Environ Monit Assess (2017) 189:490
DOI 10.1007/s10661-017-6209-7

The occurrence of toxic cyanobacterium *Cylindrospermopsis raciborskii* and its toxin cylindrospermopsin in the Huong River, Thua Thien Hue province, Vietnam

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Received: 19 May 2017 / Accepted: 27 August 2017

Abstract This research reports the presence of species *Cylindrospermopsis raciborskii* and cylindros permopsin (CYN) in the Huong River and the relationship between species with environmental factors to find a scientific basis for predicting the risk of pollution of the species and CYN in waters. Strains of *C. raciborskii* isolated from the river were also identified as potentially toxin-producing through the determination of the presence of toxins in the cultures by ELISA; the presence of the genes involved by PCR confirms the CYN-producing ability of species *C. raciborskii* from this water body. Our results have confirmed the presence of toxic cyanobacteria *C. raciborskii* in the Huong River. *C. raciborskii* from the Huong River are mostly solitary, straight trichomes. Analyses of all *C. raciborskii* strains from the Huong River by ELISA for cylindrospermopsin were positive. The contents of cylindrospermopsin (CYN) in each strain were different, ranging from 5.25 ng mg^{-1} wet weight in CR1DD to 70.83 ng mg^{-1} wet weight in CR1NY. PCR analysis confirmed that the genes involved in the production of this cyanotoxin were present in *C. raciborskii*. The relationship between densities and toxicity showed a correlation coefficient *R* of 0.88. This was a relatively high positive correlation index, indicating the close links between densities and toxins: toxin CYN concentrations increased when *C. raciborskii* densities increased.

Keywords *Cylindrospermopsis raciborskii* · Cylindrospermopsin · Huong River · Thua Thien Hue · Vietnam

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Introduction

Cylindrospermopsis raciborskii is a toxic cyanobacterium species that has repeatedly been the cause of poisoning for humans and animals in the world (Hawkins et al. 1985). Its populations are distributed within the surface water and should rarely cause a blooming phenomenon, so it is difficult to detect even when they exist in high density. This species is known to have the ability to produce cylindrospermopsin toxins (CYN) (Griffiths and Saker 2003), a group of toxic alkaloids which are classified as toxic cytotoxins (Codd et al. 2005). CYN can cause damage to the liver, kidneys, lungs, and intestines of mammals. Other studies have also noted CYN likely to cause neurological damage or cause proliferation of cancer cells. The cause of genetic damage is well known, such as chromosomal deletions or DNA breaks (Apeldoorn et al. 2007; Neumann et al. 2007).

Cylindrospermopsis raciborskii species, previously considered as tropical species, was first reported by

Published online: 07 September 2017

Woloszynska (1912) in the Lake Java in India. However, in the past decade, the large biomass development of this species has been reported to spread from tropical to subtropical and temperate regions (Shafik et al. 2001). It was suggested that climate change and ecological selection are the causes of the spread of this species (Briand et al. 2004). *Cylindrospermopsis raciborskii* has also been found in some water bodies in Vietnam (Duong 1996; Nguyen et al. 2007). However, the studies of this species and the presence of toxin cylindrospermopsin in Vietnam are still rare.

The Huong River is the largest river flowing through the middle of the city of Hue, Thua Thien Hue province, Vietnam. It has a length of 104 km. The river system has a fan-shaped basin with an area of 2830 km^2, nearly 60% of the natural area of the province. The Huong River is the source of drinking water and water for the people of Hue and the surrounding areas. It also is one of the main sources of water for agriculture, aquaculture, industry, and recreational activities, for existence of aquatic ecosystems and wildlife of the city. Although being the main source of water in Hue, the water distribution of the Huong River varies significantly throughout different periods of time during the year. In rainy seasons, flood and inundation occur frequently due to high discharge. A long dry season often causes low flow and a water-supplying crisis, which increases water pollution and salinity intrusion. The rapid economic growth, the strong development of infrastructure, and the pressure of population growth are the main causes of deterioration in the water quality of some locations (Peoples' committee of Thua Thien Hue province, 2005).

This research reports the presence of species *C. raciborskii* and CYN toxin in the Huong River, the toxicity of eight isolates of *C. raciborskii* from the river, and the relationship between the presence of species with environmental factors to find a scientific basis for predicting the risk of pollution of species and CYN toxin in waters.

Materials and methods

Sampling sites

The Huong River basin system with fan-shaped main tributaries include the Bo, the Huu Trach, and the Ta Trach River (mainstream). Ta Trach and Huu Trach join at the Tuan junction, becoming one main flow that goes slowly through the city of Hue and many villages, then joining with the Bo river at the Sinh junction before discharging into the Tam Giang-Cau Hai Lagoon. The Thao Long dam was built for stopping salt intrusion. The length from the Tuan junction to the Thao Long dam is about 30 km. The sampling sites are located in this stretch of the main river, consisting of SH1 (Tuan), SH2 (Kim Long), SH3 (Phu Van Lau), and SH4 (Thao Long). A downstream tributary of the river, also known as *Nhu Y River*, is about 10 km long. It starts from the Dap Da Dam and flows from east and northeast, through the territory of the Huong Thuy district. Due to being separated from the river by the Dap Da Dam, the flow of this branch into the Nhu Y River is often unstable and not continuous throughout the year. In the rainy season (from September to December), the river water level rises and the Dap Da Dam will spill and flow into the Nhu Y River. While in the dry season (from January to August), the Nhu Y River mostly gets water from groundwater sources and rainwater in the neighborhood and it also is the destination of domestic wastewater of the surrounding residential area. Therefore, the water body has a low water level, and the flow is weak and often in a state of pollution. On these branches, there are three sampling sites: DD (Dap Da), CB2 (Chiet Bi 2), and CB1 (Chiet Bi 1) (Fig. 1).

Field sampling

Water and phytoplankton samples were collected monthly at seven sampling sites including the Huong River (SH1, SH2, SH3, and SH4) and the Nhu Y River (DD, CB2, and CB1 stations), a tributary of the Huong River, during the period from April to August 2010.

Qualitative samples were collected using a 20-μm plankton net and preserved with formaldehyde to a 4% final concentration. At each sampling site, without fixing for isolation, a live sample was collected, using a plastic bottle.

Quantitative samples were collected with a plastic tube, 2 m long and 10 cm in diameter. Then the water samples (0–2 m depth) were mixed in a bucket and 100-mL subsamples were transferred to glass bottles. All quantitative samples were preserved with acid Lugol's solution.

Subsamples of 1.5 mL were taken for toxin analysis. They were kept in eppendorf vials and frozen at − 18 °C until analysis.

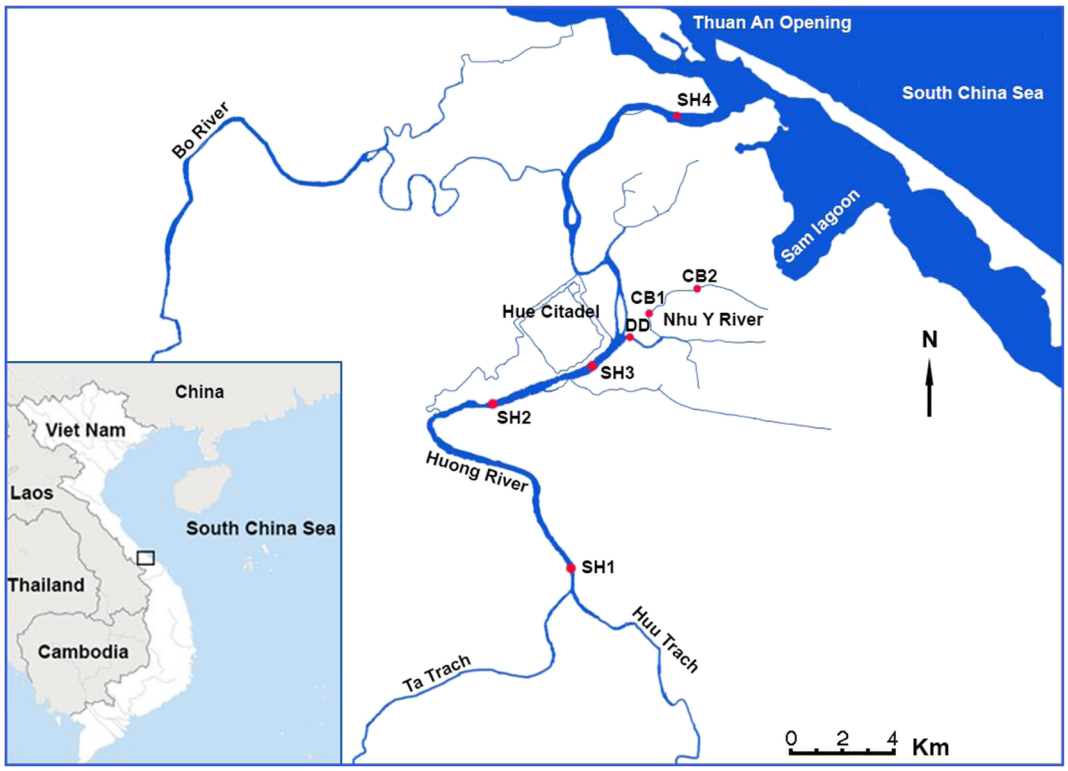

Fig. 1 Map of the Huong River with sampling sites

The 500-mL water sample of each sampling site, for nutrient determinations (PO_4^{3-}-P, NH_4^--N, NO_3^--N,), were collected from the surface layer (0–35 cm), kept in polypropylene bottles at 4 °C, and analyzed within 24 h at the Lab of Biotechnology, College of Sciences, Hue University. All analyses were conducted according to the American Public Health Association (APHA 1998). Physical factors (temperature, pH, dissolved oxygen (DO)) were measured in situ using a multi-parameter probe (HORIBA, Japan). Depths and transparences were measured using a Secchi disc.

Examination of samples

Both live and preserved material as well as cultures (see below) were examined by light microscopy, and species of cyanobacteria were identified and photographed. Examination was on CK40; photographs were taken with an Olympus DP12 digital camera. At least 20 morphometric measurements were made. The measurements were made from both live and preserved materials that were relevant for identification. Cultures were established by pipetting of filaments, and maintained in Z8 medium (Kotai 1972).

Cell counting and estimation of biomass

Direct counts of preserved samples were carried out with the Utermöhl counting technique (Lund 1958) using 5-, 10-, or 25-mL counting chambers and an Olympus CK90 inverted microscope with phase contrast. Sedimentation time was 4–12 h depending on the size of chambers, and cells were counted at ×100, ×200, or ×400 magnification. The microscope was fitted with a special eyepiece graticule, which had a grid divided into equal-sized squares (square grid) covering the whole field of view.

When counting filamentous algae, the filaments were measured using the square grid. The total lengths of filaments per diagonal of chamber or field of view were estimated (Cronberg 1982).

Cultures

The cultured strains were obtained by isolating single filaments by micro-pipetting. They were maintained in liquid Z8 medium (Kotai 1972). The cultures were kept in fluorescent light at 24 ± 4 °C in a 12:12-h light/dark cycle with an intensity of 2000–3000 lx.

Cylindrospermopsin analysis

Enzyme-linked immunosorbent assay (ELISA) The cylindrospermopsin concentrations in natural water samples and in cultures were analyzed by the ELISA test using Abraxis Cylindrospermopsin ELISA kits (Microtiter Plate) (Abraxis, USA). All steps were carried out according to the manufacturer's instructions. The kits were calibrated with a non-toxic cylindrospermopsin-HRP surrogate at levels equivalent to 0.05, 0.1, 0.25, 0.5, 1, and 2 ng mL^{-1} cylindrospermopsin-HRP. The water samples and cultures were sonicated for 3 min to lyse the cells, followed by centrifugation for 10 min at 10000 *g*. The optical density of the supernatant was measured at 450 nm on an automated ELISA system (CODA, Bio-Rad, USA), and the cylindrospermopsin concentrations (μg L^{-1}) in the samples were determined from the standard competitive curve of cylindrospermopsin-HRP. If the cylindrospermopsin concentrations in the samples were higher than levels equivalent to the standard calibration (2 μg L^{-1}), the samples were diluted until inside the range of the standard curve.

Amplification of the genes involved in cylindrospermopsin production

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). The pellets were grinded in preheated (65 °C) 500 mL 2× CTAB buffer and 5 μL β-mercaptoethanol and then incubated at 65 °C for 1 h. DNA was extracted twice with chloroform/isopenthylethanol (24:1) solution. The DNA was then precipitated by 100% ethanol and rinsed with 70% ethanol. Precipitated DNA was collected by centrifuging at 20000 rpm at 4 °C for 10 min, dried at room temperature, then re-suspended in 25 μL of double-distilled water at 37 °C overnight.

Amplification of the genes The PKS and PS gene fragments were amplified by PCR, using the specific primers:

M4 1F: 5′-GAAGCTCTTGGAATCCGGTAA and
M5 1R: 5′-AATCCTTACGGGATCCGGTGC
M13 1F: 5′-GGCAAATTGTGATAGCCACG AGC and M14 1R: 5′-GATGGAACATCGCT CACTGGTG (Schembri et al. 2001)

PCR conditions included preheating for 4 min at 94 °C, followed by 30 cycles: 10 s at 94 °C, 20 s at 55 °C, 1 min at 72 °C, and the final elongation of 6 min at 72 °C. 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).

Statistical analysis of data

To reveal relationships between environmental factors and relative abundance of species *C. raciborskii* and their toxin collected during the study period, principle correspondence analysis (PCA) was carried out based on data concerning relative abundances of *C. raciborskii* species and environmental variables (pH, DO, temperature, $N\text{-}NO_3$, $N\text{-}NH_4$, and $P\text{-}PO_4$) using SPSS (version 23). One-way analysis of variance (ANOVA) was applied for testing significant differences at $p < 0.0$ by SPSS statistical software.

Results

Species *Cylindrospermopsis raciborskii* and the cylindrospermopsin-producing ability of some strains from the Huong River

Morphology Trichomes of *C. raciborskii* from the Huong River are solitary, straight or slightly curved, free-floating, about 100–250 μm long, sometimes up to 400 μm, slightly constricted at the cross walls, slightly tapering towards the end of the trichome with tapering spire terminal cells. The length varies with the trichome from CB1, CB2, and DD, ranging between 100 and

400 μm, while trichomes from SH1, SH2, and SH3 are just in the range of 150–250 μm (Fig. 2). The vegetative cells are cylindrical with aerotopes and visible cross walls, 2.5–18 × 2.5–4.0 μm (Fig. 2). The heterocytes are solitary, arrow-shaped, 6–7 × 2–3.5 μm. They arise from the terminal cells on one or both ends (Fig. 2). The akinetes are long oval, 10–18 × 3.5–5 μm, solitary or up to three in row, next to the heterocytes or with one to three vegetative cells between an akinete and a heterocyte (Fig. 2).

Cylindrospermopsin-producing abilities

Forty-two phytoplankton samples were collected from April to August 2010. The isolating samples where clean and selected randomly from different sites. Eight isolates of *C. raciborskii* strains were chosen for further analysis on toxicity. Details on the origin of the strains are shown in Table 1. Analyses of all *C. raciborskii* strains from the Huong River by ELISA for cylindrospermopsin were positive. However, the contents of cylindrospermopsin (CYN) in each strain were different, ranging from 5.25 ng mg^{-1} WW in CR1DD to 70.83 ng mg^{-1} WW in CR1NY.

PCR analysis confirmed that the genes involved in the production of this cyanotoxin were present in *C. raciborskii* (Table 3). All eight strains isolated from three locations (DD, NY, and CB) found PS and PKS genes in cells. This is in accordance with several studies suggesting that these genes are the genes involved in the biosynthesis of cylindrospermopsin toxin (Harada et al. 1994; Mihali et al. 2008; Pearson et al. 2016; Schembri et al. 2001; Yılmaz et al. 2008). This confirmed the CYN-producing ability of *C. raciborskii* species in the Huong River.

The occurrence of this species and cylindrospermopsin concentrations in the Huong River

The species *C. raciborskii* was found in the river from April to August 2010, but at different sites and with different densities (Table 2). The density of *C. raciborskii* in the main river was highest in May SH1 at 48 × 10^3 cells L^{-1}. The lowest density was 200 cells L^{-1} in June at SH1 and in August at SH2 and SH3. The cell densities of *C. raciborskii* at DD (in the Nhu Y River) were much higher than at other sites in the Huong River. The highest was 22 × 10^6 cells L^{-1} at CB2 in April and the lowest was 17 cells L^{-1} at CB1 in August. The species *C. raciborskii* occurred at six sampling sites including SH1, SH2, SH3, CB1, CB2, and DD. This species was not found at the SH4 site although cyanobacterial composition here was not much different than the others (the data of cyanobacterial composition in the river were not shown). In addition, at SH4, the species of diatom was in abundance with a higher density than the remaining sites. For cyanobacteria, at SH4 in July, dense *Annamia* and *Arthrospira* are highly obtained in this study period (*Annamia toxica* species density reaches 9 × 10^6 cells L^{-1} and *Arthrospira spiroides* density reaches 8.5 × 10^5 cells L^{-1}).

Simultaneously with the analysis of the presence and density of *C. raciborskii* cells in the river, we have also analyzed the levels of CYN toxins in water samples. The results of ELISA analysis show that the toxin CYN in the river appeared in June and July in the main river

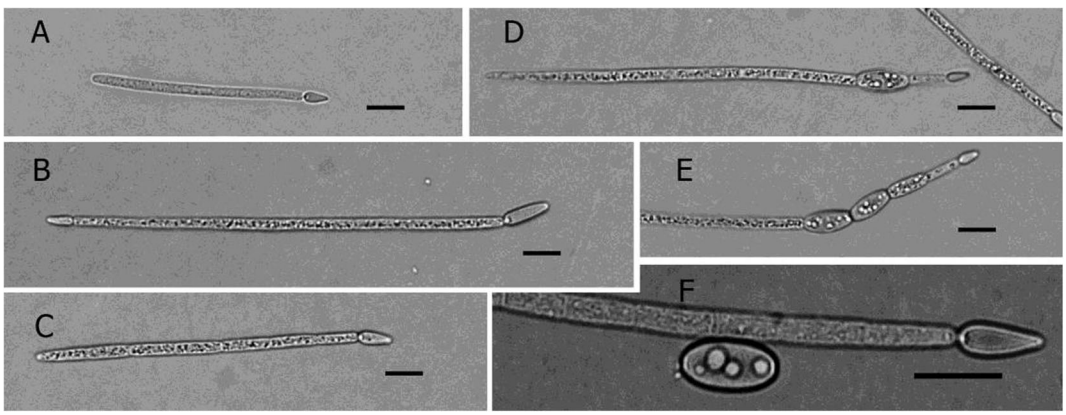

Fig. 2 Morphology of *Cylindrospermopsis raciborskii* from the Huong River (in nature and in cultures). **a**–**c** Filaments with heterocytes. **d**–**f** Filaments with heterocytes and akinetes. Scale bars = 10 μm

Table 1 List of *Cylindrospermopsis raciborskii* strains and their CYN-producing abilities

Strains	PKS/PS	CYN concentrations (ng mg^{-1} wet weight)	Origin of strains
CR1DD	+/+	5.25	Dap Da Dam
CR2DD	+/+	6.05	Dap Da Dam
CR3DD	+/+	6.74	Dap Da Dam
CR4DD	+/+	20.32	Dap Da Dam
CR5DD	+/+	5.72	Dap Da Dam
CR1CB	+/+	6.14	Nhu Y River
CR1NY	+/+	70.82	Nhu Y River
CR2NY	+/+	20.95	Nhu Y River

(SH1, SH2, SH3) and in April and August in the Nhu Y River (CB1, CB2, and DD). The highest toxin levels recorded were 1.58 μg L^{-1} from samples collected at the Dap Da Dam in April, higher than the proposed warning for drinking water at 1 μg L^{-1}. The other two sides of the Nhu Y River in April also have a higher value than the alert level. In the main river, compared to the criteria, the toxin levels measured were lower than the alert level and safe for drinking (Table 3).

The results of the analysis show that at the location without the presence of *C. raciborskii*, toxin CYN was not found (except the sample collected at SH2 in June). Toxin CYN only appeared in the location where the species was present, and toxin concentrations were not proportional to the cell densities. There were some cases that CYN appeared, but species were not found and vice versa. At the SH2 location in June, although the results of qualitative analysis did not find any *C. raciborskii* and other CYN potential species, the toxin CYN persisted in the environment. It could be explained by the species being present before the sampling, or other toxins from the outside move by the flow (Table 3).

The relationship between densities and toxicity showed a correlation coefficient R of 0.88. This was a relatively high positive correlation index, indicating the close links between densities and toxins: toxin CYN concentrations increased when *C. raciborskii* densities increased.

Environmental characteristics associated with the occurrence of *C. raciborskii* and their concentration in the Huong River

A summary of the physical and chemical conditions at seven sampling stations in the Huong River are presented in Table 4. During the study period (from April to

Table 2 The occurrence and cell densities of *C. raciborskii* in Huong River from April to August 2010

Sites	Cell densities of *C. raciborskii* (cells L^{-1})				
	April	May	June	July	August
SH1	–	–	200	48,000	**9000**
SH2	–	–	–	7500	**200**
SH3	–	–	–	8000	**200**
SH4	–	–	–	–	–
CB1	270,000	–	–	–	**17**
CB2	300,000	–	–	–	**50**
DD	**600,000**	**17,000**	–	–	–

– species absent

Table 3 Cylindrospermopsin (CYN) concentrations in the Huong River during the studied period

Sites	Toxin concentrations (μg/L)				
	April	May	June	July	August
SH1	–	–	0.23	0.75	0
SH2	–	–	0.15[a]	0.22	0
SH3	–	–	–	0.49	0
SH4	–	–	–	–	–
CB1	1.32	–	–	–	0
CB2	1.46	–	–	–	0.58
DD	1.58	0	–	–	–

0: presence of *C. raciborskii* but not CYN

– no presence of *C. raciborskii* and CYN

[a] No presence of *C. raciborskii* but CYN

Table 4 Physical and chemical characteristics of the seven sampling sites SH1, SH2, SH3, SH4, DD, CB1, and CB2 during the study period (April–August 2010)

Sites	T (°C)	pH	DO (mg/L)	N-NH_4 (mg/L)	N-NO_3 (mg/L)	P-PO_4 (mg/L)
SH1	30.7 (28–32.4)	6 (5.5–6.3)	5.1 (3.8–6)	0.18 (0.07–0.43)	0.17 (0.01–0.58)	0.34 (0.25–0.49)
SH2	32 (29.3–33.4)	6.1 (5.5–7.3)	5.3 (3.8–6.6)	0.14 (0.06–0.24)	0.18 (0.01–0.58)	0.34 (0.27–0.43)
SH3	32.3 (39–33.5)	6.1 (5.4–7.3)	5.5 (4.0–6.8)	0.09 (0.04–0.16)	0.16 (0–0.4)	0.37 (0.29–0.48)
SH4	31.1 (28.9–32.6)	5.94 (5.7–6.3)	4.7 (3.8–6.6)	0.11 (0.01–0.19)	0.08 (0.04–0.13)	0.35 (0.29–0.59)
DD	35.1 (32.1–37.3)	7.5 (6.3–8.81	9.9 (5.8–18.7)	0.69 (0.16–1.23)	0.09 (0.01–0.15)	0.86 (0.35–1.33)
CB1	34.04 (31.2–35.6)	6.5 (5.7–7.1)	5.57 (3.01–10.4)	0.67 (0.29–1.27)	0.21 (0.03–0.4)	0.71 (0.41–0.83)
CB2	34.5 (32.1–36.2)	6.82 (6–7.9)	7.36 (5,2–11.3)	0.74 (0.43–0.88)	0.21 (0.07–0.43)	0.79 (0.38–1.04)

August 2010), all stations presented average water temperature from 30.7 to 34.5 °C and pH range from 5.9 to 7.5. There was no significant difference in dissolved concentration (DO) between the sampling stations, and the mean value of DO was 5.3 mg L^{-1}. Among the whole set of sampling stations, SH1 SH2, SH3, and SH4 showed lowest values of ammonia concentration (varying from 0.09 to 0.18 mg L^{-1}), whereas in branches of the Huong River, mean ammonia values were significantly higher at sampling stations DD, CB1, and CB2 (recorded from 0.67 to 0.74 mg L^{-1}). Branches of the Huong River (DD, CB1, and CB2) stand out with high dissolved orthophosphate-P concentrations. Domestic wastewater from catchment and low water current could be associated to high nutrient levels in these sampling sites.

In order to identify the environmental factors associated with occurrence of *C. raciborskii* and their toxin in the Huong River, the principal component analysis (PCA) was applied based on the data of environmental characteristics and biological parameters (Fig. 3). Study stations appear clearly separated in the PCA plan with the Huong River stations (SH1, SH2, SH3, and SH4) located in the left area and the Nhu Y River (tributary of

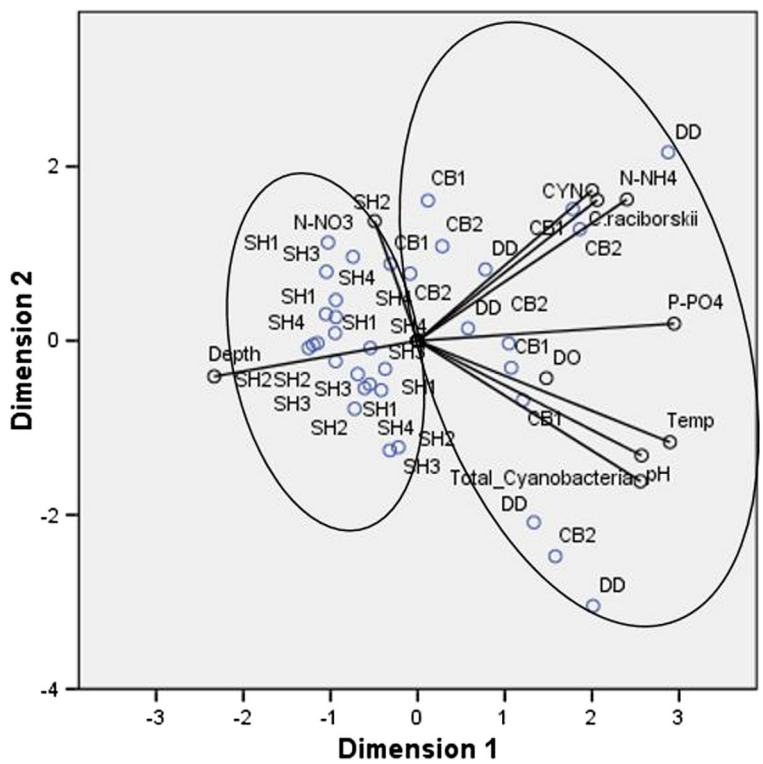

Fig. 3 Principal component analysis (PCA) based on biotic and abiotic factors during the period of April 2010 to August 2010 in the Huong River

the Huong River including DD, CB1, and CB2) grouped in the right area. The relationships between relative abundance of Cyanobacteria, *C. raciborskii*, and environmental variables are presented in Table 5. *C. raciborskii* biomass and CYN toxin concentration were positively correlated in temperature, ammonia, and phosphate concentrations. High values for water temperature and nutrient concentrations (ammonia and phosphate) were also found in the Huong River (Table 4).

Discussion

Cylindrospermopsis raciborskii is a tropical and subtropical toxic cyanobacterium that is currently spreading globally (Sinha 2012). Species *C. raciborskii* has been reported to occur in some freshwaters in Vietnam (Nguyen et al. 2007). However, there are no reports of cylindrospermopsin-producing cyanobacteria in Vietnam. Particularly for the Huong River, Nguyen et al. (2007) had introduced their presence in their surveys on planktic cyanobacteria from freshwaters in Thua Thien Hue. In our current study, we identified the occurrence of this species during the year 2010. In some sampling stations, eight strains were isolated to confirm the toxin production of this species.

The morphology of this species has been known to occur in the form of straight or coil (Chonudomkul 2004). Our nature samples are mostly straight in form with variation in length. The shape of terminal heterocytes and the size of the vegetative cells in the filaments have also changed significantly in the same sample or between the different samples. The difference in cell size and filament length was the visible feature of the *C. raciborskii* population in nature (Hawkin et al. 2001). Willis et al. (2016) proved that there is the substantial intraspecific variation when studying the population dynamics and toxicity of *C. raciborskii* in Lake Wivenhoe, Australia, in 2013. All strains isolated in the small lake also showed differences in growth rates, toxin concentration, and morphology.

All isolated strains of *C. raciborskii* from the Huong River are also in straight form. The average length of the filaments in the culture is longer than that in nature and sometimes forms a bun. Although all strains are capable of producing toxin cylindrospermopsin, defined levels of toxins in the strains from the Huong River are different (ranged from 5.25 ng mg^{-1} WW to 70.83 ng mg^{-1} WW). Some authors have suggested that the biosynthesis of toxins is unrelated to genetic characteristics or geographic area (Chonudomkul et al. 2004; Haande et al. 2008). Given evidence shows that the production of toxins and geographical factors are relevant: *C. raciborskii* species populations in the Americas produce saxitoxin while *C. raciborskii* populations in Asia and Australia produce cylindrospermopsin. The results of our research are in accordance with this: the Vietnamese strains are toxin-producing cylindrospermopsin.

The relationships between environmental factors temperature, pH, dissolved oxygen, nutrient availability, and structure and distribution of cyanobateria

Table 5 Pearson correlations between relative abundance of *C. raciborskii* and environmental factors in the Huong River from April to August 2010

	Temp	DO	pH	N-NH4	N-NO3	P-PO4	Depth	*C. raciborskii*	CYN	Total_Cyanobacteria
Temp	1									
DO	0.179	1								
pH	0.625**	0.605**	1							
N-NH4	0.475**	0.176	0.316	1						
N-NO3	−0.454**	0.063	−0.124	−0.004	1					
P-PO4	0.763**	0.292	0.476**	0.702**	−0.044	1				
Depth	−0.393*	0.026	−0.373*	−0.566**	−0.057	−0.518**	1			
C. raciborskii	0.389*	0.160	0.327	0.604**	−0.147	0.555**	−0.226	1		
CYN	0.357*	0.154	0.331	0.574**	−0.186	0.421*	−0.153	0.896**	1	
Total_Cyanobacteria	0.582**	0.371*	0.720**	0.086	−0.109	0.483**	−0.262	0.136	0.050	1

*Correlation is significant at the 0.05 level (two-tailed); **correlation is significant at the 0.01 level (two-tailed)

communities have been well documented in various ecosystems (Paerl and Huisman 2008). This study has demonstrated that high water temperature can play an important role in regulating the presence of cyanobacterial *C. raciborskii* species and their toxin in the Hue River. The positive correlation between *C. raciborskii* abundance and water temperature suggests the preference of the *C. raciborskii* for high temperature, which is agreeable with the findings of previous studies (Moustaka et al. 2007; Wiedner et al. 2007; Yamamoto et al. 2013). The cyanobacterial *C. raciborskii* is a bloom-forming species common in lakes, reservoirs, and rivers from tropical (Briand et al. 2004; Gemelgo et al. 2009, Figueredo and Giani 2009), subtropical (Everson et al. 2011), to temperate climatic zones (Padisak 1997; Messineo et al. 2010; Sinha et al. 2012). It has been proposed that *C. raciborskii* has a water temperature range of 25.5–32.7 °C for bloom development (Padisak 1997; Recknagel et al. 2014). High water temperature values in our study varied from 30.7 to 34.5 °C, and high density of *C. raciborskii* was observed in the early summer period. Many studies have been suggested that *C. raciborskii* exhibited high abundance and was most likely to bloom during the summer months (Briand et al. 2002; Saker et al. 2004), but this species was also tolerant to a wide range of climates (Figueredo and Giani 2009; Bonilla et al. 2012). Climate warming is an expected occurrence; in the result of this, there will be a likely invasion of *C. raciborskii* (Wiedner et al. 2007). It has also been shown that harmful algal blooms are a complex phenomenon, typically not caused by a single environmental factor but rather multiple potential drivers occurring simultaneously. Water temperature was identified as a significant factor for the proliferation of cyanobacteria bloom, but it may work in combination with other factors such as nutrients. In nutrient-rich freshwater ecosystems, communities may reach high abundance being a predominant cyanobacteria species in summer. In the present study, the environmental factor involved in the change in biomass of cyanobacteria, *C. raciborskii*, and their toxin concentration was the level of nutrients (dissolved phosphate and ammonium concentrations) in the Huong River. Our results were consistent with those of other studies, which found that density of *C. raciborskii* was positively correlated with phosphorus and temperature (Tonetta et al. 2013; Lei et al. 2014). Although a correlation between Cyanobacteria, *C. raciborskii*, and environmental factors (nutrients, nitrogen (N), and phosphorus (P)) was evident and discussed in many studies, this information is not completely defined and such relationship was not consistent. For example, Burford et al. (2006) have reported P limitation as determinant for blooms of *C. raciborskii* in the subtropical North Pine Reservoir (Australia). Kokociński and Soininen (2012) have revealed that a higher biomass of *C. raciborskii* can be expected in lakes with high TP and TN concentration but with low levels of nitrate and phosphate. However, other studies revealed that DIP addition in lake bioassay experiments has promoted *C. raciborskii* dominance (Posselt et al. 2009). In addition, Padisák (1997) showed that *Cylindrospermopsis* blooms occurred in lakes that have high amounts of DOP. The presence and dominance of *C. raciborskii* in a wide range of aquatic ecosystems and climates have attributed to their flexible behaviors including high phosphorus and ammonium uptake affinity and high storage capacity for phosphorus (Briand et al. 2002; Hong et al. 2013; Amaral et al. 2014;; Tonetta et al. 2015; Burford et al. 2016). Further information is needed to increase our knowledge of the ecological requirements of isolated *C. raciborskii* strains from the Huong River.

Summary

To our knowledge, there is no published literature of the distribution of CYN and *C. raciborskii* in Vietnamese freshwaters up to now. Our first results have confirmed the presence and cylindrospermopsin-producing ability of toxic cyanobacteria *C. raciborskii* in the Huong River. The positive correlation between biomass and environmental factors has shown the potential blooms of this toxic species. The presence of *C. raciborskii* and their toxin in the Huong River highlighted the potential risk for human health in the basin. There is a need for regular monitoring of *C. raciborskii*, bloom production of cyanobacteria species, and their toxin in lakes and reservoirs, which are used for drinking water supplies, aquaculture, and recreation purposes. This information should be of grave concern for water management.

Acknowledgements This work was supported by the Vietnamese National Foundation for Science and Technology Development (NAFOSTED) grant 196.06.73.09. We appreciate the effort of an anonymous reviewer and the useful comments and suggestions for improving the manuscript.

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