Chapter 9

BIOCHEMICAL HOMEOSTASIS OF TISSUE

9.1 Introduction

Molluscs are proven experimental models for detecting and evaluating the safety of toxic chemicals (Melo et al., 2000; Downs et al., 2001). Biochemical profiles of the gill and digestive tissue provide vital information towards estimation of ecotoxicological threat imposed by a toxin on the bivalves. Bivalves filter phytoplankton, bacteria and particulate organic matter from the water column (Vaughn and Hakenkamp, 2001). Since the bivalve gills have simple epithelium with specialised cells, gill filaments are suitable for histopathological analyses, in which the effects of water-soluble pollutants are easily be estimated (Sunila, 1988). The digestive diverticula of bivalve molluscs accumulate variety of toxins and actively participate in detoxification processes (Widdows et al., 1983). Cytological, histological and histochemical studies indicate that the digestive cells of the mollusc 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; Auffret, 1988). In addition to their respiratory function, the gill surfaces of serve to collect inhaled particles, facilitated by mucous secretion, followed by food-string transport along food grooves (Bayne et al., 1976). Chemical pollutants in the aquatic environment, whether in soluble form or associated with sedimentary particles or in suspension in the water, can penetrate into marine organisms in various ways and then easily cross cell membranes via the outer epithelial layer of the gills and other organs located in the paleal cavity (i.e. mantle, gonad, digestive gland); (LePennec and LePennec, 2001). After absorption, chemical pollutants accumulate and can interact with endogenous molecules thus causing disturbances in cell functions (Livingstone and Pipe, 1992; Bigas et al., 2001). The gills of bivalves are often studied as models to assess and evaluate the effects of exposure to various toxins and heavy metals, in relation to their antioxidant and immunological properties (Moraga et al., 2005; Box et al., 2009). Kinetic characterisation of gill and digestive gland glutamate oxaloacetate aminotransferase (GOT) in the clam Ruditapes philippinarum has been reported (Puppo et al., 1992). The inhibitory effects on glutamate pyruvate aminotransferase (GPT) activity in the gill and digestive gland by heavy metals like mercury, lead, cadmium, chromium, nickel and copper are in report (Blasco and Puppo, 1999). Li et al. (2009) demonstrated the inhibitory effects of copper on the activities of some metabolic enzymes like glutamate oxaloacetate transaminase (GOT), glutamate pyruvate
transaminase (GPT) acid phosphatase (ACP), alkaline phosphatase (ALP) and antioxidative enzymes including catalase (CAT) in the hepatopancreas of marine mollusc. The inhibition of acetylcholinesterase (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., 2005). The decrease in 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 used as biomarkers for the persistent organic pollutants biomonitoring of aquatic environments (Binelli et al. 2005). 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). This necessitates investigation of participation of nitric oxide in cell mediated immune response of more insect species to substantiate this hypothesis. Since the haemocytes of mussel parallel the function of vertebrate macrophages, they are the cell types of choice for such studies. Glutathione-s-transferase (GST), an enzyme system which conjugates glutathione to a variety of xenobiotics with electrophilic centres is often reported in the digestive glands of variety of snails and mussels like Littorina littorea, Myllus edulis (Lee, 1988). The prophenoloxi-dase (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; Soderhall and Cerenius, 1998). 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 beta-1,3-glucans from invading micro-organisms. PO is the final enzyme in this cascade and the bottleneck in the melanization reaction (Soderhall and Cerenius, 1998), which is a common response to parasite entry in many invertebrates. During a successful immune reaction, melanin encapsulates the parasites (including pathogens and parasitoids) and kills them. The insect phenoloxidase (PO) cascade is switched on inside the open haemocoel of insects in response to nonself. The cascade produces a number of cytotoxic by-products (e.g., phenols) that attack the pathogen (Rolf and Siva-Jothy, 2003). PO activity in non-arthropod system such as annelids is also in report (Bilej et al., 2001). The function of PO as potent immune molecule in bivalves is in report (Asokan et al., 1997).

The effects of arsenic toxicity on the biochemical profile of the gill and digestive tissue of L. marginalis have not been reported earlier. Exposure to a toxic substance can lead to permanent damage in the biochemical homeostasis of various vital tissues of aquatic organisms (Li et al., 2009). In this investigation, the gill and digestive tissue of L. marginalis
were studied in relation to activities of various enzymes and reactive molecules under the exposure inorganic sodium arsenite. The observations would provide basic information about the nature of toxicity imposed by inorganic arsenic contamination on the gills and digestive tissue of the filter feeders of the natural freshwater-bodies in the arsenic affected zones of the Gangetic basin. Moreover, the investigation would evaluate the credibility of *L. marginalis* to be chosen as a model species for biomonitoring surface water arsenic toxicity.

### 9.2 Materials and methods

#### 9.2.1 Preparation of tissue lysates

After dissection of the control and sodium arsenite exposed large sized *L. marginalis*, gill and digestive gland were aseptically collected with the help of a fine, sharp and sterile scalpel. The tissue samples were washed in chilled sterile snail saline (SSS) composed of 5mM HEPES (Himedia), 3.7mM NaOH (Merck), 36 mM NaCl (Merck), 2 mM KCl (Merck), 2mM MgCl₂, 2H₂O (Merck), 4mM CaCl₂, 2H₂O (Merck) at pH 7.8 (Adema et al., 1991a). The washed tissue samples were homogenized in SSS and the homogenized tissue fraction were then collected in 2 ml vials and centrifuged at 3000 rpm. The supernatants were discarded and the pellets were resuspended in chilled SSS and centrifuged. The pellet was then suspended in 1ml of 0.1% Triton X -100 and kept over ice for 30 min. The pellet was then centrifuged at 8000 rpm for 30 min and the supernatants were stored in labelled vials.

#### 9.2.2 Activity of phosphatases

##### 9.2.2.1 Estimation of ACP activity

The activity of ACP (EC 3.1.3.2) in the gill and digestive tissue was estimated after Michell *et al.* (1970) and the protein was estimated after Lowry *et al.* (1951). The enzyme acted on 5mM p-nitrophenol phosphate (PNPP) (Merck) in 50 mM sodium acetate buffer (pH 5.0) for 30 min at 37°C in a humid chamber. The reaction was halted with 0.1(N) NaOH for 30 min and the absorbance was recorded at 420 nm in a spectrophotometer (CECIL-CE 4002, Germany). The enzyme activity was determined by using a standard curve of p-nitrophenol (PNP) (Merck). The ACP activity was expressed as dephosphorylation of PNPP to PNP (Merck) in µM/mg protein/min. The entire experiment was repeated for at least 5 times.

##### 9.2.2.2 Estimation of ALP activity

The activity of ALP (EC 3.1.3.1) in the gill and digestive tissues was estimated after Michell *et al.* (1970) and the protein was estimated after Lowry *et al.* (1951). The enzyme
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reacted with 5mM PNPP in 50mM Glycine buffer (pH 10.0) and 10 mM MgCl₂ for 30 min at 37 °C in a humid chamber. The reaction was stopped with 0.02 (N) NaOH for 30 min and the absorbance was recorded spectrophotometrically (CECIL-CE 4002, Germany) at 420 nm. The enzyme activity was determined by using a standard curve of PNP (Merck). The ALP activity was expressed as dephosphorylation of PNPP to PNP in μM/mg protein/min. The entire experiment was repeated for at least 5 times.

9.2.3 Activity of transaminases

9.2.3.1 Estimation of GOT activity

The activity of the GOT (EC. 2.6.1.1) in the gill and digestive tissue was estimated after Bergmeyer and Bernt (1965a) and the protein was estimated after Lowry *et al.* (1951). The enzyme in the sample catalyses reaction of α-oxaloacetate and L-aspartate in substrate buffer solution (0.1 M phosphate buffer, pH 7.4; 0.01 M L-aspartate; 2 x 10⁻³ M α-oxaloacetate) with production of oxaloacetate. The oxaloacetate was determined with the indicator reaction catalysed by malic dehydrogenase with oxidation of ketone reagent (10⁻³ M 2, 4-Dinitrophenylhydrazine) which was proportional to the amount of oxaloacetate formed. The reaction was stopped with 0.4M NaOH and the optical density was recorded spectrophotometrically (CECIL-CE 4002, Germany) 546 nm. A standard curve of sodium pyruvate (Merck) was used to estimate the enzyme activity. The enzyme activity was expressed in terms of formation of pyruvate in (U)/mg protein. The entire experiment was repeated for at least 5 times.

9.2.3.2 Estimation of GPT activity

The activity of the GPT (EC 2.6.1.2) in the gill and digestive tissue was estimated after Bergmeyer and Bernt (1965b) and the protein content was estimated after Lowry *et al.* (1951). The enzyme in the sample catalyses reaction of α-oxaloacetate and L-alanine in substrate buffer solution (0.1 M phosphate buffer, pH 7.4; 0.2 M D L-alanine; 2 x 10⁻³ M α-oxaloacetate) with production of pyruvate. The pyruvate was determined with the indicator reaction catalysed by lactic dehydrogenase with oxidation of ketone reagent (10⁻³ M 2, 4-Dinitrophenylhydrazine) which was proportional to the amount of pyruvate formed. The reaction was stopped with 0.4M NaOH and the optical density was recorded spectrophotometrically (CECIL-CE 4002, Germany) 546 nm. A standard curve of sodium pyruvate (Merck) was used to estimate the enzyme activity. The enzyme activity was expressed in terms of formation of pyruvate in U/mg protein. The entire experiment was repeated for at least 5 times.

9.2.4 Estimation of AChE activity

The activity of the enzyme AChE (E.C. 3.1.1.7) in the gill and digestive tissue was estimated after Hestrin (1949) and the protein was estimated after Lowry *et al.* (1951). The sample was made to react with alkaline hydroxylamine reagent (fresh v/v solution of 2M
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hydroxylamine hydrochloride (Merck) and 3.5 N NaOH) and 0.004 M acetylcholine chloride solution for a minute and the reaction was stopped as the pH was brought to 1.2 ± 0.2 with 1.0 ml of concentrated hydrochloric acid (sp. gr. 1.18, diluted with 2 parts by volume of water) and 1.0 ml. of the iron solution (0.37M Ferric chloride, hexahydrate in hydrochloric acid (0.1 N). The optical density of the purple-brown colouration developed was recorded spectrophotometrically (CECIL-CE 4002, Germany) at 540nm. A standard curve of acetylcholine chloride (Merck) was used to estimate the enzyme activity. The enzyme activity was expressed in terms of hydrolysis of acetylcholine chloride in µM/mg protein/min. The entire experiment was repeated for at least 5 times.

9.2.5 Estimation of nitric oxide generation

The generation of nitric oxide in the gill and digestive tissue was estimated as the amount of the nitrite released from the sample with Griess reagent after Green *et al.* (1982) and the protein was estimated after Lowry *et al.* (1951). Every 1ml of the tissue lysate was incubated with equal volume of Griess reagent (1% Sulphanilamide, 0.1% naphthyl ethylenediamine dihydrochloride and 5% orthophosphoric acid) at 37°C for 30 min in a humid chamber. The absorbance was recorded in a spectrophotometer (CECIL-CE 4002, Germany) at 550nm against a standard blank. The generation of nitric oxide was determined using a standard curve of sodium nitrite (Merck). The generation of nitric oxide was expressed in terms of formation of nitrite in µM/mg protein/min. The entire experiment was repeated for at least 5 times.

9.2.6 Estimation of GST activity

The activity of GST (EC 2.5.1.18) in the gill and digestive tissue was estimated using the method described by Habig *et al.* (1974) using 1-chloro-2, 4-dinitrobenzene (CDNB) (Merck) as substrate and the corresponding protein content was estimated after Lowry *et al.* (1951). The reaction was conducted at 25°C and initiated by the addition of GSH. The assay was conducted by monitoring the appearance of the conjugated complex of CDNB and GSH and optical density was recorded spectrophotometrically (CECIL-CE 4002, Germany) at 340 nm. The enzyme activity was expressed as µM/mg protein/min. The entire experiment was repeated for at least 5 times.

9.2.7 Estimation of PO activity

The PO (EC 1.14.18.1) activity in the gill and digestive tissue was assayed after Sung *et al.* (1994) using 1,3,4-dihydroxyphenyl alanine (L-Dopa) (Sigma) as the substrate and the protein was estimated after Lowry *et al.* (1951). 100µl of sample was preincubated at 30°C for 15 min, after which 200 µl of L-Dopa (1.6 mg ml⁻¹ in cacodylate acid citrate buffer) was added and reacted for 1 min. Each reaction mixture was further diluted with 200µl of cacodylate acid citrate buffer, and then absorbance was recorded
spectrophotometrically (CECIL-CE 4002, Germany) at 490nm. The enzyme activity was expressed in terms of increase in absorbance as 0.001 Unit (U)/min/mg protein. The entire experiment was repeated for at least for 5 times.

9.2.8 Estimation of CAT activity

The CAT (EC 1.11.1.6) activity in the gill and digestive tissue was estimated according to Aebi et al. (1984) and the protein was estimated after Lowry et al. (1951). The CAT activity was assayed spectrophotometrically at 25°C. The decomposition of 10mM H₂O₂ in 50mM sodium phosphate buffer (pH 7) was followed directly by the decrease in absorbance at 240 nm. The difference in absorbance was recorded spectrophotometrically (CECIL-CE 4002, Germany) per unit time as a measure of the CAT activity. The enzyme unit was defined as the amount of enzyme that catalyzes the oxidation of one mole of H₂O₂/min under the assay condition. The enzyme activity was expressed as k/mg protein/min. The entire experiment was repeated for at least for 5 times.

9.2.10 Estimation of protein

The estimation of the protein content of all the samples were done after the method of Lowry et al. (1951) using a standard curve of bovine serum albumin.

9.2.11 Statistical analysis

The statistical data analysis was carried out using Student’s t-test (Sokal and Rohlf 1973). Differences were considered significant at P < 0.05, P < 0.01, P < 0.001. Data was presented as the mean ± standard error (S.E).

9.3 Results

9.3.1 Activity of phosphatases

An initial elevation in the activity of ACP in the gill of large sized L. marginali exposed to 1 ppm of sodium arsenite for 24 h was exhibited and the highest activity was recorded as 0.49 ± 0.005 µM /mg protein /min against a control activity of 0.425 ± 0.013 µM /mg protein /min (Figure 1a). The lowest activity was recorded as 0.10 ± 0.0725 µM /mg protein /min in the gill of the animals exposed to 5 ppm of the toxin for 30 days. The enzyme activity in the digestive tissue of the animals exposed to the 1 ppm of sodium arsenite for 24 h exhibited an elevation (Figure 1b) to 0.513 ± 0.0376 µM /mg protein /min against a control of 0.425 ± 0.0133 µM /mg protein /min. The lowest activity was recorded as 0.083 ± 0.0801 µM /mg protein /min for the tissue of the animals exposed to 5 ppm of the toxin for 30 days.
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Figure 1. Acid phosphatase (ACP) activity in the (a) gill and (b) digestive tissue of large sized L. marginalis exposed to sodium arsenite. Data presented as mean ± SE. *P < 0.05, **P < 0.01, ***P < 0.001.

The ALP activity in the gill of large sized L. marginalis exposed to all the experimental concentrations of sodium arsenite (Chapter 4) for 24 h remained unchanged with respect to the control. The lowest activity was exhibited as 0.0108 ± 0.0271 μM/mg protein/min for the animals exposed to 3 ppm of sodium arsenite for 30 days (Fig 2a). The ALP activity in the digestive tissue of L. marginalis exposed to sodium arsenite for 24 h remained unchanged with respect to the control activity of 0.416 ± 0.0143 μM/mg protein/min. The lowest activity was recorded as 0.018 ± 0.0271 μM/mg protein/min for the animals exposed to 5 ppm of sodium arsenite for 30 days (Figure 2b). The enzyme
activity was inhibited both in gill and digestive tissue in a time dependent exposure to sodium arsenite.

![Graph showing ALP activity in gill and digestive tissue](a)

**Figure 2.** Alkaline phosphatase (ALP) activity in the (a) gill and (b) digestive tissue of large sized *L. marginalis* exposed to sodium arsenite. Data presented as mean ± SE. *P < 0.05, **P < 0.01, ***P < 0.001.

### 9.3.2 Activity of transaminases

The GOT activity in the gill of large sized *L. marginalis* exposed to sodium arsenite for 24 h exhibited an initially elevation to 57.9 ± 0.155 U/mg protein/min at an exposure of 4 ppm of sodium arsenite against a control activity of 53.97 ±1.477 U/mg protein /min. The lowest activity was recorded as 16.7 ± 1.342 U/mg protein /min for the tissue of the animals exposed to 5 ppm of sodium arsenite for 30 days (Figure 3a). The enzyme activity in the digestive tissue
of *L. marginalis* exposed to sodium arsenite for 24 h exhibited highest activity as $57.9 \pm 0.155$ U/mg protein /min at an exposure of 4 ppm of sodium arsenite against a control activity of $65.1 \pm 1.021$ U/mg protein /min. The lowest activity was recorded as $18.5 \pm 0.827$ U/mg protein /min in the tissue of the animals exposed to 5ppm of sodium arsenite for 30 days (Figure 3b).

The highest GPT activity in the gill was recorded as $53.5 \pm 0.489$ U/mg protein /min for the animals exposed to 5 ppm sodium arsenite for 24 h against a control activity of $46.9 \pm 0.733$ U/mg protein /min. The lowest activity was recorded as $13 \pm 3.6429$ U/mg protein /min in the tissue of the animals exposed to 5ppm of sodium arsenite for 30 days (Figure 4a). The highest

![Figure 3. Glutamate oxaloacetate transaminase (GOT) activity in the (a) gill and (b) digestive tissue of large sized *L. marginalis* exposed to sodium arsenite. Data presented as mean ± SE. *P* < 0.05, **P* < 0.01, ***P* < 0.001.](image)
GPT activity in the digestive tissue was recorded as $58.4 \pm 0.489 \text{ U/mg protein/min}$ for the animals exposed to 5 ppm sodium arsenite for 24 h against a control activity of $50.2 \pm 0.8528 \text{ U/mg protein/min}$. The lowest activity was exhibited as $17.3 \pm 0.717 \text{ U/mg protein/min}$ for the tissue of the animals exposed to 5 ppm of sodium arsenite for 30 days (Figure 4a).

![Graph showing GPT activity in gill and digestive tissue of L. marginalis exposed to sodium arsenite.](image)

**Figure 4.** Glutamate pyruvate transaminase (GPT) activity in the (a) gill and (b) digestive tissue of large sized *L. marginalis* exposed to sodium arsenite. Data presented as mean ± SE. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.**

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9.3.4 Activity of AChE

The activity of the enzyme AChE in the gill of large sized \textit{L. marginalis} remained unaltered in 24h of exposure of sodium arsenite against a control activity of $0.2666 \pm 0.0206 \text{ pM/mg protein/min}$. The lowest activity was recorded as $0.0747 \pm 0.024 \text{ pM/mg protein/min}$ for the tissue of the animals exposed to 5ppm of sodium arsenite for 30 days (Figure 5a). The activity of the enzyme in the digestive tissue of the animals remained unaltered in 24h of exposure of sodium arsenite against a control of $0.2318 \pm 0.0157 \text{ pM/mg protein/min}$. The lowest activity was recorded as $0.0641 \pm 0.0409 \text{ pM/mg protein/min}$ in the tissue of the animals exposed to 5ppm of sodium arsenite for 30 days (Figure 5b). The activity of the enzyme was inhibited by sodium arsenite in a time dependent manner.

\textbf{Figure 5.} Acetylcholinesterase (AChE) activity in the (a) gill and (b) digestive tissue of large sized \textit{L. marginalis} exposed to sodium arsenite. Data presented as mean $\pm$ SE. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. 

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9.3.5 Generation of nitric oxide

The activity of nitric oxide in the gill of large sized *L. marginalis* was elevated to $0.1213 \pm 0.0171 \text{ M} / \text{mg protein/min}$ in 24 h of exposure of 1 ppm of sodium arsenite against a control of $0.0835 \pm 0.0115 \text{ M} / \text{mg protein/min}$. But when exposed to sodium arsenite persistently, activity of the enzyme was inhibited in a time dependent manner. The lowest activity was exhibited as $0.02 \pm 0.0031 \text{ M} / \text{mg protein/min}$ in the animals exposed to 5 ppm of sodium arsenite for 30 days (Figure 6a). The generation of nitric oxide in the digestive tissue of *L. marginalis* was elevated to $0.1287 \pm 0.0143 \text{ M} / \text{mg protein/min}$ under the exposure of 1 ppm of sodium arsenite for 24 h against a control value of $0.0964 \pm 0.0147 \text{ M} / \text{mg protein/min}$. The lowest activity was recorded as $0.0453 \pm 0.0133 \text{ M} / \text{mg protein/min}$ in the tissue of the animals exposed to 5 ppm of sodium arsenite for 30 days (Figure 6b).

**Figure 6.** Nitric oxide (NO) generation in the (a) gill and (b) digestive tissue of large sized *L. marginalis* exposed to sodium arsenite. Data presented as mean ± SE. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$**
9.3.6 Activity of GST

The activity of GST in the gill of large sized *L. marginalis* exposed to 1 ppm of sodium arsenite for 48 h exhibited an elevation with the highest activity as 8.37 ± 0.329 μM /mg protein/min against a control activity of 6.25 ± 0.204 μM /mg protein/min. The lowest activity was recorded at 1.55 ± 0.5243 μM /mg protein/min for the tissue of the animals exposed to 5 ppm of sodium arsenite for 30 days (Figure 7a). The activity of GST in the digestive tissue of *L. marginalis* exposed to 1 ppm of sodium arsenite for 48 h surged to the highest value of 7.977 ± 0.419 μM /mg protein /min against a control value of 5.71 ± 0.1309 μM /mg protein /min. The lowest activity was recorded at 1.35 ± 1.024 μM /mg protein/min in the tissue of the animals exposed to 5 ppm of sodium arsenite for 30 days (Figure 7b).

![Glutathione-s-transferase (GST) activity in the gill of large sized *L. marginalis* exposed to sodium arsenite](image)

**Figure 7.** Glutathione-s-transferase (GST) activity in the (a) gill and (b) digestive tissue of large sized *L. marginalis* exposed to sodium arsenite. Data presented as mean ± SE. *P < 0.05, **P < 0.01, ***P < 0.001.
9.3.7 Activity of PO

The activity of PO in the gill of large sized \textit{L. marginalis} increased dose dependently to the highest value of 10.61 ± 0.457 U/mg protein/min till the 96 h exposure of 5 ppm sodium arsenite. But the activity of the enzyme was inhibited under prolonged exposure and a feeble activity of 0.77 ± 0.556 U/mg protein/min was recorded for the animals exposed to 5 ppm of sodium arsenite for 30 days (Figure 8a). The activity of PO in the digestive tissue of the animal increased dose dependently to the highest value of 11.24 ± 0.457 U/mg protein/min till 96 h of exposure of 5 ppm of sodium arsenite. But the activity of the enzyme was reduced drastically under prolonged exposure of the sodium arsenite and exhibited a feeble activity of 1.463 ± 0.617 U/mg protein/min for the exposure of 5 ppm of sodium arsenite for 30 days (Figure 8b).

\textbf{Figure 8.} Phenoloxidase (PO) activity in the (a) gill and (b) digestive tissue of large sized \textit{L. marginalis} exposed to sodium arsenite. Data presented as mean ± SE. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. 

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9.3.8 Activity of CAT

The activity of CAT in the gill of large sized *L. marginalis* exposed to the different concentrations of sodium arsenite progressively diminished in a dose and time dependent manner. The lowest activity was recorded as $0.00025 \pm 0.00043 \text{ Unit/mg protein/min}$ for the gill of the animals exposed to 5 ppm of sodium arsenite for 15 days against a control value of $0.00978 \pm 0.001214 \text{ Unit/mg protein/min}$ (Figure 9a). The activity of CAT in the digestive tissue of the animal exposed to the different concentrations of the toxin progressively diminished in a dose and time dependent manner. The lowest activity was recorded as $0.00025 \pm 0.00043 \text{ Unit/mg protein/min}$ for the gill of the animals exposed to 5 ppm of sodium arsenite for 15 days against a control value of $0.01085 \pm 0.00125 \text{ Unit/mg protein/min}$ (Figure 9b).

![Graph showing the activity of CAT in the gill (a) and digestive tissue (b) of *L. marginalis* exposed to different concentrations of sodium arsenite.](image_url)

**Figure 9.** Catalase (CAT) activity in the (a) gill and (b) digestive tissue of large sized *L. marginalis* exposed to sodium arsenite. Data presented as mean ± SE. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.**
9.4 Discussion

Alterations of the immunosurveillance have been reported for bivalve molluscs exposed to metals (Cheng and Sullivan 1984; Cheng 1988; 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). Being a filter feeder, the gill forms a vital organ for L. marginalis. Gills of the bivalves remain in direct interaction with the surrounding aquatic environment and as such, the structural pattern of the gill tissues and their biochemical profiles reflect the adverse environmental impact on the animal (Gregory et al., 2002) with reference to biochemical homeostasis. Phosphatases are enzymes, which catalyze the liberation of orthophosphate from complex organic phosphorus compounds and are thus believed to have an essential function in the nutrient dynamics. The activities of both ACP and ALP were suppressed in the gill and digestive tissue of L. marginalis. ACP is considered as a major hydrolytic enzyme that acts in phagocytic vesicles to degrade endocytosed particles. It is reported that on the brush bordered cell membrane, ACP inhibits the membrane attached NADPH oxidase activity and thereby suppresses oxidative burst (generation of hydrogen peroxide and uperoxide anion) by the immune cells (Glew et al., 1988). Besides, ACP plays an important role in the detoxification process of toxic compounds entering the body (Zheng et al., 2007). On the other hand, ALP has often been implicated in phosphorylative transfer of extracellular molecules against concentration gradients at cell membranes (Monin and Rangneker, 1974). ALP being an enzyme supposedly involved in transfer of extracellular substances, it is probable that this enzyme was either utilized as extracellular hydrolytic enzyme or are involved in secretion of other hydrolytic enzymes at the cell membrane. Suppression of the activity of ACP and ALP in the tissues by arsenite might cripple the immune status and biochemical stasis in the gill and digestive tissue of L. marginalis. The activities of GOT and GPT were inhibited both in the gill and digestive tissue of L. marginalis. For interconversion of amino acids, the conversion of amino acids to keto acids and synthesis of 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. These transminases function as link enzymes between protein and carbohydrate metabolism. GOT and GPT play an important role in aerobic and anaerobic metabolism by bivalve molluscs. In aerobiosis, they are involved in oxidative amino acid catabolism. In anaerobiosis, these enzymes are especially important during the initial period of emersion, when aspartate appears to be a main energy substrate and alanine one of the principal end products (Baginski and Pierce, 1978; Meinardus and Gade, 1981). Since the activity of both GOT and
GPT is considered as the marker of health of hepatic functions, their inhibition is indicative of development of perturbation in the natural transformations of the vital bio-components that might impose a state of disorder in the tissue structure and functions. In both gill and digestive tissue, suppression in the activity of AChE was recorded. The decrease in AChE activity in oyster has been considered as a biomarker of exposure to neurotoxic compounds (Bocquene et al., 1997). Inhibition in AChE activity in the gill and digestive tissue of *L. marginalis* is suggestive to the neurotoxic behaviour of sodium arsenite. The situation may lead to 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 behaviour. During the formation of L-citruline from L-arginine by nitric oxide synthase, nitric oxide is produced as a reactive nitrogen intermediate (Ischiropoulos et al., 1992; Bogdan, 2001). Nitric oxide is an important agent of nonspecific immunity in invertebrates; its generation in the tissue lysates of arsenic exposed *L. marginalis* reflects an important aspect of the immunological status of the challenged animals. The initial surge of nitric oxide following short term (24 h) of exposures was indicative of the attempt the animal to respond and adapt to the arsenite induced stress. But the gradual suppression of nitric oxide generation reflects a compromise in the immune strategy of the animal against persistent arsenite mediated stress. GST catalyses the conjugation of glutathione with xenobiotic compounds containing electrophilic centres. It is important for organisms to deal with active electrophiles since they can react with macromolecules controlling cell growth. GST plays an important role in detoxifying strong electrophiles having toxic, mutagenic and carcinogenic properties. GST occurs in cytosol of tissues in a number of marine invertebrates (Balabaskaran et al., 1986). Among aquatic invertebrates, pentachlorophenol and butylated hydroxytoluene have been shown to increase GST activity in freshwater cladocerans and marine crustaceans, respectively (LeBlanc and Cochrane, 1985). In the present study, the activity of this important xenobiotic biotransformer seemed to have been initially activated in presence of strong electrophile like arsenic. Persistent exposure of arsenite resulted in a decrease in GST activity in the gill and digestive tissue of *L. marginalis* which indicates the susceptibility of detoxification machinery of the animal to a toxic metalloid like arsenic. The active form of pro-phenoloxidase catalyses two successive biochemical 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 PO is an early step in the biosynthesis of melanin which bears an important role in wound healing, and in the encapsulation of foreign materials for host defence.
Studies on the toxicity of arsenic in *Lamellibrachia marginalis* (Lamarck) (Cerenius and Söderhäll, 2002). Cellular studies also have been carried out in crustaceans, and several proteins that, in conjunction with the proPO system, are involved in encapsulation, phagocytosis, and cytotoxic reactions have been characterized (Cerenius and Söderhäll, 2004). The initial burst of PO activity in a dose and time dependent mode reflects the instinctive biogenesis of immune-surveillances of the arsenite exposed animals till 96 h of exposure. However, persistent arsenite stress seemed to have exhausted the animals’ immune adaptations and the PO activity was severely depleted in the gill and digestive tissue of *L. marginalis* against 30 days of exposure. A decrease in CAT activity indicates a reduced ability to protect cells against hydrogen peroxide toxicity (Papagiannis et al., 2004; Li et al., 2009). Such vulnerability was observed in the animals exposed to the arsenite which is indicative of development of oxidative stress in the gill and digestive tissue of *L. marginalis*.

In the present study, arsenic induced alterations in the activities of the studied enzymes and generation of reactive molecules were not linear. At lower concentrations and short duration of arsenite exposures, the activities of ACP, GOT, GPT, GST, PO and the generation of nitric oxide exhibited an increasing trend followed by a decrease against higher concentrations and longer time of exposure of sodium arsenite. The initial surge in the activities of the enzymes and reactive molecules probably suggests the physiological reflex of the animal to respond to the arsenite insult; depletion in the activities of the same under long span of exposure of the toxin indicates a state of exhaustion of the animals. Such non-linear responses in activities against low and high concentrations of sodium arsenite are indicative to a deviation from the state of physiological homeostasis of the animal. As a contaminant, sodium arsenite proved to be a potent inhibitor of vital enzyme and biomolecular functions of gill and digestive tissue of *L. marginalis*. It imposed a perturbation in immune capabilities of the animal by restricting the activities of ACP, ALP. The activity of the enzyme PO, a vital component of internal defense in invertebrates (Söderhäll and Cerenius, 1998; Jordan et al., 2005), was inhibited by the toxic metalloid. Sodium arsenite suppressed the generation of nitric oxide which is an important cytotoxic molecule. Arsenic induced inhibition in GOT and GPT activity resulted disarray in biochemical homeostasis of the tissue with respect to the functions of gill and digestive tissue. The decrease in AChE activity in the oyster has often been considered as a biomarker of exposure to neurotoxic compounds (Bocquene’ et al., 1997). Sodium arsenite had suppressed the activity of AChE and can be considered as a neurotoxicant. It restricted the activity of the vital xenobiotic cleanser, GST. All the more its suppression of CAT activity might provide an opportunity of imposition of oxidative stress on the animal. The
natural habitat of *L. marginalis* has susceptibility to be contaminated naturally by inorganic arsenite. Under such challenges, the threat to the animal might be cumulatively augmented in field conditions resulting in an increased invasion by opportunistic parasites in *L. marginalis*. Such a situation may lead to gradual decline in the population of the species in its natural habitat. The loss of an efficient filter feeder is apprehended which may affect the biodiversity and cause disturbance in the ecological balance of static freshwater ecosystems of India.

9.5 Bibliography

Studies on the tenacity of arsenic m ImneDniats JMrgtmth (Lamarck) Chapter 9


