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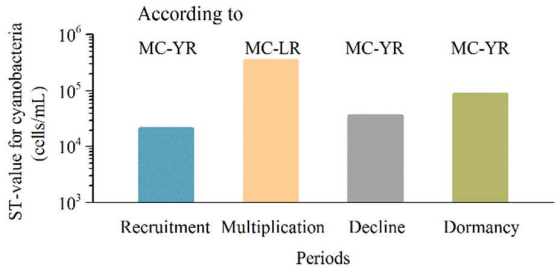

Environmental Impact

Cyanobacterial blooms have caused serious problems to water ecosystem and human health by producing cyanotoxins. To reduce cyanotoxins concentration in drinking water, one of the most basic approaches is to control the cyanobacterial abundance in source water, which is needed to establish the "safety threshold (ST)" for cyanobacteria. The ST-value was established in Chaohu Lake, the fifth largest lake in China, which is served as drinking water source but always suffering from cyanobacterial blooms. This work has highlighted the importance of classifying the ST series into different periods referring to different microcystin congeners, which makes it effective and accurate to guide the cyanobacteria control.

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ARTICLE

Establishment of preliminary safety threshold values for cyanobacteria based on periodic variations in different microcystin congeners in Lake Chaohu, China

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As harmful cyanobacterial proliferation threatens the safety of drinking water supplies worldwide, it is essential to establish a safety threshold (ST) for cyanobacteria to control cyanobacterial density effectively in water sources. For this purpose, cyanobacterial abundance, microcystins (MCs) production, and environmental parameters were monitored monthly from September 2011 to August 2012 in one drinking water source of Lake Chaohu. The cyanobacterial density ranged from 1400 to 220 000 cells/mL with the succession of two dominant species *Microcystis* and *Dolichospermum*, which was determined by water temperature and nutrients loading. The MCs concentrations were correlated significantly with the cyanobacterial density and they varied between 0.28 and 8.86 μg/L. Therefore, the characteristics of MC cell quotas were classified according to four stages of the development of cyanobacteria, namely: recruitment, multiplication, decline and dormancy. The ST for cyanobacteria was established for different periods based on MC cell quota and its guideline wherein three commonly monitored MC congeners (MC-LR, -RR and -YR) were considered in the present study. Its reliability was verified in the water source using data collected between June 2013 and May 2014. The results highlighted the necessity to classify the ST-values in different periods referring to main MC congeners rather than MC-LR, which will facilitate the management and control of toxic cyanobacterial proliferation in drinking water sources.

1. Introduction

Cyanobacterial blooms in water sources threaten drinking water safety, especially in water scarce areas, making them a serious global ecological problem.[1-3] Apart from contributing to total organic carbon and turbidity (e.g., algae cells), cyanobacteria are well known for their ability to produce diverse hepatotoxins, neurotoxins, gastrointestinal toxins and skin irritants, which are the most hazardous for public health during cyanobacterial blooms.[4-7]

Microcystins (MCs), a family of potent liver toxins, are considered to be the most resistant to degradation of cyanotoxins because of their stable cyclic peptide structure.[8] MCs have more than 90 known variants with the median lethal dose (LD_{50}) ranging from 25 μg/kg to greater than 1000 μg/kg.[9] Among them, microcystin-LR (MC-LR) is the most toxic and consistently identified MC congener.[10] Due to the strongly hepatotoxic and tumor-promoting activity, the World Health Organization (WHO) has set a provisional guideline value of 1.0 μg/L for MC-LR in drinking water.[11] Other MC congeners such as microcystin-RR (MC-RR) and microcystin-YR (MC-YR) deserve more attention as they are also commonly monitored worldwide, while MC-RR is the most frequently encountered congener in China.[12] As the acute and chronic toxic effect on people linked to MCs have been established worldwide, the toxins pose a risk to public water supplies due to their ubiquitous presence in raw water feeding into water treatment plants.[3,13]

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In drinking water treatment, cell-bound MCs could be released under mechanical and chemical stress, but conventional water treatment techniques are ineffective in removing dissolved MCs.[13,14] In order to reduce cyanotoxin concentration in treated water, controlling cyanobacterial abundance in source water will be an efficient way, which can be achieved by: 1) reducing the nutrient input (a long-term strategy); 2) salvaging and/or killing algae in water source to reduce cyanobacterial density (a practical risk mitigation strategy).[7,14,15] The approaches require the establishment of a "safety threshold (ST)" for cyanobacteria in raw water to reduce potential health risk caused by cyanotoxins effectively.

Previously, to assess the potential hazard from a cyanobacterial bloom, Alert Level Framework (ALF) proposed a monitoring and management action sequence for water treatment plant operators and managers.[1] The ALF is based on data from cyanobacterial cell counts, equivalent cell biovolume and the relevant drinking water guideline for toxins.[1] The ALF concept was first developed for algal management in South Australia in 1991, and has been applied internationally and adapted for specific regions.[1,16] Recently, Izydorczyk *et al.*[17] established an ALF based on cyanobacterial Chl *a* monitoring by an algae online analyser. Schmidt *et al.*[18] proposed the "maximum tolerable values" as a supplement of the common ALF by taking performance of drinking water treatment processes into consideration. Although the researchers have suggested that the associated monitoring program for cyanobacteria is site and season specific,[16,17] the periodic variations of toxin production were not incorporated into ALF system. The concentration and cell quota of MCs show obvious seasonal patterns in water sources, which are affected by the category of dominant species in blooms and environmental factors such as temperature, light, nutrients, salinity, pH and micronutrient concentrations.[12,15,19,20] The MC production also changed during the periodic life cycle of blooming-forming cyanobacteria, which could be classified into four periods with an irregular seasonal cycle into the four-stage hypothesis (recruitment, multiplication, decline and dormancy stage) summarized by Tan.[21] The classification of the safety threshold in different periods should be established according to the variation in cyanobacterial abundance and MCs production. On the other hand, previous studies generally used the most toxin MC-LR as reference surrogate or assumed that the other MC congeners had similar toxicity to MC-LR. The exaggeration of the toxicity of cyanobacterial bloom aimed to reduce the risk of cyanobacterial bloom, but it would increase the difficulty and cost of treatment to meet the controlling target in water sources. Therefore, it is necessary to establish the ST series relating to the periodic variations of different MC congeners production to guarantee the safety of raw water.

In the present study, the seasonal variations of cyanobacterial abundance and MC production were determined from September 2011 to August 2012 in the eastern drinking water source of Lake Chaohu, China. The influence of environmental parameters on the variations of cyanobacterial species and MCs were studied to gain insights into the processes of cyanobacterial growth and MC production. Finally, the ST-values for cyanobacteria involving different MC congeners were established for different periods of the year, the practicability of which was then verified using the data from June 2013 to May 2014.

2. Materials and Methods

2.1. Reagents and glassware

Microcystin standards including MC-LR, -RR and -YR (purities ⩾ 95%) were purchased from Alexis Biochemicals (Lausen, Switzerland). The CNWBOND LC-C18 extraction cartridges (500 mg, 6 mL) were obtained from CNW Technologies (Anpel Scientific Instrument Co., China). HPLC grade methanol, trifluoracetic acid, acetic acid, acetonitrile and acetone were purchased from TEDIA Company (Fairld, OH, USA). A stock solution was prepared by dissolving each 50 μg MC congener standard in 1 mL methanol solution and kept at -18 ℃ for later use. Milli-Q water used for the study was obtained from a Milli-Q water purification system (Millipore, USA). All other solvents and chemicals used in the analysis were of analytical reagent grade.

All glassware and plastic containers were previously soaked into 1:2 (v/v) HCl overnight and rinsed thoroughly with deionized distilled water before use.

2.2. Study sites and sample collection

Lake Chaohu, a shallow and turbid lake, is the fifth largest freshwater lakes in China. Nitrogen, phosphorus and organic matter loading from urban sewerage and agricultural non-point

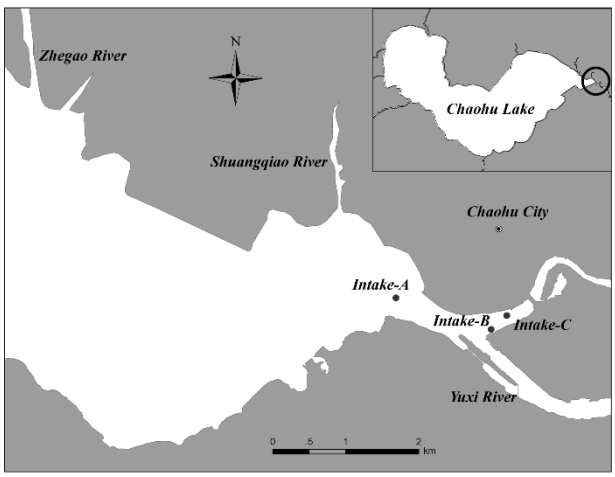

Fig.1 Location of the sampling sites in the eastern drinking water source of Lake Chaohu

source pollution speeded up the process of eutrophication of Lake Chaohu.[22] Although suffering from cyanobacterial blooms, the eastern end of Lake Chaohu still serves as a drinking water source for around 4.23 million people in Chaohu City. There are about seven drinking water intake points with different water supply rates from 3000 m^3/d to 100 000 m^3/d in this area. Three major water intakes (Intake-A: 117°50′15.13″E, 31°35′36.34″N; Intake-B: 117°50′54.32″E, 31°35′24.82″N;

Intake-C: 117°50′58.40″E, 31°35′32.30″N) were chosen as sampling sites in this study (Fig. 1).

Water samples were collected monthly from September 2011 to August 2012. Subsequently, samples were collected to check the applicability of ST series from June 2013 to May 2014. Integrated water samples were taken from the entire water column at 0.5 m intervals using a 2.5 L sampler. Water samples were stored in high-density polyethylene bottles, which were rinsed with the sample three times before filling. The samples were placed on ice in the dark and transported immediately to the laboratory for analysis. All samples were analyzed for physicochemical parameters, phytoplankton cell counting and toxin concentrations. Each measurement was carried out in triplicate.

2.3. Physicochemical water quality analysis

Physicochemical parameters, such as water temperature, pH, dissolved oxygen (DO) and conductivity were measured in field with a portable instrument (Horiba U-53, Japan). The environmental parameters including total phosphorus (TP), total nitrogen (TN), nitrate, nitrite, ammonium, orthophosphate and potassium permanganate index (COD_{Mn}) were determined according to the standard methods.[23] Dissolved organic carbon (DOC) was analyzed with a TOC carbon analyzer (Torch, USA). For Chl *a* measurement, pigments were extracted with 90% acetone at 4 ℃ in the dark for 24 h. After centrifugation, the absorbance of the supernatant was measured spectrophotometrically against 90% acetone at 630, 645, 663 and 750 nm, respectively. Concentrations of Chl *a* were calculated using the equations from Jeffrey and Humphrey.[24]

2.4. Phytoplankton analysis

Phytoplankton samples collected in 1000 mL plastic bottles were preserved in Lugol's iodine solution and deposited in 48 h, then condensed to 30 mL and then 1 mL was ultrasonicated to disperse the cells or filaments. Phytoplankton were determined in concentrated samples by a phase contrast microscope (Nikon, TS100F, Japan) according to taxonomic keys based on cell structure and dimension.[25] At least 400 individual cells or filaments were enumerated in an algal counting chamber (20 × 20 mm^2) to reduce the error to less than 10%.

2.5. MC analysis

Three MC congeners (MC-LR, -RR and -YR) including extra- and intra-cellular fractions were detected in water samples using the method modified according to Barco *et al.*[26] Water samples (500 mL) were filtered through a glass microfiber filter (GF/C, Whatman) to separate extra- and intra-cellular MCs. For extracellular part, 1 L samples of filtered water were subjected to C 18 solid phase extraction cartridges for concentration, and the toxins were eluted using 10 mL methanol (0.1% trifluoracetic acid, v/v). The eluent fraction was rotary-evaporated to dryness and re-dissolved in 200 μL of 50% aqueous methanol before high-performance liquid chromatography (HPLC) analysis. The filters with intra-cellular MCs were first freeze-thawed then extracted in 5% acetic acid, then in 75% methanol twice (with ultrasonication). The extracts were centrifuged at 11,000 *g* for 10 min and the supernatants were combined. Subsequent procedures were conducted as for extracellular MCs above.

Identification and quantification of MCs were performed by HPLC equipment (Agilent 1200, USA) with quaternary pump (G1311A), autosampler (G1329A), thermostated column compartment (G1316A), diode-array detector (G1315D), and an Agilent Eclipse XDB-C 18 column (4.6 × 150 mm *i.d.*; 5 μm particle size). Gradient elution of water/0.05% trifluoracetic acid (A) and acetonitrile (B) was used by varying the volume percentage of B from 30% to 40% over 15 min, and held constant for an additional 5 min. Injection volume was 20 μL, and chromatograms were analyzed and integrated at 238 nm. Microcystins in the samples were compared with MC-LR, -RR and -YR standards based on peak areas and retention times.

2.6. The method for ST-value establishment and verification

Although about ninety MC variants have been identified so far, the WHO only set provisional guideline for MC-LR in surface waters but lack data with respect to other congeners.[27] According to the concept of "toxicity equivalent factor" (TEF), LD_{50} values could be used to convert other MC congeners into MC-LR equivalent by defining $TEF_{MC\text{-}LR}$ as 1.0.[4,28] Then the guideline value of other MC congeners was calculated using the guideline of MC-LR (1.0 μg/L) divided by TEF_{MC}. The ST-value for cyanobacteria was the minimum value of cyanobacterial densities which were equivalent to the guideline value of each MC congener in each period. The ST-values for cyanobacteria were calculated by Eqs. (1) ~ (5):

$$ST = Min\ (G_{cya_1},\ G_{cya_2},\ G_{cya_3}, \dots\dots) \quad (1)$$

$$G_{cya} = G_{MC}/c'_{MC} \quad (2)$$

$$G_{MC} = G_{MC_0}/TEF_{MC} \quad (3)$$

$$TEF_{MC} = TEF_{MC_0}/\left(\frac{LD_{50(MC)}}{LD_{50(MC_0)}}\right) \quad (4)$$

$$c'_{MC} = c_{MC}/c_{cya} \quad (5)$$

where ST, the ST-value for cyanobacteria; G_{cya}, the cyanobacterial density equivalent to the guideline of MC; G_{MC}, guideline of MC concentration in drinking water; c', MC cell quota, *e.g.* in fg/cell; MC_0, reference MC, *e.g.* MC-LR; TEF, toxicity equivalent factor; LD_{50}, the median lethal dose of MC; c_{MC}, the concentration of MC; c_{cya}, the density of cyanobacteria in water.

The application of ST-value in this study was then verified by the data involving the concentrations of MC congeners and cyanobacterial densities using the data collected in another period, *e.g.* from June 2013 to May 2014 in this study.

2.7. Quality assurance and quality control

Quality control procedures were applied in the chemical analysis. A solvent blank, a standard and a procedure blank were included once every 20 samples to check for background contamination, peak identification and quantification.

As for the MC analysis, the recovery of the complete analytical procedure was measured by spiking standard solution at level of 1.0 μg/L in triplicate. Recoveries of dissolved MC-LR, -RR and -YR in filtered water were 102.4% ± 3.5%, 106.3% ± 1.4% and 113.2% ± 5.8%, respectively. Recoveries of filter extracts with intracellular MCs were 97.8% ± 2.7% for MC-LR, 89.3% ± 4.3% for MC-RR, 103.1% ± 4.9% for MC-YR, respectively. The limits of detection (LODs) of the MCs were calculated as the lowest concentrations resulting with a signal-to-noise (S/N) ratio of 3, while the limits of quantification (LOQs) were calculated with a S/N ratio of 10 from the same chromatograms. After concentrating the samples, the LODs for MC-LR, -RR and -YR were 0.004, 0.004 and 0.005 μg/L, while

the LOQs for MC-LR, -RR and -YR were 0.011, 0.012 and 0.015 μg/L, respectively.

2.8. Statistical analysis

Correlation analysis was conducted to determine the relationship between MCs, cyanobacterial density and physicochemical parameters using the SPSS 19.0 (Chicago, IL, USA). All statistical analyses were considered significant at $p < 0.05$.

Redundancy analysis (RDA) was performed to identify the key water quality variables affecting cyanobacterial species (the predictor variables including water temperature, pH, DO, conductivity, TN, nitrate, nitrite, ammonia nitrogen, TP and orthophosphate) and the seasonal changes of intra- and extra-cellular MCs (the predictor variables including *Microcystis* and *Dolichospermum* density and parameters used for cyanobacterial species above) using the multivariate data analysis software CANOCO 4.5 (Microcomputer Power, Ithaca, USA). The above variables were ln (x+1) transformed before performing the analysis. To avoid collinearity between independent variables, redundant environmental variables (indicated by variance inflation factors above 10.0) were removed by a primary RDA. Then the intercorrelations were re-examined and all the correlations between the selected predictors were less than 0.7. To choose the variables with significant $p < 0.05$ shown in the triplot diagram, the significance of physical chemical indicators in RDA was tested using Monte Carlo simulations with 499 unrestricted permutations.[29]

3. Results

3.1. Physicochemical parameters

In the eastern drinking water source of Lake Chaohu, the subtropical monsoon climate of the region was characterized by the lowest water temperatures (6 ℃) observed in January and the highest water temperatures (31 ℃) in July (Fig. 2). The pH values varied from 7.3 to 8.8 with relatively higher values in summer corresponding to the rapid growth of phytoplankton. The source water was well aerated, and the DO concentrations were above 7.05 mg/L during the sampling period with relatively higher values detected in winter. The electrical conductivity ranged from 293 to 416 μs/cm with a mean value of 329 μs/cm, which was demonstrated an opposite seasonal variation trend to DO.

The concentrations of nutrients showed different seasonal patterns. The concentrations of TN varied from 0.48 to 3.29 mg/L with three peaks recorded in March, August and November. On average, the nitrate, nitrite and ammonia nitrogen accounted for 35.9%, 1.0% and 13.1% of TN, respectively. Nitrate concentrations fluctuated around 0.34 mg/L in autumn and winter, and then increased in spring, with a peak at 1.22 mg/L in August. The concentrations of nitrite were below 0.03 mg/L except in July (0.07 mg/L). The concentrations of ammonia nitrogen averaged around 0.17 mg/L except that two peaks were observed in April and July with the concentrations of 0.49 and 0.66 mg/L, respectively. The concentrations of TP exhibited considerable variation during the whole year with peaks in April, July and December. The average TP concentration was 0.08 mg/L, while the orthophosphate concentrations were relatively low (< 0.02 mg/L) in the water source.

The value of COD_{Mn} ranged from 3.82 to 8.34 mg/L, which decreased slowly during winter and spring then increased dramatically in summer with the highest value detected in July, then decreased in autumn. The average concentration of DOC was 4.22 mg/L. The relatively high concentrations of DOC were observed in summer and autumn with the highest value of 5.82 mg/L in August.

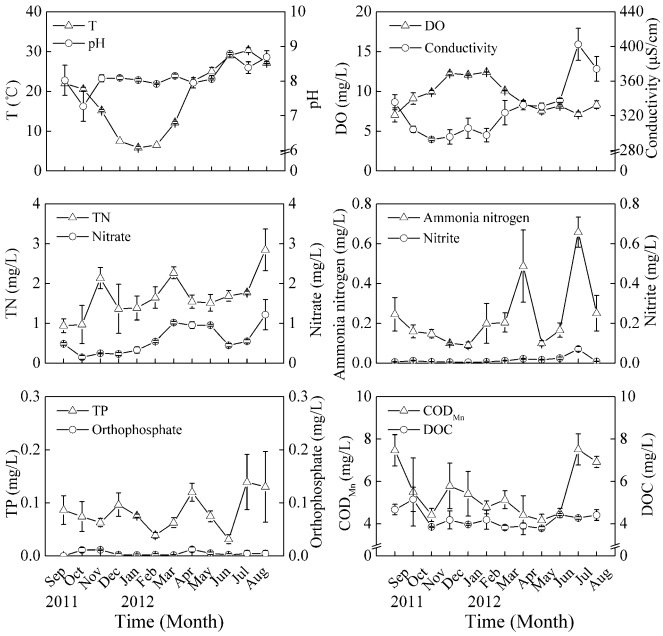

Fig.2 Seasonal variations of physicochemical parameters in the eastern drinking water source of Lake Chaohu. Error bars indicate the standard deviation of three sampling sites.

3.2. Phytoplankton population and Chl *a*

Phytoplankton divisions including Cyanophyta, Chlorophyta, Bacillariophyta, Euglenophyta, Cryptophyta were recorded in the studied area (Fig. 3a). Among them, cyanobacteria contributed more than 94% to total phytoplankton density except in March (52%) and April (60%), wherein the percentage of *Chlorophyta* density was around 37%. Two genera, namely *Microcystis* and *Dolichospermum* represented more than 98% of the total cyanobacterial density except in March (85%). In March and April, the cyanobacterial densities varied from 1400 to 4300 cells/mL, which were the lowest of the whole year. The abundance of cyanobacteria increased from the beginning of May, while the dominant cyanobacterial species *Dolichospermum* decreased until July and was succeeded by *Microcystis* from July to October. In November, the abundance of *Microcystis* decreased to 30 000 cells/mL and the *Dolichospermum* remained within a certain range of cell density around 21 000 cells/mL. In winter, the dominant cyanobacterial species changed gradually from *Microcystis* to *Dolichospermum* reaching the highest cyanobacterial cell densities (220 000 cells/mL) in December.

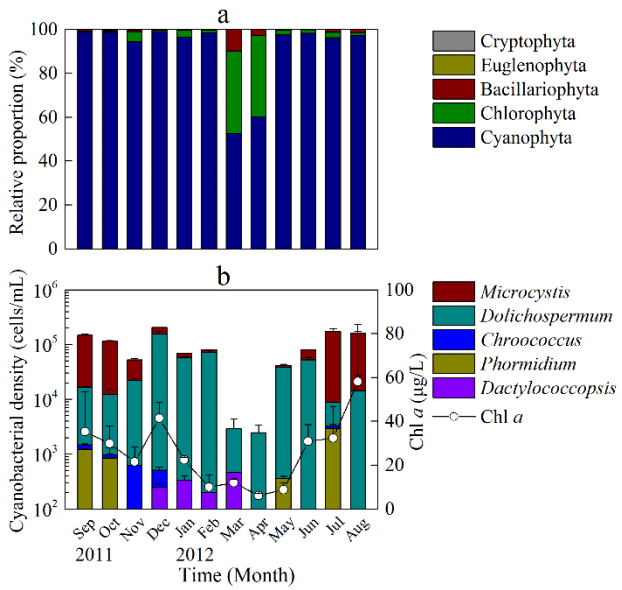

Fig.3 Seasonal variations of relative proportion of phytoplankton species (a) and Chl *a* and cyanobacterial density (b) in the eastern drinking water source of Lake Chaohu. Error bars indicate the standard deviation of three sampling sites.

During the studied period, Chl *a* concentrations varied from 5.3 to 75.6 μg/L with a mean value of 25.7 μg/L (Fig. 3b). There were two distinct peaks, the first in July and the second in December, coinciding with the maximum density of *Microcystis* and *Dolichospermum*, respectively. The abundant phytoplankton frequently appeared under specific environmental conditions with proper lighting and calm winds in winter, which were also observed in 2012 and 2013 in Lake Chaohu (data not shown) requiring immediate attention as the cyanobacteria could induce high toxic concentration in source water.

3.3. MC production

In the eastern drinking water source of Lake Chaohu, the concentrations of MCs (MC-LR, -RR and -YR) exhibited significant seasonal dynamics, and total concentrations of the three MC congeners ranged from 0.28 μg/L in March to 8.86 μg/L in September. (Fig. 4a-c). The dominant MC congeners varied in different months. In August and December, the proportion of MC-LR was 54% and 38% of the total MCs, respectively. In January, October, and November, the dominant congener changed to MC-YR, accounting for more than 50% of the total MCs. In the rest months, MC-RR was the most abundant MC congener (42% to 73% of the total MCs), which was also found in other fresh waters in China.[12]

The seasonal variations of three MC congener concentrations were in agreement with those of cyanobacterial abundance. When high *Microcystis* density appeared in summer and autumn, the concentrations of MCs also reached the peak with the dominant fraction as extracellular MCs. When the abundance of *Dolichospermum* accumulated, the intracellular fractions of MCs peaked in December. In detail, high concentrations of MC-LR (from 0.75 to 1.90 μg/L) were observed from August to October and December and relatively low concentrations of MC-LR (0.07 to 0.31 μg/L) were found in other months. The MC-LR contributed to high concentration of extracellular MCs (MC-LR 0.81 μg/L to 1.77 μg/L) in water from August to October and to high concentration of intracellular MCs (MC-LR 1.31 μg/L) in December, respectively. A similar monthly fluctuation was found for MC-RR, with the exception that the highest concentration of MC-RR occurred in September (3.57 μg/L). In addition, high concentrations of MC-RR (2.24 μg/L and 1.08 μg/L) were detected in October and December, respectively. Relatively high concentrations of MC-YR were observed until late summer with the maximum value (3.23 μg/L) in October and values remained close to 1.0 μg/L in December and January.

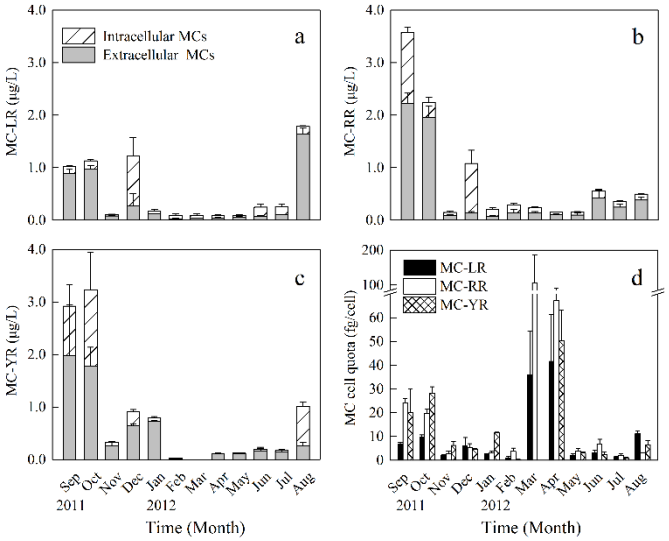

Fig.4 Seasonal variations of intra- and extra-cellular microcystin concentrations (a, b and c) and toxin quotas (d) in the eastern drinking water source of Lake Chaohu. Error bars indicate the standard deviation of three sampling sites.

The toxin cell quotas (the amount of toxin per cell) of the three MC congeners were calculated monthly, which showed different patterns from those of the MC concentrations (Fig. 4d). We classified the 12 months into four periods not only based on the seasonal cycle, but also according to the variances of dominant cyanobacterial species and the growth phase of cyanobacteria in the present study: 1) in March and April, the lowest cyanobacterial density was found at recruitment stage,[21] the cell quota of MC-LR, -RR and -YR reached the peak value of 53, 196 and 62 fg/cell, respectively; 2) from May to July, cyanobacteria was at multiplication stage with the dominant species being *Microcystis*, the mean cell quota of MC-LR, -RR and -YR was 2, 4 and 2 fg/cell, respectively; 3) from August to October, while the dominant cyanobacterial species was *Microcystis* at decline stage, the mean cell quota of MC-LR, -RR and -YR was 9, 15 and 17 fg/cell, respectively; 4) from November to February, when the dominant species changed to *Dolichospermum* and was considered to be at dormancy stage, the mean cell quota of MC-LR, -RR and -YR was 3, 4 and 6 fg/cell, respectively.

3.4. Relationships between MCs, cyanobacterial density and water quality

To identify the factors influencing toxin production, the correlations between MCs, cyanobacterial density and physicochemical parameters were determined in the eastern

water source of Lake Chaohu (Table 1). The density of *Microcystis* yielded significant positive correlations with cyanobacterial abundance and Chl *a*. In addition, the concentrations of MC congeners were positively correlated with *Microcystis* and cyanobacterial density and Chl *a*. The cell quotas of MC-LR correlated negatively with the density of *Dolichospermum*, while those of MC-RR correlated negatively with *Microcystis*, *Dolichospermum* and cyanobacterial density. Physical parameters (water temperature, pH, DO and conductivity) had effect on the abundance of *Microcystis* and *Dolichospermum*, but had no relationship with MC production except the negative relationship between pH and MC-YR cell quota. The concentrations of nitrate and orthophosphate showed negative correlations with cyanobacterial density and MC production, while the COD_{Mn} and DOC values always correlated significantly with them.

Table 1 Correlation coefficients between concentrations of MCs, MC cell quotas, the biomass of dominant cyanobacteria and selective physicochemical parameters from September 2011 to August 2012 in the eastern drinking water source of Lake Chaohu

				MC concentrations			MC cell quotas		
	Mic	Dol	Cya	MC-LR	MC-RR	MC-YR	MC-LR	MC-RR	MC-YR
Mic	1	0.004	0.877**	0.812**	0.650**	0.719**	-0.172	-0.418*	0.152
Dol	0.004	1	0.31	0.068	0.113	0.103	-0.540**	-0.365*	-0.189
Cya	0.877**	0.310	1	0.783**	0.745**	0.619**	-0.214	-0.366*	0.006
Chl *a*	0.779**	0.138	0.749**	0.811**	0.633**	0.638**	0.020	-0.233	0.099
T	0.389*	-0.422*	0.176	0.224	0.107	0.041	0.038	-0.087	-0.025
pH	0.344*	-0.103	0.254	0.247	0.101	-0.016	-0.002	-0.234	-0.360*
DO	-0.373*	0.438**	-0.119	-0.190	-0.142	-0.176	-0.030	-0.008	-0.126
Cond	0.336*	-0.502**	0.180	0.239	0.183	-0.020	0.190	0.017	-0.076
TN	-0.035	-0.234	-0.149	-0.198	-0.341*	-0.343*	0.003	-0.293	-0.431**
NO3	-0.313	-0.227	-0.332*	-0.339*	-0.331*	-0.544**	0.264	0.110	-0.326
NH4	0.201	-0.544**	0.078	0.055	0.212	-0.084	0.190	0.192	0.032
TP	0.323	-0.261	0.301	0.277	0.091	0.297	0.223	-0.029	0.384*
SRP	-0.154	-0.304	-0.333*	-0.175	-0.401*	-0.066	0.069	-0.042	0.296
COD_{Mn}	0.616**	-0.230	0.584**	0.490**	0.484**	0.377*	0.131	-0.12	0.033
DOC	0.631**	0.030	0.621**	0.702**	0.738**	0.614**	0.119	0.035	0.290

**. Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed). Mic: the density of *Microcystis*; Dol: the density of *Dolichospermum;* Cya: the density of cyanobacteria; Cond: conductivity; NO3: Nitrate; NH4: ammonia nitrogen; SRP: orthophosphate

Redundancy analysis was used to identify the key environmental factors influencing the seasonal dynamics of the abundance and structure of cyanobacteria and MC congeners. Results of RDA for the composition of cyanobacteria (including *Microcystis*, *Dolichospermum*, *Chroococcus*, *Phormidium*, and *Dactylococcopsis*) illustrated that water temperature and nitrate were the best predictors accounting for 19.9% (p = 0.004) and 15.1% (p = 0.002) of the variability, respectively (Fig. 5a). The model explained 85.7 % of the total variability. The densities of *Microcystis*, *Chroococcus*, and *Phormidium* were positively correlated with water temperature and negatively correlated with nitrate, while the reverse relationships were obtained for *Dolichospermum* and *Dactylococcopsis*.

Another RDA diagram (Fig. 5b) showed that the extra- and intra-cellular MC congeners correlated significantly with each other and their relationship with cyanobacteria and physicochemical characteristics of water. The *Microcystis* density was the best predictor of variability in MC concentrations, accounting for 25% of the variability (p = 0.002). Total nitrogen, conductivity and *Dolichospermum* density were also significant pedictors accounting for 22% (p = 0.002), 10% (p = 0.004) and 7% (p = 0.002), respectively. The model explained 64% of the total variability, which showed MC congeners were positively correlated with *Microcystis* density, but negatively correlated with TN.

3.5. The ST-values for cyanobacteria and the verification

Since the LD_{50} value (intraperitoneal application) for MC-RR is about 10 times higher than that for MC-LR and the LD_{50} for MC-YR is similar to that for MC-LR, the $TEF_{MC\text{-}RR}$ and $TEF_{MC\text{-}YR}$ were 0.1 and 1.0, respectively, by defining $TEF_{MC\text{-}LR}$ as 1.0.[4] Then the guideline value for MC-RR and -YR of 10.0 μg/L and 1.0 μg/L was calculated using the guideline of MC-LR (1.0 μg/L) divided by $TEF_{MC\text{-}RR}$ and $TEF_{MC\text{-}YR}$, respectively. Then the cyanobacterial density equivalent to the guideline of MC was calculated every month and the ST-values for cyanobacteria in four periods were obtained according to Eqs. (1) ~ (5). In the present study, the ST-values for cyanobacteria were $(2.1 \pm 0.6) \times 10^4$ cells/mL at the recruitment stage (March - April), $(3.4 \pm 1.0) \times 10^5$ cells/mL at the multiplication stage (May - July), $(3.6 \pm 0.3) \times 10^4$ cells/mL at the decline stage (August - October), and $(8.6 \pm 0.2) \times 10^4$ cells/mL at the dormancy stage (November - February), respectively (Fig. 6a).

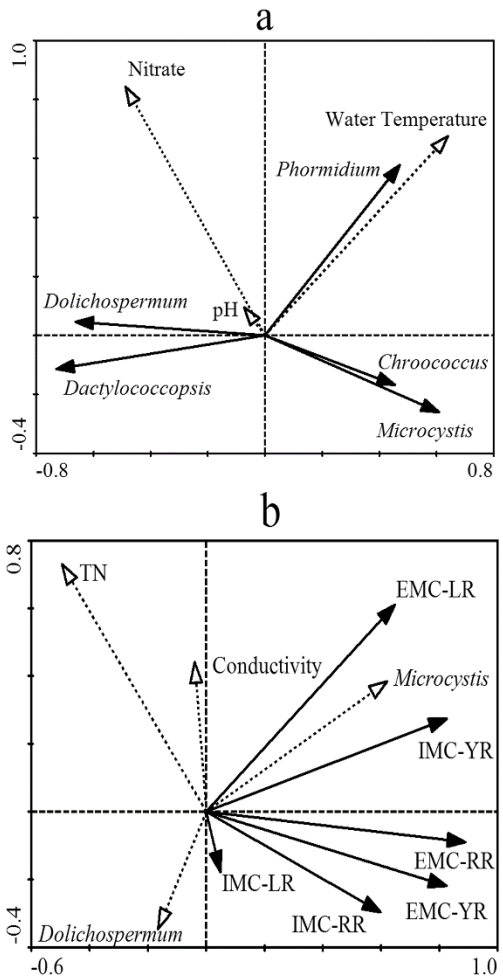

Fig. 5 Redundancy analysis (RDA) biplots showed (a) the compositions of cyanobacteria in relation to environmental factors including water temperature, pH, DO, conductivity, TN, nitrate, nitrite, ammonia nitrogen, TP and orthophosphate; and (b) the concentrations of extra- and intra-cellular MCs in relation to *Microcystis* and *Dolichospermum* density and environmental factors mentioned above in the eastern drinking water source of Lake Chaohu. E: extracellular MCs; I: intracellular MCs.

To check the applicability of the ST-value, we took the concentrations of three MC congeners and cyanobacterial densities at the water intakes from June 2013 to May 2014 for example (Fig. 6b). In August, November and December 2013, the densities of cyanobacteria were 1.3×10^5, 1.1×10^5 and 1.5×10^5 cells/mL, respectively, which were higher than the corresponding ST-values. Accordingly, the concentrations of MC-LR were 1.60, 1.24 and 1.28 μg/L, respectively, which exceeded the WHO guideline of 1.0 μg/L. When the cyanobacteria densities were lower than the corresponding ST-values in other months, the MC concentrations were below the guideline value of each MC congener. These results indicated the ST-values for cyanobacteria were a reliable indicator for a potential toxin hazard in the water source of Lake Chaohu.

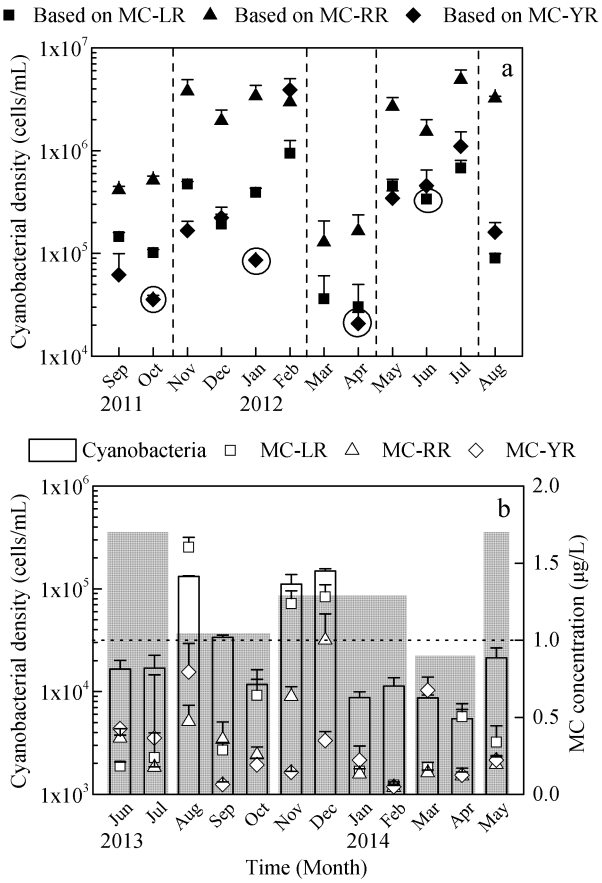

Fig.6 The establishment of ST values (the circle) for cyanobacteria in four periods (vertical dashed lines) based on the data collected from September 2011 to August 2012 (a), and the verification of ST values based on data from June 2013 to May 2014 (b) in the eastern drinking water source of Lake Chaohu. Gray shading refers to the ST-value in corresponding period and the horizontal dotted line indicates the MC guideline (1.0 μg/L). Error bars indicate the standard deviation of three sampling sites.

4. Discussion

In the eastern drinking water source of Lake Chaohu the abundance and composition of cyanobacteria showed significant seasonal variations. The dominant cyanobacterial species was *Microcystis* in summer and autumn, which changed to *Dolichospermum* gradually with the decrease of water temperature in winter and spring. The concentrations of MCs exhibited strong seasonal dynamics and the maximum concentrations were found in the late summer and the whole autumn in the source water. The concentrations of MC-LR were observed above 1.0 μg/L from August to October 2011 and in December 2011, which should be of concern since the lake serves as a direct source of drinking water for several million people all year round.

4.1. Environmental factors influencing the cyanobacterial composition and MCs

Environmental factors affect MCs production in surface waters by regulating the phytoplankton composition, the proportion of toxic-producing cyanobacteria and/or the MC production by toxigenic strains.[15,30-33] During the study period, the abundance of *Microcystis* was highly correlated with total cyanobacterial density and Chl *a*. Moreover, the concentration of various MC congeners correlated positively with Chl *a*, total cyanobacterial abundance and especially with the density of *Microcystis*, indicating that *Microcystis* spp. was the main MC producing taxon in Lake Chaohu. However, a weak relationship was found between cyanobacterial abundance and MC levels in some lakes, in which the peak of cyanobacterial biomass might not coincide with that of MC concentration.[34] This could be explained by coexistence of several cyanobacterial generas or *Microcystis* strains with different toxicity in water column and different factors responsible for the cyanobacterial biomass and MC production.[35,36] Nevertheless, in the lakes dominated by potentially MC-producing genera cyanobacteria including Lake Taihu in China,[7] Daechung Reservoir in Korea,[37] 18 Eutrophic Czech Reservoirs[38] and Lake Erie in North America[20], highly significant relationships between cyanobacterial abundance and MC concentrations were found to be consistent with the result in this study. In such circumstances, the establishment of ST values according to cyanobacterial abundance would be applicable in waters relating to MCs.

In the present study, the negative correlations were obtained between MC-RR cell quota and cyanobacterial density and between MC-LR and MC-RR cell quotas and *Dolichospermum* density. This could be explained by the fact that there is a shift in the dominance of MC-producing and non-producing cells to make a better adaptation to environmental conditions for cyanobacteria.[31,39] Toxic cells have an advantage under suboptimal conditions for growth, which could enhance growth efficiency or act as a grazing deterrent.[40] Although there was no correlation between MCs and *Dolichospermum* density, the contribution of *Dolichospermum* to MCs concentrations was obvious in December. This phenomenon was also proved by the RDA result that *Dolichospermum* abundance correlated with intracellular MC-LR (Fig. 5b).

The results of RDA showed that water temperature was the most important environmental variable to determine the abundance and structure of dominated cyanobacterial genera (Fig. 5a). Water temperature accelerated the growth of *Microcystis*, but had a negative effect on *Dolichospermum*, which was in the line with the succession of two cyanobacterial species observed in Lake Chaohu. The *Dolichospermum* was at growth advantage in winter, because *Dolichospermum* was still photosynthesizing at 76% of the maximum rate, while the photosynthetic rate of *Microcystis* declined to 4% at 15 ℃.[30] However, *Microcystis* could obtain competitive advantage at high temperatures synergistically with other environmental factors. Although temperature has a pronounced effect on both growth and toxicity of cyanobacteria,[32,41] there was no significant correlation between water temperature and MC production in Lake Chaohu. It was suggested that optimal conditions for growth should not always coincide with those for toxin production. Westhuizen and Eloff[41] found that toxicity was markedly reduced at growth temperatures above 28 ℃, which were consistent with our findings that MCs concentrations were low in July when cyanobacterial abundance peaked. The succession of dominant cyanobacterial genera was affected by water temperature and resulted in the significant seasonal variations of MCs concentrations and cell quotas. Therefore, the establishment of ST in different periods would make the management of controlling cyanobacteria more practical and effective in water source.

Nutrients, such as nitrogen and phosphorus were also key environmental factors to predict the phytoplankton species composition and MC production. As shown in RDA diagrams, nitrate and TN were correlated negatively with the composition of cyanobacteria and the concentrations of extra- and intra-cellular MCs, respectively, which agrees with the results obtained in other lakes.[7,42] However, the finding was contrary to that of monoculture experiments in labs which indicated that high nitrogen concentrations favored toxin production of studied cyanobacterial strains.[19,32] But the opposite result was obtained for co-culture experiments containing MC-producing and non-MC-producing strains, closer to natural circumstance as the present study, suggesting that the benefits of producing MC outweigh the costs under growth-limiting conditions.[43,44] As a non-nitrogen-fixing cyanobacterial genus, *Microcystis* depleted nitrate in the surface water during blooms in early summer, which resulted in the decrease of nitrate in the eastern Lake Chaohu. Orthophosphate also showed negative correlation with the cyanobacterial density and the concentration of MC-RR. In addition, the concentrations of nutrients in the lake were affected both by internal loading released by sediment and the external loading from surface runoff and other non-point sources, which increased the uncertainty of the relationships between cyanobacterial density, MC productions and nutrients.[45] The peak of nutrient loadings in raw water was coincident with rainfall peak and agricultural fertilization according to local historical pollution sources data.[22] As nutrients have definite impacts on cyanobacterial structure and abundance and MC production, great effort should be made to control nutrient inputs to reduce cyanobacterial biomass and MCs concentrations in the lake.

Environmental factors, like pH, DO and conductivity, showed no correlation with cyanobacterial density but could regulate the predominant cyanobacterial species and MC production. Positive correlation between pH and *Microcystis* abundance in natural samples has been reported previously.[46] With the help of efficient carbon concentration mechanisms, cyanobacteria have a competitive advantage over other phytoplankton species under high pH conditions.[46,47] In addition, the negative correlation between MC-YR cell quota and pH agrees with other studies, wherein decreasing MC cellular quotas were observed at increasing pH due to the succession of nontoxic cells over toxic cyanobacteria under suitable conditions.[33] The relatively low values of DO in summer reflected the comprehensive effect of water temperature and cyanobacterial proliferation on water quality. Conductivity, indicating ion concentration in water, showed contrasting seasonal patterns with DO, which was influenced by exogenous sources through storm erosion and overwash, and internal loading from sediment and cyanobacterial metabolization.[22,48] In this study, conductivity was chosen as a high weighted parameter in RDA to explain the variability of extra- and intra-cellular MC concentrations, which revealed integrated effects of individual environmental factors on MC productions.

The MCs concentrations and cyanobacterial density had significant positive correlation with COD_{Mn} and DOC. High values of COD_{Mn} and DOC were observed in summer and autumn along with the proliferation of cyanobacteria. A large number of diverse cyanobacterial secondary metabolites

including MCs constitute a significant component of organic matter in natural water bodies, and are significant sources of disinfection by-products precursors for drinking water treatment facilities.[49] Therefore, the control of cyanobacterial density below the ST-value can further reduce algal derived organic matters and lower public health risk.

4.2. The establishment and verification of ST series for cyanobacteria in different periods

The ST-value for cyanobacteria was established based on the periodic variations of MC cell quotas according to the significantly positive correlations between the concentrations of MCs and cyanobacterial densities. In this study, it is important to emphasize that the ST-values were obtained according to MC-LR during the multiplication stage, but referring to MC-YR in the other periods. When the density of cyanobacteria in source water exceeds the corresponding ST-value in each period, the concentration of certain MC would exceed its guideline.

The ST-values for cyanobacteria oscillated over a wide range in different periods because of the impact of various biotic and abiotic environmental factors on MC production. At the recruitment stage, the lowest ST-value among the whole year was obtained due to the highest MC cell quota. That might be explained by the inhibitory effect of MC against either competitor or predator being enhanced under suboptimal environmental condition.[2] From May to July, increasing water temperature and adequate nutrients led to fast growth of cyanobacteria. The highest ST-value accompanied with the lowest MC cell quota might be due to the increase in the relative proportions of non-MC-producing cells and the decreasing of MC productions by toxic cells.[40] During this period, cyanobacterial density was lower than the ST-value with MC concentrations not exceeding the guideline. From August to October, when the bloom was at the decay phase with cell lysis, the MC cell quota increased slightly and the ST-value decreased compared with that in the previous stage. As the MC concentrations were always at high level over this period, the significant chance of MC concentrations exceeding guideline requires attention. At the dormancy stage, when cyanobacteria cells usually maintain a low level of metabolic activities to acclimatize to adverse environments,[21] the ST-value was moderate compared to the values in other periods. However, frequent monitoring should be conducted in the winter of Lake Chaohu, since the likely appearance of abundant *Dolichospermum* might induce high toxic concentration. The different characteristics of cyanobacteria and MCs production in four periods highlighted the importance of establishing the ST-value for each period, which could improve the operability and efficiency in controlling toxic cyanobacterial density in water supply.

The ST-value obtained in this study was generally higher than that obtained in previous studies, wherein the threshold value was proposed as the worst case with relatively high toxin cell quota (200 fg toxins per cell) and/or assuming other cyanotoxins with the same toxicity as MC-LR.[1,17,18] The value might not reflect actual situation with the coexistence of a great variety of cyanobacterial species in source water, wherein the MC cell quotas generally vary by an order of magnitude (0-700 fg/cell) in cyanobacterial blooms.[6,50,51] Moreover, as the mixture of cyanotoxins was always detected in lakes or reservoirs worldwide,[4] different MC congeners and their degree of toxicity should be considered in the establishment of ST to obtain comprehensive and accurate assessment of the harmful cyanobacterial blooms.

Although the cyanobacterial abundance and MCs production fluctuated annually, the successful application of ST values in the water source of Lake Chaohu suggested that the proposed ST values are reliable indicators of the hazard of cyanobacterial toxin and for evaluating water quality under the influence of cyanobacterial proliferation. Thus, the proposed ST values can be used to optimize management scenarios to guarantee the safety of drinking water sources with harmful cyanobacteria through appropriate measures. For example, when the cyanobacteria densities are lower than the ST-values and the MC concentrations are below the WHO guideline value of each MC congener, the raw water meets the required quality of source water used for drinking water treatment plants and no remedial action is required. In contrast, when the densities of cyanobacteria are at or above the ST-values, it is an alert of toxin hazard that the MC concentrations exceed the guideline in water source. In this case, algal control measures, such as salvaging algae in raw water and improving water treatment efficiency, should be used for emergency control. The water supply should not be directly employed until that cyanobacteria density declines under the ST value. Lastly, in the case that the density of cyanobacteria is close to the ST-value but MC concentrations are lower than the WHO guideline of 1.0 μg/L such as in September 2013, samples representative for raw water intake should be collected and analyzed to confirm the safety of source water. Although the preliminary ST value for cyanobacteria has been confirmed to be effectiveness in assessing toxin hazard and in planning treatment strategies of water source, it has to be noted that the more investigate data including more toxins are involved to refine the ST values, the greater the reliability of the ST values for MC alert. Further management strategy such as reducing diffusive nutrient inputs is necessary to control the proliferation of cyanobacteria below ST-value.

5. Conclusions

In the eastern drinking water source of Lake Chaohu, the MC productions and cyanobacterial abundance exhibited strong seasonal dynamics due to the combined influence of environmental factors. The dominant cyanobacterial species was *Microcystis* in summer and autumn, which was succeeded by *Dolichospermum* gradually with the decrease of water temperature in winter and spring. The maximum concentrations of MC-LR, -RR and -YR were found in last part of summer and the whole autumn in the water source and the concentrations of MC-LR were even above the guideline value (1.0 μg/L). According to the positive correlation between the concentrations of MCs and the density of cyanobacteria, the ST-value was established and its applicability was checked by subsequent inspection. This work has highlighted the importance of establishment of the ST series relating to the periodic variations of different MC congeners production, which could improve the operability and efficiency in controlling toxic cyanobacterial density in water supply. This is very important for protecting the source water quality from toxic cyanobacterial pollution, especially for the emergency management. Although the presented ST-values are specific for these drinking water intakes, the principles and procedures can be applied to other water bodies under the negative effect of harmful cyanobacteria.

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References

1 I. Chorus and J. Bartram, *Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management*, Spon Press, London, 1999, pp. 150-220.
2 J. Leflaive and L. Ten-Hage, *Freshwater Biol.*, 2007, **52**, 199-214.
3 A. Zamyadi, S. Dorner, M. Ndong, D. Ellis, A. Bolduc, C. Bastien and M. Prévost, *Environ. Sci.: Processes Impacts*, 2014, **16**, 313-323.
4 H. U. Wolf and C. Frank, *Environ. Toxicol.*, 2002, **17**, 395-399.
5 R. Amer, B. Dféz and R. El-Shehawy, *J. Environ. Monitor.*, 2009, **11**, 126-133.
6 V. Vasconcelos, J. Morais and M. Vale, *J. Environ. Monitor.*, 2011, **13**, 668-672.
7 S. W. Wilhelm, S. E. Farnsley, G. R. LeCleir, A. C. Layton, M. F. Satchwell, J. M. DeBruyn, G. L. Boyer, G. Zhu and H. W. Paerl, *Harmful Algae*, 2011, **10**, 207-215.
8 D. P. Botes, P. L. Wessels, H. Kruger, M. T. Runnegar, S. Santikarn, R. J. Smith, J. C. Barna and D. H. Williams, *J. Chem. Soc., Perkin Transactions 1*, 1985, 2747-2748.
9 J. L. Graham, K. A. Loftin, A. C. Ziegler and M. T. Meyer, *Guidelines for design and sampling for cyanobacterial toxin and taste-and-odor studies in lakes and reservoirs*, U.S. Geological Survey Scientific Investigations Report 2008-5038, U.S.G.S., Reston, 2008.
10 W. J. Fischer, I. Garthwaite, C. O. Miles, K. M. Ross, J. B. Aggen, A. R. Chamberlin, N. R. Towers and D. R. Dietrich, *Environ. Sci. Technol.*, 2001, **35**, 4849-4856.
11 World Health Organization, Guidelines for drinking-water quality: incorporating first addendum, WHO, Geneva, 3rd ed., 2006, vol. 1: recommendations, pp. 407-408.
12 W. Chen, L. Peng, N. Wan and L. Song, *Chemosphere*, 2009, **77**, 1585-1593.
13 T. Jurczak, M. Tarczynska, K. Izydorczyk, J. Mankiewicz, M. Zalewski and J. Meriluoto, *Water Res.*, 2005, **39**, 2394-2406.
14 S. Zhou, Y. Shao, N. Gao, Y. Deng, J. Qiao, H. Ou and J. Deng, *Sci. Total Environ.*, 2013, **463**, 111-119.
15 G. P. Horst, O. Sarnelle, J. D. White, S. K. Hamilton, R. B. Kaul and J. D. Bressie, *Water Res.*, 2014, **54**, 188-198.
16 G. Newcombe, J. House, L. Ho, P. Baker and M. Burch, *Management strategies for cyanobacteria (blue-green algae): a guide for water utilities*, Research Report No 74, W.Q.R.A., Adelaide, 2010.
17 K. Izydorczyk, C. Carpentier, J. Mrówczynski, A. Wagenvoort, T. Jurczak and M. Tarczynska, *Water Res.*, 2009, **43**, 989-996.
18 W. Schmidt, K. Bornmann, L. Imhof, J. Mankiewicz and K. Izydorczyk, *Environ. Toxicol.*, 2008, **23**, 337-345.
19 K. Sivonen, *AEM*, 1990, **56**, 2658-2666.
20 J. M. Rinta-Kanto, E. A. Konopko, J. M. DeBruyn, R. A. Bourbonniere, G. L. Boyer and S. W. Wilhelm, *Harmful Algae*, 2009, **8**, 665-673.
21 X. Tan, *J. Food Agric. Environ.*, 2012, **10**, 929-934.
22 H. Yu, B. Xi, J. Jiang, M. J. Heaphy, H. Wang and D. Li, *Environ. Sci. Pollut. Res.*, 2011, **18**, 1333-1342.
23 X. Huang, W. Chen and Q. Cai, *Analysis and ecological investigation of lake*, Standards Press, Beijing, 1999, pp. 27-62.
24 S. W. Jeffrey and G. F. Humphrey, *Biochemie Und Physiologie Der Pflanzen*, 1975, **167**, 191-194.
25 H. Hu and Y. Wei, *The freshwater algae of China: systematics, taxonomy and ecology*, Science Press, Beijing, 2006, pp. 23-903.
26 M. Barco, L. A. Lawton, J. Rivera and J. Caixach, *J. Chromatogr. A*, 2005, **1074**, 23-30.
27 A. F. Roegner, B. Brena, G. González-Sapienza and B. Puschner, *J. Appl. Toxicol.*, 2014, **34**, 441-457.
28 S. H. Safe, *Environ. Health Perspect.*, 1998, **106**, 1051.
29 C. t. Braak and P. Šmilauer, *CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5)*, Biometris, Wageningen, 2002.
30 R. D. Robarts and T. Zohary, *N. Z. J. Mar. Freshwater Res.*, 1987, **21**, 391-399.
31 R. Akcaalan, F. M. Young, J. S. Metcalf, L. F. Morrison, M. Albay and G. A. Codd, *Water Res.*, 2006, **40**, 1583-1590.
32 T. W. Davis, D. L. Berry, G. L. Boyer and C. J. Gobler, *Harmful Algae*, 2009, **8**, 715-725.
33 M. Tao, P. Xie, J. Chen, B. Qin, D. Zhang, Y. Niu, M. Zhang, Q. Wang and L. Wu, *PloS one*, 2012, **7**, e32020.
34 K. Ozawa, H. Fujioka, M. Muranaka, A. Yokoyama, Y. Katagami, T. Homma, K. Ishikawa, S. Tsujimura, M. Kumagai and M. F. Watanabe, *Environ. Toxicol.*, 2005, **20**, 270-276.
35 S. C. Sinang, E. S. Reichwaldt and A. Ghadouani, *Environ. Monit. Assess.*, 2013, **185**, 6379-6395.
36 J. M. Jacoby, D. C. Collier, E. B. Welch, F. J. Hardy and M. Crayton, *Can. J. Fisheries. Aquat. Sci.*, 2000, **57**, 231-240.
37 H.-M. Oh, S. J. Lee, J.-H. Kim, H.-S. Kim and B.-D. Yoon, *AEM*, 2001, **67**, 1484-1489.
38 P. Znachor, T. Jurczak, J. Komárková, J. Jezberová, J. Mankiewicz, K. Kaštovská and E. Zapomělová, *Environ. Toxicol.*, 2006, **21**, 236-243.
39 K. Vareli, G. Pilidis, M.-C. Mavrogiorgou, E. Briasoulis and I. Sainis, *J. Environ. Monitor.*, 2009, **11**, 1506-1512.
40 M. Sabart, D. Pobel, E. Briand, B. Combourieu, M. Salençon, J.-F. Humbert and D. Latour, *AEM*, 2010, **76**, 4750-4759.
41 A. J. v. d. Westhuizen and J. N. Eloff, *Planta*, 1985, **163**, 55-59.
42 H. Yang, P. Xie, J. Xu, L. Zheng, D. Deng, Q. Zhou and S. Wu, *Bull. Environ. Contam. Toxicol.*, 2006, **77**, 367-374.
43 E. Briand, M. Gugger, J.-C. François, C. Bernard, J.-F. Humbert and C. Quiblier, *AEM*, 2008, **74**, 3839-3848.
44 E. Briand, M. Bormans, C. Quiblier, M.-J. Salençon and J.-F. Humbert, *PloS one*, 2012, **7**, e29981.
45 R. Sugimoto, T. Sato, T. Yoshida and O. Tominaga, *Limnol. Oceanogr.*, 2014, **59**, 37-47.

46 B. G. Kotak, A. K. Lam, E. E. Prepas and S. E. Hrudey, *Can. J. Fisheries. Aquat. Sci.*, 2000, **57**, 1584-1593.
47 R. J. Wicks and P. G. Thiel, *Environ. Sci. Technol.*, 1990, **24**, 1413-1418.
48 X. Chuai, W. Ding, X. Chen, X. Wang, A. Miao, B. Xi, L. He and L. Yang, *Ecol. Eng.*, 2011, **37**, 842-849.
49 E. C. Wert, J. A. Korak, R. A. Trenholm and F. L. Rosario-Ortiz, *Water Res.*, 2014, **52**, 251-259.
50 J.-F. Briand, S. Jacquet, C. Flinois, C. Avois-Jacquet, C. Maisonnette, B. Leberre and J.-F. Humbert, *Microb. Ecol.*, 2005, **50**, 418-428.
51 G. Fahnenstiel, D. Millie, J. Dyble, R. Litaker, P. Tester, M. McCormick, R. Rediske and D. Klarer, *Aquat. Ecosyst. Health.*, 2008, **11**, 190-195.