CHAPTER 5

Discussion

This study focused on the diversity of euglenoids in Northern Thailand and their application for water quality assessment. The samples were collected from different water bodies located in Northern Thailand during the period of April 2009 to March 2010. The diversity of the euglenoids and the physico-chemical parameters were recorded, and the discussion of which is as follows:

5.1 Diversity of euglenoids

In this study, 402 taxa of euglenoids belonging to 272 species, 108 varieties, and 22 forms were recorded. Among them, *Trachelomonas* (136 taxa) were found in the highest number. Besides, several euglenoids were recorded as follows: *Phacus* (64 taxa), *Strombomonas* (58 taxa), *Euglena* (46 taxa), *Lepocinclis* (35 taxa), *Petalomonas* (14 taxa), and *Peranema* (10 taxa). Genus *Trachelomonas* and *Strombomonas* always showed a high diversity because of the high variability of lorica. These findings were supported by several studies conducted by Conforti and Tell (1986b), Conforti and Nudelman (1994), Conforti (1999), and Wołowski and Walne (2007), as a high diversity of taxa were recorded in these studies. At present, classifications of the euglenoids have been based on variations within the cell construction, cytological and molecular studies (Wołowski, 2011). Thus, some genus were separated, such as Triemer *et al*. (2006) who described the genus *Discoplastis*, and Linton *et al*. (2010) who also described the genus *Euglenaria* from the genus *Euglena* (Brodie and Lewis, 2007; Ciugulea and Triemer, 2010). This study also observed the genus *Discoplastis* (2 taxa) and *Euglenaria* (5 taxa). Furthermore, few representatives of other genera were observed, such as *Anisonema* (6 taxa), *Menoidium* (5 taxa), *Entosiphon* (4 taxa), *Heteronema* (4 taxa), *Notosolenus* (4 taxa), *Cryptoglena* (3 taxa), *Monomorphina* (2 taxa), and *Urceolus* (2 taxa).

Only one taxon of the genus *Astasia*, *Eutreptia*, and *Rhabdomonas* were observed and these were acknowledged as rare genus in this study. However, this research was focused only in Northern Thailand. Greater diversity will be able to be recorded if there were studies conducted throughout most areas of Thailand.

This study revealed a high diversity of euglenoids due to the fact that fresh materials were used for examination. Certainly, good details of chloroplasts, pyrenoids, paramylon bodies, shapes, pellicle, their movement, and flagella (number and direction) were given. Moreover, some loricated groups, such as *Trachelomonas* and *Strombomonas*, were studied by using a scanning electron microscope for lorica ultrastructure details following the method of Bozzola and Russell (1991) and Wołowski and Walne (2007). Thus, a greater diversity of euglenoids was recorded. In addition, the samples were taken with 3 methods as follows: (1) taken from open water with a plankton-net (10 µm pore size) that showed most green euglenoids in plankton form; (2) taken from the sediment with a slime aspirator that showed mostly colorless euglenoids; (3) scraped from aquatic plants that showed the member of euglenoids in attached form or euglenoids in plankton form on aquatic plants. All were good methods for the collection of samples because they revealed complete groups of euglenoids, especially colorless euglenoids. Since this group lacked chloroplasts and always lived on the sediment, organic matter, such as saprotrophic, osmotrophic, and phagotrophic, collecting samples from sediment should be done. Besides, scraping samples from aquatic plants showed a high opportunity for the presence of euglenoids. Previously, this phenomenon had been observed and reported by Salazar (2004). He collected materials by scraping off the periphyton of the roots, stems, and submersed leaves of *Hymenachne amplexicaulis* (plants). 80 taxa of euglenoids, including *Euglena* (5 taxa), *Lepocinclis* (12 taxa), *Phacus* (29 taxa), *Strombomonas* (16 taxa), and *Trachelomonas* (18 taxa) were found.

This study found 238 taxa of euglenoids as being newly recorded in Thailand, comprising *Trachelomonas* (107 taxa), *Strombomonas* (44 taxa), *Euglena* (28 taxa), *Phacus* (27 taxa), *Lepocinclis* (20 taxa), *Cryptoglena* (3 taxa), *Euglenaria* (3 taxa), and *Eutreptia* (1). Besides, the colorless euglenoids, *Anisonema* (6 taxa), *Astasia* (1 taxon), *Entosiphon* (3 taxa), *Heteronema* (3 taxa), *Menoidium* (5 taxa), *Notosolenus* (4 taxa), *Peranema* (8 taxa), *Petalomonas* (13 taxa), *Rhabdomonas* (1 taxon), and *Urceolus* (2 taxa) were also found to be present. The newly recorded species were compared with the check-list of freshwater algae, various other publications and related books on Thailand (Lewmanomont *et al.*, 1995; Peerapornpisal, 2005, 2013; Hanpongkittikul and Wongrut, 2005; Yamagishi, 2010; Chaimongkhon and Peerapornpisal, 2012).

It was surprising that five new species and one new variety of loricate euglenoids were described as being new for science (Duangjan and Wołowski, 2013). These included 2 taxa of *Strombomonas* and 3 taxa of *Trachelomonas* as follows: *Strombomonas starmachii* Duangjan & Wołowski, and these were named to honor the memory of the eminent phycologist and hydrobiologist Karol Starmach; *Strombomonas chiangmaiensis* Duangjan, the epithet 'chiangmaiensis' refers to Chiang Mai where the materials were collected; *Trachelomonas peerapornpisalii* Duangjan & Wołowski were named in honor of Associate Professor Yuwadee Peerapornpisal for her many and varied contributions to world algological research. Regarding *Trachelomonas thailandicus* Duangjan & Wołowski, the epithet 'thailandicus' refers to Thailand where the materials were collected. With regard to *Trachelomonas reticulato-spinifera* Duangjan, the epithet 'reticulate-spinifera' refers to the lorica ultrastructure and with *Trachelomonas paucispinosa* (Prowse) Duangjan & Wołowski, specimens were described by Prowse (1958) as *Trachelomonas hystrix* var. *paucispinosa* referring to the type of taxa. Good details of lorica are important for the identification of these groups, especially details collected under a scanning electron microscope. Moreover, cytological and molecular studies are also important for the classification of euglenoids.

Information of euglenoids has also been reported worldwide and the diversity of euglenoids by approximately 2000 taxa, which belonged to 900 species, has also been reported on (Wołowski and Hindák, 2005; Wołowski, 2011; www.algaebase.org). All the early results have proven that the diversity of euglenoids in Thailand require further investigation, especially with regard to the colorless euglenoids. Collected data on these groups are still poorly known throughout the entire world. Certain data can be found in previously published elaborations (Pringsheim, 1942; Vetrova, 1980; Wołowski, 1991, 2011; Wołowski and Walne, 1997; Angeler *et al.*, 2002; Cavalier-Smith, 2013; Jeuck and

Arndt, 2013). Very detailed taxonomical and ecological studies have also been done (Larsen, 1987; Larsen and Patterson, 1990). Besides, euglenoids from brackish and marine waters have previously been reported (Triemer and Farmer, 1991; Ekebom *et al*., 1995; Alves-da-Silva and Friedrich, 2009).

5.2 Euglenoid distribution and physico-chemical parameters

According to the diversity index, the highest values of species richness were found in the water body near the Cabbages & Condoms Inn and Restaurant (CC1-9), where a total of 101 species and a diversity index of 4.158 (evenness = 0.901) were presented. Moreover, this water body also presented two new species of the loricated group, including *Strombomonas starmachii* Duangjan and Wołowski, and *Strombomonas chiangmaiensis* Duangjan. The pond typically had a constant level of water and received organic matter from the restaurant and the toilets of the restaurant. Thus, the pond contained several nutrients considered to be important factors for the development of algae, but only a small amount of algae, including euglenoids, were found (Table 31). High levels of turbidity could be the important factor that limited the development of algae (Rosowski, 2003). Moreover, the highest values of species richness was found in the following sites: BY2-5, BY1-7, CC1-7, CC1-8, CC1-10, BY1-8, CC1-4, CC1-11, BY1-3, and CC1-5 with diversity index readings of 4.141, 4.057, 4.025, 4.017, 4.009, 3.982, 3.944, 3.937, 3.924, and 3.891, respectively. Similar characteristics of these ponds were that they contained brown water and were middle ponds.

Among the recorded euglenoids, *Euglena* was the most abundant genus and it has been described as one of the most tolerant genus of organic pollution (Palmer, 1969; Slàdeček and Sládečková, 1996; Peerapornpisal *et al*., 2007). It is commonly found in farm ponds, sewage ponds, and other bodies of water with high levels of organic matter. Sometimes, *Euglena* can form blooms as green or red scum (Leedale, 1967; Ciugulea and Triemer, 2010; Wołowski, 2011). There have been several studies that have reported on the blooming of euglenoids (Rosowski, 2003; Kočárková *et al*, 2004; Rahman *et al*., 2007; Ciugulea and Triemer, 2010; Ligeza and Wilk-Woz´niakb, 2011; Jeong *et al*, 2011; Wołowski, 2011; Park *et al*, 2013). The results of the present study confirm the validity of the above-mentioned data. Several blooms of euglenoids, such as *Euglena agilis*, *E. chlorophoenicea*, *E. sanguinea*, *E. haematodes*, *Euglenaria anabaena*, *Lepocinclis fusiformis*, *Phacus triqueter*, *Trachelomonas volvocinopsis*, and *T. playfairiana*, were observed in waste ponds (ST, 1 time), ditches (LL, 5 times; AS, 1 time), garden ponds (AG, 3 times, BY1, 1 time), fish ponds (MJ1, 1 time; MJ4, 1 time); field ponds (BL, 1 time).

Besides, 23 taxa of euglenoids were recorded as being among the most highly abundant taxa, including *Euglena agilis* (45.36%) followed by *Euglenaria anabaena* (13.88%), *Trachelomonas volvocinopsis* (9.29%), *E. haematodes* (6.30%), *Phacus salina* (5.24%), *Ph. longicauda* var. *tortus* (3.96%), *Ph. triqueter* (3.86%), *E. splendens* (3.38%), *Lepocinclis fusiformis* (3.11%), *Strombomonas borystheniensis* (2.50%), *L. acus* (2.50%), *E. sanguinea* (2.45%), and *E. proxima* (2.24%), respectively.

At the same time, euglenoids usually bloom along with other species, such as those found in the Division Cyanophyta: *Microcystis aeruginosa* (Kützing) Kützing, *Merismopedia minima* Beck, *Oscillatoria* sp.; Division Chlorophyta: *Actinastrum hantzschii* Lagerheim, *Chlamydomonas* sp., *Coelastrum astroideum* De Notaris, *Golenkinia paucispina* West and G.S. West, *Pandorina morum* (O.F.Müller) Bory de Saint-Vincent, *Staurastrum polymorphum* Brébisson; Division Bacillariophyta: *Cyclotella meneghinniana* Kützing; Division Pyrrhophyta: *Peridinopsis* sp. However, *Microcystis aeruginosa* was the only species that developed together with a high abundance of euglenoids, especially those of the genus *Euglena*. Previously, data of relevant interest on this situation had been reported by Wołowski and Grabowska (2007) and Grabowska and Wołowski (2014). They observed the developed taxa of *Trachelomonas* sp. in the shallow reservoir Siemianowka in Northern Poland during a cyanobacteria bloom, *Planktothrix agardhii* (Gomont) Anagnostidis et Komarek. Development of *P. agardhii* caused a rapid decreased of *Trachelomonas* biomass because of lower water transparency and oxygen concentration. In addition, lower water temperature was the important factor in decreasing *Trachelomonas* taxa and its biomass.

Moreover, the domination of other species was also found, such as those in the Division Cyanophyta: *Aphanocapsa* sp., *Coelomoron pusillum* (Van Goor) Komárek, *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya and Subba Raju, *Dolichospermum planctonicum* (Brunnth.) Wacklin, L. Hoffm. and Komárek, *Pseudoanabaena* sp.; Division Bacillariophyta*: Aulacoseira granulata* (Ehrenberg) Simonsen; and Division Chlorophyta: *Pediastrum duplex* Meyen, *Pediastrum tetras* (Ehrenberg) Ralfs, *Dictyosphaerium* sp., *Micractinium quadrisetum* (Lemmermann) G.M. Smith, *Staurastrum pingue* Teiling, *Acutodesmus acuminatus* (Lagerheim) Tsarenko, *Desmodesmus quadricauda* (Turpin) Hegewald, *Desmodesmus opoliensis* var. *carinatus* (Lemmermann) E. Hegewald.

The N:P ratio was often limited to the growth of the algae because of the nitrogen-fixing ability. Species of cyanophytes can better compete for nitrogen than other phytoplankton when N is limited. Thus, cyanophyte blooms are usually present when the N:P ratio is low, whereas higher N:P ratios promote the development of chlorophytes (green algae and flagellates), along with diatoms (Smith, 1983; AWWARF, 1995; Hindák, 2009). Moreover, Wang *et al*. (2010) also reported that higher ammonium nitrogen concentrations, as well as the availability of organic nutrients and trace elements, may play a more significant role in stimulating or restricting phytoplankton growth when inorganic N and P are not limited for phytoplankton. This was similar to the results of this study, which found that ammonium nitrogen was significant in the stimulation of euglenoid growth (Table 4.1).

A comparison of euglenoid communities that occurred in 4 types of water bodies (garden ponds: AG, BY1; field ponds: BL, LP1; ditches: LL, AS; fish ponds: TJ, CC1) were observed over the annual cycle. The highest value of euglenoid diversity was observed in the field ponds. A total 338 taxa of euglenoids were found in 4 types of water bodies. Among the recorded euglenoids, *Trachelomonas* (117 taxa) were recorded as being the highest in diversity and it was observed in both garden ponds (99 taxa, 29.3%) and field ponds (71 taxa, 21%). These ponds usually presented both brown water and a range of aquatic plants. Additionally, *Lotuses* sp. *Phacus* (57 taxa) and were frequently observed in fish-ponds. *Strombomonas* (48 taxa) were frequently occurred in ditches, whereas

Euglena (41 taxa) were typically observed in abundance and typically bloomed in fish-ponds. A low range of diversity of *Euglenaria* (5 taxa) was observed but it was presented as being abundant. Small numbers of *Cryptoglena* (2 taxa), *Monomorphina* (2 taxa), *Eutreptia* (1 taxon), and *Discoplastis* (1 taxon) were observed. Furthermore, several taxa of colorless groups (30 taxa) were also recorded.

Euglena agilis was frequently found to be in abundance and occasionally bloomed in ditches (LL) in December and January. During certain months, high levels of ammonium nitrogen (5.62, 4.00 mg/L), biochemical oxygen demand (42.8, 45.6 mg/L), conductivity (731, 927 µS/cm), and pH (8.49, 8.55) were observed. The blooming of *E. sanguinea* was observed in a garden pond (AG, April) and ditch (LL, February), both of which presented high pH values (9.40, 9.05). *Phacus triqueter* was observed blooming in a garden pond (AG, June) that was found to have 0.37 mg/L of SRP and a pH of 7.01. *Euglenaria anabaena* was observed blooming in a ditch (LL, September) that presented 0.37 mg/L, SRT; 0.27 mg/L, nitrate nitrogen, and 0.68 mg/L, ammonium nitrogen. *Lepocinclis fusiformis* was observed blooming in a field pond (BL, May) that presented low levels of nutrients, with a high level of biochemical oxygen demand (28.0 mg/L). *T. playfairiana* and *T. volvocinopsis* were observed blooming in a garden pond (AG, May) that presented a high level of nitrate nitrogen (0.40 mg/L).

It was of interest that some taxa were found to be in abundance only once but they later disappeared at that site, such as *E. chlorophoenicea* which was found in a garden pond (AG, April); *E. haematodes* and *E. sanguinea* (LL, February) and *S. eurystoma* which were found in a ditch (LL, October); and *Euglenaria anabaena* var. *minima* which was found in a ditch (AS, April). No complete evidence has been reported to sufficiently explain the single occurrence of these taxa in these ponds.

Normally, phytoplankton development in freshwater could be limited by phosphorus rather than nitrogen availability (Vollenweider, 1968; Dillon and Rigler, 1974; Schindler, 1977). However, *Euglena*, *Trachelomonas* and *Phacus* are easily developed and in abundance in places that were rich in faeces and urine (Lackey, 1968; Wołowski 1989a, b, 1992). This was similar to the findings of Nędzarek and Pociecha (2010) who reported that euglenoids grew as the dominant species in waters which contained a high level of ammonium nitrogen. Payne *et al.* (2013) studied the Ombrotrophic Peat Lands, which were naturally oligotrophic and highly sensitive to atmospheric depositions of acids, nutrients, and other pollutants. This study showed that ammonia nitrogen promoted algal biomass, including *Euglena* cf. *mutabilis*. Many products from household laundry detergents, commercial fertilizers used for agriculture, water runoff, and the organic pollution from leaky livestock waste were typically the greatest sources of the inorganic compounds reported in this study. These findings were supported by this research in which high levels of ammonium nitrogen and soluble reactive phosphorus promoted the growth of euglenoids (Table 2). These results showed that nitrate nitrogen did not affect the growth of euglenoids. This seemed to indicate that if phytoplankton were supplied along with both nitrate and ammonium nitrogen, the rate of uptake of nitrate nitrogen may be depressed (Bienfang, 1975). Moreover, the results also described small levels of nitrate nitrogen at most sampling sites.

At present, climate change is having an impact on terrestrial and water ecosystems, including the physico-chemical parameters of the water. Climate change has had the effect of generating higher temperatures of these water bodies, which is a positive condition for the biodegradation of bacteria. The ideal temperature for the biodegradation process lies between 4-35 ºC under aerobic conditions and between 10-65 ºC for anaerobic conditions (Cabridenc, 1985). Besides, the process of degradation also induces the growth of algae, including euglenoids. This can also be clearly seen in a report from Montien-art *et al*. (2011). They reported on the influence of climate change on the physico-chemical parameters of the water quality, as well as the growth of the Nile Tilapia fish in commercial ponds. The results indicated that the amount of rain, the air and water temperatures had a significant effect on nutrient levels, such as nitrate-nitrogen, nitrite-nitrogen, and ammonium-nitrogen. Conversely, they had a non-significant influence on the growth of the Nile Tilapia. In this study, climate change also had an influence to the quality of the water. The results showed that each season presented significant differences in the physico-chemical parameters of the water body, such as the water levels and the air temperature, conductivity, pH, alkalinity, nitrate nitrogen, and ammonium nitrogen. This indicated that different seasons had significant effects on the levels of these physico-chemical parameters. Conversely, the seasons had a non-significant influence on euglenoid taxa (Table 3). The highest abundance of euglenoids was usually recorded during the rainy season, such as at the sites AG, UM, TJ, BL, and BY1. These places received greater amounts of nutrients from the floodable soil and organic matter that were present in nearby places. The cold-dry season was also observed to reveal the highest abundance of euglenoids in sites MK, LL, CC1, and AS. These sites are located in places that typically saw tourism activities during this season, like restaurants, dams, and various water bodies in the city, so they also generated more organic matter that supported the glowing of euglenoids. Moreover, in three sites, MJ1, KL, and LP1 revealed the highest abundance of euglenoids in the hot season. The temperature of the water, the temperature of the air, and the smaller water bodies presented high concentrations of nutrients as one of the parameters for higher growth rates of algae. However, the PS1 recorded a similar number of euglenoids over the three annual seasons. The seasons had little effect on euglenoids, but had a high effect on the presence of *Peridinopsis* sp. as the dominant species. This species was found to bloom in December and February.

Moreover, there have been several investigations on the seasonal dynamics on euglenoid development and the effective influence of the seasons on the physico-chemical parameters. For example, Alves-da-Silva *et al*. (2008) investigated a shallow, acid lake, which presented the genus *Trachelomonas*, occurring in the largest amounts. Besides, the highest diversity of euglenoids was found to occur during the spring and the lowest was recorded during the summer. However, Kočárková *et al*. (2004) reported that the seasonal dynamics of euglenoids were usually found in the spring and summer (or autumn) and a high diversity of *Trachelomonas* and *Euglena* was then observed. 12 taxa of *Trachelomonas* were found in the Guadalupe Dam, a eutrophic reservoir. Most of these taxa were found during the dry season with a high concentration of nutrients and a moderate amount of mineral contents (Solórzano *et al*., 2011). Khuantrairong and Traichaiyaporn (2008) reported that high an abundance of euglenoids were present in the summer at Doi Tao Lake, Chiang Mai Province, Thailand.

The water quality in all water sampling sites was classified through use of the AARL-PC score as altering certain physico-chemical parameters, such as dissolved oxygen, conductivity, ammonium nitrogen, nitrate nitrogen, soluble reactive phosphorus, and chlorophyll *a* (Peerapornpisal, 2005). Water quality values were generally classified into 5 trophic statuses. The water quality ranged from clean-moderate water (oligo-mesotrophic status) to highly polluted water (hypereutrophic status). Most of the sampling sites, e.g. garden ponds (AG, BY2), field ponds (BL, LP2), ditches (LL, AS), and fish ponds (UM, MJ1, KL, TJ, CC1, PS1), were classified in the meso-eutrophic status followed by the mesotrophic, eutrophic, oligo-mesotrophic, and hypereutrophic statuses, respectively. Euglenoids prefer to develop in water that has high organic matter, and especially occur in moderately polluted water (meso-eutrophic status) and always occur in high abundance by one or two species in very polluted water (hypereutrophic status).

The AARL-PC score is routinely used in the assessment of water quality in various types of water bodies in Thailand. An example of this would be three peat swamps located in southern, Thailand that were investigated by Ngearnpat *et al* (2008). Different desmid compositions were found in the three peat swamps, which were classified as being in the oligo-mesotrophic status. Moreover, Leelahakriengkrai and Peerapornpisal (2011) investigated the water quality and trophic status in the main rivers from region 6 of Thailand. Clean-moderate water quality (oligo-mesotrophic status) was found at most of the sampling sites. Besides, Chaimongkhon and Peerapornpisal (2012) studied the diversity of non-loricated euglenoids in Saraphi District, Chiang Mai Province and the water quality was classified as being of mesotrophic status. With regard to reservoirs, Nakkaew *et al* (2013) also studied the quality of the water by collecting samples in the main rivers and reservoirs of the Wang River, Thailand. Most of the water was classified as being clean to moderate (oligo-mesotrophic status, excluding sampling sites in urban areas, which were found to possess moderate water quality (mesotrophic status).

5.3 Using euglenoid index as a bioindicator for the assessment of water quality

Thirty-two taxa of euglenoids were selected from three groups, including the ones with the highest relative abundance (>1%), 30% occurrence of euglenoid taxa in the algae total from each site, and euglenoid taxa blooms in each site, were selected to establish a euglenoid index. Euglenoids are known as bioindicators of polluted water. According to the Pollution Index (Palmer, 1969), euglenoids were described as the genera that were most tolerant to organic pollution including *Euglena* (5), *Lepocinclis* (1), and *Phacus* (2), while the most tolerant was point 5 and the lowest was point 1.

According the results, the range of the bioindicator values was 2.8-7.0. Among them, *Euglena agilis* was present in the highest value, while *Euglena navicula* presented the lowest value. Therefore, *E. agilis* could be used to indicate the highest level of pollution and *E. navicula* could indicate the lowest level of pollution in the euglenoid index. The results showed that *Euglena agilis*, *E. ehrenbergii*, *Strombomonas scabra,* and *S. urceolata* could be used to classify water by a range of bioindicator values between 6.3 - 7.0 and were usually abundant in highly polluted waters, which were classified as hypereutrophic status.

Moreover, *Euglena granulata*, *E. haematodes*, *Euglenaria anabaena*, *Euglenaria caudata*, *Euglenaria clavata*, and *Phacus longicauda* var. *tortus* showed a range of bioindicator values between 5.3-5.5 and were usually abundant in polluted waters which have been classified as being of the eutrophic status. Several studies proved that euglenoids were largely restricted to eutrophic and hypereutrophic statuses (Dasí *et al*, 1998; Lepistö and Rosenström, 1998). Moreover, *Phacus* was usually recorded in the water that was classified as being of β-mesosaprobic to α-mesosaprobic (Pereira *et al*., 2003).

Several euglenoids, including *Euglena chlorophoenicea, E. geniculata, E. hemichromata, E. proxima, E. sanguinea, E. splendens, Lepocinclis acus, L. fusiformis, Phacus horridus, Ph. orbicularis* fo. *communis, Ph. salina, Ph. acuminatus, Ph. longicauda, Ph. triqueter, Strombomonas borystheniensis, S. ensifera, S. acuminata, Trachelomonas playfairiana, T. mirabilis, T. oblonga,* and *T. volvocinopsis,* showed bioindicator values of between 4.3-5.0 and were usually abundant in moderately polluted water that was classified as being of meso-eutrophic status. The taxa of euglenoids included in the saprobes index by Slàdeček and Sládečková are typically classified as bioindicators of moderately polluted water. Conversely, *Euglena geniculata* and *E. proxima* were often present in water that had been exposed to heavy organic pollution levels and *E. agilis* was usually present in seriously polluted water, but was still found to be within the classification of "permissible" levels of pollution (Slàdeček and Sládečková, 1996). Alves-da-Silva *et al*. (2007) pointed to the relationship between some groups of euglenoids and the place which was strongly changed by human actions with high levels of organic contamination and ammonium nitrogen, as well as nitrate nitrogen and soluble reactive phosphorus, indicating moderately polluted water (β-α mesosaprobic).

Besides, *Euglena navicula* was the only species that showed a bioindicator value of 2.8 and was usually abundant in water of moderate quality classified as being of mesotrophic status. Wołowski (1998) found that *Phacus* and *Trachelomonas* were usually found in clean and β-mesosaprobic waters. Moreover, the occurrence of *Astasia klebsii*, *Menoidium minimum, M. pellucidum* var. *pellucidum,* and *M. pellucidum* ver. *steinii* were observed in strongly polluted polysaprobic water.

The comparisons of the trophic status between the euglenoid index with the AARL-PC score and the AARL-PP score from all the investigated sites revealed the trophic status. The trophic status was classified by the AARL-PC score in the range of oligo-mesotrophic status to hypereutrophic status. Most results from the euglenoid index showed a trophic status higher than the AARL-PC score, while they were similar with the AARL-PC score at 41 %. The AARL-PC score usually showed lower trophic status or better water quality than the euglenoid index, due to rains or other events that could contribute to high levels of water and actually diluted the nutrient levels or the physico-chemical parameters of the water. Thus, algae or the euglenoid group is a good indication for the real or earlier quality of water. This has relevance to the study of bioindicators, though the AARL-PC score was often a suitable method because of the rapid assay and the unnecessary algae skills. Furthermore, the trophic status was classified by the AARL-PP score in a range of oligo-mesotrophic status to hypereutrophic status, as well. Most results from the euglenoid index were similar to the AARL-PP score as 45 %, while showing a trophic status to be lower than that of the AARL-PP score. The AARL-PP score used several groups of algae for the assessment of water quality and was found to be better than any one group of algae. However, the euglenoid index is a good bioindicator for polluted water, is an easy assay to use and it becomes unnecessary to use other algae groups.

5.4 The efficiency of the euglenoid index

A total of 32 taxa of euglenoids from various types of water bodies in Northern Thailand were scored and listed in the euglenoid index. This could be used to assess the quality of freshwater, which had euglenoids as the dominant species and greater algae development; however, the use of a euglenoid index has been limited. This was similar with the AARL-PP score that was used to assess water quality and is considered a good index in places which reveal an abundance of algae. However, this study focused only on sampling sites in Northern Thailand. It can be considered a highly significant index and could be widely used if the sampling sites covered most areas of Thailand. Thus, more samples should be collected from various water bodies of different water quality (oligotrophic to hypereutrophic) throughout Thailand. This would reduce any errors that may occur during the qualification of the euglenoid species into the index.

Moreover, the numbers of euglenoid cells developing in the investigated water bodies are related to the amount of pollution and are representative of the determination that *Euglena* and *Euglenaria* are good bioindicators for the contamination of organic substances. This finding was supported by the study of Sen and Sonmez (2006), in which the *Euglena* species had better growth during the summer and autumn when higher levels of organic matter were found in the pond. The abundant development of a few species of *Euglena* indicate a strong level of contamination with organic substances, whereas multi-species communities of euglenoids usually indicate moderately polluted water.