HUE UNIVERSITY
INSTITUTE OF BIOTECHNOLOGY

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SPECIES COMPOSITION AND CYLINDROSPERMOPSIN PRODUCING ABILITY OF CYANOBACTERIA IN SOME RESERVOIRS IN DAK LAK

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PREAMBLE

Dak Lak is known as the "**Land of Lakes"** with most of them being reservoirs. Besides the natural roles of the lake such as regulating the climate and regulating the flow, the lake is also the main source of surface water for living activities such as: providing drinking water and domestic water for livestock, farming, aquaculture and tourism suervice (Department of Agriculture and Rural Development of Dak Lak Province, 2018). Recently, due to climate change, Dak Lak has experience extreme weather phenomena such as: heavy rain in a short time, severe prolonged drought, which has reduced water volume and increased water retention time in the reservoir system. In addition, the change of area and land use purpose and unreasonable agricultural cultivation around the basin area has brought into the lake a large amount of fertilizer residues and chemical pesticides. Along with the amount of domestic wastewater, this is considered a cause of water quality deterioration, causing eutrophication in lake-shaped water bodies in Dak Lak. This phenomenon leads to increased turbidity, increased nutrient content and increased biomass of phytoplankton, especially cyanobacterial toxins.

Cylindrospermopsin (CYN) is one of the most commonly studied cyanotoxins due to it's global distribution, bioaccumulation and multi-organ toxictiy in humans and animals (Wang et al., 2020). Most of cyanotoxins exist mainly intracellularly and are released when the cell is broken. However, as for cyanotoxins, most of the toxin is released into the aquatic enviroment when the daughter cells are intact. Besides, CYN has high chemical stability, strong solubility in water and slow decomposition rate under the influence of abiotic factors in nature (Stefanova et al., 2020). This may increase the risk of exposure to and absorption of toxins by aquatic species, causing many potential risks and difficulties in the use and management of water resources.

Recently, the phenomena of water changing color and appearance of unpleasant odors often occur in the dry season in some reservoirs in Dak Lak Province. In addition, the appearance of potentially CYN-producing cyanobacteria has been observed in some reservoirs here, but no toxicological data is available (Le, 2010). These water bodies require an effective biological monitoring program. However, previous studies mainly focused on investigating phytoplankton species composition and

changes in the structure of phytoplankton communities (Le, 2010; Dao, 2016). There have not been studies on the group of toxic cyanobacteria and the CYN production ability of this species in the water bodies in Dak Lak. Therefore, with the "Identification of Species composition and cylindrospermopsin-producing ability of cyanobacteria in some reservoirs in Dak Lak. " besides the provision of CYN-producing potential cyanobacteria species composition, the results will serve as abasis for forecasting pollution risks and potential risks from the group of harmful cyanobacteria in the use and management of water resources.

Objectives of the study

Evaluation of the diversity of cyanobacterial species composition and CYN-producing potential cyanobacteria in Ea Nhai and Buon Phong reservoirs in Dak Lak.

Evaluation of the potential risk of the group of CYN-producing potential cyanobacteria in Ea Nhai and Buon Phong reservoirs in Dak Lak.

Identification of the key environmental factors affecting the population fluctuation of CYN-producing potential cyanobacteria and CYN concentrations in Ea Nhai and Buon Phong reservoirs.

Content of the study

Identification of the cyanobacterial species composition and CYN-producing potential cyanobacteria in Ea Nhai and Buon Phong reservoirs in Dak Lak.

Analysis of seasonal fluctuations in biovolume of the group of CYN-producing potential cyanobacteria and the CYN concentrations in Ea Nhai and Buon Phong reservoirs.

Isolation and identification of CYN production ability of strains by identifying the presence of genes related to CYN biosynthesis and the CYN concentrations of cyanobacterial strains isolated in two studied reservoirs.

Determination of the correlation between natural environmental conditions and presence of CYN-producing potential cyanobacteria in Ea Nhai and Buon Phong reservoirs in Dak Lak.

Scientific and practical significance

Provide a list of cyanobacterial species composition and CYN-producing potential cyanobacteria in two Ea Nhai and Buon Phong reservoirs in Dak Lak, contributing to the list of cyanobacterial species composition and CYN-producing potential cyanobacteria in the water bodies in Vietnam.

Evaluation of the variation of CYN-producing cyanobacteria populations and the CYN concentrations in two reservoirs, whereby identifying the key environmental factors controlling the growth of

CYN-producing cyanobacteria populations in nature in order to take measures for controlling and curbing the outbreak of this harmful cyanobacteria group.

The results will serve as a basis for forecasting the pollution risk as well as proposing measures for managing the group of harmful cyanobacteria, contributing to the protection of water resources and the protection of public health.

New points of the thesis

This is the first study published on the species composition of CYN-producing potential cyanobacteria in Ea Nhai reservoir, Dak Lak, and the cyanobacterial species composition and CYN-producing potential cyanobacteria in Buon Phong reservoir, Dak Lak.

The study has provided data on CYN concentrations in nature, toxin concentrations and biosynthesis gene of CYN in cyanobacterial strains isolated from Ea Nhai and Buon Phong reservoirs in Dak Lak for the first time.

The study has successfully identified the key environmental factors ($P\text{-}PO_4$, $N\text{-}NH_4$, TP, TN and temperature) affecting the population fluctuations of the group of CYN-producing potential cyanobacteria in these water bodies.

CHAPTER 1. DOCUMENTARY OVERVIEW

1.1. Introduction to cyanobacteria

1.1.1. General characteristics of cyanobacteria

Cyanobacteria are prokarytoes, appearing as unicellular, conglomerate or multicellular filamentous. Cyanobacterial cells may be spherical, elliptical, barrel-shaped, cylindrical, conical or disc-shaped. They have no flagella and their cell walls are made of peptidoglycan-like bacteria. In addition to the main form of nutrition which is photoautotrophs, cyanobacteria are also capable of photoheterotrophs and heterotrophs, using organic substances in the environment as an additional energy source. Cyanobacteria has the ability to fix nitrogen from the air through heterocytes. Cyanobacteria does not reprocedure sexually, but only reprocedures by vegetative cell division or fission, and reprocedures asexually by endospores and exospores. Resting spores (akinetes) are also formed to help cyanobacteria overcome adverse environmental conditions.

1.1.2. Taxonomy of cyanobacteria

For cyanobacteria, the taxonomy is still complicated because there are two different taxonomic nomenclature systems

coexisting: International Code of Nomenclature for Algae, Fungi and Plants; and International Code of Nomenclature for Bacteria. Due to the prokaryotic nature of cyanobacteria, Stainer et al. (1978) proposed to use polyphasic method to classify cyanobacteria. This method is based on the evaluation of morphological, physiological, cytological and biochemical characteristics using sterile culture strains as the basic taxon. The taxonomy based on a combination of molecular, biochemical, physiological, morphological and ecological characteristics is called polyphasic taxonomy, in which genetic assessment is the basis and is combined with other taxonomic features from morphological, physiological and ecological analysis (Chorus et al., 2021). Hoffmann et al. (2005a, b) introduced a new cyanobacteria taxonomy system. This is the first taxonomy system with a polyphasic approach based on the combination of genetic traits, superstructural features and phenotypic data in taxonomy. The entire cyanobacteria taxonomy system has been reconstructured and revised in the new taxonomy system of Komárek et al. (2014) based on botanical binominal nomenclature. This system is largely based on whole-genome sequencing, superstructural features and data from many published phylogenetic trees (Komárek et al., 2014).

1.2. Cylindrospermopsin toxin

1.2.1. Chemical structure

Cylindrospermopsin is a hepatotoxic cyclic toxin, with the molecular formular of $C_{15}H_{21}N_5O_7S$ and a mass of 415.43 Dalton. This toxin is an alkaloid with a tricyclic guanidine moiety center linking a sulfate group and a hydroxymethyl uracil. Four isomers of CYN in nature having been identified: 7-epi cylindro-spermopsin (7-epi-CYN), 7-deoxy-cylindrospermopsin (7-deoxy-CYN), 7-deoxydesul-phocylindro-spermopsin and 7-deoxydesulpho-12-acetylcy-lindrospermopsin (Chorus and Welker, 2021).

1.2.2. Properties

CYN is a white powder, strongly soluble in water to a transparent solution. CYN is also soluble in dimethylsulfoxide and methanol. CYN is relatively stable in the dark and under sunlight. CYN is highly chemically stable and sustainable under various light, temperature and pH conditions. CYN decomposition rate is slow under the influence of abiotic factors in nature (Stefanova et al., 2020).

1.2.3. CYN concentrations in water bodies around the world

The average CYN concentration was 0.54; 0.70; 2.25; 1.12; 2.5 and 2.35 μg/L in Europe, Asia, Oceania, North America, South America and Africa, respectively. The reported highest total CYN (granular and soluble) concentration was 1050 μg/L from farm water supplies in the central Queensland. Water bodies with CYN concentrations greater than or equal to 1 μg/L accounted for 40.0%, 39.4%, 68.8%, 52.4%, 66.7% and 75% in Europe, Asia, Oceania, North America, South America and Africa, respectively (Yang et al., 2021).

1.2.4. The influence of environmental factors on the CYN concentration and production

Saker and Griffiths (2000) found a negative correlation between CYN generation capacity of *R. raciborskii* and temperature. At 35°C, all strains grew well but none produced CYN. In contrast, at 20 °C, the growth of the strains was stopped but the CYN concentration was higher. When exposed to light with intensities of 15–340 μE/m/s, C. ovalisporum produced total CYNs ranging from 1.32 μg/mg DW at 340 μE/m/s to 6.37 μg/mg DW at 60 μE/m/s. Although there is a 4-fold variation in CYN concentration, no linear correlation was observed between these two parameters in this species (Cirés et al., 2011). The effects of phosphorus (P) and nitrogen (N) on CYN production have been studied extensively. Bacsi et al. (2006) showed that P and sulfate deficiency in *C. ovalisporum* cultures resulted in a decrease in CYN concentration of 48% and 65%, respectively, in two days. When P and S were reintroduced in the medium, the toxin levels began to increase significantly (Yang et al., 2021).

1.2.5. CYN biosynthesis

Biosynthesis is initiated through the transfer of guanidine group from arginine to glycine catalyzed by *Cyr*A (*AoaA*) gene forming the first intermediate product of guanidinoacetate. Next, *CyrB* (*AoaB*) gene recognizes guanidinoacetate and catalyzes the formation of a N-containing heterodimer. Four PKS genes, from *CyrC* to *CyrF* further catalyze the elongation of the polyketide chain resulting in a tricyclic structure. *CyrG* and *CyrH* catalyze the formation of the uracil ring. The tuning reactions are catalyzed by *CyrI, CyrJ* and *CyrN* for sulfation at C12 at hydroxylation at C7. *CyrK* is hypothesized to be a CYN transporter. The two transport enzymes of *CyrL* and *CyrM* may be responsible for the horizontal transport of *cyr* genes. *CyrO* may be involved in transcriptional regulation and DNA binding in *cyr* genes. These three protenies together partly determine the virulence of strains (Yang et al., 2021).

1.2.6. Toxicity of CYN

1.2.6.1. Toxicity to humans

The studies have delved into genomic damage. DNA fragmentation, micronucleation and chromosomal deletions induced by CYN were observed in various cell lines at different concentrations and exposure times. For example: the human lymphoma cell line WIL2-NS with the concentrations of 1 – 10 mg/mL for 48 hours of exposure; the cell line Caco-2 with the concentrations of 0.5 – 2 mg/mL for 24 hours of exposure; the human liver tumor cell line HepG2 with the concentration of 0.5 mg/mL after 12 hours of exposure and wih the concentrations lower than 0.01; 0.05 and 0.1 mg/mL after 24 hours of exposure (Poniedziałek et al., 2014). CYN also exhibits immunotoxicity and endocrine toxicity. Zegura et al. (2011a) demonstrated that the exposure to CYN at a concentration of 0.5 mg/mL could alter the expression of genes involved in apoptosis (BAX and BCL-2), oxidative stress response (GPX1, GSR, GCLC and SOD1) and toxin metabolism in neutrophils. Endocrine toxicity of CYN was also detected in human granulosa cells derived from in vitro fertilization (IVF). After 24 hours of exposure to 1 g/ml of CYN, basal progesterone production was inhibited ($p < 0.01$). Similarly, 6 hours of exposure to 1 g/ml of CYN inhibited hcG-stimulated progesterone production ($p < 0.01$).

1.2.6.2. Toxicity to animals

Many studies have shown that cell extracts containing CYN and purified CYN both cause liver and kidney dysfunction in rats after intrapertoneal and oral administration. The mean lethal dose after intrapertoneal administration (LD50) of purified CYN in rats was 2.1 mg/kg and 0.2 mg/kg in 24 hours and 5 – 6 days, respectively (Ohtani et al., 1992). CYN is also suspected of causing fish and bird deaths. Toxic blooms of *R. raciborskii* occurred frequently in Aleksandrovac lake in Serbia with the highest abundance of 2.38×10^6 fibers/mL and three tons of carp died in the lake in December, 2012. One month prior to this event, the extracellular CYN content reached a maximum concentration of 24.28 μg/L, which implies that CYN was involved in the fatal episode of this fish (Đorđević et al., 2015).

1.2.6.3. Toxicity to plants

For *Azolla filiculoides*, an aquatic fern, the growth was 99.8% inhibited by increased GST activity after seven days of exposure to 5000 μg/L of CYN, whereas it was unchanged at lower concentrations (Santos et al., 2015). Csaba et al. (2015), when stuyding the exposure of two acquatic plants of *Lemna minor* and *Wolffia arrhiza* to CYN for 5 days, found a significant reduction in the growth of *Lemna minor*

as demonstrated by the number of leaves exposed to CYN in the crude extract and to purified CYN at a concentration of 1 – 20 µg/ml. Contrary to the above results, the stimulation of root growth in *Hydrocotyle verticillata* when exposed to 400 µg/L of CYN was observed (Kinnear et al., 2008). In addition, the stimulation of fresh weight and shoot length of *Egeria densa* within the first 14 days of exposure to 2.5 µg/L and 25 µg/L of CYN was observed in the study of Flores-Rojas et al. (2020) (Flores-Rojas et al., 2020).

1.2.6.4. Toxicity to zooplankton

The toxicity of CYN-producing potential cyanobacteria and the toxicity of CYN to freshwater zooplankton species is heterogeneous. Significant inhibition of growth and reproduction as well as reduced carnivores of zooplankton were observed in cultures where the diet was pure *R. raciborskii*, or when the species was predominant significant proportion in mixed diets (Soares et al., 2010; Bednarska and Slusarczyk, 2013; Lei et al., 2020). In contrast, both field and laboratory studies have shown that some zooplankton species can still grow and reproduce in water containing R. raciborskii (Soares et al., 2010; Weithoff et al. ., 2017). They found that *R. raciborskii* was less harmful to *Brachionus calicyflorus* than *Microcystis aeruginosa.*

1.3. Method for identifying CYN-producing potential cyanobacteria

1.3.1. CYN biosynthesis gene cluster

In 2008, the entire *cyr* gene cluster responsible for CYN biosynthesis in *R. raciborskii* AWT205 strain was first proposed by Mihali et al. (2008), the *cyr* gene cluster spans 43 kb and contains 15 open reading frames encoding all the enzymes required for biosynthesis, regulation and toxin secretion. The two *cyrC* and *cyrB* genes have been shown to be the main genes involved in the production of CYN in the cyanobacterial species capable of producing this toxin. The presence of these two genes is sufficient evidence to probe and evaluate the CYN production ability of the cyanobacterial species theoretically. However, according to Schembri (2001), the presence of one or two of these genes is sufficient, because the simultaneous presence or the absence of either genes is still capable of producing toxins. Therefore, these genes can be used as molecular markers to identify the presence of species capable of producing CYN in the water bodies.

1.3.2. Method for identifying and classifying CYN-producing potential cyanobacteria

Polymerase chain reaction (PCR) is a commonly used method for rapid screening of cyanobacteria capable of producing CYN. DNA is

initially extracted from environmental or culture samples. The *cyr* gene was then amplified by using specific PCR primers designed in accordance with the *cyr* sequence in the GenBank. In addition, qPCR (quantitative real-time PCR) can also be used in this process, based on the assumption that the copy number of CYN biosynthetic gene reflects toxicity. Campo et al. (2013) first developed the TaqMan qPCR assay to detect *Chr. Ovalisporum* producing CYN in the environmental samples.

1.3.3. Method for identifying CYN

1.3.3.1. Immune method

Enzyme-linked immunosorbent assay (ELISA) is a promising method due to its sensitivity, specificity and ease of manipulation. The principle of ELISA technique is based on antigen and antibody specificity. Direct competitive ELISA is a very effective ELISA technique for the quantification of elements present in a sample in small amounts. This technique quantifies CYN based on specific antibodies.

1.3.3.2. Chemical method

Liquid chromatography (LC) is an efficient method for the separation and quantification of CYN, displaying high accuracy and specificity. The most common applications of liquid chromatography are high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC) and liquid chromatography – mass spectrometry (LC-MS or LC-MS/MS). UPLC offers a significant advantage over conventional HPLC as it allows very fest separation of analytes and great reduction of solvent usage. Mass spectrometry can provide an indication of the elemental composition and the structure of analyte along with the identification of the amount of analytes for which the reference material is available with high sensitivity. MS is the only technique clearly distinguishing and quantifying these isoforms (Yang et al., 2021), which is a significant advantage over ELISA. LC-MS/MS systems are more complex, incorporating more than one mass spectrometer. This makes LC-MS/MS trở a highly specific analytical technique.

1.4. Research situation on CYN and CYN-producing potential cyanobacteria in the world and in Vietnam

1.4.1. In the world

In order to identify cyanobacterial species capable of producing CYN in 10 reservoirs in Northeastern Brazil, Lorenzi et al. (2015) used two pairs of Schembri primers and found that CYN was detected only in water samples containing both *cyrB* and *cyrC* gene segments (Lorenzi et al., 2015). Similarly,

the M13 and M14 pairs of primers were also used to identify the presence of CYN-producing genes in the sediments of the Limpopo river basin. The results showed that the *cyrB* gene segment only appeared in the sediment samples containing CYN and *R. raciborskii*. Cordeir et al. (2021) also used these two pairs of primers to screen for CYN-producing strains in a culture collection of 157 algae strains. The results showed that the two strains of BACA0025 and BACA0031 containing both *cyrB* and *cyrC* gene segments were screened and were toxic when tested by ESI-LC-MS/MS. The *cyrB* and *cyrC* genes have become indicators to quickly and accurately probe and control CYN-producing cyanobacterial speicies in the water sources.

In the world, many cyanobacterial species have been identified to produce CYN, but ecological studies on this species group are limited, mainly focusing on common bloom-causing species in the water bodies around the world. With a few exceptions, most of *Ch. ovalisporum* blooms occur at water temperatures above 25 ^{o}C in waters of low to moderate salinity and low to moderate water transparency (Secchi disc depth from 0.2 to 2.5 m), and in waters of mild to moderate alkaline with pH values from 7.2 to 9.0. Blooms may occur in deeply stratified water bodies and in shallow ponds. In addition, some blooms have been reported from seas with high nitrogen and phosphorus content, while other blooms occur where dissolved nutrient contents may be lacking (Bowling et al., 2018).

Besides *C. ovalisporum*, *R. raciborskii* is also listed as a common harmful cyanobacterial species. The global dominance of *R. raciborskii* is on the one hand because of its phenotypic plasticity in response to dominant environmental factors such as temperature, light, and nutrients. Mobility in response to available nutrient in species has expanded the ecological niche of *R. raciborskii*. They have adapted to high levels of volatility for the nutrients of nitrogen and phosphorus. In the wild, *R. raciborskii* populations are preferred over other species with daily phosphorus supplementation (Muhid et al., 2013). Meanwhile, Kokocinski et al. (2017) found that this population still thrived in low phosphorus environment. *R. raciborskii* can still form the blooms when phosphorus concentrations are often near to or below the detection limit (Burford et al., 2006; Prentice et al., 2015), which is a result of its superior P absorption and retention ability (Burford et al., 2016). Numerous studies have shown that *R. raciborskii* favors the use of soluble inorganic nitrogen (ammonium, nitrate) and organic nitrogen (urea) sources over nitrogen fixation with a clear preference for ammonium based on both growth rate and absorption rate (Burford et al., 2018). Some studies have shown that the biomass of *R. raciborskii* in the condition of complete nitrogen

was 20 – 50 times higher than that in the condition of nitrogen deficiency and the growth rate of *R. raciborskii* increased with nitrogen concentration (Yema et al., 2016). Water temperature and light conditions in the area are important factors driving the distribution of *R. raciborskii* (Bonilla et al., 2016). Many studies have demonstrated that the optimal water temperature for growth ranges from 25 °C to 35 °C. Common bloom temperatures are usually greater than 25 °C. However, the blooms also occur at low temperatures in tropical (13 °C – 20 °C) and subtropical (11 °C) lakes (Jia et al., 2021). Even their blooms have been observed in winter in lakes and dams in Northern Taiwan; Lago Javier; Uruguay and Rio Grande do Sul, Southern Brazil, when the temperatures were 16.3 ºC, 11.2 ºC and 11 ºC, respectively (Wener et al., 2020). *R. raciborskii* strains from Australia, Europe, South America and Africa favor low light for growth, with optimal photon concentrations (flux) for growth ranging from 50 to 120 mmol photons (PAR) m^2 s^1. However, the growth can be maintained at low light, even at a photon flux < 10 mmol photons (PAR) m^2 s^1. In contrast, Carneiro et al. (2013b) showed a strain (Australia) with a high growth rate in a photon flux as high as 348 mmol photons (PAR) m^2 s^1. The researchers previously agreed that *R. raciborskii* grows in alkaline waters and does not occur in acidic waters. However, the pH of water in the study of Wener et al. (2020) ranged from 5.4 to 8.7 and the highest density of *R. raciborskii* reached 99,994 ind/mL at the pH of 6 and the lower density reached 61,400 ind/mL at the pH of 8,7. In a study in Pequeno river (São Paulo, Brazil), the pH of 5.4 was recorded during a bloom of *R. raciborskii*. In Brazilian waters, the high density of *R. raciborskii* usually occurs in alkaline waters (pH = 8 – 9,4). However, the blooms have also been recorded in slightly acidic to alkaline waters, with the pH of 6 to 10 (Wener et al., 2020). These results indicate that *R. raciborskii* is tolerant to a wide range of pH.

Gin et al. (2021) discovered new cyanobacterial species, *Synechococcus* sp., capable of producing CYN, they can also endure long-term nitrogen deficiency through the degradation of pigments including chlorophyll a. *Synechococcus* sp. can also survive a wide temperature range from regions near Antarctica to the tropics. The tolerance of *Synechococcus* to temperature and nutritional conditions is likely to be favorable under environmental fluctuations caused by climate and urbanization, which will lead to a rapid increase in future toxic blooms of *Synechococcus* (Gin et al., 2021).

1.4.2. In Vietnam

Nguyen et al. (2007) described and classified 33 cyanobacterial species in Huong river and some water bodies in Hue, in which the CYN

content was quite high in *R. raciborskii* Hcy90 strain (6.7 µg/mg). Four *R. raciborskii* strains isolated in Bien Ho and Duc An, Gia Lai also showed the CYN production ability with different concentrations. Next, Nguyen Thi Thanh investigated the large-scale occurrence of cyanobacterial species with the potential CYN production in 35 freshwater bodies of 10 provinces (cities) in the North and the Central coastal provinces. The results of the investigation have identified 12 species with the potential CYN production. In 2015, when conducting isolation and culture of *R. raciborskii* strain in Dau Tieng reservoir, Pham et al. showed that the cell size, especially the cell length of *R. raciborskii* strains in this reservoir was larger than the cell size and the cell length of these in Tri An reservoir and other areas in Hue (Pham et al., 2015). In 2017, Dao Thanh Son et al. announced the occurrence and ecological toxicity of *R. raciborskii* species on *Daphnia magna* microcustacean in Xuan Huong Lake and the survey results showed that the extract from *R. raciborskii* at a concentration of 1 mg/L and 5 mg/L stimulated reproduction and had a light effect on survival of *D. magna*. However, the concentrations of 25 mg/L and 100 mg/L resulted in high mortality and reduced the cumulative number of juveniles of this crustacean (Dao et al., 2017). Unlike strains in Dau Tieng reservoir, *R. raciborskii* strains isolated in some water bodies in Hue and Gia Lai produce CYN when tested by HPLC and ELISA methods. In addition, potential CYN-producing species such as: *R. curvata*; *R. mediterranea; Aphanizomenon* sp. were also isolated but did not produce CYN (Nguyen et al., 2012, 2017).

1.4.3. In Dak Lak

The studies on harful cyanobacterial species in general and CYN-producing cyanobacterial species in particular have not been interested, although this group of species has been present at a relatively high rate in the phytoplankton species composition in some water bodies in Dak Lak. Furthermore, the data on cyanotoxins and CYN toxin have not been identified in the reservoir system here.

CHAPTER 2. RESEARCH SUBJECTS AND METHODS

2.1. Research subjects, location and time

2.1.1. Subjects

Cyanobacteria and CYN-producing potential cyanobacteriain the two Ea Nhai and Buon Phong reservoirs.

2.1.2. Research location

Sampling locations at 2 reservoirs were selected in Dak Lak Province as shown in Figure 2.1.

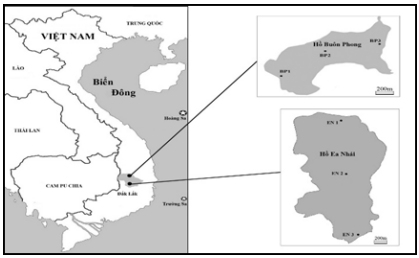


Figure 2.1. Map of sampling locations of 2 reservoirs in Dak Lak Province

2.1.3. Research time

Sampling in the two lakes was carried out monthly, for one year from May 2019 to April 2020. Collected samples were collected at 6 sampling locations located in the two lakes.

2.2. Research methods

2.2.1. Field research methods

Means of sampling: In both reservoirs, motorboats of fishermen are used.

Qualitative samples were collected using a plankton net (20 µm mesh size) and immediately fixed with formaldehyde solution at a final concentration of 4%.

Quantitative samples were collected using a plastic tube fitted with a Luppe device, 2 m in length and 10 cm in diameter. Then, water samples (0 – 2 m in depth) were mixed in a bucket. One liter of sample water was taken out, fixed with 1% Lugol acid solution, left to settle for 48 hours, and aspirated with a siphon tube to leavel the remaining 100 mL.

Culture samples and toxin analysis samples were collected according to Nguyen et al., (2017).

Samples for analysis of environmental parameters: Subsurface water samples were collected 3 times at each sampling location using cleaned polypropylene plastic bottles. Samples were kept in the dark at 4 °C before being transported to the laboratory for analysis.

2.2.2. Laboratory research methods

2.2.2.1. Qualitative analysis

Cyanobacterial species were identified by morphological comparison method. The taxonomy is based on the references of Duong 1996; Komárek and Anagnostidis, 1989; Komárek et al., 1999, 2005, 2014.

2.2.2.2. Quantitative analysis

The number of cyanobacterial cells was counted using a Sedgewick-Rafter counting chamber. The biovolume was calculated by the method of Safi et al. (2009) and Chorus and Welker. (2021).

2.2.2.3. Isolation and culture methods

Isolated using a modified Pasteur pipette. The isolates were cultured in Z8 medium at 24 ± 4 °C in a 12:12 hour dark/light cycle with 2,000-3,000 lux light intensity.

2.2.2.4. Analysis of toxins by ELISA technique

CYN concentrations in the samples were checked by ELISA technique using Abraxis Cylindrospermopsin ELISA kit (Microtiter Plate) (Abraxis, United States of America). The CYN concentrations was identified in accordance with the instructions of manufacturer.

2.2.2.5. Analysis of toxins by HPLC technique

Toxins were extracted from biomass samples of cyanobacteria by the method of Nguyen et al. (2007). CYN was analyzed in the HPLC Thermo system. In the column, the components were separated and the Detector (UV) detected CYN at the wavelength of 262 nm. CYN was qualified by retention time and quantified by the correlation between concentration and peak area at the wavelength of 262 nm using the CYN standard (CRM-CYN, PESTANAL®, Sigma-Aldrich Pte. Ltd.) as the external standard.

2.2.2.6. Analysis of environmental parameters

The physicochemical factors as: Temp. and pH were measured in situ using a multi-parameter handheld tester PCSTestr 35. Turbidity was measured directly in the field by Lovibond meter – Germany. Chemical analyses were conducted in accordance with Vietnamese standards.

2.2.2.7. Analysis of the presence of genes involved in CYN production

Total DNA extraction was performed in accordance with the CTAB protocol of Doyle and Doyle (1987) with some modifications.

The *cyrB* and *cyrC* gene fragments were amplified by PCR with two pairs of oligonucleotit M4/M5 and M13/M14 primers (Schembri et al., 2001). Thermal cycling conditions for PCR were 1 cycle at 94 °C for 4 minutes, 30 cycles at 94 °C for 10 seconds, at 55 °C for 20 minutes, at 72 °C for 1 minute and 1 cycle at 72 °C for 7 minutes. DNA amplification was carried out in thermal cycling (iCyler, Bio-Rad).

2.2.3. Data processing

Use statistical description in Microsoft Excel software.

Principal component analysis (PCA) and Pearson correlation analysis were used to evaluate the relationship of environmental parameters on CYN-producing cyanobacterial biovolume and the CYN concentration in the studied reservoir. IBM-SPSS Statistics software version 22.0 was used to analyze the results at 5% significance level.

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Cyanobacterial species composition of Ea Nhai and Buon Phong reservoirs, Dak Lak

3.1.1. Cyanobacterial species composition

Survey results of cyanobacterial species composition have recorded 34 species of 14 genera, 6 families and 3 orders (Chroococcales, Oscillatoriales and Noctoscales). The list of species composition of cyanobacteria is arranged in accordance with the taxonomy of Komárek & Anagnostidis (1999, 2005).

Table 3.1. Cyanobacterial species composition in Ea Nhai and Buon Phong reservoirs in Dak Lak.

No.	Science name	Ea Nhai (EN)		Buon phong (BP)	
		Rainy	dry	Rainy	dry
Chroococcales					
Merismopediaceae					
1	*Aphanocapsa holsatic*	-	+	+	+
2	*Merismopedia tenuissima*	+	+	+	+
3	*Woronichinia compacta*	-	-	+	+
4	*Woronichinia naegelian*	-	-	+	+
5	*Snowella fennica*	-	-	+	-
Microcystaceae					
6	*Microcystis aeruginosa*	-	+	+	+
7	*Microcystis wesenbergii*	-	+	+	+
8	*Microcystis botrys*	-	+	+	+
9	*Microcystis flos-aquae*	-	+	+	+
10	*Microcystis panniformis*	-	+	+	+
11	*Microcystis novacerkii*	-	-	+	+
12	*Microcystis natans*	-	-	+	+
13	*Microcystis* sp.1	-	-	-	+
14	*Microcystis* sp.2	-	-	-	+
Oscillatoriales Oscillatoriaceae					
15	*Lyngbya* sp.	+	+	-	-
16	*Oscillatoria limosa*	-	+	-	+
17	*Oscillatoria sancta*	-	-	-	+
18	*Oscillatoria* sp.1	-	-	-	+
19	*Oscillatoria* sp.2	-	+	-	+
20	*Oscillatoria* sp.3	-	+	-	+
Phormidiaceae					
21	*Phormidium willei*	+	+	-	-
22	*Phormidium acticulatum*	+	+	-	-
Pseudanabaenaceae					
23	*Planktolyngbya brevicellularis*	-	+	-	-
24	*Planktolyngbya circumcreta*	-	-	+	+
25	*Planktolyngbya limnetica*	-	-	+	+
26	*Pseudanabaena minima*	-	+	-	-
27	*Pseudanabaena mucicola*	-	+	-	-
Nostocales Nostocaceae					
28	*Raphidiopsis raciborskii*	+	+	+	+
29	*Raphidiopsis mediterranea*	-	+	-	-
30	*Raphidiopsis curvata*	+	+	-	-
31	*Anabaena* sp.1	-	-	-	+
32	*Anabaena* sp.2	-	-	+	+
33	*Dolichospermum circinale*	-	-	-	+
34	*Chrysosporum ovalisporum*	-	-	-	+

In Buon Phong reservoir, there are 26 cyanobacterial species distributed in 3 orders, 5 families and 10 genera with Chroococcales order making up the most number for both families, genera and species. In Ea Nhai reservoir, there are 19 cyanobacterial species a distributed in 3 orders, 5 families and 10 genera with Oscillatoriales order having the highest number of families, genera and species in Ea Nhai reservoir. *Microcystis* is the genus with the highest number of species in the cyanobacterial communities in both Buon Phong and Ea Nhai reservoirs. This result is completely consistent with the studies of some authors who found that *Microcystis* is the most species richness genus in the cyanobacterial communities in the water bodies studied by them. The species richness of these generain freshwater bodies may be because in shallow and eutrophic lakes, many species of cyanobacterial group incapable of nitrogen fixation often appear (Nitrogen in lakes is not limted), especially Chroococales and Osillatoriales orders including *Microcystis* and *Oscillatoria* genera (Havens et al., 2013).

3.1.2. Morphological description of the studied cyanobacterial species

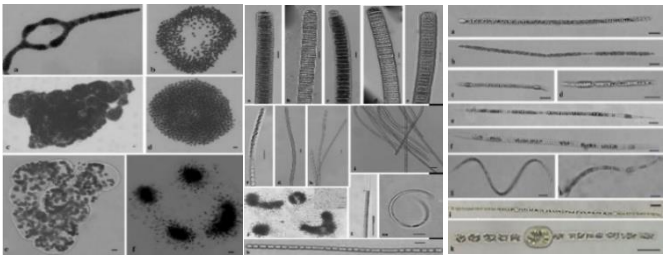


Figure 3.3; 3.6; 3.8. Cyanobacterial species in Ea Nhai and Buon Phong reservoirs

3.1.3. CYN-producing potential cyanobacteria in Ea Nhai and Buon Phong reservoirs

In the two studied reservoirs, 16 cyanobacterial species (accounting for 47,1 % of total species) have been identified in the list of species capable of producing toxins. Buon Phong reservoir has 13 CYN-producing potential cyanobacteria, in which 2 species are capable of producing CYN (*Ch. Ovalisporum*, *R. raciborskii*). In Ea Nhai reservoir, there are 11 cyanobacterial species capable of producing toxins with 3 CYN-producing potential cyanobacteria species. The results show that, the number of harful cyanobacteria in the study area is quite high, according for more than 50% of the

total species, of which there are 4 CYN-producing potential cyanobacterial species. This shows the risk of toxic contamination as well as the potential health risks.

3.2. Biovolume of cyanobacterial species and CYN concentrations in Ea Nhai and Buon Phong reservoirs

3.2.1. Biovolume of cyanobacterial species and CYN concentrations in Ea Nhai

3.2.1.1. Biovolume of cyanobacterial species in Ea Nhai reservoir

From the analysis results, we found that the biovolume of *R. raciborskii* accounted for the highest percentage, followed by *Lyngbya* sp., *R. curvata, Microcystis* spp. and *R. mediterranea*. *Merismopedia tenuissima* has the lowest biovolume in the cyanobacterial communities of Ea Nhai reservoir.

3.2.1.2. CYN concentrations in Ea Nhai reservoir

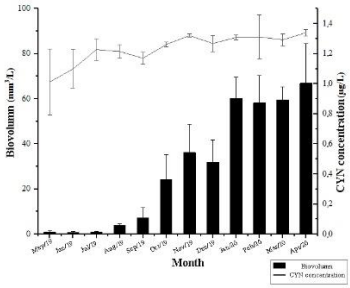
ELISA results showed the presence of CYN in all water samples during the 12 months of research, from May 2019 to April 2020 with an average value of 1.24 ± 0.08 µg/L. The higher concentrations of CYN were found in the dry season (1.27 – 1.34 µg/L) than in the rainy season (1.01 – 1.26 µg/L).

Comparing the correlation between CYN concentration and biovolume of CYN-producing potential cyanobacteria, it was shown that among the four potential CYN-producing cyanobacterial species: *R. raciborskii, R. curvata, R. mediterranea* and *Lyngbya* sp. identified in Ea Nhai reservoir, the significant correlation was found between the CYN concentration and the biovolume of the 4 species *R. raciborskii, R. curvata, R. mediterranea* and *Lyngbya* sp. ($p < 0,01$, $p < 0,01$, $p < 0,01$ and $p < 0,05$, table 3.3). However, the average biovolume of 3 species of: *R. curvata, R. mediterranea* and *Lyngbya* sp. was very low, 0.03; 0.006 and 0.61 mm^3/L, respectively. Therefore, we believe that, species *R. raciborskii*, with a relatively high biovolume (up to 66.8 mm^3/L), in the cyanobacterial communities of Ea Nhai reservoir, could be the major source of CYN production during the research period.

Table 3.3. Pearson correlation between biovolume of CYN-producing potential cyanobacteria and CYN concentrations in Ea Nhai reservoir

	R. raciborskii	*R.mediterranea*	*R. curvata*	*Microcystis*	*M.tenuissima*	*Lyngbya* sp.	CYN
R. raciborskii	1						
R.mediterranea	0,905**	1					
R. curvata	0,842**	0,928**	1				
Microcystis	0,599**	0,687**	0,702**	1			
M.tenuissima	0,663**	0,704**	0,761**	0,230	1		
Lyngbya sp.	0,615**	0,686**	0,544**	0,806**	0,066	1	
CYN	0,596**	0,506**	0,438**	0,317	0,301	0,364*	1

**. Correlation is significant at the 0.01 level; *. Correlation is significant at the 0.05 level



Hình 3.9. Seasonal variation of the *R. raciborskii; R. curvata and R. mediterranea* biovolumes and CYN concentrations in the Ea Nhai reservoir from May 2019-April 2020

3.2.2. Biovolume of cyanobacterial species and CYN concentrations in Buon Phong

3.2.2.1. Biovolume of cyanobacterial species in Buon Phong reservoir

In Buon Phong reservoir, we found that the biovolume of species of genus *Microcystis* accounted for the highest proportion in the cyanobacterial communities, over 84% of the total biovolume of cyanobacteria in the reservoir, followed by *R. raciborskii*, with the biovolume reaching the values from 0.12 – 9.14 mm^3/L, accounting for 11.9% of the total biovolume of cyanobacteria. *Anabaena* sp.2 with the potential CYN production is present all year round in the reservoir with the lowest biovolume of the reservoir from 0.08 – 3.16 mm^3/L.

3.2.2.2. CYN concentrations in Buon Phong reservoir

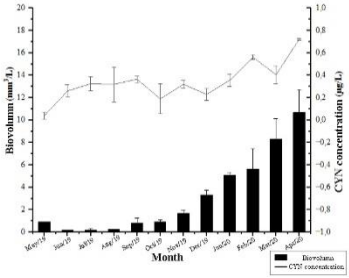
The ELISA analysis results showed that CYN in the reservoir water were present during the study 12 months, ranging from 0.04 – 0.72 µg/L, the highest concentration fell at the end of the dry season (April), and the lowest is at the beginning of the rainy season (May) (fig. 3.10).

From the Pearson correlation coefficient, we found that *R. raciborskii* is strongly correlated with the CYN concentration ($p < 0.01$) in the reservoir. Besides, species *Anabaena* sp.2 (potential CYN production) also showed the correlation with CYN when $p < 0.05$, weaker than the correlation between *R. raciborskii* and CYN. In contrast, species *Microcystis* spp. which are known to be the main group of species producing microcystin hepatotoxins (MCs) did not show any correlation with CYN concentrations in the reservoir (tab. 3.4). From there, we believe that the CYN content in the reservoir is generated by 2 species of *R. raciborskii* and *Anabaena* sp.2 but probably mostly from *R. raciborskii*.

Table 3.4. Pearson correlation between biovolume of CYN-producing potential cyanobacteria and CYN concentrations in Buon Phong reservoir

	R. raciborskii	*Anabaena* sp.2	*Microcystis*	Total Cyanobacteria	CYN
R. raciborskii	1				
Anabaena sp.2	0,566**	1			
Microcystis	0,566**	0,717**	1		
Total Cyanobacteria	0,696**	0,771**	0,985**	1	
CYN	0,538**	0,343*	0,301	0,377*	1

**. Correlation is significant at the 0.01 level; *. Correlation is significant at the 0.05 level



Hình 3.10. Seasonal variation of the *R. raciborskii and Anabaena* sp.2 biovolumes and CYN concentrations in the Buon Phong reservoir from May 2019-April 2020

3.3. Toxin production ability of isolated algae strains

3.3.1. Isolation and culture results

From 72 samples collected from May 2019 to April 2020, we isolated 24 strains of 8 species. Strain name and strain origin are shown in Table 3.5.

Table 3.5. List of isolated cyanobacterial strains from the two studied reservoirs

No.	Species	Strains	Reservoirs
1	*Dolichospermum circinale*	**AcBP2**	Buon Phong
2	*Anabaena* sp.2	**ABP1**	Buon Phong
3		**ABP3**	
4		**ABP8**	
5		**ABP10**	
6	*Raphidiopsis raciborskii*	**CENG**	Ea Nhai
7		**CEN0**	
8		**CEN7**	
9		**CEN10**	
10		**CEN11**	
11		**CBP2**	Buon Phong
12		**CBP3**	
13		**CBP4**	
14		**CBP5**	
15	*Raphidiopsis curvata*	**RCEN0**	Ea Nhai
16		**RCEN1**	
17		**RCEN2**	
18		**RCEN3**	
19	*Raphidiopsis mediterranea*	**RMEN2**	Ea Nhai
20		**RMEN3**	
21	*Lyngbya* sp.	**LyEN2**	Ea Nhai
22	*Oscillatoria* sp.3	**OsBP1**	Buon Phong
23	*Planktolyngbya circumcreta*	**PLBP1**	Buon Phong
24		**PLBP4**	

3.3.2. CYN concentration in isolated strains

The results of CYN content analysis by HPLC method showed that no CYN was detected in the strains of species *Dolichospermum circinalis, Planktolyngbya circumcreta, Lyngbya* sp. and *Oscillatori*a sp.3. The toxin was detected only in 17 strains out of 19 strains of 4 species capable of producing toxin: *Raphidiopsis raciborskii, Raphidiopsis curvata, Raphidiopsis mediterranea* and *Anabean*a sp. (tab. 3.8).

In general, in the two studied reservoirs, the toxin concentration in strains in different species was not the same, the highest in strain RMEN2 of species *R. mediterranea* (0.584 µg/g, dry weight) and the lowest in strain CBP3 of species *R. raciborskii* (0.016 µg/g, dry weight). Besides, the toxin production ability in strains in the same population was also different. There were strains producing CYN (RCEN0, RCEN1, RCEN2), there were strains not producing CYN (RCEN3), and the toxin concentration between strains was not the same (CENG, CEN0, CEN7, CEN10...).

3.3.3. The presence of genes involved in CYN synthesis in cyanobacterial strains

Out of the 21 isolated strains from the two reservoirs, 13 strains showed simultaneously both *cyrB* and *cyrC* gene fragments; 1 strain showed only *cyrB* gene fragment without the presence of *cyrC* gene fragment (ABP3). 1 strain showed only *cyrC* gene fragment without the presence of *cyrB* gene fragment (RCEN3). When analyzing on 6 strains of 4 species *Anabeana* sp.2; *R. raciborskii; Lyngbya* sp. and *Oscillatoria* sp.3, both *cyrB* and *cyrC* gene fragments were not found (ABP8, ABP10, CBP4, CBP5, LyEN2 and OsBP1) (tab.3.8).

3.3.4. Correlation between toxin synthesis genes and CYN concentrations in culture strains.

We found that out of 21 strains of 6 species *R. raciborskii, R. curvata, R. mediterrannea, Anabaena* sp.2, *Lyngbya* sp. and *Oscillatoria* sp.3, 13 toxic strains showed the presence of both *cyrB* and *cyrC* gene fragments. For the next 4 toxic strains, 1 strain (ABP3) showed only the *cyrB* gene fragment; while the remaining 3 toxic strains (ABP8, CBP4 and CBP5) lacked both these gene fragments. Among the 4 non-toxin-producing strains, 3 strains did not show both *cyrB* and *cyrC* gene fragments (ABP10, LyEN2 and OsBP1), while the remaining 1 strain (RCEN3) showed the presence of *cyrC* gene fragment. In this study, we have identified 4 cyanobacterial species capable of producing CYN in the two studied reservoirs. In which, Ea

Nhai reservoir had 3 species (*R. raciborskii*, *R. curvata*, *R. mediterrannea*) and Buon Phong reservoir had 2 species (*R. raciborskii*, *Anabaena* sp.2).

***Table 3.8. CYN concentration and the presence of* gene fragments involved in CYN synthesis in cyanobacterial strains**

No.	Strains	*CyrB/ CyrC*	CYN concentration (µg/g DW)	No.	Strains	*CyrB/ CyrC*	CYN concentration (µg/g DW)
1	ABP1	+/+	0,238 (0,234-0,241)	12	CEN0	+/+	0,504 (0,502-0,505)
2	ABP3	+/-	0,049 (0,48-0,50)	13	CEN7	+/+	0,054 (0,051-0,059)
3	ABP8	-/-	0,045 (0,044-0,046)	14	CEN10	+/+	0,444 (0,443-0,447)
4	ABP10	-/-	-	15	CEN11	+/+	0,017 (0,016-0,017)
5	RCEN0	+/+	0,267 (0,264-0,269)	16	CBP2	+/+	0,029 (0,028-0,031)
6	RCEN1	+/+	0,314 (0,311-0,318)	17	CBP3	+/+	0,016 (0,0155-0,016)
7	RCEN2	+/+	0,172 (0,169-0,176)	18	CBP4	-/-	0,345 (0,342-0,349)
8	RCEN3	-/+	-	19	CBP5	-/-	0,019 (0,019-0,020)
9	RMEN2	+/+	0,584 (0,578-0,590)	20	LyEN2	-/-	-
10	RMEN3	+/+	0,398 (0,392-0,404)	21	OsBP1	-/-	-
11	CENG	+/+	0,234 (0,229-0,243)				

3.4. Effects of environmental factors on the variation of biovolume of CYN-producing cyanobacteria and the CYN concentration in Ea Nhai and Buon Phong reservoirs

3.4.1. Effects of environmental factors on the variation of biovolume of CYN-producing cyanobacteria and the CYN concentration in Ea Nhai reservoir

3.4.1.1. The environmental factors in Ea Nhai reservoir

Water temperatures ranged from 25.5ºC to 32.0ºC with an average value of 29.0ºC during the investigation period. The pH values varied between a minimum of 7.1 to a maximum of 8.3 and did not differ significantly over the year. Higher values of turbidity were recorded during the rainy period (May to October). Mean concentrations of ammonium and nitrate across the study were 0.23±0.05 and 0.21±0.08 mg L-1, respectively. ower ammonium concentrations were found during the rainy period compared to the dry

season. While there were no clear seasonal variations in nitrate concentrations. Dissolved orthophosphate-P concentration varied from 0.06 to 0.1 mg L-1. The Ea Nhai reservoir was characterized by relatively high concentrations of TN and TP, with mean TP concentrations ranging from 0.16 to 0.4 mg L-1 and mean TN concentrations ranging from 1.4 to 3.67 mg L-1. Monthly TN:TP ratios ranged from 13.9 to 35, with a mean value of the study of 22.3. According to the trophic classification proposed by the Organization for Economic Cooperation and Development criteria (OECD, 1982), the water quality of the Ea Nhai reservoir was classified to be eutrophic (based on TP values).

3.4.1.2. Effects of environmental factors on the variation of biovolume of CYN-producing cyanobacteria and the CYN concentration in Ea Nhai reservoir

PCA and Pearson correlation analysis showed the correlation between toxic cyanobacteria and environmental factors in Ea Nhai reservoir. Abiotic parameters (Temp., N-NH_4, P-PO_4, TN and TP) were significantly correlated with biovolume of *R. raciborskii* (R = 0,66, p <0,01; R = 0,73, p <0,01; R = 0,65, p <0,05, R = 0,84, p <0,01; R = 0,34, p <0,01). Besides, biovolume of *R. curvata*, *R. mediterranea* also showed correlation with abiotic variables (CYN, Temp., N-NH_4, P-PO_4, TN, TP) (fig. 3.18).

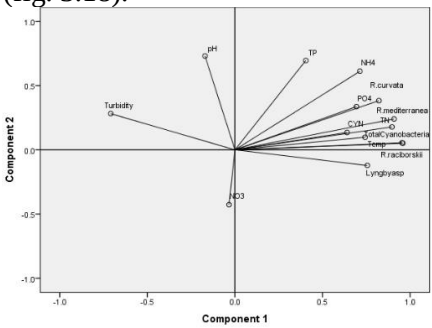


Figure 3.18. **Principal component analysis (PCA) based on biotic and abiotic factors during the period of May 2019-April 2020 in the Ea Nhai reservoir**

3.4.2. Effects of environmental factors on the variation of biovolume of CYN-producing cyanobacteria and the CYN concentration in Buon Phong reservoir

3.4.2.1. The environmental factors in Buon Phong reservoir

Water temperature varied seasonally and higher values were recorded in the dry season, ranging from 25.8°C to 32.2°C, while the

average temperature was 28.4°C. The pH values ranged from 6.7 to 7.7 and did not differ significantly during the studied period. The turbidity ranged from 15.1 to 33.0 NTU and higher values were measured in the rainy season than the dry season. The concentrations of $N\text{-}NH_4$ did not have obvious seasonal differences, which slightly changed from 0.1 to 0.26 mg L-1. The lowest value of the concentrations of $N\text{-}NO_3$ was 0.09 mg L-1 , while the highest value was 0.27 mg L-1 . The soluble orthophosphate-P concentrations varied from 0.05 to 0.09 mg L-1. The mean concentrations of total nitrogen (TN) ranged from 1.05 to 2.56 mg L-1, higher than that of total phosphorus (TP), ranging from 0.09 to 0.31 mg L-1. Based on the mean concentrations of total phosphorus (TP), the Buon Phong reservoir's water quality was classified as eutrophic (OECD, 1982).

3.4.2.2. Effects of environmental factors on the variation of biovolume of CYN-producing cyanobacteria and the CYN concentration in Buon Phong reservoir

We used PCA (Principle Correspondence Analysis) and Pearson analysis for evaluation. The results showed that *R. raciborskii* biovolume was positively correlated with abiotic variables such as temperature, $N\text{-}NH_4$, $P\text{-}PO_4$ ($R = 0,45$, $p < 0,01$; $R = 0,46$, $p < 0,01$; $R = 0,35$, $p < 0,05$). Besides, the correlation between temperature and biovolume of *Anabaena* sp.2 also observed in Buon Phong reservoir (fig. 3.19).

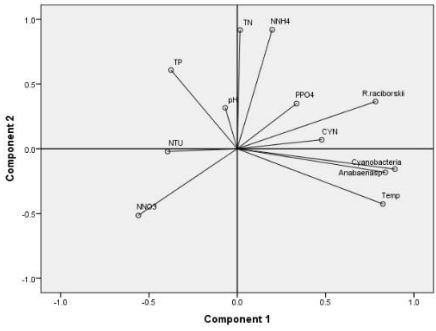


Figure 3.19. **Principal component analysis (PCA) based on biotic and abiotic factors during the period of May 2019-April 2020 in the Buon Phong reservoir**

Thus, PCA and Pearson correlation analysis showed a significant correlation between CYN-producing potential cyanobacteria and CYN concentration in water of Ea Nhai and Buon Phong reservoirs.

At the same time, it also showed a close correlation between temperature, $N\text{-}NH_4$, $P\text{-}PO_4$, TN, TP and biovolume of CYN-producing potential cyanobacteria in the two reservoirs. In which, *R. raciborskii* thrived in conditions of high temperature and nutrient concentration. We believe that, water temperature and availability of nutrients (nitrogen and phosphorus) could be the main environmental factors affecting the bloom of this toxic cyanobacterial species in both studied reservoirs.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. In the two studied reservoirs, 34 cyanobacterial species have been recorded. Buon Phong reservoir had 26 species distributed in 3 orders, 5 families and 10 genera. In Ea Nhai reservoir, 19 species were recorded distributed in 3 orders, 6 families and 9 genera. There was no significant difference in the spatial distribution of cyanobacterial species in both studied reservoirs. In terms of time, it shows a clear seasonal variation in the species composition of cyanobacteria in both reservoirs.

2. Biovolume of CYN-producing potential cyanobacteria (*R. raciborskii, R. curvata, R. mediterranea* and *Anabaena* sp.2) and CYN concentration both showed the clear seasonal fluctuations in both studied reservoirs, low in the rainy season and higher in the dry season. At the same time, the biovolume of CYN-producing potential cyanobacteria were positively correlated with the CYN concentration in water of the two reservoirs. The CYN concentration in water of Ea Nhai reservoir ranged from 1.01 – 1.34 µg/L and the CYN concentration in water of Buon Phong reservoir ranged from 0.04 – 0.72 µg/L.

3. Most strains of four CYN-producing potential cyanobacteria (*R. raciborskii, R. curvata, R. mediterrannea, Anabeana* sp.2) showed found a concordance between the presence of the toxin biosynthetic gene fragment and the toxin production capacity. However, one non-toxic strain (RCEN3) have a *cyrC* gene fragment. Meanwhile, 3 toxic strains (ABP8, CBP4 and CBP5) lack both *cyrB* and *cyrC* gene fragments.

4. Four CYN producing cyanobacterial species (*R. raciborskii, R. curvata, R. mediterranea* and *Anabaena* sp.2) have been identified in the two studied reservoirs. Ea Nhai reservoir had 3 species (*R.*

raciborskii, *R. curvata* and *R. mediterranea*) and Buon Phong reservoir had 2 species (*R. raciborskii* and *Anabaena* sp.2).

5. In both studied reservoirs, temperature and nutrition (N-NH_4, P-PO_4, TN, TP) are the key environmental factors affecting the population fluctuations of four CYN producing cyanobacterial species. In Ea Nhai reservoir, the biovolume of *R. raciborskii, R. curvata* and *R. mediterranea* were positively correlated with Temp., N-NH_4, P-PO_4, TN, TP. In Buon Phong reservoir, the biovolume of *R. raciborskii* was positively correlated with Temp., N-NH_4, P-PO_4. Meanwhile, *Anabaena* sp.2 showed only a correlation with temperature.

Recommendations

1. It is necessary to expand the scope of research to be able to accurately identify the presence of CYN-producing potential cyanobacteria as well as the key environmental factors determining the development of this group of species in freshwater bodies in Dak Lak in particular and in Vietnam in general, in order to accurately predict the risk of toxic contamination, and to curb the explosive growth of the group of CYN-producing cyanobacterial species.

2. With the potential risks of CYN in lake-shaped water bodies, it is imperative to put in place effective biological monitoring programs combined with community-based water management to ensure public health, protect water resources and aquatic resources in the study area in particular and in the freshwater bodies of Vietnam in general.

3. Besides abiotic factors, it is necessary to expand the study of the influence of biological factors on the variation in the species composition of CYN-producing potential cyanobacteria in order to have a more complete view of the aquatic ecosystem.

LIST OF PUBLISHED WORKS

1. **My Thi Diem Ngo**, Dung Manh Doan, Phap That Ton, Thuy Thi Duong, Ha Manh Bui, Lien Thi Thu Nguyen (2022). Population dynamics of *Raphidiopsis raciborskii* and cylindrospermopsin concentration in Ea Nhai reservoir in Dak Lak province, Vietnam. *Pol. J. Environ. Study* 31(4) 1-12. DOI: 10.15244/ pjoes/ 146704.
2. **Thi Diem My Ngo**, That Phap Ton, Thi Thuy Duong, Thi Phuong Quynh Le, Thi Thu Lien Nguyen (2022). Cyanobacterium *Raphidiopsis raciborskii* and its toxin in Buon Phong reservoir, Dak Lak province, Vietnam. *Vietnam Journal of Earth Sciences* 1-16. DOI: 10.15625/2615-9783/16997.
3. **Ngo Thi Diem My**, Ton That Phap, Nguyen Thi Thu Lien (2022). Blooming of harmful cyanobacterium *Raphidiopsis raciborskii* in Buon Phong reservoir, Daklak province. Hue University Journal of Science: Natural Science 131(1A) 43-49. DOI: 10.26459/ hueunijns.v131i1A.6341.
4. **Ngo Thi Diem My**, Ton That Phap, Nguyen Thi Thu Lien (2020). Cyanobacterial composition in Ea Nhai and Buon Phong reservoirs in DakLak. Proceedings of 2020 VietNam National Conference on Biotechnology, Hue University, 983-989.