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 8.08×10^4 to 1.24×10^6 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.