REVIEW OF LITERATURE

Among the several aspects of toxicity studies the bioassay constitutes one of the most commonly used methods in aquatic environmental studies with suitable organisms. The necessity of determining the toxicity of substances to commercially aquatic forms at the lower level of the food chain has been useful and accepted for water quality management.

Several studies have been conducted in assessing the toxicity of different types of pollutant to the aquatic biota. Effects have also been made to use certain bivalve species as bio-indicators of metal contamination of freshwater bodies. Acute effects are those that occur rapidly as a result of generally short-term single exposure to a chemical. In bivalve and other aquatic organisms, effects that occur within a few hours, days or weeks are considered acute. Generally acute effects are relatively severe. The most common acute effect measured in aquatic life is lethality or motility, a chemical is considered acutely toxic if by its action it kills 50% or more of the exposed population of test organisms in a relation to short period of exposure time such as 96 hrs. The objective of toxicity test is to define concentrations at which a test material is capable of producing some selected response, usually deleterious in a population under controlled conditions of exposure (FAO, 1982).

Freshwater mussels are an ecologically important fauna because they are used as sensitive biomarkers of aquatic ecosystems pollution. Bivalves, such as *Unio tumidus* are stationary, filter-feeding organisms able to bioaccumulate and concentrate most pollutants even if they are
present in fairly low concentrations (Niyogi et al., 2001). Many potentially toxic anthropogenic organic xenobiotics enter the water environment and are taken up by aquatic organisms, thus contributing to the changes in their life stages. Some reports show that used PCP can be found in phenolic compounds belonging to one of the major classes of pollutants (Choi et al., 1999). PCP, used primarily as a wood preservative, is relatively stable in the natural state and is thus a ubiquitous contaminant in the environment (Klibanov et al., 1980). This agent has been confirmed as a compound toxic to aquatic organisms as well as to mammalian cells (Klobucár et al., 1997; Jansson and Jansson, 1991). Furthermore, it has been found to be genotoxic to higher plant cells (Chand, 1980), and mutagenic to bacteria and mice (Gopalaswamy and Nair, 1992). PCP contamination is generally associated with surface soils from drying areas and with groundwater contaminated from the above source (Kieth and Telliard, 1979). Most chlorophenols, particularly PCP, have been extensively studied regarding their bio effect and biodegradability in the environment. The aqueous solubility of these substances has become an important parameter in eco toxicology and bio treatability studies. Aqueous solubility of substances like chlorophenols is useful as an indicator of their hydrophobic partitioning from water and, as a result, of the concentration, which they can achieve in biotic phases (Kaiser and Valdmanis, 1982). Molluscs have been used extensively as bioindicators of heavy metal pollution in aquatic system. The unionidae species rely on similar sensitivity to pollutants, functioning as bioindicators on the taxonomic level. They are more sensitive than the fish species from the middle and the inferior parts of the rivers. When the mussels disappear, it
means that the river is seriously affected (Fuller, 1974), leading to a decreasing in the life support capacity of the ecosystem, induced also by the elimination of these important filtering. Kulkarni, (1993) reported the acute toxicity of cadmium to the fresh water bivalve, *L. marginalis*. Talbot, (1986) reported the importance of seasonal variations of copper and zinc concentration in the oyster, *Saccostrea cucculata*. Mane and Muley, (1987) observed the seasonal variations in the toxicity of Cythion-Malathion to two freshwater bivalve molluscs, *L. marginalis* and *L. corrianus*. Khan *et al.*, (2001) studied the cadmium toxicity to the marine edible gastropod, Babylonia spirata. Piansiri and Pachanee, (2008) observed the toxicity bioassay of the Juvenile freshwater snail, *M. martensi* exposed to mercury and cadmium. Since last three decades acute toxicity bioassays in general are useful in measuring the toxicity of different pollutants to aquatic organisms. Since many workers directed the studies towards the toxicity evaluation (Lowe *et al.*, 1971; Mane *et al.*, 1979; Bhavani and Dawood, 2003; Bhomre *et al.*, 1996; Arunee S, 1986). The reaction and survival of aquatic organism, under toxic conditions depend upon several factors, such as kind, toxicity and concentration of the toxicant and the temperature, salinity, dissolved oxygen, pH and physiological factors such as reproductive cycle and seasons, in addition to the type and time of exposure to the toxicant (Holden, 1973; McKim *et al.*, 1973; Brungs *et al.*, 1977), it is necessary to carry out toxicity studies on varieties of aquatic species in different seasons. Freshwater bivalve molluscs are shown to be year around breeders or the breeding is restricted to a year (Mudkhede, 1974) and it is shown to be influence by the changes in environmental factors.
Biomarkers are defined as biological responses to environmental chemicals that give a measure of exposure and sometimes, also of toxic effect (Walker *et al.*, 1996) in an environmental context, biomarker are biological tools used as sensitive indicators, demonstrating that toxicants have entered the organisms, been distributed within the tissues, and are eliciting a toxicological effect. Biomarkers are state of the art tools used to estimate the impact of chronic exposure to specific chemicals in the environment. Biomarkers are physiological alterations or manifestations of stress in organisms. A biomarker is a biological reaction used to monitor exogenous exposure, effects of exposure, and early symptoms at the organ or organism level (Schulte, 1995). Biomarkers commonly represent biological responses of individual organism to foreign chemicals or xenobiotics. The biological responses may include, amongst others, i) enzyme alterations, ii) immune dysfunction, iii) reproductive disturbances iv) DNA changes, v) behavioral changes vi) histopathological lesion (hokas, 1993). Biomarkers have a great potential for use in environmental monitoring of both marine and freshwater ecosystems and biomarkers have been validated to be included in monitoring programs (den Besten, 1998).

Biomarkers have been classified into two groups namely biomarkers of exposure and biomarkers of effect. Biomarkers of exposure are a demonstration of chemical exposure of organism, but do not give information of any biologically important adverse effects that this exposure may have caused biomarkers of effects, or more correctly toxic effects, demonstrate that an adverse effect on the organism has occurred
due to exposure to pollutants (Molven and Goksoyr, 1993; Lowry 1995; Walker et al., 1996).

Exposing animals to xenobiotics causes alteration at the cellular level, and involves modifications of biochemical pathways. The measurable various in biological system are called biochemical markers, commonly referred to as biomarkers (Landis and Yu, 1995). The biochemical changes occurring in the body gives the important indication of stress. Different tissues and organs have different activities and metabolic rates and therefore their responses to the same toxicant may be different. Much work carried on toxic effect of heavy metals on specific target and non target aquatic invertebrate and vertebrates with respect to the biochemical changes. There is an increasing need to develop methods for the identification, estimation, comparative assessment and management of risk posed by chemical pollutants discharged into the aquatic environment. Therefore, the measuring the biological effect of pollutants in relation to body constituents is essential for assessing the quality of the freshwater environment.

A Polychlorinated phenol (PCP) is commonly used for domestic, agriculture and industrial purposes because of its potent biocide properties. Its application includes wood and textile protection in pulp mills as a bleaching agent (Gifford et al., 1996), agricultural pesticides molluscide (Tanaka and Tasji, 1997) and fungicide (Alcock and Jones, 1997). The presence of phenol compounds, especially the chlorinated forms in the aquatic environment, is of great concern as it has the potential to affect all forms of aquatic life, even at low concentrations.
(Devi and Gnudi, 1999). Chlorophenols exhibit weak aquatic properties in water. This means chlorophenols dissociate in alkaline the water but remain un-dissociated in water with low pH. Therefore, the concentration of chlorophenols in acidic water is usually higher than in nono-acidic water. PCP is the strongest acid of the chlorophenols family: chlorophenol acidic properties normally decrease with decreasing chlorine substitutes. Because of its water solubility, PCP is more available for absorption via the gills of aquatic organisms. Toxicity of chlorophenols also increases with the number of chlorine atoms, however for chlorophenols having the same number of chlorine atoms, the toxicity decreases in the order of non, mono, and di-ortho – chlorophenols.

Trace concentrations of NA-PCP compounds have a potential to cause adverse effect in aquatic organisms (Bostro and Johansson, 1972; Muir and Eduljee, 1999). Polychlorinated phenols cause an inhabitation of oxygen consumption in fish at ug/liter concentrations (Bordeur et al., 2001). Polychlorinated phenols are also known to uncouple oxidative phosphorylation (Schuurmann et al., 1997). And act as an energy transfer inhibitor in various respiration stages (Ogata et al., 1983). When bio accumulated, PCP is stored in hepatic lipid reserves and strongly bound to mitochondrial proteins (Bostrom and Hohansson, 1972). Polychlorinated phenols elevates maintenance energy demands causing a reduction of growth rate, which can be used as a sub-lethal indicator of aquatic organisms stress (Webb and Brett, 1973).

For most aquatic invertebrates tested annelids, mollusces and crustaceans as well as for fish, the acute toxicity of PCP compounds is below 1 mg/L (WHO, 1980). PCP clearly caused reduced growth rate and
inhabited swimming performance of salmon (*Oncorhynchus nerka*) (Webb and Brett, 1973). The fish chronically exposed to phenols showed a reduction in feeding rate, growth rate, delayed maturity and lower fecundity relative to control, while fish acutely exposed to phenol showed a respiratory distress, and excess mucous secretion from the skin and gill (Saha *et al.*, 1999). (LDH) increase when muscle, liver or heart is injured whether from disease or exposure to toxic compound (Singh and Sharma, 1998; Girizzle and Lovshin, 1996).

The biochemical changes occurring in the body gives the important indication of stress. Different tissues and organs have different activities and metabolic rates and therefore their responses to the same toxicant may be different. Much work carried on toxic effect of heavy metals on specific target and non target aquatic invertebrate and vertebrates with respect to the biochemical changes. Kewal Jaiswal *et al.*, (1989) observed changes in biochemical constituents such as protein, lipid and glycogen when exposed to naphthalene to freshwater prawn, *M. kistnensis*). Higher concentrations of toxicant in aquatic environment cause adverse effect on aquatic organism at cellular or molecular level and ultimately it leads to disorder in biochemical composition (Waykar and Lomte, 2001). Machale *et al.*, (1991) reported depletion in protein, lipid, and glycogen due to cuprous oxide stress in various tissues of freshwater crab, *B. guerini*. Khan *et al.*, (1990) observed changes in levels of protein, lipid and glycogen in the muscle of freshwater crab, *B. guerini* exposed to copper sulphate. Further they reported that increased metabolism may be the possible reason to the initial rise in various metabolites which might be well accompanied by higher enzyme levels in the muscles. Copuzzo and
Lancaster, (1981) investigated accumulation of compounds in oyster and its effects on their different tissues and biochemical constituents. Bayne and Thompson, (1970) determined the biochemical composition of mantle, gonad and somatic tissues of *M. edulis*. Veena Shaktivel, (2002) studied effect of phosphomidan on glycogen, protein and lipids of *G. affinis*. Kumar and Gopal, (2001) observed changes in protein and glycogen contents in, *C. punctatus* due to impact of distillery effluent. Seasonal variations in the biochemical composition have also been reported for *Teredo pedicellata* (Lane *et al.*, 1952; Greenfield, 1953). Seasonal changes in biochemical composition of *M. edulis* in British waters have been reported by Williams, (1969). Freshwater and marine bivalves display marked seasonal variations in weight and biochemical content of the soft tissue (Giese *et al.*, 1967; Ansell, 1972; Gabbott and Bayne, 1973; Beukema and de Bruin 1977; Dietz and Stern 1977; Zandee *et al.*, 1980; Williams and McMahon, 1989) that are related to the considerable energetic demand of gametogenesis. Bivalves build up stores of energy in the body tissues and then deplete these stores during the production and eventual release of gametes if reproduction occurs during the summer months. Ansell *et al.*, (1964) determined seasonal changes in biochemical composition of adductor muscle, mantle, siphon, digestive gland and foot from *Mercenaria mercenaria*. Seasonal changes in biochemical composition of different body parts of a few Indian species hade been reported by Nagbhushanam and Mane, (1975) on *Mytilus edulis*. Nagbhushanam and Talikhedkar, (1977) observed seasonal variation in protein, fat and glycogen content in *Donax cuneatus*. 
The key substances, carbohydrates, proteins and lipids act as sensitive indicators of stress (Peter, 1973). Patil and Mane (2004) observed the biochemical levels in different body parts of freshwater bivalve, \textit{L. marginalis} exposed to mercury in monsoon season. Muley and Mane, (1987) also reported sublethal effect of mercury chloride on the tissues composition of bivalve mollusc, \textit{L. marginalis}. Sujatha et al., (1996) observed NA-PCP induced physiological and biochemical changes in a tropical estuarine clam. Reddy et al., (1986) studied the effect of mercuric chloride on the Carbohydrate metabolism in the soft body parts of the freshwater mussel, \textit{Parreysia rugosa}. Parate et al., (2003) observed changes involved in total protein profile in the muscles and gills of the crab, \textit{Paratelphusa jacquimontii}, exposed to the pyrethroid pesticide cypermethrin at different experimental conditions was studied. Bhavani, and Dawood, (2003) observed the absorption of metals, biochemical components like Proteins, Carbohydrate and Lipids were, the decrease of proteins, carbohydrates and lipids in the body tissue of \textit{Perna viridis}, due to metal toxicity. Hochachka et al., (1973) found an alternative scheme for anaerobic metabolism in lamellibranch molluscs, in which redox balance is maintained by the simultaneous utilization of both carbohydrates and proteins. However, on the other hand, there is a significant loss of stored energy while converting pre-stored glycogen in to lipid, (Krebs and Webber; 1972).

marginalis exposed to chromium. Zambre, (1991) studied the reflections in protein content of freshwater bivalve, C. striatella due to heavy metal exposure. Mule, (1991) observed the alteration in protein content after exposure to monocrotophos, cypermethrin and some heavy metals. Mahajan and Zambre, (2001) studied the depletion of protein levels in the different tissue such as gonad, gills and digestive gland of freshwater bivalve, Corbicula striatella, after exposure to chronic dose of copper sulphates and mercuric chloride. Depletion of the protein contents in different tissues such as gonad, gill and hepatopancreas of bivalve, after exposure to HgCl₂ and CuSO₄.

Another constituent of animal tissue is lipid which plays an important role in energy metabolism and provides energy in metabolic process, Shigmastus and Takeshita, (1959). Lipids are also important in the cellular and subcellular membranes. Lipids are used as energy resources and these are stored and transported in the form of glycerol and esters. Some toxicologist focused certain attention on the impact of pollutants on the lipid reserves of aquatic animal. Nagabushanam et al., (1972) reported decrease in lipid level in hepatopancreas of the freshwater prawn, Macrobrachium kistnensis in response to pesticidal toxicity. Villalan et al., (1990) observed the reduction in lipid content in muscle due to chromium stress in Macrobrachium idella. Lomte and Muley, (1993) reported the decrease in lipid level in the freshwater snail and bivalve, Thaira tuberculata and Parresia corrugata exposed to copper toxicity. Sarvana and Geraldine, (1997) reported the reduction in lipid content in freshwater prawn, Macrobrachium malcomsonii when the prawn exposed to endosulfan. Shah et al., (2001) reported altered lipid

The impact of xenobiotics on an organism is reflected through alteration in its physiology, cellular structure, and biochemical balances (Najle et al., 2000) Hepatic lesion may be neoplastic, preoplastic, non-neoplastic proliferative or unique degenerative /necrotic lesion. These lesion types have been positively correlated with contaminant exposure and may be promising as biomarkers predictive of pathological effects (Mayers et al., 1998). Especially early hepatic organ lesions may be a good indicator of environmental containants (Molven and Goksoyr, 1993).
Besides causing cell structure alteration, a xenobiotic may also cause alterations to glycogen and lipid storage. Glycogen is a branched polymer of glucose and increase as well as decreases in glycogenolysis. This can occur due to toxicant-induced stress, which results in either depletion or accumulation of glycogen. In most cases the stress condition causes depletion of both glycogen and lipid storage (Giese et al., 1988).

tissues of freshwater bivalve, Parreysia cylindrical. The significant decreases in total glycogen content of gill, digestive gland were observed due to pollution stress caused by nickel chloride.

Shah et al., (1998) investigated changes in glycogen contents in estuarine Anadara rhombea exposed to organotin. Rao et al., (1983) observed significant decrease in total carbohydrates and glycogen in hepatopancreas, mantle and foot in Pila globosa to sub lethal concentration of methyl parathion and also decreased phosphorylase activity. Mandal and Ghose (1970) observed the glycogen depletion in digestive gland of the snail, Achatina fulica exposed to calcium arsenate. Reddy et al, (1986) and Shah et al., (1998) also studied the glycogenolytic phenomenon during stress. The glycogen contents in molluscs after exposure to pollutant were estimated by Lomte and Alam (1982). In molluscs the glycogen content after pesticidal impact were studied by many workers (Mandal and Ghose, 1970; Ramana Rao and Ramamurthi, 1978; Ramana Rao and Rammurthi, 1980; Swami et al., 1983; Akarte et al., 1985; Patil, 1986; Nagabhushanam et al., 1987; and Tripathi and Singh, 2002).

The way for the pollutants to enter in the body of an animal is the blood system from where the pollutant gets distributed to the whole body. Pollution of water by any pollutant can have deleterious effects on aquatic organisms. The nature of the effects varies and may cause structural levels in and functional modification at both the cellular and sub cellular level in organisms. In order to understand a pattern of damage caused by particular chemical to the tissue is essential to have and insight in to the histological analysis of the tissues. This can be helpful for better
understanding of the pathological condition and abnormalities and damages of tissues under toxic stress of pollutants. Thus histopathology is an extremely useful tool for assessing effects of toxicants at individual level. Histopathology is a valuable tool for providing health assessments of individuals and of populations since it incorporates measures of reproductive and metabolic condition, and allows for the detection of a range of toxicants that may affect morbidity and mortality. In addition to its role in providing a ‘baseline’ measure of health, histopathology has been employed to investigate the changes related to heavy metal exposure in mussels (Sunila 1984, Lowe and Pipe 1987, Auffret 1988, Kluytmans et al., 1988, Marigómez et al., 2006). Histopathology can be considered as a means to provide supporting information for measures that specifically aim to assess historic exposure to, or effect of, a contaminant.

Investigations on toxic effects of pollutants to aquatic invertebrates are a field of study which has gained momentum in recent years. There has been a spectacular increase in basic and applied research connected with contamination in the fresh water sources. Marine bivalves have been used as very useful experimental organisms, to assess the toxic effects of pollutants. These animals are capable of tolerating variable conditions provided in the laboratories. The capability of bivalves to function normally under laboratory conditions have made them potential experimental animals to assess rate functions, structural modifications and accumulations of toxic substances. On exposure to contaminant fresh water, bivalves are known to take up toxicants. Through food (George, 1978). The absorption of these can occur through food or by pinocytosis of gill epithelia (Coombs and George 1978). Evidences show that toxic
substances like heavy metals could enter the body either through food or water. Therefore, the tissues that could be involved in the process are the digestive glands, mantle and the gill. It is obvious that water is a prime source of heavy metals to bivalve. Therefore, the experiments were performed using water as the major medium to supply NA-PCP to experimental animals, *L. marginalis*.

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. 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.

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 filter feeding bivalves, widely inhabiting the river waters, are well documented by workers in India.\cite{Mane1984}. 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 \cite{Moore1991}. Sedentary filter feeding bivalves, especially mussels are often used to assess the quantity of heavy metals in aquatic environment \cite{Bryan1976}. 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 \textit{et al.}\cite{Butler1971}; Phillips \cite{Phillips1976a,Phillips1976b}. 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. As the present study involves histological methods to evaluate alterations caused by selected pollutant impact, an understanding into the basic structure is considered a must.

A comprehensive knowledge of the structure and function of the digestive glands and gills 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. 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 secretory cycle.

The same configuration of cells were noted in some other bivalves like *Anodonta anatina* (Sumner, 1966a) *Mytilus edulis* (Platt, 1971), *Mya*
arenaria (Pal, 1971, 1972) and in some other fresh water lamellibranchs. studies by Lowe and Clarke (1989) on the structural alterations of the digestive epithelium exposed to toxicants. All these reports confirm that the basic structure of the digestive tubules of most bivalves is more or less the same in appearance. An important characteristic feature of digestive cells is that it is made up of various types of micro vesicles and macro vesicles. Structural and functional differentiation on micro and macro vesicles has been studied by numerous workers. (Owen, 1970; Platt, 1971; Thompson al., 1974). However, Pipe and Moore (1985) have three types of macrovesicles as hetrophagosomes, hetrolysosomes and residual bodies. Many research conclude that digestive cells in Mytilus 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 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).

Pathological disturbances in organisms due to organic and inorganic pollutants have been widely documented. (Hawkins, 1980, Moore and Clarke, 1982; Moor, 1985; Lowe, 1988). Some examples cited are the occurrence of neoplastic lesions in fishes and non-neoplastic abnormalities in crabs. (Malins et al., 1985), 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 dilation, 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 Yevich, 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 digestive cells showed degeneration of cells. Reduction in epithelial thickness of the digestive gland of gastropods and bivalves was proposed by some authors as indicator of environmental quality assessment (Tripp et al., 1984, Marigomez et al., 1990). Geometrical transformation of that shape into a hypothetical trapezium can assess the determination of the height index of the digestive tubule for both control and heavy metal exposed molluscs (Marigomez et al., 1990).

This might be confused with monophasic digestion occurred for some gastropods during the digestive cycle, where fragmentation of the digestive tubule was observed. However, tubular atrophy can be easily distinguished from thinning of epithelial cells resulted from environmental stress (Lowe et al., 1981). Bodar et al., (1990) investigated cytological changes of digestive tract and storage cells in *D. magna* exposed to cadmium and tributyltin. Usheva et al., (2006) observed histopathology of the digestive gland of bivalve molluscs, *C. grayanus*. 

The organ specific damage can often be observed at the organism level this observation is based on the fact that an animal often exhibits deformation in done structure, damage to organs, which can be easily observed. Furthermore, lesion and necrosis in tissues have been the cornerstone of much environmental pathology, and the cytogenetic examination cells can reveal damages to genetic baggage, and reflect effects of exenobiotics at the individual level (Landis and Yu, 1995). Biomarker assess the biological and ecological responses to contaminates preset in the environment. These responses can be observed at several levels of biological organization from the molecular level, where pollutants can cause damage at cellular and elicit defensive strategies such as detoxification, to the organism level, involving adverse effect on growth, reproduction, developmental abnormalities or decreased survival. Furthermore, perturbations at the individual level may possibly translate into effects at the population, community, or even at ecosystem levels (Shugart 1996; Walker *et al.*, 1996)

Lipofuscin granules are being used as a biomarker of cellular stress in association with lysosomal alterations in the digestive gland of bivalves, and have proved to be an efficient biomarker (Au, 2004; Zorita *et al.*, 2006). Kulkarni (1993) also studied the effect of cadmium chloride on histological changes in gonads, hepatopancreas of *L. marginalis*. Patil and Mane, (2001) observed histopathological changes due to mercury on the bivalve, *L. marginalis*. Clark (1989) reported on metal induced changes in the structure of digestive epithelium of *M. edulis*. Stiles *et al.*,
(1991) measured cytologically the levels of polychlorinated biphenyls (pcbs), copper and cadmium in the mature gonads of the clam, M. mercenaria. Unlu et al., (2005) studied histopathological effects in tissues of snail, L. stagnalis exposed to sublethal concentration of thiodan. Bignell et al., (2008) found the effects of season on mussel histopathology. Various workers show the similar results worked on the freshwater as well as marine species (Sumner, 1965; Walker,. 1970; Fred,. 2003; Barsiene,. and Rybackovas,. 2008).

Histopathological alterations of gonad cells have been observed in shellfish collected from contaminated areas or exposed to contaminated sediments in the laboratory, Yevich, (1987); Teh,. (1993). The observed lesions ranged from dilation of reproductive follicles in mussel, M. edulis, Sunila. (1987), to gonadal neoplasms in soft-shell clam, M. arenaria, Yevich (1983), and the American oyster, C. virginica. Machale et al., (1990) studied on the histopathological changes in the ovary of freshwater crab, B. guerini exposed to copper sulphate and reported extensive damage to oocyte. Gyananath, (1982) reported the histopathological lesion in gonads of freshwater prawn, M. lamerrii exposed to Malathion. S. Reddy et al., (1983) observed that the sumithion affects on the ovarian growth of crab, O. sensex. Victor, (1984) observed structural changes in ovary of freshwater prawn, C. rajadhari exposed to Malathion and DDT.

The histological studies not only give an early indication of pollutants hazards but also provide useful data on nature and degree of damage to cells and tissues. It is common tool for determining the deleterious effects of toxic substances in animals. Some environmental scientists are beginning to correlate the degree of cell damage to
concentrations of the toxic substance and their synergistic or antagonistic interaction, Crandall and Goodnight, (1963); Brown, (1968). The histological techniques are promising area of research in aquatic toxicology as it gives the real picture of effects imposed and the involvement of chemical pollutants in the either disturbing or destroying the vital organs of living organisms. Histological studies were also useful in evaluating the pollution potential of organotins since trace amount of these chemicals which do not bring mortality over a period were capable to producing considerable organ damage, Indira, (1989). Histological analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs, such as the gills, liver and gonads, Dutta, (1996). A histological investigation may therefore prove to be a cost effective tool to determine the health of animals populations, hence reflecting the health of an entire aquatic ecosystem in the bio-monitoring process. Histological responses may also serve as ecotoxicologically meaningful biomarkers since they form an important link between effects at the biochemical level and those measured in whole organisms, Lowe, (1988).

Histological study is valuable for providing health assessments of individuals and of populations since it incorporates measures of reproductive and metabolic condition, and allows for the detection of a range of toxicants that may affect morbidity and mortality, Hinton et al., (1992). In addition to its role in providing a ‘baseline’ measure of health, histopathology has been employed to investigate the changes related to heavy metal exposure in mussels (Sunila, 1984, Lowe and Pipe, 1987, Auffret 1988, Kluytmans, et al., 1988, Marigómez et al., 2006). Histology
can be considered as a means to provide supporting information for measures that specifically aim to assess historic exposure to, or effect of a contaminant. Histopathological study also provides a ‘phenotypic anchor’ against which this specific data can be assessed (Stentiford et al., 2005, Hines et al., 2007a).

Perusal of literature reveals paucity of information on toxicity of NA-PCP on fresh water bivalve, *L. marginalis* Hence the present study has been undertaken to evaluate the acute toxicity and its effect *L. marginalis* as bio-indicator, of local importance from Maharashtra state in Godavari river at Kaigaon village of Aurangabad district (M.S) India.