DISCUSSION

CHAPTER IV

4.1 Assessment of water quality :

The present study deals with the assessment of the religious activities like immersion of Ganesh idols during Ganesh festival on water quality of the selected water bodies from Kolhapur city namely Rajghat, Sandhyamath, Irani Khan, Kotiteertha Lake, Rajaram Lake, Panchganga Ghat and Rajaram Bandhara. The water samples from the sites were collected before, during and after the Ganesh festival. The physicochemical parameters analysed were pH, turbidity, total dissolved solids, hardness, dissolved oxygen, biochemical oxygen demand, chemical oxygen demand, nitrates, phosphates, calcium, magnesium and heavy metals such as copper, zinc, lead and chromium. The water analysis was carried out with the standard methods prescribed by A.P.H.A. (1995) for two subsequent years 2006 and 2007 and results obtained are discussed here.

In the present investigation pH of water bodies showed significant increase in values after the idol immersion as compared to values before the immersion. This may be due to carbonate and bicarbonate of calcium and magnesium dissolved in water after idol immersion (Prasada and Narayana, 2004). Hydrogen ion activity or pH is an important factor and increase in it has direct effect on human as well as aquatic life. Similarly pH results were reported by Sinha *et al.,* (2003), Madhuri *et al.,* (2004) in case of river water pollution.

In the present study turbidity of water bodies found to be increased significantly as compared to turbidity before immersion. Turbidity plays an important role in aquatic living system and increase in it affects the light penetration through water. It has been taken as a major influencing factor in deciding the water quality of pond. Similar results were reported by

Mariappan (1999). Turbidity is caused by suspended matter such as clay, silt, finely divided organic and inorganic, soluble coloured organic compounds and planktons (Mathur, 2006). Increase in turbidity interferes with the penetration of light in the water body affecting the photosynthesis process. In the present study, turbidity at Panchganga Ghat and Rajaram Bandhara showed high values due to rainy season. The observations presented in this study are supported by the results recorded by Dhote *et al.,* (2001) in case of Twin lakes situated in Bhopal after immersion of idols.

In the present study the dissolved oxygen of the water bodies decreased significantly after the immersion of idols during festival. In case of Panchganga Ghat and Rajaram Bandhara no significant decrease was observed in pH as these sites are from rivers and no marked alterations were observed for these sites. Dissolved oxygen of Irani Khan showed reduced DO i. e. DO 0.35 mg/l in 2006 whereas normal DO was observed in 2007 as the total cover of water hyacinth and silt and debris were removed from the Irani Khan by Kolhapur Municipal Corporation. Dissolved oxygen indicates physical chemical and biological activities in a water body and indicator of water quality. Dissolved oxygen affects the solubility and availability of many nutrients and therefore controls productivity of aquatic system. Dissolved oxygen of all the sites in the present study showed decreasing trend as nutrients and other organic and inorganic matter were added to the water bodies. The reduced concentration of dissolved oxygen could be attributed to the presence of organic matter in high proportions (Paka and Rao, 1997).

In the present study, a rise in BOD levels was observed after the idol immersion because constituents of idols like bamboo hay, flowers and coir, etc. provide organic matter for decomposition hence creating a demand for oxygen. The input of biological and non-biological substances deteriorates the lake water quality and enhances silt load in the lake. The floating materials released with idols, after decomposition results in eutrophication of the lake (Leland *et al.,* 1991). The higher values of BOD have direct correlation with the increase in the nutrient level of the lake due to immersion activity. Plaster of Paris is nothing but calcium sulphate and it does not dissolve in water readily. It cuts off the supply of oxygen to small organisms that are food for fish, ultimately disturbs food chain in the aquatic ecosystem. Plastic and nylons are non-biodegradable and become permanent trash, spoils river banks, seabed and beaches. Sometimes the idols and other materials like offering were removed from the water immediately by the volunteers and kept aside the water bodies. This material gets degraded there only creating unhygienic conditions. When this degraded material again washed off in the river and lake contributes to high BOD.

In the present study, total hardness of the water bodies showed increase in values after immersion of idols in water bodies as compared to values before immersion. This increase is due to introduction of Plaster of Paris in water as it contains mainly calcium which increases hardness. Total hardness is the property of water which prevents the formation of lather with soap, caused mainly by the multivalent metallic cations (Guruprasad and Narayana, 2004). Major contribution of hardness comes from calcium and magnesium. Significant increase in hardness in the present study after the immersion of idols is supported by the results obtained by Rasool *et al.,* (2003). Calcium is linked with the carbon dioxide and is an important constituent of the skeletal structure of organisms. Rasool *et al.,* (2003) showed higher values of calcium content in Rankala Lake during rainy season which may be due to dissolution of idols in the water. High calcium content is an indicator of eutrophic water. The presence of calcium and magnesium along with their carbonates, sulphates and chlorides make water hard. Like other elements magnesium is also found dissolved in water and influence the fauna (Rath *et al.,* 2000). Magnesium is essential for chlorophyll bearing organisms, since it goes into the composition of pigments. Though non-toxic magnesium contributes to increase in hardness values of water bodies (Reddy and Vijaykumar, 2001). In the present study magnesium may have contributed in increasing the hardness of water after idol immersion.

The present study showed that total dissolved solids of the water bodies increased significantly after the immersion of Ganesh idols in the water bodies. This increase is mainly due to the dissolution of idols with colours, various offerings, etc. in the water bodies. Similar results were reported by Manohar *et al.,* (2007), Srivastava and Srivastava, (2003). Total dissolved solids increase the load of chemical oxygen demand of water body.

In the present study the nitrates and phosphates of the water bodies showed increase in values as compared to values before immersion. Similar results were reported by Patil and Dongare (2006) and Manohar *et al.*,(2007) after the immersion of idols in the water bodies. Plaster of Paris used to make idols contain high amounts of phosphates along with other hazardous chemicals such as zinc oxide, calcium oxide, lead, etc. Increased organic load in the water bodies alters the nitrate content in the water. In the present study, chemical oxygen demand of water bodies showed increase in values as compared to values before immersion. In case of Panchganga Ghat and Rajaram Bandhara decrease in COD values was observed after 5^{th} day of immersion which may due to the continuous flow of river and the subsequent dilution. Also the river has many other inlets of pollutants such as agricultural run off with pesticide and fertilizer content or industrial effluents. Due to idol immersion, organic as well as inorganic load increases in the water body. Paints used for colouring the idols have constituents like acetone, butanol, lead, chromium, cadmium and mercury most of which are not soluble in water (Manohar *et al.,* 2007). The chemical oxygen demand of the water bodies found to be increased which correlates the decrease in dissolved oxygen.

In the present study the heavy metals like copper, lead, zinc and chromium in the water bodies showed increase in values after immersion of idols as compared to values before immersion. After the idol immersion in the water bodies, mainly due to colours heavy metals like copper, lead, zinc and chromium get introduced in the water. The ultimate sink of these metals is the sediments of these water bodies. In the presence of lead, the mucous in fishes is coagulated over the entire surface of the body including the gills causing the respiratory distress (Goel, 1996). The large affinity of Pb^{2+} ions for thiol and phosphate containing ligands inhibit the biosynthesis of heme and thereby affects the membrane permeability of kidney, liver and brain cells (*Koul et al.,* 1988). Zinc is one of the most abundant essential trace elements in the human body. Excess of Zinc affects the metabolism of humans by changing their mineral and enzyme budget especially of children and persons already suffering from irregular metabolism. Zinc has a strong capacity of combining with –SH and imidazole containing ligands, thereby destroying the normal metabolism. Copper is most toxic to aquatic life. Algae are very sensitive to copper and can be killed at concentrations as low as 0.5 mg/l. Copper has also shown varying toxicities towards different species of fish. In higher organism copper interfere with –SH groups of certain enzymes and can result in brain damage. Chromium concentration as low as 0.032 mg/l can inhibit the growth of freshwater algae. Lower forms of aquatic life are more sensitive than high forms (Goel, 1996). When these heavy metals move up in the food chain they show the effect of biomagnification. The toxic limit for lead is 1 ppm whereas permissible limit set by ICMR for Pb is 0.5 ppm. In the present study lead content at Irani Khan showed highest value

i. e. 8.38 ppm which is much higher than the toxic limits and can pose threat to living organisms.

In the present study, increase in pH, hardness, nitrates, phosphates, calcium, magnesium, zinc, copper levels in the water bodies was observed. Similar results have been recorded by Patil and Dongare, (2006) in case of Rajaram and Kotiteertha Lake from Kolhapur city after immersion of Ganesh idols during the festival. The findings of the study revealed that idol immersion activity have negative impact on water quality of the lake. The regular practice of idol immersion in the lake will certainly lead to irreversible damage to the water bodies and disturb the ecosystem. During idol immersion different types of materials such as clay, straw, Plaster of Paris, iron rods, woods, dyes along with offerings deteriorate the water quality. Thus, though as compared to other heavy pollution loads Ganesh festival, rather the use of chemicals and the immersion practices cause pollution leading to organic and inorganic substances mixing in water bodies. Among all the pollutants added to the water bodies during Ganesh festival colours of the idols form a major group of pollutants released in water bodies. The toxic heavy metals which enter in the aquatic ecosystem are not degradable and remain persistent in the water as well as sediments affecting the natural environment of the ecosystem. As bottom of the water bodies act as ultimate sink for all the elements, it becomes important to study the toxic effects of the colour pigments which contribute heavy metals in the water on an aquatic organism.

4.2 Accumulation study:

Aquatic and terrestrial molluscs have been shown to have a high capacity for metal accumulation (Martin and Flegal, 1975). This has made them suitable bioindicators of metal pollution in the environment. However, when these metals are present in high concentrations in the environment, this propensity to bioconcentrate them results in lethal effects in molluscs (Amin, 1972). The exposure of molluscs to sublethal concentration of metals therefore provides an opportunity to investigate biomarkers that indicate the metal induced stress in these organisms.

In the present study the bivalve, *Lamellidens marginalis* were exposed to 400 ppm and 900 ppm colour pigments for 10 days and 20 days showed significant increase in accumulated heavy metals such as copper, zinc, lead and chromium. Zinc, copper and lead are considered as conservative metals as they are not subject to biodegradation. They are incorporated in the body by aquatic organisms and their concentration gradually increases during the life span of an organism. The accumulation of heavy metals from the water columns by bivalve molluscs has been found to be relatively rapid and to reflect ambient exposure levels closely (Boyden and Phillips, 1981). Bivalves in general can withstand high levels of lead since they have a detoxifying mechanism for lead and store large quantities in the digestive glands (Bryan and Vysal, 1978). The accumulation of heavy metals in the order Pb > Cu > Zn has been reported in the heterodont bivalve species by Chaudhari and Hazra (2003).The accumulation of copper and other heavy metal is mediated by metal inducible metallothioneins, which are cysteine-rich metal binding proteins with a high affinity for divalent metals (Hamer, 1986).

Metals may occur in the aquatic environment as hydrated ions or they may form a number of soluble and insoluble complexes with different organic and inorganic ligands. They are also found to be associated with colloidal particles and suspended particulate matter. It has been reported that the amount of copper accumulated in the organs of fish *Labeo rohita* exposed to copper increased with increase in exposure period by Radhakrishnaiah, 1988. Similar observations have been reported in the present study in the bivalve, *Lamellidens marginalis* exposed to colour pigments.

Episodic increments of copper and zinc in shells of *Cerastoderma edule* (Price and Pearce, 1997) and accumulation of zinc, lead and copper in the outer calcitic layer of *Mytilus edulis* shell (Fuge *et al.*,1993) has been observed. A rapid accumulation of lead and zinc in shells of *Perna viridis* has been studied by Yap *et al.*, 2003. Bellotto and Miekeley (2007) have shown a significant concentration enhancement in soft tissue of *Perna perna* with the magnitude of this enhancement following the order Cr > Ni > Cd > Cu > Pb.

Essential metals like copper and zinc are regulated to some degree by particular molluscs so that tissue concentrations may be largely independent of exposure concentration and duration. Partial regulation of zinc has been reported for mussels (Phillips and Rainbow, 1994; Rainbow, 1995). However, copper is apparently less well regulated than zinc in mussels (Langston *et al.,* 1998). Zinc may occur in relatively high concentrations in the natural environment and is the least toxic of the three experimental metals; elevated concentrations are known to impede physiological processes (Abel, 1976; Watling and Watling, 1982; Depledge *et al.,* 1994).

In the present study the stress exerted by the accumulated heavy metals exhibit various biological effects in the bivalve. Rate of oxygen uptake may be expected to be elevated with increasing metal exposure as a result of an increase in metabolic demand to excrete or molecularly bind metals; an increase in filtration to meet increased metabolic demands; compensation for insufficient functioning of structurally impaired gas exchange surfaces and repayment of oxygen debt in event of recent valve closure. However, oxygen uptake may be depressed due to ongoing valve closure to avoid metal stress and the utilization of anaerobic pathways; severe destruction of epithelial surfaces inhibiting gaseous exchange;

metal inhibition of enzyme systems and a compensatory lowering of heart rate and associated gill perfusion in attempt to reduce the rate of metal distribution throughout the body . Similar observations have been reported by Anandraj *et al.,* (2002) in bivalves when exposed to heavy metals. In the present study significant increase in heavy metal values in soft body of the bivalves was observed after exposure to colour pigments. Though individual level of a heavy metal seems low but the synergistic effect of all metals lead, copper, zinc and chromium has altered the biochemistry of the bivalves as discussed further.

4.3 Biochemistry and metabolism:

The results on the toxicity of colour pigments to the freshwater bivalve, *Lamellidens marginalis* showed that there are marked differences in the sensitivity to colour pigments. In the present study the freshwater bivalve, *Lamellidens marginalis* exposed to 400 ppm and 900 ppm colour pigments for 10 days and 20 days showed significant decrease in protein content as compared to control. As the concentration and exposure period increases the protein content decreases. Proteins are the important cellular constituents forming a major part of the cell boundaries along with lipids. During stress conditions the protein synthesis and inter-conversion between amino acids, glucose and fatty acids to liberate energy get affected (Mane *et al.,* 1986). Protein metabolism involves interaction between proteins, amino acids, several enzymes and co-enzymes (Harper *et al.,* 1978). Under proteolysis, enhanced breakdown dominates over synthesis, while in the case of anabolic process; increased protein synthesis dominates the protein breakdown. Depletion in protein content has been reported by Chaudhari *et al.* (1998) in Gastropods after exposure to herbicide which showed stress condition of the organisms as in the present study. Depletion in protein in the present investigation may constitute as a physiological mechanism and play a comprehensive role under stress, to provide intermediates to Kreb's cycle. Under proteolysis, enhanced breakdown dominates over synthesis, while in case of anabolic process; increase protein synthesis dominates the protein breakdown.

It is well known fact that bivalve molluscs during anaerobiosis have capacity for alternate fermentation pathways. During anaerobiosis, the fate of fermentatively generated ATP is hydrolysis for the support of various cellular work functions. Mantle tissue of *Lamellidens marginalis* exposed to abnormal pH might have an additional demand of proteins. The use of the protein as a substrate is indicated by a quantitative increase in ammonia excretion in case of bivalves (Muley and Mane, 1995).

In the present study reduced protein synthesis may be due to the inhibition of the enzymes involved in protein synthesis. Lead is known to reduce cell respiration and oxidative phosphorylation. This impairment of electron transport mechanism might reduce the availability of ATP. Exposure to zinc sulphate decreased the protein content in the liver and gill of fish *Labeo rohita* fingerlings which is due to the utilization of protein for energy through proteolysis and a dearrangement in protein synthesis machinery to some extent as reported by Indira (1995). In the present investigation similar condition must have occurred due to which protein contents showed a decrease. Metal affects the protein metabolism by decreasing the protein content which affects the nutritive value of organism (Meenakshi, *et al.*, 1998).

In the present study reduction in protein content suggests that they may be channelised into TCA cycle through aminotransferase system to cope up with excess demand of energy during toxic stress. Similar results were reported in freshwater bivalve, *Corbicula striatella* after exposure to carbaryl (Jadhav *et al.,* 1995).

Protein which serves as energy source, during stress of condition in the present study decreased as compared to control as the concentration of the colour pigments and the exposure period increased. The progressive decrease in protein content of the tissue with duration of exposure indicates a gradual accumulation of toxicant resulting in gradual increase in toxicity (Tilak, 2002). More energy to mitigate any stress conditions may be obtained from carbohydrates, proteins and lipids. Organisms try to detoxify the toxicant by spending more energy and thereby showed reduction in protein level. *Lamellidens marginalis* showed shifts in its metabolism for survival and maintenance. Additional demand of energy leads to accelerated catabolism of proteins (Muley and Mane, 1995). Dearrangement in protein synthetic mechanism may be the cause of its depletion (Indira, 1995).

The heavy metals in general interfere with protein synthesis. Further under stress conditions, the dietary protein consumed by the organism is not stored in body tissue and hence the treated organisms meet out their extra energy requirements from body proteins which are mobilized to produce glucose, the instant energy which is made available for the organism by the process of gluconeogenesis (Syversen, 1981, Vasanthi *et al.,* 1990, Kasthuri and Chandran, 1997). Thus, undesirable effects caused by heavy metals to the organisms and their hazards are elegantly reviewed by many workers Ghosh and Shrotri,1992, Durairaj and Selvarajan, 1992, Radhakrishnaiah *et al.,*1992, Rajasubramaniam, 2006.

Proteins can be expected to be involved in the compensatory mechanisms of stressed organisms. In the present study an acceleration of protein catabolism and the possibility of impaired protein synthesis may be the reason for decrease in proteins. This has been reported in *Pila globosa* after exposed to Nickel by David *et al.,* 2003. Decrease in total protein content was also reported in prawn due to toxicity of carbaryl by Bhavan and Geraldine (2002).

Proteins after being synthesized are usually deposited in the cytoplasm. These are rapidly used to replace the loss of protein that occurs during physiological stress. This may alternately be due to the utilization of amino acids through transamination and deamination which might have supplied necessary keto acids. Another possibility is that there might have occurred the blocking of protein synthesis and proteolysis. Such decrease in proteins has been reported in *Heteropneustes fossilis* due to nickel by Nanda *et al.,* (2000). Krishnamohan *et al.,* (1985) and Chandravathy and Reddy (1994) have suggested that decline in the muscle protein content may be due to reduced protein synthesis, increased proteolysis and also due to utilization for metabolic processes under lead toxicity. Reduction in protein content could be due to its utilization to mitigate the energy demand when the organism is under stress. Similar results were reported in fishes by Rao *et al.,* (1987) and Baskaran *et al.,* (1989).

In the present investigation, as compared to controls, a significant decrease in glycogen was observed in all the tissues for both the exposure periods. Mechanisms of accumulation, storage and loss of polluting substances in bivalves consist of several alternative pathways, varies with species and chemical form of substance and mode of assimilation (Gokhale, 1990). Glycogen and glucose play a key role in carbohydrate metabolism of animals which are used for the intermediate metabolic requirements to meet the energy demand caused by stress. In the present study decrease in glycogen is due to the high energy demands of the organism under toxic stress which leads to hyperglycemia.

The effect of various heavy metal salts on the oxygen binding and transporting properties of the respiratory pigments. The essential copper and zinc act pathologically on the respiratory system primarily by disruption of gill function resulting in internal hypoxia, although respiration can be accomplished even at "high" sub-lethal concentrations (Spicer and Weber, 1991).

A marked decrease in hepatopancreas glycogen in the present study indicates an extensive utilization of stored glycogen to meet the extra demands of energy necessitated under toxic stress. The stress of acute hypoxia (Heath and Pritchard, 1965), physical disturbance (Nakano and Thomson, 1967) or exposure to pollutants (Villanan *et al.,* 1988) is accompanied with rapid depletion of muscle and liver glycogen reserves. The reduction in stored glycogen content in the liver and muscle of treated fish indicate that during the exposure periods of stress the demand for the extensive utilization of energy is met by glycogen, mediated through glycogenolysis (Meenakshi, 1998).

Reduction in glycogen in the present study is probably due to its more rapid breakdown which releases glucose into circulatory system to meet the increased energy requirements in a stressed condition. Lack of oxygen to active muscles leads to anaerobic metabolism of glycogen causing its depletion. Decrease in glycogen content in *Scylla serrata* after exposed to Thiodan has been reported by Ghosh and Shrotri (1992). Decrease in glycogen is an induction of hyperglycemia which might have occurred by stepping up of glycogenolysis.

Decrease in glycogen with an increase in lactic acid levels indicates the diversion of pyruvate, the end product of glycolysis, for anaerobic metabolism instead of incorporating it into aerobic reactions of Kreb's cycle. Decrease in glycogen, increase in lactic acid and decrease in lactate dehydrogenase has been reported in freshwater bivalve, *Lamellidens marginalis* exposed to copper sulphate by Satyaparameshwar *et al.*, (2006) which supports the observations in the present study.

Decrease in glycogen was also found in all the tissues of fish, *Channa punctatus* when exposed to phenyl mercuric acetate (Karuppasamy, 2000). Decrease in glycogen content have been reported *Labeo rohita* to $HgCl_2$ (Jagadeesan, 1994), *Catla catla* to Chromium (Vincent *et al.*, 1995). Decrease in glycogen and increase in lactic acid contents has been also reported by Ramalingam, 2002 due to $CuSO_4$ toxicity (Tilak, 2002). Mane *et al.*, (1986) reported a decrease in gill glycogen levels in *Indonaia caeruleus* after exposure to Fenithion. Tort *et al.*, (1984) showed that zinc induced significant decrease in gill glycogen in *Scyliorhinus canicula* which is indicative of an increased rate of glycolysis due to a hypoxic situation (Ferrando and Andreu-Moliner, 1991). Decrease in liver and muscle glycogen due to increased demand for energy during stress. The increase in lactic acid, the end product of anaerobic glycolysis has been reported in *Labeo rohita* due to ammonia toxicity by Acharya *et al.*, 2005. Decrease in muscle and liver glycogen was also reported in *Labeo rohita* due to toxicity of Selenium by Rajasubramaniam (2006). As pointed out by Mc Leay and Brown (1979); Veeraiah and Prasad (1998) the decline in muscle glycogen could have resulted because of anaerobic stress. In the present investigation due to stress of the colour pigments bivalves closed their valves most of the times which lead to insufficient oxygen uptake and increase in anaerobic condition which may have altered the carbohydrate metabolism in bivalves.

Cholesterol has multiple effects on biological membrane and can modulate its fluidity, phospholipids spacing, enzyme activity within the membrane. Decrease in cholesterol during stress can substantially affect the membrane fluidity. Decrease in total proteins and ATPase activity has been reported in rats after exposure to perchlorate (Vijayalakshmi and Motlag, 1992). Decrease in cholesterol has been reported in different tissues with pesticide treated fishes (Ghosh and Chatterjee, 1989, Piska *et al.,* 1992), which in correlation with our experiment carried out with colour toxicity as well as PoP and shadoo clay exposure.

The enhanced glycolytic mechanism would indicate the animal's dependency on the anaerobic breakdown of glucose to alternate the increased anoxic condition. Conversion of glucose to lactic acid leads to increase in lactic acid content (Karuppasamy, 2000). The stress of acute hypoxia, physical disturbance or exposure to pollutants is accompanied with rapid depletion of muscle and liver glycogen reserves. The reduction in stored glycogen content in the liver and muscle of treated organisms indicates that during the exposure periods of stress the demand for the extensive utilization of energy is met by glycogen mediated through glycogenolysis. The increase in lactic acid content is possibly due to glycogenolysis and operation of glycolytic pathway in liver and muscle accounting for hyperglycemia. Excess utilization of these metabolites indicates the possible arrival of anoxic conditions (Meenakshi, 1998). Glycogenolysis was also reported during exposure to various pollutants considered stressful (Narendra and Srivastava, 1981, Gupta, 1982) due to hypoxic situation. Similar results were obtained by Grant and Mehrle, (1973), Bhavan (2002). In the present study also similar condition has occurred due to hypoxia. Increase in lactic acid whereas decrease in lactate dehydrogenase was observed during the experiment.

In the present investigation increase in lactic acid was observed in the bivalves exposed to colour pigments. The liver and muscle tissues always show higher lactate content. The formation and accumulation of lactic acid in tissues are due to insufficient supply of oxygen (hypoxia) and due to functioning of lactate dehydrogenase enzyme in reverse pathway to convert more pyruvate to lactate. Increase in lactic acid and subsequent decrease in protein and glycogen was reported in fishes exposed to endosulfan by Ramalingam (1990). The increase in lactic acid content is possibly due to glycogenolysis and operation of glycolytic pathway in liver and muscle accounting for hyperlactimia (Meenakshi, 1998). The anaerobic and aerobic scope of an animal depends upon the residual level of lactate content in the body tissues and the consequent clearance of accumulated lactate after short burst of activity. The tissues exhibit differential ability to accumulate as well as to eliminate the lactate through specific pathways during stress (Ramalingam and Subramaniam, 2000). Increase in lactic acid levels in crabs has been reported after exposure to lead by Tulsi *et al.,* (1992). In the present investigation lactic acid accumulated in freshwater bivalve, *Lamellidens marginalis* exposed to colour pigments which indicates the "lactic acidosis" in the bivalve.

In the present study acid phosphatase and alkaline phosphatase showed significant increase in values as compared to control. Phosphatases are key lysosomal enzymes known to play a pivotal role in cytolysis and differentiation processes and acute energy crisis. They also serve as markers for the evaluation of disease or pathological conditions in biological systems. Acid phosphatase is a lysosomal marker enzyme and can be used as a reliable tool for the biological assessment of heavy metal pollution. The potential of this biomarker in freshwater biomonitoring has been demonstrated in zebra mussels (Giambrini and Cajaravalli, 2005). A close and complex relationship exists between lysosomal response and antioxidant responses to metals and exposure to copper and mercury greatly decreased lysosomal membrane stability (Regoli *et al.,* 1998). Hyperactivity of acid phosphatase occurs only at the time of stress. Mussels from the most heavily polluted stations exhibit reduced lysosomal membrane stability and relatively higher lysosomal heavy metal content compared to mussels from less polluted areas. The intermittent high and low enzyme activity in both gills and hepatopancreas in mussels exposed to the different concentrations of metals shall also be attributed to valve closure, often reported in mussels exposed to high concentrations of metals. Oxidative stress is reported to be more in gills as inhibition of antioxidants is more pronounced in the gills (Subramanian and Ambat, 2007). Induced lysosomal destabilization in both gills and hepatopancreas revealed by fluctuation in ACP activity has been reported in *Lamellidens corrianus* after exposure to copper for different periods by Rajalaksmi and Mohandas (2005). Increase in acid and alkaline phosphatase activity has been reported in fish, *Rasbora daniconius* after exposure to Malathion by Patil *et al.,* (1990).

Any alteration in the internal membrane permeability can have severe consequences such as leakage of hydrolase enzymes, which perturb the internal environment of the cell. Environmental contaminants exert their effects through direct toxic action on the tissues or through more subtle alterations in homeostatic mechanisms such as immune system. Gills are the main interface between the organism and its environment and hence these tissues are frequent targets of environmental pollutants. Exposure to copper significantly reduces antioxidants in the gill tissue of molluscs; the high enzyme activity can also be attributed to oxidative stress caused by metal. Digestive cells are an important target of metals and that accumulation of metals may result in impairment of lysosomal structure and function.

Acid phosphatase 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 in the biological system. ALP splits various phosphorus esters at alkaline pH, mediates membrane transport and is involved in glycogen metabolism. ALP is also involved in permeability processes and forms a part of the enzyme system involved in the active synthesis of protein from the nucleic acid complex. The occurrence of inflammatory and necrotic changes in tissues due to toxic effects of endosulfan resulted in the rupture of cellular and lysosomal membranes leading to increased ACP and ALP levels in hepatopancreas of prawns has been reported by Bhavan and Geraldine (2000). In the present investigation increased phosphates activities may be due to oxidative stress forced by colour pigments.

The transaminases serve as strategic link between carbohydrates and protein metabolism under environmental stress. GOT, a key enzyme in nitrogen metabolism and energy immobilization in invertebrates is often used as biological index of stress. Increased activities of GOT and GPT during exposure to toxicants indicates an active transamination of amino acids which provide keto to serve as precursors in the synthesis of essential organic constituents under heavy metal stress (Knox and Greengard, 1965). Enzymes are very sensitive macromolecules and are easily affected by even smaller changes either in internal or external medium.

In the present investigation GOT and GPT in the bivalves exposed to colour pigments was found to be increased significantly. The observed elevation of aminotransferases in experimental bivalves may be due to increased mobilization of proteins needed under stressful conditions. Increase in GOT has been reported in freshwater bivalve *Lamellidens corrianus* exposed to iron and manganese by Ingle *et al.,* (1994).

Elevations of GOT and GPT have long been used as indicators of damage to hepatic and cardiac tissue. These aminotransferases have both a mitochondrial and cytosolic localization. It has been proposed that elevations in these enzymes in tissues are related to increased energy demand as organisms attempt to overcome the toxic effects of pollutants

(Reddy and Yellamma, 1991). In general, alterations in enzyme activities in organisms exposed to pollutants reflect homeostatic adjustments in metabolic pathways as part of general adaptation syndrome (Giesy *et al.,*1988). It is also possible that increase in activities of these enzymes were partly due to a need to deaminate the amino acids resulting from possible increased tissue breakdown as a result of toxic effects of pollutants (Masola, 2003). GOT and GPT are key enzymes in amino acid metabolism. Their substrates and products are linked to energy generating pathways. In bivalve molluscs it has been shown that environmental stress can result in increased α- amino acid excretion (Bayne, 1973), which can be correlated with aminotransferase activity. A high degree of GOT and GPT was observed in tissues of the bivalve mollusc, *Mytilus edulis*, particularly in the gills and mantle, in response to exposure to organic pollutants (Narvia, 1997). Similarly, GOT and GPT were increased in the gill tissue of *carp* when exposed to copper sulphate (Karan *et al.,* 1998).

Aminotransferases are important as they convert amino acid into ketoacids and incorporate them into TCA cycle. Both GOT and GPT in *Lamellidens marginalis* were found to be increased after exposure to chromium (Satyaparameshwar *et al.,* 2006). After exposed to the acid stress increase in GOT, GPT and ALP has been reported in freshwater catfish by Singh and Reddy (1990). An increase in the activity of GPT and decrease in protein content has been reported in *Heteropneustes fossilis* due to manganese by Garg *et al.,*(1989).

Changes in transaminases are stress mediated. The increase in GOT activity represents necrosis or damage of the organs. This indicates preponderance of anaerobic nature of carbohydrate metabolism possibly to meet the increased energy demands under sustained and prolonged toxic stress. Such changes have been studied in fish, *Cyprinus carpio* due to Cypermethrin toxicity by Sivakumari *et al.,* (1997).

The Dissolved Oxygen levels less than 2 or possibly 3 ppm will result in death of fishes (Swann, 2000). Increased activities of GPT and GOT, LDH found in fishes of more polluted water are a sign of some functional damage in such tissues as the heart and liver, which in turn lead to the leakage of these cellular enzymes. It is also known to be very symptomatic of hepatic cytotoxic injury and elevated activities are also thought to be associated with disease multiplicity. GOT is mainly located in liver and any change in its activity is suggested as reflecting the functional state of liver. Such changes in enzymes have been studied in catfishes in polluted water by Adham, 2001. Decrease in glycogen and increase in GOT and GPT has been studied in *Labeo rohita* under the stress of pesticides by Rajamannar and Manohar (2000). GOT and GPT can be useful as biomarkers of water pollution due to copper only in the sublethal range of concentrations where they are induced. The fall in these enzymes at higher concentrations of copper results in the death of organisms (Masola, 2003).

Elevated levels of GOT and GPT have been observed in freshwater fish, *Clarias batrachus* due to toxicity of carbaryl by Jyothi and Narayan, 2000. Mukhopadhyay *et al.,* (1982) reported increase in GOT and GPT in fish *Clarias batrachus* under the stress of Carbfuran. Similar observations were made by Goel *et al.,*(1984) in the serum GOT and GPT levels of *Clarias batrachus* due to alachlor toxicity and attributed to liver damage. In the present investigation also increased transaminase activities are observed which may have altered the histological structures of gills and hepatopancreas in the bivalve, *Lamellidens marginalis.*

In the present investigation decrease in ATPase activity was observed after exposure to colour pigments for 10 days and 20 days. ATPase, a membrane bound enzyme plays an important role in the homeostasis mechanism of calcium and is found to be ubiquitous in animal cell. *Lamellidens marginalis* distributed throughout the Indian subcontinent is an important freshwater mussel. Filter feeding bivalves have high bioaccumulation potential for heavy metals which at higher concentration can cause serious metabolic, physiological and structural impairments. Toxicity of heavy metals is due to alteration in enzymatic activities by binding the functional groups or displacing the metal associated with the enzyme and is also associated to structural protein modifications leading to increase in membrane permeability (Pattnaik *et al.*, 2007). Induced ATPase activity by heavy metal ions has been reported in the gills of *Mytilus galloprovincialis* by Viarengo *et al.,* (1993). Considering the role of ATPase in shell formation and divalent ion transport, its inhibition by heavy metal ions present in the surrounding water may directly or indirectly influence the mussel health. Heavy metals tend to react with essential protein sulfhydryl group and structural protein and cause modifications, resulting in membrane permeability. As polluted water bodies contain most of the heavy metals in different concentrations, the combined effect of these may lead to much more pronounced effect on mussel health. The main mechanism underlying the lactic acid production is the hypoxic condition induced by the toxicants in the intracellular atmosphere. Increase in lactic acid and alteration in ATPase activity has been studied in *Lamellidens marginalis* under methyl parathion stress by Reddy *et al.,* (1994). In the present investigation ATPase activity in bivalve, *Lamellidens marginalis* exposed to colour pigments was decreased significantly indicating the extra energy demands for the stressed animals.

4.4 Histological study:

Histopathological biomarkers at the tissue level of the biological organization are intermediate between molecular and individual levels (Hinton, 1997). They follow biological changes and resultant lesions can be observed histologically (Teh *et al.,* 1997). Anthropogenic and natural stressors may contribute to the histopathological and histochemical alterations. Alterations in the enzymatic reactivity and increases in prevalence and severity of histopathologic biomarkers in clams tended to align with increasing with body burdens of metals.

In the present study, the gills of the bivalves exposed to different concentrations of colour pigments for 10 days and 20 days showed increase in the gill filament cavity and interfilament cavity. Vacuolation in connective tissue was observed due to disintegration. Clams collected from sites polluted with heavy metals showed digestive gland atrophy and gill inflammation (Teh *et al.,* 1999). This supports the observations made in the present study. Brown and Newell (1972) concluded that the decrease in oxygen consumption they observed for *Mytilus edulis* was due principally to the action of copper on gill cilia rather than the inhibition of cellular respiration. The respiratory surfaces are most likely the first target of water borne heavy metals. The death of animals exposed to lethal concentrations of heavy metals to the coagulation or precipitation of mucous on the gills which increased the diffusion distances for oxygen and resulted in death by suffocation. The cytological damage occurred takes the form of separation of intact epithelial cells and cellular necrosis. Acute exposure of molluscs to heavy metals results in profound changes in the gills and other respiratory surface ultrastructure and the production of "slime" on the outer surface (Cheng and Sullivan, 1975). Injury to the gill epithelium is a common response to the pollutants followed by separation of gill epithelium from basal membrane. In the present investigation the gills of the bivalves showed damaged epithelium with vacuolation in the connective tissue. Also the altered structure of the basal filament showed the effect of colour pigments on the gills of bivalve, *Lamellidens marginalis.*

In the present study the digestive glands of the bivalves exposed to 400 ppm and 900 ppm concentrations for 10 days and 20 days showed elongation with decreased lumen and vacuolation in the connective tissue. In gastrpods and bivalves, the digestive gland is the major site of heavy metal storage. Molluscs possess an efficient detoxification mechanism chelating the heavy metals specially Cd and Cu with specific amino acids, the metallothioneins (Simkiss and Mason, 1983, Pozzi and Merlini, 1977). Light structure of the digestive gland of molluscs reveals the toxic effects at the cellular level induced by acute and chronic exposure to lethal and sublethal concentrations of metal contaminants. Increase in tubular lumen diameter, irregular shape of digestive cells, presence of vacuoles, detachments of tubular teguments have been reported by Tripp and Fries, (1987).

Reduction in mean epithelial thickness of the digestive gland of gastropods and bivalves was proposed as an indicator of environmental quality assessment. Changes in the histological structure of the gill as a result of long term exposure to sublethal concentrations of pollutants have not been related to death of the organism but rather, are a measure of the metal uptake and release of the organism. Copper showed more remarkable toxic effect on the digestive tubules compared to lead nitrate as cell necrosis and vacuoles were clearly demonstrated. The combined metals exhibited a destructive effect on the digestive tubule, where tubular deterioration and disintegration of the digestive cells were observed which pointed out the synergistic interaction effect of copper and lead (AbdAllah). Hepatopancreas serve as a sink for heavy metals in the bivalves. In the present investigation it has been observed that the synergistic effect of different heavy metals present in the colour pigments used for colouring the idols damaged the digestive gland structure in the freshwater bivalve, *Lamellidens marginalis.*

From the results of the present study it is clear that the colour pigments used for colouring the Ganesh idols for the festival affect the biology of the freshwater bivalve, *Lamellidens marginalis.* The biochemicals like protein, glycogen, cholesterol showed decrease in values when exposed to these colour pigments. Lactic acid showed increase after exposure to colour pigments. Enzyme activities also showed alterations when bivalves exposed to 400 ppm and 900ppm colour pigments for 10 days and 20 days. Histopathological study of the bivalve also supported the damage caused to the respiratory system and the digestive glands of the bivalves.

Thus, various types of pollutants enter in the water body after the idol immersion. Among all these pollutants heavy metals are of more concern as they show tendency to accumulate in various life forms. In the present investigation it was observed that heavy metals pose various biological altrations in the freshwater bivalve, *Lamellidens marginalis.* Apart from colours used for decorating the idols many physicochemical parameters of water such as pH, turbidity, reduced DO, increased BOD and COD have a major role in the life processes in an aquatic ecosystem.