# OBSERVATION

## Observations

### 3.1 Water analysis :

Experimental water samples and river water samples collected during collection of animals were analyzed in the laboratory for their physico-chemical characteristics and the observed results are documented here. The main objective of studying physico-chemical parameters is to understand difference in the characteristics of water used for experiment which will influence the experimental animals. All the parameters were copared with MPCB standard limits for river water.

Physical parameter like temperature, and chemical parameters like pH, dissolved oxygen, hardness, BOD and COD of water and important nutrients like nitrate have been studied along with heavy metals Ca and Pb from both water samples.

#### 3.1.1 Physical parameter :

##### 3.1.1.1 Temperature: (Table 1, Graph 1)

The heat transmitted with light is responsible for thermal stratification in water bodies and it influences the respiration rate in aquatic organisms. The temperature fluctuates from season to season and it is highest during summer months and drops down during and after monsoon.

In the present study, the temperature of collection site water ranged between 26.1 to 28.5°c which was within tolerance limit of bivalves. There was no much fluctuation in temperature of experimental water through out the experimental period.

### 3.1.2 Chemical parameters:

#### 3.1.2.1 pH: (Table 1,Graph 1)

The pH is an important chemical factor in the freshwater bodies. pH maintains the acidity or alkality of water which is determined by concentration of hydrogen ions(H+) and hydroxyl ions(OH+). The pH is dependent on the biological processes in the aquatic bodies and is linked within the compositions and life processes of organism present there. In present study, the pH value of experimental water was recorded at 8.1 and collection site water was 7.4 which is within standard limits.

#### 3.1.2.2 Dissolved Oxygen (DO): (Table 1, Graph 1)

It is the most important chemical factor in freshwater for existence of life. The dissolved oxygen is responsible for the biological and biochemical procsses.The freshwater bodies receive oxygen from atmospheric absorption at the surface and as by-product of photosynthesis from the aquatic plants. In the present study, Dissolved Oxygen in experimental water was 8.02 mg/l and collection site water sample was 7.44 mg/l both these values of DO were within standard limits.

#### 3.1.2.3 Hardness: (Table 1, Graph 1)

Hardness is the total of soluble calcium and magnesium salts present in water and expressed as its equivalent to carbonates. Hardness of water may be temporary, caused by soluble calcium and magnesium carbonate or permanent by predominant bicarbonates and sulphates of calcium and magnesium. In the present study, hardness of experimental water showed 74 mg/l and collection site water showed 21 mg/l. These values of hardness were within standard limits

#### 3.1.2.4 Biochemical Oxygen Demand (Table 1, Graph 1)

Biochemical Oxygen Demand of experimental water showed 1.39 mg/l. and collection site water showed 1.27 mg/l. Both these values of BOD were within standard limits.

**3.1.2.5 Chemical Oxygen Demand (Table 1,Graph 1)**

COD at experimental water showed 26.25 mg/l. and collection site water showed 55.75 mg/l COD of both samples were within standard limits.

**3.1.2.6 Nitrates (Table 1,Graph 1)**

In an aquatic ecosystem nitrate are formed on biological oxidation of organic nitrogenous matter received from domestic sewage, agricultural run off and industrial effluents. In addition to this metabolic waste, excretory product and dead organisms add organic nitrogen.

In the present study, nitrates at experimental water showed 10.37 mg/l and collection site water showed 0.68 mg/l. The recorded nitrate was within standard limits.

**3.1.2.7 Calcium: (Table1, Graph 1)**

Calcium content of collection site water and experimental water were 32.06 ppm and 5.72 ppm respectively. The experimental water showed minimum quantity of calcium both these values of calcium were within standard limits.

**3.1.3 Heavy metal**

**3.1.3.1 Lead: (Table1, Graph 1)**

Lead is a neurotoxic heavy metal, mainly adds to aquatic ecosystem by Lead mines, Lead smelters, storage battery factories, brass foundries, glass workers, vehicular emission etc. In the present study, lead content in water of experimental water and collection site water 0.0045 and 0.002 mg/l respectively.

**3.2 Animal Behavior:**

**3.2.1 Behaviors of bivalves after acute exposure:**

Different doses of lead were used for acute toxicity test. Bivalves exhibited restricted movement. No such abnormal behaviour was observed in control. Excreta appeared in all test containers for first 24 hours but later on, only mucus secretion was observed which increased with the concentration and period of exposure.

Bivalve did not show any response to touch from 300 ppm to 330 ppm. After 96 hours, there was 50% mortality at 280 ppm. In normal group (without toxicant) there was no mortality at the end of 96 hrs, indicates healthy group of test bivalves during acute toxicity test. On exposure of freshwater bivalve, *Lamellidens marginalis* to lead , there was 10% mortality at 180 ppm as well as 200 ppm after 96 hrs. As the concentration of toxicant increased, there was increase in the percent mortality at 220 ppm, after 96 hrs whereas at 240, 260, 280 and 300 ppm there was 30%, 40%, 50% and 80% mortality respectively at the end of 96 hrs. The observed $LC_0$ and $LC_{50}$ values were 60 ppm and 280 ppm respectively.

**Percentage mortality of the freshwater bivalve, *Lamellidens marginalis* exposed to acute toxicity of Lead.**

| Concentration in ppm | No. of bivalves used | Mortality No. according to different hrs. | | | | %mortality at96 hrs. |
|---|---|---|---|---|---|---|
| | | 24hrs. | 48hrs. | 72hrs. | 96hrs. | |
| Normal | 10 | 0 | 0 | 0 | 0 | 0 |
| 60 | 10 | 0 | 0 | 0 | 0 | 0 |
| 80 | 10 | 0 | 0 | 0 | 10% | 10% |
| 100 | 10 | 0 | 0 | 0 | 10% | 10% |
| 120 | 10 | 0 | 0 | 0 | 10% | 10% |
| 140 | 10 | 0 | 0 | 0 | 20% | 20% |
| 160 | 10 | 0 | 0 | 0 | 20% | 20% |
| 180 | 10 | 0 | 0 | 10% | 20% | 30% |
| 200 | 10 | 0 | 0 | 10% | 20% | 30% |
| 220 | 10 | 0 | 10% | 10% | 20% | 40% |
| 240 | 10 | 0 | 10% | 10% | 20% | 40% |
| 260 | 10 | 0 | 10% | 10% | 20% | 40% |
| 280 | 10 | 10% | 10% | 10% | 20% | 50% |
| 300 | 10 | 10% | 20% | 20% | 20% | 70% |
| 320 | 10 | 20% | 20% | 20% | 30% | 90% |
| 340 | 10 | 20% | 20% | 30% | 30% | 100% |

For each test 10 animals were used.

$LC_0$ = 60 ppm is the concentration for 0% mortality.

$LC_{50}$ = 280 ppm is the concentration for 50% mortality.

### 3.2.2 Behaviors of bivalves after chronic toxicity:

For the chronic toxicity study $1/10^{th}$ concentration of $LC_{50}$ value (acute) of lead have been selected. This value for lead was 28 ppm for *Lamellidens marginalis*. Also for detoxification, different concentrations of Calcium have been selected.

#### 3.2.2.1 8 days and 16 days exposure :

**Control group:**

In the control group (without toxicant), no mortality of the bivalve was observed. The bivalve showed normal behaviour patterns of respiration (by siphon), mucous secretion and movement of foot.

**Experimental 1:**

**a) Lead :**

Following behavioral patterns were observed in *Lamellidens marginalis.*

i Excessive secretion of mucus was observed on surface and bottom of container.

ii At first two days of exposure the bivalves normally move in container by exposing foot regularly.

iii From third day of exposure, the effects were manifested by restricted movement and also they were not trying to expose foot from shell.

iv From fifth day of exposure no one bivalve can expose its siphon outside from shell for respiration. The time period of siphon opening was also reduced with time of exposure to Lead acetate.

v Loss of reflex was observed.

vi From ninth day, muscles were also unable to close the shells tightly (means when we had taken the bivalves for scarification, the shells were easily opened by fingers ).

vii On $16^{th}$ day, bivalves never show any response to touch.

viii The mortality was evident because of the absence of any motion or respiration.

**b) Calcium :**

Behaviour of bivalves in calcium exposed group was similar as like control. In this groups no mortality of the bivalves were observed. The bivalves showed normal bihaviour patterns of respiration, mucus secretion and foot and siphon movements throughout the experiment. The shell secretion process was faster as compared to control and lead treated bivalves.

**c) Both Lead and Calcium doses :**

Three groups of bivalves (each contain ten animals) were introduced in different container having different concentration of Calcium acetate and same concentration of Lead acetate ($1/10^{th}$ concentration of ($LC_{50}$) which is given below,

B = 28ppm Calcium acetate and 28ppm Lead acetate

C = 56ppm Calcium acetate and 28ppm Lead acetate

D = 84ppm Calcium acetate and 28ppm Lead acetate

- **Behavior study of group B :**

i In this group, bivalves behaved normally up to the fourth day.

ii After four days the effects were manifested by restricted movement and they were not tried to take foot out of shell.

iii From eighth day of exposure no bivalve exposed its siphon outside from its shell for respiration.

iv Time period of siphon opening was also reduced from $8^{th}$ day onwards was inversely proportional to the time period of exposure.

Bivalves never gave any response to touch till $16^{th}$ day.

- **Behavior study of group C:**

i In this group, effect was observed on the movement of animals

ii Bivalves tightly closed their shells but respiration was proper, through opening of siphon throughout the experiment (up to 16 days).

- **Behavior study in group D :**

i The bivalves from this group, closed their shells tightly immediately after addition of Calcium and Lead doses.

ii Up to three days they were not behaved properly but from fourth day of exposure they were slowly moved in container through foot and respire properly by siphon.

iii The time period of siphon opening was increased with exposure period.

iv Mucus secretion was proper.

### 3.3 Accumulation Study:

The bivalves, *Lamellidens marginalis* were exposed to the various doses of Lead and Calcium for 8 days and 16 days. After completion of the exposure period whole bodies of these animals were analyzed for the heavy metals like lead and calcium for accumulation study. Different organs like gill and mantle of the animals were used for detection of heavy metals. These elements were detected by Atomic Absorption Spectrophotometry and results are documented here.

### 3.3.1 Gills (Table 2, 3 and Graph 2, 3)

The second and third tables and graphs shows the accumulation of lead and effect of calcium on 8 and 16 days exposure period in gill tissue. Accumulation of lead was more in group A after 8 days while this accumulation was increased after 16 days. The calcium level was more in group B, C and D after both exposure periods. The remedial groups showed decrease in Pb accumulation with increasing $Ca^{++}$. After 16 days this groups showed less Pb accumulation.

**3.3.2 Mantle: (Table 4, 5 and Graph 4, 5)**

The fourth and fifth tables and graphs shows the accumulation of lead and effect of calcium on 8 and 16 days exposure period in mantle tissue. Mentle also showed similar observations like gills. Accumulation of lead was more in group A after 8 days while accumulation was increased after 16 days. The calcium level was more in group B, C and D after both exposure periods as compared to control one. The remedial groups showed decrease in Pb accumulation with increasing $Ca^{++}$ as compared to 28 ppm Pb treated group. After 16 days this groups showed less Pb accumulation.

**3.4 Biochemical Study :**

**3.4.1 Protein**

**3.4.1.1 8 days Exposure :**

**A. Gills :( Table 6, Graph 6)**

The protein concentrations in the gills of control and experimental bivalve under study have been recorded in Table No.6 and have been graphically illustrated in Graph No.6. As compared to control significant decrease in protein content was observed in group A and B which was exposed to 28 ppm of Pb and 28 ppm $Ca^{++}$. Also there was nonsignificant decrease in protein content in C and D group. In these B, C and D groups the protein level was within control level.

**B. Mantle :( Table 9, Graph 9)**

The protein concentrations in the mantle of control and experimental bivalve under study have been recorded in Table No.9 and have been graphically illustrated in Graph No.9. The protein content in the gill of the mussel, *Lamellidens marginalis* exposed to various concentrations of Pb and $Ca^{++}$ for 8 days showed significant decrease in group A, C and D. These groups were treated with 28 ppm Pb, 56 ppm $Ca^{++}$ and 84 ppm $Ca^{++}$ respectively whereas groups G showed nonsignificant decreases to that of control which was treated by 28 ppm Pb. Whereas there was significant increase in protein level in first calcium B group, but there was significant decrease in protein levels in C and D.

### 3.4.1.2 16 days Exposure

**A. Gills :( Table 6, Graph 6)**

The protein concentration in the gills of 16 days of controlled and experimental bivalve groups under study have been recorded in Table No.6 and have been graphically illustrated in Graph No.6. The protein was significantly decreased in $LC_{50}$ dose of $Pb^{+}$. The percent increase was highly significant in group B, C, D, E and F. These groups were treated with 28 ppm, 56 ppm, 84 ppm $Ca^{++}$, 28 ppm $Pb^{+}$ 28 ppm $Ca^{++}$, 28 ppm $Pb^{+}$ 56 ppm $Ca^{++}$ respectively. In group G non significant increase was observed.

**B. Mantle :( Table 9, Graph 9)**

The protein concentration in the mantle for 16 days of control and experimental bivalve groups under study have been recorded in Table No.9 and have been graphically illustrated in Graph No.9. The level was significantly decreased in $LC_{50}$ dose of lead. The percent increase was significant in first calcium treated group B, this level of protein content was gradually decreased from B group to the D group non significant in mantle. In both calcium and lead treated groups there were significant difference in group E and F. The percent depletion was significant at for group E while in group F increase was significant. In G group the protein content was nonsignificantly decreased.

**3.5 Enzyme activity**

**3.5.1 Acid Phosphatase Activity**

**3.5.1.1 8 days exposure**

**A. Gills: (Table 7, Graph 7)**

The enzyme activity in the control animal and in animals exposed to various doses of lead and calcium are recorded in Table No.7 and they are shown graphically in Graph No.7. Acid phosphatase activity in terms of µg p – nitrophenol phosphate per gram wet weight of tissue per hour. In exposure to 28 ppm of Pb significant increase was observed. In the groups C and D treated by 28 ppm Pb + 56 ppm $Ca^{++}$ and 28 ppm Pb + 84 ppm $Ca^{++}$. It was observed that ACP activity significantly decreased. In the B group non-significant depletion of acid phasphatase activity was observed as compared to control. On the exposure of three different doses of both calcium and lead (group E, F and G) no significant difference was observed but as compared the values of three groups were within control limit.

**B. Mantle: (Table 10,Graph10)**

The enzyme activity in the mantle of control animal and in animals exposed to various doses of Lead and calcium are recorded in Table No.10 and they are shown graphically in Graph No.10. The acid phosphatase activity in terms of µg p – nitrophenol phosphate per gram wet weight of tissue per hour. Group A, E and F showed significant increase. These groups were treated with 28 ppm Pb, 28 ppm Pb + 28 ppm $Ca^{++}$ and 28 ppm Pb + 56 ppm $Ca^{++}$ respectively. While significant decrease was observed in group C and D. These groups were treated with 56 ppm $Ca^{++}$ and 84 ppm $Ca^{++}$. The activity of acid phosphatase in the mantle for 8 days at both Pb and calcium treated group was highest. A significant decrease in acid phosphatase activity was observed in group treated with $Pb^{+}$ and maximum dose of $Ca^{++}$.

**3.5.1.2 16 days exposure**

**A. Gills: (Table 7, Graph 7)**

The enzyme activity in the gill of control animal as well as experimental animals under study have been recorded in Table No.7 and have been graphically presented in Graph No.7. The acid phosphatase activity presented in Graph ug P - nitrophenol phosphatase per gram wet weight of tissue per hour. The activity of acid phosphatase for 28 ppm Pb exposure group A was significantly decreased as compared to that of the control. As compared to control, significant decrease was observed from group B to group G.

**B. Mantle :( Table 10, Graph10)**

The enzyme activity in the mantle in control as well as experimental animals under study have been recorded in Table No.10 and have been graphically presented in Graph No. 10. The acid phosphatase activity presented in ug P - nitrophenol phosphatase per gram wet weight of tissue per hour. Only group A showed significant increase. This group was treated with 28 ppm Pb, where as significant decrease was observed in group C, D and E. These groups were treated with 56 ppm $Ca^{++}$, 84 ppm $Ca^{++}$ and 28 ppm Pb + 28 ppm $Ca^{++}$.

**3.5.2 Alkaline Phosphatase Activity**

**3.5.2.1 Exposure for 8 days period :**

**A. Gills: (Table 8,Graph 8)**

The enzyme activity in the control animals and in the animals exposed to various doses of Lead and calcium are recorded in Table No.8 and they are shown graphically in Graph No.8 in which alkaline phosphatase activity in terms of µg p – nitrophenol phosphate per gram wet weight of tissue per hour. In group A treated by 28 ppm of Pb and group C treated by 56 ppm $Ca^{++}$ showed nonsignificant increase. In the group B, D, E and G non-significant depletion of alkaline phasphatase activity was observed. On the exposure of three different doses of both calcium and lead, only in group F showed significant difference while in remaining two (E and G) no significant difference was observed as compare to control.

**B. Mantle :(Table11,Graph11)**

The enzyme activity in the mantle of control animal and in animals exposed to various doses of lead and calcium are recorded in Table No.11 and they are shown graphically in Graph No11 in which alkaline phosphatase activity in terms of µg p – nitrophenol phosphate per gram wet weight of tissue per hour. Groups D and E showed significant increase. These groups were treated by 84 ppm $Ca^{++}$ and 28 ppm Pb + 28 ppm $Ca^{++}$. On exposure to $LC_{50}$ dose of Pb i.e. 28 ppm, nonsignificant increase was observed. Non significant increase was observed in E, F and G group.

**3.5.2.2 16 days exposure :**

**A. Gills : ( Table 8, Graph 8)**

The enzyme activity in the gills of control as well as experimental animals under study have been recorded in Table No.8 and graphically presented in Graph No.8. The alkaline phosphatase activity presented in ug P- nitrophenol phosphatase per gram wet weight of tissue per hour. In group A treated by 28 ppm Pb the activity of alkaline phosphatase was slightly increased by 1.003 folds. In the case of different calcium doses group B, C and D this activity of alkaline phosphatase was showed significant decrease difference due to calcium supplementation. In both calcium and lead treated groups E, F and G the alkaline phosphatase activity showed significant decrease as compared to control.

**B. Mantle :( Table11, Graph11)**

The enzyme activity in the mantle of control animal and animals exposed to various doses of lead and calcium are recorded in Table No.11 and shown graphically in Graph No.11 in which alkaline phosphatase activity in terms of µg p – nitrophenol phosphate per gram wet weight of tissue per hour. As compared to control, in group B and C significant decrease was observed where as group A, D, E, F and G showed non significant increase to that of control. But ALP activity in group E and G was within control.

## 3.6 Histological Observations:

### 3.6.1 Gills:

#### 3.6.1.1 Control:

Gills in the fresh water bivalve, *Lamellidens marginalis* was on the either side of the body in the mantle cavity. Each gill was formed of two laminae as inner and outer lamella. Each gill lamina consists of an outer and inner lamella, were elongated plate like structure (fig. 1 and 9). The cavity between gill lamellae was divided by vertical septa; interlamellar junctions into a number of compartments i.e. water tubes. Each gill lamella was formed of numerous thin, vertical and parallel gill filaments. Adjacent gill filament of a lamella remains connected by small horizontal bars called basal filaments. A gill filament was covered by ciliated epithelium. The epithelial cells were elongated with prominent nucleus. The cytoplasm showed secretary material. The connective tissue was present between the lamella and basal filament. The cavity in gill filament was normal as well as space between two gills was also moderate (fig. 1 and 9).

#### 3.6.1.2 Exposure for 8 days period:

**a) Pb treated:**

The light microscopic structure of gills of fresh water bivalve, *Lamellidens marginalis* showed changes in histological structure. After 8 days exposure gill filaments were ruptured and lined by epithelial cells with cilia. Gill rods were prominently observed. The cells show accumulation of some dark material. The disintegration of connective tissue and basal filament was also observed. Water tubes were elongated with long and obliquely placed inter lamellar junction (fig.2).

**b) Calcium treated:**

The light microscopic structure of gills of bivalve, *Lamellidens marginalis* exposed to 28 ppm (B), 56ppm(C) and 84 ppm (D) doses of $Ca^{++}$ for 8 days exposure period showed long and uniform gill filaments and epithelial cells covered with cilliary mass. Normal filament cavity and inter filament cavity was observed. Basal filament was clearly seen and size of water tubes was small. Proper inter lamellar junctions were observed in all

the $Ca^{++}$ treated groups. Gill bars and little amount of dark granules in the cytoplasm were dominantly observed in 56 ppm $Ca^{++}$ group (fig. 4). The dark secreting granules were enormously increased in 84 ppm $Ca^{++}$ treated gill (fig.5). The 28 ppm $Ca^{++}$ treated groups showed no much changes (fig. 3).

**b) Remedial:**

The light microscopic structure of gills of bivalve, *Lamellidens marginalis* exposed to 28 ppm Pb + 28 ppm $Ca^{++}$ (grcup E), 28 ppm Pb + 56 ppm $Ca^{++}$ (group F) and 28 ppm Pb +84 ppm $Ca^{++}$ (group G) concentration for 8 days exposure period. In group E gill filament lined by epithelial cells was not properly observed. The inter lamellar space was fulfilled by accumulation of dark material in the cytoplasm. The basal filament was not clearly seen. Water tubes were observed but inter lamellar junctions were reduced (fig.6). While in group F, gill filament lined by epithelial cells was clearly observed. The inter lamellar space was with dark material accumulated in the cytoplasm. The basal filament was not clearly seen. Length of gill filament was increased and filaments with gill bars and opening of vessel were seen. Water tubes and inter lamellar junctions were properly observed (fig.7). In the group G gill filament lined by epithelial cells was properly observed. The inter lamellar space was with dark material accumulated in the cytoplasm. The basal filament was clearly seen. Length of gill filament was increased as compared to Pb treated gills. Short water tubes and inter lamellar junctions were also observed (fig.8).

**3.6.1.3 Exposure for 16 days period:**

**a) Pb treated :**

The light microscopic structure of gills of fresh water bivalve, *Lamellidens marginalis* showed lot of changes in histological structure. After 16 days alterations in gill filaments were prominent. Gill filaments were ruptured and lined by epithelial cells with cilia. Gill filaments and laminae were totally loose their identity. Cytoplasm also stained blue due to acid screation. Inter lamellar junction was totally ruptured. Gill rods were also disintegrated. Vacuolization of connective tissues were observed (fig. 10).

**b) Calcium treated:**

The light microscopic structure of gills of bivalve, *Lamellidens marginalis* exposed to 28 ppm (B), 56 ppm(C) and 84 ppm (D) coses of $Ca^{++}$ for 16 days exposure period showed gill filaments and epithelial cells covered by frontal, lateral and fronto-latral cilia. Gill bars were not observed properly. Histological architecture of filament was normal. Amount of connective tissue was highly increased. Water tube, inter-lamellar junction and gill bars showed normal architecture (fig. 11, 12, 13).

**c) Remedial:**

The light microscopic structure of gills of bivalve, *Lamellidens marginalis* exposed to 28 ppm Pb + 28 ppm $Ca^{++}$ (group E) , 28 ppm Pb + 56 ppm $Ca^{++}$ (group F) and 28 ppm Pb + 84 ppm $Ca^{++}$ (group G) concentration for 8 days exposure period.

In group E gill filaments and epithelial cells were covered by small amount of ciliary mass. Connective tissue showed vacuolation. Histological architecture of filament some what normal. The inter-lamellar space was having with less pigmentation. Architecture of water tube, inter-lamellar junction and gill bars showed fewer disturbances (fig. 14). In group F gill filaments and epithelial cells were covered by small amount of ciliary mass. Connective tissue content was less. Histological architecture of filament was normal. The inter-lamellar space was with less pigmentation. Architecture of water tube, inter-lamellar junction and gill bars showed minimum changes disturbances (fig. 15). In group G gill filaments and epithelial cells were covered by normal amount of ciliary mass and also observed clearly. Connective tissue content was normal. Ostia were properly seen. Histological architecture of filament showed positive curative alteration. These alterations were similar with architecture of control gills. The inter-lamellar space was having with normal pigmentation. Architecture of Water tube, inter-lamellar junction and gill bars showed normal (fig. 16).

As compared to Pb treated gills these groups showed curative alterations. These alterations were positively increased from group E to G with increasing $Ca^{++}$ doses.

**3.6.2 Mantle :**

**3.6.2.1 Control:**

In *Lamellidens marginalis* two mantle was bilobed thin, transparent and bounded by single layer of epithelial on the both sides facing shell and mantle cavity, Mantle consists of outer and inner ciliated epithelium resting on basement layer with loose connective tissue. At the free margin the epithelial cells were columnar while cuboidal in the remaining regions. The mucous cells were noticed in the marginal folds as well as amongst the inner epithelial cells. In the ventral edge of the mantle the mucous cells were mainly concentrated and large in size as against those present in the lateral walls of inhalant and exhalent apertures. The mucous cells become prominent towards the pallial line and were smaller in size. Some of the mucous cells, which were very close to the epithelium of the inner marginal folds, possess neck like extensions which extend between the epithelial cells. The mucous cells from the ventral edge of the mantle were more or less oval with granular cytoplasm; their nuclei were pressed against the inner surface of the cell membrane. The outer marginal fold was devoid of mucous cells (fig. 17, 25).

**3.6.2.2 Exposure for 8 days period :**

**a) Pb treated :**

The light microscopic structure of mantle of fresh water bivalve, *Lamellidens marginalis* showed many changes in histological structure. After 8 days connective tissue was ruptured. The epithelial cells from outer fold were showed dark staining. The disintegration of connective tissue, internal fold and middle fold were prominently observed (fig.18).

**b) Calcium treated:**

The light microscopic structure of mantle of bivalve, *Lamellidens marginalis* exposed to 28 ppm (B), 56 ppm (C) and 84 ppm (D) doses of $Ca^{++}$ for 16 days exposure period showed normal histological structure. After 8 daysexposure to $Ca^{++}$ connective tissue was proper. The epithelial cells from outer fold were shows dark staining and were increased in number. No disintegration of connective tissue. Outer and inner epitheliums were normal (fig.19, 20, and 21).

**c) Remedial:**

The light microscopic structure of mantle of bivalve, *Lamellidens marginalis* exposed to 28 ppm Pb + 28 ppm $Ca^{++}$ (group E), 28 ppm Pb + 56 ppm $Ca^{++}$ (group F) and 28 ppm Pb + 84 ppm $Ca^{++}$ (group G) concentration for 8 days exposure period. In group E showed some changes in histological structure. After 8 days exposure connective tissue was proper and having with some secretions. Epithelial cells were proper. No dark pigmentation was observed. No disintegration of connective tissue. Outer epithelium showed dark secretion (fig. 22). In group F connective tissue was normal observed in the central portion of mantle. Inner and outer epitheliums were proper. No dark pigmentation was observed. There was no disintegration of connective tissue (fig. 23). In group G showed minor changes in histological structure. Connective tissue was normal in the central portion of mantle. All types of folds were proper in architecture. Inner and outer epitheliums were normal. Dark pigmentation was observed in inner epithelium region. No disintegration of connective tissue was seen. The numbers of mucous cells were seen (fig.24). As compare to Pb treated group, the histological architecture in all remedial groups were better. Recovery of the damaged connective tissue was observed. The positive changes were increased with increasing $Ca^{++}$ doses.

**3.6.2.3 Exposure for 16 days period:**

**a) Pb treated :**

The light microscopic structure of mantle of fresh water bivalve, *Lamellidens marginalis* showed many changes in histological structure. After 16 days connective tissue was ruptured. All the types of folds lost their identity. Connective tissue was completely ruptured. The epithelial cells were also badly affected (fig.26).

**b) Calcium treated:**

The light microscopic structure of mantle of bivalve, *Lamellidens marginalis* exposed to 28 ppm (B), 56 ppm(C) and 84 ppm (D) doses of $Ca^{++}$ for 16 days exposure period showed increase in connective tissue. The epithelial cells were also normal in architecture and showed some secretary

activity. Mucus film was also observed in all groups (fig.27, 28 and 29). As compare to Pb treated group the histological architecture was proper in these $Ca^{++}$ exposed animals all cells were normal in their structure.

**c) Remedial:**

The light microscopic structure of mantle of bivalve, *Lamellidens marginalis* exposed to 28 ppm Pb +28 ppm $Ca^{++}$ (group E) , 28 ppm Pb + 56 ppm $Ca^{++}$ (group F) and 28 ppm Pb +84 ppm $Ca^{++}$ (group G) concentration for 16 days exposure period. In all these groups connective tissue was proper and having enormously increased mucus secreting cells. Epithelial cells showed normal structure. There was no disintegration of connective tissue. In group E dark pigmentation was not observed but this pigmentation was observed in groups F and G.

As compare to mantle of Pb treated animal group E showed remedial changes in histological structure. But these positive changes were increased in Group F and G. Compare to mantle of remedial group of 8 days exposure period remedial changes after 16 days were increased. (fig.30, 31 and 32).

**3.6.3 Collagen fibers:**

For the demonstration of collagen fibers in tissue section, Van Gieson's Picric acid Acid fuchsin stain (Thompson, 1966), was used.

**3.6.3.1 Gills**

**Control:**

In this control group gills of *L. marginalis* showed large amount of collagen fibers. They were normal, long bundles arranged properly in all the parts of gill. In interlamellar junctions, gill filament and inter-lamellar spaces collagen was prominently seen (fig.33, 41).

**3.6.3.1.1 Exposure for 8 days period:**

**a) Pb treated :**

The light microscopic structure of collagen from gills of fresh water bivalve, *Lamellidens marginalis* showed many changes in histological structure. After 8 days collagen in the gill filaments were ruptured. In the

epithelial cell region collagen fibers were markly observed. Due to damage of collagen architecture gill structure was disturbed. The disintegration of connective tissue and basal filament was also seen prominently (fig.34).

**b) Calcium treated:**

The light microscopic structure of collagen fibers in gills of bivalve, *Lamellidens marginalis* exposed to 28 ppm (B), 56 ppm(C) and 84 ppm (D) doses of $Ca^{++}$ for 8 days exposure period, showed increase in collagen fibers in all regions of gill filaments. The epithelial cells were covered with large mass of cilia. As compared to normal collagen contents of this group were high (fig.35, 36 and 37).

**c) Remedial:**

The light microscopic structure of collagen in gills of bivalve, *Lamellidens marginalis* exposed to 28 ppm Pb + 28 ppm $Ca^{++}$ (group E) , 28 ppm Pb + 56 ppm $Ca^{++}$ (group F) and 28 ppm Pb + 84 ppm $Ca^{++}$ (group G) concentration for 8 days exposure period showed alterations in collagen synthesis. The amount of collagen was increased in filamentary region as compared to lamellar space. Also it was present around the cells. The amount of collagen was increased in filamentary region (fig. 38, 39 and 40). As compared to Pb exposed group collagen in the gills of above groups showed curative alterations. These alterations were positively increased from group E to G with increasing $Ca^{++}$ doses.

**3.6.3.1.2 Exposure for 16 days period:**

**a) Pb treated :**

The light microscopic structure of collagen in gills of bivalve, *Lamellidens marginalis* exposed to 28 ppm Pb showed alterations in collagen. These alterations were increased after 16 days. Collagen fibers from this group were totally disintegrated. The gill filaments and laminae totally loose their identity. The Inter lamellar junction were totally ruptured (fig. 42).

**b) Calcium treated :**

The light microscopic structure of collagen fibers in gills of bivalve, *Lamellidens marginalis* exposed to 28 ppm (B), 56 ppm (C) and 84 ppm (D)

doses of $Ca^{++}$ for 16 days exposure period similar effects were observed. Gill filaments and epithelial cells totally covered by frontal, latral and fronto-lateral ciliary mass. Collagen content was dominantly increased. Histological architecture of filament was normal. Amount of connective tissue was increased (fig.43, 44 and 45).

**c) Remedial:**

The light microscopic structure of collagen in gills of bivalve, *Lamellidens marginalis* exposed to 28 ppm Pb + 28 ppm $Ca^{++}$ (group E) , 28 ppm Pb + 56 ppm $Ca^{++}$ (group F) and 28 ppm Pb + 84 ppm $Ca^{++}$ (group G) concentration for 16 days exposure period similar effects were observed. Gill filaments and epithelial cells were totally covered by frontal, latral and fronto-lateral ciliary mass. In these groups the collagen content was increased and mostly it was present at the basal region of gill filaments. As compared to Pb treated animals this amount of collagen shows some remedial changes (fig.46, 47 and 48).

**3.6.3.2 Mantle :**

**Control:**

The light microscopic structure of collagen in mantle of fresh water bivalve, *Lamellidens marginalis* showed large number of collagen fibers. They were normal, long bundles arranged properly in all the parts of mantle. Large space of connective tissue was occupied by collagen fibers (fig.39, 57).

**3.6.3.2.1 Exposure for 8 days period**

**a) Pb treated :**

The light microscopic structure of collagen in mantle of fresh water bivalve, *Lamellidens marginalis* showed lot of changes in histological structure. After 8 days of exposure connective tissue was ruptured due to damage of collagen fibers. Very less amount of collagen fibers were observed (fig.50).

**b) Calcium treated:**

The light microscopic structure of collagen fibers in mantle of bivalve, *Lamellidens marginalis* exposed to 28 ppm (B), 56 ppm (C) and 84 ppm (D) doses of $Ca^{++}$ for 8 days exposure period showed changes in the structure of collagen. After 8 days connective tissue was normal. The epithelial cells from outer fold showed dark staining and surrounded by collagen fibers (fig.51, 52 and 53).

**c) Remedial:**

The light microscopic structure of collagen in mantle of bivalve, *Lamellidens marginalis* exposed to 28 ppm Pb +28 ppm $Ca^{++}$ (group E) , 28 ppm Pb +56 ppm $Ca^{++}$ (group F) and 28 ppm Pb +84 ppm $Ca^{++}$ (group G) concentration for 8 days exposure period. After 8 days connective tissue was normal and it largely contains collagen proteins. All part of mantle showed distribution in collagen fibers. The central portion of mantle shows large amount of collagen fibers (fig.54, 55 and 56).

**3.6.3.2.2 Exposure for 16 days period**

**a) Pb treated :**

The light microscopic structure of collagen fibers in mantle of bivalve, *Lamellidens marginalis* exposed to 28 ppm Pb showed alterations which were increased after 16 days. Collagen fibers were badly affected by lead toxicity. Connective tissue was totally ruptured. The epithelial cells were also badly affected (fig.58).

**b) Calcium treated:**

The light microscopic structure of collagen fibers in mantle of bivalve, *Lamellidens marginalis* exposed to 28 ppm (B), 56 ppm (C) and 84 ppm (D) doses of $Ca^{++}$ for 16 days exposure period the structure of collagen was increased and stained with dark pink colour. As compared to 8 days exposure, collagen was increased in 16 days treatment (fig.59, 60 and 61).

**c) Remedial:**

The light microscopic structure of collagen in mantle of bivalve, *Lamellidens marginalis* exposed to 28 ppm Pb +28 ppm $Ca^{++}$ (group E) , 28 ppm Pb + 56 ppm $Ca^{++}$ (group F) and 28 ppm Pb +84 ppm $Ca^{++}$ (group G) concentration for 16 days exposure period the amount of collagen was increased enormously and it stained darkly. As compared to 8 days exposure, collagen was increased in 16 days treatment. As compared to mantle of Pb treated animal this group showed curative alterations in histological architecture. (fig.62, 63 and 64).

**Table No.1**

**Physico-chemical parameters of Experimental and Collection site water**

| Sr. No. | Parameters | Experimental Water | Collection Site Water | MPCB Standards |
|---|---|---|---|---|
| 1 | pH | **8.1** | **7.4** | **5.43** |
| 2 | Temperature | **27.5** | **27.2** | **22** |
| 3 | DO | **8.02** | **7.44** | **7** |
| 4 | Hardness | **74** | **21** | **75** |
| 5 | BOD | **1.39** | **1.27** | **1.20** |
| 6 | COD | **26.25** | **55.75** | **55** |
| 7 | Nitrate | **10.37** | **0.68** | **0.72** |
| 8 | Calcium | **5.72** | **32.06** | **5.6** |
| 9 | Lead | **0.0045** | **0.002** | **0.001** |

* All values are in mg/l except pH and temperature

**Table No.2**

**Bioaccumulation of Calcium and Lead in the gills of freshwater bivalve, *Lamellidens marginalis* after 8 days exposure**

| Sr. No. | Abbreviations | Doses | Calcium | Lead |
|---|---|---|---|---|
| 1 | N | Control (NO dose) | 1.038 | 0.204 |
| 2 | A | $Pb^{+}$ 28 ppm | 0.770 | 3.300 |
| 3 | B | $Ca^{++}$ 28 ppm | 1.321 | 0.216 |
| 4 | C | $Ca^{++}$ 56 ppm | 0.938 | 0.121 |
| 5 | D | $Ca^{++}$ 84 ppm | 0.861 | 0.083 |
| 6 | E | $Pb^{+}$+ $Ca^{++}$ 28 ppm | 0.941 | 2.938 |
| 7 | F | $Pb^{+}$ + $Ca^{++}$ 56 ppm | 1.029 | 1.324 |
| 8 | G | $Pb^{+}$ + $Ca^{++}$ 84 ppm | 1.031 | 0.98 |

* All values are expressed in mg/ml

**Table No.3**

**Bioaccumulation of Calcium and Lead in the gill of freshwater bivalve, *Lamellidens marginalis* after 16 days exposure**

| Sr. No. | Abbreviations | Doses | Calcium | Lead |
|---|---|---|---|---|
| 1 | N | Control (NO dose) | 1.038 | 0.204 |
| 2 | A | $Pb^{+}$ 28 ppm | 0.678 | 4.129 |
| 3 | B | $Ca^{++}$ 28 ppm | 1.249 | 0.138 |
| 4 | C | $Ca^{++}$ 56 ppm | 0.774 | 0.070 |
| 5 | D | $Ca^{++}$ 84 ppm | 2.049 | 0.062 |
| 6 | E | $Pb^{+}$ + $Ca^{++}$ 28 ppm | 1.048 | 1.620 |
| 7 | F | $Pb^{+}$ + $Ca^{++}$ 56 ppm | 1.321 | 1.401 |
| 8 | G | $Pb^{+}$ + $Ca^{++}$ 84 ppm | 1.496 | 0.986 |

* All values are expressed in mg/ml

**Table No.4**

**Bioaccumulation of Calcium and Lead in mantle of freshwater bivalve, *Lamellidens marginalis* after 8 days exposure**

| Sr. No. | Abbreviations | Doses | Calcium | Lead |
|---|---|---|---|---|
| 1 | N | Control (NO dose) | 0.788 | 0.158 |
| 2 | A | $Pb^{+}$ 28 ppm | 1.857 | 2.381 |
| 3 | B | $Ca^{++}$ 28 ppm | 1.311 | 0.106 |
| 4 | C | $Ca^{++}$ 56 ppm | 1.475 | 0.079 |
| 5 | D | $Ca^{++}$ 84 ppm | 1.965 | 0.033 |
| 6 | E | $Pb^{+}$ + $Ca^{++}$ 28 ppm | 1.002 | 2.302 |
| 7 | F | $Pb^{+}$ + $Ca^{++}$ 56 ppm | 1.170 | 1.870 |
| 8 | G | $Pb^{+}$ + $Ca^{++}$ 84 ppm | 1.369 | 1.581 |

* All values are expressed in mg/ml.

**Table No.5**

**Bioaccumulation of Calcium and Lead in mantle of freshwater bivalve, *Lamellidens marginalis* after16 days exposure**

| Sr. No. | Abbreviations | Doses | Calcium | Lead |
|---|---|---|---|---|
| 1 | N | Control | 0.788 | 0.158 |
| 2 | A | $Pb^{+}$ 28 ppm | 0.498 | 2.938 |
| 3 | B | $Ca^{++}$ 28 ppm | 2.133 | 0.090 |
| 4 | C | $Ca^{++}$ 56 ppm | 1.868 | 0.066 |
| 5 | D | $Ca^{++}$ 84 ppm | 2.252 | 0.013 |
| 6 | E | $Pb^{+}$ 28 ppm + $Ca^{++}$ 28 ppm | 1.250 | 1.342 |
| 7 | F | $Pb^{+}$ 28 ppm + $Ca^{++}$ 56 ppm | 2.299 | 1.225 |
| 8 | G | $Pb^{+}$ 28 ppm + $Ca^{++}$ 84 ppm | 1.314 | 1.20 |

* All values are expressed in mg/ml.

**Table No. 6**

**Protein contents in the Gills of *Lamellidens marginalis* at different concentrations of Lead and Calcium for different exposure periods**

| Sr. No. | Abbreviation | Dose | 8 days | 16 days |
|---|---|---|---|---|
| 1 | N | Control | 20.55 ± 4.82 | 23.05 ± 0.324 |
| 2 | A | $Pb^{+}$ ( 28 ppm) | 13.70 ± 4.71* | 11.71 ± 1.88 |
| 3 | B | $Ca^{++}$ ( 28 ppm) | 14.94 ± 0.779* | 50.76 ± 3.22* |
| 4 | C | $Ca^{++}$ (56 ppm) | 11.89 ± 9.85 | 50.18 ± 4.73* |
| 5 | D | $Ca^{++}$ (84 ppm) | 20.5 ± 10.8 | 45.09 ± 0.34* |
| 6 | E | $Pb^{+}$ 28 ppm + $Ca^{++}$ 28 ppm | 27.4 ± 12.9 | 32.8 ± 3.368* |
| 7 | F | $Pb^{+}$ 28 ppm + $Ca^{++}$ 56 ppm | 25.67 ± 5.12 | 29.6 ± 4.39* |
| 8 | G | $Pb^{+}$ 28 ppm + $Ca^{++}$ 84 ppm | 23.0 ± 11.4 | 28.2 ±59.3 |

Values are Mean ± S.D. of 4 Estimations of protein
Activity expressed in micro gm / mg tissue wt.
*Significant at $P < 0.05$ by t - test

**Table No.7**

**Acid Phosphatase activity in Gills of *Lamellidens marginalis* at different concentrations of Lead and Calcium for different exposure periods**

| Sr. No. | Abrreviation | Dose | 8 days | 16 days |
|---|---|---|---|---|
| 1 | N | Control | 97.50 ± 18.3 | 98.5 ± 19.3 |
| 2 | A | $Pb^{+}$ ( 28 ppm) | 177.5 ± 1.90* | 329.1 ± 3.570* |
| 3 | B | $Ca^{++}$ ( 28 ppm) | 95.8 ± 20.0 | 21.12 ± 1.54* |
| 4 | C | $Ca^{++}$ (56 ppm) | 37.4 ± 17.3 * | 21.87 ± 3.93* |
| 5 | D | $Ca^{++}$ (84 ppm) | 32.0 ± 17.3* | 28.7 ± 10.7* |
| 6 | E | $Pb^{+}$ 28 ppm + $Ca^{++}$ 28 ppm | 108.86 ± 9.95 | 32.13 ± 6.65 * |
| 7 | F | $Pb^{+}$ 28 ppm + $Ca^{++}$ 56 ppm | 85.4 ± 45.0 | 27.76 ± 8.88* |
| 8 | G | $Pb^{+}$ 28 ppm + $Ca^{++}$ 84 ppm | 74.4 ± 57.0 | 21.84 ± 1.86* |

Values are Mean ± S.D. of 4 Estimations
Activity expressed in mg/mg wt
* Significant at $P < 0.05$ by t - test

Table No.8

Alkaline Phosphatase activity in the Gills of *Lamellidens marginalis* at different concentrations of Lead and Calcium for different exposure periods

| Sr. No. | Abbreviations | Dose | 8 days | 16 days |
|---|---|---|---|---|
| 1 | N | Control | 89.5 ± 27.0 | 90.0 ±27.5 |
| 2 | A | $Pb^{+}$ ( 28 ppm) | 202.9 ±170.6 | 90.3 ±19.5 |
| 3 | B | $Ca^{++}$ ( 28 ppm) | 77.8 ± 16.3 | 27.15 ± 2.46* |
| 4 | C | $Ca^{++}$ (56 ppm) | 118.1 ± 55.6 | 27.20 ±6.03* |
| 5 | D | $Ca^{++}$ (84 ppm) | 88.5 ± 26.0 | 30.95 ±1.98* |
| 6 | E | $Pb^{+}$ 28 ppm + $Ca^{++}$ 28 ppm | 62.1 ± 37.6 | 38.18 ±6.35 * |
| 7 | F | $Pb^{+}$ 28 ppm + $Ca^{++}$ 56 ppm | 50.0 ± 4.90* | 52.9 ±12.2* |
| 8 | G | $Pb^{+}$ 28 ppm + $Ca^{++}$ 84 ppm | 80.0 ± 11.7 | 40.35 ±6.32* |

Values are mean ± S.D. of 4 Estimations
Activity expressed in mg Pi librated/hr/mg protein
* Significant at $P < 0.05$ by t - test

Table No.9

Protein contents in the mantle of Lamellidens *marginalis* at different concentrations of Lead and Calcium for different exposure periods

| Sr. No. | Abbreviations | Dose | 8 days | 16 days |
|---|---|---|---|---|
| 1 | N | Control | 14.56 ± 1.59 | 14.77 ± 1.710 |
| 2 | A | $Pb^{+}$ ( 28 ppm) | 10.41 ± 21.84* | 4.58 ± 1.6* |
| 3 | B | $Ca^{++}$ ( 28 ppm) | 40.82 ± 8.33 * | 31.91 ± 1.45 * |
| 4 | C | $Ca^{++}$ (56 ppm) | 9.08 ± 1.62 * | 28.30 ± 13.5 |
| 5 | D | $Ca^{++}$ (84 ppm) | 8.74 ± 1.59 * | 20.83 ± 7.39 |
| 6 | E | $Pb^{+}$ 28 ppm + $Ca^{++}$ 28 ppm | 16.74 ± 8.07 | 9.58 ± 2.85 * |
| 7 | F | $Pb^{+}$ 28 ppm + $Ca^{++}$ 56 ppm | 29.16 ± 8.77* | 17.91 ± 1.60* |
| 8 | G | $Pb^{+}$ 28 ppm + $Ca^{++}$ 84 ppm | 13.49 ± 2.73 | 13.04 ± 4.47 |

Values are Mean ± S.D. of 4 estimations of Protein
Activity expressed in micro gm/ mg wt.
* Significant at $P < 0.05$ by t - test

Table No. 10

Acid Phosphatase activity in the mantle of *Lamellidens marginalis* at different concentrations of Lead and Calcium for different exposure periods

| Sr. No. | Abbreviations | Dose | 8 days | 16 days |
|---|---|---|---|---|
| 1 | N | Control | 41.08 ± 9.0 | 41.23 ± 9.3 |
| 2 | A | $Pb^{+}$ ( 28 ppm) | 141.7 ±20.6* | 492.2 ±152.3* |
| 3 | B | $Ca^{++}$ ( 28 ppm) | 4.35 ± 1.04* | 26.5 ± 15.2 |
| 4 | C | $Ca^{++}$ (56 ppm) | 20.62 ± 8.33* | 14.7 ± 4.49 * |
| 5 | D | $Ca^{++}$ (84 ppm) | 12.70 ± 3.76* | 18.53 ± 7.16* |
| 6 | E | $Pb^{+}$ 28 ppm + $Ca^{++}$ 28ppm | 158.6 ± 31.5* | 18.49 ± 7.9* |
| 7 | F | $Pb^{+}$ 28 ppm + $Ca^{++}$ 56 ppm | 133.3 ±72.4 * | 38.9 ± 13.6 |
| 8 | G | $Pb^{+}$ 28 ppm + $Ca^{++}$ 84 ppm | 31.0 ±15.0 | 24.1 ± 23.0 |

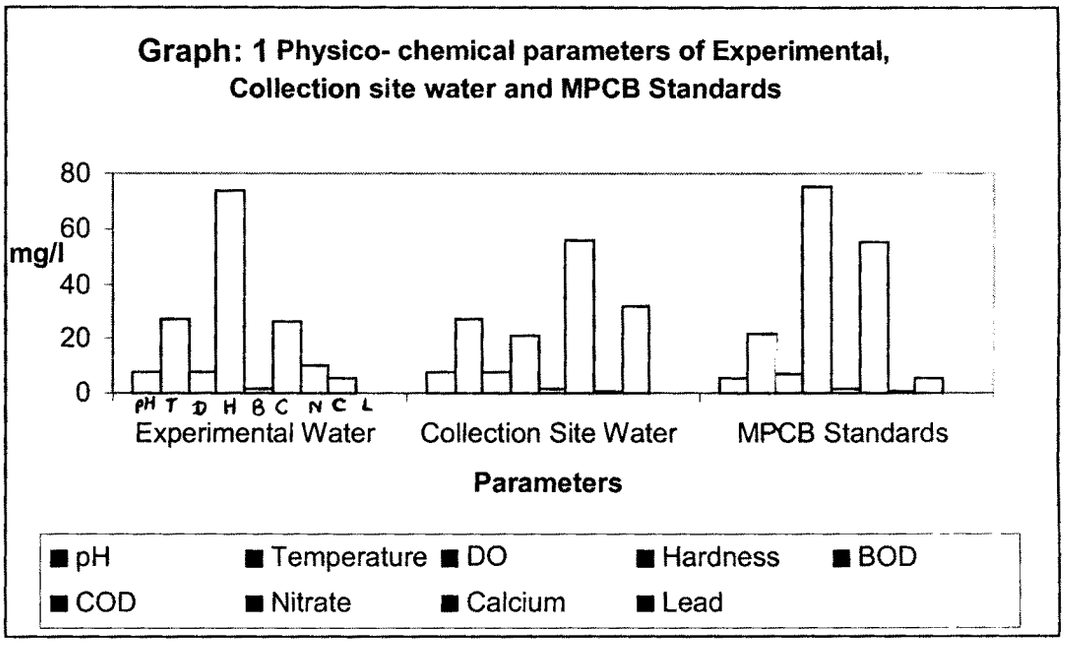
Values are Mean ± S.D. of 4 estimations
Activity expressed in mg Pi librated/hr/mg protein
* Significant at $P < 0.05$ by t - test

Table No. 11

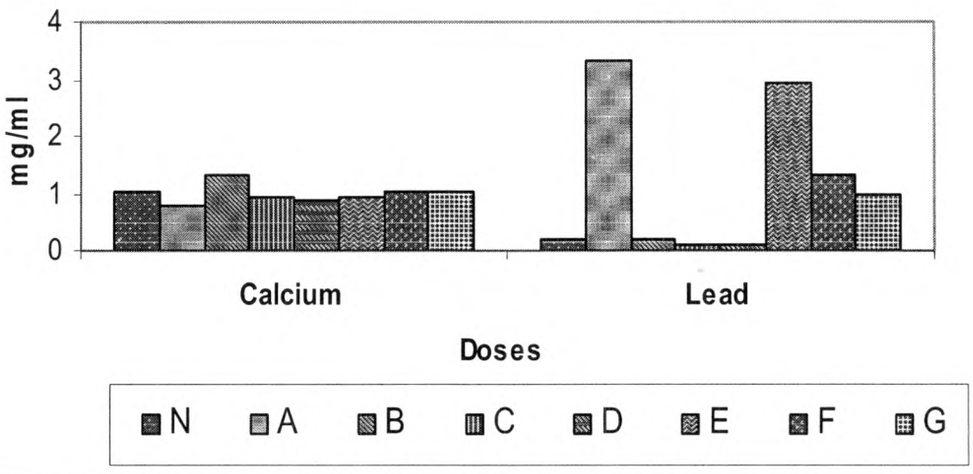
Alkaline Phosphatase activity in the Mantle of *Lamellidens marginalis* at different concentrations of Lead and Calcium for different exposure periods

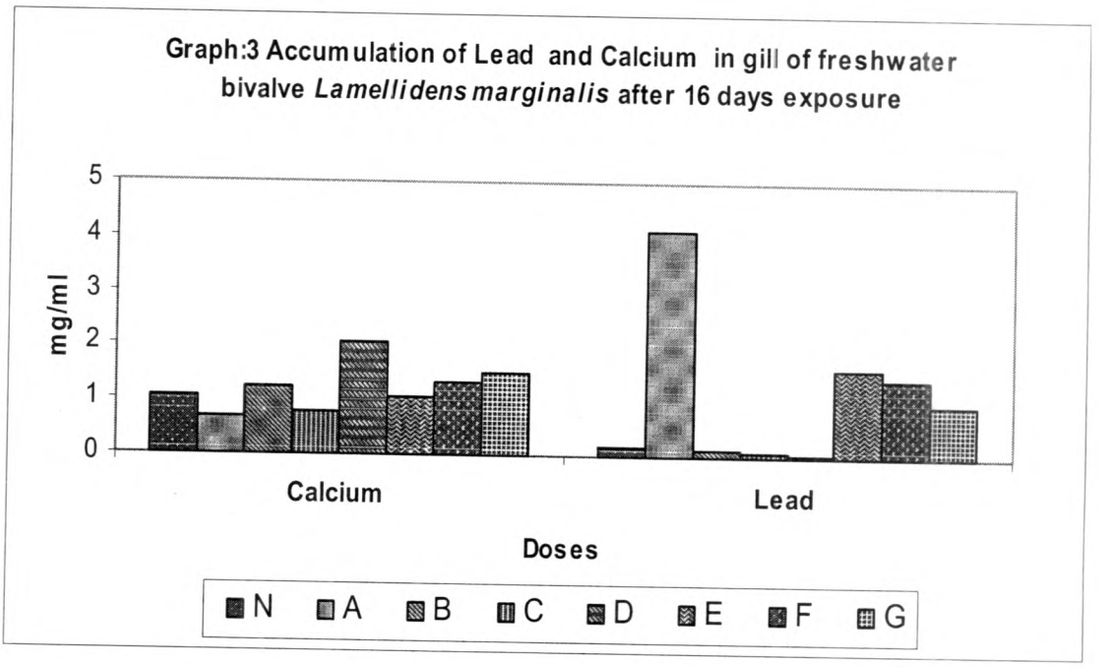
| Sr. No. | Abbreviations | Dose | 8 days | 16 days |
|---|---|---|---|---|
| 1 | N | Control | 53.1 ± 23.1 | 41.23 ±9.3 |
| 2 | A | Pb treated | 242.8 ± 141.5 | 68.1 ±23.2 |
| 3 | B | $Ca^{++}$ ( 28 ppm) | 36.6 ± 28.9 | 17.13 ±1.31* |
| 4 | C | $Ca^{++}$ (56 ppm) | 54.1 ± 5.12 | 4.850 ±0.975* |
| 5 | D | $Ca^{++}$ (84 ppm) | 79.6 ± 13.1* | 115.3 ±88.5 |
| 6 | E | $Pb^{+}$ 28 ppm + $Ca^{++}$ 28 ppm | 73.2 ± 59.5 * | 49.8 ±3.76 |
| 7 | F | $Pb^{+}$ 28 ppm + $Ca^{++}$ 56 ppm | 16.83 ± 7.26* | 112.3 ±49.4 |
| 8 | G | $Pb^{+}$ 28 ppm + $Ca^{++}$ 84 ppm | 77.9 ± 10.7 | 85.6 ±54.0 |

Values are Mean ± S.D. of 4 Estimations
Activity expressed in mg Pi librated/hr/mg protein
* Significant at $P < 0.05$ by t - test

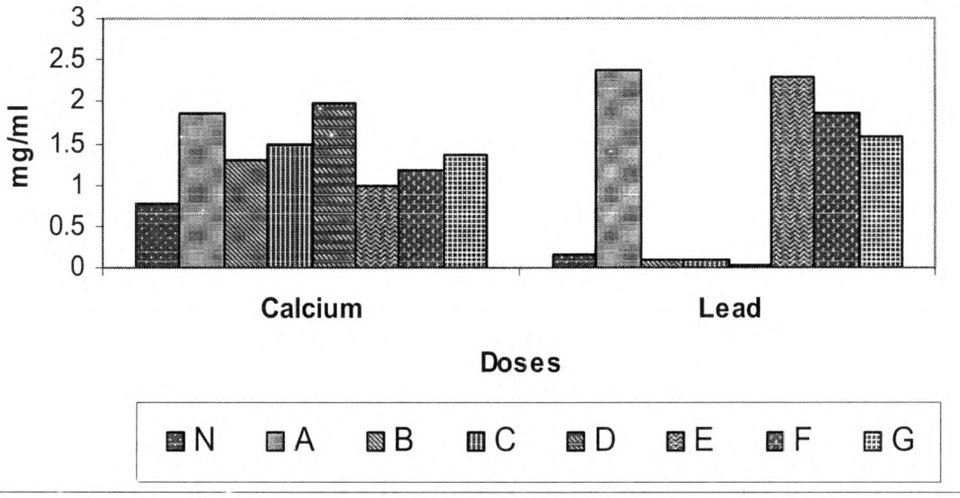

Graph: 1 Physico- chemical parameters of Experimental, Collection site water and MPCB Standards
80
60
40
20
0
mg/l
pH T D H B C N C L
Experimental Water
Collection Site Water
MPCB Standards
Parameters
pH
Temperature
DO
Hardness
BOD
COD
Nitrate
Calcium
Lead

**Graph: 2 Accumulation of Lead and Calcium in gill of freshwater bivalve *Lamellidens marginalis* after 8 days exposure.**

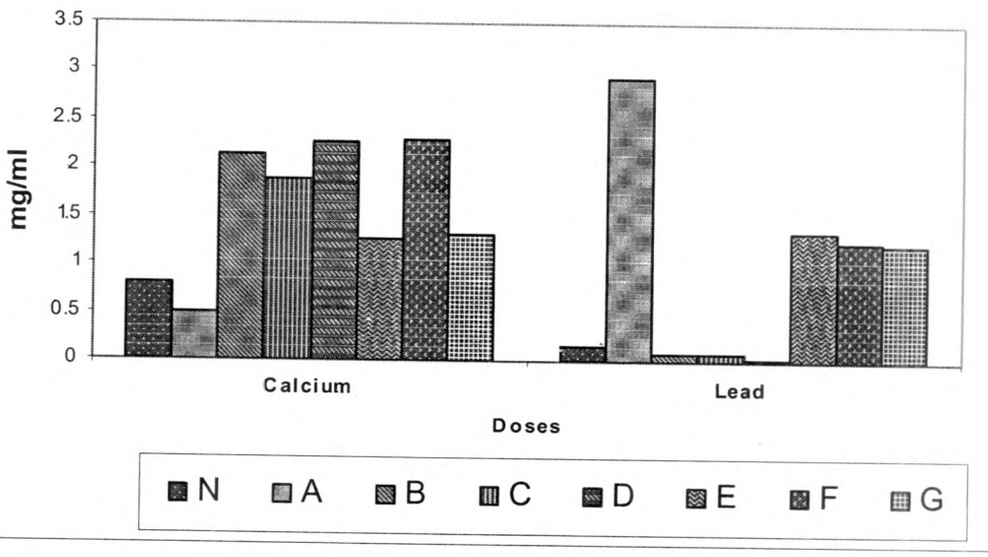



Graph:3 Accumulation of Lead and Calcium in gill of freshwater bivalve *Lamellidens marginalis* after 16 days exposure
mg/ml
5
4
3
2
1
0
Calcium
Lead
Doses
N
A
B
C
D
E
F
G

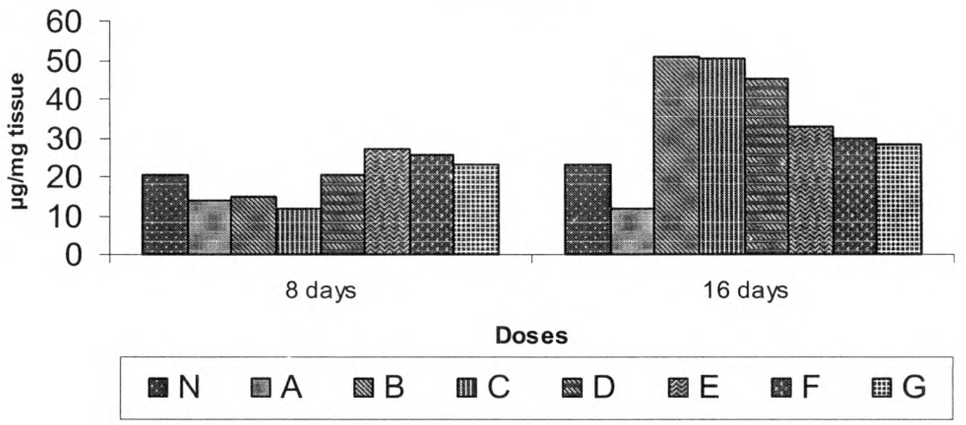
**Graph: 4** Accumulation of Lead and Calcium in mantle of freshwater bivalve *Lamellidens marginalis* after 8 days exposure.

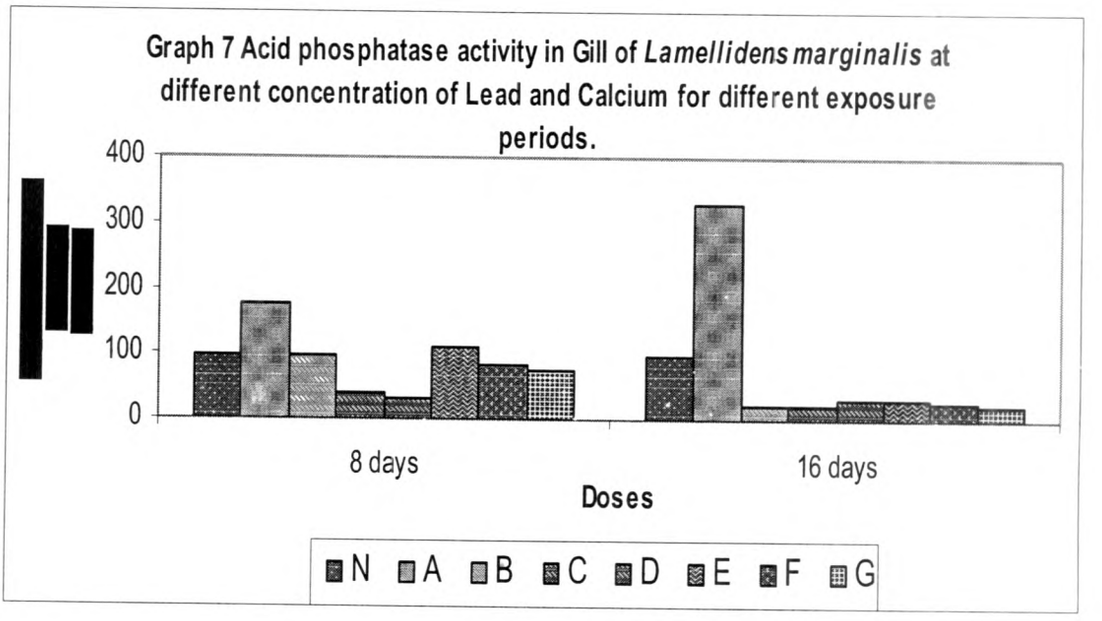


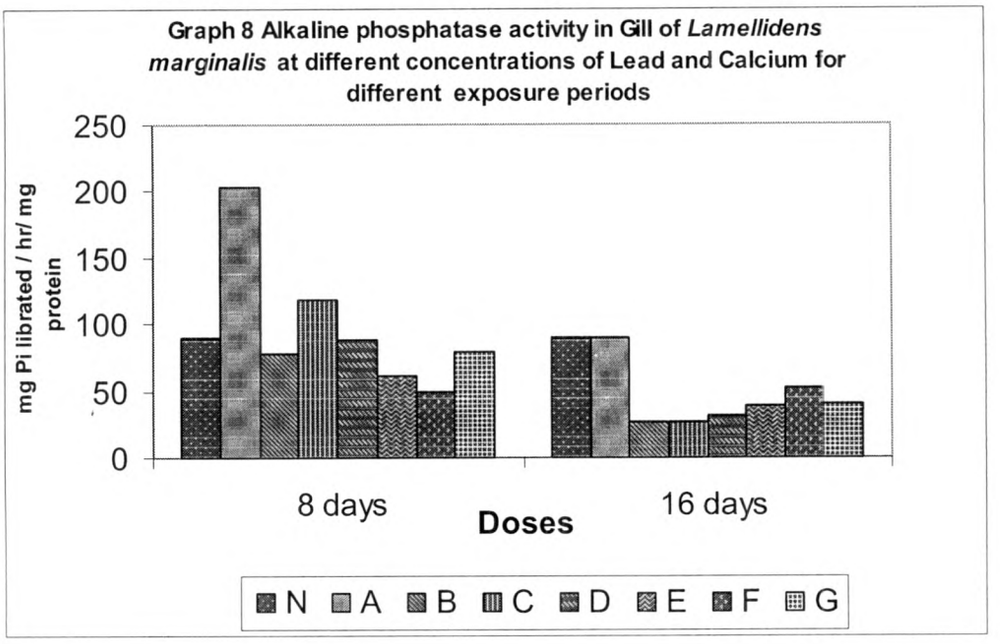
**Grapgh: 5 Accumulation of Lead and Calcium in mantle of freshwater bivalve *Lamellidens marginalis* after 16 days exposure**

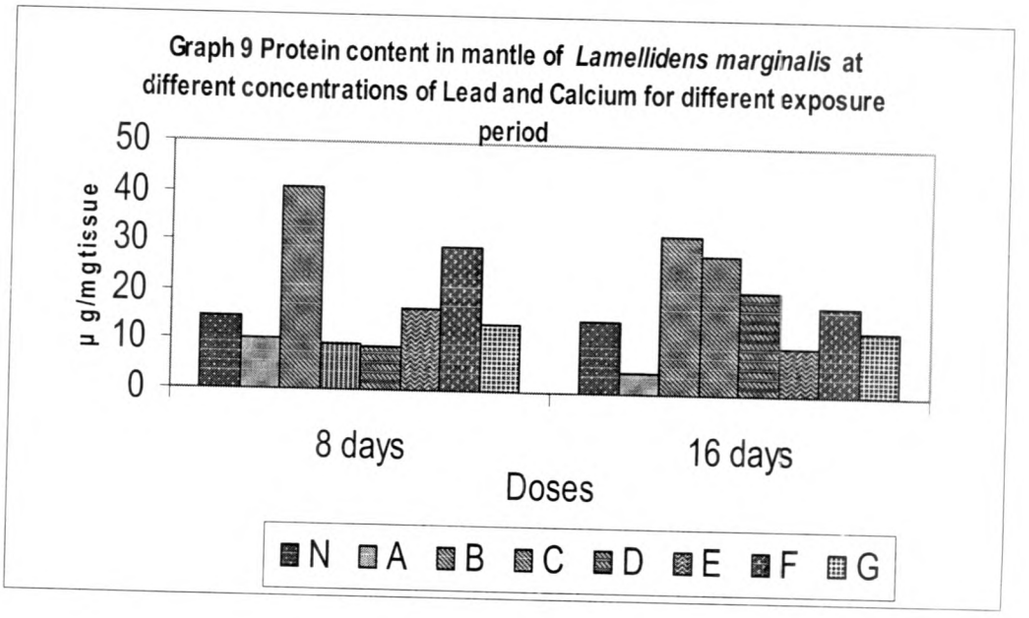


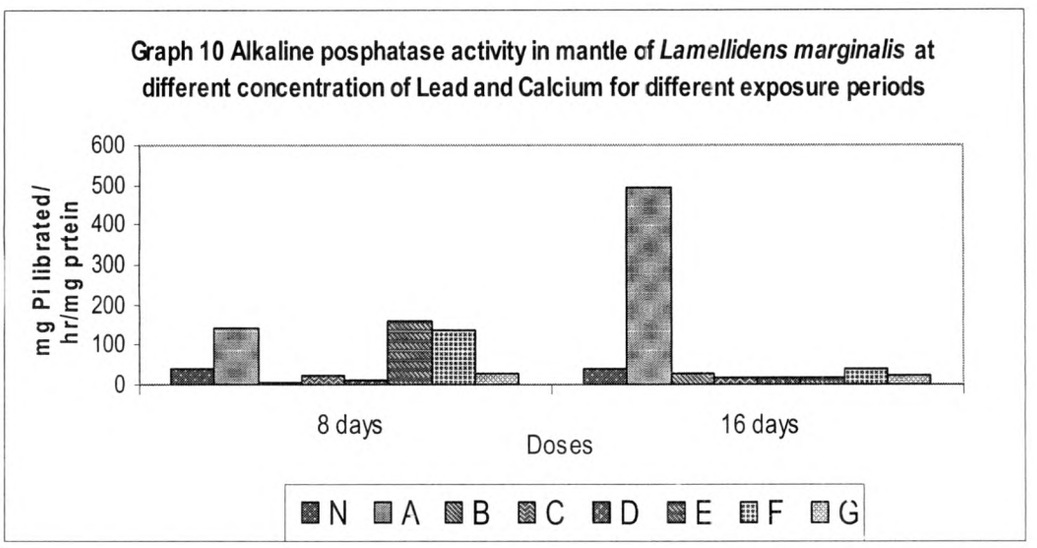
**Graph 6 Show Protein content in gill of *Lamellidens marginalis* at different concentrarion of Lead and Calcium for different exposure periods**




Graph 7 Acid phosphatase activity in Gill of *Lamellidens marginalis* at different concentration of Lead and Calcium for different exposure periods.
400
300
200
100
0
8 days
16 days
Doses
N A B C D E F G


Graph 8 Alkaline phosphatase activity in Gill of *Lamellidens marginalis* at different concentrations of Lead and Calcium for different exposure periods
mg Pi librated / hr/ mg protein
250
200
150
100
50
0
8 days
16 days
Doses
N A B C D E F G


Graph 9 Protein content in mantle of Lamellidens marginalis at different concentrations of Lead and Calcium for different exposure period
50
40
30
20
10
0
µ g/mgtissue
8 days
16 days
Doses
N A B C D E F G


Graph 10 Alkaline posphatase activity in mantle of *Lamellidens marginalis* at different concentration of Lead and Calcium for different exposure periods
mg Pi librated/ hr/mg prtein
600
500
400
300
200
100
0
8 days
16 days
Doses
N A B C D E F G

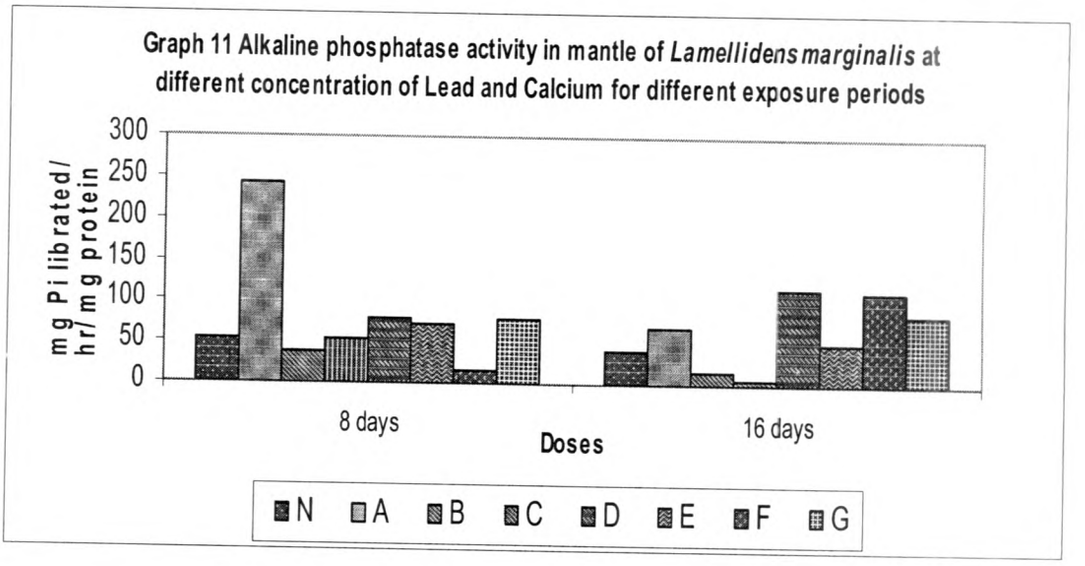
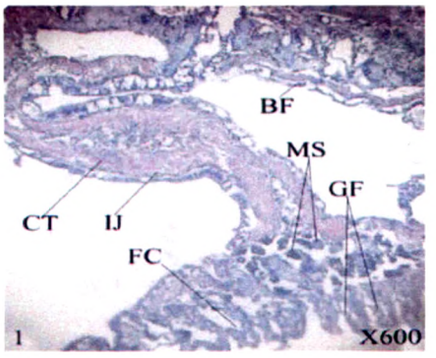

Graph 11 Alkaline phosphatase activity in mantle of *Lamellidens marginalis* at different concentration of Lead and Calcium for different exposure periods
mg Pi libratedl/ hr/ mg protein
300
250
200
150
100
50
0
8 days
16 days
Doses
N A B C D E F G

**PLATE 1**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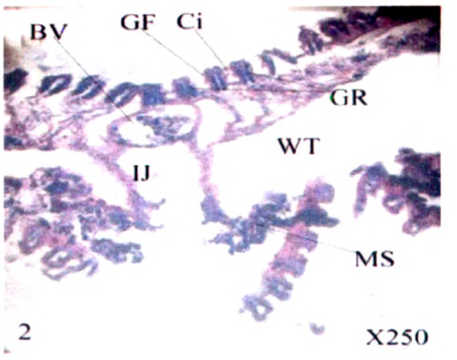
MS
GF
CT
IJ
FC
1
X600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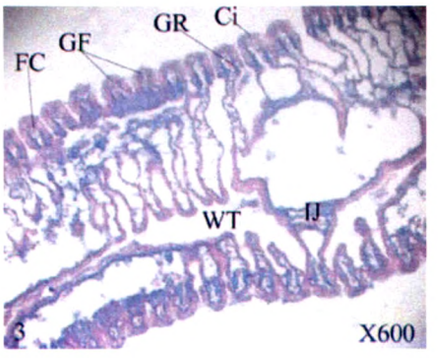
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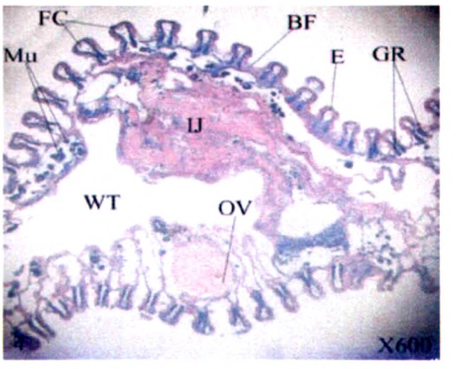
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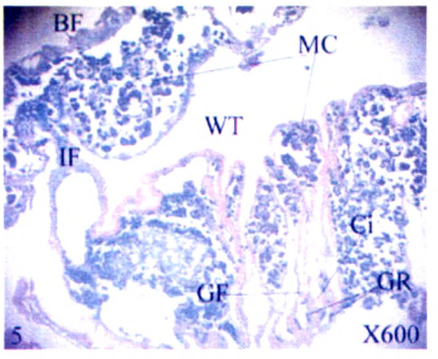
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GF
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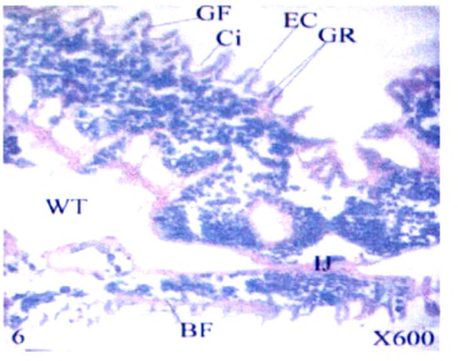
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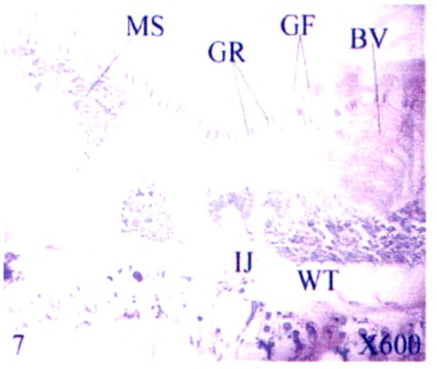
X600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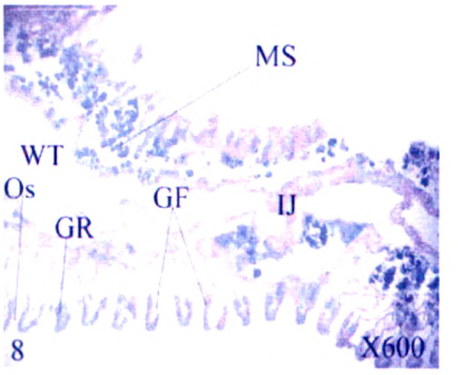
7
X600


MS
WT
Os
GF
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GR
8
X600

## PLATE 1

**Fig. 1:** Light microphotograph of gills of control bivalve, *Lamellidens marginalis* showing gill lamellae (GL), epithelial cells (E), cilia (Ci), basal filament (BF), lamellar cavity (LC), inter lamellar cavity (I), inter lamellar junction (LJ), water tubule (WT), Connective tissue(CT) and mucus secretion (MS). X600

**Fig. 2:** Light microphotograph of gills of bivalve, *Lamellidens marginalis* exposed to 28 ppm concentration of Pb for 8 days exposure period showing gill lamellae (GL), epithelial cells (E) with cilia (Ci), vacuolation in connective tissue (CT), basal filament (BF), increased lamellar cavity (LC) and inter lamellar cavity (I), water tubule (WT) with long obliquely placed inter lamellar junction (LJ) and mucus secretion (MS). X250

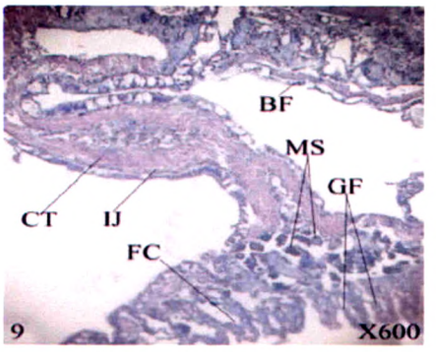
**Fig. 3, 4, 5:** Light microphotograph of gills of bivalve, *Lamellidens marginalis* exposed to 28ppm , 56 ppm and 84 ppm concentrations of $Ca^{++}$for 8 days exposure period showing gill lamellae (L), epithelial cells (E) with accumulation of dark material, cilia (Ci), connective tissue (CT), increased lamellar cavity (LC), ostia (Os), water tubule (WT), gill rod or bar(GR) and inter lamellar junction (LJ) X600

**Fig. 6:** Light microphotograph of gills of bivalve, *Lamellidens marginalis* exposed to 28ppm concentrations of $Ca^{++}$ with 28 ppm concentration of Pb for 8 days exposure period showing gill lamellae (L), epithelial cells (E) with accumulation of dark material, cilia (Ci), connective tissue (CT), increased lamellar cavity (LC), ostia (Os), water tubule (WT). gill rod or bar(GR) and inter lamellar junction (LJ) X600

**Fig. 7:** Light microphotograph of gills of bivalve, *Lamellidens marginalis* exposed to 56 ppm concentrations of $Ca^{++}$ with 28 ppm concentration of Pb for 8 days exposure period showing gill lamellae (L), epithelial cells (E) with accumulation of dark material, cilia (Ci), connective tissue (CT), increased lamellar cavity (LC), ostia (Os), water tubule (WT). gill rod or bar(GR) and inter lamellar junction (LJ) X600

**Fig. 8:** Light microphotograph of gills of bivalve, *Lamellidens marginalis* exposed to 84 ppm concentrations of $Ca^{++}$ with 28 ppm concentration of Pb for 8 days exposure period showing gill lamellae (L), epithelial cells (E) with accumulation of dark material, cilia (Ci), connective tissue (CT), increased lamellar cavity (LC), ostia (Os), water tubule (WT). gill rod or bar(GR) and inter lamellar junction (LJ) X600

**PLATE 2**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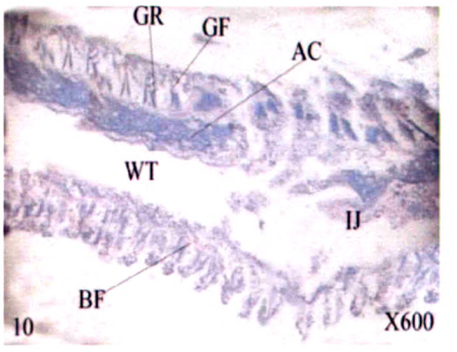
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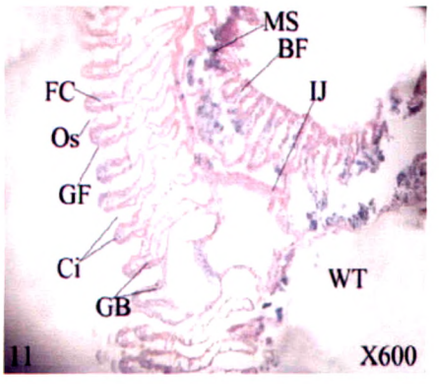
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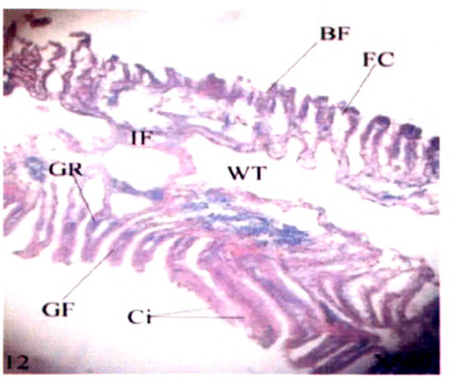
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WT
GF
Ci
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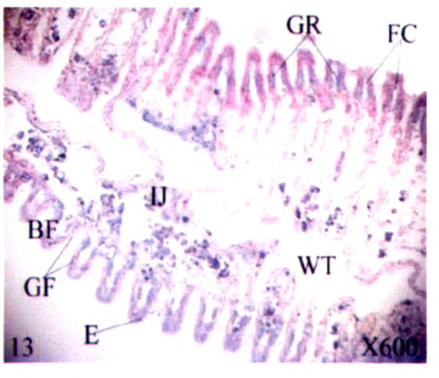
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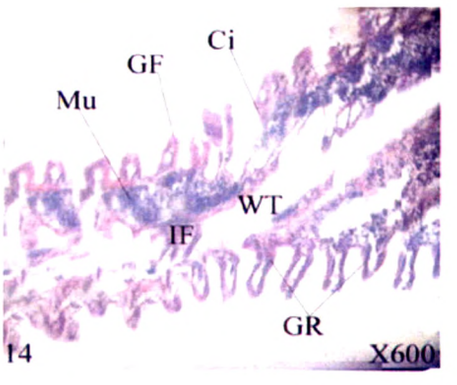
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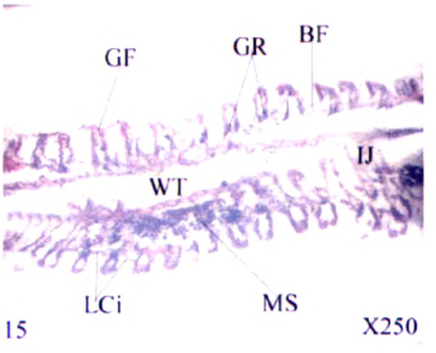
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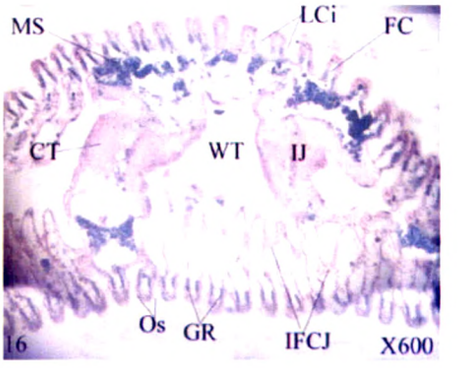
BF
IJ
WT
LCi
MS
15
X250


MS
LCi
FC
CT
WT
IJ
Os
GR
IFCJ
16
X600

## PLATE 2

**Fig. 9:** Light microphotograph of gills of control bivalve, *Lamellidens marginalis* showing gill lamellae (GL), epithelial cells (E), cilia (Ci), basal filament (BF), lamellar cavity (LC), inter lamellar cavity (I), inter lamellar junction (LJ), water tubule (WT), Connective tissue(CT) and mucus secretion (MS) X600

**Fig. 10:** Light microphotograph of gills of bivalve, *Lamellidens marginalis* exposed to 28 ppm concentration of Pb for 16 days exposure period showing gill lamellae (GL), epithelial cells (E) with cilia (Ci), vacuolation in connective tissue (CT), basal filament (BF), increased lamellar cavity (LC) and inter lamellar cavity (I), water tubule (WT) with long obliquely placed inter lamellar junction (LJ) and mucus secretion (MS) X600

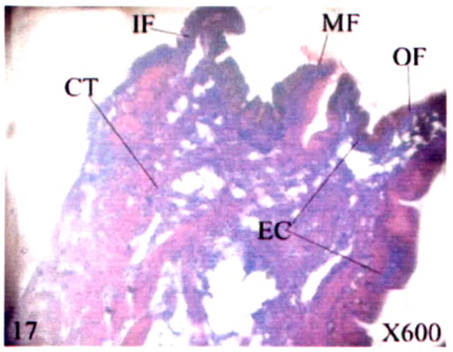
**Fig. 11,12, 13:** Light microphotograph of gills of bivalve, *Lamellidens marginalis* exposed to 28ppm , 56 ppm and 84 ppm concentrations of $Ca^{++}$for 16 days exposure period showing gill lamellae (L), epithelial cells (E) with accumulation of dark material, cilia (Ci), connective tissue (CT), increased lamellar cavity (LC), ostia (Os), water tubule (WT), gill rod or bar(GR) and inter lamellar junction (LJ) X600

**Fig. 14:** Light microphotograph of gills of bivalve, *Lamellidens marginalis* exposed to 28ppm concentrations of $Ca^{++}$ with 28 ppm concentration of Pb for 16 days exposure period showing gill lamellae (L), epithelial cells (E) with accumulation of dark material, cilia (Ci), connective tissue (CT), increased lamellar cavity (LC), ostia (Os), water tubule (WT). gill rod or bar(GR) and inter lamellar junction (LJ) X600

**Fig. 15:** Light microphotograph of gills of bivalve, *Lamellidens marginalis* exposed to 56 ppm concentrations of $Ca^{++}$ with 28 ppm concentration of Pb for 16 days exposure period showing gill lamellae (L), epithelial cells (E) with accumulation of dark material, cilia (Ci), connective tissue (CT), increased lamellar cavity (LC), ostia (Os), water tubule (WT), gill rod or bar(GR) and inter lamellar junction (LJ)), latral cilia(LCi) and mucus secretion (MS) X250

**Fig. 16:** Light microphotograph of gills of bivalve, *Lamellidens marginalis* exposed to 84 ppm concentrations of $Ca^{++}$ with 28 ppm concentration of Pb for 16 days exposure period showing gill lamellae (L), epithelial cells (E) with accumulation of dark material, cilia (Ci), connective tissue (CT), increased lamellar cavity (LC), ostia (Os), water tubule (WT). gill rod or bar(GR) and inter lamellar junction (LJ), latral cilia(LCi) and inter filamentary ciliary junction(IFCJ) X600

**PLATE 3**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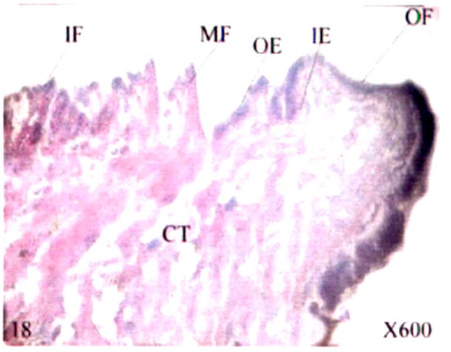
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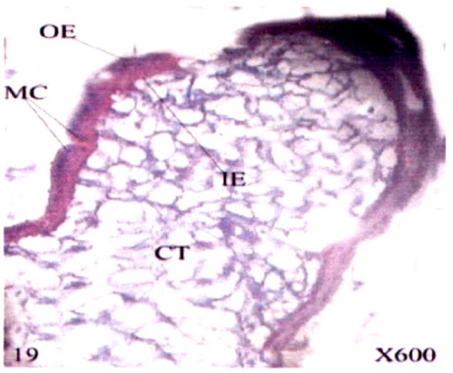
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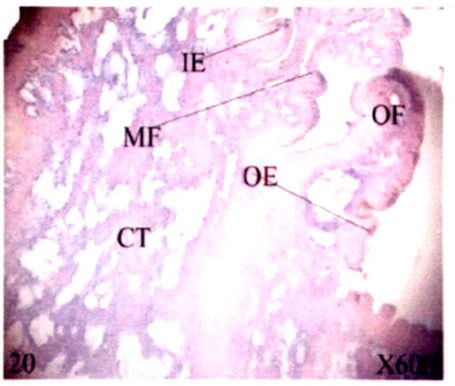
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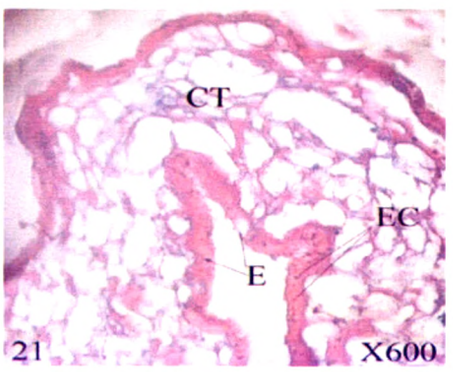
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EC
E
21
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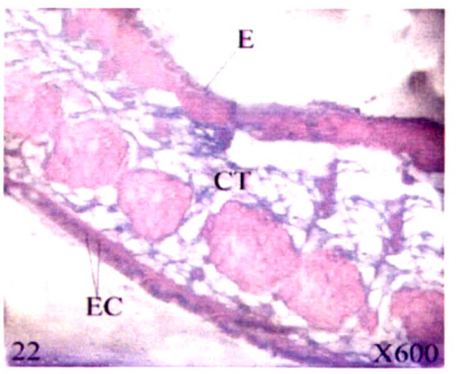
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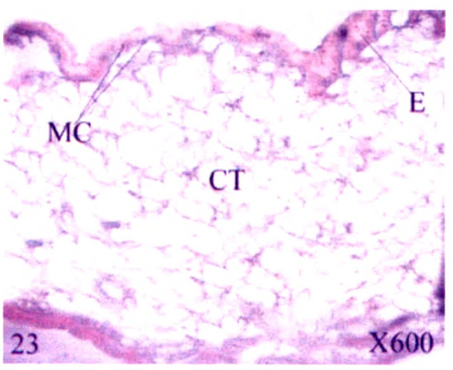
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MC
CT
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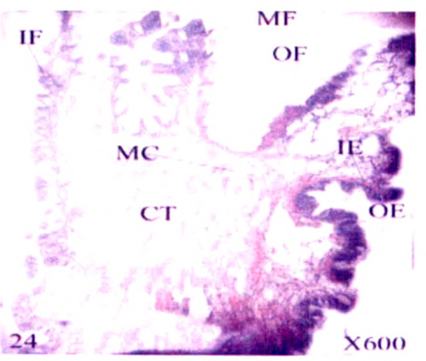

MF
IF
OF
MC
IE
CT
OE
24
X600

## PLATE 3

**Fig. 17:** Light microphotograph of mantle of control bivalve, *Lamellidens marginalis* showing middle filament (MF), outer filament (OF), inner filament (IF) epithelial cells (EC), connective tissue (CT) X600

**Fig. 18:** Light microphotograph of mantle of bivalve, *Lamellidens marginalis* exposed to 28 ppm concentration of Pb for 8 days exposure period showing middle filament (MF), outer filament (OF), inner filament (IF) epithelial cells (EC), vacuolation in connective tissue (CT) X600

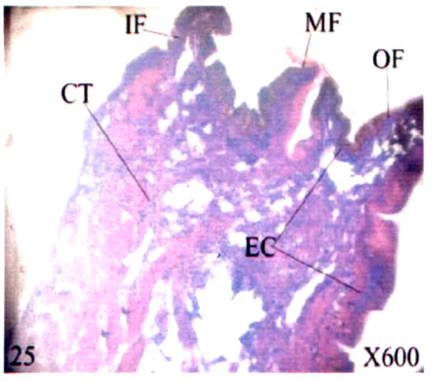
**Fig. 19, 20, 21:** Light microphotograph of mantle of bivalve, *Lamellidens marginalis* exposed to 28ppm, 56 ppm and 84 ppm concentrations of $Ca^{++}$ for 8 days exposure period showing central portion of mantle with epithelial cells (EC), inner epithelial cells (IE), outer epithelial cells (OE), connective tissue (CT) X600

**Fig. 22:** Light microphotograph of mantle of bivalve, *Lamellidens marginalis* exposed to 28ppm concentrations of $Ca^{++}$ with 28 ppm concentration of Pb for 8 days exposure period showing central portion of mantle with epithelial cells (EC), inner epithelial cells (IE), outer epithelial cells (OE), connective tissue (CT) X600

**Fig. 23:** Light microphotograph of mantle of bivalve, *Lamellidens marginalis* exposed to 56 ppm concentrations of $Ca^{++}$ with 28 ppm concentration of Pb for 8 days exposure period showing central portion of mantle with epithelial cells (EC), inner epithelial cells (IE), outer epithelial cells (OE), connective tissue (CT) X600

**Fig. 24:** Light microphotograph of mantle of bivalve, *Lamellidens marginalis* exposed to 84 ppm concentrations of $Ca^{++}$ with 28 ppm concentration of Pb for 8 days exposure period showing middle filament (MF), outer filament (OF), inner filament (IF) epithelial cells (EC), connective tissue (CT) X600

**PLATE 4**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CT
EC
25
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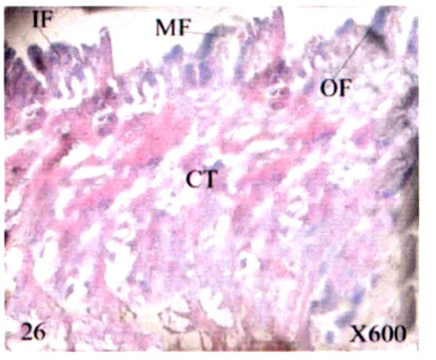
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OF
CT
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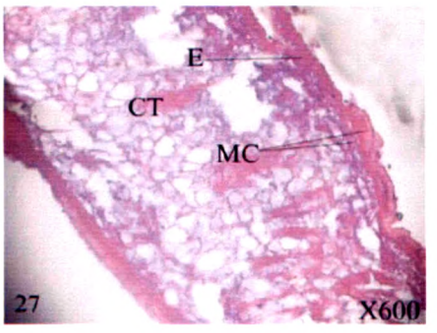
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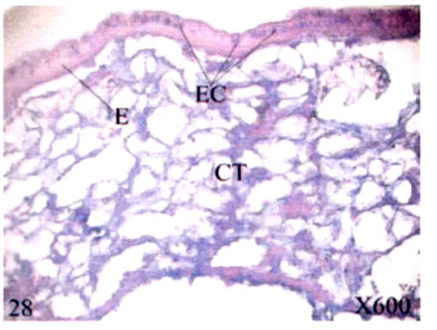
CT
MC
27
X600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CT
28
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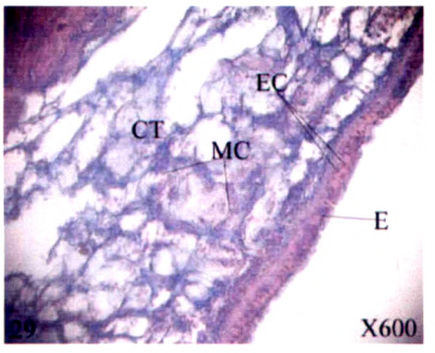
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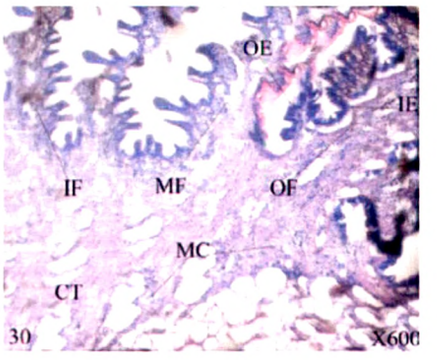
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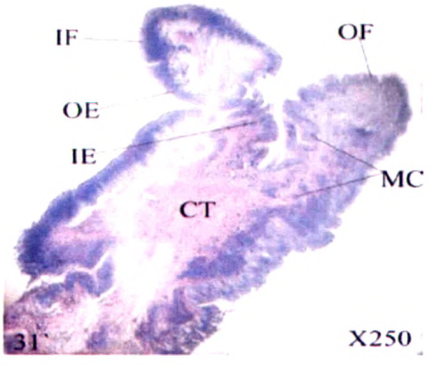
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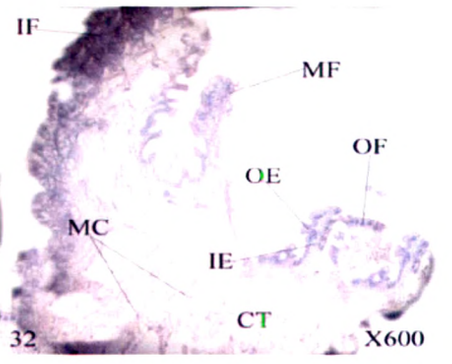
X250


IF
MF
OF
OE
MC
IE
CT
32
X600

## PLATE 4

**Fig. 25:** Light microphotograph of mantle of control bivalve, *Lamellidens marginalis* showing middle filament (MF), outer filament (OF), inner filament (IF) epithelial cells (EC), connective tissue (CT) X600

**Fig. 26:** Light microphotograph of mantle of bivalve, *Lamellidens marginalis* exposed to 28 ppm concentration of Pb for 16 days exposure period showing middle filament (MF), outer filament (OF), inner filament (IF) epithelial cells (EC), vacuolation in connective tissue (CT) X600

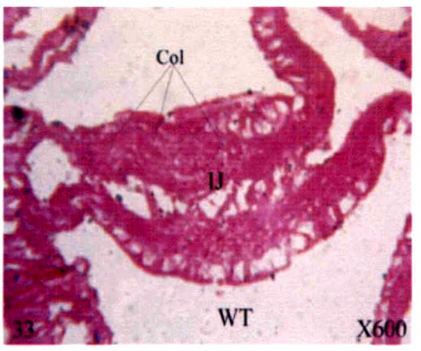
**Fig. 27, 28, 29:** Light microphotograph of mantle of bivalve, *Lamellidens marginalis* exposed to 28ppm, 56 ppm and 84 ppm concentrations of $Ca^{++}$ for 16 days exposure period showing central portion of mantle with epithelial cells (EC), inner epithelial cells (IE), outer epithelial cells (OE), connective tissue (CT) X600

**Fig. 30:** Light microphotograph of mantle of bivalve, *Lamellidens marginalis* exposed to 28ppm concentrations of $Ca^{++}$ with 28 ppm concentration of Pb for 16 days exposure period showing with middle filament (MF), outer filament (OF), inner filament (IF) epithelial cells (EC), vacuolation in connective tissue (CT). epithelial cells (E), inner epithelial cells (IE), outer epithelial cells (OE), connective tissue (CT) and mucus cells(MC) X600

**Fig. 31:** Light microphotograph of mantle of bivalve, *Lamellidens marginalis* exposed to 56 ppm concentrations of $Ca^{++}$ with 28 ppm concentration of Pb for 16 days exposure period showing middle filament (MF), outer filament (OF), inner filament (IF) epithelial cells (EC), vacuolation in connective tissue (CT). epithelial cells (E), inner epithelial cells (IE), outer epithelial cells (OE), connective tissue (CT) and mucus cells(MC) X250

**Fig. 32:** Light microphotograph of mantle of bivalve, *Lamellidens marginalis* exposed to 84 ppm concentrations of $Ca^{++}$ with 28 ppm concentration of Pb for 16 days exposure period showing middle filament (MF), outer filament (OF), inner filament (IF) epithelial cells (EC), vacuolation in connective tissue (CT). epithelial cells (E), inner epithelial cells (IE), outer epithelial cells (OE), connective tissue (CT) and mucus cells(MC) X600

**PLATE 5**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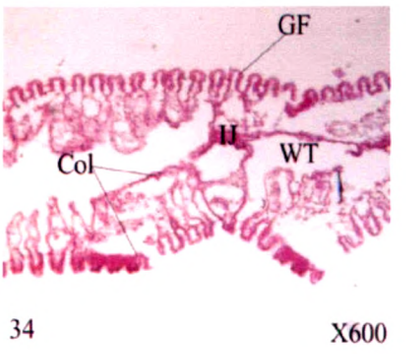
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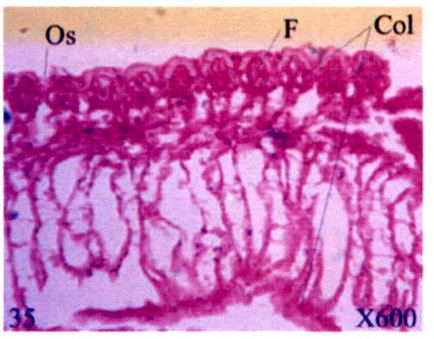
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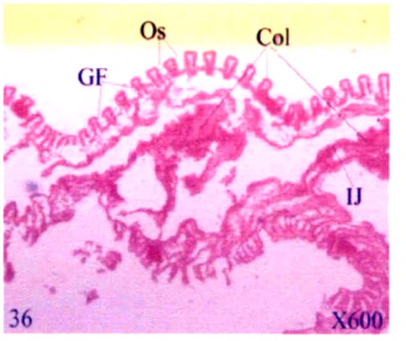
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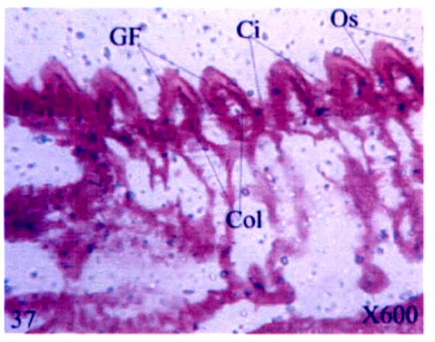
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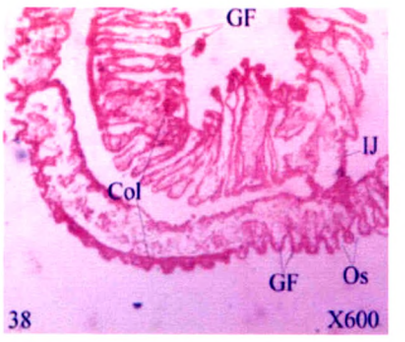
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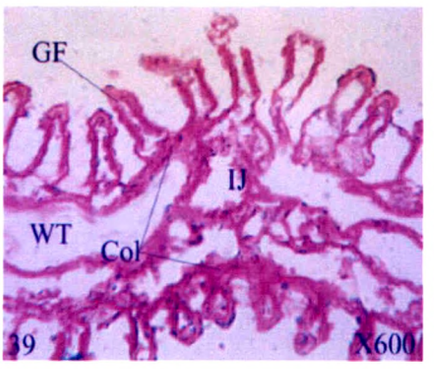
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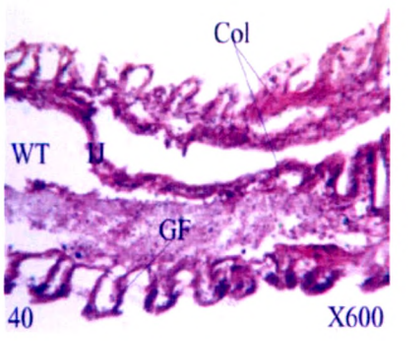

Col
WT
IJ
GF
40
X600

## PLATE 5

**Fig. 33:** Light microphotograph of gills of control bivalve, *Lamellidens marginalis* showing inter lamellar junction (IJ), water tubule (WT) and collagen(Col) X600

**Fig. 34:** Light microphotograph of gills of bivalve, *Lamellidens marginalis* exposed to 28 ppm concentration of Pb for 8 days exposure period showing water tubule (WT), inter lamellar junction (IJ), gill filament (GF) and collagen (Col) X600

**Fig. 35, 36,37:** Light microphotograph of gills of bivalve, *Lamellidens marginalis* exposed to 28ppm , 56 ppm and 84 ppm concentrations of $Ca^{++}$for 8 days exposure period showing inter lamellar junction (IJ), ostia (Os), cilia (Ci), gill filament (GF) and collagen(Col) X600

**Fig. 38:** Light microphotograph of gills of bivalve, *Lamellidens marginalis* exposed to 28ppm concentrations of $Ca^{++}$ with 28 ppm concentration of Pb for 8 days exposure period showing inter lamellar junction (IJ), water tubule (WT), ostia (Os), cilia (Ci), gill filament (GF) and collagen(Col) X600

**Fig. 39:** Light microphotograph of gills of bivalve, *Lamellidens marginalis* exposed to 56 ppm concentrations of $Ca^{++}$ with 28 ppm concentration of Pb for 8 days exposure period showing inter lamellar junction (IJ), water tubule (WT), ostia (Os), cilia (Ci), gill filament (GF) and collagen(Col) X600

**Fig. 40:** Light microphotograph of gills of bivalve, *Lamellidens marginalis* exposed to 84 ppm concentrations of $Ca^{++}$ with 28 ppm concentration of Pb for 8 days exposure period showing inter lamellar junction (IJ), water tubule (WT), ostia (Os), cilia (Ci), gill filament (GF) and collagen(Col) X600

## PLATE 6

**Fig. 41:** Light microphotograph of gills of control bivalve, *Lamellidens marginalis* showing inter lamellar junction (IJ), water tubule (WT), basal filament (BF), cilia (Ci) and collagen(Col) X250

**Fig. 42:** Light microphotograph of gills of bivalve, *Lamellidens marginalis* exposed to 28 ppm concentration of Pb for 16 days exposure period showing gill filament (GF) and collagen (Col) X600

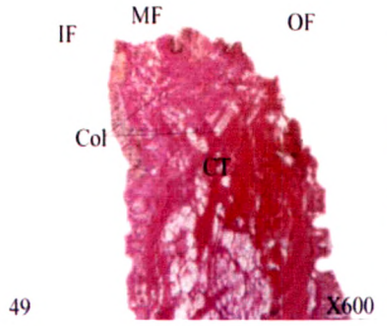
**Fig. 43, 44, 45:** Light microphotograph of gills of bivalve, *Lamellidens marginalis* exposed to 28ppm , 56 ppm and 84 ppm concentrations of $Ca^{++}$for 16 days exposure period showing inter lamellar junction (ILJ or IJ), ostia (Os), cilia (Ci), gill filament (GF), calcium (Ca) and high amount of collagen(Col) X600

**Fig. 46:** Light microphotograph of gills of bivalve, *Lamellidens marginalis* exposed to 28ppm concentrations of $Ca^{++}$ with 28 ppm concentration of Pb for 16 days exposure period showing inter gill filament (GF) and collagen (Col) X600

**Fig. 47:** Light microphotograph of gills of bivalve, *Lamellidens marginalis* exposed to 56 ppm concentrations of $Ca^{++}$ with 28 ppm concentration of Pb for 16 days exposure period showing inter lamellar junction (IJ), water tubule (WT), gill filament (GF) and collagen(Col) X600

**Fig. 48:** Light microphotograph of gills of bivalve, *Lamellidens marginalis* exposed to 84 ppm concentrations of $Ca^{++}$ with 28 ppm concentration of Pb for 16 days exposure period showing inter lamellar junction (LJ), water tubule (WT), gill filament (GF) and collagen(Col) X600

**PLATE 7**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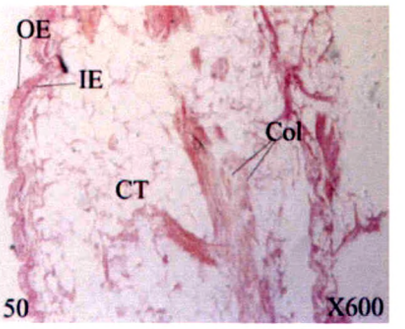
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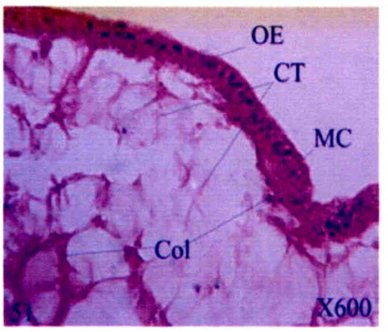
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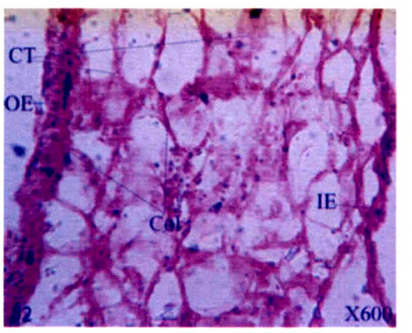
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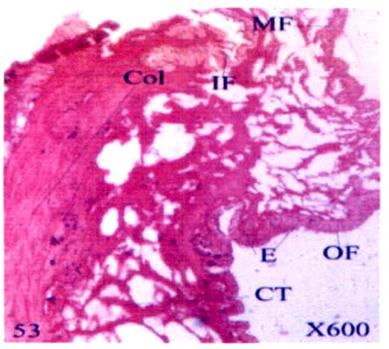
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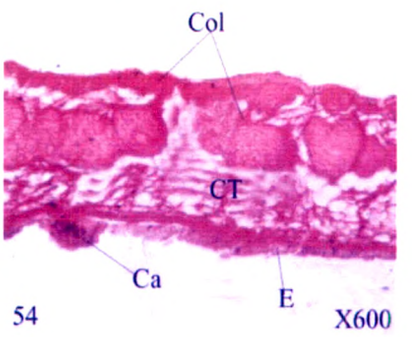
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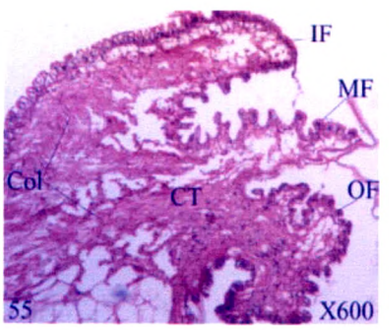
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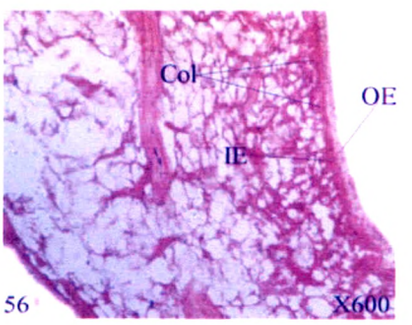

Col
OE
IE
56
X600

## PLATE 7

**Fig. 49:** Light microphotograph of mantle of control bivalve, *Lamellidens marginalis* showing middle fold (MF), outer fold (OF), inner fold (IF) epithelial cells (EC), connective tissue (CT) and collagen (Col) X600

**Fig. 50:** Light microphotograph of mantle of bivalve, *Lamellidens marginalis* exposed to 28 ppm concentration of Pb for 8 days exposure period showing outer epithelial cells (OE), inner epithelial cells (IE), epithelial cells (EC), connective tissue (CT) and less amount of collagen (Col) X600

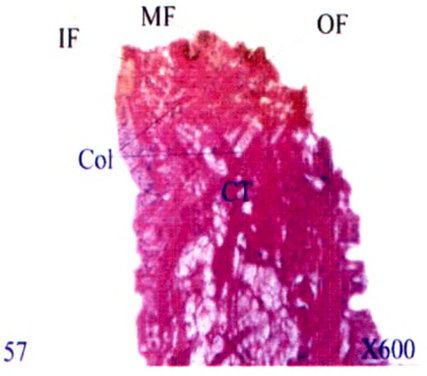
**Fig. 51, 52, 53:** Light microphotograph of mantle of bivalve, *Lamellidens marginalis* exposed to 28ppm, 56 ppm and 84 ppm concentrations of $Ca^{++}$ for 8 days exposure period showing central portion of mantle with epithelial cells (EC), inner epithelial cells (IE), outer epithellium (OE), connective tissue (CT), mucus cells (MC) and high amount of collagen (Col) X600

**Fig. 54:** Light microphotograph of mantle of bivalve, *Lamellidens marginalis* exposed to 28ppm concentrations of $Ca^{++}$ with 28 ppm concentration of Pb for 8 days exposure period showing central portion of mantle with epithelium (E), connective tissue (CT), calcium (Ca) and collagen (Col) X600

**Fig. 55:** Light microphotograph of mantle of bivalve, *Lamellidens marginalis* exposed to 56 ppm concentrations of $Ca^{++}$ with 28 ppm concentration of Pb for 8 days exposure period showing middle fold (MF), outer fold (OF), inner fold (IF), connective tissue (CT) and collagen (Col) X600

**Fig. 56:** Light microphotograph of mantle of bivalve, *Lamellidens marginalis* exposed to 84 ppm concentrations of $Ca^{++}$ with 28 ppm concentration of Pb for 8 days exposure period showing central portion of mantle with outer epithelium (OE), inner epithelium (IE), connective tissue (CT) and collagen (Col) X600

**PLATE 8**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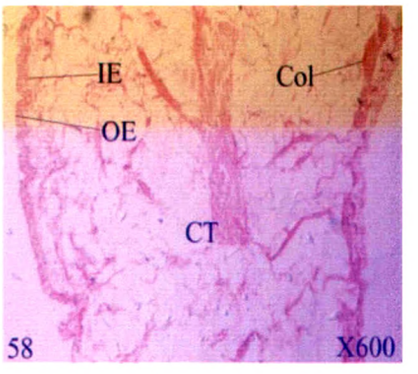
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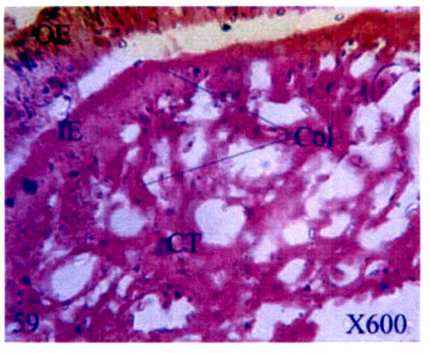
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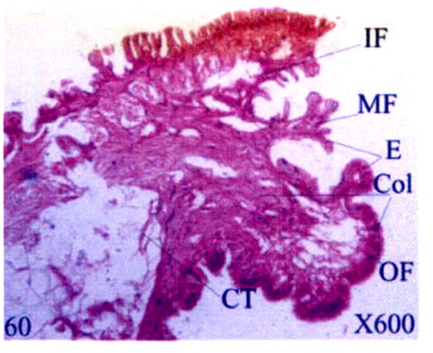
CT
59
X600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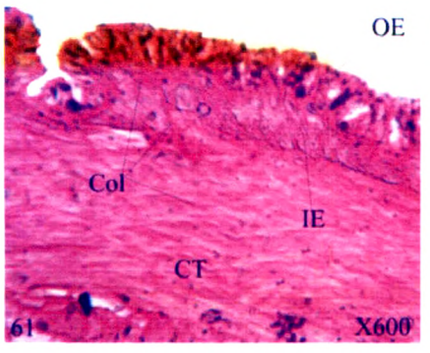
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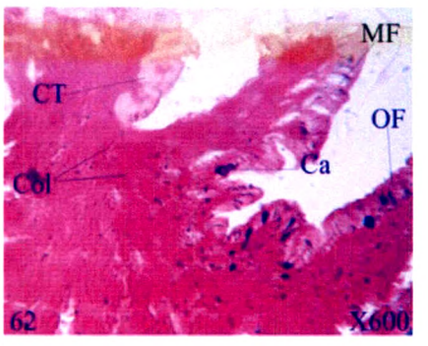
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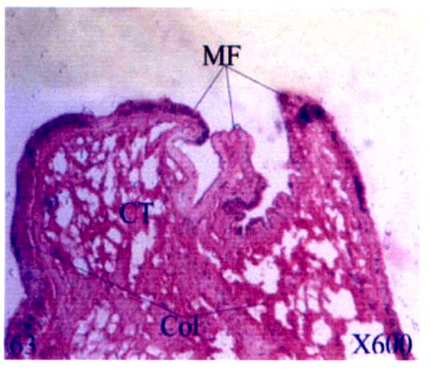
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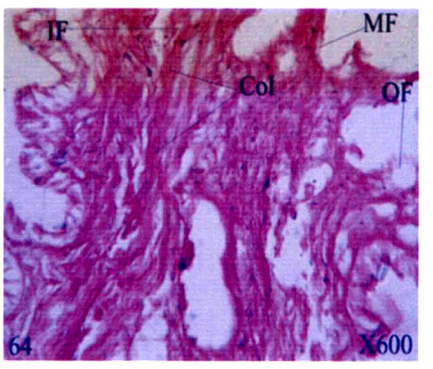
IF
MF
Col
OF
64
X600

## PLATE 8

**Fig. 57:** Light microphotograph of mantle of control bivalve, *Lamellidens marginalis* showing middle fold (MF), outer fold (OF), inner fold (IF) epithelial cells (EC), connective tissue (CT) and collagen (Col) X600

**Fig. 58:** Light microphotograph of mantle of bivalve, *Lamellidens marginalis* exposed to 28 ppm concentration of Pb for 16 days exposure period showing outer epithelium (OE), inner epithelium (IE), epithelial cells (EC), connective tissue (CT) and less amount of collagen (Col) X600

**Fig. 59, 60, 61:** Light microphotograph of mantle of bivalve, *Lamellidens marginalis* exposed to 28ppm, 56 ppm and 84 ppm concentrations of $Ca^{++}$ for 16 days exposure period showing central portion of mantle with epithelium (EC), inner epithelium (IE), outer epithelium (OE), middle fold (MF), outer fold (OF), inner fold (IF), connective tissue (CT) and high amount of collagen (Col) X600

**Fig. 62:** Light microphotograph of mantle of bivalve, *Lamellidens marginalis* exposed to 28ppm concentrations of $Ca^{++}$ with 28 ppm concentration of Pb for 16 days exposure period showing central portion of mantle with epithelium (E), connective tissue (CT),middle fold (MF), outer fold (OF), calcium (Ca) and collagen (Col) X600

**Fig. 63:** Light microphotograph of mantle of bivalve, *Lamellidens marginalis* exposed to 56 ppm concentrations of $Ca^{++}$ with 28 ppm concentration of Pb for 16 days exposure period showing middle fold (MF), outer fold (OF), inner fold (IF), connective tissue (CT) and collagen (Col) X600

**Fig. 64:** Light microphotograph of mantle of bivalve, *Lamellidens marginalis* exposed to 84 ppm concentrations of $Ca^{++}$ with 28 ppm concentration of Pb for 16 days exposure period showing central portion of mantle with connective tissue (CT), middle fold (MF), outer fold (OF), inner fold (IF),and collagen (Col) X600