CHAPTER 1

INTRODUCTION

Environmental pollution with toxic heavy metals has become a global problem phenomenon. Because of the growing apprehension about the potential effects of the heavy metal contaminants on various fauna and flora, the research on fundamental, applied and health aspects of trace heavy metals in the environment has paramounted to the point that it has become difficult to keep track of recent developments in particular topics (Lorris and Barbara, 1994).

Heavy metals are conservative pollutants in that they are not broken down, so that they effectively become permanent additions to the aquatic environment. They accumulate in organisms and some may become biomagnified in food chains, though biomagnifications is the exception rather than the rule. The major uptake route for many aquatic organisms is by direct uptake from the water so that tissue concentrations reflect metals concentration into water. Carnivores at the top of food chain, however, such as many aquatic bird and mammal species, including humans, obtain most of their pollutant burden from aquatic ecosystems by ingestion, especially from fish, so there exists the potential for considerable biomagnifications (Vernet, 1991). Much attention has been paid to heavy metals contamination in the water environment and their potential hazards to organisms and human beings. Sediments are the most often used matrix to monitor the heavy metals pollution in water. However, the determination of total heavy metal contents in sediments have not been satisfied up to now because the behavior of heavy metals are closely related to their chemical forms, and only those with high bioavailability could be absorbed by organisms and did great harm to organisms and human beings. Therefore, the investigation of heavy metal contamination in organisms could be more directly reflect the hazards to human health and the potential heavy metal pollution in water. Lying in the second trophic level in the water ecosystem, mollusks have long been known to accumulate both essential and non-essential trace elements in aquatic ecosystems (Dallinger and Rainbow, 1993).

The ability of aquatic organisms to accumulate pollutants in their tissues to elevate levels reaching concentrations that are much higher than that of ambient water concentrations makes these biota useful for assessment purposes. The use of organisms to monitor pollutants relies on the fact that a contaminant, if present in the water column, may not be recorded in the sediment, but may accumulate in biota, which become recorders of the contaminants. The Mussel Watch project in the USA has been developed using mussels and oysters to monitor spatial and temporal trends of contaminant concentrations in coastal and estuarine regions (O' Conner, 1996). Aquatic organisms are also time integrating, since they can indicate the presence of contaminants that are no longer in the water, or those whose presence or use is intermittent (Eisler, 1980).

The term "biomonitor" describes species that accumulate trace metals or other substances in their tissues and therefore can be used to monitor the bioavailability of these substances in a particular environment. Biomonitors exclusively provide time-integrated measures of the available levels of substances (Camusso *et al*., 1994; Rainbow *et al*., 2000), a feature that makes them superior compared to water or sediment samples. Moreover, concentrations in water or sediment samples can vary widely due to small temporal variations (Cunningham, 1979). Biomonitors generally accumulate trace metals to concentrations that are relatively easy to measure (Rainbow and Phillips, 1993), since they concentrate metals continuously, often several orders of magnitude above ambient water concentrations. Essential characteristics required of biomonitors include the capacity to accumulate pollutants without being killed by the encountered levels, and sedentariness in order to be representative of the area. Furthermore, biomonitors should be abundant in the area of study, sufficiently long-lived and be of a reasonable size to provide enough tissue for analysis. They should be easy to sample and possess high concentration factors for the elements investigated. The best-studied indicator types include macroalgae and bivalve mollusks, which are commonly used as biomonitors of trace metals and have widely been used as monitoring organisms for aquatic environments (Phillips, 1977). Generally, freshwater mollusks are widely distributed in nearly all types of surface-water systems and they are frequently very abundant (Elder and Collins, 1991).

Electron microscope (EM) can be divided into scanning electron microscope (SEM) and transmission electron microscope (TEM) which are used to study impact of heavy metals to cells of mollusk tissues. Danilatos and Robinson (1979) studied principles of scanning electron microscope (SEM) at high specimen chamber pressures. Pipe (1986) used an azo dye technique to investigate localization of the acid hydrolase, β-glucuronidase, at light and electron microscope level in the stomach and digestive gland of the marine periwinkle *Littorina littorea*. In addition, Desouky (2006) studied tissue distribution and subcellular localization of trace metals in the pond sail *Lymnaea stagnalis* with special reference to the role of lysosomal granules in metal sequestration. The microscopical examinations in this study revealed that metal detoxification from the digestive gland cells may occur via faeces or via basal exocytosis towards haemocytes dispersed by the connective tissue in the visceral mass. In the kidney, one type of granules, the excretory concretion, was identified in the nephrocytes. The significant increase in the number of these concretions in the snail *L. stagnalis* upon exposure to metals may give further evidence for their role in metal excretion. Moreover, Nudelman *et al*. (2008) studied forming nacreous layer of the shells of the bivalves *Atrina rigida* and *Pinctada margaritifera* that they use environmental and cryo- scanning electron microscopy to investigate the organic matrix structure at the onset of mineralization in the nacre of two mollusk species. These two techniques allow the visualization of hydrated biological materials coupled with the preservation of the organic matrix close to physiological conditions.

Much of the advances in toxicology over the last two decades have been focused on the identification and characterization of specific proteins expressed in response to toxic heavy metals and xenobiotic (Viarengo, 1989; Lauwerys *et al*., 1995; Soni and Mehendale, 1998). Various responses of enzyme have been observed in animals exposed to metallic contaminants in both field and laboratory experiments which indicated an increase or a decrease in the activity depending on the dose, species and route of exposure (Wong and Wong, 2000; Jiraungkoorskul *et al*., 2003; Sanchez *et al*., 2005). It had been documented through electrophoretic studies that allozyme polymorphisms and heterogeneities of animal and plant populations and species are affected by environmental stress (Nevo *et al*., 1986; Ben-Shlomo and Nevo, 1988; Gillespie and Guttman, 1999). Recently, Yab *et al*. (2004) found a positive relationship between metal levels and allozyme polymorphisms in the soft tissues of the green-lipped mussel *Perna viridis* collected from contaminated and uncontaminated sites. In addition, Yab and Tan (2007) studied changes of allozymes of *P. viridis* subjected to zinc stress, and found an indication of changes in the enzymes glutamate oxaloacetate (GOT), esterase (EST) and malic enzyme (ME), which are complemented by reductions of filtration rate and condition index. However, it is not yet known for sure whether the enzymes GOT, EST and ME are inducing behavioral and other changes in *P. viridis*. This is because the possible subtle interactions could occur between different environmental stresses.

Acclimation might be revealed directly by the genome (Stürzenbaum *et al*., 1998) or by gene expression of allozymes (Frati *et al*., 1992; Aziz *et al*., 1999; Gillespie and Guttman, 1999; Kammenga *et al*., 2000). It is demonstrated that earthworms, *Lumbricus rubellus*, were harboring DNA fragments, which were expressed as mRNA in worms from a heavy metal contaminated site but not transcribed in worms from a clean site (Stürzenbaum *et al*., 1998). Some allozymes are responding to environmental factors (Gillespie and Guttman, 1999). The response of allozymes seemed not to be universal but species dependent (Tranvik *et al*., 1994) and no sample mechanistic relationship was revealed. However, esterases are known to respond to organophosphates (Raymond *et al*., 1991). Simonsen (1996) observed *in vitro* effect of Cd, Cu and Zn on esterase from *Eisenia fetida*. No difference in response of various alleles was observed in that study. Labrot *et al*. (1996) observed reduced acethylcholinesterase activity following exposure to uranium (U) and lead (Pb) in *E. andrei*. On the other hand, Scaps *et al*. (1997) did not observe any reduction of this enzyme when *E. fetida* was exposed to Pb and Cd. These two heavy metals did only affect phosphoglucose isomerase among several enzymes tested and the impact was the disappearance of one fraction of the zymogram (Scaps *et al*., 1997). Zinc was shown to inhibit phosphomonoesterase and phosphodiesterase from L. *terrestris* (Park *et al*., 1992). However, the impact of heavy metals on esterases in mollusks has only been studied to a limited extent.

As discussed previously, mollusks are important biomonitors and could be used as an indicator of aquatic environment. Thus, this study will focus on mollusks in contaminated area with heavy metals. Bueng Jode Wetland, a reservoir receiving effluent from factories before flowing down to a main river (Pong River) in northeastern Thailand, is chosen as our contaminated study site. The study is conducted to firstly establish diversity of mollusks in the area down to species level while water quality is extensively determined. Accumulation of some heavy metals both in selected edible mollusks and on sediment is studied using Inductively-Coupled Plasma (ICP) spectroscopy. Impacts of heavy metals on cells and tissues in the mollusks are examined using electron microscopes. To further understand the impacts, an experiment is designed to study influence of heavy metals which are directly treated to mollusks from unpolluted area and verify the effect that heavy metals have on activity of esterases enzyme.

1.1 Research Objectives

The objectives of this research are as follows:

1.1.1 To study the biodiversity and distribution of mollusks in the Bueng Jode wetland.

1.1.2 To study physical and chemical properties of the water in Bueng Jode wetland.

1.1.3 To study the accumulation of some heavy metals on sediment, cell and tissue samples of selected edible mollusks in the Bueng Jode wetland.

1.1.4 To study the toxicity and cytotoxicity of selected heavy metals on selected edible mollusk in the Bueng Jode wetland.