CHAPTER 2

Literature reviews

2.1 Life history and general characteristics of euglenoids

Euglenoids are microscopic, solitary, freely swimming organisms, occurring all over the world in various types of polluted water bodies. Their characteristics that are visible in light microscopy include: flagella, chloroplasts, eyespot, paramylon bodies, striations of the pellicle, cell shapes, euglenoid movements and envelopes of some genera. The euglenoids contain about 54 free - living genera. Most genera are green and phototrophic. Typically, they live in freshwater more than brackish and marine water (Leedale, 1967; Ciugulea and Triemer, 2010; Wołowski, 2011). Euglenoid systematics began when Ehrenberg first recognized and described the major photosynthetic taxa: *Euglena* (Ehrenberg, 1830), *Cryptoglena* (Ehrenberg, 1832), *Colacium* (Ehrenberg, 1833) and *Trachelomonas* (Ehrenberg, 1833), as well as, *Phacus* (Dujardin, 1841). The genera *Lepocinclis, Eutreptia* (Perty, 1852) and *Monomorphina* were described by Mereschkowsky (1877). Several genera were established in the twentieth century, such as *Eutreptiella* according to Da Cunha (1914) followed by *Strombomonas* Deflandre (1930).

The turn of the nineteenth to the twentieth centuries resulted also in the release of a number of original monographs on euglenoids. Elder assembling which served as the keys for identification of algae includes relevant information about euglenoids in Czech Republic (Hansgirg, 1886) Germany (Klebs, 1883; Lemmermann, 1910, 1913) and Poland (Dreżepolski, 1925). Monographs of Huber-Pestalozzi (1955) and Starmach (1983) assembled species of euglenoids from around the world.

Partial compilations, in which one can find information about different areas, are included in the Popova (1966), Popova and Safonova (1976) monographs on the flora of the former Soviet Union, for which material collected in Western Siberia was used. Moreover, Vetrova (1980, 1991, and 1993) assembled information about colorless euglenoids and green *Eutreptia*, *Euglena*, *Strombomonas* and *Trachelomonas* from Ukrainian continental waters. The twentieth century also resulted in several detailed elaborations on the genus *Euglena* (Mainx, 1927; Chu, 1947; Gojdics, 1953; Pringsheim, 1956); *Lepocinclis* (Conrad, 1935); *Trachelomonas* (Playfair, 1915; Deflandre, 1926; Balech, 1944); *Phacus* (Pochmann, 1941).

The last four decades have resulted in a number of studies in the field of physiology by Walne *et al*. (1998), and Iseki *et al.* (2002); cytology by Walne (1971), Gomez *et al*. (1974), Palisano and Walne (1976), and Zakryś and Walne (1998); ultrastructure by Dunlap *et al*. (1983), Dunlap and Walne (1987), Couté and Iltis (1981), Couté and Thérézien (1985), Conforti and Nudelman (1994, 1997), Nudelman *et al.* (1998), Conforti and Ruiz (2001), Wołowski and Hindák (2004, 2005), and Wołowski and Walne (2007); genetic and molecular studies by Brosnan *et al*. (2003), Marin *et al*. (2003), Triemer *et al*. (2006), Kosmala *et al*. (2005, 2007), Karnkowska-Ishikawa *et al*. (2010), Linton *et al.* (1999, 2000, 2010), and Ciugulea and Triemer, (2010). There is no doubt that the results of all these studies have had significant influences on a significant number of changes on various levels of the taxonomy of Euglenophyceae.

Presently about 900 species are known, of which just over 250 are colorless. They are usually placed in the phylum Euglenophyta by botanists, who have recognized a single class having two (Bourrelly, 1985), three (Asaul, 1975) or six (Leedale, 1967) orders. Zoologists placed the euglenoids in the Protozoa and divided the Order Euglenida into five suborders (Lee *et al*., 1985; Leedale and Vickerman, 2000). The general taxonomic system followed that of Leedale (1967) and Walne and Kivic (1989). Moreover, Triemer *et al*. (2006) described the genus *Discoplastis* and Linton *et al*. (2010) also described the genus *Euglenaria* (Brodie and Lewis, 2007; Ciugulea and Triemer, 2010). Recent classifications of the euglenoids have been based on variations within the cell construction, cytological and molecular studies (Wołowski, 2011). The phylogenetic studies have been associated with taxonomic revisions (Marin *et al*., 2003; Brosnan *et al*., 2003, 2005; Triemer *et al*., 2006; Kosmala *et al*., 2007; Ciugulea *et al*., 2008; Kosmala *et al*., 2009).

The very important data about euglenoids from sub-tropical and tropical regions of Australia can be found in one of the basic elaborations done by Playfair (1915, 1921), Ling and Tyler (2000). Similar zones were elaborated on in South America, such as from Brazil, 130 taxa of euglenoids in habitats strongly changed by human actions were recorded by Alves-da-Silva *et al*. (2007), Alves-da-Silva and Friedrich (2009). Lately, 26 taxa of *Trachelomonas* were found in a shallow subtropical reservoir and rain water storage in the municipality of Triunfo (Alves-da-Silva *et al*., 2013). More information about 90 taxa of *Trachelomonas* and 43 taxa of *Strombomonas* were recorded from Camaleão Lake, near Manaus, Brazil (Conforti, 1993; 1993a). In Mexico, 12 taxa of *Trachelomonas* were found in the Guadalupe Dam, a eutrophic reservoir. Most of them were found during the dry season with a high concentration of nutrients and a moderate amount of mineral content (Solórzano *et al*., 2011). In the Caura River, a major tropical watershed, and in its floodplain, in the Bolivar State of Venezuela, 21 taxa of euglenoids in various types of water bodies were found in the lower basin (Delgado and Sánchez, 2007). Furthermore, Salazar (2004) also reported on 80 taxa of euglenoids by scraping off the periphyton from the roots, stems and submerses leaves of *Hymenachne amplexicaulis*. In Bolivia, 35 taxa of *Trachelomonas* were recorded from the Ibare River (Couté and Thérézien, 1985). Thérézien (1989) found 64 taxa of euglenoids in the Amazon region. In addition, Tolivia *et al*. (2012) also found 37 taxa of naked euglenoids in several water bodies in Columbia. From some warm regions of North America, especially in the South-Eastern USA, the data of Dillard (2000) have included a general elaboration. Zakryś and Walne (1994) reported 56 taxa of green euglenoids. Afterwards, Wołowski and Walne (2007), Wołowski *et al*. (2013) published several new reports on the pigmented euglenoids from this region. Very interesting data have been included in the paper by Wołowski and Walne (2007), in which detailed studies on 5 taxa of *Strombomonas* and 63 taxa of *Trachelomonas* that occur in polluted environments have been included.

Information on euglenoids in Africa has included just several papers, which have been elaborated on by Bourrelly and Gayral (1951), Bourrelly (1961), Compére (1975). Only a minimal number of detailed studies on euglenoids, especially those on *Trachelomonas*, have been published by Couté and Iltis (1981) and Zongo *et al*. (2006). Da *et al* (2009) reported on 58 taxa of *Trachelomonas* that were found in the samples of the Agnéby River and two small ponds in the Ivory Coast. Recently, Wołowski (2012) described 16 taxa of euglenoids from a drying fishpond in Cameroon, West Central Africa.

In Asia, different pond ecosystems of Bangladesh have presented 17 taxa of euglenoids: *Lepocinclis* (7 taxa), *Strombomonas* (7 taxa), *Astasia* and *Menoidium* (3 taxa) (Khondker *et al*., 2008). In Taiwan, euglenoids involving 7 genera and 165 species were found by Yamagishi (1992) in several water bodies. Moreover, several samples of plankton algae from lakes, ponds, fishponds, ditches of the road-sides and paddy fields of Southeast Asia, Thailand, Cambodia and Malaysia were reported by Yamagishi (2010). Among other algae, 316 taxa of euglenoids were also found. In Korea, there have also been many studies on euglenoids. In the Han River, Beom-Jun (1980) collected samples from 1929 to 1980, and found 101 taxa of Euglenophyceae. Additionally, 22 taxa of *Euglena* were found in 58 sites of Korean freshwaters by Kim *et al*. (1998). Furthermore, taxonomic and floristic accounts of the genus *Euglena* (Kim *et al*, 1998), *Trachelomonas* (Kim *et al*., 2000) and *Phacus* (Kim *et al*., 2000) were studied. In the Chunam Reservoir, 57 taxa of *Trachelomonas* and 39 taxa of naked euglenoids, including *Euglena* (9 taxa), *Lepocinclis* (11 taxa) and *Phacus* (19 taxa) were found by Conforti and Ruiz (2001, 2002). In the Philippines, 28 taxa of euglenoids were recorded from the fishponds and fish pens of Mayondon, but were found more frequently in fishponds with highly eutrophic water (Martinez, 1978). From Malaysia, Prowse (1958) also provided information on 125 species of euglenoids. This publication serves as documentation and as a key for the identification of this group.

The study about euglenoids in Thailand has not yet been fully elaborated on, due to a lack of basic information, a limitation in species identification, and the limited relevant ecological data. The main sources of information on euglenoids are references given by

Lewmanomont *et al.* (1995) and Peerapornpisal (2005, 2013) in publications on different taxonomic groups of algae. Moreover, Hanpongkittikul and Wongrut (2005) also recorded the plankton communities that included 30 taxa of euglenoids from the Pasak Jolasid Reservoir. Yamagishi (2010) also published documentation on *Gyroppaigi* (1 taxon), *Lepocinclis* (4 taxa), *Phacus* (19 taxa), *Trachelomonas* (31 taxa), *Strombomonas* (7 taxa) and *Peranema* (1 taxon) euglenoid species that occurred in Thai ponds. Chaimongkhon and Peerapornpisal (2012) presented 34 taxa of non - loricate euglenoids.

2.2 Cell structure and biology of euglenoids

2.2.1 Euglenoid cells

Euglenoid cells are of various shapes, elongated, spindle-shaped, lance-shaped to ovoid or leaf-shaped, with or without cell flattening. They can be either naked or enclosed within an envelope (Wołowski and Hindák, 2005; Wołowski, 2011). The anterior end of the cell has an anterior invagination, which consists of a narrow canal (the gullet) leading to a flask-shaped reservoir. The contractile vacuole and eyespot are placed around the reservoir. The contractile vacuole can be almost spherical before discharging into the reservoir, and is frequently reported as being surrounded by several vacuoles (Figure 2.1).

A naked cell is surrounded by a prominent pellicle. The pellicle has a complex structure formed by flat interlocking strips, which pass helically along the cell. Each strip is associated with several microtubules and muciferous bodies. The pellicle ranging from the presence is very flexible to semi - or completely ridged, and is usually striated. The striations are frequently twisted in the longitudinal axis (Leedale, 1967; Wołowski and Hindák, 2005).

2.2.2 Lorica (extracellular matrix, envelope or test)

Lorica (extracellular matrix, envelope or test) is a protective envelope surrounding the protoplast in some euglenoid genera, such as *Strombomonas*, *Trachelomonas* and *Ascoglena*. The lorica is first secreted as a mucilaginous layer, which is then mineralized. The amount of the deposited mineral depends on the amount of mucilage secreted (West and Walne, 1980). Ferric, silica and manganese are the major chemical components of the lorica (Dunlap *et al.*, 1983). In *Trachelomonas*, iron is the main mineralizing component of the envelope, with lesser amounts of silicon and manganese, while in *Strombomonas*; silica plays the main role (Conforti *et al.*, 1994). They give orange to black coloration to the lorica; the color depends on the mineral concentration.

Lorica development was studied and described in cultured *Trachelomonas* species by Pringsheim (1953), Singh (1956) and Leedale (1975). Moreover, the details of the lorica ultrastructure were studied (Conforti and Tell, 1986b; Conforti, 1999; Conforti and Perez, 2000; Wołowski and Hindák, 2005; Wołowski and Walne, 2007; Da *et al.*, 2009; Ciugulea and Triemer, 2010). Recently, Brosnan *et al.* (2003, 2005) and Ciugulea *et al.* (2008) have addressed problems related to the separation of those genera based on differences in lorica morphology and development. Ciugulea *et al.* (2008), Linton *et al.* (2010) established the very high investigative value of cell morphology (number of chloroplasts, type of pyrenoids).

2.2.3 Flagella

Flagella are mobile threads, which extend from anterior invagination, on the lateral or bottom of the reservoir (Figure 2.1). Most euglenoids possess two flagella of equal length, one emergent and the second reduced and not emerging; although there can may be one to several which are each often associated with swelling (Leedale, 1967).

2.2.4 Eyespot (stigma)

Eyespots are possessed by all green euglenoid species and there are a few that are colorless. The eyespot contains a variable number of lipid droplets containing carotenoid pigments (ß-carotene, xanthophylls) and flavins. The eyespot and flagella swelling form the photosensory transduction system, which controls phototactic movement. They usually move from strong light to darkness (negative phototaxis) or from darkness to weakly or moderately illuminated areas (positive phototaxis) (Leedale, 1967; Wołowski and Hindák, 2005).

2.2.5 Chloroplasts (chromatophores)

Chloroplasts are the organelle in which the photosynthetic process takes place. They are grass green, 1 or 2 in numbers to being very numerous in a cell. They vary in shape they are: disc-, plate-, star- or ribbon-shaped (Figure 2.1). Chloroplasts contain chlorophylls *a* and *b*, ß carotene, diatoxanthin, diadinoxantin, neoxantin. Chloroplasts of many algae usually consist of pyrenoids (Leedale, 1967; Wołowski and Hindák, 2005).

2.2.6 Pyrenoids

Pyrenoids are a specialized area of chloroplast that contains high levels of RuBisCo (ribulose-1, 5-bisphosphate carboxylase/oxygenase), the key enzyme in carbon dioxide fixation. They are usually associated with an accumulation of materials derived from photosynthesis (Leedale, 1967; Wołowski and Hindák, 2005). Three types of pyrenoids are found in euglenoids: (1) naked pyrenoids with no paramylon bodies, (2) haplopyrenoids with a paramylon sheath only on one side of the pyrenoids, and (3) diplopyrenoids with a sheath of paramylon on each side of the pyrenoid, which are also known as double - sheathed pyrenoids. Sometimes, they are associated with paramylon, e.g., in *Euglena viridis*, *E. geniculata* and *E. tristella* containing 1-3 star-shaped chloroplasts (Ciugulea and Triemer, 2010).

2.2.7 Paramylon bodies

Paramylon bodies are storage products or energy reserves for euglenoids. They were first recognized to appear as β 1-3-like glucans by Kreger and Meeuse (1952) on the basis of X-ray diffraction studies. Paramylon bodies differ from most starches, “paramylon bodies always form in the cytoplasmic matrix of the cell, never inside the chloroplast” and are insoluble in boiling water (Leedale, 1967). Moreover, they also differ from the true starch because they do not present the blue color reaction with iodine (Gojdics, 1953). Morphology and chemical properties were investigated (Leedale, 1967; Zakryś and Walne, 1998; Shin and Triemer, 2004; Monfils *et al*., 2011; Shibakami *et al*., 2012). They are found in a large variety of shapes and sizes (disc-, rod-, or ring-shaped, linked or granular) (Figure 2.1).

2.2.8 Nucleus

Nucleus lies in the central or posterior part of the cell, but may move during euglenoids movement. It is usually spherical, ovoid or elongated (Figure 2.1). Euglenoids are eukaryotes so they have a true nucleus that contains one or more endosomes (nucleoli). Nuclear division in euglenoids is mitosis (Leedale 1967; Wołowski and Hindák, 2005).

2.2.9 Locomotion

Locomotion is controlled by helical waves of the emergent flagellum combined with helical rotation of the cell (pellicle permitting). The major movements of euglenoids are swimming, contraction, creeping, and gliding. Most euglenoids can bend or contract to a greater or lesser degree. This movements are known as contractile body movements, euglenoid movements or metabolic (rapid change of body shape) (Bovee, 1982; Wołowski and Hindák, 2005).

2.2.10 Nutrition

Nutrition consists of two basic types, photosynthetic and heterotrophic. Most photosynthetic euglenoids can supplement photosynthesis by absorbing some organic compounds (osmotrophic), such as vitamins. So, they probably combine phototrophic and osmotrophic properties, but they never present phagotrophic properties. In Addition, colorless euglenoids are phagotrophic and ingest particulate food. Some species combine osmotrophic and phagotrophic characteristics (Leedale, 1967, Wołowski and Hindák, 2005).

2.2.11 Reproduction

Reproduction is asexual by longitudinal division of motile or non-motile cells, usually in the evening. Cell division starts at the anterior end of a cell after the nucleus has divided and processes posteriorly. At the same time, its organelles are doubled. Then, the two daughter cells are parent cell clones. The sporadic meiosis that occurs in some euglenoids is generally believed to result from non-sexual autogamy (Pringsheim, 1956; Leedale, 1967; Ciugulea and Triemer, 2010; Wołowski, 2011).

2.2.12 Resting stage

Resting stage is a general phenomenon for euglenoids. Many representing types of *Euglena* are able to change the characteristic unicellular motile stage to non-motile mucilaginous colonies (cf. Hindák *et al*., 2000). Monads of euglenoids during the encystation stage change their original shape to that of ovoid or oval (in flattened forms), to spherical forms, while losing flagella. The mucus bodies eject mucilage that forms layers around the cell. Mucilaginous envelopes (cysts) are usually noticeable, spherical, or pentagonal. These mucilaginous colonies are called palmelloid or gloeocystis stages (Wołowski and Hindák, 2005).

Three basic types of cysts are formed by the *Euglena* species: (1) Protective ones-generally have a heavy, sometimes stratified wall containing one cell; (2) Reproductive ones (palmella formation)-have a thin elastic, permeable membrane that increases in diameter as the cell divides, the cell is non-flagellated; (3) Temporary ones, resting or transitory cysts-have a wall that is impervious to water, but which contains a small pore (Hindák *et al* 2000; Wołowski and Hindák, 2005).

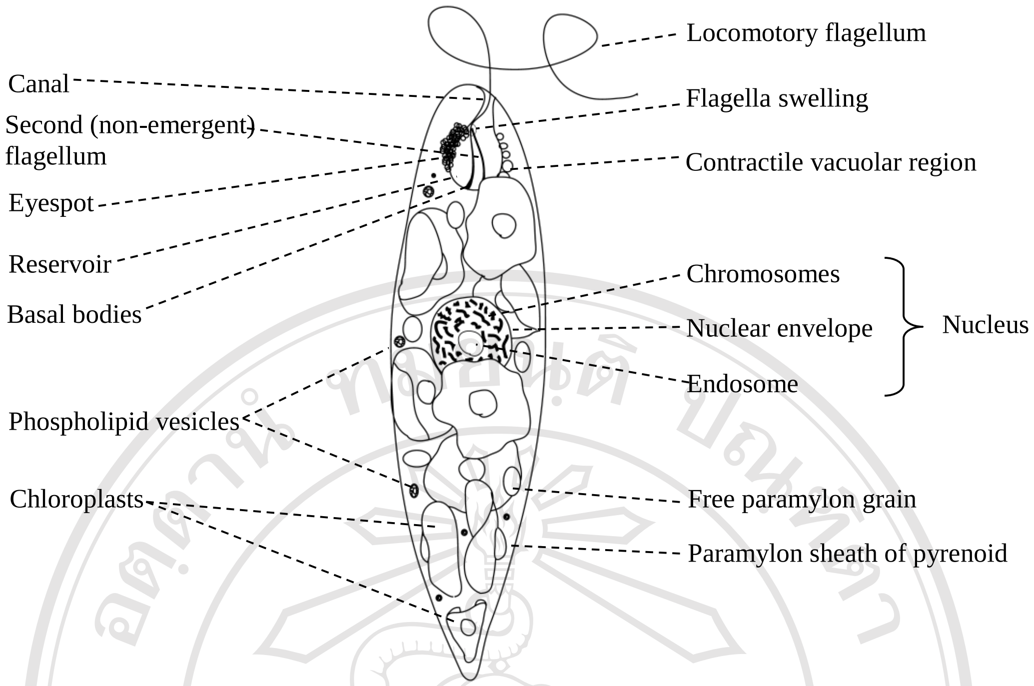

Figure 2.1 Cell structures of *Euglena gracilis*
Source: Leedale (1967)

2.3 Ecology and distribution

Euglenoids occur worldwide especially in fresh water, but are also found in marine or brackish water. They are present in various types of water bodies. Usually, photosynthetic euglenoids are rare in clean waters. They usually occur in small water bodies that are influenced by organic matter, such as ditches, puddles, and ponds or shallow parts of lakes (Wołowski and Grabowska 2007) and slow-flowing rivers. In large and deep-water bodies they occur along the banks in the shallow parts that easily warm up. Most of them are free swimming, but several can either creep on mud or be attached by the posterior end to a substrate (*Euglena mutabilis*, *E. adhaerens*) or can form colonies that are often attached to invertebrates (*Colacium*) (Wołowski 1998, Wołowski and Hindák, 2005).

They can form green (*E. viridis*), red (*E. sanguinea*, *E. rubra*) (Figure 2.2), or brown (*Trachelomonas*) blooms. Usually, the blooming of algae have more negative impacts on the environment, modifying the color, odor, and taste of the water, especially causing massive deaths of the fish, and even bird populations (Ciugulea and Triemer, 2010). Fish deaths caused by *E. sanguinea* and *E. granulata* have resulted in important economic losses in fisheries in the United States (Zimba *et al*., 2004). It has even been suggested that *E. sanguinea* could be the species responsible for turning the Nile River red in ancient Egypt.

Some euglenoids can occur in most unusual habitats, e. g., *Euglena* in the Great Salt Lake, *Notosolenus* in snow, while several taxa can tolerate heavy metals, phenol, herbicides and insecticides, and intense radioactivity. Several taxa are also tolerant to extremes of pH, e.g., *E. gracilis* which can grow well at any pH from 3.0 to 9.0, *E. mutabilis* has an even wider pH tolerance ranging from just over 1.0 to nearly 9.0, though the evidence indicates this range is partially related to diverse, genetically different strains. They can play a major role during the purification of sewage ponds and oxygenation of strongly polluted waters (Wołowski 1989a, 1989b, 1992, 1998; Wołowski and Hindák, 2005).

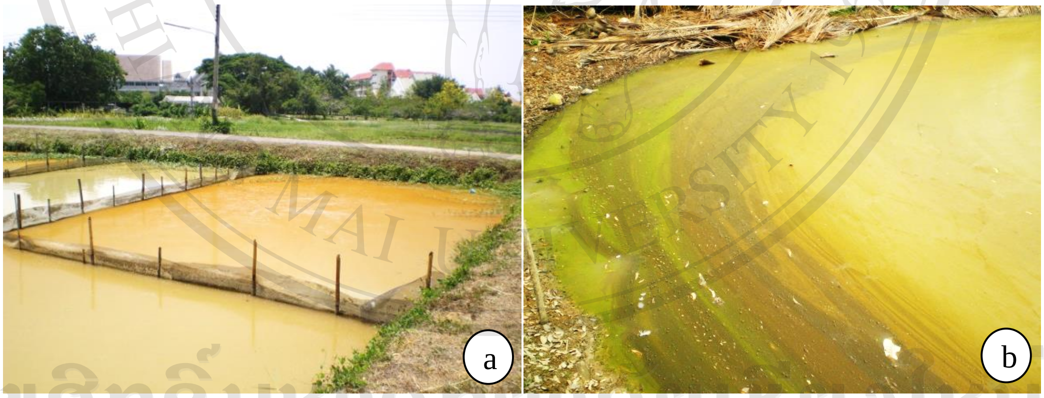

Figure 2.2 Bloom of *E. sanguinea* and other taxa of *Euglena* in a fish-pond at Mae Jo University (a) and the blooming of *Euglena* species in a garden pond at the Pa Ko Dam Tobacco Station, Chiang Rai (b)

2.4 Algae and water assessments

Water resources can be fully characterized by three components, including hydrology, physico-chemistry, and biology. Biological analysis is one of the tools for the assessment of water quality, based on five main approaches, such as **ecological methods:** investigation of the biological communities (biosensors) in the water body, investigation of the biosensors on artificial substrates placed in the water body, and the presence or absence of specific species, **physiological and biochemical methods**: oxygen production and consumption, stimulation or inhibition, respiration and growth of organisms living in the water, and studies of the effects on enzymes, **the use of organisms in controlled environments**: assessment of the toxic (or even beneficial) effects of trials on organisms under settle laboratory conditions (toxicity tests or bioassays) and an assessment of the effects on defined organisms (e.g. behavioral effects) of waters and effluents in situ, or on-site, under controlled situations (continuous, field or “dynamic” tests), **biological accumulation**: investigation of the bioaccumulation of substances by organisms living in the environment (passive monitoring), and studies by organisms deliberately exposed in the environment (active monitoring), and **histological and morphological methods**: observation of histological and morphological modifications, and embryological development or early life-stage tests. Some of these methods are generally used in freshwaters although other methods have been developed for use in specific environments, or in relation to particular environmental impacts (Chapman, 1996).

Algae also have long been used to assess environmental conditions in aquatic habitats throughout the world. Algae have been explored as indicators of organic pollution in European streams and rivers (Kolkwitz and Marsson, 1908). Besides, the uses of algae indicators of environmental conditions have been enlarged based on the environmental sensitivities and tolerances of individual taxa and species composition of assemblages (Slàdeček, 1986; Lowe, 1974; Lange-Bertalot, 1979). Palmer (1969) published the information on pollution-tolerant algae assembled from reports made by 165 authors. Among them, the top of the genera are *Euglena, Oscillatoria, Chlamydomonas, Scenedesmus, Chlorella, Nitzschia, Navicula,* and *Stigeoclonium*. Furthermore, Coesel

(1983, 2001) established the method for using desmids as bio-indicators in freshwater. Also, in Thailand, Peerapornpisal *et al.* (2007) proposed a method by using phytoplankton for water quality assessment based on 35 limnology research studies in Thailand. These water quality assessments were called the "AARL-PP Score" or "Applied Algal Research Laboratory-Phytoplankton Score". Euglenoids groups, such as *Euglena, Strombomonas and Trachelomonas*, occur in polluted water. Algae have been used successfully in the environmental assessment of many streams, larger rivers, lakes and wetland areas around the world (Sheath and Wehr, 2003).

Algae are appropriate to water quality assessment because of their nutrient needs, rapid reproduction rate, and short life cycle. Algae are valuable indicators of ecosystem conditions because they respond quickly both in species composition and densities to a wide range of water conditions due to changes in water chemistry. Basic features of algal assemblies that can be measured and potentially used to assess environmental conditions include **(1) biomass**: chlorophyll *a*, ash free dry mass, cell density, cell biovolume; **(2) taxonomic composition**: species relative abundances, species relative biovolume, functional group biovolume; **(3) diversity**: species richness, genus richness, evenness; **(4) chemical composition**: chlorophyll *a* (phaeophytin and chlorophyll *a*), chlorophyll a: ash free dry mass ratio, P or N/ ash free dry mass, N:P ratio of algal assemblages; **(5) photosynthesis rates**: respiration rates, net primary productivity, nutrient uptake rates (McCormick and Cairns, 1994).

Several publications have included information on the importance of euglenoids in the evaluation of water quality, such as Cyrus and Slàdeček (1973), which gave 91 taxa of colored and 42 taxa of colorless euglenoids. The individual taxa were described morphologically, and their occurrence and saprobiological classification were reported. In addition, Simić *et al.* (2006) recorded 4 groups of phytoplankton and 4 groups of zooplankton from Bovan Reservoir, Serbia. Relative abundance and qualitative composition values of plankton showed saprobic index pointing β-mesosaprobic. Several indices were used by Jekatierynczuk-Rudczyk *et al.* (2012) including Carlson's ISI (Carlson, 1997), Crustacean Index (CI) and a number of zooplankton indices to assess the trophic state of the lakes in the Suwałki Landscape Park (NE Poland). Furthermore, Cvetkovic and Chow-Fraser, (2001) used ecological indicators to assess the quality of the Great Lakes coastal wetlands: the Water Quality Index (WQI) (Chow-Fraser, 2006), the Wetland Fish Index (Seilheimer and Chow-Fraser, 2007) and the Wetland Macrophyte Index (Seilheimer *et al*. 2009).