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CHECKLIST OF MICROALGAE COLLECTED FROM DIFFERENT HABITATS IN PENINSULAR MALAYSIA FOR SELECTION OF ALGAL BIOFUEL FEED-STOCKS

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ABSTRACT A survey of 30 collections sites in Peninsular Malaysia, representing five types of habitats, namely, hot springs, eutrophicated freshwater lake, palm oil mill effluent ponds, brackish and marine water habitats, yielded 79 environmental samples. The total number of algal taxa and genera identified were 73 and 52 respectively; with 9 genera and 11 taxa of Cyanophyta; 25 genera and 33 taxa of Bacillariophyta; 13 genera and 16 taxa of Chlorophyta and 5 genera and 13 taxa of Euglenophyta. In terms of cell numbers, the samples from the oil palm mills had the highest cell density. Of the isolates obtained from the samples collected, only six genera, namely the Cyanophyte *Cyanosarcina*, the Chlorophytes *Chlorella*, *Chlamydomonas, Chlorococcum, Scenedesmus* and the Euglenophyte *Euglena*, are discussed in terms of their growth and biochemical profiles in this paper. *Chlorococcum* and *Euglena* had the highest specific growth rate, μ, followed by *Chlorella* and *Scenedesmus*. Biomass productivity at day 12 was generally higher than that at day 8 for all genera. Highest biomass productivity was from *Chlorella* followed by *Chlamydomonas*. Highest lipid productivity was from *Chlorella* and *Chlamydomonas* on day 12. *Chlorella* had the highest carbohydrate productivity followed by *Chlamydomonas*. *Chlorella* and *Chlamydomonas* had the highest protein productivity. In this short survey, some interesting algae were identified and isolated, which proved to have potential for use as feedstocks for biofuel production.

ABSTRAK *Satu tinjauan keatas 30 tapak pesampelan di Semenanjung Malaysia, mewakili lima jenis habitat,iaitu kolam air panas, tasik air tawar yang mengalami eutrofikasi, kolam kumbahan kilang kelapa sawit, habitat air payau dan habitat air masin, menghasilkan 79 sampel persekitaran. Jumlah keseluruhan 73 taxa dan 52 genera alga dikenalpasti, dengan 9 genera dan 11 taxa Cyanophyta; 25 genera dan 33 taxa Baccilariophyta; 13 genera dan 16 taxa Chlorophyta dan 5 genera dan 13 taxa Euglenophyta. Dari segi bilangan sel, sampel daripada kilang kelapa sawit adalah paling tinggi bilangan selnya. Daripada sampel dipencilkan yang diperolehi daripada sampel yang dikumpulkan, hanya enam genera, iaitu Cyanophyta* Cyanosarcina*, Chlorophyta* Chlorella*,* Chlamydomonas*,* Chlorococcum*,* Scenedesmus *dan Euglenophyta* Euglena *akan dibincangkan dari segi pertumbuhannya dan profil biokimia didalam kertas kerja ini.* Chlorococcum *dan* Euglena *mempunyai kadar pertumbuhan spesifik, μ, paling tinggi diikuti oleh* Chlorella *dan* Scenedesmus*. Produktiviti biojisim pada hari ke-12 secara amnya adalah lebih tinggi daripada hari ke-8 untuk semua genera. Produktiviti biojisim paling tinggi adalah daripada* Chlorella *diikuti oleh* Chlamydomonas*. Produktiviti lemak paling tinggi adalah daripada* Chlorella *pada hari ke-12.* Chlorella *mempunyai produktiviti karbohidrat paling tinggi diikuti* Chlamydomonas. Chlorella *dan* Chlamydomonas *mempunyai produktiviti protein paling tinggi. Dalam tinjauan pendek ini, terdapat alga tertentu yang telah dikenalpasti dan dipencilkan yang dapat membuktikn ia mempunyai potensi untuk digunakan sebagai bahan asas utama untuk penghasillan bahan api biologi.*

(**Keywords**:microalgae, checklist, specific growth rate, carbohydrate, protein, lipid productivity)

INTRODUCTION

Malaysian phycology was initiated in the 1930's with the discovery of the algal resources and their uses (Patrick 1936; Prowse 1957, 1958, 1960, 1962a, b, 1969). The economic uses of indigenous algae, primarily as food, were published till the 1970's (Zaneveld 1959; Burkill 1966; Johnson 1970). From 1970 till 1990, inventories, checklists of Malaysian algae, including ecological studies and applied phycology publications were produced. The advancement of gene technology encouraged the

development of algal systematics and phylogenetics (Hanagata et al. 1998; Krienitz et al. 2004; Wu et al. 2001; Luo et al. 2010; Vello et al. 2014). The diverse products and processes that can be derived from the algae, brought Algae Biotechnology to the forefront. There has been numerous publications on the use of algae for food, feed, medicine, fuel and energy, as well as for bioremediation (Phang and Ong 1988; Phang et al. 2000; Vairappan 2008; Chu et al. 2009; Mustafa et al. 2012; Lim et al. 2010). In spite of the increased interest in utilization of algal resources for the diverse applications, the algal flora, especially the freshwater algae, is relatively unknown. There have been monographs and checklists on the freshwater algae, namely the desmids (Williamson 1998), diatoms (Wah et al. 1987; 1992), flagellates (Prowse 1958; 1960; 1962a), freshwater red algae (Kumano 1978; Kumano and Ratnasabapathy 1982; Kumano and Phang 1987; 1990), *Trentepohlia* (Salleh and Milow 1999) and blue-green algae (Johnson 1970). Algal flora of the Taiping Lakes (Prowse and Ratnasabapathy 1970), Gunong Jerai (Ratnasabapathy 1972), Tioman Island (Ratnasabapathy 1977; Ratnasabapathy and Kumano 1982), Tasik Bera (Ratnasabapathy et al. 1982), Ulu Endau area (Phang and Leong 1987), Maliau River, Sabah (Anton et al. 1998) and the Kenyir Lake (Abdur Rouf et al. 2008, 2009, 2010) have been published.

Annotated guides to the freshwater and marine diatoms of Malaysia have been published (Shamsudin 1990; 1991). The most recent monograph gives an annotated list of 195 algal species collected from Carey Island, Selangor (Salleh and Tajuddin 2006). The survey of Carey Island consisted of eight sampling stations with salinity ranging from 2 to 30 ppt; pH (5.21 to 7.70); dissolved oxygen (5.92 to 7.23 mg/L); total phosphorus (0.05 to 1.96 mg/L); nitrate (0.1 to 7.4 mg/L); silicate (0.491 to 8.785 mg/L); while the algae biomass in terms of chlorophyll *a* (chl *a*) ranged from 0.006 to 0.926 mg/L. The 195 algal species identified comprised of 63 genera from six divisions as follows: Bacillariophyta (50 genera, 132 spp.); Chlorophyta (13 genera, 31 spp.); Chrysophyta (1 genus, 1 sp.); Cyanophyta (2 genera, 3 spp.); Euglenophyta (4 genera, 11 spp.); and Pyrrophyta 93 genera, 6 spp.). Two new records for Malaysia were reported, namely the diatom *Nitzschia clausii* and the green desmid *Cosmarium humile.* The University of Malaya Algae Culture Collection (UMACC) was established in 1999 (Phang and Chu 1999). The UMACC is a collection of algal strains isolated from the Malaysian environment and serves as a depository as well reference culture collection for teaching and research.

In recent years, there has been increased interest in searching for lipid rich microalgae as feedstocks for biofuel production (Chisti 2007; Harun et al. 2010; D'oca et al 2011; Doan et al 2012; Vello et al. 2014). A collaboration between the University of Malaya, Aerospace Malaysia Innovation Centre (AMIC) and AIRBUS Group, was initiated in 2013, with focus on selecting suitable tropical strains that are suitable for aviation bio-kerosene production in Malaysia. The selection of algal strains for potential use as feedstock for biofuel production is based on their ability to reproduce fast and have high lipid and carbohydrate productivities (Thi et al. 2011; Pribyl et al. 2012; Hempel et al. 2012). This paper contributes the checklist of microalgae collected and identified in a survey of various habitats, in the search for potential tropical algal strains for use as feedstocks for biodiesel production. Strain selection is an important variable in the development of indigenous algal resources, and the aim of the survey was to produce a list of local strains with high potential for biofuel production. Algal strains will be isolated for growth and biochemical profiling studies, and suitable strains will be further studied to optimize their biomass and lipid productivity.

MATERIALS AND METHODS

Sampling from diverse habitats (freshwater and marine), including polluted habitats (palm oil mill factory) was carried out. The samples were divided into two sub-samples; one fixed with 4% formalin for microscopic examination and the 2nd sub-sample was incubated on illuminated shelves designed for growing algal cultures, for isolation of dominant strains. The algal collections were characterized qualitatively in terms of species composition (genus level) and relative abundance, while the environmental profiles (water quality) were also determined.

1) Collection of Algae Samples

Planktonic and flagellate algae were collected and concentrated using a phytoplankton net (10 µm mesh size). A known volume of water sample was filtered through a phytoplankton net and the water sample containing the concentrated algae was stored in a screw-cap plastic bottle.

Attached algae growing on substrate such as rocks, and epiphytic algae, especially diatoms, growing on other macroalgae were also collected. The collected samples were transported to the laboratory for processing as soon as possible. It is important that the sample is observed as soon as possible because populations change rapidly. Before culturing the samples, they were examined by light microscopy to determine the identity and relative abundance of the species present. A sub-sample was preserved in 4% formalin for future reference, especially for identification purposes.

2) Algae Isolation

Several techniques can be used to isolate algae of single species from a mixture to obtain unialgal cultures. To obtain clonal cultures, the culture should be propagated from a single cell or single filament of a few cells. Water samples collected were subjected to manipulations before isolation was carried out. The following techniques of algae isolation was used in this study:

a) Selective enrichment media technique (Table 1)

The purpose of using selective enrichment media is to promote growth of particular species in order to get enough materials for isolation.

b) Techniques for isolation of algae

Several techniques were used, including the centrifuge-washing and streak-plating technique and the taxis technique (Phang and Chu 1999). The isolates were purified using antibiotic Kanamycin and Penicillin G) treatment as described in Phang and Chu (1999).

3) Microalgae Stock Culture Maintenance.

The purified microalgal cultures on agar slants (2% w/v) were maintained in sterile Bold's Basal Medium (BBM) for freshwater algae and Provasoli 50 (Prov50) Medium for marine algae. The slants were placed on illuminated shelves, incubated at 25+1 oC under 12:12 h light-dark cycle with irradiance of 40 – 60 µmol photon/s/m2. Sub-culturing was done every two months.

4) Algal Biomass Determination

a) Determination of chlorophyll a (chl a) and carotenoid content

The chl a content was determined using the spectrophotometric method (APHA, 1998). A known volume of algal culture collected on a filter paper (glass fiber cellulose, 0.45µm, GF/C-47mm) was mashed and mixed homogenously with 10 ml of 100% acetone in a screw-cap centrifuge tube. The tubes were kept overnight at 4±1°C in the dark before centrifugation (using Kubota 2100 centrifuge, Kubota Corporation, Japan) at 3000 rpm, for 10 minutes. The optical density of the supernatant at 630, 645 and 665nm (for chl *a*) and 452nm (for carotenoid) was measured using Spectrophotometer (Shimadzu UV-160A Spectrophotometer, Shimadzu Corporation, Japan). Acetone was used as the blank.

The chl *a* content was calculated through the following equation (Parsons & Strickland,1963):

$$\text{Chl } a \text{ (mgm}^{-3}) = \frac{\text{Ca X acetone vol. (ml)}}{\text{Algae culture vol. (L)}}$$

$$\text{Where Ca} = 11.6\,(OD_{665}) - 1.31(OD_{645}) - 0.14\,(OD_{630})$$

Total carotenoid content can be calculated using the formula below;

$$\text{Total carotenoid (µg/mL)} = OD_{452} \times 3.86 \times \frac{\text{Vol.of acetone (ml)}}{\text{Vol. of sample (ml)}}$$

Table 1. Types of Enrichment Media

No	Type of algae/ habitats	Enrichment media used
1.	Freshwater algae	Bold's Basal Medium (BBM) (Nichols and Bold, 1965)
2.	Marine algae	Provasoli 50 Medium (Prov50) (Guillard, 1975)
3.	Blue-green algae	Kosaric Medium (modified after Zarrouk, 1996)
4.	Diatoms	Diatom Medium (Beakers et al., 1988)

b) Algal cell count

The microalgal cell density were determined by counting cells using an Improved Double-Neubauer Haemacytometer (Germany). Appropriate dilution and homogenization of the algal samples was performed prior to counting, to limit the cell number to below 100 cells per field counted.

c) *Determination of Dry Weight (DW)*

A known volume of the sample containing the algae was filtered through a pre-weighed dried 0.45 µm glass fiber cellulose (GF/C-47 mm) filter paper. The pre-weighed filters were pre-combusted prior to use to remove any organic contaminants. The filters containing algae were dried at 100±1°C in an oven (ULM-600 Memmert Oven, Schwabach, W. Germany) for 24 hours, cooled in a desiccator filled with silica gel and weighed. The algal dry weight is determined through the following equation:

$$DW\ (mg/L) = \frac{A - B}{C}$$

A = weight of filters with dried algal biomass (mg)

B = weight of blank filters (mg)

C = Volume of algal culture (L)

5) Water Quality Measurement at the Site of Collection

Water quality analyses were carried out at the site of algal sample collection (habitats). The water samples were collected in 1.5 to 25.0 L plastic bottles and kept in the cold room temperature at 18±1°C. Analyses were done to determine the basic physical-chemical characteristics at the collection site. The results from these analyses were used as the baseline parameters for the growth studies. Physical parameters such as Global Positioning System (GPS) coordinates, time of sampling, irradiance, pH, air temperature, water temperature, depth, dissolved oxygen, salinity, conductivity, total dissolved solid (TDS), total solid (TS) and total suspended solid (TSS) were determined. The nutrient/chemical content such as ammoniacal-nitrogen (NH_3-N), orthophosphate (PO_4^{3-}), chemical oxygen demand (COD), nitrate (NO_3-N) and nitrite (NO_2-N) were also determined. The analytical methods for this parameter are as described.

a) *Determination of pH, temperature, dissolved oxygen, salinity, conductivity, total dissolved solid and irradiance*

These parameters were obtained via instrumental measurement. Waterproof Cyberscan PCD650 (Eutech Instruments Pte Ltd, Singapore) was used to measure all the environmental parameters such as pH, dissolved oxygen, salinity, conductivity, total dissolved solid, air temperature and water temperature which were taken on-site during sample collection. Irradiance (mol photon/s/m^2) was determined by a light meter (L1-250A, LI-COR® Biosciences, USA). In the laboratory, pH of the water samples was measured again using a pH meter (Delta 320 Mettler Toledo, Mettlerr-Toledo Group, Shanghai China).

b) Determination of Chemical Oxygen Demand (COD), Ammoniacal-Nitrogen (NH_3-N), Nitrate (NO_3-N), Nitrite (NO_2-N) and Orthophosphate (PO_4) content

The nutrients such as COD, NH_3-N, NO_3-N, NO_2-N and PO_4^{3-}, content in water samples were determined using the Method 8155, Method 8048, Method 8171 and Method 8000 respectively as shown in the Hach HandBook Odyssey DR/2500 Spectrophotometer Procedure Manual (Hach Company, USA, 2001).

The COD content of the seawater sample was determined by the 8000 method (APHA, 1998). An amber colour solution will develop if the COD is present and the absorbance of the colour was read at 620 nm using Hach spectrophotometer (Hach Odyssey DR/2500, Hach Company, USA). Based on COD value, the carbon (C) content can be calculated using the formula given by Edwards *et al*., (1980):

$$\text{Carbon (C) in mg/L} = \text{COD (mg/L)} \times \frac{12}{32}$$

The NH_3–N content of the seawater sample was determined by the salicylate method [Method 8155] (APHA, 1998), A green colour solution will develop if the ammoniacal-nitrogen is present and the absorbance of the colour was read at 665 nm using Hach spectrophotometer (Hach Odyssey DR/2500, Hach Company, USA).

The nitrate content of the seawater sample was determined by the cadmium reduction method [Method 8171] (APHA, 1998). An amber colour solution is developed if the nitrate is present and

the absorbance of the colour was read at 507 nm using the Hach spectrophotometer (Hach Odyssey DR/2500, Hach Company, USA).

Orthophosphate is referred to as phosphate that responds to colorimetric tests without prior hydrolysis or oxidative digestion of the sample (APHA, 1998). The assay employed was the ascorbic acid method [Method 8048], based on the molybdenum blue colour development from phosphomolybdic acid and the absorbance of the colour was read at 880 nm using Hach spectrophotometer (Hach Odyssey DR/2500, Hach Company, USA).

6) Biochemical Composition of Algae (Lipid, Carbohydrate and Protein)

a) Determination of total carbohydrate content

The total carbohydrate content in microalgae was determined using Kochert (1978) and Dubois (1956) methods, based on colorimetric measurement. A known volume of filtered algae (filtered on 0.45 µm glass fiber cellulose, GF/C-47 mm filter paper) was mashed in 5ml 2 M hydrochloric acid (HCL) using glass hand-homogeniser and transferred into a plastic screw-cap centrifuge tube. The tube was incubated in the water-bath (BS-11 Lab Companion, Jeio Tech, Korea) for one hour at 80±1 °C and mixed regularly. The sample was then centrifuged at 3000 rpm for 10 minutes using Kubota 2100 centrifuge (Kubota Corporation, Japan). The residue was re-extracted again in 5ml 2M HCL as followed in above procedures. The supernatant obtained was pooled to make up to 10mL. A volume of 500 µL of the supernatant was transferred into a wide-mouth glass tube and mixed with 1.5 mL distilled water. 100 µL of phenol reagent (100%) was added slowly into the glass tube followed by 5mL of concentrated sulphuric acid (H_2SO_4) and mixed homogenously using Vortex mixer (S0200-230-UK, Labnet International Inc, USA). The sample was incubated for 30 minutes at 25±1 °C and the absorbance at 485nm was read using Shimadzu UV-160 spectrophotometer (Shimadzu Corporation, Japan). A standard curve was prepared by using a series of glucose solution at concentrations of 0- 50 µg as reference.

b) *Determination of total protein content*

The total protein content in microalgae was determined using dye-binding method (Bradford, 1976). A known volume of filtered algae (filtered on 0.45 µm glass fiber cellulose, GF/C-47mm filter paper) was mashed in 5ml 0.5M Sodium hydroxide (NaOH) using glass hand-homogeniser and transferred into a plastic screw-cap centrifuge tube. The tube was incubated in the water-bath (BS-11 Lab Companion, Jeio Tech, Korea) for 20 minutes at 80±1 °C and mixed regularly. The sample was then centrifuged at 3000 rpm for 10 minutes using Kubota 2100 centrifuge (Kubota Corporation, Japan). The residue was re-extracted again in 5ml 0.5 M NaOH as followed in above procedures. The supernatant obtained was pooled to make up to 10 mL. A volume of 100 µL of the supernatant was transferred into a glass test tube and mixed with 3mL protein reagent. The sample was incubated for 30 minutes at 25±1 °C and the absorbance at 595 nm was read using Shimadzu UV-160 spectrophotometer (Shimadzu Corporation, Japan). A standard curve was prepared by using a series of Bovine Serum Albumin (BSA) solution at concentrations of 0-100 µg/mL as reference. Protein reagent was prepared by (1) mixing 20 mL Bio-Rad solution with 80mL distilled water or (2) dissolving 100 mg of Coomasie Brilliant Blue G-25 (Sigma-Aldrich) in 50 mL ethanol. This solution was then filtered on the filter paper and the supernatant obtained was mixed with 100 mL of 85 %v/v phosphoric acid. Additional distilled water was added slowly until 1.0 L level was reached. The solution were mixed homogenously before transferring into the 1.0 L dark glass bottle for storage at 4±10 °C.

c) *Determination of total lipid content*

The total lipid content in microalgae was determined using Bligh and Dyer (1959) method, based on gravimetric measurement. A known volume of filtered algae (filtered on 0.45 µm glass fiber cellulose, GF/C-47 mm filter paper) was mashed in 5 ml Methanol-Chloroform (2:1 v/v) solution using glass hand-homogeniser and transferred into a plastic screw-cap centrifuge tube. The sample was then centrifuged (using Kubota 2100 centrifuge, Kubota Corporation, Japan) at 3000 rpm for 10 minutes. The residue was re-extracted again in 5 ml Methanol-Chloroform (2:1 v/v) solution as followed in above procedures. The supernatant obtained was pooled in new centrifuge tube and 2 mL of chloroform was added followed by 2 mL of distilled water. The tube were shaken thoroughly using the vortex (S0200-230-UK Vortex mixer, Labnet International Inc,

USA) until the mixture turned milky green colour. The sample was then centrifuged at 3000 rpm for 10 minutes (using Kubota 2100 centrifuge, Kubota Corporation, Japan). The lower layer (green colour) was removed using a special drawn out Pasteur pipette and transferred into a new screw-cap glass tube. The sample was then blow-dried with a gentle stream of nitrogen gas. After drying, the sample. extract was redissolved in 1 mL of chloroform and transferred into a pre-weight 3.5 mL borosilicate glass vial. The extract was blow-dried again and the dry extract was kept in a desiccator containing silica gel for 24 hours before weighing and retained for the fatty acid transesterification. The lipid content in percentage of lipid per dry weight was calculated using the following equation:

$$\text{Lipid content, \% DW} = \frac{[\text{Wt. of lipid + vial (}\textit{final weight}\text{)}] - [\text{Wt. of empty vial }]}{\text{DW algae sample}} \times 100\%$$

RESULTS

1) Characteristics of Algal Habitats (Collection Sites)

A total of 30 collection sites (Table 2) from freshwater (15, of which 9 are considered polluted), brackish water (5) and marine (10) habitats provided 79 environmental samples. The freshwater sites included three hotsprings locations. Polluted sites included the vicinity of a palm oil mill and the highly eutrophicated lake of the University of Malaya. Marine sites were from Port Dickson and Kelantan including mangroves and an estuary. The locations are indicated in the map (Figure 1). Figure 2 shows the sampling sites during the survey.

2) Physical Characteristics of the Samples and Sampling Sites (Table 3)

Physical Characteristics of Habitats and Water Samples gives the physical characteristics of the collection sites. The pH of the water samples ranged from 4.4 to 10.4, with only 2 sites, Site 10 and 11, which are the cooling pond and the acidification pond used for palm oil mill effluent (POME) treatment, being acidic. The water from the settling pond containing POME (482 uS/cm), and the hot springs have very high conductivity (255 to 264 uS/cm) compared to the other sites. Total solids content was highest in the ponds treating the POME.

3) Water Quality of Collection Sites (Table 4)

a) Hotsprings: Water temperature ranged from 40.5 to 52.0 ºC during the time of collection; pH ranged from 6.52 to 7.37. Nitrogen was present mainly as ammoniacal-nitrogen (up to 3.47 mg/L); phosphate content was high (up to 0.53 mg/L).

b) Negeri Sembilan coast (Pantai Dickson, Pantai Purnama, Pantai Tg. Biru): Salinity ranged from 32 to 34 ppt. pH ranged from 6.88 to 7.49. COD (up to 2633 mg/L) and TSS (up to 69.8 mg/L) was high. Nitrate level reached 0.3 mg/L and phosphate level up to 0.21 mg/L).

c) University of Malaya Lake: The pH was 9.47 and high DO (14.44 mg/L) indicated high primary productivity due to the eutrophicated condition of the lake. COD (1175.67 mg/L), nitrate (0.6 mg/L), ammoniacal-nitrogen (0.47 mg/L) contents were high.

d) Tenamaram Palm Oil Mill (raw POME tank, acidification ponds, anaerobic ponds, aerobic ponds, final discharge pond): pH ranged from 4.91 to 10.37. Conductivity was highest (482.37µS) in the settling pond. COD ranged from 225 to 7193 mg/L. NH_3N (0.8 to 124.0 mg/L), NO_3N (1.0 to 45.3 mg/L) and PO_3 (5.2 to 430 mg/L) were abundant. Total solids (0.43 to 43.13 g/L) were high.

e) Labu Palm Oil Mill: pH ranged from 4.42 to 7.78. COD (9243 to 96,666 mg/L) was higher than the Tennamaram Palm Oil Mill samples. NH_3N (1.10 to 35.33 mg/L), NO_3N (27.33 to 264.67 mg/L and PO_3 (22.3 to 146.6 mg/L) were abundant. Total solids contents (7.19 to 69.46 g/L) were high.

f) Kelantan coast: Salinity ranged from 0.01 to 32.08 ppt, since the sites were of brackish water to marine environments. pH ranged from 7.00 to 9.23. Conductivity was high around Pantai Sg. Golok (49.00 µS), Pantai Genting (48.54 µS), Pantai Cahaya Bulan (48.85 µS) and at the Bachok Marine Research Station (48.763 µS). COD ranged from 83 to 1116 mg/L. NH_3N (0.01 to 1.73 mg/L), NO_3N (0.1 to 0.73 mg/L) and PO_3 (0.247 to 1.567 mg/L) were low.

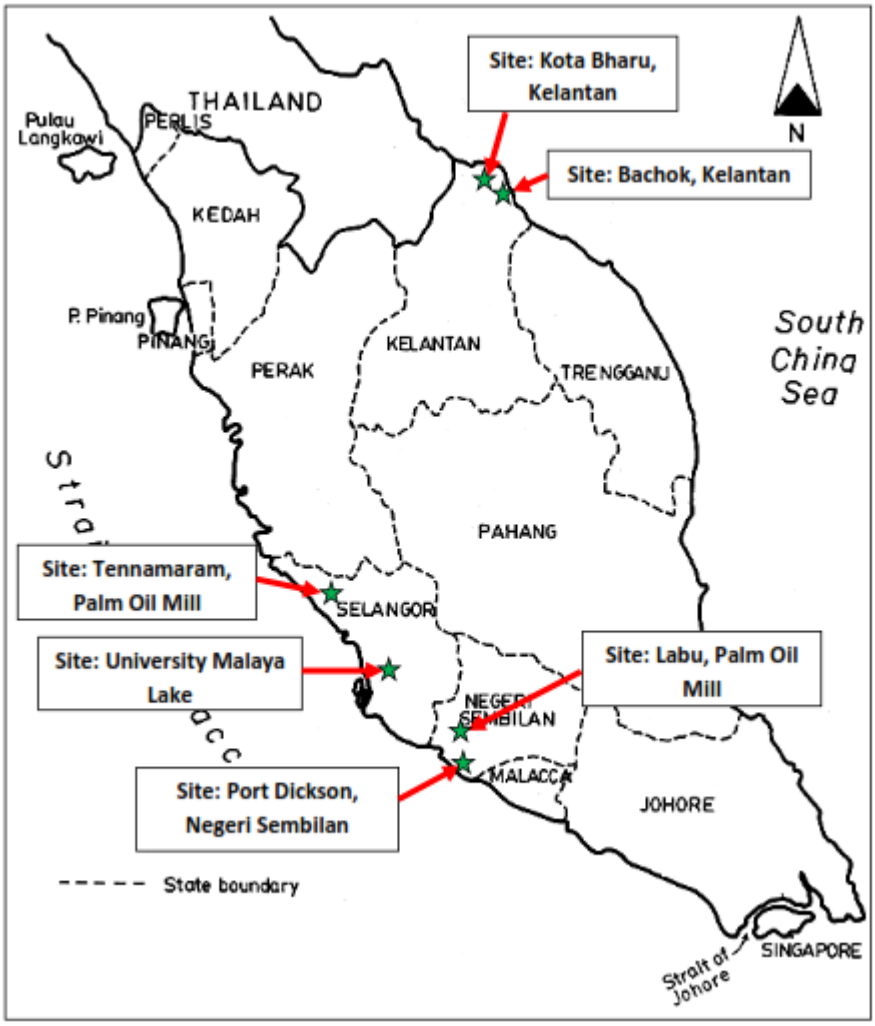
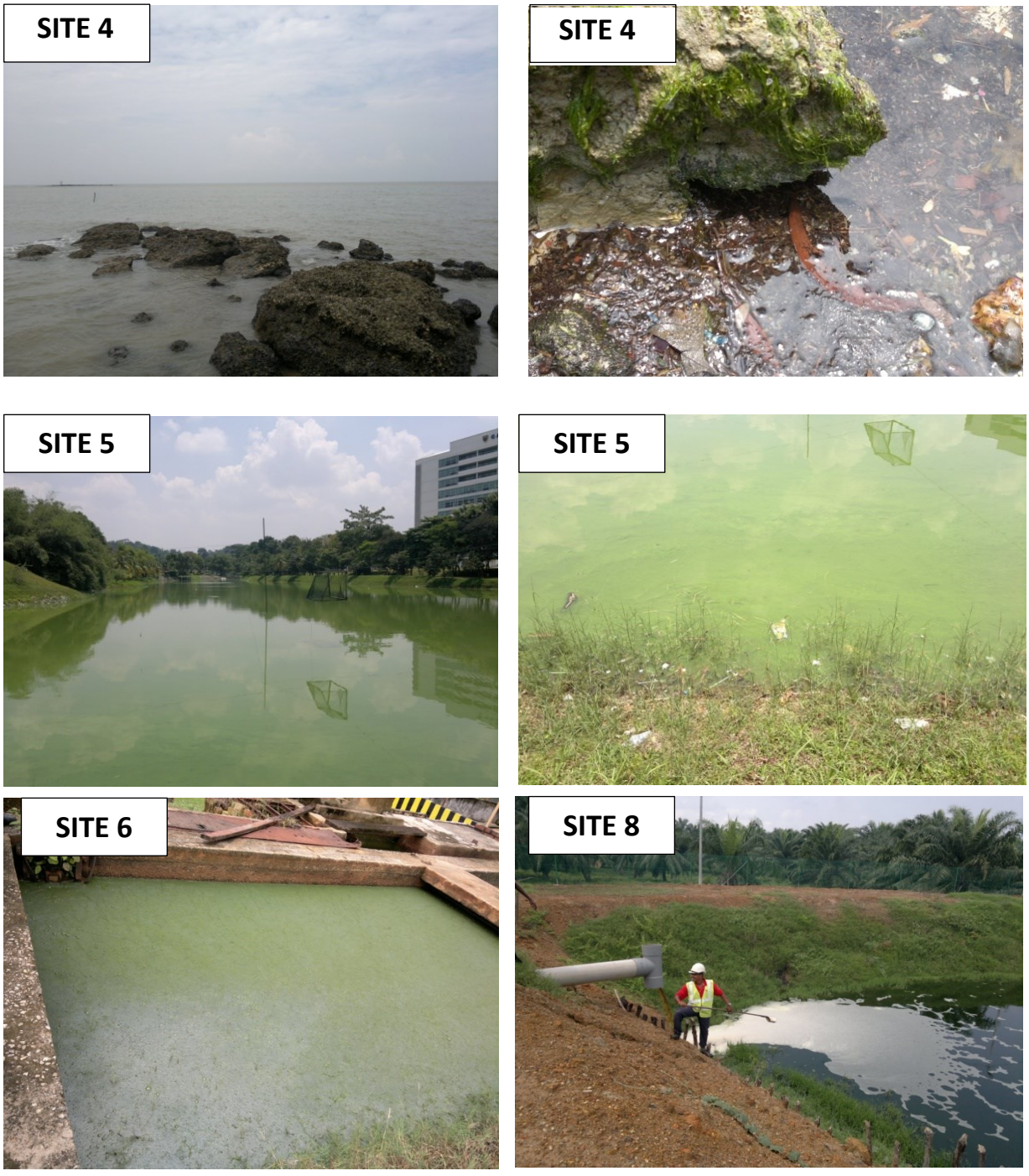


Figure 1. Map of Peninsular Malaysia Showing Collection Sites of Algae

Notes :
Site 1 - Hotspring at Kg. Sungai Serai, Batu $11^{1/2}$ Hulu Langat
Site 2 - Hotspring at Kg. Dusun Tua, Batu $15^{1/2}$ Hulu Langat
Site 3 - Hotspring at Selayang, Selangor

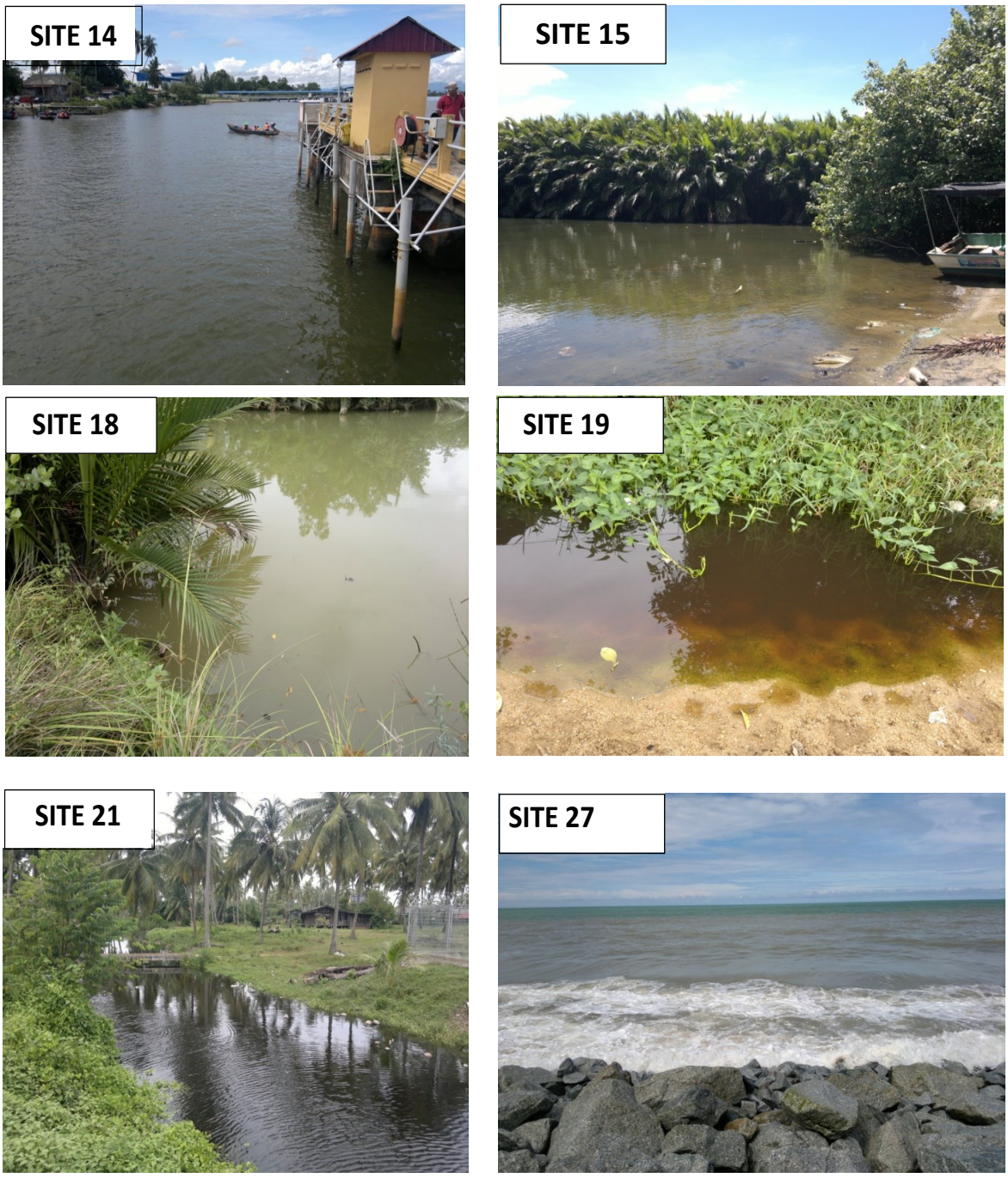
Figure 2. Collection Sites during the Algae Survey



Site 4: Port Dickson, Negeri Sembilan

Notes :
Site 4 - Port Dickson, Negeri Sembilan
Site 5 - University of Malaya Eutrophicated Lake
Site 6 - Tenamaram Palm Oil Mill, Effluent Settling Pond
Site 8 - Tenamaram Palm Oil Mill, Aerobic Pond

Figure 2. Collection Sites during the Algae Survey (Continued)



Notes :
Site 14 - Jetty Pengkalan Kubor, Kelantan
Site 15 - Sungai Golok Stream, Kelantan
Site 18 - Sungai Tumput Stream, Kelantan
Site 19 - Stream at Batik Factory, Kota Bharu
Site 21 - Stream in front of Bachok Marine Station, Kelantan
Site 27 - Pantai Cahaya Bulan, Kota Bharu, Kelantan

Figure 2. Collection Sites during the Algae Survey (Continued)

Table 2. List of Collection Sites and Sample Type

Habitat	Date	Location	GPS	Collection Site	Origin
Hotspring	3 Apr 2013	Pond A, Kampung Sungai Serai Hotspring, Batu 11 ½, Hulu Langat, Selangor	N03° 05' 26.4" E101 °47' 40.6"	Site 1	FW
	3 Apr 2013	Dusun Tua Hotspring,, Batu 15 ½, Dusun Tua, Hulu Langat, Selangor	N03° 08' 20.8" E101 °50' 10.5"	Site 2	FW
	3 Apr 2013	Selayang Hotspring,, Selangor	N03° 15' 32.8" E101 °38' 46.z1"	Site 3	FW
Port Dickson, Negeri Sembilan	8 Apr 2013	Pantai Dickson, Negeri Sembilan	N02° 25' 02.3" E101° 53' 41.7"	Site 4	MW
Eutrophicated Lake	22 Apr 2013	University Lake, University of Malaya Kuala Lumpur	N03° 07' 10.1" E101° 39' 31.0"	Site 5	FW
Palm Oil Mill Factories	29 Aug 2013	Settling Pond A, Tenamaram Palm Oil Mill Factory, Selangor	N03° 23' 43.39" E101° 25' 08.78"	Site 6	FW
	29 Aug 2013	Anaerobic Pond, Tenamaram Palm Oil Mill Factory, Selangor	N03° 23' 50.71" E101° 24' 55.45"	Site 7	FW
	29 Aug 2013	Aerobic Pond, Tenamaram Palm Oil Mill Factory, Selangor	N03° 23' 50.71" E101° 24' 55.45"	Site 8	FW
	29 Aug 2013	Final discharge tank, Tenamaram Palm Oil Mill Factory, Selangor	N03° 23' 50.71" E101° 24' 55.45"	Site 9	FW
	12 Sep 2013	Cooling Pond, Labu Palm Oil Mill Factory, Negeri Sembilan	N02° 45' 3.1" E101° 48' 19.4"	Site 10	FW
	12 Sep 2013	Acidification Pond, Labu Palm Oil Mill Factory, Negeri Sembilan	N02° 45' 3.1" E101° 48' 19.4"	Site 11	FW
	12 Sep 2013	Anaerobic Pond, Labu Palm Oil Mill Factory, Negeri Sembilan	N02° 45' 3.1" E101° 48' 19.4"	Site 12	FW
	12 Sep 2013	Aerobic Pond, Labu Palm Oil Mill Factory, Negeri Sembilan	N02° 45' 3.1" E101° 48' 19.4"	Site 13	FW
Kelantan	22 Oct 2013	Jetty JKR Pengkalan Kubor, Kelantan	N06°13' 56.86" E102°53' 45.96"	Site 14	BW
	22 Oct 2013	Sungai Golok Stream, Pengkalan Kubor, Kelantan	N06°14' 5.53" E102°5' 55.86"	Site 15	BW

Table 2. (Continued)

Habitat	Date	Location	GPS	Collection Site	Origin
Kelantan	22 Oct 2013	Water Reservoir along Pantai Genting, Tumpat, Kelantan	N06°13'49.87" E102°6'25.56"	Site 16	BW
	23 Oct 2013	Sungai Tumpat estuary, Jalan Station, Tumpat, Kelantan	N06°12'5.67" E102°10'4.98"	Site 17	BW
	23 Oct 2013	Sungai Tumpat Stream, Jalan Station, Tumpat, Kelantan	N06°12'4.75" E102°10'6.71"	Site 18	FW
	24 Oct 2013	Stream at Batik factory, Kg. Sirih Bawah Lembah, Bunut Payong, Kota Bharu, Kelantan	N06°6' 24.62" E102°13'49.04"	Site 19	FW
	24 Oct 2013	Sungai Kelantan, Kg. Pulau Pisang, Kota Bharu, Kelantan	N06°11'11.26" E102°14'22.24"	Site 20	FW
	24 Oct 2013	Stream in front of Bachok Marine Station, Kg. Kuala Rekang, Bachok, Kelantan	N06°0.5' 32.8" E102°25'28.27"	Site 21	BW
	22 Oct 2013	Pantai Sungai Golok, Pengkalan Kubor, Kelantan	N06°14'16.12" E102°6'1.51"	Site 22	MW
	23 Oct 2013	Pantai Genting, Tumpat, Kelantan	N06°13'35.72" E102°6'56.92"	Site 23	MW
	23 Oct 2013	Lagoon 1 Pantai Tanjung Tujuh, Tumpat, Kelantan	N06°13'14.7" E102°7'31.11"	Site 24	MW
	23 Oct 2013	Lagoon 2 Pantai Tanjung Tujuh, Tumpat, Kelantan	N06°13'6.78" E102°7'55.96"	Site 25	MW
	24 Oct 2013	Pantai Mek Mah, Kota Bharu, Kelantan	N06°12'58.93" E102°14'35.56"	Site 26	MW
	24 Oct 2013	Pantai Cahaya Bulan, Kota Bharu, Kelantan	N06°11'47.22" E102°16'26.29"	Site 27	MW
	24 Oct 2013	Pantai Sabak, Pengkalan Chepa, Kelantan	N06°10'22.15" E102°19'56.35"	Site 28	MW
	24 Oct 2013	Pantai Kg. Kelawang, Bachok, Kelantan	N06°8' 3.08" E102°22'2.06"	Site 29	MW
	24 Oct 2013	Pantai Melawi, in front of Bachok Marine Station, Kg. Kuala Rekang, Bachok, Kelantan	N06°0.5' 32.47" E102°25'35.54"	Site 30	MW

Note: FW : Fresh water

BR : Brackish water

MW : Marine water

Table 3. Physical Chracteristics of Habitats and Water Samples

Habitat	Collection Site	Water Temperature (°C)	Irradiance (µmol/s/m²)	pH	Dissolved Oxygen (mg/L)	Salinity (ppt)	Conductivity (µS/cm)	Total Dissolved Solids (mg/L)	Total Solids (g/L)
Hotsprings	Site 1	40.5±1.3 - 45.0±0.1	1080.3±45.7	7.0±0.1	3.6±0.1	0.0	264.4±0.1	334.0±0.1	na
	Site 2	48.7±0.6	1621.9±24.6	7.6±0.1	3.9±0.1	0.0	257.8±0.6	379.3±2.3	0.2±0.1
	Site 3	45.0±0.1 - 52.0±0.1	233.8±10.1	6.5±0.1 - 7.4±0.1	1.4±0.1- 4.2±0.1	0.0	255.6±2.9 - 257.3±0.2	376.3±0.2 - 435.6±0.1	0.2±0.1 – 0.3±0.1
Port Dickson, Negeri Sembilan	Site 4	30.7±0.1 - 36.0±0.1	389.4±41.2 - 899.8±21.9	6.9±0.1 - 7.5±0.1	5.9±0.1 - 7.4±0.1	32.0±0.1 - 34.0±0.1	46.2±0.1 - 47.6±0.1	0.1±0.1 - 42.4±0.1	na
Eutrophicated Lake	Site 5	38.0±0.1	1758.5±7.2	9.5±0.1	14.4±0.2	0.0	142.8±0.1	157.5±0.2	0.8±0.1
Palm Oil Mill Factories	Site 6	28.0±0.4	966.9±0.8	7.4±0.1	5.4±0.2	0.0	482.4±0.3	393.8±0.1	43.1±0.4
	Site 7	31.0±0.1 - 34.1±0.3	1213.8±8.7	8.5±0.7 - 10.4±0.1	1.6±0.1 - 1.7±0.1	0.0	6.0±0.1 - 13.6±0.2	5.6±0.1 - 14.0±0.1	4.9±0.1 - 14.4±1.3
	Site 8	30.9±0.6 - 33.0±0.5	1213.8±8.8	7.9±0.1 - 9.1±0.1	1.1±0.2 - 8.5±0.6	0.0	5.6±0.1 - 10.4±0.1	5.2±0.1 - 10.3±0.1	4.5±0.1 – 9.1±0.2
	Site 9	32.6±0.1	1213.8±8.8	7.7±0.1	0.7±0.1	0.0	10.8±0.1	10.7±0.1	9.7±0.1
	Site 10	48.0±0.1	1019.4±56.6	4.4±0.1	na	0.0	na	na	69.5±2.8
	Site 11	30.0±0.1 - 45.0±0.1	924.1±3.4 - 1393.3±174.1	5.3±0.1 - 7.1±0.1	na	0.0	na	na	7.2±0.1 - 37.9±3.0
	Site 12	35.0±0.1 - 37.0±0.1	1294.1±55.4 - 1553.2±72.7	6.9±0.1 - 7.3±0.1	na	0.0	na	na	12.6±0.4 - 63.7±0.5
	Site 13	33.0±0.1	1981.8±27.9	7.4±0.1	na	0.0	na	na	28.6±1.3
Kelantan	Site 14	29.2±0.1	867.6±10.1	7.0±0.1	3.8±0.1	7.7±0.2	12.7±0.1	8.5±0.1	na
	Site 15	30.4±0.1	2050.3±78.8	7.2±0.1	6.0±0.1	9.4±0.1	16.1±0.1	10.3±0.1	na

Table 3. (Continued)

Habitat	Collection Site	Water Temperature (°C)	Irradiance (µmol/s/m²)	pH	Dissolved Oxygen (mg/L)	Salinity (ppt)	Conductivity (µS/cm)	Total Dissolved Solids (mg/L)	Total Solids (g/L)
	Site 16	39.7±0.1	1733.8±31.8	7.3±0.1	10.5±0.1	10.1±0.1	17.3±0.1	11.0±0.1	na
	Site 17	29.0±0.1	496.6±16.2	8.0±0.1	6.1±0.1	6.1±0.1	10.8±0.1	6.9±0.1	na
	Site 18	28.6±0.1	208.6±43.1	7.7±0.1	6.4±0.1	8.0±0.1	13.8±0.1	8.9±0.1	na
	Site 19	26.7±0.1	383.9±4.8	9.6±0.1	1.2±0.1	0.3±0.1	0.7±0.1	0.4±0.1	na
	Site 20	26.4±0.1	659.3±97.9	7.5±0.1	5.8±0.1	0.1±0.1	0.1±0.1	0.1±0.1	na
	Site 21	30.3±0.1	143.5±8.2	7.5±0.1	3.2±0.1	9.6±0.3	13.6±2.3	10.7±0.3	na
	Site 22	31.7±0.1	1797.8±12.6	8.5±0.1	5.7±0.1	32.1±0.1	49.0±0.1	31.4±0.1	na
	Site 23	29.6±0.1	1455.6±133.7	na	5.8±0.1	31.8±0.1	48.5±0.1	31.1±0.1	na
	Site 24	31.4±0.1	153.9±28.3	9.2±0.1	7.6±0.1	14.8±0.1	24.4±0.1	15.7±0.1	na
	Site 25	28.4±0.1	675.9±6.2	8.3±0.1	6.9±0.1	13.4±0.1	22.2±0.1	14.3±0.1	na
	Site 26	29.8±0.1	1966.2±48.2	8.5±0.1	6.1±0.1	27.5±0.1	42.6±0.1	27.3±0.1	na
	Site 27	29.9±0.1	1471.7±21.9	8.5±0.1	5.9±0.1	32.0±0.1	48.8±0.1	na	na
	Site 28	29.8±0.1	278.0±39.5	8.4±0.1	5.9±0.1	30.6±0.3	46.7±0.1	29.9±0.1	na
	Site 29	29.5±0.1	335.9±3.0	8.4±0.1	6.0±0.1	26.9±0.1	41.7±0.1	26.9±0.1	na
	Site 30	29.8±0.1	119.8±1.2	8.5±0.1	5.9±0.1	31.8±0.1	48.6±0.1	31.3±0.3	na

Table 4. Chemical and Biological Characteristics of Habitats and Water Samples

Habitat	Collection Site	COD (mg/L)	Ammonia-N (mg/L)	Nitrate-N (mg/L)	Nitrite-N (ug/L)	Ortho-Phosphate (mg/L)	Chl-*a* (µg/L)	Carotenoids (ng/mL)	Biomass (DW) (based on TSS) (mg/L)	Biomass (DW) (based on Chl-*a*) (mg/L)
Hotsprings	Site 1	22.3±0.6 - 75.7±1.5	0.10±0.01 - 3.47±0.06	0.1±0.1 - 0.4±0.1	2.7±0.6 - 6.3±1.5	0.2±0.1 - 0.5±0.1	0.42±0.07 - 5.20±0.57	0.11±0.03 - 2.12±0.38	3. 7±1.5 - 6.3±3.2	0.03±0.01 - 0.35±0.04
	Site 2	58.7±0.6	0.07±0.01	0.2±0.1	2.3±0.6	0.4±0.1	0.08±0.02	0.08±0.01	0.9±0.6	0.01±0.00
	Site 3	62.7±0.6 - 101.7±0.6	0.04±0.01 - 1.18±0.03	0.1±0.1 - 0.5±0.1	1.3±0.6 - 7.3±0.6	0.3±0.1 - 0.5±0.1	0.07±0.01 - 5.49±0.19	0.03±0.01 - 1.72±0.05	0.6±0.3 - 4.0±2.8	0.01±0.01 - 0.37±0.01
Port Dickson, Negeri Sembilan	Site 4	1473.3±11.6 - 2633.3±11.5	0.01±0.01 - 0.05±0.01	0.0 - .3±0.1	0.0 - 7.0±1.0	0.1±0.1 - 0.2±0.1	0.86±0.05 - 1.66±0.14	0.59±0.03 - 1.08±0.09	46.8±3.0 - 69.8±1.6	0.06±0.01 - 0.11±0.01
Eutrophicated Lake	Site 5	1175.7±12.4	0.47±0.06	0.6±0.1	17.0±1.7	0.2±0.1	518.53 ±94.42	200.40±35.43	361.1±174.0	34.74±6.33
Palm Oil Mill Factories	Site 6	225.3±0.6	0.80±0.10	1.0±0.1	10.0±1	2.1±0.3	15.65±2.24	3.07±0.27	93.3±15.1	1.05±0.15
	Site 7	507.7±2.1 - 1145.3±3.1	11.67±0.58 - 124.00±4.00	3.3±0.6 - 21.3±2.3	40.0±0.1 - 466.7±46.2	6.6±0.3 - 107.9±0.9	42.85±4.61 - 73.59±3.31	44.91±2.08 - 243.95±10.92	301.7±16.0 - 6227.8±1286.1	2.87±0.31 - 4.93±0.22
	Site 8	482.7±0.6 - 825.0±2.0	2.17±0.21 to 92.00±6.93	4.0±0.1 - 4.7±0.6	26.7±5.8 - 33.3±5.8	5.2±0.4 - 80.7±3.8	40.17±15.85 - 103.10±5.36	40.11±13.80 - 80.42±2.87	348.3±36.2 - 753.3±64.3	2.69±1.06 - 6.91±0.36
	Site 9	815.3±1.5	116.00±6.93	4.7±0.6	73.3±11.5	98.7±1.2	111.06±2.05	41.50±0.82	1183.3±211.3	7.44±0.14
	Site 10	23200.0 ±200.0	19.67±1.16	30.0±1.7	270.0±26.5	146.7±1.2	18570.0 ±201.0	182.32±4.13	75083.3 ±4775.3	1243.84 ±134.94
	Site 11	9243.3±28.9 - 73466.7±115.5	1.10±0.01 - 1.87±0.06	27.3±1.2 - 264.7±3.1	183.3±5.8 - 1170.0±10.0	24.1±1.4 - 61.7±2.1	8850±60 - 16240±2130	50.18±7.46 - 764.67±5.76	2713.3±613.3 - 32116.7±404.2	593.08±3.80 - 1088.32±142.83
	Site 12	11283.3±5.8 - 96666.7±115.5	1.00±0.01 - 26.67±2.08	33.3±0.6 - 152.7±3.1	376.7±5.8 - 1506.7±250.1	22.3±2.0 - 67.3±4.6	2720±620 - 26750±4990	167.14±1.09 - 1024.06±100.62	4486.7±75.7 - 60433.3±8489.0	182.16±41.22 - 1792.18±334.26
	Site 13	97433.3 ±404.2	28.03±20.73	220.3±14.0	1073.3±80.2	275.7±1.2	22830±3930	937.72±62.15	70733.3±1632.7	1529.41±263.11
Kelantan	Site 14	562.0±0.1	0.04±0.01	0.1±0.1	3.3±0.6	0.4±0.1	0.04±0.01	0.05±0.01	15.2±1.7	0.01±0.01
	Site 15	651.0±0.1	0.01±0.01	0.2±0.1	7.3±1.2	0.5±0.1	0.81±0.02	0.98±0.05	21.6±1.9	0.05±0.01
	Site 16	661.0±0.1	0.01±0.01	0.1±0.1	2.7±1.5	0.4±0.1	1.28±0.02	1.55±0.01	18.4±1.2	0.09±0.01
	Site 17	622.3±7.5	0.05±0.01	0.2±0.1	4.3±0.6	0.6±0.1	0.12±0.01	0.16±0.01	52.1±4.0	0.01±0.01
	Site 18	680.0±0.1	0.03±0.01	0.1±0.1	4.3±0.6	0.7±0.1	0.35±0.01	0.46±0.01	46.1±4.2	0.02±0.01
	Site 19	83.0±3.5	1.73±0.29	0.2±0.1	6.7±0.6	8.1±0.2	155.61±8.24	249.69±15.35	48.2±19.6	10.43±0.55
	Site 20	112.0±1.0	0.30±0.01	0.3±0.1	4.0±1.0	1.1±0.2	15.28±10.84	14.48±4.09	230.0±25.2	1.02±0.73
	Site 21	434.3±0.6	0.13±0.01	0.2±0.1	1.0±0.1	1.6±0.4	8.78±0.10	9.09±0.15	31.5±5.4	0.59±0.01
	Site 22	987.0±0.1	0.01±0.01	0.4±0.1	10.3±0.6	0.5±0.1	1.94±0.02	2.60±0.07	92.8±14.5	0.13±0.01
	Site 23	1116.0±0.1	0.01±0.01	0.6±0.1	10.3±1.2	0.3±0.1	5.01±0.19	5.54±0.07	139.6±5.9	0.34±0.01
	Site 24	882.3±1.5	0.01±0.01	0.3±0.1	2.7±1.2	0.3±0.1	1.04±0.06	0.94±0.03	50.6±2.4	0.07±0.01

Table 4. (Continued)

Habitat	Collection Site	COD (mg/L)	Ammonia-N (mg/L)	Nitrate-N (mg/L)	Nitrite-N (ug/L)	Ortho-Phosphate (mg/L)	Chl-*a* (µg/L)	Carotenoids (ng/mL)	Biomass (DW) (based on TSS) (mg/L)	Biomass (DW) (based on Chl-*a*) (mg/L)
Kelantan	Site 25	841.0±0.1	0.01±0.01	0.3±0.1	4.3±0.6	0.4±0.1	0.39±0.02	0.73±0.01	51.9±0.9	0.03±0.01
	Site 26	928.0±0.1	0.03±0.01	0.5±0.1	12.0±2.0	0.6±0.1	22.18±7.24	39.89±1.82	97.0±5.7	1.49±0.49
	Site 27	921.7±0.6	0.02±0.01	0.7±0.1	8.7±1.2	0.5±0.1	0.76±0.01	1.07±0.01	90.4±10.5	0.05±0.01
	Site 28	949.3±1.2	0.07±0.01	0.7±0.2	23.3±1.2	1.0±0.1	1.02±0.06	1.53±0.03	309.3±57.2	0.07±0.01
	Site 29	922.00±1.0	0.08±0.01	0.6±0.2	24.0±1.7	0.9±0.1	2.83±0.05	3.59±0.21	316.7±39.1	0.19±0.01
	Site 30	975.0±0.1	0.02±0.01	0.7±0.2	10.3±0.6	0.8±0.1	3.16±0.07	4.71±0.02	142.3±12.7	0.21±0.01

4) Algae biomass (chl a)

The algal biomass based on chl a, determined from the different habitats are shown in Table 5.

Table 5. Algae Biomass at Collection Sites

	Collection Site	Algae Biomass, mg/L (based on chl a)
1	Hotsprings	0.01 to 0.37
2	Marine sites of Port Dickson, Negri Sembilan	0.06 to 0.11
3	Eutrophicated freshwater lake in the University of Malaya (1 site)	34.74
4	Tennamaram Palm Oil Mill	1.05 to 7.44
5	Labu Palm Oil Mill	182.16 to 1792.18
6	Brackish water and marine sites, Kelantan	0.01 to 1.49

5) Algal Composition at Collection Sites (habitats)

The taxonomic identification of the algae was conducted using selected monographs as listed in the Introduction and other monographs (Prescot 1981; Shamsudin 1990; 1991; Shihira and Krauss 1965; Yamagishi 2010; Salleh and Tajuddin 2006). The algae were identified to species where possible. The algae identified from the sampling sites together with their relative abundance (cells per mL) are given in Table 6. Figure 3 shows selected algae observed in the samples. The number of taxa observed for each location is as follows: Hotsprings (8); Port Dickson, Negeri Sembilan coast (15); University of Malaya Lake (20); Tennamaram Palm Oil Mill & Labu Palm Oil Mill (25) and Kelantan coast (43). The highest number of taxa were identified from Kelantan sites, while the lowest was from the hot springs areas. The total number of taxa and genera identified were 73 and 52 respectively; with 9 genera and 11 taxa of Cyanophyta; 25 genera and 33 taxa of Bacillariophyta; 13 genera and 16 taxa of Chlorophyta and 5 genera and 13 taxa of Euglenophyta. In terms of cell numbers, the samples from the oil palm mills had the highest cell density.

6) Growth and Biochemical Properties of Selected Algal Genera

During the isolation process, the fast-growing, dominant algae that grew out of the samples collected during the survey, were isolated and purified into axenic cultures. This paper only reports the preliminary biochemical characterization of selected algal genera isolated from the samples. Of the 73 algal taxa identified in this paper, only six genera, namely the Cyanophyte *Cyanosarcina*, the Chlorophytes *Chlorella*, *Chlamydomonas, Chlorococcum, Scenedesmus* and the Euglenophyte *Euglena* are reported. Table 7 gives the data on growth (specific growth rate, μ and biomass productivity) and biochemical characteristics (range of lipid, carbohydrate and protein productivities) of these genera.

In terms of specific growth rate, *Chlorococcum* and *Euglena* had the highest μ, followed by *Chlorella* and *Scenedesmus*. Biomass productivity at day 12 was generally higher than that at day 8 for all genera. Highest biomass productivity was from Chlorella followed by Chlamydomonas. Lipid productivity was generally higher on day 12 than on day 8.

Highest lipid productivity was from Chlorella and Chlamydomonas on day 12. In general carbohydrate productivity was higher on day 12, except for Chlorella. Chlorella had the highest carbohydrate productivity followed by Chlamydomonas. In general protein productivity was higher on day 8. Chlorella and Chlamydomonas had the highest protein productivity.

Table 6. Checklist of Algae Collected during the Survey

Division	Taxa	Hot Springs	Eutrophicated UM Lake	Palm Oil Mills	Port Dickson, Negeri Sembilan Coast	Kelantan
Cyanophyta (Blue-green algae)						
1	*Anabaena* Bory ex Bornet & Flahault	0	0	0	nd	0
2	*Anacystis* Meneghini	nd	9	nd	nd	nd
3	*Gloeocapsa* Kuetzing	nd	nd	0	nd	nd
4	*Lyngbya* Agardh ex Gomont	0	nd	0 - 5000	0	0 - 1
5	*Cyanosarcina chroococcoides* Geitler Kovacik	nd	nd	1932 - 5000	nd	nd
6	*Microcystis* Kuetzing ex Lemmermann	nd	114 - 280	nd	nd	nd
7	*Oscillatoria* Vaucher ex Gomont	3 - 18	nd	70 – 15,000	nd	0 - 5
8	*Oscillatoria* (thick) Vaucher ex Gomont	nd	0	nd	nd	nd
9	*Oscillatoria* (thin) Vaucher ex Gomont	nd	5950 - 1655	nd	nd	0 - 2
10	*Spirulina* Turpin ex Gomont	nd	210	nd	nd	nd
11	*Synechococcus* Naegeli	1835 - 4902	nd	0	nd	nd
	Total Cyanophyta Taxa Observed	4	6	6	1	4
Bacillariophyta (Diatom)						
1	*Achnanthes* Ehrenberg	nd	nd	nd	nd	0 - 2
2	*Amphiprora* Ehrenberg	nd	nd	nd	nd	0
3	*Asterionella* Hassal	nd	nd	nd	nd	0 - 1
4	*Bacillaria* (very long) Gmelil	nd	nd	nd	0	nd
5	*Bacteriastrum* Shadboli	nd	nd	nd	nd	0 - 1
6	*Biddulphia* Gray	nd	nd	nd	1	2 - 4
7	*Coscinodiscus* Ehrenberg	nd	0	nd	5 - 8	0 - 5
8	*Chaetoceros* Grunow	nd	nd	nd	nd	0 - 2
9	*Cyclotella* (Kutzing) Brebisson	nd	nd	0	0	0 - 2
10	*Cyclotella* (small) (Kutzing) Brebisson	nd	nd	nd	nd	nd
11	*Cyclotella* (Kutzing) Brebisson	nd	nd	nd	nd	nd
12	*Cymbella* (Kutzing) Brebisson	nd	nd	nd	3	0 - 20
13	*Diploneis weissflogii* (A.W.F. Schmidt) Cleve	nd	ND	ND	ND	0 - 1

Table 6. Continued

Division	Taxa	Hot Springs	Eutrophicated UM Lake	Palm Oil Mills	Port Dickson, Negeri Sembilan Coast	Kelantan
Bacillariophyta (Diatom)						
14	*Diploneis* Ehrenberg ex Cleve	nd	nd	nd	0	0
15	*Fragilaria* Lyngbye	nd	nd	nd	1	0
16	*Gomphonema* Ehrenberg	nd	nd	nd	nd	0 - 2
17	*Gyrosigma* Hassall	nd	nd	nd	nd	0 - 15
18	*Melosira* C.Agardh	nd	nd	nd	5	0 - 2
19	*Navicula* Bory	6 - 80	9	nd	1	0 - 245
20	*Navicula* Bory (small)	nd	nd	nd	nd	0 - 28
21	*Navicula* Bory (very small)	nd	nd	nd	nd	0 - 3
22	*Navicula* Bory (long and thin)	nd	nd	nd	nd	0
23	*Nitzschia* Hassal	nd	nd	nd	0	nd
24	*Pinnularia* Ehrenberg	0	nd	0	3	0 - 1
25	*Pinnularia* (big) Ehrenberg	nd	nd	nd	nd	0 - 7
26	*Pleurosigma* W.Smith	nd	nd	nd	3	0 - 1
27	*Rhizosolenia* Ehrenberg	nd	nd	nd	nd	0 - 1
28	*Rhizosolenia* Ehrenberg (very big)	nd	nd	nd	nd	0
28	*Skeletonema* Greville	nd	nd	nd	0	0
30	*Surirella* Turpil	nd	nd	nd	nd	0
31	*Synedra* Ehrenberg	nd	nd	nd	nd	0
32	*Thalassiosira* Cleve	nd	nd	nd	nd	0 - 4
33	*Triceratium* Ehrenberg	nd	nd	nd	nd	0
	Total Bacillariophyta Taxa Observed	2	2	2	13	29
Chlorophyta (Green algae)						
1	*Actinastrum* Lagerheim	0	nd	nd	nd	nd
2	*Chlorella ellisoidea* Gerneck	nd	nd	0	nd	nd
3	*Chlorella vulgaris* Beyerinck	462 - 1975	44 - 350	400 – 770,000	13	1 - 700
4	*Chlorococcum* Meneghini	nd	nd	0	nd	nd
5	*Closterium* Nitzsch ex Ralfs	nd	nd	0 - 9660	nd	1 - 7
6	*Cosmarium* Corda ex Ralfs	nd	70	nd	nd	0 - 6

Table 6. Continued

Division	Taxa	Hot Springs	Eutrophicated UM Lake	Palm Oil Mills	Port Dickson, Negeri Sembilan Coast	Kelantan
Chlorophyta (Green algae)						
7	*Dictyosphaerium* Naegeli	nd	nd	0 - 40000	nd	nd
8	*Eudorina* Ehrenberg	nd	nd	0 - 350,035	nd	nd
9	*Kirchneriella* Schmidle	nd	0	nd	nd	nd
10	*Netrium* Nageli	nd	140	nd	nd	nd
11	*Pandorina* Bory St. Vincent	nd	nd	0 – 50,000	nd	nd
12	*Pediastrum* Meyel	nd	560	nd	nd	nd
13	*Scenedesmus arcuatus*	nd	0 - 70	nd	nd	nd
14	*Scenedesmus obliquus* (Turpin) Kuetzing	nd	nd	0 – 105,000	nd	nd
15	*Scenedesmus* Meyel	nd	nd	nd	nd	nd
16	*Selenastrum* Reinsch	nd	nd	nd	nd	0
	Total Chlorophyta Taxa Observed	2	6	8	1	4
Euglenophyta (Euglenoids)						
1	*Euglena sanguine* Ehrenberg	nd	0 - 560	nd	nd	nd
2	*Euglena* Ehrenberg	nd	nd	0 – 1,120,000	nd	0 -28
3	*Euglena* Ehrenberg *(colourless)*	nd	nd	0 – 600,000	nd	nd
4	*Peranema* Dujardin	nd	0	nd	nd	nd
5	*Phacus longicauda* (Ehrenberg) Dujardin	nd	nd	0	nd	nd
6	*Phacus cf. curvicauda* Swirenko	nd	nd	0	nd	nd
7	*Phacus* Dujardin	nd	0	0 – 20,000	nd	0 - 131
8	*Phacus* (small) Dujardin	nd	70	nd	nd	0 - 11
9	*Strombomonas* Deflandre	nd	nd	0	nd	nd
10	*Trachelomonas armata* (Ehrenberg) Stein	nd	nd	0	nd	nd
11	*Trachelomonas scabra* Playfair	nd	nd	0 – 35,000	nd	0
12	*Trachelomonas volvocina* Ehrenberg	nd	0	0 – 200,000	nd	0 - 2
13	*Trachelomonas* Ehrenberg emend. Deflandre	nd	140	nd	nd	0 - 1
	Total Euglenophyta Taxa Observed	0	6	9	0	6
	Total All Taxa Observed	8	20	25	15	43
Grand Total Taxa: 73						
Grand Total Genera: 52						

Numbers: Cells per mL
nd: Not observed
0: Observed but not counted in Haemacytometer chamber

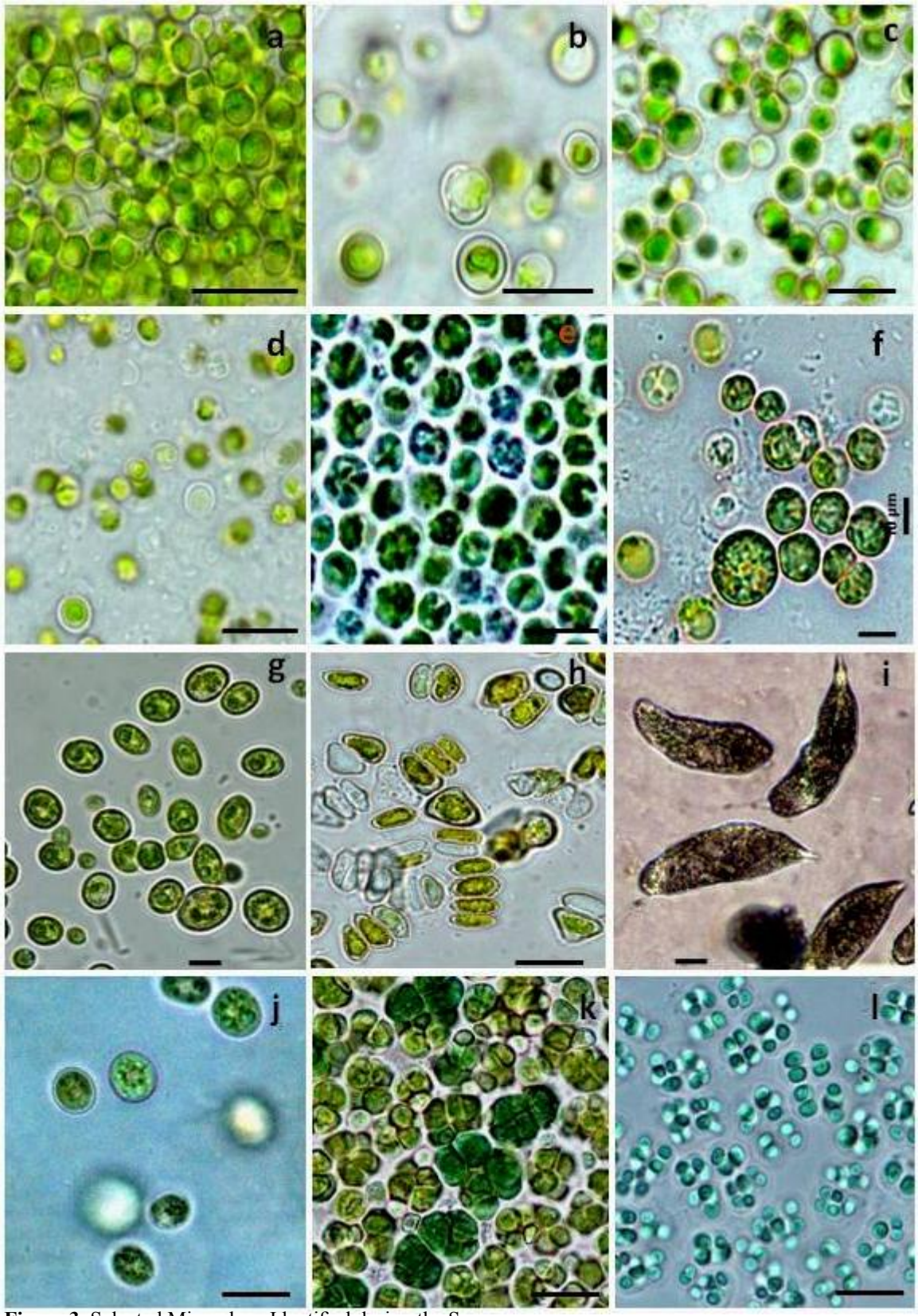


Figure 3. Selected Microalgae Identified during the Survey
a.Chlorella vulgaris; b. Chlorella volutis; c. Chlorella vulgaris var. autotrophica; d.Chlorella rotunda; e.Chlorella vulgaris var. viridis; f. Chlorella sorokiana; g. Chlamydomonas; h.Scenedesmus bijugatus; i.Euglena; j.Chlorococcum vitiosum; k.Cyanosarcina chroococcoides; l.Aphanocapsa planctonica. Scale bars: 10 µm

Table 7. Growth and Biochemical Composition (range) of Selected Algal Genera

Taxa	*Cyanosarcina*	*Chlorella*	*Chlamydomonas*	*Chlorococcum*	*Scenedesmus*	*Euglena*
Habitats	Eutrophicated Lake; Palm Oil Mill	Hot Springs; Eutrophicated Lake; Palm Oil Mill; Kelantan Coast	Palm Oil Mill	Eutrophicated Lake	Eutrophicated Lake	Eutrophicated Lake
Specific growth rate, μ (per day)	0.18 – 0.44	0.27 – 0.77	0.57 – 0.62	0.69 – 0.83	0.67 – 0.75	0.827
Biomass Productivity, day 8 (mg/L/d)	1.44 – 1.88	9.99 – 56.70	23.68 – 33.05	6.89 – 18.33	10.58 – 18.92	4.99
Biomass Productivity, day 12 (mg/L/d)	2.56 – 3.07	12.46 – 42.91	37.59 – 48.48	12.31 - 38.31	18.32 – 18.62	12.14
Lipid Productivity, day 8 (mg/L/d)	0.21 – 0.44	2.14 – 16.85	4.19 – 11.77	1.62 – 5.04	3.45 – 3.73	1.48
Lipid Productivity, day 12 (mg/L/d)	0.37 – 0.39	2.62 – 18.65	7.45 – 21.00	6.00 – 14.33	4.75 – 5.07	4.89
Carbohydrate Productivity, day 8 (mg/L/d)	0.0 – 0.04	0.23 – 4.42	0.56 – 0.72	0.25 – 0.44	0.36 – 0.45	0.17
Carbohydrate Productivity, day 12 (mg/L/d)	0.05 – 0.06	0.24 – 4.38	0.54 – 0.93	0.40 – 0.63	0.26 – 0.93	0.27
Protein Productivity, day 8 (mg/L/d)	0.09 – 0.12	0.85 – 5.90	2.42 – 3.88	1.48 – 2.49	1.55 – 2.40	0.95
Protein Productivity, day 12 (mg/L/d)	0.12 – 0.14	1.64 – 7.69	1.5- - 1.88	1.12 – 1.31	0.75 – 2.24	0.67

DISCUSSION

The survey of tropical algae from different habitats in Peninsular Malaysia occurred over seven months from April 2013 till October 2013. With the objective of isolating tropical algae with potential to be used as feedstocks for aviation biofuel production, suitable properties like fast growth, high biomass productivity, lipid, carbohydrate and protein productivities, were considered (Griffiths and Harrison 2009; Hempel et al. 2012). The initial approach was to isolate the algal colonies which were quickly established and those that dominated the agar plates during isolation. This papers reports only the initial growth and biochemical profiling of selected genera. The detailed results will be published in following publications.

The collections sites represented habitats ranging from freshwater eutrophicated lake, to the very polluted palm oil mill ponds, to hot springs and the coastal sites of Kelantan which had freshwater, brackish water and marine habitats. As expected diatoms formed the main taxa identified for the marine and brackish water habitats of Kelantan and Port Dickson. Kelantan had the highest number of taxa (43) identified. In the polluted and highly organic POME ponds, the samples were dominated by the Euglenophytes, Chlorophytes, while the Chlorophytes (*Pediastrum, Chlorella*) and Cyanophytes (*Oscillatoria*) dominated the eutrophicated lake. The Euglenophytes are tolerant of high organic content while the diatoms and desmids are characteristic of oligotrophic waters (Phang and Leong 1987). The hot springs with water temperatures ranging from 40 to 68ºC, presented an extreme environment and although the conductivity was highest, indicating rich mineral contents, only eight taxa were identified, with *Synechococcus* (Cyanophyte) and *Chlorella* dominant. The Sorenson's Similarity Index, S, was used to compare the taxa identified from the different collection sites (Table 8). The two marine habitats, Port Dickson and Kelantan, share the highest similarity (S=0.448) in algal taxa identified, followed by Port Dickson and the Hot Springs (S=0.348). However, it must be noted that the checklist used for the calculation of the Sorensen's Similarity Index is based on identification up to the genus level only.

Of the six genera profiled for growth and biochemical composition, the genera with potential to be used as biofuel feedstocks may be the Chlorophytes *Chlamydomonas* (21.00 mg/L/d) and *Chlorella* (18.65 mg/L/d), with high lipid productivities, for production of biodiesel. A true *Chlorella* UMACC 050 was reported to have the highest biomass productivity (600 mg/L/d) day^{-1}) amongst 29 *Chlorella* strains isolated from Malaysia (Vello et al. 2014). The lipid productivity of the strains at exponential phase ranged from 34.53 to 230.38 mg/L/d, with *Chlorella* UMACC050 attaining the highest lipid productivity with high lipid (38.18±2.88 % dw), protein (35.4±1.0 %) and carbohydrate (15.0±0.7 % dw) contents. Lipid productivities have been reported to range from 70 (Talebi et al. 2013), to 97 (Griffiths and Harrison 2009), to 155 mg/L/d (Tang et al. 2011) in laboratory cultures and to 576 mg/L/d in outdoor photobioreactor cultures. The *Chlorella* strains in the present study, have highest carbohydrate productivities amongst the algae studied, and may be suitable for bioethanol production as well (Doan et al. 2012; Harun et al. 2010).Renaud et al. (1994), reported that Chlorophytes contain 20 to 67 % dw protein. In general the protein productivities obtained from the present preliminary study were low (up to 7.69 mg/L/d), and these algae may not be very good protein sources for nutritional purposes. It should be noted that the results are from a preliminary study based on

Table 8. Sorenson's Similarity Index, S for Comparison of Algal Flora in the different Collection Sites.

	Hot Springs	Eutrophicated Lake	Palm Oil Mill	Port Dickson	Kelantan
Hot Springs		0.214	0.242	0.348	0.235
Eutrophicated Lake			0.178	0.171	0.317
Palm Oil Mill				0.200	0.324
Port Dickson					0.448
Kelantan					

flask cultures, without optimization, only with the objective of profiling the strains isolated from the diverse habitats of Peninsular Malaysia. Optimization of biomass and lipid productivities have been conducted and will be published later.

CONCLUDING REMARKS

In this short survey, some interesting algae were identified and isolated, which proved to have potential for use as feedstocks for biofuel production. Further studies to determine the fatty acid profiles will allow further assessment of their potential use. As reported in numerous studies, the Chlorophytes, namely *Chlorella*, have high photosynthetic productivity (Morita et al. 2000) and have always been shown to be amongst the best producers of lipids and carbohydrates (Feng and Zhang 2011; Pribyl et al. 2012; Vello et al. 2014).

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