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BENTHIC CYANOBACTERIA COMPOSITION AND MICROCYSTINS CONCENTRATION IN THE SEDIMENT OF THE TRI AN RESERVOIR

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SUMMARY

The occurrence of cyanobacterial blooms (CYBs) associated with cyanotoxins has been reported worldwide. While planktonic cyanobacteria and the contamination of microcystin (MC) in the water column have been the subject of many investigations, little attention is paid to the benthic cyanobacterial communities and MC concentration in the benthic environment. This study aimed to investigate the benthic cyanobacterial composition and MC concentration accumulated in sediment from the Tri An Reservoir (TAR), Dong Nai province. Benthic cyanobacterial communities and sediment were collected at five sites in dry and rainy seasons. The benthic cyanobacterial communities were morphological observation and identification. Different nitrogen, phosphorus species, and chlorophyll-a (Chl-a) in sediment were analyzed. MCs concentration in sediment was quantified by High-Performance Liquid Chromatography (HPLC). Results indicated that the sediment from the TAR was contaminated with nitrogen. A total of 19 species belonging to 12 genera, 3 orders of cyanobacteria were identified. In which, *Anabaena*, *Oscillatoria*, and *Microcystis* were the three most commonly found in the samples. Surface sediment samples contained high densities of colonial *Microcystis*. The Chl-a concentration in sediment in dry and rainy seasons ranged from 1.96–3.45 and 0.71–3.15 µg/g fresh weight (FW), respectively. MC was detected in sediment at all sites in both dry and rainy seasons with a concentration up to 191.4±14.2 ng/g FW. The outcome of this study provides basic knowledge on MC and potential MC-producing cyanobacteria in a drinking water supply. Further investigation on the toxic producing ability of the benthic cyanobacteria species from the Tri An Reservoir is required.

Keywords: accumulation, benthic cyanobacteria, cyanotoxins, eutrophication, microcystin

INTRODUCTION

The occurrence of cyanobacterial blooms (CYBs) associated with cyanotoxins has been reported worldwide, and their frequency has been increasing in the last two decades due to eutrophication and climate (Bormans *et al.*, 2020; Harke *et al.*, 2016). Among cyanotoxins, the hepatotoxins, especially microcystins (MCs) are among the most commonly detected and widespread in freshwater ecosystems (Merel *et al.*, 2013; Harke *et al.*, 2016). MCs are produced by various species within the genera *Dolichospermum* (planktic *Anabaena*), *Microcystis*, *Oscillatoria*, *Planktothrix*, *Nostoc*, *Aphanizomenon*, *Arthrospira*, *Lyngbya*, *Pseudanabaena*, and *Anabaenopsis* in freshwaters (Chorus, Bartram, 1999). The contamination of MCs in freshwater sources has led to a serious threat to drinking water and recreational lakes worldwide. The chemical structure of MCs includes seven peptide-linked amino acids, with the two terminal amino acids of the linear peptide being condensed (joined) to form a cyclic compound. The general structure is cyclo-(D-alanine-R1-D-MeAsp-R2-Adda-D-glutamate-Mdha) in which R1 and R2 are variable L amino acids, D-MeAsp is D-erythro-b-methylas-partic acid, and Mdha is N-methyldehydroalanine (Chorus, Bartram, 1999). The R1, R2 variable amino acids for MC-LR, MC-RR, and MC-YR are leucine (L), arginine (R), and tyrosine (Y). The amino acid Adda, (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid, is the most unusual structure in this group of cyclic peptide toxins. There are over 100 structure variants that have so far been reported and the three most common are MC-LR, -RR, and -YR (Harke *et al.*, 2016; Pham *et al.*, 2017).

In recent centuries toxic CYBs have been reported worldwide and intensively reviewed in literature (Blaha *et al.*, 2009; Merel *et al.*, 2013). The most dominant bloom-forming cyanobacterial genera in freshwaters are *Microcystis*, *Dolichospermum*, *Aphanizomenon*, *Oscillatoria*, *Cylindrospermopsis*, *Phormidium*, and *Nostoc* (Merel *et al.*, 2013). The blooms of cyanobacteria have been reported from many lakes and reservoirs in Europe, Canada, the United States, and Asian countries, in many cases reported that the MCs contents higher than the WHO guideline (1 µg/L) and related to human exposure and animal deaths (Chorus and Bartram, 1999). In Vietnam, many studies on toxic cyanobacteria and their toxins have been undertaken. The bloom of *Microcystis* spp. associated with MCs concentration up to 1116 µg/L in cyanobacterial scum samples have been reported in the Nui Coc, Dau Tieng, and Tri An reservoirs that have been used for drinking water and recreational activities (Duong *et al.*, 2014; Dao *et al.*, 2016; Pham *et al.*, 2017).

Benthic cyanobacteria are growing on the surface of the sediment in water bodies, especially on the surface sediment and substrates along the banks. Previous studies have been reported some of the known MCs produced by benthic cyanobacterial species (Catherine *et al.*, 2013; Gaget *et al.*, 2017; Pham, Utsumi, 2018). However, planktonic cyanobacteria and MCs concentration in the water column have been the subject of many previous studies in terms of risk assessment, little is known about benthic species and MCs concentration in the benthic sediment as well as how their impact on water quality or human and animal health.

The Tri An Reservoir (TAR) is the largest man-made reservoir in Southern Vietnam. It supplies potable water to Ho Chi Minh City, Thu Dau Mot, and Bien Hoa, where the total populations exceed 10 million people. Although blooms of toxic cyanobacteria in the TAR have been reported during the last decade, where *Microcystis* was dominant in samples collected from the bloom samples (Dao *et al.*, 2016), the quantitative reports on the benthic cyanobacteria and MCs concentration in the benthic sediment have not been conducted yet. This study aimed to investigate the community structure of the benthic cyanobacteria and measure the MCs concentration in the sediment of the Tri An Reservoir. Results of this study will support evaluating the risk associated with the production of highly potent hepatotoxins MCs by benthic cyanobacteria in benthic environments.

MATERIALS AND METHODS

Sample collection

Benthic cyanobacteria and sediment were collected at 5 stations (TA1, TA2, TA3, TA4, and TA5) in the TAR (Fig. 1) in dry (April) and rainy seasons (October), 2019 and transported to the laboratory using a cool box with ice. The surface sediment samples were collected using the Ponar Grab and kept in 1-L plastic bottles. The sediment samples were dried completely under sunlight and sieved with a standard 100 mesh sieve for further experiments.

Green biofilms, presumed to contain abundant cyanobacteria, were collected on hard substrates in triplicate by scraping three stones or hard surfaces with three toothbrushes over a surface area of 10 cm^2. Samples were preserved in 100 mL plastic bottles and fixed in Lugol solution. Cyanobacterial samples were identified to the species or sub-species level using the identification books of Komárek and Anagnostidis (1989, 1999, 2005). All samples were collected along the banks at the depth between 0.5 m and 1.0 m.

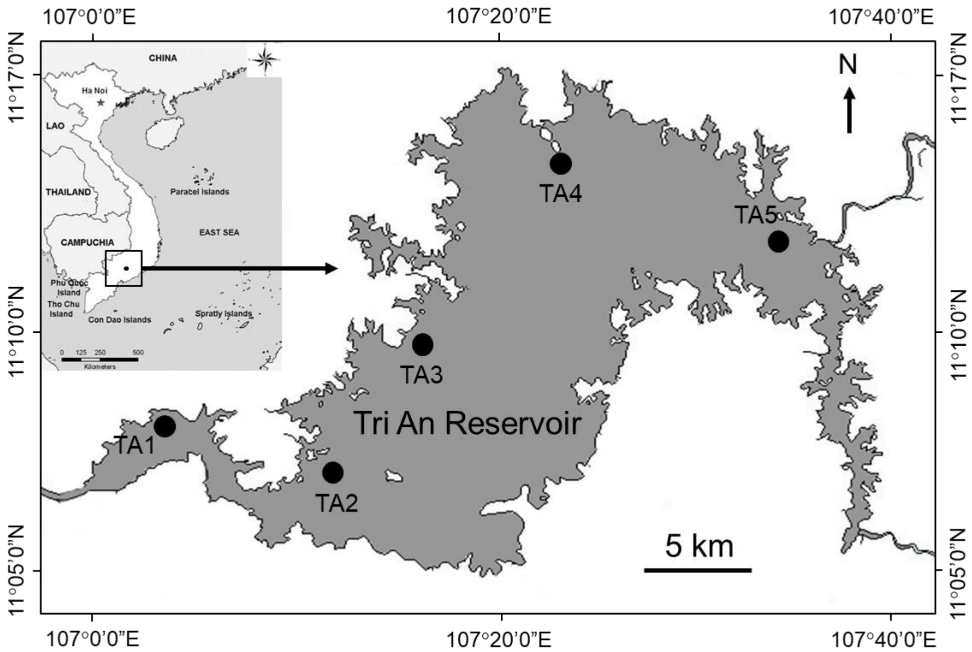

Figure 1. Map of the Tri An Reservoir with five sampling sites.

Analysis of nitrogen and phosphorus in sediment

The concentration of $N\text{-}NH_4^+$ (mg/100g), $N\text{-}NO_3^-$ (mg/100g), $P\text{-}PO_4^{3-}$ (%), TP (%), and TN (%) were measured in the sediment according to the method of Li et al. (2017). Ammonium, nitrate, and TN were determined using a spectrometric technique according to Nessler's method, while TP and $P\text{-}PO_4^{3-}$ were determined from the $H_2SO_4\text{-}HClO_4$ digestion through the dinitrophenol colorimetric technique.

Chlorophyll-a extraction and measurement

To measure the chlorophyll-a (Chl-*a*) concentration in sediment, about 15 g of the wet weight of sediment was extracted with 10 mL acetone 90% overnight with shaking in darkness. After centrifugation at 3000 rpm for 15 min, the supernatant was collected and the Chl-*a* concentration was measured at 630–750 nm using a spectrophotometer (UV-VIS, Harch, 500), and calculated using the trichromatic equations (APHA, 2005).

Microcystins extraction and measurement

To extract the MCs content in the sediment, about 10 g fresh weight (fw) of sediment were individually homogenized and extracted with 10 mL of 100% MeOH for 60 min at room temperature, followed by two 60-min extractions in 10 mL of 80% (vol/vol) aqueous MeOH. Each extraction step was completed with the aid of sonication for 3 min and centrifugation at 1,800×*g* for 20 min at 4°C. The supernatants of all extractions were collected and diluted with MQ water. And then the filtrate was purified on an HLB cartridge (60 mg, Waters, MA, USA) for solid-phase extraction. The MC fraction in the cartridge was collected using 3 mL of MeOH (100%) and dried. The residue was then diluted in 0.5 mL of methanol. Samples were then passed through a Minisart filter membrane into a vial and kept at –20°C before high-performance liquid chromatography (HPLC) analysis. Samples were prepared in triplicate.

To measure the MC concentration, the HPLC system equipped with a UV-VIS detector (Dionex UltiMate 3000, Thermo Scientific, Waltham, MA, USA) was used. Samples were carried with a mobile phase consisting of methanol:0.05 M phosphate buffer (pH 2.5; 50:50 v/v) at a flow rate of 0.65 mL/min. The MCs were separated with a silica-based reverse-phase C18 column (Acclaim^{M} 120 C18 5 µm, 4.6 × 150 mm, Waltham, MA, USA) maintained at 40°C. The MC congeners were distinguished by UV at 238 nm and identified based on retention time and UV spectra. Three MC variants were used as standards, including an MC-LR, MC-RR, and MC-YR purchased from Enzo Lifesciences (Farmingdale, NY, USA). The HPLC system had a detection limit of 0.12 µg/L.

Statistical analyses

Samples of MCs were performed in triplicate. The data are expressed as means ± SD. The relationships between the cyanobacterial metrics and the sediment variables were assessed by Pearson correlations. Differences between dry and rainy seasons were tested for significance using a one-way analysis of variance (ANOVA). When the ANOVAs were significant, we conducted a pairwise comparison using Tukey's HSD post-hoc test to detect the significant differences. *P*-values less than 0.05 were considered statistically significant. All variables were log-transformed to normalize their distributions before analysis.

RESULTS

Nitrogen and phosphorus concentration in sediment

The concentration of different nitrogen and phosphorus species including $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, $P\text{-}PO_4^{3-}$, TP, and TP in sediment in dry and rainy seasons were shown in Table 1. The sediment was contaminated with nitrogen species; $N\text{-}NH_4^+$ ranged from 8.7 to 23.5 mg/100 g and from 13.6 to 22.7 in dry and rainy seasons, respectively; $N\text{-}NO_3^-$ concentration ranged from 0.3 to 2.2 mg/100 g and 0.9 to 2.3 mg/100 g in dry and rainy seasons, respectively; TN ranged from 0.23 to 0.29% and 0.24 to 0.38% in dry and rainy seasons, respectively; while $P\text{-}PO_4^{3-}$ ranged from 0.003 to 0.022% and 0.01 to 0.018% in dry and rainy seasons, respectively; and TP ranged from 0.018 to 0.035% and 0.075 to 0.083% in dry and rainy seasons, respectively.

Benthic cyanobacteria composition and density

The benthic cyanobacteria composition recorded in the Tri An Reservoir was shown in Table 2. In total 19 species belonging to 12 genera, 3 orders of cyanobacteria were identified. In which the three genera of *Anabaena*, *Oscillatoria,* and *Microcystis* were the most common found in the samples. The total number of species recorded in the dry and rainy seasons was 17 and 14 species, respectively.

The present survey of the benthic cyanobacteria revealed the dominance of the order Oscillatoriales along the two fieldtrips with 9 species, the Nostocales come the next with 6 species and the Chroococcales come the last with 4 species. In total, 13 species were present in both seasons (Table 1).

Table 1. Nitrogen and phosphorus concentration in dry and rainy seasons.

Season	Species	TA1	TA2	TA3	TA4	TA5
Dry season	$N\text{-}NH_4^+$ (mg/100 g)	9.8	8.7	11	19.3	23.5
	$N\text{-}NO_3^-$(mg/100 g)	2.2	2	1.2	0.3	2.1
	$P\text{-}PO_4^{3-}$ (%)	0.005	0.003	0.005	0.007	0.022
	TP (%)	0.022	0.018	0.02	0.028	0.035
	TN (%)	0.29	0.23	0.23	0.24	0.27
Rainy season	$N\text{-}NH_4^+$ (mg/100 g)	15.0	21.3	18.2	13.6	22.7
	$N\text{-}NO_3^-$(mg/100 g)	2.0	1.4	0.9	0.9	2.3
	$P\text{-}PO_4^{3-}$(%)	0.010	0.014	0.011	0.011	0.018
	TP (%)	0.075	0.078	0.075	0.077	0.083
	TN (%)	0.29	0.31	0.24	0.25	0.38

Table 2. The benthic cyanobacteria composition recorded in the Tri An Reservoir.

No.	**Species name**	**Dry**	**Rainy**
	Chroococcales		
1	*Merismopedia* sp.	+	
2	*Microcystis aeruginosa*	+	+
3	*Microcystis botrys*	+	+
4	*Microcystis wesenbergii*	+	+
	Oscillatoriales		
5	*Geitlerinema splendidum*		+
6	*Lyngbya* sp.	+	+
7	*Oscilatoria perornata*	+	
8	*Oscillatoria kawamurae*	+	+
9	*Oscillatoria limosa*	+	+
10	*Oscillatoria tenuis*	+	+
11	*Phormidium* sp.		
12	*Planktolyngbya limnetica*	+	+
13	*Pseudanabaena linetica*	+	
	Nostocales		
14	*Anabaena affinis*	+	+
15	*Anabaena circinalis*	+	
16	*Anabaena flos-aquae*	+	+
17	*Arthrospira massartii*	+	+
18	*Cylindrospermopsis raciborski*	+	+
19	*Fischerella* sp.	+	+
	Total	17	14

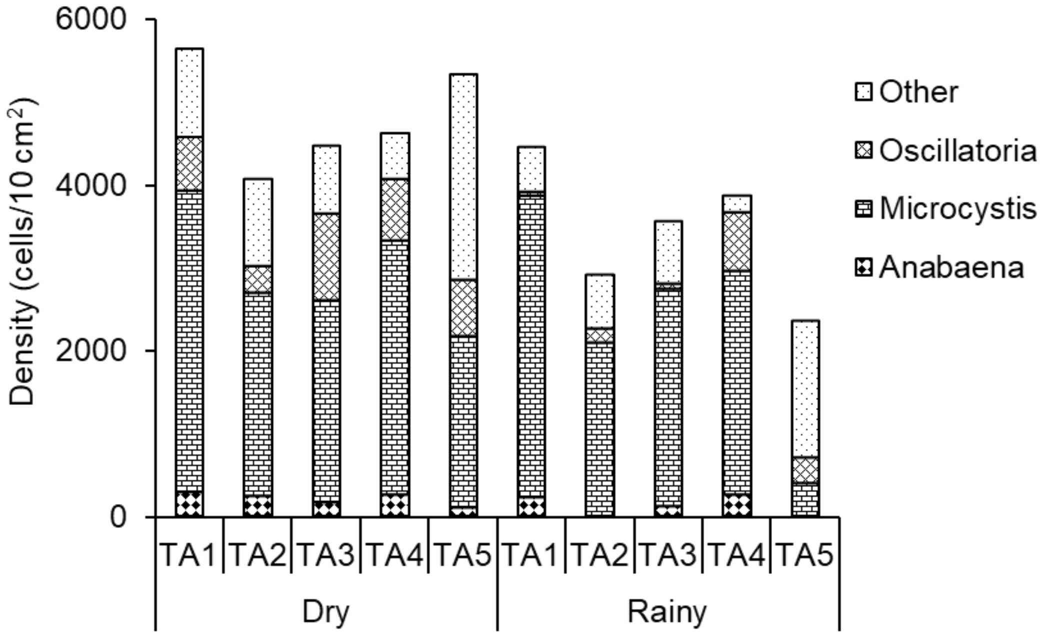

Figure 2. The abundance of benthic cyanobacteria in the Tri An Reservoir.

The abundance of the benthic cyanobacteria was different in both seasons and ranged from 4,065–5,646 to 3,430–4,455 cells/10 cm^2 in dry and rainy seasons, respectively (Fig. 2). The abundance of benthic cyanobacteria at most sites in the dry season was higher than in the rainy season. The abundance of *Microcystis* was dominant in the samples at all sites.

Chlorophyll-a concentration in sediment

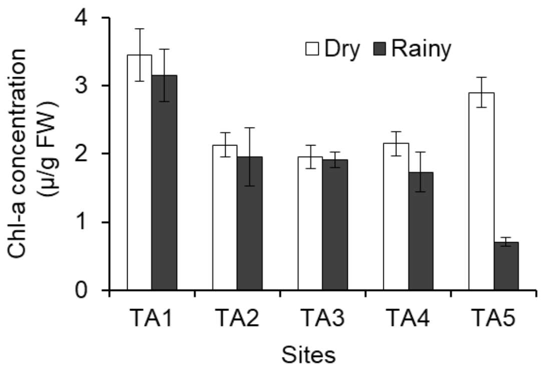

Figure 3. Chlorophyll-a concentration in sediment in the Tri An Reservoir.

The concentration of Chl-a in sediment in the Tri An Reservoir in both seasons was shown in Fig. 3. The Chl-a concentration in dry and rainy seasons ranged from 1.96–3.45 and 0.71–3.15 µg/g FW, respectively. The Chl-a concentration was higher at the TA1 than in the other site in both seasons, while the Chl-a at sites of TA2, TA3, TA4 were almost the same in both seasons. And the Chl-a at TA5 in the dry was higher than in the rainy season.

Microcystins concentration in sediment

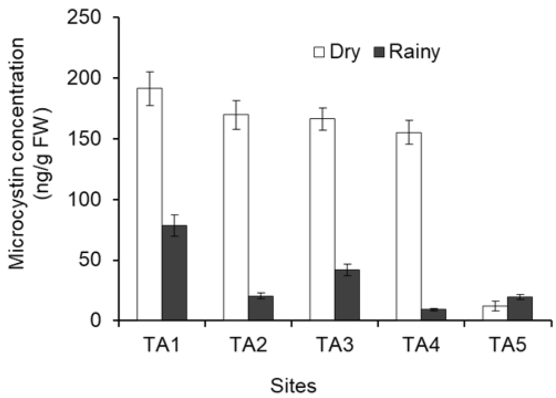

Figure 4. Microcystins concentration in sediment in the Tri An Reservoir.

The results of the HPLC analysis revealed the presence of MCs in the sediment with a concentration of up to 191.4±14.2 ng/g FW. The MCs concentration in the sediment in dry and rainy seasons ranged from 12.1–191.4 and 9.1–78.6 ng/g FW, respectively (Fig. 4). In most stations, MCs concentration detected in the dry season was higher than in the rainy season. And the concentration of MCs detected at site TA5 was often lower than other sites.

DISCUSSION

Nitrogen and phosphorus (P) are essential nutrients for aquatic organisms for primary production in lake ecosystems. The excessive of nitrogen (N) and phosphorus (P) is a key factor for eutrophication (Wan et al., 2020). Lake sediment plays an important role in water quality by acting as a nutrient source and sink (Lee et al., 2019). The accumulation of different N and P species in the sediment can be a reduction of nutrient loading to the overlying water column, but the release of N and P from sediment could be increased the internal load to the water column. Therefore, measurement the concentration of different N and P species in the sediment is important to understand the effect of sediment release on nutrients and contaminants on water quality as well as the interaction on benthic communities. This study indicated that the sediment in the Tri An Reservoir was contaminated with nitrogen, especially the ammonium concentration at the TA5 station in both seasons. This may be the result of the fish farming activities in this area.

Most previous studies investigated only planktonic cyanobacteria in aquatic ecosystems because of the difficulties in sampling and quantification cyanobacterial cells in sediments (Pham, Utsumi, 2018). Quantifying cyanobacterial cells in sediments is more difficult than in the water column, thus few investigations have been conducted on the benthic cyanobacterial communities. The presence of *Anabaena*, *Oscillatoria,* and *Microcystis* were observed in surface sediments at all sites in the Tri An Reservoir confirmed they are the three most common genera present in both planktonic and benthic environments in the Tri An Reservoir (Pham et al., 2020). The high number of cyanobacterial species recorded in the dry season may result from the light availability along the banks. The abundance of *Microcystis* and other cyanobacteria in sediment at the TA1 was often higher than in other stations in both seasons. In contrast, the cell number of cyanobacteria at the TA5 was high in the dry season but not in the rainy season. Previous studies indicated that the density of cyanobacteria in lake sediment depends on numerous factors such as water flow, water depth, light availability, sediment composition as well as redox conditions (Kravchuk *et al.,* 2011; Legrand *et al.*, 2016; Bormans *et al.*, 2020). The high abundance of cyanobacteria at the TA1 may result from high transparency in this area while the low density of cyanobacteria at the TA5 may result from the high concentration of total suspended solids (TSS) in the rainy season. Our results also indicated that the presence of cyanobacteria in sediments is consistent with the dominant species in the water column (Pham *et al.,* 2020).

Due to the difficulties in extraction, little attention has been given to the quantification of cyanotoxins in benthic sediments. Analysis of MCs in the aquatic environment has been limited to determining the MC content in water in previous studies (Pham, Utsumi, 2018). However, MCs have been detected in the sediments where no benthic cyanobacteria were observed (Umehara *et al.,* 2017). Although the concentration of MCs in sediment was temporal and spatial differences, the MC content in sediment often corresponds to the MC in the water column and the abundance of cyanobacteria in sediment (Gurbuz *et al.*, 2016; Umehara *et al.*, 2017). The concentration of MC has been reported from 20.4 to 168.1 ng/g in Lake Taihu, China (Chen *et al.,* 2008). Gurbuz *et al.* (2016) reported that several MC variants were detected in sediment from Lake Eğirdir, Turkey, in which MC-YR was present in samples between 7.0 and 17.6 µg/g dw, especially in October, November, and

December when no MC-YR was recorded in water, followed by MC-LW. When monitoring the co-occurrence of cyanobacteria and MC in both the water column and surface sediments at five stations along the Saint Eloi River from a freshwater reservoir to the coastal area in Brittany, Pen Lann estuary, France, Bormans *et al.* (2020) reported that MC was detected in sediment at all sites with the concentration up to 2315 µg/kg DW. Thus, toxic cyanobacteria with their toxin can travel through the water flow, sometimes into the sea (Umehara *et al.*, 2017). MC concentration has been reported in water from the Tri An Reservoir (Dao *et al.*, 2016; Pham *et al.*, 2020). In the present study, we reported the MC contaminated in benthic sediment, we confirmed that MC was present in different components of the aquatic ecosystems. Therefore, MC in sediment should be considered when performing investigations of cyanotoxins in aquatic environments.

The presence of MC in sediments could come from several processes including the sedimentation of toxic cyanobacterial cells or other detritus contained MC from water column, transfer or/and adsorption of dissolved MCs to benthic phase, and the production of benthic cyanobacteria (Pham, Utsumi, 2018; Bormans *et al.*, 2020). Bormans *et al.* (2020) reported that MC in sediment could come from preserving both colonial cyanobacteria from cells lysing and probably also MC from degradation. Our results showed that the MC concentration in sediment is a positive correlation with the abundance of benthic cyanobacteria. Probably, several benthic cyanobacterial species from the Tri An Reservoir can produce MCs. These results are in agreement with Izaguirre *et al.* (2007) that several MC-producing benthic filamentous cyanobacteria were isolated from several drinking-water reservoirs in southern California, USA. Nevertheless, these results are still preliminary and there is still a need to further investigation on the toxic producing ability of the benthic cyanobacteria species from the Tri An Reservoir.

CONCLUSION

The results of this study confirm the occurrence of benthic cyanobacteria and the accumulation of MC in benthic sediment. The presence of several genera of cyanobacteria and MC in the surface sediment indicates that sediment could play as a potential source of MC in the lake ecosystems. *Anabaena*, *Oscillatoria*, and *Microcystis* are the three most common genera present in benthic environments in the Tri An Reservoir. The outcome of this study provides basic knowledge on MC and potential MC-producing cyanobacteria in a drinking water supply. Further investigation on the toxic producing ability of the benthic cyanobacteria species from the Tri An Reservoir is required.

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