Chapter 10

GENERAL DISCUSSION

10.1 Introduction

In the middle and lower Gangetic basin of India, ground water pollution by arsenic has become a devastating threat for environment and mankind (Acharyya, 1999; Chakraborti et al., 2003; Islam et al., 2004; Neumann et al., 2010; Roberts et al., 2010). It poses a threat not only to the ground water but to the surface water of the selected plains of West Bengal too (Acharyya, 1999). Bivalves are aquatic invertebrates considered as bioindicators in environmental monitoring (Sanders, 1993). In the tropical freshwater reserves of India, pearl-producing mussel L. marginalis is distributed abundantly (Annandale and Prashad, 1919; Ghosh and Ghosh, 1972; Rao and Dey, 1989; Chakraborty et al., 2008) as an important component of freshwater ecosystem. As filter feeder, this species is known to be a general indicator of chemical contamination (Mohan et al., 1987; Sivaramakrishna et al., 1990; Hameed and Raja, 1990; Satyaparameshwar et al., 2006; Mukherjee et al., 2007; Pattnaik et al., 2007). No detailed analysis has been made till date to evaluate the effect of arsenic toxicity on the semi-sessile inhabitants of the freshwater reserves of the region (Chakraborty et al., 2008). An in depth toxicological study on L. marginalis appears to be imminent to assess the environmental risk of arsenic in freshwater invertebrate distributed in the arsenic affected zones of West Bengal. In the present study, a detailed investigation on arsenic induced toxicity in L. marginalis in relation to the immunological and biochemical functions of its haemocytes and other tissues was carried out to analyse and evaluate the probable arsenic induced toxicity in the freshwater ecosystem.

10.2 Haemocyte structure and density

Haemocytes are the circulating cells of the open circulatory system of the molluscs. In bivalves, the internal defence system is based on the structural and functional integrity of haemocytes which display phagocytic and microbicidal activities (Cooper and Knowler, 1992). They act as the major immune effector cells (Cheng, 1977; Adema et al.,
1991b) and mediate non-self phagocytosis that provides natural immunity in the bivalves (Lopez et al., 1997a,b). Haemocytes also remain associated with a variety of physiological and pathological functions including nutrient transport, digestion, wound and shell repair, internal defence as well as excretion (Cheng, 1981; Bayne, 1983; Fisher, 1986; Gliniak and Jarosz, 1997). Haemocytes are considered to be the main cellular mediators of the defence system in bivalves (Volety and Chu, 1995; Cheng, 1996), responsible for recognition, phagocytosis, and elimination of non-self particles by microbicidal activities (Pipe, 1992; Cheng, 2000; Chu, 2000). It is reported that the efficiency of haemocytes may be affected by environmental contaminants (Anderson et al., 1981). The haemocytes of L. marginalis exposed to sodium arsenite exhibited formation of numerous cytoplasmic vacuoles of diverse shape and size. The abundance of vacuoles in the haemocytes of the animals may be linked to extensive degranulation of the electron-dense granules from the cell cytoplasm under the toxic exposure. The density of vacuoles is the highest in the granulocytes which contained the highest granular content. Micrometric analysis revealed that the exposure to the natural toxicant caused swelling of the haemocytes which is indicative of probable malfunction of the membrane channels responsible for maintenance of cellular osmolarity. One approach to assessing immune responses of oysters is to measure haemocyte parameters (Delaporte et al., 2007). The analysis of haemocyte population dynamics involves the simultaneous approach to a series of parameters, namely, total haemocyte count (THC) and differential haemocyte count (DHC) (Jones, 1962; Arnold and Hinks, 1976). Marked variations in the density of haemocytes may be mainly related to haemocyte release from haemocytopoietic organs into the circulation (Hoffmann, 1973; Feir, 1979). Experimental exposures of bivalves to all the experimental concentrations of sodium arsenite resulted in a dose responsive suppression in total count of haemocytes. Suppression in the density of haemocytes signifies two major probabilities: (1) a major immune compromise on the part of the animal and (2) development of a probable haematopoietic stress in the animal. Both conditions would affect the normal physiology of the animal in an adverse manner; as such, weak immune surveillance will invite opportunistic parasitic/microbial infections and suppressed haematopoiesis might perpetuate the physiological weakness. Under the experimental conditions, the density of the blast-like cells was depleted whereas the densities of granulocytes and hyalinocytes were increased under the higher concentration of sodium arsenite. The data is indicative of two probabilities: (1) suppression in density of blast like cells signifies development of haematopoietic stress in the animal, or (2) their transformation from prohaemocyte condition into functional granulocyte and
hyanocytes population. Although exposure to sodium arsenite resulted in elevation of the relative density of major immune haemocyte subpopulations like granulocytes and haalinocytes in a dose responsive manner, suppression in the THC would probably nullify the immune vigour of the animals which might cause depletion in the ability of the bivalve to defend against the natural hostile environment of the animals including opportunistic parasitism (Chakraborty et al., 2008).

Lysosomes are multifunctional cellular organelles which contain various hydrolases. On phagocyte stimulation, lysosomal hydrolases are released from the cells to degrade foreign materials (Mohandas et al., 1985) or into phagosomes, thus participating in the degradation of internalized foreign particles (Cheng, 1981). Alteration of the integrity of lysosomal membranes may initiate undesired release of hydrolases into the cytosol, resulting consequent damage of self-cells (Lowe et al., 1995). These hydrolases are involved in intracellular digestion of endocytosed food particle and macromolecules as well as the accumulation and sequestration of xenobiotic substances and metals (Moore, 1988). The intactness of the lysosomal membrane is of critical importance to the cell because leakage of lytic enzymes into the cytosolic compartment can result in autogenous degradation of cellular macromolecules and other components, the result of which may lead to severe cell damage or death (Decker et al., 1980). Role for ROS in lysosomal damage associated with xenobiotic exposure has been reported by Singal et al. (1985). The retention of the cationic probe neutral red within the lysosomal compartment over time is determined as a measure of damage to the lysosomal membrane. In the present study, a steady decline in the NNR time was recorded in the haemocytes of the animals exposed to sublethal concentrations of sodium arsenite in a dose responsive manner. By reducing the NRR time of the haemocytes, sodium arsenite as a natural pollutant at sublethal concentrations shows the potential to impose perturbation in intrahaemocyte homeostasis in L. marginalis. Such leaking of lysosomal vesicle might prove to be detrimental for the health and viability of the haemocytes as the lysozyme bears the probability of inflicting damage to the self cellular components. Such derogatory effect of the toxin not only signals a threat to L. marginalis only, but to other inhabitants of the freshwater aquasystems in the arsenic affected zone (Chakraborty and Ray, 2009a). Micronucleus (MN) test is a promising test in environmental genotoxicity studies, has served as an index of cytogenetic damage (Fenech et al., 2003). There are many compounds that induce genotoxic effects with or without direct damage to DNA. Micronuclei can be formed from chromosomal fragments or the from the whole chromosome that lags behind during cell division due to the lack of a centromere, centromere damage or a defect in cytokinesis
(Heddle et al., 1991). Frequency of MN and binucleus (BN) were enumerated in haemocytes of *L. marginalis* exposed to sublethal concentrations of sodium arsenite in controlled laboratory condition. The increase in the nuclear abnormalities against higher concentration of arsenic appears to be dose responsive with mild hormesis at some sublethal exposures. Hormesis is a natural cellular response under low level toxin exposure. The exposed cells seems to be vulnerable to nuclear damage and result of the studies provide acceptability of the micronuclei assay as an efficient method for detecting mutagenic effects of sodium arsenite in *L. marginalis in situ*. This biomarker can serve as an early warning signal for health deterioration of freshwater ecosystem (Chakraborty and Ray, 2009a).

10.3 Haemocyte behaviour: adhesion and aggregation

Among variety of functions exhibited by the motile blood cells of higher organisms, hemostasis and defence against invading pathogens are the most prominent (Salt, 1970; Zweifach et al., 1973). Both functions depend on the ability of circulating cells to display nonself surface recognition, adhesion and active motility. In molluscs, it is postulated that clump formation induces hemostasis and wound healing (Bang, 1961; Sparks, 1972; Sminia, 1981). Although the phenomena of clump formation and haemocyte adhesion and spreading have been described both *in vivo* and *in vitro* in bivalves (Dundee, 1953; Bang, 1961; Sparks, 1972; Narain, 1973; Cheng, 1981), the phenomena have been least explored in *L. marginalis* under the exposure of arsenic, a natural pollutant of freshwater ecosystem. The motile characteristics of the haemocytes have been reported by various workers (Armstrong 1979 a, b). Following trauma or extravasations, the cells transform into a motile and adhesive form which can participate in phagocytosis (Armstrong and Levin, 1979) and which can seal leaks in the vascular system by forming a plug of adhesive cells (Bang, 1979). In aquatic environment, uncontrolled bleeding may lead to a precarious situation. The haemocyte adhesion on glass surface progressively diminished with increasing sodium arsenite concentration indicating inhibitory effect of the toxin on this important cell behaviour. The toxin seems to impair the property of cell adhesion which is vital for attachment to self or nonself surface. Increase in the density of non-adherent haemocyte population in the animals exposed to sodium arsenite indicates that the natural contaminant imposed significant inhibition on this innate tendency of cell adhesion in a dose responsive manner. Inhibition in cellular interaction might prove detrimental for such benthic invertebrates where cellular kinetics and mobility are important from immunological point of view. The observation is predictive of a probable
Studies on the toxicity of arsenic in *Lamell户ura marginalis* (Lamarck) Chapter 10

immune compromise for the species in sodium arsenite contaminated environment. Xenobiotics may alter the aggregative behaviour (Auffret and Oubella, 1997) and adherence (Chen and Bayne, 1995) of molluscan haemocytes. Divalent cations promote attachment to and spreading on protein-coated surfaces for a variety of vertebrate cells (Taylor, 1961; Grinnel, 1974; Maroudas, 1977; Rabinovitch and DeStefano, 1973). Sublethal concentrations of sodium arsenite imposed significant inhibition on the aggregating behaviour of *L. marginalis*. The inhibitory effect was progressive with increasing concentrations and its span of exposure. In some concentrations, the nature of such aggregation was weak. Sucrose in all the studied concentrations imposed significant interference on the aggregating behaviour of the haemocytes. This result indicates that sugar may play a key role in the cell adhesion. Application of EDTA as a chelating agent caused mild interference in the aggregation of the haemocytes and the nature of aggregation appears to be weak. Anticoagulant heparin had feeble effect on haemocyte aggregation which probably indicates absence of any related clotting factor in the animal. Combined effect of sodium arsenite and the aggregation interfering agents increased inhibitory effect on haemocyte aggregation and it was significant for sucrose and in some doses with EDTA. Sodium arsenite thus seems to have palpable role in inhibiting haemocyte aggregation and interfere cell-cell communication. Reduced haemocyte aggregation signifies lack of cellular coordination to deal pathogenic challenge as well as clogging of wounds in the animals, both of which might prove to be detrimental for the survival of the species in contaminated natural habitat. Altered agglutination titer of the haemolymph fraction of molluscs during interaction with parasites (Goldman and Honinberg, 1968; Loker et al., 1994) is in report. But the effect of metals and metalloids on cell agglutination response with the haemocytes of freshwater mollusc is scanty (Ray and Chattopadhyay, 2002). Sodium arsenite probably caused a shift in haemocyte surface character that resulted in steady fall in the agglutination titter with all the immune sera. The pattern of agglutination response reflects probable existence of conserved reactive domains shared by the haemocytes, murine lymphocyte and rabbit cell antigen. The findings suggest: (1) close hierarchy of cell surface epitopic profiles of mollusc haemocytes and rabbit lymphocytes and (2) the ability of the natural toxin to alter the cell surface topology in the exposed animals. The result indicate that exposure of *L. marginalis* to 3 and 5 ppm of sodium arsenite for 96 h yielded an alteration in the surface characteristics of the circulating immunocytes resulting in a significant shift in agglutination response against the control. The animals distributed in arsenic contaminated habitat may often be subjected to similar alteration in surface
characteristics of haemocytes. This arsenic induced shift in surface characteristics of haemocytes may alter the normal immunological status of the animal which may reduce its adaptibility to survive in contaminated habitat.

10.4 Functional attributes of haemocyte

In bivalve molluscs, the haemocytes are responsible for cell mediated immunity through phagocytosis and various cytotoxic reactions, such as the release of lysosomal enzymes and antimicrobial peptides, and the respiratory burst that involves production of oxygen metabolites (Canesi et al., 2002a). Phagocytes are believed to be ancient immune defence cells which are widely distributed throughout the body of multicellular animals (Bayne et al., 1979). Toxin mediated modulation of haemocyte number and function in relation to nonself recognition, phagocytosis, respiratory burst activity etc. are in report by various workers (Adema et al., 1991a,b; Chakraborty et al., 2009b). Phagocytosis by haemocytes is the major line of defence against invading foreign materials including toxic xenobiotics. In the marine mussel *Mytilus*, haemocytes have been shown to represent a sensitive target for a number of environmental contaminants, including heavy metals and organic xenobiotics, with consequent immunotoxicological effects or stimulation of immune parameters, leading to inflammation, depending on the compound and on the conditions of exposure (Lowe et al., 1995; Wootton et al., 2003). The present study provides a proof of inhibitory effect of sodium arsenite on the phagocytic potency of the haemocytes of *L. marginalis* at varied sublethal concentrations. Being an important immune weaponry in the arsenal of the bivalve, crippling its strength signifies a major immune compromise on the part of the animal. *In vivo* as well as *in vitro* PI of the haemocytes of *L. marginalis* was markedly suppressed. Such inhibition might transform the animal vulnerable to parasitic and microbial invasions. The study not only establishes the inhibitory effect of the toxin *in vivo* and *in vitro* but also strengthens the candidature of phagocytosis as a biomarker of arsenic contamination (Chakraborty et al., 2009b). The observation is predictive of a probable immune compromise for the species in sodium arsenite contaminated environment. Phagocytosis being an important mechanism of defence and sustenance of *L. marginalis*, the role of acid phosphatase (ACP) and alkaline phosphatase (ALP) appears to be vital. Being important hydrolytic enzymes, they aid to digest phagosomal particles and membrane transport, thereby maintain the health and vigour of the animal. The activities of phosphatases in the haemocyte of *L. marginalis* increased significantly in the first 24 h of exposure to the sublethal concentrations of sodium arsenite. This is indicative
of the tendency of the haemocyte to augment its immune components against xenobiotic challenge. But exposure to sodium arsenite for longer time span exhibited a significant fall in the activity of ACP and ALP in the same tissue fraction. Suppression of the activity of ACP and ALP in the haemocytes by arsenite might cripple the immune status and nutrient mobility of *L. marginalis*. The conversion of amino acids to keto acids and for oxidative deamination of amino acid by coupled reactions are all among the potential functions of transamination and this is essentially the mechanism by which interconversion of protein with carbohydrates and fat occurs. Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) are important transaminases and selected heavy metals exhibited inhibitory effect on their activity in molluscs (Das and Jana, 2004). Following exposure to sublethal concentrations of sodium arsenite, the level of activity GOT and GPT in the haemocyte increased progressively in the first 24 h. Such increase in the enzyme level is indicative of the effort exerted by the cells to cope with the insult of the toxin. But prolonged exposure nullified the effort with decrease in the activity of the enzymes indicating a condition of vulnerability of the animals under persistent arsenic exposure. Thus, sodium arsenite at sublethal concentrations can perturb the normal functioning of the detoxification machinery of *L. marginalis* and makes it susceptible to possible microbial attack and other natural contaminants in its natural habitat. The decrease in acetylcholinesterase (AChE) activity in the oyster was efficiently used as a biomarker of exposure to neurotoxic compounds (Bocquene' et al., 1997). The inhibition of AChE in Zebra mussels from the Italian Great Lakes was considered as biomarker (Binelli et al., 2005). The AChE plays a significant role in nerve conduction processes at myoneural junction of the nerve ending of muscle tissue (Cajaraville et al., 2000). Inhibition in AChE activity in the haemocytes of *L. marginalis* suggests the neurotoxic behaviour of sodium arsenite. Lack of proper nervous coordination in the animal might affect the animal in diverse ways that might range from affected movement and locomotion, inadequate foraging as well as perturbed reproductive behaviours. In the present study, sodium arsenite inhibited the activity of AChE significantly. Inhibition in the activity of such an important enzyme can affect the natural reflexes of *L. marginalis* in the contaminated habitat that might include effective locomotion, foraging, escape from predator, etc. This might put the species under threat of microbial and parasitic attack. A number of studies provide evidence for the participation of nitric oxide in innate defense responses in invertebrates (Radomski et al., 1991; Conte and Ottaviani, 1995; Weiske and Wiesner, 1999; Nappi et al., 2000; Foley and O'Farrell, 2003). Since the hemocytes of mussel parallel the function of vertebrate
Studies on the toxicity of arsenic in *Lamellidens marginalis* (Lamarck) Chapter 10

macrophages, they are the cell types of choice for similar type of studies. The result is indicative of a dose dependent inhibition on nitric oxide generation by sodium arsenite. Since nitric oxide is involved with production of bactericidal peroxynitrite molecules in association with superoxide anion (O2·−), low production of the immune molecule affects the immune profile of the animal rendering the animal vulnerable to opportunistic microbial attack (Chakraborty *et al.*, 2009b). Glutathione-S-transferase plays a significant role in the process of xenometabolism. The entire process of biotransformation of toxin involves phase I and phase II reactions which facilitates the elimination of non-toxic or less toxic metals from the body. The enzyme GST is a phase II biotransformer and its activity has been documented in several bivalve species (Perendija *et al.*, 2009). GST catalyzes the conjugation of GSH to a wide variety of xenobiotics with an electrophilic site, yielding xenobiotics more water soluble and facilitating their excretion (Mannervik and Danjelson, 1988). As a phase II biotransformation enzyme, GST has been considered as a biomarker of organic industrial effluents (Sheehan *et al.*, 1995). In the present study, the activity of GST was initially activated in presence of strong electrophile like arsenic. Persistent exposure of sodium arsenite resulted in a suppressed GST activity which indicates the susceptibility of detoxification machinery of *L. marginalis* to the contaminant itself. The prophenoloxidase (ProPO) activating system plays several roles in invertebrate immunity and is considered to be one of the most important defence mechanisms. The oxireductase phenoloxidase (PO) is part of a complex system of proteinases, pattern-recognition proteins and proteinase inhibitors that constitute the ProPO activating system (Millar and Ratcliffe, 1994; Söderhäll and Cerenius, 1998). Activation of PO is an innate immune response under the challenge of toxic microorganisms. It is thought to be part of the invertebrate’s immune response against parasites because the conversion of ProPO to active enzyme can be initiated by molecules such as lipopolysac-charide, peptidoglycan and β-1,3-glucans from invading micro-organisms. PO is the final enzyme in this cascade involved in the melanization reaction (Söderhäll and Cerenius, 1998), a common response to parasite entry in many invertebrates. During a successful immune reaction, melanin encapsulates the parasites (including pathogens and parasitoids) and destroys them. Cellular studies also have been carried out in crustaceans which suggest that the proPO system is involved in encapsulation, phagocytosis, and cytotoxic reactions have been characterised (Cerenius and Söderhäll, 2004). This phenomenon is in report in annelids too (Bilej *et al.*, 2001). This important immune parameter was investigated under the exposure of arsenic to screen the possible effect of the toxin on the innate immunity of *L. marginalis*. Exposure of arsenic resulted an abrupt increase in production of 173
intrahaemocyte PO followed by a steady decrease. The observation was indicative to onset of arsenic induced physiological stress. The highest activity of PO was documented under the exposure of 5 ppm of sodium arsenite for a span of 96 h. The initial increase in the PO activity upto 96 h appeared to be dose dependent followed by an abrupt decrease which possibly indicates the “exhaustive phase” of the cells on prolonged exposure to arsenic. The superoxide anions impart antipathogenic activity in invertebrate innate immune system. They are generated in the immunocytes under proper immunogenic stimulations. A rise in the generation of superoxide anions in the haemocytes was recorded till 96 h of exposure. But by 15 and 30 days of exposure, the generation of the cytotoxic molecule reduced significantly indicating suppression of the cytotoxicity by sodium arsenite. The situation might cause a condition of immunological weakness in the animal under arsenic exposure. Catalase (CAT) is a major scavenger of reactive oxygen species (ROS) like hydrogen peroxide and thereby acts as an oxidative stress reliever in a living system (Saint-Denis et al., 1998). Thus, CAT is regarded as an enzyme presenting a clear and early response to contamination (Wenning et al., 1988). A decrease in CAT activity indicates a reduced ability to protect cells against oxidative stress mediated by hydrogen peroxide. Such vulnerability is indicative of development of oxidative stress in the circulatory haemocytes of the animals with a possibility of reduction of immunocompetence.

10.5 Restoration of haemocyte number, function and NOEL

Haemocytes are characterised by diverse subpopulation of cells of morphological and functional discreteness namely granulocyte, agranulocyte, hyalinocyte, blast-like cell, asterocyte, reticulocyte and vesicular haemocytes. Partial restoration of total haemocyte count in post-treated animals in toxin free water suggests that the toxic effect is persistent in nature and apparently irreversible for the considered span of experimental observation. The alteration in the total count of haemocyte of L. marginalis implies a possible immune compromise in the animal that reflects a potential risk to the species in arsenic contaminated habitat. Such shift in immunological status of L. marginalis may lead to decline of the population of the species due to possible opportunistic growth of microbes and parasites. Haemocytes are reported as chief phagocytes capable of generating nitric oxides - a potential cytotoxic agent. Exposure to inorganic arsenic affected the phagocytic efficiency and generation of nitric oxide in haemocytes. Sublethal concentration of sodium arsenite had suppressed these primary defence responses in the bivalve leading to a state of immune compromise. The effect of the natural toxin in all the
studied concentrations appears to be threatening over prolonged exposures and efforts to restore the normal parameters proved futile even after 30 days. Sublethal concentrations of sodium arsenite not only suppressed the nitric oxide generation in *L. marginalis*, its residual toxic effect enabled the post-treated animals to restore the normal activity – a condition of immune suppression of *L. marginalis*. Opportunistic microbial attack on these immune compromised species may lead to population reduction and gradual loss of biodiversity in the freshwater ecosystem of selected regions of India. Immune effector subpopulation of haemocytes of the *L. marginalis* appears to be an important biomarker of aquatic pollution in relation to phagocytosis and generation of nitric oxide (Chakraborty et al., 2009b). Aquatic ecosystem of this subcontinent supports a wide range of biodiversity which is under threat of environmental contamination. The alteration in the total count of haemocytes of *L. marginalis* implies a possible immune compromise in the animal. Phagocytosis and generation of intrahaemocyte nitric oxide are major immune parameters of the species that failed to attain 100% recovery even in 30 days. Although the toxicity of sodium arsenite was persistent, the resilience exhibited by the animals provides hope that in restored environments they are likely to be surviving by maintaining homeostasis. The increase in population density of these efficient filter feeders would stabilize the biodiversity of the freshwater habitats of India and conserve the health and habitability of the water bodies in a sustainable manner. In this study, the NOEL values of sodium arsenite for the THC, haemocyte dimension and generation of intrahaemocyte cytotoxic molecules of *L. marginalis* was found to be very low signifying the sensitivity of the parameters at feeble concentrations of arsenic exposure.

10.6 Histopathology and biochemical homeostasis of tissue

*L. marginalis* is a filter feeding freshwater mussel and filter feeding is a vital phenomenon for maintenance of turbidity and mineral dynamics in an aquatic ecosystem. The gill epithelium is a major barrier against environmental pollutant injury and pathological agents (Bigas et al., 2001). The structure of bivalve gills is suitable for histopathological analysis, since they consist of a simple epithelium with various cell types, in which the effects of water-soluble pollutants can easily be observed (Sunila, 1988). The digestive gland acts as the site of detoxification and a source of several digestive enzymes. The digestive diverticula of bivalve molluscs accumulate different pollutants and actively participate in detoxification processes (Widdows et al., 1983). Cytological, histological and histochemical studies indicate that the digestive cells of the molluscan digestive tubules appear to be a sensitive target for the injurious action of
many pollutants under field and experimental conditions (Moore, 1979, 1985, 1988; Lowe et al., 1981; Lowe, 1988). The pericardial cavity of molluscan heart may be the primary target of metals circulating in the haemolymph (Motley, 1933; Hill and Welsh, 1966). The gill and digestive gland of molluscs are the target organs of environmental toxicity assessment. It accumulates miscellaneous pollutants and actively participates in the detoxification process (Marigo mez et al., 2002). The study of the histopathological conditions of the gills of sodium arsenite exposed *L. marginalis* exhibits damage and destruction of the organ. The gill filaments of the sodium arsenite exposed animals exhibited sign of inflammation, swelling and tissue lysis which signifies a state of compromise in the tissue health. Gills remain in direct exposure of contaminants and are subjected to tissue damage. The clogging of the water channels with infiltrated hyperchromatic anaplastic cells and fibrolitic tissues, deformed and punctured branchial epithelium probably signifies a condition of possible perturbation of the normal functioning of the organ that might include inadequate water filtration, insufficient gaseous exchange and reduced in filter-feeding. The compromise appears to be detrimental for the sustenance of the species in contaminated habitat (Chakraborty et al., 2010). Digestive lysosomal response in the zebra mussel, *Dreissena polymorpha*, has been proposed as a biomarker for the assessment of freshwater pollution (Giamberini and Cajaraville, 2005). Li et al. (2009) has reported suppression of ACP, ALP, GOT and GPT activities and elevation in CAT activity the muscle and digestive tissue of mollusc under exposure of copper. The natural contaminant induced pronounced adverse effect on the tissue architecture of the digestive glands of *L. marginalis*. With respect to the control animals, the digestive tubules of the sodium arsenite exposed animals attained thinning in its epithelial girth with appearance of numerous vacuoles, formation of necrotic tissue in the tubule channel accompanied with frequent lysis of the tubules. The tissue features are suggestive of probable inflammation and disrupted cellular homeostasis that might affect the functioning of the organ accordingly. It might affect the nutritional vigour of the animals and induce a condition of stress and immune compromise that might be deleterious for the animal in long run. Exposure to high metal concentrations reduces the heart rate of mytilids and bradycardia may be induced due to dysfunction of the nerves responsible for mediating heart function (Grace and Gainey, 1987; Curtis et al., 2001). As the heart lacks an endothelial lining and is bathed directly in the haemolymph (Motley, 1933) toxic humoral substances would rapidly affect cardiac physiology. Any major perturbation of heart rate can, therefore, reasonably be attributed to either alterations in environmental parameters or exposure to toxicants. The condition of tissue destruction
was also noted in the heart muscle tissue of *L. marginalis* exposed to sodium arsenite which implies the vulnerability of the organ under the toxic insult. The collapse and disarray of the ventricular chamber of the heart of the exposed animals suggest a probable loss in efficiency in the pumping action of the heart that can in turn produce physiological and nutritional stress on the animals. It is reported that localized cellular patches in the pericardium of the bivalves are potential sites of haematopoiesis (Cooper *et al.*, 1982; Hartenstein, 2006). The pericardial tissue of the control animals contained prohaemocyte like agranular haemocyte, the probable site of haematopoiesis. The scarce distribution of prohaemocyte like agranular haemocyte patches from the meshwork of the pericardial tissue of the exposed animals and the decrease in total haemocyte count of the same animals probably indicates a condition of haematopoietic stress induced by the toxicant. The contaminant seemed to have developed cardiac stress in the exposed animals that signifies the vulnerability of the organism’s vital organ and its functioning in arsenic contaminated habitats. The biochemical profile of the gill and digestive tissue of the animals exposed to sodium arsenite was simultaneously destabilized. As a contaminant, sodium arsenite acted as a potent inhibitor of vital enzyme and biomolecule functions. The activities of enzymes namely ACP, ALP, GOT, GPT and AChE was suppressed by sodium arsenite. It restricted the activity of the vital “xenobiotic cleanser”, GST. The activity of the enzyme PO, a vital component of innate defense in invertebrates (Söderhäll and Cerenius, 1998), was inhibited by arsenic. The toxin suppressed the generation of nitric oxide which is an important cytotoxic molecule involved in intracellular killing of pathogen. The CAT activity was suppressed in a dose responsive manner, suggesting development of oxidative stress in the tissues of the animals exposed to sodium arsenite. The combined biochemical and oxidative stress might render *L. marginalis* less competent to combat against invading pathogens and parasites. Situation may lead to population decline and gradual dwindling of biodiversity in the freshwater ecosystem of selected regions of India.

Freshwater molluscs play a vital role in the economy and dietary tradition of West Bengal in India - serving as a food for numerous people and supplementing the feed in fish and prawn culture industry. The importance of these molluscan species in mitigating the protein deficiency in poor countries like India cannot be overlooked. Moreover, the species has been reported for its medicinal value against certain cardiac and neurological problem (Prabhakar and Roy, 2009). The shell of the animal is the source of valuable calcium, often used as supplements for bone ailment. This freshwater mussel naturally
produces ornamental pearls which indicate its potentiality in the industry of artificial pearl culture. Besides, as a filter feeder, it helps to maintain the turbidity and nutrient dynamics of the aquatic habitat that benefits the rest of the inhabitants of the water body. The natural habitat of this important species is under the potential threat of arsenic contamination due to natural and anthropological activities. In India, the contamination of wetland by various pollutants affects the aquatic ecosystem adversely. The current study was aimed to assess and predict the health of *L. marginalis* as a representative species of the freshwater environment under the threat of arsenic toxicity. The data would provide the baseline information about the nature of toxicity of arsenic contamination in freshwater aquasystem in relation to the structural and functional response of the circulating blood cells of a sentinel invertebrate organism. The study is reflective of a state of serious threat induced by sublethal concentrations of sodium arsenite on the behaviour and function of the circulating cells, tissue structure, physiological and immunological homeostasis. The contaminant suppressed phagocytic potency of the haemocytes, impaired their self-nonself recognition efficacy and perturbed the activity of vital enzymes of physiological and immunological significance. Moreover, the structure of gill, digestive gland and heart of the animal was severely damaged by arsenic which implies the precarious condition of *L. marginalis* under arsenic insult. The data is indicative of the degree of damage the toxicant can inflict on the members of the freshwater community upon potential arsenic contamination. The situation demands strict monitoring of these natural freshwater bodies for possible risk of arsenic contamination. In this scenario, development of a suitable biomarker to assess the quality of the environment appears to be an imminent scientific challenge of India. Establishment of an inexpensive biomarker is necessary to assess the health of the aquatic ecosystem and to protect its important bioresource of India. *L. marginalis* is a widely distributed aquatic mussel sensitive to diverse forms of xenobiotics and holds valid candidature to be studied as a model for determination of aquatic health. The studied parameters bear high feasibility to be selected for effective biomonitoring of arsenic contamination and can be considered as significant tool for estimation of toxin induced stress in aquatic molluscs. Of all the cell biological and biochemical parameters studied, phagocytic potentiality of the *L. marginalis* haemocytes and their nuclear abnormalities and lysosomal stability were reported to be important biomarkers of arsenic exposure and toxicity (Chakraborty *et al.*, 178)
2009b; Chakraborty and Ray, 2009a). Moreover, in depth analyses of haemocyte density of *L. marginalis* was screened under the exposure of arsenic. Relative density of haemocytes, its possible modulations sublethal arsenic exposure and its recovery was indicative to its suitability to be selected as biomarker of arsenic toxicity (Chakraborty et al., 2008). Observations related to the present investigation are suggestive to consider *L. marginalis* as a suitable indicator species of arsenic toxicity.

10.7 Bibliography


