



Seasonal and spatial variations of microcystins in Poyang Lake, the largest freshwater lake in China

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Abstract

Poyang Lake is the largest freshwater lake in China and an important drinking water source. Since the year 2000, toxic cyanobacteria have been observed frequently in Poyang Lake. In the present study, spatial and seasonal variations of microcystins (MCs; MC-RR, MC-YR, and MC-LR) in water column were examined monthly from January to December (except the months of March, May, and November) in 2013, by using ultra-high-performance liquid chromatography-electrospray ionization tandem triple quadrupole/mass spectrometry (UPLC-MS/MS). MC-RR was the most dominant variant, followed by MC-LR, while MC-YR was detected in low concentration. Total MC concentrations (intracellular + extracellular MCs) ranged from 1.26 to 9916.05 ng/L, with an average of 469.99 ng/L, and only 3.14% (6 out of 192 samples) of the water samples contained MC concentrations that exceeded the drinking water guideline level of 1 µg/L for MC-LR proposed by the World Health Organization (WHO). MC concentrations in water column showed obvious seasonal variations in Poyang Lake. Intracellular and extracellular MCs were both at a low level from January to April but increased quickly from June to August and decreased dramatically thereafter. Intracellular MCs exhibited similar spatial distribution pattern with extracellular MCs. Both intracellular and extracellular MC concentrations in eastern bays and around Songmen Mountain of Poyang Lake were higher than other regions. Intracellular MC concentrations were positively correlated with Chl *a* ($r = 0.46$, $P < 0.01$), pH ($r = 0.25$, $P < 0.01$), cyanobacterial biomass ($r = 0.40$, $P < 0.01$), and temperature ($r = 0.36$, $P < 0.01$) but negatively correlated with TN ($r = -0.28$, $P < 0.01$), suggesting that TN, cyanobacteria biomass, pH, and temperature might be regulating factors for MC production in Poyang Lake.

Keywords Cyanobacteria · Microcystins · Seasonal variation · Spatial distribution · Poyang Lake

Introduction

The frequent occurrence of cyanobacteria blooms in freshwater has become a serious problem worldwide (Brookes and Carey 2011). Several species of cyanobacteria (e.g., *Microcystis*, *Planktothrix*, *Oscillatoria*, and *Anabaena*) can

produce an impressive range of toxins including neurotoxins, hepatotoxins, cytotoxins, and lipopolysaccharide endotoxins (Metcalf and Codd 2012). Among these cyanotoxins, microcystins (MCs) are the most common and dangerous groups in freshwater (Chorus and Bartram 1999). Until now, more than 90 MC variants have been recognized (Pantelic et al. 2013).

Toxicological studies indicated that MCs mediate their toxicity by uptake into hepatocytes via a carrier-mediated transport system, and then inhibits the activity of serine/threonine protein phosphatases 1 and 2A (Falconer et al. 1981). This inhibition leads to protein phosphorylation imbalance, followed by the disruption of the liver cytoskeleton, finally resulting in massive hepatic hemorrhage and thus the cause of death (Pacagadhi and Balasubramanian 2013). Exposure to MCs represents potential health risks to aquatic organisms and humans (Malbrouck and Kestemont 2006). Previous studies have evidenced that the high incidence of primary liver cancer

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in the eastern region of China is attributed to MC contaminations in drinking water (Yu 1995). In addition, MCs were identified in the serum from fishermen at Lake Chaohu, China (Chen et al. 2009).

Many research have reported that occurrences of MCs in natural environment are highly variable due to the tremendous differences of cyanobacterial biomass in freshwater with different tropic status and environmental factors (Dai et al. 2016; Sinang et al. 2013; Wu et al. 2006), and these relationships between MCs and environmental parameters are sometimes different or even contradictory (Dai et al. 2016; Sinang et al. 2013; Salmaso et al. 2014; Zhang et al. 2015; Singh et al. 2015).

This might be due to the complicated interactions of various environmental factors in different water bodies. And thus, environmental drivers of MC variability are needed to study extensively, to explore the mechanism of MC occurrence in the field.

Poyang Lake, the largest freshwater lake, as well as a typical Yangtze-connected lake in China, is located in northern Jiangxi province. Five main tributaries (Ganjiang River, Xiushui River, Raohe River, Xinjiang River, and Fuhe River) drain into the lake from the south and freely exchanges water with the Yangtze River in Hukou (Fig. 1). The water level varies largely in different seasons, depending on the balance between Poyang Lake and Yangtze River (Shankman et al. 2006). In recent years, the environment of Poyang Lake has undergone great changes due to the rapid development of economy and increased human activity, of which eutrophication is the most serious (Liao et al. 2017). Meanwhile, MCs were found in Poyang Lake many years ago (Sui et al. 2007; Wu et al. 2006; Zhang et al. 2015). Due to the special geographical conditions and unique hydrological regime, studies on seasonal and spatial variations of MCs and its relationship with driving factors are essential.

The aims of the present study are to (a) examine seasonal and spatial variations of MCs in Poyang Lake and (b) determine the potential environmental drivers of MC variability.

Materials and methods

Study area

Poyang Lake (28°4′–29°46′N, 115°49′–116°46′E) lies on the southern bank of the middle and lower reaches of the Yangtze River. The lake is an important source for drinking water, as well as water for irrigation, aquaculture, and the industries. This lake serves all the main functions of an ecosystem, including provisioning, supporting, and regulating environmental functions as well as providing cultural and recreational functions for local residents (Li et al. 2009). The floodplains are inundated and form a large lake with an inundation area

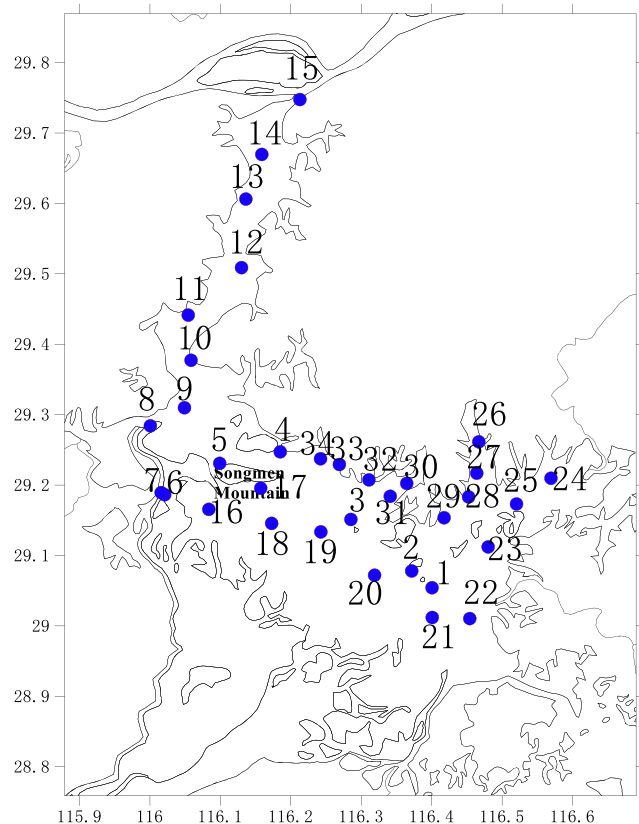


Fig. 1 The sampling sites in Poyang Lake during the study period

reaching >4000 km² during the wet season from April to September (Shankman et al. 2006). During the dry season from October to March, the inundation area of the lake can shrink to <1000 km² to form a narrow, meandering channel.

Reagents and standards

MC-RR and MC-LR standards were purchased from Taiwan Algal Science Inc. (Taiwan, China), and MC-YR standard was purchased from Alexis Biochemical Corporation (Lausen, Switzerland). Methanol, formic acid, and ammonium acetate were of HPLC grade. Methanol was obtained from Tedia company Inc. (OH, USA), formic acid was purchased from Aladdin Industrial Corporation (Shanghai, China), and ammonium acetate was obtained from Alfa Aesar (MA, USA). Other reagents used were of analytical grade.

Sample collection

Thirty-four sampling stations (numbered 1–34) were established according to the geographic characteristics of Poyang Lake (Fig. 1). According to the variation of water level in Poyang Lake and the occurrence of cyanobacteria in this lake, field samples were collected from January to December (except months of March, May, and November) 2013. Sites 1–15 were sampled all the studied months, while

water samples of other sites (sites 16–34) were collected from June to August during the rainy season. Each sample was a mixture collected from the top (0–0.5 m surface water) and the bottom (0–0.5 m over sediment) of the water column with a 5-L Schindler sampler.

Water quality parameters

In the field, transparency was measured by a Secchi disk, and the water depth was measured using a digital ultrasonic echosounder (Umwet and Wissenschafts Technik, Mondsee, Austria). Water temperature, conductivity, turbidity, and pH were determined using a five-star portable multi-parameter measuring instrument (Orion, CO, USA). Chlorophyll *a* (Chl *a*) was analyzed by using hot-ethanol methods (Chen and Gao 2000). Chemical oxygen demand (COD_{Mn}), total nitrogen (TN), and total phosphorus (TP) contents were analyzed according to APHA (1998).

Phytoplankton

One-liter integrated water sample for phytoplankton was preserved with 1% acidified Lugol's iodine solution and concentrated to 30 mL after sedimentation for 48 h (Chen et al. 2003). After mixing, 0.1 mL concentrated samples were counted directly under ×400 magnification. Taxonomic identification was made according to Hu et al. (1979) and biomass was estimated from approximate geometric volumes of each taxon, assuming that 1 mm³ equals 10⁻⁶ μg fresh weight (Shei et al. 1993). The geometric dimensions were measured on 10–30 individuals for each dominant species.

MC extraction and analysis

MC extraction and analysis were conducted according to the method of Zhang et al. (2015). The limit of detection (LOD) for the MC-RR, MC-YR, and MC-LR were 0.04, 0.03, and 0.02 ng/mL, respectively.

Recovery experiment

Milli-Q water was filtered with GF/C membrane and used as blank matrix. One-liter blank water was spiked with mixed standards of MCs (MC-RR, MC-YR, MC-LR) solution to a final concentration of 10 ng/L. Samples were enriched, purified, and analyzed according to the method of Zhang et al. (2015). Recovery experiments were carried out in triplicate, and the recovery and the relative standard deviation of the analytical method were calculated.

Statistical analysis

The values below the detection limit can be represented in the dataset by half of the detection limit, while the values for the target compounds that were not detected in the samples were set by zero.

The SPSS (Chicago, IL, USA) for Windows (Ver 13.0) statistical software was used for all analyses. The spatial distribution maps of extracellular and intracellular MCs were constructed using Surfer 8.0 (Golden, Greensboro, NC, USA).

Results

Recovery test

The mean recoveries ($n = 3$) of MC-RR, MC-YR, and MC-LR were 78.21% (ranging from 74.25 to 84.10%), 90.25% (ranging from 85.75 to 94.38%), and 96.57% (ranging from 92.40 to 98.32%), respectively. The relative standard deviations (RSDs) of MC-RR, MC-YR, and MC-LR were 5.98, 6.12, and 6.97%, respectively.

Environmental parameters

The annual mean and ranges of the physicochemical and biological variables of Poyang Lake in 2013 are presented in Table 1. Water depths varied from 0.60 to 19.34 m with an average of 5.53 m. Water temperature varied from 3.37 to 33.70 °C with an average of 22.41 °C. pH, Secchi depth, conductivity, Chl *a*, COD_{Mn}, and turbidity ranged from 6.97 to 8.59, from 0.10 to 1.50 m, from 60.30 to 388.00 μs/cm, from 0.15 to 34.04 μg/L, from 1.10 to 4.82 mg/L, and from 8.20 to 543.30 NTU, respectively, and the average values of these parameters were 7.71, 0.43 m, 149.19 μs/cm, 5.51 μg/L, 2.26 mg/L, and 74.85 NTU, respectively. In this study, average concentrations of nutrient salts, including TN and TP, were 1.82 and 0.09 mg/L, respectively, which ranged from 0.17 to 7.79 mg/L and from 0.02 to 0.48 mg/L, respectively. Cyanobacterial biomass ranged from 0.00 to 8.07 mg/L, with an average value of 0.45 mg/L.

MC concentrations

Three variants (MC-RR, MC-YR, and MC-LR) were identified in water samples of Poyang Lake, among which MC-RR and MC-LR were the main components, while MC-YR was detected in low concentrations (Table 2). Intracellular MC-RR, MC-YR, and MC-LR ranged from below detectable limit to 8067.09 ng/L, from below detectable limit to 30.63 ng/L, and from below detectable limit to 1975.07 ng/L, respectively, and the average values were 372.19, 2.41, and 70.27 ng/L, respectively. Dissolved MC-RR, MC-YR, and MC-LR ranged

Table 1 Mean and ranges of the environmental parameters during the study period of Poyang Lake

Parameters	Min	Max	Mean
pH	6.97	8.59	7.71
Secchi depth (m)	0.10	1.50	0.43
Water depth (m)	0.60	19.34	5.53
Temperature (°C)	3.37	33.70	22.41
Conductivity (µs/cm)	60.30	388.00	149.19
TN (mg/L)	0.17	7.79	1.82
TP (mg/L)	0.02	0.48	0.09
Chl <i>a</i> (µg/L)	0.15	34.04	5.51
COD _{Mn} (mg/L)	1.10	4.82	2.26
Turbidity (NTU)	8.20	543.30	74.85
Cyanobacterial biomass (mg/L)	0.00	8.07	0.45

from below detectable limit to 162.37 ng/L, from below detectable limit to 2.71 ng/L, and from below detectable limit to 44.24 ng/L, respectively, and with mean values of 21.05, 0.32, and 7.38 ng/L, respectively. Total MC concentrations (intracellular MCs + extracellular MCs) ranged between 1.26 and 9916.05 ng/L, with an average of 469.99 ng/L.

Seasonal variations

Seasonal variations of intracellular and extracellular MCs are demonstrated in Fig. 2. Intracellular and extracellular MCs showed similar seasonal changes. Intracellular and extracellular MCs were both at a low level from January to April but increased quickly from June to August while decreased dramatically thereafter. The highest intracellular and extracellular MC concentrations (9871.50 and 166.81 ng/L, respectively) were observed in August, and the lowest values were found in February.

Table 2 Intracellular and extracellular MC levels in Poyang Lake (ng/L)

		RR	YR	LR	Total
Intracellular MCs	Min	0.00	0.00	0.00	0.00
	Max	8067.09	30.63	1795.07	9871.50
	Mean	372.19	2.41	70.27	444.50
Extracellular MCs	Min	0.00	0.00	0.00	0.00
	Max	162.37	2.71	44.24	166.81
	Mean	21.05	0.32	7.38	28.52
Total MCs	Min	0.00	0.00	0.00	1.26
	Max	8083.57	33.35	1823.14	9916.05
	Mean	392.60	2.72	77.13	469.99

Spatial distributions

Both intracellular and extracellular MC concentrations (MC-RR + MC-YR + MC-LR) showed great spatial distributions in Poyang Lake during wet season, and MCs in June, July, and August exhibited a similar spatial distribution pattern, and thus, spatial distribution maps of MCs in Poyang Lake were depicted according to the data collected from August, due to the maximum MC value occurrence in this month. The highest extracellular (166.81 ng/L) and intracellular MC (9871.50 ng/L) concentrations were found at sites 31 and 28, respectively (Fig. 3), and these two sites were located in eastern bays. Generally, intracellular and extracellular MCs showed a similar spatial distribution pattern, namely higher MC concentrations that appeared in eastern bays and around Songmen Mountain of Poyang Lake, while lower concentrations occurred in the west, center, and north region (Fig. 3).

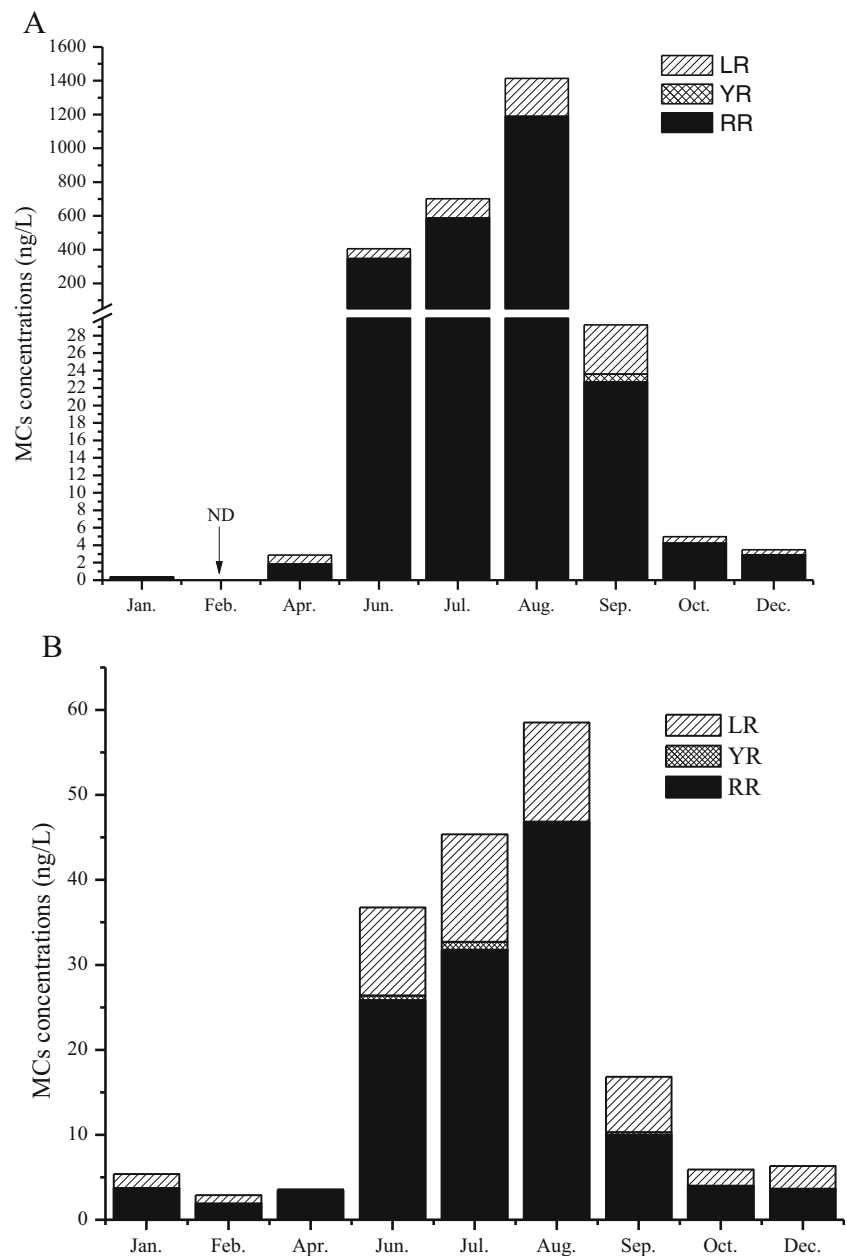
Relationship between MC concentrations and environmental factors

As showed in Table 3, intracellular MC concentrations were positively correlated with Chl *a* ($r = 0.46, P < 0.01$), pH ($r = 0.25, P < 0.01$), cyanobacterial biomass ($r = 0.40, P < 0.01$), and temperature ($r = 0.36, P < 0.01$) but negatively correlated with TN ($r = -0.28, P < 0.01$). No significant correlations were observed between intracellular MC concentrations and TP ($P > 0.05$), turbidity ($P > 0.05$), and COD_{Mn} ($P > 0.05$).

Discussion

It has been well documented that MC production was influenced by a complex suite of interacting environmental factors, such as nutrient inputs (Tao et al. 2012; Yu et al. 2014), hydrodynamics (i.e., turbulence, wind-driven currents, and stratification) (Zhou et al. 2016; Elliott 2012), Chl *a* (Yu et al. 2014), cyanobacteria biomass (Zhang et al. 2015), temperature, and pH (Tao et al. 2012). In the present study, intracellular MC concentrations were positively correlated with Chl *a*, pH, cyanobacterial biomass, and temperature but negatively correlated with TN, and no significant correlations were found between intracellular MC concentrations and TP, conductivity, turbidity, suggesting that TN, cyanobacterial biomass, pH, and temperature might be regulating factors for MC production in Poyang Lake. The not significant correlation between MC concentrations and TP could be due to the fact that TP in Poyang Lake (annual mean of TP was 0.09 mg/L) always exceeded the minimum requirement for cyanobacteria growth of 10 µg/L (Chorus and Bartram 1999). A similar result was also found in Taihu Lake (Tao et al. 2012). Until now, there have been extensive studies on the relationship between MC content and environmental factors, while such relationship

Fig. 2 Seasonal variations of intracellular (a) and extracellular (b) MC concentrations in Poyang Lake, 2013 (ND not detected)



was not always similar among different water bodies (Wu et al. 2006; Yu et al. 2014; Tao et al. 2012; Duong et al. 2013). For instance, MCs were significantly correlated with Chl *a*, cyanobacterial biomass, and NO_3^- in 30 subtropical shallow lakes in China, but MC concentrations showed no significant correlations with water temperature, TN, and TP (Wu et al. 2006). In contrast, MCs exhibited a significant correlation with temperature but had poor relationships with other variables (i.e., relative abundance of cyanobacteria, Chl *a*, *Microcystis*, nutrients) (Duong et al. 2013). It has also been reported that conductivity, water temperature, and PH presented significant correlations with MC cellular quotas in Taihu Lake (Tao et al. 2012). However, no correlations were found

between MC concentrations and all the measured variables in a Germany reservoir (Jungmann et al. 1996). These contradictory relationships can be attributed to different responses of MC-producing cyanobacteria to various environmental parameters (Duong et al. 2013).

In the present study, though intracellular MCs exhibited significant correlations with various environmental factors, the absolute value of coefficients was low ($r < 0.46$). This suggests that various environmental factors (i.e., hydrological parameter) which are not included in our study might be involved in the regulation of MC production. Previous studies pointed out that turbidity, water velocity, and fluctuating water level can extensively affect the phytoplankton abundance and

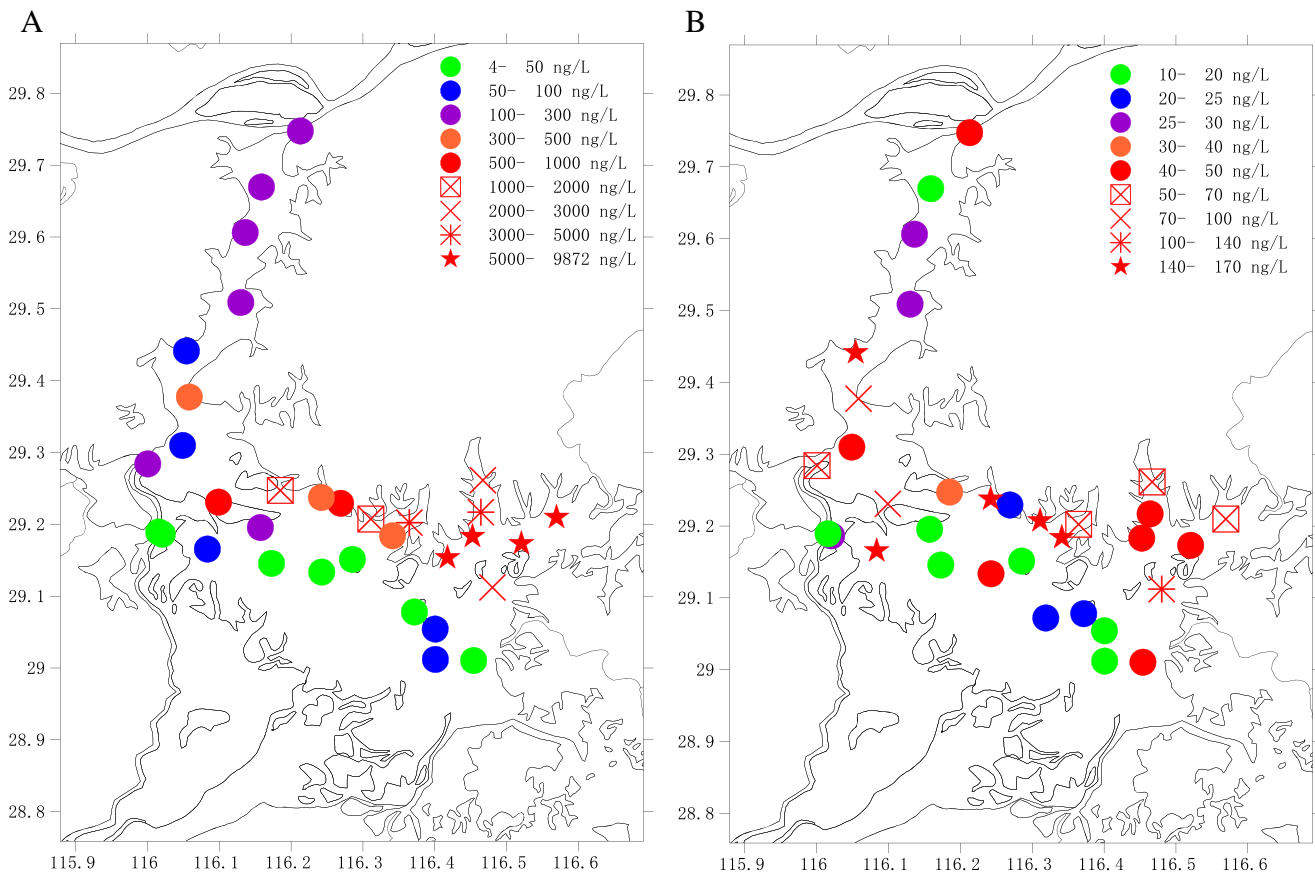


Fig. 3 Spatial distributions of intracellular (a) and extracellular (b) MC concentrations in Poyang Lake

composition in water bodies (Sharma et al. 2007; Crayton and Sommerfeld 1979), and Liu et al. (2016) revealed that hydrological variables play important roles in the cyanobacterial

Table 3 Correlation matrix between intracellular MC concentrations and environmental parameters in Poyang Lake

	MC-RR	MC-YR	MC-LR	MCs
MC-RR	1			
MC-YR	0.77 ^b	1		
MC-LR	0.98 ^b	0.72 ^b	1	
MCs	1.00 ^b	0.77 ^b	0.99 ^b	1
pH	0.25 ^a	0.33	0.29 ^a	0.25 ^b
Temperature	0.35 ^b	0.39 ^b	0.38 ^b	0.36 ^b
Conductivity	-0.15	-0.23 ^b	-0.11	-0.14
Turbidity	-0.063	-0.11	-0.038	-0.060
TN	-0.28 ^b	-0.32 ^b	-0.27 ^b	-0.28 ^b
TP	-0.048	-0.068	-0.04	-0.047
COD _{Mn}	0.11	-0.036	0.12	0.11
Chl <i>a</i>	0.45 ^b	0.39 ^b	0.47 ^b	0.46 ^b
Cyanobacterial biomass	0.41 ^b	0.31 ^b	0.37 ^b	0.40 ^b

^a Correlation is significant at the 0.01 level (two-tailed)

^b Correlation is significant at the 0.05 level (two-tailed)

bloom accumulation in the Yangtze-connected eutrophic lakes of eastern China. Unlike other large shallow eutrophic lakes (i.e., Lake Taihu and Chaohu) along the Yangtze River, Poyang Lake is characterized with its connection to Yangtze River. Its size of water body (>4000 km² during rainy season and <1000 km² during dry season) undergoes great change within a year, and water velocity can reach to 1.48–2.85 m/s when the water level in Xingzi Station of Poyang Lake is below 15 m (Gu and Wan 2011). The above results indicate that hydrological parameter might play an important role in regulating MC production in Poyang Lake, and the effect of hydrological parameters on MC production in Poyang Lake is needed to be examined in our future study.

Previous studies confirmed that seasonal and spatial distributions of MCs are caused by variances of the toxic strains in cyanobacteria blooms (Chorus 2001), and water currents are regarded as the dominant variable determining phytoplankton distribution in some water bodies (Webster 1990; Liu et al. 2016). In the present study, intracellular MCs were closely correlated with cyanobacterial biomass, and extracellular and intracellular MC concentrations exhibited a similar distribution pattern, that is, the MC content in eastern bays and around Songmen Mountain region were higher than that in other regions. The higher MC concentrations in eastern

bays of Poyang Lake might be due to the higher amount of toxic cyanobacteria in these bays, since Liu et al. (2016) reported that a large amount of cyanobacteria are maintained for a longer time in the eastern bays of Poyang Lake due to local clockwise water recirculation. In addition, water in the main part of Poyang Lake must flow through a narrow channel between Songmen Mountain and Duchang County to the Yangtze River, and then a relatively high amount of cyanobacterial biomass accumulated in this region around Songmen Mountain, finally resulting in higher MC concentration in this area. The spatial distribution pattern in our present study was similar with that in Poyang Lake in 2012 (Zhang et al. 2015) and can be attributed to the cyanobacteria distribution driven by water currents and wind (Liu et al. 2016). Meanwhile, in our previous study, we also found that wind speed is considered an important factor for spatial distribution of MCs in Poyang Lake (Zhang et al. 2015). The above results demonstrated that water currents and wind play important roles in spatial distributions of MCs in Poyang Lake and should be examined extensively in the future.

In the present study, intracellular MC contents in Poyang Lake were one to two orders of magnitude higher than extracellular concentrations. The mean annual total MC content in the water column of Poyang Lake was 469.99 ng/L, which is considered to be low when compared with previous studies. The MC level in our present study was comparable with those given from Erhai Lake (Yu et al. 2014) but generally lower to the MC level in Taihu Lake (Tao et al. 2012), Chaohu Lake (Yang et al. 2006), in China; Lake Vancouver in USA (Lee et al. 2015); Lake Biwa in Japan (Ozawa et al. 2005); and Kovada Lake in Turkey (Gurbuz et al. 2009).

In order to avoid potential health risk of MCs to humans, the World Health Organization (WHO) has proposed a provisional guideline level of 1 µg/L for MC-LR equivalent (MC-LReq) in drinking water (Chorus 2001). In the present study, MC-RR and MC-YR were converted to MC-LReq according to the intraperitoneal median lethal dose in mice for MC-RR, MC-YR, and MC-LR reported by Gupta et al. (2003). Though only 3.14% (6 out of 192 samples) of the water samples in Poyang Lake contained MC concentrations above the safety limit of 1 µg/L MC-LReq required for drinking water, the highest MC concentration reached as high as 3.44 µg/L MC-LReq in August. Hence, long-term monitoring of MC contamination in Poyang Lake is needed.

Conclusions

The present study shows that MCs in Poyang Lake may vary by orders of magnitude both seasonally and spatially due to changes in both cyanobacterial biomass and physicochemical/hydrological parameters. TN, pH, and temperature might be regulating factors of MC production in Poyang Lake. It seems

that hydrological characteristics such as water currents play a very important role in spatial distributions of MCs. The effect of hydrological parameters on spatial and temporal variations of MCs in Poyang Lake is needed to be studied extensively in our future work.

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