CHAPTER 1

INTRODUCTION

The availability of adequate and safe water is of serious concern in many parts of the world. A recognition of this has led to an emphasis on the need to provide appropriate safe water facilities in developing countries. Thailand is also encountering problems from many adverse impacts on the quality of their natural water sources. This has been caused by an increasing economic growth rate since the late 1980s as Thailand has attempted to develop the country industrially (Eamsakulrat *et al.*, 1994). This has produced a huge demand for water supplies for industrial processes. For decades now, the demand for water in Thailand has exceeded the reliable supply of surface water and renewable groundwater. In addition, there are other environmental issues including pollution of the aquatic environment by toxic compounds from various sources. These aggravated pollution problems can cause public health hazards (Cheevaporn and Menasvete, 2003; Fatoki and Awofolu, 2003; Thongra-ar and Parkpian, 2002).

Arsenic contamination of natural waters is a worldwide problem. It has become a challenge for many scienctists across countries such as Argentina, China, Thailand and Hungary (Kamal and Parkpian, 2002; WHO, 1981). It is a result of mining and anthropogenic activities. Mining activities are considered to be a major cause of arsenic contamination of surface waters and groundwater (Williams *et al.*, 1996). Tin was once one of the leading minerals and in the 1980's tin mining activities were conducted in the southern peninsula of Thailand (LePoer, 1987). Significant deposits of tin were located in Ranong, Phuket, Pangnga, etc. (Karnasuta, 1985). The Ron Phibun district of Nakhon Si Thammarat province, Thailand has had tin mining and mineral processing for centuries. During the processing of tin ores, arsenic can be released into natural waters. For those who consume these waters, they can be threatened with serious illnesses (Rattanachongkiat *et al.*, 2004; Suwanmanee, 1991). However, the toxicity of arsenic is particularly difficult to measure because the effects of long-term exposure causes damage to human health that may not show up for years (Varathorn, 1997; WHO, 1981).

Arsenic is one of the metalloid elements that can exhibits extreme toxicity even in trace amounts. It is found naturally in rocks and soil, surface water, groundwater, aquatic animals, agricultural products, and especially in phytoplankton (Boonchalermkit *et al.*, 1996; Chaffin, 2003;

Katsoyiannis and Zouboulis, 2004; Pinto *et al*., 2003; Thirunavukkarasu *et al*., 2003). Phytoplankton absorb and accumulate arsenic in their cells resulting in bioaccumulation in the food web (Chen *et al*., 2000). There are numerous laboratory studies dealing with the effects of arsenic on phytoplankton (Fujiwara *et al*., 2000; Howard *et al*., 1995; Riedel, 1993; Sanders and Windom, 1980; Sanders and Riedel, 1993; WHO, 1981). However, changes in phytoplankton communities resulting from arsenic contamination are not particularly understood because of lack of prior information on the initial structure of these communities. The amount of arsenic that can be tolerated varies with different phytoplankton. The degree of tolerance of these algal species can make them potential indicator organisms (Perez and Sumugat, 2001). With this information it may be possible to assess the changes of water quality using the phytoplankton flora as indicative parameters of arsenic contaminated waters.

There have been very few studies on the flora and taxonomy of phytoplankton in arsenic contaminated waters. This research project was designed to examine the changes of phytoplankton communities in arsenic contaminated waters in the Ron Phibun district of Nakhon Si Thammarat province and assess the environmental factors that may cause a change of species and abundance of the phytoplankton communities. The information obtained from this study will also be used to assist with the management of the aquatic environment.

Literature reviews

Water is an essential resource for human survival. Water is used for public supply, recreation, tourism, fisheries, industrial, commercial, domestic purposes etc. (Ntengwe, 2005). As the amount of freshwater on the earth is limited, the importance of assessing the quality of the available water must be considered. Because of a concern for human health and the habitat of aquatic life, and an increasing global awareness of the need to maintain a clean world, may people have come to realize the importance, to a nation's economy, of having an adequate clean, water supply (Chaibu, 2000).

The popularity of recreational activities involving contact with water is continuing to grow. Pollution by municipal sewage and (in some cases) industrial effluents has given rise to concern by public health authorities worldwide regarding the human health risks involved in such uses. Moreover, ease of travel and changes in human behaviour has altered the use of water for recreational purposes. Users should be aware that recreational exposures to toxic compounds may have increased risks to human health and the environment (Nriagu, 2006).

As Thailand becomes more industrialized, and increased pressure to improve productivity and yields of agricultural products using chemical fertilizers, pesticides, and toxic organic compounds causes adverse environmental effects, the country faces increasing problems related to water pollution and environmental degradation (Chaibu, 2000). Surface and groundwater in the Ron Phibun district have been contaminated with arsenic and the source of the contamination is the consequence of old mining activities that continue to cause arsenic contamination of the environment. Background arsenic concentrations in these waters are usually measured at the μg/L (ppb) level with levels being generally higher in contaminated areas (Jianjun, 2000; JICA, 2000). The waters in the Ron Phibun district contaminate aquatic organisms, particularly phytoplankton, thereby allowing arsenic to enter the food chain (Suwanmanee, 1991). There have been many studies that have attempted to present a comprehensive overview of arsenic emissions in air, water, and soil and their specific sources. Arsenic emissions may be considered to arise from either point sources from anthropogenic activities or natural processes (Duker *et al*., 2005).

Phytoplankton populations and changes to the environment are often used to determine the impact of natural processes and anthropogenic factors on aquatic ecosystems and to forecast their possible evolution (Reynolds, 1984). As a result of mining activities from now

abandoned tin mines in the Ronphibun district, in some areas there have been environmental problems resulting in serious dermatological symptoms and cancer to the Ronphibun's villagers. In order to assess the relationship between the types of living organisms and the presence of arsenic in the waters, phytoplankton may be one of the best group of organisms to study to act as an indicator for monitoring this relationship.

1.1 The important roles of phytoplankton in aquatic ecosystems

1.1.1 Role of phytoplankton in the aquatic food web

Phytoplankton have many important roles such as their beneficial and detrimental effects on other aquatic organisms. They are the basis of all food webs in nature and are the ultimate source of animal and bird food. Since phytoplankton are photosynthetic or autotrophic organisms and produce oxygen evolvement. They are highly important to aquatic organisms as a primary producer in aquatic environments. Gross primary production refers to the total carbon fixed (or energy stored) by the phytoplankton. Net primary production is the amount of fixed carbon available to the plant for synthesis of its own organic matter after its respiratory requirements have been satisfied (Boney, 1989).

1.1.2 Agriculture and medicine

Among free-living and nitrogen-fixing microorganisms, the filamentous heterocystous cyanobacteria make the largest global contribution to biological nitrogen fixation. In the orient, they grow naturally, or are cultured and applied to rice fields as green fertilizers. It has been reported that certain strains of cyanobacterium, *Anabaena variabilis,* are capable of excreting the ammonia. They produce via nitrogenase into the environment (Peerapornpisal, 2005; Spiller *et al*., 1986). When these cultures were grown in association with rice in a greenhouse, ammonia excreted by the mutant strain supported growth of the rice plants (Latorre *et al*., 1986). Some species are regionally popular edible delicacies. Some phytoplankton such as *Nostoc* produce antibacterial substances which are considerably useful for human's health. Some human pathogens (fungal and bacterial) are inhibited by phenolic extracts from them; it is possible that *Nostoc* some

day may provide biotechnologists with unique medicinal compounds. *Nostoc commune* has been shown to lower cholesterol in the serum of rats and, thus, has potential to become a "health food." (Dodds *et al.*, 1995).

1.1.3   Waste water treatment and use as a biomonitor of water quality

In wastewater treatment processes, the organic materials are mineralized producing carbon dioxide, phosphate, nitrate, ammonia and others. The phytoplankton can utilize these minerals and some organic compounds and produce oxygen by photosynthesis. This improves the quality of the water. Moreover, phytoplankton can grow in water of different conditions. *Microcystis aeruginosa* Kutzing, *Euglena* spp. and *Oscillatoria* spp. can grow in water with both high organic content and in polluted water, whereas desmids, a group of green algae i.e., *Cosmarium* spp. and *Staurastrum* spp. can only grow in clean water with low nutrients. Therefore, the type of phytoplankton could be used as bioindicators of the water quality, especially with respect to water with organic pollution. The approach followed so far for determining water quality clearly reflects the dated concept that biological data, being so variable, are only good to act as indicators for more precise chemical analyses. In the coming years the more widespread use of aquatic environmental biomonitoring in addition to the usual physical or chemical examination of water will surely improve the knowledge of the characteristics of environmental waters (Ariyadej *et al.*, 2004a; Baudo, 2001; Marneffe *et al.*, 1996; Pereira *et al.*, 2003; Peerapornpisal, 2005).

The use of bioindicators in water quality assessment has been employed for ages (Liebmann, 1962). Phytoplankton are considered to be sensitive indicators of environmental stresses. In addition, on account of their widespread occurrence and range of environmental preferences, phytoplankton can be used as bioindicators of the trophic state of water bodies. For example, *Cyclotella meneghiniana* Kutzing and *Melosira varians* Agardh were normally found in low nutrient water and such could be used as bioindicators of an oligo-mesotrophic reservoir (Ariyadej, 2005; Ariyadej *et al.*, 2004b; Ryding and Rast, 1989). There are several good reasons for using phytoplankton for biomonitoring. Because phytoplankton are the base of the trophic chain in many rivers and lakes, an impact on these communities will have broad repercussions on the fuctioning of the entire ecosystem. Many environmental problems, like cyanophyte blooms, result directly from changes in the algal community. Rapid reproductive rates make phytoplankton very responsive to

changes in water quality. Siliceous remains of diatoms and chrysophyceae can also be used in paleolimnological studies to detect past pollution episodes and/or to establish the composition of pre-pollution communities (Louise *et al.*, 1997).

1.1.4   Biosorption of arsenic by algae

Contamination of freshwater ponds by toxic metals continues to be a problem in many regions of the world. Arsenic is one of toxic elements widely distributed in marine, freshwater and soil environments. It is known, however, that some algal species can concentrate arsenic to much higher levels than in the surrounding water (Fujiwara *et al.*, 2000). Some species of microalgae such as *Chlorella*, *Cryptomonas*, *Hymenomonas*, *Synechococcus*, *Phormidium*, and *Anabaena* have been reported to be resistant to concentrations of arsenic that are orders of magnitude greater than those found in natural waters (Bottino *et al.*, 1978; Budd and Craig, 1981; Csonto *et al.*, 2004; Planas and Healey, 1978). It is therefore necessary to understand both the nature of arsenic concentrations that are adsorbed and absorbed by algal cells and how they may be released back into the water.

It is known that arsenic compounds can be accumulated by marine algae and seaweed. For example, there have been a number of attempts to determine the arsenic concentrations in marine algae. It was found that marine algae contain considerable amounts of arsenic (10-100 mg/kg dry weight) from the Norwegian coast (WHO, 1988; PCD, 1998; Schaeffer *et al.*, 2005). Algae have the ability to regulate, reject, or sequester some compounds, and can play an important role in the geochemistry, transport, and toxicity of many trace elements. However, many heavy metals are also toxic to organisms, and changes in their chemical form and reactivity can be of considerable importance to the ecosystem as a whole (Sanders and Riedel, 1993)

Arsenic dissolved in the water occurs as different chemical species (ions, complexes, etc.) in equilibrium with inorganic and organic complexing agents. The results of laboratory algal uptake experiments involving arsenic have been explained by many authors (Fujiwara *et al.*, 2000; Hsu *et al.*, 2001; Pinto *et al.*, 2003; Raab *et al.*, 2005). Moreover, algal species associated with arsenic can be used for treating metal contaminated wastewater (Knaver and Hemond, 1999). Jahan *et al.* (2006) reported that *Scenedesmus abundans* and *Chlorella vulgaris* are common species can be used to remove a high percentages of arsenic from contaminated wastewater.

The aforementioned paragraphs have cited briefly some beneficial activities or uses of phytoplankton. However, there are negative aspects that should also be taken into consideration. Certain phytoplankton or their products are toxic to animals and man (Fenner *et al*., 1997; Burgess and Shaw, 2001). For instance, many species of phytoplankton can grow rapidly following eutrophication caused by run off from agricultural fertilizers, degraded land, and disposal of domestic sewage and industrial effluents. This causes phytoplankton bloom and the widespread occurrence of toxic and anoxic aquatic ecosystems all over the world, can be harmful to public health and the environment (Lehimaki *et al*., 1997).

1.2 Arsenic contamination in the aquatic environment

1.2.1 Arsenic and its compounds

Arsenic (As) appears in Group V of the periodic table. Elemental arsenic is a gray, crystalline material characterized by atomic number 33, atomic weight of 74.9216, melting point of 817 ^{0}C and its chemical properties are similar to those of phosphorus. Arsenic can exist in several oxidation states: As^{3-}, $As(0)$, As^{3+}(arsenite); and As^{5+}(arsenate). Being a natural element, arsenic is widely distributed in a number of minerals, mainly as the arsenicals of copper, nickel, and iron or as arsenic oxide or sulfide. In water, arsenic is usually found in the form of arsenate (V) or arsenite (III) (NAS, 1977; WHO, 1981). Arsenic is also mobilized by dissolution not only in water, but also with soil or sediment with concentrations being controlled by a variety of input and removal mechanisms (Nair *et al.*, 2003).

Arsenic is ubiquitous in the environment, and is present in both solid and liquid phases. It is ranked as the 20th most common trace element in the earth's crust, 14th in seawater, and 12th in the human body (Cullen and Reimer, 1989; Eisler, 1988; Koopetngarm, 1978). It has been estimated that about one-third of the atmospheric flux of arsenic is of natural origin. In nature, even though there are 150 species of arsenic bearing minerals, only 3 of them, i.e., arsenic sulphide or realgar (As_2S_2), arsenic tri-sulphide or orpiment (As_2S_3) and ferrous arsenic sulphide or arsenopyrite ($FeAsS_2$) are considered arsenic ores because the amount of arsenic is higher in these three compounds and when exposed to weathering this may lead to arsenic being continuously released into the environment (Hossain, 2006). Natural phenomena such as the metabolism of phytoplankton,

decomposition of organic matter by bacteria, and microbial reduction of iron and manganese oxides in sediments, are responsible for the variation of arsenic species present in lake water (Sohrin *et al.*, 1977). High concentrations of arsenic are found in soils/sediment and water affected by mining operations. Mining, smelting of non-ferrous metals and burning of fossil fuels are the major activities that cause arsenic contamination in air, water and soil. Moreover, the use of arsenic-containing pesticides has also led to contamination of the environment (Sophary, 2003).

Detection of arsenic contaminated water has become one of the main responsibilities for protecting the environmental. It is clear that there are many countries in the world where arsenic in drinking water has been detected at concentrations greater than the WHO guideline value of 0.01 mg/L or the prevailing national standards, but reliable data on exposure and health effects are rarely available. Due to the problem of arsenic contamination of waters used for drinking water, it makes sense to develop a treatment technology with sensitive detection methods. Furthermore, a reduction in the acceptable consumption levels of arsenic by the regulatory agencies is forcing water utilities to identify and implement cost effective arsenic removal technologies (Thirunavukkarasu *et al.*, 2003).

1.2.2 Usage of Arsenic Compounds

Arsenic occurs naturally in all environmental media and is usually present in the form of compounds with sulfur and with many metals (copper, cobalt, lead, zinc etc.). The average concentration in the earth's crust is about 2.0 mg/kg. In some localized geographic areas, commercial use and production of arsenic compounds have resulted in a significant elevation in the amounts of environmental arsenic above natural background levels (Flora *et al.*, 2007).

With the passing of time, human beings have found many beneficial properties of arsenic. The major use of arsenic is still for use in agricultural such as in pesticides, insecticides, in cattle and sheep dips, primarily on cotton fields and in orchards. It has also been used in the glass and ceramics industry, in drugs and feed additives and it is still used as a desiccant, rodenticide, and for veterinary applications (Azcue and Nriagu, 1994; PCD, 1998; WHO, 1981). Various arsenate containing compounds such as chromate copper arsenate and pentavalent arsenic compounds are still used for the preservation of wood (PCD, 1998). Manufactured arsenic (III) oxide has been used for medicinal purposes for ages. It has been used to cure for acute promyelocytic leukemia (APL), and

there has been promising activity noted against other hematologic and solid tumors (Evens *et al.,* 2004).

1.2.3   Sources and occurrence of arsenic in the aquatic environment

The concentration of arsenic in freshwater shows considerable variation associated with the geological composition of the drainage area and the extent of anthropogenic activities (Cullen and Reimer, 1989), but such enrichment is often magnified significantly by the mining and processing of arsenic bearing ores. In many historically important mining provinces, contamination of surface drainage and groundwater systems by arsenic is a major toxicological and environmental concern. Although the mine has been settled for a long time, a small headwater stream flowing adjacent to the former mine site were contained a level of arsenic that is high potentially toxic and may have an effect on aquatic communities (Williams *et al.*, 1995).

The analysis of toxic elements in water samples is a complicated task, as they are present at low concentration and can be subject to a variety of chemical modifications after sampling (Oliveira *et al.*, 2006). The occurrence and detection of arsenic in natural water has been given significantly attention for many years (Michel *et al.*, 1998). Arsenic varies with the chemical and physical forms of the compound, and occurs in a number of valence states in the aquatic environment. The valence state of arsenic plays an important role in its behaviour and toxicity in aqueous systems (Hossain, 2006; WHO, 1981). The organic arsenic compounds are not as toxic as the inorganic forms. As (III) and As (V) which are very toxic (Li and Lee, 2004). The form of arsenic present in water depends on Eh, pH, organic content, suspended solids, dissolved oxygen, and other variables (EPA, 1985). Sohrin and Matsui (1997) showed that the distribution of arsenic in lake water largely depends on oxygen, while its presense in sediments normally depends on iron oxides and manganese oxides (Nair *et al.*, 1997; Sohrin and Matsui, 1997)

The activities of microorganisms and microbiological oxidation/reductions enhance the mobility and speciation of arsenic in the environment (Duker *et al.*, 2005). The contribution provided by microorganisms to the biogeochemistry of arsenic in the environment is extensive and

detailed as it involves various oxidation, reduction, and demethylation reactions of the dominant chemical species (Sohrin and Matsui, 1997). Mono Lake is an extreme environment, in which abundant of arsenic cycling occurs in the region of the chemocline (Figure 1). Arsenite reduction is mediated by dissimilatory arsenate-reducing prokaryotes (DARPs) that use released organic matter from dying plankton to fuel their respiration. Arsenite oxidation (aerobic and anaerobic) is mediated by chemolithoautotrophic arsenite oxidizers (CAOs) that also contribute to secondary production by "fixing" CO_2 into organic matter. Arsenic first enters this alkaline (pH=9.8), saline (~90 g/L) lake as a dissolved component contained in the discharge from hydrothermal springs. Arsenic, as well as other dissolved constituents, reaches high concentrations because of the predominance of evaporation over precipitation in this arid region (Oremland and Stolz, 2003; Oremland *et al*., 2004).
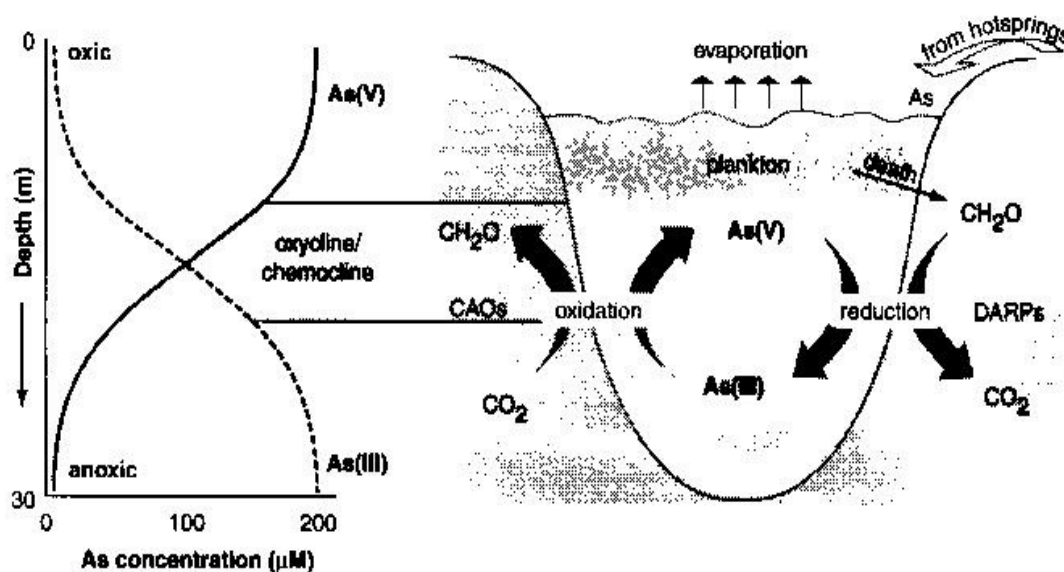
Figure 1. The chemical speciation of arsenic in the stratified water column of Mono Lake California (left) as explained by the metabolism of arsenic by microbial populations present in the water column (right).
Source: Oremland and Stolz (2003)

Groundwater and phosphorus fertilizers enhanced arsenic accumulation in plants. At present arsenic in groundwater and plants are considered a major source of arsenic in the food chain. Arsenic accumulation in plants and phyto-toxicity due to increased arsenic in soil and water may have long term impacts on agricultural yield and subsequent effects on human health (Rahman and Parkpian, 2004). In natural water, arsenic transferred through aquatic food webs to algae, fish, bivalves, human, and other terrestrial animals are of environmental and health concern. Investigations of the chemical constituents of aquatic organisms can provide useful information about the environment as well as toxicologically data relevant to the composition of biological species consumed by humans. This means that the relatively small percentage of the population who are now known to be exposed to the risk of arsenic disease due to arsenic poisoning would possible increase significantly (Chen *et al.,* 2000; Schaeffer *et al*., 2005).

Recent studies have emphasized the need to understand the accumulation and fate of metal contaminants at different trophic levels. Phytoplankton have been mainly used as suitable aquatic organisms for testing the bioaccumulation of arsenic under growing conditions (Bottino *et al.*, 1978; Lamai *et al*., 2005). The uptake of As(V) by phytoplankton is the first step of arsenic transformation in any aerobic aquatic environment. Many studies have demonstrated that algae are able to accumulate arsenic from the aquatic environment in their cells (Sanders, 1979; Sanders, 1980; Sanders and Windom, 1980). In addition, *Chlorella* sp. are reported to survive at 500 mg/L. As(V) in the medium and approximately 35% As(V) of this was removed within the 1st day of exposure (Sanders and Riedel, 1993; Bunnag, 2000; Knaver and Hemond, 2000). Arsenite however will inhibit the growth of *Chlorella vulgaris* at concentrations of more than 10 mg/L (Cullen and Reimer, 1989).

1.2.4   Factors that influence partitioning of arsenic in waters

Arsenic occurs in several chemical forms in an aquatic environment i.e., inorganic and organic forms, dissolved form and arsine gas. The trivalent arsenic (As^{3+}) and the pentavalent arsenic (As^{5+}) are widely distribute in natural water (Tongboriboon, 1997). The formation of inorganic pentavalent arsenic, typically the dominant species in freshwater, is favored under condition of high dissolved oxygen, high pH, high redox potential, and reduced organic material. The opposite conditions usually favor the formation of arsenite (http://2the4.net/redox.htm). There

are several physical factors that influence the mobility of dissolved arsenic oxyanions in the environment. For example, arsenate as H_3AsO_4 and arsenite as H_3AsO_3 through porous media is controlled primarily by sorption reactions with metal hydroxides (Redman *et al*., 2002). pH and Eh are considered to be important factors that have an effect on the distribution and speciation of arsenic in any aquatic environment (Tallman and Shaikh, 1980; Wittayawarawat, 1994; http://2the4.net/redox.htm).

1.2.4.1   Rodox potential (Eh)

Eh (redox potential) is an important parameter of overall redox potential in the system. It does not characterize the capacity of the system for specific oxidation or reduction reactions. Its process plays a crucial role in the geochemical organization, present in the water system. Redox reactions measure the mobility of many inorganic compounds and the tendency for a solution to either receive and donate electrons when it is subjected to change by introduction of a new species (http://2the4.net/redox.htm).

At the high Eh values encountered in oxygenated waters, arsenic acid species (H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$, and AsO_4^{3-}) are stable. At Eh value characteristic of mildly reducing conditions, arsenious acid species (H_3AsO_3, $H_3AsO_3^-$, and AsO_3^{2-}) become stable. Under conditions where S^{2-} is stable, realgar (AsS), and orpiment (As_2S_3) have low solubilities and occur as stable solids at pH values below about 5.5 and Eh values about -0.45. $HAsS_2$(aq) is the predominant species at low pH in the presence of sulfide. At still lower Eh values arsenic metals are thermodynamically stable. At very low Eh values, AsH_3, arsine may be formed (Meihong, 1995).

1.2.4.2   pH

pH is probably the most important factor governing metal speciation, solubility from mineral surfaces, transport and eventual bioavailability of metals in aqueous solutions. pH affect both solubility of metal hydroxide minerals and adsorption-desorption processes. Most metal hydroxide minerals have very low solubilities under the pH conditions in natural water. Because hydroxide ion activity is directly related to pH, the solubility of metal hydroxide minerals increases with decreasing pH, and more dissolved metals become potentially

available for incorporation in biological processes as the pH decreases (Calzada *et al.,* 1998; Salomons, 1995)

Large amounts of arsenic are tightly bound to sediments (Nikolaidis *et al.* ,2004). The amount of arsenic leaching out of sediments to the water column is substantially decreased due to iron/arsenic coprecipitation at the water-sediment interface. Overall, it is found that arsenic accumulates at the ground water/lake interface where it forms insoluble precipitates (http://2the4.net/redox.htm). Olias *et al.* (2006) found that the arsenic concentration near to the mining area is very low due to sorption and/or coprecipitation processes together with Fe oxyhydroxides. The highest concentrations (close to 0.1 mg/L) occur during low water in the lower part of the river due to the highest pH values. When pH rises above 7.5, desorption of the arsenic contained in the river sediments starts. Empirical studied have indicated that pH is one of the environmental factors with the greatest potential to remove arsenic. The optimum pH observed was at 5-7 (Bunnag, 2000).

In the pH range of natural waters, the predominant aqueous arsenate species are $H_2AsO_4^-$ and $HAsO_4^{2-}$. The predominant arsenite species is H_3AsO_3 (Aurillo *et al*., 1994). Waters emanating from acid rock drainage areas have arsenic below drinking water limits. Hence, adsorption of arsenic is expected to decrease as the pH rises above the natural range of pH 3-5, and increased mobilization of arsenic is expected under a rising pH. Leaching experiments and theoretical predictions indicate that minimum leaching of arsenic occurred at pH 1, and the amount of arsenic mobilized increased by an order of magnitude at pH 6. Leaching of arsenic in the strongest acid solution was higher than expected from the theory. This high arsenic mobilization may be due to acid attack of more than just adsorption sites, releasing arsenic from the material (Craw, 2005)

According to the experiment of Chantanachunlaka (1990), arsenic is relatively easy to remove from water. The adsorption ability of arsenite and arsenate by coagulation process was limited at pH 6.5 to 8.5. Ferric sulphate was the most effective coagulant to remove arsenite in synthetic water at pH 6.5-8.0. In natural water, ferric sulphate could reduce arsenic to less than 0.05 mg/L. The efficiency of arsenic removal at more than 90% occurred when using alum hydroxide over 120 mg/L at the optimum pH of 6.6-6.9 (Intrakaroonvate, 1988) or chitosan beads at the pH 6 (Netvichain, 2000). Moreover, >78% efficiency of arsenic removal was obtained with calcium hydroxide at the optimum pH of 11.1-11.4 (Intrakaroonvate, 1988).

The optimum pH for arsenic precipitation by arsenic-resistant bacterial strains of both of an obligately anaerobic bacterium and a facultative anaerobic bacterium named AsR-17, AsR-19 and AsR-20 was 7. Percentages of removal of arsenic were 35.02 and 42.07, respectively. Arrykul *et al.* (1996) also reported that ferric chloride could precipitate arsenic at a ratio of Fe:As > 4:1. Enhanced flocculation occurred when the pH was raised to 7 when more than 90% of arsenic precipitated out (Ittisupornrat,1999).

1.3 Problems of arsenic contaminated waters in the Ron Phibun district

A center for the mining of tin from primary Tin-Wolfram-Arsenic (Sn-W-As) deposits and secondary placer tin deposits is located in the Ron Phibun district, Nakhon Si Thammarat province. It has been exploited in for over 100 years (Jianjun, 2000; Williams *et al.*, 1996). The mining and mineral processing adopted in this area were the main causes of arsenic contamination of the environment. Ponds dug for mining activities were also located in the Ron Phibun district (Wattanasen *et al.*, 2006). Arsenic contamination arises from tin mining activities particularly leachates from dumped mine tailings running into tributary streams resulting in bioaccumulation in the food web. The outcomes showed that humans who are the top consumers were clearly affected by arsenic accumulation in their cells. The adverse health effects of this accumulation are well documented (Hayashi *et al.*, 2002; Li *et al.*, 2001). Symptoms of chronic arsenic toxicity include vomiting, oesophageal and abdominal pain and long term exposure from arsenic in drinking water causes cancer of the skin, lungs, urinary, and kidney (Chatchawet, 2001; Vitayavirasak, 2005).

This area was selected because it is the most serious case of arsenic poisoning ever reported in Thailand. There are a number of research reports on this area (Bunnag, 2000; Jianjun, 2002; Williams, 1996). The reports showed that the concentration of arsenic in aquatic plants was between 0.23 and 2.97 mg/kg wet weight. The highest levels were found in *Eichornia crassipes*. The concentrations of arsenic in aquatic animals were between a non detected level and 2.45 mg/kg wet weight. The highest levels were found in *Sinotaia ingallsiana* (Tongboriboon, 1997). The concentration of arsenic in water was between 70 and 1,000 μg/L, with the highest value in the Ron Na stream (Bunnag, 2000). The arsenic values in five pond water samples ranged from 0.04 to 1.1 mg/L. It must be mentioned that high concentrations of arsenic in pond water would have been

another polluting source of arsenic discharge back into groundwater (Jianjun, 2002). Additionally, arsenic from the tin mining area in the Ron Phibun district has been flowing via the Pak Pa-Nang river into Pak Pa-Nang bay in the southern part of Thailand. This is an important area for aquatic organism culture, but arsenic accumulation in mussel, fish and shrimp indicated that the arsenic levels would have no adverse harmful effects on the consumer (Boonchalermkit *et al.*, 2004; Rattanachongkiat *et al.,* 2004). However, some sites in the Ron Phibun district are high risk areas that may cause people ultimately to die from cancers caused by chronic arsenic poisoning (Rakwong, 1999)

A JICA survey (Aug1998-Aug1999) revealed that arsenic accumulated in the soil during mining activities in the past is continously being released into the groundwater. In some areas, the arsenic concentrations in the groundwater are several hundred times more than the maximum permissible limit in potable water. The survey area has relatively abundant precipitation and during the rainy season the groundwater is recharged and flows out of the area. It may cause arsenic contamination to become more wide-spread, because the contaminated ground water may be transported out of the area by its flow. It is urgent and important to remedy the contaminated zone, to prevent further casualities and improve the natural environment (JICA, 2000)

The long term investigation of water showed the presence of arsenic at concentrations exceeding the exceptional (0.01 mg/L) as recommend by the WHO (Williams *et al.,* 1996). However, a progressive decrease in arsenic level in surface waters has been recorded, but dissolved arsenic is still present at concentration that exceed the WHO potable water standard of 0.01 mg/L. So, arsenic contamination of the surface water in Ron Phibun continues to be a matter of concern and requires environmental monitoring to ensure that the arsenic contamination is within the standard limit of WHO (Bunnag, 2000; Williams *et al.*, 1996).

Objectives

1. To investigate the species diversity of phytoplankton in arsenic contaminated waters
2. To study the changes of phytoplankton communities in relation to certain environmental factors in arsenic contaminated waters