CHAPTER 5
DISCUSSIONS

5.1 Diversity of *Pediastrum* spp.

A total of 26 species which consisted of 60 taxa were found from 68 sampling sites in some freshwater resources of Thailand and this study revealed new record species in Thailand. The new record species were compared with the checklist of freshwater algae and publications of Thailand (West and West, 1902; Hirano, 1967; Hirano, 1975; Lewmanomont *et al.*, 1995 and Peerapornpisal *et al*., 1996). The new record species in this study were found to be higher than those in other reports in Thailand. Because samples were collected from all 6 of regions the country. In addition, the details of the cell wall under SEM were clearer, which is an important criterion for identification (Kowalska and Wolowski, 2010). Additionally, more species were found than previously reported by Kowalska and Wolowski (2010) who found 15 taxa of *Pediastrum* spp. in Poland. This might be due to the fact that Thailand is situated in the tropical zone which is considered an appropriate location for species biodiversity (Mutke and Barthlott, 2005) in view of its topography and climate, as high temperature is a limiting factor for the distribution of phytoplankton in tropical areas (Perumal *et al*., 2009).

In this study, *P. boryanum, P. duplex, P. simplex and P. tetras* were the dominent species which were similar to that reported in Europe and Asia by Komárek and Jankovská (2001) and Rai and Misra (2012). *P. duplex* was found to have the highest

number of taxa (12 taxa) followed by *P. boryanum* and *P. simplex* (8 taxa) and *P. tetras* (4 taxa). This study revealed the highest numbers of those dominant species in the Chiang Mai Moat in Chiang Mai province and the highest number of taxa (13 species, 30 taxa) was obtained at this sampling site.

5.2 Water quality in some freshwater resources of Thailand

The water qualities are generally classified into 5 trophic status levels i.e. oligo-mesotrophic, mesotrophic, meso-eutrophic, eutrophic and hypereutrophic status. Meso-eutrophic status was found in most sampling sites (28 sampling sites) followed by mesotrophic status (25 sampling sites), oligo-mesotrophic status (9 sampling sites), eutrophic status (4 sampling sites) and hypereutrophic status (2 sampling sites). This is due to the fact that different activities were taking place along the reservoir. The majority of sites with oligo-mesotrophic status were surrounded by deciduous forests, so there was not much contamination in the water bodies, whereas most sampling sites with mesotrophic and meso-eutrophic status were contaminated by the general community, restaurants, fish ponds, and agricultural activities. Most sampling sites with eutrophic status were contaminated with wastewater from drainage pipes. Some sampling sites were situated in water treatment areas, which were directly affected. This situation was similar to that which was previously reported in other countries. In special case, hypereutrophic status were found in 2 sampling sites i.e. SMP1 (Samut Prakan province) and SKN1 (Sakon Nakhon province). SMP1 is located near the roadside and received wastewater from drainage pipes which gives dark green color to surface water. When water sample was studied under light microscope and *Pediastrum* spp. were not found but

just found blue green algae and euglenoid group were observed. SKN1 is the wastewater treatment pond and the water is green under light microscope but *Pediastrum* was not found only *Microcystis* was observed which was similar to that reported by Para (1979) who mentioned that *Pediastrum* spp. were found in mesotrophic to eutrophic water but not in hypereutrophic water.

Pediastrum spp. can be generally found in oligo-mesotrophic, mesotrophic, meso-eutrophic and eutrophic conditions but they were most commonly found in meso-eutrophic and mesotrophic conditions. So, they are used to assess water quality in the meso-eutrophic status. Some species e.g. *P. alternans* Nygaard, *P. angulosum* Ehrenberg ex Meneghini, *P. angulosum* var. *coronatum* (Raciborski) J.Komárek & V.Jankovská and *P. braunii* Waetm. Schweiz were found in oligo-mesotrophic condition. They are used to assess the water quality in oligo-mesotrophic status (Sladecek *et al.,* 1981). However, to determine the ecological situation in the lake, the whole assemblage of more species in one biotope should be studied (Komarek and Jankovska, 2001).

5.3 Cultivation of *Pediastrum* spp.

5.3.1 Effect of media

Cultivation of dominant species; *P. boryanum*, *P. duplex*, *P. simplex* and *P. tetras* in 3 media: JM, AM and BBM indicated that *P. boryanum* exhibited highest growth in BBM followed by those in JM and AM respectively. *P. duplex, P. simplex* and *P. tetras* grew best in JM followed by those in BBM and AM media respectively. Cultivation in BBM and JM gave higher growth than in AM because BBM and JM contain many

macronutrients and micronutrients (Appendix D). Macronutrients are required in large quantities which are used generally as building materials. Whereas, micronutrients comprise mainly of vitamins and minerals which are required in minute quantities. However, both macronutrients and micronutrients are essential for algal growth (McElroy and Nason, 1954). The elements required for the growth of green algae are N, P, K, Mg, Ca, S, Fe, Cu, Mn, and Zn. These elements are added in the form of salts (Oh-Hama and Miyachi, 1988). The lack of micronutrients in AM gave the lowest growth.

5.3.2 Effective of pH

P. boryanum was found to grow best at pH 7.5 in BBM. *P. duplex, P. simplex* and *P. tetras* grew better at pH 8.0 in JM. Most microalgal species favored neutral pH, whereas some species are tolerant to higher pH e.g. *Spirulina platensis* at pH 9 or lower pH e.g. *Chlorococcum littorale* at pH 4 (Celia and Edward, 1994). The pH range for most cultured algal species is between 7 to 9 depending on species and media (Azov, 1982). pH is the major determinant of relative concentrations of carbon dioxide, carbonate, and bicarbonate ions in water and could affect the availability of carbon for algal photosynthesis in intensive cultures (Celia and Edward, 1994).

5.3.3 Effect of temperature

The four dominant species were found to grow best at room temperature. During the cultivation period the minimum temperature was 26.0-28.5 ºC and the maximum temperature was 29.0-33.0 ºC. Temperature is an important factor for growing algae. It strongly influences cellular chemical composition, the uptake of nutrients, carbon dioxide fixation, and the growth rates. It is known that the growth rate increases with the increase

in temperature up to its optimum and decreases drastically. Most commonly cultured species of microalgae tolerate temperatures between 16 and 27°C. Temperatures lower than 16°C slow down the growth, whereas those higher than 35 ˚C are lethal for a number of species (Quinn and Williams, 1983). The optimum temperature for *Chlorella vulgaris* ranges from 25 to 30°C and for *Scenedesmus sp.* is between 20-40°C (Sanchez *et al*.,2008). Thailand is situated in the tropical zone and the average temperature is 26.0-28.5 ºC which is suitable for algal growth. The benefit of room temperature is that it is not controlled temperature which can reduce production costs.

5.4 Nutritional value of dominant species of *Pediastrum* spp.

Proximate composition of protein contents of *P. boryanum, P. duplex, P. simplex and P. tetras* were 30.17, 36.45, 35.93 and 30.44 g/100g respectively; carbohydrate contents were 43.49, 32.88, 34.41 and 43.86 g/100g respectively, fat contents were 7.50, 14.06, 13.56 and 10.29 g/100 g respectively, ash contents were 8.80, 8.74, 8.73 and 6.50 g/100g respectively and the moisture contents were 10.04, 7.84, 7.73 and 8.91 g/100 g respectively. The composition of protein in *Pediastrum duplex* of this study were less than those previously reported by Lee *et al*. (2009) i.e. protein 46.3 g/100g. However, carbohydrate, fat and moisture content of this study were higher than the previous report i.e. carbohydrate 30.4 g/100g, lipid 2.4 g/100g and moisture 6.1 g/100g due to the difference in culture condition and strain. Protein and carbohydrate were the major components in these algae that provide high protein close to that found in *Chlorella* sp. and *Spirulina* sp. They were known to provide higher protein than other natural food.

They can be applied as food supplement for human and animal feeds. Carbohydrate value was interesting as polysaccharide for sources of antioxidant which can be applied in food and pharmaceutical industries. In addition, lipid value from these studies might be used in the production of biodiesel which is becoming an efficient alternative source of fuel.

5.5 Phylogenetic analysis of the 26S rDNA and rbcL sequence

Pediastrum spp. are not supported as monophyletic in the 26S and rbcL rDNA which was similar to previous study by Buchheim *et al.* (2005) who indicated that *P. duplex* Meyen 1829 was not supported as monophyletic, which analysis of combination between 18S, 26S, and ITS-2 rDNA. 26S and rbcL study indicated that the genus *Pediastrum* is paraphyletic. *Pediastrum duplex* var. *gracillimum* was separated from *Pediastrum duplex* group which is sister to *Pediastrum simplex* group. The processi of *Pediastrum duplex* var. *gracillimum* are very long and slender, diameter of the perforations is usually larger than the cell diameter which is similar to *Pediastrum simplex* var. *biwaense* (*P. biwae*). Phylogenetic analyses demonstrated that *Pediastrum simplex* is not monophyletic. In pervious study, *Pediastrum simplex* was changed to *Monactinus simplex* (Buchheim *et al*. 2005). *Pediastrum simplex* exhibits cell wall ultrastructure consisting of granules, mostly lacking intercellular perforations, with rosettes at the cell wall corners, and bristles are present (Buchheim *et al*. 2005). In this study, it was found that *Pediastrum* could be divided into two groups as considered by morphological characteristic that the cell wall is different. Group I; *Pediastrum simplex* var. *simplex* Meyen, *P. simplex* var. *sturmii* (Reinsch) Wolle, *P. simplex* var. *echin*ulatum

Wittrock are densely regularly granular and intercellular perforation are small. Group II; *P. biwae* Negoro and *P. simplex* var. *pseudoglabrum* Parra Barrientos are smooth and very fine granular, intercellular perforations with diameter usually lager than cell diameter and the process of this group are longer and more slender than those of Group I.