CHAPTER 4

RESULTS

4.1 Descriptions of study sites

Hypersaline area in Nakhon Ratchasima province (NP):

This area is located at Ban Kumpaya, Kham Tale So district, is natural hypersaline area which whole areas are covered by salt crystals (Figure 5). During the first time of survey, this area was wild, without agricultural activities. However, local people used this area for feeding their animals (buffalo, cows and others). It was found only few species of halophyte plants and weeds. This area is very interesting and cyanobacteria are more or less presented. Unfortunately, this area was later drastically modified and developed for culturing the *Eucalyptus* plants (family Myrtaceae) and no one is allowed to go inside that area.

Udon Thani (UT), Sakon Nakhon (SN) and Chanthaburi (CP) saltworks:

The first two are located in Northeastern part of Thailand and the third located (CP) at eastern part of Thailand. All these areas are used for the same purpose, that is for salt productions, but the difference is that UT and SN are used for rock-salt productions and CP used for sea-salt productions. The main characteristic of all these localities is the same; the soil surface of all areas were crushed many times until soil particles, which were tightly packed on the soil surface. Cyanobacteria are not present even in rainy season in these areas.

Petchaburi solar saltern:

Petchaburi is a province, located in center part where connected with the gulf of Thailand. The seasons of this area can be separated into 3 periods: (1) Summer (middle of February-middle of May); (2) the rainy season (middle of May-middle of October) and (3) cold dry season (middle of October-middle of February). Because this province is located nearby the Gulf of Thailand, it is affected by west-south monsoon in rainy season and north-east monsoon in cold dry season. Cyanobacteria are always present throughout the year.

The information about climatology of the main study area (Petchaburi) are important as well. In the year 2006-2010, the lowest and highest temperature of the whole areas are 15 °C (2009) and 37.5 °C (2009) respectively and the average temperature of 5 years is around 28 °C (Figure 9). The statistics of precipitation between 2008-2010 of Petchaburi area are as follows: the average of rainy-days is 105 days . In addition, total rain-falls was more than 850 mm, and ranged from 871-1,169 mm per year (Figure 10-11). In October was the highest amount of precipitations (Figure 12). The relative humidity of this area in various years is not a great difference. The average mean of relative humidity is around 74 % (Figure 13). All climatic data of these areas were modified from the original data by Department of Meteorology (www.tmd.go.th).

All areas in Petchaburi are used for agriculture and the whole area nearby the sea coast is used for sea-salt production. Concerning about the Petchaburi solar saltern, the study area is approximately 14,400 m^2 and 1 km far from the sea (Chao Sam Ran beach). It was surrounded from one side by the road to Ban Laem village

and also by Laempukbia district, from the other by mangrove forest. The salt is produced throughout the whole year exceptionally in the periods of heavy rains. The salinity gradients of this area were 0-35 ppt in rainy periods and gradually increased in salt–producing periods from more than 35 ppt up to more than 400 ppt, when salt crystals are formed. In the sites with salt producing ponds, there is no occurrence of any higher plant species, however, the surrounding areas with salt fields, many species of mangrove forest were found including *Rhizophora apiculata* Blume, *Avicennia marina* (Forsskål) Vierhapper., *Thespesia populnea* (Linnaeus) Solander ex Correia and with *Suaeda maritima* (Linnaeus) Dumort. Moreover, sometimes the birds, *Ardea alba* Linnaeus and others were also found in the areas (Figure 14). Cyanobacteria were observed along the whole areas during the study.

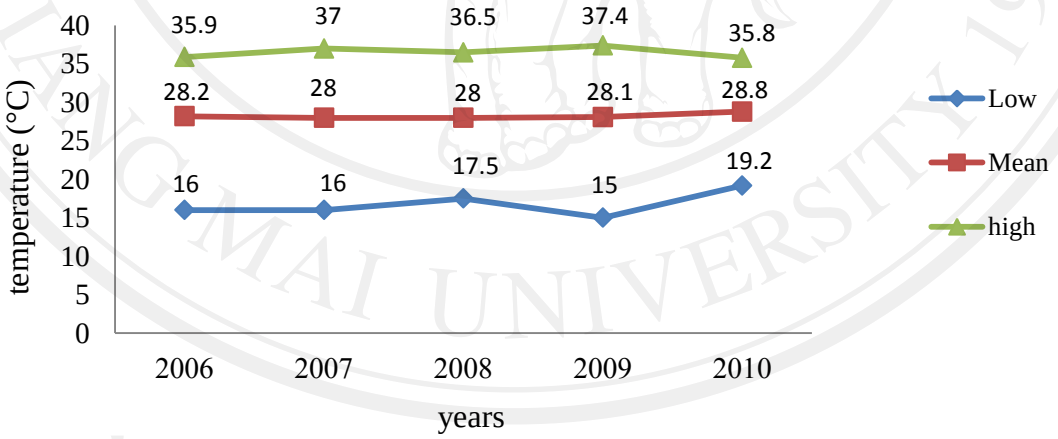

Figure 9 The highest, lowest and average temperature of Petchaburi province between the years 2006-2010 (www.tmd.go.th).

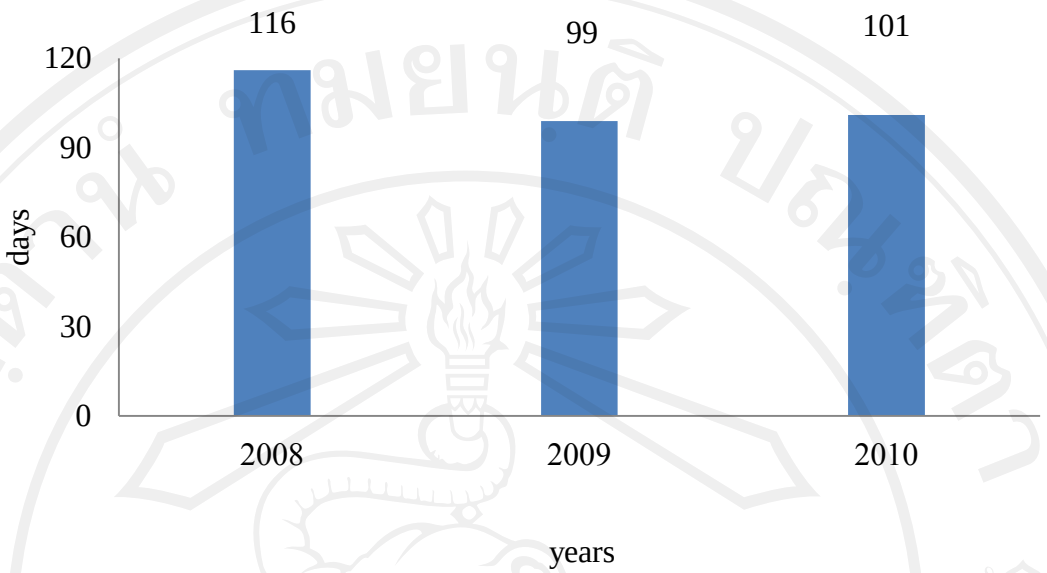

Figure 10 Rainy-days of Petchaburi province between the year 2008-2010 (www.tmd.go.th).

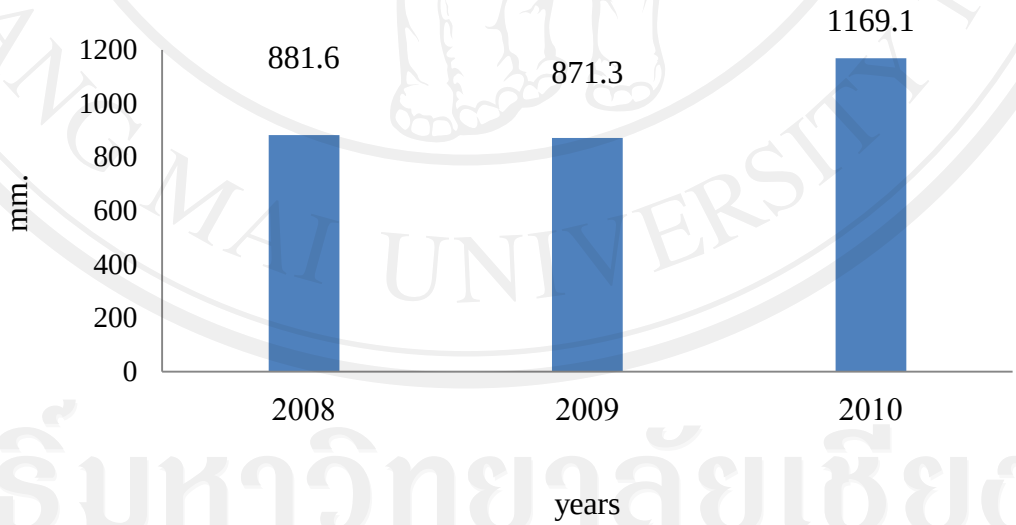

Figure 11 Total rainfalls of Petchaburi province between the year 2008-2010 (www.tmd.go.th).

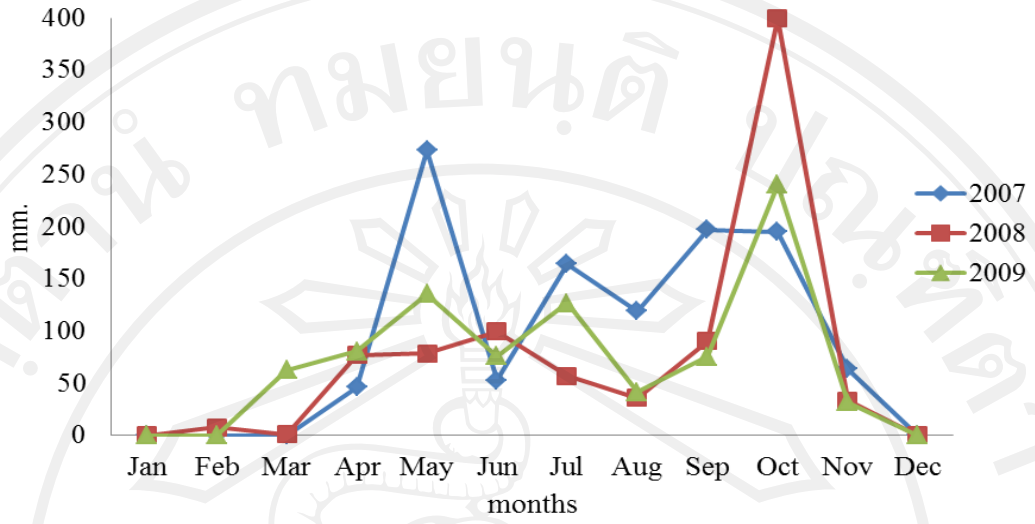

Figure 12 Monthly rainfalls of Petchaburi province between the year 2007-2009 (www.tmd.go.th).

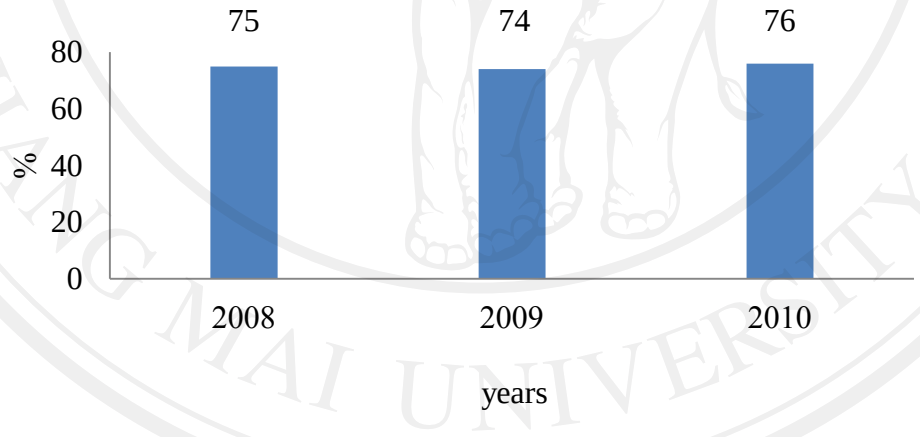

Figure 13 Percent of relative humidity of Petchaburi province between the year 2008-2010 (www.tmd.go.th).

Figure 14 Some species of plants and bird found surrounding the study areas of Petchaburi solar salterns.

(1) *Suaeda maritima* (local name is Cha - khram), (2) *Avicennia marina* (local name is Samae Talay), (3) *Rhizophora apiculata* (local name is Kongkang Bai Lek), (4) *Thespesia populnea* (local name = Pho-Talay) (5) *Ardea alba*
(Sources of illustrations: http://trnerr.org /invasives /plant.html&plantID=46[(2)]; www.niobioinformatic.in/mangroves/MANGCD/indo/p22.htm[(3)];www.wellgrowhorti.com/page/landscapeplants/Trees/Tree%20Images%20T.htm[(4)]).

Samut Songkhram solar salterns (SS):

Located in Mueang Samut Songkhram district, also in the centre part of Thailand, mainly purposed for sea-salt productions. This area is 6 km from sea coast (the Gulf of Thailand) and the characteristic features, the occurrence of higher plant species and cyanobacteria in these area is more or less similar to Petchaburi salt field.

At each study site in Petchaburi and Samut Songkhram salt fields, cyanobacteria occurred predominately onto soil surface (dry, moisture, soaking under water). Their natural characteristics was mostly presented in various forms of microbial mats, filaments and thalli were tightly packed with the soil surfaces. Sometimes they can be found underneath the soil surface and also among colonies of filamentous green alga *Oedogonium* sp. (Figure 15). The occurrence of these organisms were presented in salinity gradients ranged from 0 ppt up to 300 ppt, when salt crystals were formed.

4.2 Soil physical and chemical properties

The physico–chemical properties of soil samples from 14 sampling sites of 4 study areas were determined during February-May and October-December 2009. Each sampling site was selected according to the developement of cyanobacteria communities. It was remarkable that the site PP_2A and PP_3A occurred in the same area, but they were separated conditions because the PP_2A site is distinctly dryer than PP_3A, which was flooded by water. The texture and compositions of soils from almost all areas were similar. The soils from all study sites were classified as clay loam which composed with sand, silt and clay (Table 3).

Almost all sampling sites in this study are characterized by wet soils. The moisture contents ranged from 29.2-215.9 % of water in soils. The moisture is lowest in CP_1A and SN_1A. The mean average of moisture in Petchaburi areas is 107.17 % and Samut Songkhram is 62.75 % (Figure 16).

The pH values of soils from each sampling site are only slightly different. The pH of all areas is in range of neutral to weak base (7.2-8.1) except only 2 sites: CP_1A and SN_1A where the pH is distinctly lower (5.03 and 4.98 respectively). The pH value

of other sampling sites in the same areas does not differ substantially from the others (Figure 17).

The salinity of most sampling sites was determined by salinometer (salt refractometer). It varied independence on the process of salt productions, namely at Petchaburi saltern areas, where salinity ranged from 53-249 ppt. In the first periods of sampling, salinity is lower than in the second, because it was in the early step of salt productions (the time of preparing areas and early of evaporization steps). In Samut Songkhram, salinity is 35 ppt up to saturated (300 ppt) and in CP_1A and SN_1A are 355 ppt and 400 ppt, respectively (Figure 18).

In soil sciences, electrical conductivity (EC) is a method used to classify levels of salt concentration in soils samples. This value also refers to salinity or ions in soils such as Na^+, Ca^{2+}, Cl^-. The soils that have EC more than 2 dS/m, are classified as saline soils (U.S. soil salinity laboratory Staff, 1954). The EC of all sampling areas are higher than 2 ds/m. In Petchaburi areas EC ranged from 164-398 dS/m, and 84-346 dS/m in Samut Songkhram, 296.1 ds/m in CP_1A and SN_1A 30 ds/m respectively (Figure 19).

The organic matter in soils derived from decaying of animals or plants, is necessary for the growth of plants and algae. In this study, organic matters of each area is not highly different. It presented from 1.5-3.74 g/100 g and the highest value is in PP_2B (Figure 20).

Figure 15 The natural characteristics of cyanobacterial mats presented at Petchaburi and Samut Songkhram areas.

(1-5) mats and thalli of cyanobacteria (soaking); (6-7) dry mats and cyanobacterial colonies under the soil surface (red arrow); (8) filaments of green alga *Oedogonium* sp. (red arrow).

Table 3 Textures and composition of soil samples from 4 study sites during February-May and October to December 2009.

Study sites	Sand (%)	Silt (%)	Clay (%)	Texture
Petchaburi (PP)	34	28.4	37.6	clay loam
Samut Songkhram (SS)	25.1	36.3	38.6	clay loam
Chantaburi (CP)	36.6	35.2	28.2	clay loam
Sakhon Nakhon (SN)	26.5	44.6	28.9	clay loam
Nakhon Ratchasima (NR)	ND	ND	ND	ND
Udon Thani (UT)	ND	ND	ND	ND

Notes: the texture and composition of soil samples were analyzed by the samples from Febuary-May 2009 only because the changing process in soil texture and compositions will take more than 50 years; ND = non detected.

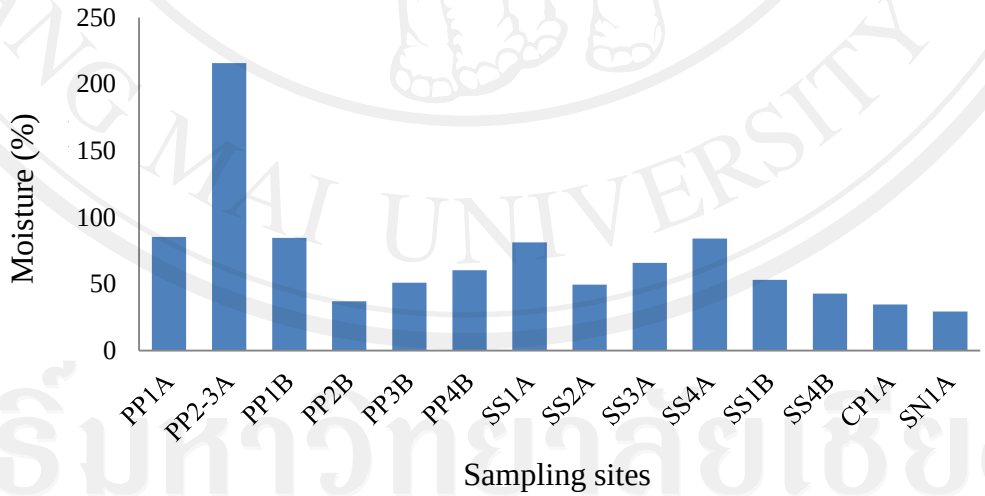

Figure 16 Moisture content (% values of water in soil samples) of 14 sampling sites from 4 study areas during February-May (PPA, SSA, CPA and SNA) and October-December (PPB and SSB) 2009.

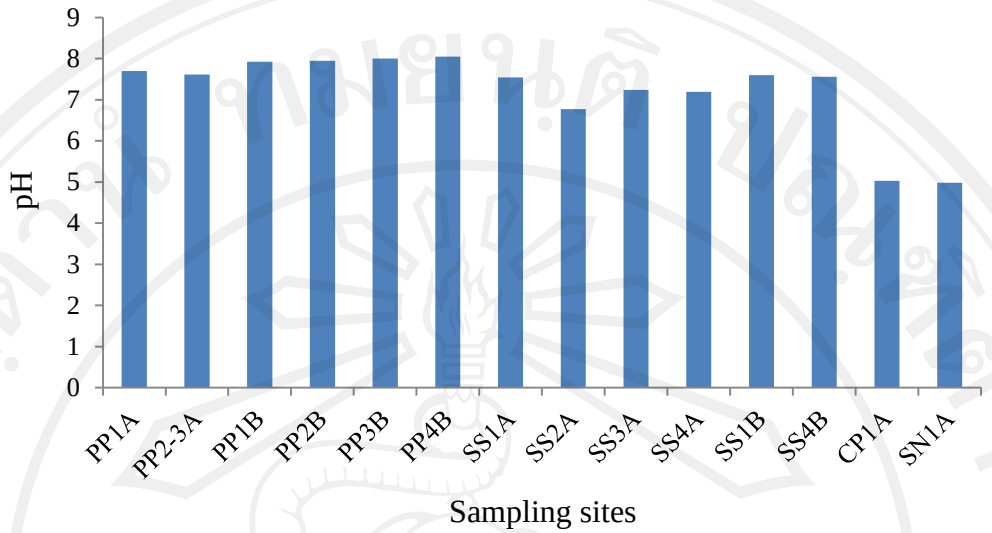

Figure 17 The pH values of soil samples of 14 sampling sites from 4 study areas during February-May (PPA, SSA, CPA and SNA) and October-December (PPB and SSB) 2009.

The nitrogen is essential for all life forms (animals, plants, algae and cyanobacteria). Cyanobacteria can utilize nitrogen only in inorganic forms such as NO_3^- and Ammonium (NH_3^+) that is usually presented as NH_4^+ form. Nitrate is the most abundant source of combined nitrogen for cyanobacterial nutrition (Roger and Kulasooriya, 1980). In this study NH_4^+-N and NO_3^--N were measured. The results of both values were reported in Figure 21 and 22.

Phosphorus (P), Potassium (K) and Sulfur (S) are also essential nutrients for cyanobacteria. They require these elements for their metabolic pathways. Phosphorus is constituent of high energy compound: Adenosine triphosphste or ATP that important for all organisms on earth. This element helps in the biosynthesis of nucleotides, nucleic acids, lipids and covalently modified proteins in cyanobacteral metabolism (Roger and Kulasooriya, 1980; Roger *et al*, 1987) Sulfur is needed for

amino acid synthesis and K is necessary for proteins, starch and sugar synthesis. The results of P, K and S of this study showed that P ranged from 2 mg/kg up to more than 130 mg/kg.

The P values are very high in Petchaburi areas but lower in CP_1A and SN_1A areas (Figure 23). On the contrary to potassium, this element showed higher value in all sampling sites which ranged from 545-4,349 mg/kg (Figure 24). About sulfur, it ranged from 1.9-12.42 mg/kg. S value in Petchaburi areas between each sampling site is not much different. However, in Samut Songkhram sampling sites, the S value vary from lowest to highest (2 to 12 mg/kg) (Figure 25).

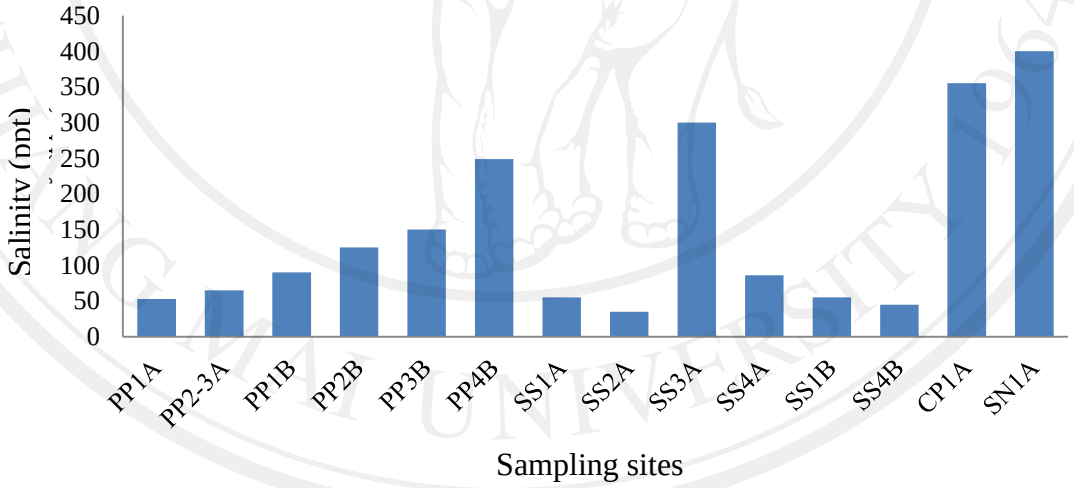

Figure 18 Salinity levels of soil samples from 14 sampling sites in 4 study areas during February-May (PPA, SSA, CPA and SNA) and October-December (PPB and SSB) 2009.

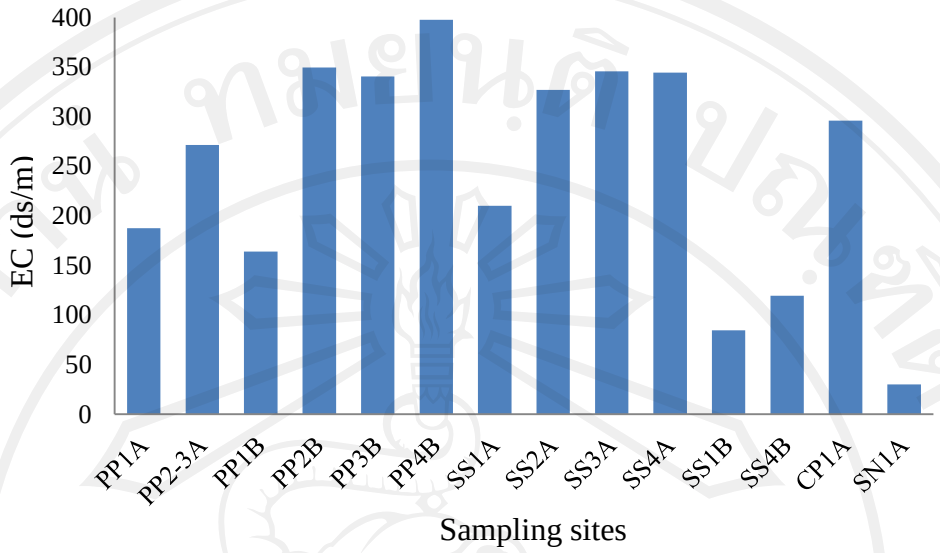

Figure 19 The electrical conductivity (EC) of 14 soil sampling sites from 4 study areas during February-May (PPA, SSA, CPA and SNA) and October-December (PPB and SSB) 2009.

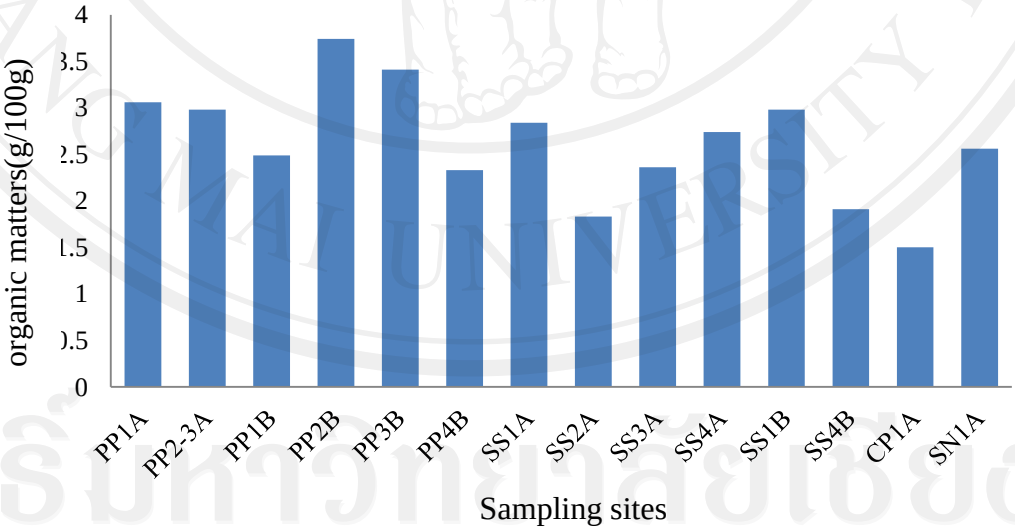

Figure 20 The organic matters values of 14 soil sampling sites from 4 study areas during February-May (PPA, SSA, CPA and SNA) and October-December (PPB and SSB) 2009.

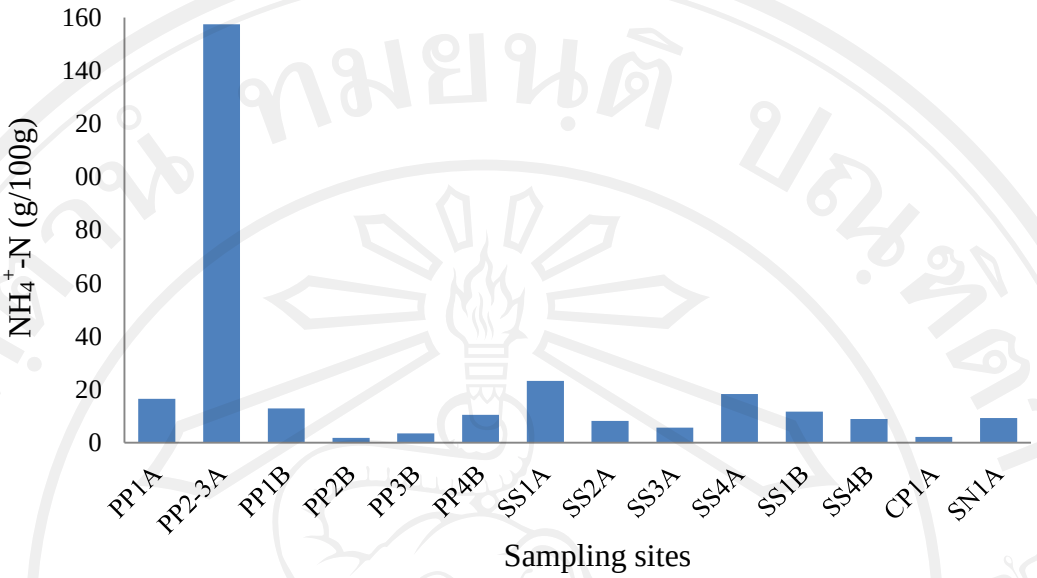

Figure 21 Ammonium nitrogen (NH_4^+-N) values of 14 soil sampling sites from 4 study areas during February-May (PPA, SSA, CPA and SNA) and October-December (PPB and SSB) 2009.

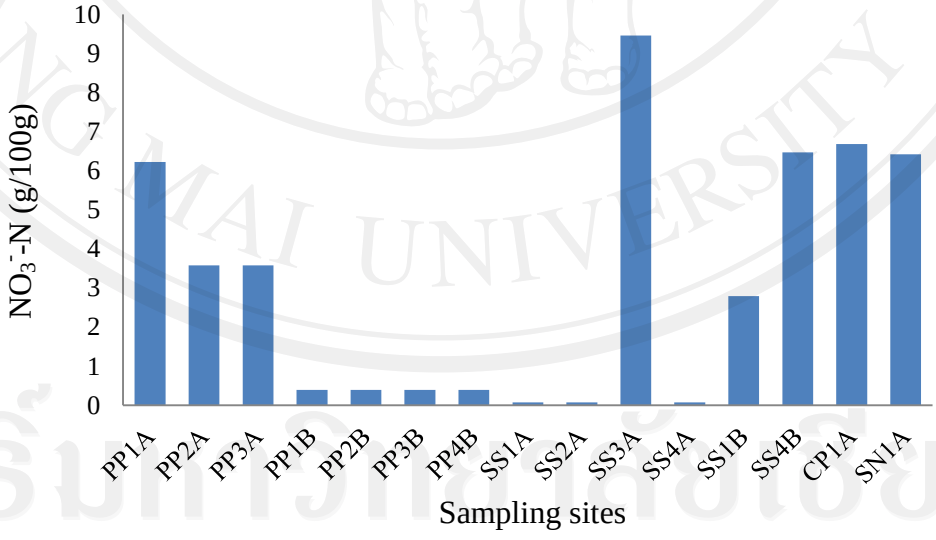

Figure 22 Nitrate nitrogen (NO_3^--N) values of 14 soil sampling sites from 4 study areas during February-May (PPA, SSA, CPA and SNA) and October-December (PPB and SSB) 2009.

Concerning about Manganese (Mn) and Iron (Fe), both of them are micronutrients which cyanobacteria require only in small amounts. However, all of these elements still necessitate for cyanobacterial metabolisms. The Mn is high in Petchaburi sampling sites but lower in CP_1A and SN_1A (Figure 26). Irons ranged from 14-102 mg/kg. The value of Fe ions does not differ substancially in all sampling sites except in SN_1A that the value is very high (102.03 mg/kg) (Figure 27).

Sodium ion (Na^+), calcium ion (Ca^{2+}) and magnesium ion (Mg^{2+}) are also important for cyanobacteria and other algae. Several cyanobacteria are considerable halophilic or halotolerant tosalt (NaCl) (Padhi *et al*, 1998). They usually require Na^+ for their growth, nitogen fixation, nitrate reduction and intracellular pH regulation. However, for non-halophilic cyanobacteria, high concentration of NaCl inhibitory effect on the cyanobacterial cells, inhibition of growth, declining rate of photosynthesis and respiration (Allen and Arnan, 1955; Apte and Thomas, 1980; Batterton and Van Baalen, 1971; Vonshak *et al*, 1988).

Other ions including Ca^{2+} and Mg^{2+} are also the main components of salt compound in saline soils (similarly to Na^+) but in different areas are present in different ratio of ions. From this study sampling sites showed very high values of Na^+ than Ca^{2+} and Mg^{2+} respectively ($Na^+ > Ca^{2+} > Mg^{2+}$) . The exception is in PP_2B, PP_4B and SS_4B where Ca^{2+} is higher than Na^+ ($Ca^{2+} > Na^+ > Mg^{2+}$) and in SS_2A is $Na^+ > Mg^{2+} > Ca^{2+}$ (Figure 28-30).

Chlorides ranged from 4.4-8.37 g/100g in Petchaburi sampling sites and the greatest difference between lowest and highest of Cl^- values is in Samut Songkhram localities. It was ranged from 1.95-12.42 g/100g. The Cl^- concentration is very similar in CP_1A and SN_1A (Figure 31).

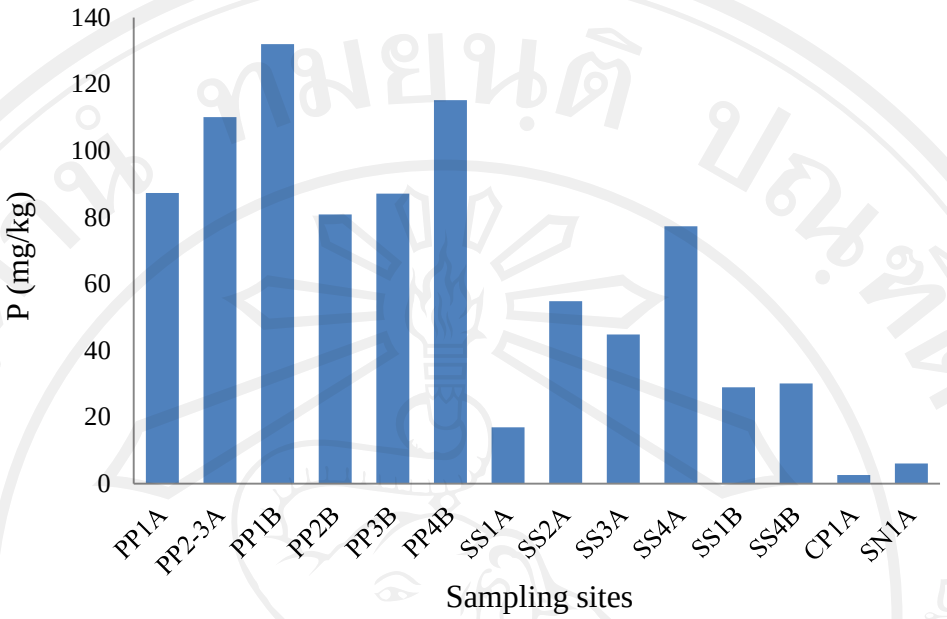

Figure 23 Phosphorus (P) values of 14 soil sampling sites from 4 study areas during February-May (PPA, SSA, CPA and SNA) and October-December (PPB and SSB) 2009.

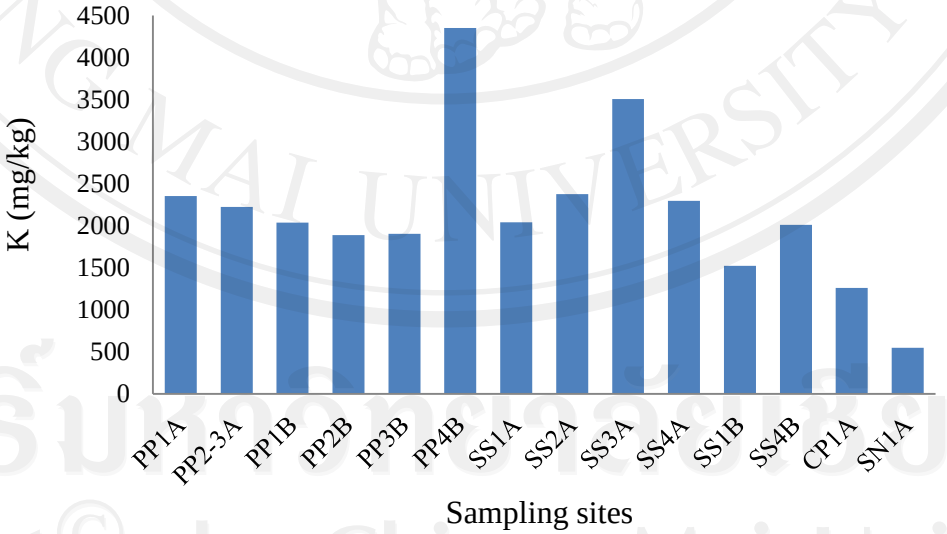

Figure 24 Potassium (K) values of 14 soil sampling sites from 4 study areas during February-May (PPA, SSA, CPA and SNA) and October-December (PPB and SSB) 2009.

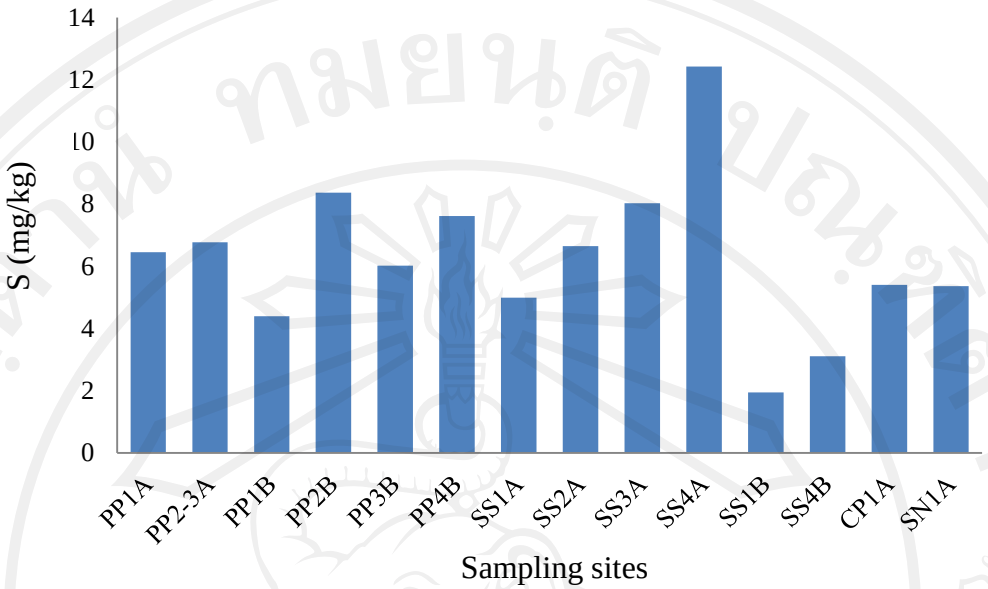

Figure 25 Sulfur (S) values of 14 soil sampling sites from 4 study areas during February-May (PPA, SSA, CPA and SNA) and October-December (PPB and SSB) 2009.

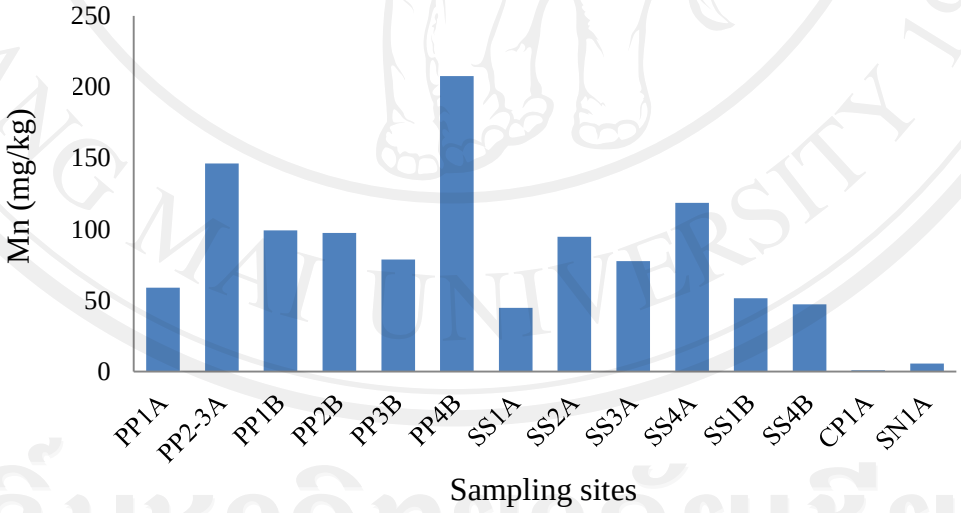

Figure 26 Manganese (Mn) values of 14 soil sampling sites from 4 study areas during February-May (PPA, SSA, CPA and SNA) and October-December (PPB and SSB) 2009.

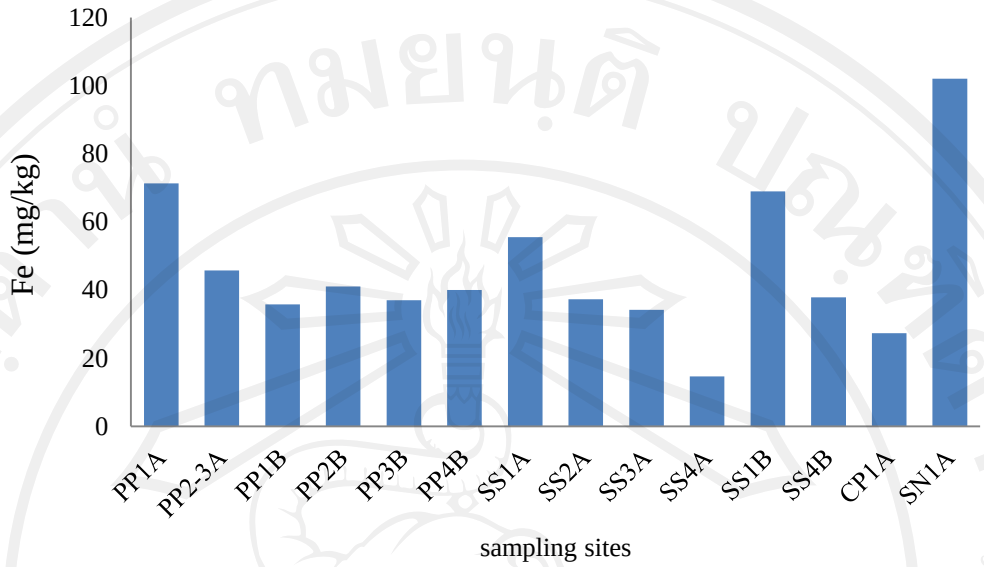

Figure 27 Iron (Fe) values of 14 soil sampling sites from 4 study areas during February-May (PPA, SSA, CPA and SNA) and October-December (PPB and SSB) 2009.

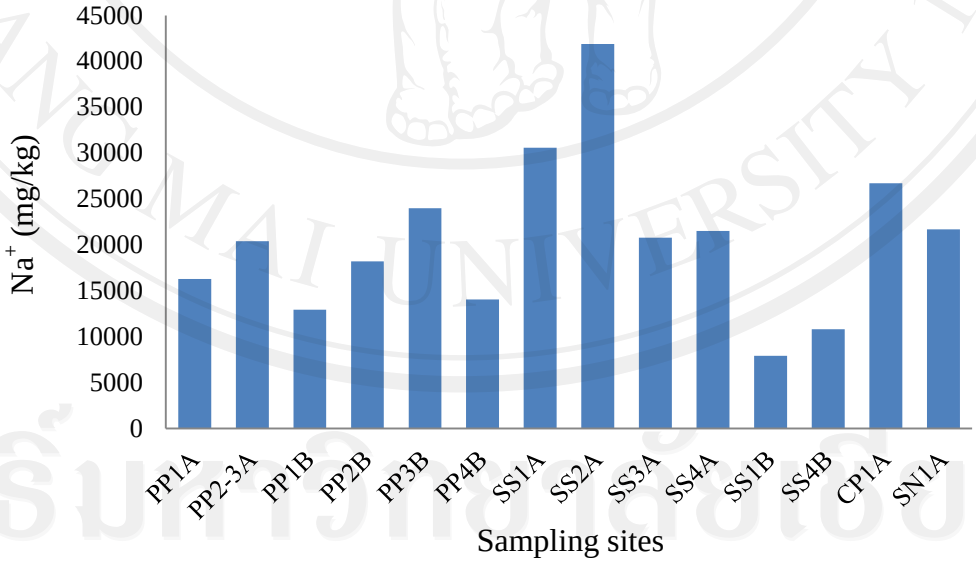

Figure 28 Sodium ion (Na^+) values of 14 soil sampling sites from 4 study areas during February-May (PPA, SSA, CPA and SNA) and October-December (PPB and SSB) 2009.

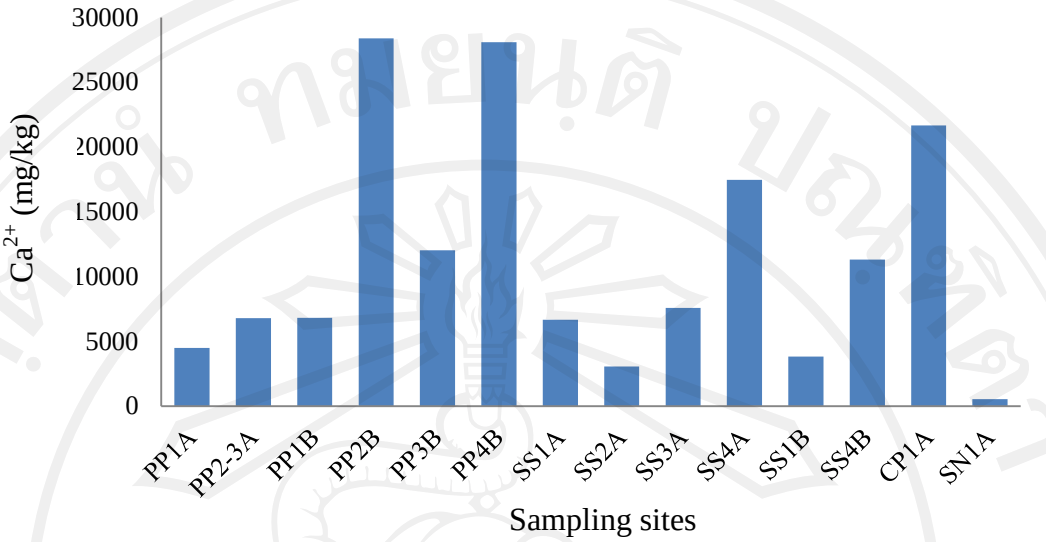

Figure 29 Calcium ion (Ca_2^+) values of 14 soil sampling sites from 4 study areas during February-May (PPA, SSA, CPA and SNA) and October-December (PPB and SSB) 2009.

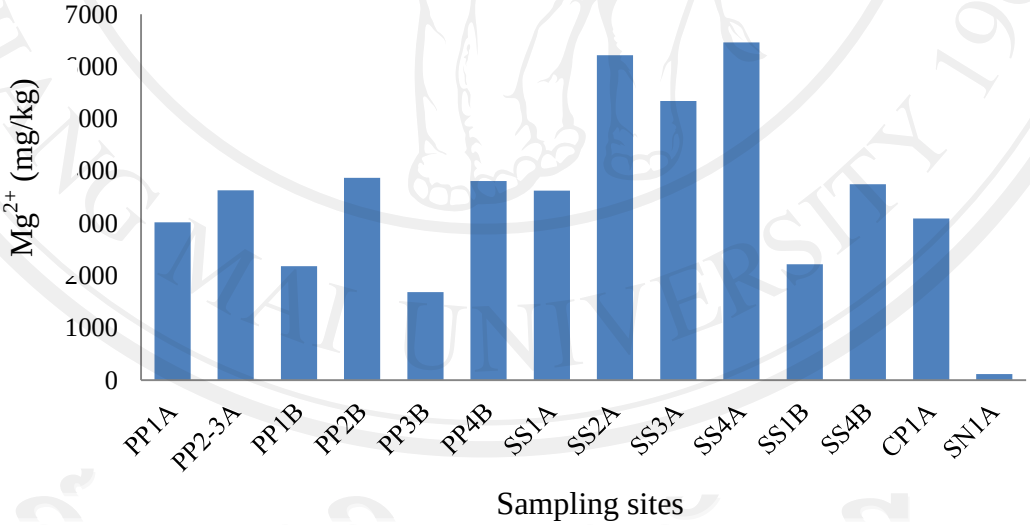

Figure 30 Magnesium ion (Mg^{2+}) values of 14 soil sampling sites from 4 study areas during February-May (PPA, SSA, CPA and SNA) and October-December (PPB and SSB) 2009.

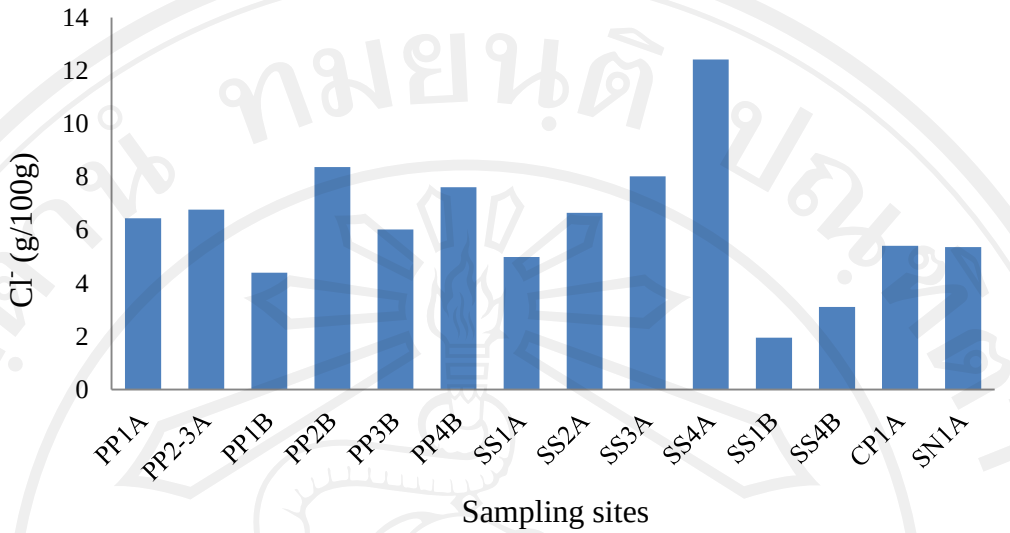

Figure 31 Chloride ion (Cl^-) values of 14 soil sampling sites from 4 study areas during February-May (PPA, SSA, CPA and SNA) and October-December (PPB and SSB) 2009.

4.3 Evaluation of cyanobacterial morphotypes

Numerous forms (colonies, mats, thallus, filaments, etc.) of cyanobacterial samples were collected from 3 localities with different salinities (from 4 up to 300 ppt - see also Table 4) namely from Petchaburi solar salterns (P_1A, $P_{2\text{-}3}A$, P_1B, P_2B and P_3B), Samut Songkhram saltworks (S_1A, S_3A, S_1A, S_1B and S_4B) and Sakon Nakhon solar saltern (SN). In total, twenty-three genera with thirty-three taxa of cyanobacteria were found (Table 4). They belong to order Chroococcales (39.4 %), Synechococcales (6.1 %) Pseudanabaenales (15.2 %), Oscillatoriales (30.3 %), and Nostocales (9 %), respectively. Most of them were commonly found in filamentous forms. The dominant species were *Coleofasciculus chthonoplastes*, *Oxynema thaianum* and *Spirulina subsalsa*. Moreover, most of cyanobacterial species found during this study were revealed to be new taxa records of Thailand when comparing to Lewmanomont

et al, (1995) and Peerapornpisal (2005). It included *Johannesbaptistia pellucida*, *Aphanocapsa* cf. *salina*, *Geitlerinema lacus-solaris*, *Xenococcus schousboei*, *Halomicronema* sp. *Halothece* sp. and *Wollea* sp. In addition, the polyphasic approaches combined with morphology, ecophysiological characteristics, molecular and ultrastructure evaluations were applied to study some important strains namely *Phormidium lloydianum* (= *Oxynema thaianum*). The results are evidently that this species does belong to *Phormidium* and *Oscillatoria* group, and therefore it should be transferred to a new genus. In this case, we designated the new genus *Oxynema* (see more details in *Oxynema thaianum* part).

Sixteen taxa have been reported in Chatchawan *et al*. (2011) already and reviews of taxonomy of new taxa recorded found from salterns of Thailand is published in AGRC conference's proceedings. Among these, 1 genus (*Oxynema*) was new to science, with the type species. *Oxynema thaianum* **comb. nov**., **spec. nov.** [Cryptogamie/Algologie, Chatchawan *et al*., 2012]. The morphological and taxonomic characteristics of all species found in this study were described in taxonomic part of this chapter. The number of cyanobacterial species declined with increasing of salinities (Table 4).

However, not only cyanobacteria but also some algal species from the Division Chlorophyta and Division Bacillariophyta (Table 5) were found during this study. In nature, cyanobacteria commonly were found mixed with other group of algae such as green algae and diatoms. In this study, it was found that cyanobacteria usually composed their colonies or mats together with many species of diatoms. In the case of green alga *Dunaliella* sp., it occurred in high numbers, and after caused the

orange mucilaginous layer on the surface of water (only in Petchaburi study sites), especially when salinities excess approximately 150 ppt.

4.3.1 Taxonomic part

All species found during this study (Table 4) were described (morphology, other criteria and occurrence) as follows:

1. *Aphanocapsa* cf. *salina* Voronichin, 1929 [Figure 32(1), 45(2)].

Descriptions: Colonies spherical, microscopic. Cells, blue-green, spherical, about 0.7-1.2 μm in diameter, more clustered.

Occurrence: It was found in blue-green mats on wet soil surface where salinity around 50 ppt.

Notes/remarks: This species probably belongs to *Aphanocapsa salina* from description of Komárek and Anagnostidis (1998) according to the size, shape and ecology. However, it identification still needs further information.

2. *Aphanothece halophytica* Frémy in Hof et Frémy , 1933 [Figure 33(2-3), 45(1)].

Descriptions: Colonies multicellular, macroscopic. Envelopes present, colourless, firm. Cells blue-green to olive-green, widely oval to cylindrical, arranged loosely and distributed in many direction, 6-7 μm long and 3-4 μm wide. Cell content net-like, granulated. Cell division in one plane perpendicular to longitudinal of cell axis.

Occurrence: It usually grows among other cyanobacteria in blue-green to brownish mats or wet soil surface. This species was found only in Petchaburi salterns where salinity ranged from 90-150 ppt.

Table 4 Diversity and abundance of cyanobacterial species from nature and cultures (from soils) found in Petchaburi, Samut Songkhram and Sakon Nakhon solar salterns in evaporization periods with different salinities.

Cyanobacterial morphotypes	PP_1A	$PP_{2-3}A$	PP_1B	P_2B	P_3B	P_4B	S_1A	S_3A	S_4A	S_1B	S_4B	SN*	Remarks
	53‰	65‰	90‰.	125‰	150‰	249‰	55‰	300‰	86‰	55‰	45‰	4 ‰	
Order Synechococcales													
Aphanocapsa cf. *salina* Voronichin	+	-	-	-	-	-	-	-	-	-	-	-	N
Aphanothece halophytica Hof et Frémy	-	+	+	+	+	-	-	-	-	-	-	-	N
Order Chroococcales													
Chroococcus cf. *submarinus* (Hansgirg) Kováćik	++	+	-	-	-	-	-	-	-	-	-	-	N
Chroococcus sp.1	+	+	-	+	-	-	-	-	-	-	-	-	N
Chroococcus sp.2	+	+	-	-	-	-	-	-	-	-	-	-	N
Cyanobium sp.	-	-	-	-	-	-	++	-	-	-	++	-	C
Cyanosarcina sp.1	+	-	+	-	-	-	+	-	-	-	-	-	N
Gloeocapsa sp.	+	-	-	-	-	-	-	-	-	-	-	-	N
Halothece sp.1	-	+	+	-	-	-	-	-	-	+	+	-	N
Halothece sp.2 (*Halothec*e-like)	-	-	-	-	-	-	++	-	-	+	-	-	C
Johannesbaptistia pellucida (Dickie) Taylor et Drouet	+	+	++	-	-	-	-	-	-	-	-	-	N
Merismopedia sp.	-	-	-	-	-	-	-	-	-	-	+	-	N
Pseudacapsa sp.2	+	-	-	-	-	-	+	-	-	-	-	-	N
Xenococcus schousboei Thuret	-	-	-	-	-	-	-	-	-	++	-	-	N
Spirulina subsalsa Oersted ex Gomont	++	+++	+++	++	++	+	+	-	+++	-	-	-	N

Notes: +++ = dominant, present > 30 % of population; ++ = common, present > 10 – 30 % of population; + = present 1 – 10 % of population; - = not present; * = not salinic locality (the samples were collected from SN salterns after rainy); N = species found in nature only; C = species found in culture only; NC = species found in both nature and culture; ‰ = salinity (ppt).

Table 4 (continue).

Cyanobacterial mophotypes	P_1A	$P_{2-3}A$	P_1B	P_2B	P_3B	P_4B	S_1A	S_3A	S_4A	S_1B	S_4B	SN*	Remarks
	53‰	65‰	90‰.	125‰	150‰	249‰	55‰	300‰	86‰	55‰	45‰	4‰	
Order Pseudoanabaenales													
Geitlerinema lacus-solaris (Campbell et Golubic) Anagnostidis	++	+	-	-	-	-	-	-	-	-	-	-	N
Geitlerinema sp.	+	-	-	-	-	-	-	-	-	-	-	-	C
Halomicronema sp.	+	+	+	+	+	-	-	-	-	-	-	-	NC
Pseudanabaena sp.	+	+	-	-	-	-	-	-	-	-	-	-	NC
Trichocoleus sp.	-	-	-	-	-	++	-	-	-	-	-	-	NC
Order Oscillatoriales													
Coleofasciculus chthonoplastes (Thuret es Gomont) Siegesmund *et al*.	+	+++	+++	++	+	+	++	+++	++	++	+++	-	N
Komvophoron cf. *skujae* Anagnostidis	-	-	-	-	-	-	-	-	-	-	-	+++	N
Komvophoron sp.	+	+	+	+	-	-	-	-	-	-	+	-	N
Leptolyngbya sp.1	++	+	+	-	-	-	-	-	-	-	-	-	NC
Leptolyngbya sp.2 (spiral form)	+	+	+	+	-	-	-	-	-	-	-	-	NC
Leptolyngbya sp.3	-	-	-	-	-	-	-	-	-	-	+	-	N
Lyngbya cf. *aestuarii* Liebman ex Gomont	-	-	-	-	-	-	-	-	-	+	-	-	N
Lyngbya sp.	+	+	+	+	-	-	-	-	-	-	+	-	N
Oxynema thaianum Chatchawan et al.	++	++	+++	++	++	-	++	-	++	+	+	-	NC
Tychonema sp.	-	-	-	-	-	-	-	-	-	++	-	-	N
Order Nostocales													
Nostoc sp.1	-	-	+	+	-	-	-	-	-	-	-	-	C
Nostoc sp.2	-	-	+	-	-	-	-	-	-	-	-	-	C
Wollea sp.	-	-	+	-	-	-	-	-	-	-	-	-	C
Total number of species	**19**	**16**	**15**	**10**	**5**	**3**	**7**	**1**	**3**	**7**	**8**	**1***	

Table 5 Diversity and abundance of other algal species from nature and cultures (from soils) found in Petchaburi, Samut Songkhram and Sakon Nakhon solar salterns in evaporization periods with different salinities.

Morphotypes	P_1A 53‰	$P_{2\text{-}3}A$ 65‰	P_1B 90‰.	P_2B 125‰	P_3B 150‰	P_4B 249‰	S_1A 55‰	S_3A 300‰	S_4A 86‰	S_1B 55‰	S_4B 45‰	SN* 4‰	Remarks
Division Chlorophyta													
Oedogonium sp.	++	+	-	-	-	-	-	-	-	-	-	-	N
Dunaliella sp.	-	-	-	-	++	+++	-	-	-	-	-	-	N
Division Bacillariophyta													
Amphora cf. *oligotrophenta* Lange - Bertalot	+++	-	-	-	-	-	-	-	-	-	-	-	N
*Gyrosygma scalproide*s Rabenhorst	-	+	-	-	-	-	-	-	-	-	-	-	N
Nitzschia sp. 1	-	+	-	-	-	-	+	-	-	-	-	-	N
Nitzschia sp. 2	-	+	-	-	-	-	+	-	-	-	-	-	N
Navicula sp.1	++	-	-	-	-	-	-	-	-	+	-	-	N
Navicula sp.2	+	-	-	-	-	-	-	-	-	+	-	-	N

Notes: (+++) = dominant, present > 30 % of population; (++) = common, present > 10 – 30 % of population; + = present 1 – 10 % of population; (-) = not present; * = not salinic locality (the samples were collected from SN salterns after rainy); N = species found in natural only; ‰ = salinity (ppt).

Notes/remarks: This species is similar to *Aphanothece halophytica* of Hof and Frémy. However, this species is designated and published by many authors in different senses and conceptions such as Yopp *et al*., 1978; Golubić 1980; Dor and Hornoff, 1985; Campbell and Golubić, 1985 and others. The taxonomy of this species is still unclear and need revision.

3. *Chroococcus* cf. *submarinus* (Hansgirg) Kováćik, 1988 [Figure 32(4), 45(3)].

Synonyms: *Chroococcus turgidus* var. *submarinus* Hansgirg 1889; *Chroococcus turgidus* var. *hookeri* Lagerheim 1883.

Descriptions: Cells olive-green, spherical, hemispherical or subspherical, approximately 11-16 μm in diameter with sheaths. Cell contents homogeneous with net-like structure with granules. Sheaths colourless, fine, firm, narrow and delimited, usually two cells together in a sheath.

Occurrence: It was found in the blue-green mats on wet soil surfaces.

Notes/remarks: The population from this study are quite similar to the description of Komárek and Anagnostidis 1998. This species was reported in many hypersaline biotopes throughout the world.

4. *Chroococcus* sp.1 [Figure 32(5), 45(4)].

Descriptions: Solitary, microscopic colonies. Cells olive-green, hemispherical, 8-9.5 μm in diameter with sheaths up to 18 μm in diameter. Cell contents homogeneous. Sheaths firm, delimited, colourless, usually two gathered cells in a sheath.

Occurrence: It was rarely found in blue-green mats on wet soils surface, solitary among other cyanobacteria.

Notes/remarks: This species does not in agreement with any described species.

5. *Chroococcus* sp.2 [Figure 32(6), 45(5)].

Descriptions: Solitary, very microscopic colonies. Cells pale blue-green, subspherical, approximately 14.6 μm in diameter with sheaths. Cell contents homogeneous. Sheaths narrow, firm, delimited, usually with gathered cells inside the sheaths.

Occurrence: It was found in blue-green mats on wet soil surfaces among other algae and cyanobacterial species, solitary.

Notes/remarks: This species occurred rarely in our localities and could not be assigned to any known described taxon.

6. *Coleofasciculus chthonoplastes* (Thuret ex Gomont) Siegesmund *et al*, 2010 [Figure 33, 48(1)].

Synonym: *Microcoleus chthonoplastes* Thuret ex Gomont, 1982.

Descriptions: Thallus very densely compact but smooth and soft mucilaginous, dark green to olive-green, usually tightly attached on soil surface, sometimes mixed with other cyanoprokaryotes or other algae, particularly with diatoms. Sheaths present, colourless, thick, usually open at the ends. Trichomes bright blue-green, 3.2-4.3 μm wide, ± straight but sometimes slightly curved. There are many trichomes in the same sheath, usually packed in dense fascicles, with slight constrictions at cross-walls. Cell longer than wide, 3.5-4.5 μm long. Apical cells acute-conical. Hormogonia present.

Occurrence: It was usually found in blue-green mats on a wet soil surface, or sometimes in green thallus soaking in water. Thick filaments are attached to the soil surface and soaking in water. It was occurred throughout the whole study areas.

Notes/remarks: This species is cosmopolitan species (Garcia-Pichel *et al*, 1996) and distribute worldwide.

7. *Cyanobium* sp. [Figure 32(7), 46(3)].

Descriptions: Colonies blue-green. Cells usually in groups and sometimes solitary, very microscopic, oval, ± cylindrical, pale blue-green, 1.5-3 x 0.7-1.5 μm. Cell contents not clearly visible. Cells division transverse to the long axis. Mucilage not observed. Immotile.

Occurrence: It was found from (on/under) wet to little dry soil surfaces from both Petchaburi and Samut Songkhram study sites.

Notes/remarks: This species was found only in culture after the isolation. Morphological characteristics are quite similar to *Cyanobium bacillare* (Butcher) Komárek *et al*. when comparing the description of Komárek and Anagnostidis 1998 (especially range of cell sizes). But the ecology is different, therefore this species is probably not *C. bacillare*. The group of *Cyanobium* from hypersaline biotopes is still little known up to now.

8. *Cyanosarcina* sp.1 [Figure 32(8), 45(6)].

Descriptions: Colonies microscopic, hemispherical, composed of densely arranged cells, forming packet-like aggregates, to 15 μm in diameter. Sheaths around colonies thin, up to 2 μm thick, firm, tightly surrounding cell clusters. Cells irregularly rounded, blue-green, 0.7-1.5 μm in diameter.

Occurrence: It was found in pale to dark blue-green mats on/under soil surfaces with soaking water, intermixed with other cyanobacterial species.

Notes/remarks: This species was rarely found in our localities. Its morphology does not agree with any reported taxon.

9. *Geitlerinema lacus-solaris* (Campbell et Golubić) Anagnostidis, 2001 [Figure 34(1), 48(2)].

Synonyms: *Oscillatoria lacus-solaris* Campbel et Golubić 1985; *Oscillatoria salina* Biwas sensu krumbien *et al*. 1977.

Descriptions: Trichomes thin, solitary, sometimes intermixed with other cyanoprokaryotes, pale blue-green, 1.5-1.9 μm wide, straight, ± slightly curved, not constricted at cross-walls. Cells longer than wide. Sheaths absent. Motile. Apical cells conical, rounded at apex.

Occurrence: The species is found in blue-green mats on wet soil surface among other cyanobacteria and diatoms. It occurred rarely in both Petchaburi and Samut Songkhram salterns.

Notes/remarks: The population from this study is close to description of Campbell and Golubić (1985) Komárek and Anagnostidis (2005) but it differs a little in the size of cells. This species was first described from Solar lake, Sinai in Egypt. It is very similar to *Halomicronema* (very thin filaments) and need more study in future.

10. *Geitlerinema* sp. [Figure 34(2), 48(3)].

Descriptions: Trichomes very long, blue-green, about 3 μm wide, fine, not attenuated at the end, solitary or in clusters, straight to slightly flexible. ± motile. Apical cells rounded.

Occurrence: This species was found only in culture. It was isolated from soils from Petchaburi salterns.

Notes/remarks: This species does not correspond to any previous described species and it still needs more information and further study.

11. *Gloeocapsa* sp. [Figure 35(1), 46(4)].

Descriptions: Colonies microscopic**,** with numerous aggregated cells. Cells always spherical to irregular oval, blue-green, 4.8-6.7 μm in diameter, usually 2 cells together in thin and colourless mucilaginous sheaths and many sub-colonies packed tightly together.

Occurrence: This species was found only in Samut Songkhram salt fields, on wet soil particles and among other groups of algae and cyanobacteria.

Notes/remarks: This species was found rarely in Samut Songkhram saltworks and it did not correspond to any known taxon.

12. *Halomicronema* sp. [Figure 34(3-4), 48(4)].

Descriptions: Non heterocytous filaments, not branched. Trichomes blue-green, fine, thin, cylindrical, approximately 0.7-1.8 μm wide, not constricted at cross-wall. Cells blue-green, longer than wide, 1-3 μm long. Sheaths not distinct. Apical cells rounded. Immotile.

Occurrence: It was found mix with other cyanobacteria in green filament mats on wet soils and occurred in study sites where salinity was lower than 100 ppt.

Notes/remarks: *Halomicronema* was first described by Abed *et al*. in the year 2002 from benthic microbial mats in a man-made hypersaline pond in Eilat (Israel), but it is probably more widely distributed. The specimens from Petchaburi saltern differ little in width in comparison with the original description of *Halomicronema excentricum*

Abed *et al.*, 2002, therefore, the identification on species level was omitted in this study.

13. *Halothece* sp.1 [Figure 35(4), 46(1)].

Descriptions: Cells solitary, blue-green to olive-green, 8 x 6 μm, widely oval. Cell contents net-like with granules. Motility not observed.

Occurrence: It was found in mats with other cyanobacteria on wet to dry soil surfaces.

Notes/remarks: This organism is currently still little known. The species from this study is similar to the description of *Halothece californica* Margheri *et al*., 2008, but the *Halothece* probably contains more than one species from various hypersaline habitats (Garcia-Pichel *et al*., 1998) and the identity was not yet confirmed.

14. *Halothece* sp.2: *Halothece*-like [Figure 35(2-3), 46(2)].

Descriptions: Colonies blue-green, microscopic. Cells very small, pale blue-green, oval to rod-like, 2.3-2.9 x 1.9 μm. Mucilaginous envelopes absent. Living usually in colonies, only sometimes ± solitary; cells tend to arrange in short (several-celled) filamentous forms. Cell contents homogeneous.

Occurrence: This species was found only in cultures and was isolated from soil samples from Samut Songkhram saltfields.

Notes/remarks: This species does not correspond to the previous described taxon of *Halothece* and it needs more study. However, the morphology of cells and tendency to form short filamentous colonies, correspond to this genus.

15. *Johannesbaptistia pellucida* (Dickie) Taylor et Drouet, 1938 [Figure 36(2-5), 47(1)].

Synonym: *Hormospora pullucida* Dickie, 1874.

Descriptions: Solitary pseudofilaments, unbranched, straight, sometimes slightly curved to various curved or little wavy. Cells bright blue-green, olive-green, sometimes yellow-green to brownish, shorted discoid or rounded lenticular, 6.6 x 2(4.6) μm, arranged in one row. Cell enveloped by a wide mucilage, colourless, tube-liked shaped, and rounded closed at the end. The spaces between cells are distinct and clear. Cell content finely granular; numerous necridic cells are present in pseudofilaments.

Occurrence: It was usually found in a green-brownish mats on dry or little wet soil surface. It occurred only in Petchaburi salterns where salinities range from 50-90 ppt.

Notes/remarks: The population from our localities close to the description of Komárek and Anagnostidis (1998). This species is considered cosmopolitan, it was reported from several saline biotopes around the world.

16. *Komvophoron* cf. *skujae* Anagnostidis, 2001 [Figure 37(1-5), 49(1)].

Synonym: *Pseudanabaena minuta* sensu Skuja 1956.

Descriptions: Trichomes solitary, straight or ± slightly curved, 35-140 μm long, deeply constricted at cross-walls. Motile. Sheaths absent. Cells blue-green, barrel-shaped, ± isodiametric or slightly longer than wide, 1.5-3 μm long and approximately 2.3 μm wide, sometimes cells distinctly joined by "hyaline bridges". Cell contents homogeneous with distinct separated chromoplasma from centroplasma. Apical cells obtuse-conical, rarely more rounded.

Occurrence: This species was found mixed with diatoms. It was in pale blue-green mats attached on soil surface soaking in water.

Notes/remarks: This species was found only in Banmaung salterns in Sakhon Nakhon province when salinity is lowest (4 ppt.). This species was found in this area only in our locality. These population is very similar to *Komvophoron skujae* which was found only from Sweden in comparison to the description of Komárek and Anagnostidis (2005).

17. *Komvophoron* sp. [Figure 38(4-5), 49(2)]**.**

Descriptions: Trichomes solitary among other cyanobacterial species, microscopic, always straight, short, up to 60 μm long, deeply constricted at cross-wall. Immotile. Sheaths not observed. Cells pale blue-green, mostly barrel-shaped, little longer than wide, 0.1-0.8 x 0.1 x 1.2 μm. Cell contents homogeneous. Apical cells rounded without thickened cell walls and calyptra.

Occurrence: It was usually found in pale to dark blue-green mats with soaking water and always occurred (few filaments) mixed with other cyanobacteria, presented only in the areas with salinity lower than 150 ppt.

Notes/remarks: This populations do not correspond to the description of any known species. *Komvophoron* species from hypersaline localities are still little known.

18. *Leptolyngbya* sp.1 [Figure 35(5-6), 49(4)].

Descriptions: Filamentous, filaments long, not branched, usually in clusters, slightly curved, sometimes straight with colourless facultative sheaths. Trichomes blue-green to olive-green, fine, very thin, up to 2 μm wide. Cell blue-green, longer than wide,

approximately 1-2 μm long . Apical cells rounded. Hormogonia not present. Heterocytes and akinetes absent.

Occurrence: It usually grows in blue-green mats on wet soil surfaces, but also sometimes in dry mats mixed with other cyanobacteria.

Notes/remarks: Not identifiable according to literature available.

19. *Leptolyngbya* sp.2: spiral form [Figure 38(1), 50(1)].

Descriptions: Filamentous, filaments long, in form of thin spiral, not branched, usually living solitary, rarely in clusters, slightly waved with colourless facultative sheaths. Trichomes blue-green, quite thin up to 3 μm wide. Cells blue-green, longer than wide. Hormogonia not observed. Heterocytes and akinetes absent.

Occurrence: It grow in blue green mats attached on the wet soil surfaces and usually occurs among other cyanobacteria. This species was found only in Petchaburi salterns where salinities ranged from 50 – 120 ppt.

Note/remarks: This species does not correspond with any description of halophilic *Leptolyngbya* species.

20. *Leptolyngbya* sp.3 1988 [Figure 38(2-3), 50(2)].

Descriptions: Filaments slightly curved, thin, approximately 3-4.6 μm wide, blue-green. Trichomes about 1.5 μm wide, constricted at cross-walls. Cells 0.7-3.0 μm long, blue-green. Sheaths thin, colourless.

Occurrence: This species was found only in Samut Songkhram sites and presented among others cyanobacteria .

Note/remarks: This species does not correspondwith any described halophilic *Leptolyngbya* species.

21. *Lyngbya* cf. *aestuarii* Liebman ex Gomont 1982 [Figure 39(4-6), 50(4)].

Synonyms: *Lyngbya aestuarii* f. *limicola* Gomont 1982; *Lyngbya aestuarii* f. *natans* Gomont 1982; *Lyngbya aestuarii* f. *symplocoidea* Gomont 1982; *Lyngbya aestuarii* f. *ferruginea* Gomont 1982; *Lyngbya aestuarii* f. *aeruginisa* Gomont 1982; *Lyngbya aestuarii* f. *spectabilis* Gomont 1982.

Descriptions: Thallus in clusters, thick, dark blue-green, attached to soil surfaces. Filaments macroscopic, almost straight or slight curved, not branched, 24-30 μm wide. Sheaths smooth, thin, colourless but yellow-brownish in old stage. Trichomes blue-green, olive-green or yellow-brownish, 18-21 μm wide, cylindrical, not constricted at granulated cross-walls, not attenuated at the ends. Cells shorter than wide, 3.3-4.7 μm long. Apical cells rounded. Necridic cells observed. Hormogonia present.

Occurrence: It occurred on the soil surface with soaking water, attached and tightly packed to soil surfaces.

Notes/remarks: There are many species of *Lyngbya* found in marine or hypersaline habitats including *Lyngby salina*, *Lyngby agardhii*, *Lyngby meneghiniana*, *Lyngby sordida*, *Lyngby semiplena*, *Lyngby confervoides*, *Lyngby majuscula*. However, the population from this study was designated to *Lyngbya* cf. *aestuarii* because their size and other morphology are in the range of *Lyngby*a aestaurii (Komárek and Anagnostidis 2005).

22. *Lyngbya* sp. [Figure 39(2-3), 50(3)].

Descriptions: Filaments straight or ± slightly curved, long, not branched, living solitary, rarely in clusters, 20 μm wide. Sheaths present, thin, firm, colourless to sometimes yellowish-brown, only with one trichome in a sheath. Trichomes blue-green, olive-green to yellowish-brown, cylindrical, 11-15 μm wide, not constricted at cross-wall. Cells discoid, usually shorter than wide, mostly 1/3 as long as wide, 3-5 μm long. Apical cells widely rounded without thickened outer cell walls. Hormogonia and necridic cells present.

Occurrence: This species was found in pale to dark blue-green mats on wet soil surfaces, sometimes on soils with soaking waters. It occurred usually with other cyanoprokaryotes and also diatoms, It occurred in both Petchaburi and Samut Songkhram saltworks where salinity is lower up to 150 ppt.

Notes/remarks: This *Lyngbya* does not morphologically correspond to any described taxon.

23. *Merismopedia* sp. [Figure 39(1), 46(5)].

Descriptions: Colonies microscopic, usually consist of 16 cells. Colonies mucilaginous, envelopes colourless. Cells blue-green, oval, 3-3.8 μm in diameter, arranged closely in perpendicular rows. Cell with distinct fine net-like contents with granules.

Occurrence: Found among other cyanobacterial species.

Notes/remarks: Not correspond to any described species.

24. *Nostoc* sp.1: black colony [Figure 40(1-3), 53(2)].

Descriptions: Colonies brown to dark green with irregular shape, approximately up to 1 cm in diameters with rough surface. Sheaths little yellowish-brown to colourless. Filaments, not branched, densely agglomerated and coiled within individual sheaths. Trichomes brown, yellow-brownish to olive-green. Cells spherical, slightly oval to irregular oval (variable in shape), 3-4.5 μm in diameter. The morphology of apical cells is not different from other cells. Heterocytes and akinetes were not observed under culture conditions.

Occurrence: This species was found only in culture isolated from the salinic soils. It was also found among other cyanophytes in the first period of isolation, but the growth time of this species is very slow.

Notes/remarks: It can not identifiable according to any present literature.

25. *Nostoc* sp.2 [Figure 40(4-6), 53(3)].

Descriptions: Colonies (macroscopic) green with a rough surface, mucilaginous of irregular shape. Sheaths colourless. Trichomes green,coiled, sometimes wavy, not branched and loosely packed in sheaths. Cells with similar size along the whole filaments, spherical to slightly oval, very small, less than 1 μm in diameter. Heterocytes oval, sometimes presented in culture.

Occurrence: This species was registered only from culture, isolated from soils. It also grew together with other cyanobacterial species in the first period of isolation.

Notes/remarks: Not identifiable according to present literature.

26. *Oxynema thaianum* Chatchawan *et al*. 2012 [Figure 41(2-6), 51]**.**

Descriptions: Thallus dark blue-green, tightly packed with soil particles, sometimes mixed with other cyanoprokaryotes. Trichomes blue-green to olive-green, very long, solitary or sometimes clustered, trichomes without sheaths, not constricted at cross-walls, 7.5-9 μm wide, a little arcuated at the end of trichomes. Cells blue-green, shorter than wide, 2.2-2.8 μm long. Apical cells pointed. Reproduction by hormogonia.

Occurrence: This species was usually found in bright to dark blue-green mats, tightly packed on the wet soil surfaces, sometimes bubbles occurred in mats. Distributed in the areas where salinities ranged from 45 up to 150 ppt.

Notes/remarks: This species was firstly described and isolated from Thailand. It was previously designated by the name *Phormidium lloydianum*, which was described originally from saltworks in France. However, the study by polyphasic approaches (morphology, TEM and molecular sequencing) showed that it does not belong to the genus *Phormidium* (see special chapter about *O. thaianum* and also enclosed publication).

27. *Pseudanabaena* sp. [Figure 42(1-2), 49(3)].

Descriptions: Trichomes solitary, straight, very thin, blue-green, distinctly constricted at cross-walls, approximately 96 μm long and 1.4 μm wide, of the same width along the whole trichome. Sheaths not observed. Cells blue-green or slightly grey, 0.9-1.9 μm long. Apical cells rounded, not attenuated.

Occurrence: It appeared in pale to dark blue-green mats soaking under water with other microalgae and cyanobacteria.

Notes/remarks: This species was found only in few filaments and its morphology does not correspond to any previous described taxa. The group of *Pseudanabaena* was reported from several habitats worldwide and seem to be heterogeneous. However, species from hypersaline environments are still little known and need more study including species from Petchaburi salterns.

28. *Pseudocapsa* sp. [Figure 32(9), 45(7)].

Descriptions: Colonies microscopic, spherical, with radially oriented, composed of densely arranged cells, 13-15 μm in diameter with sheaths. Cells irregularly rounded, blue-green, 2-5 μm. Sheaths firm, delimited, colourless, tightly surrounding cell clusters.

Occurrence: It was found in bright to dark blue-green mats on wet to little dry soil surface, mixed also with other cyanobacteria.

Notes/remarks: This species is not identifiable with any previous described species.

29. *Spirulina subsalsa* Oersted ex Gomont, 1892 [Figure 42(3-5), 52(1)].

Synonyms: *Spirulina tenuissima* Kützing 1836; *Oscillatoria oceanica* Crouan 1852; *Spirulina subsalsa* f. *genuine* Gomont 1892; *Spirulina subsalsa* f. *oceanica* (Crouan) Gomont 1982; *Spirulina neumannii* Schmidle 1901; *Spirulina compacta* Perfilev 1923; *Spirulina tenuissima* var. *salina* Wisłouch 1924; *Arthrospira subsalsa* Crow in Croasdale 1935; *Spirulina* sp. (marina) sensu Schwabe 1947; *Spirulina supersalsa* Schiller 1956; *Spirulina condessata* Welsh 1964; *Oscillatoria subsalsa* (Oersted) Bourrelly 1970; *Oscillatoria seumannii* (Schmidle) Iltis 1972.

Descriptions: Trichomes pale to bright blue-green, loosely clustered among other cyanoprokaryotes, sometimes they occurred as solitary, spirally coiled, straight or

variously curved, short to very long, from 50 up to more than 300 μm long. Coils with right-handed screw-like rotation, each coil regularly tightly joined to another, nearly parallely arranged, 2.5-4.5 μm wide, 1.8-2 μm high. Sheaths absent. Apical cells rounded.

Occurrence: Found in blue-green mats on soaked soil surfaces, rarely in a dry mats. It occurred in all sampling sites in Petchaburi salterns areas, but rarely in Samut Songkhram area.

Notes/remarks: This species correspond to the description of Komárek and Anagnostidis (2005). It was published by many authors and probably has a cosmopolitan distribution.

30. *Trichocoleus* sp. [Figure 43(4-6), 52(2)].

Descriptions: Filaments very long, nearly straight, unbranched, 10-11 μm wide with sheaths. Sheaths distinctly firm, colourless. Trichomes densely agglomerated in fascicles in a sheath, always straight, slightly constricted at cross-walls. Cells blue-green, (2)3-5 μm wide, a little longer than wide. Apical cells 6-8 μm long, conical to acute conical without calyptras.

Occurrence: In dark blue-green mats on soil surface soaking in water and mixed with other cyanobacterial species.

Notes/remarks: It could not be assigned to any known taxa.

31. *Tyconema* sp. [Figure 43(7-8), 52(3)].

Descriptions: Trichomes usually solitary, blue-green to olive-green, straight, cylindrical, not narrowed toward ends, not constricted at cross-walls. Sheaths not present. Cells nearly isodiametric or slightly longer than wide, 3-6.9 μm. Cell

contents net-like with granules, near cell walls of each cell are present densely arranged several granules.

Occurrence: It was found in blue-green mats on wet soil surface soaking in water and found only in Samut Songkhram salt field, where salinity is 55 ppt.

Notes/remarks: This species could not be identified according to literature.

32. *Wollea* sp. [Figure 44(1-5), 53(1)].

Descriptions: The colony is filamentous, blue-green, rough, waved. Trichomes usually packed, ± parallel in fascicles, or rarely solitary, straight to slightly curved, with the same width along the whole filament, not branched, slightly constricted at the cross walls. Individual sheaths not distinct. Cells blue-green, cylindrical to barrel-shaped, nearly isodiametric to slightly longer than wide, 2.3 x 3.0 μm. Apical cells acute-rounded. Heterocytes occurred intercalary. Akinetes elongate, larger than vegetative cells, about 10 x 4.6 μm and usually attach to one side of heterocytes, less frequently from both sides developing usually in unsuitable condition.

Occurrence: This species was registered only from culture, isolated from the soil samples. It lived together with other cyanophytes in the first isolation.

Notes/remarks: This species corresponds to the genus *Wollea*, but it differs ecologically and morphologically from up to date described species. In the early step of cultivation, this species does not form heterocytes and appear morphologically similar to *Komvophoron* spp.

33. *Xenococcus schousboei* Thuret 1880 [Figure 43(1-3), 47(2)].

Synonyms: *Coleonema arenifera* Schousboe 1880; *Dermocarpa schousboei* (Thuret) Bornet in Batters 1889.

Descriptions: Cells blue-green, almost spherical and sometimes flattened, usually densely arranged into groups in one layer, usually attached to filaments of other cyanobacteria, in this study to *Lyngbya aestuarii*. The cells are 3.8-8.6 μm in diameter, all more or less of the same size. Mucilaginous sheaths distinct, thin, colourless. The baeocytes formation not observed.

Occurrence: Found only in attached form to the filaments of *Lyngbya aestuarii*.

Notes/remarks: The morphological criteria of this species is agreed with the description of Komárek and Anagnostidis (1998).

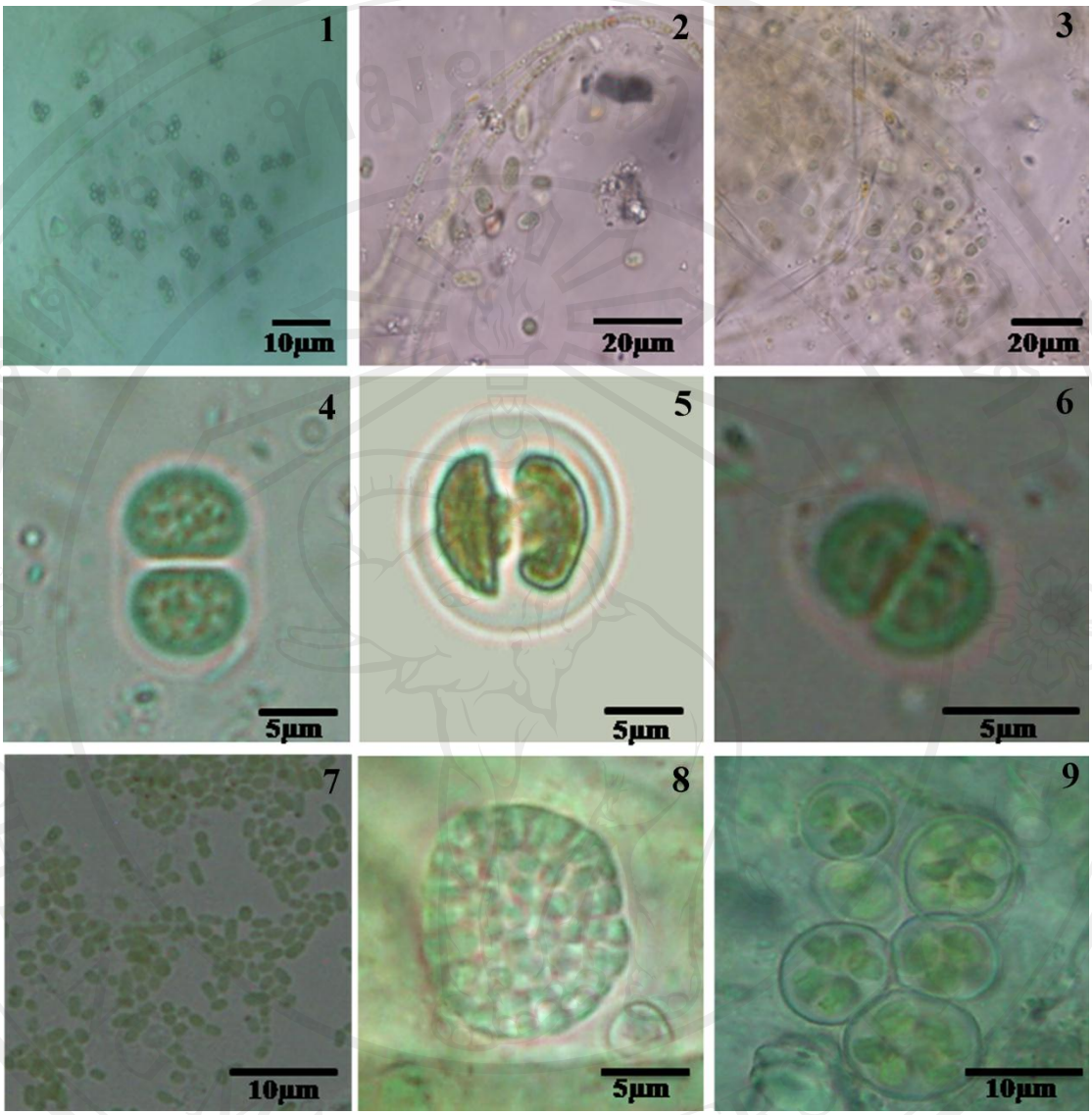

Figure 32 Light microscope photographs of cyanobacteria found in this study (Petchaburi and Samut Songkhram solar salterns).

(1) *Aphanocapsa* cf. *salina* Voronichin, (2-3) *Aphanothece halophytica* Frémy in Hof et Frémy, (4) *Chroococcus* cf. *submarinus* (Hansgirg) Kováćik, (5) *Chroococcus* sp.1, (6) *Chroococcus* sp.2, (7) *Cyanobium* sp., (8) *Cyanosarcina* sp.1, (9) *Pseudocapsa* sp.

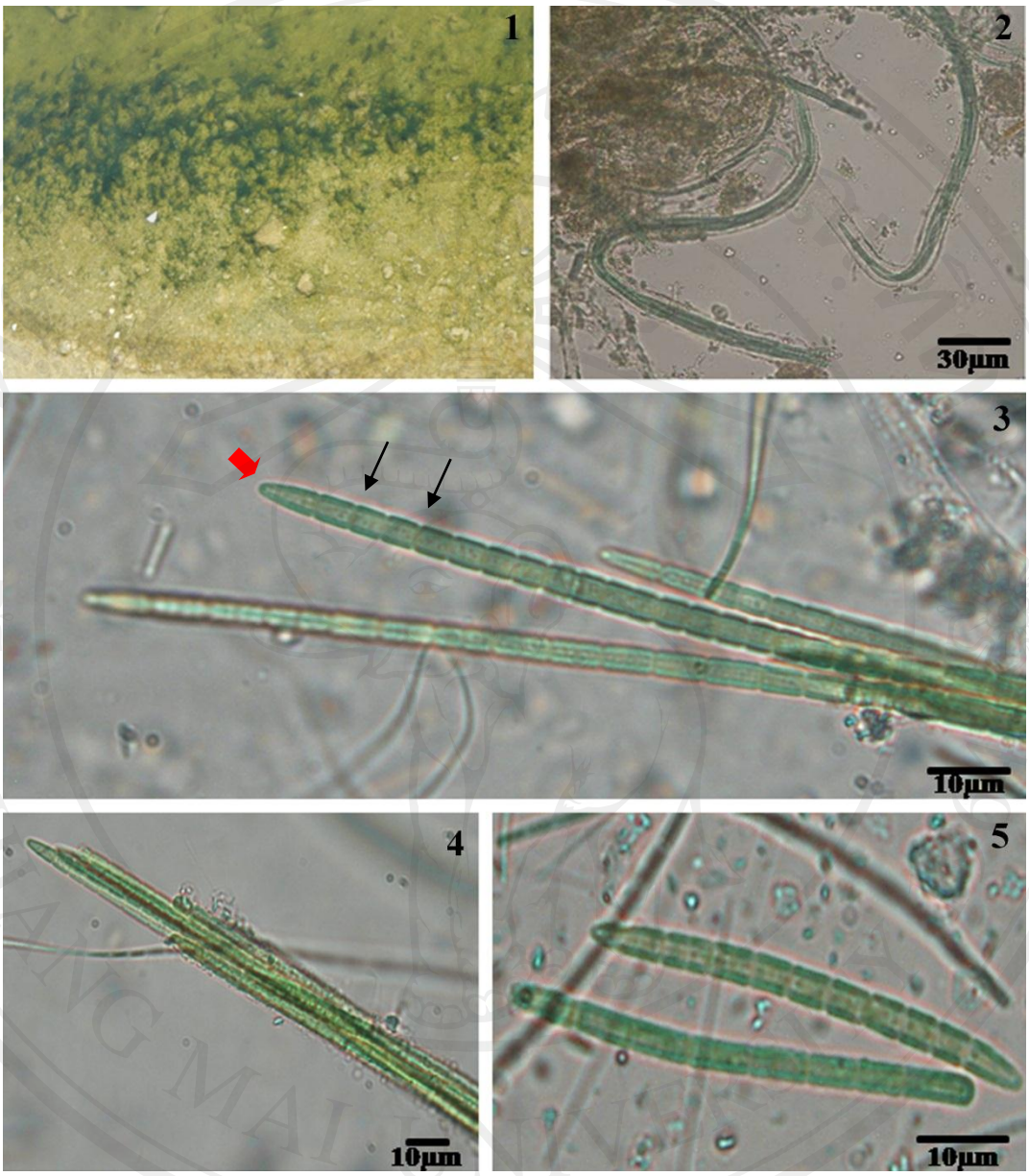

Figure 33 Light microscope photographs of cyanobacteria found in this study (Petchaburi and Samut Songkhram solar salterns).

Coleofasciculus chthonoplastes (Thuret ex Gomont) Siegesmund *et al*: (1) Mats of *C. chthonoplastes* from Nature (mixed with other cyanoprokaryotes and diatoms), (2) trichomes in fascicles in sheaths, (3-4) trichomes (with constricted at cross-wall = black arrow) and conical apical cells (red arrow) of *C. chthonoplastes*, (5) hormogonia of *C. chthonoplastes*.

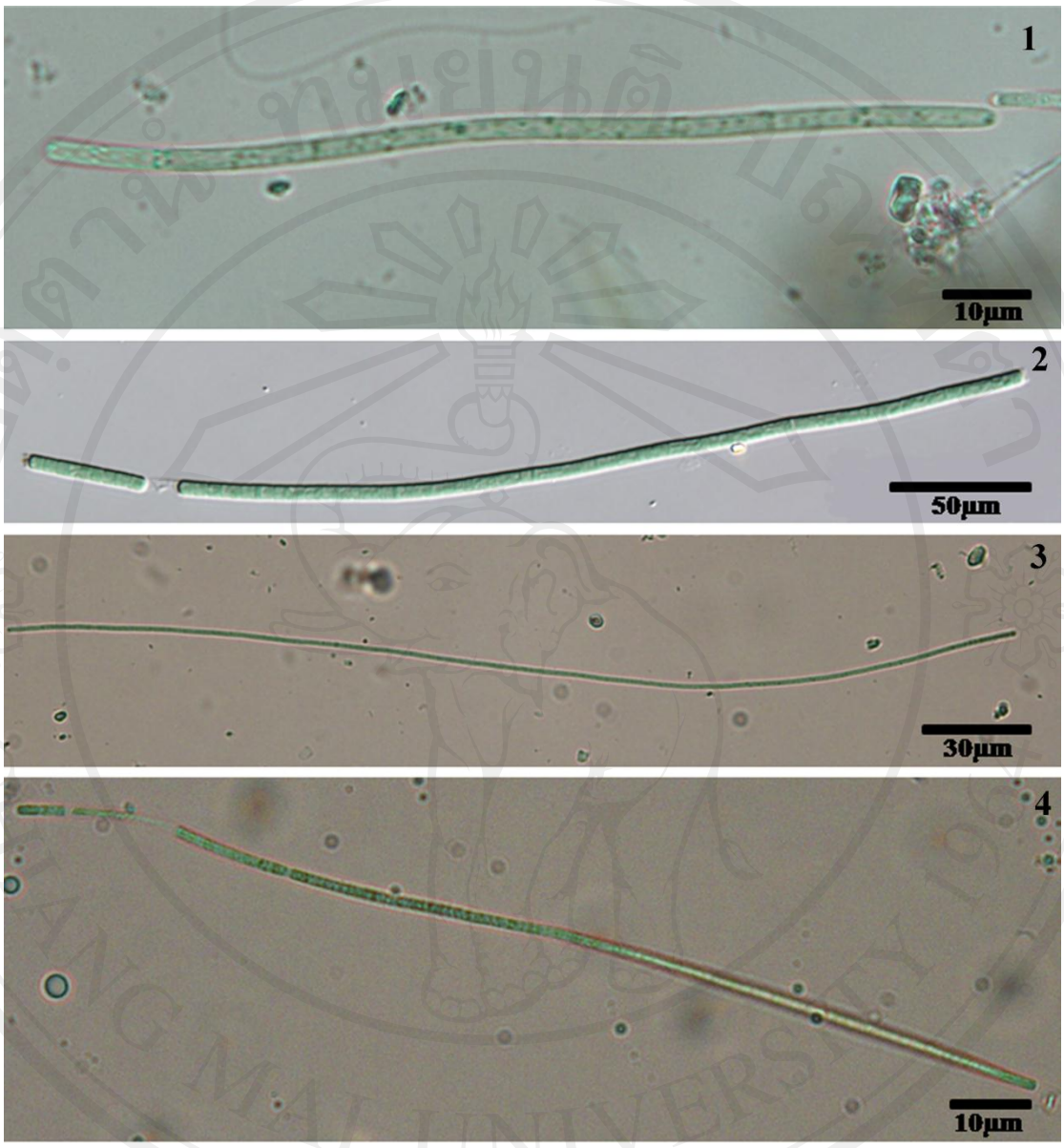

Figure 34 Light microscope photographs of cyanobacteria found in Petchaburi and Samut Songkhram solar salterns.

(1) *Geitlerinema lacus-solaris* (Campbell et Golubić) Anagnostidis,

(2) *Geitlerinema* sp., (3-4) *Halomicronema* sp.

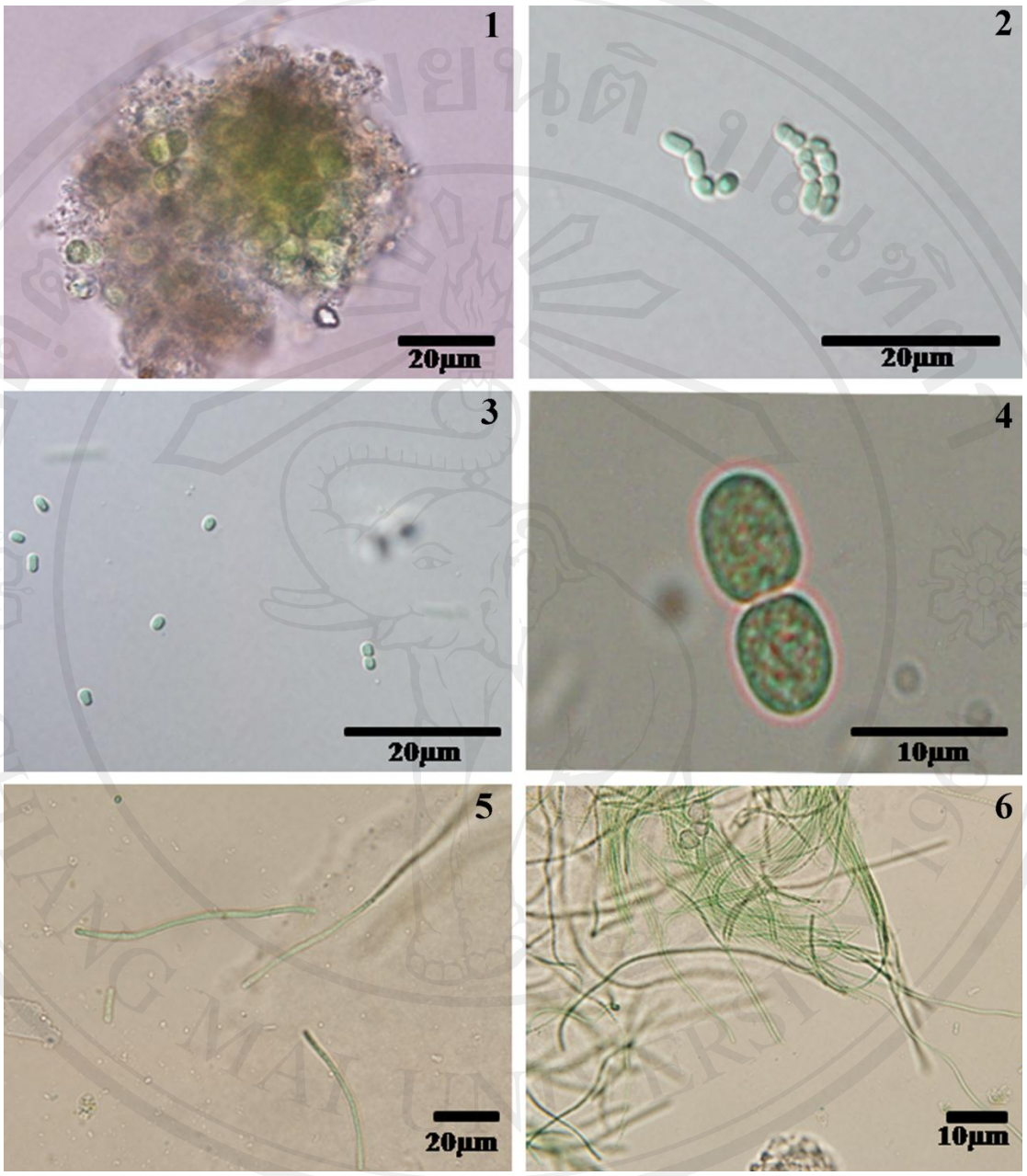

Figure 35 Light microscope photographs of cyanobacteria found in Petchaburi and Samut Songkhram solar salterns.

(1) *Gloeocapsa* sp., (2-3) *Halothece* sp.2: *Halothece*-like, (4) *Halothece* sp.1, (5-6) *Leptolyngbya* sp.1.

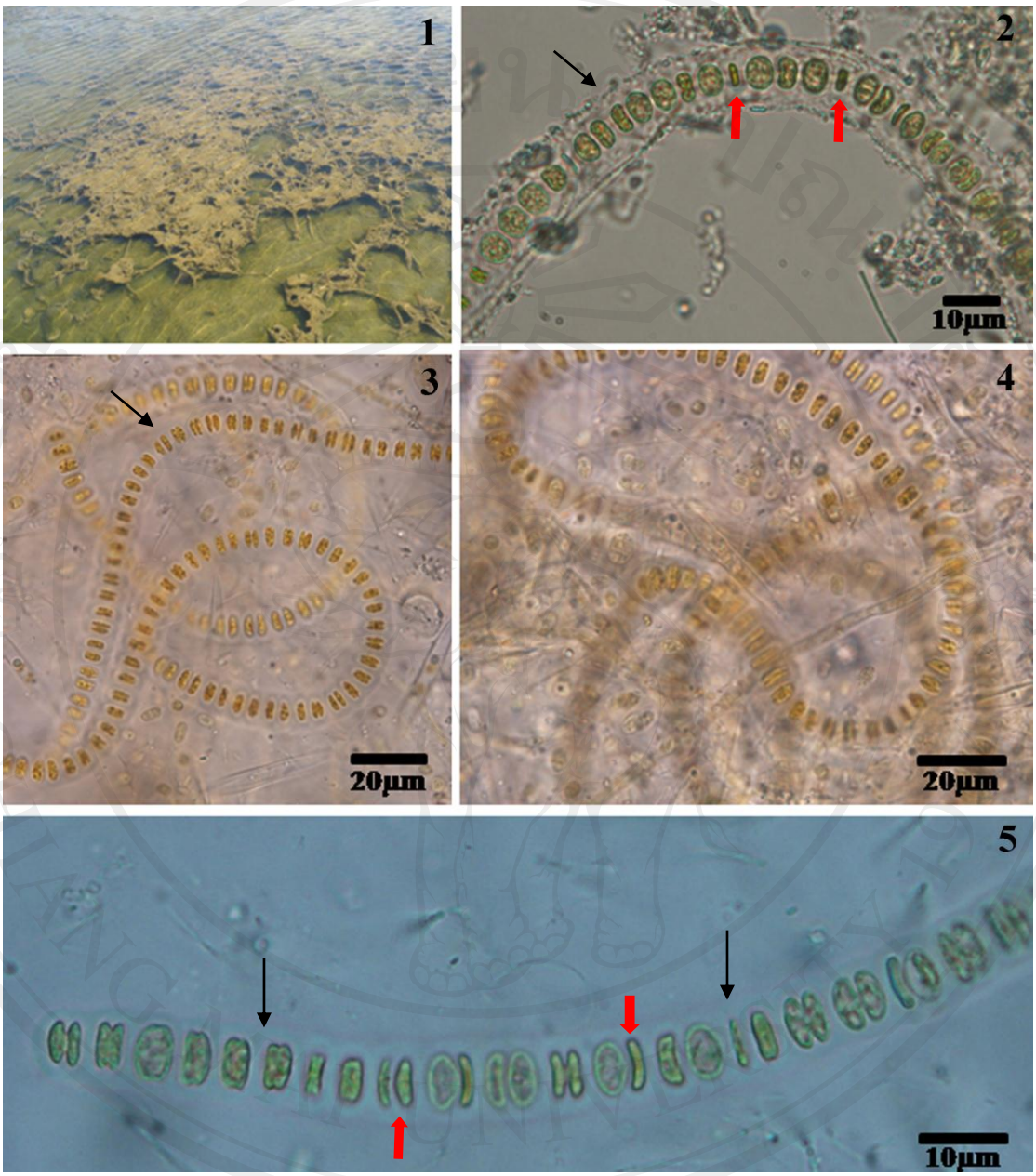

Figure 36 Light microscope photographs of cyanobacteria found in Petchaburi and Samut Songkhram solar salterns.

Johannesbaptistia pellucida (Dickie) Taylor et Drouet: (1) Mats of *J. pellucida* in nature, (2-5) pseudofilaments of *J. pellucida*, in which some necridic cells occurred (red arrows); sheaths presented (black narrow arrows).

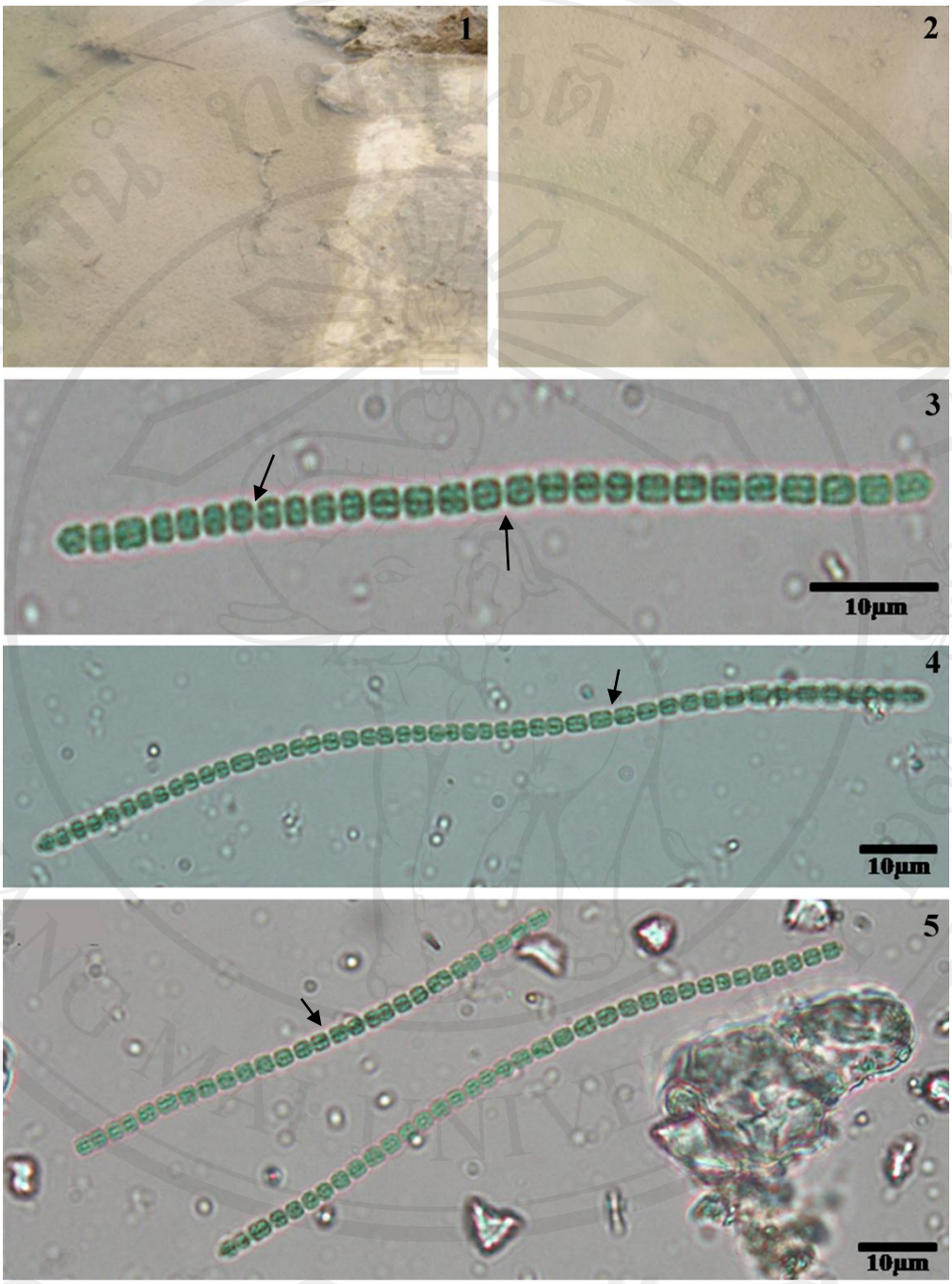

Figure 37 Locations and light microscope photographs of cyanobacteria found only in Sakon Nakhon solar salterns (not salinic areas).

Komvophoron cf. *skujae* Anagnostidis: (1-2) Mats of *K.* cf. *skujae* occurred in nature, (3-5) trichomes of *K.* cf. *skujae*; all trichomes with distinct constrictions (black arrows).

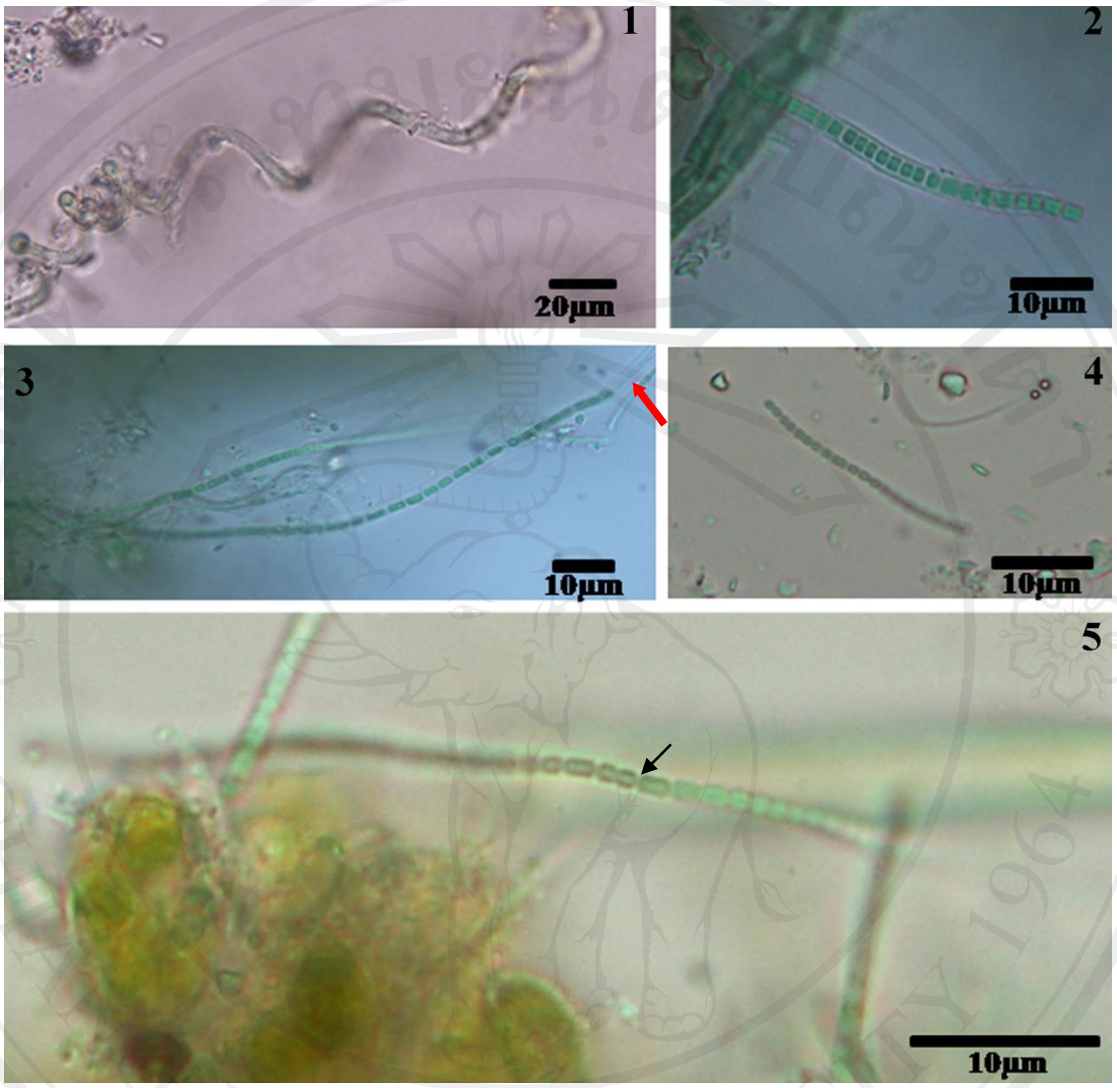

Figure 38 Light microscope photographs of cyanobacteria found in Petchaburi and Samut Songkhram solar salterns.

(1) *Leptolyngbya* sp.2 (spiral form), (2-3) *Leptolyngbya* sp.3 -sheaths presented (red arrow), (4-5) *Komvophoron* sp. (cells barrel shaped and trichomes with distinct constrictions (black arrow).

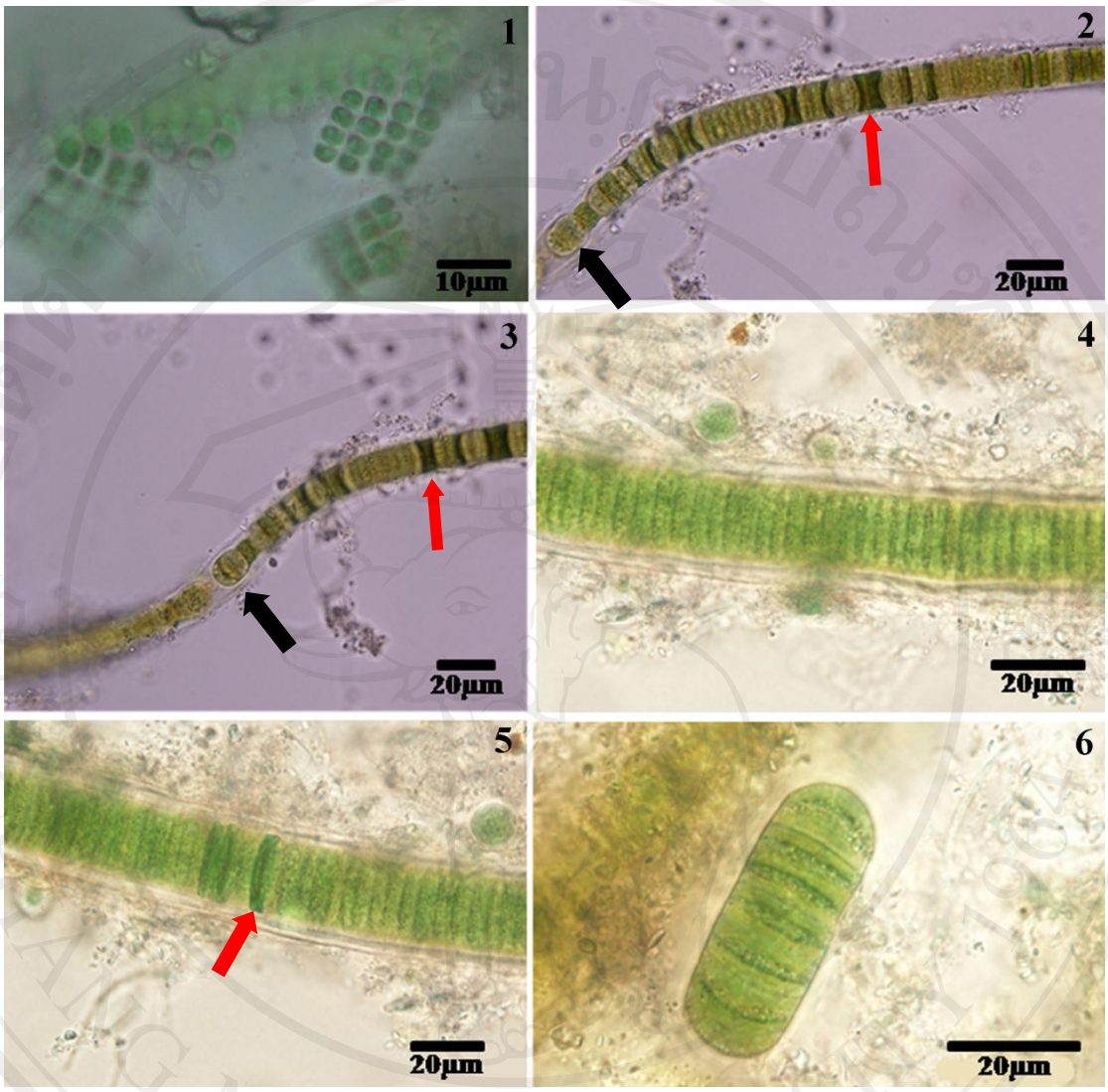

Figure 39 Light microscope photographs of cyanobacteria found in Petchaburi and Samut Songkhram solar salterns.

(1) *Merismopedia* sp., (2-3) *Lyngbya* sp. (Hormogonium = black arrows), (4-5) *Lyngbya* cf. *aestuarii* Liebman ex Gomont, (6) hormogonium of *L.* cf. *aestuarii*. (necridic cells shown with red arrows).

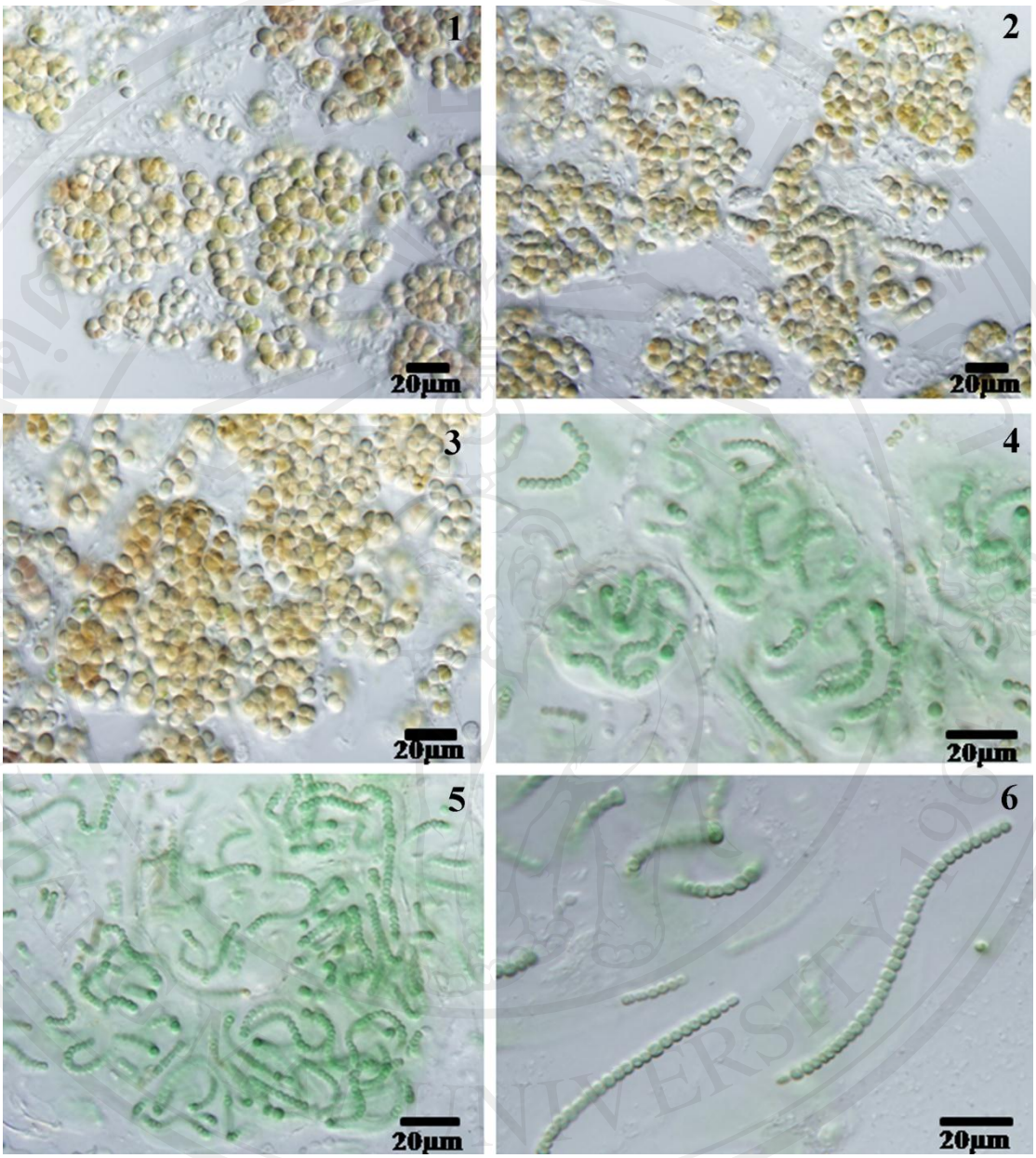

Figure 40 Light microscope photographs of cyanobacteria found in Petchaburi and Samut Songkhram solar salterns.

(1-3) Colonies of *Nostoc* sp.1 (black colonies on agar media), (4-6) colonies and filaments of *Nostoc* sp.2 (green colonies on agar media).

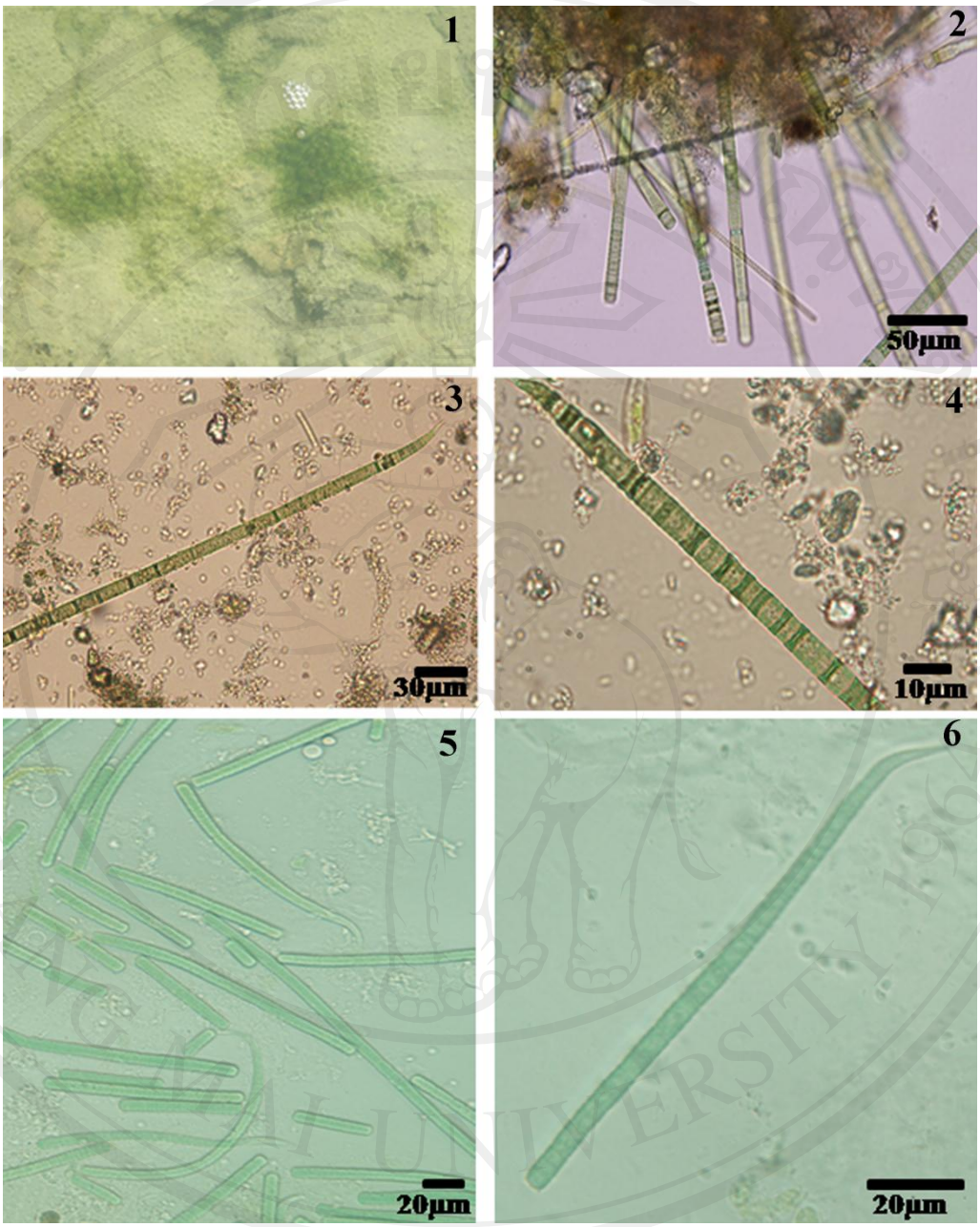

Figure 41 Light microscope photographs of cyanobacteria found in Petchaburi and Samut Songkhram solar salterns.

Oxynema thaianum: (1) Mats of *O. thaianum* from nature, (2) filaments of *O. thaianum* in nature, (3-4) *O. thaianum* – solitary filaments, (5-6) *O. thaianum* in culture (BG-11 with 3 % NaCl liquid media).

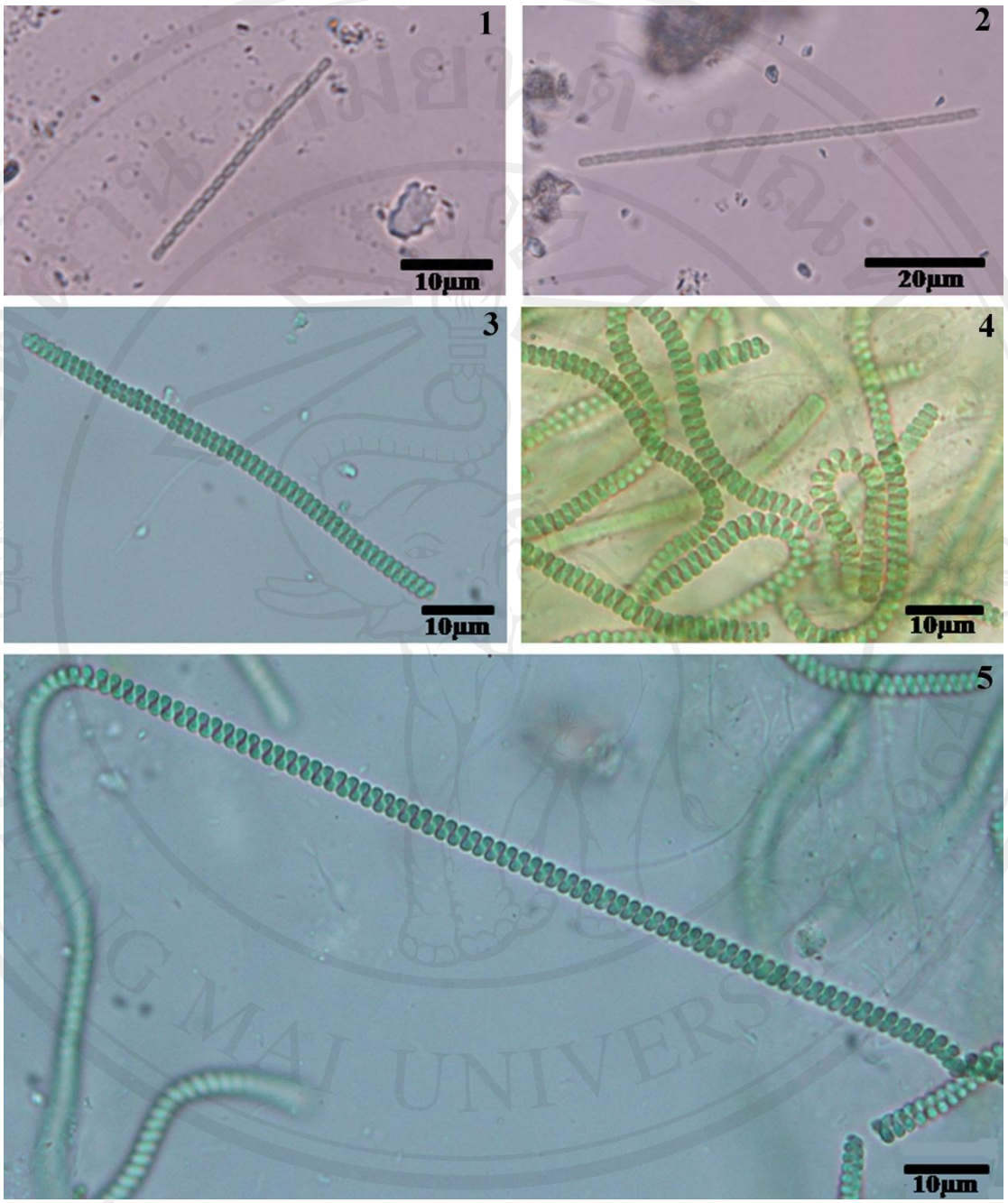

Figure 42 Light microscope photographs of cyanobacteria found in Petchaburi and Samut Songkhram solar salterns.

(1-2) *Pseudanabaena* sp., (3-4) *Spirulina subsalsa* Oersted ex Gomont.

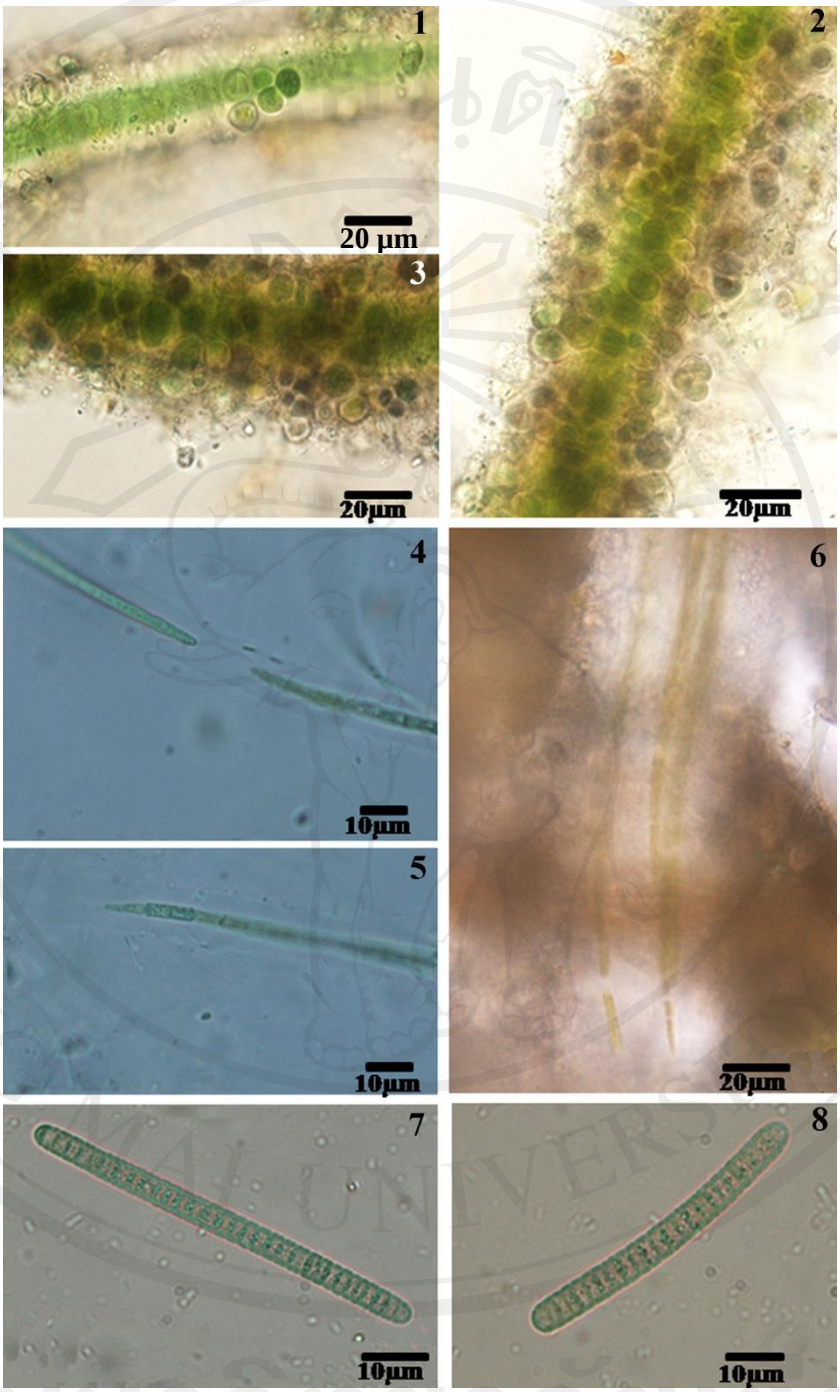

Figure 43 Light microscope photographs of cyanobacteria found in this study (Petchaburi and Samut Songkhram solar salterns).

(1-3) *Xenococcus* cf. *schousboei* Thuret (colonies attached on *Lyngbya* sp.), (4-6) *Trichocoleus* sp., (7-8) *Tychonema* sp.

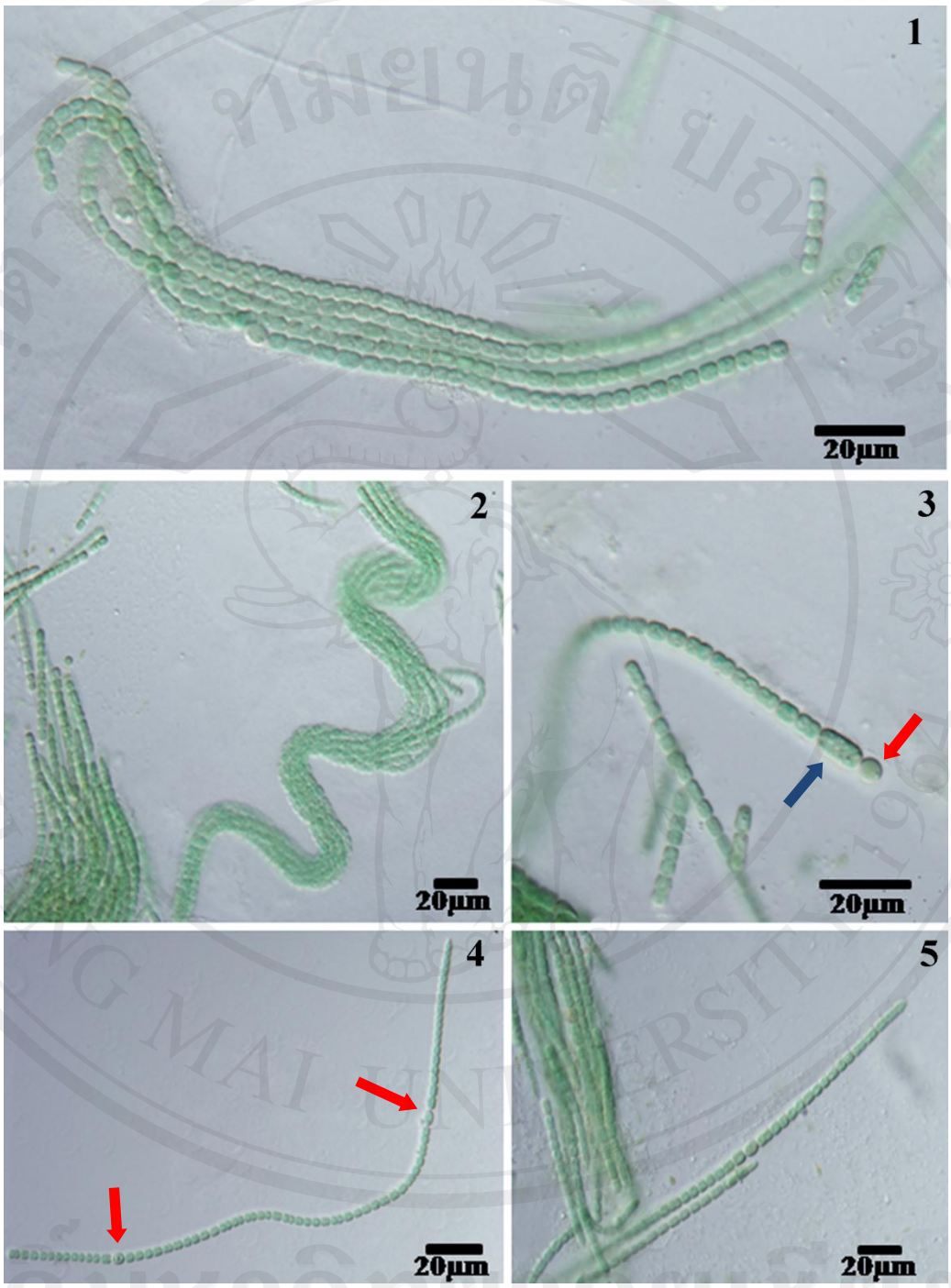

Figure 44 Light microscope photographs of cyanobacteria found in Petchaburi and Samut Songkhram solar salterns.

Wollea sp.: (1-2) trichomes of *Wollea* sp. (in fascicles), (3-5) solitary filaments, presence of akinete (elongated akinete = blue arrow) and heterocytes = red arrow.

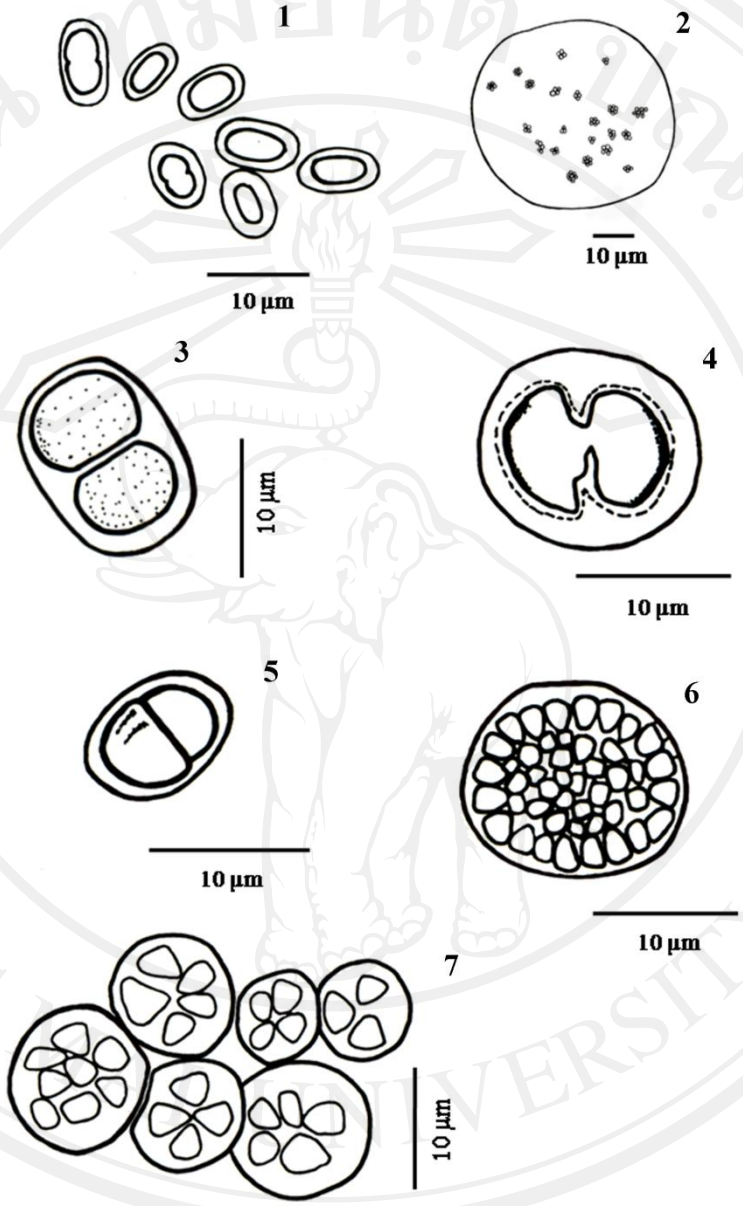

Figure 45 Illustrations of coccoid cyanobacteria found in Petchaburi and Samut Songkhram solar salterns.

(1) *Aphanothece halophytica* Frémy in Hof et Frémy, (2) *Aphanocapsa* cf. *salina* Voronichin, (3) *Chroococcus* cf. *submarinus* (Hansgirg) Kováćik, (4) *Chroococcus* sp. 1, (5) *Chroococcus* sp. 2, (6) *Cyanosarcina* sp.1, (7) *Pseudocapsa* sp.

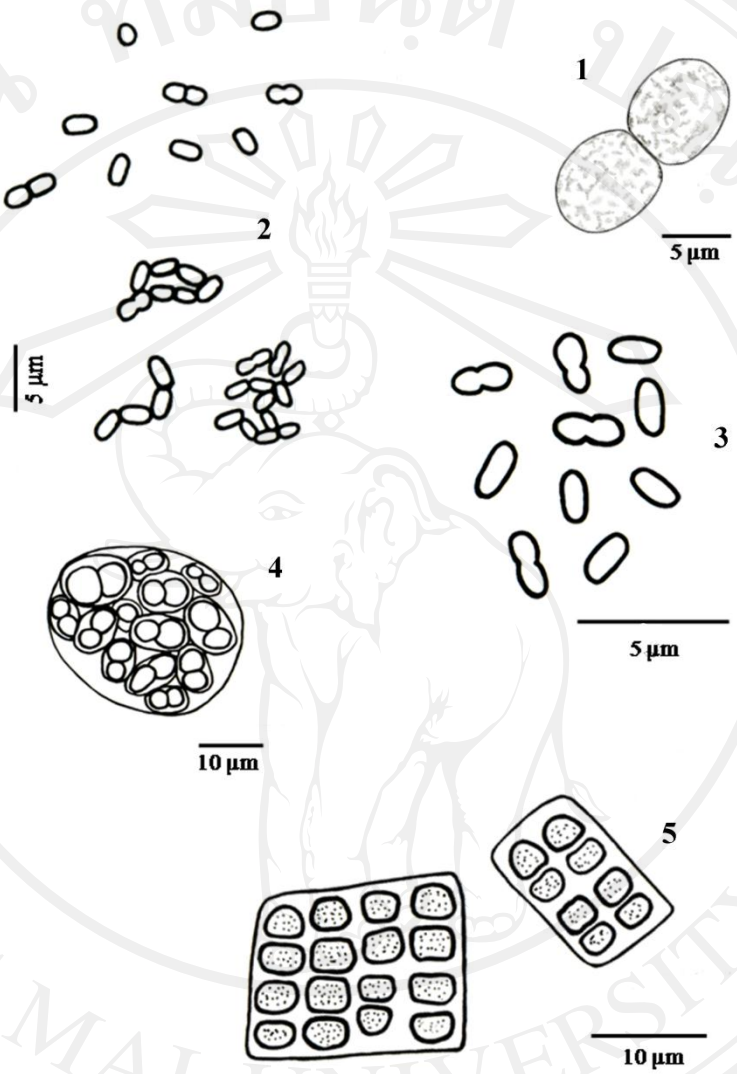

Figure 46 Illustrations of coccoid cyanobacteria found in Petchaburi and Samut Songkhram solar salterns.

(1) *Halothece* sp.1, (2) *Halothece* sp.2: *Halothece*-like of Garcia-Pichel *et al*. (3) *Cyanobium* sp., (4) *Gloeocapsa* sp., (5) *Merismopedia* sp.

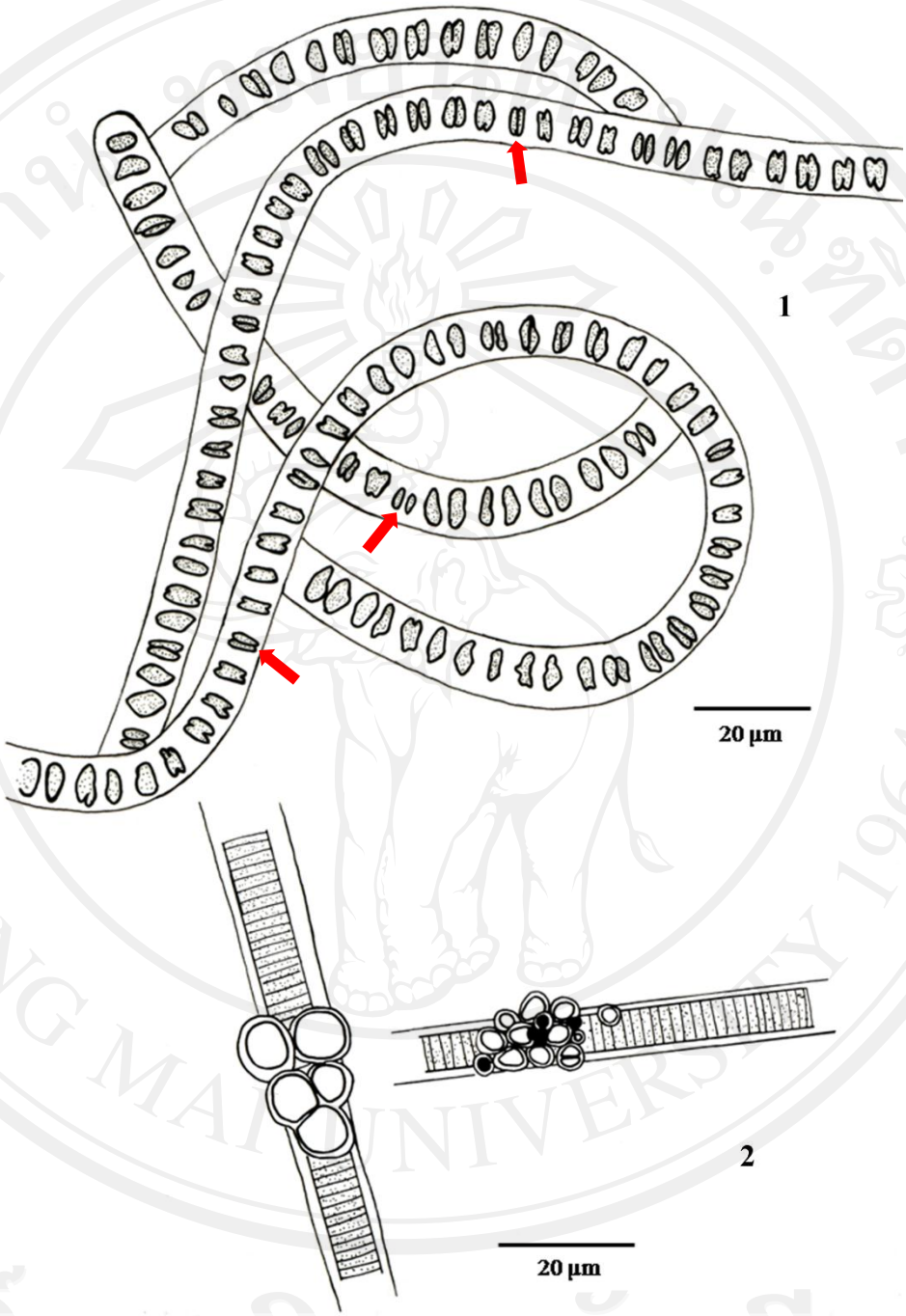

Figure 47 Illustrations of coccoid cyanobacteria found in Petchaburi and Samut Songkhram solar salterns.

(1) *Johannesbaptistia pellucida* (Dickie) Taylor et Drouet (red arrows = dead cells), (2) *Xenococcus* cf. *schousboei* Thuret.

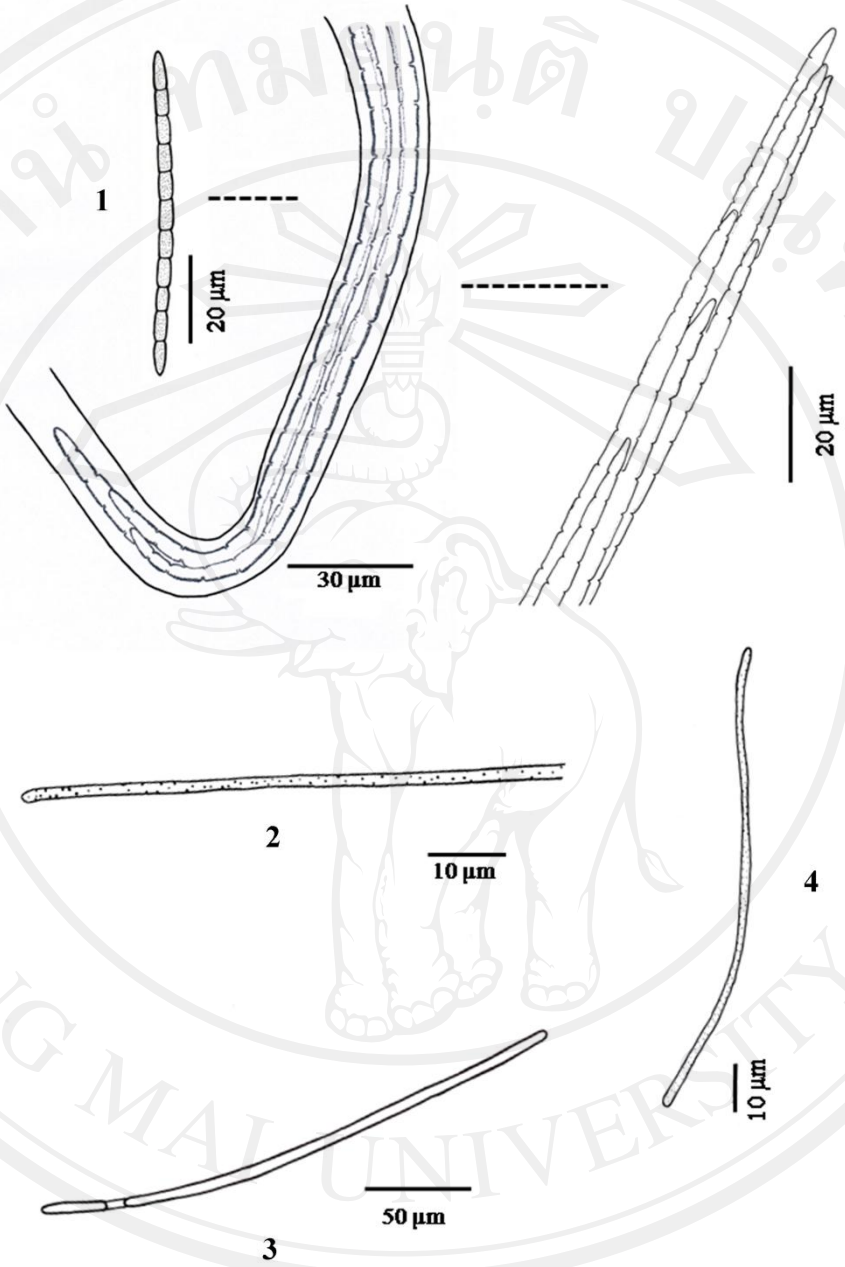

Figure 48 Illustrations of filamentous cyanobacteria found in Petchaburi and Samut Songkhram solar salterns.

(1) *Coleofasciculus chthonoplastes* (Thuret ex Gomont) Siegesmund *et al*. (H = hormogonium), (2) *Geitlerinema lacus-solaris* (Campbell et Golubić) Anagnostidis, (3) *Geitlerinema* sp., (4) *Halomicronema* sp.

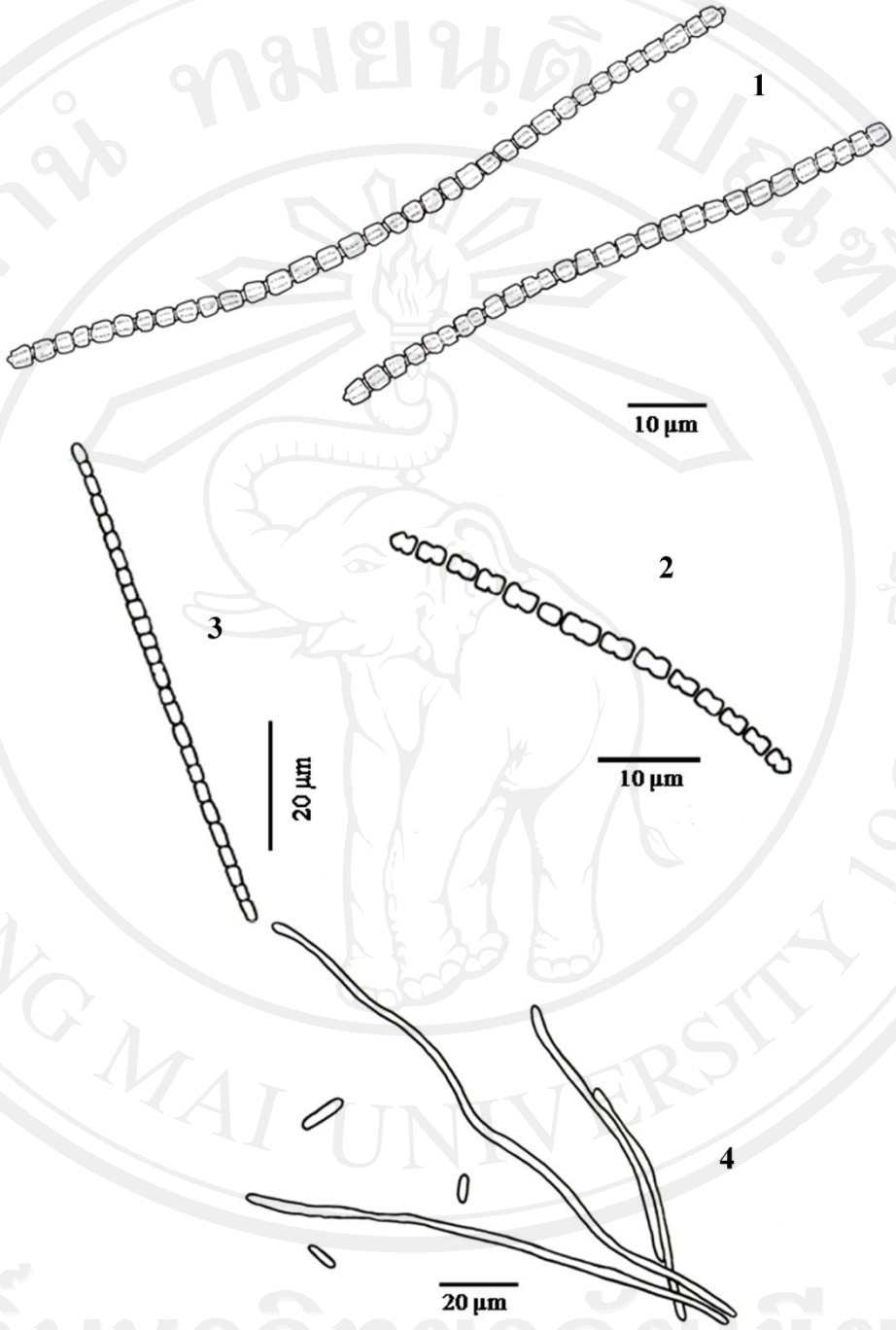

Figure 49 Illustrations of filamentous cyanobacteria found in Petchaburi, Samut Songkhram and Sakhon Nakhon solar salterns.

(1) *Komvophoron* cf. *skujae* Anagnostidis, (2) *Komvophoron* sp., (3) *Pseudanabaena* sp., (4) *Leptolyngbya* sp.1.

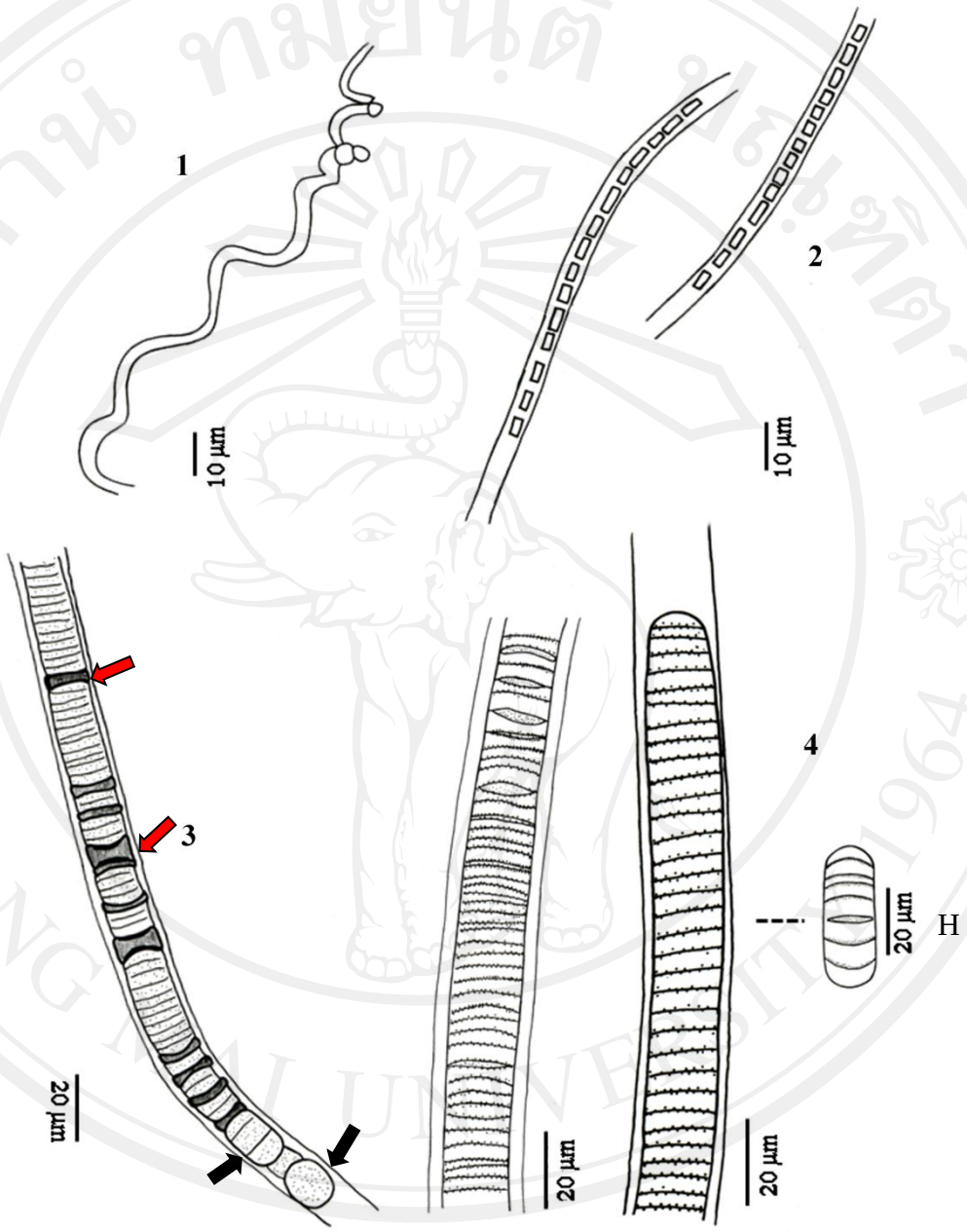

Figure 50 Illustrations of filamentous cyanobacteria found in Petchaburi, Samut Songkhram and Sakhon Nakhon solar salterns.

(1) *Leptolyngbya* sp.2, (2) *Leptolyngbya* sp.3, (3) *Lyngbya* sp. (hormogonia = black arrows, necridic cells shown with red arrows), (4) *Lyngbya* cf. *aestuarii* Liebman ex Gomont (H = hormogonium).

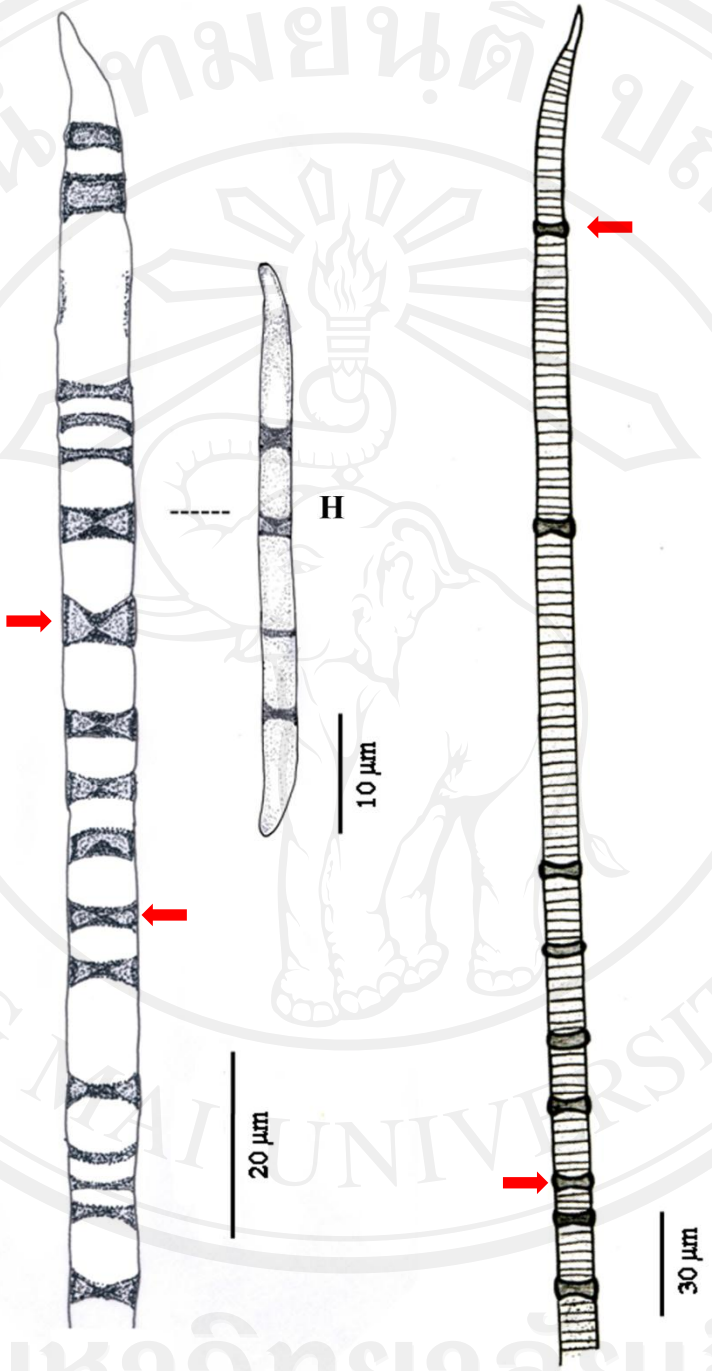

Figure 51 Illustrations of filamentous cyanobacteria found in Petchaburi, Samut Songkhram.

Filaments of *Oxyenema thaianum* from nature: H = homogonium, red arrow = necridic cells.

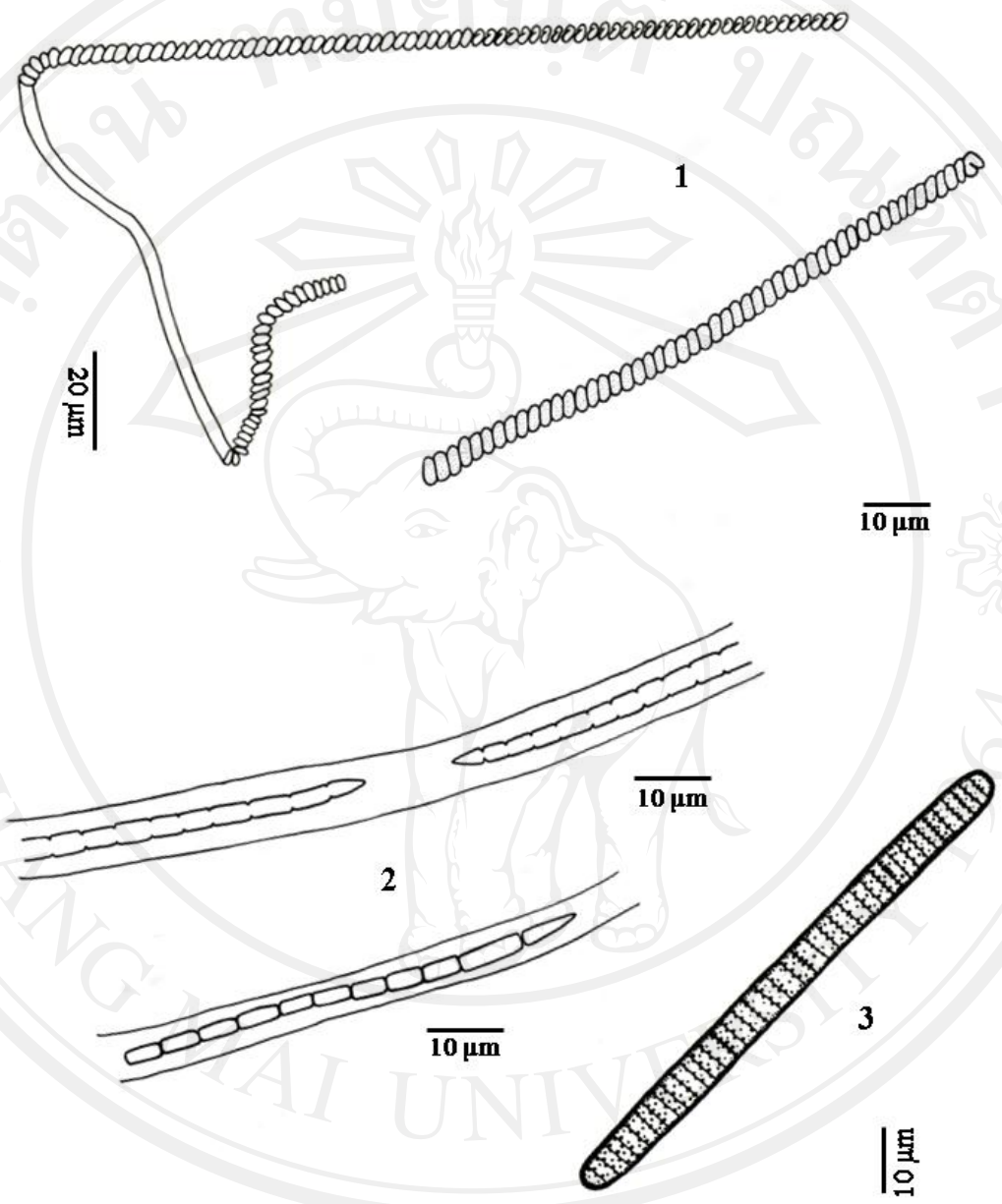

Figure 52 Illustrations of filamentous cyanobacteria found in Petchaburi, Samut Songkhram and Sakhon Nakhon solar salterns.

(1) *Spirulina subsalsa* Oersted ex Gomont, (2) *Trichocoleus* sp. Anagnostidis, (3) *Tychonema* sp.

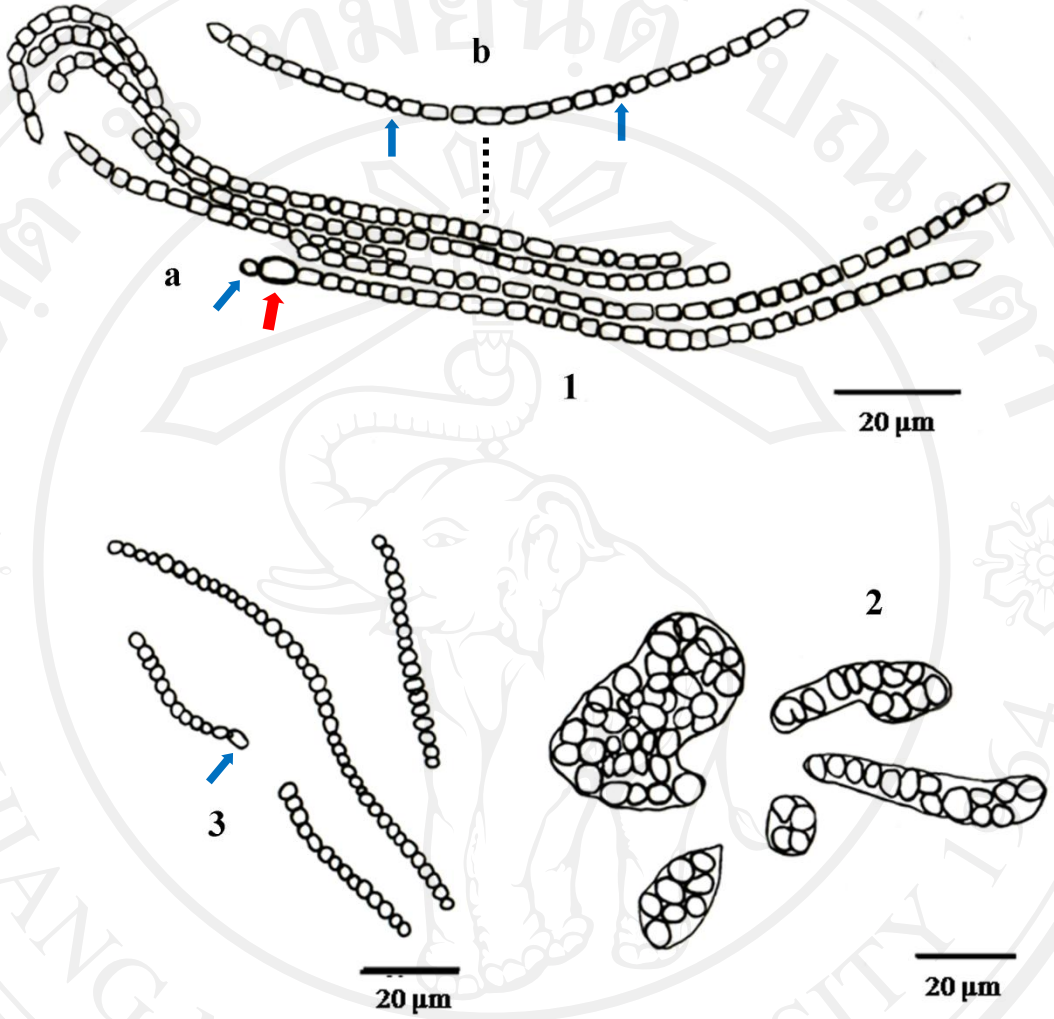

Figure 53 Illustrations of heterocytous cyanobacteria found in Petchaburi, Samut Songkhram and Sakhon Nakhon solar salterns.

(1) *Wollea* sp.: (a) trichomes of *Wollea* sp. (in fascicles), (b) solitary (red arrows = akinetes, blue arrows = heterocytes) (2) *Nostoc* sp.1, (3) *Nostoc* sp. 2 (blue arrow = heterocyte).

4.4 Isolation and cultivation of cyanobacteria

Isolation and cultivation are important for other laboratory studies (molecular, eco-physiology, life cylces, etc.) of cyanobacteria therefore, cyanobacterial samples were isolated and cultivated in this study. The process for isolation and cultivation consumed approximately 8-12 months until monospecific species were obtain and not all cyanobacteria found in natural environments could be grown in culture. This study found BG-11 media are better than Zarrouk's mediua and Castenholtz D media, modified. Cyanobacteria grow very well in BG-11 media with 3 % of NaCl whereas they are not present in others mentioned media. Some of the dominant species, such as *Spirulina subsalsa* and *Coleofasciculus chthonoplastes* were not successfully cultured. In contrary, some species which could not be found in nature can grow in culture, especially from the Nostocales group. However, total 12 monospecific strains were successfully obtained in BG-11 agar media (Table 6). The important and interested strains were selected and studied experimentally their ecophysiological demands (growth dependence on salinity) and continued their phylogenetic position of molecular sequencing.

4.5 Ecophysiological studies

The ecophysiology studies were separated into 2 parts including growth dependence of selected cyanobacterial strains under salinity gradients and crossed gradients experiments. The results from 2 experiments were shown as follows:

4.5.1 Growth dependence under salinity gradients

The dependence on gradients of salt concentration was studied in several isolated strains including *Leptolyngbya* sp.1, *Nostoc* sp.1 (black colonies), *Nostoc* sp.2 (green colonies), *Wollea* sp. and *Oxynema thaianum*. In majority of studied strains

were found the maximum growth in small salt concentrations, and the decreasing growth intensity was detected with increasing salinity. It means that studied strains belong rather to halotolerant types than to the halophilic. The exception is *Oxynema thaianum*. This strain evidently belongs to halotolerance types.

***Leptolyngbya* sp.1:** the strain can grow when salinity ranged from 0-25 %. and then stopped to grow when salinity excessed 30 % (Figure 54).

***Nostoc* sp.1:** the strains grows in broad salinity range from 0-25 % and the growth rate was stopped in salinity higher than 30 %, similar as in the case of *Leptolyngbya* sp.1 (Figure 55).

***Wollea* sp.:** the results of growth under various salt concentrations of *Wollea* sp. strain is quite similar with *Leptolyngbya* sp.1 and *Nostoc* sp.1.This strain can also grow in salinity ranged from 0-25 % and at salt concentrations excess than 30 %, growth rate stopped (Figure 56).

***Oxynema thaianum*:** the growth rates of this strain is quite restricted. They grow better in salinity around 15 % and 20 %. They do not grow in lower salinity (0-10 %) and relatively higher salinity (25-40 %) (Figure 57).

***Nostoc* sp.2:** The strain shows growth very different from the others. It can grow very well in salinity about 0-2 % and when salinity increasing the growth rate stopped (Figure 58). It means, that it is not adapted to salinic environments.

Table 6 Main characteristics of cultured cyanobacterial morphotypes.

Name	Morphotypes	Notes
Cyanobium sp.	cells microscopic, oval to cylindrical, solitary or in group	Třeboň, CZ
Halothece sp.2 (*Halothece* - like)	cells very small, oval to rod-like, cells arranged in short filaments	Třeboň, CZ
Geitlerinema sp.	trichomes very long, solitary or in clusters, straight to slightly flexuous	Třeboň, CZ
Halomicronema sp.	non heterocytous filaments without sheaths, trichomes thin, cylindrical	Třeboň, CZ
Leptolyngbya sp.1	short to long, thin filaments with facultative sheaths, non heterocytous	Třeboň, CZ
Leptolyngbya sp.2	long, spiral filaments, facultative sheaths, non heterocytous	Třeboň, CZ
Oxynema thaianum	trichomes with pointed apical end cells, without sheaths	CCALA960,BRNM/HY2363
Pseudanabaena sp.	trichomes solitary, straight, highly constricted at cross walls	Třeboň, CZ
Trichocoleus sp.	very long filaments, nearly straight, not branching, without sheaths	Třeboň, CZ
Nostoc sp.1	heterocytous, black colonies, cell spherical, oval to irregularly oval, trichomes packed tightly in sheaths	Třeboň, CZ Třeboň, CZ
Nostoc sp.2	heterocytous, green colonies, cells spherical to little oval	Třeboň, CZ
Wollea sp.	heterocytous, trichomes in fascicles, akinetes elongated	Třeboň, CZ

Notes: CCALA = Culture Collection of Autotrophic Organisms (Institute of Botany, Academy of Sciences of the Czech Republic, Centre of Phycology, Czech Republic); CCALA 960 = the monospecific strain was deposited in CCALA collection in Třeboň (CZ) under designation CCALA 960; BRNM/HY 2363 = the exsiccated sample (dry material) was maintained in herbarium under designation BRNM/HY 2363 at Moravian Museum Brno, Czech Republic; Třeboň, CZ = the species, which were isolated and cultivated in laboratory at Institute of Botany, Academy of Sciences of the Czech Republic at Třeboň. All strains were also maintained in CMU culture collection.

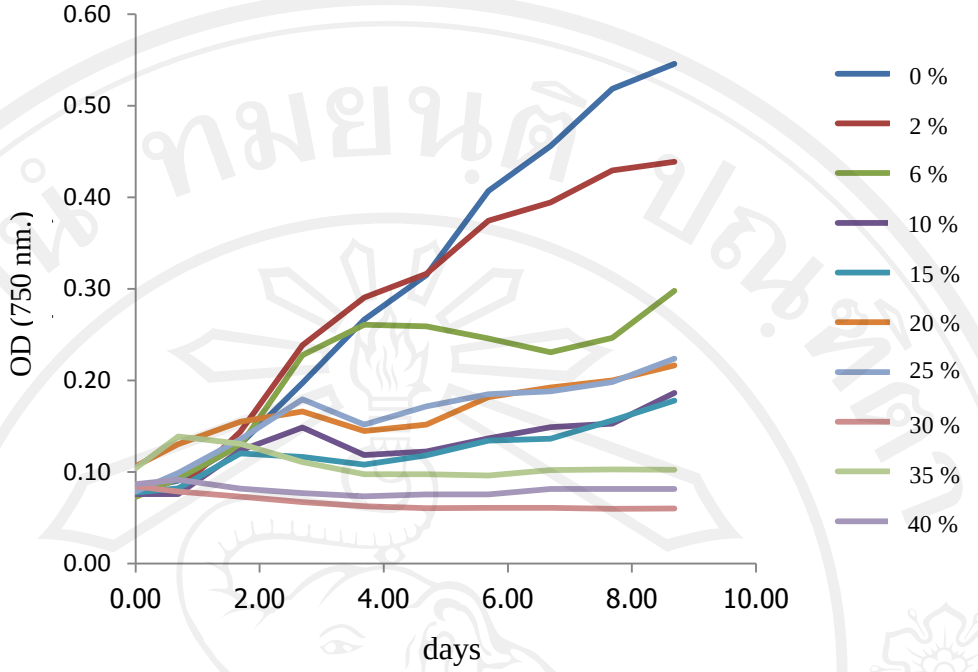

Figure 54 The growth of *Leptolyngbya* sp. 1 strain, cultured in various concentrations of salt under temperature around 30 °C and light intensity 32 µmol.m^{-2}.s^{-1}.

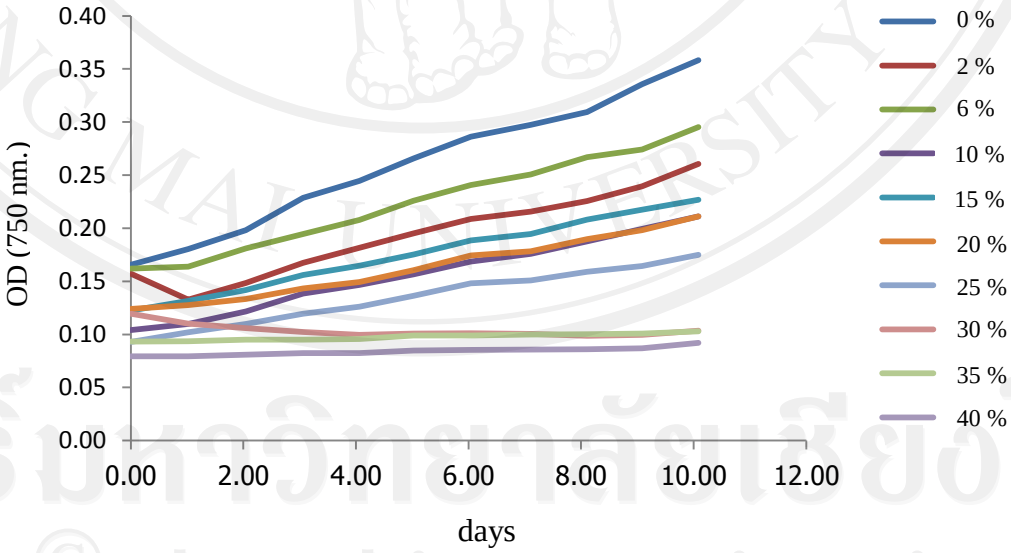

Figure 55 The growth of *Nostoc* sp.1 strain, cultured in various concentrations of salt under temperature around 30 °C and light intensity 32 µmol.m^{-2}.s^{-1}.

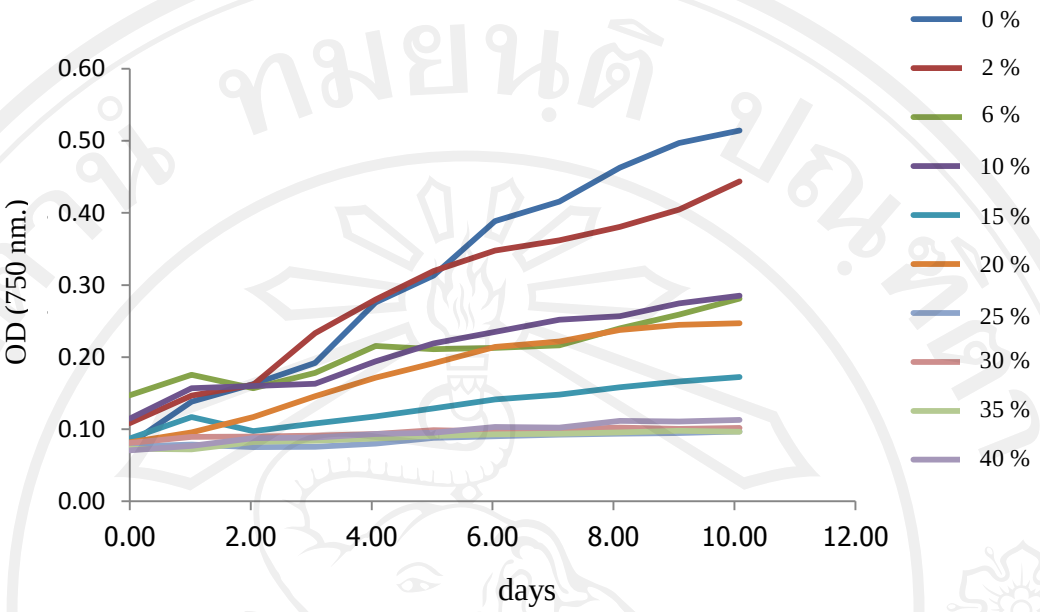

Figure 56 The growth of *Wollea* sp. strain isolated from this study, cultured in various concentrations of salt under temperature around 30 °C and light intensity 32 µmol.m^{-2}.s^{-1}.

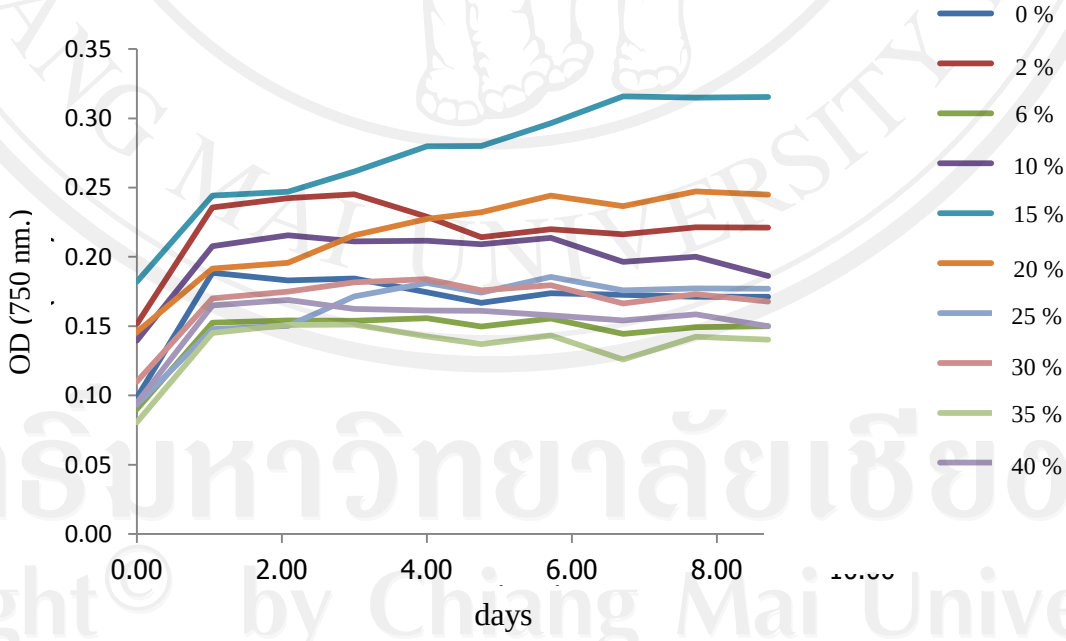

Figure 57 The growth of *Oxynema thaianum* strain, cultured in various concentrations of salt under temperature around 30 °C and light intensity 32 µmol.m^{-2}.s^{-1}.

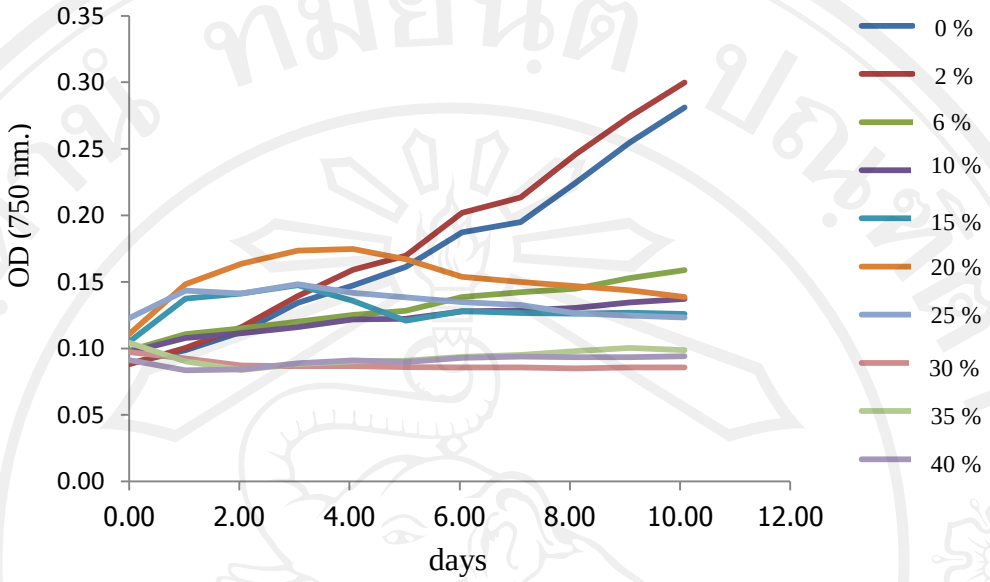

Figure 58 The growth of *Nostoc* sp.2 strain, cultured in various concentrations of salt under temperature around 30 °C and light intensity 32 µmol.m^{-2}.s^{-1}.

The morphology (colonies, cell sizes, shaped, sheaths, branching and etc.) of each strain growing under salt concentration gradients were investigated as well. It was found that the morphology of *Leptolyngbya* sp.1 and *Oxynema thaianum* does not change in all salt concentrations. In the case of *Nostoc* sp.1 and *Wollea* sp., their morphology slightly changed;

(1) ***Nostoc* sp.1:** at 0 %, filaments are tightly packed in sheaths (also described in taxonomic part) and when salinity increasing the filaments loosely packed in sheaths and the cell sizes are smaller than growth under lower salinities [Figure 59(1-3)].

(2) ***Wollea* sp.:** when salinity was increased(2-6 %), lots of akinetes were produced and the filaments tend to be shorter when salinity increasing (15-20 %) [Figure 59(4-6)].

However, in ***Nostoc* sp.2**, the morphology of this strain were very different from the normal conditions, namely, when the salinity is higher, the size of cells are bigger than usual condition 2-3 times, the occurrence of colonies forming was changed and each filament tend to produce its own sheaths (Figure 60).

4.5.2 Crossed gradients experiments

Continuation of the influence of light and temperature is only methodologically. Two strains of *Nostoc* spp.: *Nostoc* sp.1 and *Nostoc* sp.2 were used for this study. The strains were cultivated on the crossed gradients unit of light and temperature which temperature was performed from 16-38 °C and light irradiance ranged from 30-750 $\mu mol.m^{-2}.s^{-1}$. The results showed that both *Nostoc* sp.1 and *Nostoc* sp.2 tend to grow better in range of low temperature and irradiance. The strain of *Nostoc* sp.1 can grow in broader ranges of temperature and light better than *Nostoc* sp.2. *Nostoc* sp.1 displayed growth rates between 17-34 °C and light irradiance between 100-494 $\mu mol.m^{-2}.s^{-1}$ (Figure 61). While, the growth rates of *Nostoc* sp.2 was slightly limited. It can grow in range of temperature around 20-32 °C and light irradiance approximately 69-100 $\mu mol.m^{-2}.s^{-1}$ (Figure 62).

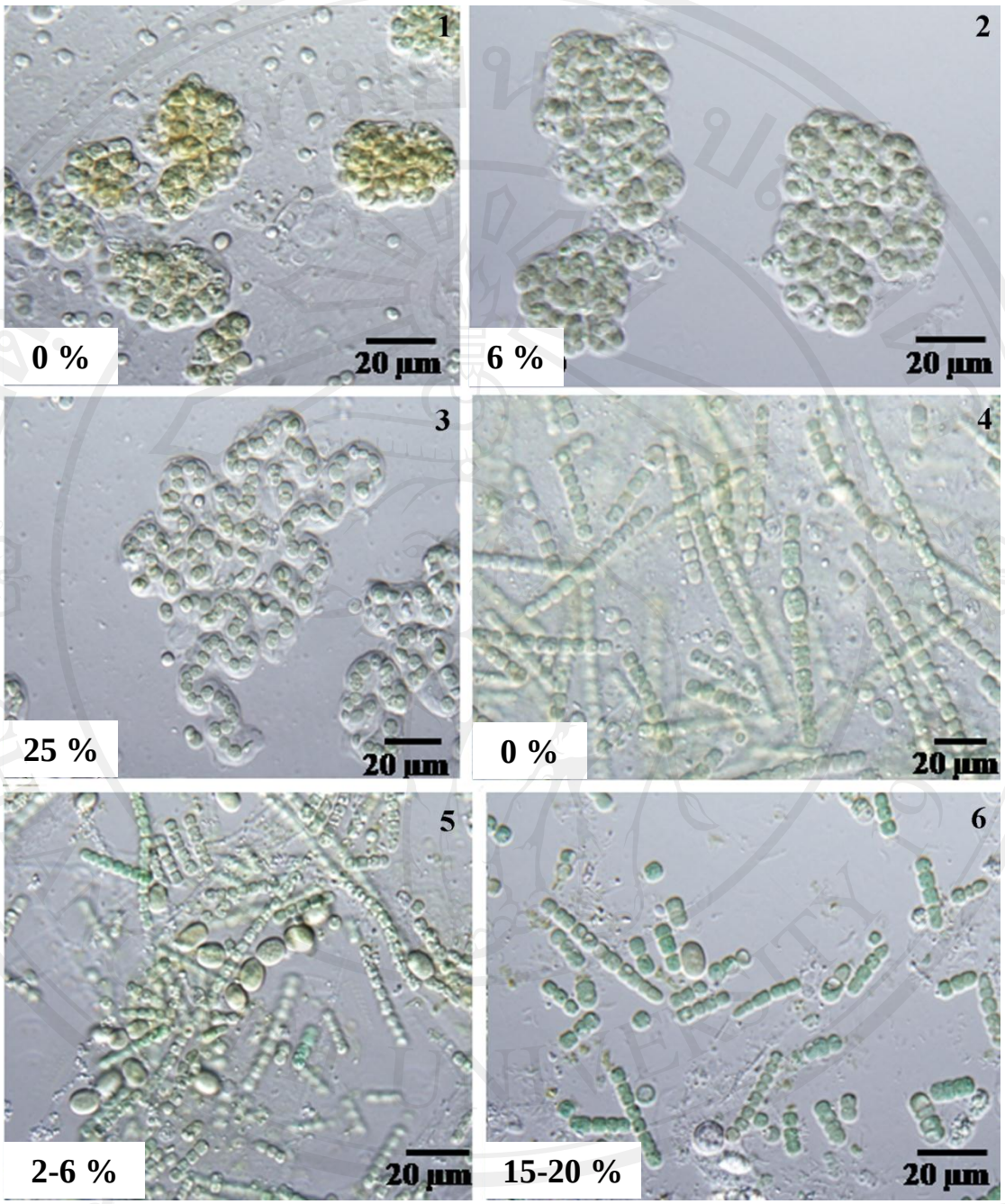

Figure 59 Morphology changing of the strains under gradients of salt concentrations. (1-3) changing morphology of *Nostoc* sp.1 strain under salinity gradients (at salinity 0 %, 6 % and 25 % respectively); (4-6) changing morphology of *Wollea* sp. strain under salinity gradients(at salinity 0 %, 2-6 % and 15-20 % respectively).

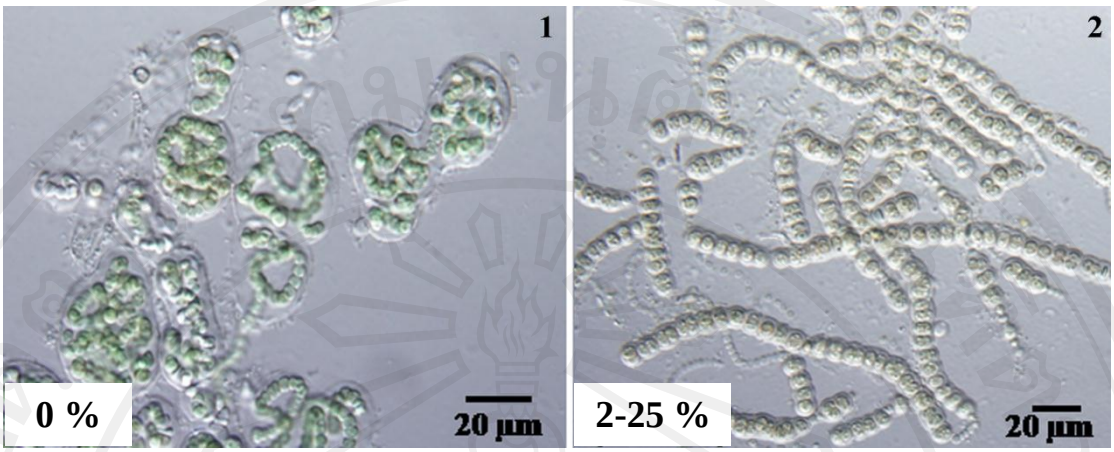

Figure 60 Morphology changing of the strains of *Nostoc* sp. 2 growing under gradients of salt concentrations.

(1) the morphology of *Nostoc* sp.2 under non-salinic condition: (2) the morphology of *Nostoc* sp.2 under semi up to extremely hypersaline conditions

4.6 *Oxynema* genus novum

Halophilic strain of "*Oxynema thaianum"* was selected for more studies. The polyphasic approaches include morphology, eco-physiology, molecular and ultrastructure evaluations, which were applied to the intensive study of this strain.

Our strain *Oxynema thaianum* CCALA 960 was previously designated as *Phormidium lloydianum* (Gomont) Anagnostidis et Komárek 1988 (Synonym: *Oscillatoria lloydiana* Gomont 1899), but according to phylogenetic evaluation it must be transferred into a new taxon. Therefore, we proposed to describe it as new genus "***Oxynema***" gen. nov., and transferred the traditional *Phormidium lloydianum and Oscillatoria acuminata* to the new taxon "*Oxynema lloydianum*" and "*Oxynema acuminatum*" comb. nov., respectively. The details are included in the publication in

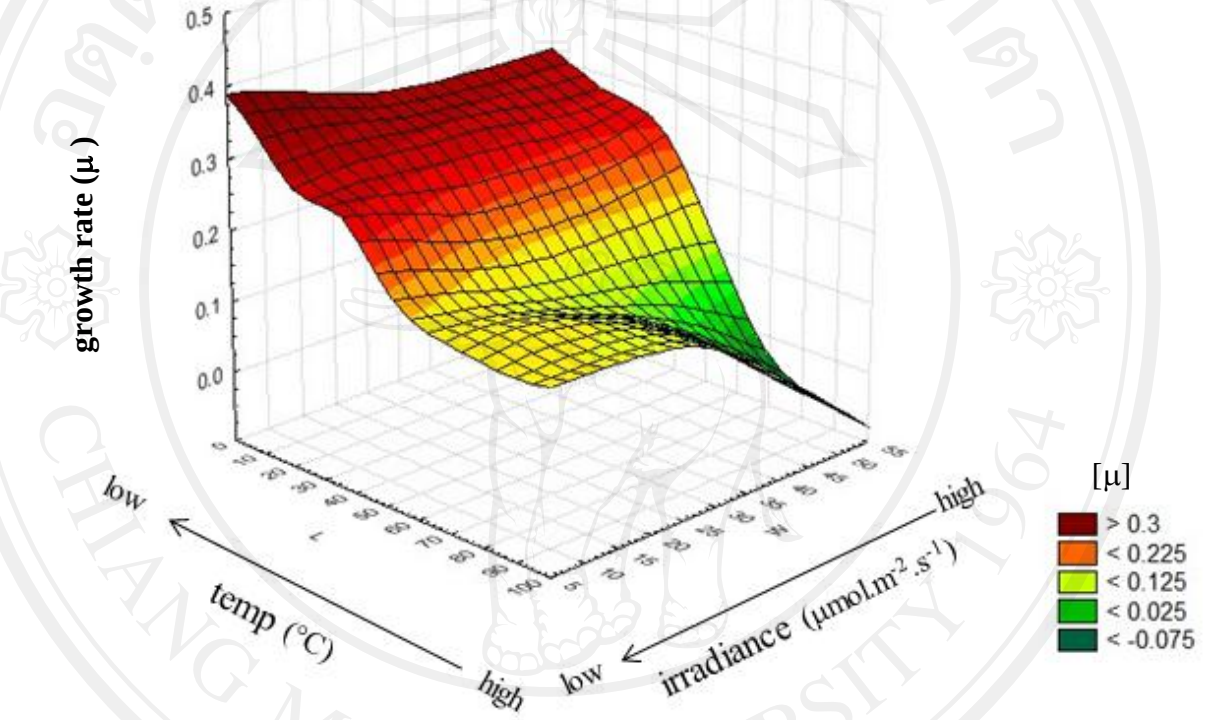

Figure 61 Growth rates of *Nostoc* sp.1 strain in crossed gradients of temperature and irradiance.

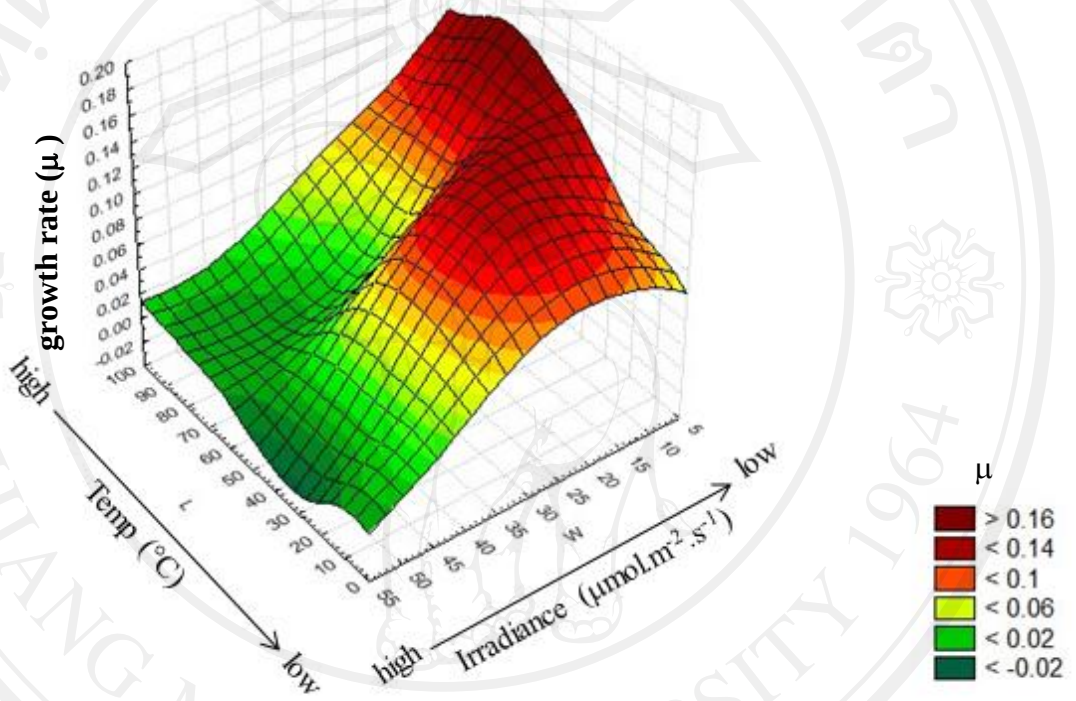

Figure 62 Growth rates of *Nostoc* sp.2 strain in crossed gradients of temperature and irradiance.

Cryptogamie/Algologie by Chatchawan *et al*., 2012. Some information and results of this strain are as follows:

Morphology:

During the study of cyanobacterial flora in hypersalinic areas (solar salterns in Petchaburi province, Thailand), was found the population of filamentous, aheterocytous cyanobacterium, which morphologically similar to the traditional species *Phormidium lloydianum* (Gomont) Anagnostidis et Komarek 1988 (Figure 63(1), Table 7).The first determination was only based on phenotype taxonomy. The monospecific cultures were isolated and deposited in CCALA collection in Třeboň, Czech Republic under designation CCALA 960.

The traditional filamentous, nonheterocytous and non akinetes cyanophytes genus *Phormidium* Kützing ex Gomont (1892) was characterized by the following morphological characters: cylindrical trichomes with facultative envelope. The taxonomy of *Phormidium* is still unclear, and therefore it was separated by the purpose of Komárek and Anagnostidis (2005), based on their morphological characters included presence of calyptra, morphology of terminal cells, terminal segment of trichomes, morphology of vegetative cells and others into 8 groups. From this morphological criteria, our *Oxynema thaianum* strain CCALA 960 corresponds more or less with the description of all species in *Phormidium* group-I which the main characteristic including attenuated at the end of trichomes and pointed apical cells. The resulting morphological characteristic are as follows: Thallus in the forms of mats, bright-dark blue green or blackish green, flat or layered in old parts and usually tightly packed with soil surface (particles). Fine sheaths are sometimes visible under electron microscope [Figure 67(2)]. Trichomes are always blue-green, long, not or

later (before disintegration to short hormogonia) constricted at the cross-walls, 7.5-9 µm wide, not or slightly granulated at the cross-walls, shortly attenuated at the end and usually slightly bent; the terminal cells are more elongated, conically narrowed and sharply pointed, always without calyptra.

The morphology of the type material of *Oscillatoria lloydiana* (=*Phormidium lloydianum*) Gomont and *Oscillatoria acuminate* Gomont, derived from PC herbarium, Paris, were studied for comparison with our strain CCALA 960. The most morphologically similar to *Oxynema thaianum* is *Phormidium* (*Oxynema*) *lloydianum* which differs by not having constriction and granulated at the cross-walls. *O. thaianum* also differs by facultative presence of fine sheaths [Figure 65(1-5), Table 7] and by length of cells, which can be shorter than wide up to isodiametric in the population from Thailand. *O. acuminatum* is a species which also closely similar to our *O. thaianum*. It was found only from thermal springs and differ from both species by morphologically with the length of cells and width of trichomes and ecologically [Figure 66(6-10), Table 9]. The *O. thaianum* CCALA 960 was also morphologically compared with other similar species in *Phormidium* group I described in Komárek and anagnostidis 2005 (Figures 63-64, Table 7).

Table 7 Morphology of other species in *Phormidium* group I according to Komárek and Anagnostidis (2005) and other related/similar species with *Oxynema thaianum*.

Species	Morphotypes	
Oxynema thaianum Chatchawan *et al*., 2012	**Trichomes:**	cylindrical, 7.5-9 µm. wide, not or later slightly constricted at cross-walls, ± slightly granulated at cross-walls, shortly attenuated at the ends and always slightly bent.
	Cells:	mostly shorter than wide, 2.2-3 µm. long, rarely isometric.
	Apical cells:	elongated, conically narrowed and sharply pointed, usually without calyptra.
	Sheaths:	can be observed under electron microscope.
	Occurrence:	salterns Thailand (Petchaburi province).
Oxynema lloydianum (Gomont) Chatchawan *et al*.,2012 (synonyme: *Oscillatoria lloydiana* Gomont 1899) [Figure 63(1)]	**Trichomes:**	cylindrical, 8-9 µm. wide, not constricted at cross-walls, shortly attenuated and bent at the ends.
	Cells:	mostly shorter than wide, 2.5-3 µm. long.
	Apical cells:	strongly pointed.
	Sheaths:	not observed.
	Occurrence:	**s**altwork, saline and backish waters, France (Bretangne).

**description of each species derived from Komárek and Anagnostidis (2005).

Table 7 (continued).

Species	Morphotypes	
Oxynema acuminatum (Gomont) Chatchawan *et al*., 2012 [syn: *Oscillatoria acuminata* (Gomont 1899) sensu Anagnostidis 1961] [Figure 63(2)]	**Trichomes:**	3-5 µm wide, not or slightly constricted at the granulated cross-walls, at the ends abruptly briefly attenuated.
	Cells:	mostly longer than wide, rarely nearly isodiametric or shorter, 4-7 µm long.
	Apical cells:	acute-conocal, pointed.
	Occurrence:	thermal springs with higher levels of salinity, probably cosmopolitan.
Oscillatoria salina Biwas 1926 [Figure 63(3)]	**Trichomes:**	3-5 µm wide, not constricted at cross-walls, attenuated at the ends.
	Cells:	up to 2 µm. long.
	Apical cells:	elongated, arcuated and pointed.
	Occurrence:	saline lakes from India (near Calcutta).
Oscillatoria brevis sensu Claus 1961 [Figure 63(4)]	**Trichomes:**	5.4-6.3µm wide, straight, not constricted at cross-walls, shortly tapering and bent on apex.
	Cells:	1.3-1.8 2 µm. long.
	Occurrence:	Salzlacken-gebiet, Austria.

**descriptions of each species derived from Komárek and Anagnostidis (2005).

Table 7 (continued).

Species	Morphotypes	
Oscillatoria janthiphora (Fiorini-Mezzetti) Gomont 1982 [Figure 63(5)]	**Trichomes:**	3.4-5.8 µm wide straight or slightly curved, not or very slightly at the usually finely granulates cross-walls, ± long attenuated at the ends and slightly bent or spiral coiled.
	Cells:	nearly isodiametric, 3.3-6.7 µm. long.
	Apical cells:	acute-conical or rounded-conical without calyptra.
	Occurrence:	thermal and warm springs; Greece, Hungary, Italy.
Oscillatoria acuminata sensu Rina 1972 [Figure 63(6)]	**Trichomes:**	± 4 µm wide.
	Cells:	shorter than wide 3.5-4 µm long.
	Occurrence:	not thermal waters; Mozambique.
Oscillatoria paulseniana Boye-Peterson 1930 [Figure 63(7)]	**Trichomes:**	7-8 µm wide, very slightly constricted at the cross-walls, shortly attenuated towards ends.
	Cells:	shorther than wide.
	Apical cells:	pointed.
Oscillatoria sonorensis Drouet 1942 [Figure 64(1)]	**Trichomes:**	3-5 µm wide, not very slightly constricted at cross-walls.
	Cells:	isodiametric to 2x longer than wide.
	Apical cells:	hooked, conically-narrowed and pointed.
	Occurrence:	in brackish tide pools; Sonora, Mexico.

**descriptions of each species derived from Komárek and Anagnostidis (2005).

Table 7 (continued).

Species	Morphotypes	
Oscillatoria animalis sensu Borge 1933 [Figure 64(2)]	**Trichomes:**	± 4 µm wide, districtly constricted at cross-walls.
	Cells:	3.5-5 µm long.
	Apical cells:	pointed.
	Occurrence:	Argentina, China.
Oscillatoria rostrata Borge 1933 [Figure 64(3)]	**Trichomes:**	5.7-7 3-5 µm wide, not constricted at cross-walls, hooked at the ends.
	Cells:	very short cells.
	Apical cells:	acute-pointed.
	Occurrence:	mineral water in Mongolia.
Oscillatoria animalis sensu Anagnostidis 1961 [Figure 64(4)]	**Trichomes:**	2.5 µm wide.
	Occurrence:	Hot spring in Greece.
Oscillatoria karakalpenensis Muzafarov [Figure 64(5)]	**Trichomes:**	4.6-6 µm wide, not constricted at the cross-walls.
	Cells:	1-2.5 µm long.
	Apical cells:	conical and pointed-rounded.
	Occurrence:	described from Lake Dzaman-Saj (salinic lakes), Azerbaijan.

**descriptions of each species derived from Komárek and Anagnostidis (2005).

Table 7 (continued).

Species	Morphotypes	
Oscillatoria acuminata var. *longe-attenuata* Geitler et Ruttner 1935 [Figure 64(6)]	**Trichomes:**	3.7-4 µm. wide with continually narrowed and pointed ends.
	Occurrence:	hot spring; Indonesia (Kadjaj).
Phormidium anabaenoides Drouet 1961 [Figure 64(7)]	**Trichomes:**	cylindrical, 2-5 µm wide,constricted at cross-walls.
	Apical cells:	conical and sharply pointed.
	Sheaths:	colorless, diffluent.
	Occurrence:	in hot springs; USA. (California).
Phormidium richardsii Drouet 1942 [Figure 64(8)]	**Trichomes:**	3-7 µm wide, not or very indistinctly constricted at the cross-walls.
	Cells:	shorter than wide.
	Apical cells:	conical elongated and pointed-rounded.
	Occurrence:	in periodically wetted soils; USA. (Montana, New Mexico).

**descriptions of each species derived from Komárek and Anagnostidis (2005).

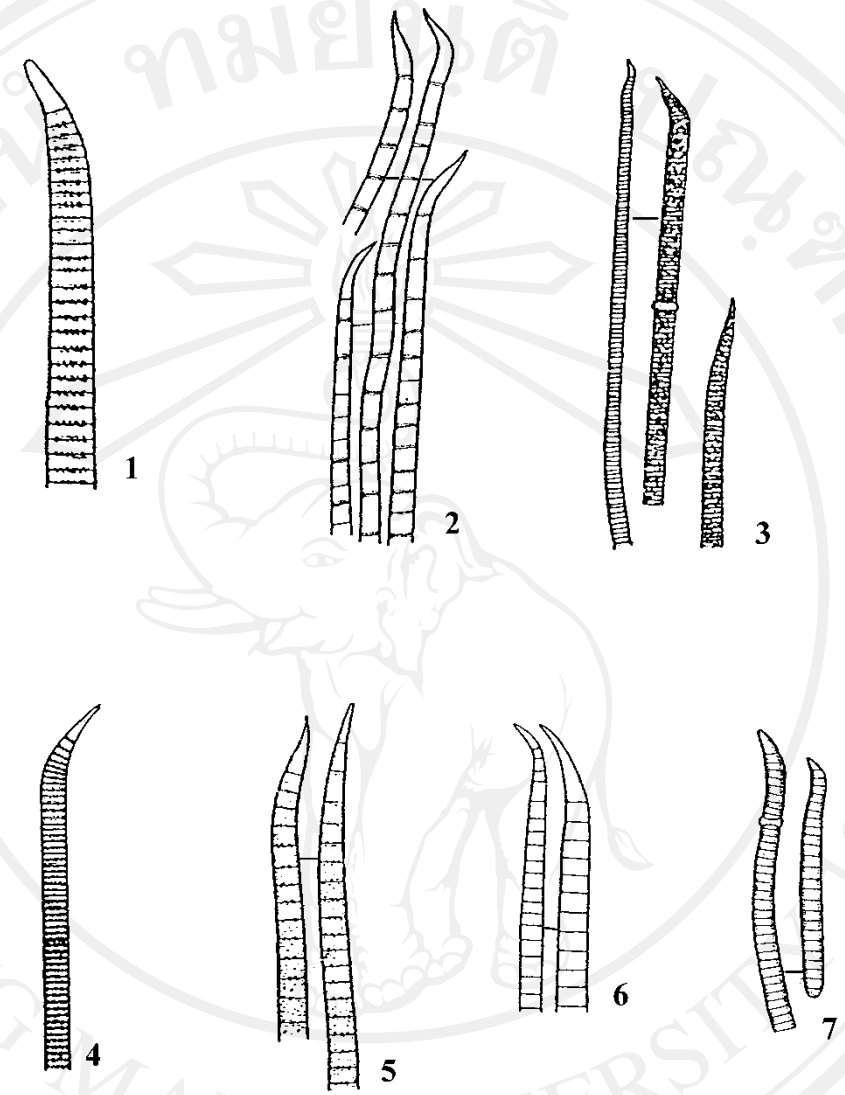

Figure 63 Illustration of other species in *Phormidium* group I and other similar species with *Oxynema thaianum* [derived from Komárek and Anagnostidis (2005)].

(1) *Oxynema lloydianum* (*Oscillatoria lloydiana* Gomont 1899). (2) *Oxynema acuminatum* (*Oscillatoria acuminate* Gomont 1899), (3) *Oscillatoria salina* Biwas, (4) *Oscillatoria brevis* sensu Claus 1961, (5) *Oscillatoria janthiphora* (Fior.-Mazz.) Gomont, (6) *Oscillatoria acuminata* sensu Rino, (7) *Oscillatoria paulseniana* Boye-Petersen.

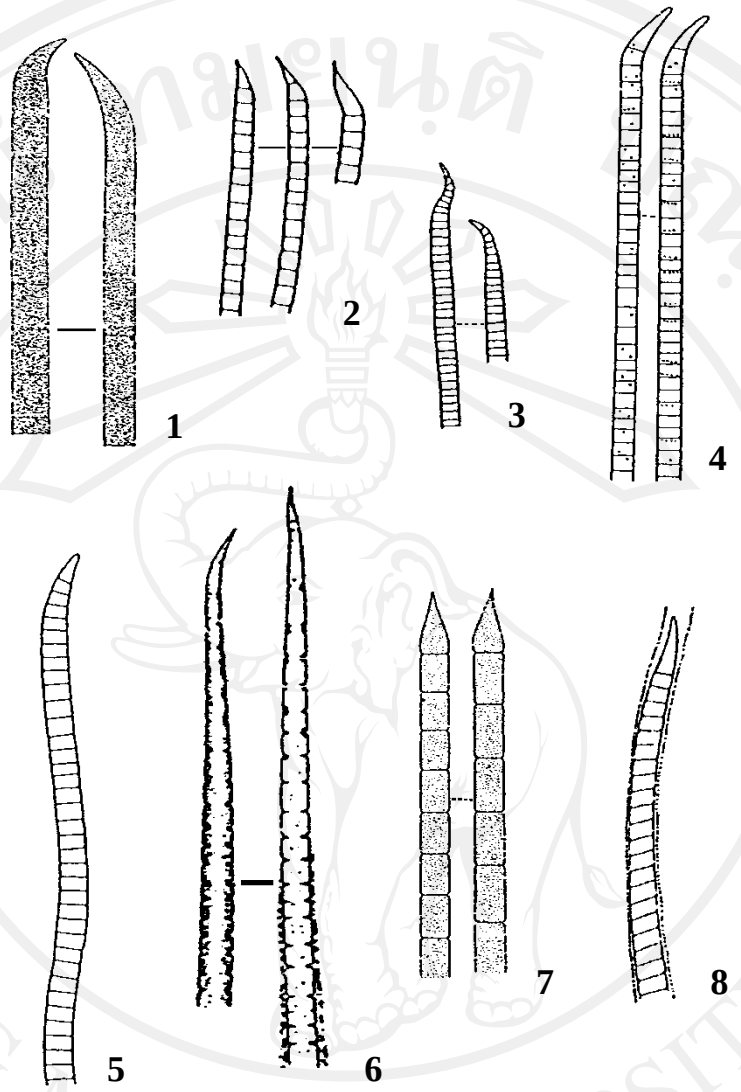

Figure 64 Illustration of other species in *Phormidium* group I and other similar species with *Oxynema thaianum* [derived from Komárek and Anagnostidis (2005)].

(1) *Oscillatoria sonorensis* Drouet, (2) *Oscillatoria animalis* sensu Borge 1933, (3) *Oscillatoria rostrata* Borge, (4) *Oscillatoria animalis* sensu Anagnostidis 1961, (5) *Oscillatoria karakalpanensis* Muzafarov, (6) *Oscillatoria acuminata* var. *longe-attenuata* Geitler et Ruttner, (7) *Phormidium anabaenoides* Drouet, (8) *Phormidium richardsii* Drouet.

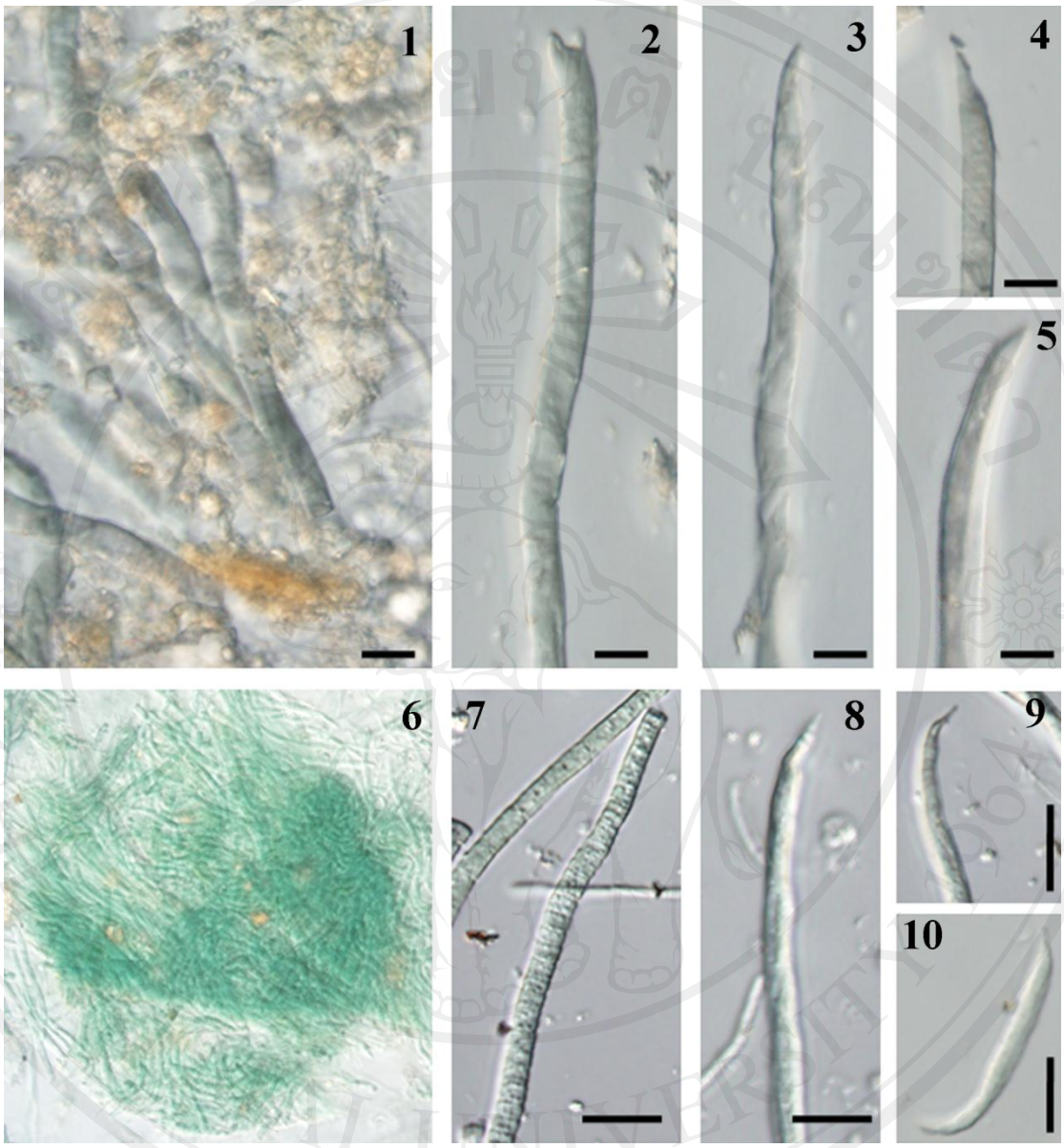

Figure 65 Photos of type specimens of *Oxynama lloydianum* and *Oxynema acuminatum*.

(1-5) Type specimens of *Oxynema lloydianum* (originally *Oscillatoria lloydiana* PC0655991) from coastal salt marshes in France (1959). (6-10) Type specimens of *Oxynema acuminatum* (originally *Oscillatoria acuminata* PC0655992) from Euganean thermal springs, Italy (without date of collection). Scale bars = 10 µm.

Molecular evaluation (Phylogeny):

From the 16S rRNA gene sequencing, the *Oxynema thaianum,* strain CCALA 960 (JF729323) belongs in an isolated cluster in the phylogenetic tree (less than 93% similarity to the nearest cluster). The GenBank Blast search revealed only 2 similar sequences of 16s rDNA; the other sequences had less than 93 % similarity. The two strain derived from GenBank are included: (1) Strain EF 44714, designated as thermal species *Oscillatoria acuminata* (syn.: *Phormidium acuminatum*). This species is known from thermal springs, often with higher conductivity [see, e.g., Anagnostidis 1961(Figure 64(4))]. (2) Strain HQ 730084 identified as *Phormidium* sp. CENA 135 which the morphology of this species is closely similar to *Oscillatoria lloydiana* (=*Phormidium lloydianum*) (Figure 66A) and isolated from mangroves in Brazil; it means evidently from halophilic localities and also with similar morphology. It is possible to conclude that all members of this isolated generic cluster have very similar and specific morphological characters (cylindrical trichomes without heterocytes and akinetes, characteristical termination in form of elongated narrowed and bent cells with pointed ends, without calyptra) and also a similar ecology (mineral or halophilic localities, or in thermal habitats, in warm regions). The cluster, containing the strains of the type species of *Phormidium* or *Oscillatoria* (the cluster C in Figure 66) is distinctly far from the group with *Oxynema thaianum.* From this point of view, the cluster with *Oxynema thaianum* must be separated as a special generic entity, characterized by phylogenetic position as well as by phenotype specificity.

Taxonomy:

From molecular results, the cluster containing *Oxynema thaianum* is very separated from the nearest clades by less than 93 % of similarity. Therefore, the whole

cluster should be defined as a separated genus: ***Oxynema*** genus novum (Chatchawan *et al*., 2012).

Diagnosis: Filamenta cyanoprocaryotica, in stratum planum aerugineum vel aerugineo-viride agglomerata, recta vel paucim flexuosa, simplicia, cylindrica, sine ramificatione. Vaginae facultativae, tenues, incolores, diffluentes vel adsunt. Trichoma cylindrica, uniseriata, fragilia, ad genicula non vel interdum leviter constricta, in parte apicali ad extremum attenuata et interdum flexa, cum cellula terminali plus minusve acuta vel acute-rotundata. Cellulae isodiametricae vel longiores vel breviores quam latae. Thylakoidae radialiter dispositae. Heterocytae akinetesque carentes. – Plantae hydrophilae vel subaerophyticae, plerumque halophilae vel thermales.

Thallus in the form of mats, bright blue-green, blackish green or dark green, flat, layered in old parts, sometimes tightly packed with soil or salt particles. Sheaths facultative, thin, coulorless and diffluent. Trichomes are blue-green, less frequently olive-green or dark green, long, sometimes fasciculated on the surface, not constricted or later constricted at the cross-walls shortly attenuated at the ends and usually slightly bent; the terminal cells are acute to acute-rounded. Cells were mostly shorter than wide rarely up to isodiametric. Thylakoid radially arrangement. Heterocytes and akinentes are not presented.

Typus generis: ***Oxynema thaianum*** **spec. nova.**

Diagnosis: Filamenta in stratum planum aerugineo-viride agglomerata, plus minusve recta vel flexuosa. Vaginae facultativae, tenues, diffluentes. Trichoma fragilia, ad

genicula leviter constricta, 7.5-9 µm lata, ad extremum breve attenuata et flexa, cum cellula apicali subacuta. Cellulae breviores quam latae ad isodiametricae.

Diagnosis: Thallus in the form of mats, bright blue-green, blackish green or dark green. Sheaths facultative, thin, coulorless and diffluent. Trichomes constricted at the cross-walls, 7.5-9 µm wide, not or slightly granulated at the cross walls, shortly attenuated at the ends and usually slightly bent; the terminal cells are more elongated, conically narrowed and sharply pointed, always without calyptra. Cells shorter than wide, rarely isodiametric.

Habitatio: Bentice in solis salinis; salinae in Provincia Petchaburi Dicta, Thailandia.

Habitats: Benthic from in saline soils, found in Petchaburi province, Thailand.

Typus: Exsiccatum BRNM HY 2363; iconae typicae figurae nostrae 41,51,67-68.

Types: Exsiccate BRNM HY 2363; typical pictures 41,51,67-68.

Reference strain: CCALA 960, deposited in the CCALA collection, Třeboň, Czech Republic; accession number = JF729323. The type exsiccate is deposited under the number: BRNM HY2363 in the Moravian Museum Brno, Czech Republic.

Etymology: oxys (όξυς) = sharp, pointed; nema (νημα) = thread, filament.

Ultrastructure patterns:

The thylakoid pattern (Figure 68) of this strain is similar to cyanobacteria from the traditional family Phorimidiaceae according to Komárek and Anagnostidis (2005). It is mostly similar to the thylakoid pattern of the genus *Microcoleus chthonoplastes* (comparing with Hernández-Mariné, 1996) which was transferred recently to the

genus *Coleofasciculus* (Siegesmund *et al*., 2008) (Figure 69). Thylakoids are arranged characteristically radially in cells, in the lateral view irregularly situated in the whole cell volume along the long axis of cells. In the apical narrowed cells were found commonly the widened thylakoids. Among thylakoids are visible carboxysomes, phycobilisomes and ribosomes, but gas vesicles were never found (Figure 68). Morphology of terminal parts of the filaments is clearly visible from SEM preparations (Figure 67). A fine mucilage is always present, but it sometimes disappears during SEM treatment. Mucilaginous material is always visible at the end of pointed terminal cells. The trichomes of *Oxynema* have no calyptras and the terminal cells are more or less pointed (rounded-pointed). However, the net-like mucilaginous material occurs obligatory at the ends of apical cells [Figure 67(3-4)].

From this results, cyanophycin found along whole filaments of *Oxynema thaianum*. Cyanophycins are largely insoluble granules which heterocytous mainly used as nitrogen storage compound and also serves as a dynamic buffer for fixed nitrogen (Krehenbrink *et al*, 1998; Steinbüchel *et al*, 2002). Therefore, *Oxynema thaianum* may be possible to have nitrogen fixation ability. However, the intensive studies about nitogen fixation of this cyanobacteria still need in future.

Ecology and distribution:

In Thailand, *O. thaianum* occurred in Petchaburi saltworks in the form of mats (mixing with other cyanoprokaryotes from the genus *Leptolyngbya* and *Coleofasciculus*) on wet salty soils, or sometimes on the bottom in shallow water. It is typical halophilic species.

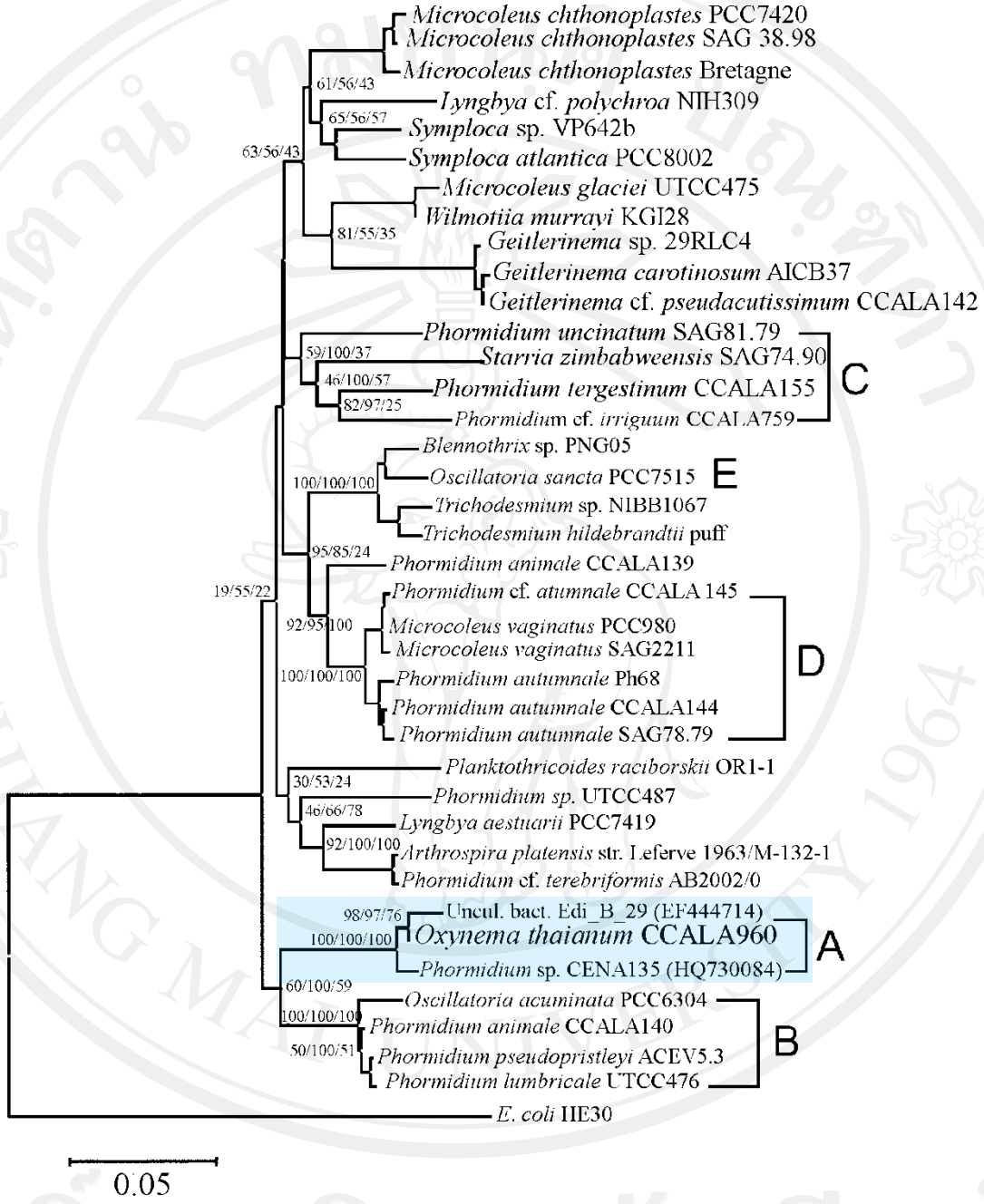

Figure 66 Position of the *Oxynema thaianum* strain CCALA 960 (printed by bold font) in the phylogenetic tree.

(A) In comparison with other strains of Oscillatoriales, selected from GenBank. The evolutionary (molecular phylogenetic) analyses were conducted in MEGA 5, using

the Maximum Likelihood method based on the Jukes-Cantor model. Topology was validated by Bayesian analysis in MrBayes 3.1.2. and maximum pasimony search in Paup*. Numbers at internal branches indicate bootstrap values from 1,000 replicates of ML, posterior probabilities from Bayesian analyses and bootstrap values from 1,000 MP searches. Values corresponding to partitions reproduced in less than 50% in ML and MP bootstrap replicates are not shown. The nearest cluster (B) differs by less than 93% of similarity from *Oxynema*. The typical *Phormidium* strains are included in the cluster (C) Complex of *Phormidium autumnale* forms separate cluster with *Microcoleus vaginatus* (D) The typical *Oscillatoria* are contained in the cluster (E).

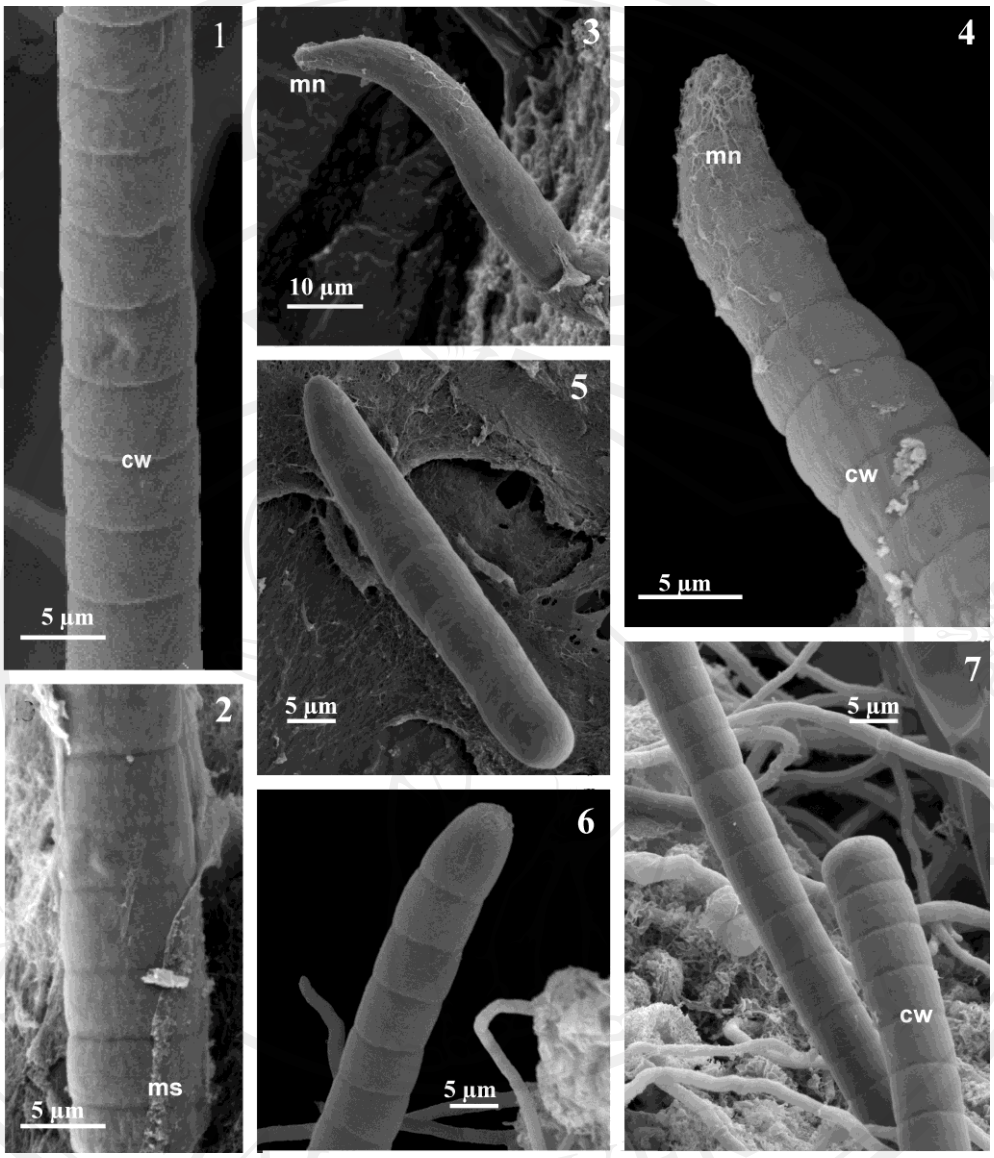

Figure 67 Ultrastructure (SEM) of trichomes from the type strain CCALA 960 of *Oxynema thaianum*.

(1-2) part of a filament (2 with fine mucilaginous sheath), (3-5) terminal parts with pointed end cells, (5) hormogonium liberated from the terminal part of a trichome, (6-7) ends of disintegrated trichomes. Explanations: **cw** = cross walls; **ms** = mucilaginous sheaths; **mn** = mucilaginous net-like material at the end of apical cells (according to Šmarda in Chatchawan *et al*., 2012).

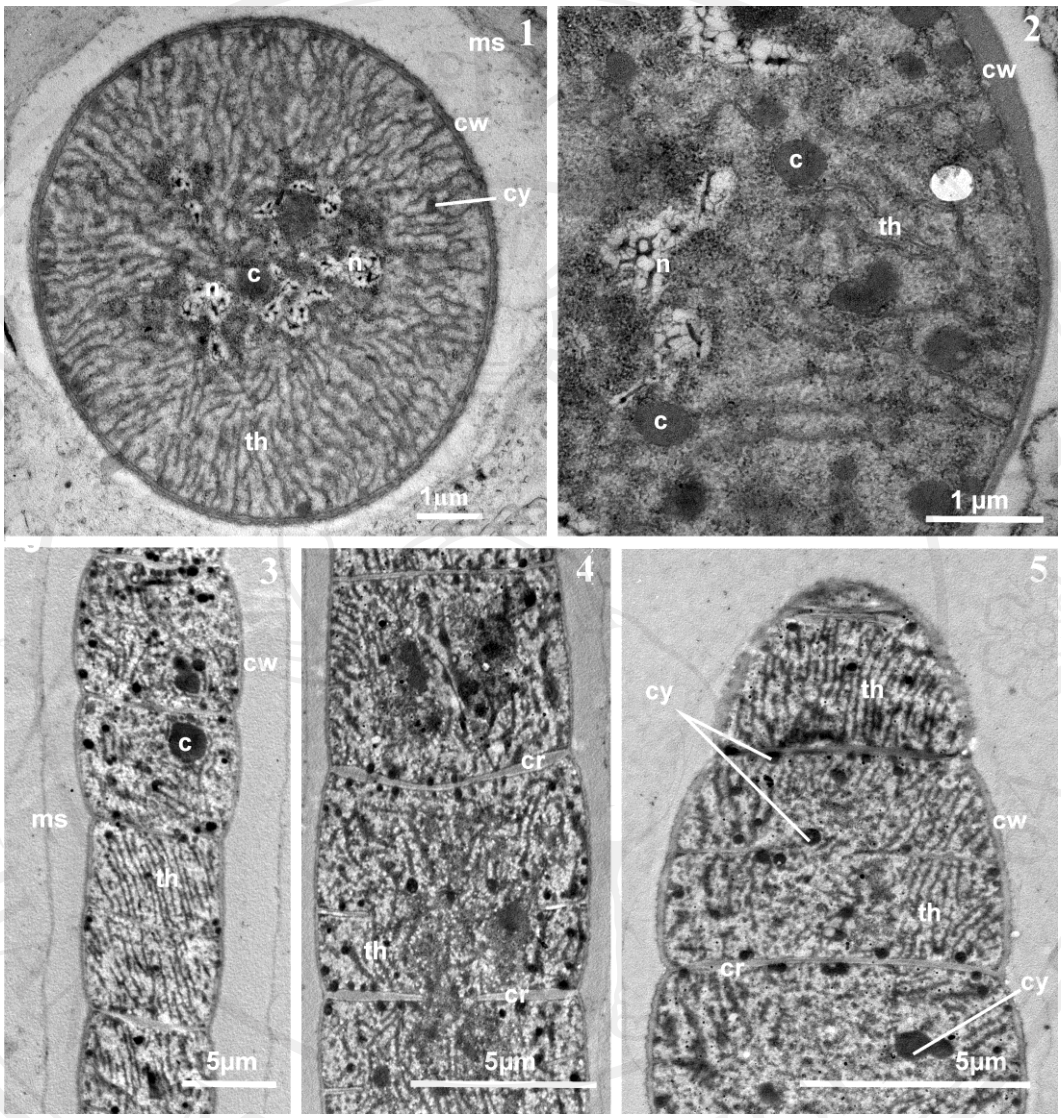

Figure 68 Ultrastructure (TEM) of filaments of *Oxynema thaianum* (the type strain: strain CCALA 960).

(1-2) fixed by osmium, (3-5) fixed by glutaraldehyde. Characteristical are particularly the radially arranged, flexuous thylakoids, scatered nucleoid, high number of carboxysomes, small cyanophycin granules and diffluent mucilaginous envelopes. Explanations: **cr =** cross-walls; **cw** = cell wall; **cy** = cyanophycin; **ms** = mucilaginous sheaths; **n** = nucleoplasma; **th** = thylakoids; **c** = carboxysomes; **r** = ribosomes (after Šmarda in Chatchawan *et al*., 2012).

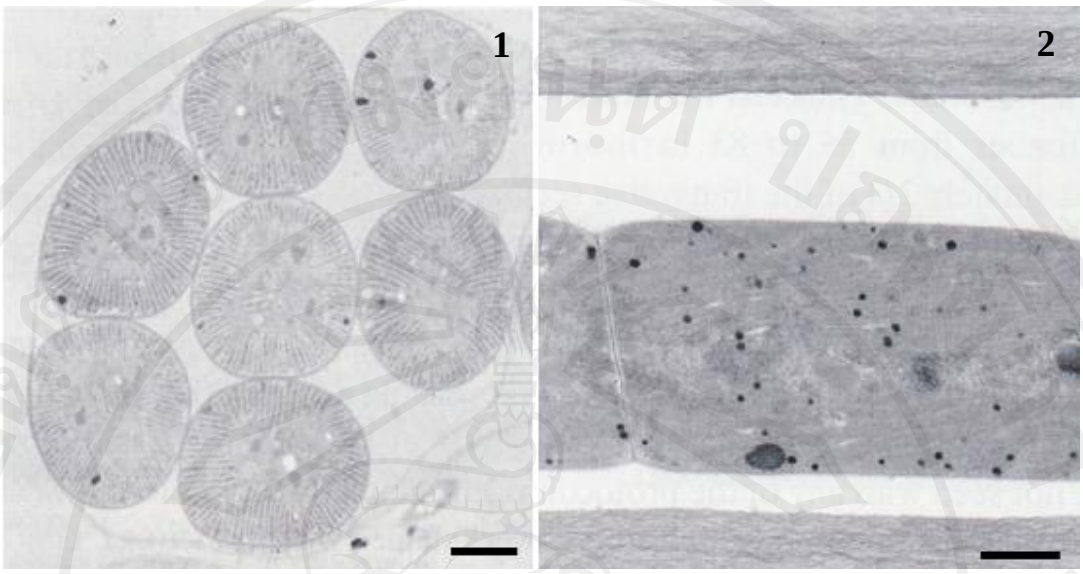

Figure 69 Transmission electron micrographs of *Microcoleus chthonoplastes* (after Hernández-Mariné, 1996).

(1) rapid freeze fixation-cross section through a bundle inside a common sheath; (2) conventional method-longitudinal section of a filament with fasciculated thylakoids (scale bar = 1 μm).