DISCUSSION AND CONCLUSION

Our industry and agriculture are the two main sources of chemical pollution. Chemical pollution is becoming particularly worrying issue in developing India because thousands of their companies continue to dump untreated industrial waste water filled with different toxic materials and other chemicals in rivers. In India, for instance, more than 40% of their rivers are considered unsafe for drinking. Sodium pentachlorophenate (NA-PCP) is a kind of organic-chlorine pesticides, which is widely used all over the world. In India, NA-PCP is used as biocide, fungicide ,molluscide , paper and wood preservative acquiring the obvious effects. NA-PCP has a good water-solubility, but it degrades slowly in nature.

NA-PCP can cause pollution by long-term and unsuitable use. To protect the environmental safety and aquatic animal health, it is very important to study the entry in the environment and organisms of NA-PCP, clarify the mechanism of its toxicity and mortality. The aims of this study were to investigate the effects NA-PCP contaminant on its acute toxicity in freshwater bivalve Lamelldens marginalis and to discuss of this traits that make them useful as potential indicators of pollution. For decades, bivalve have been used as a sentinel species to monitor pollution in the aquatic environment (Foster 1978, Farrington 1983, Colombo 1995, Peven 1996, Blackmore 2003). In an attempt to overcome these obstacles, native mussels frequently are collected worldwide, extracted, and analyzed for pollutant tissue burdens to provide preliminary information at sites suspected of contamination, or to monitor chemical/waste discharge effluents. However, in order to effectively understand and correlate the relationship between concentrations of pollutants in the aquatic environment to potential toxic
effects, it is best to have an understanding of the toxicity tolerance behavior changes, oxygen uptake, histological and biochemical alteration by/from mussel tissues. (Neff, 1996). Traditionally, mussels have been used for environmental monitoring due to concern for pollution in coastal and estuarine areas (Farrington 1983, Salanki 1989, Beliaeff 2002,). However, freshwater bivalve have been increasingly utilized in order to assess the quality of lakes, rivers, and streams of concern, not only for the protection of human health, but also to better explain recent major declines of many freshwater mussel populations (Jacobson, 1997).

Toxic substances at very low concentrations might be perceived organism’s sensory system. If the stimulant is recognized harmful, avoidance may follow leading to escape in motile organisms stationary such as freshwater bivalves, avoidance may take in the form of reducing exposure external body surfaces through mucus production. In the present study, L. marginalis when exposed to NA-PCP, at different lethal concentrations, test animals showed behavioral changes like closing of shell, secretion of mucus and as exposure period increases, foot comes out from the shell indicated death of the animal. Shell closing mechanism might be the protective device against the toxicant used and provides good tolerance in the molluscs (Nagaratnamma and Ramamurthi, 1982b). Chaudhari et al., (1988) reported many behavioral changes in pesticide exposed snail, Bellamya bengalensis like sudden withdrawal of foot inside the shell, closing of operculum and mucus secretion. Mucus secretion was also observed in Corbicula striatella on exposure to pesticides (Jadhav, 1993) and in Parreysia favidens against heavy metal exposure (Bhamre et al., 1996). Avoidance reaction is an example of a stereotyped behavior i.e a trait that may be quantified objectively and that shows little variation between individuals that has been
shown to be sensitive to pollution. In a field experiment Saunders and Sprague (1967) showed that Atlantic salmon avoided localities that were contaminated with copper and zinc. Also, in laboratory experiments avoidance of pesticide by carp (Ishida and Kobayashi, 1995) and avoidance of acidified water in *Rhinichthys atratulus* and *Salvelinus fontinalis* (Newman and Dulloff, 1995) were documented. Furthermore, behavior has also been used in ecotoxicological investigations. Warner and co-workers (1966) were among the first to use behavior in the investigation of effects of pollution when they studied movement and avoidance behavior in fish exposed to toxaphene. The main aim of the work by Warner *et al.*, (1966) was to identify behavioral variables that could be used to detect effects of sub lethal concentrations of pollution. Their hypothesis was that behavioral variables will give an early warning to pollution and that behavior is a comprehensive variable in the detection of effects of pollution since alterations in behavior is the consequence of several physiological alterations. In the present investigation of, test animals *L. marginalis* responded to NA-PCP exposure at lethal concentration and observed secretion of mucus, closing of valves and then opened to come out of foot indicated death of the animal.

Due to economic and ecological importance, as well as their sedentary life, molluscs have assumed as major role in monitoring contaminants worldwide (Folke and Birklund. 1986). The present investigation completes previous reports made on the studied area concerning the trace heavy metals (*Abdenour et al.*, 2000, 2004) on *D. trunculus*. Cadmium, copper, lead and zinc are the most present trace heavy metals in the wastewater. The mean values recorded exhibited seasonal profile was observed for the different metals following decreasing order: Zn, Cu, Pb and Cd for *D. trunculus*. The concentrations measured in the soft tissues of *D. trunculus* revealed a
significant effect of season for heavy metals measured. It is assumed that heavy metal uptake occurs mainly from water, food and sediment. However, effectiveness of metal uptake from these sources may differ in relation to ecological needs and metabolism of animals and concentrations of the heavy metals in water, food and sediment as well as some other factors such as salinity, temperature, interacting agents (Roesijiadi and Robinson, 1994) Similar observation reported by (Pierce,. 1978, Tagatz, 1981, Fisher, 1986 Fisher, and Wadleigh. 1986, Haynes et al., 1997, Fishelson et al., 1999; Usero et al., 2005 and Sidoumou et al., 2006). The objective of this study was to evaluate acute toxicity of NA-PCP concentration in bivalve *L. marginalis* collected from the river Godavari at Kaigaon Ta, Gangapur, and District: Aurangabad. In the present study, the LC$_{50}$ values for NA-PCP exposed to test animal *L. marginalis* for 24, 48, 72 and 96 hours showed significant difference. The measured values is attained their lowest values indicated high toxicity response. Variations of the lethal concentration may be due to changes of the organism’s tissues weight rather than to any variability in the absolute metal content of the organism. There are many factors which may affect the bioavailability and intake of heavy metals by the organisms, such as variations in the physicochemical parameters in the surrounding water like, temperature, pH, total suspended solids, dissolved organic carbon. Among others (Gupta, and Rao. 1982); variations in water flow, which may cause dilution of the concentrations of pollutants in water (Hedtke, *et al.*, and variations in the physiology of organisms (Naimo *et al.*, 1992). These factor remains in constant interaction in the environment and these
interactions could cause of different intake patterns of heavy metals by organisms.

Fresh water bivalves were chosen for this study because they meet many of the requirements of a good biological monitor (Phillips, 1980). They are somewhat sedentary, regionally abundant, long lived and have adequate tissue mass for analysis. They readily uptake many pollutants and their body burden seems to reflect mean exposure levels over time (Naimo, 1995). Consequently, such organisms have been largely used in programmes of biological monitoring in freshwater (Manly and George, 1977; Foster and Bates, 1978; Millington and Walker, 1983).

The determination of the LC$_{50}$ value is of immense importance since it provides fundamental data for the design of more complex disposal model. The values obtained are highly useful in the evaluation of safe level or tolerance level of a pollutant. Mary (1984) has reported that the LC$_{50}$ values depend on the concentrations of pesticides and also with the time of exposure. The 96 hours LC$_{50}$ value was the low; however the mortality scored was high. Srinivasulu Reddy et al., (1985) reported that the LC$_{50}$ values and the exposure period showed inverse relation. The result shows that the LC$_{50}$ values decreased with increase in exposure period and vice-versa and also the 95 % confidence limits. Sultana, (1995) reported the toxicity evaluation of CuSO$_4$, HgCl$_2$ and ZnSO$_4$ was conducted on the bivalve, L. marginalis and Lc$_{10}$ and LC$_{50}$ values were calculated, they observed the HgCl$_2$ was more toxic than CuSO$_4$ and ZnSO$_4$ respectively. Eldon et al., (1981) studied the effects of low concentrations of heavy metals on the bivalve. Similar kind of results were obtained by Patil, (1993) in L. marginalis when exposed to heavy
metals like CuSO₄, HgCl₂ and CdCl₂. Toxicity evaluation of ZnSO₄ to the freshwater snail, *Viviparus bengalensis*. Alam, (1984) heavy metal ions in sufficient higher concentration might kill organisms or cause adverse effects that change aquatic community structure. Catherine and Jayapaul (1993) studied the acute toxicity of Zn to the green mussel, *Perna viridis* and recorded the highest values of Zn accumulated in the viscera followed by gills and mantle. Ong *et al.* (2001) studied the toxicity of cadmium, copper and zinc in the clam, *Donax faba*, and observed that the Zn is toxic when excess amount occurs in the body.

In the present study on *L. marginalis* exposed to NA-PCP for 96h. it has been observed that the test animals showed 0.96 ppm LC₅₀ values. Thus this species revealed comparatively more sensitivity to NA-PCP. LC₅₀ values of NA-PCP in on *L. marginalis* in the present study can be accounted for the difference in the change in the physiological status of the animal in coordination with the changing environmental parameters. The rise in temperature, low oxygen content and low food availability in the water body inhabiting the animals in accounting for the physiological demand to the survival of the species (Goodnight,. 1942).

Addition of toxicant stress increased the demand and thereby the animal becomes sensitive to the toxic stress. our observation are supported by (Bose, and Fujiwara. 1978) they reported that NA-PCP compounds influences aquatic invertebrates and suggested these compound must be acting on the organized enzyme sites of the cells, in the aquatic concentration initially increases and further decreases the rate of oxygen consumption causes mortality. (Stuart, and Robertson, 1985) reported similar observation and stated NA-PCP compound are known to
cause variety of effects on mitochondrial which correlates with increase in oxygen consumption at initially and further decreases, oxygen consumption decreases as pollutant concentration increases gradually to inhibit ions translocating ATP. Similar observations were reported by many researchers (Gupta, 1983, Hanumante, and Kulkarni, 1979, Gupta, and Rao, 1982 Kaila, and Saarikoski, 1977, Stuart, and Robertson, 1985, Erben 1982 Rao, et al., 1986).

Mortality of *L. marginalis* is a more sensitive measure of toxicant. The percent survival rate of the bivalves decreased with increasing concentration and period of exposure. The evaluation of LC$_{50}$ concentration of pollutants is an important step before carrying out further studies on physiological changes in animals. The toxicity of particular pollutant depends on many factors such as animal weight and size (Macek et al., 1968), its developmental stages (Kamaldeep and Toor, 1977) period of exposure, temperature (Macek et al., 1968 Doris Feind et al., 1995) pH, and hardness of water and dissolved oxygen contents of the medium.

In the present study the *L. marginalis* exposed to NA-PCP the acute toxicity level was expressed in terms of LC$_{50}$ values. The LC$_{50}$ values were calculated. The LC$_{50}$ values for 24 h, 48 h, 72 h and 96 hrs for NA-PCP oxide were found 2.6 ppm, 2.2 ppm, 1.6 ppm, and 0.96 ppm respectively.

NA-PCP considered as major environmental pollutants causing ecotoxic, cytotoxic and mutagenic and mortality effects in animals (Conklin, and Rao, 1978). NA-PCP compounds are known to cause a
variety of effects on mitochondrial, which correlates with increase in oxygen consumption. (Wulf and Byington, 1975; Aldrich, 1976). Furthermore, NA-PCP compounds are known to inhibit ions translocating ATPase (Selvin et al., 1970; Selvin 1976 Gupta et al.,2006.). NA-PCP found to be extremely toxic to aquatic biota as demonstrated for a variety of different organisms in vivo and in vitro, Choudhury, et al., (1996). Many ecotoxicological studies on organisms of different evolutionary level have been reported. (Dalela, et al., 1980,Alzieu and Heral, 1984; . Fisher and. Wadleigh. 1986. Bryan et al., 1989; Fioramonti et al., 1997; Alzieu, 2000).

Aquatic organisms have the ability to accumulate pollutants from various sources including sediments, soil erosion and runoff, air depositions of dust and aerosol, and discharges of waste (Weinbach, 1954. Webb, and Brett. 1973; Goodwin et al., 2003). The L. marginalis species are among the top consumers of trophic pyramids in aquatic ecosystem. In consequence, they are endangered by diet-borne pollutants transferred along the food chain. Because heavy metals tend to accumulate in different body organs, these metals are dangerous for aquatic organisms and in turn they led into serious problems. Bivalves have been used for many years to determine the pollution status of water, and are thus regarded as excellent biological marker of metals in aquatic ecosystem. Godavari River is considered one of the most important water bodies in Maharashtra state, large, shallow, and exposed to high levels of pollutants from industrial, domestic and agricultural resources.

In conclusion the response of behavioral study of L. marginalis to the toxic environment of NA-PCP showed mucus secretion and closing
and opening of valve indicate death of the test animal. Lethal concentration values obtained for 24h, 48h, 72h, and 96 hrs. Clearly indicated the response of *L. marginalis* to the toxic environment of NA-PCP further it is suggested that for examining toxicity evaluation toxicity the other parameters such as physico-chemical and biological should have to be considered.

Oxygen is needed continuous throughout the life for an active animals. The rate of supply depends on anatomical and physiological characteristics of the organs of respiration and transport pigment could be a limiting factor while other environmental variation like temperature or pH and salinity may impose extra demands for oxygen or affects the rate of exchange. Each of the atmospheric gaseous gets dissolves in water according to its partial pressure, its solubility coefficient and the temperature. Rising temperature reduces the solubility of gases. In addition, the solubility of gases is also altered by the presence of dissolved solids. (Fujita *et al.*, 2003).

It is proved that increased industrialization lead to deterioration of aquatic environment particularly; freshwater ecosystems and it alter the quality of water and subsequently to health hazardous of organisms. NA-PCP are noted as one of the significant contributor to water pollution. The Pentachlorophenols and its derivatives pollution in the environment has raised to alarming proportion which has caused great concern among the environmental toxicologist as the NA-PCP residues that are released as non-biodegradable substances. The recent developments in bioinformation on these substances have brought forth the scientist to
cause remedial action to this problem. Among the various toxicants pentachlorophenols are known to be quite severe in their action Sudipta Chakraborty ((2010)). Toxicity is termed as the relative property of chemical pollutants with reference to its potential to cause harmful effects on any organism. The harmful effects depend upon the function of concentration of the chemical and its duration of exposure, These chemicals when enter into water, disturb the normal functioning of cells in flora and fauna which may result in the alteration in the biochemical and physiological mechanism of aquatic animals. Pollutants, because of their potential toxicity are known to produce morphological, behavioural and physiological changes in the vital organs such as respiratory, reproductive, nervous, osmoregulatory etc. (Gupta, et al., 1983)

Respiration is the mostly used tool for understanding the physiological action of the pollutants. The respiration rate of organism is an indicative for the physiological state and changes in the respiration rates may be an indicative for environmental stress. Biological responses of organisms to pesticides in the aquatic environment are usually understood through determining their rate of survival and changes in the levels of various physiological phenomena. Newell, (1973) stated that toxicants act as physiological stressors upon the organism. It is well known fact that the rate of oxygen consumption is used as an important tool for understanding the physiological state of metabolic activity of an organism. In this study, oxygen consumption of *L. marginalis* in normal and after exposure to NA-PCP media has been quantified. On exposure to NA-PCP the respiratory metabolism of freshwater bivalve *L. marginalis*, has been found to be directly affected. The results obtained clearly
showed that there was an increase in the rate of oxygen consumption of bivalve, *L. marginalis* after exposing for 1 h and 2 h in both (0.73 ppm and 0.22 ppm) 1/3rd and 1/10th sub lethal concentrations of 48 h LC$_{50}$ respectively of NA-PCP. The rate of oxygen consumption decreased significantly (p<0.05) after exposing for 4 h to 24 h as compared to control groups. It was also observed that at acute exposure, the bivalves showed sudden decrease in oxygen consumption followed by an initial increase and then steady decrease. There was a continuous increase in the rate of oxygen consumption up to 2 hr when the bivalves were exposed to all the concentrations of lethal and sub lethal concentration of NA-PCP. As the period of exposure increased this uptake gradually but constantly decreased severe fall after 12 h and then continued up to 24 h of exposure. These results clearly indicates that the used NA-PCP must be acting on the organized respiratory mechanism i.e. damaging epithelial cell layer of gills ultimately altering the elements involved in the respiration mechanism of the gills. The NA-PCP must be acting on the enzyme sites of cells slowly in the lower concentration initially. Where it might be acting as a stressor in higher concentrations and after prolonged exposure, interfering the physiological activities. This is speculated because there was an obvious decrease in rate of oxygen uptake after 4 h to 24 h exposure to all the concentration of NA-PCP as compared to the first 4 h of exposure to all the concentration of NA-PCP. From these observation it can be inferred that the NA-PCP disrupting enzyme-mediated process and / or disrupting cellular structures. The initial elevation in the rate of oxygen consumption showed a compensatory phase to enhance the physiological activity but the continues decrease may be due to the failure
of respiratory metabolism. The mechanism of toxic action of NA-PCP compounds appears to be through disruption of oxidative phosphorylation, by a) secondary responses caused by discharge of a hydroxyl chloride gradient across mitochondrial membrane, b) interaction with the basic energy conservation system involve in the synthesis of ATP, and c) an interaction with mitochondrial membrane to cause swelling and disruption, Weinbach., (1954), Selwyn, (1976); Aldridge, (1976). Thus the decreased rate of oxygen consumption of bivalve, L.marginalis may be expected because of toxic action of NA-PCP as reported, in some mammals.

Chlorophenols compounds are known to cause a variety of effects on the mitochondrial membranes, which correlated with the increase in oxygen consumption, (Cantelmo, et al., 1978). Kirk and Lewis (1993) stated that the NA-PCP compounds are the active in very low concentration and they are slow acting poison. Sarojini et al., (1989) observed oxygen consumption rate by Caridina weberi showed alteration like increase and decrease oxygen uptake when exposed to different concentration of copper sulphate and NA-PCP the chemicals which are used as a biocide and molluscide particularly in antifouling pains applied for ships. They reported that after 10 days exposure to NA-PCP the animals could not survive to collect further oxygen consumption data. Though the antifouling organometallic compounds at the cellular level decrease the metabolism of accumulated toxicant may demand increased oxygen, which is reflected in the upward shift of the oxygen uptake in the prawn. The inhibition of the oxygen uptake in the bivalves might be due to the penetration of the toxicant molecules and its action alters the
metabolic cycle at cellular level. Liu and Thomson, (1986) stated that n-butyltin is biologically active due to their ability to stimulate or inhibit the dehydrogenase activity and oxygen consumption. Stimulation or inhibition of the dehydrogenase activity by toxicant is harmful to a living organism as this produces deleterious effects on the organism by interfering with its energy metabolism.

Exposure of bivalve, L. marginalis to NA-PCP resulted in morphological changes in the gills, are reflected in plasma had a significant effect on the respiration, excretion and osmoregulatory functions of the gills. These changes can be regarded as primary changes, which will inevitably lead to secondary physiologically changes as well as responses that could affect various organ systems. Similar results were described by Ghate and Mulhekar, (1978); Baticodes et al., (1991). According to Piver (1973) dialkyltin and trialkyltin compounds are known to be capable of effecting the respiration. Sonawane and Lomte, (2000) studied the effect of heavy metals copper sulphate and mercuric chloride on oxygen consumption of the fresh water bivalve Lamelliden marginalis. Chinni et al., (2000) reported on changes in oxygen consumption, ammonia excretion and metal accumulation in post larva of Penaeus indicus exposed to lead. Manikumar, (1986) also observed changes in oxygen consumption in marine prawn, Penaeus merguiensis exposed to pesticides. Hiltibran, (1966) considered that the decreasing oxygen consumption rate due to herbicide brought the process of breaking the link between oxidative phosphorylative processes. This decrease can be also caused by free oxidation in the organism. Kale, (2002) observed increased in oxygen consumption rate to first 6 h and decline gradually
and steadily after 12 h and continued till they attain normalcy. She observed that stressful effect of cadmium chloride started decline after 6 h of freshwater crab *Barytelphusa cunicularis*. Vosloo et al., (2002) documented that, in attempt to move away from pollution, the animal’s oxygen consumption rate increases from pre-exposure values to support this additional activity. Ratsamee and Nongnud, (2006) documented the changes in the oxygen consumption in the blue swimming crab, *Portunus pelagicus* (Linnaeus) when exposed to the sublethal concentrations of copper. They reported that the rate of oxygen consumption per unit body weight tended to decreases when exposed to copper concentrations. He observed the copper affect and the cardiac activity in *Portunus pelagicus*. He also observed that the heart rate increased with decreasing salinities and increasing copper concentrations. Shivakumar and David, (2007) observed depletion in oxygen consumption in freshwater fish, *Catla catla* exposed to endosulfan and concluded that the decreased rate of oxygen consumption due to disrupt metabolic activities after endosulfan toxicity.

In the present probe the finding related to respiratory mechanism was initial elevation and subsequent decrease in the rate of oxygen uptake. Therefore from the above results it was suggested that the increase in rate of oxygen consumption in different contaminated media might be the reflection of an augmented physiological activity like osmosis at the cellular level in eliminating and counteracting the NA-PCP stress perhaps when exposed to the different sub lethal concentration. It is also found that bivalves have little ability to regulate their metabolic rate when faced with adverse environment and as the concentration increases the response is intensified. In conclusion, the response of an organism to
the toxic environment is quite evident from the variation in respiratory metabolism and that can also affect several parameters such as the growth rates in bivalves or exhausts the biochemical reserve.

The aquatic contaminants causes damage in vital organs and disrupt the physiological and biochemical processes of the animals. In the present study NA-PCP was used for the estimation of biochemical changes in different tissues of the freshwater bivalve, *L.marginalis*. NA-PCP is one of the most important compound used in pesticide, molluscide in paint as an antifouling agent, PVC, paper and pulp industries and wood preservative etc. The other antifouling agents like tributyltin, copper sulphate and copper oxide were also used in the paints for the application of ships and hulls to prevent attachment of microorganisms and foulers like Barnacles, Gastropods and mussels on its surface, Chi Young (2006). The Chlorophenol pollution in the environment has risen to alarming proportion which has caused great concern among the environmental toxicologist, as its residues that are released are non-biodegradable substances. The recent developments, in bio-information on these substances have brought forth scientists to cause remedial action to this problem. The harmful effects depend upon the functions of concentration of chemical and its duration of exposure, Gehring and Rao (1981).

Chlorophenol derivative like NA-PCP may induce certain biochemical changes in aquatic organisms and before the drastic cellular and systematic dysfunctions manifest themselves, appropriate biochemical parameters related to proteins, lipids and glycogen etc. could be used effectively to know the gravity of the situation and to check it at
the initial stage itself (Aldridge, 1983). Studies on energy metabolism are concerned in the way in which the major carbohydrate, lipid and protein fuels are used by an organism for energy production. Invertebrates, changes in the biochemical constituents are pronounced which are cyclic in reproduction, since a great amount of energy, must be channelised to the gonad during reproduction. This is reflected in deposition or depletion of the nutrients with the advent of departure of the reproductive period (Lambert and Dehnel, 1974). Many aquatic animals meet the metabolic expense of spawing by drawing on response materials accumulated during non-reproductive period. Seasonal changes in biochemical constituents in relation to reproductive cycle of marine invertebrates such as mollusces were studied by Giese, (1969) and bivalve molluscs (Nagbhushnam and Dhamne, 1979; and Nagbhushnam and Talikhedkar, 1977 Graney, and. Giesy, Jr. 1986).

In the past two decades NA-PCP have developed into important industrial commodities. It is unsurpassed by any other chemical in the number of its organic applications which includes uses as industrial catalyst, agricultural biocide, wood preservative and as an antifouling agents in paint industries. The chemistry and toxicity of NA-PCP compounds have been reviewed extensively, WHO, (1980); Pierce,. and Victor. (1978) Folke, and Birklund. (1986);

Due to little information available related to effects of Chlorophenol compounds on aquatic invertebrate particularly bivalves, present investigation was aimed to evaluate alterations in the biochemical changes in freshwater bivalve, *L.marginalis*, exposed to acute concentration of NA-PCP for 24, 48, 72 and 96 hrs. NA-PCP is used as a biocide in a variety of consumer and industrial products and it enters the aquatic environment
mainly via wood preservative pesticides, pulp and paper industries etc. A variety of toxic effects have been described, viz. shell malformations in oysters, reduced scope for growth, population decline, bioaccumulation, etc. (Widdows and Page, 1993).

In the present study the freshwater bivalve, *L. marginalis* was exposed to 2.6 ppm, 2.2 ppm, 1.6 ppm and 0.96 ppm LC$_{50}$ of 24 h, 48 h, 72 h and 96 h respectively for NA-PCP as a model. The aim of the experiment was to obtained biochemical constituents like total protein, lipid and glycogen, level in the tissues like gonad, digestive glands, foot and mantle exposed to NA-PCP.

The results obtained in the present study revealed that all lethal concentrations of NA-PCP produced a significant decrease in glycogen content in gonad, digestive glands, foot and mantle. Decline in glycogen content may be due to enhanced breakdown of glycogen to glucose through glycogenolysis under toxic stress of NA-PCP. Carbohydrate is an important biochemical constituent of animal tissues. It is the store material which is used as an immediate source of energy when required as an essential feature of normal organism. The disturbance in the glycogen profile is one of the outstanding biochemical lesions due to the action of many chemicals, Elumalai and Balasubramanian (1997). Similar conclusions were reported by many authors when different animals exposed to heavy metals, organopesticides and antifouling agents like copper sulphate and copper oxide etc. very few literature were available on the effect of pentachlorophenols related to biochemical studies.

Depletion in haemolymph glucose, tissue glycogen and total free sugar were observed in rat exposed to NA-PCP, Nishimura, (1984). Peer, *et*
al., (1983) reported declined glycogen content in Rhinomugil corsula and on survival, activity and metabolism exposed to NA-PCP. Similar results were observed in Scylla serrata in response to cadmium toxicity, Reddy and Bhagyalakshmi (1994). Mane and Kulkarni (1999) have reported significant decrease in the glycogen content in bivalve, Lamelidens marginalis. They exposed bivalve Lamelidens marginalis for 96 h with concentration of cadmium 1.00 ppm and 2.114 ppm. Fox, and Rao. 1978 suggested. Effects of Sodium Pentachlorophenate and 2,4-Dinitrophenol on Hepatopancreatic Enzymes in the Blue Crab, Callinectes sapidus and found decrease in carbohydrate content in different tissue stated depletion in carbohydrate level due to the increased glycogenolysis as a response to toxic stress for overcoming their needs for additional energy. Decline in glycogen content in muscle, gill and hepatopancreas may be due to utilization for combating the stress caused by toxicant action and it indicated the prevalence of anaerobic conditions such as anoxia. Decrease in carbohydrate content was observed in the tissue of marine prawn Metapenaeus monoceros following exposures to the pesticide methyl parathion, Reddy and Rao, (1991) Yeragi and Koli, (2000) showed that under acute and chronic exposure of malathion, the magnitude of glycogen decrease was directly linked to the duration of exposure. Veena shaklivel, (2002) studied the effect of phosphomidan on glycogen contents in fish, G. affinis and observed decreasing trend in glycogen content further she concluded that decrease in glycogen levels signifies their utility, possibly to meet the higher energy demand under phosphomidan induced stress conditions. This impairment may involve a shift of the equilibrium of inter conversation of glycogen, lactic acid and glucose towards the enhanced mobilization of stored polysaccharide through anaerobic glycogenolysis. K. Vijayavel et al., (2006) studied the
effect of naphthalene on carbohydrate metabolism of crab, Scylla tranquebarica. They found depletion in carbohydrate level and suggested that naphthalene can induce hypoxic condition, which results in the extra expenditure of carbohydrate metabolism. Shah et al., (1998) investigated changes in glycogen contents in estuarine edible clam, Anadona rhombea exposed to NA-PCP indicated declined glycogen levels in tested tissue.

The finding of the present study substantiate earlier findings that depletion in glycogen level might be due to its rapid utilization to meet the energy demands under stress condition exposed to NA-PCP and supply energy demand is in the form of the glucose which undergoes breakdown to produce energy rich compound ATP. According to Holwerda and Herwig (1986) exposed clam A. anatine to dibutyltin, noted decrease in weight and carbohydrate stores indicated intoxicated clams appeared to rely on anaerobic energy metabolism as indicated by elevated levels of lactate, succinate, acetate and propionate. Weinbach, 1956 reported the mechanism of toxic action of NA-PCP appears to be through disruption of oxidative phosphorylation which causes interaction with the basic energy conservation system due to anoxic conditions of the tissue. They also suggested tissues acidosis due to reduced oxygen transport must have also favored the process of glycogenolysis in tissues and the decrease in glycogen level might be due to anoxic condition since anoxia increases glycogen consumption to meet energy demand.

In the present study freshwater bivalve, L. marginalis was exposed to lethal concentrations of NA-PCP for 2.6 ppm, 2.2 ppm, 1.6 ppm and 0.96 ppm LC$_{50}$ of 24 h, 48 h, 72 h and 96 h respectively. Depletion in protein level in different tissues of L. marginalis were observed consistent
decrease as exposure period and lethal concentration increases. The maximum decrease recorded at 1.6 ppm in gonad followed by digestive gland foot and mantle for all concentrations. All the tested tissue showed significant decreased of protein level when compared to control tissue of \textit{L. marginalis}. The results obtained in the present finding were in agreement with some researchers who have showed depletion in protein content in the different tissue exposed to acute and chronic concentration of different pollutants. The decrease in protein content was reported in freshwater prawn \textit{Macrobrachium kistnensis} exposed to some pesticides Nagabhushanam \textit{et al.}, (1972). Shivprasad \textit{et al.}, (1981) revealed that methyl parathion pesticidal effects in snail, \textit{Pila ghobusa} and reported that depletion in protein level in different tissue due to enhanced proteolytic enzyme activity and decreased protein synthesis. Sarvana and Geraldine, (1997) reported decrease in protein level in \textit{Macrobrachium malcomsonii} when exposed to endosulfan and their finding suggested the depletion of tissue protein level was due to diversification of energy to meet the impending energy demand under toxic stress. Similar results were reported by Vincent \textit{et al.}, (1995) and Geraldine \textit{et al.}, (1999). While studying on freshwater prawn, \textit{Macrobrachium malcomsonii} exposed to dichlorvos. The toxic substances used in biocide and wood preservative caused several consequences in the aquatic biota when these substances discharged into environment. After the restriction on the use of toxic chlorophenols under prevention act, uses of NA-PCP have been largely increased, Dalela \textit{et al.}, (1980), In higher concentrations NA-PCP tends to damage protein metabolism. The sodium tends to damage the cell wall membrane function causing reduction in potassium ion concentration in
the cells, which inhibits protein synthesis because potassium ion is one of the important catalysts in different protein metabolic processes Huber (1982). Arillo et al., (1982) reported biochemical effect of long term exposure to heavy metals, chromium, cadmium, and nickel in Salmo gairdnerui. They further suggested increase in the protease activity, decreased in protein level and increased in amino acids level suggests proteolysis. Increased levels ALAT and AAT activities indicate that the conversion of liberated amino acids into keto acids for energy production by TCA cycle. The decrease in protein along with an increase in the levels of amino acids and increase in the levels of RNA might indicate an increase catabolism of protein and decrease synthesis, Desai et al., (2002). They also reported protease activity increase in the tissue of fish after exposure to heavy metals nickel and copper. Increase in the protease activity is reflected in the decrease in protein content and increase in the levels of amino acids.

Yeragi et al., (2000) observed the decline in protein content when crab, Uca marions was exposed to the pesticide and found that the depletion in protein level during pesticide exposure may be due to increased catabolism and decreased anabolism of protein. Kumar and Gopal (2001) found decrease in protein contents in liver, brain, kidney, and muscle of fish, Channa punctatus exposed to distillery effluent, due to the breakdown of protein into free amino acids which is used for different metabolic activities during stress condition. Monika kale (2002) also found decrease in protein level of freshwater crab, Barytelphusa cunicularis exposed to cadmium chloride. After exposure to 1 ppm and 1.5 ppm of cadmium chloride for 10 days, she observed significant
depletion in protein level in tested tissues like hepatopancreas, gills, ovary, muscle and intestine due to toxic stress influences the conversion of tissue protein into soluble fractions reaching in the blood for utilization in metabolic activities during stress conditions. Similar results were reported by Ramalingan (2003); Gupta and Bhide (2001; 2004); Jagutheesuari (2005); Vijayavel et al., (2006); Ramalingam and Ramarani (2006) and Mangala Bhide et al., (2006)

Glycogen is considered to be the major source of energy in animal tissues and maintenance of glycogen reserves is an essential feature of the normal organismal metabolism. It also plays an important role in the structural part of the cell membranes De Zwaan and Zandee, (1972). The decrease in glycogen content in the different tissues of the bivalve, L. marginalis suggests the possibility of the glycogenolysis which in turn produce energy to cope up the adverse stress conditions. It has been described that in mollusces the glycogen is the chief source to liberate energy and its increase or decrease will alter the metabolism (Chaudhari and Lomte, 1992). Any stressful condition alters the biochemical composition. The change in metabolic rate leads towards the change in biochemical composition hence, the change in biochemical composition is an indicator of stress of chemical or physical nature in the surrounding which mainly affects glycogen contents.

Possibility of another factors may influences environmental parameters on the condition reproductive activity and biochemical composition of a native population of gametogenesis proceeded slowly and spawning took place in May- June, although the predicted time of ripening was early March. Available food appeared to be a very important
factor in controlling gonad growth, once gametogenesis initiated. Changes in the main biochemical components of this bivalve were determined for a standard individual. When food was abundant, energy reserves were built up. Spawning produced a decrease in biochemical constituent levels, and recovery coincided with the phytoplankton bloom. The stored reserves, mainly lipids, were used to overcome a state of energy imbalance in winter associated with low food availability. Results show this bivalve to be an opportunist organism which concentrates its reproductive effort during a short period of favorable conditions and which is directly dependent on nutritive availability in the environment. Gabbott and Bayne, (1973), have shown that seasonal variations in biochemical composition of molluscs depend on environmental parameters such as temperature and available phytoplankton and factors such as timing of the reproductive cycle and the rate of turnover of stored energy. Changes in biochemical composition may be of great importance in relation to energy metabolism. Studies on energy metabolism in bivalve molluscs have been reviewed by Dezwaan, (1977). He discussed the formation of multiple end products and the dependence of the proportional accumulation on the duration of anaerobiosis. Over burden of

In the present investigation, reduction in lipid content was observed in selected tissue of *L. marginalis* when exposed to all concentrations at 24 h, 48 h, 72 h and 96 h. The values obtained for gonad, digestive glands, foot and mantle were significantly decreased for 2.6 ppm, 2.2 ppm, 1.6 ppm and 0.96. Among the vital organs digestive glands is chief metabolic organ in invertebrates like bivalves, crustaceans, etc. and is involved in the regulation of general metabolism by storage and release of
reserves such as lipids under normal or stress conditions. The results of the present study indicated that digestive glands suffered greater damage following mantle, gonad and foot in *L. marginalis*. Lipids not only provide energy during unfavorable circumstances but also found in various forms in the structural membrane in the cell. As lipids are insoluble, in aqueous, they are mostly found in the form of complex in case of membranes, associated lipids in the form of hydrophobic barrier that permits partition between aqueous contents of the cell and other cell organelles or in association with protein as lipoprotein. Imbalance of lipid metabolism due to toxic stress may cause major lethal problems in the body of animal. Bhilve *et al.*, (2000) stated that considerable decrease in total lipid in tested tissues might be due to drastic decrease in glycogen content in the same tissue which is an immediate source of energy during toxic stress conditions after glycogen, lipid content may be used for energy production overcome the toxic stress. Similar results were observed in different aquatic animals, Villan *et al.*, (1990); Lomte and Muley, (1993); Sarvana and Geraldine, (1997); Deshmukh and Lomte, (1998); Geraldine *et al.*, (1999); S. Amanulla, (2004); Jagatheeswari (2005) and Vijayavel *et al.*, (2006).

In the present study, total protein, lipid and glycogen percentage decreases gradually in gonads, digestive glands, foot and mantle of bivalve significantly as period of tributyltin oxide exposure increases. (Table 7-9).

The mode of action of pollutant may be responsible for cellular disorganization offering the storage and metabolism of the organic constituents. The pollutant give the heavy physical irritated stress causing
rapid movement and increased respiration rate thus increased the utilization of reserve constituents like lipids and glycogen to meet the high energy demand of body causing decrease in organic constituents content Bhagylakshmi, (1981). Lomte and Alam, (1982) observed the fall of glycogen in the digestive gland during sublethal exposure for 24hrs. to organophosphate pesticide malathion. Choudhari, (1988) studied the effect of organochlorides on biochemical composition of snail, B. bengalensis and found decrease glycogen content after treatment. Similar results were reported by (Bhamre, 1993; Jadhav, 1993; Deshmukh, 1995; Erande, 1998). Another important constituent of animal tissue is protein which plays an important role in cellular metabolism. As a constituent of cell membrane, proteins have a major role in the process of interaction between intra and extra cellular media. All enzymes are proteins in nature and they control subcellular functions. In the metabolism of protein many enzymes, co-enzymes, intermediate protein and amino acid are involved and studied in many animals (Sekeri et al; 1968).

The depletion of protein content suggests an increased proteolysis and possible utilization of the products of their degradation for metabolic purpose. They may be mobilized in to TCA cycle through amino acid metabolism system to cope up with the excess demand of energy during toxic stress conditions. Increased protease activity in the present investigation further supports the degradation of protein content in the tissues. The fall in protein level during pollutant exposure may be due to increased catabolism and decreased anabolism of proteins. Depletion in protein content in animal tissue after exposure to NA-PCP pollutant and other pesticides, heavy metals were reported many researchers, Hanes,
Isani et al., (2003) they suggested that the copper is extremely toxic to bivalve, S. inaequivalvis, when present as free metal ions, as it can generate reactive oxygen species, therefore cells are equipped with sophisticated biochemical systems to keep its concentration at very low levels. Ramana Rao and Ramamurthi, (1978) studied the protein content in the tissue of Pila globosa after exposing it to sumithon. Lomte and Alam, (1982) studied the biochemical composition of B. bengalensis exposed to Malathion and observed a marked fall in protein level. Katticaran et al., (1995) on exposure to acute concentrations of copper observed a decline in protein level in the bivalve, S. scripta after prolonged exposure. Deshmukh and Lomte, (1998) observed the depletion in protein content in all the selected tissues after acute and chronic exposures to copper sulfate. Many researchers, showed similar results (Krishnamoorthy, and Subramanian, 1995; Indira, 1989; Ramalingam, 2003).

The results of total protein contents in all tissues clearly indicated that digestive gland was the most affected organ followed by gonad and gill. The higher depletion of protein in the digestive gland might be due to high metabolic potency and efficiency of the gland under pollutant stress. The digestive gland may be the site of action of pollutant in the body of bivalve or digestive gland seems to be the main site of degradation and detoxification of toxicants and hence has the largest demand of energy for the metabolic processes resulting into increasing utilization of protein to meet energy demand. The higher degradation of protein in digestive gland

The reasons for the decrease in lipid content in the bivalve, L. marginalis after tributyltin oxide stress may be due to reduced synthesis of lipid or increased activity of lipase involved in oxidation of lipids (Hollands, 1978). The overall decrease in the lipid content of tissues indicates the pronounced lipolysis and its utilization during tributyltin exposure. The effect of mercury on oxygen consumption and body biochemical composition of a marine bivalve M. salleiwas investigated by Uma Devi et al., (1985) and results were significant decrease in oxygen consumption with increasing concentration of the toxicant. The effect of exposure time and concentration of Hg on body biochemical composition was also studied in M. sallei a decrease in the glycogen, protein and lipid was observed in time-dependent experiments. In concentration-dependent experiments, there was also a decrease in glycogen lipid and protein at all exposure concentrations.
The results of the present investigation supported and indicated that *L. marginalis* exhibited a differential preference in their utilization of biochemical constituents during time- and concentration-dependent stress of tributyltin oxide. The decrease in oxygen consumption together with the utilization of lipid and protein during tributyltin oxide exposure suggests that these bivalves might shift to anaerobic metabolism in order to encounter the heavy metal stress in the environment. Sujatha *et al.*, (1996) reported effect of tributyltin oxide induced biochemical changes in estuarine clam. They suggested tributyltin compounds are generally lipophilic and may reduces the lipid level in gemal muscle and digestive gland under stress condition of NA-PCP in clams, similar results were reported by Lundebye *et al.*, (1997); Shiva Prasad Rao and Ramana Rao (1979) stated that, considerable decrease in the total lipid in muscle might be due to drastic decrease in glycogen content in the same tissue which is an immediate source of energy during toxic stress conditions and after glycogen lipid content may be used for energy production to overcome the toxic stress. Romeo and Mauricette, (1997) in vitro experiments indicated that cadmium, which does not undergo redox cycling, was found unable to stimulate the lipid peroxidation process, where as copper and mercury may exist under different oxidation states and have detrimental effects on the antioxidant defence system of the Mediterranean clam, Ruditapes decussates. Deshmukh and Lomte, (1998) observed significant depletion in the lipid content in all the tissues tested after acute treatment of copper sulphate.

In conclusion it is observed that NA-PCP affected the biochemical constituents in tested animal tissues such as gonad, digestive glands, foot
and mantle under stress condition of NA-PCP to meet the requirement of the cell to produce the energy to perform the physiological activity of the animal under unfavourable conditions. The protein and lipid may be mobilized for energy production. The decrease in the level of organic constituents clearly indicated the mobilization of organic constituents under stress condition.

It is known that manifestation of toxic effects is better seen at cellular and sub cellular levels. This is mainly because of the fact that the pollutant employed affects the basic functions of the experimental animals and that these effects are best seen at cellular and sub cellular levels. Identification of such changes can be facilitated by histological enquiry.

In the present investigation, *L.marginalis*, one of the commonest of the *lamellidens*, encountered in realms of the Godavari river bank of India was identified as the experimental animals. Representative of this species inhabiting the river waters of Marathwada region was sampled many times stretching for a span of three years and was used as experimental materials to analyze the effect of sodium pentachlorophenate on histology digestive gland, ovary, mantle, foot and gills.

Bivalves have proved to be an excellent material for histological and histopathological assay. This quality of the material has helped in utilizing bivalves as materials to assess the effects of toxicants, mainly heavy metals phenolic and polycyclic aromatic hydrocarbons. The efficacy of histology to delineate the effects of toxicants at tissue levels probably became evident after Goldberg (1975b) developed the idea of "Mussel Watch". The mussel watch strategy is based on the concept that
bivalves are capable of accumulating reasonably higher concentration of toxicants when they are variable in the environment. When the cause and defects of heavy pollution are analyzed experimentally, various morphological, physiological and biochemical assays become useful methodologies to understand the degree of effect that can be quantified chemically and qualified structurally. Therefore, structural changes represented by way of alterations, modifications and damage, should he looked into, to employ these as basic tools to explain toxicity. Bivalves are exposed for a considerable period of time, lasting for a few days to weeks in toxicants with a view to allowing the animal to manifest the toxic effect’s in tissues involved in performing functions which are responsible (or tolerance, adaptation and successful existence in a dynamic environment. The present investigation envisaged a histopathological assay of important tissues namely, the gills, foot and mantle, which are copiously bathed by external medium resulting in considerable water tissue contact and the gastric gland and ovary, an internal organ which gets the impact of toxicity via water or food. Structural changes as envisaged in the present investigation could be the result of either contact or absorption. In this chapter therefore, a histopathological enquiry has been conducted and presented with the help of photomicrographs, expressed in terms of intensity of damage of numerous sections of these tissues prepared from animals sacrificed after varying periods of exposure to sodium pentachlorophenate.

NA-PCP a penta chlorophenols form one among the innumerable contaminants ridded to our aquatic ecosystem every day mainly due to anthropogenic activities or otherwise. Their contributions in making life
hazardous to all organisms especially the sessile lefilter feeding bivalves, widely inhabiting the river waters, are well documented by workers in India. (Mane et al., 1984). Man's increasing awareness of the impact of pollutants, which has become a threat to the stability of our biosphere, has made many a scientists to devise efficient diagnostic biomarkers to warn him of the perturbations in organisms exposed to pollutants at a very early stage. One such approach is the application of cytochemical methods which probes the alterations at the molecular and biochemical levels. This would be of advantage in that these alterations, though unnoticed at the early stage, could be detected and deleted or controlled at the onset (Moore, 1991). The capacity of the bivalves to accumulate trace metals and other toxicants has led to the selection of this group as an important bio indicator for the reason that they satisfy the basic requisites as proposed by Butler et al., (1971); Phillips (1976a,b).

The adverse effects of pollutant accumulation in river waters are often reflected as minute alterations in the structure, biochemistry and physiology of the organism inhabiting that area. In fact, though these changes are commonly classified under different categories, they are all interrelated. The prominent organs which are often prone to the effect of pollutant accumulation are the gills and digestive glands. The former organ is always in close proximity with the aquatic environment and the latter, the chief sites of intracellular digestion and detoxification. The histomorphology of the digestive gland of the bivalve *L. marginalis* subjected to the effect of NA-PCP was studied up to 96 hrs. Histological changes of the digestive tubules, channels, and connective tissue of the gland were recorded. The epithelium of the tubules and channels were
characteristic with erosive disturbances and by heavy vacuolization of digestive cells; connective tissue of the gland was specified by cells with granulocytomes and by necrosis and lysis. It was concluded that histological changes in digestive gland of bivalve *L. marginalis* might be caused by acute toxicity of the tributyltin oxide.

Although the observed histological changes can not be linked with the relatively high mortalities observed during exposure and probably due to spawning, their severity appears to be both dose and time dependent. Furthermore, the data acquired suggest that poisoning results from different mechanisms. Although there are few studies devoted to histological effects of pollutants in molluscan species, digestive diverticula’s modifications such as intensive fragmentation, vacuolization, epithelial thinning have been noted (Tripp *et al.*, 1984; Couch, 1984; Rasmussen, 1982; Rasmussen *et al.*, 1985). Such modifications could be considered as a general molluscan response to stress (Moore *et al.*, 1979; Lowe *et al.*, 1981) and have been interpreted as a physiological survival mechanism of bivalves subjected to stress.

In the present study, it is revealed that the initial impact of the NA-PCP exposed for 24 hours was less when compared with other exposure periods. The 48 hours exposure to NA-PCP shows that damage caused is at a higher rate to the tissue structure as it metabolizes into the tissues. After 72 hours exposure the damage is still there and not so high which might be due to the adaptation of the tissues to the pollutant and development of resistance to some extent. At 96 hours exposure the damage was increased which may be due to the lost of resistance of power of the tissues. This may be either due to the defense mechanism of cell becoming weak, or due to the high
accumulation of the pollutant. The degree of toxic effect depends mainly on levels of pollutant and metabolites in the target tissues.

A comprehensive knowledge of the structure and function of the digestive glands of bivalve molluscs can be obtained from the extensive literature available on them (Atkins, 1937; Owen 1955, 1956). The preliminary concept of the structure and function of digestive diverticula of bivalves was derived from the earlier works of Yonge (1926), which stated that the cells lining the digestive diverticula to be made of a single type of cell. This was constantly being replaced by single type of darkly staining undifferentiated pyramidal shaped cells; present in the crypts of tubules. These cells were believed to be concerned with intracellular digestion. Contrary to these findings, numerous reports that in some species of bivalves, the digestive cells performed secretory function were put forth.(Mansour, 1946; Mansour and Zaki, 1946; Owen, 1956; Reid, 1965). Owen (1955, 1956) reported that the darkly stained cells of many bivalves are flagellated and appear to undergo a secretary cycle.

This idea held true in the case of cells in the digestive diverticula of gastropods. Based on these findings, it was confirmed that the digestive cells of most bivalves were mainly concerned with functions of absorption, digestion and secretion. This view was supported by the works of Sumner (1966b), which is perhaps the only published account of electron microscope study of the digestive tubules of bivalves at that time. In the case of most bivalves studied, it was found that the blind ending tubules communicate with the stomach by means of partially ciliated ducts and non ciliated secondary ducts. (Owen, 1955). These digestive tubules were noted to be made of two distinct types of cells. 1) Digestive cells containing numerous membrane bounded vesicles, 2) Basophilic darkly stained cells. (Owen, 1972a
However, in Cardium edule, Owen (1970) reports the epithelium lining of digestive tubule to be composed of three cell types: 1) mature digestive cells, 2) mature secretory cells, and 3) immature flagellated cells. Many research conclude that digestive cells in bivalve are multifunctional (Moore, 1991). They are found to analogous to vertebrate liver cells as they are found to be important storage sites of glycogen and lipids. Apart from these cells, they have turned out to be the major storage sites of physiological processes like detoxification and removal of toxicants entering the system (Moore, 1985; Moore et al., 1987).

Bivalve reproduction has been extensively studied. Much of the literature is concerned with gonad development, breeding periods, and mobilization of the nutrient from the storage organ. From marine species this aspect has been nicely reviewed by Sastry, (1979) and Andrewes, (1979) and for freshwater species by Purchon, (1977) and Mackie, (1984). Reproduction in both the marine and freshwater species is cyclical, and it may be annual, semi-annual, or continuous. The changes occurring in the histology of *L. marginalis* ovary can be evaluated in five stages: 1) Beginning to gametogenesis, 2) Development, 3) Maturation, 4) Spawning and 5) Acinus regression. These phases function continuously in co-ordination with seasonal environmental changes and produce the pattern characteristics of a species. Reproductive cycle of a species is a genetically controlled response to the environment and the pattern is apparently determined through the co-ordination of successive reproductive events with changes in the external environment. Sastry, (1970) Studies carried out by several workers indicate that a reproductive response is produced through an interaction of environmental factors especially temperature, salinity, light and food, and endogenous factors within an organism. Besides, reproductive response also
affected by various types of pollutants because of the interference in the histological structure of the vital organs such as digestive glands, gonad and gills etc. (Newell et al., 1982, Paulet and Boucher, 1991).

It is well known that the spawning period corresponds to a phase of increased mollusc sensitivity to changes in the environment. In present experiment, NA-PCP-exposed specimens and histological changes occurred due to NA-PCP action and not to any experimental artifacts. Detailed results presented which shows the histological changes recorded for each group, at specific time intervals and acute concentration for each target organ histological effects were roughly similar in the different treated groups, but the time sequence and the severity of damage differed. No histological effect was observed in the control animals during the experiment.

Gills are yet another organ of much importance in bivalves in that they are structures in close proximity which next to the aquatic environment. The complicated ciliary mechanisms in the gills, make bivalves the most efficient filter feeders. The basic organization of gill in mussels is that it is composed of an ascending and descending lamellae. Each lamellae is composed of filaments which are joined by ciliary interfilamentar junctions. These lamellae are interconnected by interlamellar blood vessels. Ciliated and nonciliated cells are found in the epithelium of bronchial vein which runs through the filament. Chitinous rods are seen along with the muscles(abofrontal and frontal) which help to preserve the integrity of the structure. The arrangement of cilia follow a definite pattern (Sunila, 1986). At the frontal end of the filament are four columnar cells. These cells are provided with cilia which are called frontal cilia. Adjacent to this lies one cell which is large and ciliated
called latero frontal cell and the cilia associated with it is called latero frontal cilia. A row of lateral cells bear lateral cilia. Interfilamentar junction is present. At the abofrontal end can be seen abofrontal cells which bear abofrontal cilia. It is also noted that beneath the ciliated abofrontal cells mucous glands are present (Lucas, 1931; Satir and Gilula, 1970; Aiello and Sleigh, 1972; Paparo, 1972).

Pathological disturbances in organisms due to organic and inorganic pollutants have been widely documented. (Hawkins, 1980; Moore and Clarke, 1982; Moore, 1985; Lowe, 1988;). Some examples cited are the occurrence of neoplastic lesions in fishes and non-neoplastic abnormalities in crabs. (Malins et al., 1984), hepatopancreatic epithelial reduction in bivalve molluscs by a variety of contaminants, (Lowe et al., 1981; Conch, 1984), lysosomal disruption in response to copper and phenanthrene (Pickwell and Steinert, 1984; Moore, et al., 1984)

The digestive diverticula in some invertebrates showed the following pathological alterations namely, atrophy of cells, reduction in height of tubular epithelium, tubular dialation, necrosis and desquamation of tubular epithelium. when exposed to cadmium chloride (Establier et al., 1978b). Auffret (1988) describes severe degenerative changes in the epithelium and digestive glands of Mytilus edulis when exposed to high concentrations of a mixture of copper and diesel oil. Lesions of similar type have been reported in mussels exposed to sub lethal stress (Gonzales and Yевич, 1976). Oysters from contaminated estuaries presented atrophic epithelium, sloughing of cells and necrosis. (Couch, 1985). Martin (1971) showed histopathogical changes in digestive tubules and gills of fresh water clam C.fluminer exposed to copper.
The connections between cilia and microvilli are seen to break following uncoupling of the gill filaments due to copper and cadmium exposure. (Sunilla & Lindstroem, 1985). Short term exposures to cadmium and copper indicated swelling of endothelial cells and detachment of abofrontal cells and dilation of brachial veins (Sunilla, 1986). Pathological alterations observed in *Perna indica* when exposed to 10 ppb of mercury for twenty one days were sloughing off of lateral and frontal cilia and infiltration of blood cells in gill filaments. The digestive cells showed degeneration of cells.

The normal structure of the tubule as evidenced from the photo micrographs show a sheath of collagen fibers covered with smooth muscle fibers forming a meshwork. 'This is the normal structure of digestive tubule of majority of bivalves. 'The digestive cells which occupy the internal lining of the tubule are large and characterized by the presence of irregularly shaped empty vesicles. A comparison of this structure with that of digestive tubules of those animals exposed to sodium pentachlorophenate for various concentration and exposure period shows the extent of damage caused. The tubules have virtually lost the basic characteristic and are so bulged and enlarged. The collagenous layer has disintegrated exposing the muscular layer. The cells have been found to be dislodged and in some cases remain distributed in the lumen.

Those animals exposed to 96 hrs also possessed gastric tubules with partial or complete damage reflected in form of disruption of the muscle layer, dislodging of cells and partial or complete disintegration of secretary and absorptive cells The damage to the tubule in animals exposed for 24 and 48 hrs were not drastic as found in the case of 72 and
96 hrs exposed animals. Noticable feature was the presence of haemocytes in the lumen of the diverticula. Tubular damage involved disruption and dislocation of layer, loss of identity of tubules and heavy vacuolization in the two types of cells and sometimes obliterating the lumen.

The basic structure of the gill of bivalve shows descending and ascending lamellae. The lamellae are formed of jointed filaments at inter filamenter junction by means of cilia. Ascending and descending lamellae are connected by interlamellarhlood vessels. The blood vesselshave well defined epithelium formed of ciliated or non cilliated cells. A chitinolls rodrupports the whole soft tissue. As in the case of the study of the gills of such animals which were exposed to various concentration of sodium pentachlorophenate were examined histologically to asses the extent of damage, the basic structure of the gills evidenced in Fig was maintained only in the control animals.

The mussels gills cells are attractive models in ecotoxicological studies, since gills are the first target and uptake site for many toxicants in the aquatic environment and thus gills cells are often affected by exposure to pollutants (Sunila, 1986, Bigas et al., 2001) well established genotoxicity and cytotoxicity assays have been applied to isolated gill cells of mollusks as measures of damage by environmental chemicals (Venier et al., 1997.A.uzoux-Bordenave1995). The histological studies have shown that the NA-PCP have affected the gills of _L. marginalis_ when compared with the respective controls. The gills of the exposed bivalves showed disorganization of the tissue especially in the lamellae. Inter lamellar space was filled with hyperplasic epithelium and lamellar
shape structure were lost due to desquamation. The histological changes in the gills were mostly due to inflammatory reaction. Swelling may be caused by disturbances in osmotinc balance. Ion exchange and permeability to fluid are change when NA-PCP disturb the function of the membranes (Viarengo, 1985).

There seems to be scanty information on the histopathological effects of NA-PCP on the mantle and foot of molluscs. In the present findings it was observed that the NA-PCP acts directly on epithelial cells, mucous glands and muscle fibers of the foot. The muscles were broken and the mucocytes were concentrated. The central connective tissue and oblicmuscle fibers were disrupted and empty space enlarged in 72 and 96 hrs exposure period. Desquamation of the hyperplastic epithelium was noticed. The sub epidermal cells were concentrated and muscular layers were damaged. The damage to the foot tissue in 96 hrs exposed was extensive. In addition to the disruption of the muscle fibers, spaces and gaps appeared between fibers and the vesicular cells, and the mucous cells were swollen and lost their shape. Mane et al., (1991) reports similar results while working on B. dissimilis. They noted swelling of epithelium, reduction in the number and size of mucous glands in parenchyma and disruption of muscle fibers in B. dissimilis. Many researchers worked on effect of foot of various pollutants, Pauley, (1967); Elyakov, (1992); Hietanen et al., (1988), Mane et al., (1979).

Mantle tissue was examined because it provides the major site for the location of gonads in bivalve and it is a common side for mucous producing cell in the mantle and is termed as mucocytes (unicellular glands) (Tay et al., 2003). There was a relatively small number of mucocytes (unicellular
glands) in the mantle of control bivalves. Interestingly size and number of these cells increased after 72 and 96 hrs exposure period. Other lesions were observed in the mantle of experimental animal exposed to Na-PCP including atrophy of columnar muscle and this has been identified by other authors Weinstein, (1997) as a response to the various environmental pollutants. They observed increase in the number of adipogranula cells and aggregation of haemocytes in connective tissue. Our results are in agreement with findings of Nahala et al., (2007). They have examined the histological changes in marine mussel *M. edulis* and stated marked histological changes in various tissues of the bivalve following a long term exposure to sub lethal levels of lindane and atrazine. Jagtap, (2010) noted histological changes in the cells of gonads and digestive glands of freshwater bivalve, *L. marginalis* when exposed to TBTCl. Many researchers reported similar results Livingstone, (1993); Russo and Lagadic, (2004), Smolarz et al., (2005), Birgitta et al., (1988), Clark et al., (2000).

The present study has shown several degenerative changes in the histological structure of the ovary, digestive gland, gill, mantle and foot of *L. marginalis* exposed over 96-hr LC$_{50}$ concentrations of the NA-PCP. It was interesting to note that histological changes induced by NA-PCP were more intense in experimental when compared to control group. NA-PCP is toxic to *L. marginalis*, when various parameters were considered together. Hence, it is essential to study the histological effects, changes in metabolism and biochemical at cellular level before arriving at a definite conclusion in the toxicity of the pentachlorophenols compounds.