

CHAPTER 2

LITERATURE REVIEWS

Heavy metals are found to occur naturally, and large scale release into the aquatic environment requires human intervention. Many supposedly natural metal-rich rivers in fact are shown to have a history of mining activities, so that the river receives either contaminated mine-waters or run off from aging spoil heaps. Industrial waste water is another obvious source of heavy metals discharge. In addition, another source is the diffuse from urban runoff and leachate from solid waste disposal sites, both of which may be rich in heavy metals (Mance, 1987). The industrial source of the major heavy metals are likely to be discharged into the hydrosphere, such as fertilizers and can discharge Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn; insecticides/pesticides can also discharge Ag, Cu, Hg and paper mills can discharge also Cr, Cu, Hg, Ni, Pb, Ti and Zn. List of heavy metals such as Cr, Cu, Fe, Mn, Ni and Zn possess adverse affect on aquatic life as reported by Burrell (1977).

Chromium is frequently present in industrial wastewater and commonly used as a rust inhibitor which is highly poisonous but non accumulative. Copper is derived from many industrial processes and is a major cause of boiler corrosion. It is also toxic to fish, algae, bacteria and hence frequently used to control excessive algal bloom (eutrophication). Iron is objectionable and is the primary cause of coloring in water from staining/deposition characteristics. There are common derivatives of general corrosion, but the worst effects are frequently a by-product of acid mine drainage. Manganese compounds are more readily solubilized than those of iron, with a less reducing environment required. Objections to discharges of this heavy metals (*e.g.*, staining properties) are similar to those for iron but are not toxicologically significant heavy metals. Nickle corrosion is caused by stainless steels but is non toxic to fish. Zinc is a ubiquitous associate of industrialization having an impact closely paralleled to that of copper (Burrell, 1977).

Thus, aquatic ecosystems may receive anthropogenic pollutants originating from various sources located in the catchments area or at distant places. Many pollutants, including heavy metals, are toxic to aquatic organisms and cause their lethal or sublethal deterioration (Krenkle, 1975). Mollusks have long been regarded as promising bioindicators and biomonitoring subjects. They are abundant in many terrestrial and aquatic systems, being easily available for collection. They are highly tolerant to many pollutants and exhibit high accumulation properties, particularly for heavy metals. Mollusks living in the sea can accumulate heavy metals and may serve as excellent passive biomonitors (Wang *et al.*, 2005). Moreover, freshwater mollusks can also accumulate heavy metals and so it is a necessary step to make a culture of mollusk in order to study as well as to observe their life cycle. Chaleamwat (2001) documented a methodology for culturing economical mollusks in Thailand. To learn about the mollusk life cycle, biology, ecological, food and diseases, it is necessary to prepare lived mollusks culture.

Various heavy metals accumulating bivalve and gastropod species are abundant in coastal waters and play an important role as biomonitors for tracing heavy metals pollution in global monitoring programs (Sericano, 2000). They assimilate trace heavy metals from their food and from the ingestion of inorganic particulate material (Phillips, 1977). The development of techniques allowing the analysis of the effects of pollutions on bivalve biology may lead to the monitoring of pollutant transfer in estuarine areas. Pollutant effects were tested *in vitro* on oyster haemocytes by Gagnaire *et al.* (2004).

Most of the research works of heavy metals affecting ecology and tissue of mollusks have been mainly studied on marine and estuarine samples with relatively less research work being done on fresh water samples. Usero *et al.* (2005) studied heavy metal concentrations in mollusks from the Atlantic coast of southern Spain, and the results showed that the area near the mouth of the Huelva estuary possessed the highest heavy metal concentrations found in the sediment and in the two bivalves species (*Donax trucus* and *Chamelea gallina*). The two species of bivalves have different amounts of heavy metals concentration in their tissues. According to China

Marine Environmental Quality Communiqué in 2001 (Zhan *et al.*, 2002), most estuaries bays and seas abutting big cities had high contamination of heavy metals. Many marine mollusks have been annihilated and those marine mollusks having their habitat near the coastal sea area abutting the pollution sources are not suitable for consumption. In addition, Gagnaire *et al.* (2004) studied the effects of cadmium and mercury on Pacific oyster's haemocytes *in vitro*, and showed clearly that pollutant transfer towards the aquatic environment in estuarine areas was inevitable. Due to the way of life of bivalves, including mussels and oysters, they are considered to be ideal indicator organisms. Pollutant affects were tested *in vitro* on oyster haemocytes and the results showed that mercury may be expected to have an impact on the bivalve immune function in contaminated areas. Saha *et al.*, (2006) studied interspecific variation in heavy metal body concentrations in biota of Sunderban mangrove wetland in northeast India, with a high degree of organ specificity being evident in the bivalves where gill and mantle exhibited higher heavy metal accumulation due to ion exchange property of the mucous layer covering these organs. The results of this analysis suggested that mollusks can play a significant role in trace metal trophic transfer studies, especially as their representatives were intertidally and subtidally ubiquitous.

In the term of tissue and cell of mollusks, cell cultural techniques of marine invertebrates have been developed since the 1960s. Despite many attempts, no continuous cell lines have been reported to date. However, marine invertebrate primary cell cultures have been successfully used to investigate specific processes. For example, cultured cells of bivalve gill were used for pathology studies (Auzoux *et al.*, 1993) and tunicate cells were used to identify marine invertebrate cytokines (Raftos *et al.*, 1990 ; 1991). Invertebrate primary cell cultures are also a promising tool for sustainable production of complex marine molecules with pharmacological or industrial interest (Pomponi and Willoughby, 1994). Primary cultures of pearl oyster mantle (*Pinctada fucata*, *P. margaritifera*) have been initiated both by tissue fragmentation and by enzymatic dissociation (Macchii and Wada, 1989).

Macchii and Wada (1989) attempted to produce nacre *in vitro* by tissue and larval cell culture of *P. fucata*. At present, the study of cell culture of mollusks is on the increase. Sud *et al.* (2001) discovered the role of water soluble matrix (WSM) fraction extracted from the nacre of *Pinctada maxima*, in the regulation of cell activity in abalone mantle cell culture (*Haliotis tuberculata*), and the effect of a water-soluble extract from the organic matrix of *Pinctada maxima* (WSM) was tested on this cell culture system for an 11 days exposure. WSM reduced the global viability of mantle cells in a dose-dependent way which corresponded to a cell death. In addition, Chelomin *et al.* (1995) studied cadmium in the tissue of scallop *Mizuhopecten yessoensis* and studied the effect of cadmium accumulation on the levels of essential elements (copper, zinc and iron) in the tissue of a marine bivalve mollusk, *Mizuhopecten yessoensis*, exposed to cadmium at 250 ppb for 2 weeks. It was found that the concentration of cadmium in the tissue increased in the following orders: gonads, gills, hepatopancreas and kidney respectively during the time exposure. Cadmium had the most pronounced effects on essential trace element homeostasis in the kidney. From the literature, most studies were done on marine and estuarine bivalves, because bivalves live in sediment and accumulate heavy metals.

In this chapter, heavy metals in this study consist of cadmium (Cd), copper (Cu), mercury (Hg), lead (Pb) and zinc (Zn). Physical and chemical properties, sources in the environment, uptake, elimination, accumulation in organisms and toxicity to aquatic organisms due to the heavy metals are discussed. Inductively-Coupled Plasma Spectroscopy (ICP) techniques are described definition and general description of ICP optical emission spectrometry (ICP-OES). Mollusca is also discussed at final. This topic general describes dominant species of the Phylum. Habitats of freshwater mollusks are additionally shown in this chapter.

2.1 Heavy metals and the effects on organisms and habitats

2.1.1 Cadmium (Cd)

1) Physical and chemical properties

Cadmium is a metallic element belonging, together with zinc and mercury, to group IIb of the periodic table. Some cadmium salts, such as the sulfide, carbonate and oxide are practically insoluble in water; these can be converted to water-soluble salts in nature. The sulfate, nitrate and halides are soluble in water. The specification of cadmium in the environment is of importance in evaluating the potential hazard (WHO, 1992a). In addition, cadmium is a nonessential element and is potentially highly toxic to human, animals and plants, even at low doses (Benavidies *et al.*, 2005)

2) Source in the environment

Cadmium is released into aquatic environments from industrial sources involved in, for example, mining, ore refining and plating processes, as well as from natural sources such as rocks and soils (Choi *et al.*, 2007). The average cadmium content of sea water is about 0.1 µg/L or less. River water contains dissolved cadmium at concentrations of between less than 1 and 13.5 ng/L. In remote, uninhabited areas, cadmium concentrations in air are usually less than 1 ng/m³. In areas not known to be polluted, the median cadmium concentration in soil has been reported to be in the range of 0.2 to 0.4 mg/kg. However, much higher values, up to 160 mg/kg in soil, are occasionally found (WHO, 1992a).

3) Uptake, elimination, and accumulation in organisms

WHO (1992a) discussed environmental factors affect the uptake and, therefore, the toxic impact of cadmium on aquatic organisms. Increasing temperature increases the uptake and toxic impact, whereas increasing salinity or water hardness decreases them. Freshwater organisms are affected by cadmium at lower concentrations than

marine organisms. The organic content of water generally decreases the uptake and toxic effect by binding cadmium and reducing its availability to organisms. However, there is evidence that some organic matter may have the opposite effect. Kim, *et al.* (2004) found that cadmium has physiologically adverse effects on the growth, reproduction and osmoregulation of fish. Itai-itai disease, which is cadmium toxicosis caused by cadmium in wastewater discharged from lead and zinc refineries in Japan 50 years ago, is a representative case that illustrates the harmfulness of cadmium (Kobayashi, 1971).

Cadmium accumulates within the bodies of organisms and alters and degrades processes of enzyme activation (Sastry and Subhadra, 1982). It also causes cell damage and can result in cell death (Benavides *et al.*, 2005). In addition, cadmium is readily accumulated by many organisms, particularly by microorganisms and mollusks where the bioconcentration factors are in the order of thousands. Soil invertebrates also concentrate cadmium markedly. Most organisms show low to moderate concentration factors of less than 100 mg/kg. Cadmium is bound to proteins in many tissues. Specific heavy metal – binding proteins (metallothioneins) have been isolated from cadmium-exposed organisms. The concentration of cadmium is greatest in the kidney, gills and liver (or their equivalents). Elimination of the metal from organisms probably occurs principally via the kidney, although significant amounts can be eliminated via the shed exoskeleton in crustaceans. In plants, cadmium is concentrated primarily in roots and to a lesser extent in the leaves (WHO, 1992b).

4) Toxicity to aquatic organisms

Cadmium is one of the most toxic heavy metals and common environmental contaminant (UNEP, 1978; Nriagu, 1980). The acute toxicity of cadmium to aquatic organisms is variable, even between closely related species, and is related to the free ionic concentration of the metal. Cadmium interacts with the calcium metabolism of animals. In fish, it causes hypocalcaemia, probably by inhibiting calcium uptake from the water. However, high calcium concentrations in the water protect fish from cadmium uptake by competing at uptake sites. Zinc increases the toxicity of cadmium

to aquatic invertebrates. Sublethal effects have been reported on the growth and reproduction of aquatic invertebrates; there are structural effects on invertebrate gills. There is evidence of the selection of resistant strain of aquatic invertebrates after exposure to cadmium in the field. The toxicity is variable in fish, salmonids being particularly susceptible to cadmium. Sub-lethal effects in fish, notably malformation of the spine, have been reported. The most susceptible life-stages are the embryo and early larva, while eggs are the least susceptible. There is no consistent interaction between cadmium and zinc in fish. Cadmium is toxic to some amphibian larvae, although some protection is afforded by sediment in the test vessel (WHO, 1992b). Presence of cadmium binding metallothioneins (MTs) in some mollusk population (Viarengo *et al.*, 1993; Choi *et al.*, 2001, 2003) may be one such defensive mechanism against cadmium toxicity. MTs are one of the most important metal-binding and detoxifying ligands in marine bivalve mollusk (Roesijadi, 1992; Viarengo and Nott, 1993; Mason and Jenkins, 1995; Langston *et al.*, 1998). Moreover, Bebianno and Langston (1998) studied cadmium and metallothionein turnover in different tissues of the gastropod *Littorina littorea*. They found that the turnover of metallothionein is tissue dependent. Metallothionein has an estimated half-life of 69 and 160 days in the gills and kidney of *Littorina littorea*, respectively. From toxicology viewpoint, cadmium is easily taken in to an organism from aqueous and dietary sources, readily concentrate in the tissue and have long biological half-lives. Ivanina *et al.* (2008a) found that the stress proteins such as heat shock protein (HSPs) and metallothioneins play a key role in cellular protection against environmental stress. Furthermore, Ivanina *et al.* (2008b) studied differential sensitivity to cadmium of key mitochondrial enzymes in the eastern oyster, *Crassostrea virginica* Gmelin (Bivalvia: Ostreidae). They found that the cadmium exposure also resulted in a decrease in activation energy of mitochondrial enzymes suggesting that mitochondria from cadmium exposed oyster could exhibit reduced capacity to respond to temperature rise with an adequate increase in the substrate flux.

2.1.2 Copper (Cu)

1) Physical and chemical properties

Copper, the 29th element and the first in group IB of the periodic table, displays four oxidation states: metallic copper Cu^0 , cuprous ion Cu^+ , cupric Cu^{2+} and trivalent copper ion Cu^{3+} . Copper is found in a wide variety of mineral salts and organic compounds and can also be found naturally in the elemental or metallic form. The metal is a dull lustrous reddish-brown in colour, malleable, a good thermal conductor and an excellent electrical conductor. The metallic form is very stable to dry air at low temperatures but undergoes a slow reaction in moist air to produce a hydroxycarbonate or hydroxysulfate that forms a greenish-grey amorphous film over the surface which protects the underlying metal from further attack. The metal is sparingly soluble in water, in salts solution and in mildly acidic solution, but can be dissolved in nitric acid and sulfuric acid as well as in basic solution of ammonium hydroxide, ammonium carbonate and cyanide in the presence of oxygen (Cotton & Wilkinson, 1989). Furthermore, copper is an essential trace element required by all organisms as a vital constituent of cofactors, enzymes and proteins. At the same time, copper is also involved in toxicological interactions with cellular components, mainly originating from its ability to produce toxic hydroxyl radicals in Fenton-like reactions (Schumann *et al.*, 2002). Probably, as a consequence of this double biological role, intracellular and tissue concentrations of copper are homeostatically regulated in many animal species (Thiele, 2003), including mammals (Tapiero *et al.*, 2003), fish (Bury *et al.*, 2003), arthropods (Norum *et al.*, 2003) and mollusks (Swaileh *et al.*, 2002).

2) Source in the environment

Copper is released to water as a result of natural weathering of soil and discharges from industries and sewage treatment plants. Copper compounds may also be intentionally applied to water to kill algae. In marine environments, the sedimentation of microalgae during algal blooms has been associated with substantial

(20-70%) reductions in the level of suspended heavy metals, as well as heavy metal deposition (Luoma *et al.*, 1998). Similarly, for organic xenobiotics, algae play an important role in the dispersal (Wang *et al.*, 1998; Kowalewska, 1999), chemical transformation and bioaccumulation of many toxic compounds (Okay *et al.*, 2000; Todd *et al.*, 2002; Lei *et al.*, 2002, 2007; Murray *et al.*, 2003; Bopp and Lettieri, 2007). In addition, marine microalgae are particularly promising indicator species for organic and inorganic pollutants since they are typically the most abundant life forms in aquatic environments and occupy the base of the food chain (Torres *et al.*, 2008). The photosynthetic ciliated *Mesodinium rubrum* is a common red tide species associated with upwelling. Other species reported are *Gymnodinium splendens*, *Scrippsiella trochoidea*, *Prorocentrum dentatum*, *Noctiluca scintillans* (Cortes-Altamirano *et al.*, 1995a; Cortes-Altamirano *et al.*, 1995b) and *Stephanopyrix palmeriana* (Molina *et al.*, 1997). There have been reports of toxic species in some red tide events. These include *Gymnodinium catenatum* and *Gonyaulax polyedra* (Cortes-Altamirano *et al.*, 1996). Several processes influence the fate of copper in the aqueous environment. These include complex formation, sorption to hydrous metal oxides, clays and organic materials, and bioaccumulation. Information on the physicochemical forms of copper (speciation) is more informative than total copper concentrations. Much of the copper discharged to water is in particulate form and tends to settle out, precipitate out or be adsorbed by organic matter, hydrous iron, manganese oxides and clay in the sediment or water column. In the aquatic environment the concentration of copper and its bioavailability depend on factors such as water hardness and alkalinity, ionic strength, pH and redox potential, complexing ligands, suspended particulate matter and carbon, and the interaction between sediments and water (WHO, 1998).

3) Bioaccumulation

Despite the importance of copper as an essential trace element for all biota, aquatic organisms may suffer from exposure to concentrations, which might be 10-50 times higher than the required concentrations (Hall *et al.*, 1998). Elliott *et al.* (1985) found that the marine mussels *Mytilus edulis* exposed either continually, or in a 2 day

cycle, to copper (10µg/L) exhibited a linear accumulation over a 40-day period. Mussels exposed under cycled condition showed a lower rate of accumulation. Copper accumulation was not in direct proportion to the time exposed to the elevated concentration. The presence of cadmium reduced the accumulation factor by 50%. Moreover, biological half-lives for depuration of copper from “green” oysters (*Crassostrea gigas*) and mussels (*Mytilus smaragdium*) from 3 copper-contaminate area, and “normal” oysters were 11.6, 6.4 and 25.1 days, respectively (Han *et al.*, 1993). The acute toxicity of copper is suggested to be mainly caused by the formation of reactive oxygen species (ROS) (Manzl *et al.*, 2004). Common biomarkers based on response to oxidative stress, such as catalase activity and anti-oxidant level, were shown to be induced (Hansen *et al.*, 2006; Ansaldo *et al.*, 2000).

4) Effects on other organisms in the laboratory and field

The adverse effects of copper must be balanced against its essentiality. Copper is an essential element for all biota, and care must be taken to ensure the copper nutritional needs of organisms are met. At least 12 major proteins require copper as an integral part of their structure. A number of trace metals are used by living organisms to stabilize protein structures, facilitate electron transfer reactions and catalyze enzymatic reaction (Ash and Stone, 2003). For example, copper, zinc and iron are essential as constituents of catalytic sites of several enzymes (Allan, 1997). Other metals, however, such as lead, mercury and cadmium may displace or substitute for essential trace metals and interfere with proper functioning of enzymes and associated cofactor (Ash and Stone, 2003). It is essential for the utilization of iron in the formation of haemoglobin, and most crustaceans and mollusks possess the copper-containing haemocyanin as their main oxygen-carrying blood protein. In plants, copper is a component of several enzymes involved in carbohydrate, nitrogen and cell wall metabolism.

A critical factor in assessing the hazard of copper is its bioavailability. Adsorption of copper to particles and complexation by organic matter can greatly limit the degree to which copper can be accumulated and have elicit effects. Other

cations and pH can also significantly affect bioavailability. Jing *et al.* (2006) studied the effect of exposure to copper under laboratory-controlled conditions in pearl oysters, *Pinctada fucata*. They found that the digestive gland of *P. fucata* was the main copper accumulation organ when oysters were exposed to low concentrations, whereas gills became the target organ in oysters exposed to high concentrations.

Copper has been shown to exert adverse reproductive, biochemical, physiological and behavioral effects on a variety of aquatic organisms. Copper concentrations as low as 1-2 $\mu\text{g/L}$ can have adverse effects on aquatic organisms; however, large variations due to species sensitivity and bioavailability must be considered in the interpretation and application of this information (WHO, 1998). Moreover, copper toxicity has been studied in several fish species. It is acutely highly toxic to rainbow trout (*Oncorhynchus mykiss*) with LC_{50} (96 hours) of 210 $\mu\text{g/L}$ (De Boeck *et al.*, 2004). Chronic copper exposure for 28 days to 1000 $\mu\text{g/L}$ had no effect on growth, condition factor or food consumption of juvenile rainbow trout. Copper accumulated mainly in the liver, the gill, the gut and least in the carcass (Kamunde *et al.*, 2001; Handy *et al.*, 2002). Copper toxicity is believed to be partly due to non-specific binding of the metal ion Cu^{2+} to biologically important molecules. It binds to histidine-, cysteine- and methionine residues in proteins with high affinity, which may result in their dysfunction (Camakaris *et al.*, 1999). The influence of copper speciation on the toxic effects observed was shown in several studies. Campbell *et al.* (1999) reported that uptake of copper into rainbow trout gill follows second-order kinetics, related to the reduction of Cu^{2+} to Cu^{+} prior to membrane transport. It was found that cupric ions are reduced to cuprous ions on the extracellular membrane surface, catalyzed by sulphhydryl groups (Bogdanova *et al.*, 1999). Cu^{2+} is responsible for intracellular oxidative effects, whereas effects on membrane transport and ion permeability are mainly elicited by Cu^{+} (Bogdanova *et al.*, 2002)

2.1.3 Mercury (Hg)

1) Physical and Chemical Properties

Mercury is mostly available inorganic form (Watras *et al.*, 1998). Inorganic mercury (Hg^{2+}) and herbicides are important contaminants of world water systems with effects on aquatic organisms and human (Cheney *et al.*, 2008). In addition, mercury is a metal which is liquid at normal temperatures and pressure. It is in the forms of salts with two ionic states, mercury (I) and mercury (II). Mercury also forms organometallic compound which are stable, though some are readily broken down by living organisms, while others are not readily biodegraded. Elemental mercury gives rise to a vapour which dissolves only slightly (WHO, 1989a).

2) Source in the environment

Natural mercury arises from the degassing of the earth's crust through volcanic gases and, probably, by evaporation from the oceans. Local levels in water derived from mercury ores may also be high (up to $80\mu\text{g/L}$). Atmospheric pollution from industrial production is probably low, but pollution of water by mine tailing is significant. The burning of fossil fuels is a source of mercury. The chloralkali industry and the wood pulping industry also released significant amounts of mercury. Although the use of mercury is reducing, high concentrations of the metal are still present in sediment associated with the industrial applications of mercury. Some mercury compounds have been used in agriculture, principally as fungicides (WHO, 1989a). UNEP (2002) has reported that at many locations around the world, mercury is found at levels that adversely affect humans and wildlife. In India, Chloroalkali plants are the major source for mercury release in the atmosphere and surface water. Besides, the coal-fired plants such as thermal power plants, steel industries and cement plants, may also contribute to a significant extent to mercury pollution (Nanda, 1993). More recently, several brands of soap and creams containing up to 1% organic mercury were distributed within markets in sub-Saharan Africa. Not only has this posed a human health risk (Glahder *et al.*, 1999), but improper disposal of these

products has led to elevated levels of inorganic mercury and methylmercury (MeHg^+) in wastewaters (Grandjean *et al.*, 1999). Mercury accumulates in soil, sediment, water, marine organisms and consequently in consumers of the food chain, leading to the disruption of the local ecology and varying levels of toxicity among many species (WHO, 1991). Globally, deterioration of the environment has increased the vulnerability of various populations to mercury from contaminants such as mercury in food supply. Metals appear in all Arctic ecosystems and can impact the local freshwater and marine food supply. With natural sources of metals, plants and animals, including humans, have time to adapt over many generations. As natural sources change with time, populations can continue to change because, on a relative time scale, these physical processes are slow enough to allow biochemical and genetic adaptation. With anthropogenic sources of metals such as erosion from mining activities, negative impacts can occur over a much shorter time scale and biological adaptation of subsistence species may not be able to keep up with these rapid environmental changes. Thus, a rapid increase in contaminants can threaten the physical health of both fish species and the human populations which depend on them for their subsistence (AMAP, 1997, 2002; Nriagu, 1988; Egeland and Middaugh, 1997; Egeland *et al.*, 1998; Nobmann *et al.*, 1992; WHO, 1990, 1991; Arnold and Middaugh, 2004; Suk *et al.*, 2004).

3) Uptake, elimination, and accumulation in organisms

Mercuric salts, and to a much greater extent, organic mercury, are readily taken up by organisms in water. Aquatic invertebrates, and most particularly aquatic insects, accumulate mercury to high concentrations. Fish also take up the metal and retain it in tissues, principally as methylmercury, although most of the environmental mercury to which they are exposed is inorganic. The source of the methylation is uncertain, but there is strong indication that bacterial action leads to methylation in aquatic systems. Environmental levels of methylmercury depend upon the balance between bacterial methylation and demethylation. The indications are that methylmercury in fish arises from this bacterial methylation of inorganic mercury, either in the environment or in bacteria associated with fish gills, surface, or gut.

There is little indication that fish themselves either methylate or demethylate mercury. Elimination of methylmercury is slow from fish (which half life in the order of months or years) and from other aquatic organisms. Loss of inorganic mercury is more rapid and so most of the mercury in fish is retained in the form of methylmercury (WHO, 1991). Moreover, bioaccumulation through the food chain increases the human risk of methylmercury (MeHg) chronic exposure mainly in those populations with high intake of fish or fish products (WHO, 1990; Sexton *et al.*, 1993; Goyer and Clarkson, 2001; Macdonald *et al.*, 2002; Hansen and Gilman, 2005). A major proportion of what is known about the disease processes that are associated with environmental contaminants, such as mercury, comes from epidemiologic research in the occupational health field. A recent report by Mahaffey *et al.* (2004) suggests that greater than 300,000 newborns may have been exposed in utero to MeHg concentrations higher than those considered to be without increased risk. In addition, Riisgard *et al.* (1985) transferred mussels (*Mytilus edulis*) from clean water to an area chronically polluted with aquatic organisms. The mussels accumulated aquatic organisms. Loss of inorganic mercury is more rapid and so most of the mercury readily during 3 months of exposure. They were then transferred to clean water in the laboratory and the elimination of the aquatic organisms. The biological half-life was 293 days, but was only 53 days in the case of mussels contaminated by a temporary massive mercury contamination. In both cases, 75% of the mercury in the mussels was inorganic, but both inorganic and organic species were immobilized in the mussels from the chronically polluted area.

4) Toxicity to aquatic organisms

The organic forms of mercury are generally more toxic to aquatic organisms than the inorganic forms. Aquatic plants are affected by mercury in the water at concentrations approaching 1 mg/L for inorganic mercury but at much lower concentrations of organic mercury. Aquatic invertebrates are highly susceptible to mercury. Generally, larval stages are more sensitive than adults. The 96 hour LC₅₀s vary between 33 and 400 µg/L for freshwater fish and are higher for sea-water fish. However, organic mercury compounds are more toxic. Toxicity is affected by

temperature, salinity, dissolved oxygen and water hardness. Verlecar *et al.* (2007) studied biochemical markers of oxidative stress in *Perna viridis* exposed to mercury and temperature. A wide variety of physiological and biochemical abnormalities has been reported after fish have been exposed to sublethal concentrations of mercury, although the environmental significance of these effects is difficult to assess. Reproduction is also affected adversely by mercury (WHO, 1991). Mercury is clear that symptoms overlap with other sulfhydryl-reactive metals such as cadmium, lead and arsenic. However, in Arctic wildlife and fish these metals do vary with respect to their site of deposition and action in the organism. Mercury can build up in biological systems in its organic form; MeHg crosses the blood brain barrier (Weil *et al.*, 2005). There have been many reviews of mercury and its toxicity for example Sorensen (1991), Boening (2000), Braune *et al.* (1999), Wolfe *et al.* (1998), Morel *et al.* (1998), Grigal (2002), Goyer and Clarkson (2001) and National Academy of Sciences (NAS, 2000).

2.1.4 Lead (Pb)

1) Physical and chemical properties

Lead is a bluish or silvery-grey soft metal. With the exception of the nitrate, the chlorate and to a much lesser degree, the chloride, the salts of lead are poorly soluble in water. Lead also form stable organic compounds. Tetraethyllead and tetramethyllead are used extensively as fuel additives. Both are volatile and poorly soluble in water. Trialkyllead compounds are formed in the environment by the breakdown of tetraalkylleads. These trialkyl compounds are less volatile and more readily soluble in water. Lead is mined, most usually as the sulfides, “galena” (WHO, 1989b). Moreover, lead is a trace element occurring naturally in rock and soils. Due to a low melting point, the metal has been smelted and worked since historic ages, resulting in widespread environmental lead pollution (Davies, 1990; Nriagu, 1978). Although legislative measures have been taken to decrease lead emissions to the environment, many places in the world are still polluted with lead due to the element’s

long residence time and its tendency to accumulate in soils and sediment (Davies, 1990; Pain, 1995)

2) Source in the environment

Pollution of the environment occurs through the smelting and refining of lead, the burning of petroleum fuels containing lead additives and, to a lesser extent, the smelting of the other metals and the burning of coal and oil. Metallic lead deriving from shotgun cartridges or used as fishing weights is lost in the environment and often remains available to organisms (WHO, 1989b). In addition, lead has no known essential role in living organisms and is toxic at even low concentrations. Because lead persists in the environment, concentrations of this metal may progressively increase in water, sediments or in biological tissues to levels above natural background. Elevated metal concentrations in aquatic systems may pose health risks to marine life (e.g. primary producers, fish and filter feeders) and consumers of these organisms, including humans (Bryan and Langston, 1992). In 1990, 5.627×10^6 tonnes of lead were consumed worldwide (WHO, 1995). Furthermore, atmospheric lead concentrations have sharply decreased since the ban on leaded fuel and concentrations are well below air quality standards (recent atmospheric lead concentrations are about 15 ng/m^3) (Buijisman, 2004). Thus, lead in the aquatic environment has both natural and anthropogenic origins. Anthropogenic lead mostly from mining activities, industrial uses, coal burning and leaded gasoline because of the increasing number of automobiles on the road (Nriagu and Pacyna, 1988; Nriagu, 1998). Atmospheric fallout is the main anthropogenic input of lead to the aquatic ecosystem (Nriagu and Pacyna, 1988) and leaded gasoline may be the main emission source until its phasing out in many countries (Soto- Jiménez *et al.*, 2006).

3) Uptake, loss, and accumulation in organisms

Lead in the environment is strongly adsorbed onto sediment and soil particles reducing its availability to organisms. Because of the low solubility of most of its salts, lead tends to precipitate out of complex solutions. The uptake and accumulation

of lead by aquatic organisms from water and sediment are influenced by various environmental factors such as temperature, salinity, and pH, as well as humic and alginic acid content. In contaminated aquatic systems, almost all of the lead is tightly bound to sediment. Only a minor fraction is dissolved in the water, even interstitial water between the sediment particles (WHO, 1989b). In an earlier study, metal concentrations were investigated in a soil-plant (*Urtica dioica*) snail (*Cepaea nemoralis*) food chain in the Biesbosch and two reference locations outside this area (Notten *et al.*, 2005). This food chain was selected because stinging nettles (*U. dioica*; Urticaceae) and *C. nemoralis* Linnaeus (Pulmonata) are very common in the Biesbosch and *C. nemoralis* includes nettle leaves in its diets (Grime *et al.*, 1970; Wolda *et al.*, 1971). Besides, terrestrial snails are well known for their metal accumulating capacities (Dallinger *et al.*, 2001). Elevated lead concentrations were found in all compartments of the food chain in the Biesbosch, as well as positive relationships of lead concentrations between the snail-leaf and snail-soil compartments (Notten *et al.*, 2005). The results showed that lead transfers to snails via nettle leaves as well as directly from the soil.

4) Toxicity to aquatic organisms

Lead is unlikely to affect aquatic plants at levels that might be found in the general environment. In the form of simple salts, lead is acutely toxic to aquatic invertebrates at concentrations above 0.1 and more than 40 mg/L for freshwater organisms and above 2.5 and more than 500 mg/L for marine organisms. For the same species, the 96 hour LC₅₀s for fish vary between 1 and 27 mg/L in soft water, and between 440 and 540 mg/L in hard water. The higher values for hard water represent nominal concentrations. Available lead measurements suggest that little of the total lead is in solution in hard water. Lead salts are poorly soluble in water, and the presence of other salts reduces the availability of lead to organisms because of precipitation. Results of toxicity tests should be treated with caution unless dissolved lead is measured (WHO, 1989b). In addition, lead is known to exert toxic on various target organs mainly on central nerve, digestive system, hematopoietic, renal and immune system. Immune system is an important target. Many studies have addressed

the toxic effects of lead in immune system. In occupationally exposed human workers, lead-associated changes have been reported for T lymphocyte subpopulations (Sata *et al.*, 1998), the response to T lymphocyte mitogens (Mishra *et al.*, 2003), serum levels of immunoglobulins (Ayatollahi, 2002) and complement (Basaran and Undeger, 2000). Some report demonstrated that lead-induced immune function changes. These include elevated susceptibility to viral infection (Gupta *et al.*, 2003), bacterial challenge (Fernandez-Cabezudo *et al.*, 2007) and autoimmune disease (Hudson *et al.*, 2003). Early studies reported growth of algal mats downstream of mining areas (Gale *et al.*, 1973; Wixson, 1977) and several subsequent studies documented changes in benthic macroinvertebrate communities of streams downstream of active and inactive mines during the 1960s and 1970s (Duchrow, 1983; Ryck, 1974; Ryck and Whitley, 1974) and in recent years (Humphrey and Lister, 2004; Lister and Humphrey, 2005). Other recent studies have documented increased concentrations of lead, cadmium and zinc in plant biomass, aquatic invertebrates and fish in streams downstream of mining areas (Besser *et al.*, 2007; Schnitt *et al.*, 2007a). Fish from habitats affected by mining activities also exhibit biochemical effect of metal exposure (Schnitt *et al.*, 1984, 2007b; Dwyer *et al.*, 1988).

In communities of aquatic invertebrates, some populations are more sensitive than others and community structure may be adversely affected by lead contamination. However, populations of invertebrates from polluted areas can show more tolerance to lead than those from non-polluted areas. In other aquatic invertebrates, adaptation to hypoxic conditions can be hindered by high lead concentrations (WHO, 1989b).

2.1.5 Zinc (Zn)

1) Physical and chemical properties

Zinc metal does not occur in the natural environment. It is present only in the divalent state Zn (II). Ionic zinc is subject to salivation, and its solubility is pH and anion dependent. Zinc is a transition element and is able to form complexes with a variety of organic ligands. Organometallic zinc compounds do not exist in the environment (WHO, 2001). Moreover, zinc is known to be an essential micronutrient both in plants and animals. Zinc is involved in various metabolic pathways. It serves as a specific cofactor of several enzymes. In addition, zinc is an integral part of about 20 metalloenzymes, such as alkaline phosphatase, alcohol dehydrogenase and carbonic anhydrase. Zinc is associated with prostaglandin metabolism and also may have a structural role in nucleoproteins (Watanabe *et al.*, 1997).

2) Source in the environment

The largest natural emission of zinc to water results from erosion. Natural inputs to air are mainly due to igneous emissions and forest fires. Anthropogenic and natural sources are of a similar magnitude. The main anthropogenic sources of zinc are mining, zinc production facilities, iron and steel production, corrosion of galvanized structures, coal and fuel combustion, waste disposal and incineration, and the use of zinc-containing fertilizers and pesticides (WHO, 2001).

3) Environmental concentrations

Zinc occurs ubiquitously in environmental and biological samples. Concentrations in soil sediments and fresh water are strongly determined by local geological and anthropogenic influences and thus vary substantially. Natural background total zinc concentrations are usually more than 0.1-50 µg/L in freshwater, 0.0002-0.1 µg/L in seawater, 10-300 mg/kg dry weight (dw) in soil, up to 100 mg/kg

dry weight in sediments, and up to 300 ng/m³ in air. Increased levels can be attributed to natural occurrence of zinc enriched ores, to anthropogenic sources or to abiotic and biotic processes. In anthropogenically contaminated samples, zinc levels of up to 4 mg/L in water, 35 g/kg in soil, 15 µg/L in estuarine water, and 8 µg/m³ in air are found (WHO, 2001). In addition, zinc is found in oysters, and to a far lesser degree in most animal proteins, beans, nuts, almonds, whole grains, pumpkin seeds, sunflower seeds and blackcurrant (USDA, 2007). Soil conservation analyzes the vegetative uptake of naturally occurring zinc in many soil types. The US recommended dietary allowance of zinc from puberty on is 11 milligrams for males and 8 milligrams for females, with higher amounts recommended during pregnancy and lactation. Other sources include fortified food and dietary supplements, which come in various forms. A review by Allen (1998) concluded that zinc oxide, one of the most common supplements in the United States, and zinc carbonate are nearly insoluble and poorly absorbed, and cited studies which found low plasma zinc concentrations after zinc oxide and zinc carbonate consumption relative to the plasma concentrations seen after consumption of zinc acetate and sulfate salts.

4) Toxicity to aquatic organisms

Zinc is an essential element and *in vivo* levels are therefore regulated by most organisms. Zinc is not biomagnified. The absorption of zinc by aquatic animals tends to be from water rather than food. Only dissolved zinc tends to be bioavailable, and bioavailability depends on the physical and chemical characteristics of the environment and biological processes. Consequently, environmental assessment must be conducted on a site-specific basis. Zinc concentrations in representative organisms during exposure to water-borne zinc are in the range 200-2000 mg/kg (WHO, 2001). Recent research on zinc-gene interactions has assigned a basic role for this element in controlling growth (Chesters, 1991). Normal zinc levels in freshwater (Spry *et al.*, 1988) and seawater (Willis and Sunda, 1984) are known to be insufficient to meet the requirement of growing aquatic species. Hence, zinc is regarded as an essential nutrient in fish feeds (Lall, 1989; NRC, 1993; Wei *et al.*, 1999) and shrimp feed (Li *et al.*, 1995). The zinc requirement of rainbow trout was found to be between 15

and 30 mg/kg diet (Ogino and Yang, 1978). Gatlin *et al.* (1991) reported that dietary zinc requirement of the red drum was between 20 and 25 mg/kg diet. Whereas, the requirement of Atlantic salmon for zinc is more than and equal 67 mg/kg diet (Mage and Julshamn, 1993). All these requirements were determined with zinc sulfate ($\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$) as supplemental zinc source.

High dietary levels of zinc may, however, negatively affect the status of other elements, such as iron (Wekell *et al.*, 1986). Unnecessarily high additions of zinc and other micronutrients also increase the price of feeds, as well as increase the input of minerals to the aquatic environment (Mage and Julshamn, 1993). There is no information on the requirement of dietary zinc by any mollusks. Also, no information is available on the bioavailabilities of organic and inorganic sources of dietary zinc to these mollusk species. Declining abalone fisheries worldwide (Clavier, 1992; Farlinger and Campbell, 1992; Guzmán del Proò, 1992; Johnson *et al.*, 1992; Nie, 1992; Parker *et al.*, 1992; Prince and Shepherd, 1992; Schiel, 1992; Tarr, 1992; Tegner, *et al.*, 1992) have accelerated the development of abalone aquaculture.

2.2 Inductively-Coupled Plasma Spectroscopy (ICP) Technique

ICP optical emission spectrometry (ICP-OES) is one of the most important techniques of instrumental elemental analysis. It can be used for the determination of approximately 70 elements in a variety of matrices (Joachim, 2003). The heart of an ICP emission spectrometer is the plasma, an extremely hot “gas” with a temperature of several thousand Kelvin ($726.85 \text{ }^\circ\text{C}$). It is so hot that atoms and ions are formed from the sample to be analyzed. The very high temperature in the plasma destroys the sample completely, so the analytical result is usually not influenced by the nature of the chemical bond of the element to be determined (absence of chemical interference). In the plasma, atoms and ions are excited to emit electromagnetic radiation. The emitted radiation is spectrally resolved with the aid of diffractive optics, and the emitted intensity is measured with a detector. In ICP-OES, the wavelengths are used for the identification of the elements, while the intensities serve for the determination of their concentrations. Moreover, since all elements are excited in the plasma

simultaneously, they can be determined simultaneously or very rapidly one after another. Consequently, the analytical results for a sample can be obtained after a short analysis time. The time needed for a determination depends on the instrument used and is of the order of a few minutes. The fact that all the elemental concentrations are determined in one analytical sequence and not by measuring one series of samples for one element, another series for another element and so on usually makes the technique attractive with respect to speed (Joachim, 2003). Furthermore, samples analyzed are normally liquids, occasionally solids and (quite rarely) gases. For the determination of the element, no specific equipment (such as the lamp used in atomic absorption spectrometry) is needed. As a rule, one only needs a calibration solution of the element to be analyzed and a little time for method development. Hence, an existing analytical method can easily be extended to include another element. This makes ICP emission spectrometry very flexible (Joachim, 2003).

Some element analysis applications require concentration uncertainties that are better than (less than) 0.1% relative. While short term precision in ICP-OES is often 1% or better, concentration uncertainties are often 10% or larger, even in the absence of chemical matrix effects for analyses done by experienced analysts (Mermet, 1994). Salit *et al.* (1998, 2000) described a procedure to obtain concentration uncertainties of 0.2% relative or better using ICP-OES. The procedure relies on three concepts. High precision spectrophotometry requires sufficiently long measurement times and high enough sensitivity so that counting statistics do not limit concentration uncertainties. Internal standardization using simultaneously measured line pairs that have signals which are highly correlated as a function of time is essential to account for short term drift. The deleterious effects or signal drift that would naturally result from the use of long measurement times are removed by fitting a single function to deviations for all measurements of samples and standards from the mean signal for each (Salit *et al.*, 1998). Furthermore, great care must be taken to make and handle standards and sample solution to obtain sufficient precision and accuracy. This requires gravimetric rather than volumetric based methods, carefully planned details of experimental procedures and extreme care to avoid errors due to evaporation.

The procedure described by Salit *et al.* (2001) and termed “High Performance ICP-OES ” (HP- ICP-OES) has been applied to compare single element solutions and to obtain NIST trace ability of standards (Rettberg *et al.*, 2005) with typical instrument measurement error less than 0.05% relative. The dominant source of uncertainty in this case (0.5% relative) was the concentration of the NIST Standard Reference Material solutions. When using HP- ICP-OES, precision and accuracy similar to that previously obtained only from gravity, titrimetry or isotope dilution mass spectrometry can be obtained. In addition, Rabb and Olesik (2008) reported that the assessment of high precision, high accuracy inductively coupled plasma-optical emission spectroscopy to obtain concentration uncertainties less than 0.2% with variable matrix concentrations. There have been many use ICP technique for study trace heavy metals in organisms recent years, including Wang *et al.*, 2005; Türkmen and Ciminli, 2007; Soto-Jiménez *et al.*, 2008; Kamaruzzaman *et al.*, 2008; Uysal *et al.*, 2008.

2.3 Phylum Mollusca

2.3.1 Definition and general description

The members of Phylum Mollusca are generally called “mollusk”. Mollusks are unsegmented coelomate animals that have an anterior head, a ventral foot, and a dorsal visceral mass covered by a mantle. The mantle secretes a calcareous shell and encloses a mantle cavity containing paired ctenidia or gills. The anus, excretory pores and genital pores open into the mantle cavity. Internally, the coelom is reduced and the body cavity is an expanded blood space or haemocoel (Environment Australia, 1998a). In addition, mollusks are a very diverse group of animals. Although some groups are so modified that they no longer have a dorsal shell or a mantle cavity. For example, nudibranch sea slugs, all mollusks retain the basic head-foot-visceral mass morphology diagnostic of the phylum. The head carries out sensory and feeding functions and contains, except in bivalve mollusks, a characteristic buccal mass incorporating a toothed, ribbon-like radula. The foot is basically a muscular structure which functions as a locomotory organ. The visceral mass comprises the major

internal organ systems-dorsal heart, gonads, kidneys, digestive system. The cavities surrounding the gonads and kidneys, and the pericardial cavity surrounding the heart, are coelomic cavities. The general internal body cavity surrounding the heart, are coelomic cavities. The general internal body cavity is a haemocoel. The circulation of blood through the haemocoelic system is maintained by the pumping action of the heart and by the general muscular contractions of the soft body wall (Environment Australia, 1998a). Furthermore, more than half of all molluscan species are gastropods. Not only are gastropods speciose and numerous, they rank also among the most diverse of all animal groups and second only to the Insecta in terms of diversity as a class. Gastropods represent an extremely plastic group of organisms. They range from primitive marine species to highly evolved terrestrial air-breathing snail and slugs, and are found in almost all terrestrial, freshwater and marine habitats (Environment Australia, 1998b).

The chemical concentration in the environment may not be enough to kill the organism. However, sub-lethal concentrations often affect the biochemistry of an organism. It is well known that, trace contaminants in aquatic ecosystem pose environmental hazard because of their great toxicity or persistence (Meador *et al.*, 1995). In recent years, the emphasis on toxicity testing in marine organisms has been moving towards sub-lethal tests, as they can provide much more relevant information in assessing the long-term effects of pollutions imposed on the ecosystem. Various sub-lethal tests and indices from cellular to physiological levels within an organism have been developed (Bayne *et al.*, 1985). Physiological parameters are considered to identify integrated responses to diverse stressors imposed by trace metals, other pollutions and natural environment factor such as temperature, salinity and pH (Depledge *et al.*, 1995). According to Hebel *et al.* (1997), many physiological variables must be measured to obtain the impact of toxicants in the whole organism which determine the survival potential of individuals. Two methods that have received much attention are the scope for growth and the oxygen and nitrogen ratio. There have been many studies on trace metals concentrations in mollusks. Among sea organisms, filter-feeding sea mussels such as *Mytilus galloprovincialis*, a widely distributed mollusk in the Mediterranean Sea, have been used as bio-indicators for the

presence of heavy metals in sea water, since they are able to accumulate and concentrate them in a detoxified form, providing a direct correlation with the ecosystem's degree of contamination (Popham and D'Auria, 1981; Cossa, 1989; Regoli and Orlando, 1993). In addition, mussels are widely used as bioindicators of heavy metal pollution in coastal areas because they can accumulate various elements as filter-feeders (Regoli, 1998). They also show the other parameters for being bioindicators such as suitable dimensions, easy identification and collection of organisms, abundance in an ecosystem and accumulation of the elements to a degree suitable to measure (Wagner and Boman, 2004). Moreover, mussels are well known to accumulate a wide range of contaminants in their soft tissue (Goldberg *et al.*, 1978). For example, the Pacific oyster, *Crassostrea gigas*, is the most economically valuable mollusk species in the world (FAO, 2004). This species is often reared in coastal ecosystems where pollutants degrade environmental quality. Thus, they are often employed to monitor metal pollution in sea. Size has sometimes been shown to be an important variable, but contradictory results have been found. In order to monitor metal content in the mussels, the number of size classes according to shell length as well as the number of samples must be taken into account to obtain a representative sampling (Saavedra *et al.*, 2004). Additionally, the total cost of collection, preparation and analysis of each sample have to be considered in the analysis.

The open circulatory systems of mollusks are continually exposed to fluctuations of environmental factors including contaminants (Cheng, 1981). In bivalves, the cellular defense system consists of haemocytes which have various modes of action including phagocytosis, intracellular degradation of pathogens with hydrolytic enzymes, production of reactive oxygen metabolites and phenoloxidase activity (Cheng and Rodrick, 1975; Cheng, 1988; Pipe, 1992).

2.3.2 Classification of Mollusca

Mollusks can be identified into 2 classes; class Gastropoda and class Bivalvia

1) Class Gastropoda

The class Gastropoda is the largest class of mollusks. About 35,000 existing species have been described and to this total should be added some 15,000 fossil forms. The class has had an unbroken fossil record beginning with the early Cambrian period and has undergone the most extensive adaptive radiation of all the major molluscan groups. Considering the wide variety of habitats the gastropods have invaded, they are certainly the most successful of the molluscan classes. Marine species have become adapted to life on all types of bottoms as well as to a pelagic existence. They have invaded fresh water and the pulmonate snails and several other groups have conquered land by eliminating the gills and converting the mantle cavity into a lung (Barnes, 1986).

2) Class Bivalvia

The class Bivalvia, also called Pelecypoda or Lamellibranchia, comprises mollusks known as bivalve and includes such common forms as clams, oysters and mussels. Bivalves are laterally compressed and possess a shell with two valves, hinged dorsally, that completely enclose the body. The foot, like the remainder of the body, is also laterally compressed, hence the origin of the name *Pelecypoda*- hatchet foot. The head is very poorly developed. The mantle cavity is the most capacious of any class of mollusks and the gills are usually very large, having assumed in most species a food collecting function in addition to that of gas exchange. Most of these characteristics represent modifications that enabled bivalves to become soft bottom burrowers, for which the lateral compression of the body is better suited. Although modern bivalves have invaded other habitats, the original adaptations for burrowing in mud and sand have taken bivalves so far down the road of specialization that they have become largely chained to a sedentary existence (Barnes, 1986).

2.3.3 Freshwater Mollusks

1) Definition of Freshwater Mollusks

Within the large group of animals known as mollusks, three subgroups, snail, mussels and clams, have representatives that live in freshwater. Because all freshwater mollusks have hard shells that are often washed up on shores, they are some of the most conspicuous of the freshwater invertebrates. Moreover, freshwater mollusks are an integral part of the complex web of life that supports biodiversity. Like other invertebrates, they play an important role in nutrient cycling, functioning as decomposers and as critical links in the food chain (Jacqueline, 2000).

2) Habitat of Freshwater Mollusks

Freshwater mollusks live in all types of wet habitats from large lakes and rivers to roadside ditches and backyard ponds. They are most commonly found along the shallow edges of water bodies where warmer temperatures and additional light provide them with more food than is available in deeper water. Some species of freshwater mollusks can be found in temporary wet areas, where they survive by burrowing into the substrate to wait out dry periods. Burrowing is also a strategy used by some mollusks to survive winter freezing. Others are able to avoid the ice by retreating to unfrozen areas. All freshwater mollusks evolved from marine ancestors. The shift between radically different environments required the development of specialized adaptations. For example, the movement of freshwater is very different than that of oceans. In the ocean, tides result in complex water flow patterns, whereas inland waters are always ultimately flowing in one direction-toward the ocean. In order to live in fresh water, mollusks have to evolve reproductive adaptations that would ensure their eggs and sperm could meet and that larvae and juveniles could disperse without being drawn into that one way flow to the sea (Jacqueline, 2000).

Freshwater ecosystems contain approximately 12% of Earth's animal diversity and most other species depend on them indirectly for their survival

(Abramovitz, 1996). Despite their importance, freshwater ecosystems are rapidly being modified or destroyed (Allan and Flecker, 1993; Green *et al.*, 2002) and many species have consequently become endangered (Master *et al.*, 1998; Duncan and Lockwood, 2001; Perez-Losada *et al.*, 2002; Abellan *et al.*, 2005). The global freshwater biodiversity crisis is most striking in tropical Asia (Dudgeon, 2003), which has the highest relative rates of deforestation in the world (Laurance, 1999; Achard *et al.*, 2002). At the current rates, the region's terrestrial and freshwater species will likely suffer catastrophic levels of extinction (Brook *et al.*, 2003; Sodhi *et al.*, 2004; Sodhi and Brook, 2006). Therefore, it is imperative that remaining freshwater ecosystems are assessed for conservation priorities. This process, however, is beleaguered by the paucity of sound biological information on tropical Asian freshwater habitats (e.g., less than 0.1% of 4579 freshwater biology paper involves conservation within the region: Dudgeon, 2003).

Most toxic substances generated by industrial, agricultural and domestic activities are released into natural freshwater courses (Nriagu, 1992; Timbell, 2000). In natural aquatic environments, invertebrates constitute more than 90% of the present species (Jha, 1998). It is important to have reliable test methods using invertebrate organisms at successive stages of their life-cycle for ecological assessment of chemicals (Gourmelon and Ahtiainen, 2007). Disturbances in their metabolism, development and reproduction are of concern for the preservation of biodiversity of ecosystems. In fact, the reproduction of aquatic organisms is one of the end-points currently employed to evaluate the effect of toxicants (Gomot, 1998; Cheung and Lam, 1998; Coeurdassier *et al.*, 2003, 2004, 2005; Cervera *et al.*, 2004). In addition, the use of mollusks as test organisms is becoming general since they are able to accumulate contaminants and facilitate their detection, even when minute amounts of trace contaminants are present (Moriarty, 1990; Cooney, 1995). Freshwater mollusks of the genus *Biomphalaria* can be considered good indicators for biomonitoring studies (Münzinger, 1987; Abd-Allah *et al.*, 1999; Nakano *et al.*, 2003; Ansaldo *et al.*, 2006).

3) Nutrients Availability to Mollusks

The contents of the stomach are rotated by the style sac cilia. The rotation winds up the mucous food strings, drawing them along the esophagus and into the stomach. The rotating mucous mass is called a protostyle. The size and consistency of particle within the string vary greatly and the chitinous lining of the anterior part of the stomach protects the wall from damage by sharp surfaces. The acidity of the stomach fluid (pH of 5 to 6 in living mollusks) decreases the viscosity of the mucus and aids in freeing the contained particles. Such particles are eventually swept against the sorting region, in which they are graded by size. Lighter and finer particles are driven by the cilia of the ridges to the duct opening of the digestive diverticula. Heavier and larger particles are carried in the grooves between the ridges to a large groove running along the floor of the stomach to the intestine (Barnes, 1986).

Particles utilized as food pass into the ducts of the digestive glands and digestion occurs intracellularly within the cells of the distal tubules. Although intracellular digestion appears to be primitive in mollusks, at least some extracellular digestion occurs within the stomach cavity of most living species.

The long, coiled intestine functions largely in the formation of fecal pellets. The anus opens middorsally at the posterior margin of the mantle cavity and wastes are swept away by the exhalant current (Barnes, 1986).