DISCUSSION

CHAPTER IV

The present investigation aims at the understanding of the effect of tannery wastewater on a bivalve, *Lamelliidens marginalis* using acute and subacute toxicity tests. This chapter deals with the discussion on the results obtained during the investigation, analysis of tannery wastewater, general toxicity, biochemical changes, enzyme activities, metal accumulation and histopathological changes observed in gills and hepatopancreas.

4.1 physico chemical parameters of tannery wastewater:

The physico chemical properties of tannery wastewater used in this investigation are in agreement with the studies made by earlier investigator (Bosnic *et al.*, 2000). The properties of tannery wastewater are due to chemicals present in it. These chemicals alter pH, suspended and dissolved solids and chlorides of normal water, which leads to water pollution. Untreated tannery wastewater is analyzed for its physicochemical parameters which are collected from tannery located in Jawahar Nagar, Kolhapur. The physico chemical characteristics of tannery wastewater determined were pH, Turbidity, TS, TDS, TSS, Chlorides, COD, Chromium and Oil and Grease etc. methods used for the determination of these parameters were as per A.P.H.A., (1995).

In the present investigation pH of tannery wastewater detected was highly acidic and did not meet the general standards recommended by Central Pollution Control Board for the discharge of effluent into inland surface water for irrigation purposes. Discharge of untreated effluents with such a low pH into ponds, rivers or on lands for any purpose may be detrimental to fauna and aquatic biota such as zooplankton and fishes, since low pH level may affect the physiology of fishes (Fromn, 1980).

During leather manufacture, natural oils and grease are released from the skin. If fat liquor exhaustion is poor, some fatty substances may be produced through reaction when wastewater mingles. Floating grease and fatty particle collected to form a covering which then bind other materials, thus causing a potential blockage problem especially in effluent treatment systems. If the surface waters are contaminated with grease or thin layers of oil, oxygen transfer from the atmosphere is reduced (Bosnic *et al.,* 2000). In the present investigation oil and grease is within the permissible limit for wastewater discharge suggesting that the tannery wastewater does not get much contaminated with oil.

The suspended solid component of an effluent is the quantity of insoluble matter contained in the wastewater. These insoluble materials cause a variety of problems when discharged from tannery to fresh water bodies. In the present investigation total suspended solids level was found beyond the permissible limit (100 mg/lit) for effluent discharge. Moreover, presence of total suspended solids leads to turbidity resulting in poor penetration of light in aquatic system, thereby curtailing the light for photosynthetic activity. Further, the settling of suspended particles might lead to clogging off gills and respiratory surface of fishes (Alabasetr and Lioyd, 1980). These observations suggest that the release of tannery wastewater in to the water bodies which results in contamination of it.

Total dissolved solid level in the present studies was found to be exceeded the permissible limit of 2100 mg/lit prescribed by CPCB (1995). Tannery wastewater contains high dissolved and suspended solids because lime is used for loosening of hairs. High level of TDS in the effluent renders it unsuitable for irrigation and release in water bodies. According to Manivasakam (1984), high amounts of total dissolved solids were recorded in the tannery effluent could be attributed to processes like soaking, liming, dehairing, defleshing and deliming. Ramasubramanian *et al.,* (2004) recorded that total dissolved solids and total solids were high in untreated tannery effluent. Such

water bodies contaminated with high level of total dissolved solids are not suitable as habitat for aquatic animals.

In the present investigation Chemical Oxygen Demand of tannery wastewater was found to exceed the permissible limit of 250 mg/lit. This indicates that the tannery wastewater is unsuitable for existence of aquatic organisms due to reduction in the dissolved oxygen content (Raj *et al.,* 1996). High COD may be due to vegetable tanning and high amount of inorganic compounds, which are not affected by the bacterial decomposition (Nagarajan and Ramachandramoorthy, 2002). This depends on the chemicals used in the different leather making processes and their rate of biodegradability. This high chemical oxygen demand adversely affects on the dissolved oxygen content in the water sources. In such water aquatic animals could not survive due to which aquatic ecosystem get disturbed.

The total chromium content was low in the tannery wastewater because the work was carried out on vegetable tannery wastewater. If chrome discharges are excessive, the chromium might remain in the water (Bosnic *et al.,* 2000). Even in low concentrations, chromium has a toxic effect on aquatic animals, thus disrupting the food chain for fish life and possibly inhibiting photosynthesis. In the present investigation tannery waste used has chromium contents within the permissible limit therefore, discharge of tannery wastewater in to water bodies will not contaminate it with chromium, as this wastewater is from vegetable tannery.

In the present investigation chloride contents observed were beyond the permissible limit for wastewaters. Chloride is introduced into tannery effluents as sodium chloride usually on account of the large quantities of common salt used in hide and skin preservation or the pickling process. Increased salt content in groundwater, especially in areas of high industrial density, is now becoming a

serious environmental hazard. Chlorides inhibit the growth of plants, bacteria and aquatic animals in surface waters; high levels can lead to breakdowns in cell structure (Bosnic *et al.,* 2000). Therefore, water source where these wastewater released will be without any biotic component if proper dilution has not been allowed.

4.2 Accumulation study

Chromium has been used by industry and sources to the environment include metal plating and refining, the leather industry etc. In the present investigation chromium content were estimated in *Lamelidens marginalis* exposed to tannery wastewater at different concentrations for different exposures. The chromium content in the animal tissues is dependent on concentration of dose and exposure period. Chromium compounds are frequently encountered as environmental pollutants and have been known to produce toxic, mutagenic and carcinogenic effects in biological systems (Parlak *et al.*, 1999). According to these results, it is necessary to continue the studies on the effects of chemical speciation of Cr on mussels using biochemical and histopathological methods to illuminate the difference in their accumulation, loss and distribution in tissues. Industrial effluent contributing to aquatic pollution contains a vast array of toxic substances, which include heavy metals. Indiscriminate discharge of these wastes alters the quality of water and cause harm to aquatic animals. Bivalve molluscs circulate large amount of water through their bodies to obtain oxygen and food by ciliary mode of feeding. They are known to accumulate metal ions from the surrounding environment to a very high level relative to the concentration of water and serve as bioindicators of metal pollution (Nambison *et al.*, 1977).

High amount of heavy metals in mantle may be due to more surface area of the organ and in gills as it circulates more water for respiration (Satyaparameshwar *et al.,* 2006). Metabolic processes like respiration in mussels

are reported to be adversely affected by the presence of certain heavy metals even at sublethal concentrations (Scott and Major, 1972; Baby and Menon, 1986). The short-term effects of chromium and nickel on macromolecular variations in fishes have been reported by number of investigators (Jha and Jha, 1995; Sornarej *et al.,* 1995). Khangarot and Tripathi (1990) reported affected gill histology in air breathing fish after immersion in hexavalent chromium for 7 days. In the present investigation chromium was below detectable limit in the control animals but the animals exposed to different concentration of tannery wastewater accumulated enough amount of chromium may be responsible for the physiological changes in aquatic animals.

4.3 Biochemistry and Metabolism

The aquatic animals in polluted water show some physiological and histopathological changes. This results in the alterations in the biochemical parameters and enzyme activities. Therefore, it is necessary to assess the impact of tannery wastewater, which highly pollutes fresh water bodies used for the disposal of it. In the present study, fresh water bivalve, *Lamellidens marginalis* was used to study the impact of tannery wastewater. The biochemical paramters studied were protein, glycogen, lactic acid and cholesterol. Similarly enzymatic alterations in acid phosphtase, alkaline phosphtase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, adenosene triphosphtase and lactate dehydrogenase were also studied.

In the present investigation the protein contents in different tissues of fresh water bivalve, *Lamellidens marginalis* exposed to different concentrations of tannery wastewater to different exposure periods showed significant decrease. Satyaparameshwar (2006) reported similar observations in bivalve, *Lamellidens marginalis* along with an increase in the level of RNA might indicate an increased catabolism of protein and decreased synthesis after exposure to chromium. Depletion in protein level may be due to diversification of energy to meet the

impending energy demand when animal is under stress (Ambrose *et al.,* 1994). Fall in protein content in tissues under stress of pollutants may be due to altered enzyme activities (Jadhav and Lomate 1982; Chaudhari *et al.*, 1989). Depletion in protein also suggests increased proteolysis and possible utilization of the products of their degradation for metabolic purposes (Chandravarthy 1994). Somnath (1991) have reported protein in fish under tannic acid stress are likely to undergo hydrolysis and oxidation through TCA cycle to meet increased demand for energy caused by stress. Heavy metals in general could interfere in protein synthesis. Another possibility for the observed protein reduction might be due to the blocking of protein synthesis or protein denaturation or interruption in the amino acid synthesis (Vijayavel *et al.,* 2006). The gradual accumulation of toxicants present in the industrial effluent has been shown to cause drastic reduction in the protein content in the fish tissues (Roger 1980). The decreased trend in protein content observed may be due to inhibition of its biosynthesis (Tripathi and Verma 2004; Yadav *et al.*,2007) or enhanced degradation of protein and metabolic utilization of the ketoacid into gluconeogenesis pathway for the synthesis of glucose (Tilak *et al.*, 2005) under the fertilizer industry effluent induced stress. It may be said that effluent have stimulated the breakdown of carbohydrates and proteins resulting to amino acids leading to its accumulation and thereby elevating transaminase levels reaching a maximum after exposure to pollutants indicating toxication mechanism in animal (Dhanapakiam *et al.*, 2006). Further close relationship exists between the mitochondrial intensity and transaminases level (Suhasini *et al.,* 1979) and any change in the function of this organelle will reflect on the aminotrasferases. The decrease in protein content of fish after exposure to nickel (Desai *et al.*, 2002; David, *et al.,* 2003) and copper (Indira and Ramalingam, 1996) suggest an acceleration of protein synthesis (David, Mushigiri and Prashanth, 2003). Increased protease activity in the tissues of nickel exposed snails could be due to lysosomal instability or cellular destruction by high concentrations of the metal (Sternlieb and Goldfischer, 1976). Increased free amino acids indicates stepped up proteolysis and fixation of

ammonia to keto acids resulting in amino acids may contribute to the regulation of ionic imbalance and to the production of energy during stress (Lowenstein, 1972). Increased levels of ammonia in the exposed snails during lethal concentration corroborate the increased level of protein hydrolysis, since ammonia is the main product of protein catabolism (Martin *et al.,* 1983). In the present investigation decrease in protein contents in animals exposed to tannery wastewater was directly proportional to concentration of tannery wastewater and exposure periods.

In the present investigation glycogen contents in *Lamellidens marginalis* exposed to tannery wastewater at different concentrations and different exposure periods showed significant decrease. The work carried out by Kulkarni and Utkar (1983) supported these observations and this might be due to glucose stores decreases remarkably as the rate of utilization of glucose increased during stress condition in freshwater snail. The glycogen content of the tissue was considerably lower than control level indicating the impaired tissue glycogen metabolism (Reddy *et al.,* 1988). An impaired secretion of insulin agrees well with the observation that muscle glycogen was slightly decreased due to stress. Thus, glucose uptake in muscle and subsequent muscle glycogen formation seemed to be retarded (Larsson, 1976). In the white muscle fibers glucose is stored in the form of glycogen and that despite high levels of NADH - tetrazolium reductase present, the energy for intermittent firing activity is provided by glycolysis (Graaf *et al.*, 1991). Glucose in circulation is used as an instant energy source (Nagabhushanam *et al.*, 1983). Saxena and Saxena (1996) have been reported the decline in the protein and glycogen content in ovaries of fish exposed with pesticides and reduction in the ovarian glycogen may be due to inhibition of glycogenesis. Depletion in the muscle glycogen of fish might be due to the rate of utilization exceeds the rate of supplementation when exposed to pesticides (Khillare, 1990). Glycogen is known to be ready to break down for the energy requirement of the fish. Awari and Gaikwad (1990) have been reported

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cadmium stress on fish and suggested that fish must have put very heavy demand on glycogen for excess energy requirements and in spite of some possible replenishment through glyconeogenesis. In the present investigation glycogen decreased in bivalve, *Lamellidens marginalis* exposed to different concentrations of tannery wastewater for different exposure periods may be due to the stress exerted by tannery wastewater on the important organs like gills involved in exchange of respiratory gases.

In the present study lactic acid contents were increased in the tissues of *Lamellidens marginalis* exposed to different concentrations of tannery wastewater for different exposure periods. Similar results were found in the same animal exposed to copper sulfate (Satyaparameshwar *et al.*, 2006). Decrease in glycogen with increase in lactate level indicates the diversion of pyruvate, the end product of glycolysis for anaerobic metabolism instead of incorporating it into aerobic reactions of Krebs cycle. Increase in lactic acid content in fish has been reported by Singhal (1994) and Narayan (1993) after lead exposure during anoxic condition. Alternatively the accumulation of lactic acid in the digestive gland, in absence of glycogen breakdown can be attributed to the transport of this acid to the digestive gland from foot and mantle. Adaptive mechanism to mobilize the accumulating lactates for oxidation in liver and conversion into glucose might occur into fishes to meet the energy demand during fasting. Walton and Cowey (1979) have also reported that lactate is the best precursor for gluconeogenesis in most of the species examined and thus the enhanced level of lactate in the tissues clearly shows the hepatic gluconeogenesis from this metabolite during fasting (Premakumari and Shantha, 1992). Other investigator during heavy metal toxicity in rainbow trout has reported similar hyperlactemic conditions (Larsson and Haux 1982, Spry and Wood 1984). Further, hyperlactemia, due to utilization of hepatic glycogen seems to be a common phenomenon in fish following muscular exertion (Black *et al.*, 1962). Anitha and Ramkumar (2006) found increased level of blood lactate in fishes suffering from

pollution stress might be due to release of lactic acid into blood following cellular damage and hypoxic conditions. In the present investigation lactic acid accumulates in fresh water bivalve, *Lamellidens marginalis* exposed to tannery wastewater but these results are insignificant as compared to control bivalves.

In the present study cholesterol content in *Lamellidens marginalis* exposed to tannery wastewater decreases significantly, similar results for cholesterol content has been reported in different tissues with pesticide treated fishes (Piska *et al.*, 1992). Cholesterol is a precursor of steroid synthesis and the cholesterol esters are thought to be the storage from the free cholesterol utilized in biosynthesis of steroids (Saxena and Saxena, 1996). Brycesmith and Waddson (1974) emphasized that reduced ability to metabolize pyruvate would also results in less acetyl co-enzyme 'a', being available from the synthesis of fatty acids and cholesterol than a loss of lipids. It is due to inhibited pyruvate metabolism. Tewari *et al.*, (1987) reported lead exposure brought a decline in de novo synthesis of cholesterol and the mobilization of cholesterol for conversion in to bile acids in fish. Jyoti and Narayan (2001) however, attributed the decline in serum cholesterol and inturn a disturbed steriodogenesis to liver dysfunction due to carbaryl stress in fish. On the other hand, Gill and Pant (1988) reported that the decrease in serum cholesterol was due to the heavy metal stress affecting absorption of dietary cholesterol in fish. The cholesterol level in crab of all tissues studied after chronic exposure to nickel showed a significant decrease suggesting its role in the supply of fuel to meet the metabolic demands (Mayekar, 2007). In the present investigation the cholesterol contents in animals exposed to tannery wastewater decreased significantly and these are concentration and dose dependent.

In the present work acid phosphtase and alkaline phophtase activity increased in different tissues of fresh water bivalve, *Lamellidens marginalis* exposed to tannery wastewater at different concentrations and exposure periods.

Acid phosphtase plays a vital role in the autolytic degradation of tissues and dissociation of dead cells. It acts as a good indicator of environmental stress condition of the biological system (Verma *et al.,* 1980; Murti *et al.,* 1984). Alkaline phophtase splits various phosphorous esters at alkaline pH, mediates membrane transport and involved in glycogen synthesis (Gupta and Rao, 1974). Saxena and Saxena (1996) have been reported increased acid phosphatase activity and decreased alkaline phosphatase activity in fresh water fish, murrel exposed with pesticides. Acid phosphatase is a lysosomal enzyme, which hydrolyses the phosphorous esters in acidic medium and thus, it is logical that the enzyme is hydrolytic in its function and acts as one of the several acid hydrolases in the autolysis process of the cell after its death. Alkaline phosphatase is a brush border enzyme, which splits various phosphorous esters at an alkaline pH. It is well known that phosphatases are involved in carbohydrate metabolism, growth and differentiation, protein synthesis, synthesis of certain enzymes, secretary activity and transport to phosphorylated intermediates across the cell membranes. Elevation in the acid phophtase level has also been reported In the fish exposed to pesticides (Ansari and Kumar, 1987; Gill *et al.,* 1990), nickel plating factory effluent (Ramesh *et al.*, 1993). Similarly increase in alkaline phophtase activity has also been reported in shrimp and freshwater fish due to exposure to cadmium (Thaker and Haritos, 1989). It has been observed that acid phosphtase and alkaline phosphtase activity in animals exposed to tannery wastewater increased significantly so the metabolic activities in animals may change.

Enzymes play an important role at the junction between the carbohydrate and protein metabolism by interconnecting strategic compounds viz. ketoglutarate, pyruvate and oxaloacetate on one hand and alanine asparate and glutamate on other hand. A close relationship exists between the mitochondria intensity and transaminases level (Baimtenico, 1974., Suhasini *et al.*, 1979) and any modification in the organization of mitochondria might alter the enzyme

associated with it. In view of this it may be suggested that chemicals that are present in the effluent might be acting on the carbohydrate metabolism. The increased transaminase activity might be due to increase in transamination reaction i.e. is transferring of NH_2 group from amino acid to a keto acid. It may be said that effluents have stimulated the breakdown of carbohydrate and proteins resulting to amino acids leading to its accumulation and thereby elevation in transaminase levels reaching maximum after exposure to tannery effluent indicates the toxification mechanism in the animal. The glutamate pyruvate transaminase and glutamate oxaloacetate transaminase found to be elevated in fish after lead exposure (Chandravathy and Reddy, 1994). Heavy metals induce hypoxic conditions (Dalavankatasuppaiah, 1984) offer evidence to the comparative role of glutamate oxaloacetate transaminase and glutamate pyruvate transaminase under lead induced manifestations.

In the present investigation glutamate oxaloacetate transaminase and glutamate pyruvate transaminase activity increases after exposure to tannery wastewater. Adhan (2002) reported increased activity of glutamate pyruvate transaminase and glutamate oxaloacetate transaminase from more polluted fresh water were a sign of some functional damages in tissues. John (2007) and Dhanatakian *et al.*, (2006) have been reported increase in glutamate oxaloacetate transaminase and glutamate pyruvate transaminase activity in fresh water fish due to pesticide exposure. Increased activity of glutamate oxaloacetate transaminase and glutamate pyruvate transaminase in *Pila globosa* during exposure to sublethal and lethal concentration indicates an active transamination of amino acids which provide keto to serve as precursor in the synthesis of essential organic constituents under heavy metal stress (Venkatramana and Radhakrishnan, 1987; David *et al.*, 2003). Observations by these investigators support the observation in the present study that the stress produced by tannery wastewater exposure in bivalves found significantly increased glutamate oxaloacetate transaminase activity mostly in 20 days exposure where as

glutamate pyruvate transaminase activity increased significantly to both 10 and 20 days exposure periods.

In the present investigation Adenosine tirphosphatase activity in bivalve, *Lamellidens marginalis* exposed to tannery wastewater was decreased. An inhibition of ATPase in tissue of the clams exposed to various pollutants including heavy metal was also reported by investigator Wang and Fisher (1999) support the findings of the present investigation in the bivalve, *Lamellidens marginalis*. Cellular Na+ regulation and solute transport based on the cell transmembrane Na+ gradient depend on the activity of the Na,K-ATPase, a key enzyme in the interactions between the environment and the animal cell. The evaluation of the activity of this enzyme in bivalve molluscs resistant to environmental changes may allow us to relate biochemical parameters to metabolic, seasonal and environmental factors, and may even provide new tools for detecting anomalous environmental conditions. Low enzyme activity levels are tentatively related to hypoxia, the lower oxygen content (Borgatti *et al.*, 2003). Disruption of cellular ion regulation as a result of heavy metal exposure may be related to inhibition of sodium-potassium adenosine triphosphatase (Na-K ATPase) activity (Renfro *et al.,* 1974). The bulk of cellular energy in normal cell is derived from ATP. Mitochondrial ATPase is responsible for the production of ATP by mitochondrial oxidative phosphorylation. Any impairment of ATPase activity therefore results in less availability of cellular energy in the form of ATP (Dosalah *et al.,* 1979). In the present investigation ATPase activity in bivalve, *Lamellidens marginalis* exposed to tannery wastewater was decreased but the changes are insignificant which accounts for the energy demand in stressed animals.

In the present study Lactate dehydrogenase activity was decreased in *Lamellidens marginalis* exposed to tannery wastewater and similar observations were reported by Satyparameshwar *et al.,* (2006) in same animal exposed to copper sulfate. It might be due to glycolysis under anaerobic conditions where

pyruavate is reduced to lactate and lactate oxidation is inhibited so as to supply NAD+ for glyceraldehyde- 3- phosphate dehydrogenase activity. Decrease in the activities of enzyme shows the inhibition of oxidative metabolism of the mussels appears to be shifted from aerobic to anaerobic type of metabolism. Carbohydrate metabolism is a major source of energy production of many fish species and activity of lactate dehydrogenase has been an easy target for the action of various xenobiotics. The decrease in LDH activity has been reported in fish exposed to fertilizer effluent (Yadav *et al.*, 2007). The inhibitory effects of industrial effluent on the activity of lactate dehydrogenase may be mediated via formation of enzyme-inhibitor complex leading to impairment of carbohydrate metabolism (Sharma and Gopal 1995). Singhal (1994) has been reported inhibited lactate dehydrogenase activity in lead induced fishes. Lactate dehydrogenase and pyruvate dehydrogenase are generally associated with cellular metabolic activity. Lactate dehydrogenase is present in most of the animal tissues and is involved in the interconversion of lactic acid to pyruvic acid that acts as a pivotal enzyme between glycolytic pathway and tricarboxylic acid cycle. Decreased activity of lactate dehydrogenase towards the formation of lactate in animal tissues proves that role of glycolysis in this organ is more likely to supply precursor for biosynthetic process like gluconeogenesis rather than to provide pyruvate for oxidation of NADH (Walton and Cowey, 1982). Shaffi (1993) discussed lactate dehydrogenase variations in relation to the breakdown of gas exchange at the lamellar level, visceral hypoxia, hypoglycemia, impaired aerobic and anaerobic pathways, and formation of a metalloenzyme complex. In the present investigation lactate dehyrogenase decreased due to exposure to tannery wastewater more significant changes found in 20 days exposure periods, which leads to histological and physiological changes.

4.4 Histopathological study

In recent years, industrial development has resulted in the increased levels of toxic pollutants in environment. Histopathology is used to study the impact of toxic materials as it provides the real picture of the toxic effects of xenobiotics in vital functions of a living organism. A histopathological study with light microscopy is necessary for the description and evaluation of potential lesion in aquatic animals exposed to various toxicants (Meyers and Hendricks, 1985). Toxic effects are often due to physical changes in the tissue at the cellular and ultra structural levels. Toxic chemicals with water current enter in the gills of bivalves and find their way in to different tissues of the body where they affect normal metabolism.

In the present investigation bivalve, *Lamellidens marginalis* exposed at different concentrations of tannery wastewater for different exposure periods showed changes in the structure of the gills followed by the disintegration and vacuolization of connective tissues. Similar kinds of observations were reported in gills of bivalve, *Parreysia cylindrica* exposed to pesticides (Lomate and Wayekar, 1998). Bivalve gills are unique organs that show continuous terminal growth by the addition of new elements in correlation with the lifelong increase in shell size (Neumann and Kappes, 2003). In aquatic vertebrates and invertebrates gills are the organs directly exposed and the most susceptible to environmental variations hence they are target tissues for different pollutants (Rodriguez *et al.,* 2005). Moreover, the gills are the main organs involved in respiration, maintaining a constant current, filtering the water and collecting and retaining food particles. Once water is filtered, the trapped food particles are absorbed by gill mucus. If these trapped particles have been exposed to metals (Cheung and Wong, 1992) and chemicals, the gill tissue will show a high content of these contaminants. In bivalve molluscs such as the mussel, *Mytilus galloprovincialis*, the gills are key organs involved in nutrient uptake, digestion and respiration (Mendikute *et al.,* 2005). Damage to the gill lamellae is potentially serious

because of the possible effects on respiration. Chromium induced the marked ultrastructural alterations in the gill epithelia of fishes (Khangarot and Tripathi, 1990). Jadhav (1993) reported hyperplasia regeneration of respiratory epithelial cells, fusion of secondary gill lamellae, vacuolization in epithelium and connective tissue and damage to chitinous rods of gill lamellae of bivalve after exposure to pesticides. Franchini and Ottavianic (2000) noted the similar observations in molluscs after exposure to hebicide. In bivalves, the gills are the most important organs for respiration and osmoregulation and it is the first organ that comes into contact with the pollutant first. There is increasing evidence that toxic compounds have a potential to cause the most harm to tissues and organs that contact first (Timbrell, 1991). Increased amount of mucus production has some detoxification properties (Bender and Westman, 1976). It is thought to be highly appropriate physiological response. Dalela *et al.,* (1979) have also been reported injury to gill epithelium is a common response to extreme levels of environmental pollutants followed by separation of gill epithelium from basement membrane, necrosis, fusion of gill lamellae, erosion at distal end of gill lamellae. The reason is that the gills are very important absorption place for the toxic chemicals. These histopathological changes reduce the respiratory area thereby reducing the respiratory and osmoregulatory potential. It also indicates a decrease in energy metabolism due to degeneration of respiratory epithelium and the damage to gill tissue may finally result in tissue hypoxia. These pathological changes in respiratory gill might have resulted in such shift from aerobic to anaerobic pathway in tissue under pollutant stress. In the present investigation gills of the bivalves exposed to tannery wastewater showed accumulation of dark material. The pollutants present in tannery wastewater may be accumulated in the gills of these animals and adversely affect the respiratory processes. Damage to the gill filament and epithelial cells may be the reason for the changes in the metabolic activities in the animals.

Hepatopancreas of the bivalve, *Lamellidens marginalis* exposed to different concentrations of tannery wastewater for different treatment periods, report that there were major changes in histological architecture. Disintegration and sometimes vacuolization in connective tissue have been observed. The lumen of the digestive tubule has reduced and many times seen irregularly shaped. These changes were increased with increase in exposure period as well as dose. Similar type of observations have been reported by Moore *et al.*, 1982; Mohite, 2002; Bayne *et al.*, 1978. In hepatopancreas the epithelial cells are involved in the secretion of digestive enzymes by secretary cells as well as in absorption and storage of nutrients by absorptive cells. Miywaki *et al.*, 1962 have been reported similar light microscopic changes in hepatopancreas of clam exposed to heavy metal suggesting metabolic activity i.e. TCA cycle and lipid metabolism has suffered. The examination of microscopic changes in the hepatopancreas of *Lamellidens marginalis* due to sub acute toxicity of tannery wastewater is important to determine the effects of pollutants found in tannery wastewater on cellular structure in 10 and 20 days exposure period. The severe damage in the hepatopancreas may be due to breakdown of proteins. The increase in the ACP activity is partially responsible for the destruction of proteins and also results in impairment of RNA and protein synthesis thus results in depletion of proteins content. In the present investigation bivalve, *Lamellidens marginalis* exposed to tannery wastewater showed structural changes in the hepatopancreas. In these animals changes observed in the hepatopancreas are like vacuolation in connective tissues and reduction in the lumen cavity. The secretary cells showed accumulation of dark material with large vacuolations and this material may contain pollutants from tannery wastewater. The changes in the hepatopancreas of animals exposed to toxicants interfere the metabolic processes.

The foregoing discussion suggests that the tannery wastewater is highly polluted and not suitable to discharge directly in the natural the water bodies

without any treatment. It also seen that the animal model fresh water bivalve, *Lamellidens marginalis* exposed at 0.5% and 2% concentrations of tannery wastewater for 10 and 20 days exposure period showed accumulation of the pollutants as well as chromium metal. It also alters biochemical composition as well as metabolism which are clearly shown by enzyme studies. It also had its impact on the histology of the animal which is shown by the study of gills and hepatopancreas by light microscopy. Overall it can be assumed that the exposure of the animals in the freshwater is released are not suitable for the growth and development of biota.