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# Seasonal dynamics of water bloom-forming *Microcystis* morphospecies and the associated extracellular microcystin concentrations in large, shallow, eutrophic Dianchi Lake

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ABSTRACT

The increasing occurrence of *Microcystis* blooms is of great concern to public health and ecosystem due to the potential hepatotoxic microcystins (MCs) produced by these colonial cyanobacteria. In order to interpret the relationships between variations of *Microcystis* morphospecies and extracellular MC concentrations, the seasonal dynamics of phytoplankton community composition, MC concentrations, and environmental parameters were monitored monthly from August, 2009 to July, 2010. The results indicated that *Microcystis* dominated total phytoplankton abundance from May to December (96%–99% of total biovolume), with toxic *Microcystis viridis* and non-toxic *Microcystis wesenbergii* dominating after July (constituting 65%–95% of the *Microcystis* population), followed by *M. viridis* as the sole dominant species from November to January (49%–93%). Correlation analysis revealed that water temperature and nutrient were the most important variables accounting for the occurrence of *M. wesenbergii*, while the dominance of *M. viridis* was related with nitrite and nitrate. The relatively low content of MCs was explained by the association with a large proportion of *M. viridis* and *M. wesenbergii*, small colony size of *Microcystis* populations, and low water temperature, pH and dissolved oxygen. The extracellular MC (mean of 0.5 ± 0.2 μg/L) of water samples analyzed by enzyme-linked immunosorbent assay (ELISA) demonstrated the low concentrations of MC in Dianchi Lake which implied the low potential risk for human health in the basin. The survey provides the first whole lake study of the occurrence and seasonal variability of *Microcystis* population and extracellular MCs that are of particular interest for water quality monitoring and management.


## Introduction

Harmful cyanobacterial blooms (CyanoHABs) are proliferating and expanding globally due to anthropogenic nutrient enrichment and climatic-induced change, and they represent a serious threat to the ecological integrity and sustainability of our freshwater resources (Heisler et al., 2008; Paerl et al., 2011, 2012; O'Neil et al., 2012). *Microcystis*, the most frequently reported cyanobacterial genus responsible for freshwater cyanobacterial blooms worldwide (Visser et al., 2005), has long been a primary focus of attention because of its potential to produce a potent cyanotoxin called microcystins (MCs) (Haider et al., 2003). Special attention

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has been given to hepatotoxic MCs due to their ability to lead to serious poisoning but also due to their cancer promotion potential to humans which is caused by chronic exposure to low MC concentrations in drinking water (de Figueiredo et al., 2004). Research interest in this genus has increased steadily for the problems associated with *Microcystis* blooms and MCs.

Dianchi Lake is the largest lake in Yunnan Province and the sixth largest freshwater lake in China. It is characterized as having a subtropical climate, and is very important as a water supply for municipal, agricultural, and industrial purposes. In addition, it is also used for aquaculture, tourism, and shipping, and helps to regulate the regional climate, all of which have contributed to the social and economic development of Kunming City. However, the water quality of Dianchi Lake has deteriorated rapidly since the 1980s, and cyanobacterial blooms have broken out frequently in the past 20 years (Liu et al., 2006). Because the lake is downstream of Kunming City, large amount of municipal sewage, industrial wastewater, and high nonpoint loads of nutrients are discharged into the lake, particularly in the rainy season. The warm plateau climate and anthropogenically induced nutrient enrichment favor the extended duration of the cyanobacterial blooms, which may start in March and persist until December, or in some cases, may even be continuous throughout the year.

Preliminary studies on phytoplankton compositions in Dianchi Lake indicated that the *Microcystis* spp. were the dominant phytoplankton assemblages; however, the morphospecies of *Microcystis* was still problematic and even contradictory. The previous results showed that the phytoplankton community was dominated by *Microcystis aeruginosa*, *Microcystis viridis* and *Microcystis wesenbergii* in western Dianchi Lake (Li et al., 2005; Pan et al., 2006), whereas Wan et al. (2008) revealed that *M. aeruginosa* was the most common species, and the percentage sometimes reaches 100% from 2001 to 2002. According to the latest research carried out in the northern lake, Dai et al. (2012) found that dominant species were *M. viridis* and *M. wesenbergii* in December and June. Furthermore, the response of dominated *Microcystis* morphospecies to environmental factors was still blank at present. Meanwhile, the extracellular MC concentrations and the influencing factors have not been reported upon the whole lake.

Descriptions of the presence and seasonality of *Microcystis* morphospecies and the corresponding MC concentrations are of particular value for the future prediction and mitigation of cyanobacterial blooms, especially the presence of *Microcystis* populations. The explanation of the seasonal variation of dominant *Microcystis* species and the associated MC concentration is potentially important for better understanding of the mechanism of cyanobacterial blooms, developing management and utility strategies for protecting water quality. Hence, the purpose of this study was: (1) to investigate the seasonal variation of *Microcystis* populations and the environmental factors favoring their formation, and (2) how the MC concentrations were influenced by the external conditions and *Microcystis* morphospecies. This study also provides the whole lake, a year around, information on the abundance and composition of cyanobacterial blooms and their toxin.

## 1. Materials and methods

### 1.1. Study area and sampling

Dianchi Lake (latitude 24°51′N, longitude 102°42′E) is located in a plateau area of the southwestern part of China, with a water surface of 300 km$^2$, watershed area of 2920 km$^2$, average depth of 4.4 m and a maximum depth of 11 m, basin average length of 114 km, average width of 25.6 km, and an altitude of 1886.5 m above sea level. The lake basin is in a northern subtropical, humid, moist monsoon climate, with average annual temperature of approximately 14.5°C, average annual precipitation of approximately 1000 mm, relative humidity of 74%, and mean wind speed of about 2.5 m/sec (Liu et al., 2006). Variations of *Microcystis* populations, extracellular MC concentrations and water quality were monitored monthly from August 2009 to July 2010. Surface water samples collected in 1000 mL plastic bottles were fixed with acidic Lugol iodine solution at a final concentration of 1%. An aliquot of 30 mL was transferred to a sedimentation chamber for analysis. Water samples were collected between 1000 and 1500 hr at the surface at ten stations as shown in Fig. 1.

### 1.2. Environmental parameters

Water temperature (WT), dissolved oxygen (DO), and pH were measured *in situ via* a multiparameter meter (YSI 660, Yellow Spring Instruments, Yellow Springs, Ohio, USA). Water transparency (SD) was measured with a 10-cm diameter black and white Secchi disk. Chemical analyses of water samples included total nitrogen (TN), total dissolved nitrogen (TDN), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), total phosphorus (TP), total dissolved phosphorus (TDP), and soluble reactive phosphorus (SRP). TP, TDP, SRP (GB 11893-89), TN, TDN (GB 11894-891990), $NH_4^+$ (HJ 535-2009), $NO_3^-$ (HJ/T 346-2007), and $NO_2^-$ (GB 7493-87) concentrations were measured according to Chinese standard methods for monitoring lake eutrophication.

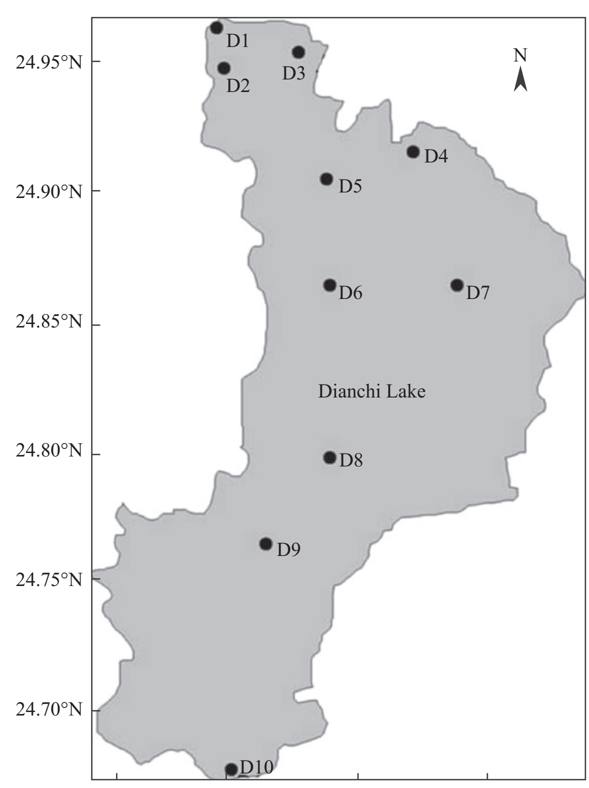


**Fig. 1 – Location of Dianchi Lake, China and the sampling sites (D1–D10).**

### 1.3. Chlorophyll and phytoplankton population analyses

To determine chlorophyll *a* (Chl-*a*) concentration, 100 mL water sample was filtered through a 47-mm glass fiber filter (GF/C; Whatman, Buckinghamshire, UK), and the filters were then extracted with 90% acetone for 24 hr in the dark (4°C). The Chl-*a* concentrations were spectrophotometrically determined after extraction in acetone (Nusch, 1980). Phytoplankton were identified and counted using the method described following the method of Hu and Wei (2006). *Microcystis* morphospecies were classified using their morphology with fresh samples collected through a plankton net (25 μm diameter). The different morphospecies and geometries of *Microcystis* colonies in each group were identified according to the morphological descriptions given by Yu et al. (2007) and Komárek and Komárková (2002).

### 1.4. Determination of extracellular MCs

Extracellular MC concentrations were determined using a 96-well microcystin enzyme-linked immunosorbent assay (ELISA). For analysis of MCs, an aliquot of 100 mL of water samples, collected in glass bottles at the surface, was filtered through a glass microfiber filter (GF/C; Whatman, UK) (1.2 μm diameter) to remove plankton cells and the filtered water was stored frozen at −20°C until analysis. The preparation of water samples and their analysis by ELISA were performed using the method of Ou et al. (2005). The samples were analyzed in triplicates and compared with 0.1 to 2.0 μg/L calibration curve of MC-LR standard (provided by Institute of Hydrobiology, Chinese Academy of Sciences) performed on each individual plate.

### 1.5. Statistical analysis

All the data presented in the study are the combined results of the ten different sampling sites. The correlations between cell abundances of *Microcystis* morphospecies, extracellular MCs, and environmental variables were analyzed using a Pearson correlation with the SPSS statistical software, version 18.0 for Windows (Chicago, USA). Before the correlation analysis, a logarithmic transformation was conducted for the factors that were not normally distributed.

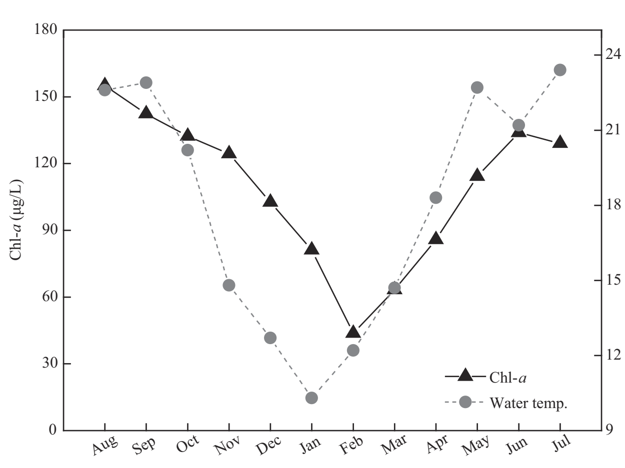


**Fig. 2 – Changes in Chl-*a* concentrations and water temperature of the surface water, June 2009 to March 2010.**

## 2. Results

### 2.1. Chl-a and environmental parameters

Fig. 2 shows the seasonal changes of Chl-*a* concentration and WT at the surface of Dianchi Lake from August 2009 to July 2010.

Chl-*a* concentration varied with seasons (fluctuated greatly between 43.7 and 155 μg/L) with high values in summer and low values in winter season. Chl-*a* concentration reached the maximum value in August, 2009, and then gradually decreased until February, 2010. The WT also followed a seasonal pattern in the lake. Temperature ranged from 10.3 to 23.4°C with the highest temperatures recorded in July and the lowest in January. The WT had climbed from 14.7°C in April to 18.3°C in May, yet it dropped sharply from 20.2 to 14.8°C in November. It is worthwhile to note that the WT in Dianchi Lake fluctuated between 20.3 and 23.4°C from May to October during the study period, with an average of 22.2°C. The results of physical and chemical characteristics (DO, pH, Secchi disc transparency, SRP, TDP, TP, $NH_4^+$-N, $NO_3^-$-N, $NO_2^-$-N, TDN and TN) during the study period are summarized in Table 1.

During the study period, DO ranged from 5.44 to 12.0 mg/L with the lowest and highest value recorded in February and May 2010, respectively. The pH scale did not differ significantly over the year and varied between a minimum of 8.02 and the maximum of 9.82. SD oscillated many times and ranged between 12 and 36 cm. The concentrations of TP were high from May to November (from 0.223 to 0.350 mg/L), and were generally low in winter (December–March). The tendencies of variations of TDP and SRP were similar, and they oscillated continuously. The maximum value and the minimum value of TDP and SRP were 0.088, 0.038 mg/L and 0.028, 0.013 mg/L, respectively. The concentrations of TN showed an increasing trend with the study, and the average value was as high as 2.68 mg/L. TDN concentrations were around 1.65 mg/L, except for the maximum value (2.94 mg/L), which occurred in February, 2010. $NH_4^+$ concentrations increased slowly and ranged between 0.104 and 0.541 mg/L. The $NO_3^-$ concentrations were less than 0.100 mg/L from August to December, but a sudden increase was recorded at the end of January, with the highest $NO_3^-$ concentration (1.18 mg/L) recorded in April. The $NO_2^-$ concentrations were low and varied between 0.010 and 0.098 mg/L.

### 2.2. Phytoplankton composition

The variation in phytoplankton composition during the investigation period in Dianchi Lake is shown in Fig. 3.

The phytoplankton community was mainly dominated by the Cyanobacteria during summer and autumn seasons, accounting for more than 96% of the total phytoplankton abundance during the study period. In winter, the relative abundance of phytoplankton composition was dominated by Cyanobacteria, Chlorophyceae, and Bacillariophyceae. From January to April, Chlorophyceae dominated the phytoplankton

**Table 1 – Environmental parameters recorded during one-year study period (from August 2009 to July 2010) in Dianchi Lake.***

| Date | DO (mg/L) | pH | Transparency (cm) | SRP (mg/L) | TDP (mg/L) | TP (mg/L) | $NH_4^+$-N (mg/L) | $NO_3^-$-N (mg/L) | $NO_2^-$-N (mg/L) | TDN (mg/L) | TN (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| August | 10.0 | 9.40 | 13 | 0.014 | 0.088 | 0.350 | 0.205 | 0.0740 | 0.018 | 1.33 | 1.87 |
| September | 5.76 | 9.79 | 36 | 0.033 | 0.052 | 0.264 | 0.0530 | 0.127 | 0.020 | 1.26 | 1.93 |
| October | 6.96 | 9.82 | 15 | 0.014 | 0.061 | 0.246 | 0.104 | 0.100 | 0.020 | 1.40 | 1.95 |
| November | 8.17 | 9.57 | 13 | 0.038 | 0.088 | 0.245 | 0.292 | 0.0440 | 0.010 | 1.44 | 2.11 |
| December | 7.21 | 9.00 | 12 | 0.024 | 0.071 | 0.156 | 0.186 | 0.0870 | 0.016 | 1.48 | 3.24 |
| January | 6.07 | 8.34 | 14 | 0.020 | 0.035 | 0.187 | 0.324 | 0.150 | 0.019 | 1.55 | 2.86 |
| February | 5.44 | 8.02 | 27 | 0.036 | 0.084 | 0.191 | 0.239 | 0.422 | 0.075 | 2.94 | 3.06 |
| March | 7.27 | 8.38 | 19 | 0.038 | 0.039 | 0.138 | 0.415 | 0.713 | 0.076 | 2.27 | 3.31 |
| April | 10.0 | 9.18 | 23 | 0.013 | 0.036 | 0.200 | 0.346 | 1.18 | 0.091 | 1.25 | 2.27 |
| May | 12.0 | 9.56 | 23 | 0.024 | 0.028 | 0.245 | 0.335 | 0.572 | 0.098 | 1.74 | 2.90 |
| June | 8.40 | 9.18 | 24 | 0.038 | 0.067 | 0.296 | 0.237 | 0.209 | 0.036 | 1.59 | 3.67 |
| July | 7.63 | 9.49 | 14 | 0.010 | 0.042 | 0.223 | 0.541 | 0.140 | 0.027 | 1.56 | 3.05 |

* The data are average values of the sampling sites.

community with a relative numerical abundance that ranged from 17% to 43%. Bacillariophyceae were only dominant in January (accounting for 13%), and the other algae was relatively low during the whole study period. The most abundant cyanobacteria found during the study period belonged to *Aphanizomenon flos-aquae* and *Microcystis* spp., but *A. flos-aquae* occurred only in March and April.

### 2.3. Seasonal variation of *Microcystis* populations

Fig. 4a shows the seasonal variations in the morphology of *Microcystis* species. *Microcystis* made up almost all of the total phytoplankton in Dianchi Lake's surface water, and there was clear seasonal dynamics in the *Microcystis* abundance and morphospecies.

The total cell density of *Microcystis* was low in February and March, but *Microcystis* existed in the water body throughout the year during the study period, with the minimum value of $1.3 \times 10^7$ cells/L. The abundance of *Micocystis* began to increase in April, and the cell density of *Microcystis* was relatively high from May to December (ranging between $1.12 \times 10^8$ and $7.87 \times 10^8$ cells/L); then the population densities of *Microcystis* decreased from $3.8 \times 10^8$ to $2.75 \times 10^7$ cells/L in January. *Microcystis ichthyoblabe*, *M. viridis* and *M. wesenbergii* predominated in April, but *M. viridis* and *M. wesenbergii* increased rapidly from April, and showed a remarkable dominance from May to December, with the relative abundance of *Microcystis* between 65% and 95% (Fig. 4b). The amounts of *M. viridis* and *M. wesenbergii* were similar in summer, but *M. viridis* was the most abundant species since December — representing almost 90% of the total cell density of *Microcystis* in January. For the meantime, the seasonal dynamics of colony size of *Microcystis* populations was recorded as showed in Fig. 4b. *Microcystis* morphospecies were divided into five size fractions: the smallest size class (<100 μm), the smaller colony size fractions (100–200 μm), the middle colony size classes (200–300 μm), the larger colony size classes (300–400 μm), and the largest colony size classes

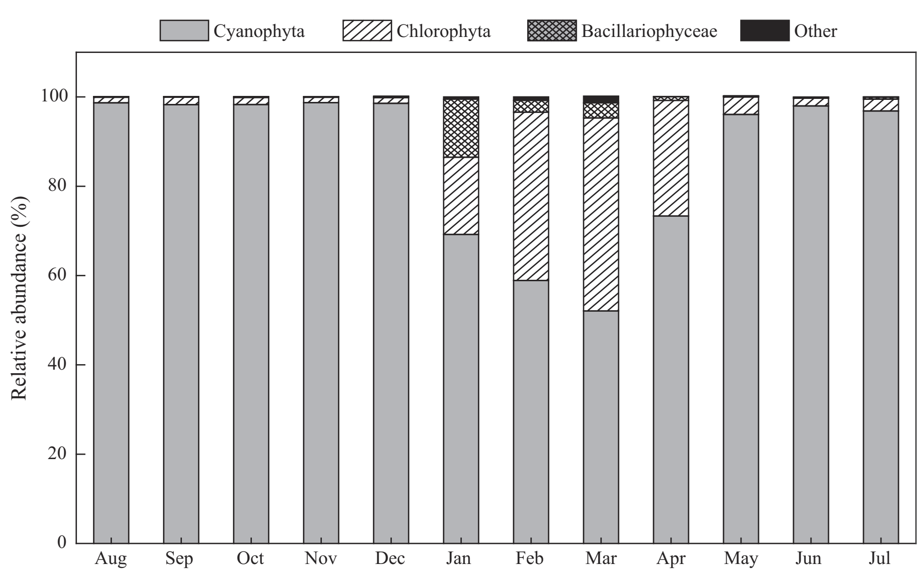


**Fig. 3 – Seasonal variation of relative abundance of phytoplankton in Dianchi Lake during the study period from September 2009 to August 2010.**

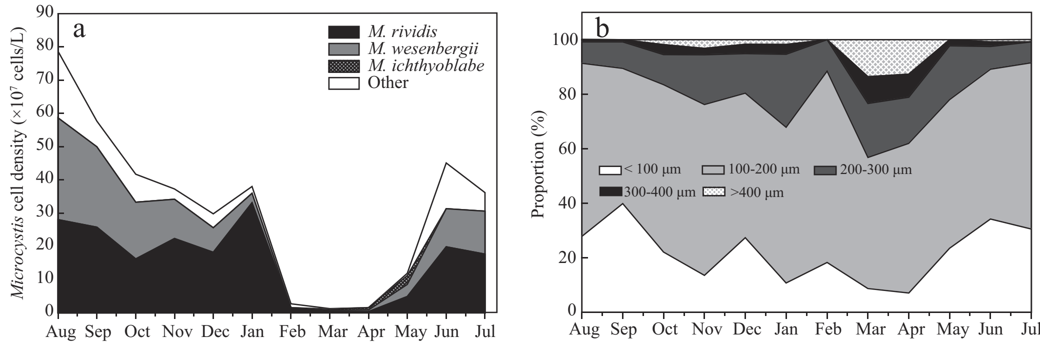


**Fig. 4 – Changes in cell density (a) and colony size (b) of *Microcystis* populations in Dianchi Lake during the study period from August 2009 to July 2010.**

(>400 μm). The smallest and smaller colony size classes always had a clear advantage during the study period, and varied between 62% and 92% of *Microcystis* populations. The proportion of the smaller colony size fractions and the middle colony size classes fluctuated narrowly, accounting for 58% and 14%, respectively. The proportion of the smallest size class decreased during early *Microcystis* bloom formation (in March and April), whereas the proportions of the larger and largest colony size classes, which were rather low during *Microcystis* blooms, began to increase and accounted for 9.2% and 13%, respectively.

### 2.4. *Cyanobacterial toxicity*

The monthly changes in MC concentrations analyzed by ELISA in the water column of Dianchi Lake are shown in Fig. 5.

The measurable MC levels were detected for all of the investigated months with concentrations in the water column ranging from 0.166 to 1.03 μg/L. The average MC concentration was 0.447 μg/L, and the maximum concentration in the lake was 1.03 μg/L, occurring in May at station D6, located in the middle of the lake. Only one raw water sample contained MC concentrations exceeding 1 μg/L, the WHO provisional guideline value for one of the MC variants, MC-LR (WHO, 1998a). Generally, higher levels of MC were observed during the warmer months and lower levels during the cooler months (from January to March). When the cyanobacterial blooms occurred from April to December, the MC concentrations in the water column were approximately 0.55 μg/L, and the dissolved MC concentrations were around 0.20 μg/L during the decline of the water blooms.

### 2.5. *Corrections between the* ***Microcystis*** *species, MCs and environmental factors*

Environmental factors associated with the variation of *Microcystis* populations and MC production were investigated using a correlation analysis (Table 2).

In the entire lake, the total abundance of *Microcystis* was strongly positively correlated with TP ($p < 0.01$) and negatively correlated with various forms of nitrogen ($p < 0.05$), including $NO_3$-N, $NO_2$-N and TDN. Especially, the cell density of *Microcystis* showed a significant negative correlation with the size of the *Microcystis* colony ($p < 0.05$), suggesting that the colony size was smaller during *Microcystis* blooms. The cell density of *M. wesenbergii* was more closely related with the WT and TP ($p < 0.05$), and exhibited a negative relationship with $NO_3^-$-N, $NO_2^-$-N ($p < 0.05$) and TN ($p < 0.01$), suggesting that eutrophication together with global warming could benefit to this kind of *Microcystis*. WT was not a very important factor for the proliferation of *M. viridis*. The abundance of *M. viridis* was only significantly negatively related with $NO_3^-$-N and $NO_2^-$-N ($p < 0.01$), but not to the other environmental factors. *M. wesenbergii* and *M. viridis* were strongly related ($p < 0.05$), implying that their growth may be governed by certain common environmental factors. The concentrations of MCs correlated with neither the total abundance of *Microcystis* nor the cell density of *M. viridis* and *M. wesenbergii* individually. The most important environmental factor affecting MC concentrations was pH ($p < 0.01$). However, WT and DO also correlated with the seasonal variation of total MC concentrations, implying that the increasing temperature may lead to the rise of MC concentrations.

## 3. Discussion

### 3.1. *Explanation of the dynamics of* ***Microcystis*** *morphospecies*

Harmful *Microcystis* blooms have increased globally in frequency and intensity in recent decades, and it also occurred

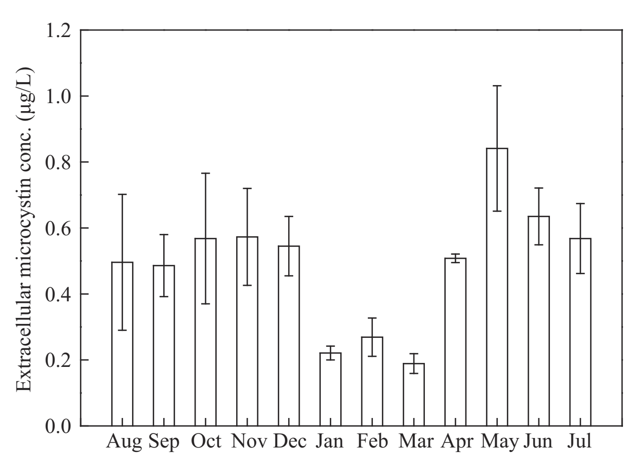


**Fig. 5 – Variation of total microcystins concentrations in the surface water of Dianchi Lake.**

Table 2 – Pearson correlations between *Microcystis* populations, MC, and environmental factors in Dianchi Lake from August 2009 to July 2010 ($n$ = 12).

| | M. v | M. w | Total | Size | MC | WT | DO | pH | Transparency | SRP | TDP | TP | $NH_4^+$-N | $NO_3^-$-N | $NO_2^-$-N | TDN | TN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M. v | 1.0 | | | | | | | | | | | | | | | | |
| M. w | 0.65[a] | 1.0 | | | | | | | | | | | | | | | |
| Total | 0.87[a] | 0.92[a] | 1.0 | | | | | | | | | | | | | | |
| Size | −0.48 | −0.60[a] | −0.61[a] | 1.0 | | | | | | | | | | | | | |
| MC | 0.024 | 0.28 | 0.20 | −0.46 | 1.0 | | | | | | | | | | | | |
| WT | 0.069 | 0.61[a] | 0.43 | −0.43 | 0.66[a] | 1.0 | | | | | | | | | | | |
| DO | −0.24 | 0.025 | −0.047 | 0.097 | 0.68[a] | 0.49 | 1.0 | | | | | | | | | | |
| pH | 0.30 | 0.66[a] | 0.52 | −0.40 | 0.78[b] | 0.77[b] | 0.40 | 1.0 | | | | | | | | | |
| Transparency | −0.28 | −0.020 | −0.17 | −0.15 | 0.0050 | 0.27 | −0.15 | 0.010 | 1.0 | | | | | | | | |
| SRP | −0.15 | −0.22 | −0.21 | 0.076 | −0.21 | −0.33 | −0.30 | −0.32 | 0.42 | 1.0 | | | | | | | |
| TDP | 0.26 | 0.44 | 0.42 | −0.44 | −0.024 | −0.14 | −0.209 | 0.014 | −0.18 | 0.29 | 1.0 | | | | | | |
| TP | 0.47 | 0.80[b] | 0.77[b] | −0.62[a] | 0.50 | 0.70[a] | 0.38 | 0.60[a] | 0.091 | −0.14 | 0.39 | 1.0 | | | | | |
| $NH_4^+$-N | −0.33 | −0.50 | −0.46 | 0.41 | −0.096 | −0.054 | 0.27 | −0.28 | −0.34 | −0.20 | −0.45 | −0.36 | 1.0 | | | | |
| $NO_3^-$-N | −0.80[b] | −0.62[a] | −0.76[b] | 0.69[a] | −0.11 | −0.050 | 0.38 | −0.28 | 0.32 | −0.04 | −0.52 | −0.39 | 0.37 | 1.0 | | | |
| $NO_2^-$-N | −0.88[b] | −0.65[a] | −0.80[b] | 0.46 | 0.019 | 0.014 | 0.43 | −0.33 | 0.41 | 0.094 | −0.48 | −0.32 | 0.37 | 0.88[b] | 1.0 | | |
| TDN | −0.57 | −0.54 | −0.59[a] | 0.12 | −0.48 | −0.44 | −0.32 | −0.75[b] | 0.22 | 0.48 | 0.12 | −0.44 | 0.22 | 0.22 | 0.49 | 1.0 | |
| TN | −0.30 | −0.61[a] | −0.46 | 0.12 | −0.12 | −0.30 | −0.091 | −0.57 | −0.022 | 0.34 | −0.22 | −0.46 | 0.45 | 0.13 | 0.29 | 0.51 | 1.0 |

M. v: the cell density of *M. viridis*, M. w: cell density of *M. wesenbergii*, Total: total cell density of *Microcystis*, Size: the size of *Microcystis* colony.
[a] Correlation is significant at the 0.05 level.
[b] Correlation is significant at the 0.01 level.

widely in a large number of lakes, reservoirs, and rivers in China (Visser et al., 2005). Dianchi Lake is a typical large shallow lake which has suffered a pronounced and prolonged toxic *Microcystis* blooms annually during the last two decades, causing serious problems in the management of water quality. In general, *Microcystis* blooms often consist of multiple species, and dominant species changes frequently during a bloom (Watanabe et al., 1986). In Dianchi Lake, various morphospecies of *Microcystis* were observed, and ten common species of them, including *M. aeruginosa*, *M. botrys*, *M. firma*, *M. flos-aquae*, *M. ichthyoblabe*, *M. novacekii*, *M. pseudofilamentosa*, *M. smithii*, *M. viridis* and *M. wesenbergii*, have already been morphologically described (Yu et al., 2007). However, quantitative reports on the distribution of *Microcystis* morphospecies composition are rare based on the research of the whole lake. Insufficient information is available on the seasonal dynamics of *Microcystis* species and the driving factor(s). Meanwhile, there are no reports about the relationships between MC concentrations and *Microcystis* morphospecies in the very lake.

The results of this study indicated that the phytoplankton community was dominated quantitatively by the Cyanobacteria group all year round, and several *Microcystis* morphospecies were the overwhelming species in the warmer period from April to December. Although *Microcystis* species frequently occur in the same habitat, the mechanisms determining the dominance of the several *Microcystis* species are not yet clearly understood. The growth and succession of the *Microcystis* complex is determined by multiple factors (various physico-chemical and biological factors), and dominant factors can differ depending on *Microcystis* species as well as habitat characteristics, such as nutrients, solar radiation, pH, temperature, primary production and oxygen saturation (Takamura, 1988; Wicks and Thiel, 1990; Kotak et al., 2000; Janse et al., 2005). Honma and Park (2005) reported that $NO_3^-$ concentration affected the composition of populations containing *M. aeruginosa* and *M. viridis*, while *M. ichthyoblabe* dominated under low phosphate conditions. In this study, we found that the cell density of *M. viridis*, *M. wesenbergii* and total *Microcystis* were correlated with $NO_3^-$ and $NO_2^-$, implying the variation of nitrogen may have tremendous effect upon the seasonal dynamics of *Microcystis* morphospecies in Dianchi Lake. *M. wesenbergii* was also significantly correlated with TN, TP and WT, indicating that the dynamic of *M. wesenbergii* was probably influenced extensively by both nutrients (nitrogen and phosphorus) and temperature. Previous studies have demonstrated that the WT had significant influence on the growth and succession of *Microcystis* species (Krüger and Eloff, 1978; Imai et al., 2009; Zhai, 2013). Imai et al. (2009) revealed that the WT during the period when *M. aeruginosa* was dominant was higher (24.7–33.9°C) than that when *M. wesenbergii* was (19.6–28.6°C). Temperature probably also plays an important role in the population dynamics of *Microcystis*. The WT ranged from 10.3 to 23.4°C throughout this study, and fluctuated between 20.3 and 23.4°C from May to October in plateau Dianchi Lake. This feature may explain why *M. aeruginosa* was less popular while *M. wesenbergii* was relatively abundant.

*M. viridis* widely existed even in winter with WT of 12.7°C, and Takamura and Watanabe (1987) also found that the dominant *Microcystis* sp. was *M. viridis* in the winter season in

Lake Kasumigaura, Japan. *M. viridis* is able to survive at lower WT, showing that more rigorous management strategies are needed for the control of *Microcystis* bloom in Dianchi Lake. *M. viridis* only exhibited negative relations with $NO_3^-$ and $NO_2^-$, so the changes of nitrogen source rather than temperature and phosphorus possibly affect the abundance and succession of *M. viridis*.

### 3.2. Explanation of the dynamics of extracellular MC concentrations

The present study focuses on concentrations of extracellular MCs, as they may represent the major risk with respect to drinking water production. Although the highest portion of MCs is present inside cyanobacterial cells, this kind of toxin fraction can easily be removed by regular filtration processes during water treatment (Bláhová et al., 2007). Based on the results, the MC concentrations ranged from 0.166 to 1.03 μg/L in surface water samples. Most of the values (98.9%) were below the guideline values of the World Health Organization for drinking water supply (WHO, 1998b). An earlier study result of Pan et al. (2006) also revealed a similar result that the MC concentrations of all the samples were lower than 1 μg/L in western Fubao bay of the Dianchi Lake.

Previous studies have reported that MCs in water samples were strongly correlated with environmental factors, such as TN (Graham et al., 2006), TP (Kotak et al., 2000), low stream flow and high WT (Lehman, 2008; Duong et al., 2012) and $NO_3^-$ concentration (Mitsuhiro et al., 2007). In contrast, according to Jungmann et al. (1996), MC concentration was not correlated with any measured variables in a hypereutrophic reservoir in Germany. While it is apparently impossible to explain these contradictory results using a single factor, the different results were likely to depend on a combination of both the environmental factors and characteristics *Microcystis* strain.

The MC concentrations were often positive related with the *Microcystis* cell abundance (Joung et al., 2011), but there was no significant correlation observed between the MC concentrations and the cell density of *Microcystis* in our study despite low MC concentrations often observed in winter. Interestingly, although the abundance of *Microcystis* was relatively high in Dianchi Lake (>$1.0 \times 10^9$ cells/L), the cell-bound and extracellular MC concentrations were significant lower than the MC concentrations in raw drinking water samples from Taihu Lake and Chaohu Lake in China (Peng, 2012; Zheng et al., 2004; Song et al., 2007; Xu et al., 2008a, 2008b). Although our research indicated that MC was correlated positively with WT, pH and DO, the MC concentrations could be probably associated with *Microcystis* morphospecies and colony size in Dianchi Lake.

The study of Chen et al. (2009) had shown that the frequent variations of MCs in both concentrations and toxin species were associated with the frequent alteration of *Microcystis* colonies or/and species from the monitoring results of *Microcystis* colonies in Taihu Lake. High concentration of MCs in the blooms was always associated with *M. flos-aquae* and *M. aeruginosa*, whereas when *M. wesenbergii* was the dominant colonial type, the MC production was low. Meanwhile, most colonies (>75%) of *M. aeruginosa* and *M. botrys* contained the *mcy* genes, whereas <20% of the colonies identified as *M. ichthyoblabe* and *M. viridis* gave a PCR product of the *mcy* genes or MCs oligopeptide (Kurmayer, 2002; Ozawa et al., 2005; Fastner et al., 2001). However, the most common *M. aeruginosa* in other aquatic environment was not the dominant species during the study period. The study above had indicated the dominant *Microcystis* species were identified as *M. wesenbergii*, *M. ichthyoblabe* and *M. viridis*. *M. wesenbergii* was one of the most dominant waterbloom-forming microcystis morphospecies from May to December (ranged between 25% and 42%), but were not shown to contain MC synthesis gene or MC by both molecular and chemical methods (Xu et al., 2008a, 2008b; Ozawa et al., 2005). *M. viridis* dominated almost throughout the study, and the proportion of total *Microcystis* varied between 35% and 87%. Even though *M. viridis* was predominant, the amounts of MCs it produced were low (Harada et al., 2001; Ozawa et al., 2005), and our results generally were consistent with observations from above studies.

Furthermore, a positive relationship was found between the size of the colony and the frequency of those containing the *mcy* genes. The smallest colonies (<200 μm) showed the lowest proportion of *mcy* and MC producers, and the largest colony size class (>1101 μm) had a maximum proportion of the *mcy* (83%) (Via-Ordorika et al., 2004). Kurmayer et al. (2003) revealed that 42% to 73% of the large colonies (>500 μm) belonged to the MC-producing genotype, compared to only 10% to 15% of the small colonies (<500 μm). Our findings were in accordance with the results of Kurmayer et al. (2003) from Wannsee Lake implying that the observation that the larger colonies of *Microcystis* sp. are the chief MC producers may be more generally valid. Since the colony size fractions (<200 μm) accounted for 62%–92% of *Microcystis* populations in the study, the MC concentrations should be probably low according to this result. By comparison, the fractions of over 200 μm were more common in Taihu Lake (unpublished data).

## 4. Conclusions

This is the first report of dynamics of *Microcystis* populations, extracellular MC concentrations and the relationships with environmental factors in the whole lake monitoring of hypertrophic Dianchi Lake, China. Based on our experimental results, it is clear that there was significantly seasonal succession in both *Microcystis* cell density and morphospecies. *M. viridis* and *M. wesenbergii* dominated during cyanobacterial blooms, and *M. viridis* was the only dominant species in winter. The dominance of *M. wesenbergii* was influenced by both nutrients and temperature, while the dominance of *M. viridis* was associated with nitrogen. The MC concentrations were correlated with WT, pH and DO. The relatively low content of MCs in water samples poses a slight public safety risk in the study period, which could be due to the a large proportion of *M. viridis* and *M. wesenbergii*, along with small colony size of *Microcystis* populations. These findings are of particular meaning with respect to the ecological characteristics of *Microcystis* morphospecies and MC concentrations, which could be helpful in the prediction and management of *Microcystis* blooms under adverse conditions or fluctuating environments.

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