

# Distribution and isolation of microalgae for lipid production in selected freshwater reservoirs of northern Thailand

TIPPAWAN PRASERTSIN<sup>1,\*</sup> YUWADEE PEERAPORNPISAL<sup>2,\*\*</sup>

<sup>1</sup>Program in Biology, Faculty of Science and Technology, Chiang Rai Rajabhat University, Phaholyothin Rd, Chang Wat Chiang Rai 57100 Thailand. Tel.: +66 53-776012, +66 85-4594633; Fax: +66 53-702758, \*email: tippawan\_pra@hotmail.com

<sup>2</sup>Department of Biology, Faculty of Science, Chiang Mai University, Su Thep, Chiang Mai 50200, Thailand. Tel.: +66-53-94-3346, Fax.: +66-53- 89-2259, \*\*email: yuwadee.p@cmu.ac.th

Manuscript received: 14 October 2017. Revision accepted: 31 January 2018.

**Abstract.** Prasertsin T, Peerapornpisal Y. 2018. Distribution and isolation of microalgae for lipid production in selected freshwater reservoirs of northern Thailand. *Biodiversitas* 19: 343-350. Nong Bau Reservoir and Chiang Saen Lake are considered important freshwater reservoirs of Chiang Rai Province located in northern Thailand. The surrounding areas of these water bodies are host to a range of human activities that influence water quality. Moreover, to date, there have not been any studies on the water quality and the distribution of microalgae in these places. The physical and chemical parameters of the water quality and microalgae were carried out in Nong Bua Reservoir and Chiang Saen Lake during the months of May, July, and October of 2015. Microalgae were isolated in order to investigate the lipid-producing abilities. Samples collected from Nong Bua Reservoir have revealed the presence of seven divisions, 90 species of algae. Six divisions, 55 species of algae were found in Chiang Saen Lake. The trophic status of the water was evaluated from the main parameters (AARL-PC Score), and it was determined that Nong Bua Reservoir was of meso-eutrophic status and Chiang Saen Lake was of mesotrophic status. Microalgae were isolated from Nong Bua Reservoir and Chiang Saen Lake for the purposes of studying lipid content; 25 and 6 isolations were identified, respectively. The lipid content was highest in *Botryococcus braunii* ( $39.25 \pm 0.32\%$  dry weight) followed by *Ankistrodesmus* sp. ( $26.80 \pm 0.44\%$  dry weight) and *Coelastrum microsporum* ( $24.95 \pm 0.55\%$  dry weight). The lowest lipid content was found in *Planktolyngbya* sp. ( $7.25 \pm 0.43\%$  dry weight). The indigenous strains of microalgae of Nong Bau Reservoir and Chiang Saen Lake's can be considered quite promising as model strains in terms of the production of biofuel within the country.

**Keywords:** AARL-PC Score, isolation, microalgae, trophic status, water quality

## INTRODUCTION

Algae are known to be the main oxygen-producing organisms in aquatic environments. They are a diverse group of aquatic organisms and are considered the foundation of the food chain in aquatic environments. They are responsible for more than 40% of global carbon fixation and are thought to be effective in regulating global warming and climate change by controlling pollution levels (Wetzel 2001). Algae can live in various environments including saline water, freshwater and brackish water. They can be found throughout a wide variety of water bodies that display a range of water quality. Algae possess different physical and chemical requirements whereby each species has a different set of favorable conditions that promote its growth and reproduction. The algae communities are sensitive to changes in their environment, and therefore phytoplankton total biomass and certain designated species are often used as indicators of water quality (Reynolds et al. 2002; Brettum and Andersen 2005). Algae range in size from unicellular (microalgae) to very large multicellular species (macroalgae). Microalgae are microscopic photosynthetic organisms that are found in marine and freshwater environments (Brennan and Owende 2010; Demirbas 2010). Notably, there exists a potential for these organisms to fix 1.83 tons of atmospheric CO<sub>2</sub> while

producing one ton of algae biomass (Chisti 2008). Microalgae are considered to be good candidates for biofuel production because of their higher photosynthetic efficiency, higher biomass production, and faster growth rate when compared to other energy crops such as rapeseed and soybean (Miao and Wu 2006). Moreover, it is easy to cultivate microalgae with wastewater even in lands that are unsuitable for agriculture (Mata et al. 2010). The lipid accumulation in the cells of microalgae ranges from 25-75% of its dry weight (Malcata 2011). Microalgal lipids, commonly referred to as crude lipids or total fatty acids of microalgae, are extracted and then arranged by transesterification during the production of microalgal biofuels. The key processes involved in biodiesel production from microalgae include cultivation, harvest, lipid extraction, and transesterification of the lipids (Jungmin 2013).

Chiang Rai Province is located in the northern part of Thailand and is home to many important fresh bodies of water such as lakes, reservoirs and ponds. Nong Bau Reservoir is a potential source of freshwater and is located within the grounds of Chiang Rai Rajabhat University. This reservoir resource provides water for the general public. The areas surrounding this reservoir are home to various restaurants and communities, which have both direct and indirect effects on the water body, particularly during the

months of October to December of every year. During this period, the water of this reservoir is often green in color and sometimes a scum of algae can be found on the surface of the water. The area comprising Chiang Saen Lake is one of the areas that have been selected for implementation of the project entitled Ramsar Convention Management and Protection of Wetland Areas. This water body is also influenced by a range of human activities that take place in the immediate area of the lake. It is understood that these two locations are exposed to various human activities. There have been only a few studies that have been conducted on water quality, distribution of phytoplankton and production of microalgal lipids in these two locations. This research study has two main objectives; i.e., to study the water quality based on the physical and chemical parameters of these water sources in collaboration with the distribution of microalgae and isolation of lipid producing microalgae in search of potential sources of biofuel. □

## MATERIALS AND METHODS

### Study areas

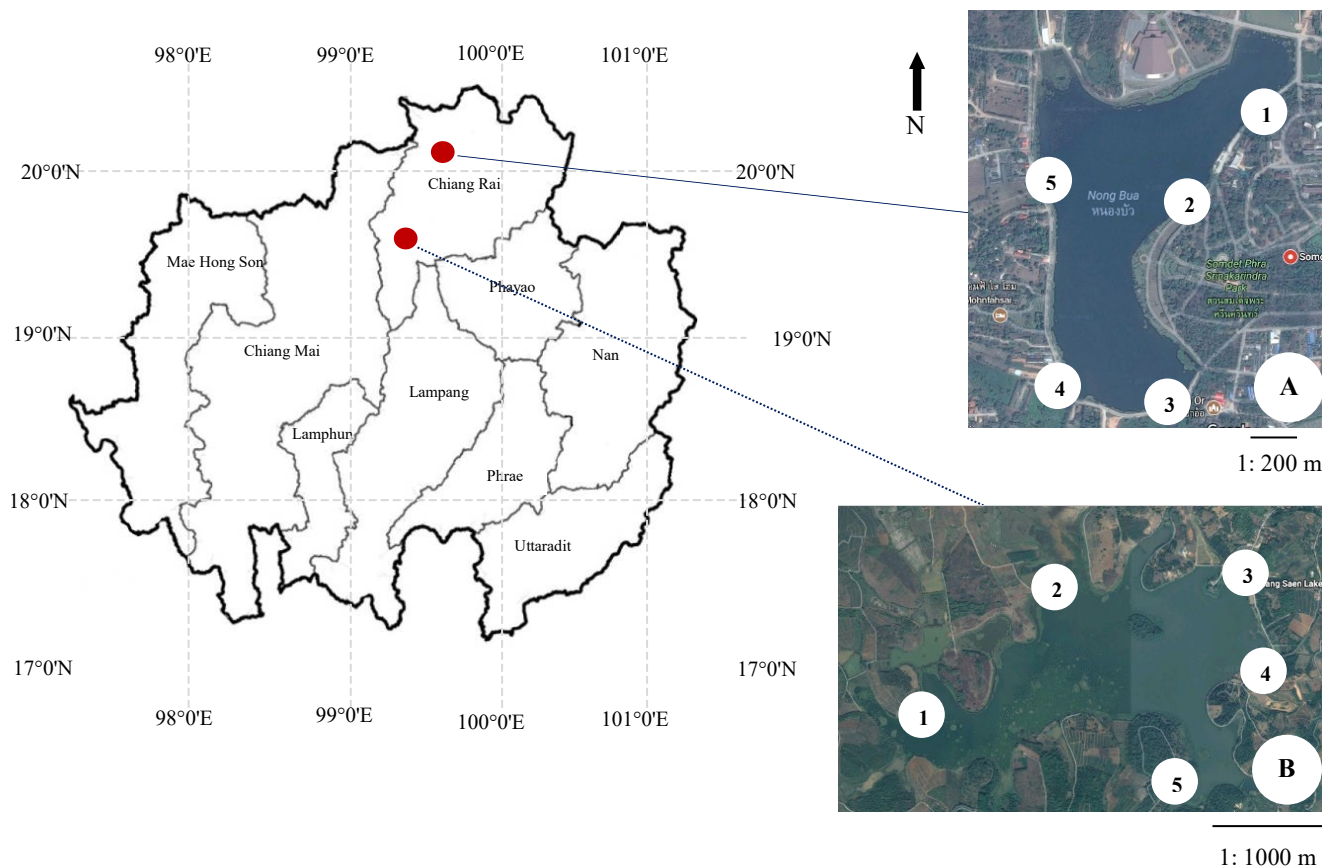
Microalgae and water samples were collected from two sampling sites that were located in the northern part of Thailand: 1. Nong Bua Reservoir (19°58'44.3"N 99°50'38.7"E) located in Chiang Rai Rajabhat University,

Mueang District, Chiang Rai Province and 2. Chiang Saen Lake (Nong Bong Kai; 20°15'43.3"N 100°02'49.8"E) located in Chiang Saen District, Chiang Rai Province. Water samples were collected from five stations at each sampling site and were chosen based on the community activities and agricultural areas that cover the surrounding areas (Figure 1) during May, July and October 2015 (samples were collected once per month).

### Procedures

*Water quality of physicochemical properties and distribution of microalgae* □

**Determination of physicochemical properties of water.** A determination of the relevant physicochemical properties of the water in each reservoir was done at each sampling site. The depth to which sunlight could penetrate the water body was measured with a Secchi disc. The temperature was measured with a thermometer, while pH and conductivity were measured with the use of a multiparameter (Eutech CyberScan CD 650). Dissolved oxygen (DO) was measured using the azide modification method (Eaton et al. 2005). Water samples were then collected at a depth of 30 centimeters from the surface of each water resource using polyethylene bottles, which were then kept in a cool box (5-7 °C) for later analysis in the laboratory.



**Figure 1.** Map of northern Thailand showing 2 sampling sites (A) Nong Bua Reservoir and (B) Chiang Saen Lake

Some physical and chemical properties of the water in the reservoirs were measured in the laboratory as follows. Total alkalinity was measured using the phenolphthalein methyl orange indicator method (Eaton et al. 2005). Biochemical oxygen demand (BOD) was measured using the azide modification method (Eaton et al. 2005). Water turbidity was measured using a turbidity meter. Nutrient content, especially with regard to ammonium nitrogen, nitrate nitrogen and soluble reactive phosphorus (SRP), were determined using the nesslerization method, cadmium reduction method and ascorbic method, respectively (Eaton et al. 2005). Chlorophyll content was determined by employing the method developed by Saijo (1975) and Winterman and de Mots (1965).

The trophic status of the water was determined by evaluation of the main parameters which included: conductivity, DO, BOD, ammonium nitrogen ( $\text{NH}_4^+$ ), nitrate nitrogen ( $\text{NO}_3^-$ ), soluble reactive phosphorus ( $\text{PO}_4^{3-}$ ) and chlorophyll *a* according to the method of Peerapornpisal et al. (2004). This method was based on those of Wetzel (2001) and Lorraine and Vollenweider (1981).

**Collection of microalgae.** Twenty liters of water samples from each station at the two sampling sites were collected and filtered with a 10  $\mu\text{m}$  pore size plankton net. The 100 mL of remaining water in the plankton net collected from each station were pooled together and refiltered to obtain 100 mL. The samples were then divided into two equal portions. One portion was preserved by adding 0.7 mL of Lugol's solution to 100 mL of the sample (Eaton *et al.*, 2005). The other portion was kept at a natural temperature for morphological observation, identification, and isolation of microalgae in order to determine lipid content in the laboratory (Wetzel 2001).

**Identification of microalgae.** The microalgae were observed, and the details of the cells were identified under a 40X and 100X light microscope. The identification of microalgae was based on a number of relevant characteristics such as the color of the cells, the size of the cell or colony or filament, the shape of the chloroplast, the number and position of the flagella, with or without the spine and details of the granular characteristics of the cell wall which were clearly visible with a light microscope (John and Brook, 2002). Each microalga was measured in terms of the size of the cell or colony or filament, and the details of the cells were observed and photographed using an Olympus Normaski microscope. Species identification was conducted according to Prescott (1970); Huber-Pestalozzi (1983); Hindák (1990); Komarek and Jankovska (2001) and John et al. (2011). For detailed identification of the genera and species, special publications on tropical environments were used Lewmanomont (1995); Yamagishi and Kanetsuna (1987); Hirano (1975, 1992).

#### *Isolation and lipid extraction of microalgae*

**Isolation of microalgae.** Microalgae in the water samples were examined under a microscope, and single cells or colonies were isolated with a glass micropipette using the micropipet washing technique (Daniel, 2012). Single cells or colonies were washed with sterile

Jaworski's medium (JM) at least five times. Each cell or colony was cultivated in 5 mL of sterile JM medium at 25 °C for at least 7 days. They were purified using the streak method on JM agar plate and incubated at room temperature until growth appeared to initiate. Sub-culturing was carried out until a monoculture was obtained and transferred to JM broth as the stock culture.

The monoculture of microalgae was inoculated in 250 mL Erlenmeyer flasks containing 150 mL JM medium at room temperature under  $10.8 \mu\text{mol.m}^{-2}.\text{s}^{-1}$  with a 16: 8 h photoperiod for 10 days. It was then transferred to 500 mL Erlenmeyer flasks containing 300 mL of JM medium, and the seed cultures were scaled up to 20 L of JM medium. Ten percent (V/V) of each seed culture was inoculated in 20 L of JM medium and was then cultivated at room temperature under  $10.8 \mu\text{mol.m}^{-2}.\text{s}^{-1}$  with a 16: 8 h photoperiod and aerated using a bubbling air-line in a plastic carboy tank (Daniel, 2012). When growth reached a stationary phase (about 14 days), the cells were harvested by sedimentation, and the supernatant was siphoned off and then centrifuged at 3,000 rpm for five minutes. The cells were dried at 60°C in an oven for 48 hr and ground thoroughly with a mortar and pestle for the purposes of lipid extraction (Stemmler et al. 2016).

**Lipid extraction.** The lipids obtained from the microalgae was extracted using a procedure modified from the method of Bligh and Dyer (1959). Each microalgal cell powder sample of 0.5 g was put in a 50 mL centrifuge tube and 15 mL of a solution of chloroform: methanol (2: 1, v/v) was added. The samples were then mixed well on a vortex mixer. Each sample was then placed in an ultrasonic bath at 40 kHz, 40°C for 1 hr. The solvent layer was separated by centrifugation at 6000 rpm for 10 min, and the samples were evaporated to dryness in a fume hood until constant weight was obtained. The lipid content was determined gravimetrically. This research was conducted according to Mubarak et al. 2015, who reported that a mixture of chloroform/methanol was used as a solvent for increased extraction yields of lipids from microalgae than those that were extracted using other solvents. The ultrasonic extraction assisted the thick cell walls of the microalgae blocks in the release of intra-lipids that were present in Neto et al. (2013). □

## RESULTS AND DISCUSSION

### **Water quality and physicochemical properties** □

The results of the analysis of the physicochemical parameters of the Nong Bua Reservoir and Chiang Saen Lake revealed certain differences. The values of the parameters were found to be higher in the Nong Bua Reservoir. The trophic status of the water was evaluated using the main parameters, which included conductivity, DO, BOD, ammonium nitrogen ( $\text{NH}_4^+$ ), nitrate nitrogen ( $\text{NO}_3^-$ ), soluble reactive phosphorus ( $\text{PO}_4^{3-}$ ) and chlorophyll *a*. These parameters were assessed according to the method of Peerapornpisal et al. (2004), and it was determined that Nong Bua Reservoir was meso-eutrophic in status and Chiang Saen Lake was mesotrophic in status (Table 1).

**Table 1.** Certain physical and chemical parameters of water quality (average) in Nong Bua Reservoir and Chiang Saen Lake, northern Thailand

Parameters	Nong Bua Reservoir	Chiang Saen Lake
Water temperature (°C)	30.00 ± 0.71	30.80 ± 0.76
Air temperature (°C)	32.00 ± 0.71	31.20 ± 1.44
Secchi dept (m)	0.94 ± 0.03	1.44 ± 0.10
Turbidity (NTU)	8.07 ± 1.70	5.26 ± 2.25
pH	7.42 ± 0.11	6.64 ± 0.13
Alkalinity (mg/L as CaCO <sub>3</sub> )	149.00 ± 2.65	138.00 ± 2.59
Conductivity (μS/cm)*	94.80 ± 2.39	82.60 ± 2.39
Dissolved oxygen (DO) (mg/L)*	7.10 ± 0.79	6.30 ± 0.77
Biochemical oxygen demand (BOD) (mg/L)*	3.20 ± 1.43	1.50 ± 0.69
Nitrate nitrogen (mg/L)*	1.12 ± 0.33	0.74 ± 0.29
Ammonium nitrogen (mg/L)*	0.36 ± 0.05	0.20 ± 0.04
Soluble reactive phosphorus (mg/L)*	0.52 ± 0.28	0.31 ± 0.27
Chlorophyll <i>a</i> (μg/L)*	46.00 ± 0.23	18.00 ± 0.30
AARL-PC Score	4.00	3.20
Trophic status	Mesotrophic-eutrophic	Mesotrophic

Note: \* = Parameters used to evaluate water quality

#### Distribution of microalgae in Nong Bua Reservoir and Chiang Saen Lake

Seven divisions, 90 species of algae were found in Nong Bua Reservoir. The most diverse division was Chlorophyta (39 species) followed by Euglenophyta (17 species), Bacillariophyta (14 species), Cyanophyta (13 species), Chrysophyta (four species), Pyrrophyta (two species) and Cryptophyta (one species), respectively. Six

divisions, 55 species of algae were found in Chiang Saen Lake. The most diverse division was Chlorophyta (25 species) followed by Bacillariophyta (12 species), Cyanophyta (seven species), Euglenophyta (five species), Chrysophyta (two species) and Pyrrophyta (two species), respectively (Table 2).

The dominant species of microalgae present in the two reservoirs were found to be different. Nong Bua Reservoir was home to *Botryococcus braunii* Kützinger, *Lepocinclis oxyuris* (Schmarda) Marin & Melkonian and *Pediastrum duplex* var. *duplex* Meyen, while *Kirchneriella lunaris* (Kirchner) Möbius, *Peridinium* sp. and *Staurastrum* sp.1 were the dominant species of microalgae found in Chiang Saen Lake (Figure 1).

#### Isolated of microalgae and lipid contents

Ninety species and 55 species of microalgae were isolated from Nong Bua Reservoir and Chiang Saen Lake, respectively. However, only 25 isolates of microalgae were isolated from Nong Bua Reservoir, and 6 isolates were isolated from Chiang Saen Lake. Most of the isolates (26 isolation) were green microalgae followed by blue-green algae (5 isolation), but the microalgae in another group were not able to grow in JM medium. The lipid content was highest in *Botryococcus braunii* ( $39.25 \pm 0.32\%$  dry weight) followed by *Ankistrodesmus* (NB) ( $26.80 \pm 0.44\%$  dry weight) and *Coelastrum microsporum* ( $24.95 \pm 0.55\%$  dry weight), and the lowest lipid content was found in *Planktolyngbya* sp. ( $7.25 \pm 0.43\%$  dry weight). Isolates collected from the two reservoirs revealed differing lipid contents (Table 3).



**Figure 3.** A-C. Dominant species of microalgae in Nong Bua Reservoir, northern Thailand: A. *Botryococcus braunii* Kützinger, B. *Lepocinclis oxyuris* (Schmarda) Marin & Melkonian and C. *Pediastrum duplex* var. *duplex* Meyen. D-F. Dominant species of microalgae in Chiang Saen Lake, northern Thailand: D. *Kirchneriella lunaris* (Kirchner) Möbius, E. *Peridinium* sp. and F. *Staurastrum* sp.1 (scale bar = 10 μm)

**Table 2.** List of microalgae found in Nong Bua Reservoir and Chiang Saen Lake, northern Thailand

Species	I	II
<b>Division Cyanophyta</b>		
<i>Anabaena</i> sp.	+	+
<i>Aphanocapsa</i> sp.	+	+
<i>Chroococcus</i> sp.	+	+
<i>Cylindrospermopsis raciborskii</i> (Woloszynska) Seenayya & Subba Raju	+	+
<i>Coelomorion pusillum</i> (Van Goor) Komárek	+	+
<i>Merismopedia punctata</i> Komárek	+	-
<i>Microcystis aeruginosa</i> Kützing	+	-
<i>Microcystis wesenbergii</i> (Komárek) Komárek ex Komárek	+	-
<i>Oscillatoria</i> sp.	+	-
<i>Planktolyngbya</i> sp.	+	+
<i>Pseudanabaena mucicola</i> Naumann&Huber-Pestalozzi	+	-
<i>Pseudanabaena</i> sp.	+	+
<i>Spirulina</i> sp.	+	-
<b>Division Chlorophyta</b>		
<i>Actinastrum hantzschii</i> Lagerh	+	+
<i>Acutodesmus acuminatus</i> (Lagerheim) Chodat	+	+
<i>Acutodesmus javaensis</i> Chodat	+	-
<i>Ankistrodesmus</i> sp.	+	-
<i>Botryococcus braunii</i> Kützing	+	+
<i>Closteriopsis</i> sp.	+	+
<i>Closterium parvulum</i> Nägeli	+	-
<i>Coelastrum microsporum</i> Nägeli	+	-
<i>Coelastrum reticulatum</i> var. <i>cubanum</i> Komárková	+	-
<i>Cosmarium moniliforme</i> (Turpin) Ralfs	+	+
<i>Cosmarium punctulatum</i> Brébisson	+	-
<i>Cosmarium subtumidum</i> Nordstedt	+	+
<i>Crucigeniella crucifera</i> (Wolle) Komárek	+	+
<i>Desmodesmus opoliensis</i> (P.G.Richter) E.H.Hegewald	+	+
<i>Dictyosphaerium granulatum</i> Hindák	+	+
<i>Dictyosphaerium tetrachotomum</i> Printz	+	+
<i>Euastrum turneri</i> W. West	+	+
<i>Golenkinia</i> sp.	+	+
<i>Groenbladia</i> sp.	+	-
<i>Kirchneriella lunaris</i> (Kirchner) Möbius	+	+
<i>Micrasterias foliacea</i> Bailey	+	-
<i>Monoraphidium tortile</i> (West et G.S.West) Komárková-Legnerová	+	+
<i>Nephrocystium</i> sp.	-	+
<i>Oocystis</i> sp.	+	+
<i>Pediastrum duplex</i> var. <i>duplex</i> Meyen	+	+
<i>Pediastrum duplex</i> var. <i>gracillimum</i> West & G.S.West	+	+
<i>Pediastrum duplex</i> var. <i>punctatum</i> (Willi Krieger) Parra	+	-
<i>Pediastrum simplex</i> var. <i>simplex</i> Meyen	+	-
<i>Pediastrum tetras</i> (Ehrenberg) Ralfs	+	-
<i>Radiococcus</i> sp.	+	+
<i>Scenedesmus</i> sp.	+	+
<i>Stauroastrum</i> cf. <i>longbrachiatum</i> (Borge) Gutwinski	+	+
<i>Stauroastrum smithii</i> Teiling	+	+
<i>Stauroastrum</i> sp.1	+	+
<i>Stauroastrum</i> sp.2	+	+
<i>Stauroastrum</i> sp.3	+	-
<i>Staurodesmus convergens</i> var. <i>labportei</i> Teiling	+	+
<i>Staurodesmus</i> sp.1	+	-
<i>Staurodesmus</i> sp.2	+	-
<i>Staurodesmus</i> sp.3	+	-
<b>Division Euglenophyta</b>		
<i>Euglena spirogyra</i> var. <i>abrupte-acuminata</i> Lemmermann	+	-
<i>Euglena</i> sp.	+	-
<i>Lepocinclis ovum</i> (Ehrenberg) Lemmermann	+	+
<i>Lepocinclis ovum</i> var. <i>gracilicauda</i> Deflandre	+	+
<i>Lepocinclis oxyuris</i> (Schmarda) Marin & Melkonian	+	-
<i>Lepocinclis playfairiana</i> Deflandre	+	-
<i>Lepocinclis truncata</i> A.M.Cunha	+	-
<i>Lepocinclis</i> sp.	+	-
<i>Phacus lismorensis</i> Playf.	+	-
<i>Phacus longicauda</i> (Ehrenberg) Dujardin	+	-
<i>Phacus ranula</i> Pochmann	+	+
<i>Trachelomonas armata</i> var. <i>steinii</i> Lemmermann	+	+
<i>Trachelomonas hispida</i> (Perty) Stein	+	-
<i>Trachelomonas volvocina</i> var. <i>subglobosa</i> Lemmermann	+	-
<i>Trachelomonas volvocinopsis</i> Swirenko	+	+
<i>Trachelomonas</i> sp.1	+	-
<i>Trachelomonas</i> sp.2	+	-
<b>Division Chrysophyta</b>		
<i>Dinobryon sertularia</i> Ehrenberg	+	+
<i>Isthmochloron gracile</i> Chodat	+	-
<i>Centritractus belanophorus</i> Lemmermann	+	+
<i>Mallomonas</i> sp.	+	-
<b>Division Bacillariophyta</b>		
<i>Achnanthes</i> sp.	+	+
<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen	+	+
<i>Cyclotella meneghiniana</i> Kützing	+	+
<i>Cymbella</i> sp.	+	+
<i>Encyonema</i> sp.	+	+
<i>Fragilaria</i> sp.	+	+
<i>Frustulia</i> sp.	+	+
<i>Gomphonema</i> sp.	+	+
<i>Gyrosigma scalproides</i> Rabenhorst	+	+
<i>Meloseira varians</i> Agardh	+	+
<i>Navicula</i> sp.	+	+
<i>Nitzschia</i> sp.	+	-
<i>Pinnularia</i> sp.	+	+
<i>Synedra ulna</i> (Nitzsch) Ehrenberg	+	-
<b>Division Cryptophyta</b>		
<i>Cryptomonas</i> sp.	+	-
<b>Division Pyrrophyta</b>		
<i>Peridinium inconspicuum</i> Lemmermann	+	+
<i>Ceratium</i> sp.	+	+
Note: I = Nong Bua Reservoir, II = Chiang Saen Lake, + = detected, - = not detected		
<b>Discussion</b>		
<i>Water quality and physicochemical properties</i> □		
The water quality of the two water resources was found to be different. Chiang Saen Lake revealed a lower value in terms of the physicochemical properties than Nong Bua Reservoir. Most of the surrounding area of Chiang Saen Lake was made up of forests and agricultural areas. This area was selected for the implementation of the Ramsar Convention Management and Protection of Wetland Areas project and consequently was subject to fewer human activities. The trophic status of Chiang Saen Lake was classified as mesotrophic. Nong Bua Reservoir was classified as meso-eutrophic status because the area was surrounded by communities, restaurants, fish ponds and was home to various types of fishing activities. The influence of these activities resulted in higher BOD and nutrient levels (ammonium nitrogen, nitrate nitrogen,		



**Table 3.** Isolates of microalgae and their lipid contents

Isolation of microalgae	Lipids content (% dry weight)	
	Nong Bua Reservoir	Chiang Saen Lake
<i>Aphanocapsa</i>	10.02 ± 0.36	-
<i>Chroococcus</i>	15.07 ± 0.22	-
<i>Cylindrospermopsis raciborskii</i>	8.24 ± 0.09	-
<i>Coelomon pusillum</i>	10.73 ± 0.25	-
<i>Planktolyngbya</i>	7.25 ± 0.43	-
<i>Acutodesmus acuminatus</i>	10.27 ± 0.05	-
<i>Ankistrodesmus</i>	26.80 ± 0.44	24.48 ± 0.25
<i>Botryococcus braunii</i>	39.25 ± 0.32	-
<i>Chlorella</i>	14.92 ± 0.56	-
<i>Closteriopsis</i>	13.20 ± 0.24	-
<i>Closterium parvulum</i>	21.28 ± 0.05	-
<i>Cosmarium punctulatum</i>	17.25 ± 0.45	13.33 ± 0.67
<i>Coelastrum microsporum</i>	24.95 ± 0.55	-
<i>Desmodesmus opoliensis</i>	21.56 ± 0.30	-
<i>Dictyosphaerium granulatum</i>	17.36 ± 0.22	12.89 ± 0.34
<i>Euastrum turneri</i>	17.68 ± 0.10	13.08 ± 0.54
<i>Kirchneriella lunaris</i>	24.30 ± 0.34	-
<i>Micrasterias foliacea</i>	10.45 ± 0.07	-
<i>Pediastrum duplex</i>	14.50 ± 0.20	-
<i>Pediastrum simplex</i>	13.25 ± 0.65	-
<i>Pediastrum tetras</i>	10.46 ± 0.44	-
<i>Radiococcus</i>	16.96 ± 0.87	-
<i>Scenedesmus</i>	23.00 ± 0.47	-
<i>Staurastrum</i>	8.20 ± 0.33	5.20 ± 0.39
<i>Staurodesmus</i>	10.45 ± 0.50	7.42 ± 0.87

Note: - = not isolated, = microalgae isolated from Nong Bua Reservoir

soluble reactive phosphorus) being present in Nong Bua Reservoir than in Chiang Saen Lake, where nitrates and phosphates present in the water body contributed to higher BOD levels. Nitrates and soluble reactive phosphorus contamination resulted from the degradation of animal and plant carcasses. Additionally, agricultural fertilizers and wastewater from the community that were discharged into the lake caused plant life and algae to grow quickly. However, when plants and algae grow quickly, they also die quickly. This can contribute to the amount of organic waste present in the water, which would then decompose resulting in high BOD levels (Wetzel 2001). The value of BOD was recorded in a range of 2.5 -5.0 mg/L indicating  $\beta$ -mesosaprobic (Eaton et al. 2005).

#### *Distribution of microalgae in Nong Bua Reservoir and Chiang Saen Lake*

The number of species of microalgal found in Nong Bua Reservoir was found to be greater than in Chiang Saen Lake. A wide variety of algae was found to possess differing physical and chemical requirements (John 2011). Each species responded to different sets of favorable conditions that could promote growth and reproduction. The two most important nutrients, nitrogen (N) and phosphorus (P), were present in the soluble form. Phosphorus and nitrogen present in natural waters are usually found in the form of phosphorus ( $\text{PO}_4^{3-}$ ) and nitrate ( $\text{NO}_3^-$ ), and are present in a soluble form that plants and algae can use for growth. In freshwater lakes and rivers,

phosphorus is often found to be the growth-limiting nutrient because it occurs in a form that is least relative to the needs of plants and algae. If excessive amounts of phosphorus and nitrogen are added to the water, algae and aquatic plants can be produced in large quantities (Wetzel 2001). The dominant species of microalgae in Nong Bua Reservoir were used to assess the water quality as being of meso-eutrophic status. These species were *Pediastrum duplex* var. *duplex* Meyen (Prasertsin et al. 2015), while in the Nong Bua Reservoir, the euglenoid group were found to be present in higher numbers than in Chiang Saen Lake. The euglenoid group was indicative of water quality in the eutrophic status (Wetzel 2001 and Peerapornpisal et al. 2007).

#### *Isolated of microalgae and lipid contents*

A total of 25 species and 6 isolates of microalgal were isolated from Nong Bua Reservoir and Chiang Saen Lake, respectively. The conditions of the Nong Bua Reservoir were suitable and similar to the conditions of the media used for the isolation and cultivation of algae, which was in agreement with the findings of Rosenberg et al. (2008). It was reported that suitable or similar conditions were responsible for the fast adaptation and growth of the algae. Jaworski's medium (JM) contains many macronutrients and micronutrients which are essential for algal growth (Mcelroy and Nason 1954). In this study, the highest isolates were green microalgae (26 isolates) followed by blue-green algae (5 isolates). The elements required for the growth of green algae are N, P, K, Ca, Cu, S, Fe, Mg, Mn, and Zn. These elements are typically added in the form of salts (Oh-Hama and Miyachi 1988). All of these elements were detected by Jaworski's medium (JM). Additionally, Jaworski's medium could not be used to isolate euglenoid, dinoflagellate and diatom groups. These groups may require special elements such as silicon (Si) as a fundamental inorganic nutrient that is required for diatom growth (Jickells 1998). Notably, a source of silicon must be provided for continued good growth. The dinoflagellate requires yeast extract, thiamine, and proteose peptone for the highest possible level of growth (Daniel 2012).

Algae can be photosynthesised as a first step in the conversion of light energy to chemical energy and is ultimately responsible for supporting all biofuel synthetic processes that are used to convert solar energy into biomass, carbon storage products (carbohydrates and lipids) and hydrogen (Perrine et al. 2012). The synthetic processes of blue-green algae can convert solar energy into carbohydrates and a number of lipids. Green algae can double their biomass in less than 24 hours and can also yield high lipid contents, usually those of over 50%. The lipid content depends on the specific algal strain and their growth conditions with an average content ranging from 2 to 75% of dry weight (DW) under exceptional circumstances. However, these circumstances would typically be between 10 and 30% DW (Chisti 2007). Notably, the algae used in biodiesel production are usually aquatic unicellular green algae (Chlorophyceae) (Demirbas 2010). In this study, it was determined that the highest crude lipid content of *Botryococcus braunii* (39.25 ± 0.32%

dry weight) was more than that which had been previously reported by Ashokkumar et al. (2014) as  $23.5 \pm 0.40\%$  dry weight. Moreover, *Scenedesmus* ( $23.00 \pm 0.47\%$  dry weight) content was more than what had been previously reported by Shin et al. 2014 at  $14.5 \pm 0.50\%$  dry weight when using the Bligh and Dyer method. In this study, the extraction of lipids from microalgae biomass was done by using non-polar solvents such as chloroform, and polar solvents such as methanol (Bligh and Dyer 1959) that were combined with the ultrasonic extraction effect in achieving higher crude lipid contents.

The lipid composition of microalgae mainly consists of non-polar (neutral) and polar molecules. The polarity of a molecule is determined by the presence of fatty acids. The charge of neutral lipids occurs mainly in the form of triacylglycerols, which are used to determine their structure (Shahidi and Wanasundara 2002). A combination of the polar and non-polar solvents such as hexane, benzene, toluene, diethyl ether, chloroform, and polar solvents such as acetone, ethyl acetate, and ethanol and methanol, are recommended for higher lipid extraction efficiency from algal biomass. These are usually used to extract both polar lipids (from membranes) and neutral lipids (from lipid droplets that are highly desirable for biodiesel feedstock) (Dos Santos et al. 2015). Moreover, the ultrasonic extraction assists the thick cell walls of the microalgae in blocking the release of intra-lipids that are present inside (Neto et al. 2013). The principle behind the ultrasound-assisted extraction method involves the intense sonication of liquid that generates sound waves to be propagated into the liquid media resulting in alternate high-pressure and low-pressure cycles. During the high-pressure cycle, the small vacuum bubbles that are produced in a low-pressure cycle, collapse violently and result in a phenomenon called cavitation. The high pressure and high speed liquid jets then form shearing forces around the algae cells during cavitation and break the cell structure mechanically and improve material transfer in supporting the extraction of lipids (Yoon et al. 1998).

In conclusion, algae can be found in a wide variety of water qualities that possess different physical and chemical requirements. The lipid contents of the two water bodies in this study could be used in the production of biodiesel, which has been recognized as an efficient alternative source of fuel.

## ACKNOWLEDGEMENTS

The authors would like to thank the Biology Program, Faculty of Science and Technology and the Research and Development Institute, Chiang Rai Rajabhat University, Thailand for providing financial support.

## REFERENCES

- Ashokkumar V, Rengasamy R, Deepalakshmi S, Sivalingam A, Sivakumar P. 2014. Mass cultivation of microalgae and extraction of total hydrocarbons: a kinetic and thermodynamic study. *Fuel* 119: 308-312.
- Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. *Can J Biochem Phys* 37: 911-917.
- Brennan L, Owende P. 2010. Biofuels from microalgae a review of technologies for production, processing, and extractions of biofuels and co-products. *Renew Sust Energ Rev* 14: 557-577.
- Brettum P, Andersen T. 2005. The use of phytoplankton as indicators of water quality. The Research Council of Norway, Norway.
- Chisti Y. 2007. Bio-oil from microalgae. *Biotechnol Adv* 25: 294-306.
- Chisti Y. 2008. Biodiesel from microalgae beats bioethanol. *Trends Biotechnol* 26: 126-131.
- Daniel E. James. 2012. *Culturing Algae*. Carolina biological supply company printed, USA.
- Demirbas A. 2010. Use of algae as biofuel sources. *Energy Convers Manage* 5: 2738-2749.
- DOS Santos RR, Moreira DM, Kunigami CN, Gomes Aranda DA, Luz Lapa Teixeira CM. 2015. Comparison between several methods of total lipid extraction from *Chlorella vulgaris* biomass. *Ultrason Sonochem* 22: 95-99.
- Eaton AD, Clesceri IS, Rice EW, Greenberg AE. 2005. *Standard Method for Examination of Water and Wastewater*. 21<sup>st</sup> ed. Virginia: American Public Health Association (APHA), Washington DC.
- Hindák F. 1990. Studies on the chlorococcal algae (Chlorophyceae). V. Slovak Academy of Science, Veda, Slovakia. □
- Hirano M. 1992. Desmids from Thailand and Malaysia. *Contr Boil Lab Kyoto Univ* 28: 1-98.
- Hirano M. 1975. Phytoplankton from Lake Boraphet in the Central Plain of Thailand. *Centr Biol Lab Kyoto Univ* 24 (4): 187-203.
- Huber-Pestalozzi G. 1983. *Das Phytoplankton des Subwassers: Chlorophyceae (Grünglgen) Ordnung Chlorococcales*, 7. Teil. 1. Hälfte, E. Schweizerbart, sche Verlags Buch Handlung, Stuttgart.
- Jickells TD. 1998. Nutrient biogeochemistry of the coastal zone. *Science* 10: 217-221.
- John DM, Whitton BA, Brook AJ. 2011. *The Freshwater Algal Flora of the British Isles*. Cambridge University Press, Cambridge.
- Jungmin Kim GY, Lee H, Lim J, Kim K, Kim CW, Park MS, Yang JW. 2013. Methods of downstream processing for the production of biodiesel from microalgae. *Biotechnol Adv* 31 (6): 862-876.
- Komarek J, Jankovska V. 2001. Review of the Green Algal Genus *Pediastrum*; Implication for Pollen-Analytica Research. *Bibliotheca Phycologica*. Band 108, Berlin.
- Lewmanomont K, Wongrat L, Supanwanid C. 1995. *Algae in Thailand*. Bangkok: Integrated Promotion Technology, Bangkok.
- Lorraine LJ, Vollenweider RA. 1981. Summary Report, the OECD Cooperative Programme on Eutrophication. National Water Research Institute, Burlington.
- Malcata FX. 2011. Microalgae and biofuels: a promising partnership. *Trends Biotechnol* 29: 542-549.
- Mcelroy WD, Nason A. 1954. Mechanism of action of micronutrient elements in enzyme systems. *Annu Rev Plant Physiol* 5: 1-30.
- Miao X, Wu Q. 2006. Biodiesel production from heterotrophic microalgal oil. *Bioresour Technol* 97: 841-846.
- Mubarak M, Shaija A, Suchithra TV. 2015. A review on the extraction of lipid from microalgae for biodiesel production. *Algal Res* 7: 117-123.
- Neto AMP, de Souza RAS, Leon-Nino AD, de Costa JDA, Tiburcio RS, Nunes TA, Mello TCS, Kanemoto FT, Saklanha-Correa FMP, Giancesella SMF. 2013. Improvement in microalgae lipid extraction using a sonication-assisted method. *Renew Energy* 55: 525-531.
- Oh-Hama T, Miyachi S. 1988. *Chlorella*. In: Borowitzka MA, Borowitzka LJ (eds.) *Microalgal Biotechnology*. Cambridge University Press, Cambridge. □
- Peerapornpisal Y, Chaiubol C, Pekkoh J, Kraibut H, Chorum M, Wannathong P, Ngernpat N, Jusakul K, Thammathiwat A, Chuanunta J, Inthasotti T. 2004. Monitoring of water quality in Ang Kaew Reservoir of Chiang Mai University using phytoplankton as bioindicator from 1995-2002. *Chiang Mai J Sci* 31 (1): 85-94. □
- Peerapornpisal Y, Pekkoh J, Powangprasit D, Tonkamdee T, Hongsirichat A, Kunpradit T. 2007. Assessment of water quality in standing water by using dominant phytoplankton (AARL-PP Score). *J Fish Tech Res* 1: 71-81.
- Perrine Z, Negi S, Sayre RT. 2012. Optimization of photosynthetic light energy utilization by microalgae. *Algal Res* 1 (2): 134-142.
- Prasertsin T, Peerapornpisal Y. 2015. Diversity of phytoplankton and water quality in some freshwater resources in Thailand. *Intl J Applied Environ Sci* 10 (4): 1101-1123.
- Prescott GW. 1970. *How to Know Fresh Water Algae*, The Picture Key Nature Series W.M.C. Brown Company Publishers, Dubuque, Iowa. □

- Reynolds C, Huszar V, Kruk C, Naselli-Flores L, Melo S. 2002. Towards a Functional Classification of the Freshwater Phytoplankton. *J Plankton Res* 24: 417-428.
- Rosenberg J, Oyler G, Wilkinson L, Betenbaugh M. 2008. A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution. *Biotech* 19: 430-436.
- Saijo Y. 1975. A method for determination of chlorophyll. *Jap J Limnol* 36: 103-109.
- Shahidi F, Wanasundara U. 2002. Method for measuring oxidative rancidity in fats and oils. *Food Sci. Technol* 4650-4688.
- Stemmler K, Massimi R, Kirkwood AE. 2016. Growth and fatty acid characterization of microalgae isolated from municipal waste-treatment systems and the potential role of algal-associated bacteria in feedstock production. *Peer J* 4: 1-14.
- Wetzel RE. 2001. *Limnology*. Academic Press, London.
- Wintermans JF, de Mots A. 1965. Spectrophotometric characteristics of chlorophyll a and b and their pheophytins in ethanol. *Biochim Biophys Acta* 109: 448-453.
- Yamagishi T, Kanetsuna Y. 1987. The planktonic Chlorophyceae from Lake Boraphet (The Central Plain, Thailand). *Gen Educ Rev Coll Agr Vet Med, Nihon Univ* 23: 19-38.
- Yoon BJ, Changkook R, Jong-Ki J, Junhong P, Dong J, Young-Woong S. 2011. The characteristics of bio-oil produced from the pyrolysis of three marine macroalgae. *Bioresour Technol* 102: 3512-3520.