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Influence of environmental factors on cyanobacterial biomass and microcystin concentration in the Dau Tieng Reservoir, a tropical eutrophic water body in Vietnam

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Abstract – Cyanobacterial blooms can be harmful to environmental and human health due to the production of toxic secondary metabolites, known as cyanotoxins. Microcystins (MCs), one of the most widespread class of cyanotoxins in freshwater, have been found to be positively correlated with cyanobacterial biomass as well as with nitrogen and phosphorus concentrations in temperate lakes. However, in tropical water bodies, cyanobacterial density and cyanotoxin correlation to environmental factors is not fully understood. In the present study, we examined the effects of total nitrogen and total phosphorus (TP) concentrations among other environmental parameters on cyanobacterial community structure and MC concentrations in the Dau Tieng reservoir, a tropical, eutrophic water body in Southern Vietnam. Cyanobacterial biomass and MC content were monitored monthly from March 2012 to February 2013, when MCs were present in the Dau Tieng Reservoir. The highest concentrations of intracellular MCs were found in September and February when cyanobacteria biomass reached maximum values, with 2.50 and 2.13 μg MC.L^{-1}, respectively. Principle component analysis and redundancy analysis showed that MC concentration was positively correlated with the biomass of the cyanobacterial order Chroococcales, whereas TP was the primary abiotic factor influencing cyanobacterial biomass and MC concentrations in the Dau Tieng Reservoir. In addition, Bayesian model average analysis was used to construct a prediction model of MCs using cyanobacterial biomass and environmental variables revealing a suite of useful predictive factors for MCs in the Dau Tieng Reservoir, including water temperature, TP and the biomass of Chroococcales.

Key words: Cyanotoxins / harmful cyanobacterial blooms / total phosphorus / Bayesian model average

Introduction

The eutrophication of freshwaters, mainly due to the increasing input of nitrogen (N) and phosphorus (P), is often accompanied by harmful cyanobacterial blooms (HCBs). The frequent occurrence of toxin-producing HCBs poses a serious threat to environmental and human health because of their ability to produce a wide variety of toxic secondary metabolites including hepatotoxins, neurotoxins and dermatotoxic compounds throughout the world (Blaha *et al.*, 2009). The impacts of these toxins on drinking water and fisheries-related food supplies have been exacerbated as a result of climate change and global warming (El-Shehawy *et al.*, 2012; Paerl and Paul, 2012; He *et al.*, 2016; Visser *et al.*, 2016). In addition, cyanotoxins can be accumulated in various aquatic organisms and transferred via food webs, presenting potential risks to human health (Martins and Vasconcelos, 2009; Paerl and Paul, 2012).

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Microcystins (MCs) are considered to be the most commonly detected and widely distributed toxins compared with other cyanotoxins, such as anatoxin and cylindrospermopsin (Dolman *et al.*, 2012). MCs are small hepatotoxic peptides that inhibit protein phosphatases 1 and 2A in both plants and animals and are produced by various species of the genera *Anabaena* (now *Dolichospermum*), *Microcystis*, *Planktothrix* (*Oscillatoria*), *Nostoc*, *Anabaenopsis*, *Merismopedia* and *Leptolyngbya* (MacKintosh *et al.*, 1990; Chorus and Bartram, 1999). Due to growing concern about health effects of MCs especially *via* drinking water, the World Health Organization (WHO) has adopted a provisional guideline value of MC-LR of 1.0 $\mu g.L^{-1}$ for drinking water (Chorus and Bartram, 1999). To date, more than 100 structural variants of MCs have been identified from cyanobacterial blooms and cultures worldwide. Among them, MC-LR, -RR and -YR are the three most commonly reported in natural waters (Puddick *et al.*, 2014).

MCs are produced intracellularly by cyanobacteria genera, including *Microcystis*, *Dolichospermum* and *Planktothrix* spp. Numerous studies have shown that an increase in the biomass of MCs producing species is typically associated with elevated total MC concentrations in the water column (Kim *et al.*, 2010; Joung *et al.*, 2011; Monchamp *et al.*, 2014). However, MC production is also affected by physico-chemical and biological factors such as competition and grazing (Su *et al.*, 2015). Despite the contradictory results, environmental parameters such as temperature, pH, light intensity, nutrients, trace metals and TN:TP were found to influence production of cyanobacterial toxins (Sivonen, 1990; Rapala *et al.*, 1993; Ame and Wunderlin, 2005). In addition, there is evidence that toxin production is regulated by a complex set of environmental conditions rather than by a single factor (Lukac and Aegerter, 1993).

In Lake Vancouver (Canada) and Lake Taihu (China), phosphate was found to be the main factor influencing MC concentrations (Lee *et al.*, 2015; Su *et al.*, 2015), whereas N availability increased the amount of toxin produced by *Microcystis aeruginosa* in Lake Erie (USA) (Horst *et al.*, 2014). Light availability was reported to be the key factor influencing cyanobacterial bloom formation in the Kasumigaura lakes of Japan (Tomioka *et al.*, 2011), while water temperature, pH and total nitrogen (TN) were reported to be positively correlated with cyanobacterial community dynamics and concentrations of MCs in the Daechung Reservoir of Korea and the Tiegang and Shiyan Reservoirs of China (Joung *et al.*, 2011; Luo *et al.*, 2014). Given these varied results, the determination of the key environmental drivers affecting cyanobacterial proliferation and MC production continues to be actively discussed. In addition, these studies have been conducted in temperate or subtropical freshwater bodies where warmer temperature during summer is thought to be a key driver in the regulation of cyanobacterial growth.

In tropical regions, nutrient levels may be the most important factors influencing cyanobacterial blooms (Mowe *et al.*, 2015). In Garças Reservoir, Brazil, internal loading of P, water stability and anoxia at the lake bottom have been positively correlated with cyanobacterial blooms (Oliveria *et al.*, 2010). However, stability of water temperature and the constant mixed water column have been reported to be the most important factors driving the long-term dominance and persistent bloom of *Cylindrospermopsis raciborskii* in lake Lagoa Santa, Brazil (Figueredo and Giani, 2009). In Vietnam, toxic cyanobacteria and their MCs have been reported mainly in lentic waters (Hummert *et al.*, 2001; Nguyen *et al.*, 2007a; Dao *et al.*, 2010; Duong *et al.*, 2013; Pham *et al.*, 2015). Temperature and phosphate concentrations were the key factors influencing the occurrence of cyanobacterial density and MCs in the Nui Coc Reservoir in Northern Vietnam (Duong *et al.*, 2013). In contrast, MC concentrations were correlated positively with BOD_5, TN:TP ratio and cyanobacterial biomass in the Tri An Reservoir in Southern Vietnam (Dao *et al.*, 2016). Thus, there remains a lack of quantitative data to determine the key environmental parameters affecting cyanobacterial biomass and MC production in tropical environments with sustained high temperatures.

Considering the variety of factors that influence cyanobacterial growth and permanence, the identification of the local environmental conditions that allow *Microcystis*, *Dolichospermum* or *Oscillatoria* to be dominant and produce toxins is a valuable piece of information. Thus the objective of this study was to investigate environmental variables influencing MC concentrations and cyanobacterial biomass in the Dau Tieng Reservoir. In addition, we used a Bayesian model average (BMA) approach to predict the measured MC concentrations using cyanobacterial biomass and environmental variables.

Materials and methods

Sampling site

The Dau Tieng Reservoir is located in Southern Vietnam, about 85 km northwest of Ho Chi Minh City. It has 24 m maximum depth, 3 m mean depth, 264 km^2 surface area and a volume of 1.08×10^8 m^3 (Fig. 1). It has a total watershed of 2700 km^2 that is mainly covered by agricultural cropland (mostly near the lakeshore) and brushwood and forest (mostly upstream). Its elevations are in the range of 24–100 m above mean sea level and it receives annual rainfall of about 1800 mm, of which more than 75% occurs between July and November (Ngoc *et al.*, 2014). The climate of the Dau Tieng Reservoir is typical for a tropical monsoonal zone with two distinct seasons. The dry season is from November to April and the rainy season from May to October (Ngoc *et al.*, 2014).

The Dau Tieng Reservoir serves multiple purposes such as drinking water supply, crop irrigation, flood control, recreation and tourist attractions. The reservoir falls into the eutrophic condition according to the water

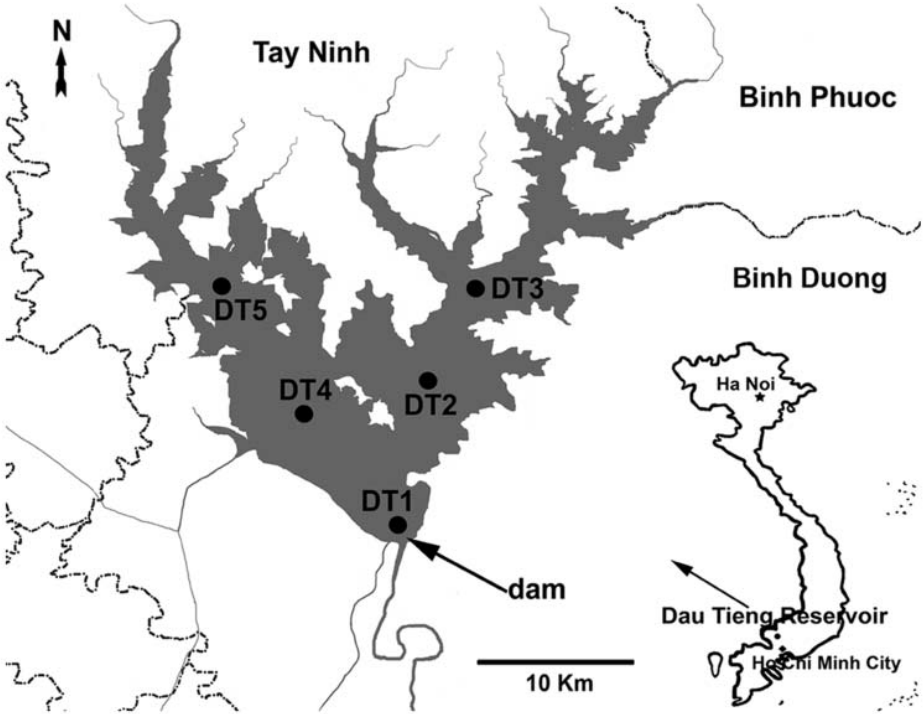

Fig. 1. Map of the Dau Tieng Reservoir showing the location of sampling stations (DT1–DT5).

quality classification described by Ye *et al.* (2009) based on the TP concentrations of 25–100 μg.L^{-1} and TN concentrations of 600–1500 μg.L^{-1} (Nguyen *et al.*, 2007b; Pham *et al.*, 2015). The Dau Tieng Reservoir supplies drinking water for millions of people in Ho Chi Minh City and nearby provinces. Nutrient enrichment, especially P and N, has created favorable conditions for cyanobacterial growth in the reservoir.

Sample collection

Surface water samples for physico-chemical parameters, cyanobacteria and MCs were collected monthly on the same day for each sampling event at five different sites (DT1–DT5) in the Dau Tieng Reservoir (Fig. 1). All the sampling sites are at deep locations and represent the characteristics of the water body. The DT1 site is located toward the end and near the outlet of the reservoir and its water quality is closely related to that at the water works near the river downstream. Sites DT2 and DT4 could be considered as the middle areas of the reservoir reflecting most of the lacustrine characteristics. The DT3 and DT5 sites are characterized by inflowing water from the rivers and streams. pH and temperature were measured *in situ* with a WTW Oxi197i multi-detector. Transparency was measured with a Secchi disk. Samples for nutrient analyses (inorganic N and P) were collected 2–3 m around the sampling spots in 2-L plastic vessels and kept on ice in the field until analyzed in the laboratory on the same day. Samples for MCs quantification were collected in 1-L plastic vessels. Field water was passed through a GF/C glass-fibre filter (Whatman, Kent, England), dried at 50 °C overnight and stored at –70 °C prior to MCs analysis, duplicate samples were prepared from one sample taken in the field for each sampling sites. Cyanobacterial samples for qualitative analysis were collected with a conical plankton net with a mesh size of 25 μm. Samples for quantitative analysis of cyanobacteria were collected using 500 mL of surface water fixed with neutral Lugol iodine solution in the field (Sournia, 1978).

Chemical analysis and cyanobacterial determination and enumeration

Chemical parameters were analyzed colorimetrically in triplicate with a spectrophotometer (Hach DR/2010) using the following APHA (2005) methods: nitrate 4500NO_3^- (A), ammonium 4500NH_4^+ (B), TN Kjeldahl, 4500N (C) and total phosphorus (TP) 4500P (D). Briefly, for TN Kjeldahl, to 400 mL of water sample, 50 mL digestion reagent was added and briskly boiled until the water became transparently pale green. After cooling to room temperature, the sample was diluted with 300 mL distilled water, adding 50 mL sodium hydroxide-thiosulfate reagent and distillated. The vapor containing the ammonia was absorbed in borate buffer solution. The ammonia (calculated as mg N.L^{-1}) was determined by titration with a standard mineral acid (H_2SO_4). For ammonium analysis, to 25 mL of water sample, 1 mL phenol solution, 1 mL sodium nitroprusside solution and 2.5 mL oxidizing solution were added. The sample was left for color development at room temperature in subdued

light for 2 h before extinction was determined at 640 nm. Sample concentrations (calculated as mg $N.L^{-1}$) were determined based on a calibration curve using NH_4Cl. For nitrate measurement, to a 25 mL water sample, 0.5 mL sodium nitrate and 0.2 mL acetic acid were added. The sample was dried at 80 °C, cooled, dissolved in 1 mL sodium salicylate and dried again. It was then dissolved in 1 mL saturated sulfuric acid, diluted with 10 mL distilled water, 10 mL sodium hydroxide solution and cooled. Finally, the absorbance of the sample was measured with a spectrophotometer (Hach DR/2010) at the wavelength of 415 nm. Sample concentrations (mg $N.L^{-1}$) were determined based on standard sodium nitrate ($NaNO_3$) concentrations. TP characterization was conducted by adding sulfuric acid (1 mL) and potassium persulfate (0.5 g) to a 50 mL water sample. The sample was heated to convert phosphate compounds into dissolved P and to concentrate it to 5–10 mL, then cooled. In succession, 2 mL of molybdate and 5 drops of stannous chloride reagents were added to the sample and left for 10 min prior to photometric measurement (Hach DR/2010) at the wavelength of 690 nm. Sample concentrations (mg $P.L^{-1}$) were determined based on standard KH_2PO_4 concentrations. The detection limits of these parameters were 0.02 $mg.L^{-1}$ (nitrate), 0.04 $mg.L^{-1}$ (ammonium), 0.06 $mg.L^{-1}$ (TN Kjeldahl) and 0.03 $mg.L^{-1}$ for TP.

Living and Lugol-fixed cyanobacterial species were morphologically identified under a light microscope (Olympus BX51) equipped with a digital camera and DP71 software (Olympus, Tokyo, Japan). Taxonomic classification was based on the system described in Komárek and Anagnostidis (1989, 1999, 2005). For counting, 10 mL of sample was settled over night in a tubular counting chamber (Utermöhl-chamber). Cyanobacteria were counted using an inverted microscope (Olympus CKX 41) equipped with a square grid and a scale to measure the dimensions of cells and trichomes. The biomass of cells and/or trichomes was calculated based on geometrical formulas and the cyanobacterial biomass was estimated according to Olrik *et al.* (1998). Cyanobacterial density was determined based on the Utermöhl technique (Utermöhl, 1958). Biovolume was calculated based on geometrical cell- or colony volumes (Olrik *et al.*, 1998; Hillebrand *et al.*, 1999) and subsequently converted to biomass (wet weight) by assuming a specific gravity of 1 $mg.mm^{-3}$ (Wetzel and Likens, 2000).

MCs extraction and analysis

Due to a very low extracellular MC fraction that was only detectable at the end of the bloom after cell lysis (Park *et al.*, 1998; Sinang *et al.*, 2013), we measured only intracellular MCs in this study. Intracellular MC extraction and analysis were conducted as described by Barco *et al.* (2005) with minor modifications. Briefly, MCs from the dried filters were first extracted in 5 mL of 100% methanol (MeOH) followed by two 60-min extractions in 3 mL of 75% (vol/vol) aqueous MeOH. Each extraction step was followed by sonication (3 min, 150 W) and centrifugation (1800 *g*, 30 min, 4 °C). The supernatants of all extractions from each sample were pooled, dried at room temperature, re-dissolved in 0.5 mL MeOH (100%) and centrifuged at 6000 *g*, 4 °C for 5 min. The supernatant was passed through a Minisart RC4 filter membrane (0.2 µm pore size, Sartorius Stedim Biotech, Göttingen, Germany) and kept at –20 °C prior to reverse-phase HPLC analysis.

A reverse-phase HPLC with UV-visible photodiode array (PDA) detector (Shimadzu 10A series, Kyoto, Japan) was equipped with a silica–based, reverse-phase C18 column (Waters SunFireTM 5 µm, 3 × 250 mm, Milford, Massachusetts, USA) and maintained at 40 °C. The samples were separated with a mobile phase consisting of methanol: 0.05 M phosphate buffer (pH 2.5; 50:50 v/v) at a flow rate of 0.58 $mL.min^{-1}$. MC congeners were detected by UV detection at 238 nm and identified on the basis of both their retention time and characteristic UV spectra. MCs (MC-LR, MC-RR and MC-YR) purchased from Wako Pure Chemical Industries, Ltd. (Chuoku, Osaka, Japan), were used as standards. The HPLC system had a detection limit of 0.1 $\mu g.L^{-1}$. Duplicate samples with duplicate analysis were used for each sampling site.

Statistical analysis

First, all the numeric variables including measured MC concentrations, cyanobacterial biomass and environmental variables were transformed (*i.e.,* square root) to approach the normal distribution assumption. Principle component analysis (PCA) was used to investigate the association of MC concentrations and cyanobacterial biomass. Redundancy analysis (RDA), a constrained linear ordination method, was applied to further understand the complex relationship between species biomass, MCs and environmental data. The measured environmental factors, including temperature, transparency, T-inorganic N, TN, TP and N:P ratio were treated as explanatory variables, whereas MC concentrations and cyanobacterial biomass were adopted as response variables. To test the significance of each environmental variable in RDAs, permutation tests were performed with 999 permutations, using the *anova.cca* function of the *Vegan* package. The BMA method was applied to search for the most parsimonious models (*i.e.,* models with the minimum number of explanatory variables and the maximum discriminatory power) that predict measured MC concentrations using cyanobacterial biomass and environmental variables (Hoeting *et al.*, 1999). In summary, if there are k explanatory variables, then 2^k possible models (not including interaction models) can be constructed. Among the possible models, the best models are suggested on the basis of the Bayesian information criterion (BIC), in which a smaller BIC value indicates a better model. Recently, BMA has been shown to be a better method for model selection compared with the stepwise method, which has been widely used in ecological

Table 1. Physical, chemical and biological parameters measured in the Dau Tieng Reservoir between March 2012 and February 2013.

	Mean	Min	Median	Max	SD	C.V.
Temperature (°C)	31.03	27.5	31.2	34.0	1.33	0.04
pH	7.04	6.30	6.90	8.70	0.44	0.06
Transparency (cm)	115.5	40	115.0	210	39.0	0.34
Total inorganic nitrogen (mg.L^{-1})	0.248	0.06	0.105	1.55	0.37	1.49
Total nitrogen (mg.L^{-1})	3.22	0.28	1.78	16.4	3.54	1.1
Total phosphorus (mg.L^{-1})	0.06	0.03	0.06	0.36	0.04	0.67
TN:TP ratio	55.7	6.0	33.0	274.0	58.8	1.05
Chroococcales (mg.L^{-1})	0.35	0.0	0.08	7.54	1.01	2.87
Nostocales (mg.L^{-1})	4.71	0.01	1.00	192.62	24.73	5.25
Oscillatoriales (mg.L^{-1})	0.03	0.0	0.01	0.60	0.08	2.47
Total cyanobacterial biomass (mg.L^{-1})	5.09	0.02	1.20	200.15	25.69	5.04
Microcystins (μg.L^{-1})	0.47	0	0.23	2.50	0.55	2.46

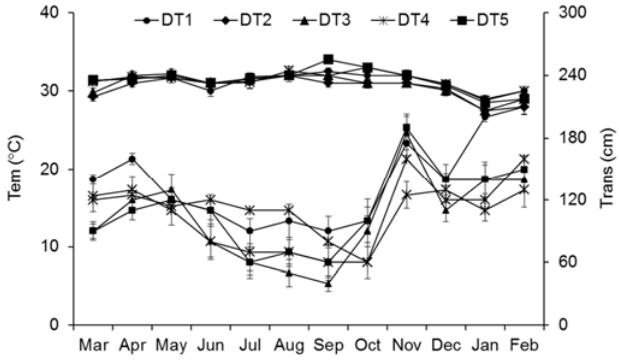

Fig. 2. Temporal and spatial variations of temperature (Tem) and Transparency (Trans) from March 2012 to February 2013.

studies (Wang *et al.*, 2004). All analyses were performed using R software version 3.2.2 (The R Foundation for Statistical Computing).

Results

Physical and nutrient characteristics

The concentration ranges and averages of water quality variables between March 2012 and February 2013 are shown in Table 1. Water temperature had a low variation in the Dau Tieng Reservoir during the sampling period and ranged from 27.5 to 34.0 °C (Fig. 2). pH ranged from 6.3 to 8.7. Transparency, which strongly influences the light available for algae photosynthesis, exhibited wide variation, ranging from 40 to 210 cm among the five sampling sites. Higher water transparency was recorded during the dry season (November–April; Fig. 2). Water temperature was not significantly different among sites, while transparency levels at DT5 were significantly higher than those at DT1 (Fig. 2).

With regard to the trophic condition of the Dau Tieng Reservoir, TN and TP covered a wide range of concentrations (from 0.28 to 16.4 mg.L^{-1} and from 0.03 to 0.36 mg.L^{-1}, respectively). The variation of TN and TP exhibited a bell-shaped curve during the study period (Fig. 3(a) and (b)). Both TN and TP concentrations exhibited almost the same trend, with a peak in September 2012 (rainy season). The TN:TP ratio in the Dau Tieng Reservoir ranged from 6 to 274, with higher values during the rainy season (Table 1). Both TN and TP concentrations during the rainy season (May–October) were higher than those during the dry season (November–April). TN concentration was not significantly different among sites, while TP levels at DT3 and DT5 were significantly higher than those at the other sites (Fig. 3(a) and (b)).

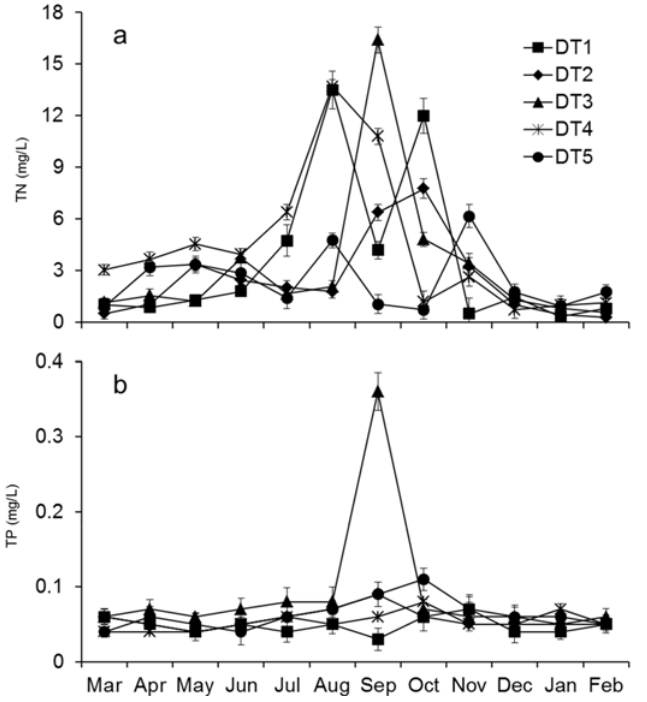

Fig. 3. Temporal and spatial variations of (a) total nitrogen (TN) and (b) total phosphorus (TP) from March 2012 to February 2013.

Table 2. List of cyanobacterial species recorded from the Dau Tieng Reservoir (March 2012–February 2013).

Taxon	Species
Chroococcales	
Aphanocapsa	*A. delicatissima*, *A.* sp.
Chroococcus	*C. limneticus*
Cyanodictyon	*C. imperfectum*
Merismopedia	*M. glauca*, *M. tenuissima*
Microcystis	*M. aeruginosa*, *M. botrys*, *M. flos-aquae*, *M. novacekii*, *M. panniformis*, *M. smithii*, *M. wesenbergii*
Snowella	*S. lacustris*
Woronichinia	*W. naegeliana*
Oscillatoriales	
Geitlerinema	*G. splendidum*, *G.* sp.
Limnothrix	*L.* sp.
Lyngbya	*L.* sp.
Oscillatoria	*O. kawamurae*, *O. limosa*, *O. princeps*, *O. tenuis*, *O. agardhii*, *O.* sp.
Planktolyngbya	*P. circumcreta*, *P. limnetica*
Pseudanabaena	*P. limnetica*, *P. mucicola*
Spirulina	*S. major*, *S. princeps*, *S. subsalsa*
Nostocales	
Cylindrospermopsis	*C. raciborskii*
Dolichospermum	*D. affinis*, *D. bothai*, *D. circinalis*, *D. flos-aquae*, *D. mucosa*, *D. smithii*, *D. viguieri*, *D. torques-reginae*, *D.* sp.

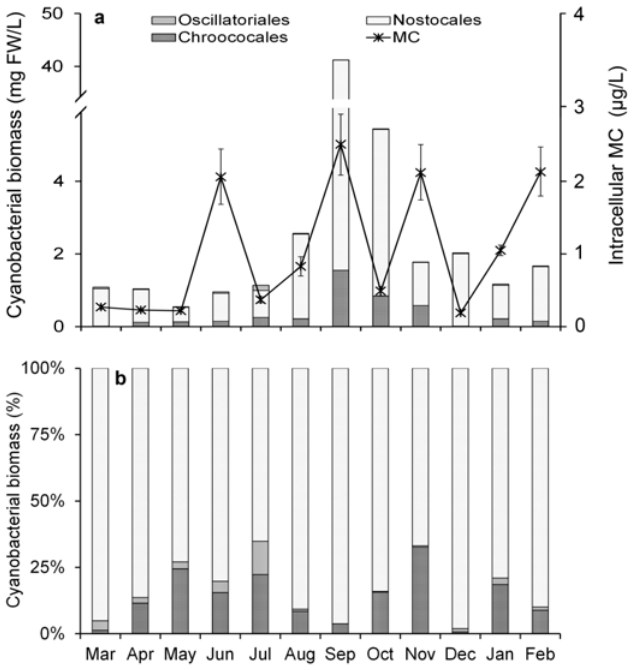

Fig. 4. Temporal variations of (a) cyanobacterial biomass and intracellular microcystin (MC) (data were given by mean value of five sampling stations) and (b) proportion of Nostocales, Oscillatoriales and Chroococcales in the total cyanobacterial biomass.

Cyanobacterial species composition

Over 1 year of monitoring, a total of 3 orders, 16 genera and 42 cyanobacterial species were indentified in the Dau Tieng Reservoir (Table 2). The relative percentages of individual cyanobacterial genera were *Dolichospermum* 21.4%, *Microcystis* 16.7%, *Oscillatoria* 14.4%, *Spirulina* 7.1% and others (*e.g., Cylindrospermopsis, Snowella, Woronichinia, Lyngbya, Planktolyngbya*, etc.) 40.5%. The identified cyanobacteria included potentially toxic species such as *Microcystis* spp., *Dolichospermum* spp., *Cylindrospermopsis* spp. and *Oscillatoria agardhii*. The number of species identified per sampling event ranged from 22 to 28 species. The maximum number of species found within a single survey occurred in March 2012 and the minimum number of species was found in October 2012 (online Supplementary data). The species belonging to *Dolichospermum*, *Microcystis* and *Oscillatoria* were the main components of the cyanobacterial community.

Cyanobacterial biomass

The cyanobacterial biomass of Chroococcales, Oscillatoriales and Nostocales are shown in Table 1 and Figure 4. The mean total biomass ranged between 0.55 and 41.72 mg fresh weight (FW).L^{-1} and was especially high in September 2012 due to a bloom of *Dolichospermum flos-aquae* (Fig. 4(a)). The biomass was higher during the rainy season (from May to October). The biomass of Nostocales was the dominant component of the cyanobacteria, contributing 65.2–98.5% of the total biovolume (ranging from 0.40 to 40.16 mg FW.L^{-1}) (Fig. 4(b)). The biovolume of Chroococcales ranged from 0.013 to 1.55 mg FW.L^{-1} with a peak in September 2012. Oscillatoriales accounted for a small proportion of total cyanobacterial biomass in many sampling events (ranging from 0.006 to 0.14 mg FW.L^{-1}) (Fig. 4(a)). The biomass of Nostocales and Chroococcales were not significantly different among sites, while the biomass of Oscillatoriales at DT3, DT4 and DT5 was significantly higher than at the other sites (Anova, $P < 0.05$).

Intracellular MC concentrations

The HPLC results revealed that intracellular MCs were detected in all samples except two samples (DT2 and DT3) in December 2012. The concentrations of intracellular MCs (only the three most dominant MC variants MC-RR, -LR and -YR were measured) ranged from 0.19 to 2.50 µg.L^{-1}. The maximum concentration was measured in September 2012 (2.50 µg.L^{-1} at station DT3) when a thick mat of *D. flos-aquae* bloom was observed during sampling (Fig. 4(a)). The most common congener in the sample was MC-RR (60%) followed by MC-LR (26%) and MC-YR (14%). Variant dominance followed the order MC-RR > MC-LR > MC-YR across all sites and times.

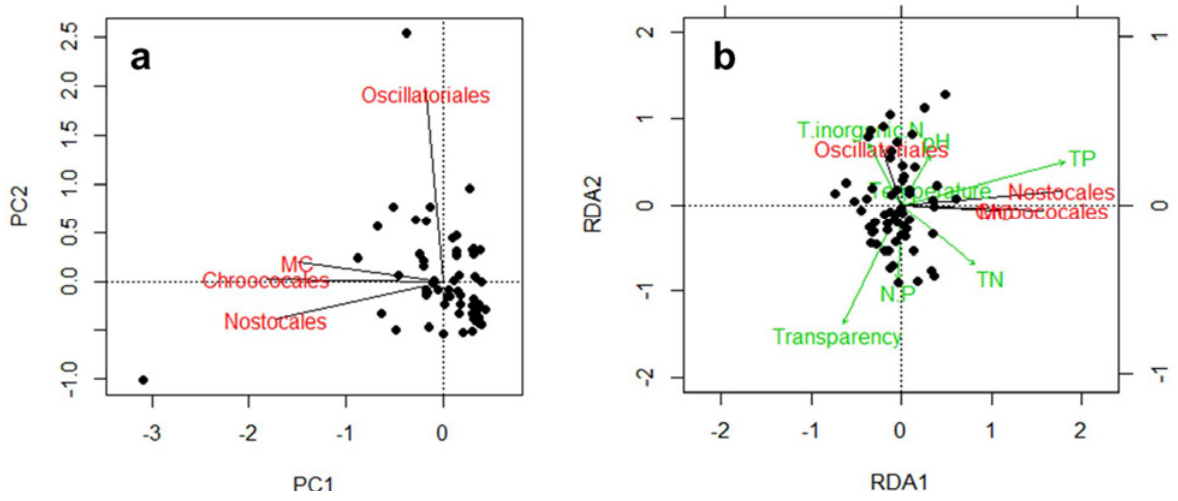

Fig. 5. Ordination analysis including (a) principle component analysis (PCA) of cyanobacterial biomass and microcystin (MC) concentrations and (b) redundancy analysis (RDA) of environmental variables, cyanobacterial biomass and MC concentrations.

Relation between abiotic and biotic variables

A PCA correlation biplot revealed the first axis (PC1) represented by the biomass of Chroococcales, Nostocales and MC concentrations and explained 55.7% of total variation, whereas the second axis (PC2) was mainly related to biomass of Oscillatoriales and explained 25.5% of total variation. Measured MC concentrations were closest related to the biomass of their producing group Chroococcales (Fig. 5(a)).

We used RDA to evaluate the combined associations between cyanobacteria variation, measured MC concentrations and environmental variables. RDA results clearly showed that both TP and TN were positively correlated with MC concentrations and cyanobacterial biomass including Chroococcales and Nostocales, in which TP was the most important factor (permutation test *P* values for both TP and TN were 0.001). T-inorganic N was positively associated with the biomass of Oscillatoriales at *P* value 0.008, whereas transparency was negatively related to the biomass of Oscillatoriales at *P* value 0.006 (Fig. 5(b)).

BMA for prediction MC concentration

We used a BMA approach to determine whether MC concentrations could be predicted by cyanobacterial biomass, and environmental variables. There were five best models suggested by the BMA process, in which the most parsimonious model (*i.e.,* minimum explanatory variables and maximum discrimination power) contained two variables: temperature, and biomass of Chroococcales. The other possible models included temperature, biomass of Chroococcales and one of the following environmental variables: TP, T-inorganic N, TN, or transparency (Table 3). The later environmental variables were discovered in RDA analysis, whereas temperature was not identified in RDA. One possible reason that temperature was not identified in RDA is that temperature was highly associated with other environmental variables, and was therefore preferentially selected by the BMA process as parsimonious.

Discussion

The Dau Tieng Reservoir is a large tropical water body; hence the temperature did not vary much over the seasons and was favorable for cyanobacterial growth (Davis *et al.*, 2009; Dao *et al.*, 2016). Studies from temperate and sub-tropical regions have shown that water temperature is a critical factor influencing the bloom of toxic *Microcystis* (Davis *et al.*, 2009; Joung *et al.*, 2011; Liu *et al.*, 2011). The pH values ranged from slightly acidic to slightly alkaline and were within a similar range observed in the Nui Coc and Tri An Reservoirs in Vietnam (Duong *et al.*, 2013; Dao *et al.*, 2016) and in the Juturnaiba, Garcas and Botafogo Reservoirs, Brazil (Marinho and de Moraes Huszar, 2002; Lira *et al.*, 2009). Nitrogen leaching from the catchment area and nitrate concentrations have contributed to episodic acidification of the water (Nguyen *et al.*, 2007b). Although transparency in the reservoir revealed a low trophic state, the TN and TP concentrations (0.28–16.4 mg.L^{-1} and from 0.03 to 0.36 mg.L^{-1}, respectively) are characteristic of eutrophic conditions according to Reynolds (2006) and were not limiting factors for cyanobacterial development at any time during the sampling period. Intensive fish cage aquaculture, livestock farming, sand mining and agricultural activities have adversely affected water quality (especially at sites DT2, DT3, DT4 and DT5 in the rainy season) and have contributed significantly to nutrient enrichment and cycling within the Dau Tieng Reservoir (Nguyen *et al.*, 2007b). In addition, cyanobacterial blooms in moderately deep, stratified eutrophic lakes are typically comprised of N_2-fixing taxa, including *Dolichospermum* and *Aphanizomenon* (Paerl *et al.*, 2001). In contrast,

Table 3. Cyanobacterial biomass and environmental factor models in prediction of MC concentrations using the BMA approach.

Model	Explanatory variables	Coefficient	R^2 (%)[a]	BIC[b]	Posterior probability[c]
1	Temperature	− 0.83	41.1	− 23.6	0.443
	Chroococcales	0.45			
	Intercept = 5.06				
2	Temperature	− 0.89	42	− 20.4	0.09
	TP	0.85			
	Chroococcales	0.37			
	Intercept = 5.18				
3	Temperature	− 0.77	41.5	− 19.9	0.068
	T inorganic N	− 0.07			
	Chroococcales	0.45			
	Intercept = 4.74				
4	Temperature	− 0.89	41.4	− 19.8	0.065
	TN	0.02			
	Chroococcales	0.44			
	Intercept = 5.39				
5	Temperature	− 0.86	41.2	− 19.6	0.059
	Transparency	− 0.05			
	Chroococcales	0.44			
	Intercept = 5.29				

[a] R^2: Coefficient of determination in linear regression indicates how much variation of the outcome can be explained by the linear model.
[b] BIC stands for Bayesian Information Criteria. BIC smallest suggests the model with maximum parsimony (*i.e.,* minimum explanatory variables and maximum discriminatory power).
[c] Posterior probability is the probability of a model being a "correct" model in the BMA process.

shallow eutrophic lakes are typically dominated by cyanobacterial taxa that do not fix N_2 because N is not limiting, in particular the families *Chroococcales* and *Oscillatoriales*, including *Microcystis* and *Oscillatoria* (Havens *et al.*, 2003).

In the Dau Tieng Reservoir, the cyanobacterial community includes both non-N_2-fixing cyanobacteria (Chroococcales and Oscillatoriales) and N_2-fixing ones (Nostocales). Although the main representatives were species belonging to non-N_2-fixing cyanobacteria (Chroococcales and Oscillatoriales), the N_2-fixing cyanobacteria (Nostocaless) accounted for the largest proportion of biomass. *Microcystis* and *Dolichospermum* are the primary cyanobacteria that cause cyanobacterial blooms in various lakes and reservoirs worldwide (WHO, 1998; Krienitz *et al.*, 2002; Zhang *et al.*, 2014). Blooms of *Microcystis* spp. associated with MCs were reported throughout Vietnam (Nguyen *et al.*, 2007a; Dao *et al.*, 2010; Duong *et al.*, 2013; Pham *et al.*, 2015). However, in this study we observed for the first time a bloom of *Dolichospermum flos-aquae* in this reservoir. Under light-limiting conditions, the non-N_2-fixing cyanobacteria *Microcystis* is able to completely exclude other phytoplankton via buoyancy regulation (Reynolds *et al.*, 1987), whereas under N-limiting conditions, N-fixing cyanobacteria have an advantage of competition over *Microcystis* and other non-N fixing bacteria (Findlay *et al.*, 1994; Dolman *et al.*, 2012). Our results showed that light, temperature and nutrient availability support both *Microcystis* and *Dolichospermum* growth. Therefore, other factors may govern the bloom of *Dolichospermum*, *e.g.,* high light intensity, iron or other micronutrient limitation, water column mixing and extracellular allelochemicals (Figueredo and Giani, 2009; Monchamp *et al.*, 2014; Zhang *et al.*, 2014). The exact environmental factors that favor *Dolichospermum* blooms at certain times need further investigation.

Both N and P concentration regulate cyanobacterial proliferation (Wilhelm *et al.*, 2011), of which P is the main fundamental regulating factor (Joung *et al.*, 2011). Our results showed that both N and P concentrations during the rainy season were higher than those during the dry season. Inorganic nutrient inputs due to erosion from agricultural land surrounding the lakeshore during the rainy season are probably rapidly assimilated in the reservoir (Nguyen *et al.*, 2007b). This results in a significant turnover of N and P in the system. Phosphorus is critical for cellular function and influences cyanobacteria dynamics, including growth, life cycle stages, and toxin production (Jacoby *et al.*, 2000; Dyhrman, 2008; Joung *et al.*, 2011). Our results indicated that cyanobacterial proliferation and measured MC concentrations in the Dau Tieng Reservoir were positively correlated with P and N, with the strongest correlation found for TP. Increased PO_4–P availability positively influences cyanobacteria populations (Rinta-Kanto *et al.*, 2009; Yu *et al.*, 2014) and more specifically abundance of toxin and non-toxin producing *Microcystis* sp. populations (Davis *et al.*, 2009; Li *et al.*, 2012). It also correlates strongly with increasing MCs and has been shown in some cases to yield higher amounts of MCs per cell count (Rapala *et al.*, 1997; Jacoby *et al.*, 2000; Rinta-Kanto *et al.*, 2009). Our observations were also consistent with Lee *et al.* (2015) who found that TP was the main

environmental variable influencing the abundance of *Dolichospermum*, total cyanobacteria abundance and intracellular MC concentrations from *Microcystis* sp. in temperate Vancouver Lake.

In a tropical reservoir, the Kranji Reservoir in Singapore, the abundance of total *Microcystis* and toxigenic *Microcystis* was positively correlated with TN and TP (Te and Gin, 2011). The MC-producing genotypes were also found to increase with the TP concentration in cold-temperate Finnish lakes (Rantala *et al.*, 2006) and Lake Erie (Rinta-Kanto *et al.*, 2009). Phosphorus limitation for phytoplankton biomass may become severe at TN:TP ratios above 20:1 (Hecky *et al.*, 1993) while TN:TP ratios below 20:1 may become N limited until N-fixation can balance N-deficits (McEachern *et al.*, 2002). The Dau Tieng Reservoir has displayed a wide range of TN:TP ratios and has infrequent blooms of N_2-fixers such as *D. flos-aquae*. Our results were contrary to the hypothesis that potentially N_2-fixing Nostocales taxa would be favored in a tropical system where TN concentration was less than TP concentration. Our observations agree with the findings reported by Dolman *et al.* (2012) and Muhid *et al.* (2013) that some N_2-fixing Nostocales often reach their highest biovolume in lakes with higher N concentrations relative to P concentrations. Thus the question arises of why some N-fixing cyanobacterial species occur at higher abundance in lakes that are P-limited. Some possibilities are that Nostocales are not always able to compensate fully for N_2-deficiency, particularly in high productivity systems and not all species of Nostocales may be able to overcome N_2-deficiency (Dolman *et al.*, 2012). However, in order to fully answer this question, more information is needed about the N_2-fixation capacity and *in situ* N_2-fixation rates of different Nostocales species in tropical environments.

The amount of MCs has been suggested to be a result of cyanobacterial biomass and MC production, which are both influenced by environmental factors such as temperature and nutrients in some studies (Davis *et al.*, 2009; Rinta-Kanto *et al.*, 2009; Liu *et al.*, 2011). In contrast, other studies reported a negative or lack of correlation (Wu *et al.*, 2006; Wilhelm *et al.*, 2011; Srivastava *et al.*, 2012). In the present study, intracellular MC concentrations were strongly associated with the abundance of Chroococcales, and to a lesser extent with the biomass of Nostocales. *Microcystis* bloom was observed from July to September, 2010 in the Dau Tieng Reservoir in Vietnam. A previous study has shown that *Microcystis* is the main MC producer in the Dau Tieng Reservoir, since no *mcy* gene fragments nor MCs were detected from several species of Nostocales and Oscillatoriales (Pham *et al.*, 2015). In the present study, we recorded a bloom of *D. flos-aquae* (*D. flos-aquae* $>95\%$ of the cyanobacteria biovolume) in September 2012, however, the MC concentration was not very high (2.5 $\mu g.L^{-1}$) compared with the biomass of Nostocales. Krienitz *et al.* (2002) reported MC concentrations (<1 $\mu g.L^{-1}$) during a *Dolichospermum* bloom (*Dolichospermum* spp. $>90\%$ of the phytoplankton biovolume with 8×10^5 $cells.mL^{-1}$) from Lake Victoria, Kenya. The same authors stated that it was unclear whether the detected MCs could be attributed to all of the cyanobacterial species found and that the identification of the species responsible for MC production requires further investigation. In contrast, the results from several Ugandan waters including Lake Victoria and the Nyanza Gulf showed that *Microcystis* was the only MC producer (Okello *et al.*, 2010a, 2010b; Sitoki *et al.*, 2012). Several species of *Dolichospermum* producing MCs have been reported (Harada *et al.*, 1991; Sivonen *et al.*, 1992; Namikoshi *et al.*, 1998; Sotero-Santos *et al.*, 2008). Since no MC production was detected from a culture of *D. flos-aquae* isolated from the Dau Tieng Reservoir (unpublished results), we concluded that the detected MCs could be attributed to the toxic *Microcystis* species, as proven in a previous investigation by Pham *et al.* (2015).

According to WHO guideline values, the cyanobacterial cell abundance and biomass measured in the present study falls within alert level 3 for drinking water and level 2 for recreational waters (WHO, 1998, 2003). MC concentrations in raw water from the Dau Tieng Reservoir were sometimes higher than the WHO guideline value of 1 $\mu g.L^{-1}$. The concentrations of MCs in tap water (310–570 $ng.L^{-1}$) corresponded to the concentrations in the raw water (740–820 $ng.L^{-1}$) after sand filtration and flocculation (Hoeger *et al.*, 2004). Risks associated with exposure to MCs present in surface waters and reservoirs are a serious concern for human health worldwide because of the potent hepatotoxicity of MCs and their capacity as tumor promoters (Chorus and Bartram, 1999). Hence, during periods of high MC concentrations in the reservoir (especially at sites DT3 and DT5), local residents may be prone to suffer from hepatotoxic effects via their daily consumption of MC-contaminated drinking water or contaminated field products from spray irrigation. In addition, the presence of other cyanotoxins such as anatoxin, cylindrospermopsin and saxintoxins and their potential toxic effects warrant further investigation. Monitoring of toxic cyanobacterial abundance, cyanotoxin concentrations, as well as other water quality parameters in the Dau Tieng Reservoir are strongly recommended to minimize the toxic effects on humans and ecosystems. Our model including temperature and biomass of Chroococcales can be used as a simple tool to predict MC concentrations in the reservoir, though further studies are warranted to validate the models.

Conclusion

Our results suggest that cyanobacterial biomass and nutrient content in the Dau Tieng Reservoir varied temporally with water transparency. *Microcystis* spp., *Dolichospermum* spp. and *Oscillatoria* spp. were the main components of the cyanobacterial community where *Microcystis* was the main toxin producer in the reservoir. Our findings reveal that the biomass of Chroococcales could be used as an indicator of increased phosphorus levels and measured MC concentrations in the reservoir,

whereas Nostocales could be used in more productive waters. We propose that phosphorus supports the growth of the potentially toxic cyanobacteria, *Microcystis* spp., and may be the limiting factor in measured MC production during the growing season. Further studies on the presence of other cyanotoxins such as anatoxin, cylindrospermopsin and saxintoxins and their potential toxic effects should be implemented. Monitoring of toxic cyanobacterial abundance, cyanotoxin concentrations, as well as water quality in the Dau Tieng Reservoir are strongly recommended to minimize the toxic effects on humans and ecosystems.

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