OBSERVATION

CHAPTER III

The chapter deals with the observations found through out the experimental work and include observations of site from where tannery wastewater was collected, characteristics of tannery wastewater, animals response to tannery wastewater, metal accumulation, acute and subacute toxicity tests, biochemical and enzymological analysis, AAS and histopathological logical study.

3.1 Tannery industry

As tannery wastewater was collected from the small scale, household vegetable tannery industry located in Jawahar nagar of Kolhapur city. In the past there were many tanneries in Kolhapur city but with decentralization of industry of nearby places, today there is much decrease in the number of tannery industries. The wastewater from these tanneries having highly disagreeable odour, dark brown in color and the wastewater generated is released through the drainage pipes without any treatment, which gets mixed into Jayanti nalla which further joins Panchganga river.

3.2 Characteristics of tannery wastewater

The parameters analyzed were pH, turbidity, total suspended solids, total dissolved solids, total solids, chloride, hardness, chemical oxygen demand, oil and grease, calcium, magnesium and chromium.

3.2.1 pH (Table 1 and Graph 2)

Tannery wastewater was acidic in nature with pH 4.17 whereas the permissible limit of MPCB for pH is 5.5 to 9.0 in case of wastewater.

3.2.2 Turbidity (Table 1 and Graph 2)

Turbidity of tannery wastewater was 144 NTU, which is very high as compared to the permissible limit that is 5NTU.

3.2.3 Total Solids (Table 1 and Graph 1)

Total solids found in tannery wastewater were (44090 mg/lit) which were very high as compared to the permissible limit (2100 mg/lit).

3.2.4 Total Suspended Solids (Table 1 and Graph 1)

Total suspended solids found in tannery wastewater were 2062 mg/lit which is also very high as compared to permissible limit (600 mg/lit).

3.2.5 Total Dissolved Solids (Table 1 and Graph 1)

Total dissolved solids found in tannery wastewater were 42028 mg/lit, while the standard limit of MPCB for total dissolved solids is 2100 mg/lit.

3.2.6 Chlorides (Table 1 and Graph 1)

Chlorides in tannery wastewater were 3685 mg/lit that are very high as compared to standard limit (600 mg/lit) for tannery wastewater.

3.2.7 Chemical Oxygen Demand (Table 1 and Graph 1)

Chemical oxygen demand of tannery wastewater was 36320 mg/lit while the permissible limit of MPCB for it is 250 mg/lit.

3.2.8 Oil and Grease (Table 1 and Graph 1)

Oil and grease contents in tannery wastewater were 0.823 mg/lit. The permissible limit of MPCB for the oil and grease is 10 mg/lit.

3.2.9 Chromium (Table 1 and Graph 1)

The chromium contents in tannery wastewater were 0.563 mg/lit. The permissible limit of MPCB for the chromium is 2 mg/lit in case of vegetable tanning industry.

3.3 Bivalve behavior

The acclimatized bivalves when exposed to tannery wastewater with different concentrations of effluent showed some changes in their behavior with respect to doses. Initially bivalves did not respond to the dose at very first day of exposure, but from second day bivalves started giving response to the dose. The bivalves were completely suffocated and did not show any response to touch. The mortality was evident because of the absence of any motion or respiration

since shells were closed tightly. After the death of bivalves shell valves remained open. There was abundant discharge of mucus through the shells.

3.4 Acute Toxicity

In the acute toxicity test bivalves showed mortality at different concentrations of tannery wastewater. The acute toxicity study was carried out for 96 hours and the mortality was recorded after every 24 hours. LC_0 and LC_{50} values of tannery wastewater estimated were 5% and 20% respectively. The LC_0 is the lethal concentration at which animal does not show mortality while LC_{50} is the lethal concentration at which 50% animal's shows mortality. Dead bivalves were removed from the tank. The concentration of tannery wastewater was maintained by replacing wastewater after every 24 hrs.

3.5 Percentage mortality of the bivalve, *Lamellidens marginalis* exposed to different concentrations of tannery wastewater at different time intervals.

Concentration %	24hrs	48hrs	72hrs	96hrs	Total Mortality(%)
1	-	-	-	-	-
2	-	-	-	-	-
5	-	-	-	-	-
10	-	-	-	10%	10%
15	-	-	10%	10%	20%
20	-	10%	20%	20%	50%
25	-	10%	20%	30%	60%
30	10%	10%	20%	40%	80%
40	10%	10%	30%	40%	90%
50	10%	20%	30%	40%	100%
60	20%	30%	50%	-	100%

For each test 10 bivalves were used

LC_0 = 5% is the concentration for 0% mortality.

LC_{50} = 20% is the concentration for 50% moratlity.

0.5% = $1/10^{th}$ of LC_0 i.e. sublethal concentration.

2% = $1/10^{th}$ of LC_{50} i.e. sublethal concentration.

3.6 Subacute Toxicity

Subacute concentrations used for the subacute toxicity test were 0.5% and 2% which were $1/10^{th}$ values of LC_0 and LC_{50} values respectively. Bivalves were exposed to wastewater for two exposure periods i.e. 10 and 20 days. The concentrations of tannery wastewater were replaced after every 24 hrs and after completion of exposure period animals were sacrificed and tissues were used for various tests.

3.7 Atomic Absorption Spectrophotometry (Table 2, Graph 3)

Animals exposed to different concentrations of tannery wastewater were used for the detection of accumulation of heavy metal chromium through the whole body of animal. Chromium level was found very high in the exposed animals while in control animal chromium was absent. Chromium level was increased significantly in the bivalves, exposed for 20 days.

3.8 Biochemical Observations

3.8.1 Protein (Table 3, Graph 4)

Gills: The protein contents in the gills of the bivalve, *Lamellidens marginalis* exposed at 0.5% concentration of tannery wastewater for 10 days declined insignificantly, whereas at 2% concentration for 10 days exposure period and at 0.5% and 2% concentrations of tannery wastewater for 20 days showed a significant decrease as compared to control.

Gonads: The protein contents in gonads of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant decrease as compared to control.

Hepatopancreas: The hepatopancreas of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant decrease in protein contents as compared to control. At 0.5% concentration of tannery wastewater for 10 days exposure period protein contents in hepatopancreas declined insignificantly.

Muscles: The protein contents in the muscles of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed significant reduction as compare to control. At 0.5% concentration of tannery wastewater for 10 days exposure period, protein contents in muscles showed insignificant decrease as compared to control.

Mantle: The protein contents in mantle of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant decrease, whereas at 0.5% concentration of tannery wastewater for 10 days exposure period, protein contents in mantle observed insignificant decrease as compared to control.

Foot: The protein contents in the foot of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant decrease as compare to control. At 0.5% concentration of tannery wastewater for 10 days exposure period, protein contents in gills showed insignificant decrease as compared to control.

3.8.2 Glycogen (Table 4, Graph5)

Gills: The Glycogen contents in the gills of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods decreased significantly as compared to control.

Gonads: The Glycogen contents in the gonads of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant reduction as compared to control.

Hepatopancreas: The Glycogen contents in the hepatopancreas of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant decrease as compare to control. At 0.5% concentration of tannery wastewater for 10 days exposure periods, glycogen contents in hepatopancreas showed insignificant decrease as compared to control.

Muscles: The glycogen contents in muscles of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant decrease. At 0.5% concentration of tannery wastewater for 10 days exposure period, glycogen contents in muscles showed insignificant decrease as compared to control.

Mantle: The Glycogen contents in the mantle of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant decrease. At 0.5% concentration of tannery wastewater for 10 days exposure period, glycogen contents in foot showed insignificant decrease as compared to control.

Foot: The glycogen contents in the foot of bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods declined significantly. At 0.5% concentration

of tannery wastewater for 10 days exposure period, glycogen contents in foot showed insignificant decrease as compared to control.

3.8.3 Lactic Acid (Table 5, Graph6)

Gills: The Lactic Acid contents in the gills of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed insignificant increase as compared to control.

Gonads: The lactic acid contents in gonads of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods, showed insignificant increase. Similarly at 0.5% and 2% concentration for 10 days exposure lactic acid contents in gonads showed insignificant decrease as compared to control.

Hepatopancreas: The Lactic Acid contents in the hepatopancreas of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed insignificant increase as compared to control.

Muscles: The lactic acid in muscles of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed insignificant increase as compared to control.

Mantle: The lactic acid in mantle of bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed insignificant increase as compare to control.

Foot: The Lactic Acid contents in the foot of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed insignificant increase as compared to control.

3.8.4 Cholesterol (Table 6, Graph 7)

Gills: The cholesterol contents in the gills of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant decrease as compared to control, except at 0.5% of tannery wastewater for 10 days exposure.

Gonads: The cholesterol contents in the gonads of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant decrease as compared to control animals.

Hepatopancreas: The cholesterol contents in hepatopancreas of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods observed a significant decrease in cholesterol as compared to control bivalves.

Muscles: The cholesterol contents in the muscles of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods, noticed significant decrease as compared to control animals.

Mantle: The cholesterol contents in the mantle of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant decreased as compared to control animals, except at 0.5% of tannery wastewater for 10 days exposure.

Foot: The cholesterol contents in foot of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant increase as compared to control.

3.9 Enzyme Observations

3.9.1 Acid Phosphatase (Table 7, Graph 8)

Gills: The acid phosphatase activity in the gills of the bivalve, *Lamellidens*

marginalis exposed to 0.5% and 2% concentrations of tannery wastewater for two distinct (10 and 20 days) exposure periods observed a significant increase as compared to control bivalves.

Gonads: The acid phosphatase activity in the gonads of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods, showed a significant increase as compared to control.

Hepatopancreas: The acid phosphatase activity in hepatopancreas of bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant increase as compared to control.

Muscles: The acid phosphatase activity in the muscles of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods revealed a significant increase as compared to control animals.

Mantle: The acid phosphatase activity in the mantle of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant increase as compared to control bivalves.

Foot: The acid phosphatase activity in the foot of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant increase as compared to control.

3.9.2 Alkaline Phosphatase (Table 8, Graph 9)

Gills: The alkaline phosphatase activity in the gills of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant increase as compared to control.

Gonads: The alkaline phosphatase activity in gonads of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant increase as compared to control.

Hepatopancreas: The alkaline ahosphatase activity in the hepatopancreas of bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant increase as compared to control.

Muscles: The alkaline phosphatase activity in the muscles of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods observed a significant increase as compared to control.

Mantle: The alkaline phosphatase activity in mantle of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant increase as compared to control bivalves.

Foot: The alkaline phosphatase activity in the foot of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant increase as compared to control bivalves.

3.9.3 Glutamate Oxaloacetate Transaminase (Table 9, Graph 10)

Gills: The Glutamate Oxaloacetate Transaminase activity in the gills of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for 10 days exposure showed insignificant increase, whereas at 0.5% and 2% concentration for 20 days exposure period showed significant increase as compared to control.

Gonads: Glutamate Oxaloacetate Transaminase activity in gonads of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of

tannery wastewater for two different (10 and 20 days) exposure periods observed an insignificant increase as compared to control animals.

Hepatopancreas: Glutamate Oxaloacetate Transaminase activity in the hepatopancreas of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant increased. However, at 0.5% concentration for 10 and 20 days exposure, GOT activity increases insignificantly as compared to control animals.

Muscles: Glutamate Oxaloacetate Transaminase activity in the muscles of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for 10 days exposure periods showed a insignificant increase, but at 0.5% and 2% concentration for 20 days exposure, GOT activity increased significantly as compared to control.

Mantle: The Glutamate Oxaloacetate Transaminase activity in the hepatopancreas of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant increase. However, at 0.5% concentration for 10 and 20 days exposure, GOT activity increased insignificantly as compared to control.

Foot: Glutamate Oxaloacetate Transaminase activity in the foot of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for 10 days exposure period showed a insignificant increase. However, for 20 days exposure showed significant increase as compared to control.

3.9.4 Glutamate Pyruvic Transaminase (Table 10, Graph 11)

Gills: The Glutamate Pyruvic Transaminase activity in the gills of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for 10 and 20 days exposure period showed significant increase, but

insignificantly increased at 0.5% concentration for 10 days exposure as compared to control.

Gonads: The Glutamate Pyruvic Transaminase activity in the gonads of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for 20 days exposure period, showed a significant increase, but insignificantly increased at 0.5% and 2% concentration for 10 days exposure as compared to control bivalves.

Hepatopancreas: The Glutamate Pyruvic Transaminase activity in the hepatopancreas of bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant increase as compare to control bivalves.

Muscles: The Glutamate Pyruvic Transaminase activity in muscles of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods, increases significantly as compared to control animals.

Mantle: The Glutamate Pyruvic Transaminase activity in the mantle of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentration for 10 days exposure showed insignificant increase and significantly increased at 0.5% and 2% concentrations of tannery wastewater for 20 days exposure as compared to control.

Foot: The Glutamate Pyruvic Transaminase activity in foot of the bivalve, *Lamellidens marginalis* exposed at 0.5% and 2% for 10 and 20 days exposure period, increases significantly as compared to control.

3.9.5 Adenosine Triphosphatase (Table 11, Graph 12)

Gills: The Adenosine Triphosphatase activity in the gills of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant decrease as compared to control.

Gonads: Adenosine Triphosphatase activity in gonads of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant decrease as compared to control bivalves.

Hepatopancreas: The Adenosine Triphosphatase activity in the hepatopancreas of bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant decrease whereas insignificantly increased at 0.5% concentration for 10 days exposure as compared to control bivalves.

Muscles: The Adenosine Triphosphatase activity in the muscles of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant decrease as compared to control.

Mantle: The Adenosine Triphosphatase activity in mantle of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods, decreased insignificantly, whereas insignificantly increased at 0.5% concentration for 20 days exposure as compared to control.

Foot: The Adenosine Triphosphatase activity in the foot of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant decrease as compared to control.

3.9.6 Lactate Dehydrogenase (Table 10, Graph 10)

Gills: The Lactate Dehydrogenase activity in gills of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods showed a significant decrease as compared to control bivalves.

Gonads: The Lactate Dehydrogenase activity in the gonads of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery

wastewater for two different (10 and 20 days) exposure periods showed a significant decrease as compared to control animals.

Hepatopancreas: The Lactate Dehydrogenase activity in the hepatopancreas bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for 10 days exposure periods showed an insignificant decrease. However, at 0.5% and 2% concentrations for 20 days exposure LDH in hepatopancreas increased significantly as compared to control bivalves.

Muscles: The Lactate Dehydrogenase activity in the muscle of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for 10 days exposure periods showed an insignificant decrease. However, at 0.5% and 2% concentrations for 20 days exposure LDH in muscle increases significantly as compared to control bivalves.

Mantle: The Lactate Dehydrogenase activity in mantle of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods, *showed* a significant decrease, whereas insignificantly increases at 0.5% concentration at 10 days exposure as compared to control bivalves.

Foot: The Lactate Dehydrogenase activity in the foot of the bivalve, *Lamellidens marginalis* exposed to 0.5% and 2% concentrations of tannery wastewater for two different (10 and 20 days) exposure periods decreased insignificantly, except at 2% concentration for 20 days as compared to control bivalves.

3.10 Histological observations:

Histology of fresh water bivalve, *Lamellidens marginalis* was studied by light microscopy using hematoxylene stain and counter stained by eosin for the tissues gills and hepatopancreas. Histology of these tissues was studied for control bivalve as well as the bivalves exposed at two different concentrations of tannery wastewater for two different exposure periods.

3.10.1 Gills

A) Control:

Gills in the fresh water bivalve, *Lamellidens marginalis* lies on the either side of the body in the mantle cavity. Each gill is formed of two laminae as inner and outer lamella. Each gill lamina consists of an outer and inner lamella, are elongated plate like structure (fig. 1 and 2). The cavity between gill lamellae is divided by vertical septa, interlamellar junctions into a number of compartments i.e. water tubes. Each gill lamella is 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 is covered by ciliated epithelium. The epithelial cells are elongated with laterally located prominent nucleus. The cytoplasm shows secretary material. The connective tissue lies between the lamella and basal filament. The cavity in gill filament is normal as well as space between two gills is also moderate (fig. 1 and 2).

B) 0.5% tannery wastewater for 10 days:

The light microscopic structure of gills of fresh water bivalve, *Lamellidens marginalis* showed some changes in histological structure. Gill filaments were short, ruptured and lined by epithelial cells with cilia. The epithelial cells shows centrally located prominent nucleus. The cells show accumulation of some dark material. The disintegration of connective tissue and basal filament was also observed (fig. 3,4). Increased filamental cavity and interfilamental cavity was observed. Water tubes were elongated with long and obliquely placed inter lamellar junction (fig. 3,4).

C) 0.5% tannery wastewater for 20 days:

The light microscopic structure of gills of bivalve, *Lamellidens marginalis* exposed to 0.5% concentration of tannery wastewater for 20 days exposure period showed short gill filaments and epithelial cells with accumulation of dark material in the cytoplsm. Increased filament cavity and inter filament cavity was

observed with the vacuolisation of connective tissues (fig. 5,6). Basal filament was not clearly observed and water tubes were broadened with obliquely placed inter lamellar junctions (fig. 5,6).

D) 2% tannery wastewater for 10 days:

The light microscopic structure of gills of bivalve, *Lamellidens marginalis* exposed to 2 % concentration of tannery wastewater for 10 days exposure period showed some changes in histological architecture. Gill filaments observed were elongated and lie uniformly. The epithelial cells showed normal structure with prominent nucleus and cilia (fig. 7, 8). The cells showed some dark granules in the cytoplasm. The vacuolated connective tissues, increased filament cavity and inter filament cavity was also observed. The water tubule elongated with obliquely placed inter lamellar junction (fig. 7, 8).

E) 2% tannery wastewater for 20 days:

The light microscopic structure of gills of bivalve, *Lamellidens marginalis* exposed to 2 % concentration of tannery wastewater for 20 days exposure period showed gill filament lined by epithelial cells with cilia. The epithelial cells show central nucleus with accumulation of dark material in the cytoplasm. The basal filament was not clearly seen. The disintegration in connective tissue was observed with elongated water tubes and inter lamellar junctions (fig. 9, 10).

3.10.2 Hepatopancreas

A) Control

Light microscopic structure of hepatopancreas of control bivalve, *Lamellidens marginalis* showed loosely arranged digestive tubules. Two types of cells namely absorptive cell and secretary cell were lining the digestive tubules (fig. 11, 12). Absorptive cells showed column like structure while secretary cells were dark and oval in shape. The secretary cells were with secretary material in the cytoplasm.Each digestive tubule consisted of wide lumen with secretary of

material. Digestive tubules were lied inter connected with each other by connective tissue (fig. 11, 12).

B) 0.5% tannery wastewater for 10 days:

Light microscopic structure of hepatopancreas of bivalve, *Lamellidens marginalis* exposed to 0.5% concentration of tannery wastewater for 10 days exposure period showed loosely arranged digestive tubules with elongated absorptive cells and secretary cells. The absorptive cells are tall and elongated. The secretary cells showed vacuolation. The lumen was reduced and devoid of secretary material. Connective tissue was disintegrated and also showed some vacuolations. (fig 13, 14).

C) 0.5% concentration wastewater for 20 days:

Light microscopic structure of hepatopancreas of bivalve, *Lamellidens marginalis* exposed to 0.5% concentration of tannery wastewater for 20 days exposure periods showed loosely arranged and inter linked digestive tubules. The absorptive cells were elongated with basal nucleus. The secretary cells were big with centrally located nucleus and showing darkly stained material in the cytoplasm. There was disintegration of connective tissue and reduction in size of lumen cavity with devoid of secretion (fig 15, 16).

D) 2% tannery wastewater for 10 days:

Light microscopic structure of hepatopancreas of bivalve, *Lamellidens marginalis* exposed to 2% concentration of tannery wastewater for 10 days exposure period showed loosely arranged digestive tubules with absorptive cells and secretary cells. The secretary cells were with prominent nucleus and stained material in the cytoplasm. The cytoplasm shows vacuolations. The lumen was irregularly shaped and smaller in size. (Fig. 17, 18). An inter-tubular space was increased and showed vacuoles in the connective tissues.

E) 2% tannery wastewater for 20 days:

Light microscopic structure of hepatopancreas of bivalve, *Lamellidens marginalis* exposed to 2% concentration of tannery wastewater for 20 days exposure period showing loosely inter linked, irregularly shaped digestive tubules. The elongated absorptive cells with basal nucleus and secretary cells with central nucleus were prominent. The secretary cells showed accumulation of darkly stained material with many large vacuolations. There was disintegration of connective tissue and reduction in size of lumen cavity (fig 19, 20).

Table No. 1

Analysis of tannery wastewater

Sr. No.	Parameter	Results	MPCB standards
1	pH	4.17 ±0.51	7.5
2	Turbidity	144.75 ±8.42	5*
3	TS	44090 ±64.08	2100
4	TDS	42028 ±48.19	2100
5	TSS	2062 ±110	600
6	COD	36320 ±127	250
7	Oil and grease	0.8235 ±0.55	10
8	Chloride	3685.5 ±32.27	600
9	Chromium	0.563 ±0.21	2

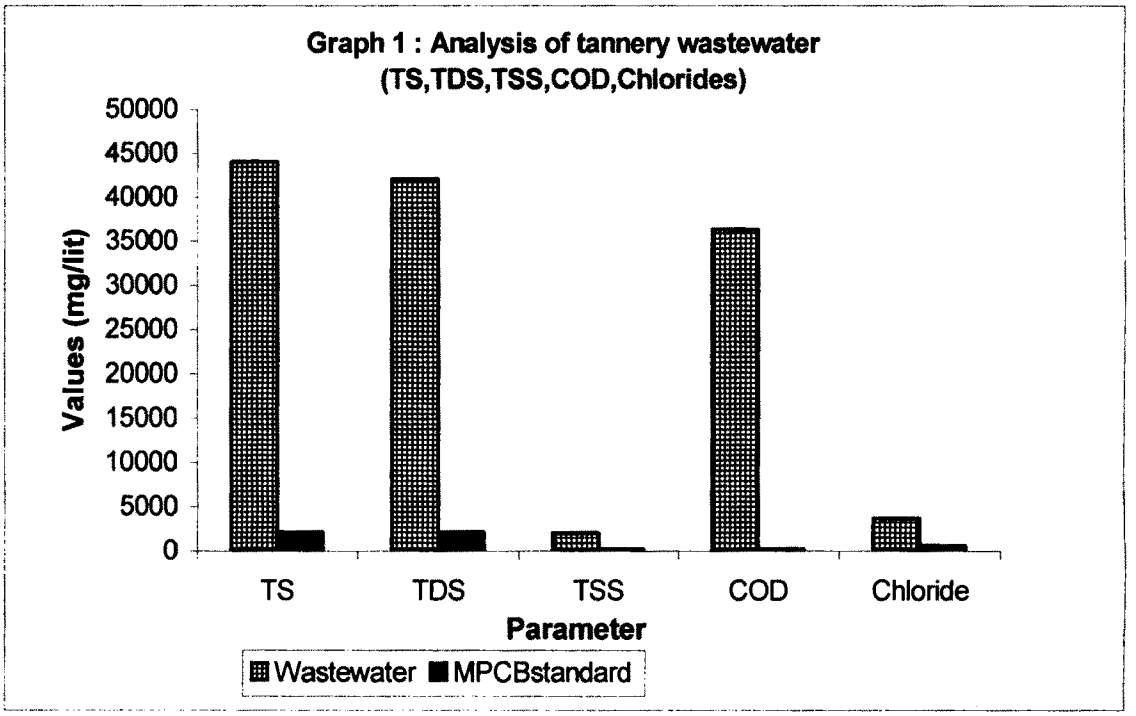
All parameters are in mg/lit except pH and Turbidity
*ISI standard.

Table 2

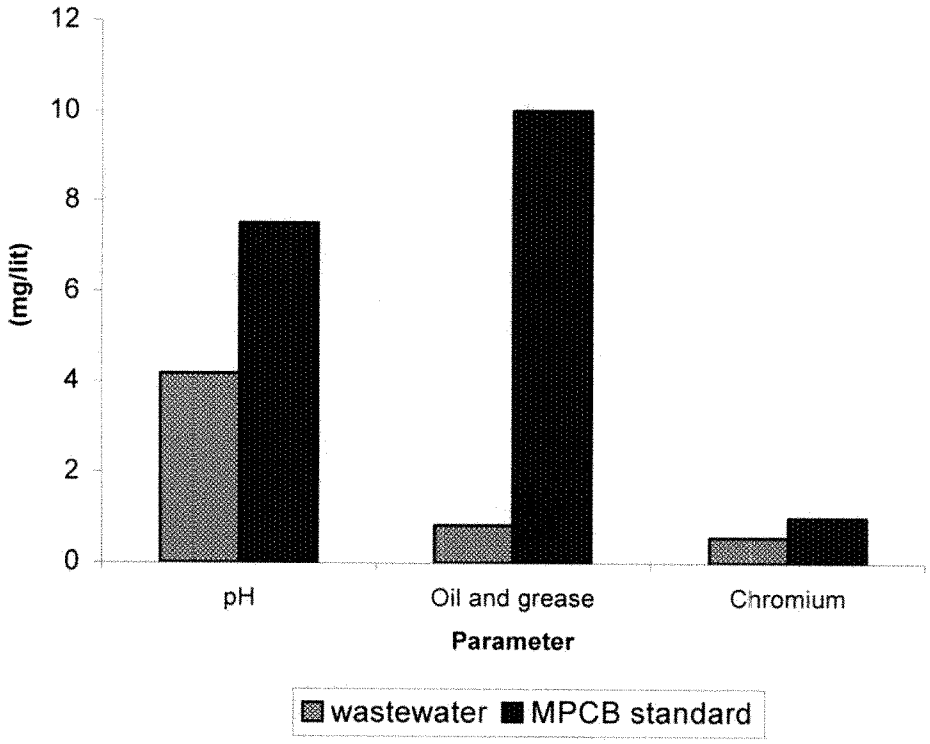
Chromium accumulation in *Lamellidens marginalis* at different concentrations of tannery wastewater

Metal	Control	0.5% 10 days	2% 10 days	0.5% 20 days	2% 20 days
Chromium	BDL	0.003 ±0.0001	0.016* ±0.001	0.012* ±0.001	0.032* ±0.001

All values are expressed in microgram/ gm wt. of tissue.
BDL- below detectable limit.
Values are ± S.D. of 4 Estimations
*Significant at P<0.05 by t- test


Graph 1 : Analysis of tannery wastewater
(TS,TDS,TSS,COD,Chlorides)
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
Values (mg/lit)
TS
TDS
TSS
COD
Chloride
Parameter
Wastewater
MPCBstandard

Graph 2: Analysis of tannery wastewater (pH, oil & grease, chromium)



Graph 3 : Chromium content in fresh water bivalve, *Lamellidens marginalis* exposed to different concentrations of tannery wastewater

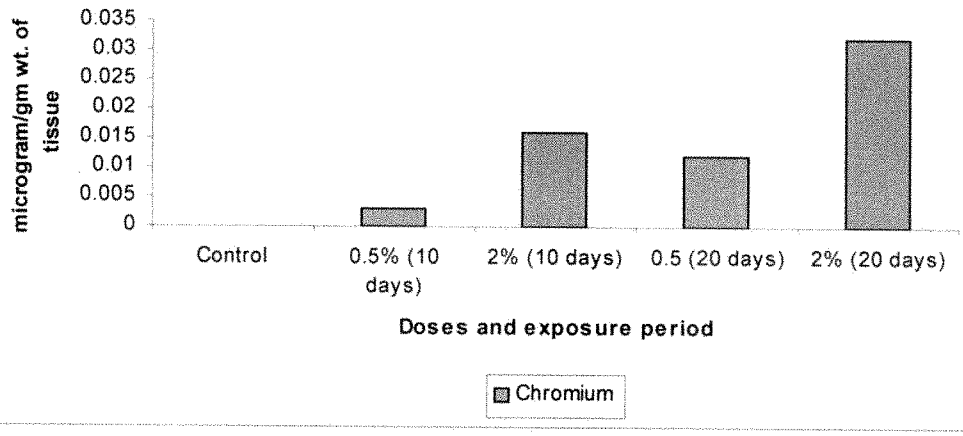


Table No. 3

Protein content in *Lamellidens marginalis* at different concentrations of tannery wastewater for different exposure periods

Concentration	Control	0.5% (10 days)	2% (10 days)	0.5% (20 days)	2% (20 days)
Gills	35.12 ±7.97	32.00 ±1.95	15.87* ±2.860	14.37* ±1.430	6.37* ±1.100
Gonads	50.75 ±1.25	26.875* ±1.790	24.25* ±3.270	19.37* ±1.180	10.37* ±1.100
Hepato pancreas	35.50 ±5.21	31.15 ±1.87	26.00* ±2.850	24.62* ±0.750	17.37* ±1.930
Muscle	31.00 ±4.52	28.62 ±1.03	20.75* ±2.660	11.87* ±0.470	8.50* ±1.290
Mantle	32.75 ±4.64	31.75 ±0.866	25.87* ±3.470	15.37* ±1.400	11.37* ±1.290
Foot	44.5 ±2.2	40.62 ±2.92	35.75* ±1.650	16.5* ±0.70	13.00* ±1.470

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

Graph 4 : Protein content in fresh water bivalve, *Lamellidens marginalis* exposed to different concentrations of tannery wastewater

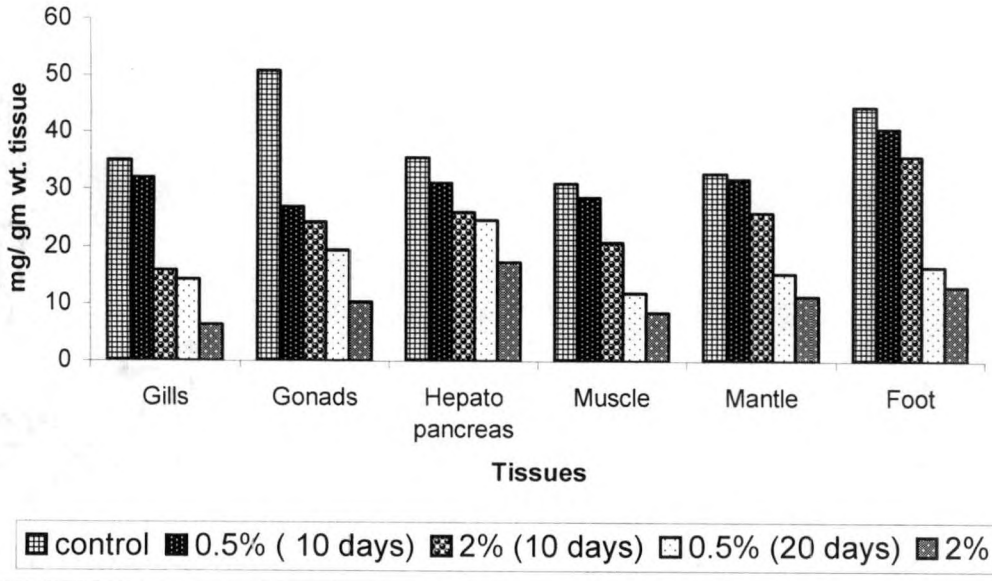


Table No. 4

Glycogen content in *Lamellidens marginalis* at different concentrations of tannery wastewater for different exposure periods

Concentration	Control	0.5% (10 days)	2% (10 days)	0.5% (20 days)	2% (20 days)
Gills	23.12 ±1.86	17.51* ±0.690	16.47* ±0.050	5.60* ±0.130	3.58* ±0.050
Gonads	45.90 ±2.88	18.82* ±0.080	15.26* ±1.260	7.23* ±0.320	3.08* ±0.160
Hepato pancreas	50.67 ±15.94	40.32* ±0.07	32.87* ±0.520	18.14* ±0.060	14.91* ±0.170
Muscle	26.08 ±2.19	17.19* ±22.4	13.66* ±0.100	8.64* ±0.140	4.66* ±0.150
Mantle	28.10 ±0.13	24.90 ±1.990	12.85* ±0.080	9.94* ±0.100	6.46* ±0.110
Foot	23.21 ±4.31	19.37 ±0.16	13.06* ±0.070	10.86* ±0.110	6.74* ±0.110

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

Graph 5: Glycogen content in fresh water bivalve, *Lamellidens marginalis* exposed to different concentrations of tannery wastewater

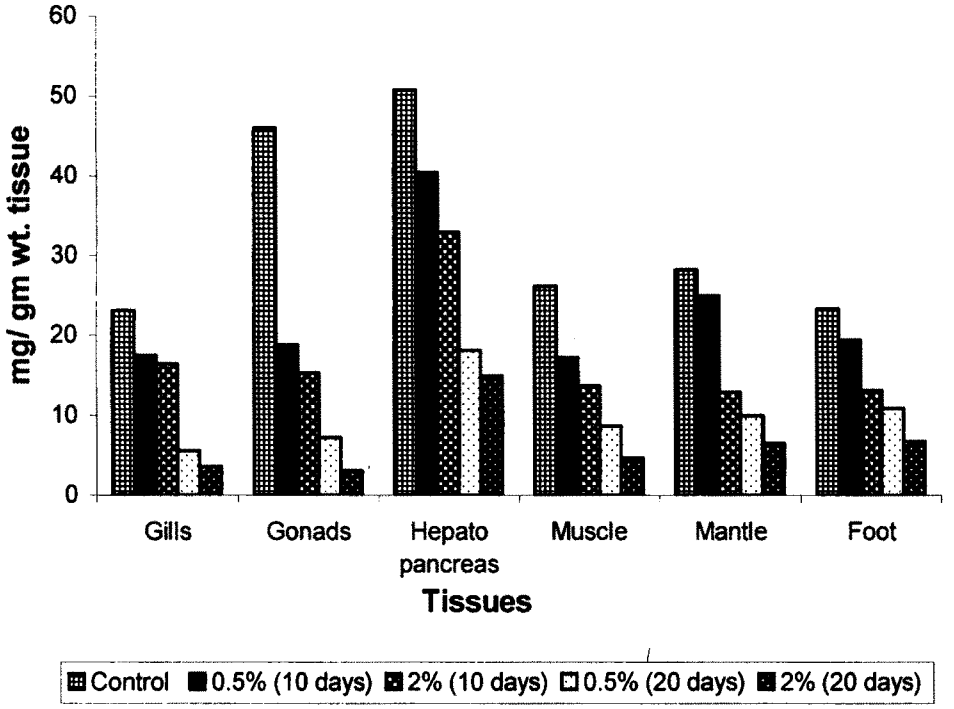


Table No. 5

Lactic Acid contents in *Lamellidens marginalis* at different concentrations of tannery wastewater for different exposure periods

Concentration	Control	0.5% (10 days)	2% (10 days)	0.5% (20 days)	2% (20 days)
Gills	0.601 ±0.39	0.641 ±0.39	0.680 ±0.39	0.873 ±0.80	0.959 ±0.83
Gonads	0.494 ±0.31	0.533 +0.25	0.602 ±0.16	0.686 ±0.43	0.708 ±0.48
Hepato pancreas	0.360 ±0.19	0.434 ±0.23	0.533 ±0.28	0.514 ±0.25	0.838 ±0.25
Muscle	0.387 ±0.20	0.468 ±0.26	0.336 ±0.19	0.384 ±0.22	0.960 ±0.50
Mantle	0.309 ±0.16	0.465 ±0.25	0.474 ±0.25	0.453 ±0.26	0.682 ±0.36
Foot	0.298 ±0.16	0.559 ±0.31	0.511 ±0.28	0.771 ±0.51	0.798 ±0.59

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

Graph 6 : Lactic Acid content in fresh water bivalve, *Lamellidens marginalis* exposed to different concentrations of tannery wastewater

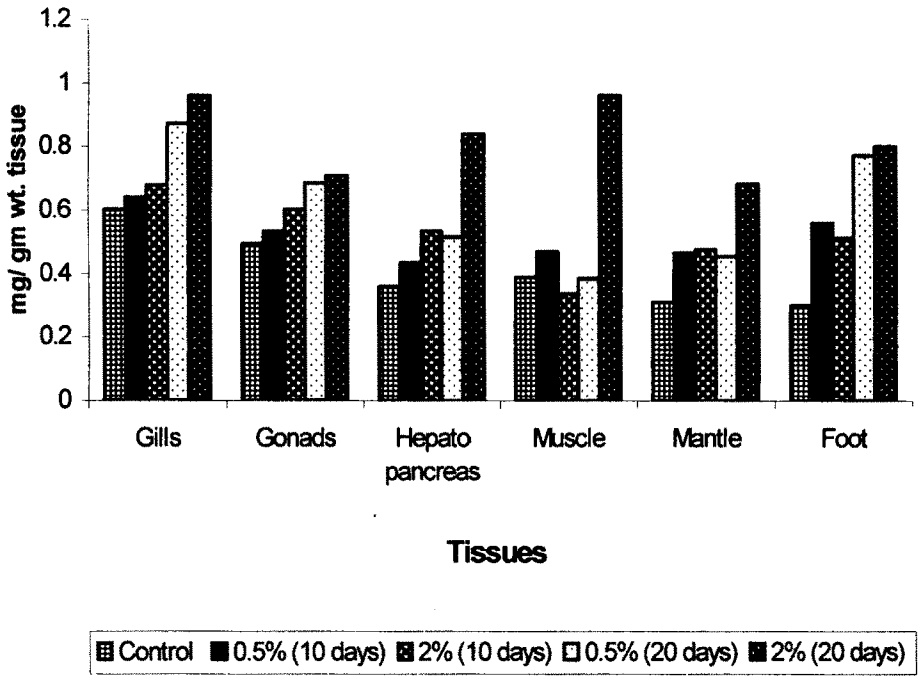


Table No. 6

Cholesterol contents in *Lamellidens marginalis* at different concentrations of tannery wastewater for different exposure periods

Concentration	Control	0.5% (10 days)	2% (10 days)	0.5% (20 days)	2% (20 days)
Gills	0.98 ±0.22	0.922 ±0.01	0.86* ±0.005	0.58* ±0.006	0.47* ±0.004
Gonads	0.97 ±0.01	0.765* ±0.007	0.745* ±0.004	0.566* ±0.01	0.595* ±0.013
Hepato pancreas	0.34 ±0.04	0.263* ±0.003	0.266* ±0.01	0.195* ±0.02	0.13* ±0.06
Muscle	0.58 ±0.02	0.47* ±0.009	0.44* ±0.01	0.277* ±0.01	0.26* ±0.044
Mantle	0.68 ±0.21	0.659 ±0.01	0.585* ±0.01	0.448* ±0.020	0.4* ±0.004
Foot	0.530 ±0.007	0.46* ±0.018	0.231* ±0.01	0.159* ±0.010	0.13* ±0.003

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

Graph 7 : Cholesterol content in fresh water bivalve, Lamellidens marginalis exposed to different concentrations of tannery wastewater

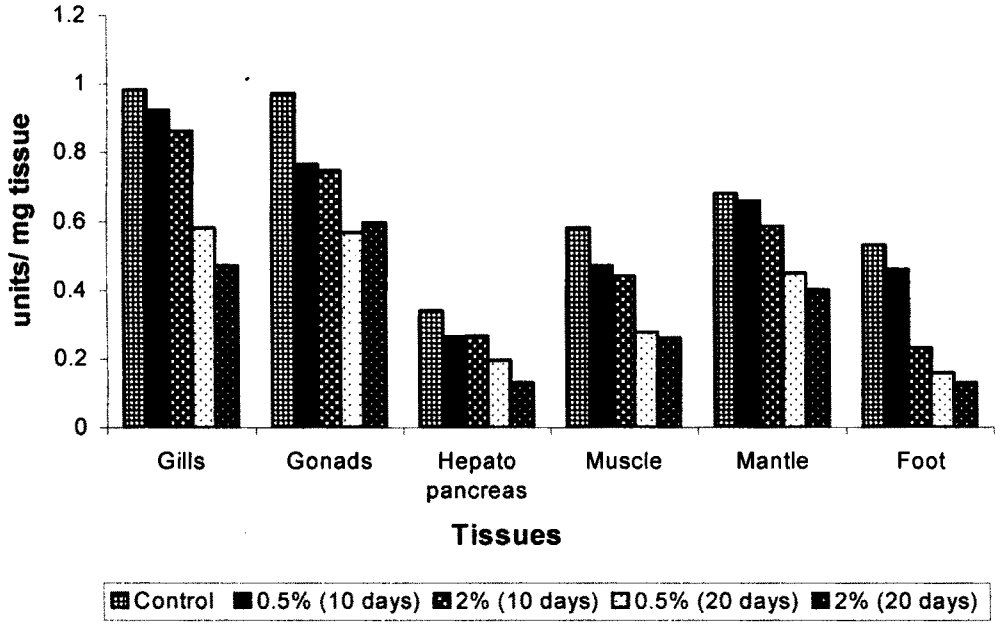


Table No. 7

Acid Phosphatase activity in *Lamellidens marginalis* at different concentrations of tannery wastewater for different exposure periods

Concentration	Control	0.5% (10 days)	2% (10 days)	0.5% (20 days)	2% (20 days)
Gills	1.55 ±0.06	3.87* ±0.24	4.62* ±0.18	6.73* ±0.17	4.62* ±0.18
Gonads	1.42 ±0.21	5.37* ±0.22	7.37* ±0.20	7.12* ±0.12	8.51* ±0.70
Hepato pancreas	0.98 ±0.15	5.28* ±0.24	5.39* ±0.33	6.57* ±0.18	6.50* ±0.10
Muscle	1.44 ±0.82	4.50* ±0.30	4.7* ±0.23	6.07* ±0.20	6.39* ±0.09
Mantle	0.69 ±0.23	4.73* ±0.18	5.30* ±0.15	5.12* ±0.06	4.55* ±0.08
Foot	1.50 ±0.17	4.48* ±0.06	4.71* ±0.01	5.44* ±0.10	4.60* ±0.25

Values are ± S.D. of 4 Estimations

Activity expressed in mg Pi liberated/hr/mg protein tissue.

* Significant at $P<0.05$ by t- test

Graph 8 : Acid phosphatase activity in fresh water bivalve, *Lamellidens marginalis* exposed to different concentrations of tannery wastewater

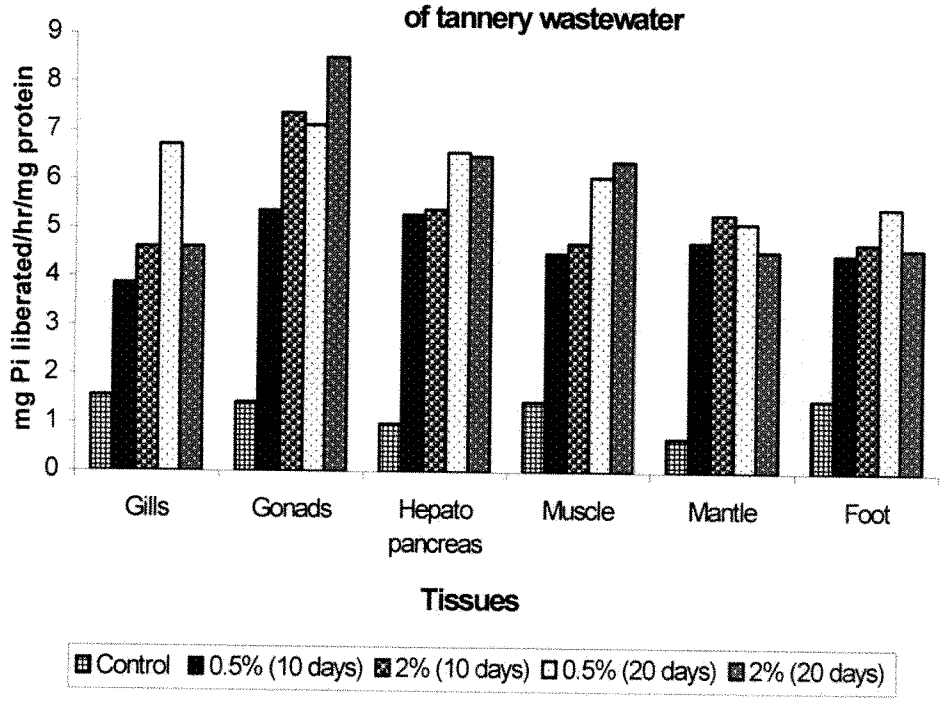


Table No. 8

Alkaline Phosphatase activity in *Lamellidens marginalis* at different concentrations of tannery wastewater for different exposure periods

Concentration	Control	0.5% (10 days)	2% (10 days)	0.5% (20 days)	2% (20 days)
Gills	2.07 ±0.29	4.50* ±0.10	4.48* ±0.13	5.80* ±0.34	6.89* ±0.38
Gonads	1.55 ±0.03	8.50* ±0.58	9.82* ±0.94	10.14* ±0.12	12.83* ±0.25
Hepatopancreas	1.92 ±0.24	9.17* ±0.09	10.41* ±0.41	13.09* ±0.20	13.50* ±0.13
Muscle	1.16 ±0.12	6.73* ±0.12	6.55* ±0.37	8.07* ±0.10	8.64* ±0.42
Mantle	1.46 ±0.04	5.35* ±0.06	6.42* ±0.29	6.98* ±0.29	7.5* ±0.48
Foot	1.83 ±0.27	6.71* ±0.15	6.82* ±0.31	7.85* ±0.10	9.03* ±0.37

Values are ± S.D. of 4 Estimations

Activity expressed in mg Pi liberated/hr/mg protein tissue.

* Significant at $P<0.05$ by t- test

Graph 9: Alkaline phosphatase activity in fresh water bivalve, *Lamellidens marginalis* exposed to different concentrations of tannery wastewater

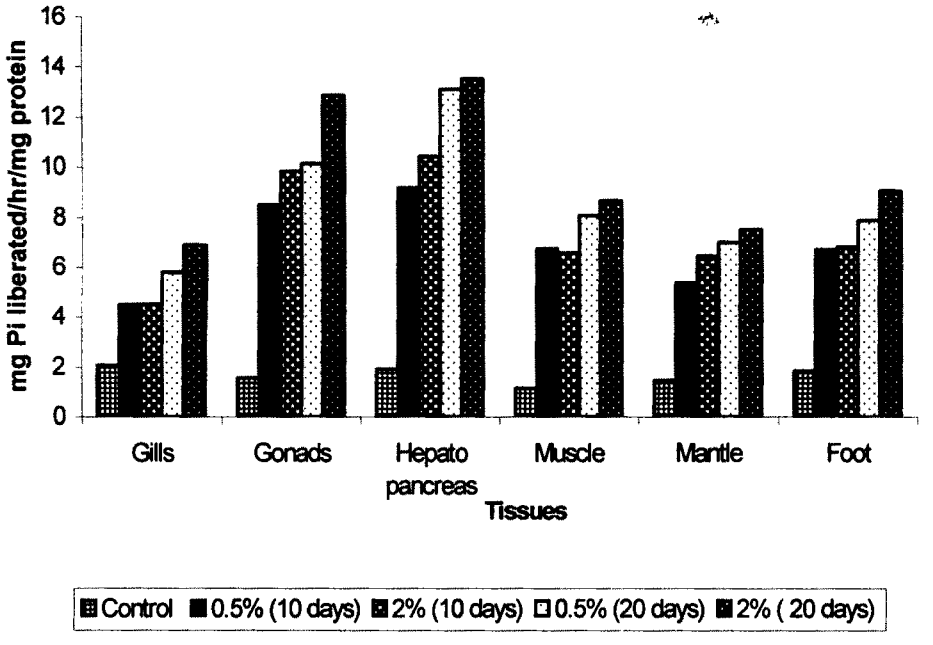


Table No. 9

Glutamate oxaloacetate transaminase activity in *Lamellidens marginalis* at different concentrations of tannery wastewater for different exposure periods

Concentration	Control	0.5% (10 days)	2% (10 days)	0.5% (20 days)	2% (20 days)
Gills	0.0135 ±0.003	0.014 ±0.003	0.017 ±0.002	0.036* ±0.013	0.048* ±0.01
Gonads	0.0183 ±0.008	0.018 ±0.004	0.019 ±0.005	0.019 ±0.008	0.023 ±0.003
Hepatopancreas	0.030 ±0.001	0.031 ±0.002	0.036* ±0.001	0.033 ±0.002	0.036* ±0.001
Muscle	0.023 ±0.002	0.025 ±0.003	0.025 ±0.003	0.029* ±0.002	0.03* ±0.001
Mantle	0.029 ±0.002	0.03 ±0.003	0.035* ±0.001	0.033 ±0.002	0.036* ±0.003
Foot	0.027 ±0.003	0.032 ±0.11	0.031 ±0.003	0.035* ±0.002	0.04* ±0.005

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

Graph 10 :Glutamate oxaloacetate transaminase activity in fresh water bivalve, *Lamellidens marginalis* exposed to different concentrations of tannery wastewater

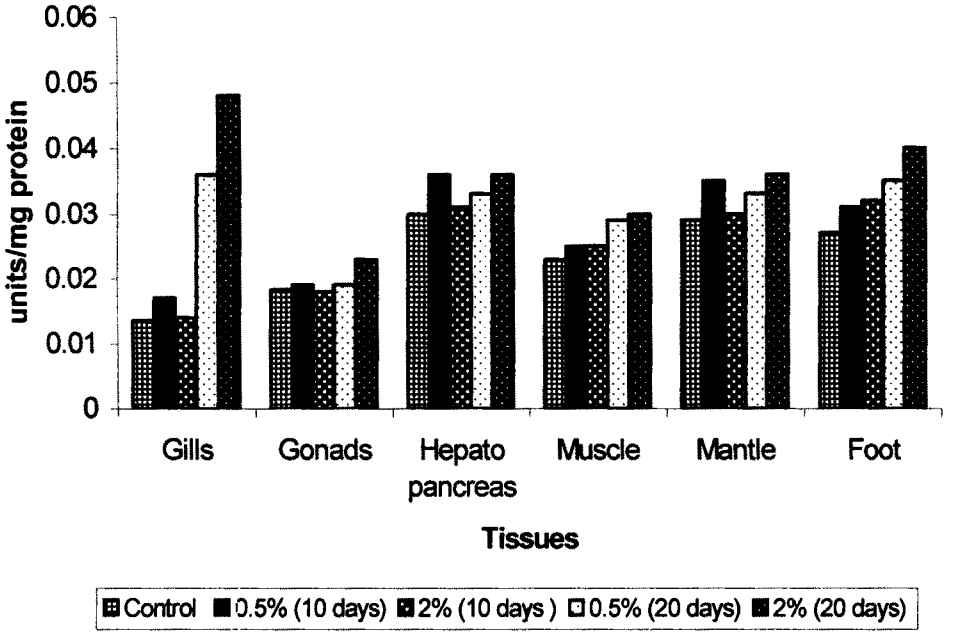


Table No. 10

Glutamate pyruvate transaminase activity in *Lamellidens marginalis* at different concentrations of tannery wastewater for different exposure periods

Concentration	Control	0.5% (10 days)	2% (10 days)	0.5% (20 days)	2% (20 days)
Gills	0.014 ±0.002	0.015 ±0.001	0.029* ±0.004	0.039* ±0.08	0.063* ±0.001
Gonads	0.013 ±0.003	0.014 ±0.0006	0.019 ±0.002	0.03* ±0.007	0.048* ±0.001
Hepatopancreas	0.018 ±0.007	0.028* ±0.001	0.029* ±0.001	0.035* ±0.0006	0.036* ±0.001
Muscle	0.014 ±0.007	0.023* ±0.002	0.028* ±0.002	0.03* ±0.003	0.033* ±0.001
Mantle	0.022 ±0.01	0.023 ±0.005	0.027 ±0.001	0.045* ±0.011	0.055* ±0.002
Foot	0.022 ±0.009	0.031* ±0.003	0.037* ±0.002	0.036* +0.001	0.032* ±0.003

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

Graph 11: Glutamate pyruvate transaminase activity in fresh water bivalve, *Lamellidens marginalis* exposed to different concentrations of tannery wastewater

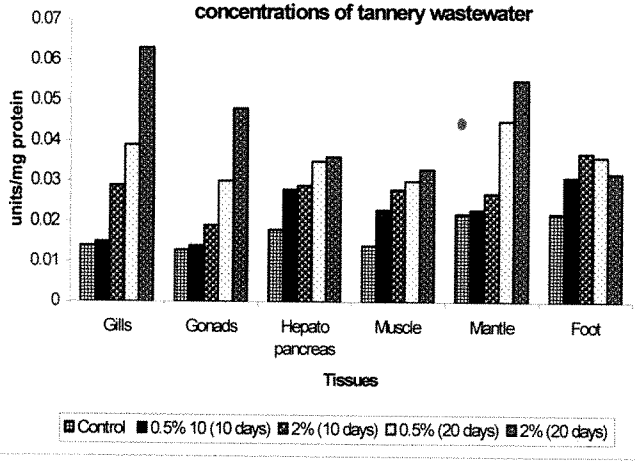


Table No. 11

ATPase activity in *Lamellidens marginalis* at different concentrations of tannery wastewater for different exposure periods

Concentration	Control	0.5% (10 days)	2% (10 days)	0.5% (20 days)	2% (20 days)
Gills	19.16 ±1.23	12.20 ±0.11	11.08 ±1.08	8.04 ±0.11	6.77 ±0.06
Gonads	16.14 ±2.66	11.68 ±0.10	10.40 ±0.5	8.35 ±0.07	7.66 ±0.03
Hepatopancreas	14.90 ±1.53	14.99 ±1.15	12.85 ±0.16	10.24 ±0.125	9.23 ±0.09
Muscle	14.41 ±1.43	14.29 ±1.11	12.68 ±0.17	10.06 ±0.11	7.72 ±0.06
Mantle	13.60 ±1.25	13.89 ±1.47	12.12 ±0.36	14.16 ±0.23	11.49 ±1.08
Foot	15.47 ±1.15	14.26 ±1.08	8.39 ±0.34	13.19 ±0.07	12.61 ±0.29

Values are ± S.D. of 4 Estimations

Activity expressed in mg Pi liberated/hr/mg protein tissue.

* Significant at $P<0.05$ by t- test

Graph 12: Adenosine triphosphatase activity in fresh water bivalve, *Lamellidens marginalis* exposed to different concentrations of tannery wastewater

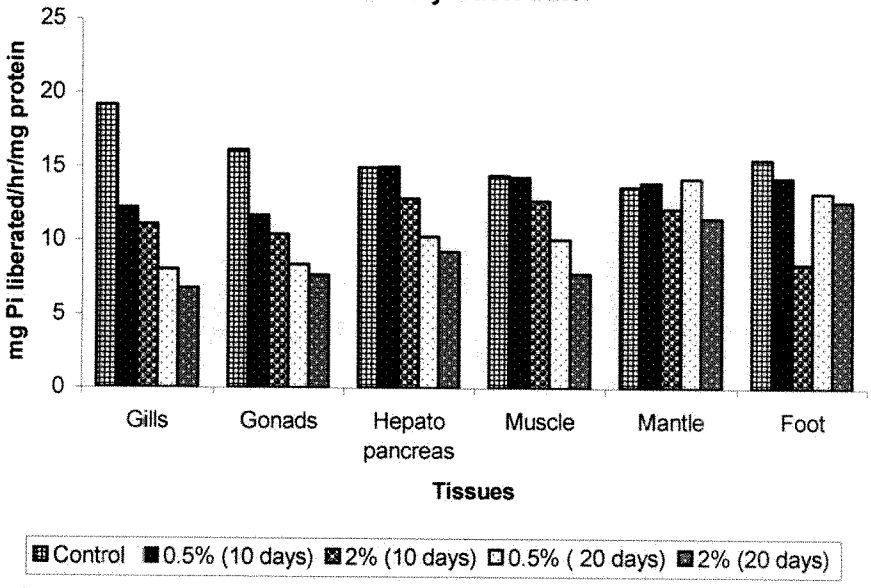


Table No. 12

Lactate dehydrogenase activity in *Lamellidens marginalis* at different concentrations of tannery wastewater for different exposure periods

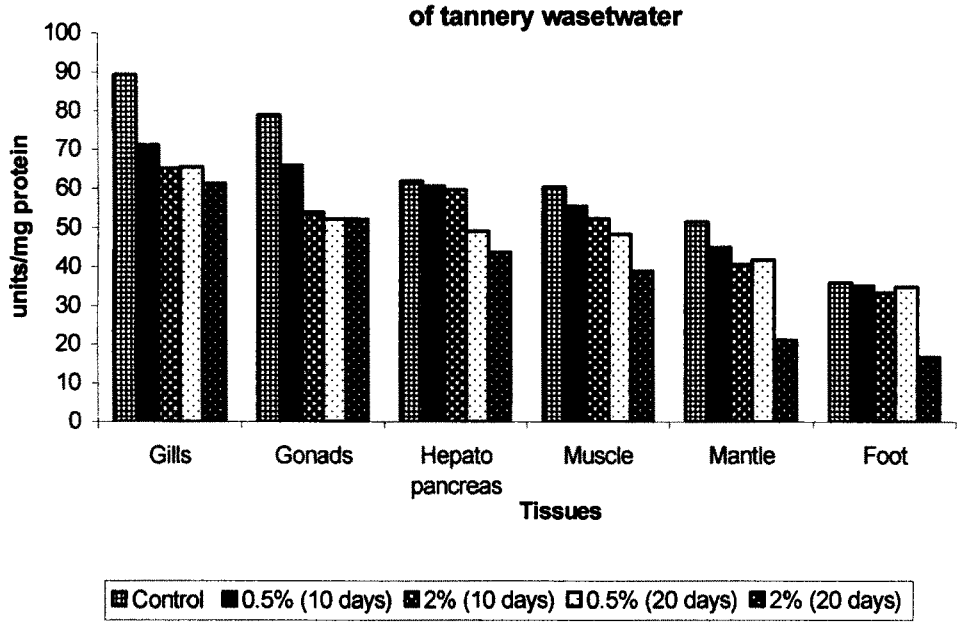
Concentration	Control	0.5% (10 days)	2% (10 days)	0.5% (20 days)	2% (20 days)
Gills	89.2 ±16.67	71.2* ±0.97	65.2* ±0.69	65.5* ±2.83	61.3* ±2.04
Gonads	78.9 ±17.96	65.9* ±1.14	54* ±1.50	52.1* ±8.48	52.2* ±1.14
Hepato pancreas	61.8 ±11.79	60.7 ±1.14	59.5 ±1.14	49.1* ±1.8	43.6 ±2.19
Muscle	60.3 ±10.74	55.5 ±3.58	52.3 ±1.14	48.3* ±3.53	38.7* ±3.96
Mantle	51.3 ±16.98	44.8 ±4.27	40.5* ±0.69	41.7* ±1.14	21* ±2.29
Foot	35.7 ±14.74	34.9 ±3.30	33.1 ±0.97	34.6 ±1.54	16.5* ±1.14

Values are ± S.D. of 4 Estimations

Activity expressed in units/mg protein.

* Significant at $P<0.05$ by t- test

Graph 13: Lactate dehydrogenase activity in fresh water bivalve, *Lamellidens marginalis* exposed to different concentrations of tannery wasetwater



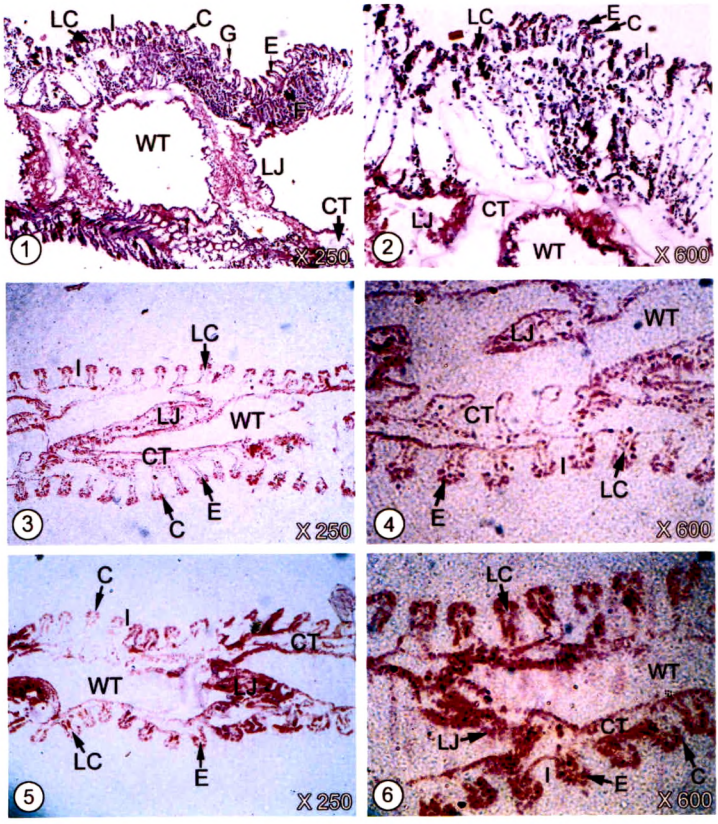


Fig. 1,2: Light microphotograph of gills of control bivalve, *Lamellidens marginalis* showing gill lamellae (L), epithelial cells (E), cilia (C), basal filament (F), lamellar cavity (LC), inter lamellar cavity (I), inter lamellar junction (LJ). Note presence of normal connective tissue and water tubule (WT).

Fig. 3,4: Light microphotograph of gills of bivalve, *Lamellidens marginalis* exposed to 0.5% concentration of tannery wastewater for 10 days exposure period showing gill lamellae (L), epithelial cells (E) with cilia (C), vacuolation in connective tissue (CT), basal filament (F), increased lamellar cavity (LC) and inter lamellar cavity (I). Note presence of elon-gated water tubule (WT) with long obliquely placed inter lamellar junction (LJ).

Fig. 5,6: Light microphotograph of gills of bivalve, *Lamellidens marginalis* exposed to 0.5% concentration of tannery wastewater for 20 days exposure period showing gill lamellae (L), epithelial cells (E) with accumulation of dark material, cilia (C), vacuolation in connective tissue (CT), increased lamellar cavity (LC) and inter lamellar cavity (I). Note presence of elongated water tubule (WT) with long obliquely placed inter lamellar junction (LJ)

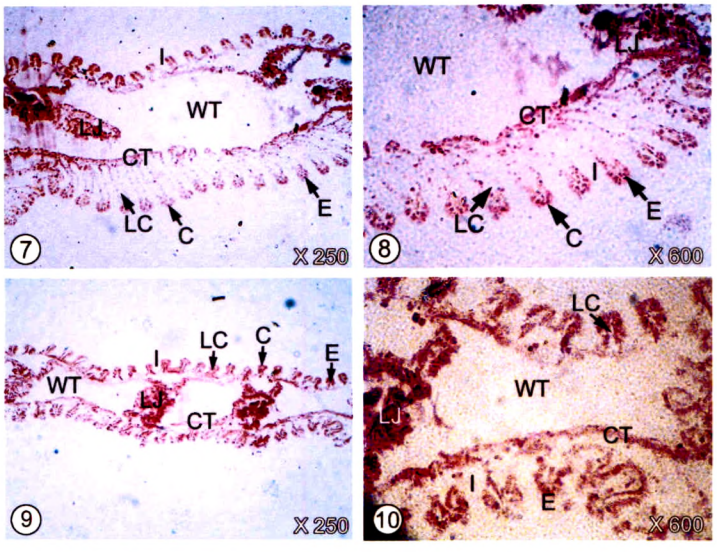


Fig. 7,8: Light microphotograph of gills of bivalve, *Lamellidens marginalis* exposed to 2% concentration of tannery wastewater for 10 days exposure period showing gill lamellae (L), epithelial cells (E), cilia (C), vacuolated in connective tissue (CT), increased lamellar cavity (LC) and inter lamellar cavity (I). Note presence of elongated water tubule (WT) with long obliquely placed inter lamellar junction (LJ) .

Fig. 9,10: Light microphotograph of gills of bivalve, *Lamellidens marginalis* exposed to 2% concentration of tannery wastewater for 20 days exposure period showing gill lamellae (L), epithelial cells (E), cilia (C), vacuolated in connective tissue (CT), lamellar cavity (LC) and inter lamellar cavity (I). Note presence of elongated water tubule (WT) with long obliquely placed inter lamellar junction (LJ) .

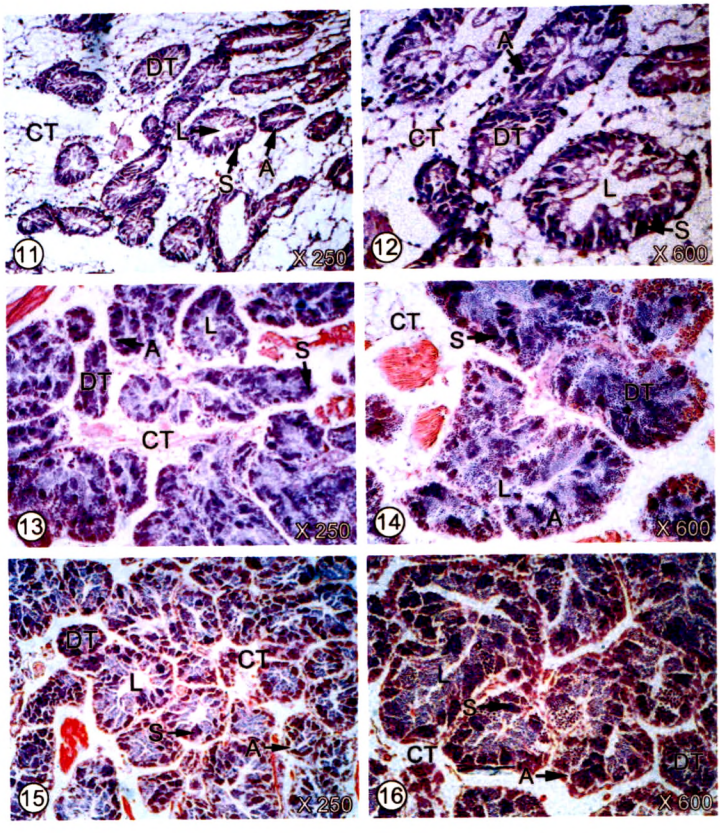


Fig. 11, 12: Light microphotograph of hepatopancreas of control bivalve, *Lamellidens marginalis* showing digestive tubule (DT) lined by absorptive cells (A) and secretary cells (S) with secretary material, wide lumen (L) with secretion. Note presence of normal connective tissue.

Fig. 13, 14: Light microphotograph of hepatopancreas of bivalve, *Lamellidens marginalis* exposed to 0.5% concentration of tannery wastewater for 10 days exposure period showing digestive tubule (DT) with elongated absorptive cells (A) and secretary cells (S) with vacuolation. Note disintegration of connective tissue (CT) and reduced lumen cavity (L).

Fig. 15, 16: Light microphotograph of hepatopancreas of bivalve, *Lamellidens marginalis* exposed to 0.5% concentration of tannery wastewater for 20 days exposure period showing digestive tubule (DT) with absorptive cells (A) and secretary cells (S) with accumulation of dark material. Note disintegration of connective tissue (CT) and reduction in size of lumen cavity (L)

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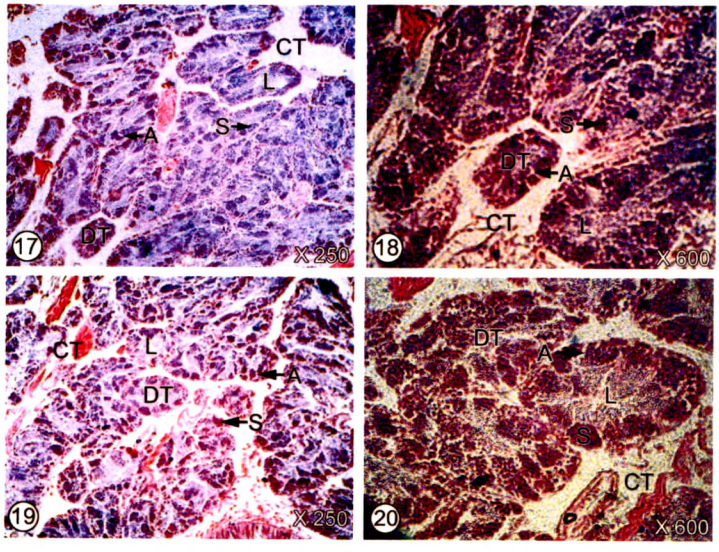

Fig. 17, 18: Light microphotograph of hepatopancreas of bivalve, *Lamellidens marginalis* exposed to 2% concentration of tannery wastewater for 10 days exposure period showing digestive tubule (DT) with absorptive cells (A) and secretary cells (S) with secretary material. Note disintegration of connective tissue (CT) and reduction in size of lumen cavity (L).

Fig. 19, 20: Light microphotograph of hepatopancreas of bivalve, *Lamellidens marginalis* exposed to 2% concentration of tannery wastewater for 20 days exposure period showing digestive tubule (DT) with absorptive cells (A) and secretary cells (S) with accumulation of dark material. Note disintegrated connective tissue (CT) and reduced lumen cavity (L).