Chapter 2

REVIEW OF LITERATURE

2.1 Arsenic contamination of ground and surface water

Over the last few decades, a majority of the population of Bangladesh and West Bengal, India switched their water supply from surface water to groundwater. As many as 10 million new domestic wells were installed, providing drinking water for over 100 million people. This large-scale transition to groundwater as the source for domestic water was motivated by the necessity of providing water free of pathogens. The transition to well water was adopted because of the convenience of having a water supply in close proximity to homes and the ease of drilling in the high-yielding aquifers of the region. Simultaneously, along with the drinking wells, irrigation wells were also installed across the region and groundwater pumping for irrigation greatly increased food production in the area (Harvey, 2005).

Disastrously, much of the groundwater resource of the region is dangerously contaminated by naturally occurring arsenic (Chatterjee et al., 1995; Acharyya, 1999; Chakraborti et al., 2003; Islam et al., 2004; Harvey, 2005; Neumann et al., 2010; Roberts et al., 2010). Groundwater arsenic contamination was reported back in 1976 in Punjab, Haryana, Himachal Pradesh and Uttar Pradesh of Northern India (Datta and Kaul, 1976). In 1984, groundwater arsenic contamination was discovered in lower Gangetic Plain of West Bengal (Garai et al., 1984). In 1992, groundwater contamination by arsenic in Bangladesh was identified (Dhar et al., 1997). In the year 1995, the arsenic threat in West Bengal and consequent suffering of people was reported (Chakraborti et al., 2002). By 2001, groundwater arsenic contamination in the lower Plain area (Terai) of Nepal was reported (Shrestha et al., 2003). In 2002, arsenic contamination in middle Ganga plain and upper Ganga plain was reported (Chakraborti et al., 2003).

In the middle and lower Gangetic basin of India, ground water pollution by arsenic has become a devastating threat for mankind (Acharyya, 1999; Das et al., 1996; Acharyya, 2002; Chakraborti et al., 2003; Islam et al., 2004; Neumann et al., 2010; Roberts et al., 2013). Alarming, it poses a threat not only to the ground water but also to the surface water of the selected plains of West Bengal (Acharyya, 1999). In the vast arsenic affected flood plains of Gangetic basin of India and Bangladesh, the irrigational groundwater carries huge load of dissolved arsenic that not only contaminate the agricultural fields but also the
agricultural runoffs have high probability to contaminate the field adjacent freshwater bodies (Islam et al., 2004; Neumann et al., 2010; Roberts et al., 2010).

Moreover, arsenic sediments of lakes and ponds often release arsenic which contaminates natural habitat of diverse invertebrates including molluscs (Aggett and O'Brien, 1985) and accumulation of arsenic in hypolimnion with temporary increase in concentration of arsenic during summer is reported. The arsenite is regarded as the prevalent chemical form of arsenic which exhibits 90% of occurrence in lake water (Aggett and Kriegman, 1987). Thus, chemical weathering and anthropogenic activities have been identified as the causes of arsenic contamination of the surface water of the lower Gangetic plains (Islam, 2000; Neumann et al., 2010; Roberts et al., 2010).

2.2 Arsenic toxicity

The first two epidemiological studies of arsenic-induced human dermatoses from consuming arsenic-contaminated water were conducted by Tseng (1977) in Taiwan and Saha (1984) in West Bengal, India. Subsequently, an extensive survey during the period of 1983–1987 from 61 villages of 7 districts of West Bengal (WB) and 1214 cases of chronic arsenical dermatoses (melanosis and keratosis), having skin cancer in 6 cases were detected (Saha, 1995). This report was further substantiated by other workers (Mandal et al., 1996; Dhar et al., 1997). Arsenic exhibits a complex metabolism and is possibly the most abundant pollutant as well and a potential human carcinogen. Arsenic acid (AsV) is the least toxic of the inorganic forms and arsenous acid (AsIII) is more toxic in vivo than arsenic acid. Toxicity of arsenic in mammalian system is the focal point of study for a long period of time. Cytotoxicity of arsenite (AsIII) was found to be greater than that of arsenate (AsV) (Jacobson-Kram and Montalbano, 1985). Arsenate accelerates the rate of inosine triphosphate (ITP) hydrolysis and inhibits both Ca2+ and Sr2+ uptake (Alves and Meis, 1987). This perturbation of intracellular Ca2+ homeostasis activates protein kinase C (PKC) activity, which may play an important role in arsenite-induced genotoxicity (Liu and Huang, 1997). Arsenic is a pro-oxidant and thus may cause lipid peroxidation, protein and enzyme oxidation and GSH depletion, DNA oxidation and DNA adducts (Lynn et al., 1997). Further, arsenic generates reactive oxygen species (Vehter et al., 1996; Wang et al., 1997; Lynn et al., 1998) like nitric oxide. As a result, arsenite may induce DNA strand-breaks and NAD depletion (Lynn et al., 1997). Hence the genotoxic effects of arsenic compounds may be connected with an inhibition of DNA repair or the induction of oxidative stress (Gebel, 1997). It is thus likely that arsenic-mediated DNA-protein interactions may play a major role in arsenic carcinogenesis and the induced protein associated DNA-strand breaks could provide an explanation for chromosome aberration (Uong and Luo, 1993). It is convincingly established that arsenicosis is mediated through chromosome abnormalities, modification of gene expression, and cell proliferation due to
oxidative stress and other uncharacterised or poorly defined physiological modifications or aberrations (Roy and Saha, 2002). Vutukuru et al., (2007) reported severe hepatic damage while studying the effect of arsenic exposure on the activity of aminotransferases in the serum of Indian major carp \textit{Labeo rohita}. Report on the toxicity of arsenic in aquatic invertebrates is limited (Phillips and Depledge, 1986). Phillips \textit{et al.} (1982) reported presence of high concentrations of total arsenic in the spindle shells of large marine gastropods \textit{Hemifusus tuba} and \textit{H. ternatanus} which are fished commercially by trawlers operating from Hong Kong.

2.3 Molluscs as indicators of aquatic pollution

The features of an aquatic ecosystem appear to be intricate in nature. Biological indicators are considered as useful means to obtain effective information about the condition of an ecosystem. Worldwide concern over the threats to natural resources and public health has led to increased efforts to monitor and assess environmental conditions. This has stimulated the need for development and application of select biological and ecological measurements, or indicators, that are responsive to environmental stress. Pollution is a major threat to the worldwide degradation of the freshwater habitats (Naiman and Turner, 2000; Jackson \textit{et al.}, 2001; Malmqvist and Rundle, 2002; Rachel, 2002). Bivalves are aquatic invertebrate organisms widely considered as bioindicators in environmental monitoring (Sanders, 1993). As filter feeders, these species are known to be ideal indicators of chemical contamination (Fournier \textit{et al.}, 2001).

Filter-feeding bivalves accumulate chemical contaminants and may acquire tissue concentrations of anthropogenic chemicals thousands of times higher than those in the water column. Chemical analysis of bivalve tissues can then be used to describe contamination profiles for different sites. This exposure monitoring approach was used in northern Europe and the United States in the 1960s and 1970s under the International Mussel Watch Program (National Academy of Sciences 1980), and continues as the National Mussel Watch Program conducted by the National Oceanographic and Atmospheric Administration.

The measures of bivalve mollusc defence activities, such as haemocyte density, phagocytic activity, locomotion, production of cytotoxic molecules and haemolymph constituents have potential as indicators and appear to be responsive to xenobiotic chemical insults in the aquatic environment. It has been proposed that the measurement of physiological and biochemical responses of individual bivalves may be used as indicators of the health of the larger population and community (Bayne \textit{et al.}, 1980). Such measurements today are termed ‘biomarkers’ (McCarthy and Shugart, 1990; Huggett \textit{et al.} 1992).
2.3.1 Molluscan haemocyte

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). They 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; Glinski and Jarosz, 1997). In molluscs, bivalves in particular, haemocytes represent the major component of their immune system. The types of haemocytes in molluscs, as well as their specific functions, are not fully understood. In this context, substantial information is with haemocytes of bivalves (Cheng, 1984; Adema et al., 1991a, b; Jing and Wenbin, 2003). It has been demonstrated that apparently different types of haemocyte (hyalinocyte or granulocyte) may actually represent the same type of cell during different functional or maturational stages and it is not clear, if the variety of haemocytes described in the literature represents distinct cell lineages, or due to the differences in the maturation and/or physiology of the haemocytes, or variations in the techniques being applied (Cheng, 1975).

Bivalve haemocytes are believed to be responsible for the transport of contaminants from the organ of entry (e.g. gill, mantle, digestive gland) to the kidneys or other tissues where detoxification or accumulation may occur (Pirie et al., 1984). Alterations of the immunosurveillance have been reported for bivalve molluscs exposed to metals (Cheng and Sullivan 1984; Cheng 1988b; Pipe et al., 1999) and xenobiotics (Fries and Tripp 1980; Alvarez and Friedl 1992; Beckmann et al., 1992; Coles et al., 1994; Cima et al., 1998). It has been established that the efficiency of haemocytes may be affected by environmental contaminants (Anderson et al., 1988; Renwrantz, 1990; Canesi et al., 2003).

Xenobiotics may alter functional profiles in molluscan haemocytes, such as phagocytosis (Fries and Tripp, 1980; Anderson, 1988; Cima et al., 1998), lysosomal enzyme activity and lysosomal membrane stability (Lowe et al., 1995; Grundy et al., 1996). Consequently, toxic effects on haemocytes potentially affect the survival of these animals. Haemocytes act as the major immune effector cells in invertebrates including molluscs (Cheng, 1977; Adema et al., 1991b). Characterisation of haemocyte function is an important step towards understanding their immune capacity and its potential failure during toxic exposure and disease development.
2.3.1.1 Morphological attributes and enumeration and of haemocyte

Morphological analyses and functional consequence of molluscan blood cells under toxic exposure is poorly understood. Leydig (1850) studied circulatory haemocytes of mollusc and provided the baseline information in understanding the function of haemocyte in health, disease and toxic exposure. An ideal analysis of haemocyte population dynamics involves the simultaneous approach to a series of parameters throughout the animal’s life, namely, total haemocyte count (THC) and differential haemocyte count (DHC) (Jones, 1962; Wheeler, 1963; Shapiro, 1968, 1979; Arnold and Hinks, 1976). Marked variations in the density of haemocytes may be related to irregular haemocyte release from haemocytopenic organs into the open circulation (Hoffmann, 1973; Crossley, 1975; Feir, 1979). Detoxification and elimination have been attributed primarily to granular haemocytes and the proportion of this cell type is reported to be elevated in polluted environment (Pirie et al. 1984). The types of haemocytes of molluscs often reported as:

a. Blast like cells:
   - Small cells; non-spreading (Hine, 1999; Chang et al., 2005; Chakraborty et al., 2008).
   - Often called as pro-haemocytes/stem cells (Cheng, 1984; Hine, 1999; Cima et al., 2000; Martin et al., 2007; Chang et al., 2005).

b. Agranulocytes:
   - Cells are large and have ovoid to round nuclei (Martin et al., 2007; Chang et al., 2005)
   - Cytoplasm with scarce secretory granules (Auffret 1988; Chakraborty et al., 2008).

c. Hyalinocytes:
   - These cells are ovoid in shape (Hine, 1999; Martin et al., 2007).
   - Pale hyaline cytoplasm with small and distinct nucleus (Hine, 1999; Chang et al., 2005).
   - Cytoplasm with scattered secretory granules (Hine, 1999; Cheng, 1984).

d. Granulocytes:
   - Cells have variable in size and shape — spherical or oval (Cheng, 1981; Hine, 1999).
   - Small nucleus; highly granular cytoplasm (Cheng, 1984; Hine, 1999; Martin et al., 2007).

e. Asterocytes:
   - Cells are spreading (Chang, 2005; Mahilini and Rajendran, 2008; Chakraborty et al., 2009a).
   - Variable in their morph (Hine, 1999; Chang, 2005; Chakraborty et al., 2008)
   - Projects pseudopodia/filopodia (Mahilini and Rajendran, 2008; Chakraborty et al., 2008).
   - Cytoplasm contains few granules (Chakraborty et al., 2008).
An understanding of the types of hemocytes in molluscs is essential in studying basic cell responses to (1) environmental changes (Fisher et al., 1987; Fisher and Tamplin, 1988; Fisher et al., 1989; Burge et al., 2002; Pamparinin et al., 2002); (2) handling of the animals (Malham et al., 1998; Ballarin et al., 2003; Malham et al., 2003); and (3) infections (Beckmann et al., 1992; Ford, et al., 1993; Matricon-Gondran and Letocart, 1999; Canesi et al., 2002; Cochenec-Laureau et al., 2003).

In freshwater ecosystem, bivalves are dominant filter-feeders that make up most of the biomass and exert control over ecosystem structure and function (Strayer et al., 1999). Considerable data obtained from controlled exposures have demonstrated that oyster defence activities do respond to anthropogenic chemicals such as heavy metals. Cheng (1988 a, b) reported lower percentage of hyalinocytes in oysters exposed to 1 ppm copper sulphate and significantly higher percentage of hyalinocytes in oysters exposed to 1 ppm of cadmium chloride. Coles et al. (1995) reported a significant increase in circulating haemocyte numbers in mussels *Mytilus edulis* resulting from exposure to 400 ppb of cadmium for 7 days. Haemocytes of freshwater zebra mussels (*Dreissena polymorpha*) exposed to lead and zinc contained enlarged and/or more numerous lysosomes compared with controls (Giamberini and Pihan, 1997). Exposure to 40 ppb of cadmium suppressed the release of degradative enzymes from the haemocytes during phagocytosis. Mussels (*Mytilus edulis*) exposed to copper also had increased granular blood cells by factors of 3-4 over unexposed controls (Pickwell and Steinert, 1984).

### 2.3.1.2 Haemocyte adhesion and aggregation

Cell aggregation and cellular clumping in bivalves was first reported by Geddes (1880), cited by Narain (1973). 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 under exposure of arsenic, a natural pollutant of freshwater ecosystem. In multicellular animals, cell adhesion is a fundamental property for establishing tissue and organ architecture. Any abnormality in the adhesive properties of cells is believed to cause pathological lesions such as cancer metastasis (Nicolson and Winkelhake, 1975), ontogenic malformations (Ede and Flint, 1975; Yanagisawa and Fujimoto, 1977) etc. Among the variety of functions served 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 adhesion and active motility.

Xenobiotics may alter behavioural parameters in molluscan haemocytes, such as aggregation (Auffret and Oubella, 1997), and adherence (Chen and Bayne, 1995). 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). Divalent cations promote attachment to and spreading on protein-coated surfaces for a variety of vertebrate cells (Grinnel, 1974; Maroudas, 1977; Rabinovitch and DeStefano, 1973; Takeichi and Okada, 1972; Taylor, 1961). Reports are there on altered agglutination titer of the haemolymph fraction of molluscs during interaction with parasites (Loker et al., 1994; Goldman, M. and Honinberg, B. M., 1968; Honinberg et al., 1971) but effect of metals and metalloids on cell agglutination response with the haemocytes of freshwater bivalves is scanty (Ray and Chattopadhyay, 2002).

2.3.1.3 Nuclear aberrations

A micronucleus (MN) test as an endpoint of cytogenic damage is a dependable assay (UNEP/RAMOGE, 1999). Appearance of micronucleated apoptotic cell in mussels from polluted areas has been attributed to exposure of the animals to the hazardous environmental contaminants (Bihari et al., 1990; Steinert, 1996; Steinert et al., 1998; Busch et al., 2004; Baršiene et al., 2006). The micronuclei assay is simple and relatively rapid, and is suitable for routine screening and monitoring purposes (Heddle et al., 1983). Micronuclei are produced from chromosome fragments whose occurrence may be due to the defect in cytokinesis or centromere damage (Heddle et al., 1991).

Toxic chemicals can cause genotoxic impacts on organisms by modifying the structure of DNA, consequently resulting in irreversible damage to the integrity of chromosome (Hus, 1982). These responses can be considered as biomarkers of adverse effects on the scale of cellular changes from normal to transformed cells and thus, can be applied as biological endpoints in genotoxicity assays (Shugart et al., 1992). The assay have been utilised as a biomarker of genotoxicity in marine monitoring programme (Brunetti et al., 1988; Dailianis et al., 2003; Kalpaxis et al., 2004; Baršiene et al., 2006; Schiedek et al., 2006).

2.3.1.4 Lysosomal stability

A reduction in lysosome membrane stability has been reported in mussels and oysters exposed to heavy metals and proposed as an indicator of cell damage (Regoli, 1992; Ringwood et al. 1998). Lysosomes play an important role in the immune responses of bivalve molluscs. On phagocyte stimulation, lysosomal hydrolases are released out of cells to degrade foreign materials (Mohandas et al., 1985) or into phagosomes, thus participating in the degradation of internalized foreign particles (Cheng, 1981). It is known that the hemocytes of bivalve molluscs may accumulate high levels of metals, mainly in lysosomes (Pauley and Nakatini, 1968; Viarengo et al., 1981; Moore, 1990; Bordin et al., 1996). Alteration of the integrity of lysosomal membranes may cause undesired release of hydrolases into the cytosol, resulting consequent damage of self-cells (Lowe et al., 1995).
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Lysosomal hydrolytic phosphatase enzymes remain compartmentalised within electrondense specific granules of haemocytes and granular cells (Pipe, 1990). Cytochemical studies have demonstrated the occurrence of several lysosomal enzymes associated with the cytoplasmic granules in haemocytes of several bivalve molluscan species (Bayne et al., 1979; Moore, 1985; Gelder and Moore, 1986). Degranulations are associated with the release of lysosomal enzymes in the serum during phagocytosis and are therefore vital for maintenance of tissue homeostasis (Cheng and Dougherty, 1989).

The neutral red retention assay (NRR) is a useful technique applied for monitoring alterations in the permeability of the lysosomal membrane caused by environmental pollutants. The utility of the NRR test for environmental monitoring has been established as a sensitive indicator to estimate and assess the period of contaminant exposure (Femley et al., 2000). It has been reported that the NRR assay is least affected by natural factors, such as temperature and salinity, but is mainly influenced by pollutants (Ringwood et al., 1993). This assay has been applied to several studies to examine the effects of diverse toxins (Femley et al., 2000; Lowe and Pipe, 1994; Wedderburn et al., 2000) including heavy metals (Svendsen and Weeks, 1995). Such studies were carried out on molluscs reared in the laboratory (Lowe et al., 1995; Chakraborty and Ray, 2009a) as well as specimens collected from their natural habitat (Femley et al., 2000).

2.3.1.5 Phagocytosis

In cell-mediated immune responses, nonself phagocytosis by circulating haemocytes is the main defence reaction against pathogens and foreign materials (Cheng, 1981). 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., 1991; Oliver and Fisher, 1999; Chakraborty et al., 2009b). Phagocytosis by haemocytes is the major line of defence against invading foreign materials including xenobiotics. In coelomate metazoans such as molluscs, haemocytes form the primary line of defence through phagocytosis and respiratory burst activities (Renwrantz, 1990; Cooper and Knowler, 1992; Sami et al., 1992).

Heavy metals constitute one of the major contaminants of the aquatic ecosystem that may yield adverse biological effects, including changes in immune function of vertebrate species (Lawrence, 1981; Zelikoff et al., 1994). In invertebrate species such as earthworm (Lumbricus terrestris) heavy metals have been shown to impair phagocytic activity of
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coelomocytes (Fuge’re et al., 1996). The central role of phagocytosis in immune defence and the sensitivity of this biological function to environmental xenobiotics in several molluscan species (Loose et al., 1981; Kollner et al., 2002; Hillyer et al., 2003) have made it a major function to be assessed for evaluating the immunosuppressive effects of pollutants.

2.3.1.6 Enzymatic activities

Acid phosphatase (ACP) is considered as a major hydrolytic enzyme that acts in phagocytic vesicles to degrade endocytosed particles whereas alkaline phosphatase (ALP) has often been implicated in phosphorylative transfer of extracellular molecules against concentration gradients at cell membranes (Monin and Rangneker, 1974). Clams (Sunetta scripta and Villorita cyprinoides var. cochinensis) exposed to copper exhibited elevated level of ACP of the haemolymph, presumably released by haemocytes due to destabilization of lysosomal membranes (Suresh and Mohandas, 1990b). 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 transaminase enzymes and cadmium has been reported to have inhibitory effect on their activity in molluscs (Das and Jana, 2004).

The measurement of acetylcholinesterase (AChE) activity in marine organisms is widely used for biomonitoring of marine pollution by environmental neurotoxins (Sen Gupta et al., 1991; Galgani et al., 1992; Escarting and Porte, 1997; Pfeifer et al., 2005; Cajaraville et al., 2000). The inhibition of AChE by neurotoxin substances such as copper, lead, carbamate pesticides, organophosphorous compounds, polyaromatic hydrocarbons and cadmium, have been established (Sarkar 1992; Tabche et al., 1997; Sturm et al., 1999; Cajaraville et al., 2000; Wells et al., 2001; Matozzo et al., 2001). The inhibition of AChE in Zebra mussels from the Italian Great Lakes was used 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). The decrease in AChE activity in the oyster was efficiently used as a biomarker of exposure to neurotoxic compounds (Bocquene’ et al., 1997).

Glutathione peroxidase binds glutathione (GSH) with high affinity and oxidizes it to oxidized glutathione (GSSG). This enzyme can detoxify hydrogen peroxide and organic hydroperoxides which are formed during tissue oxidative stress. 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
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effluents (Sheehan *et al.*, 1995). Catalase (CAT) is a major scavenger of reactive oxygen species (ROS) like H$_2$O$_2$ and thereby acts as an oxidative stress reliever in a living system (Saint-Denis *et al.*, 1998). These studies involved a hierarchical organization of the cellular activity and more specifically of the antioxidant enzymes. Thus, CAT is regarded as an enzyme presenting a clear and early response to contamination (Wenning *et al.*, 1988).

2.3.1.7 Generation of cytotoxic agents

Nitric oxide (NO) was described as a component of the vertebrate immune system (Colasanti *et al.*, 2002) and it has been reported in invertebrates and plants, where it provides effective protection against bacterial infections (Zeidler *et al.*, 2004). At present, nitric oxide is considered as a dependable immune molecule for invertebrates including bivalve molluscs (Conte and Ottaviani, 1995; Bogdan, 2001) as it has the ability to kill pathogens itself or by combining with superoxide (O$_2^-$) to form peroxynitrite, a strong bactericidal agent (Arumugam *et al.*, 2000). Chakraborty *et al.* (2009b) reported the toxicity of arsenic in molluscan haemocytes in relation to phagocytosis and nitric oxide generation. In many bivalve species, phagocytic cells can be activated by foreign particles, or organisms and their products, to release oxidative chemicals; this response is often referred to as an ‘oxidative burst’. An oxidative burst leads to production of reactive oxygen species (ROS), catalyzed by the membrane-associated enzyme NADPH oxidase. The initial metabolite O$_2^-$ is dismutated to hydrogen peroxide (H$_2$O$_2$), which may then be converted to other toxic ROS, such as hydroxyl radical (OH$^-$) and singlet oxygen (¹O$_2$) (Bugge *et al.*, 2007). ROS act as killing agents, either alone or in combination with lysosomal enzymes and are important in the elimination of viruses, bacteria, yeast, fungi, and protozoa (Chu, 2000). Production of ROS in a number of molluscan species like *Crassostrea virginica*, *Crassostrea gigas*, *Ostrea edulis*, *Mytilus edulis*, *Mytilus galloprovincialis*, *Pecten maximus* and *Mercenaria mercenaria* has been documented where luminol-dependent chemiluminescence was measured in a liquid scintillation counter or the optical density of the reduction of nitroblue tetrazolium (NBT) to measure production of ROS (Bachere *et al.*, 1991; Pipe, 1992; Larson *et al.*, 1989; Bugge *et al.*, 2007).

The active form of prophenoloxidase, catalyzes two successive reactions (a) hydroxylation of a monophenol to an O-diphenol, followed by (b) oxidation of O-diphenol to O-quinone (Sugumaran, 2002). The production of toxic quinone intermediates and O-quinones by phenoloxidase (PO) is an early step in the biosynthesis of melanin which is important in wound healing, and in the encapsulation of foreign materials for host defence (Cerenius and Söderhäll, 2004). Phenoloxidase, which has been detected in a wide range of invertebrates (Smith and Soderhall, 1991; Jackson *et al.*, 1993) is activated by several microbial polysaccharides including β-1,3 glucan from fungal cell walls (Unestam and Soderhall, 1977; Soderhall and Unestam,1979) and peptidoglycans (Ashida *et al.*, 1983) or lipopolysaccharides (Soderhall and Hall, 1984; Soderhall *et al.*, 1990) from bacterial cell walls or zymosan (Sahoo *et al.*, 2005). Additional factors found to activate the proPO
system include calcium, sodium dodecyl sulfate (SDS), trypsin and high temperature (Ashida et al., 1983; Duloray and Lackie, 1985; Sugumaran and Nellaiappam, 1991). Melanisation is a common manifestation in the haemocyte mediated encapsulation reactions, exhibited by arthropods against intrahaemocoelic infections, and in cuticular wound healing responses, that are generated at the site of integumental assault (Ashida and Brey, 1995). Knowledge of the cellular and humoral activation processes that localize cytotoxic elements of the melamin proteolytic cascade is crucial to a better understanding of the non-self recognition mechanism of organisms that employ catecholamine-derived pigment intermediates in their defence against infectious agents. Presently, the mechanism(s) by which injury or pathogen invasion activates melanogenic enzyme cascades remain virtually unexplored (Nappi and Ottaviani, 2000). The function of NO and phenoloxidase (PO) as potent immune molecules in bivalves is well established (Conte and Ottaviani, 1995; Cerenius and Söderhäll, 2004). It is generally believed that contaminant exposure leads to suppression of defence activities and thereby reduces the ability of bivalves to defend against invading parasites and pathogens (Fisher et al., 1999). However, some studies have shown that relatively low concentrations of certain chemical contaminants can create a hormesis and may initiate activation or enhancement of haemocyte defence activities (Fisher et al., 1989). Enhanced activity may permit bivalves to survive in a polluted environment.

2.3.2 Molluscan gill, digestive tissue and heart as target of toxicity

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 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; Auffret, 1988; 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). Uptake and accumulation of a pollutant can provoke measurable changes in volume, surface, size and number of lysosomes of digestive glands (Lowe et al., 1981; Marigo’mez et al., 1989; Cajaraville et al., 1995b; Marigo’mez et al., 1996). Changes in these cellular organelles therefore may be considered as biomarkers environmental stress
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(Cajaraville *et al.*, 2000). 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. Metals can interfere with gill function due to the inhibition of ciliary function (Abel, 1976). 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. GST is a sensitive biomarker of environmental pollution and its activity in an organism has been reported to increase as a function of the xenobiotic concentration (Stein *et al.*, 1998). In addition, activity of GST in gills and digestive glands of bivalves has been used as a biomarker of exposure to anthropogenic organics (Fitzpatrick *et al.*, 1997). The measurement of the oxidative stress has been examined through estimation of activities and interaction of CAT and GSH in specific organs like gills and digestive tissues (Mandizuo *et al.*, 2005; Chakraborty *et al.*, 2010).

### 2.3.3 Aquatic invertebrates and bio-safety level of toxins

Acute toxicity is involved in estimation of LC50 or the concentration which has been proved to be lethal to 50% of the test group of animals under observation in a given time of exposure. Determination of acute toxicity is usually an initial screening step in the assessment and evaluation of the toxic characteristics of all compounds. Data from the acute study may: (a) serve as the basis for classification and labelling; (b) provide initial information on the mode of toxic action of a substance; (c) help to arrive at a dose or concentration of a new compound; (d) help in dose determination in animal studies (Akhila *et al.*, 2007). No observed effect level (NOEL) is a method commonly applied in ecological risk-based screening assessments (ESI, 1999a). The NOEL value is widely regarded as the standard value or the start point of a toxin concentration that triggers toxicity in model animals under environmental monitoring. NOEL is generally described as that exposure level at which there is no statistically or biologically significant increase in frequency or severity of effects between the exposed population and its appropriate control (Crane and Newman, 2000). NOEL of a range of toxins and xenobiotics have been established in bivalves, gastropods polychaetes etc. to estimate the physiological risks of the inhabitants in the aquatic environment (ESI, 1999a).
2.4 *L. marginalis* and its importance in toxicological study

In the tropical freshwater reserves of India, pearl-producing mussel *L. marginalis* (Kingdom-Animalia; Phylum-Mollusca; Class-Bivalvia; Superorder-Eulamellibranchia; Order-Unionoida; Family-Unionacea; Genus: *Lamellidens*) is distributed abundantly (Annandale and Prashad, 1919; Bloomer, 1931; Ghosh and Ghosh, 1972; Rao and Dey, 1989; Chakraborty et al., 2008) as an important component of freshwater ecosystem. The bivalve is characterized by possessing two shells secreted by a mantle that extends in a sheet on either side of the body. The oldest part of the shell, the umbo, can be recognized as a large hump on the anterior end of the dorsal side of each shell (Bloomer, 1931). The two shells are joined at the dorsal end by ligament. The ligament is comprised of the tensilium and resilium (Purchon, 1968). Together they open the shells at rest. A bivalve closes its shells by contracting its powerful adductor muscles (an anterior and a posterior) (Rao and Dey, 1989). The body of the animal is compressed laterally. The bivalve also possesses two ctenida and a muscular foot. The edges of the mantle are fused to form tube-like siphons. One inhalant siphon carries water to the mantle cavity and one exhalent siphon carries water in the opposite direction. A bivalve uses its muscular foot to attach itself to a substratum (Ghosh and Ghosh, 1972; Rao and Dey, 1989). The bivalve is a filter feeder in which the ctenida trap the food particles in their mucous coating and transfer the food to the labial palps by ciliary action. Radula is absent in the species. *L. marginalis* develops through a larval stage known as the glochidium. A glochidium attaches to the gill of fish or other objects for further development (Rao and Dey, 1989).

*L. marginalis* earns its importance as a common dietary item of rural human population and also as poultry feed (Rao and Dey, 1989; Chakraborty et al., 2008; Prabhakar and Roy, 2009). Few scientific attempts have been made to culture this animal in captive condition for commercial purposes (Rao and Dey, 1989; Gayatri et al., 1995; Barik et al., 2004). Notably, Padmanabhanaidu and Ramamurthy (1961) have observed the influence of sex and size on the osmotic pressure, the chloride and the free amino acids of the blood of *L. marginalis*. Effects of acclimatization to high temperature on the blood chloride, free amino acids and osmotic pressure in the animal have been reported by Rao and Ramachandra (1961). Verute (1969) reported beta-glucuronidase activity of crystalline style of the *L. marginalis*. Rahim et al. (1985) has carried out a survey on the relative abundance of faecal coliform bacteria in *L. marginalis* while Mandal et al. (2007) has reported an examination on the gut content of the animal. The nature of adaptations of freshwater mite *Unionicolora* sp. on *L. marginalis* has been reported by Majumdar and Pal (1988). Pattnaik et al. (2002) reported the L-asparaginase activity in *Aeromonas* sp. isolated from the freshwater mussel. Das and Jana (2003) evaluated oxygen uptake and filtration rate in *L. marginalis* as animal health biomarker. Carbohydrate metabolism in selected tissues of the animal has been studied by Satyaparameshwar et al. (2006).
L. marginalis has been used as a model for examination of the toxicity of diverse chemical compounds like pesticides, heavy metals etc. for a long time. Sublethal effect of methyl parathion on tissue proteolysis in L. marginalis was observed by Rao et al. (1980) while Moorthy et al. (1984) investigated the kinetics of succinic dehydrogenase inhibition by methyl parathion and its recovery by oximes and L-cysteine in hepatopancreas of the animal. Moorthy et al. (1984) and Mohan et al. (1987) reported the metabolic consequences of methyl parathion in L. marginalis. Acute toxicity of endosulf was reported by Mane and Muley (1984). Hameed and Raja (1990) tried to establish L. marginalis as an indicator of river pollution. Alterations in the tissue lipid profiles of L. marginalis under ammonia stressor were investigated by Indira and Chetty (1994). Moorthy et al. (1983) has reported alterations in carbohydrate metabolism in tissues of the bivalve under the exposure of phosphamidon. Impairment of immunoadhesion of the haemoocytes of the bivalve under the exposure of a neem base pesticide has been reported by Mukherjee et al. (2007). Mishra et al. (2008) has studied differential growth of the freshwater mussel in relation to certain xenobiotics. Observations have also been made on the effects of diverse metals on L. marginalis. Sivaramakrishna et al. (1990) assessed the effect of mercury toxicity on the oxygen consumption and ion levels in the L. marginalis. Effect of nickel on some aspects of protein metabolism in selected organs of the bivalve has been reported (Sreedevi et al. 1992). Some aspects of the effect of polonium in the animal were studied (Shaheed et al., 1997). Das and Jana (2003, 2004) reported suppression of some enzyme activity like acid phosphatase, alkaline phosphatase, glutamate oxaloacetate transaminase, glutamate oxaloacetate transaminase etc. in L. marginalis under the exposure of cadmium. Satyaparameshwar et al. (2006) studied the effect of chromium on protein metabolism of the bivalve. Modulation of Ca²⁺-ATPase activity in gill microsomes of L. marginalis under the exposure of heavy metals was reported (Pattnaik et al., 2007).

In the lower Gangetic basin of West Bengal, India and Bangladesh, the freshwater reservoirs which form the natural habitat of L. marginalis, are under potential threat of arsenic toxicity (Islam et al., 2004; Neumann et al., 2010; Roberts et al., 2010). The effects of arsenic toxicity in freshwater invertebrates including L. marginalis in relation to haemocyte density, structure, behaviour and selected functions is in report (Chakraborty et al., 2008; Chakraborty and Ray, 2009a; Chakraborty et al., 2009 b). An elaborate study on the toxicity of sodium arsenite in the gill of L. marginalis exhibited the derogative effect of the toxin damaging the gill structure and crippling the biochemical homeostasis of the organ (Chakraborty et al., 2010). An in depth study on a model organism of the freshwater habitat of this region seems to be imminent in order to assess and estimate the probable threat looming on the natural freshwater aquifers of arsenic affected zones of southern West.
Bengal. Above discussion suggests that *L. marginalis* holds an ideal candidature in this respect. A detailed investigation on *L. marginalis* in relation to its immunological and biochemical functions of haemocytes and selected target tissues under the exposure inorganic arsenic in standard laboratory conditions would provide the base line information to evaluate arsenic induced toxicity in the freshwater biota of West Bengal.

### 2.5 Bibliography


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