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Cyanobacterium *Raphidiopsis raciborskii* and its toxin in Buon Phong reservoir, Dak Lak province, Vietnam

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ABSTRACT

This study investigated the variation of the cyanobacterium *Raphidiopsis raciborskii* population under the influence of physicochemical parameters from May 2019 to April 2020 in the Buon Phong reservoir. The correlations between these parameters were explored by using Principal Component Analysis (PCA) and Pearson correlation analysis. The cylindrospermopsin (CYN) toxin in isolated strains from the reservoir was confirmed by using High-performance liquid chromatography (HPLC) analyses. *R. raciborskii* presented throughout the year in the reservoir with a biovolume from 0.12 to 9.14 mm^3 L^{-1}. Four *R. raciborskii* strains (CBP2, CBP3, CBP4, and CBP5) were successfully isolated and confirmed to produce CYN by the HPLC results. The highest concentration in isolated strains was 0.345 µg g^{-1} DW in the CBP4 strain. The PCR results of genes responsible for CYN biosynthesis showed that the PCR amplicons of *cyrB* and *CyrC* were amplified in two toxic strains (CBP2 and CBP3), while the amplicons of both *cyrB* and *cyrC* were not observed in two remaining toxic strains. In addition, such abiotic factors as temperature and nutrients played major roles in the abundance of *R. raciborskii*. Moreover, the biovolume of *R. raciborskii* positively correlated with the CYN concentrations in the Buon Phong reservoir.

Keywords: toxic cyanobacteria, cylindrospermopsin, Buon Phong reservoir, Dak Lak province, Vietnam.

1. Introduction

Raphidiopsis raciborskii (previously *Cylindrospermopsis raciborskii*) is a freshwater, planktonic filamentous and potentially invasive cyanobacterium that has been known worldwide for forming cyanobacterial harmful algal blooms (cyanoHABs) (Werner et al., 2020). Eutrophication and global warming were likely to increase the bloom frequency, intensity and duration of the cyanobacterium in many aquatic ecosystems globally (O'Neil et al., 2012; Baxter et al., 2020). Blooms of *R. raciborskii* have the potential to alter the function of freshwater ecosystems and contribute to water quality degradation through the release of toxins (Willis et al., 2018). Several *Raphidiopsis* strains can

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produce CYN, other trains can build SXTs, while many different strains do not make either CYN or SXTs. So, the toxin productions are strain-specific and independent of geographical characteristics (Antunes et al., 2015; Kokocinski et al., 2017). This species has been recorded worldwide under different environmental conditions, such as freshwater, brackish water, and various areas from tropical subtropical to temperate regions (Bittencourt et al., 2014; Burford et al., 2016; Wener et al., 2020). The success and rapid expansion of *R. raciborskii* in various climates are partly attributed to intra-population strain variability, enhancing the potential of populations to rapidly adapt to changing environmental conditions (phenotypic plasticity). In addition, the existence of multiple ecotypes and diazotrophic activities have promoted the further spread of this species (Xiao et al., 2017; Jiang et al., 2014).

CYN is a toxin produced by several cyanobacterial species such as *Raphidiopsis raciborskii, Umezakia natans*, *Chrysosporum ovalisporum, Aphanizomenon flos-aquae, Aphanizomenon gracile*, *Raphidiopsis curvata*, *Raphidiopsis mediterranea*, *Anabaena bergii*, *Lyngbya wollei, Phormidium ambiguum*.... *R. raciborskii* was first deemed a harmful bloom species after a toxic bloom event in 1979 when 148 people were hospitalized with symptoms of food poisoning, including vomiting and tender hepatomegaly, after consumption of water from the local reservoir (Byth, 1980; Hawkins et al., 1985). CYN is an alkaloid ($C_{15}H_{21}N_5O_7S$; 415,43 Da) with a tricyclic guanidine, a sulfate group and a cyclic ring. CYN is toxic to cells, genes, immune system, nervous system and endocrine system. Its mechanism of toxicity is mainly by inhibiting protein synthesis, interacting with cytochrome P450 (CYP450), causing oxidative stress and DNA strand breaks, linking to estrogen receptors and affecting acetylcholinesterase (AchE) functioning (Puerto et al., 2018; Yang et al., 2020). Unlike microcystin (MC) toxin, most CYN toxin is released into the external aquatic environment, is strongly soluble in water, stable to sunlight, heat and exists over a wide pH range (Stefanova et al., 2020). In addition, the CYN decomposition rate in the natural environment is prolonged, thereby causing many potential risks and difficulties in the use and management of water resources. The complete cyr gene cluster responsible for CYN toxin biosynthesis was first proposed by Mihali et al. (2008). This *cyr* gene cluster spans 43 kb and contains 15 open reading frames encoding all the enzymes required for biosynthesis (*cyrA-J* and *cyrN*), regulation (*cyrL, cyrM* and *cyrO*), and secretion (*cyrK*) of CYN. Complete sequences of *cyr* gene clusters from several toxic cyanobacterial strains have been published to date: *R. raciborskii* AWT205, CS-505, CS-506, CHAB3438, CHAB358; *Aphanizomenon* sp. 10E6 (GQ385961.1); *Oscillatoria* sp. PCC 6506; *Raphidiopsis curvata* CHAB1150, CHAB114, HB1 and *Raphidiopsis mediterranea* FSS1-150/1 (Sinha, 2015; Pearson et al., 2016; Yang et al., 2020).

According to DWR figures from 2017, Vietnam has a total of 6,648 reservoirs with a total capacity of 12,000 million m^3 of water, of which lakes are utilized for a variety of purposes, including hydropower and providing drinking water for inhabitants, tourist visitors, and other activities. It is also imperative that these reservoirs' water quality be properly managed and monitored at all times (Pham et al., 2019). The Buon Phong an artificial reservoir located in Dak Lak province plays an important role in ensuring water safety as it supplies water for domestic use, irrigation, livestock and aquaculture. The water changing color and having an unpleasant odor is regularly observed during the dry season. The reservoir requires an effective biomonitoring program, but at present little is known about phytoplankton

diversity in this system. Although the *R. raciborskii* was observed in several freshwater bodies in the Dak Lak, no data on cyanotoxin is available (Le et al., 2010). Therefore, this study aims to investigate the influence of environmental factors on biovolume *R. raciborskii* and the CYN concentration in the Buon Phong reservoir. In addition, the CYN concentration and presence of CYN synthesis genes in cultured strains of *R. raciborskii* are also determined.

2. Materials and methods

2.1. Study site

In Dak Lak province, the study was conducted at the Buon Phong reservoir (12°92'01", 108°16'24"). The sampling site is determined as shown in Fig. 1. The Buon Phong is an artificial reservoir with 3.3 million m^3. The catchment area of the reservoir is 13 km^2. The average depth of the reservoir is 10 m, with the most profound site at total supply capacity being 20m. The climate of the basin is both characterized by a humid tropical climate and a hot, and dry southwest monsoon that divides this area into two distinct seasons: the dry season from November of the last year to April of the following year; the rainy season from May to October with cool and humid climate (90% of the annual rainfall is in the cool and humid climate zone). The primary use of the reservoir is as a public water supply, providing agricultural irrigation. Intensive agriculture (mainly coffee and rice crops) is the predominant land use in the catchment. The reservoir receives pollutants from domestic and agricultural wastewater in the basin (Department of Agriculture and Rural Development, Dak Lak).

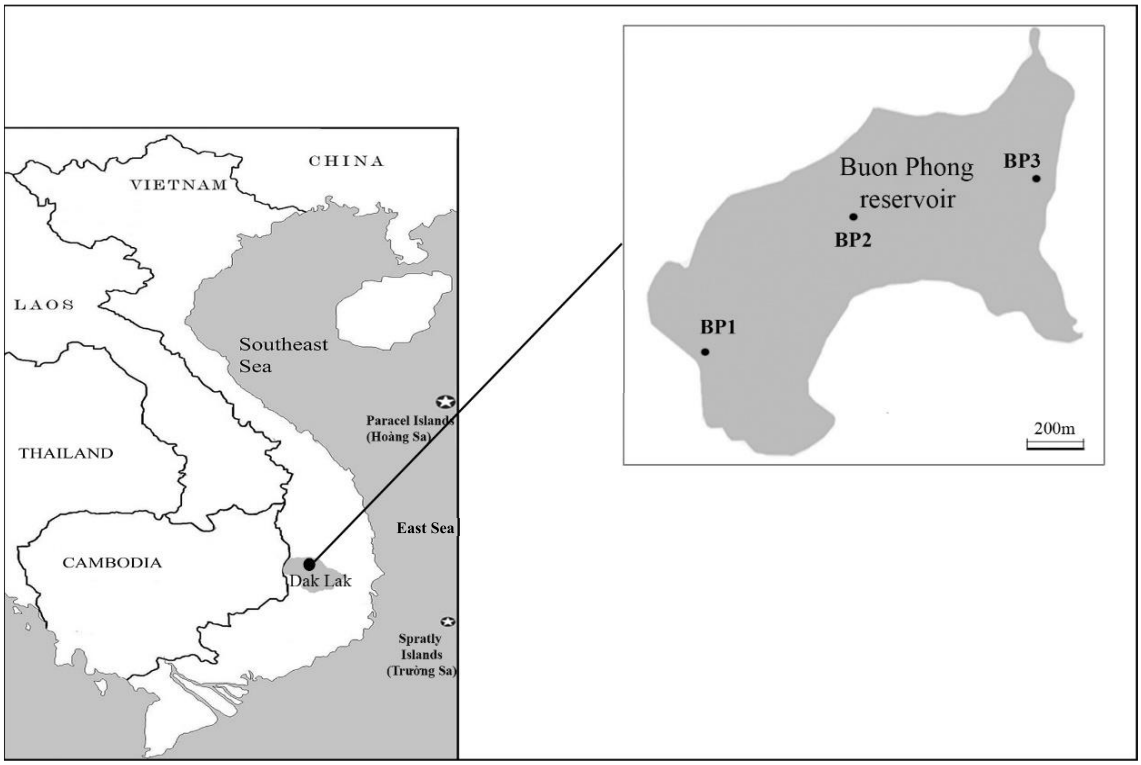

Figure 1. Sampling location in the Buon Phong reservoir, Dak Lak province

2.2. *Sample collection and analysis*

Water and cyanobacteria samples were collected monthly at 3 sampling sites BP1, BP2 and BP3 in the Buon Phong reservoir from May 2019 to April 2020. The physicochemical factors (temperature, and pH) were measured on the spot with a PCSTestr 35 Multi-Parameter Pocket Tester, Eutech brand (Singapore). Dissolved oxygen (DO) was measured on the spot with a portable oxygen meter (Hana HI9147). Turbidity (NTU) was measured directly at the field by a Lovibond - Germany meter. The subsurface water was collected with cleaned polypropylene bottles for analyzing nutritional parameters (P-PO_4, N-NH_4, N-NO_3, TP, and TN). Samples were kept in the dark at 4°C before being transported to the laboratory for analysis during the day. Chemical analyses were conducted in accordance with the International Standards (ISO) at the laboratory of the Institute of Biotechnology, Tay Nguyen University.

A plastic tube with a length of 2 m and a diameter of 10 cm was used to gather quantitative samples. After mixing the water samples (0-2 m depth), 100 ml of the subsample was taken into a glass bottle and fixed with Lugol's iodine solution. Qualitative samples were collected by using a 20-µm mesh plankton net and promptly preserved with formaldehyde solution at a final concentration of 4%.

Cyanobacteria cells counts were conducted using light microscopes (Olympus BX51) under 400× magnification. The number of cyanobacteria filamentous was counted using Sedgewick-Rafter counting chamber under a light microscope (Olympus BX51) (Karlson et al., 2010). Cyanobacterial biovolume was calculated by multiplying the mean cell volume of each taxon by the cell counts in the sample (Chorus et al., 2021). Cyanobacteria species were identified using light microscopes (Olympus BX51) using a morphological comparison method based on the standard references including Duong, 1996; Komárek and Anagnostidis, 1989; Komárek et al., 1999).

To isolate cyanobacteria, live samples (sample without formaldehyde fixation) were collected at the sampling sites. A modified single-cell isolation method was used to separate filaments of *R. raciborskii* (Kotai, 1972). The isolated strains were grown at 24±4°C in a 12:12 hour dark/light cycle with 2,000-3,000 lux light intensity. Toxins of the cultured strains were determined by obtaining the biomass at the end of the exponential growth phase by centrifugation for 10 minutes at 6,000 rpm at room temperature. The pellets were then freeze-dried at -55°C for 24 hours and kept at -20°C before toxicity analysis (Nguyen et al., 2017).

2.3. CYN determination by High-performance liquid chromatography (HPLC)

The biomass samples of the lyophilized strains were extracted in 2.5 mL of methanol (MeOH-99.9%) containing 0.1% trifluoroacetic acid (TFA) in an ultrasonic tank for 15 minutes. The samples were then sonicated on ice for 1 minute. The extracted fluid was filtered through chromatography column C_{18} which was cleaned with methanol. After filtration, the filtered fluid was evaporated at a low temperature (30°C) for 5 minutes. The remaining after evaporation was re-suspended in 250 µL ml of deionized twice distilled water and filtered by centrifugation (4,000 rpm for 30 min) before HPLC analysis (Meriluoto and Codd, 2005; Nguyen et al., 2007). The HPLC Thermo system consists of UltiMate 3000 autosampler and UltiMate 3000 variable wavelength detector (VWD); BDS Hypersil C18 column (250 × 4.6 mm, 5.0 µm), mobile phase: MeOH (A) 30% - water containing 10 mM of ammonium acetate (B) 70% (v/v), flow rate: 0.8 mL min^{-1}; sample volume: 10 µL; column chamber temperature: 30°C; analysis time: 7 minutes; the samples

were injected into the mobile phase in front of the column. In the column, the components were separated and the detector measured the absorbance of CYN at 262 nm wavelength. Cylindrospermopsin was determined by absorption spectra and retention times and quantified at 262 nm wavelength using the pure cylindrospermopsin standard (CRM-CYN, PESTANAL®, Sigma-Aldrich Pte. Ltd.) as an standard external substance.

2.4. Amplification of the genes involved in cylindrospermopsin production

2.4.1. DNA extraction

Exponentially growing cultures (10 mL of each) were centrifuged at 1500 rpm for 15 min at room temperature. The pellets were transferred to 1.5 mL Eppendorf tubes and frozen at -18°C until DNA extraction. Extraction of total genomic DNA was carried out according to the CTAB protocol of Doyle and Doyle (1987) with some modifications. The pellets were ground in preheated (65°C) 1mL 2× CTAB buffer and 10 µL β-mercaptoethanol and then incubated at 65°C for 1h. DNA was extracted with phenol - chloroform - isopenthylethanol (25:24:1) solution, then centrifuged at 13000 rpm at 4°C for 10 min to collect the supernatant. The supernatant was aspirated into a new Eppendorf tube, added isopropanol in a 1:1 ratio, and placed in a deep refrigerator for one hour. Then, centrifuge at 13,000 rpm at 4°C for 15 min to collect the precipitated DNA. The pellets were dissolved entirely by adding 1 volume of ethanol (70%). DNA was collected by centrifuging at 13,000 rpm at 4°C for 10 min, dried at room temperature, then re-suspended in 20 µL of double-distilled water at 37°C overnight.

2.4.2. Amplification of the genes

The *cyrB* and *cyrC* gene fragments involved in the cylindrospermopsin (CYN) biosynthesis were amplified by PCR using the oligonucleotide primer pairs M4/M5 and M13/M14 (Schembri et al., 2001). Thermal cycling conditions for PCR were 1 cycle at 94°C for 4 min, 30 cycles at 94°C for 10s, at 55°C for 20s, at 72°C for 1 min, and 1 cycle at 72°C for 7 min. DNA amplification reaction was carried out in the thermal cycler (iCyler, Bio-Rad). PCR products were examined by agarose gel electrophoresis on 1.4% at 50 V in 1× TAE buffer and the electrophoresis image analysis by a gel documentation system (Bio-Rad).

2.5. Data analysis

Principal component analysis (PCA) and Pearson correlation analysis were used to evaluate the relationship of environmental parameters (pH, DO, water temperature, turbidity, $N\text{-}NO_3$, $N\text{-}NH_4$, $P\text{-}PO_4$, TN and TP) on the biomass of *R. raciborskii* and the CYN concentration in the researched reservoir (SPSS statistics (version 22)). Analysis of variance (ANOVA) was used to test the significant differences at $p < 0.05$.

3. Results and discussions

3.1. Raphidiopsis raciborskii and its biovolume in the Buon Phong reservoir

The trichomes of the *R. raciborskii* were straight. Detailed morphological characteristics of this species have been described in the previous study (Ngo et al., 2022) (Fig. 2). In Vietnam, *R. racirskii* occurs in Xuan Huong Lake, Tri An reservoir and some water bodies in Hue (Dao et al., 2010; Nguyen et al., 2017). In the current study, *R. raciborskii* occurred throughout the year in the Buon Phong reservoir with its biovolume varying considerably with the seasons, low in the months of the rainy season and higher in the months of the dry season. The biovolume ranged from 0.12 to 9.14 $mm^3\ L^{-1}$ (Fig. 3) Blooms of *R. raciborskii* occurred in the dry months of the year when its biovolume ranged from 1.56 to 9.14 $mm^3\ L^{-1}$ and reached the maximum biovolume at the end of the dry

season, 9.14 mm^3 L^{-1}. Dense blooms of *R. raciborskii* were also observed in shallow ponds in northern Taiwan, when its biovolume reached the highest value of 102.5 mm^3 L^{-1} at the end of September 2009 (Yamamoto and Shiah, 2012, 2016). In a study on the influence of environmental factors on cyanobacterial blooms in 20 reservoirs in semi-arid regions in Brazil, Barros et al. observed that *R. raciborskii* prevailed and bloomed in 8 reservoirs with the highest biovolume above 10 mm^3 L^{-1} in the BO reservoir in Ceará state (Barros et al., 2019). CYN concentration in the Buon Phong reservoir (0.04-0.72 µg L^{-1}, Ngo et al., 2022) was equivalent to that in the Jordan Lake, North Carolina (0-0.83 µg L^{-1}, Wiltsie et al., 2018), the Macau reservoir, Macau (0-1.3 µg L^{-1}, Zhang et al., 2014) and in the Huong River, Vietnam (0-1.58 µg L^{-1}, Nguyen et al., 2017), but still higher than those of the Harris Chain Lake, Florida; the Nero Lake, Yaroslavl and the Tai-Hu reservoir, Taiwan with toxic contents were 0.05-0.2 µg L^{-1}; 0.12-0.36 µg L^{-1} and 0.14 µg L^{-1}, respectively (Williams et al., 2007; Babanazarova et al., 2015; Marbun et al., 2012). However, CYN concentration in our study was significantly lower than those of the Saudi Arabia Gazan Dam Lake (0.03-23.3 µg L^{-1}, Mohamed and Al-Shehri, 2013), the Saudi Arabia Gazan Lake (4-173 µg L^{-1}, Mohamed and Al-Shehri, 2013) and the water supply farm in central Queensland (1050 µg L^{-1}, Shaw et al., 2004). The CYN concentration in our reservoir was still within the permitted threshold for lifetime drinking water guideline values by the World Health Organization (WHO), 0.7 µg L^{-1} (Chorus et al., 2021). Indeed, the presence of the CYN in the reservoir shows the potential risks in the future water source when it is used for domestic purposes, livestock and aquaculture.

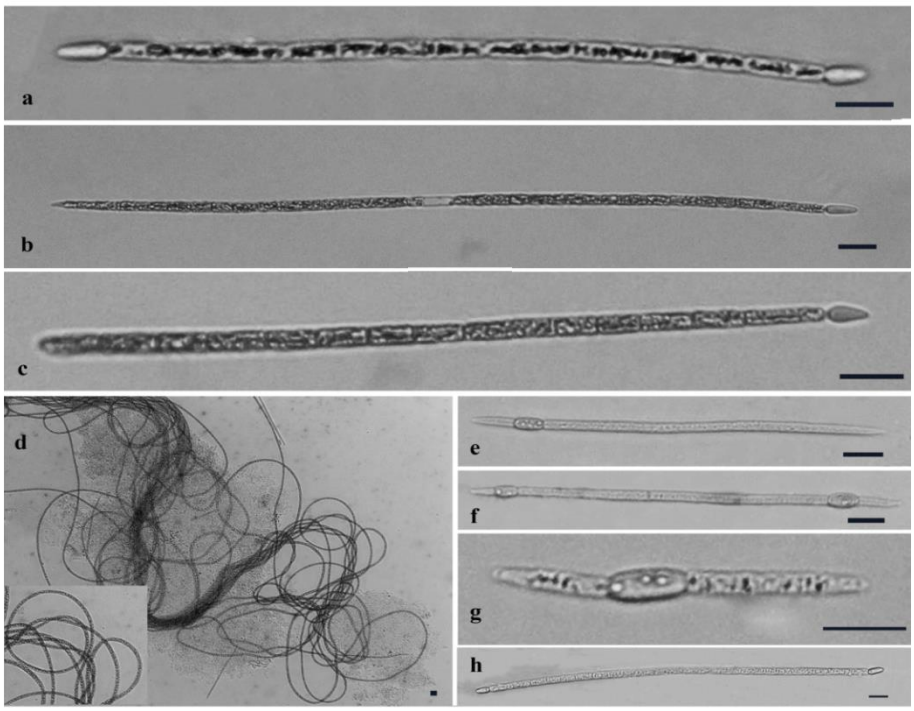

Figure 2. The morphology of *Raphidiopsis raciborskii* in the Buon Phong reservoir: a-c. Filaments with heterocytes in nature; d-h. Filament with heterocytes and akinetes in cultures. Scale bars = 10 µm

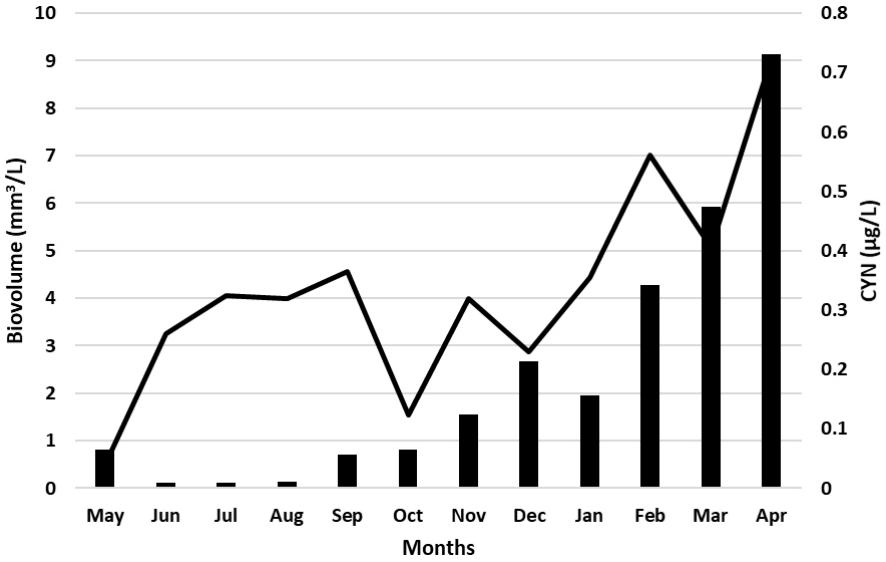

Figure 3. Seasonal variation of the *Raphidiopsis raciborskii* biovolume and CYN concentrations in the Buon Phong reservoir during the study period from May 2019 - April 2020

3.2. The ability to produce CYN toxin and genes involved in CYN production in cultured strains

In this study, four potential CYN-producing strains of *R. raciborskii* (CBP2, CBP3, CBP4 and CBP5) were successfully isolated from water samples taken from the Buon Phong reservoir. These isolated strains were cultured in Z8 medium and harvested in the exponential growth stage. The results from HPLC analysis of biomass extracts of the four strains showed that all of them produced CYN and toxic concentrations varied from 0.016 to 0.345 µg g^{-1} DW (Table 1). The highest concentration was 0.345 µg g^{-1} DW, which was much lower than those of *R. raciborskii* cyDB-1 strain in America (0.85 mg g^{-1}, Jiang 2014; Jiang et al., 2014), *R. raciborskii* CHAB3438 strain in China (2.6 mg g^{-1}, Jiang 2014; Jiang et al., 2014) and *R. raciborskii* QHSS/NR/Cyl/03 strain in Australia (6.73 mg g^{-1} (LC/MS), Yilmaz et al., 2008). On the other hand, previous studies have noted that some *R. raciborskii* isolated strains in Europe and Africa did not produce CYN toxin (Berger et al., 2006; Piccini et al., 2013; Rzymsk et al., 2018; Falfushynska et al., 2018; Stefanova et al., 2020). Willis et al. (2016) demonstrated significant differences in each individual when studying the population variation and toxicity of *R. raciborskii* in Wivenhoe Lake, Australia. All strains isolated in the small lake also showed differences in growth rate, toxin content and trichome morphology (Willis et al., 2016). A recent study also showed that strain variability could be just as significant as species differences in determining the growth response to light and temperature (Xiao et al., 2017). *R. raciborskii* exists in many forms (straight, curved, coiled) natural environments. Strains in the Buon Phong reservoir all exist in straight form, with the average length of trichomes in the culture medium (from 97 µm to several centimeters) more extended than in the wild (100-250 µm) and will sometimes form into tufts. Although all strains could produce CYN, toxin content in strains was different. The difference in toxin content in the strains is probably due to the number, sequence, and organization of genes in the CYN toxin gene cluster between different strains. This could lead to a change in toxicity in these strains.

Table 1. Cylindrospermopsin (CYN) concentrations in cultured *Raphidiopsis raciborskii* isolated strains from the Buon Phong reservoir

Species	Strain codes	CYN concentrations μg g^{-1} DW
Raphidiopsis raciborskii	CBP2	0.029
	CBP3	0.016
	CBP4	0.345
	CBP5	0.019

Previous studies suggested that CYN producing cyanobacterial species must have homologs for *cyrA, cyrB*, and *cyrC* to produce the toxin (Fergusson and Saint, 2003; Rasmussen et al. 2008; Lorenzi et al. 2015). The first three steps in CYN biosynthesis involve the *cyrA, cyrB* and *cyrC* genes. *CyrB* recognizes guanidinoacetate and catalyzes the formation of the first N-containing heterocycle. *CyrC* further catalyzes elongation of the polyketide chain, resulting in a tricyclic structure. *cyrB (PKS)* and *cyrC (PS*) genes as markers to detect and control toxin-producing *R. raciborskii* strains in water sources quickly and accurately (Saint et al., 2007; Wiedner et al., 2007; Nguyen et al., 2017). Our PCR results showed that two (CBP2, CBP3) out of four toxic strains were amplified both *cyrB* and *cyrC* gene fragments. While the *cyrB* and *cyrC* gene fragments were not observed in the remaining two toxic strains (CBP4, CBP5). The presence of both these gene fragments was also observed in 8 toxic *R. raciborskii* strains in water bodies in Hue (Nguyen et al., 2017), 12 toxic *R. raciborskii* strains in aquatic bodies in Thailand (Tawong et al., 2019) or a toxic strain (*R. raciborskii* QHSS/NR/Cyl/03) in Australia (Yilmaz et al., 2008). The absence of toxin synthesis genes in the toxic strains was also detected in the study of Tawong et al. (2019). The study results showed a lack of PCR products of the *cyrA* gene in seven toxic *R. raciborskii* strains and *cyrC* gene in one toxic *R. raciborskii* strain in Thailand. The absence of the *cyrB* genes in the toxic strain *R. raciborskii* Boczowskie also presented in some lakes in Poland (Tawong et al., 2019). We think that the absence of *cyrB, cyrC* gene fragments in toxic strains was probably due mismatches in the primer target sites (genetic variations that occur on the CYN synthesis genes to each strain). Besides, the *cyr* gene cluster consists of 15 genes from *cyrA* to *cyrO*, each gene performs a specific function in the process of the biosynthesis of CYN. In which *cyrA* gene participates in the initiation of toxin synthesis and *cyrJ* gene catalyzes the sulfation process and completes CYN structure. In many studies, *cyrJ* has been considered a suitable genetic marker in identifying CYN-producing cyanobacteria (Hoff-Risseti et al. 2013; Kokociński et al. 2012; Lorenzi et al., 2013; Tawong et al., 2019). Therefore, we suggest that further determination of *cyrA, cyrJ* gene fragments in toxic strains will have more accurate results.

3.3. Environmental factors affecting biovolume of Raphidiopsis raciborskii and CYN concentration in the Buon Phong reservoir

3.3.1. Physical and chemical characteristics

The results of environmental characteristics from May 2019 to April 2020 of the Buon Phong reservoir are shown in Table 2. 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 higher values of DO were observed in the dry season and its values varied between a minimum of 3.8 to a maximum of 6.6 mg L^{-1}. 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-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). This was also the cases of the Nui Coc reservoir and the Suoi Hai Lake where the TN and TP concentrations were characteristic of eutrophic conditions (Duong et al., 2013; Nguyen et al., 2021).

Table 2. Environmental variables in the Buon Phong reservoir during the year 2019-2020 (average values and min-max values)

Month	Temp. (^{O}C)	DO (mg L^{-1})	Turbidity (NTU)	pH	N-NH_4 (mg L^{-1})	N-NO_3 (mg L^{-1})	TN (mg L^{-1})	P-PO_4 (mg L^{-1})	TP (mg L^{-1})
May-19	**26.87** *(26.7 - 27.1)*	**3.76** *(3.72 - 3.8)*	**23.59** *(23.47 - 23.78)*	**7.57** *(7.52 - 7.61)*	**0.23** *(0.229 - 0.232)*	**0.1** *(0.099 - 0.107)*	**2.34** *(2.31 - 2.38)*	**0.08** *(0.079 - 0.088)*	**0.22** *(0.21 - 0.226)*
Jun-19	**26.83** *(26.5 - 27)*	**4.67** *(4.6 - 4.74)*	**21.37** *(21.22 - 21.5)*	**6.71** *(6.65 - 6.77)*	**0.19** *(0.182 - 0.193)*	**0.09** *(0.088 - 0.091)*	**2** *(1.98 - 2.03)*	**0.07** *(0.064 - 0.068)*	**0.19** *(0.174 - 0.189)*
Jul-19	**25.83** *(25.5 - 26)*	**4.6** *(4.54 - 4.64)*	**20.87** *(19.3 - 22.8)*	**7.62** *(7.52 - 7.7)*	**0.11** *(0.11 - 0.12)*	**0.24** *(0.22 - 0.26)*	**1.47** *(1.4 - 1.56)*	**0.05** *(0.045 - 0.053)*	**0.17** *(0.163 - 0.182)*
Aug-19	**27.83** *(27.5 - 28)*	**4.81** *(4.72 - 4.9)*	**22.80** *(21 - 24)*	**7.63** *(7.5 - 7.7)*	**0.1** *(0.084 - 0.112)*	**0.14** *(0.141 - 0.145)*	**1.40** *(1.29 - 1.52)*	**0.06** *(0.056 - 0.065)*	**0.13** *(0.124 - 0.137)*
Sep-19	**27.33** *(27 - 27.5)*	**4.57** *(4.44 - 4.76)*	**33** *(28.1 - 42.3)*	**7.43** *(7.3 - 7.58)*	**0.14** *(0.126 - 0.14)*	**0.27** *(0.238 - 0.294)*	**1.12** *(1.17 - 1.19)*	**0.06** *(0.056 - 0.06)*	**0.31** *(0.3 - 0.327)*
Oct-19	**26.67** *(26.5 - 27)*	**5.25** *(5.06 - 5.38)*	**32.7** *(28 - 37.5)*	**6.75** *(6.71 - 6.8)*	**0.14** *(0.137 - 0.146)*	**0.21** *(0.207 - 0.216)*	**1.33** *(1.32 - 1.33)*	**0.08** *(0.078 - 0.079)*	**0.13** *(0.128 - 0.132)*
Nov-19	**27.83** *(27.5 - 28)*	**5.73** *(5.66 - 5.8)*	**20.5** *(17 - 24.4)*	**6.81** *(6.79 - 6.83)*	**0.1** *(0.095 - 0.106)*	**0.19** *(0.187 - 0.193)*	**1.33** *(1.26 - 1.4)*	**0.09** *(0.09 - 0.094)*	**0.13** *(0.123 - 0.127)*
Dec-19	**28.83** *(28.5 - 29)*	**5.21** *(5.18 - 5.26)*	**15.07** *(12.6 - 17.5)*	**7.06** *(7.01 - 7.1)*	**0.14** *(0.135 - 0.148)*	**0.12** *(0.117 - 0.12)*	**1.23** *(1.18 - 1.29)*	**0.09** *(0.086 - 0.093)*	**0.12** *(0.098 - 0.138)*
Jan-20	**32.17** *(32.1 - 32.2)*	**5.63** *(5.52 - 5.72)*	**23.24** *(22.91 - 23.7)*	**6.97** *(6.92 - 7.01)*	**0.13** *(0.12 - 0.135)*	**0.17** *(0.168 - 0.175)*	**1.32** *(1.27 - 1.38)*	**0.07** *(0.061 - 0.073)*	**0.13** *(0.12 - 0.134)*
Feb-20	**30.9** *(30.8 - 31)*	**6.62** *(6.54 - 6.7)*	**21.36** *(20.93 - 22.01)*	**6.66** *(6.61 - 6.7)*	**0.14** *(0.137 - 0.141)*	**0.17** *(0.158 - 0.177)*	**1.35** *(1.23 - 1.42)*	**0.06** *(0.052 - 0.064)*	**0.09** *(0.087 - 0.096)*
Mar-20	**31.37** *(31.2 - 31.5)*	**6.13** *(6.08 - 6.18)*	**23.49** *(23.38 - 23.7)*	**7.70** *(7.63 - 7.78)*	**0.13** *(0.126 - 0.134)*	**0.11** *(0.103 - 0.117)*	**1.05** *(1.03 - 1.09)*	**0.08** *(0.072 - 0.091)*	**0.14** *(0.128 - 0.149)*
Apr-20	**27.93** *(27.3 - 28.3)*	**5.6** *(5.42 - 5.89)*	**22.8** *(22.29 - 23.1)*	**7.46** *(7.4 - 7.5)*	**0.26** *(0.237 - 0.282)*	**0.1** *(0.098 - 0.105)*	**2.56** *(2.37 - 2.8)*	**0.09** *(0.081 - 0.089)*	**0.25** *(0.231 - 0.264)*

3.3.2. Influence of environmental factors on the biovolume of R. raciborskii

To determine the influence of environmental factors on *R. raciborskii* biovolume during the research period from May 2019 to April 2020 at the 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 the CYN concentration (R=0.54, p<0.01). Abiotic variables such as temperature, DO, N-NH_4, P-PO_4 were also correlated with the *R. raciborskii* biovolume in the Buon Phong reservoir (R=0.45, p<0.01; R=0.58, p<0.01; R=0.46, p<0.01; R=0.35, p<0.05, respectively) (Fig. 4, Table 3). *R. raciborskii* is a common bloom species in natural lakes, reservoirs and rivers from tropical, subtropical to temperate regions (Padisák et al., 1997; Burford et al., 2016, 2018; Fu et al., 2019). Numerous studies have suggested that the abundance of *R. raciborskii* could be influenced by environmental factors such as light, temperature and nutrients (Antunes et al., 2015; Burforf et al., 2016; Pagni et al., 2020). The correlation between temperature and *R. raciborskii* in the Buon Phong reservoir showed that high temperature stimulated the growth of *R. raciborskii.* A significant correlation between *R. raciborskii* and water temperature was also observed in lakes in different climate zones and *R. raciborskii* biomass reached its highest value during periods of the warmest water

temperatures (e.g., Briand et al., 2004; Mehnert et al., 2010). Similar results were observed when researching the population variation in lakes from tropical, subtropical to temperate regions indicating that *R. raciborskii* was strongly associated with temperature and preferred high temperature for its growth, but it also tolerated a wide range of climates (Bonilla et al., 2012; Recknagel et al., 2019). In this study, although *R. raciborskii* was present all year round, blooms occurred only in the months of the dry season (1.56-9.14 mm^3 L^{-1}) when the temperature was high, ranging from 27.83-32.17°C. Indeed, field and laboratory studies have indicated that the preferred temperature for blooming was above 25°C (Mehnert et al., 2010; Kokocinski et al., 2017). However, blooms of this species were also found in lakes with low temperatures (Everson et al., 2011; Bonilla et al., 2012; Soares et al., 2013; Jia et al., 2021). Even, their blooms have been observed in winter in lakes and dams in Northern Taiwan, Lago Javier, Uruguay and the Rio Grande do Sul when the temperatures were at 16.3°C, 11.2°C and 11°C, respectively (Fabre et al., 2010; Yamamoto et al., 2012; Wener et al., 2020). Wener et al. (2020) found that the blooms of *R. raciborskii* formed yellow streaks on the surface at temperatures between 12.6-15.5°C, still its biomass reached its maximum value in late summer when the temperature was high, at 26.6°C. The tolerance to low temperatures may also be important in creating favorable conditions for this species to prevail in winter.

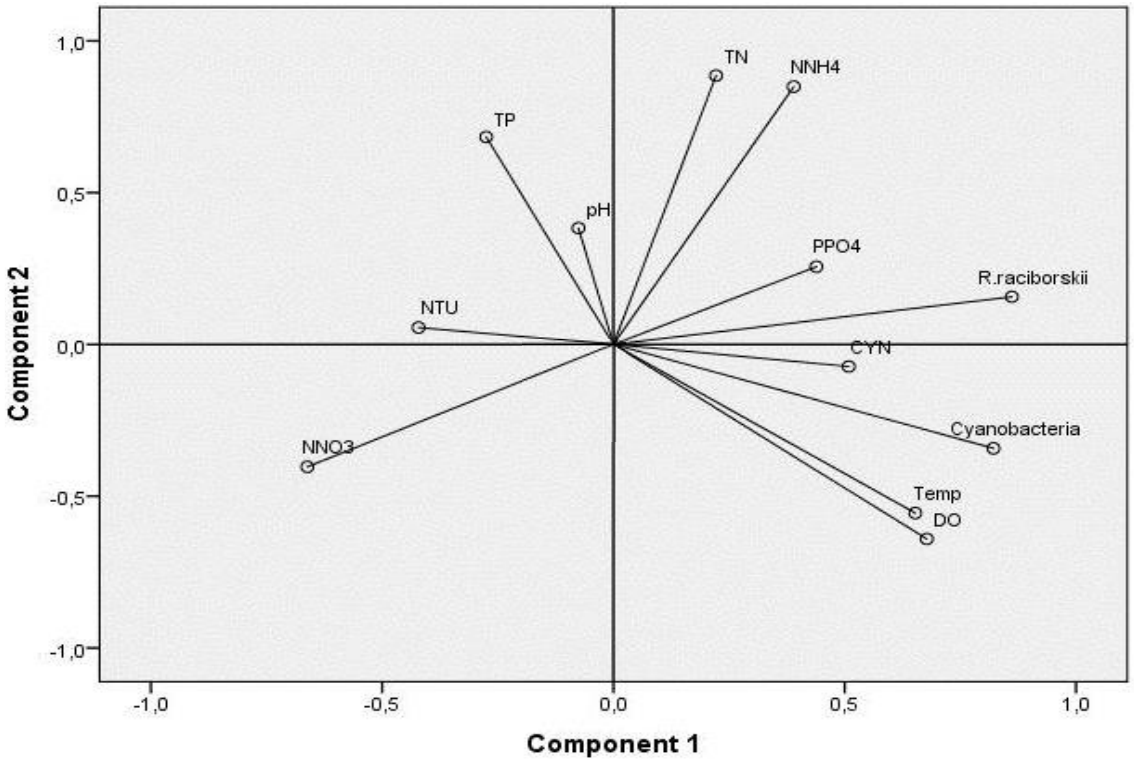

Figure 4. Principal component analysis (PCA) based on biotic and abiotic factors during the period of May 2019-April 2020 in the Buon Phong reservoir

Table 3. Pearson correlation between relative abundance of species *R. raciborskii* and environmental factors in Buon Phong reservoir from May 2019 to April 2020

	Temp	DO	Turbidity	pH	N-NH_4	N-NO_3	P-PO_4	TN	TP	*R. raciborskii*	CYN	Total Cyanobacteria
Temp	1											
DO	***0.717*****	1										
Turbidity	-0.176	-0.173	1									
pH	-0.135	***-0.386****	0.051	1								
N-NH_4	-0.188	-0.237	0.003	0.112	1							
N-NO_3	-0.227	-0.053	***0.475*****	-0.049	***-0.546*****	1						
P-PO_4	0.066	0.108	-0.193	-0.059	0.313	***-0.482*****	1					
TN	***-0.378****	***-0.389****	-0.102	0.129	***0.877*****	***-0.508*****	0.190	1				
TP	***-0.447*****	***-0.549*****	***0.439*****	***0.434*****	***0.497*****	0.186	-0.093	***0.429*****	1			
R. raciborskii	***0.445*****	***0.580*****	-0.138	0.136	***0.462*****	***-0.412****	***0.347****	0.286	0.043	1		
CYN	0.300	***0.476*****	-0.154	0.038	0.115	-0.047	-0.140	0.081	0.056	***0.538*****	1	
Total Cyanobacteria	***0.795*****	***0.765*****	-0.195	-0.056	0.057	***-0.340****	0.176	-0.175	-0.308	***0.696*****	***0.377****	1
**. Correlation is significant at the 0.01 level (2-tailed).												
*. Correlation is significant at the 0.05 level (2-tailed).												

In addition to temperature, the abundance of these species is also correlated with other environmental factors such as nutrients (nitrogen (N) and phosphorus (P)) (Mohamed et al., 2018). Abiotic variables affecting the biovolume of *R. raciborskii* in this study were dissolved nitrogen and phosphorus (N-NH_4, P-PO_4). The positive correlation between *R. raciborskii* and N-NH_4 and P-PO_4 in our study was also observed in previous studies (Kokocinski et al., 2012; Nguyen et al., 2017; Burford et al., 2016, 2018; Werner et al., 2020). These studies suggested that N-NH_4 and P-PO_4 were considered as the preferred source of N, and P to stimulate the growth of the *R. raciborskii* populations. The biomass of this species is higher in lakes with high N-NH_4, and P-PO_4 concentrations (Kokocinski et al., 2012; Nguyen et al., 2017; Burford et al., 2016, 2018; Werner et al., 2020). On the contrary, a number of studies noted that *R. raciborskii* can still prevail even under the low N and P conditions (Burford et al., 2018 Recknagel et al., 2019; Galvanese et al., 2019; Xiao et al., 2020; Werner et al., 2020; Li et al., 2020). This is partly explained by the high storage and the absorption capacity for N-NH_4, and P-PO_4 of *R. raciborskii* (Burford et al., 2006; Kenesi et al., 2009; Kokoscinski et al., 2012). The intraspecific variation occurs in the *R. raciborskii* strains within a population or among the populations in geographical areas that differ in N, P uptake and storage capacity and dissolved organic phosphorus (DOP), dissolved organic nitrogen (DON) and utilization capacity have been shown in recent studies (Bai et al., 2014; Bolius et al., 2017; Willis et al., 2017; Burford et al., 2020). Studies on isolated strains of *R. raciborskii* in Australia showed higher P uptake rates than other continents (Willis et al., 2017). The biovolume of *R. raciborskii* in the Buon Phong reservoir was negatively correlated with N-NO_3 concentration ($R=-0.41$, $p<0.05$). This observation has also been reported in the studies in Dongquan city, Egypt and western Poland) where shows that the strong growth of *R. raciborskii* can still be detected in reservoirs with low N-NO_3 concentrations (Briand et al., 2002; Mohamed et al., 2007; 2013; Kokociński and Soininen, 2012; Lei et al., 2014). Indeed, studies have indicated that *R. raciborskii* can utilize nitrogen sources, including nitrogen-soluble inorganic form (N-NH_4, N-NO_3) and organic form (urea) with a clear preference for N-NH_4 based on both growth rate (Amaral et al., 2014) and absorption rate (Burford et al., 2016). Moreover, the Pearson analysis showed that the biovolume of *R. raciborskii* was positively correlated with the CYN concentration, and the toxic concentration increased when *R. raciborskii* biomass increased. We identified CYN toxin in the isolated strains of *R. raciborskii* in the Buon Phong reservoir using the HPLC method,

which further confirmed the correlation between biovolume and the CYN concentration of *R. raciborskii* in the reservoir. From that, the potential hazard of the water sources with the presence of the toxic *R. raciborskii* cyanobacterium and CYN toxic should be noticed.

4. Conclusions

R. raciborskii (straight) occurred throughout the year in the reservoir, but bloomed only in the dry and warmer months. Four strains of *R. raciborskii* that were able to produce CYN have been successfully isolated. Among them, CBP2 and CBP3 strains were amplified by both *cyrB* and *cyrC* gene fragments. Abiotic factors such as temperature and nutrients play a key role in the presence and predominance of *R. raciborskii* species in the reservoir. In addition, the biovolume of *R. raciborskii* was positively correlated with the CYN concentration indicating that the cause of CYN contamination in this waterbody was the presence of *R. raciborskii*. Furthermore, the presence of *Microcystis* spp. and *Anabaena* sp. in the reservoir indicates that the development of *R. raciborskii* was regulated by environmental factors and can be completed by the cyanobacterial species in the water body.

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References

Amaral V., Bonilla S., Aubriot L., 2014. Growth optimization of the invasive cyanobacterium Cylindrospermopsis raciborskii in response to phosphate fluctuations. Eur J. Phycol., 49, 134-41.

Antunes J.T., Leão P.N., Vasconcelos V.M., 2015. Cylindrospermopsis raciborskii: review of the distribution, phylogeography, and ecophysiology of a global invasive species. Front. Microbiol, 6, 1-13.

Babanazarova O.V., Sidelev S.I., Fastner J., 2015. Northern expansion of Cylindrospermopsis raciborskii (Nostocales, Cyanoprokaryota) observed in shallow highly eutrophic lake Nero (Russia). Int. J. Algae, 17, 131-141.

Bai F., Liu R., Yang Y., 2014. Dissolved organic phosphorus use by the invasive freshwater diazotroph cyanobacterium, Cylindrospermopsis raciborskii. Harmful Algae, 39, 112-20.

Barros M.U., Wilson A.E., Leitão J.I., Pereira S.P., Buley R.P., Fernandez-Figueroa, E.G., Capelo-Neto, J., 2019. Environmental factors associated with toxic cyano-bacterial blooms across 20 drinking water reservoirs in a semi-arid region of Brazil. Harmful Algae, 86, 128-137.

Berger C.B.N., Muriel G., Marc B., Filippo R., Alain C., Marc T., Ce´cile B., 2006. Seaso-nal dynamics and toxicity of Cylindrospermopsis raciborskii in Lake Guiers (Senegal, West Africa). FEMS Microbiol Ecol, 57, 355-366.

Bernard C., Harvey M., Briand J.F., Biré R., Krys S., Fontaine J.J., 2003. Toxicological comparison of diverse Cylindrospermopsis raciborskii strains: evidence of liver damage caused by a French C. raciborskii strain. Environ. Toxicol, 18, 176-186.

Bolius S., Wiedner C., Weithoff G., 2017. High local trait variability in a globally invasive cyanobacterium. Freshwater Biology, 62, 1879-1890.

Bonilla S., Aubriot L., Soares M.C.S., González-Piana M., Fabre A., Huszar V.L.M., Lürling M., Antoniades D., Padisák J., Kruk C., 2012. What drives the distribution of the bloom-forming cyanobacteria Planktothrix agardhii and Cylindrospermopsis raciborskii? FEMS Microbiology Ecology, 79, 594-607.

Briand J.F., Leboulanger C., Humbert J.F., Bernard C., Dufour P., 2004. Cylindrospermopsis raciborskii (cyanobacteria) invasion at mid-latitudes: selection, wide physiological tolerance, or global warming. J. Phycol, 40, 231-238.

Burford M.A., Beardall J., Willis A., Orr P.T., Magalhaes V.F., Rangel L.M., Neilan B., 2016. Understanding the winning strategies used by the bloom-forming cyanobacterium Cylindrospermopsis raciborskii. Harmful Algae, 54, 44-53.

Burford M.A., Carey C.C., Hamilton D.P., Huisman J., Paerl H.W., Wood S.A., Wulff A., 2020. Perspective: Advancing the research agenda for improving under-standing of cyanobacteria in a future of global change. Harmful Algae, 91, 101601.

Burford M.A., McNeale K.L., McKenzie-Smith F.J., 2006. The role of nitrogen in promoting the toxic cyanophyte Cylindrospermopsis raciborskii in a subtropical water reservoir. Freshwater Biol., 51, 2143-53.

Burford M.A., Willis A., Chuang A., Man X., Orr P., 2018. Recent insights into physiological responses to nutrients by the cylindrospermopsin producing cyanobacterium Cylindrospermopsis raciborskii. Chinese Journal of Oceanology and Limnology, 36, 1032-1039.

Chorus I., eds., 2021. Toxic Cyanobacteria in Water, 2nd edition. CRC Press, Boca Raton (FL), on behalf of the World Health Organization, Geneva. CH, 859p.

Dao T.S., Cronberg G., Nimptsch J., Do-Hong L.C., Wiegand C., 2010. Toxic cyano-bacteria from Tri An Reservoir, Vietnam. Nova Hedwigia., 90, 433-448.

Doyle J.J, Doyle J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin, 19, 11-15.

Duong D.T., 1996. Taxonomy of Cyanobacteria of Vietnam. Hanoi: Agriculture Publishing House (in Vietnamese), 189p.

Duong T.T., Le T.P.Q., Dao T.S., Pflugmacher T., Rochelle-Newall E., Hoang T.K., Vu T.N., Ho TC., Dang D.K., 2013. Seasonal variation of cyanobacteria and microcystins in the Nui Coc Reservoir, Northern Vietnam. Journal of Applied Phycology, 25, 1065-1075.

Everson S., Fabbro L., Kinnear S., Wright P., 2011. Extreme differences in akinete, heterocyte and cylindrospermopsin concentrations with depth in a successive bloom involving Aphanizomenon ovalisporum (Forti) and Cylindrospermopsis raciborskii (Woloszynska) Seenaya and Subba Raju. Harmful Algae, 10, 265-276.

Fabre A., Carballo C., Hernández E., Piriz P., Bergamino L., Mello L., González S., Pérez G., León J.G., Aubriot L., Bonilla S., Kruk C., 2010. El nitrógeno y la relación zona eufótica/zona de mezcla explican la presencia de cianobacterias en pequeños lagos subtropicales, artificiales de Uruguay. PanAmerican Journal of Aquatic Sciences, 5, 112-125.

Falfushynska H., Horyn O., Brygider A., Fedoruk O., Buyak B., Poznansky D., Poniedziałek B., Kokociński M., Rzymski P., 2018. Is the presence of Central European strains of Raphidiopsis (Cylindrospermopsis) raciborskii a threat to a freshwater fish? An in vitro toxicological study in common carp cells. Aquatic Toxicology, 30901-9.

Fergusson K.M., Saint C.P., 2003. Multiplex PCR assay for Cylindrospermopsis raciborskii and cylindrospermopsin-producing cyanobacteria. Environ Toxicol, 18, 120-125.

Fu L.Q., Yeung C.Y., Fujii M., Neilan B.A., T. David, Waite T.D., 2019. Physiological responses of the freshwater N2-fixing cyanobacterium Raphidiopsis raciborskii to Fe and N availabilities. Environmental Microbiology, 21, 1211-1223.

Galvanese L.F., André A.P., Luis A., 2019. Acclimation at high temperatures increases the ability of Raphidiopsis raciborskii (Cyanobacteria) to withstand phosphate deficiency and reveals distinct strain responses. Journal of Phycology, 54, 359-368.

Hoff-Risseti C., Dörr F.A., Schaker P.D., Pinto E., Werner V.R., Fiore M.F., 2013. Cylindrospermopsin and saxitoxin synthetase genes in Cylindrospermopsis raciborskii strains from Brazilian freshwater. PLoS One, 8, e74238.

Jiang Y., Xiao P., Yu G., Shao J., Liu D., Azevedo S.M.F.O., Li R., 2014. Sporadic distribution and distinctive variations of cylindrospermopsin genes in cyano-bacterial strains and environmental samples

from Chinese freshwater bodies. Appl. Environ. Microbiol., 80, 5219-5230.

Karlson B., Cusack C., Bresnan E., 2010. Microscopic and molecular methods for quantitative phytoplankton analysis (IOC Manuals and Guides, no. 55) (IOC/2010/MG/55). UNESCO, Paris.

Kenesi G., Shafik H.M., Kovács A.W., Herodek S., Présing M., 2009. Effect of nitrogen forms on growth, cell composition and N2 fixation of Cylindrospermopsis raciborskii in phosphorus-limited chemostat cultures. Hydrobiologica, 623, 191-202.

Kokocinski M., Jasser I., Karosiene J., Kasperoviciene J., Kobos J., Koreiviene J., Soi-ninen J., Szczurowska A., Woszczyk M., 2017. Distribution of invasive Cylindrospermopsis raciborskii in the East-Central Europe is driven by climatic and local environmental variables. FEMS Microbiol. Ecol., 93, 127-137.

Kokocinski M., Soininen J., 2012. Environmental factors related to the occurrence of Cylindrospermopsis raciborskii (Nostocales, Cyanophyta) at the north-eastern limit of its geographical range. Eur. J. Phycol., 47, 12-21.

Komárek J., Anagnostidis K., 1989. Modern approach to the classification system of cyano-phytes 4-Nostocales. Archiv für Hydrobiologie/Supplements, 56, 247-345.

Komárková J., Laudares-Silva R., Senna P.A.C., 1999. Extreme morphology of Cylindro-spermopsis raciborskii (Nostocales, Cyanobacteria) in the Lagoa do Peri, a freshwater coastal lagoon, Santa Catarina, Brazil. Algol. Stud., 94, 207-222.

Kotai J., 1972. Instructions for preparation of modified nutrient solution Z8 for algae Publication B-11/69. Norwegian Institute for Water Research, Blindern. Oslo 3 Norway.

Le Thuong, 2010. Variation in species composition and number of phytoplankton in EaNhai and EaSup reservoirs, Dak Lak province. PhD thesis in Biology. Vietnam Institute of Science and Technology, Institute of Oceanography, 147p.

Lei L., Peng L., Xianghui H., Bo-Ping H., 2014. Occurrence and dominance of Cylindrospermopsis raciborskii and dissolved cylindrospermopsin in urban reservoirs used for drinking water supply, South China. Environ Monit Assess., 186, 3079-3090.

Li X., Shouliang H., Jingtian Z., Xiao Z., Beidou X., Renhui L., 2020. Factors related to aggravated Cylindrospermopsis (cyanobacteria) bloom following sediment dredging in an eutrophic shallow lake. Environmental Science and Ecotechnology, 2, 100014.

Lorenzi A.C., Chia M.A., Piccin-Santos V., 2015. Microcystins and cylindrospermopsins molecular markers for the detection of toxic cyanobacteria: a case study of northeastern Brazilian reservoirs. Limnetica, 34, 269-282.

Marbun Y.R., Yen H.K., Lin T.F., Lin H.L., Michinaka A., 2012. Rapid on-site monitoring of cylindrospermopsin-producers in reservoirs using quantitative PCR. Sustain. Environ. Res., 22, 143-151.

Mehnert G., Leunert F., Cires D., 2010. Competitiveness of invasive and native cyanobacteria from temperate freshwaters under various light and temperature conditions. J. Plankton Res., 32, 1009-21.

Meriluoto J., Codd G.A., 2005. Toxic: Cyanobacterial Monitoring and Cyanotoxin Analysis. Åbo Akademi University Press, Åbo, Finland.

Mohamed Z.A., 2007. First report of toxic Cylindrospermopsis raciborskii and Raphidiopsis mediterranea (Cyanoprokariota) in Egyptian fresh waters. FEMS Microbiol. Ecol., 59, 749-761.

Mohamed Z.A., Al-Shehri A.M., 2013. Assessment of cylindrospermopsin toxin in an arid Saudi lake containing dense cyanobacterial bloom. Environ. Monit. Assess., 185, 2157-2166.

Mohamed Z.A., Bakr A., 2018. Concentrations of cylindrospermopsin toxin in water and tilapia fish of tropical fishponds in Egypt, and assessing their potential risk to human health. Environ. Sci. Pollut. Res., 25, 36287-36297.

Ngo T.D.M., Ton T.P., Nguyen T.T.L., 2022. Bloom of harmful cyanobacterium Raphidiopsis raciborskii in Buon Phong reservoir, Dak Lak province. Journal of Science: Natural Science: Hue University, 131, 28-35.

Nguyen T.L., Nguyen T.T.H., Nguyen T.P.T., Pham Q.V., 2021. Assessing trophic status of Suoi Hai

Reservoir using Carlson's Trophic State Index. Vietnam Journal of Earth Sciences, 43(4), 509-523. https://doi.org/10.15625/2615-9783/16562.

Nguyen T.T.L., Cronberg G., Larsen J., Moestrup Ø., 2007. Planktic cyanobacteria from freshwater localities in Thua Thien-Hue province, Vietnam. I. Morphology and distribution. Nova Hedwigia, 85, 1-34.

Nguyen T.T.L., Hoang T.H., Nguyen T.K., Duong T.T., 2017. The occurrence of toxic cyanobacterium Cylindrospermopsis raciborskii and its toxin cylindrospermopsin in the Huong River, Thua Thien Hue province, Vietnam. Environ Monit Assess., 189, 490p.

OECD, 1982. Eutrophication of Waters monitoring, Assessment and Control. Organization for Economic Cooperation and Development, Paris.

Padisák J., 1997. Cylindrospermopsis raciborskii (Woloszynska) Seenayya et Subba Raju, an expanding, highly adaptive cyanobacterium: Worldwide distribution and review of its ecology. Archiv Für Hydrobiologie. Supplementband. Monographic Studies, 107, 563-593.

Pagni R.L., Falco P.B., André C.A.S., 2020. Autecology of Cylindrospermopsis raciborskii (Woloszynska) Seenayya et Subba Raju. Acta Limnologica Brasiliensia, 32, e24.

Pham Q.V., Nguyen T.T.H., Nguyen T.B., Nguyen N.T., La T.O., Nguyen T.P.T., 2019. Developing algorithm for estimating chlorophyll-a concentration in the Thac Ba Reservoir surface water using Landsat 8 Imagery. Vietnam Journal of Earth Sciences, 41(1), 10-20. https://doi.org/10.15625/0866-7187/41/1/13542.

Piccini C., Aubriot L., D'Alessandro B., Martigani F., Bonilla S., 2013. Revealing toxin signatures in cyanobacteria: report of genes involved in cylindrospermopsin synthesis from saxitoxin-producing Cylindrospermopsis raciborskii. Adv. Microbiol, 03, 289-296.

Puerto M., Prieto A.I., Maisanaba S., Gutiérrez-Praena D., Mellado-García P., Jos Á., Cameán A.M., 2018. Mutagenic and genotoxic potential of pure Cylindrospermopsin by a battery of in vitro tests. Food Chem. Toxicol, 121, 413-422.

Rasmussen J.P., Giglio S., Monis P.T., Campbell R.J., Saint C.P., 2008. Development and field testing of a real-time PCR assay for cylindrospermopsin-producing cyanobacteria. J. Appl. Microbiol, 104, 1503-1515.

Recknagel F., Zohary T., Rücker J., Orr P.T., Castelo C., Nixdorf B., Max M.M., 2019. Causal relationships of Raphidiopsis (formerly Cylindrospermopsis) dynamics with water temperature and N:P-ratios: A meta-analysis across lakes with different climates based on inferential modelling. Harmful Algae, 84, 222-232.

Rzymski P., Horyn O., Budzyńska A., Jurczak T., Kokociński M., Niedzielski P., Klimaszyk P., Falfushynska H., 2018. A report of Cylindrospermopsis raciborskii and other cyanobacteria in the water reservoirs of power plants in Ukraine. Environ. Sci. Pollut Res., 25, 15245-15252.

Shaw G.R., McKenzie R.A., Wickramasinghe W.A., Seawright A.A., Eaglesham G.K., Moore M.R., 2004. Comparative toxicity of the cyanobacterial toxin cylindrospermopsin between mice and cattle: human implications. Harmful Algae, 465-467.

Soares M.C.S., Lürling M., Huszar V.L.M., 2013. Growth and temperature-related phenotypic plasticity in the cyanobacterium Cylindrospermopsis raciborskii. Phycological Research, 61, 61-67.

Stefanova Katerina., Mariana R., Blagoy U., Georg G., Maya S., 2020. Pilot search for cylindrospermopsin producers in nine shallow Bulgarian waterbodies reveals nontoxic strains of Raphidiopsis raciborskii, Raphidiopsis mediterranea and Chrysosporum bergii. Biotechnology & biotechnological equipment, 34, 384-394.

Tawong W., Pongcharoen P., Nishimura T., Adachi M., 2019. Molecular characterizations of Thai Raphidiopsis raciborskii (Nostocales, Cyanobacteria) based on 16S rDNA, rbcLX, and cylindrospermopsin synthetase genes. Plankton Benthos Res., 14, 211-223.

Werner V.R., Andréa T., Lisangela M.S., João S.Y., Emanuel B.N., David E.B., Dail H.L., 2020. Morphological, ecological and toxicological aspects of Raphidiopsis raciborskii (Cyanobacteria) in a eutrophic urban subtropical lake in southern Brazil. Iheringia Serie Botanica, 2446-8231.

Wiedner C., Ruecker J., Brueggemann R., Nixdorf B., 2007. Climate change affects timing and size of

populations of an invasive cyanobacterium in temperate regions. Oecologia, 152, 473-484.

Williams C.D., Aubel M.T., Chapman A.D., D'Aiuto P.E., 2007. Identification of cyanobacterial toxins in Florida's freshwater systems. Lake Reserv. Manage, 2, 144-152.

Willis A., Chuang, A.W., Woodhouse, J.N. 2016. Intraspecific variation in growth, morphology and toxin quotas for the cyanobacterium, Cylindrospermopsis raciborskii. Toxicon, 119, 307-10.

Willis A., Posselt A.J., Burford M.A., 2017. Variations in carbon-to-phosphorus ratios of two Australian strains of Cylindrospermopsis raciborskii. European Journal of Phycology, 52, 303-310.

Willis A., Woodhouse J.N., Ongley S.E., Jex A.R., Burford M.A., Neilan B.A., 2018. Genome variation in nine cooccurring toxic Cylindrospermopsis raciborskii strains. Harmful Algae, 73, 157-166.

Wood S.A., Pochon X., Luttringer-Plu L., Vant B.N., Hamilton D.P., 2014. Recent invader or indicator of environmental change? A phylogenetic and ecological study of Cylindrospermopsis raciborskii in New Zealand. Harmful Algae, 39, 64-74.

Xiao M., Willis A., Burford M. A., 2017. Differences in cyanobacterial strain responses to light and temperature reflect species plasticity. Harmful Algae, 62, 84-93.

Yamamoto Y., Shiah F.K., 2012. Factors relating to the dominance of Cylindrospermopsis raciborskii (Cyanobacteria) in a shallow pond in northern Taiwan. J. Phycol., 48, 984-991.

Yang Y., Gongliang Y., Youxin C., Nannan J., Renhui L., 2020. Four decades of progress in cylindrospermopsin research: The ins and outs of a potent cyanotoxin. Journal of Hazardous Materials, S0304-3894, 326431.

Yilmaz M., Phlips E.J., Szabo N.J., Badylak S., 2008. A comparative study of Florida strains of Cylindrospermopsis and Aphanizomenon for cylindrospermopsin production. Toxicon, 51, 130-139.

Zhang W., Lou I., Ung W.K., Kong Y., Mok K.M., 2014. Analysis of cylindrospermopsin- and microcystin-producing genotypes and cyanotoxin concentrations in the Macau storage reservoir. Hydrobiologia, 741, 51-68.