromium (Anusuya et al, 2005).

Continuous water is circulated through the mantle cavity formed within mantle region of *L. acuminata*, for purpose of gaseous exchange. During chromium toxicity study, this region is continuous in contact with the metal chromium dissolved in surrounding water naturally got affected drastically, showing histological lesions like, rupture of unicellular glands and derangement within arrangement of
longitudinal and transverse muscular bands. Sesha Srinivas (1998) observed effect of hexavalent chromium on haematology and gill epithelium of the freshwater teleost fish, *Labeo rohita*. After exposure to chromium, damage to gill architecture was noticed.

Hepatopancreas is the main depot tissue, which encircles the entire gonadal lobes of the snail. It provides nutritive substances to developing gametes within hermaphrodite follicle of gonad. Aiken and Byard (1972) observed degenerative changes in the hepatopancreas of lobster, *Homarus americanus* after exposure to yellow phosphorus. Doughtie and Rao (1984) while working on another grass shrimp, *Palaemonetes pungio* reported histopathological and ultrastructural changes in the antennal gland, midgut, hepatopancreas and gill following exposure of hexavalent chromium. In the present snail similar type of changes have been noticed after exposure to chromium. At the initial period of exposure, the secretory cells drop their secretion within lumen of hepatopancreatic tubules thereby resulting, vacuolization and necrosis within epithelial cells is observed. Similar type of vacuolization and increase in size of vacuoles and number of secretory and absorptive cells reported by aforementioned authors in *H. americanus*. 
Nuclear picnosis with darkly stained nuclear material in the absorptive cells of hepatopancreas of freshwater crab *Barytelphusa cunicularis* was noticed after exposure to the pesticide sevimol (Bodke, 1983). While working on another marine crab, *Scylla serrata*, Sambasivarao (1984) observed vacuolization, decrease in size of lumen, nuclear picnosis and damage to the tubular arrangement of hepatopancreas was resulted after pesticide treatment. In the present study, after prolonged exposure to chromium for 10 days, show abnormalities in normal histo-morphological picture of hepatopancreas such as tubular contraction, causing decrease in lumen size and increase in inter tubular space.

Literature on the effect of trace metal on the freshwater gastropods is scanty (Ravera, 1977; Halilt et al, 1987 and Gomat de valuflewry Kerhoas, 2000). The freshwater gastropod *Viviparus bengalensis* after exposure to heavy metal mercury showed release of premature young ones in various stages during monsoon season (Muley, 1985). Considerable changes also occur at cellular level in prostate, albumen, capsule gland and pallial oviduct after exposure to sub-lethal concentrations of mercury during different seasons.

Chromium induced changes have been observed within the hermaphrodite gonad of *L. acuminata*. Ravera, (1977), reported effects
of heavy metals (cadmium, copper and chromium) on developing embryos of *B. glabrata*. He has concluded that cadmium and copper were more toxic than chromium. In the present investigation the freshwater pulmonate snail, *L. acuminata* is used as a test organism to chromium toxicity tests and is advantageous in the field of freshwater toxicity testings, since it is a vector snail, invades various types of larval trematode parasities. These findings are useful to undertake snail population control programme more effectively. This is a hermaphrodite snail, breeds throughout the year with peak in breeding activity in the monsoon season. The environmental conditions are favourable for the development of young ones during this period of the year.

Chromium exerts induced changes within gonad of *L. acuminata*, when exposed to sub-lethal concentrations of the metal. There is damage to the capsule layer of hermaphroditic gonad. The very first site to get affected is proliferation zone of hermaphroditic acinus, from which gonial cells are added to lumen of acinus followed by premature ovulation observed when compared with the control animals. Similar type of abnormal development of the sex cells, their release, fertilization and embryonic development has been reported in another gastropod mollusc, *B. glabrata* after exposure to zinc,
(Munzinger and Guarducei, 1988). Fertility decreased and percentage of abnormal embryos increased with stay length of *B. glabrata* in contaminated waters during female phase maturation period.

After prolonged exposure to chromium, the effects are very profound to the gonad of *L. acuminata*. The normal picture of gonad gets changed, with severe damage to follicular epithelium and the process of degeneration of gametogenic mass/cells was at its peak. The cytoarchitecture of hepat-pancreatic tubules surrounding the follicles gets dramatically affected. Deshmukh and Kulkarni (2005) also reported that the fish, *Channa orientalis* after exposure to cadmium chloride, showed retardation of gonadal maturation and caused acute degeneration of testes. Various stages of spermatogenesis lost their characteristics and greatly reduced in number. The fibrous tissue was found to be developed in the testicular walls and the epithelial cells lining these walls were seen atrophied, are in accordance with present findings, where due to chromium treatment, there is an increase in fibrous tissue amongst the gonadal follicles and the size and follicle number also reduced.

Alterations in the neurosecretory profiles with cerebral ganglion of freshwater basomatophoran snail, *L. acuminata* studied after exposure to hexavalent chromium. The number of various cell types,
their cell and nuclear diameter and the intensity of neurosecretory material before and after exposure is summarized in Table 5.1 and Plate No. 10, 11 and 12.

Aquatic pollutants such as heavy metals and pesticides interfere with the normal functional processes and ultimately the hormonal secretory system gets disturbed. Highman and Hill (1969) reported that the activity of neurosecretory cells is affected by three factors, a) rate of synthesis of secretory material, b) rate of transport of secretory material along with axon and c) rate of release of transported material in the circulatory system. Effects of drugs and neurotransmitter substances like, acetylcholine, Novacaine, epinephrine, 5-hydroxytryptamine also alter the activity of neurosecretory cells of different invertebrates (Schmid, 1947; Nagabhushanam and Hanumante, 1977 and Kulkarni et al, 1980). According to Cooke, (1977), pollutants act on neurosecretory cells other neurons by resulting the plasma membrane more permeable to Ca++. The role of the Ca++ ion is linked with the stimulus and was demonstrated in detail by Fingerman et al, (1977).

In the present snail, *L. acuminata* after exposure to sub-lethal concentration of chromium, shows increase in cell number of both cell types ‘A’ and ‘B’ with increase in their cell and nuclear dimeters upto
48 hours of exposure. An enhanced synthetic activity of neurosecretory cells may be correlated to the heavy metal chromium, in the form of pollution stress and to maintenance of homeostasis in the milieu interior. Numerous neuro -ropharmacological agents and other chemicals provoke alterations in the neurosecretory cells of both vertebrates (Gold and Ganong, 1967 and Singh and Dominic, 1975) and invertebrates (Manser et al, 1970, and Raina, 1974).

Impact of pesticides on neurosecretory cells of various invertebrates species is well documented, VIZ, the fiddler crab, Uca Pugilator (Nagabhushanam et al, 1979); in freshwater pulmonate, I. exustus (Hanumante et al, 1979); in Macrobrachium lamerrii (Gyananath, 1982 and Mary Avelin, 1984) and in B. cunicularis (Bodke, 1983). It was observed that there is no uniformity in the responses of the neurosecretory cells of various invertebrates to pollutants and the nature of their response is probably linked to the chemical structure of the pollutant.

After prolonged exposure to chromium for 10 days period, there is decrease in cell number of both types with reduced cell and nuclear diameter. The neurosecretory material intensity is decreased in both cell types. Vacuolization within perikaryon, nuclear picnosis in all groups of cerebral ganglionic neurosecretory cells together with damage to the
perineurim covering cerebral ganglia also got affected after prolonged exposure period. Shailaja Patil (1998) is of the opinion that the heavy metals, copper, mercury and zinc on the neurosecretory cell of freshwater bivalve, *Lamellidens corianus*, have the similar type of effects. In the present study, the responses of neurosecretory cell type ‘A’ and ‘B’ in the cerebral ganglion of *L. acuminata* are similar after exposure to chromium water. The decrease in cell and nuclear diameter with less neurosecretory material within their cell perikarya is indicative of increased neurosecretory material transportation via axons and finally release into the circulatory system of the snail.