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ORIGINAL RESEARCH ARTICLE

Role of *Cylindrospermopsis* sp. in vertical nitrogen changes observed in tropical oxidation wastewater treatment ponds

M. Srichomphu[1], O. Phewnil[1,*], T. Pattamapitoon[1], R. Chaichana[2], K. Chunkao[3], W. Wararam1, N. Dampin[1], P. Maskulrath[3]

[1] Department of Environmental Science, Faculty of Environment, KasetsartUniversity, Bangkok, Thailand

[2] Department of Environmental Technology and Management, Faculty of Environment, Kasetsart University, Bangkok, Thailand.

[3] The King's Royally Initiated Laem Phak Bia Environmental Research and Development Project, Chaipattana Foundation, Ban Laem District, Phetchaburi Province, Thailand

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ABSTRACT

BACKGROUND AND OBJECTIVES: As a producer within the ecological food chain, phytoplankton provides the base energy and oxygen to the environment through photosynthesis and higher tropic levels. These benefits can be applied in five consecutive nature-by-nature oxidation ponds for the treatment of community wastewater coming through a high density polyethylene pipeline from the Phetchaburi Municipality located at the King's Royally Initiated Laem Phak Bia Environmental Research and Development Project, Ban Laem District, Phetchaburi Province (Universal Transverse Mercator 47P 1442725 North 617774 East). This study focuses on the vertical distribution of the phytoplankton *Cylindrospermopsis* sp. and its relationship with nitrogen compounds in oxidation ponds.

METHODS: Samples were collected from a community wastewater treatment system at various depths (30, 60, 90, 120, and 150 centimeters) below the water surface in April 2019 between 11:00 and 13:00 hours and analyzed for their chemical and physical properties. The analysis revealed a vertical relationship between *Cylindrospermopsis* sp. and wastewater. In the density of phytoplankton which were collected by measuring 20 liters of water and filtered using a 36-micron plankton net, calculated and counted under a high magnification microscope, as the species are classified according the taxonomy.

FINDINGS: The results of the wastewater quality were as follows: the content of suspended solids was 65–81 milligram per liter, water temperature was 31.8–33.2°C, potential of hydrogen was 8.7–9.2, total nitrogen content was 4.0–5.3 milligram per liter, ammonium content was 0.03–0.06 milligram per liter, nitrate content was 0.09–0.12 milligram per liter, total phosphorus content was 0.9–1.3 milligram per liter, and phosphate content was 0.4–0.5 milligram per liter. In the density of phytoplankton, a significant correlation was observed between the population of *Cylindrospermopsis* sp. and water depth ($R^2 = 0.9324$). The number of populations at the depths of 30, 60, 90, 120, and 150 centimeters were 3.2×10^7, 1.6×10^7, 1.1×10^7, 5.5×10^7, and 1.1×10^8 cells per cubic meter, respectively.

CONCLUSION: The different densities of *Cylindrospermopsis* sp. found at different depths throughout the treatment pond are related to the nitrogen dynamics of the water body. The results of this study revealed that organic nitrogen, including ammonium, was assimilated and converted to inorganic nutrients, which promoted the growth of other phytoplankton species. The correlation between *Cylindrospermopsis* sp. and total nitrogen and ammonium showed significance at R2 = 0.7268 and 0.797, respectively, with a confidence level of 0.05. Therefore, to ensure treatment effectiveness, the depth of wastewater treatment ponds should be considered during their construction because phytoplankton regulation plays an important role to maintain the overall treatment efficiency.

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*Corresponding Author:
Email: *onanong.p@ku.th*
Phone: +668 1824 8649

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INTRODUCTION

Phytoplankton are an important ecosystem species and primary producers of food for marine fauna larvae and dissolved oxygen (DO) in water (Cahyonugroho *et al.,* 2023). As a bioindicator for water fertility and quality, different types of plankton require different environmental factors for growth and blooming (Yossan *et al.,* 2015; Enawgaw *et al.*, 2023). For water contaminated with high levels of organic substances, such as community oxidation pond systems, phytoplankton can produce oxygen for the aerobic digestion of bacteria, which reduces contaminated organic substances and is effective in wastewater treatment (Chunkao *et al*., 2014; Gautam and Saini, 2020). Environmental factors affecting phytoplankton growth include sunlight, temperature, and chemical compounds, such as nitrogen and phosphorus (Chaichana *et al.,* 2016), which are necessary for protein synthesis and cell formation (Klotz *et al*., 2016). In addition, some phytoplankton, such as cyanobacteria and blue-green algae, including *Microcystis* sp., *Oscilatroria* sp., *Annabena* sp., and *Cylindrospermopsis* sp., are capable of fixing nitrogen from the air (Paerl, 2017; Ammar *et al*., 2022). However, some cyanobacteria produce biotoxins, such as microcystin, nodularin, cylindrospermopsin, anatoxin, and saxitoxins, which often affect the nervous system, liver, kidney, skin, and cell membranes of aquatic species, such as fish (Sivonen, 2009; Pelaez *et al.*, 2010; Boopathi *et al*., 2014; Mowe *et al*., 2015; Sotton *et al*., 2015; Mohamed *et al*., 2018). The importance of nitrogen on phytoplankton can be observed based on the nutrients that affect the growth of plankton by stimulating the production of pigments in the cells used in photosynthesis. In particular, pigments affect energy absorption at different wavelengths, facilitating photosynthesis and producing energy for cell division and growth (Cira *et al.*, 2016). In open water bodies, nitrogen fixation occurs through the transformation of organic nitrogen by ammonification into ammonia nitrogen (NH_4^+), generating nitrite (NO_2^-) and nitrate (NO_3^-), which promote the growth of phytoplankton (Wang *et al.,* 2022). In municipal wastewater treatment systems, nitrogen is transformed through nitrification without significant affects caused by both the depth and seasons. Studies on community wastewater oxidation ponds in Thailand have shown that environmental factors, such as high nutrient levels and organic contaminants, promote high growth rates of cyanobacteria. Such nutrients were measured with total nitrogen (TN) ranging between 5.0 and 7.1 milligram per liter (mg/L), total phosphorus (TP) at 0.5 and 1.0 mg/L. In terms of energy, solar radiation wavelengths ranging from 381 to 450 nm, 501 to 570 nanometer (nm), and 621 to 750 nm support the growth of cyanobacteria, and each wavelength is specifically important for individual pigments, such as chlorophyll-a, chlorophyll-b, and phycoerythrin, within cyanobacteria cells (Chunkao *et al*., 2014; Chaichana *et al*., 2016; Li *et al*., 2017; Sukchinda *et al*., 2019). However, since the wastewater treatment systems are highly turbid, solar radiation reaches only up to a water depth of around 30 centimeters (cm) below the surface. Studies have also shown that cyanobacteria, such as *Cylindrospermopsis* sp., grow well under low-light-intensity conditions and are distributed vertically. *Cylindrostermopsis* sp. is a phytoplankton species that fixes nitrogen from the atmosphere for its growth and causes eutrophication. In addition, *Cylindrostermopsis* sp. produces cylindrospermopsin (CYN), a hepatotoxin or cytotoxin that targets the liver, kidney, intestine and muscles (Fernandez *et al*., 2014; Sotton *et al*., 2014; Mohamad *et al*., 2018). Furthermore, *Cylindrospermopsis* sp. cyanobacteria are members of the *Nostocaceae* family and have a filamentous structure, with their cell structures containing pigments, such as chlorophyll-a, carotenoids, phycocyanin, and phycoerythrin. They can produce saxitoxin and cylindrospermopsin (Krienitz *et al*., 2013; Boopathi *et al*., 2014; Pierangelini *et al*., 2014; Vico *et al*., 2020; Huo *et al*., 2021; Swe, 2021), which affect fish livers and accumulate in fish muscles (Mohamed *et al*., 2018; Sotton *et al*., 2015). Thus, the knowledge gained from this study can be applied for the management of wastewater treatment systems considering the pond depth, especially in stabilization ponds, which allow light to reach the bottom of the pond for an appropriate retention period, thereby realizing an efficient and sustainable water treatment. Cyanobacteria contain phycocyanin, a type of pigment in photosystem II, as they also have the ability to fix nitrogen in low nitrogen environments (Pierangelini *et al*., 2014; Noreña-Caro *et al*., 2018). *Cylindrospermopsis* sp. grows well at temperatures ranging from 27.5 to 32.5 degrees Celsius (°C) (Antunes *et al*., 2015; Pierangelini *et al*., 2015). The TN were 1.81–3.53 mg/L, the TP were 0.046–0.119

mg/L, with all together making the TN to the TP ratio of 23:1 (Chapman *et al*., 1997). *Cylindrospermopsis* sp. does not grow well on the water surface, mainly because of the effects of hydrogen peroxide (H_2O_2) and hydroxyl radicals formed as a result of solar radiation penetrating the water surface and DO molecules. Free radicals created by photolysis damage and destroy *Cylindrospermopsis* sp. cell membranes via lipid peroxidation, resulting in ripped cell membranes that leave cells unable to exchange substances and energy, thereby leading to cell death (Pattamapitoon *et al*., 2013; Smith *et al*., 2015; Zarantonellot *et al*., 2018). Most studies on *Cylindrospermopsis* sp. were conducted in freshwater reservoir, lakes, and rivers (Antunes *et al*., 2015; Yamamoto and Shiah, 2016). This study aimed to investigate the distribution of *Cylindrospermopsis* sp. and its correlation with nitrogen compounds to demonstrate its ability to remove and fix atmospheric nitrogen into bodies of water, especially in domestic treatment ponds (Yang *et al.,* 2018), where the addition of nitrogen compounds can lead to toxicity and blooming. The study site is the community wastewater treatment system of the King's Royally Initiated Laem Phak Bia Environmental Research and Development Project: Phetchaburi Province, Thailand, in 2019, which was investigated at different depths.

MATERIALS AND METHODS

Study area

The study site of the community wastewater treatment system is located at the King's Royally Initiated Laem Phak Bia Environmental Research and Development Project, Ban Laem District, Phetchaburi Province (Universal Transverse Mercator 47 P 1442725 N 617774 E). The project site consists of five oxidation ponds used for the treatment of domestic wastewater from the Phetchaburi Municipality located near the coastal region of Thailand. The wastewater is gathered through 18.5 kilometers (km) of 40-cm-diameter high density polyethylene pipeline (HDPE) pipes that transfer 6,167 cubic meter per day (m^3/day) of wastewater to the treatment system (Figs. 1 and 2 and Table 1). The wastewater used for treatment in the system is the municipal wastewater of the Phetchaburi Municipality, which

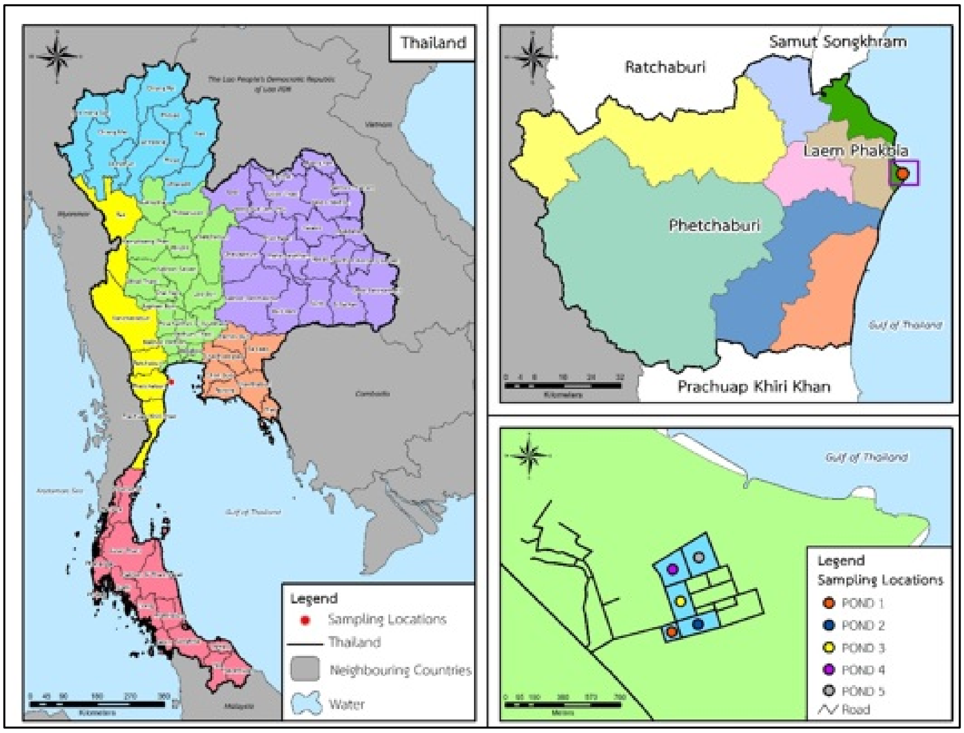


Fig. 1: Geographical location of the study area at the King's Royally Initiated Laem Phak Bia Environmental Research and Development Project, Ban Laem District, Phetchaburi Province, Thailand

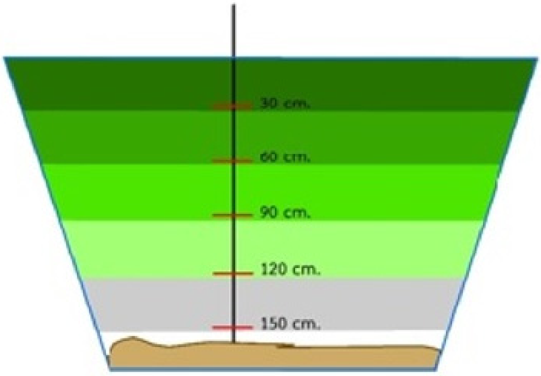


Fig. 2: Wastewater sampling collection depth in pond no. 3 at the King's Royally Initiated Laem Phak Bia Environmental Research and Development Project

Table 1: Pond capacity and dimension

Pond	Pond No. 1	Pond No. 2	Pond No. 3	Pond No. 4	Pond No. 5
Capacity (m^3)	21,970	60,906	59,007	54,011	59,155
Depth (m)	2.43	2.23	1.93	1.64	1.42
HRT (day)	5	12	15	15	16

is mainly derived from household activities, including that from fresh markets, cooking, and washing. The combined sewer system transfers the wastewater from the source into the wastewater collection pond and retains it for 26 hour (h) before pumping through HDPE pipes in a closed (anaerobic) system at a distance of 18 km and rate of 5000–6000 m^3/day (Jinjaruk *et al*., 2018). Regarding the changes in the nitrogen compounds, when community wastewater enters the system, it undergoes nature-by-nature treatment to decrease the biological oxygen demand (BOD), phosphorus, and nitrogen contents while increasing the suspended solid (SS) content owing to the growth of phytoplankton in the system (Chunkao *et al*., 2018). These processes may affect the nitrogen content as most of the growing plankton are cyanobacteria (Sukchinda *et al*., 2019), which can fix nitrogen. Moreover, when the phytoplankton die, their cells increase the amount of organic matter in the system.

Water sampling and analysis

Wastewater samples were collected from the community wastewater treatment ponds during the dry period in April 2019. This work only focused on pond no. 3, which has the highest density of *Cylindrospermopsis* sp. Before sampling, the collection depths of the pond were defined and indicated to ensure that the water was sampled from the desired depth. The samples were collected from the center of the pond at five different depths (30, 60, 90, 120, and 150 cm from the surface) (Noikondee *et al*, 2019) using water samplers in a vacuum water pump attached to a tube placed at the desired depths of 30, 60, 90, 120, and 150 cm between 11:00 a.m. and 14:00 p.m. Once the pump was operated for 60 s before obtaining the actual sample water, 1 liter (L) water sample was collected in polyethylene bottles, which were wrapped in aluminum foil and stored at 4°C to prevent exposure to light and slow down microorganism activities. The samples were then analyzed in laboratories following standard procedures (APHA, 2012) for the physical parameters temperature and total dissolved solids and the chemical parameters dissolved oxygen, pH, nitrogen compounds (Organic nitrogen, total Keldahl nitrogen, ammonium, and nitrate), phosphorus, and phosphate. The temperature and pH were measured on site; the results are shown in Table 2.

Phytoplankton analysis

Phytoplankton were collected by filtering 20 L from each depth through a 36-micrometer (µm) plankton net. Once filtered, 4% formalin solution was added

Table 2: Parameters analyzed and the methods used

Parameters	Units	Method
Temperature (Degree Celsius)	°C	Thermometer
DO (Dissolve oxygen)	mg/L	DO meter
pH (Potential of hydrogen)	-	pH meter
TN (Total nitrogen)	mg/L	Kjeldahl
TKN (Total Kjedahl nitrogen)	mg/L	Kjeldahl
NH_4^+ (Ammonium nitrogen)	mg/L	Colorimetric
NO_3^- (Nitrate nitrogen)	mg/L	Brucine
TP (Total phosphorus)	mg/L	Ascorbic acid
PO_4^{3-}(Orthophosphate)	mg/L	Colorimetric
SS (Suspended solids)	mg/L	Filter with glass microfiber filter (GF/C), dried at 103-105^{0}C
Transparency	cm	Secchi disk

to maintain the sample (Figs. 1 and 2) (Wongrat *et al*., 2017). The samples were then analyzed under a high magnification microscope to classify the phytoplankton species according to their taxon using molecular and morphological methods (Bellinger and Sigee, 2010; Li *et al*., 2017; Wongrat, 2017). The size and particles in 1 L samples were determined under a Sedgewick–Rafter chamber thrice and averaged in terms of cells per cubic meter (cells/m^3), using Eq. 1 (USEPA, 2021).

$$C = \frac{NV_2}{V_1} \tag{1}$$

Where,

C = plankton density in cells/L (a conversion (×1000) was conducted to obtain the value in cells/m^3)

N = average density of per 1 milliliter of plankton

V_1 = volume of water filtered through the plankton filter bag (liters)

V_1 = volume of sample water contained in the sample bottle (milliliters)

In the data analysis, the average value was used to create graphs in terms of the depth and its correlation with the water quality and *Cylindrospermopsis* sp. For the community wastewater treatment, pond no. 3 was used as the best representation of the treatment system of the water quality, which demonstrated the water treatment to meet standards. However, when the system processes progressed and the plankton bloomed, *Cyanobacteria* sp. grew.

Statistical analysis

All data of this study were analyzed using one-way analysis of variance, and correlation graphs were prepared for comparing the relationship between *Cylindrospermopsis* sp. and different nitrogen compounds.

RESULTS AND DISCUSSION

Type and amount of Cyanobacteria

According to the sample analysis, cyanobacteria were detected in all five oxidation ponds at the following population levels: 5.19 × 10^6, 9.00 × 10^6, 3.10 × 10^7, 1.36 × 10^5, and 1.27 × 10^4 cells/m^3. The cyanobacteria were classified into two orders, three families, five genera, and six species: *Oscillatoria* sp., *Spirulina patensis, S. subsalsa*, *Anabenopsis* sp., *Cylindorspermopsis* sp., and *Microcystis aroginosa*. The highest cyanobacteria population was found in pond no. 3 and was distributed across all depths with the population 2.9 × 10^7, 4.4 × 10^7, 1.6 × 10^7, 2.1 × 10^7, and 4.2 × 10^7 cells/m^3 at 30, 60, 90, 120, and 150 cm from the surface, respectively (Table 3 and Fig. 3).

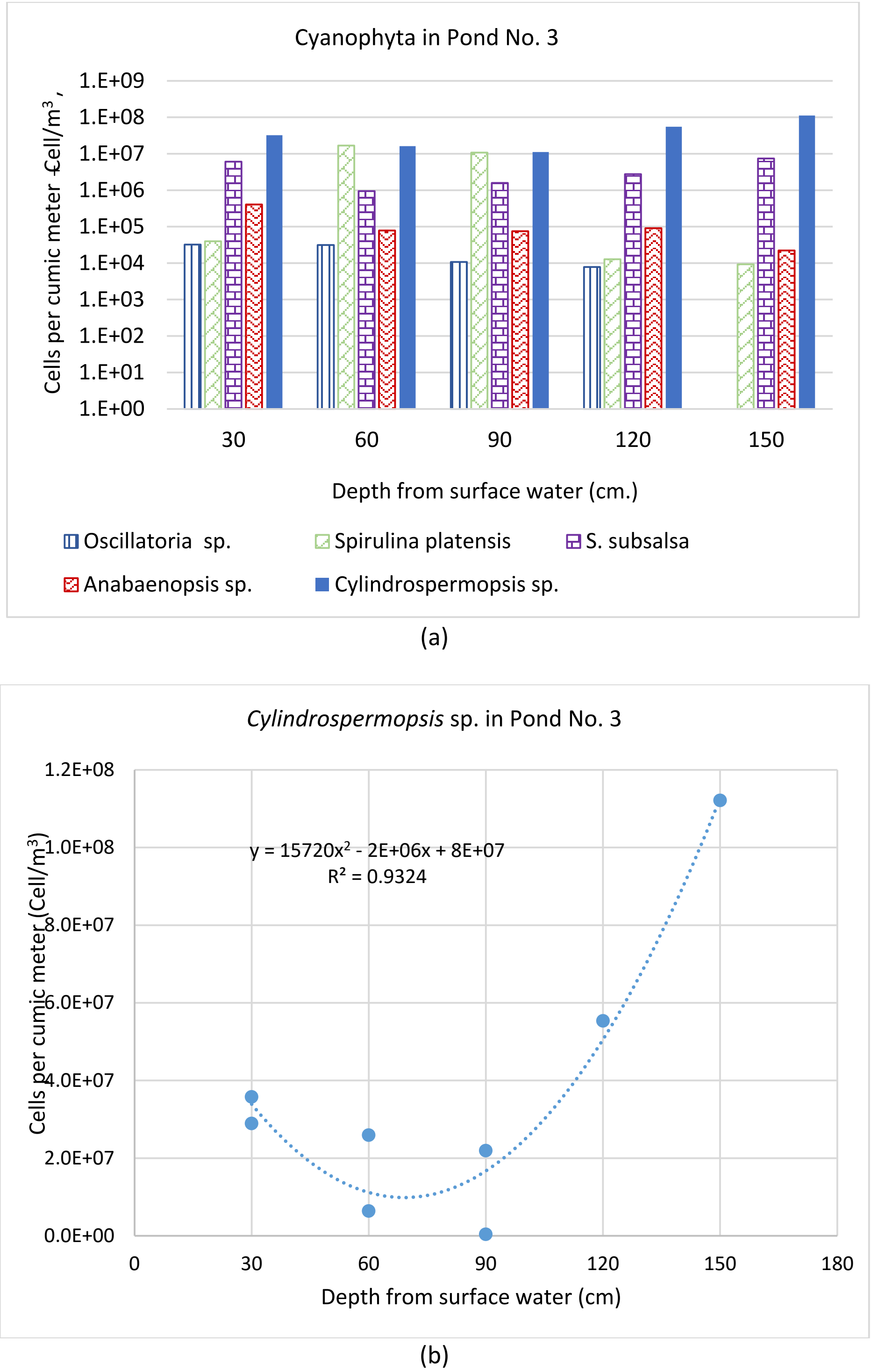
Five species of cyanobacteria were found in pond no. 3: *Oscillatoria sp.*, *Spirulina patensis*, *S. subsalsa*, *Anabenopsis* sp., and *Cylindorspermopsis* sp. In particular, *Cylindorspermopsis* sp. was distributed at all depths and tended to increase with the depth (R^2 = 0.9324). At 30, 60, 90, 120, and150 cm, the populations of *Cylindorspermopsis* sp. were 3.2 × 10^7, 1.6 × 10^7, 1.1 × 10^7, 5.5 × 10^7, and 1.1 × 10^8 cells/m^3, respectively. The density of the phytoplankton is related to the nitrogen content, as *Cylindrospermopsis* sp. was that the assimilate the nitrogen compound in which they support the removal of ammonium in the oxidation pond, however under their optimal habitat of low light and temperature (Yamamoto, and Shiah, 2016) leading to differences in density resulting in the vertical distribution of the species as well as the

Table 3: Average populations of cyanobacteria in the community wastewater treatment system oxidation ponds in April 2019

Depth (cm)	Temp (°C)	DO (mg/)	pH	TN (mg/L)	TKN (mg/L)	NH_4^+ (mg/L)	NO_3^- (mg/L)	TP (mg/L)	$[PO_4]^{3-}$ (mg/L)	SS (mg/L)
30	33.23 ± 0.11^a	10.85 ± 1.45^a	9.17 ± 0.11^a	5.17 ± 0.56^a	5.04 ± 0.56^a	0.04 ± 0.01^{ab}	0.10 ± 0.03^a	1.37 ± 0.25^a	0.50 ± 0.04^a	65.33 ± 7.63^b
60	32.80 ± 0.20^b	8.74 ± 0.35^b	9.12 ± 0.04^a	4.63 ± 0.98^a	4.48 ± 0.96^a	0.05 ± 0.02^{ab}	0.10 ± 0.01^a	0.94 ± 0.13^{ab}	0.48 ± 0.04^a	63.33 ± 3.51^b
90	32.40 ± 0.30^{bc}	5.80 ± 0.93^c	9.05 ± 0.04^a	4.04 ± 1.46^a	3.92 ± 1.48^a	0.03 ± 0.005^b	0.10 ± 0.01^a	0.97 ± 0.39^b	0.47 ± 0.02^a	68.67 ± 2.88^b
120	32.07 ± 0.30^{cd}	3.80 ± 0.58^d	8.88 ± 0.07^b	4.48 ± 2.58^a	4.29 ± 2.58^a	0.06 ± 0.02^a	0.12 ± 0.005^a	1.16 ± 0.19^{ab}	0.52 ± 0.07^a	70.00 ± 1.73^b
150	31.80 ± 0.10^d	2.21 ± 0.75^d	8.74 ± 0.06^c	5.38 ± 1.98^a	5.23 ± 1.96^a	0.03 ± 0.02^{ab}	0.12 ± 0.01^a	1.11 ± 0.24^{ab}	0.51 ± 0.04^a	81.33 ± 6.11^a
STD**	-	-	5.5–9.0*	<20*	-	-	-	<2*	-	<50*

[*] Pollution Control Department of Thailand (2010)

**STD = Standard



(a)

(b)

Fig. 3: Species of cyanobacteria in pond no. 3 relationship between *Cylindrospermopsis* sp. and depth in pond no. 3. (a) Cyanophyta density in pond no. 3. (b) *Cylindrospermopsis* sp. in pond no. 3

Table 4: Water quality in pond no.3

Depth (cm)	Temp (°C)	pH	TN (mg/L)	TKN (mg/L)	NH_4^+ (mg/L)	NO_3^- (mg/L)	TP (mg/L)	PO_4^{3-} (mg/L)	TN:TP (mg/L)	SS (mg/L)
30	33.23	9.17	5.17	5.04	0.04	0.10	1.37	0.50	3.80	65.33
60	32.80	9.12	4.63	4.48	0.05	0.10	0.94	0.48	5.06	63.33
90	32.40	9.05	4.04	3.92	0.03	0.10	0.97	0.47	4.40	68.67
120	32.07	8.88	4.48	4.29	0.06	0.12	1.16	0.52	4.22	70.00
150	31.80	8.74	5.38	5.23	0.03	0.12	1.11	0.51	4.73	81.33

nitrogen content. Furthermore, it was suggested that the phytoplankton prefer different nitrogen sources, mainly NH_4^+ over NO_3^-, followed by N-free; therefore, dissolved inorganic nitrogen was considered the main source of nitrogen for *Cylindrospermopsis* sp., which is related to the nitrogen removal efficiency (Antunes *et al*., 2015).

Water quality

The community wastewater at the study site contains high levels of organic substances contamination is rested within the sedimentation pond (pond no. 1) for 7 days to settle out the heavy sediment. The water then flows into pond no. 2, 3, and 4. Microorganisms in oxidation ponds use oxygen produced by plankton through photosynthesis and the thermosiphon process (Pattamapitoon *et al*., 2013; Chunkao *et al*., 2014; Noikondee *et al*., 2019; Kumar *et al*., 2019) to digest organic substances and produce inorganic substances, which are used by phytoplankton for growth. The wastewater is retained in oxidation pond nos. 2, 3, and 4 for 12, 15, and 15 days, respectively. After the community wastewater is treated, it flows into pond no. 5, the stabilization pond. This is the shallowest pond as the pond function were to allow for sunlight to penetrate into the bottom of the pond killing bacteria, while also adds oxygen produced by the phytoplankton to the water before it is released into natural environment. The wastewater treatment process takes 49 days, and the results of water quality sampling indicated that the water quality reached standards for community wastewater treatment system set by the Ministry of Natural Resource and Environment, Thailand. Depicting into pond no. 3, the transparency was 27 cm, and the dissolved oxygen content was 2.2–10.8 mg/L. However, the dissolved oxygen tended to decrease with depth because of decreasing levels of sunlight. Sunlight supports phytoplankton photosynthesis at the water surface, thereby increasing the oxygen levels in water. Dissolved oxygen at deeper levels is used by microorganisms for organic digestion, resulting in higher levels of dissolved oxygen at the water surface than at the deeper levels. The suspended solid content was 65–81 mg/L, and it tended to increase with depth due to the increasing density of *Cylindrospermopsis* sp. at deeper levels (Table 4).

Relationship between nitrogen and Cylindrospermopsis sp.

The TN tended to increase with an increase in the density of *Cylindrospermopsis* sp. because of their nitrogen-fixing ability. Moreover, NH_4^+ increased with an increase in water depth due to ammonification; NH_4^+ was used by *Cylindrospermopsis* sp. for their growth (Yang *et al*., 2018). This showed a reverse relationship between the amount of NH_4^+ and the density of *Cylindrospermopsis* sp., considering the relationship between *Cylindrospermopsis* sp. to TN and NH_4^+ according to water depth. The results revealed that TN and NH_4^+ were significantly correlated (R^2 = 0.7268 and 0.797, respectively) (Fig. 4).

The water temperature ranged from 31.8°C to 33.2°C and tended to decrease with depth because of the heating by solar radiation at the surface (Noikondee *et al*., 2019). Solar radiation and wind promote evaporation and cool the surface of the pond, creating a denser water column. These changes in density promote vertical circulation of the water within the pond as there is a thermal gradient. Such phenomena makes the water temperature at the bottom of the pond suitable for the growth of

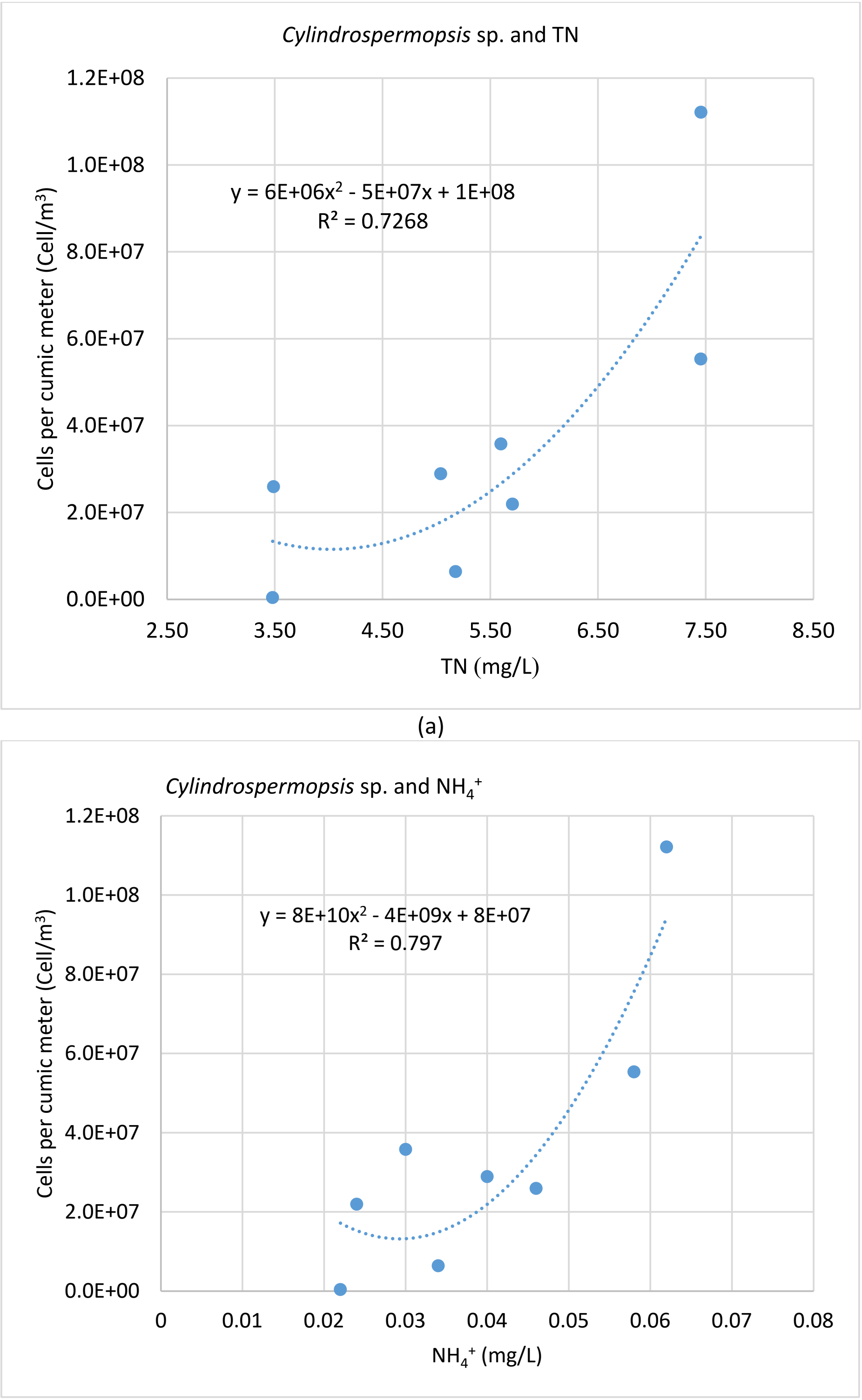


Fig. 4: Relationship between *Cylindrospermopsis* sp. and (a) total nitrogen (TN), (b) ammonium (NH_4^+)

Cylindrospermopsis sp. The results showed that the pH at the surface of water (30 cm) was 9.2; however, it decreased to 8.7 at deeper levels (150 cm) due to the release of carbon dioxide from microbial digestion. The carbon dioxide reacts with water and is dissociated into bicarbonate, which releases hydrogen ions. At the same time, nitrification turns NH_4^+ into hydrogen ions, increasing the alkalinity of water. The nutrient levels in pond no. 3 were indicated by the TN (4.04-5.38 mg/L) and TP (0.94-1.37 mg/L) levels, which decreased with water depth. However, it increased slightly at 90 cm below the water surface due to the greater distribution of *Cylindrospermopsis* sp. at deeper levels, which fix nitrogen from the air, resulting in increased organic substances in the water. NH_4^+ levels decreased from 0.03–0.06 mg/L according to the depth of the water because of the ammonification process, which decomposes nitrogen from organic substances into inorganic substances in the form of NH_4^+. At deeper levels, the nitrification process converts NH_4^+ into NO_3^-. At the bottom of the pond, where oxygen is low, *Cylindrospermopsis* sp. is capable of photosystem II that produced oxygen for the microorganisms through nitrification. This resulted in an increase in NO_3 to 0.09–0.12 mg/L. Moreover, the TP level was 0.9–1.3 mg/L and the orthophosphate (PO_4^{3-}) level was 0.4–0.5 mg/L, which decreased at the surface and middle depths of the pond but increased at deeper levels (Table 3). From (Fig. 4), the correlation between *Cylindrospermopsis* sp., TN and NH_4^+ presented a high significant relation, where this is explained through the process of nitrogen fixation by *Cylindrospermopsis* sp. from the atmosphere together with the ammonification process within the pond. *Cylindrospermopsis* sp. cyanobacteria in pond no. 3 had the highest growth rate and density. *Cylindrospermopsis* sp. was distributed at every depth without significant difference because it is capable of growing under low-light intensities, with preferable temperature ranging between 27.5°C and 32.5°C (Antunes *et al.*, 2015; Pierangelini *et al.*, 2015; Xiao *et al.*, 2017). Under high-light-intensity conditions, high levels of UVA and UVB breakdown water molecules and transform DO into a new compound, such as H_2O_2 (Pattamapitoon *et al.*, 2013), which can destroy the cell membrane of *Cylindrospermopsis* sp. It also results in vesicle leakage, leading to malformation and rendering cells incapable of cell division and growth, thereby reducing cell density and division (Pattamapitoon *et al.*, 2015; Smith *et al.*, 2015; Zarantonello *et al.*, 2018). However, at low-light intensities, *Cylindrospermopsis* sp. uses more phycocyanins for photosystem II (Pierangelini *et al.*, 2014; Noreña-Caro *et al.*, 2018). Overall, it can be concluded that the distribution of *Cylindrospermopsis* sp. was greater in low-light-intensity areas than in high-light-intensity areas at the study site. Nitrogen compounds in the municipal wastewater treatment system are transformed from organic nitrogen compounds to NH_4^+, NO_2^-, and NO_3^- through nitrification. Considering the depth, the TN and organic nitrogen contents were higher than NH_4^+ and NO_3^- at the bottom of the pond. As more than 15 types of microorganisms were found in the community wastewater treatment system, each group contributed to the transformation of nitrogen compounds together. Moreover, the growth of *Cylindrospermopsis* sp. in the pond increased the high organic nitrogen concentration due to the increased nitrogen fixation owing to the use of NH_4^+ and NO_3^- to produce more cells (Rose *et al.*, 2021; Saneha *et al.*, 2023). The process is described by the conversion of organic nitrogen into inorganic nitrogen (NH_4^+ and NO_3^-), which are the N sources for *Cylindrospermopsis* sp. growth, by nitrification. The inorganic nitrogen is then converted back into its organic form in the plankton's cells through cellular assimilation (Sprőber *et al.*, 2003). When the cells die, the amount of organic nitrogen at the bottom of the pond increases. Overall, nitrogen fixation refers to the conversion of nitrogen into NH_4^+, and cyanobacteria can photosynthesize and supply oxygen to the environment. However, *Cylidrospermopsis* sp. can adapt to nitrogen fixation under low-light conditions, such as that at the bottom of the pond (Paerl, 2017; Rose *et al.*, 2021).

CONCLUSION

In the identification of the total phytoplankton species found in the nature-by-nature (an oxidation process which is not assisted or enhanced by any artificial aeration or mixing) oxidation treatment pond for domestic wastewater at The King's Royally Initiated Laem Phak Bia Environmental Research and Development Project, Phetchaburi Province, Thailand. This work investigated the distribution in domestic wastewater treatment system. The findings suggested that the most abundant species was *Cylindrospermopsis* sp., which has the ability to move freely throughout the treatment pond and fix free atmospheric nitrogen into the water body. Furthermore, through the oxidation process in the treatment system, the conversion of organic nitrogen, including NH_4^+, into inorganic nutrients promotes the growth of other phytoplankton species and the photosynthesis process. The distribution of *Cylindrospermopsis* sp. was the highest in pond no. 3 at 150 cm below the surface (1.1×10^8 cells/m^3). This population affected the overall water quality by increasing the nitrogen and phosphorus-containing SS, TN, and NH_4^+ concentrations that were significantly correlated with the *Cylindrospermopsis* sp. population. Therefore, the depth of wastewater treatment ponds should be considered for the treatment effectiveness when constructing wastewater treatment systems, as regulation by phytoplankton plays an important role in maintaining the overall treatment efficiency with the implication of the potential impact would be through their ability to remove nitrogen from the oxidation pond as this would increase plankton bloom leading to a potential c*ylindrospermopsin* build up. This study determined the appropriate pond depth to be less than 1.5 m to eliminate low-light areas, which could decrease the density of *Cylindrospermopsis* sp.; however, further studies are recommended to determine the correlation among ponds deeper than 1.5 m, cylindrospermopsin, light, and seasonal variations.

AUTHOR CONTRIBUTIONS

M. Srichomphu performed literature review, experimental design, material preparation, data collection, analysis, and interpretation, and manuscript preparation. O. Phewnil performed study conception, experimental design, material preparation, data collection and analysis, and manuscript preparation and editing. T. Pattamapitoon performed experimental design and data collection and interpretation. R. Chaichana performed experiments and commented on a previous version of the manuscript. K. Chunkao performed experimental design and manuscript editing. W. Wararam performed data collection, analysis, and interpretation. *N*. Dampin performed some experiments and data collection. P. Maskulrath performed material preparation, data collection, and manuscript preparation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been completely observed by the authors.

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ABBREVIATIONS

%	Percent
°C	Degree celsius

µm	Micrometer
BOD	Biological oxygen demand
cm	Centimeter
$Cells/m^3$	Cell per cubic meter
CYN	Cylindrospermopsin
DO	Dissolved oxygen
GF/C	Glass microfiber filter
h	Hour
HDPE	High density polyethylene
H_2O_2	Hydrogen peroxide
km	Kilometer
L	Liter
m	Meter
m^3	Cubic meter
m^3/Day	Cubic meter per day
mg/L	Milligram per liter
mL	Milliliter
NH_4^+	Ammonium nitrogen
nm	Nanometer
No.	Number
NO_2^-	Nitrite
NO_3^-	Nitrate
pH	Potential of hydrogen
PO_4^{3-}	Orthophosphate
R^2	Coefficient of determination
SS	Suspended solid
TN	Total nitrogen
TP	Total phosphorus
TKN	Total Kjedahl nitrogen
STD	Standards

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AUTHOR (S) BIOSKETCHES

Srichomphu, M., Ph.D. Candidate. Department of Environmental Science, Faculty of Environment, Kasetsart University, Bangkok, Thailand.

- Email: manlikasri@gmail.com
- ORCID: 0009-0004-4965-5769
- Web of Science ResearcherID: NA
- Scopus Author ID: 57202059256
- Homepage: https://www.lerd.org/

Phewnil, O., Ph.D., Assistant Professor, Department of Environmental Science, Faculty of Environment, Kasetsart University, Bangkok, Thailand.

- Email: onanong.p@ku.th
- ORCID: 0000-0001-9616551X
- Web of Science ResearcherID: NA
- Scopus Author ID: 56252364600
- Homepage: https://envi.ku.ac.th

AUTHOR (S) BIOSKETCHES (continued)

Pattamapitoon, T., Ph.D., Assistant Professor, Department of Environmental Science, Faculty of Environment, Kasetsart University, Bangkok, Thailand.
- Email: thanit.pa@ku.th
- ORCID: 0009-00051664450X
- Web of Science ResearcherID: NA
- Scopus Author ID: 55822416300
- Homepage: https://envi.ku.ac.th

Chaichana, R., Ph.D., Associate Professor, Department of Environmental Technology and Management, Faculty of Environment, Kasetsart University, Bangkok, Thailand.
- Email: fscircc@ku.ac.th
- ORCID: 0000-0003-0244-9736
- Web of Science ResearcherID: NA
- Scopus Author ID: 35766295700
- Homepage: https://envi.ku.ac.th

Chunkao, K., Ph.D., Professor, The King's Royally Initiated Laem Phak Bia Environmental Research and Development Project, Chaipattana Foundation, Ban Laem District, Phetchaburi Province, Thailand.
- Email: prof.kasemc@gmail.com
- ORCID: 0009-0002-80354732
- Web of Science ResearcherID: NA
- Scopus Author ID: 54683551800
- Homepage: https://www.lerd.org

Wararam, W., Ph.D., Assistant Professor, Department of Environmental Science, Faculty of Environment, Kasetsart University, Bangkok, Thailand.
- Email: watcharapong.warar@ku.th
- ORCID: 0009-0000-1487-4284
- Web of Science ResearcherID: NA
- Scopus Author ID: 57096032600
- Homepage: https://envi.ku.ac.th

Dampin, N., Ph.D., Lecturer, Department of Environmental Science, Faculty of Environment, Kasetsart University, Bangkok, Thailand.
- Email: ecncd@ku.ac.th
- ORCID: 0009-0000-5540-5281
- Web of Science ResearcherID: NA
- Scopus Author ID: 57194972798
- Homepage: https://envi.ku.ac.th

Maskulrath, P., The King's Royally Initiated Laem Phak Bia Environmental Research and Development Project, Ban Laem District, Phetchaburi Province, Thailand.
- Email: parkin.ma@ku.th
- ORCID: 0000-0002-1352-4247
- Web of Science ResearcherID: NA
- Scopus Author ID: 57202060728
- Homepage: https://www.lerd.org/

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