Changes in Phytoplankton Communities in Arsenic Contaminated Waters at the Ron Phibun District of Nakhon Si Thammarat Province

Weeradej Meeinkuirt

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Ecology (International Program)

Prince of Songkla University

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Dean of Graduate School

ชื่อวิทยานิพนธ์	การเปลี่ยนแปลงของประชาคมแพลงก์ตอนพืชในแหล่งน้ำที่มีการปนเปื้อนสารหนู ในอำเภอร่อนพิบูลย์ จังหวัดนครศรีธรรมราช
ผู้เขียน	นายวีระเดช มีอินเกิด
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บทคัดย่อ

ศึกษาการเปลี่ยนแปลงของประชาคมแพลงก์ตอนพืชในแหล่งน้ำที่ปนเปื้อนสารหนูในอำเภอร่อนพิบูลย์ จังหวัดนครศรีธรรมราช โดยเลือกแหล่งน้ำจากเหมืองดีบุกเก่าในตำบลร่อนพิบูลย์ และตำบลหินตก จำนวน 4 แหล่ง และบ่อขุดในตำบลเสาธง และตำบลควนเกยจำนวน 2 แหล่ง โดยตรวจสอบปัจจัยทางกายภาพ เคมี และชีวภาพ จำนวน 12 ปัจจัย พร้อมทั้งวิเคราะห์ชนิดและความชุกชุมของแพลงก์ตอนพืชทุกเดือน ตั้งแต่เดือนกรกฎาคม พ.ศ. 2547 ถึงเดือนมิถุนายน พ.ศ. 2548 จากการตรวจสอบปริมาณสารหนูของน้ำตัวอย่าง พบว่ามีปริมาณการปนเปื้อนสารหนูระหว่าง 0.30±0.01 ถึง 167.85±0.96 ไมโครกรัม/ลิตร แหล่งน้ำที่มีระดับการปนเปื้อนสารหนูในระดับสูง (High Arsenic Contaminated Ponds, HACP) มี 3 แหล่ง โดยมีระดับการปนเปื้อนระหว่าง 19.00±0.03 ถึง 167.85±0.96 ไมโครกรัม/ลิตร ค่าเฉลี่ยในแต่ละแหล่งน้ำคือ 69.31±5.66, 39.06±3.31 และ 84.41±13.95 ไมโครกรัม/ลิตร สำหรับแหล่งน้ำที่ 1, 3 และ 5 ตามลำดับ ขณะที่แหล่งน้ำที่เหลือมีระดับปริมาณสารหนูปนเปื้อนที่ต่ำกว่า (Low Arsenic Contaminated Ponds, LACP) โดยมีระดับการปนเปื้อนระหว่าง 0.30±0.01 ถึง 16.08±0.20 ไมโครกรัม/ลิตร ค่าเฉลี่ยของแหล่งน้ำที่ 2, 4 และ 6 คือ 13.64±0.54, 0.92±0.35 และ 7.24±0.48 ไมโครกรัม/ลิตร

จากการศึกษาพบชนิดและองค์ประกอบของแพลงก์ตอนพืชทั้งหมด 78 สกุล ประกอบด้วย Chlorophyceae (40 สกุล) รองลงมาคือ Cyanophyceae (18 สกุล) Bacillariophyceae (11 สกุล) Euglenophyceae (4 สกุล) Chrysophyceae (3 สกุล) และ Pyrrophyceae (2 สกุล) จำนวนชนิดของแพลงก์ตอนพืชใน HACP มีมากกว่าใน LACP และชนิดของแพลงก์ตอนพืชมีความแตกต่างกันตามสถานที่และเวลา แพลงก์ตอนพืชที่พบ ได้บ่อยในแหล่งน้ำที่ศึกษาพบสาหร่ายสีเขียวแกมน้ำเงิน (cyanobacteria) เป็นกลุ่มของแพลงก์ตอนพืชที่พบได้บ่อย ได้แก่ *Cylindrospermopsis* sp. และ *Oscillatoria* spp. จากการใช้ดัชนีความหลากหลายทางชีวภาพมาช่วยประเมินผลกระทบของสารหนูรวมต่อประชาคมแพลงก์ตอนพืช พบว่ามีความแตกต่างกันไม่ชัดเจนระหว่าง HACP และ LACP ผลการศึกษาพบว่าฤดูกาลมีผลต่อชนิดและความชุกชุมของแพลงก์ตอน

พืชในทุกแหล่งน้ำ โดยเฉพาะในช่วงฤดูฝน ซึ่งสอดคล้องกับการเปลี่ยนแปลงค่าความชุกชุม สัมพัทธ์ในทุกแหล่งน้ำ ความหนาแน่นเฉลี่ยของแพลงก์ตอนพืชในแต่ละแหล่งน้ำพบอยู่ในช่วง 8.08×10^4 ถึง 1.24×10^6 เซลล์/ลิตร จากการวิเคราะห์ด้วย cluster โดยใช้ข้อมูลความชุกชุมของแพลงก์ตอนพืช พบว่าแหล่งน้ำที่ศึกษาไม่สามารถจัดกลุ่มได้เนื่องจากมีความคล้ายคลึงกันในระดับต่ำ ขณะเดียวกันการวิเคราะห์โดยใช้ข้อมูลปัจจัยแวดล้อม แสดงให้เห็นว่าคุณภาพน้ำโดยทั่วไปไม่มีความแตกต่างกันตามสถานที่ และจากการวิเคราะห์ความสัมพันธ์ระหว่างแพลงก์ตอนพืชและปัจจัยทางด้านกายภาพและเคมีของน้ำโดยใช้วิธี Canonical Correspondence Analysis (CCA) พบว่า ฟอสฟอรัสละลาย, สารหนูรวม, แอมโมเนีย-ไนโตรเจน, ไนเตรท-ไนโตรเจน และ ปริมาณของแข็งแขวนลอย มีความสัมพันธ์กับ Cyanophyceae (ได้แก่ *Raphidiopsis* sp. และ *Microcystis* spp.), ค่าความนำไฟฟ้า, บีโอดี และ พีเอช มีความสัมพันธ์กับ Cyanophyceae (ได้แก่ *Cylindrospermopsis* sp., *Cylindrospermum* sp. และ *Oscillatoria* spp.), Pyrrophyceae (ได้แก่ *Peridinium* spp.) และ Chrysophyceae (ได้แก่ *Dinobryon* spp.) ขณะที่ Chlorophyceae (ได้แก่ *Botryococcus* sp.) มีความสัมพันธ์กับออกซิเจนละลาย

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ABSTRACT

Changes in phytoplankton communities in arsenic contaminated waters at the Ron Phibun district of Nakhon Si Thammarat province were investigated. Four of the locations chosen were dredging ponds (abandoned tin mines) at Ron Phibun and Hin Tok sub-districts and two were dug ponds used by the local community at Sao Thong and Khuan Koey sub-districts. Measurements of 12 physical, chemical and biological variables, species composition and phytoplankton abundance were taken at monthly intervals between July 2004 and June 2005. Analysis of the total arsenic content of the water collected from the sampling locations showed that levels were in the range of 0.30±0.01 to 167.85±0.96 μg/L. The mean values of each sampling location were 69.31±5.66, 39.06±3.31 and 84.41±13.95 μg/L for locations 1, 3 and 5, respectively. Furthermore, three locations were similar to one another in total arsenic concentrations and as a result categorized as High Arsenic Contaminated Ponds (HACP). The ranges of total arsenic values in those sampling locations were 19.00±0.03 to 167.85±0.96 μg/L, whereas the remaining locations (Low Arsenic Contaminated Ponds, LACP) had lower arsenic concentrations (0.30±0.01 to 16.08±0.20 μg/L). The mean values of each sampling location were 13.64±0.54, 0.92±0.35 and 7.24±0.48 μg/L for locations 2, 4 and 6, respectively.

Seventy-eight genera of phytoplankton were identified. There were 40 genera in the class Chlorophyceae, 18 genera in the class Cyanophyceae, 11 genera in the class Bacillariophyceae, 4 genera in the class Euglenophyceae, 3 genera in the class Chrysophyceae and 2 genera in the class Pyrrophyceae. There was more species richness of phytoplankton flora in HACP than in LACP. In addition, the results showed that there were spatial and temporal differences in phytoplankton genera. However, the dominant phytoplankton genera in all observed locations were blue-green algae (cyanobacteria) such as *Cylindrospermopsis* sp. and

Oscillatoria spp. Diversity indices were used to estimate the effect of total arsenic on phytoplankton communities. They revealed that there were no significant differences as compared between HACP and LACP. In addition, the results showed that there was seasonal variation in phytoplankton genera and abundance in all sampling locations, particularly during the rainy period. This was also consistent with a change of relative abundance. Mean density of phytoplankton ranged from 8.08×10^4 to 1.24×10^6 cells/L. Analysis of phytoplankton abundance using cluster analysis indicated that none of the sampling locations could be grouped due to their dissimilarity level. It was also observed that all sampling locations had similar limnological behaviour. Canonical Correspondence Analysis (CCA) ordination indicated that dissolved phosphorus, total arsenic, nitrate-nitrogen, ammonia-nitrogen, and Total Suspended Solids had a relationship with Cyanophyceae (i.e., *Raphidiopsis* sp. and *Microcystis* spp.), and that conductivity, BOD and pH had a relationship with Cyanophyceae (i.e., *Cylindrospermopsis* sp., *Cylindrospermum* sp. and *Oscillatoria* spp.), Pyrrophyceae (i.e., *Peridinium* spp.) and Chrysophyceae (i.e., *Dinobryon* spp.). Chlorophyceae (i.e., *Botryococcus* sp.) was shown to have had a relationship with dissolved oxygen.

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Weeradej Meeinkuirt

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LIST OF ABBREVIATIONS AND SYMBOLS

et al.	=	Et. Ali (Latin), and others
HG-AAS	=	Hydride Generation Atomic Absorption Spectrophotometry
HACP	=	High Arsenic Contaminated Ponds
LACP	=	Low Arsenic Contaminated Ponds
BOD_5	=	Biological Oxygen Demand
DO	=	Dissolved Oxygen
NO_2^--N	=	nitrite-nitrogen
NO_3^--N	=	nitrate-nitrogen
NH_3-N	=	ammonia-nitrogen
PO_4^{3-}-P	=	dissolved phosphorus
TSS	=	Total Suspended Solids
As	=	arsenic
mg/L	=	milligram per liter
µg/L	=	microgram per liter
µS/cm	=	microsiemens per centrimeter
R	=	species richness
J	=	evenness
H'	=	Shannon-Wiener diversity
MVSP	=	multivariate statistical programme
UPGMA	=	unweighted pair group method algorithm
CCA	=	Canonical Correspondence Analysis
µm	=	micrometer
nm	=	nanometer
cm	=	centrimeter
mL	=	milliliter
mg	=	milligram
g	=	gram
L	=	liter

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).

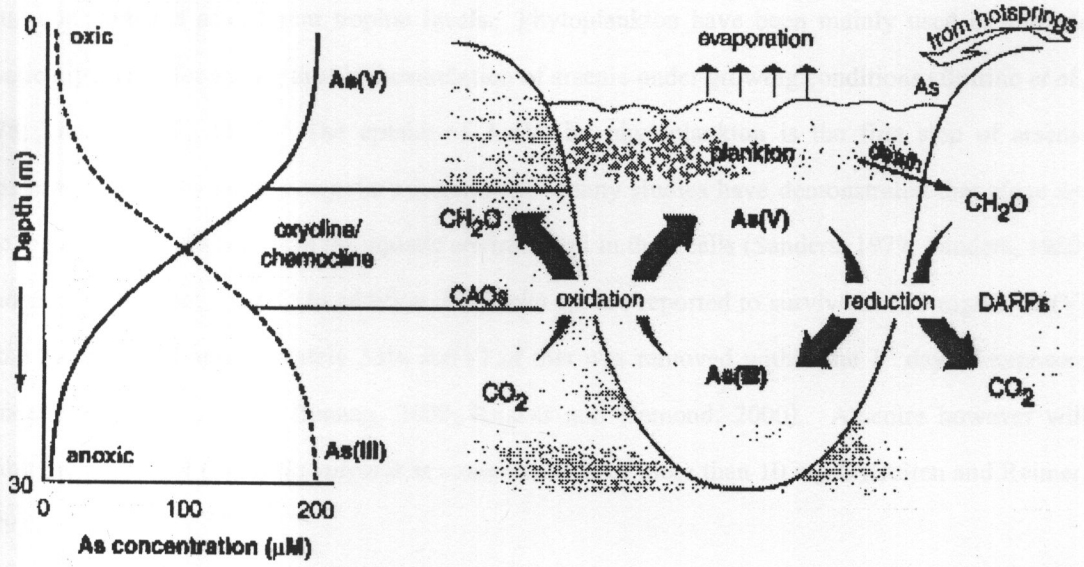


Figure 1. The chemical speciation of arsenic in the stratified water column of Mono Lake California (left) as explained by the metabolism of arsenic by microbial populations present in the water column (right).
Source: Oremland and Stolz (2003)

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

CHAPTER 2

MATERIALS AND METHODS

1. Study area and sampling sites

Nakhon Si Thammarat province is one of the 76 provinces in Thailand. It is the second largest province in the south after Suratthani province. It has a total land area of 9,942 km^2 with a population of over 1.5 million as reported in 1996. Neighboring provinces are (from south clockwise) Songkhla, Pathalung, Trang, Krabi, and Suratthani. The province is administratively divided into 23 districts (amphoe), which are further sub-divided into 163 sub-districts (tambon).

Ron Phibun district is located approximately 800 km south of Bangkok on the shore of the gulf of Thailand on the east side of the Malay Peninsula. It is one of the 23 districts in Nakhon Si Thammarat province, and composed of 6 sub-districts, i.e, Hin Tok, Sao Thong, Ron Phibun, Khuan Chum, Khuan Phang and Khuan Koey. It has an area of 504.76 km^2. The Ron Phibun sub-district is situated between longitude 99° 46′ - 99° 54′ E and latitude 8° 04′ - 8° 15′ N. It consists of 16 villages and located in the center of a tin mining area of Primary Tin-Wolfram-Arsenic (Sn-W-As) deposits and secondary placer tin deposits that were exploited 100 years ago. In the western part of the district, the Ron Na-Suang Chan mountain subrange is a wide mountanious area.

This research project was conducted at Ron Phibun district, Nakhon Si Thammarat province. Samples were collected between July 2004 and June 2005 at four locations in dredging ponds at Ron Phibun and Hin Tok sub-districts and two locations in dug ponds used by the local community at Sao Thong and Khuan Koey sub-districts. At each sampling site, a GPS was used to collect positioning data in order to allow accurate mapping of our sites. The map of the study is shown in Figure 2. These locations were selected based upon the following criteria: 1) The latest survey's findings 2) The arsenic contamination tested areas 3) Recommended areas by research papers such as Bunnag (2000), JICA (2000), Williams *et al.* (1996).

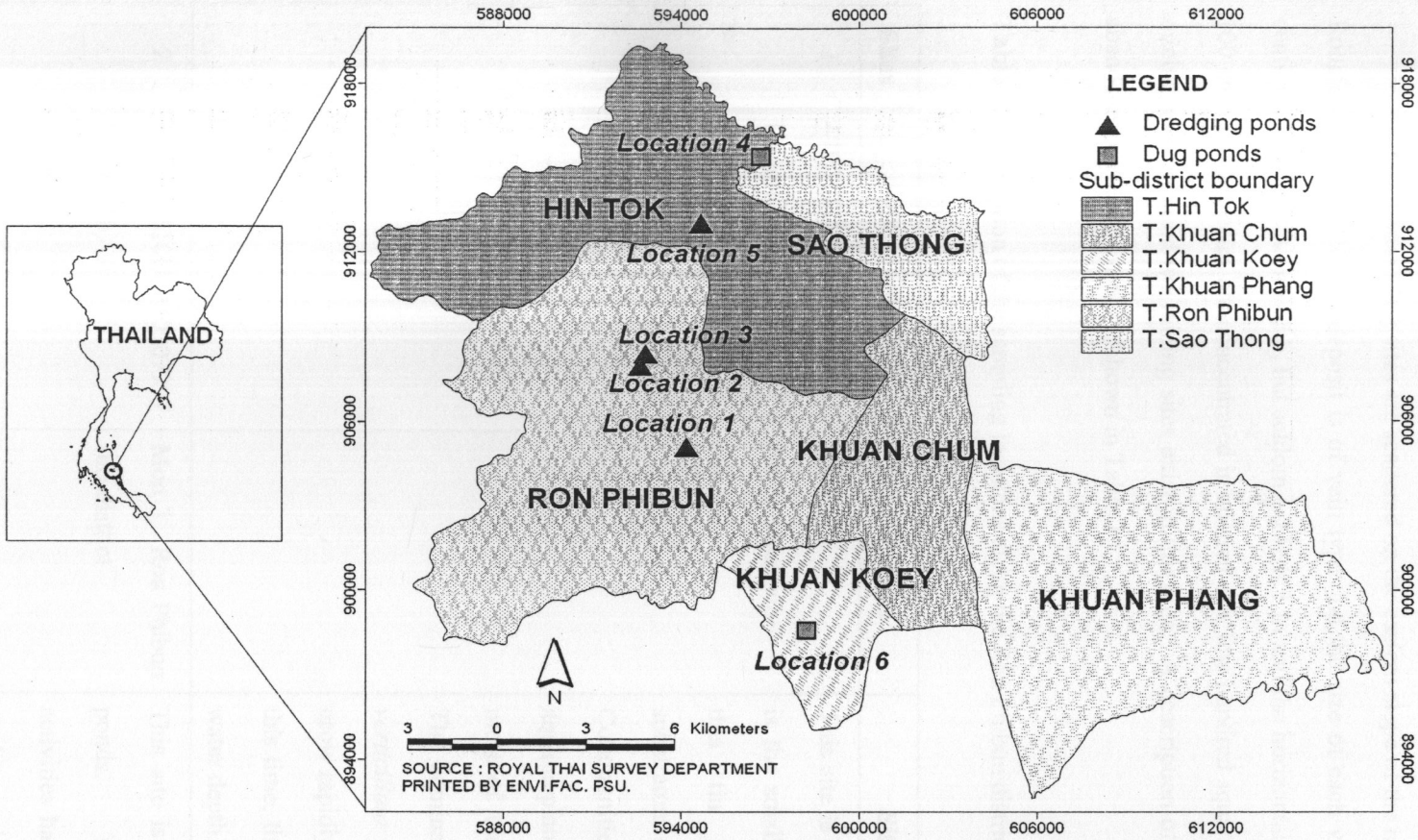


Figure 2. The 6 sampling locations along the Ron Phibun district of Nakhon Si Thammarat province.

Dredging ponds

Old mining sites are scattered on the eastern slope of the mountain encircling the Ronphibun basin. The ore deposit is of vein type and the size of each vein varies from a few centimeters to 30 cm. in width, but seldom exceeds 1 m and the horizontal length is more or less 100 cm. Several veins are concentrated in an area of a few hundred square meters from any one mining site. Around ten mining sites exist in this area. A description of selected ponds in the abandoned tin mining areas is shown in Table 1.

Table 1. Description of the sampling locations along the Ron Phibun district (dredging ponds)

Sites	Ordination	Locations	Site characters
1	47P0594173UTM0905041 (Figure 4a)	Moo 12, Ron Phibun sub-district	This site is relatively shallow and is the smallest dredging pond in this tin mining area. The substratum consists of brown silt. Communities of submerged aquatic plants are also observed in most of the water surface area. The dominant species is *Hydrilla verticillata* (LF) Royle. They grow rapidly in the dry season. At this time, they may interfere with water depth.
2	47P0592610UTM0907997 (Figure 4b)	Moo 3, Ron Phibun sub-district	This site is used for aquaculture ponds. The anthropogenic activities have an effect on water quality and may cause limits or changes in phytoplankton communities. Around the site is also planted many kinds of fruit

			such as rambutan, banana and rubber trees.
3	47P0592799UTM0908476 (Figure 4c)	Moo 3, Ron Phibun sub-district	This site is a big pond. Some part of this site has blooms of *Hydrilla verticillata* (LF) Royle. The water is relatively turbid. The substratum consists of brown silt. This site is also used for multiple purposes such as fishing, aquaculture, and agricultural irrigation.
5	47P0594657UTM0913222 (Figure 4e)	Moo 2, Hin Tok sub-district	This site is the biggest dredging pond in this tin mining area. This water is relatively turbid and brown in colour. In the surface photic zone, the sediments can change seasonally. It is possible that a high deposition rate of sediment in this area is the principle limiting factor for phytoplankton production. The substratum consists of gravel, sand and brown silt.

Dug ponds

There are many available ponds which are all performed by human. The gravel and rocks were added. Additional fencing was constructed to prevent access. The ponds were filled to capacity with water or by that recieved by precipitation. Ponds can serve as a source of irrigation water or emergency water source in the event of fire; provide recreational opportunities such as wildlife watching, skating and fishing; or can dramatically enhance the natural environment, attracting and benefitting wildlife. The water temperature of ponds is fairly even from top to bottom and changes with the outside air temperature. There is little wave action in the water body, and the pond bottom is usually mud-covered. The amount of dissolved oxygen in the pond may vary greatly during a day. Two dug ponds were selected for sampling. A description of the dug ponds is shown in Table 2.

Table 2. Description of the sampling locations along the Ronphibun district (dug ponds)

Sites	Ordination	Locations	Site characters
4	47P0596692UTM0915634 (Figure 4d)	Moo 6, Sao Thong sub-district	This site is artificially dug pond for community use. The water is clear and slow flowing and has an approximate depth of 3 m. An absence of aquatic macrophytes is also the common characteristic of this site. This site was enclosed by agricultural areas.
6	47P0598203UTM0898358 (Figure 4f)	Moo 1, Khuan Koey sub-district	This site is built for irrigationand domestic supply. It is smaller in size and protected by concrete. There is no appearance of any macrophyte species.

2. Climate and hydrology

Ron Phibun's climate is tropical with high temperature and humidity and dominated by monsoons. During each year there are two seasons, nine months rainy (May to November) and three months summer (Febuary to April). In addition, rainy season is affected by tropical monsoon, and can be divided into two seasonal periods. Wind direction is predominantly to the southwest from May to October (light rainy period) and northeast from November to January (heavy rainy period). Monthly average temperature varies from 25.8 °C to 28.5 °C. Ron Phibun has a high average rainfall around 2,381.8 mm/yr (Nakhon Si Thammarat Provisional Administration, http://www.nakhonsithammarat.go.th/air.php). The information on precipitation during 1999 to 2003 is shown in Figure 3.

Both surface and groundwater drainage systems are water sources in Ron Phibun. Surface drainage systems are orientated flowing predominantly west-east, with headwaters in the Ron Na-Suang Chan mountains. Groundwater drainage systems include two types of aquifer 1) a shallow aquifer with a depth of less than 10 m consists of unconsolidated alluvial gravel, sand, and clay, typically yielding 20-50 m^3/h and 2) a deeper carbonate-rich aquifer at a depth of more than 15 m. This aquifer generally yields 10-20 m^3/h with an easterly or southeasterly hydraulic gradient. Hydraulic interaction between the two aquifers is strictly limited due to an intervening clay bed which acts as an efficient aquiclude. The principal bedrock mining areas of the Ron Phibun district occupy the headwaters of the Huai Ron Na River, which flows southeast ward from the granite massif through areas of alluvial mining to the north of Ron Phibun town. The principal alluvial mining areas of the district are drained by the Klong Sak, Klong Rak Mai, and Klong Nam Khun systems. Its all surface drainage is slow flowing and extensively canalized in the east of the main Nakhon Si Thammarat highway (Williams *et al.*, 1996).

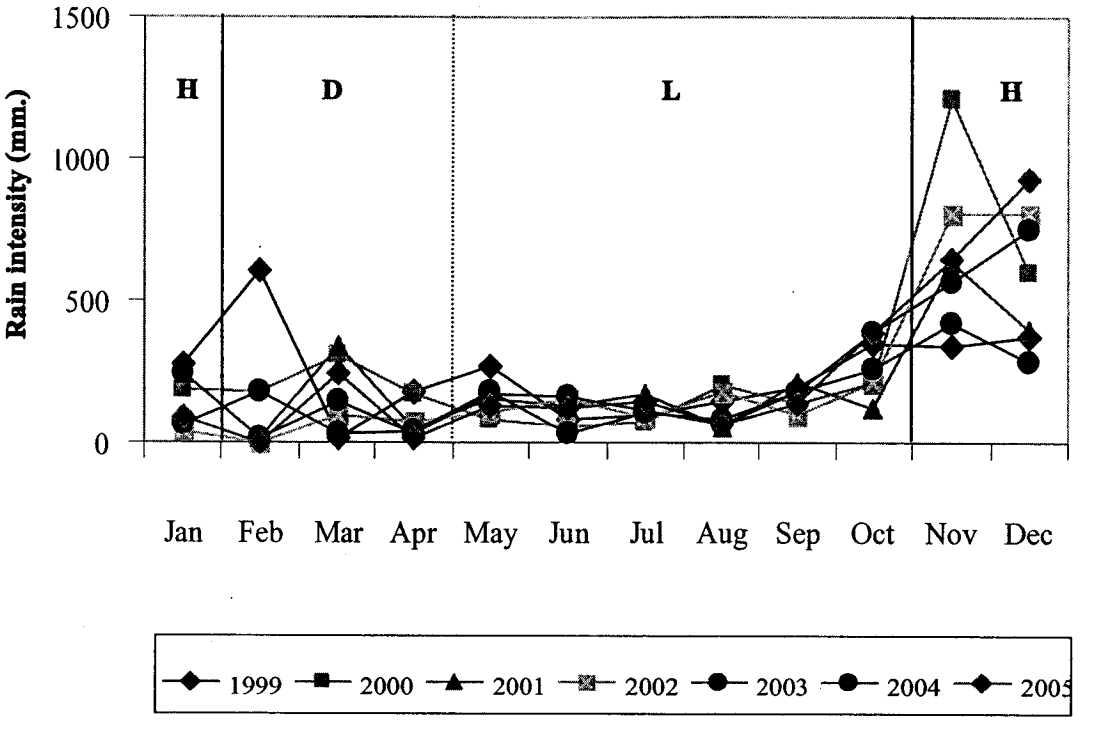
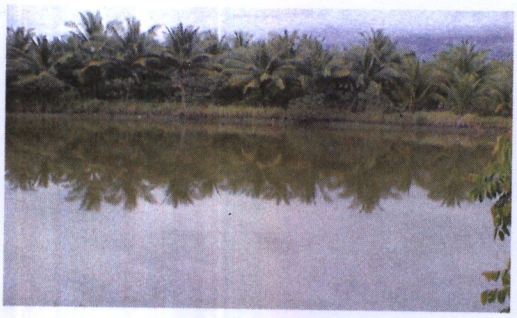


Figure 3. Temporal pattern of rain intensity from 1999 to 2005:

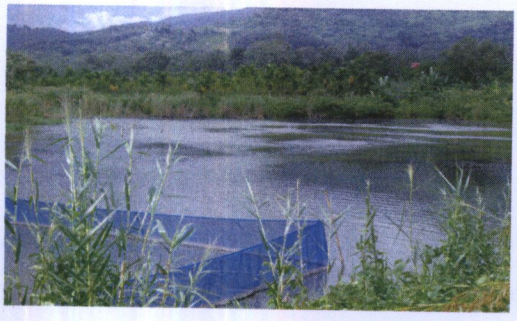
L = light rainy period, H = heavy rainy period, D = dry period.

Source: Meteorological Department (2007)

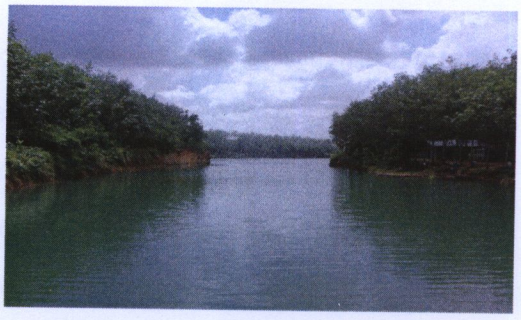


a) location 1

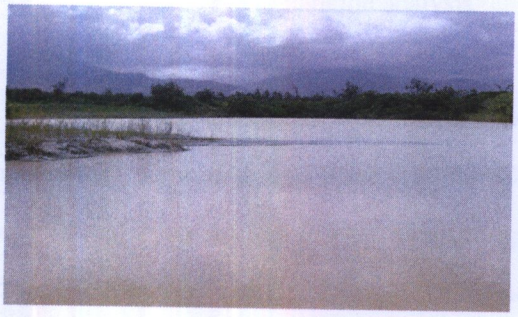
b) location 2



c) location 3



d) location 4



e) location 5

f) location 6

Figure 4. The dredging ponds and dug ponds along the Ron Phibun district

3. Sampling methods and analysis

1. Phytoplankton sampling

Samples for phytoplankton were collected at 3 points and at a depth to 30 cm. Each time thirty-five liters of water sample was filtered through a 20 μm plankton net; the retained material was preserved with buffered formalin to a final concentration of 5-10% formaldehyde for further analysis in the Plankton Research Unit Laboratory.

2. Water sampling

One liter of surface water sample was collected using polyethylene bottle at the same points as for phytoplankton sampling. All samples were stored in ice containers during their transport to the laboratory. Chlorophyll *a*, Total Suspended Solids (TSS), Biochemical Oxygen Demand (BOD_5), nitrite-nitrogen, nitrate-nitrogen, ammonia-nitrogen, dissolved phosphorus and Dissolved Oxygen (DO) concentrations were analysed as soon as possible. Moreover, total arsenic was determined by Hydride Generation Atomic Absorption Spectrophotometry (HG-AAS).

3. Phytoplankton analysis

Phytoplankton classification was conducted by the methods of Croasdale and Flint (1986a), Croasdale and Flint (1986b), Croasdale and Flint (1986c), John *et al.* (2002), Komarek and Anagnostidis (1999), Peerapornpisal (2005), Whitford and Schumacher (1973), Wongrat (2001). For determination of the abundance of phytoplankton, a micropipette was used to add the phytoplankton samples into a Sedgwick-Rafter chamber and specimens were counted with an Olympus CH-2 compound microscope (UNESCO, 1978). The Sedgwick-Rafter counting cell has no lines on the slide for measurement and is rectangular (50x20 mm), 1 mm deep with an area of 1,000 mm^2 and holds 1 mL of water. Count the target cells in the entire counting slide. Replication of counts of one mL samples is recommended for the statistical

treatments. When the counting cell in Sedgwick-Rafter is complete, the phytoplankton density is calculated for each genus (NIO, 2004) by use the following formula:

$$N = \frac{nV_2 \times 1{,}000}{V_1}$$

Where N = total number of phytoplankton cells per liter of water filtered

n = average number of phytoplankton cells in 1 ml of plankton sample

V_2 = volume of plankton concentrate (mL)

V_1 = volume of total water filtered (L)

Furthermore, some phytoplankton genera were filamentous or colonial forms such as *Anabaena* or *Microcystis*, while in others, small unicellular genera of *Chlorella* are generally also found in natural waters. These phytoplankton taxon are not easily observed and counted by Sedgwick-Rafter chamber due to their small in size. Therefore, drop count method was also used for the cell counts in this study (NIO, 2004). The common glass slide mounted with a drop of concentrated phytoplankton sample and covered with cover slip is placed under the microscope provided with a mechanical stage. The phytoplankton are then counted from the microscopic field. In this way all the plankton present in entire microscopic field are counted. The total number of cells then calculated by summing the phytoplankton numbers of all the microscopic fields. If this total number is of one drop of the concentrated phytoplankton, then total number is in 1 mL of the phytoplankton concentration has to be calculated before calculating this, number of drops which from 1 mL has to be counted by adding the drops which form 1 mL has to be counted by adding the drops of water into the graduated centrifuge tube. If one drop of concentrated phytoplankton contains some known number then cells present in 1 mL can be calculated. For example, if 16 drops forms 1 mL, and suppose 50 phytoplankton cells are counted in one drop. Then the plankton in 1 mL are calculated as follows:

Phytoplankton in 1 mL concentrate	=	16 x 50 = 80	cells
Phytoplankton per litre	=	800 x 1,000	cells
	=	800,000	cells

4. Water samples analysis

Chlorophyll *a* concentration was extracted by 90% acetone and then determined by Spectrophotometer following the methodology in APHA, AWWA and WEF (1998; Appendix AI).

Total Suspended Solids (TSS) were determined by gravimetric method (APHA, AWWA and WEF 1998; Appendix AII).

Dissolved Oxygen (DO), oxygen was determined by Winkler method (APHA, AWWA and WEF, 1998; Appendix AIII).

Biochemical Oxygen Demand (BOD_5), oxygen was determined by Winkler method (APHA, AWWA and WEF, 1998; Appendix AIV).

Dissolved phosphorus was determined by ascorbic acid method (APHA, AWWA and WEF, 1998; Appendix AV).

Nitrogen : NO_2^--N was determined by colorimetric method (APHA, AWWA and WEF, 1998; Appendix AVI).

NO_3^--N was determined by colorimetric method after cadmium reduction (APHA, AWWA and WEF, 1998; Appendix AVII).

NH_3-N was determined by phenate method (APHA, AWWA and WEF, 1998; Appendix AVIII).

During sampling, selected environmental factors were determined at all sampling locations. For physical factors, pH was determined by YSI model 60/10 FT, conductivity and water temperature were determined by YSI model 30/10 FT and light intensity was determined by Lux meter in the field at the time of sampling.

5. General procedure for the determining total arsenic and hydride generating conditions.

For estimation of arsenic compounds, 50 mL water samples were filtered through a 0.45 μm cellulose membrane filter. After filtration the water samples was immediately acidified by the addition of 0.05 mL of concentrated hydrochloric (conc. HCl), to provide a pH lower than 2. The analytical technique used for determining total arsenic concentrations was hydride generation atomic absorption spectrophotometry (HG-AAS). The samples are first reduced to As^{3+} prior to analysis. To 1 mL of sample was added 1 mL 6 M HCl and 1 mL 5% (w/v) KI and 5% (w/v) ascorbic acid. Ten percentages HCl was then added to bring the solution to 10 mL. Reduction of As(V) to As(III) occurred within 1 hour at room temperature. As (III) was converted to arsine (AsH_3) by 0.5 % (w/v) sodium borohydride ($NaBH_4$) in 0.05 % (w/v) sodium hydroxide (NaOH). The arsine gas was purged with argon gas to heated quartz cell and atomized. The atomic absorption spectrometer operated at 193.7 nm was equipped with a heated quartz cell. The flow rates in the arsine generation system were as follows; 10% HCl 9 cm^3/min flow rate, 0.5% (w/v) $NaBH_4$ 5 cm^3/min flow rate, argon 50 cm^3/min flow rate. The limit of detection of total arsenic is 0.1 μg/L. This method is modified from manufactured procedure (PEI, 1999).

4. Statistical analysis

The computer statistical package, SPSS for Windows version 12.0 was used to perform the box plot to identify the differences in total arsenic concentrations at each sampling location.

The multivariate statistical programme (MVSP version 3.0, Kovach Computing Services, UK) performs several types of eigenanalysis ordination and was used to carry out diversity indices, cluster analysis and Canonical Correspondence Analysis (CCA) (Kovach, 1998). A diversity analysis comprised the species richness, evenness and Shannon-Weiner diversity indices (log_{10}-based). Ordination analyses included a cluster analysis and CCA. Cluster Analysis was used to establish any similarities of the abundance of phytoplankton species and environmental variables. It was carried out in order to group the sampling locations. Percentage

similarity was applied to the abundance data and to obtain the clusters by the Unweighted Pair Group Method Algorithm (UPGMA). The cluster method chosen was the average lingkage. In addition, Canonical Correspondence Analysis (CCA), a direct gradient analysis technique, was used to elucidate the relationship between biological assemblages of species and their environment. Rare taxa create a large number of zero values and noise in data sets, and this in turn can cause increased distortion of ordinations. To reduce the amount of noise, rare taxa (those with an occurrence of $< 0.1\%$ of total phytoplankton number) were removed from the data set. CCA assumes the data have a multivariate normal distribution and Palmer (1993) recommended transforming environmental data into log values. Since there is no way to test for a multivariate normal distribution, the CCA was run with both log transformed and untransformed environmental data. The untransformed environmental data did not alter the results from this study in any way, so the results presented in this research are based on transformed data. The data for cluster analysis and CCA were standardized by a $\log(x+1)$ transformation to meet the basic requirement of the statistical test, except for pH the values that had skewed distributions.

CHAPTER 3

RESULTS

3.1 General characteristics of water quality in the study area

Total arsenic (As)

The total arsenic values in water samples from the six sampling locations were shown in Figure 5. Among the water samples measured, total arsenic values ranged from 0.30±0.01 to 167.85±0.96 μg/L (Appendix B). The highest concentration of total arsenic occurred in April at location 5 and the lowest in October at location 4 (Figure 6). The highest mean value was 84.41±13.95 μg/L for location 5, followed by location 1 (69.31±5.66 μg/L), and the lowest values of 0.92±0.35 μg/L were for location 4. The mean value of total arsenic was the highest in June (62.06±21.03 μg/L), followed by April (50.35±25.79 μg/L) and May (46.17±24.53 μg/L), whilst the lowest mean value was in November (23.39±6.43 μg/L) (Appendix CI). For all sampling locations, it may be observed that locations 1, 3 and 5 had high fluctuation during the sampling periods and obviously showed the highest values during the dry period. The highest value during the dry period was recorded in April at location 5 (167.85±0.96 μg/L), followed by February at location 1 (98.96±0.96 μg/L). The mean values for total arsenic in High Arsenic Contaminated Ponds (HACP) – location 1 (69.31±5.66 μg/L), location 3 (39.06±3.31 μg/L) and location 5 (84.41±13.95 μg/L) were higher than that of the Low Arsenic Contaminated Ponds (LACP) – location 2 (13.64±0.54 μg/L), location 4 (0.92±0.35 μg/L) and location 6 (7.24±0.48 μg/L). In addition, total arsenic values showed high variation in HACP and less in LACP. The HACP values ranged from 19.00±0.03 to 167.85±0.96 μg/L, whilst the LACP values ranged from 0.30±0.01 to 16.08±0.20 μg/L.

Water temperature

Temperature generally showed little variability during the time of sampling. The maximum-minimum values were in the range of 28.3±0.3 to 33.6±0.7 °C (Appendix B). Of the mean values the highest were in April (33.6±0.7 °C) and the lowest in December (28.3±0.3 °C) (Appendix CII). As for observations on seasonal variations the highest values were determined during the dry period, and the lowest in the heavy rainy period (Figure 7).

Light intensity

Light intensity ranged from 466.9±84.2 to 2,002.7±345.6 lux (Appendix B). The mean values of light intensity were also calculated and the sample mean indicated that light intensity was significantly different depending on the season. The light intensity records show discreet seasonal difference, with minimum values during in December (466.9±84.2 lux) and maximum values during in September (2,002.7±345.6 lux) and March (1,747.1±344.9 lux) (Figure 8; Appendix CIII).

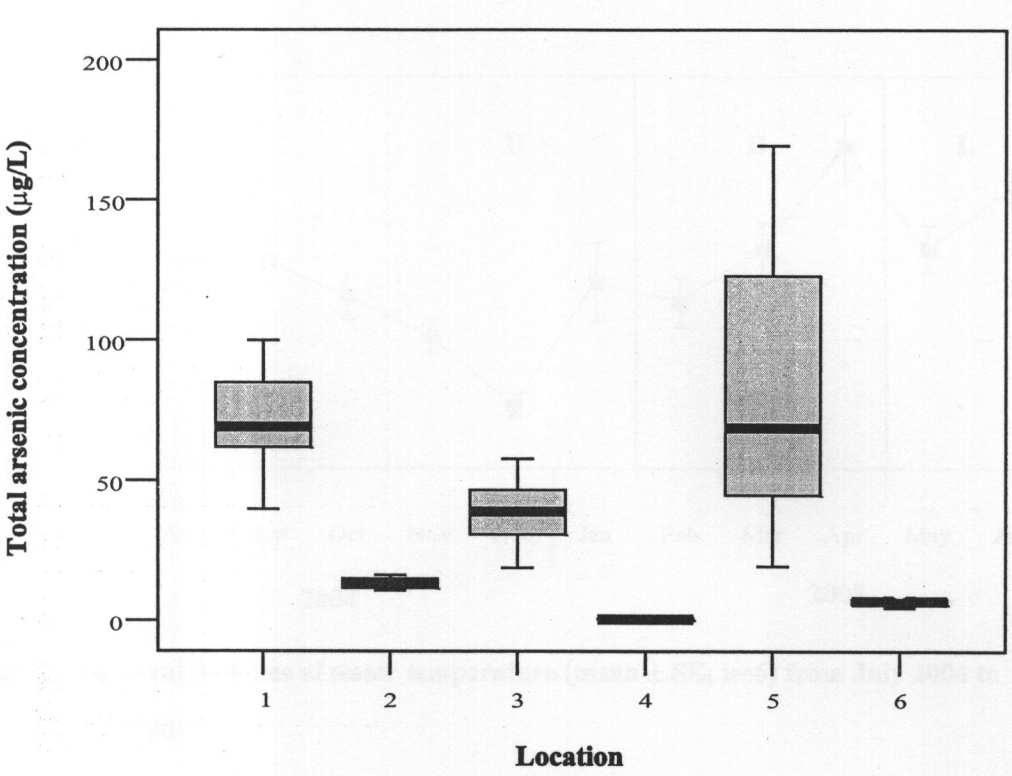


Figure 5. Boxplot of arsenic concentration presented in sampling locations in the Ron Phibun district of Nakhon Si Thammarat province.

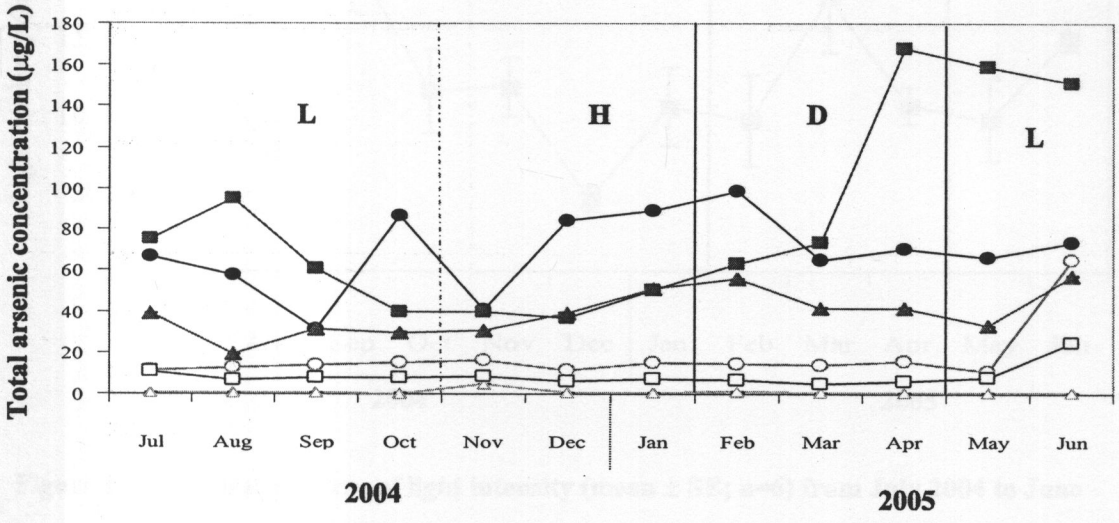


Figure 6. Temporal patterns of total arsenic (mean ± SE; n=3) from July 2004 to June 2005.

location 1, location 2, location 3, location 4,

location 5, location 6,

L = light rainy period, H = heavy rainy period, D = dry period.

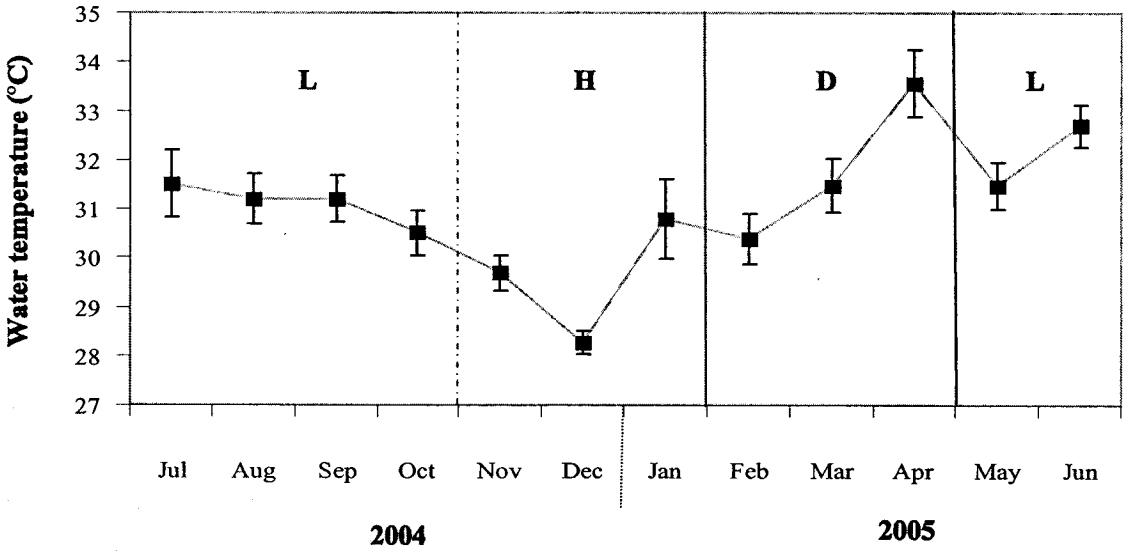


Figure 7. Temporal patterns of water temperature (mean ± SE; n=6) from July 2004 to June 2005.

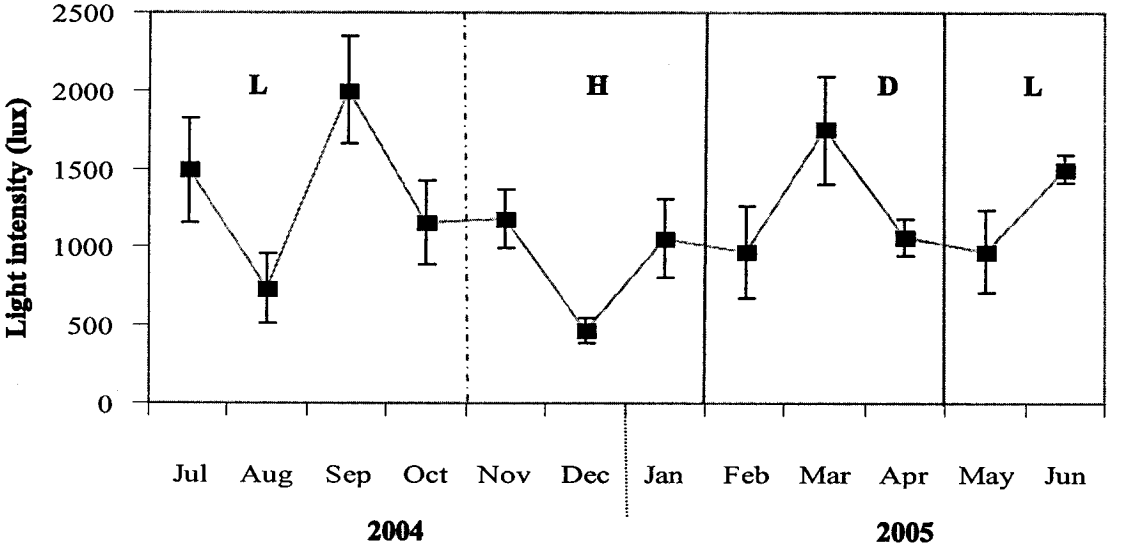


Figure 8. Temporal patterns of light intensity (mean ± SE; n=6) from July 2004 to June 2005. ■ mean values for all sampling locations, L = light rainy period, H = heavy rainy period, D = dry period.

Conductivity

The conductivity of water varied from 21.17±0.32 to 275.80±1.65 μS/cm (Appendix B). The lowest conductivity value was detected in January at location 2, whilst the highest conductivity was in June at location 6. The mean conductivity values at locations 5 (140.28±16.08 μS/cm) and 6 (183.10±18.99 μS/cm) were significantly higher than at the other locations. June and May fall in the light rainy period when conductivity values are high. The mean values in June and May were 141.39±32.48 and 126.97±34.44 μS/cm, respectively. The conductivity values showed high variability in locations 5 and 6. They ranged from 84.50±14.25 to 214.40±0.30 μS/cm with a mean value of 141.59±16.08 μS/cm and 119.13±0.73 to 275.80±1.65 μS/cm with a mean value of 185.15±18.99 μS/cm for locations 5 and 6, respectively. The observed conductivity values in locations 3 and 4 showed moderate changes. They ranged from 46.73±1.15 to 114.50±1.95 μS/cm with a mean value of 67.34±7.30 μS/cm and 38.10±0.70 to 89.33±0.90 μS/cm with a mean value of 57.71±5.58 μS/cm for locations 3 and 4, respectively. In addition, the conductivity of location 2 was the lowest and demonstrated little fluctuation, ranging from 21.17±0.32 to 50.50±1.19 μS/cm, with the mean recorded at 29.61±2.94 μS/cm (Figure 9; Appendix CIV).

pH

The pH values varied greatly from 4.32±0.12 to 8.28±0.13 (Appendix B). The lowest pH value was detected in April at location 2, whilst the highest pH value was in March at location 3. The mean pH value at location 2 (6.00±0.32) was lower than the others, whereas at location 6 the mean pH (6.67±0.28) was higher than the others. The highest mean pH levels were in March (7.86±0.12), followed by July (7.37±0.24) and the lowest in April and May (5.40±0.36 and 5.40±0.20, respectively). The changes in pH values were difficult to explain and were not totally dependent upon seasonal changes. Additionally, pH values generally had a similar pattern in all locations during the sampling period. They ranged from 5.19±0.43 to 7.60±0.21 for location 1, 4.32±0.13 to 7.88±0.12 for location 2, 4.43±0.05 to 8.28±0.13 for location 3, 4.79±0.13 to 8.05±0.10 for location 4, 5.21±0.03 to 8.10±0.08 for location 5 and 5.33±0.04 to

8.02±0.18 for location 6. The mean values for those sampling locations were 6.37±0.23, 6.00±0.32, 6.24±0.33, 6.10±0.24, 6.66±0.26, 6.67±0.28, respectively (Figure 10; Appendix CV).

Dissolved Oxygen (DO)

DO ranged from 2.02±0.55 to 7.86±0.39 mg/L (Appendix B). It was least in December at location 1, and highest in September at location 6. Values at location 4 were generally slightly higher than at the other locations, and the mean value at location 4 was 6.13±0.2 mg/L. The overall mean value of DO was highest in September (7.19±0.22 mg/L), and lowest in April (4.66±0.38 mg/L). DO levels can changed dramatically and appear not to be affected by seasonal changes. DO concentrations in water at different sampling locations during different periods of time appear to be variable. They ranged from 2.02±0.55 to 6.67±0.46 mg/L for location 1, 3.67±1.71 to 7.56±0.10 mg/L for location 2, 3.20±0.42 to 6.91±0.65 mg/L for location 3, 4.98±0.62 to 7.38±0.17 mg/L for location 4, 4.03±0.40 to 7.30±0.17 mg/L for location 5 and 4.50±0.14 to 7.86±0.40 mg/L for location 6. The mean values for those sampling locations were 4.56±0.42 mg/L, 5.97±0.33 mg/L, 5.17±0.32 mg/L, 6.13±0.20 mg/L, 5.68±0.33 mg/L, 5.70±0.30 mg/L, respectively (Appendix CVI). From these observations, it seems that the surface waters were normally saturated with oxygen (> 4 mg/L), except during July and December at location 1 when measurements showed a slightly lower value (Figure 11).

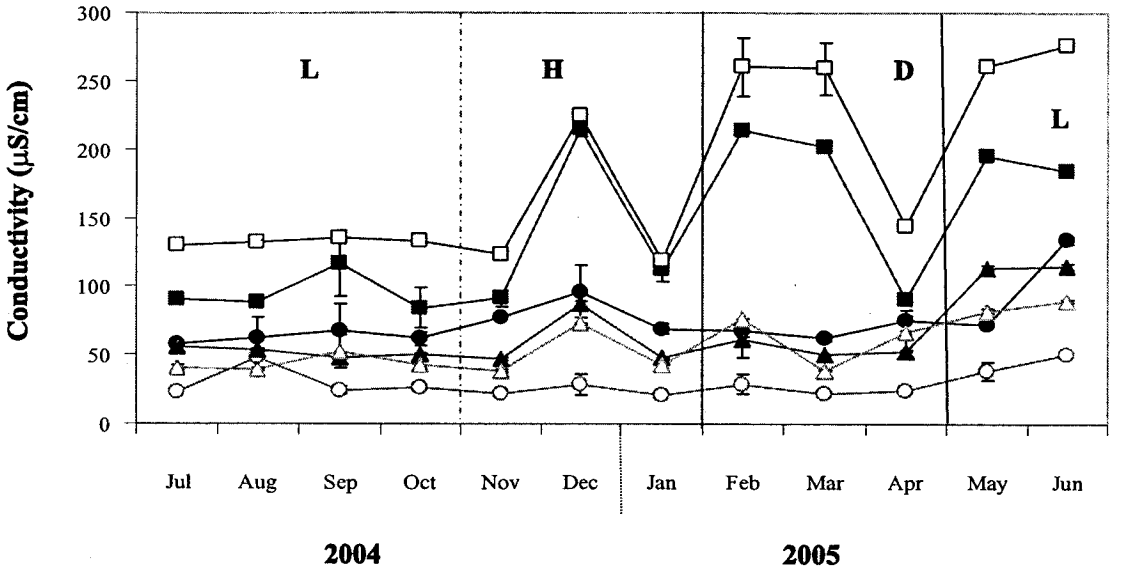


Figure 9. Temporal patterns of conductivity (mean ± SE; n=3) from July 2004 to June 2005.

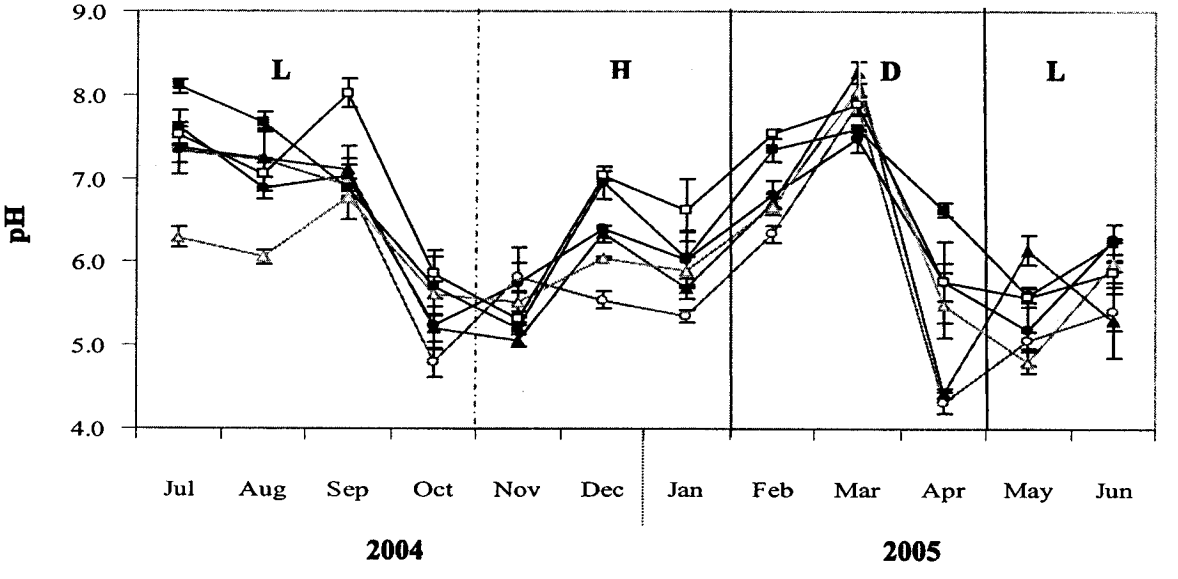


Figure 10. Temporal patterns of pH (mean ± SE; n=3) from July 2004 to June 2005.

location 1, location 2, location 3, location 4,

location 5, location 6,

L = light rainy period, H = heavy rainy period, D = dry period.

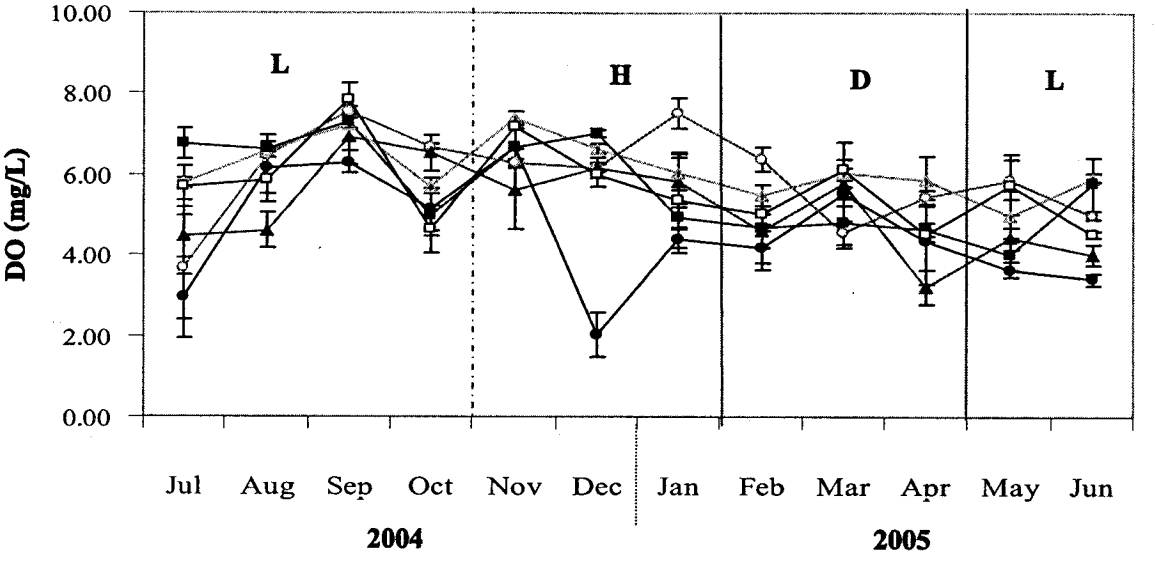


Figure 11. Temporal patterns of Dissolved Oxygen (DO) (mean ± SE; n=3) from July 2004 to June 2005.

—●— location 1, —○— location 2, —▲— location 3, —△— location 4,

—■— location 5, —□— location 6,

L = light rainy period, H = heavy rainy period, D = dry period.

Total Suspended Solids (TSS)

TSS values ranged from 1.5 to 296.5 mg/L (Appendix B). Location 5 had a high concentration in October (296.5 mg/L) and November (236.3 mg/L). A low value of 1.5 mg/L was recorded on January at location 4. The mean value of location 5 was the highest (75.4 mg/L), and this was in contrast with other locations where the means were low: 6.6, 7.1, 10.4, 2.9, 9.5 mg/L at locations 1, 2, 3, 4, 6, respectively. The mean value of TSS was highest in October (55.3 mg/L), and lowest in February (6.2 mg/L) (Appendix CVII). TSS values at the sampling locations were generally low during the study period, except at location 5 which was distinctly different to the other locations. TSS values in location 5 generally increased during the rainy periods (both in the light rainy period and the heavy rainy period), whereas there was no differences in TSS values at the other locations during the changes in season. They ranged from 11 to 296.5 mg/L, with a mean value of 75.4±30.0 mg/L for location 5, and 1.5 to 22.1 mg/L, with a mean value of 7.3±0.6 mg/L for the remaining sampling locations (Figure 12).

Biochemical Oxygen Demand (BOD_5)

Maximum-minimum values of BOD_5 were in the range of 3.36 to 5.18 mg/L (Appendix B), respectively. The results showed that the BOD_5 values were highest in May at location 6, and lowest in April at location 4. The highest mean BOD_5 value was at location 3 (2.32 mg/L), whilst location 5 had the lowest mean value (1.77 mg/L). In addition, the mean reached its highest value in May (3.57 mg/L), followed by September (2.58 mg/L) and July (2.51 mg/L), respectively. BOD_5 concentrations tended to increase during May at all sampling locations, and decreased again in June, whereas values in other months seem to have no consistency. BOD_5 values generally showed little variability in space and time. They ranged from 0.90 to 3.36 mg/L for location 1, 0.63 to 3.48 mg/L for location 2, 0.70 to 3.84 mg/L for location 3, 0.20 to 3.39 mg/L for location 4, 0.40 to 3.61 mg/L for location 5 and 0.63 to 5.18 mg/L for location 6. The mean values for those sampling locations were 2.18±0.24, 1.80±0.26, 2.32±0.25, 2.06±0.27, 1.77±0.32 and 2.07±0.34 mg/L, respectively (Figure 13; Appendix CVIII).

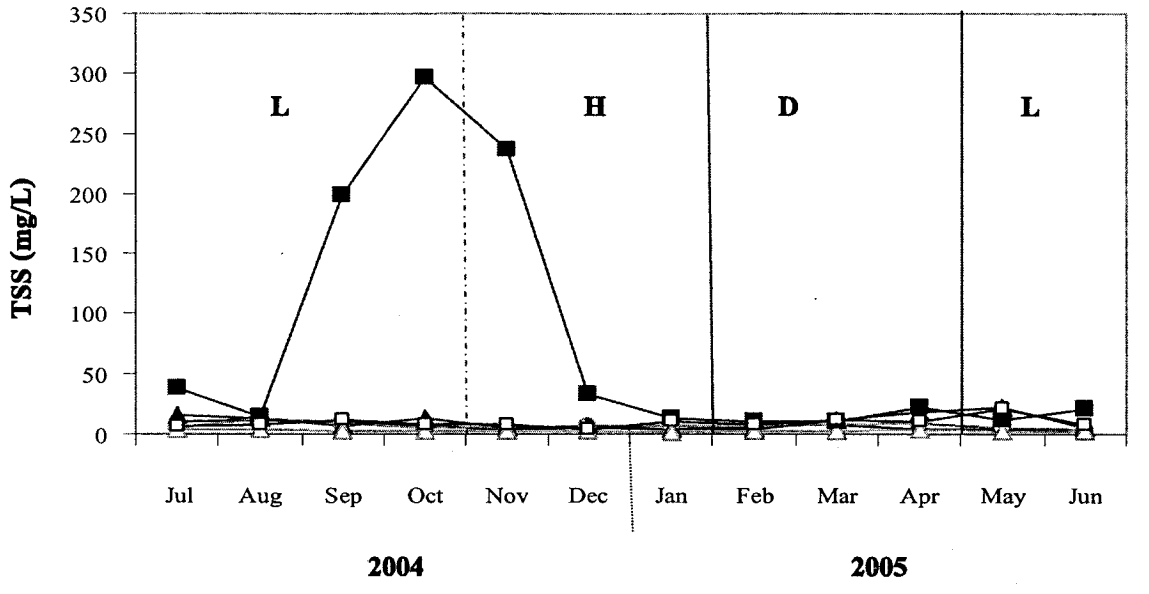


Figure 12. Temporal patterns of Total Suspended Solids (TSS) (mean ± SE; n=3) from July 2004 to June 2005.

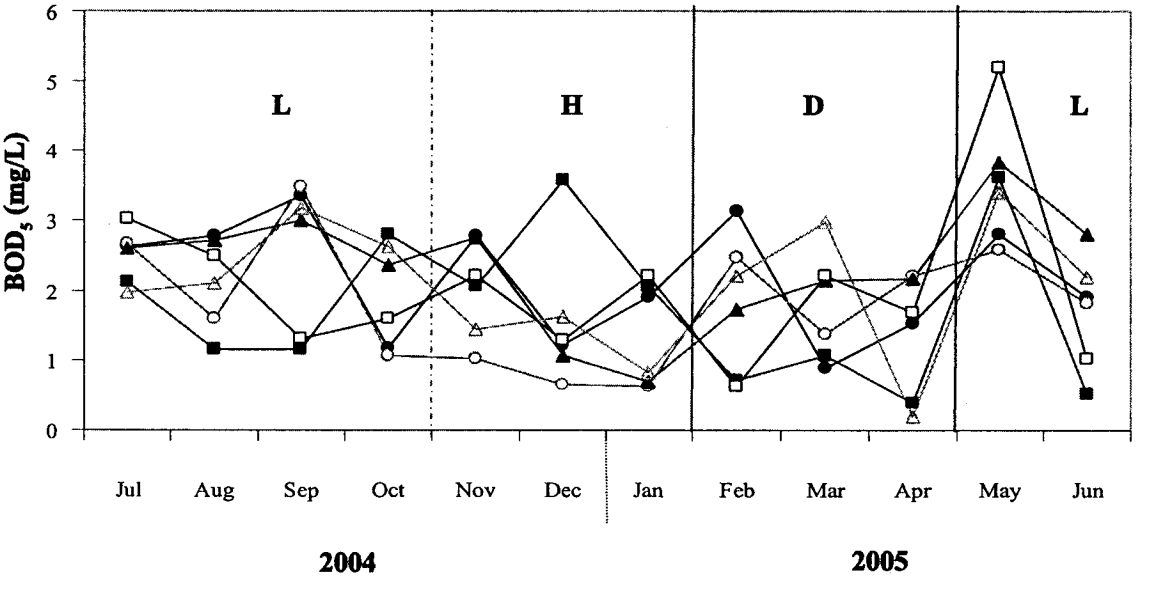


Figure 13. Temporal patterns of Biochemical Oxygen Demand (BOD_5) (mean ± SE; n=3) from July 2004 to June 2005.

location 1, location 2, location 3, location 4, location 5, location 6,

L = light rainy period, H = heavy rainy period, D = dry period.

Nitrogen

Concentrations of nitrite-nitrogen were always below the detection level of the method used (0.01 mg NO_2^--N/L).

Nitrate-nitrogen concentrations ranged from 0.01±0.000 to 0.24±0.001 mg NO_3^--N/L (Appendix B). The highest value was found in February at location 6. At location 1 values were higher than at the other locations with a mean of 0.08±0.02 mg NO_3^--N/L. The overall mean values of nitrate-nitrogen were highest in February (0.12±0.03 mg NO_3^--N/L), followed by June (0.11±0.03 mg NO_3^--N/L), and lowest in November and December (0.01±0.002 and 0.01±0.005 mg NO_3^--N/L, respectively) (Appendix CIX). The lowest values were generally found during the heavy rainy period (December 2004 to January 2005), except for location 1(Figure 14).

Ammonia-nitrogen concentrations were always low ranging from undetectable values to 0.09±0.005 mg NH_3-N/L (Appendix B). The highest concentration was found in July at location 6, whereas in some months, at all sampling locations, ammonia-nitrogen was below the detectable level. At location 1, ammonia-nitrogen concentrations were higher than at the other locations with a mean value of 0.03±0.01 mg NH_3-N/L. It was also shown that the mean value of ammonia-nitrogen was highest in December (0.06±0.006 mg NH_3-N/L), followed by July (0.04±0.016 mg NH_3-N/L) (Appendix CX). In this study, ammonia-nitrogen levels were slightly higher than those of nitrate-nitrogen. The ammonia-nitrogen tended to be higher in all sampling locations during the heavy rainy period (December), however, nitrate-nitrogen were generally low in December and increased markedly in February (Figure 15).

Overall, nitrate-nitrogen and ammonia-nitrogen are varied from time to time but not obviously from location to location. Nitrate-nitrogen ranged from 0.01±0 to 0.23±0.001, 0.01±0.001 to 0.07±0.001, 0.01±0.000 to 0.12±0.002, 0.01±0.000 to 0.08±0.001, 0.01±0.000 to 0.20±0.003 and 0.01±0.000 to 0.24±0.002 mg NO_3^--N/L for locations 1, 2, 3, 4, 5 and 6, respectively. The mean nitrate-nitrogen values for those sampling locations were 0.08±0.02, 0.04±0.01, 0.05±0.01, 0.04±0.01, 0.06±0.02 and 0.06±0.02 mg NO_3^--N/L, respectively. Additionally, ammonia-nitrogen ranged from 0 to 0.08±0.001 mg NH_3-N/L for location 1, 0 to 0.06±0.001 mg NH_3-N/L for location 2, 0 to 0.06±0.001 mg NH_3-N/L for location 3, 0 to 0.05±0.000 mg NH_3-N/L for location 4, 0 to 0.08±0.001 mg NH_3-N/L for location 5 and 0 to

0.09±0.005 mg NH_3-N/L for location 6. The mean ammonia-nitrogen values for those sampling locations were 0.03±0.01, 0.81±0.01, 0.02±0.01, 0.01±0.00, 0.02±0.01 and 0.02±0.01 mg NH_3-N/L, respectively.

Dissolved phosphorus (PO_4^{3-}-P)

Dissolved phosphorus levels ranged from 0.01±0.000 to 0.24±0.001 mg PO_4^{3-}-P/L (Appendix B). The highest value was found in May at location 5, whereas the lowest values were found in almost all months at location 4 and in some months at locations 2, 5, and 6. Maximum and minimum values of the means were 0.04±0.002, 0.04±0.008 at locations 1 and 5, and 0.01±0.001 mg PO_4^{3-}-P/L at locations 4 and 6, respectively. It was also found that the mean of dissolved phosphorus was the highest in October and May (0.04±0.01 and 0.04±0.01 mg PO_4^{3-}-P/L, respectively), followed by September and March (0.03±0.01 and 0.03±0.01 mg PO_4^{3-}-P/L, respectively) and the lowest values were in July, August, November, December, January, February, April and June (0.02±0.01, 0.02±0.01, 0.02±0.00, 0.02±0.01 0.02±0.01, 0.02±0.01, 0.02±0.01, 0.02±0.00 mg PO_4^{3-}-P/L, respectively). Dissolved phosphorus concentrations changed seasonally at all sampling locations, except location 4. They also tended to increase during the rainy periods (mainly in October and December) (Figure 16).

Of all the sampling locations, location 4 had the lowest dissolved phosphorus values (0.01±0.000 mg PO_4^{3-}-P/L) at all times. Dissolved phosphorus ranged from 0.03±0.001 to 0.06±0.001 mg PO_4^{3-}-P/L for location 1, 0.01±0.000 to 0.03±0.000 mg PO_4^{3-}-P/L for location 2, 0.02±0.001 to 0.05±0.000 mg PO_4^{3-}-P/L for location 3, 0.01±0.000 to 0.10±0.000 mg PO_4^{3-}-P/L for location 5 and 0.01±0 to 0.02±0.002 mg PO_4^{3-}-P/L for location 6. The mean values for those sampling locations were 0.04±0.002, 0.02±0.002, 0.03±0.003, 0.04±0.008 and 0.01±0.001 mg PO_4^{3-}-P/L, respectively (Appendix CXI).

Chlorophyll *a*

Chlorophyll *a* levels were highly variable during the study period ranging between 1.0 ± 0.9 and 71.0 ± 1.0 µg/L (Appendix B), and achieving a maximum value in July at location 3. The lowest value was present in May at location 5 at the time when total suspended solids at that location were at its highest value. The highest mean value of chlorophyll *a* concentrations was 28.3 ± 5.3 µg/L at location 3, whilst the lowest mean value was 4.4 ± 0.6 µg/L at location 4. July had the highest mean value of chlorophyll *a* concentrations at 29.1 ± 11.7 µg/L, and February the lowest mean value of chlorophyll *a* (8.7 ± 2.3 µg/L). Significant levels of chlorophyll *a* were found in locations 1, 2, 3 and 6 (the average values were 21.9 ± 5.3, 10.8 ± 1.1, 28.3 ± 5.3, 23.3 ± 2.5 µg/L respectively). Whereas, the average values in locations 4 and 5 were 5.4 ± 0.6, 4.4 ± 0.9 µg/L respectively. In general, chlorophyll *a* concentrations were relatively low and seemed to follow a similar pattern to the number and density of the phytoplankton during the heavy rainy period (November and December).

For chlorophyll *a* values, locations 1 and 3 varied significantly more than other locations. They ranged from 3.7 ± 0.9 to 58.7 ± 3.8 µg/L with a mean value of 21.9 ± 5.3 µg/L for location 1 and 3.0 ± 0.6 to 71.0 ± 1.0 µg/L with a mean value of 28.3 ± 5.3 µg/L for location 3. Whereas, the chlorophyll *a* of location 2 ranged from 5.0 ± 0 to 17.0 ± 2.5 µg/L, location 4 ranged from 2.0 ± 0.7 to 8.0 ± 0.0 µg/L, location 5 ranged from 1.0 ± 0.0 to 11.3 ± 1.5 µg/L and location 6 ranged from 11.3 ± 0.3 to 39.0 ± 1 µg/L. The mean values of the remaining locations were 10.8 ± 1.1, 4.4 ± 0.6, 5.4 ± 0.9 and 23.3 ± 2.5 µg/L, respectively (Figure 17; Appendix CXII).

Referring to the methods modified by the Applied Algal Research Laboratory, Chiang Mai University (Peerapornpisal *et al.*, 2004), by altering the amounts of DO, BOD, conductivity, nitrate-nitrogen, ammonia-nitrogen, dissolved phosphorus and chlorophyll *a* (Appendix D), all sampling locations seem to have a similar limnological behaviour. According to the magnitude of those parameters, locations 3 and 5 can be classified as having oligo-mesotrophic status, whilst other locations showed some differences in water quality at some sampling periods (Table 3).

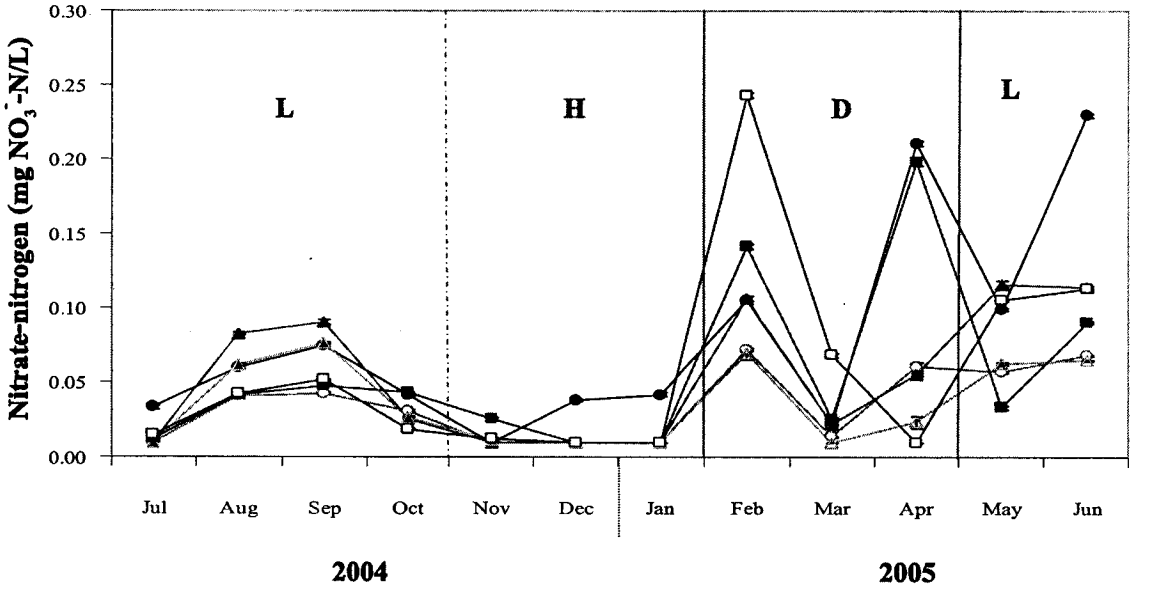


Figure 14. Temporal patterns of nitrate-nitrogen (NO_3^--N) (mean ± SE; n=3) from July 2004 to June 2005.

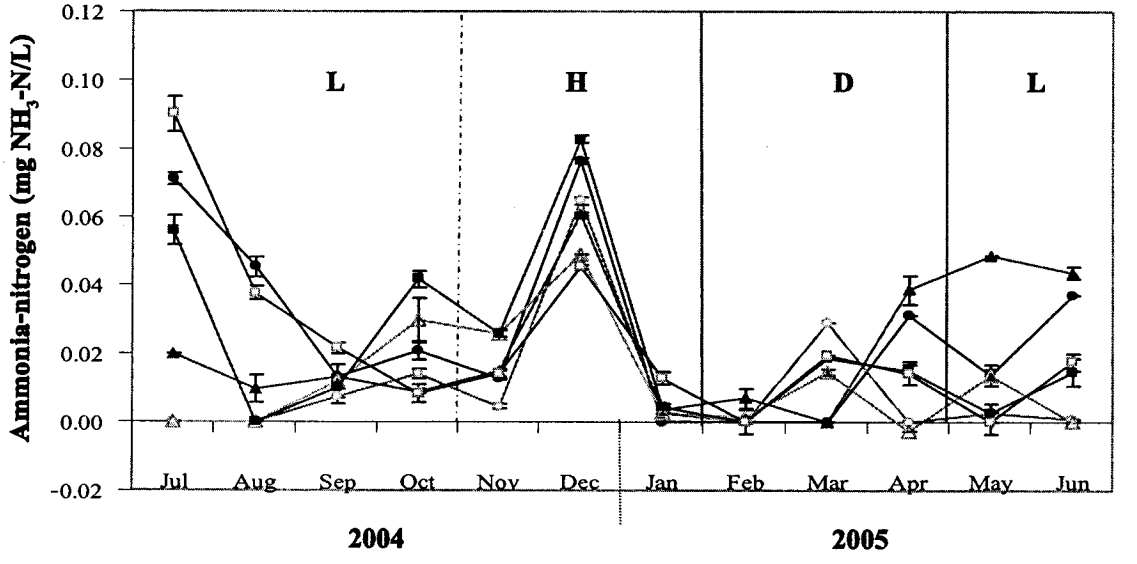


Figure 15. Temporal patterns of ammonia-nitrogen (NH_3-N) (mean ± SE; n=3) from July 2004 to June 2005.

—●— location 1, —○— location 2, —▲— location 3, —△— location 4,

—■— location 5, —□— location 6,

L = light rainy period, H = heavy rainy period, D = dry period.

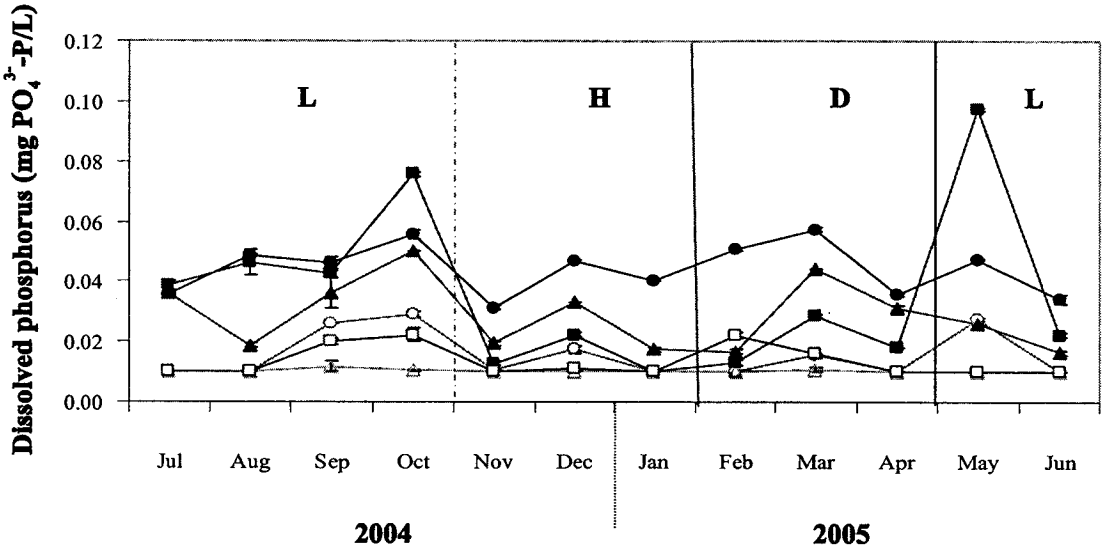


Figure 16. Temporal patterns of dissolved phosphorus (PO_4^{3-}-P) (mean ± SE; n=3) from July 2004 to June 2005.

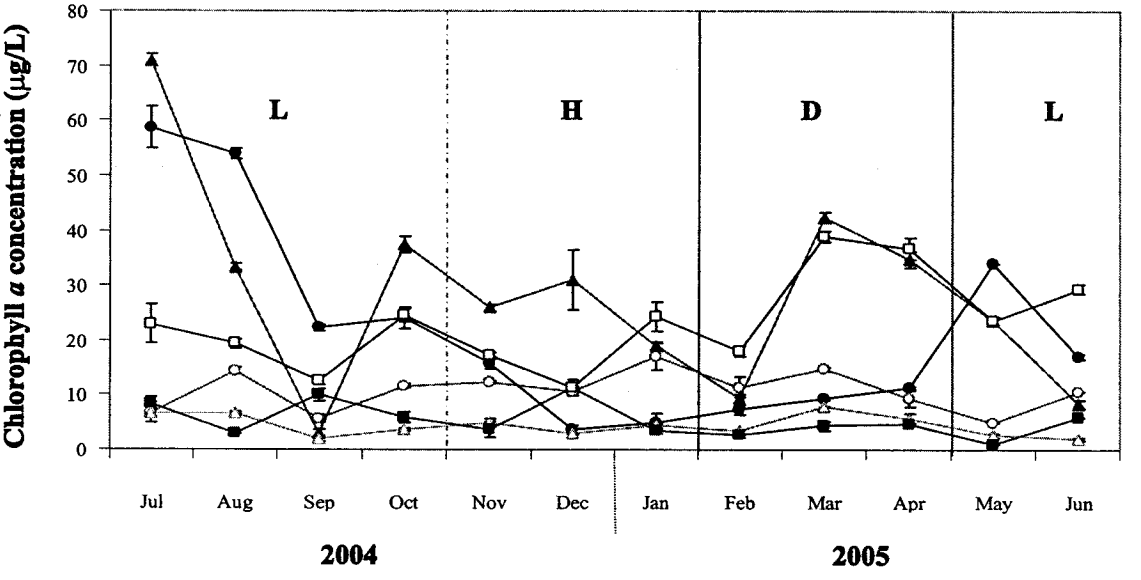


Figure 17. Temporal patterns of chlorophyll *a* (mean ± SE; n=3) from July 2004 to June 2005.

location 1, location 2, location 3, location 4,

location 5, location 6,

L = light rainy period, H = heavy rainy period, D = dry period.

Table 3. Water quality status of sampling locations in arsenic contaminated waters determined from July 2004 to June 2005.

Months	Location 1	Location 2	Location 3	Location 4	Location 5	Location 6
July	Mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic
August	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic
September	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic
October	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic
November	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligotrophic	Oligo-mesotrophic	Oligo-mesotrophic
December	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic
January	Oligo-mesotrophic	Oligotrophic	Oligo-mesotrophic	Oligotrophic	Oligo-mesotrophic	Oligo-mesotrophic
February	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic
March	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Mesotrophic
April	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligotrophic	Oligo-mesotrophic	Oligo-mesotrophic
May	Mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Mesotrophic
June	Mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Oligo-mesotrophic	Mesotrophic

3.2 Species composition and diversity of phytoplankton in arsenic contaminated waters

During these investigations, the composition of phytoplankton showed a remarkable diversity. A total of seventy-eight genera of phytoplankton were identified. Of the taxa, Chlorophyceae was the largest group with 40 genera, followed by Cyanophyceae (18 genera), Bacillariophyceae (11 genera), Euglenophyceae (4 genera), Chrysophyceae (3 genera) and Pyrrophyceae (2 genera). A floristic list is given in Table 4. The Chlorophyceae comprised of 51.3 % (Figure 18) of the total taxa, followed by the Cyanophyceae 23.1 %, Bacillariophyceae 14.1 %, Euglenophyceae 5.1 %, Chrysophyceae 3.8 % and Pyrrophyceae 2.6 %. Comparison of the genera number of the phytoplankton flora between High Arsenic Contaminated Ponds (HACP) and Low Arsenic Contaminated Ponds (LACP) revealed little differences. The number of genera in the former was 75 and the latter was 72 genera (Figure 19). The highest number of taxa (64) was recorded in location 1, whereas locations 5 and 6 had the lowest number of taxa (54) (Figure 20).

Obviously, a decrease of phytoplankton species diversity observed in this study was correlated with rain intensity (Figure 21). Chlorophytes and cyanophytes were found mainly in phytoplankton assemblage and their genera number decreased during the heavy rainy period (November to December 2004) (Figures 22-23). During the heavy rainy period, the chlorophytes constituted the richest with 27 genera or 45.8 % of the total genera number. Thirteen genera of cyanophytes were found, constituting around 22.0 % of the total genera number. In the dry period, 17 genera of cyanophytes and 29 genera of chlorophytes were identified adding up to around 25.8 % and 43.9 % of the total genera number, respectively. The number of chlorophyte genera increased remarkably during the light rainy period (40 genera), estimated at 55.6 % of the total genera number, whilst genera number of cyanophytes (13 genera) increased only slightly, estimated at 18.1 % of the total genera number (Table 5 and Figures 24a, b and c).

Table 4. Spatial and temporal occurrence of taxa registered from sampling locations in the arsenic contaminated waters from July 2004 to June 2005 : Jan=January, Feb=Febuary, Mar=March, Apr=April, May=May, Jun=June, Jul=July, Aug=August, Sep=September, Oct=October, Nov=November, Dec=December, HACP=high arsenic contaminated ponds, LACP=low arsenic contaminated ponds, exc=except.

	Spatial occurrence		Temporal occurrence
	HACP	LACP	
Division Cyanophyta			
Anabaena spp.	1,3,5	2,4,6	All months exc Mar, May
Anabaenopsis sp.	5	4,6	Jul, Aug, Feb, Apr, May, Jun
Anacystis sp.	-	6	Jul
Aphanocapsa sp.	1,3	2,4,6	All months exc Nov, Dec, May
Calothrix sp.	5	2	Apr, May
Chroococcus spp.	1,3,5	2,4,6	All months
Cylindrospermopsis sp.	1,3,5	2,4,6	All months exc Dec
Cylindrospermum sp.	1,3,5	2,4,6	All months exc Dec
Gloeocapsa sp.	1,3,5	2,4,6	All months
Microcystis spp.	1,3,5	2,4,6	All months
Merismopedia spp.	1,3,5	2,4,6	All months
Oscillatoria spp.	1,3,5	2,4,6	All months
Phormidium spp.	1,3,5	2,4,6	All months
Raphidiopsis sp.	1,3,5	2,4,6	All months exc Sep, Nov, Dec
Spirulina sp.	1,3	2,6	Oct, Dec, Jan, Feb, Mar, Apr, Jun
Synechococcus sp.	1	6	Mar, May
Tolypothrix sp.	1,5	6	Mar, Apr, May
Trichodesmium sp.	1,3,5	-	Oct, Dec, Feb, Mar, May
Division Chlorophyta			
Ankistrodesmus spp.	1,3,5	2,4,6	All months
Botryococcus sp.	3,5	2,4	All months exc Jul, Dec, May, Jun
Chlorella sp.	1,3,5	2,4,6	All months
Chlorococcum sp.	1,5	2,4,6	Jul, Sep, Oct, Nov, Jan, Apr
Chodatella sp.	1	4	Jul, May
Clamydomonas sp.	1	6	Jul
Closterium sp.	1,3,5	2,4,6	All months exc Jul, Aug, Dec
Coelastrum spp.	1,3,5	2,6	All months

Table 4. (continued)

Cosmarium spp.	1,3,5	2,4,6	All months
Crucigenia spp.	1,3,5	2,4,6	All months
Crucigeniella sp.	1,3	2,4	Oct, Nov, Jan, Feb, Mar, Apr, May, Jun
Cylindrocystis sp.	1	2,4,6	Sep, Jan, Feb, May, Jun
Dictyosphaerium sp.	1,3	4	Jul, Dec, Apr, May
Elakatothrix sp.	-	4	Aug, Feb
Euastrum spp.	1	2,6	All months exc Aug, Apr, May
Eudorina sp.	1,5	6	Feb, Mar
Gloeocystis sp.	3,5	2,4,6	Sep, Jan, Feb, Mar, Apr, May, Jun
Golenkinia sp.	1,3,5	2,4,6	All months
Gonatozygon sp.	1	4	Jan, May, Jun
Micractinium sp.	1,3	2,4	Oct, Feb, Jun
Monoraphidium spp.	1,3,5	2,4,6	All months
Mougeotia spp.	1,3,5	2,6	All months exc Jul, Oct, Jan
Nephrocytium sp.	3	-	Jul
Netrium sp.	5	-	Sep, May
Oedogonium spp.	1,3,5	2,4,6	All months exc Oct
Oocystis spp.	1,3,5	2,4,6	Jul, Aug, Sep, Oct, Feb, Apr, May, Jun
Pandorina spp.	1,3	2	Jul, Mar, Apr, Jun
Pediastrum spp.	1	2,4,6	All months exc Feb, May
Penium sp.	1,5	-	Jul
Phaeodactylum sp.	1	2,6	Aug, Sep, Jun
Scenedesmus spp.	1,3,5	2,4,6	All months
Spirogyra sp.	1,3	2	Nov, Jan, Feb, Mar, Apr, May
Spirotaenia sp.	1	-	Jul, Sep
Staurastrum spp.	1,3,5	2,4,6	All months
Staurodesmus spp.	1,5	2,4,6	Jul, Aug, Sep, Nov, Dec, Jun
Tetraedron spp.	1,3,5	2,4,6	All months
Tetralantos sp.	1	2,4	Oct, Nov, Jan
Treubaria sp.	3	4	Mar, Jun
Ulothrix sp.	1	-	Jul
Zygnema spp.	1,3,5	2,4	Aug, Sep, Jan, Feb, Apr, May, Jun
Division Pyrrophyta			
Ceratium sp.	5	2,4,6	All months
Peridinium spp.	1,3,5	2,4,6	All months

Table 4. (continued)

Division Bacillariophyta			
Caloneis sp.	-	2,4,6	Sep, Dec, Jan, Feb, May, Jun
Cymbella sp.	1,3	2	Nov, Dec, Mar, Apr
Diatomella sp.	1,3,5	2,4,6	Jul, Aug, Sep, Jan, Feb, Mar, May
Fragilaria sp.	1,3,5	2,4,6	All months
Gomphonema spp.	1,3,5	2,4,6	All months
Gyrosigma sp./*Pleurosigma* sp.	5	2	Sep, Oct, Mar
Navicula spp.	1,3,5	2,4,6	All months
Nitzchia spp.	1,3,5	2,4,6	All months
Pinnularia sp.	1,3,5	2,4,6	All months exc Aug, Jun
Surirella spp.	1,3,5	2,4	All months exc Nov, Jan
Synedra sp.	3,5	2,4	Aug, Sep, Dec, Feb, Mar
Division Euglenophyta			
Euglena spp.	1,3,5	2,4,6	All months
Lepocinclis sp.	1,3,5	2,4,6	All months exc Nov
Phacus spp.	1,3,5	2,4,6	All months
Trachelomonas spp.	1,3,5	2,4,6	All months
Division Chrysophyta			
Centritractus sp.	1,3,5	2	All months exc Jul, Oct
Dinobryon spp.	1,3,5	2,4,6	All months
Isthmochloron sp.	1,3,5	2,4,6	All months

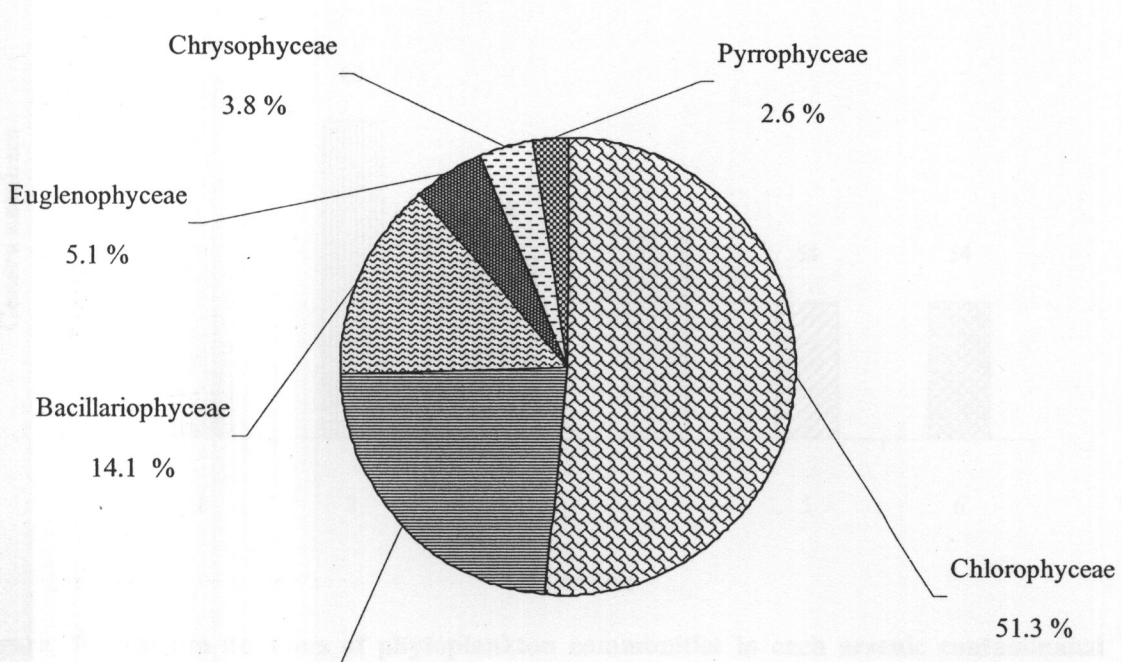


Figure 18. The percentage of the genera numbers of phytoplankton in each class detected in six water ponds designated as arsenic contaminated waters in the Ron Phibun district of Nakhon Si Thammarat province, Thailand.

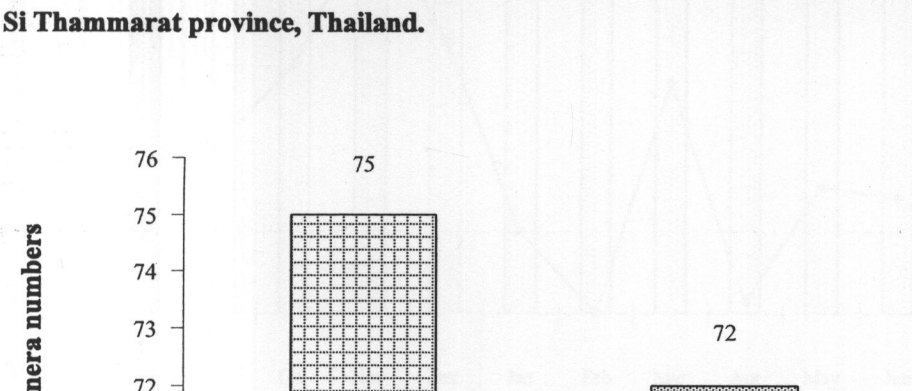


Figure 19. Phytoplankton genera numbers in HACP and LACP waters in the Ron Phibun district of Nakhon Si Thammarat province, Thailand. HACP = high arsenic contaminated ponds, LACP = low arsenic contaminated ponds.

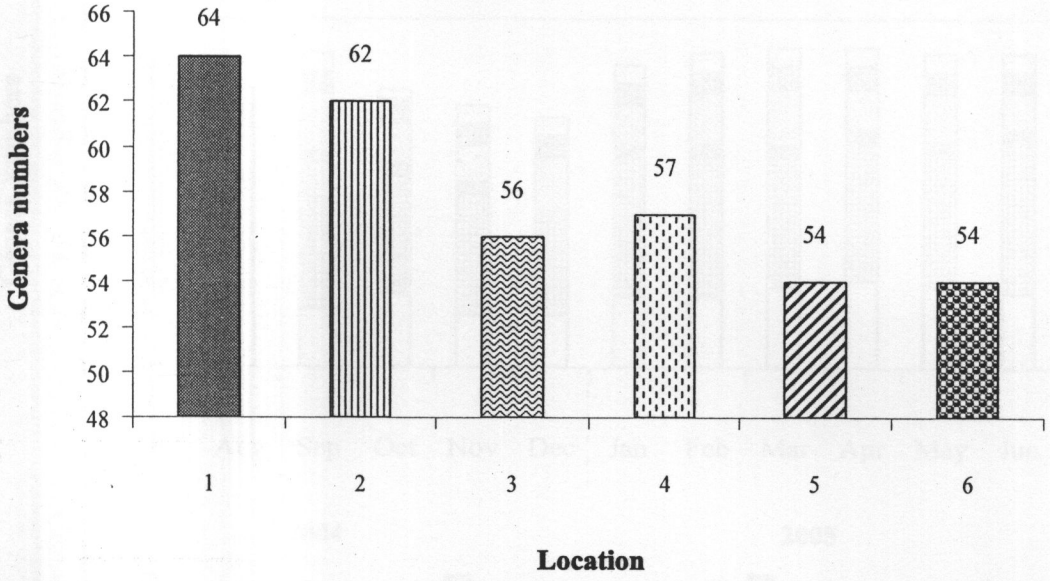


Figure 20. Genera numbers of phytoplankton communities in each arsenic contaminated water pond in the Ron Phibun district of Nakhon Si Thammarat province, Thailand.

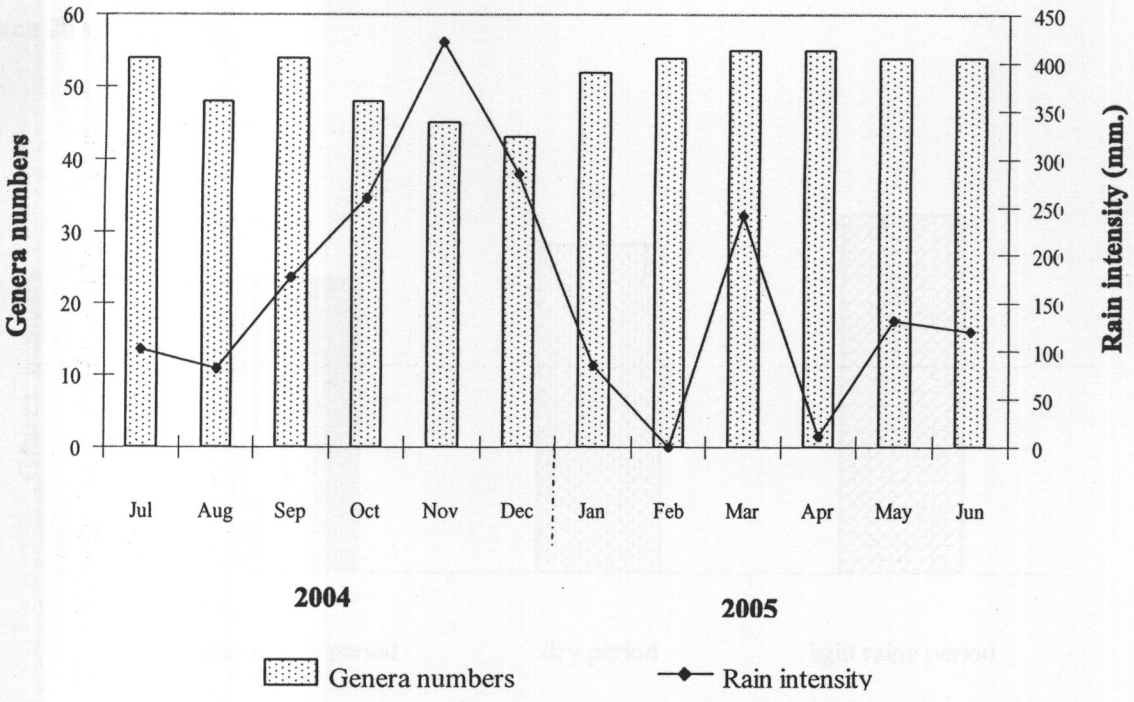


Figure 21. Seasonal occurrence of genera numbers of phytoplankton communities in six arsenic contaminated sampling ponds in the Ron Phibun district of Nakhon Si Thammarat province, Thailand.

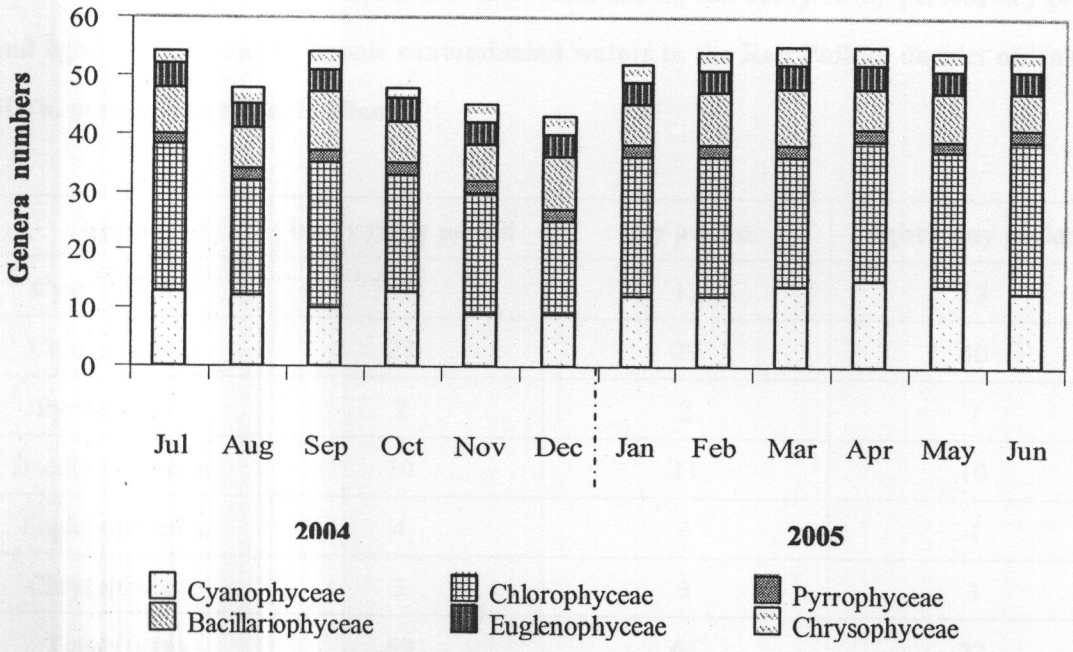


Figure 22. Genera numbers in each class of six water ponds in arsenic contaminated waters in the Ron Phibun district of Nakhon Si Thammarat province, Thailand from July 2004 to June 2005.

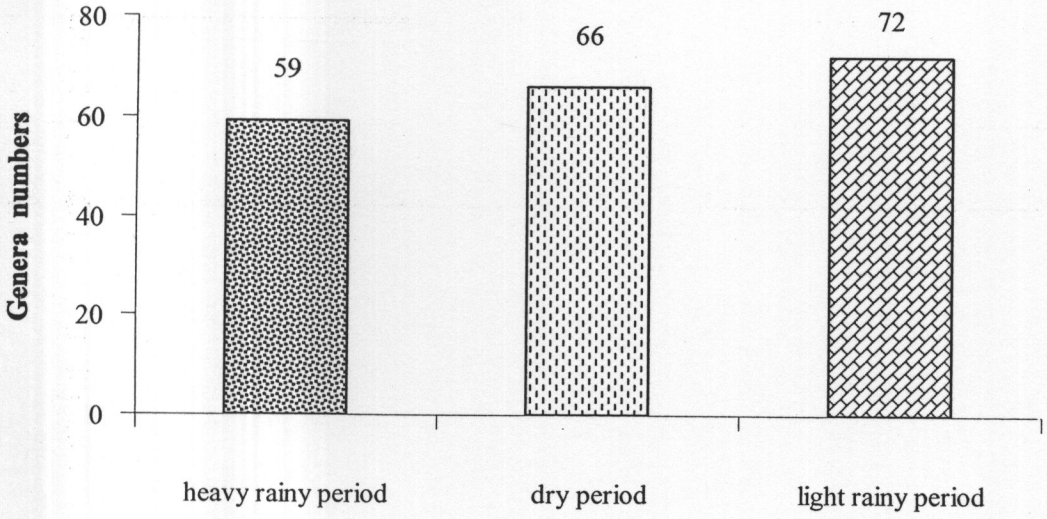
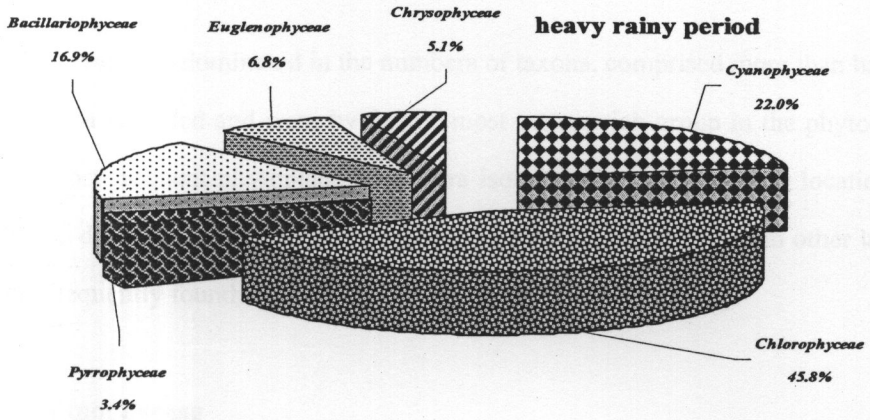


Figure 23. Genera numbers recorded in each seasonal period in arsenic contaminated waters in the Ron Phibun district of Nakhon Si Thammarat province, Thailand.

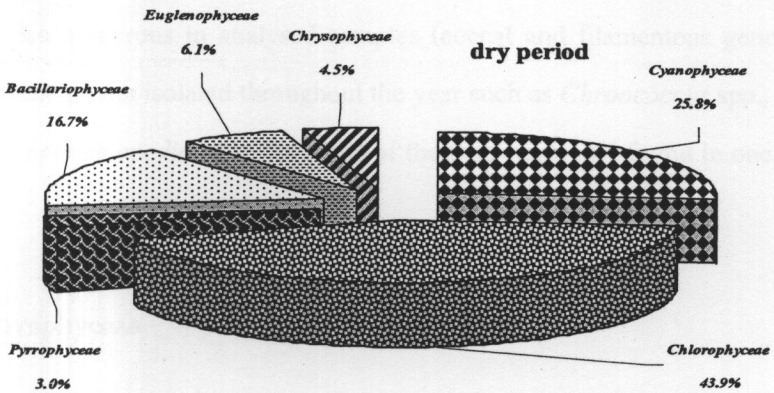
Table 5. Genera numbers recorded in each class during the heavy rainy period, dry period and light rainy period in arsenic contaminated waters in the Ron Phibun district of Nakhon Si Thammarat province, Thailand.

Class	heavy rainy period	dry period	light rainy period
Cyanophyceae	13	17	13
Chlorophyceae	27	29	40
Pyrrophyceae	2	2	2
Bacillariophyceae	10	11	10
Euglenophyceae	4	4	4
Chrysophyceae	3	3	3
Total (taxa)	**59**	**66**	**72**

a)



b)



c)

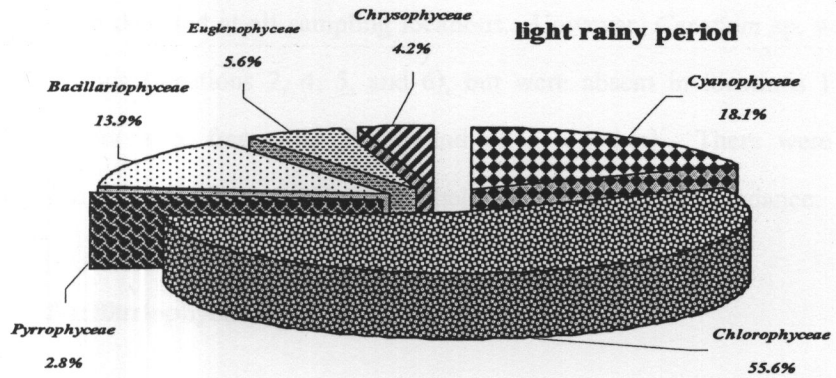


Figure 24. The percentage of the phytoplankton genera numbers in each class during the heavy rainy period, dry period and light rainy period in arsenic contaminated waters in the Ron Phibun district of Nakhon Si Thammarat province, Thailand.

Chlorophyceae

Chlorophytes dominated in the numbers of taxons, comprised more than half of the total number of genera recorded and were by far the most genera-rich group in the phytoplankton community. The most frequent phytoplankton genera isolated from all sampling locations were chlorococcacean and desmids. Desmids were more important in location 4 than in other locations. The desmids most frequently found were *Staurastrum* spp. and *Cosmarium* spp.

Cyanophyceae

Generally, the blue-greens (cyanophyceae) dominated the community. Cyanophytes were also numerous in analysed samples (coccal and filamentous genera). In this study, the most frequent genera isolated throughout the year such as *Chroococcus* spp., *Gloeocapsa* sp., *Microcystis* spp., *Merismopedia* spp., but some of the taxa were only found in one month such as *Anacystis* sp.

Pyrrophyceae

Taxons in the Pyrrophyceae were less frequently isolated from sampling locations. During the study period, the genera of pyrrophyceae isolated changed little at all sampling location. *Peridinium* spp. were detected at all sampling locations. However, *Ceratium* sp. was commonly found in four locations (locations 2, 4, 5, and 6), but were absent in locations 1 and 3. The armoured algal genera were frequently found (and often together). There were some minor significant groups in the studied ecosystem due to their high biomass and abundance.

Bacillariophyceae

Bacillariophyceae were not very significant in all sampling locations in terms of genera number. They were comprised of almost a quarter of the total number of genera. *Fragilaria* sp. *Gomphonema* spp., *Navicula* spp. and *Nitzchia* spp. were presented all through the year and other genera were found at least a few months that is *Gyrosigma* sp./*Pleurosigma* sp.

Euglenophyceae

Although, euglenophytes genera comprised only 5.0 % of the total number of genera, they are very important in the sampling locations in terms of being a bioindicative parameter. For example, *Euglena* spp., *Phacus* spp. and *Trachelomonas* spp. were present all year round.

Chrysophyceae

This phylum also showed a very low number of genera. However, chrysophytes were consistently found at all sampling locations, especially in *Dinobryon* spp. and *Isthmochloron* sp.

3.3 Species richness, evenness indices and Shannon-Weiner diversity of phytoplankton flora in arsenic contaminated waters

The algal abundance data of each sampling location and each sampling time were analyzed using diversity, evenness and richness values. A summary of the values for these indices can be found in Table 6. Species richness over the study period was the highest in June at location 2 with 41 genera and the lowest was in November at location 1 (11). Comparison of species richness values between HACP and LACP indicated that HACP had higher variation in species richness than LACP. Wide variation in species richness among the samples was found at location 1. The number varied widely from 11 to 40 between November to April.

In general, evenness values were greater in all sampling locations during some months of the rainy period (November and December). Furthermore, Shannan-Weiner's diversity index was also taken into consideration during the rainy period because of its relatively high value compared to other seasonal periods. These represent values between 0.053 and 1.165, with a mean value of 0.531 ± 0.03. However, species richness generally had lower numbers within the same sampling period. The results indicated that the rainy period generally increased the diversity of phytoplankton due to increasing evenness values. Overall there are quite a few differences in the diversity, evenness and richness values for the six sampling locations. Also, the results of these

values were more pronounced seasonally than spatially. The maximum value of Shannon-Weiner diversity and evenness indices was recorded in June, at location 4 (H' =1.165, J=0.781, respectively) and the lowest was recorded in November at location 1 (H' =0.053, J=0.051, respectively).

Table 6. Summary of species richness (R), equitability or evenness (J) and Shannon-Wiener diversity (H′) (bits/ind) indices for July 2004 to June 2005 in arsenic contaminated waters.

Location 1	R	J	H′
Jul	35	0.322	0.497
Aug	35	0.275	0.424
Sep	36	0.290	0.452
Oct	22	0.082	0.110
Nov	11	0.051	0.053
Dec	18	0.710	0.892
Jan	32	0.430	0.647
Feb	25	0.370	0.517
Mar	32	0.366	0.551
Apr	39	0.569	0.906
May	24	0.057	0.078
Jun	19	0.097	0.124
Average	27	0.302	0.438

Location 3	R	J	H′
Jul	31	0.415	0.619
Aug	20	0.361	0.470
Sep	18	0.479	0.601
Oct	26	0.261	0.369
Nov	17	0.523	0.643
Dec	13	0.316	0.352
Jan	30	0.261	0.385
Feb	34	0.098	0.150
Mar	26	0.133	0.188
Apr	28	0.221	0.320
May	29	0.441	0.645
Jun	30	0.478	0.705
Average	25	0.332	0.454

Location 2	R	J	H′
Jul	34	0.580	0.888
Aug	32	0.205	0.308
Sep	40	0.272	0.436
Oct	30	0.305	0.450
Nov	33	0.053	0.080
Dec	25	0.672	0.939
Jan	32	0.079	0.118
Feb	33	0.553	0.839
Mar	30	0.256	0.379
Apr	34	0.353	0.540
May	31	0.177	0.265
Jun	41	0.630	1.017
Mean	33	0.345	0.522

Location 4	R	J	H′
Jul	25	0.612	0.856
Aug	32	0.253	0.382
Sep	33	0.644	0.977
Oct	29	0.517	0.756
Nov	25	0.610	0.853
Dec	17	0.609	0.750
Jan	24	0.593	0.818
Feb	31	0.235	0.350
Mar	26	0.572	0.809
Apr	33	0.524	0.796
May	23	0.776	1.056
Jun	31	0.781	1.165
Mean	27	0.561	0.797

Location 5	R	J	H′
Jul	27	0.513	0.735
Aug	23	0.156	0.213
Sep	35	0.404	0.623
Oct	21	0.206	0.272
Nov	12	0.327	0.353
Dec	14	0.576	0.660
Jan	18	0.288	0.361
Feb	14	0.303	0.348
Mar	23	0.253	0.345
Apr	24	0.381	0.526
May	25	0.336	0.469
Jun	17	0.374	0.460
Mean	21	0.343	0.447

Location 6	R	J	H′
Jul	33	0.268	0.406
Aug	28	0.091	0.131
Sep	25	0.648	0.906
Oct	24	0.377	0.520
Nov	25	0.662	0.926
Dec	19	0.663	0.848
Jan	31	0.424	0.632
Feb	23	0.126	0.172
Mar	26	0.234	0.332
Apr	22	0.360	0.483
May	21	0.331	0.438
Jun	22	0.441	0.592
Mean	25	0.385	0.532

3.4 Relative abundance and density of phytoplankton

The relative abundance of phytoplankton assemblages in each sampling location studied was variable. It varied from 0% to 99.66%, among cyanophytes, from 0.04% to 98.65% for chlorophytes, from 0% to 59.94% for pyrrophytes, from 0% to 91.85% for bacillariophytes, from 0% to 18.63% for euglenophytes, and from 0% to 98.18% for chrysophytes. In most of the samples taken, cyanophytes were the most abundant group, representing 77.55% of the total phytoplankton assemblages.

Cyanophytes contributed relatively high proportions at all sampling locations and sampling times, except location 4 and during the rainy period. In location 4, chlorophytes generally were found as the most abundant group in several months (except October, November, and June), representing more than 50% of the total. Also, note that the changes in relative abundance occasionally occurred during the rainy period (mainly from November to December). During the rainy period, several algal groups were recorded with highly relative abundance such as chrysophytes in location 1 (98.18% in November), chlorophytes in locations 2, 4, and 5 (98.65% in November, 72.57% in December and 79.55% in November, respectively), and bacillariophytes in location 3 (91.85% in December).

Mean total phytoplankton density ranged from 8.08 x 10^4 to 1.24 x 10^6cells/L. The overall mean numbers per litre of phytoplankton collected throughout the study period were 1.24 x 10^6; 7.89 x 10^5; 5.16 x 10^5; 4.92 x 10^5; 9.87 x 10^4 and 8.08 x 10^4 cells/L in locations 3, 6, 1, 2, 4 and 5, respectively (Figure 25). Comparison of phytoplankton communities at each location indicated that the highest density varied depending on seasonal effect. It was apparent that the mean density of phytoplankton recorded in all locations generally were lower during the rainy period (November to December). Density also showed monthly variations, with high values and peaks sometime above 1.2 x 10^6 cells/L in September, March and May at location 1, and higher than 2 x 10^6 cells/L in July and August at location 6. The highest density occurred in March at location 3 (5.59 x 10^6 cells/L), whilst the lowest occurred in December at location 1 (976 cells/L). In addition, the highest density occurred in May (1.58 x 10^6cells/L) for location 1, January (1.84 x 10^6 cells/L) for location 2, and August (4.87 x 10^5; 2.50 x 10^5; 2.49 x 10^6 cells/L) for locations 4, 5 and 6, respectively.

Cyanophytes made up the highest density group, accounting for 89.58, 25.88, 84.73, 28.49, 81.99 and 95.6 % of the total densities at each location (locations 1, 2, 3, 4, 5 and 6, respectively). The 2nd highest density group were chlorophytes which accounted for 1.01, 60.49, 5.85, 66.06, 1.01 and 0.75 % of the total density at each location (locations 1, 2, 3, 4, 5 and 6, respectively). The 3rd highest density group were chrysophytes which accounted for 3.61, 12.57, 5.50, 1.27, 14.97 and 0.42 % of the total density at each location (locations 1, 2, 3, 4, 5 and 6, respectively). The 4th highest density group were pyrrophytes which accounted for 2.35, 0.57, 1.73, 3.92, 1.20 and 2.73 % of the total density at each location (locations 1, 2, 3, 4, 5 and 6, respectively). The 5th highest density group was bacillariophytes which accounted for 2.01, 0.16, 1.70, 0.21, 0.53 and 0.10 % of the total density at each locations (locations 1, 2, 3, 4, 5 and 6, respectively). The 6th highest density group were euglenophytes which accounted for 1.44, 0.34, 0.49, 0.05, 0.30 and 0.41 % of the total density at each location (locations 1, 2, 3, 4, 5 and 6, respectively) (Figure 26).

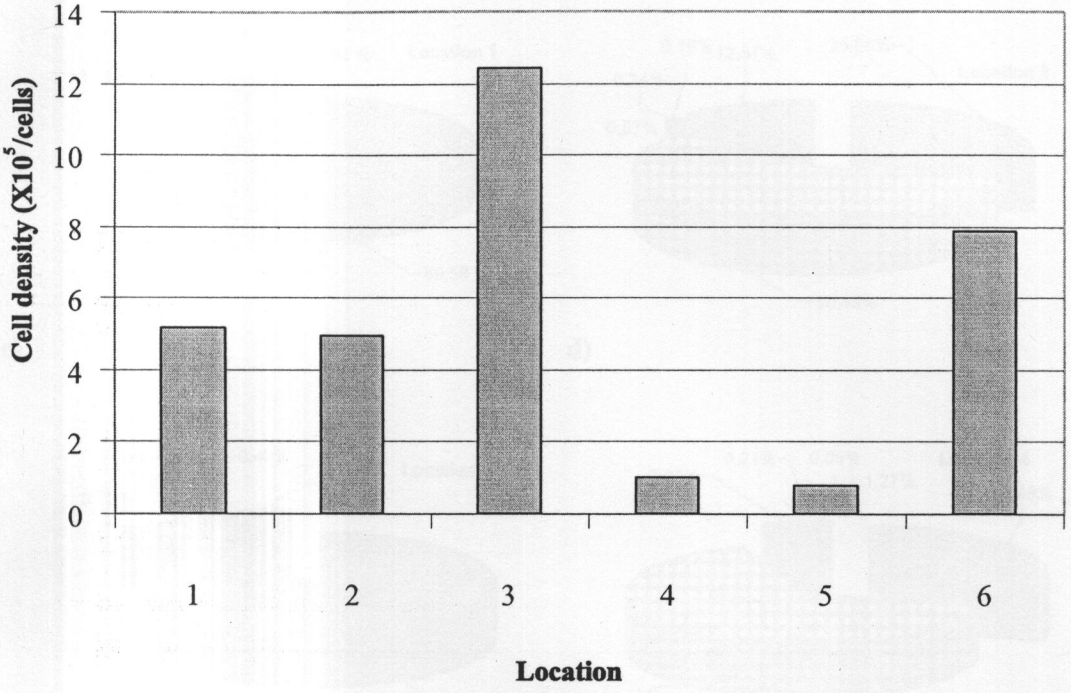


Figure 25. Chart of the phytoplankton densities of six sampling locations in the arsenic contaminated waters at the Ron Phibun district of Nakhon Si Thammarat province, Thailand.

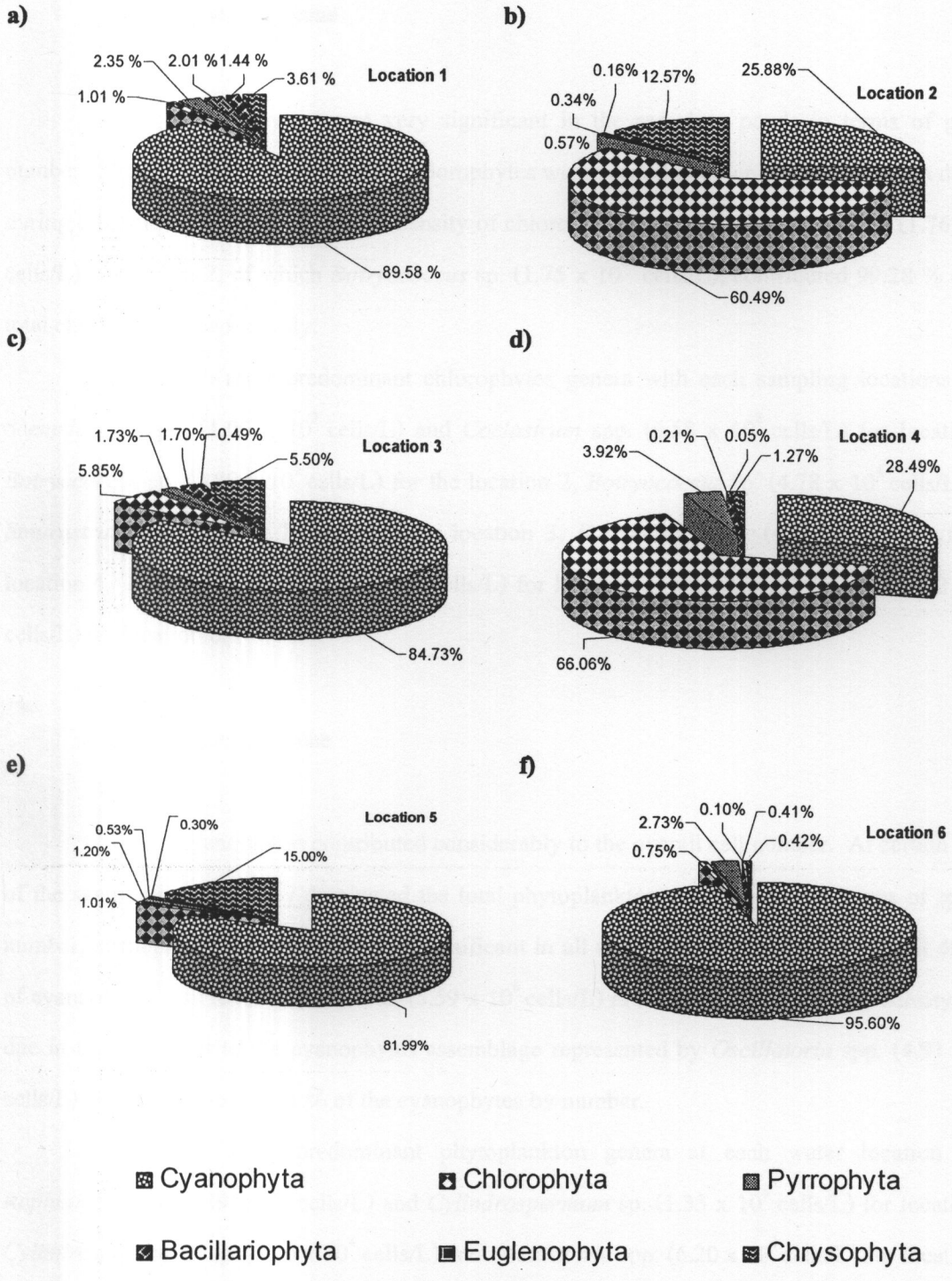


Figure 26. The percentage of phytoplankton densities in each sampling locations of arsenic contaminated waters in the Ron Phibun district of Nakhon Si Thammarat province, Thailand.

Chlorophyceae

Chlorophytes were very significant in the sampling ponds in terms of genera number. However, in terms of density, chlorophytes were second in order of phytoplankton density during this study period. The highest density of chlorophytes was recorded in January (1.76×10^6 cells/L) at location 2, of which *Botryococcus* sp. (1.75×10^6 cells/L), contributed 99.28 % of the total chorophytes, respectively.

The most predominant chlorophytes genera with each sampling locations were *Scenedesmus* spp. (1.09×10^3 cells/L) and *Coelastrum* spp. (6.58×10^2 cells/L) for location 1, *Botryococcus* sp. (2.88×10^5 cells/L) for the location 2, *Botryococcus* sp. (4.78×10^4 cells/L) and *Staurastrum* spp. (1.81×10^4 cells/L) for location 3, *Botryococcus* sp. (4.76×10^4 cells/L) for location 4, *Botryococcus* sp. (4.63×10^2 cells/L) for location 5, and *Staurastrum* spp. (1.53×10^3 cells/L) for location 6.

Cyanophyceae

Cyanophytes contributed considerably to the overall cell number. At certain times of the sampling period, they dominated the total phytoplankton cell number. In terms of species number, cyanophytes were not the most significant in all sampling locations. The highest density of cyanophytes was recorded in March (5.59×10^6 cells/L) at location 3. These high density were due in the most part to the cyanophytes assemblage represented by *Oscillatoria* spp. (4.91×10^6 cells/L), accounting for 88.91 % of the cyanophytes by number.

The most predominant phytoplankton genera at each water location were *Raphidiopsis* sp. (1.39×10^5 cells/L) and *Cylindrospermum* sp. (1.33×10^5 cells/L) for location 1, *Cylindrospermopsis* sp. (5.26×10^4 cells/L) and *Oscillatoria* spp. (6.20×10^4 cells/L) for location 2, *Oscillatoria* spp. (4.83×10^5 cells/L) and *Raphidiopsis* sp. (3.45×10^5 cells/L) for location 3, *Oscillatoria* spp. (7.58×10^3 cells/L) and *Gloeocapsa* sp. (4.94×10^3 cells/L) for location 4, *Cylindrospermopsis* sp. (2.49×10^4 cells/L) and *Oscillatoria* spp. (2.92×10^4 cells/L) for location 5, *Cylindrospermopsis* sp. (4.92×10^5 cells/L) and *Oscillatoria* spp. (1.96×10^5 cells/L) for location 6.

Pyrrophyceae

Throughout the study period, pyrrophytes were poorly diversified. However, species composition and dinoflagellate densities were one of the most important phytoplankton assemblage along the arsenic contaminated waters. Pyrrophyceae were a frequent algal group in many sampling locations during the study period. They consisted mainly of two genera, *Peridinium* and *Ceratium*. These genera made up 1.95 % of the total phytoplankton density. The highest mean value of dinoflagellate density occurred in July at location 6 (2.01×10^5 cells/L). The highest density of dinoflagellates corresponded with *Peridinium* spp., which is common in the arsenic contaminated waters and was found in 100 % of all dinoflagellate densities.

The most predominant pyrrophytes genera within all sampling locations were *Peridinium* spp. The mean density of *Peridinium* spp. was 1.22×10^4; 8.37×10^3; 2.15×10^4; 3.51×10^3; 7.00×10^2 and 2.15×10^4 cells/L for locations 1, 2, 3, 4, 5 and 6, respectively.

Bacillariophyceae

The bacillariophytes were considerable in number throughout most of the study period. Incidences of bacillariophytes densities were low for all species, except *Fragilaria* sp. and *Navicula* sp. During the study period, cell densities showed the highest value of 2.21×10^5 cells/L in October at location 3. The high contribution of bacillariophytes made by *Fragilaria* sp. was 98.95 %.

The most predominant bacillariophytes genera within each sampling locations were *Navicula* spp. (8.86×10^3 cells/L) for location 1, *Navicula* spp. (3.34×10^2 cells/L) for location 2, *Fragilaria* sp. (2.04×10^4 cells/L) for location 3, *Navicula* spp. (6.70×10^1 cells/L) for location 4, *Fragilaria* sp. (1.64×10^2 cells/L) for location 5, and *Navicula* spp. (5.97×10^2 cells/L) for location 6.

Euglenophyceae

In general, euglenophytes were found in relatively low density during the study period, but they are a very important algal group in terms of indicative parameter along contaminated waters. The highest density was observed in September (5.57×10^4 cells/L) at location 1. Fewer but appreciable numbers were collected of *Trachelomonas* spp. at 98.56 % of the total euglenophytes density. These taxa are characteristic of contaminated waters.

The most predominant euglenophytes genera within all sampling location were *Trachelomona* spp. The mean density of *Trachelomona* spp. was 6.81×10^3; 1.07×10^3, 4.79×10^3; 2.50×10^1; 1.13×10^2 and 2.47×10^3 cells/L for locations 1, 2, 3, 4, 5 and 6, respectively.

Chrysophyceae

Chrysophytes is one of the minor groups in terms of density. They were dominated numerically by *Dinobryon* spp. which collectively represented approximately 4.87 % of the total densities. The highest density of chrysophytes within the period of investigation was registered in January (8.02×10^5 cells/L) at location 3. Chrysophytes highest density due to *Dinobryon* spp. comprising 79.05 % of the total chrysophytes density.

The most predominant chrysophytes genera within all sampling locations were *Dinobryon* spp. The mean density of *Dinobryon* was 1.20×10^4; 6.16×10^4; 6.81×10^4; 1.13×10^3 ; 1.20×10^4 and 3.26×10^3 for locations 1, 2, 3, 4, 5 and 6, respectively.

Tables 7 and 8 show the dominant phytoplankton genera along arsenic contaminated waters at each location and each sampling period. Cyanophytes were the most abundant of algal flora. In addition, chlorophytes seem to be an important algal group in location 4, whilst chrysophytes were also presented as a dominant algal group during some months of the sampling period, particularly in locations 2 and 5. Changes in phytoplankton abundance were more pronounced seasonally than spatially. The diminished growth of the algal flora was noticed during the rainy period. Detailed changed in the main populations are presented separately for each sampling location as follows:

Location 1

In location 1, cyanophytes generally were the dominant group in all sampling periods, except in November (Figure 27). The highest total abundance was attained in May with growth of *Raphidiopsis* sp. comprising 97.16% of the total. Minor peaks occurred in September and March, due to a large number of *Cylindrospermum* sp. in those periods, constituting about 62.77% and 53.37% of the total, respectively. However, the pattern in the rainy period (November and December) was very different. A significant number of phytoplankton flora was detected during the rainy period. In subsequent periods, the total abundance decreased with a fall in the numbers of mostly phytoplankton assemblages. During November, chrysophytes in the genus *Dinobryon* spp. were most conspicuous with a relatively high abundance of 98.14% of the total. In December, phytoplankton assemblages seemed to decrease distinctly as the rain intensity decreased slightly. The population of dinoflagellates dominated with only small quantities of *Peridinium* spp., found (21.41%). When rain intensity sharply increased, the phytoplankton identified was mostly dominated by cyanophytes. Phytoplankton rich waters in those periods were generally dominated by *Oscillatoria* spp.

Location 2

In location 2, different phytoplankton groups alternated dominance in each period. Cyanophytes were dominant in July, August, February, April and May, and a small number were also found in December. Chlorophytes were dominant in November, January and March and chrysophytes in September (Figure 28). The investigations showed that the lowest density was observed in December. *Microcystis* spp. dominated but with only small quantities, or 37.67% of the total. In the following month, January, the highest numbers were observed with *Botryococcus* sp. achieving 61.17% of the total.

In general, all sampling locations had their highest cell density in the early rainy period. However, it was found that the highest cell density occurred in January, the rainy period. The Meteorological Department of Thailand has reported that the annual rain intensity was not high during the study period, when compared with previous investigations.

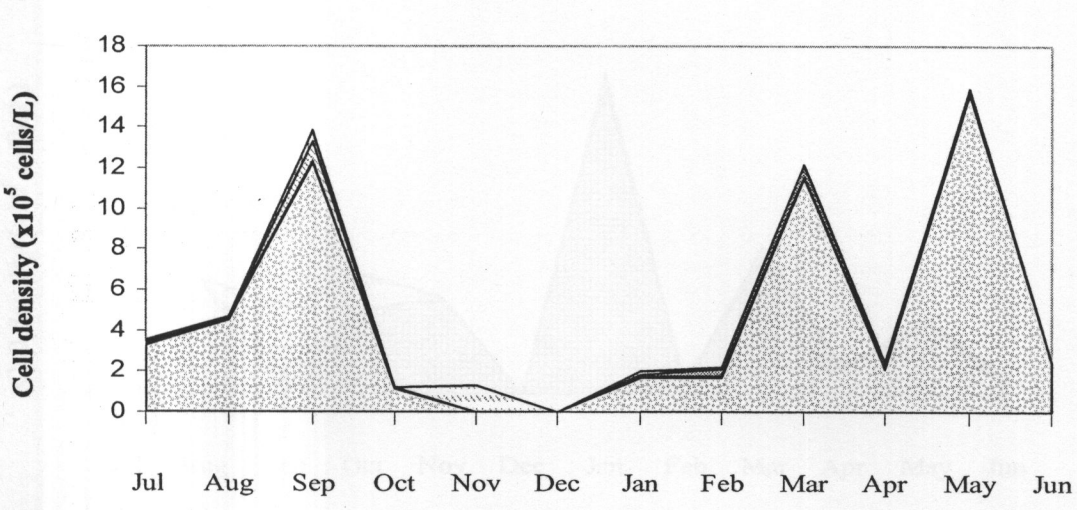
Table 7. Dominant phytoplankton genera in each location of arsenic contaminated waters at the Ron Phibun district of Nakhon Si Thammarat province, Thailand during July to December 2004.

Location	Year 2004					
	July	**August**	**September**	**October**	**November**	**December**
1	*Cylindrospermopsis* sp.	*Cylindrospermopsis* sp.	*Cylindrospermum* sp., *Oscillatoria* spp.	*Raphidiopsis* sp.	*Dinobryon* spp.	*Peridinium* spp., *Dinobryon* spp., *Oscillatoria* spp.
2	*Cylindrospermopsis* sp.	*Cylindrospermopsis* sp.	*Dinobryon* spp., *Botryococcus* sp.	*Botryococcus* sp., *Dinobryon* spp.	*Botryococcus* sp.	*Microcystis* spp.
3	*Cylindrospermopsis* sp., *Microcystis* spp. *Raphidiopsis* sp.	*Microcystis* spp. *Raphidiopsis* sp., *Oscillatoria* spp.	*Staurastrum* spp.	*Raphidiopsis* sp., *Fragilaria* sp., *Peridinium* spp.	*Anabaena* spp.	*Fragilaria* sp.
4	*Cosmarium* spp.	*Botryococcus* sp.	*Cosmarium* spp., *Staurastrum* spp., *Gloeocapsa* sp., *Chroococcus* spp.	*Raphidiopsis* sp., *Gloeocapsa* sp.	*Microcystis* spp. *Oscillatoria* spp., *Botryococcus* sp.	*Staurastrum* spp., *Ankistrodesmus* spp.
5	*Raphidiopsis* sp., *Oscillatoria* spp., *Anabaena* spp., *Chroococcus* sp.	*Cylindrospermopsis* sp.	*Cylindrospermopsis* sp., *Oscillatoria* spp., *Dinobryon* spp.	*Oscillatoria* spp.	*Botryococcus* sp.	*Phormidium* spp., *Fragilaria* sp.
6	*Cylindrospermopsis* sp., *Peridinium* spp., *Oscillatoria* spp.	*Cylindrospermopsis* sp.,	*Cylindrospermopsis* sp., *Phormidium* spp., *Chroococcus* spp., *Cylindrospermum* sp.	*Cylindrospermopsis* sp.,	*Peridinium* spp., *Oscillatoria* spp.	*Chlorella* sp., *Gomphonema* sp., *Trachelomonas* spp.

Table 8. Dominant phytoplankton genera in each location of arsenic contaminated waters at the Ron Phibun district of Nakhon Si Thammarat province, Thailand during January to June 2005.

Location	Year 2005					
	January	**Febuary**	**March**	**April**	**May**	**June**
1	*Oscillatoria* spp.	*Oscillatoria* spp.	*Cylindrospermum* sp., *Oscillatoria* spp., *Phormidium* spp.	*Oscillatoria spp., Phormidium* spp.	*Raphidiopsis* sp., *Phormidium* spp.	*Oscillatoria* spp.
2	*Botryococcus* sp.	*Oscillatoria* spp., *Cylindrospermopsis* sp.	*Botryococcus* sp.	*Oscillatoria* spp., *Cylindrospermopsis* sp.	*Oscillatoria* spp.	*Dinobryon* spp., *Oscillatoria* spp.
3	*Dinobryon* spp.	*Oscillatoria* spp.	*Oscillatoria* spp., *Botryococcus* sp.	*Raphidiopsis* sp., *Oscillatoria* spp.	*Cylindrospermopsis* sp., *Oscillatoria* spp.	*Peridinium* spp., *Oscillatoria* spp.
4	*Peridinium* spp., *Cosmarium* spp.	*Botryococcus* sp.	*Ankistrodesmus* spp., *Oscillatoria* spp.	*Botryococcus* sp.	*Chroococcus* sp., *Ankistrodesmus* spp.	*Oscillatoria* spp., *Anabaenopsis* sp.
5	*Dinobryon* spp.	*Dinobryon* spp., *Oscillatoria* spp.	*Oscillatoria* spp., *Dinobryon* spp.,	*Oscillatoria* spp., *Raphidiopsis* sp., *Dinobryon* spp.,	*Oscillatoria* spp., *Peridinium* spp.,	*Oscillatoria* spp., *Dinobryon* spp., *Raphidiopsis* sp.
6	*Cylindrospermopsis* sp., *Oscillatoria* spp.	*Oscillatoria* spp.	*Oscillatoria* spp.	*Cylindrospermopsis* sp., *Cylindrospermum* sp.	*Oscillatoria* spp., *Cylindrospermopsis* sp.	*Cylindrospermopsis* sp.

a)



b)

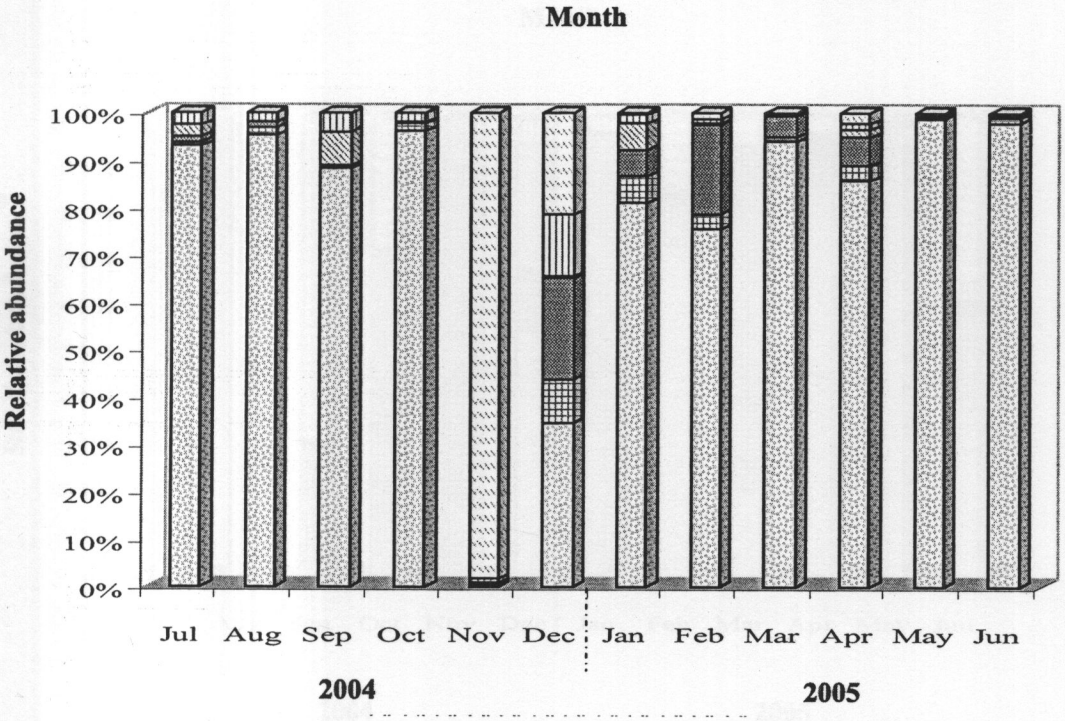
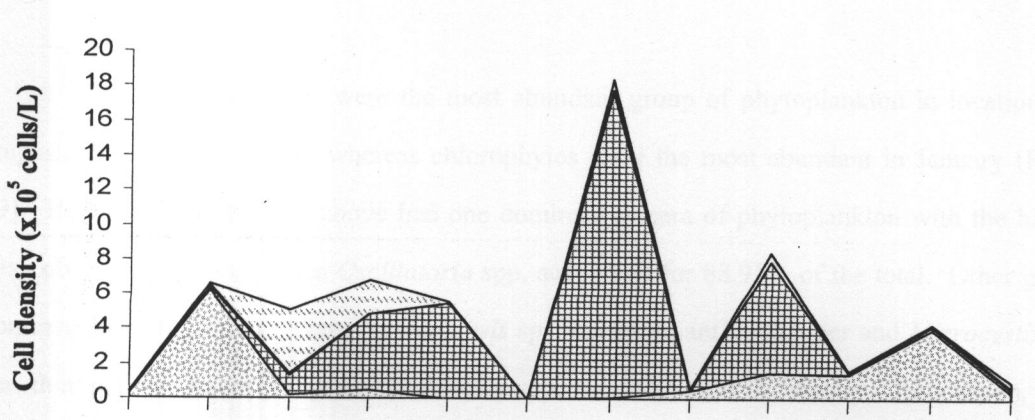


Figure 27. Changes in phytoplankton densities (a) and relative abundance (b) of phytoplankton assemblages in location 1 at the arsenic contaminated waters, during the period July 2004 to June 2005.

a)



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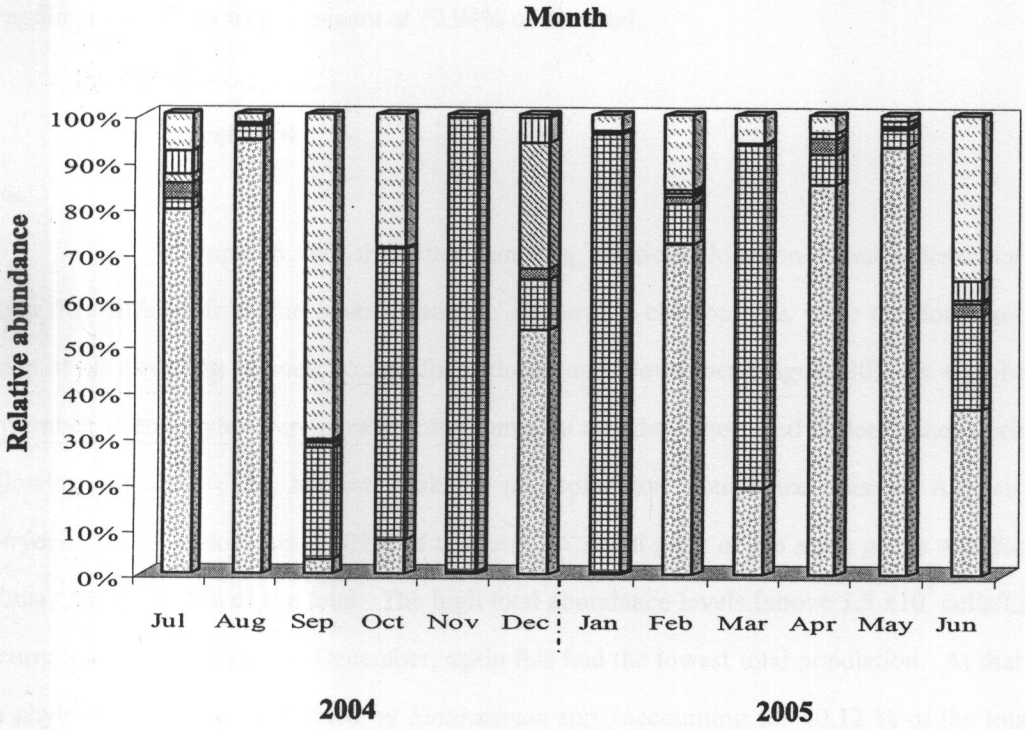


Figure 28. Changes in phytoplankton densities (a) and relative abundance (b) of phytoplankton assemblages in location 2 at the arsenic contaminated waters, during the period July 2004 to June 2005.

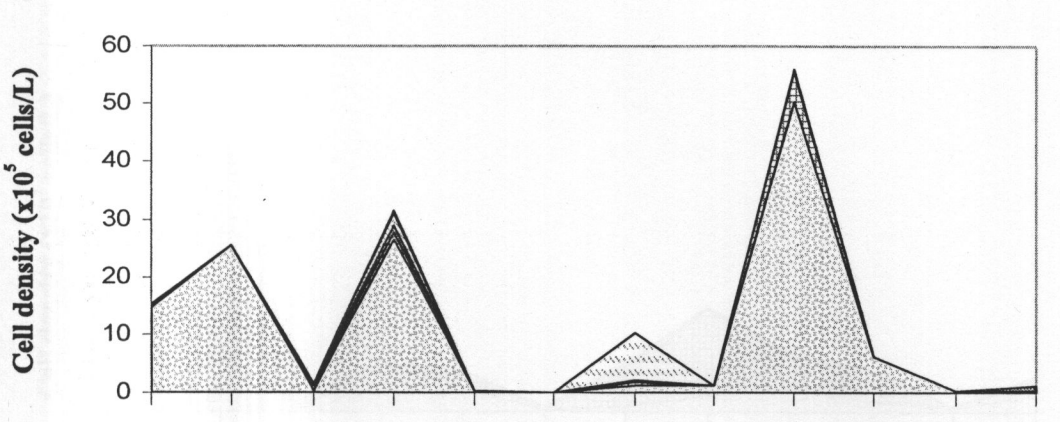
Location 3

Cyanophytes were the most abundant group of phytoplankton in location 3 in August, October and March, whereas chlorophytes were the most abundant in January (Figure 29). Each sampling periods above had one dominant genera of phytoplankton with the highest peak observed in March when *Oscillatoria* spp. accounted for 88.91% of the total. Other genera dominated at other times. Thus, *Raphidiopsis* sp. was dominant in October and *Microcystis* spp. was dominant in August, whereas *Dinobryon* spp. was dominant in January. In addition, there was a noticeable decrease in the total cell densities of phytoplankton assemblages during the rainy period (November and December). In December, cell densities were at their lowest level when *Fragilaria* sp., which was dominant at 79.94% of the total.

Location 4

Compared with the other sampling locations, location 4 had a dominant algal group that differed from the other locations. Generally, chlorophytes were the dominant algal group at all sampling periods, except for October and November (Figure 30). In October and November, cyanophytes were occasionally dominant and they alternated in dominance during the following months. The highest peak of phytoplankton abundance was in August, with *Botryococcus* sp. making up 79.82% of the total. A small peak of the same genus was found in Febuary, with 79.82% of the total. The high total abundance levels (above 1.5 $x10^5$ cells/L) only occurred in those periods. In December, again this had the lowest total population. At that time, the phytoplankton was dominated by *Staurastrum* spp., accounting for 40.12 % of the total. In January pyrrophytes in the genus *Peridinium* spp. and chlorophytes in the genus *Cosmarium* spp. had small increases. In particular, the phytoplankton present during the remaining months in 2005 was frequently characterized by the presence of chlorophytes and cyanophytes such as *Botryococcus* sp., *Ankistrodesmus* spp. and *Oscillatoria* spp.

a)



b)

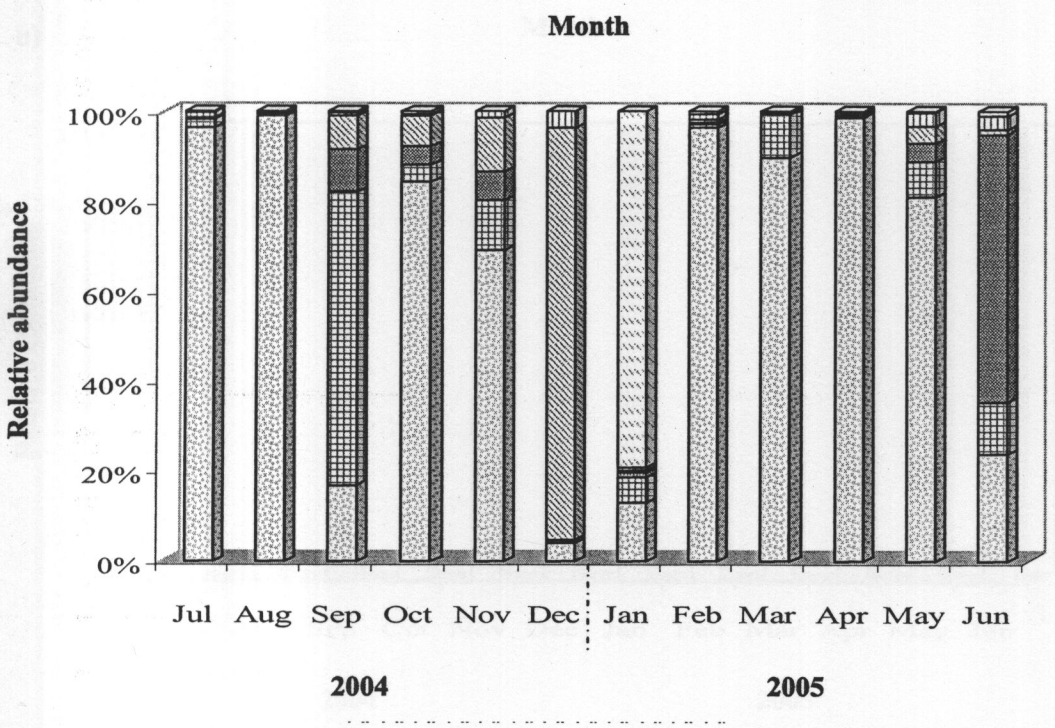
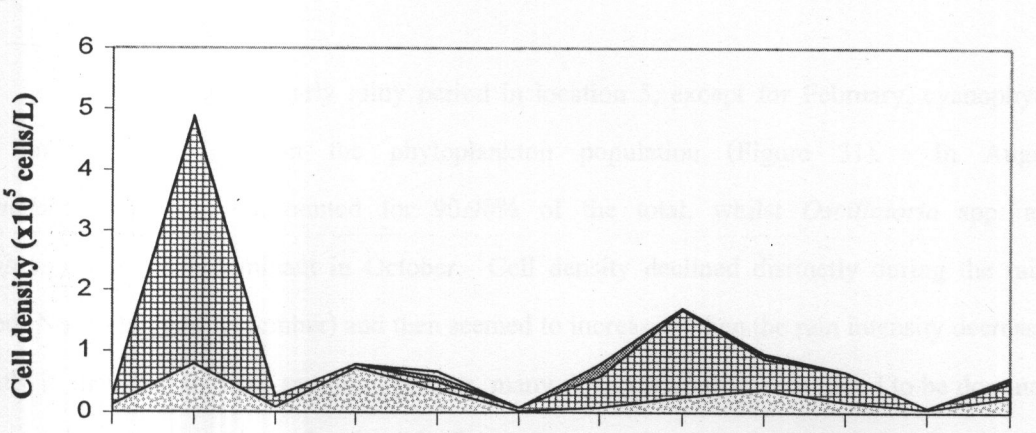


Figure 29. Changes in phytoplankton densities (a) and relative abundance (b) of phytoplankton assemblages in location 3 at the arsenic contaminated waters, during the period July 2004 to June 2005.

a)



b)

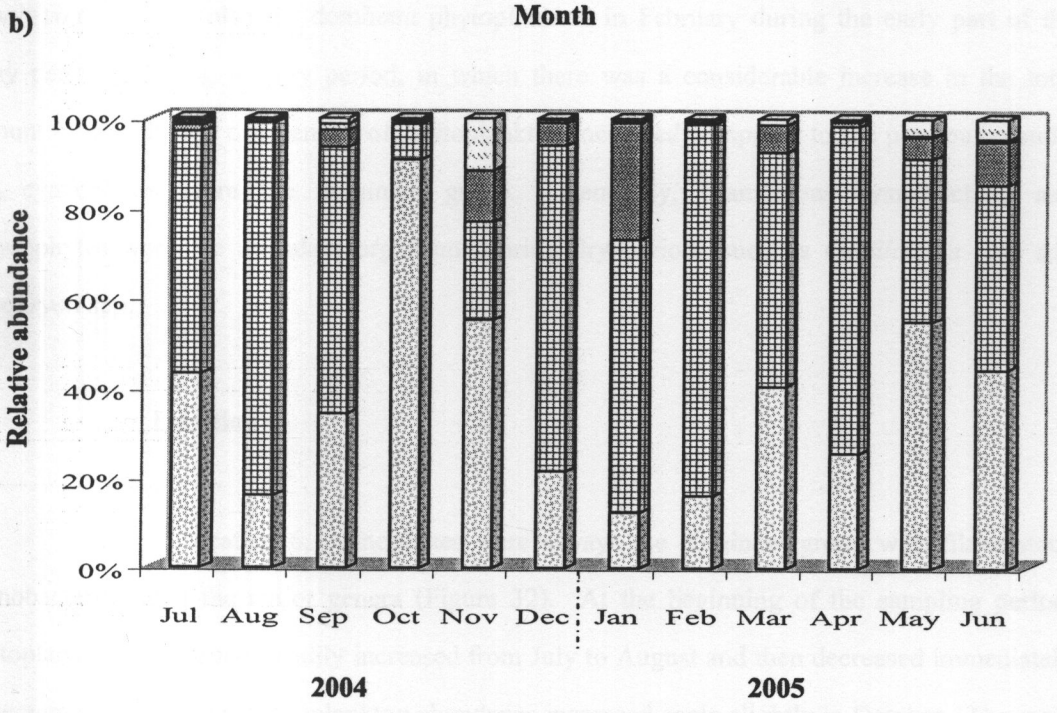


Figure 30. Changes in phytoplankton densities (a) and relative abundance (b) of phytoplankton assemblages in location 4 at the arsenic contaminated waters, during the period July 2004 to June 2005.

Location 5

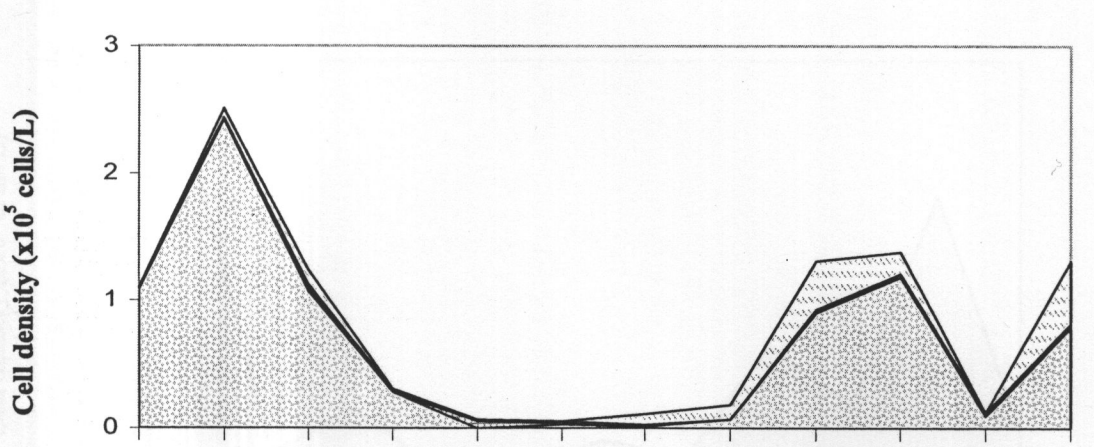
During the early rainy period in location 5, except for February, cyanophytes play an important role in the phytoplankton population (Figure 31). In August *Cylindrospermopsis* sp. accounted for 90.90% of the total, whilst *Oscillatoria* spp. and *Dinobryon* spp. were dominant in October. Cell density declined distinctly during the rainy period (November and December) and then seemed to increased when the rain intensity decreased slightly in January. In those sampling periods, many different groups were found to be dominant such as chlorophytes in November (mainly *Botryococcus* sp.), cyanophytes in December (mainly *Phormidium* spp.) and chrysophytes in January (mainly *Dinobryon* spp.). In addition, *Dinobryon* spp. was also the dominant phytoplankton in February during the early part of the rainy period. During the dry period, in which there was a considerable increase in the total amount of arsenic, the cell density of phytoplankton increased compared to the previous months and cyanophytes were the dominant group. Generally, filamentous cyanobacteria and chrysophytes were the abundant organisms during dry periods such as *Oscillatoria* spp. and *Dinobryon* spp.

Location 6

In location 6, cyanophytes were always the dominant group with filamentous cyanobacteria being the major genera (Figure 32). At the beginning of the sampling period, phytoplankton abundance steadily increased from July to August and then decreased immediately in September. However, phytoplankton abundance increased again slightly in October. The main genera found in those periods was *Cylindrospermopsis* sp. with the highest peak occurring with 94.98% of the total population. A later peak was found in May and the present study also shows filamentous cyanobacteria still being the dominant genera with *Oscillatoria* spp. and *Cylindrospermopsis* spp. constituting 46.10% and 44.43% of the total respectively. During November and December, the proportion of phytoplankton assemblages seemed to have changed considerably and the phytoplankton density also declined, compared with other months. Many other genera were encountered during November and December in small numbers such as

pyrrophytes, chlorophytes, cyanophytes and euglenophytes. In the genus *Peridinium* spp. a member of the pyrrophytes were dominant in November, accounting for 31.87% of the total, whilst chlorophytes in the genus *chlorella* sp. were dominant in December, accounting for 30.19% of the total.

a)



b)

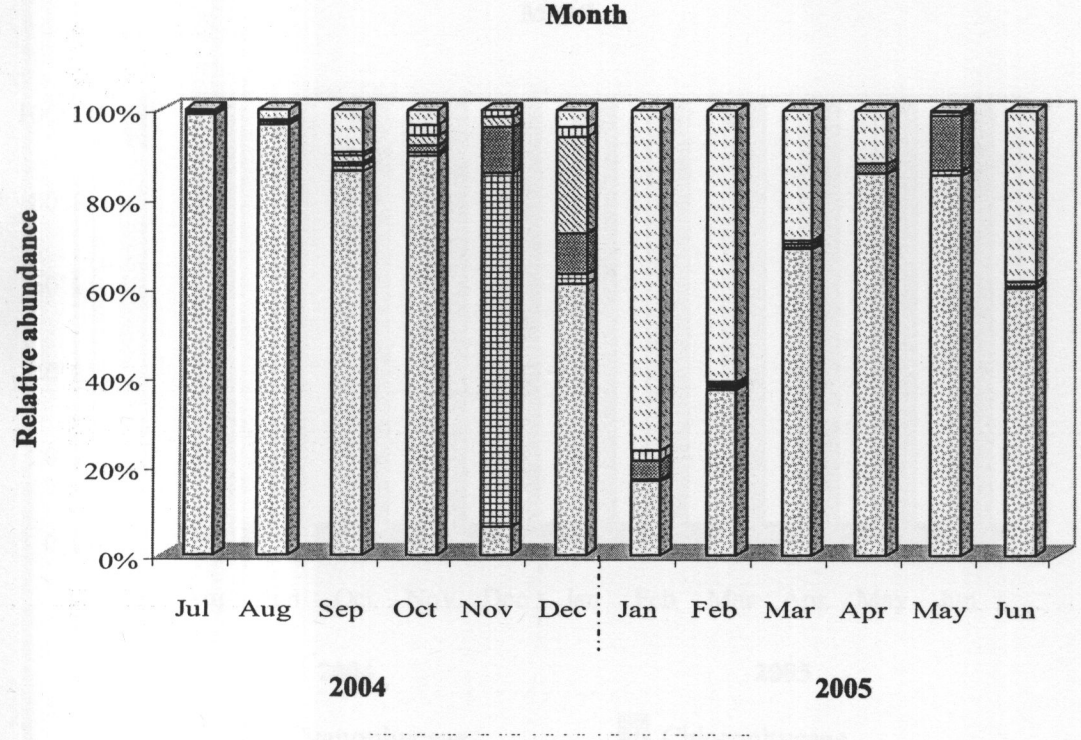


Figure 31. Changes in phytoplankton densities (a) and relative abundance (b) of phytoplankton assemblages in location 5 at the arsenic contaminated waters, during the period July 2004 to June 2005.

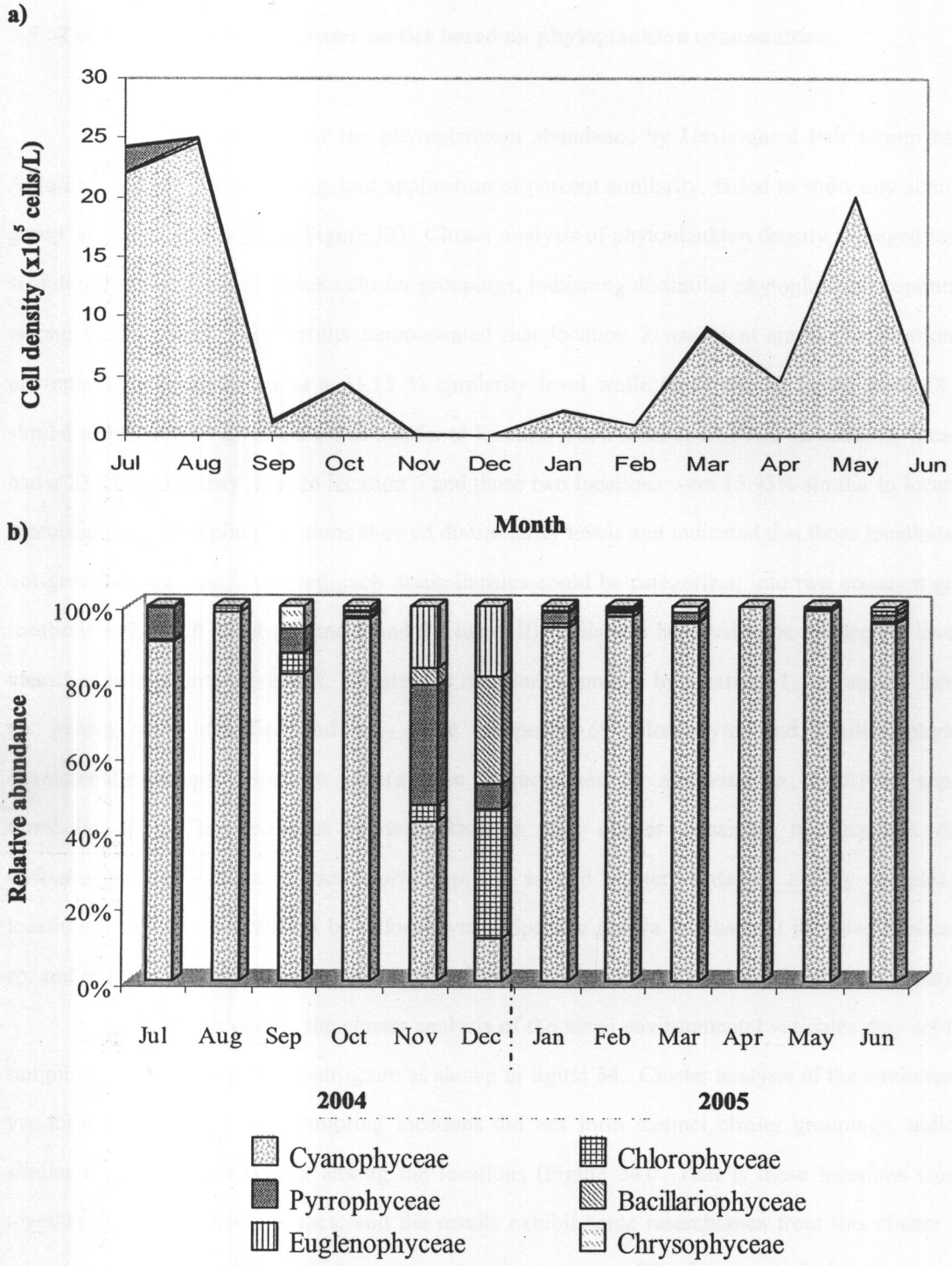


Figure 32. Changes in phytoplankton densities (a) and relative abundance (b) of phytoplankton assemblages in location 6 at the arsenic contaminated waters, during the period July 2004 to June 2005.

3.5 The classification of six water bodies based on phytoplankton communities

The analysis of the phytoplankton abundance by Unweighted Pair Group Method Algorithm (UPGMA), clustering, and application of percent similarity, failed to show any significant group with 50% of similarity (Figure 33). Cluster analysis of phytoplankton density averaged for each sampling location showed distinct cluster groupings, indicating dissimilar phytoplankton communities among the locations. The results demonstrated that location 2 was most similar to location 3 in phytoplankton communities at a 33.15 % similarity level while those two locations were 28.08 % similar to location 6. Location 6 was similar to location 2 at a level of 25.10%. In addition, location 4 had a 23.21% similarity level to location 5 and those two locations were 15.95% similar to location 2. Accordingly, all sampling locations showed dissimilarity levels and indicated that those locations were not grouped together. However, such dissimilarities could be categorized into two common groups; locations 1, 2, 3& 6 (cluster I) and 4 and 5 (cluster II). This was believed to be the density level that identified two distinct categories. Cluster I is represented mainly by locations 1, 2, 3 and 6. Seventy-six genera were identified and they were composed of chlorophytes and bacillariophytes as characteristic groups. Specific genera were characterized by *Anacystis* sp., *Euastrum* spp. and *Cymbella* sp. The dominant phytoplankton in this cluster consisted of members of the *Cylindrospermopsis* sp. and *Oscillatoria* spp. A second cluster contained mainly samples from locations 4 and 5, characterized by chlorophytes. Specific genera in cluster II included *Elakatothrix* sp. and *Netrium* sp. This cluster showed a lower number of phytoplankton species with 65 genera.

Additionally, the cluster analysis of the mean environmental variables data set for all sampling locations gave the dendrogram as shown in figure 34. Cluster analysis of the environmental variables averaged for each sampling locations did not form distinct cluster groupings, indicating similar limnological behaviour among the locations (Figure 34). That is those locations clustered together will share characteristics, and the results exhibit some resemblance from this cluster being related to cluster grouping of phytoplankton abundance above. The first one includes the samples in locations 1, 2, 3 and 6, whereas the second belongs to the remaining samples from other locations (locations 4 and 5). Accordingly, locations 1, 2, 3 and 6 were quite similar to one another in environmental variables and as a result categorized in cluster I due to their adjacent similarity level. In addition, the outcome indicated that locations 4 and 5 were obviously the same and were grouped as

cluster II. The mean environmental variables from cluster I were as follows: pH 6.32±0.15, DO 5.26±0.19 mg/L, BOD 2.09±0.14 mg/L, total arsenic 34.26±3.94 μg/L, nitrate-nitrogen 0.06±0.01 mg/L, dissolved phosphorus 0.03±0.002 mg/L, conductivity 88.30±9.84 μS/cm, TSS 8.31±0.63 mg/L, ammonia-nitrogen 0.02±0.003 mg/L. In the meantime, the mean of environmental variables in cluster II is shown as follows: pH 6.43±0.26, DO 5.90±0.27 mg/L, BOD 1.96±0.30 mg/L, total arsenic 40.58±15.48 mg/L, nitrate-nitrogen 0.05±0.01 mg/L, dissolved phosphorus 0.02±0.01 mg/L, conductivity 99.74±16.78 μS/cm, TSS 37.87±22.93 mg/L, ammonia-nitrogen 0.02±0.01 mg/L. Of 11 environmental variables, TSS values differ considerably between cluster I and II.

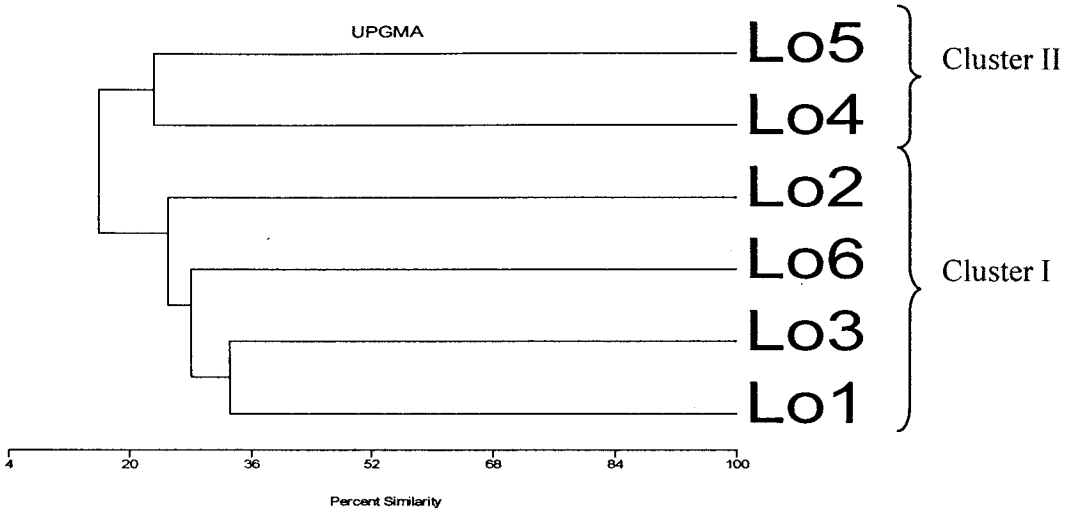


Figure 33. Cluster of dissimilarity (Percent similarity) among phytoplankton samples averaged for each sampling locations obtained by UPGMA: Lo = location.

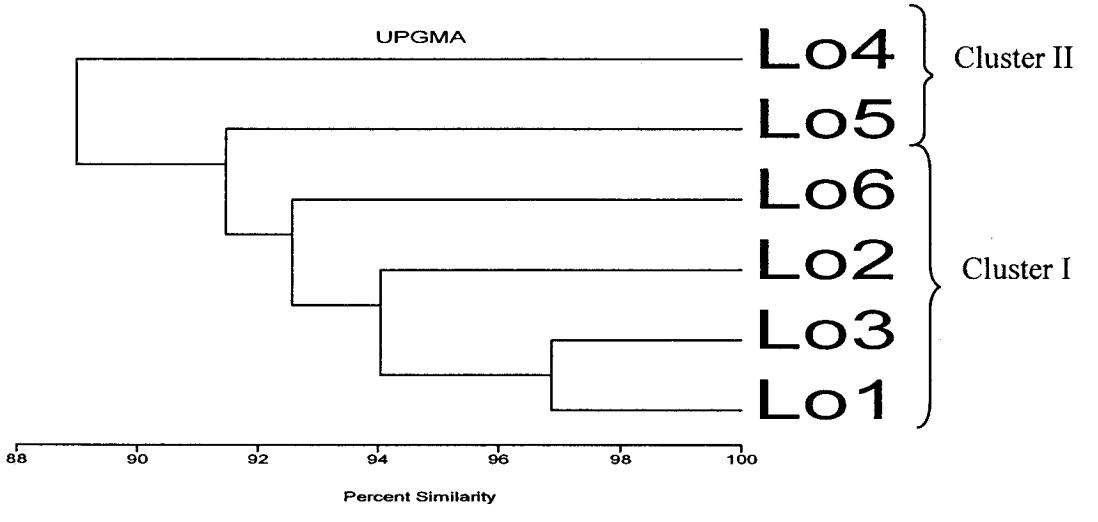


Figure 34. Cluster of dissimilarity (Percent similarity) among environmental variables averaged for each sampling locations obtained by UPGMA: Lo = location.

3.6 Canonical Correspondence Analysis (CCA)

Canonical Correspondence Analysis (CCA) is used to produce bi-plots for sample scores and was performed on the selected environmental and phytoplankton species datasets. Eigenvalues of axes 1 and 2 were 0.61 and 0.54, respectively. The CCA explained a small proportion of the variance in the genera data (Table 9). The first two dimensions of the CCA accounted for 29.29 % of the total variance of phytoplankton species and environmental data. The first axis accounted for 15.23 % of the total variance and the second axis for 14.07 % of the total variation in the data set. This low percentage is typical for noisy datasets containing many zero values. Specie and environmental correlations showed 0.88 and 0.83 explained by axes 1 and 2, respectively. The outcome from CCA analysis showed that pH, DO, BOD, total arsenic, nitrate-nitrogen, dissolved phosphorus, conductivity, TSS and ammonia-nitrogen were found to correlate with phytoplankton flora in arsenic contaminated waters (Figure 35).

In the CCA diagram of environmental variables, conductivity and BOD were strongly associated with Axis 1 (right hand side of ordination). pH, total arsenic, nitrate-nitrogen, TSS, and dissolved phosphorus were moderately associated. Ammonia-nitrogen was weakly associated. Dissolved oxygen had moderate negative associations with this axis. The variables with positive loading on Axis 2 (left hand side of ordination) were dissolved phosphorus (strongly associated), total arsenic (moderately associated), nitrate-nitrogen and ammonia-nitrogen (weakly associated). In addition, pH and conductivity had moderately negative associations and DO, BOD and TSS had weak negative associations with this axis (Table 10).

Many phytoplankton assemblages were reported to coincide with the following environmental variables as per below:

1. Group I consisting of cyanophytes (e.g. *Raphidiopsi* sp., *Microcystis* spp.), coincided with high dissolved phosphorus, total arsenic, ammonia-nitrogen, nitrate-nitrogen and TSS.

2. Group II consisting of cyanophytes (e.g. *Cylindrospermopsis* sp., *Cylindrospermum* sp., *Oscillatoria* spp.) and Pyrrophytes (e.g. *Peridinium* spp.) associated with high conductivity, BOD and pH.

3. Group III was situated at the higher part of the centre on the left hand side, characterized by lower conductivity and more acid. This group consisted of chrysophytes (i.e. *Dinobryon* spp.).

4. Group IV consisted of chlorophytes (i.e. *Botryococcus* sp.). It seemed to prefer an environment with more dissolved oxygen.

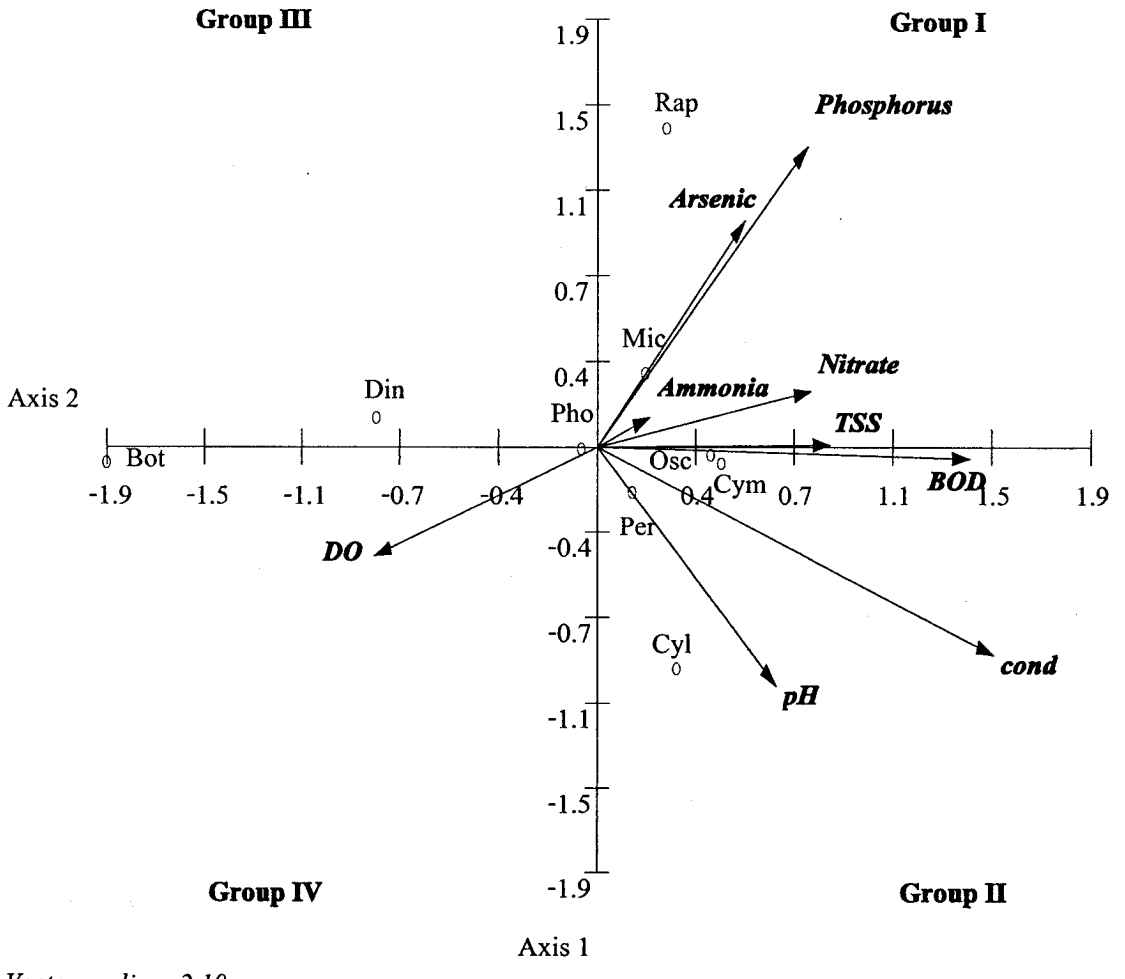
Comparing phytoplankton genera with the spatial and temporal dynamics of phytoplankton communities, all groups seemed to indicate both spatial and temporal effects with those phytoplankton assemblages.

Table 9. Summary of the results from the CCA (Canonical Correspondence Analysis). Eigenvalues, % of variance explained and species environmental correlation.

	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.579	0.535	0.230	0.192
Percentage	15.225	14.069	6.060	5.036
Cum. Percentage	15.225	29.294	35.353	40.390
Cum.Constr.Percentage	36.156	69.568	83.959	95.919
Spec.-env. correlations	0.867	0.829	0.676	0.595

Table 10. Biplot scores for environmental variables.

	Axis 1	Axis 2	Axis 3	Axis 4
pH	0.324	-0.498	0.687	-0.192
DO	-0.401	-0.228	0.005	-0.342
BOD	0.671	-0.024	-0.122	0.443
Arsenic	0.268	0.470	0.096	-0.482
Nitrate	0.386	0.116	0.076	0.004
Phosphorus	0.381	0.624	0.305	-0.426
Cond	0.715	-0.434	-0.307	0.092
TSS	0.421	0.005	0.230	0.420
Ammonia	0.095	0.062	-0.240	0.307



Vector scaling: 2.10

Figure 35. Ordination biplot of phytoplankton genera and environmental variables in the arsenic contaminated waters. The codes for the genera are shown in Table 11.

Table 11. Genera code of phytoplankton communities in arsenic contaminated waters at the Ron Phibun district of Nakhon Si Thammarat province.

Taxa	Genera code
Division Cyanophyta	
Cylindrospermopsis sp.	Cyl
Cylindrospermum sp.	Cym
Microcystis spp.	Mic
Oscillatoria spp.	Osc
Phormidium spp.	Pho
Raphidiopsis sp.	Rap
Division Chlorophyta	
Botryococcus sp.	Bot
Division Pyrrophyta	
Peridinium spp.	Per
Division Chrysophyta	
Dinobryon spp.	Din

CHAPTER 4

DISCUSSION

1. Environmental variables in the arsenic contaminated waters

Total arsenic concentration of water samples collected from dredging ponds (locations 1, 2, 3 and 5) were relatively high as compared to dug ponds (locations 4 and 6). This is due to the fact that water bodies have received arsenic contamination directly from tin mining activities for many years. Also, it is probably due to the additional effects of discharged water from domestic areas and agricultural land (JICA, 2000; PCD, 1998). With regard to arsenic concentrations, it can be observed that the concentrations of arsenic in dredging ponds are above the WHO standard for arsenic of 10 μg/L (WHO, 1981) all year round, whilst the situation of arsenic concentrations in dug ponds are still within a safe level, except in June 2005 for location 6.

Total arsenic at locations 1, 3 and 5 (HACP) were observed to have been affected by rain intensity. Arsenic concentrations in surface waters decreased with increasing rainfall and flow conditions (Sultan and Dowling, 2006). This is because increased levels of arsenic are released back from the bottom sediment, whilst low arsenic levels usually occur during the wet season as a result of the diluting effects of rain. Waste discharge may also interfere with the surface water concentration to some extent (Jianjun, 2002). Previous investigations in arsenic contaminated waters in the Ron Phibun district of Nakhon Si Thammarat province have reported that higher arsenic concentrations occured in the dry season, rather than in the rainy season (Jianjun, 2002). In addition, the remaining locations with low total arsenic concentrations were found to fluctuate less year round. These patterns were similar to other studies carried out by Stoner *et al.* (1977) and Bu-Olayan and Thomas (2001).

According to the study, the DO contents of a sampling location were not found to be stable. However, all sampling locations had enough dissolved oxygen to support aquatic organism life. Extremely low levels of dissolved oxygen were recorded in July and December at location 1 (< 3 mg/L). This location is more likely to have low oxygen levels than other locations. The causes of low DO may be a combination of the following factors: high organic production and respiration, slow flushing, nutrient enrichment, vegetative decay, decomposition of slowly decaying organic matter,

benthic oxygen demand (Frisk, 1982; Lee and Jones-Lee, 2003; McFall, 2003; Parr and Mason, 2003). Phytoplankton produce oxygen during daylight hours, but consume oxygen during the night. To a lesser extent, oxygen is also absorbed from the atmosphere at the water's surface. Wave action or other disturbances will increase the water's dissolved oxygen concentration by expanding its surface area for oxygen to enter (Conte and Cubbage, 2000). Phytoplankton populations will produce enough dissolved oxygen to support life in a pond throughout the day time. However, dying populations may consume more dissolved oxygen at night then they produce during the day. When phytoplankton release less dissolved oxygen during cloudy days than they consume at night, low dissolved oxygen conditions may occur, particularly during the day time in December. Sensitivity to low levels of dissolved oxygen is species specific, however, most species of aquatic organisms, particularly in fish are distressed when DO falls between 2 and 4 mg/L. Mortality usually occurs at concentrations less than 2 mg/L (Francis-Floy, 2003). An increase in BOD is also considered to be a potential problem as this will result in sudden DO depletion (Frisk, 1982; Watananugulkit *et al.*, 2003). However, BOD concentrations were mostly below the established water quality standard (not more than 4 mg/L) in this study (UNEP, 1999). Such BOD levels still provided appropriate conditions for the water ponds during the sampling period. In addition, natural sources such as leaf fall from vegetation near the water's edge, aquatic plants, drainage from organically rich areas and anthropogenic sources of organic matter can increase the BOD concentrations along the water column (Jha *et al.*, 2007; Watananugulkit *et al.*, 2003). Organic matter also comes from sources that are not easily identifiable such as agricultural runoff, urban runoff and livestock operations. These sources can significantly reduce oxygen demand in all sampling locations if not properly regulated and controlled.

It was found that nutrient availability (ammonia-nitrogen) tended to be high during the rainy period (December) in all sampling locations and those nutrients decreased considerably in January. However, this event is in contrast with nitrate-nitrogen. Nutrients in this study were dominated by agricultural runoff and soil erosion. They are always common in waters and have been affected by anthropogenic activities (Chaibu, 2000). They may be considered to be arising from either sources from heavy rainfall or agricultural runoff. A significant increase of conductivity concentrations were also observed during the rainy period (December), suggesting an important mineralization process occurring close to the lake bottom, probably because of anaerobic, heterotrophic bacterially mediated processes that are releasing ions back into the water column

(Kemka *et al.*, 2006). In addition, increasing conductivity was probably due to sewage discharge, which also increased nutrient concentrations (Borges *et al.*, 2003).

2. Phytoplankton communities in the arsenic contaminated waters

Phytoplankton communities represent a highly diversified flora as compared to other studies on heavy metal contaminated wetlands (Yan, 1979). From all taxa, chlorophyceae were higher in diversity than other groups. This is apparently consistent with another study by Bunnag (2000) and Chankaew *et al.* (2007) whose observed locations were nearby the study areas. The study in mine drainage areas showed that metals decreased the diversity of phytoplankton flora. Also, Cyanophyceae and Bacillariophyceae were less diverse than members of the Chlorophyceae (Pongswat *et al.*, 2004; South and Whittick, 1987). Additionally, the diversity of phytoplankton in this study was greater than that found previously. To some extent, only fifteen phytoplankton genera were identified from the old mining areas of Ron Phibun district. Of these, eleven genera were green algae and the others were cyanobacteria and diatom (Bunnag, 2000). Given the same district, the survey of phytoplankton in sago palm forest waters showed that sixty-one phytoplankton genera were identified. The most diverse group was Chlorophyceae (27 genera), followed by Bacillariophyceae (15 genera), Cyanophyceae (10 genera), Euglenophyceae (5 genera), Chrysophyceae (3 genera) and Pyrrophyceae (1 genus) (Chankaew *et al.*, 2007). Referring to Bunnag (2000), a lesser number of phytoplankton might be as a result of insufficient filtration of water samples. In the meantime, the lower genera numbers of phytoplankton were found to be influenced by water flow and dilutional effect in those lotic habitats of sago palm forest waters.

Regarding phytoplankton abundance, Cyanophyceae was significant in many sampling locations. Of six sampling locations, Cyanophyceae in location 4 was generally found to be below a relative abundance of 50%. The high abundance of Cyanophyceae recorded during the sampling locations was due to several similar taxa with filament buoyancy. Similarity of cyanophytes assemblage in many sampling locations might be caused by similar environmental conditions such as nutrient supply, mean light intensity, water temperature, etc. In addition, this was obviously clear from the cluster analysis result. In addition, location 4 which was a newly dug pond was found to be dominated by Chlorophyceae, particularly desmids. This was consistent with Alam *et al.* (1987). It

has been suggested that plankton of oligotrophic water bodies are characterized by a large number of desmid species (Brook, 1965; Opute, 2000). However, the appearance of desmids seems to depend upon the age of pond-water rather than whether the water is eutrophic or oligotrophic (Michiyasu, 1954). The present study also found some level of succession in phytoplankton abundance, such that as the Cyanophyceae abundance increases to the maximum, the population of other groups tend to decrease and/or increase gradually as compared with Cyanophyceae during the period of sampling times. It is well known that some phytoplanktonic algae are better adapted than others to stress environments (Reynolds *et al.*, 1994). Cyanophyceae can assimilate ammonia and this affords it a competitive advantage over other species (Akin-Oriola, 2003). In this study, many buoyant cyanophytes species such as *Cylindrospermopsis* sp., *Cylindrospermum* sp., *Phormidium* spp. were found. In an aquatic ecosystem, the motility and buoyancy regulation of filamentous cyanobacteria may offer considerable advantages in gaining dominance and may allow enhanced levels of light and nutrient to be received in comparison with other phytoplankton (Mitrovic *et al.*, 2001; Steinberg and Hartman, 1988; Walsby *et al.*, 1997). This may advantage buoyant cyanophytes by providing for the high energy requirements of nitrogen fixation (Smith, 1990), or by compensating for poor competitive growth rates due to the colonial habitat diminishing antenanal efficiency in comparison with many chlorophytes and diatoms (Reynolds, 1994).

Comparison of attributes and diversity indices were also taken into consideration in this study. The analysis of data over this period showed relatively low diversity (less than 1.5 bits individual^{-1}). Low diversity environments normally correspond with Shannon-Weiner indices lower than 2.5 bits individual^{-1} (Margalef, 1972). Additionally, the diversity index did not showed a low value in all sampling locations during the rainy period as compared to other seasonal periods, though during this period there was low species richness. This may be due to the results showing increasing evenness values which are considered a dependent factor of the diversity index (Chittapun, 2003; Magurran, 1987). The rainy period presented a high uniformity in the evenness index as compared to other seasonal periods due to the lower density values of the several phytoplankton groups. Moreover, it indicated that all species generally distributed evenly. Although the diversity index in many sampling locations was found to be similar due to the seasonal effects, the number of species was the more sensitive parameter. During the rainy period, using species richness as an index, all sampling locations were significantly different from all other seasonal periods. The result was probably

influenced by seasonal effect. Furthermore, a comparison between HACP and LACP revealed slight differences in diversity indices. Thus, such aquatic ecosystems may not be affected by total arsenic.

3. Correlations between phytoplankton and environmental variables

3.1 Spatial and temporal patterns of phytoplankton communities in arsenic contaminated waters

There were remarkable differences in relative abundance among sampling locations, although cyanophytes seemed to be more prolific in many sampling locations as compared to other algal groups. In fact, cyanophyceae represented a large portion of the assemblage in the epilimnion lake, an environment that may shelter species which can adapt to quite different ecosystems (Round, 1984). Nutrient addition might have affected the species composition of the phytoplankton communities (Seppala *et al.*, 1999). This study found that all sampling locations had moderate nutrient. Cyanophyceae have been frequently associated with high trophic environment (Harrer, 1992; Huszar and Reynolds, 1997), but they are also important components of phytoplankton in oligo and mesotrophic waters (Blomqvist *et al.*, 1994; Canfield *et al.*, 1989; Hecky and Kling, 1987; Huszar and Caraco, 1998). However, the other environmental variables such as high temperature, low light intensity and a degree of environmental constancy supported cyanobacteria blooms (Beyruth, 2000; Padisak and Reynolds, 1998; Paerl, 1988; Trimbee and Prepas, 1987; Watson *et al.*, 1997). Chlorophyceae were also significant in location 4 mainly due to desmids. However, such desmids were of considerably less importance in other locations. Beyruth (2000) stated that the growth of chlorococcales and cyanobacteria is favored in lentic and eutrophic tropical environments such as Guarapiranga during periods of high temperature, mixing and nutrient input. However, the domination of desmids in location 4 indicates oligotrophic or mesotrophic lake conditions, conforming to the observations by Nweze (2006), Round (1977) and Yan (1977) in that the abundance of desmids is common in low nutrient lakes and pH. Desmids can be dominant under oligotrophy, mesotrophy, and eutrophy, since they have a wide spectrum (Coesel, 1983; Coesel and Blokland, 2006; Nygaard, 1991). A high number of different species in this study was apparent to support the suggestion of Beadle's study that the pond exhibits a low content of nutrients along the water (Beadle, 1974). In addition, the

oligo-mesotrophic species were *Dinobryon* spp. (Alves-de-Souza *et al.*, 2006; Anneville *et al.*, 2002), which was found to be a dominant species in locations 2 and 5. Nutrient enrichment-related changes in the taxonomic composition of phytoplankton are widely documented (Kemka *et al.*, 2006; Levkov, 2005). According to Tilman *et al.* (1986), diatoms and chrysophytes can better utilize phosphorus than cyanobacteria, and because of that in waters with lower phosphorus content chrysophytes are the dominant group in locations 3 and 5 during January. The difference in occurrences of different algal groups in water bodies was the base for the concept of nutrient limitation. Tilman *et al.* (1982) stated that there was not a nutrient limitation of a particular water body, but that only individual algae were limited by a particular nutrient.

It was also discovered that the trophic status of the sampling locations was not so different from each other during the sampling time and indicated that all sampling locations generally had moderate levels of nutrients and phytoplankton diversity. The enrichment of nutrition often induces the loss of biodiversity (Miyazaki *et al.*, 2004). In addition, its variability may be caused by the significant input of nutrient rich effluent into the sampling locations, which induced ecosystem modification on the level of phytoplankton diversity (Trifonova, 1998). On the other hand, there is no precise answer about the existence of typical indicators for oligotrophic waters. From many investigations in to natural ecosystems and cultures, it is know that increased production and biomass occurs with increased nutrient content. Also it is known that many species can be found in higher trophic levels (Peerapornpisal, 2005; Wetzel, 1983) but cannot be found in lakes with a lower nutrient content (Lange-Bertalot and Metzeltin, 1996). Nevertheless, it is slightly unusual that species which exist in lower nutrient concentrations will suffer in lakes with higher nutrient concentrations. Experiments suggest that the species do not increase nutrient uptake in case of higher nutrient concentrations (Rosenstrom and Lepisto, 1996). The most probable explanation is that oligotrophic indicators could have a wider distribution, but in the case of higher nutrient content, they are out-competed by eutrophic species (Levkov, 2005).

Total suspended solids were notably higher in location 5 during the rainy periods. During such a period of time, buoyant cyanophytes such as *Cylindrospermopsis* sp., *Phormidium* spp. and flagellate chrysophytes such as *Dinobryon* spp. were found to be the dominant genera. This finding may imply that these phytoplankton genera can live under environmental stresses, particularly in turbid environments. Holz *et al.* (1997) recorded a shift from high phytoplankton turbidity to high

sediment turbidity as a response to aging of the Pawnee reservoir. At the same time, the phytoplankton assemblage shifted away from buoyant cyanophytes, toward flagellates, which were better able to avoid the shading caused by sediments and optimize their position in the euphotic zone via active phototaxic swimming. Furthermore, the diversity reduction in the worst samples is in agreement with a simplification of the entire community, or part of it, commonly observed in most forms of extreme pollutional stress (Chittapun, 2003). In location 5, qualified as very turbid and very rich in arsenic concentration, algal species adapted to such prevailing conditions are likely to be found. Some of these algae are resistant to heavy metals (e.g. *Oscillatoria* sp., *Phormidium* sp.) (Badmus *et al.*, 2007; Bhattacharya *et al.*, 1989), as was demonstrated for these aquatic ecosystems with respect to total arsenic. Therefore, they are capable of great proliferation when the total arsenic level increases.

Obviously, phytoplankton abundance in location 5 is generally low all year round. Location 5 which is categorized as HACP was generally contaminated with total arsenic level of more than 30 μg/L. Such contamination had an adverse effect of arsenicals on phytoplankton organisms due to chronic arsenic poisoning (Eisler, 1988; Sanders, 1986; Sander and Cibik, 1985). NRCC (1978) reported that growth and biomass in freshwater and marine algae was reduced at 75 μg As^{+5}/L. Arsenic was also considered to be the key factor driving the change in the phytoplankton community due to high arsenic concentrations (Kalin *et al.*, 2001; Price and Pichler, 2005; Sanders and Vermersh, 1982). Therefore, addition of arsenic was expected to result in differential mortality of the phytoplankton communities. On the other hand, arsenic in trace amounts may exhibit a high degree of toxicity, yet in a suitable range level, could be beneficial to phytoplankton growth (Knaver and Hemond, 2000). Growth stimulation by arsenic has been described by Sanders (1979) who stated that the growth of the diatom *Skeletonema costatum* increased with the addition of arsenic (80 μM). Thus, in some aquatic environments such as LACP that contaminated with arsenic, it might not affect the phytoplankton population.

Certain dominant genera of *Cylindrospermopsis* sp. and *Microcystis* spp., for example, synthesized hepatoxic alkaloids and peptides, respectively. Whereas, *Cylindrospermum* sp. synthesized neurotoxin. Cyanobacterial toxins were commonly classified according to their toxicological effect from cyanobacterial toxins (Beasley *et al.*, 1989). They can occur within the cyanobacterial cell or be released into the water after cell lysis. A possible biological function of cyanotoxins, such as microcystins, is that they might provide cyanobacteria an advantage by reducing

the losses associated with grazing and competition. Because grazing and competition pressures are not constant, the production of cyanotoxins could be induced or promoted only when necessary to avoid dispensable costs. Several cyanobacterial genera and strains can be examined on induced chemical (toxin production) and morphological (colony formation) defenses when exposed to grazers or competitors. Several functional groups on the cyanobacteria surface can interact with metals and play a major role in heavy metal contaminated waters (Ledin, 2000). These functional groups commonly exist on polysaccharide and some proteins which cover the cell surface. They are useful to cyanobacterial genera by increasing their ability to live well in heavy metal contaminated waters. Many cyanobacteria are known to be able to synthesize outermost slimy layer and to release polysaccharidic material into the outside of cells (De Philippis and Vincenzini, 1998; Geesey and Jang, 1990). With increased cyanobacterial age, the amounts of polysaccharides and proteins on the cell surface were also assumed to increase resulting in the existence of more functional groups (Ruangsomboon *et al.*, 2006). Furthermore, nutrients (especially phosphorus) are usually thought to be one of the factors most responsible for cyanobacterial blooms. The restriction of other phytoplankton genera following reduced phosphorus has allowed cyanobacteria to make use of organic phosphorus to improve their competitiveness (Jacquet *et al.*, 2005; Sarnelle and Wilson, 2005). However, in contrast to planktonic algae, some cyanobacteria are able to escape nitrogen limitation by fixing atmospheric nitrogen. The lack of nitrate or ammonia, therefore, favours the dominance of these organisms (Chorus and Bartram, 1999).

All the water pond studies lie in the same geographical area, they are subject to broadly an identical climate and, on average, to the same seasonal meteorological variability (Jianjun, 2000). However, with regard to all sampling locations, it may be observed that seasonal variations, and especially, the rainy period, interfere with species numbers and phytoplankton densities. A distinctive seasonal pattern in phytoplankton communities was observed in December at all sampling locations. There is no field or experimental evidence offering a clear mechanism for the responses in richness and abundance observed along the arsenic contaminated waters. This study is among the first to explore regional phytoplankton richness and abundance with respect to a major well-documented seasonal impact.

Most previous investigations on tropical waters have reported that higher phytoplankton populations occur in dry, rather than in rainy period (Egborge, 1979; Zhang *et al.*,

2006). However, this conclusion was not the case for all ecosystems in this study. The highest phytoplankton population was particularly noticeable in location 3 during the dry period which could have been attributed to increased temperature and light during the sampling period. This was consistent with Ghavzan and Gunale's study (2007). On the other hand, it was found at other locations that the mean total phytoplankton population density during the early rainy period was significantly higher than that in the dry period. This is also consistent with the observations of Nweze (2006). This finding was correlated with the rains, which caused appropriate quantities of nutrients to enter the water ponds. The generated rainfall from the surrounding agricultural land might have induced the phytoplankton growth. The observed number and density of phytoplankton dramatically decreased with the progression of precipitation during the rainy period (November to December) in all sampling locations, which was possibly attributable to reduced water transparency, wind effect, cloud cover and the dilutional effects of rain (Evurunobi, 1984; Gurung *et al.*, 2006). From November to December, heavy rains occurred in the sampling ponds, resulting in large volumes of water over a short period of time. This increased water volume might have diluted the phytoplankton in the sampling ponds, and the rate of basin flushing restricts the flora to small, fast-growing and invasive species with the potential rate of growth to be able to resist dilution from the waters (Melo and Huszar, 2000; Reynolds and Lund, 1988). During such a period of time, the water ponds cool and low phytoplankton abundance coincides with low temperature and light. The population collapse was probably brought about by light and temperature conditions, which are therefore of great importance in controlling phytoplankton growth (Bleiker and Schanz, 1989; Perez *et al.*, 1999). Needoba and Harrison (2004) stated that the light regime influences the relative uptake, assimilation and efflux rates of nitrate, whilst decreases in phytoplankton density with falling water temperature were probably due to slow reproduction, rather than an increased death rate (Biswas, 1992). In the meantime, species number and density apparently increase in January which could be as a result of the steady level of precipitation and a lack of any monsoon effect from the northeast as compared to the pass few years. Thus, climatic events and seasonal impact have a strong influence on the hydrodynamics and on the structure of aquatic communities in ponds, agreeing with Cowan *et al.* (1999), mainly through interference with the nutrient balance (Anneville *et al.*, 2005). The stability and diversity of the phytoplankton community with respect to nutrients is also discussed.

The present study clearly shows that low cell densities were found during the rainy periods (November and December), and that they dramatically increased in the following month (January). This fluctuation pattern was well-matched to all sampling locations that were determined during the same time. In addition, if nutrient depletion occurred because of its consumption by phytoplankton, it is likely that phytoplankton had accumulated in the water in abundant numbers even if their growth rates were limited (Gurung *et al.,* 2006). In support of this conclusion, short-term experiments showed that nutrient supplies limit the phytoplankton growth rate in the lake (McEachern, 1996). Nitrate-nitrogen is also an important nitrogen source for phytoplankton. During the rainy period, nitrate-nitrogen concentrations seemed to be low at all sampling locations, these values possibly being related to phytoplankton uptake and denitrification (Akunna *et al.*, 1994; Egborge, 1974; Findley *et al.*, 1973). Increases in the transformation of nitrogen were sufficient to offset the increasing inputs throughout the production cycle at low intensity (Cowan *et al.*, 1999). Kietpawpan (2002) stated that relatively low concentrations of nitrate and/or other nutrients in the waters probably resulted from their losses by natural processes and by anthropogenic activities. Furthermore, the result in February was vice versa to December. This may be as a result of the denitrification process along the water column in this study, which is consistent with Dummee's research during the dry period (Dummee, 2006). In addition, nitrite-nitrogen could not be detected in all sampling times because it is easily oxidized (Lemmel and Cape, 1996).

The present study has shown some heterogeneity of phytoplankton biomass. Chlorophyll *a* content of the algae in the studied ponds seems to depend on species composition, TSS, rain intensity effect and nutrient availability. Hunter and Laws (1981) documented low chlorophyll *a* content under nutrient limitation and elevated chlorophyll *a* content under light limitation, the latter connection has also been demonstrated in lake Kinneret (Berman *et al.*, 1992). High chlorophyll *a* occurred when a large proportion of the chlorophyll *a* was made up of coccal and filamentous forms such as *Microcystis, Oscillatoria, Cylindrospermopsis, Phormidium* which belong to cyanophyceae. This situation was observed, particularly in July. Significant levels of chlorophyll *a* were found in locations 1, 2, 3 and 6. Such chlorophyll *a* levels were obviously consistent with mean phytoplankton abundance as shown in cluster analysis. Locations 1, 2, 3 and 6 were surrounded by agricultural areas. The nutrient loading from domestic waste and fertilizers entering those locations should provide the pond with enough nutrients for phytoplankton growth. For decades, there has been a series of

proposals indicating that many algal species could be used as indicative parameters along the aquatic ecosystems, e.g. with one or more cyanophytes species representing a large portion of the assemblage in those locations, they could be used to indicate that the water has a moderate/or high nutrient loading into the ecosystems (Wetzel, 1983). Moreover, the content of chlorophyll *a* in locations 4 and 5 seems to be low in general, which comprised mainly phytoplankton assemblages such as desmids and dinoflagellates. Such algal groups indicated that those ecosystems would place it in moderated nutrient loading in those water ponds (Wetzel, 1983). Low chlorophyll *a* in location 4 occurred because there is little discharge of domestic waste or fertilizers from anthropogenic sources even in those sampling locations enveloped by agricultural areas. The low content of chlorophyll *a* in location 5 was due to the pond turbidity. It is suggested that suspended solids entering the pond or recurrent from the bottom are a crucial factor affecting the phytoplankton abundance and composition (Kwang-Guk and Jones, 2000; Holz et al., 1997), as occurred particularly at location 5. Furthermore, there was some evidence of reductions of biomass in lakes with the highest heavy metal concentrations (Yan, 1977). This event may cause low chlorophyll *a* concentrations along the water column.

The seasonal dynamics of the phytoplankton biomass in the studied ponds was generally found to be consistent, particularly during the rainy period. The very low biomass in November and December can then also be considered as a temporary phase, possibly connected to the heavy rainfalls. In contrast, the sharp increase in phytoplankton biomass accompanying heavy rainfalls (identified as physical disturbance) was observed in the nearby Thale Noi (Noi lake) (In-pang, personal contact). Additionally, the ecosystem found in location 5 has unique properties and entailed an unpredictably low production of phytoplankton biomass. Despite high nutrient supplies, the water transparency period observed in the rainy period (November to December) seemed to be an important feature in the functioning of location 5. Gin *et al.* (2000) found that there was some seasonal variation in nutrients and chlorophyll *a* due to the different monsoons. In general, slightly higher values were recorded during the south-west monsoon compared with the north-east monsoon. As a consequence, the actual algal response is far less predictable in nature than in laboratory assays. For example, although nutrients increased downstream in April, with increasing algal biomass in bioassays, the weak response of phytoplankton in the study areas concided with an increase in the turbidity, which may then have limited potential production (Olguin *et al.*, 2004). In the meantime, coincidence of the highest levels of both organic minerals and heavy metals in samples was an impediment for the

discrimination of their isolated effects (Olguin *et al.*, 2004). and may be difficult to explain in this study. To avoid misidentification of pollution effects, when several substances occur in common concentration gradients at concentrations high enough to have a potential effect on the community, then all substances should be tested (Wangberg, 1995).

3.2 Canonical Correspondence Analysis (CCA)

Of the measured environmental variables, CCA analysis showed that nine parameters were related to phytoplankton flora in arsenic contaminated waters. Phytoplankton are known to be reliable indicators of changes in environmental conditions, most notably changes related to nutrient loading (Henrikson *et al.*, 1980; Jeppeson *et al.*, 2005; Novales-Flamarique *et al.*, 1993). Furthermore, phytoplankton strongly influences food quality for higher trophic levels and thus plays an important role in energy transfer within food webs (Gaedke *et al.*, 2002). Changes in phosphorus concentrations have a strong influence on phytoplankton (Hejzlar *et al.*, 1998), agreeing with this research. However, it is often difficult to predict how identical changes in phosphorus concentrations will affect different waters. In some cases, phytoplankton biomass concentrations were controlled by other environmental variables, and even though the phosphorus levels are high, the lakes are still not very productive. The higher value for total phosphorus unfiltered in the arctic tundra lake as well as in a lake a few meters away may be related to the relatively high iron concentrations (Ruhland *et al.*, 2003). Iron is known to form complexes with phosphorus, leaving the measured phosphorus unavailable for biological uptake (Jones *et al.*, 1988). This appears to be the case for these lakes, as low algal biomass, as inferred from chlorophyll *a*, and high TP values was also recorded (Ruhland *et al.*, 2003).

The result demonstrates that not only dissolved phosphorus, but also nitrate-nitrogen and ammonia-nitrogen had a relation to phytoplankton flora, particularly in Group I. Nutrients entering into waters probably result from higher domestic runoff, higher rain intensity, and agricultural discharge loading (Declerck *et al.*, 2006; Neonov and Nazarov, 2001). In addition, the nutrients present may be from resuspended sediment maintained during the study. Resuspension can introduce sediment-derived nutrients (Cotner *et al.*, 2000; Schallenberg and Burns, 2004) and also be of benefit to viable, or resting cells, from the sediments during their convertion into plankton while being transported through the waters (Ishikawa and Furuya, 2004). The lack of response of phytoplankton

flora to nutrient decreases can be explained by compositional changes in phytoplankton communities, grazing by zooplankton, sedimentation, light, temperature, turbulence and changes in self-shading (Agusti, 1991; Banse, 1994; Schwartzkopf and Hergenrader, 1978; Tallberg and Heiskanen, 1998). These factors might therefore distort the theoretically linear relationship between nutrients and phytoplankton. Additionally, the cause of increased arsenic concentration at all times may result from an increased amount of suspended solids in the water, particularly noticeable in HACP at high values. Also, it is possibly linked to the decreased water level during the dry season. The above-mentioned phenomena may be one of the possible causes for the observed increase in total arsenic concentration. Arsenic is strongly adsorbed by several common minerals including suspended solids (Koyama *et al.*, 1989; Oremland and Stolz, 2003). A significant increase in total arsenic concentration correlated with the dissolution of manganese and iron on the sediment surface and this could have played a significant role in the dissolution of arsenic from the sediment (Takamatsu *et al.*, 1985). The processes that influence arsenic accumulation in sediments are also at least partly responsible for the control of aquatic arsenic concentrations. The redistribution of arsenic by natural phenomena has important environmental consequences (Cornett *et al.*, 2004). Arsenic released from the water sediment also has a big effect on the total arsenic concentration in surface waters and it may cause change in aquatic organism communities, particularly phytoplankton (Cullen and Reimer, 1989; Linge and Oldham, 2002).

Some phytoplankton genera in water samples preferred an increased of total arsenic. The concentrations of total arsenic had a correlation with phytoplankton flora, especially cyanophytes. Environmental stress tolerance has been demonstrated for that phytoplankton group in many papers (Fiore and Trevors, 1994; Ruangsomboon *et al.*, 2007). Additionally, removal of arsenic contaminated water has become one of the main responsibilities for protecting the environment (Bunnag, 2000). In recent years it is interesting to note the development of programs using microalgal for water and wastewater treatment, heavy metal control in natural waters and industrial waste streams, and even biological detoxification (Pinto *et al.*, 2003). The microalgae can utilize the minerals and some organic compounds and produce oxygen by photosynthesis. This improves the quality of the water (Peerapornpisal, 2005). These findings showed the importance of considering the tolerant algal group, perhaps suggesting that some phytoplankton genera have adapted in some other way to high arsenic concentrations and may be used for bioremediation of arsenic pollution.

The outcomes also indicate that some phytoplankton genera correlate with pH. pH is one of the environmental variables that can potentially limit the growth of phytoplankton in natural water (Findlay and Kasian, 2004). Findley and Kasian (2004) stated that acidification of freshwater ecosystems changes phytoplankton biomass and reduces species composition. The presence of *Dinobryon* spp. from Group III indicated that this genus preferred lower BOD and conductivity and was well adapted to an environmental condition with low pH, whereas some genera of pyrrophytes such as *Peridinium* spp. and cyanobacterial genera such as *Cylindrospermopsis* sp., *Cylindrospermum* sp. and *Oscillatoria* spp. from Group II preferred an environment with high BOD, conductivity and pH. However, members of dinoflagellate and cyanobacteria have different levels of acid tolerance (Belkin and Boussiba, 1991; Niesel *et al.*, 2007). Up until the present time in this study, it is not known whether acid levels in the water will affect the dinoflagellate and cyanobacteria described here. Furthermore, chlorophytes in the genus *Botryococcus* sp. were presented in Group IV, and that genus correlated with DO. Such coccal green algal genus has no clearly evidence to be concided with DO. However, some paper stated that the phytoplankton blooms were found in chlorococcales and was observed when DO was as its maximum (Kumawat and Jawale, 2004). In addition, Valecha *et. al.* (1990) has shown that unicellular chlorophytes including *Botryococcus* sp. are the species found in organically polluted waters. They can live in a wide range of environmental habitat. It was therefore indicated that *Botryococcus* sp. could also live well in low DO conditions. Some phytoplankton genus such as *Phormidium* spp. plotted near the centre of the graph was not correlated to any environmental variables. However in general environmental variables had the most influence on phytoplankton communities as seen from the CCA data.

CONCLUSIONS

1. The average density of phytoplankton flora ranged between 8.08 x 10^4 to 1.24 x 10^6 cells/L. Cyanophyceae were the dominant group with a relative abundance of more than 50 % except at the sampling location in Saothong sub-district due to a high abundance of chlorophyceae.

2. Phytoplankton in the waters investigated were characterized by numerous forms of different algal groups. Cyanophyceae were of quite a high importance in many studied locations, but the dominating genera were different. The dominant cyanophyceae genera that were generally found in all sampling locations were *Cylindrospermopsis* sp. and *Oscillatoria* spp. In addition, chlorophyceae were the most abundant component of the present phytoplankton community in sampling location of Saothong sub-district, particularly desmids flora.

3. The composition of both HACP and LACP did not show great differences in diversity indices. Furthermore, changes in the phytoplankton community along the gradient of increasing rain intensity became more prominent when the species richness (R) was taken into consideration. Also, it was clear that the amplitude of the phytoplankton relative abundance responded more to seasonal effects than to spatial factors. Heavy rain in the sampling locations limited the phytoplankton abundance during the rainy period.

4. CCA indicated that nine environmental variables correlated with the phytoplankton communities e.g., dissolved phosphorus, total arsenic, nitrate-nitrogen, ammonia-nitrogen, BOD, TSS, conductivity and dissolved oxygen.

RECOMMENDATIONS

1. Not only does total arsenic interfere with phytoplankton communities, but it also affects other environmental variables. Thus, further information from laboratory experiments regarding sensitivity, selection and tolerance is needed to explain the impact on natural communities as a result of arsenic contaminated waters.

2. Further investigations need to determine the effectiveness of removing total arsenic in laboratory and natural waters, with a further suggestion that the dominant phytoplankton genera used should be one of the alternative bioadsorbers for total arsenic in bioremoval systems. The recommended cyanophytes are, *Phormidium* spp. a phytoplankton genus is considered to be effective in removing arsenic from the water column (Wang and Weissman, 1998). In addition, the phenomenon of heavy metal tolerance in aquatic plants has attracted considerable attention from environmental biologists. Arsenic accumulation by aquatic plants may make them valuable tools for bioindication and phytoremediation. Suitable aquatic plants must be selected for polluted locations. For example, *Colocasia esculenta* is the selected aquatic plant for removing arsenic (Aksorn and Visootiviseth, 2004). Additionally, *Pityrogramma calomela* has been discovered in Thailand to be an arsenic-hyperaccumulating fern which can be used for phytoextraction of arsenic-contaminated soil (Visootiviseth *et al.*, 2002). It can be planted around the banks of ponds. The effectiveness of these biological plants makes their use the preferred method of removing arsenic from the environment.

3. The speciation of arsenic in an aquatic environment is affected partly by indiscriminate biological uptake. Each one of the arsenic species has its own property and affects a biological system in its own way. Therefore, arsenic speciation is needed to identify. Another factor that needs to be determined is arsenic accumulation by phytoplankton cells. The question as to how phytoplankton accumulate arsenic could be partially resolved by conducting microanalyses on the tissues to determine if arsenic is present inside the cells or simply bound to the cell walls.

4. Nowadays, the input of waste water to the sampling locations increases daily due to an increasing human population and the expansion of agricultural land, thus threatening water quality. Measures must be undertaken to counter this deteriorating trend. Also, it is necessary to prohibit the discharge of waste water with high nutrient concentrations into natural ponds.

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APPENDICES

Appendix A

I. Chlorophyll *a*

Reagents:

1) Distilled water
2) Aqueous acetone solution:

   Carefully measure 100 mL of water into the 1 L graduated cylinder. Transfer the contenet to a 1 L flask or storage bottle. Measure 900 mL of acetone into the graduated cylinder and transfer it to the flask or bottle containing the water (90 % acetone: 10 % distilled water). Mix, label and store the liquid.

Procedure:

1) The samples were filtered as soon as possible after collection as chlorophyll pigments react with light and oxygen.
2) A known volume of sample water was vacuum filtered through a glass fibre filter. When the analysis cannot proceed immediately, samples can be stored in this state at -20 °C for approximately 3 to 4 weeks if wrapped in aluminium foil to keep the light out.
3) The filter was broken up to facilitate extraction before placing it into a 15 mL centrifuge tube. A 90 % ethanol solution was added and the mixture was shaken thoroughly. The tube should be wrapped in foil to keep out any light and refrigerated at 4 °C for at least 2 hours but not longer than 24 hours. The tube was shaken three times during this period.
4) After it had been left to stand, the contents of the tube was centrifuged for 10 minutes at which point a clear solution remained. This time can be as short as 5 minutes depending on the speed of the centrifuge.

5) The optical density of the supernatant should ideally be less than 0.05 abs at 750 nm for a 1 cm cuvette.
6) In subdued light the supernatant was poured into a 1 cm cuvette and measured immediately. The concentration of the solution must be determined spectrophotometrically using a multiwavelenght spectrophotometer. The instrument was zeroed on a 90 % ethanol solution and the sample was measured at wavelengths 750, 664, 647 and 630 nm.

Calculation:

The amount of chlorophyll *a* was calculated by inserting the 750 nm corrected absorbances into the equation:

Chlorophyll *a* = 11.85 (Abs 664)-1.54 (Abs 647)-(Abs 630)

The amount of chlorophyll *a* pigment in the water sample was determined using the following equation:

$$\text{Chlorophyll } a\ (\mu g/L) = \frac{(\text{Chl a}) \times V\ (mL)}{v(L) \times \text{cell length (cm)}}$$

Where; V = volume of sample filtered in litres

v = volume of extract solvent in mL

II. Total Suspened Solid (TSS): gravimetric method

Reagents:

Distilled water or de-ionized water.

Procedure:

Filter preparation

1) Pre-wash glass fiber filter disks in Gooch crucibles. With vacuum operating wash the disks with three 20 mL portions of distilled water.
2) When all water has been vacuumed through the filter disks, place the Gooch crucible in a 103-105 °C oven to dry. Then, place the crucibles into a desiccator to cool.
3) Cool the filters thoroughly in a desiccator before use.
4) Weigh the Gooch crucible and filter (at room temperature) on an analytical balance.
5) Record the weight of the crucible and filter.

Sample analysis

1) Place prepared crucible and filter on the vacuum manifold or side-arm Erlenmeyer flask with vacuum gasket. Wet the filter with distilled water in order to seat the filter against the crucible. Turn on the vacuum. If there is a hole in the filter, it may hear an abnormal hissing or whistling. Use a different weighed crucible and filter.
2) A well-mixed sample is filtered in a glass fiber filter. The volume of water sample used was at least 250 mL.

3) Rinse the filter with three successive 10 mL portions of distilled water. If the sample takes excessive time to filter (longer than 10 minuits), begin again with a different weighed crucible and filter using a smaller volume of sample for filtering.

4) Allow the vacuum to continue until no traces of moisture are present. If solids are present on the side of the funnel, rinse the sides gently with distilled water.

5) Place the crucible in the oven to dry for at least 1-2 hours at 103-105 °C.

6) Transfer the dried crucible to a desiccator to cool. When the crucible has cooled sufficiently it should not feel warm to the touch on the inside of your forearm.

7) Weigh the dried and cooled crucible on an analytical balance. Record the weight. If the sample is not going to be used for regulatory purposes, it may be acceptable to use this weight as the final dry weight.

8) Return the crucible to the drying oven for another thirty minutes. Cool, reweigh and record its weight. Repeat this procedure until the change in the weight of the residue remains within 4 % or less than 0.5 mg from one weighing to the next (this is referred to as constant weight). Record the final weight and calculate the total suspended solids.

Calculation:

$$\text{mg total suspended solids/L} = \frac{(A-B) \times 1{,}000}{\text{sample volume, mL}}$$

where: A = weight of filter + dried residue, mg, and

B = weight of filter, mg.

III. Dissolved Oxygen (DO): Winkler method

Reagents:

1) Manganous sulfate reagent

Dissolve 36.5 g manganous sulfate monohydrate ($MnSO_4 \cdot H_2O$) in 100 mL distilled water.

2) Alkaline iodide solution

Dissolve 50 g sodium hydroxide (NaOH) in 50 mL distilled water. Add to 30 g potassium iodide (KI) and dissolved in 45 mL distilled water. This reagent should not show a color with starch solution when diluted and acidified.

3) 0.5 N Standard thiosulfate solution

Dissolve 145 g sodium thiosulphate ($Na_2S_2O_3 \cdot 5\ H_2O$) and 0.1 sodium carbonate (Na_2CO_3) in 1 L distilled water.

4) Starch indicator solution (0.1-0.2 % solution)

Dissolve 1 g laboratory-grade soluble starch in 150-200 distilled water. Gradually add 20 % NaOH and carefully stir it until it becomes transparent. To see the pH paper change, the concentrated sulfuric acid (conc. HCl) needs to be dropped until it turn to acid. Finally, add 1 mL glacial acetic acid.

5) 0.1 N Iodate solution

- Incubate potassium iodate reagent grade (KIO_3) at 105 $^{\circ}C$ for an hour.

Leave it at the room temperature to cool down.

- Dissolve 0.3567 KIO_3 in distilled water and diluted to 100 mL.

6) Conc. Sulfuric acid (H_2SO_4)

Procedure:

1) Add to the sample collected in a 300 mL bottle, first 1 mL manganous sulphate solution, and then 1 mL alkaline iodide solution. If pipets are dipped into ample, rinse them before returning to reagent bottles. Alternatively, hold pipet tips just above liquid surface when adding reagents. Stopper carefully to exclude air bubbles and mix by inverting bottle a few times. When precipitates have settled sufficiently (to approximately half the bottle volume) to leave clear supernate above the manganous hydroxide floc, add 1 mL conc H_2SO_4. Restopper and mix by inverting several times until dissolution is complete.

2) Add 50 mL water sample in Erlenmeyer flask. Titrate with 0.01 N Na-thiosulphate to a pale straw color. Add a few drops of starch solution and continue titration to the first disappearance of blue color. If the end point is overrun, back-titrate with 0.01 N Na-thiosulphate added dropwise, or by adding a measured volume of treated sample. Repeat titration until the value is constant within 0.05 mL.

Blank:

Add to the distilled water in BOD bottle. Add 1 mL conc. H_2SO_4. Followed by, 1 mL alkaline iodide solution and 1 mL manganous sulphate solution, respectively. Mix them thoroughly. If the color appears, titration is needed to find out the blank value.

Standardization:

Add 5 mL 0.01 N KIO_3 in Erlenmeyer flask. Add 50 mL blank solution and mix thoroughly. Titrate with the 0.01 N sodium thiosulphate, adding starch toward the end of titration, when a pale straw color is reached. Repeat this procedure until a constant value is less than 0.05 mL.

IV. Biochemical Oxygen Demand (BOD_5): Winkler method

Reagents:

See in DO

Procedure:

1) Collect sample with BOD bottle
2) Measure DO_1 (see in DO)
3) Collect sample with BOD (dark) bottle and keep them in BOD incubator (20 $^{\circ}C$) for 5 days. Measure DO_5 by using the same method.

Calculation:

BOD_5 (mg/L) = $DO_1 - DO_5$

V. Dissolved phosphorus: ascorbic acid method

Reagents:

1) Ammonium molybdate solution:

   Dissolve 15 g ammonium paramolybdate in 500 mL de-ionized water.

2) Sulfuric acid:

   Dilute 70 mL. conc. H_2SO_4 to 450 mL with de-ionized water. Leave it at the room temperature to cool down.

3) Ascorbic acid solution:

   Dissolve 27 g L-ascorbic acid in 500 mL deionized water. This solution is not stable; prepare daily.

4) Potassium antimonyl-tartrate solution:

Dissolve 0.34 g potassium antimonyl tartrate [K(SbO) C_4 H_4O_6 . ½ H_2O] in 250 mL deionized water. This solution can be warmed when its use.

5) Composit reagent:

Mix the above reagents in the following proportions for 500 mL of the combined reagent: 100 mL ammonium molybdate solution, 250 mL sulfuric acid, 100 mL ascorbic acid and 50 mL potassium antimonyl tartrate solution. Mix after addition of each reagent. Let all reagents reach room temperature before mixing them in the order given. If turbidity forms in the combined reagent, shake and let it stand for a few minutes until turbidity disappears before proceeding. The reagent is stable for 4 hours. (1 mL standard phosphate solution = 50 μg PO_4^{3-}-P).

Procedure:

1) Pipet 100 mL sample into a clean, dry test tube or 125 mL erlenmeyer flask.
2) Add 0.05 mL (1 drop) phenolphthalein indicator. If a red color develops add 5N H_2SO_4 solution dropwise to just discharge the color.
3) Add 10 ± 0.5 mL composite reagent and mix thoroughly.
4) After at least 10 minutes but less than 2 hours, measure absorbance of each sample at 880 nm, using reagent blank as the reference solution.
5) Prepare individual calibration curves from a series of six standards within the phosphate ranges such as 50, 100 and 500 μg PO_4^{3-}-P/L.
6) Use a deionized water blank with the combined reagent to make photomethic readings for the calibration curve. Plot absorbance and phosphate concentration to check whether it yields a straight line passing through the origin. Test at least on phosphate standard with each set of samples.

Calculation:

Obtain a standard curve by plotting absorbance of standards against PO_4^{3-}-P concentration. Compute sample concentrations directly from standard curve. Report as milligrams oxidized PO_4^{3-}-P per liter.

VI. Nitrite-nitrogen: colorimetric method

Reagents:

1) deionized water
2) Sulphanilamide:

   Dissolve 10 g sulfanilamide in a mixture of 100 mL concentrated HCl and 600 mL deionized water. Dilute to 1 L with deionized water. The solution is stable for many months.
3) N-(1-naphthyl)-ethylenediamine dihydrochloride (NED dihydrochloride) solution:

   Dissolve 1 g N-(1-naphthyl)-ethylenediamine dihydrochloride in deionized water and dilute to 1 L. Store in a dark bottle. Replace monthly or as soon as a brown color appears.
4) Stock nitrite solution:

   Dissolve 0.4926 g $NaNO_2$ or 0.6072 g KNO_2 (dried in a desiccator for 24 hours) and dilute to 1 L. Preserve with 2 mL chloroform ($CHCl_3$) and refrigerate. This is stable for approximately 3 months (1 mL = 0.1 mg NO_2^--N).
5) Standard nitrite solution:

   Dilute 5 mL stock nitrite solution to 500 mL with nitrite-free water; 1 mL = 0.001 mg NO_2^--N.

Procedure:

1) To a 50 mL sample in a 125 mL flask, add 2 L sulfanilamide and mix thoroughly. Add 2 mL NED-dihydrochloride solution and mix immediately.
2) Between 10 minutes and 2 hours afterward, measure absorbance at 543 nm.
3) Using the standard NO_2^--N solution, prepare standards in the range 0.01 to 0.5 mg NO_2^--N/L by diluting the following volumes of standard to 100 mL in volumetric flasks: 1, 2, 5, 10, 20, 50 mL.

Calculation:

Obtain a standard curve by plotting absorbance of standards against NO_2^--N concentration. Compute sample concentrations directly from standard curve. Report as milligrams oxidized NO_2^--N per liter.

VII. Nitrate-nitrogen: colorimetric method

Reagents:

1) Deionized water
2) Copper-Cadmium (Cu-Cd) granules:

   Wash 0.5 to 2.0 mm. Cd granules with 6N HCl and rinse with water. Swirl Cd with 2 % $CuSO_4$ solution for 5 minutes or until blue color partially fades. Decant and repeat with fresh $CuSO_4$ until a brown colloided precipitate develops. Wash Cu-Cd copiously with water (at least 10 times) to remove all precipitated Cu.
3) Ammonium chloride-EDTA solution:

   Dissolve 125 g ammonium chloride (NH_4Cl) and 17 g disodium ethylenediamine tetraacetate (EDTA) in 400 mL deionized water. Adjust pH to 8.5 with concentrated NH_4OH and dilute to 500 mL.

4) Dilute ammonium chloride-EDTA solution:

Dilute 25 mL NH_4Cl-EDTA solution to 1 L with deionized water.

5) Concentrated ammonium hydroxide (conc. NH_4OH)

6) 6 N sodium hydroxide (NaOH):

Dissolve 240 g NaOH in deionized water and dilute to 1 L.

7) 6 N hydrochloric acid (HCl):

Dissolve 50 mL HCl in deionized water and dilute to 100 mL.

8) 2% copper sulfate solution:

Dissolve 20 g $CuSO_4 . 5H_2O$ in deionized water and dilute to 1 L.

9) Zinc sulfate solution:

Dissolve 100 g $ZnSO_4 . 7H_2O$ in deionized water and dilute to 1 L.

10) Stock nitrate solution:

Dissolve 0.7218 g KNO_3 (dried in a desiccator for 24 hours) in deionized water and dilute to 100 mL (1 mL = 1 mg NO_3^--N). Add 0.2 mL $CHCl_3$. Store refrigerated; allow reagent to come to room temperature before use.

11) Standard nitrate solution:

Dilute 1 mL stock nitrate solution to 100 mL with deionized water; 1 mL = 0.01 mg NO_3^--N.

Procedure:

1) Preparation of reduction column: Insert a glass wool plug into the bottom of the reduction column and fill it with water. Add sufficient Cu-Cd granules to produce a column 18.5 centrimetres long. Maintain water level above Cu-Cd granules to prevent entrapment of air. Wash column with 200 mL dilute NH_4Cl-EDTA solution. Activate column by passing through it, at 7-10 mL/minute, 100 mL of a solution composed of a 1 mg NO_3^--N/L standard and 75 mL NH_4Cl-EDTA solution.

2) Treatment of sample:

2.1 Turbidity removal: If turbidity or suspended solids are present, remove by filtering through a 0.45 μm pore diameter membrane or glass fiber filter.

2.2 pH adjustment: Adjust pH to between 7 and 9, as necessary, using a pH meter and dilute HCl or NaOH. This insures a pH of 8.5 after adding NH_4Cl-EDTA solution.

2.3 Sample reduction: To 25 mL sample or a portion diluted to 25 mL, add 75 mL NH_4Cl-EDTA solution and mix. Pour mixed sample into column and collect at a rate of 7 to 10 mL/minute. Discard first 25 mL. Collect the rest in original sample flask. There is no need to wash columns between samples, but if columns are not to be reused for several hours or longer, pour 50 mL dilute NH_4Cl-EDTA solution on to the top and let it pass through the system. Store Cu-Cd column in this solution and never allow it to dry.

2.4 Color development and measurement: As soon as possible, and not within 15 minutes after reduction, add 2 mL sulfanilamide reagent to 50 mL sample. Let the reagent react for 2 to 8 minutes. Add 2 mL NED-dihydrochloride solution and mix immediately. After 10 minutes up to 2 hours, measure the absorbance at 543 nm against a deionized water-reagent blank.

2.5 Standards: Using the standard NO_3^--N solution, prepare standards in the range 0.05 to 1.0 mg NO_3^--N/L by diluting the following volumes of standard to 100 mL in volumetric flasks: 0.5, 1.0, 2.0, 5.0 and 10.0 mL. Carry out reduction of standards exactly as described above for other samples.

Calculation:

Obtain a standard curve by plotting absorbance of standards against NO_3^--N concentration. Compute sample concentrations directly from standard curve. Report as milligrams oxidized NO_3^--N per liter.

VIII. Ammonia-nitrogen: phenate method

Reagents:

1) Sodium hypochlorite solution:

   Add 10 mL of bleach solution containing 5 % sodium hypochlorite (NaOCl) to 40 mL deionized water. Adjust pH to 6.5-7.0 with 1:1 (HCl : H_2O). Reagent is stable up to 1 week.

2) Manganoussulfate solution:

   Dissolve 50 mg 0.0003 M manganous sulfate ($MnSO_4 . H_2O$) in 100 mL deionized water.

3) Phenate solution:

   Dissolve 2.5 g sodiumhydroxide (NaOH) and 10 g phenol (C_6H_5OH) in 100 mL deionized water. Reagent is stable up to 1 week.

4) Ammonia standard solution:

   Dissolve 0.3819 g ammonium chloride (anhydrous NH_4Cl; oven dried at 80 °C) in deionized water and diluted to 1 L (1 mL = 1 mg NH_3-N)

Procedure:

1) To a 10 mL sample in a 50 mL beaker, add 1 drop (0.05 mL) $MnSO_4$ solution. Place on a magnetic stirrer and add 0.5 mL hypochlorous acid reagent.

2) Immediately add, a drop at a time, 0.6 mL phenate reagent. Stir vigorously during addition of reagents. Color formation is complete in 10 minutes and is stable for at least 24 hours.
3) Measure absorbance of each sample at 630 nm, using reagent blank as the reference solution
4) Use standard ammonia solution and water blank to prepare the calibration curve in the appropriate ammonia concentration range. Working standards in concentrations of 0.01, 0.03, 0.05 and 0.10 mg NH_3-N/L.

Calculation:

Obtain a standard curve by plotting absorbance of standards against NH_3-N concentration. Compute sample concentrations directly from standard curve. Report as milligrams oxidized NH_3-N per liter.

Appendix B

Table 1. Summarized the means and range of variation of the environmental variables and Chlorophyll *a* at the arsenic contaminated waters from July 2004 to June 2005 : As=Total Arsenic, Water=Water temperature, Light= Light intensity, Cond=Conductivity DO=Dissolved oxygen demand, TSS=Total suspended solids, BOD_5=Biochemical oxygen demand, Am=Ammonia-nitrogen, Ni=Nitrate- nitrogen, Phos=Dissolved phosphorus, Chl *a*=Chlorophyll *a*, ND.=Non detection vulue.

		Location 1		Location 2		Location 3		Location 4		Location 5		Location 6	
	Units	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
As	(μg/L)	69.31±5.66	31.21-98.96	17.92±4.30	10.80-64.94	39.06±3.31	19.00-57.46	0.92±0.35	0.3-4.69	84.41±13.95	36.88-167.85	8.68±1.51	4.76-24.55
Cond	(μS/cm)	70.17±8.73	0-134.10	29.61±2.94	21.17-50.50	65.13±7.30	46.73-114.50	56.71±5.58	38.10±89.33	140.28±16.08	84.50-214.40	183.10±18.99	119.13-275.80
pH		6.37±0.23	5.19-7.60	6.00±0.32	4.32-7.88	6.24±0.33	4.43-8.28	6.10±0.24	4.79-8.05	6.66±0.26	5.21-8.10	6.67±0.28	5.33-8.02
DO	(mg/L)	4.56±0.42	2.02-6.67	5.97±0.33	3.67-7.56	5.17±0.32	3.20-6.91	6.13±0.20	4.98-7.38	5.68±0.33	4.03-7.30	5.70±0.30	4.50-7.86
TSS	(mg/L)	6.6±0.89	3.7-12.4	7.1±0.74	4.4-13.3	10.4±1.72	3.8-22.1	2.9±0.19	1.5-3.9	75.4±30.01	11.0-296.5	9.5±1.17	4.4-20.5
BOD_5	(mg/L)	2.18±0.24	0.9-3.36	1.80±0.26	0.63-3.48	2.32±0.25	0.7-3.39	2.06±0.27	0.2-3.39	1.77±0.32	0.4-3.61	2.07±0.34	0.63-5.18
Am	(mg/L)	0.03±0.01	ND.-0.08	0.01±0.01	ND.-0.06	0.02±0.01	ND.-0.06	0.01±0.01	ND.-0.05	0.02±0.01	ND.-0.08	0.02±0.01	ND.-0.09
Ni	(mg/L)	0.08±0.02	0.01-0.23	0.04±0.01	0.01±0.07	0.05±0.01	0.01-0.12	0.04±0.01	0.01-0.08	0.06±0.02	0.01-0.20	0.06±0.02	0.01-0.24
Phos	(mg/L)	0.04±0.002	0.03-0.06	0.02±0.002	0.01-0.03	0.03±0.003	0.02-0.05	0.01±0.0	0.01	0.04±0.008	0.01-0.10	0.01±0.001	0.01-0.02
Chl *a*	(μg/L)	21.9±5.29	3.7-58.7	10.8±1.06	5.0-17.0	28.3±5.28	3.0-71.0	4.4±0.57	2.0-8.0	5.4±0.90	1.0-11.3	23.3±2.46	11.3-39.0

Appendix C

Monthly changes of environmental variables in arsenic contaminated waters were shown as follows:

I. Total arsenic

Months	Lo1	Lo2	Lo3	Lo4	Lo5	Lo6	Mean	SE
Jul	67.08	11.37	38.92	0.73	75.45	10.71	75.45	0.73
Aug	58.06	12.75	19.00	0.39	95.40	6.89	95.40	0.39
Sep	31.21	13.62	31.21	0.44	60.74	7.65	60.74	0.44
Oct	86.56	14.77	29.34	0.30	39.59	7.83	86.56	0.30
Nov	40.84	16.08	30.54	4.69	39.59	8.63	40.84	4.69
Dec	84.29	11.72	38.89	0.42	36.88	5.86	84.29	0.42
Jan	89.16	15.11	51.29	0.40	50.40	7.09	89.16	0.40
Feb	98.96	14.70	55.74	1.07	63.37	6.71	98.96	1.07
Mar	65.24	13.73	41.76	0.45	73.71	4.76	73.71	0.45
Apr	70.58	15.45	41.75	0.60	167.85	5.87	167.85	0.60
May	66.12	10.80	32.89	0.85	158.78	7.61	158.78	0.85
Jun	73.64	64.94*	57.46	0.67	151.12	24.55*	70.72	2.18
Mean	69.31	13.64	39.06	0.92	84.41	7.24		
SE	5.66	0.54	3.31	0.35	13.95	0.48		

* These values were not taken into consideration in statistical analysis, probably due to an error during the analysis process.

II. Water temperature

Months	Lo1	Lo2	Lo3	Lo4	Lo5	Lo6	Mean	SE
Jul	29.3	30.2	30.7	32.3	33.2	33.3	31.5	0.68
Aug	29.5	30.7	30.5	31.6	32.1	33.0	31.2	0.51
Sep	29.9	30.3	31.3	33.0	30.7	32.1	31.2	0.48
Oct	29.0	30.9	30.7	30.7	29.6	32.2	30.5	0.46
Nov	28.4	29.7	30.3	30.3	28.8	30.6	29.7	0.37
Dec	27.2	28.4	28.8	28.8	28.2	28.2	28.3	0.25
Jan	29.0	29.2	29.5	30.8	32.3	34.1	30.8	0.83
Feb	28.5	29.7	29.9	30.8	31.2	32.1	30.4	0.51
Mar	29.6	31.0	31.4	31.0	32.2	33.6	31.5	0.55
Apr	31.5	32.3	32.8	33.9	34.7	36.1	33.6	0.68
May	30.1	31.2	30.6	31.8	32.0	33.4	31.5	0.47
Jun	32.2	31.8	32.3	32.3	32.8	34.8	32.7	0.43
Mean	29.5	30.4	30.7	31.4	31.5	32.8		
SE	0.39	0.32	0.32	0.39	0.54	0.59		

III. Light intensity

Months	Lo1	Lo2	Lo3	Lo4	Lo5	Lo6	Mean	SE
Jul	1179.8	2397.7	2472.1	1325.4	1263.6	299.2	1489.6	335.71
Aug	69.9	1564.4	1053.8	876.5	509.3	344.0	736.3	220.29
Sep	2516.6	2502.7	2391.1	2486.1	1733.1	386.7	2002.7	345.58
Oct	729.8	1803.5	2091.0	1061.6	860.6	378.5	1154.1	269.31
Nov	1280.9	1533.2	1071.7	1330.0	1551.1	300.6	1177.9	189.76
Dec	689.9	626.8	197.9	311.4	628.8	346.6	466.9	84.17
Jan	861.2	503.3	823.4	861.9	2241.0	1039.2	1055.0	247.64
Feb	302.1	892.4	548.5	438.3	2091.6	1556.4	971.6	289.51
Mar	668.7	2000.7	1871.8	819.4	2826.7	2295.5	1747.1	344.95
Apr	950.9	1097.6	696.5	897.1	1525.2	1219.8	1064.5	117.45
May	312.8	468.1	788.2	919.0	1279.6	2073.7	973.6	260.35
Jun	1395.1	1885.8	1298.1	1588.3	1495.4	1323.4	1497.7	89.36
Mean	913.1	1439.7	1275.3	1076.2	1500.5	963.6		
SE	186.7	206.3	217.7	164.6	194.1	211.1		

IV. Conductivity

Months	Lo1	Lo2	Lo3	Lo4	Lo5	Lo6	Mean	SE
Jul	58.17	22.90	56.10	40.60	90.13	129.73	66.27	15.61
Aug	62.17	47.47	53.87	39.37	88.50	132.07	70.57	14.10
Sep	68.07	24.27	47.70	52.63	117.00	135.20	74.14	17.56
Oct	62.00	25.93	50.53	42.30	84.50	133.13	66.40	15.57
Nov	77.40	22.30	46.73	38.10	91.13	123.10	66.46	15.36
Dec	96.20	28.20	87.53	72.73	214.40	224.30	120.56	32.70
Jan	69.23	21.17	47.47	42.37	112.50	119.13	68.64	16.19
Feb	67.43	28.87	60.57	76.37	214.13	260.33	117.95	38.74
Mar	62.60	21.63	50.20	38.50	201.50	259.47	105.65	40.56
Apr	75.07	23.70	52.90	66.03	90.53	144.07	75.38	16.54
May	72.20	38.33	113.40	82.17	194.87	260.87	126.97	34.44
Jun	134.10	50.50	114.50	89.33	184.13	275.80	141.39	32.48
Mean	78.35	30.50	67.34	57.71	141.59	185.15		
SE	6.07	2.94	7.30	5.58	16.08	18.99		

V. pH

Months	Lo1	Lo2	Lo3	Lo4	Lo5	Lo6	Mean	SE
Jul	7.60	7.33	7.36	6.29	8.10	7.51	7.37	0.24
Aug	6.89	7.21	7.23	6.06	7.67	7.04	7.02	0.22
Sep	7.03	6.92	7.11	6.77	6.87	8.02	7.12	0.19
Oct	5.25	4.79	5.21	5.62	5.71	5.85	5.41	0.16
Nov	5.75	5.81	5.06	5.53	5.21	5.33	5.45	0.12
Dec	6.40	5.55	6.34	6.04	6.95	7.03	6.38	0.23
Jan	6.03	5.35	5.68	5.90	6.04	6.62	5.94	0.17
Feb	6.80	6.34	6.71	6.67	7.34	7.53	6.90	0.18
Mar	7.48	7.88	8.28	8.05	7.58	7.88	7.86	0.12
Apr	5.77	4.32	4.43	5.49	6.63	5.76	5.40	0.36
May	5.19	5.06	6.14	4.79	5.60	5.58	5.40	0.20
Jun	6.27	5.42	5.31	6.00	6.23	5.87	5.85	0.17
Mean	6.37	6.00	6.24	6.10	6.66	6.67		
SE	0.23	0.32	0.33	0.24	0.26	0.28		

VI. Dissolved oxygen

Months	Lo1	Lo2	Lo3	Lo4	Lo5	Lo6	Mean	SE
Jul	2.95	3.67	4.46	5.78	6.75	5.69	4.88	0.59
Aug	6.15	6.47	4.60	6.59	6.61	5.85	6.05	0.31
Sep	6.30	7.56	6.91	7.23	7.30	7.86	7.19	0.22
Oct	5.13	6.68	6.52	5.72	4.99	4.64	5.61	0.34
Nov	6.67	6.28	5.62	7.38	6.67	7.19	6.63	0.26
Dec	2.02	6.18	6.15	6.61	7.02	5.97	5.66	0.74
Jan	4.38	7.50	5.83	6.01	4.96	5.35	5.67	0.44
Feb	4.19	6.37	4.60	5.49	4.69	5.01	5.06	0.32
Mar	5.54	4.55	5.77	6.03	4.79	6.11	5.47	0.27
Apr	4.35	5.45	3.20	5.85	4.63	4.50	4.66	0.38
May	3.64	5.88	4.42	4.98	4.03	5.75	4.78	0.37
Jun	3.41	5.00	4.00	5.93	5.77	4.51	4.77	0.40
Mean	4.56	5.97	5.17	6.13	5.68	5.70		
SE	0.42	0.33	0.32	0.20	0.33	0.30		

VII. Total suspended solids

Months	Lo1	Lo2	Lo3	Lo4	Lo5	Lo6	Mean	SE
Jul	10.7	5.7	15.4	3.4	38.1	7.1	13.4	10.7
Aug	12.4	13.3	12.5	3.7	13.9	7.9	10.6	12.4
Sep	10.8	9.6	6.4	2.9	198.0	11.6	39.9	10.8
Oct	6.0	5.4	13.6	2.5	296.5	7.6	55.3	6.0
Nov	4.2	4.8	5.4	3.2	236.3	7.3	43.5	4.2
Dec	4.8	6.1	7.0	2.1	33.1	4.4	9.6	4.8
Jan	3.7	6.6	3.9	1.5	12.9	10.8	6.6	3.7
Feb	5.9	5.9	3.8	3.1	11.0	7.2	6.2	5.9
Mar	7.5	7.6	11.5	2.7	11.0	11.6	8.6	7.5
Apr	4.0	9.6	17.8	3.9	22.4	10.3	11.3	4.0
May	4.3	5.8	22.1	2.7	11.7	20.5	11.2	4.3
Jun	4.5	4.4	5.8	2.6	20.4	8.1	7.6	4.5
Mean	6.6	7.1	10.4	2.9	75.4	9.5		
SE	0.9	0.8	1.7	0.2	30.0	1.2		

VIII. Biochemical Oxygen Demand

Months	Lo1	Lo2	Lo3	Lo4	Lo5	Lo6	Mean	SE
Jul	2.62	2.67	2.61	1.97	2.13	3.03	2.51	0.16
Aug	2.78	1.59	2.71	2.11	1.17	2.49	2.14	0.26
Sep	3.36	3.48	2.99	3.17	1.16	1.31	2.58	0.43
Oct	1.19	1.07	2.37	2.62	2.80	1.60	1.94	0.31
Nov	2.78	1.04	2.75	1.45	2.06	2.22	2.05	0.28
Dec	1.22	0.65	1.08	1.61	3.57	1.29	1.57	0.42
Jan	1.90	0.63	0.70	0.84	2.05	2.21	1.39	0.30
Feb	3.13	2.48	1.73	2.22	0.73	0.63	1.82	0.41
Mar	0.90	1.37	2.14	2.97	1.08	2.22	1.78	0.33
Apr	1.53	2.21	2.17	0.20	0.40	1.69	1.37	0.36
May	2.80	2.59	3.84	3.39	3.61	5.18	3.57	0.38
Jun	1.90	1.82	2.80	2.18	0.53	1.02	1.71	0.33
Mean	2.18	1.80	2.32	2.06	1.77	2.07		
SE	0.24	0.26	0.25	0.27	0.32	0.34		

VIX. Nitrate-nitrogen

Months	Lo1	Lo2	Lo3	Lo4	Lo5	Lo6	Mean	SE
Jul	0.03	0.01	0.01	0.01	0.01	0.01	0.02	0.004
Aug	0.06	0.04	0.08	0.06	0.04	0.04	0.05	0.007
Sep	0.08	0.04	0.09	0.08	0.05	0.05	0.06	0.008
Oct	0.04	0.03	0.03	0.03	0.04	0.02	0.03	0.004
Nov	0.01	0.01	0.01	0.01	0.03	0.01	0.01	0.002
Dec	0.04	0.01	0.01	0.01	0.01	0.01	0.01	0.005
Jan	0.04	0.01	0.01	0.01	0.01	0.01	0.02	0.005
Feb	0.11	0.07	0.11	0.07	0.14	0.24	0.12	0.026
Mar	0.02	0.01	0.02	0.01	0.03	0.07	0.03	0.009
Apr	0.21	0.06	0.06	0.02	0.20	0.01	0.09	0.036
May	0.10	0.06	0.12	0.06	0.03	0.11	0.08	0.013
Jun	0.23	0.07	0.11	0.06	0.09	0.11	0.11	0.025
Mean	0.08	0.04	0.05	0.04	0.06	0.06		
SE	0.02	0.01	0.01	0.01	0.02	0.02		

X. Ammonia-nitrogen

Months	Lo1	Lo2	Lo3	Lo4	Lo5	Lo6	Mean	SE
Jul	0.07	0.00	0.02	0.00	0.06	0.09	0.04	0.016
Aug	0.05	0.00	0.01	0.00	0.00	0.04	0.02	0.008
Sep	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.002
Oct	0.02	0.01	0.01	0.03	0.04	0.01	0.02	0.005
Nov	0.01	0.00	0.02	0.03	0.03	0.01	0.02	0.003
Dec	0.08	0.06	0.06	0.05	0.08	0.05	0.06	0.006
Jan	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.002
Feb	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.001
Mar	0.00	0.03	0.00	0.01	0.02	0.02	0.01	0.005
Apr	0.03	0.00	0.04	0.00	0.02	0.01	0.02	0.007
May	0.01	0.00	0.05	0.01	0.00	0.00	0.01	0.007
Jun	0.04	0.00	0.04	0.00	0.01	0.02	0.02	0.007
Mean	0.03	0.01	0.02	0.01	0.02	0.02		
SE	0.01	0.01	0.01	0.00	0.01	0.01		

XI. Dissolved phosphorus

Months	Lo1	Lo2	Lo3	Lo4	Lo5	Lo6	Mean	SE
Jul	0.04	0.01	0.04	0.01	0.04	0.01	0.02	0.006
Aug	0.05	0.01	0.02	0.01	0.05	0.01	0.02	0.008
Sep	0.05	0.03	0.04	0.01	0.04	0.02	0.03	0.005
Oct	0.06	0.03	0.05	0.01	0.08	0.02	0.04	0.010
Nov	0.03	0.01	0.02	0.01	0.01	0.01	0.02	0.003
Dec	0.05	0.02	0.03	0.01	0.02	0.01	0.02	0.006
Jan	0.04	0.01	0.02	0.01	0.01	0.01	0.02	0.005
Feb	0.05	0.01	0.02	0.01	0.01	0.02	0.02	0.006
Mar	0.06	0.02	0.04	0.01	0.03	0.02	0.03	0.008
Apr	0.04	0.01	0.03	0.01	0.02	0.01	0.02	0.005
May	0.05	0.03	0.03	0.01	0.10	0.01	0.04	0.013
Jun	0.03	0.01	0.02	0.01	0.02	0.01	0.02	0.004
Mean	0.04	0.02	0.03	0.01	0.04	0.01		
SE	0.002	0.002	0.003	0.000	0.008	0.001		

XII. Chlorophyll *a*

Months	Lo1	Lo2	Lo3	Lo4	Lo5	Lo6	Mean	SE
Jul	58.7	6.7	71.0	6.7	8.3	23.0	29.1	11.7
Aug	54.0	14.3	33.3	6.7	3.0	19.3	21.8	7.8
Sep	22.3	5.7	3.0	2.0	10.0	12.7	9.3	3.1
Oct	24.0	11.7	37.3	3.7	6.0	24.7	17.9	5.3
Nov	15.7	12.3	26.0	5.0	3.7	17.3	13.3	3.4
Dec	3.7	10.7	31.0	3.0	11.3	11.3	11.8	4.1
Jan	5.0	17.0	19.0	4.3	3.3	24.3	12.2	3.7
Feb	7.3	11.3	9.3	3.3	2.7	18.0	8.7	2.3
Mar	9.3	14.7	42.3	8.0	4.3	39.0	19.6	6.8
Apr	11.3	9.3	34.7	5.7	4.7	36.7	17.1	6.0
May	34.0	5.0	23.7	2.7	1.0	23.7	15.0	5.7
Jun	17.0	10.7	8.3	2.0	6.0	29.3	12.2	4.0
Mean	21.9	10.8	28.3	4.4	5.4	23.3		
SE	5.3	1.1	5.3	0.6	0.9	2.5		

Appendix D

Each measured environmental value (Table 2) is given a standard score that ranges from 0.1 to 1.0 depending on its relationship to the recommended maximum allowable level given a score of 1.0. For each water sample these scores are summed to provide an indication of its quality. Value will range from 0.7 to 7.0 with the latter having the worst quality. According to the work of Peerapornpisal (2004) the values are then classified according to the following Table 3.

Table 2 Standard scores for each environmental variable

DO (mg/L)	Standard score
> 8	0.1
7-8	0.2
6-7	0.3
5-6	0.4
4-5	0.5
3-4	0.6
2-3	0.7
1-2	0.8
0.5-1	0.9
< 0.5	1.0

Table 2 (continued)

BOD (mg/L)	Standard score
< 0.25	0.1
0.25-0.5	0.2
0.5-1	0.3
1-2	0.4
2-4	0.5
4-10	0.6
10-20	0.7
20-40	0.8
40-80	0.9
> 80	1.0

Conductivity (μS/cm)	Standard score
< 10	0.1
10-20	0.2
20-40	0.3
40-70	0.4
70-100	0.5
100-150	0.6
150-230	0.7
230-400	0.8
400-550	0.9
> 550	1.0

Table 2 (continued)

Nitrate-nitrogen (mg/L)	Standard score
< 0.05	0.1
0.05-0.1	0.2
0.1-0.3	0.3
0.3-0.8	0.4
0.8-1.5	0.5
1.5-3.0	0.6
3.0-10.0	0.7
10.0-20.0	0.8
20.0-40.0	0.9
> 40.0	1.0

Ammonia-nitrogen (mg/L)	Standard score
< 0.1	0.1
0.1-0.2	0.2
0.2-0.4	0.3
0.4-0.8	0.4
0.8-1.5	0.5
1.5-3.0	0.6
3.0-5.0	0.7
5.0-10.0	0.8
10.0-20.0	0.9
> 20.0	1.0

Table 2 (continued)

Dissolved phosphorus (mg/L)	Standard score
< 0.05	0.1
0.05-0.2	0.2
0.2-0.4	0.3
0.4-0.8	0.4
0.8-1.5	0.5
1.5-3.0	0.6
3.0-5.0	0.7
5.0-10.0	0.8
10.0-20.0	0.9
> 550	1.0

Dissolved phosphorus (mg/L)	Standard score
< 0.05	0.1
0.05-0.2	0.2
0.2-1.0	0.3
1.0-2.5	0.4
2.5-5.0	0.5
5.0-10.0	0.6
10.0-20.0	0.7
20.0-50.0	0.8
50.0-150.0	0.9
> 150	1.0

Source: Prommana (2006)

Table 3 Score from the assessment of water quality

Scores	Trophic status
0.1-0.9	hyper oligotrophic
1.0-1.8	oligotrophic
1.9-2.7	oligotrophic-mesotrophic
2.8-3.6	mesotrophic
3.7-4.5	mesotrophic-eutrophic
4.6-5.4	eutrophic
> 5.5	hypereutrophic

Source: Prommana (2006)

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Scholarship Awards during Enrolment

- TRF/BIOTEC Special program for Biodiversity Research and Training grant BRT T_348333
- Grant from Faculty of Science, Prince of Songkla University, Hat Yai campus, Hat Yai, Songkhla province, Thailand to support traveling to Department of Biology and Ecology, University of Novi Sad, Serbia from November 10, 2005-January 18, 2006.

List of Publication and Proceeding

- **Meeinkuirt, W.**, Sirinawin, W., Angsupanich, S. and Polpunthin, P. 2007. Phytoplankton communities in arsenic contaminated waters in Ronphibun district of Nakhon Si Thammarat province, Thailand. Proceedings of the First Joint PSU-UNS International Conference on BioScience: Food, Agriculture, and the Environment, held during 17-19 August 2006. Songkhla: Faculty of Natural Science, Prince of Songkla University, pp. 154-167.
- **Meeinkuirt, W.**, Sirinawin, W., Angsupanich, S. and Polpunthin, P. 2007. Seasonal changes in phytoplankton communities and water quality in arsenic contaminated waters at the Ronphibun district, Nakhon Si Thammarat province. *J. Sci. Res. Chula Univ. (Section T)*. 6(Special Issue 1): 311-321.

- **Meeinkuirt, W.**, Angsupanich, S., Sirinawin, W. and Polpunthin, P. 2008. Changes in relative abundance of phytoplankton in arsenic contaminated waters at the Ron Phibun district of Nakhon Si Thammarat province, Thailand. *Int. J. Algae.* (*accepted for publication*)