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Spatial and temporal variations in cyanobacteria and microcystins in Aha Reservoir, Southwest China*

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Abstract Toxic cyanobacteria (TCB) are well-known worldwide for their adverse impacts on humans. Species compositions and seasonal variations of TCB in reservoirs depend on interactions between physical and chemical factors. This study was conducted to evaluate the water quality in the Aha Reservoir, Southwest China, focusing on cyanobacteria and cyanotoxins. Water samples were collected weekly or biweekly from May to September of 2015 and used to delineate temporal variations in density and distribution of toxic cyanobacteria and cyanotoxins in the reservoir. Toxic cyanobacteria identified consisted of *Aphanizomenon flos-aquae*, *Pseudanabaena limnetica*, *Cylindrospermopsis* sp., and *Microcystis* sp., with *Aphanizomenon flos-aquae* and *Pseudanabaena limnetica* being the most common and significant toxic genera. The total biomass of cyanobacteria was 17.0 mg/L. Identification and quantification of microcystin variants were conducted by high performance liquid chromatography (HPLC) using a system equipped with a photodiode array detector. Microcystin levels were between 0–3.0 μg/L, MC-RR was around 0–3.0 μg/L and MC-LR was approximately 0–0.9 μg/L. Overall, the results of this study indicate that the investigated reservoirs should be monitored regularly to minimize potential health risks to the human population.

Keyword: Cyanobacteria; *Aphanizomenon flos-aquae*; Microcystin-LR; Microcystin-RR; Aha Reservoir

1 INTRODUCTION

Cyanobacteria are considered an important water quality problem. Blooms of these organisms release toxic secondary metabolites known as cyanotoxins (Park et al., 2001; Li et al., 2008; Carmichael and Boyer, 2016), which have been shown to cause numerous animal deaths and may be a hazard to human health (Hudnell, 2010; McGregor et al., 2011; Zanchett and Oliveira-Filho, 2013; Watson et al., 2016). More than 40 species of cyanobacteria have been reported in aquatic ecosystems (Jayatissa et al., 2006; Messineo et al., 2006; Spoof et al., 2006). Hepatotoxic cyclic peptide toxins (microcystins, MCs, and nodularins), which are the most widespread cyanotoxins, are present in diverse environments. Microcystins are cyclic heptapeptides produced by different cyanobacteria genera including *Microcystis*, *Anabaena*, *Plankothrix* and *Nostoc* (Messineo et al., 2006; Spoof et al., 2006; Aráoz et al., 2010). Several incidents of wild and domestic animal poisoning and death have reportedly been caused by blooms of toxic cyanobacteria (Chapman and Schelske, 1997; Wiedner et al., 2008; Carmichael et al., 2016). Cyanobacterial blooms are currently very common in China because of eutrophication. *Microcystis* sp., *Anabaena* sp., *Planktothrix* sp. and *Oscillatoria* sp. are the most frequently reported cyanobacterial genera and microcystin-producing species that cause blooms in freshwater (Chen et al., 2008; Dong et al., 2012; Dai et al., 2012).

In southwest China, cyanobacterial blooms have been observed in reservoirs and lakes in the Yunnan-Guizhou plateau. These blooms have caused odor

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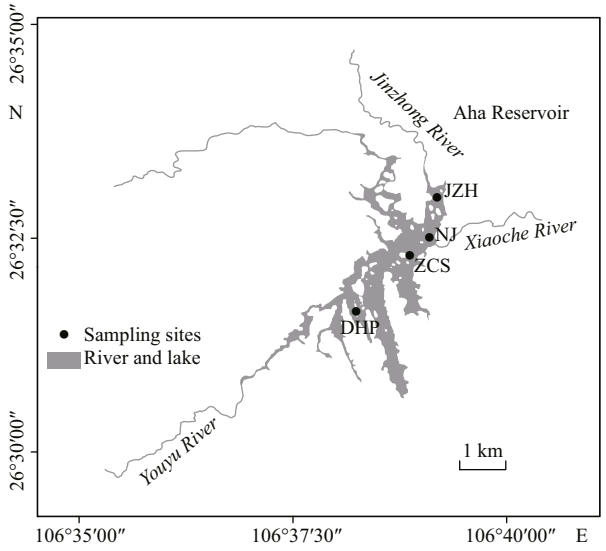

Fig.1 Map of sampling sites in Aha Reservoir

DHP: Dahuangpo; ZCS: Zhongcaosi; JZH: Jingzhonghe; NJ: Nanjiao, near the dam of the Aha Reservoir.

problems and reduced water clarity (Wu et al., 2012). The Aha Reservoir is an essential strategic water resource which, like many other water bodies in China, is primarily used for drinking purposes (He et al., 2015). The Aha Reservoir has been impacted by expansion of the surrounding city in recent years, leading to serious water quality deterioration. In this study, we will evaluate the spatial and temporal variations in cyanobacteria and cyanotoxins and the relationship of cyanobacteria with changes with environmental factors.

2 MATERIAL AND METHOD

2.1 Study area

The Aha Reservoir (AHR), which was built in 1958, has a capacity of 0.87×10^8 m^3 and a water residence time of 0.325 a. The reservoir is located near Guiyang, in Guizhou Province (Li, 2018), Southwestern China. The maximum monthly precipitation in the catchment occurs between April and September (1 140–1 200 mm). Other characteristics of the reservoir are summarized in Table 1. The primary purpose of the reservoir was changed from irrigation to drinking water supply in 2000, and it is now an important source of water to residents of Guiyang (Table 1).

Four sampling sites were established: DHP (upstream of Aha Reservoir); ZCS (near the middle of the Aha Reservoir); JZR (another important tributary to the Aha Reservoir); NJ (near the dam). Each sampling point was sampled at three depths (surface, middle and bottom). Phytoplankton samples and water samples for water chemistry determination were collected biweekly or once a week from May to September in 2015. Samples were taken with a 30-lm-mesh plankton net and a Van Dorn bottle (Fig.1).

Table 1 Geo-morphological features of the AHR

Geo-morphological features	AHR
Year of impoundment	1958
Volume ($\times10^8$) (km^3)	0.870
Water residence time (year)	0.325
Mean water level (m)	1110
Watershed area (km^2)	190
Maximum depth (m)	24
Mean depth (m)	12
Annual average precipitation (mm)	1 140–1 200
mean outflow rate (m^3/s)	5.19

2.2 Sampling

Water samples (1 L) were collected into PE bottles from different depths of the Aha Reservoir and between various treatment steps in water treatment plants for quantitative toxin determination by PDA/HPLC. For phytoplankton enumeration, take 1.5 L of water sample to the sample bottles from 5 L hydrophore on the spot. Samples were then preserved with 3% formaldehyde solution with migration tube. Solid phase extraction (SPE) of the filtrate was performed using C18 end-capped SPE cartridges (Agilent) conditioned with 15 mL methanol and then washed with 15 mL distillated water (H_2O-MQ). Samples were loaded onto the cartridges, washed with 15 mL H_2O-MQ water, and then eluted with 25 mL 80% methanol (0.02% TFA). The eluent was then dried using a rotary evaporator and resuspended in 1 mL H_2O-MQ.

2.3 Analysis methods

Phytoplankton counting was performed after sedimentation of 1.5 L of samples fixed with 3% formaldehyde solution in a graduated flask. After 48–72 h, the supernatant was syphoned off with a 2-mm diameter hose until obtaining 35 mL of residual concentrated sample. Two concentrated samples containing 0.1 mL each were allowed to settle in a Sedgewick-Rafter chamber. Taxonomical determination and counting were conducted using an Olympus-BX41 microscope at 400× magnification. One sample is made into two microscope slides,

Table 2 Distribution of dominant species

Date	Dominant species
18 May	*Aphanizomenon flos-aquae*
26 May	*Aphanizomenon flos-aquae*, *Ceratium hirundinell*, *Melosira* sp.
9 June	*Ceratium hirundinell*, *Melosira* sp., *Cyclotella* sp.
16 June	*Cyclotella* sp., *Synedra* sp.
23 June	*Cyclotella* sp., *Pseudanabaena limnetica*., *Melosira* sp.
30 June	*Pseudanabaena limnetica*., *Asterionella*sp., *Cyclotella* sp.
14 July	*Pseudanabaena limnetica*., *Cyclotella* sp.
28 July	*Pseudanabaena limnetica*.
11 August	*Pseudanabaena limnetica*., *Synedra* sp., *Cyclotella* sp.
25 August	*Pseudanabaena limnetica*
8 September	*Pseudanabaena limnetica*
22 September	*Pseudanabaena limnetica*.

averaging, and the error should not be greater than 15%. Phytoplankton was classified at the lowest taxonomical rank possible. Species names and the definition of higher taxonomic groups of eukaryotic phytoplankton and cyanobacteria were based on the most recent literature (Krienitz and Bock 2012; Guiry and Guiry, 2016). For every individual taxon, phytoplankton biomass (PB, mg/L) were calculated from abundances (cells/mL) and specific biovolumes (μm^3) estimated by approximating the phytoplankton shapes to simple geometrical solids. Specific biovolumes estimated from direct linear measurements were further verified using standard references (Druart and Rimet, 2008). External standards were prepared for MC-LR and MC-RR (both provided by ALGALCHEN Inc). Samples and standards (10 μL in 20% methanol) were injected into the HPLC (Waters e2695-2998; Column: Luna 5u C18(2) 100A, 250 mm×4.6 mm) using an auto sampler and peaks were compared to standards. The flow rate was 1.0 mL/min and a water (0.02% TFA)/acetonitrile gradient was employed as the mobile phase by transitioning from 30% acetonitrile to 70% acetonitrile over 15 min, which was then held for 5 min. With the PDA detector, the procession was set at 238 nm.

3 RESULT AND DISSCUSION

3.1 Bloom-forming cyanobacteria

At the beginning of the sampling period in May, many phytoplankton genera were found, with the phytoplankton and cyanobacterial biomass reaching 19.2 mg/L and 17.0 mg/L, respectively. The cyanobacterial biomass was high in May, then gradually decreased until the end of the season. Toxic cyanobacteria including *Aphanizomenon flos-aquae*, *Pseudanabaena limnetica*, *Cylindrospermopsis* sp., and *Microcystis* sp. dominated the phytoplankton community in May, while the dominant genus found in the water samples was *Aphanizomenon flos-aquae* in May and *Pseudanabaena limnetica* from July to September (Table 2).

A green surface scum of *Aphanizomenon flos-aquae* was observed during May and June, indicating the occurrence of an *Aphanizomenon flos-aquae* bloom. In the early stages of the bloom, the dominant species was *Aphanizomenon flos-aquae*. At the surface of the JZH sampling site, the cyanobacterial biomass reached 17.0 mg/L on 18 May, while very high biomass were also observed at sampling sites ZCS and NJ. Interestingly, no *Aphanizomenon flos-aquae* was observed in August and September, but *Pseudanabaena limnetica* was. From May 14 to May 26, the dominant species was *Aphanizomenon flos-aquae* and the cyanobacteria bloom was single. The dominant cyanobacteria was gradually replaced by diatoms and dinoflagellates and a bloom observed from June 9–23 consisted of diatoms mixed with dinoflagellates. The water color turned from green to brown and then black with bacillariophyta and pyrrhophyta (Fig.2).

3.2 Microcystins

At the DHP sampling points, the concentration of microcystins was between 0 and 1.3 μg/L, in which the MC-RR was between 0 and 1.2 μg/L, with the maximum observed on 30 June. In addition, MC-LR ranged from 0 to 0.9 μg/L, with the maximum observed on 18 May (Fig.3a). The microcystins ranged from 0 to 2.2 μg/L, the MC-RR was between 0 and 2.2 μg/L and the MC-LR was between 0 and 0.5 μg/L (Fig.3b). The highest concentration of microcystin was found in the water sample collected on 28 July at the JZH sampling point, where the microcystin level was 0–3.0 μg/L and the MC-RR was between 0–3.0 μg/L, the highest was at the bottom on 14 July. The MC-LR was between 0 and 0.5 μg/L, with the maximum being observed at the surface on 24 June at the JZH sampling point (Fig.3c). Microcystin was between 0 and 3.1 μg/L, in which the MC-RR was between 0 and 3.1 μg/L, the highest was on 25 August, while the MC-LR was between 0 and 0.9 μg/L, the maximum in the surface on 18 May at the NJ sampling points (Fig.3d).

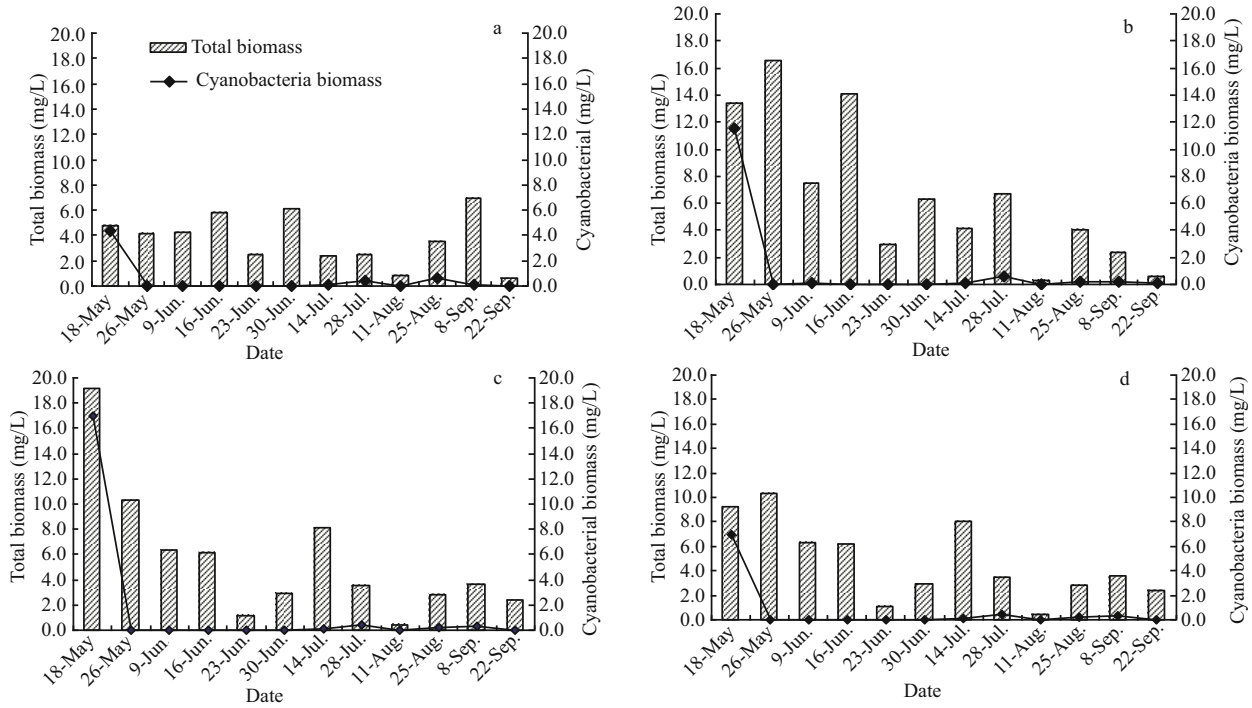

Fig.2 Total biomass and cyanobacterial biomass

a. DHP; b. ZCS; c. JZH; d. NJ.

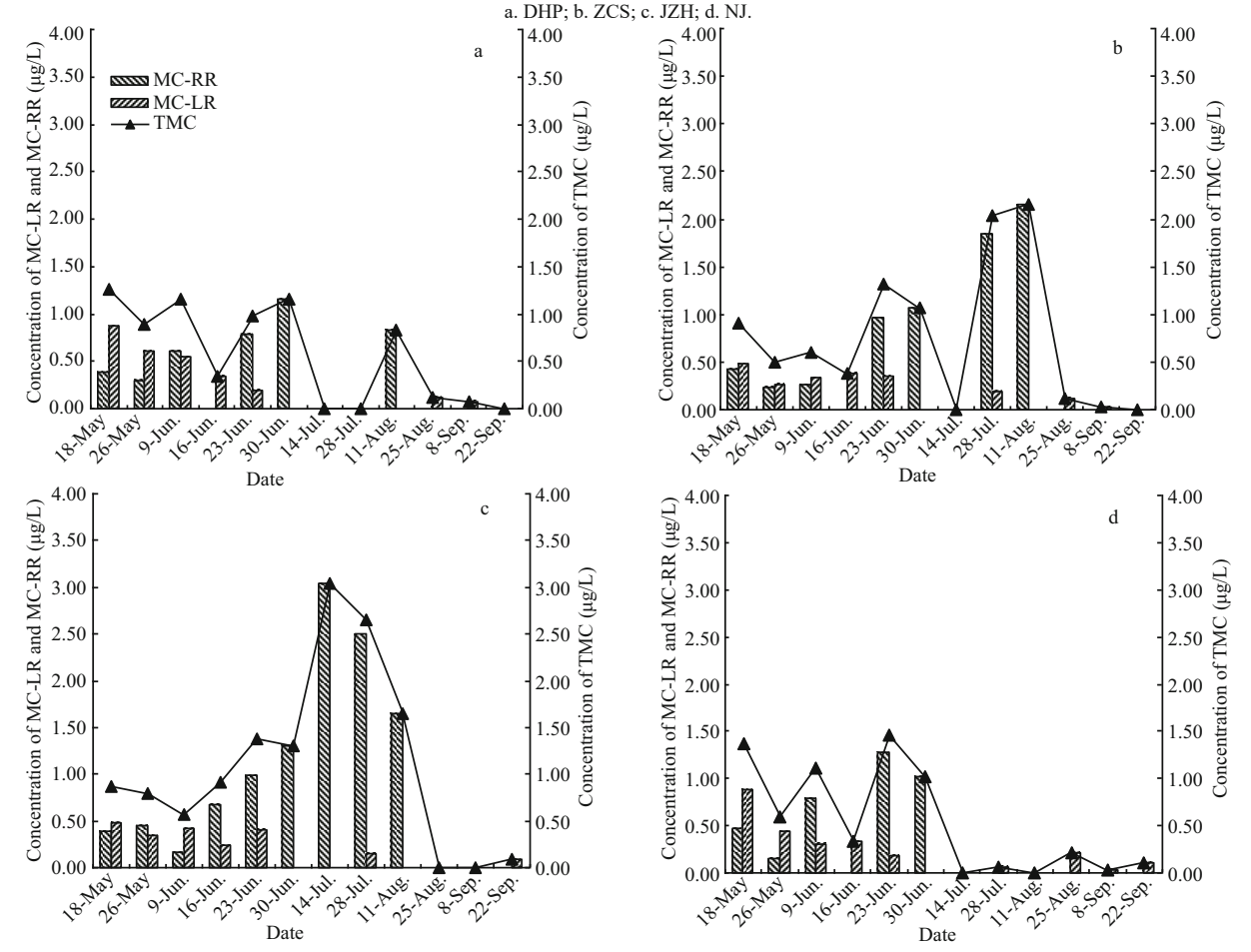

Fig.3 Concentrations of MC-LR, MC-RR and TMC

a. DHP; b. ZCS; c. JZH; d. NJ.

The total microcystin content decreased from 0.5–2.0 μg/L in each phase of the bloom to 3.0–4.0 μg/L at the middle, and then to below the detection limit at the end of the bloom. There are several possible explanations for this phenomenon. First, the dissolved oxygen in the bottom is low because of biomass degradation. The microcystins increased gradually as dead cyanobacteria cells sank and released them during algal blooms. Second, the microcystins decreased during rainfall events and when the reservoir was drained (Fig.3). The contents and proportions of MC-RR were much higher than those of MC-LR, especially at the end of the bloom in August. In nature, the half-life of microcystin is usually 15±5 d (Paerl et al., 2016); however, their degradation can increase under some conditions (Paerl and Otten, 2013). The MC-LR levels in the surface ranged from 0 to 1.0 μg/L, which is within the drinking water health standards (GB5749-2001), and the contents of MC-LR gradually approached zero. According to the World Health Organization (WHO) guidelines, MC-LR should not exceed 1.0 μg/L in raw water.

From May to September, the water temperature was between 21.20°C and 26.40°C. Indeed, temperature is considered an important factor influencing cyanobacterial dominance (Paerl, 1996). Temperatures above 25.0°C promote cyanobacterial blooms, hepatotoxic species, and production of toxins, and increases in temperature have been reported to stimulate cyanotoxin biosynthesis (El-Shehawy et al., 2012). Cyanobacterial growth rates reach their optima and continue to remain high, even when temperatures exceed 25.0°C. Moreover, cyanobacteria attain maximum growth rates at higher temperatures than green algae and diatoms (Mur et al., 1999).

Some other environmental factors have been found to have a strong relationship with the growth of cyanobacteria (Somdee et al., 2013). For example, pH and total nitrogen were associated with the growth of microcystin-producing genera in Finnish Lakes (Rantala et al., 2006). Additionally, nitric nitrogen might be a significant factor promoting *Microcystis aeruginosa* communities (Yoshida et al., 2007). The dominance of *Aphanizomenon flos-aquae* and *Pseudanabaena limnetica* in the Aha Reservoir agreed with its occurrence as the most common bloom-forming cyanobacteria in freshwater bodies worldwide (Yılmaz et al. 2008). *Aphanizomenon flos-aquae* is a cylindrospermopsin producing cyanobacterium that has been identified in eutrophic water bodies worldwide. These organisms severely endanger environmental safety and human health because they produce paralytic shellfish poisons (PSPs). *Microcystis* sp. has been replaced by this genus as the most commonly detected toxin producing species in reservoirs in recent years (Chapman and Schelske, 1997).

4 CONCLUSION

The results of this study clearly demonstrated that MC-LR and MC-RR were present in Aha Reservoir. The highest MC-LR content of 0.9 μg/Lwas found at the NJ sampling point on 18 May 2015, whereas the highest microcystin-RR content of 3.0 μg/L was found at the JZH sampling point on 14 July 2015. Microscopic examination of the phytoplankton samples showed the dominance of *Aphanizomenon flos-aquae*, *Pseudanabaena limnetica*, *Cylindrospermopsis* sp., and *Microcystis* sp. This is the first report providing evidence of the presence of cyanotoxins in Aha Reservoir, Southwest China. The results presented herein suggest that cyanobacterial toxins can be produced by several cyanobacterial species in Aha Reservoir; therefore, these compounds pose a risk to water ecological security and potential health risks to the human population.

5 DATA AVAILABILITY STATEMENT

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

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