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# Environmental abundance and microcystin-LR production ability of toxic *Microcystis* in Nanquan region of Lake Taihu

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**Abstract:** The variations of environmental abundance and microcystin-LR (MC-LR) production ability of toxic *Microcystis* in the Nanquan region of Lake Taihu are investigated by real-time quantitative PCR (RTQ-PCR) and high performance liquid chromatography (HPLC) from May to December in 2009. Simultaneously, degrees of water pollution and eutrophication are monitored. The results indicate that the water quality in the Nanquan region of Lake Taihu is in a moderate degree of pollution and eutrophication. Algal density exceeds the threshold of bloom from May to November. The environmental abundance of toxic *Microcystis* is more than 40% from May to October and then significantly declines to 5.66% due to the obvious reduction in the water temperature in December. From May to December, the MC-LR production ability of toxic *Microcystis* ranges from 1.661 to 9.293 μg/$10^8$ cells. With the significant drops in water temperature and algal density, the MC-LR production ability of toxic *Microcystis* is obviously increased from November to December. It is concluded that the lake presents *Microcystis* bloom and the toxic *Microcystis* becomes dominant during most of the year. The environmental abundance and the MC-LR production ability of toxic *Microcystis* have a close relationship with water temperature. The effective control of toxic *Microcystis* should be considered in both the bloom period and the non-bloom period of winter since the MC-LR production ability of toxic *Microcystis* obviously increases in winter.

**Key words:** eutrophication; lake pollution; algae; microcystins; environmental abundance; Lake Taihu

Eutrophication of the lakes presents one of the most serious environmental problems in China and more than 60% of the lakes have been reported to be in the situation of eutrophication and the outbreak of blooms in recent years[1]. Studies have revealed that the majority of the blooms are classified as cyanobacteria blooms. *Microcystis* usually turns out to be the most dominant species and produces different kinds of microcystins(MCs) which are cyclic nonribosomal peptides consisting of several uncommon non-proteinogenic amino acids which have strong toxicity, especially hepatotoxicity[2]. Long-term consumption of water contaminating the MCs can lead to liver damage and even liver cancer[3]. Lake Taihu is the third largest freshwater lake of China with an area of 2 250 $km^2$ and is the principal drinking water source for more than 10 million residents in the region of the lake. Dramatic increases in nutrient loading by urban and agricultural development in its watershed have fueled accelerated eutrophication, characterized by increasingly severe, toxin producing cyanobacterial blooms since the mid-1980s[4-5]. Therefore, the study of the environmental existence and the biological features of toxic *Microcystis* in Lake Taihu is necessary to understand the properties and characteristics of the bloom in the lake as a basic effort of effective control. In this study, the variation of environmental abundance and the microcystin-producing ability of toxic *Microcystis* are investigated in the Nanquan region of Lake Taihu.

## 1 Material and Methods

### 1.1 Studied area

The water is sampled from the Nanquan region of Lake Taihu in Wuxi, located in east longitude 119°21′ to 122°00′ and latitude 30°19′ to 32°00′(see Fig. 1). According to the monitoring data of the Water Environment Monitoring Center for Lake Taihu, the $COD_{Mn}$ of the entire lake has been increasing since 2003. The average $NH_3$-N concentration of the whole lake is 0.18 to 0.55 mg/L, which is significantly higher in flood season than that in non-flood season. The pH value of the lake water rises when the algae begins to aggregate. The assessment of the water quality in the Nanquan region of Lake Taihu is moderately polluted.

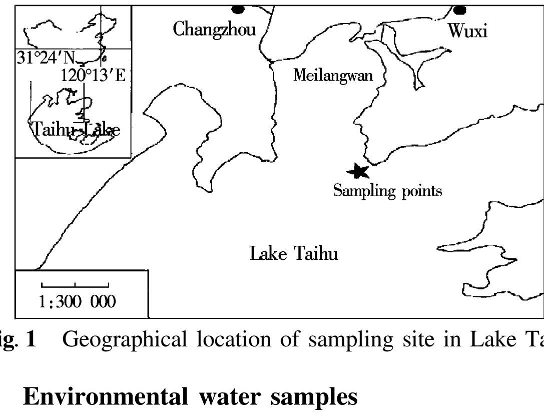


**Fig. 1** Geographical location of sampling site in Lake Taihu

### 1.2 Environmental water samples

The water is sampled from the lake at a distance of 50 m away from the shore at a frequency of three times monthly from May to December in 2009. Water samples collected from a depth of 0.5 m are filtered with a sieve(234 μm pore size), and immediately stored in dry ice for later analysis.

### 1.3 Evaluation indicators for water quality

Water temperature, pH, $COD_{Mn}$, $NH_3$-N concentration

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and algal density are monitored for the evaluation indicators of water quality. The detection methods are based on the monitoring analysis method of water and wastewater[6].

### 1.4 DNA extraction[7]

For the analysis of algal cells, 450 μL of STE buffer(10 mmol/L tris-HCl, pH = 7.5, 10 mmol/L NaCl, 1 mmol/L EDTA, pH = 8.0), 50 μL of 10% SDS, 20 μL of proteinase K(10 mg/mL) are added into the collected algal cells washed by sterile water. The entire mixture is incubated at 65 ℃ for 2 h and centrifuged at 12 000*g* for 10 min. After extraction of the DNA-containing aqueous sphase with sodium acetate(3 mol/L, pH = 5.2), DNA is precipitated with an equal volume of isopropanol, centrifuged at 12 000*g* for 10 min. Precipitated DNA is washed with 75% ethanol and resuspended in TE buffer.

### 1.5 Primers and RTQ-PCR for cyanobacteria, *Microcystis* and toxic-producing gene

The primers are designed as described in Refs. [8 − 9]. The sequences of the primers of cyanobacteria, *Microcystis* and toxic-producing genes are PCβF(5'-GGCTGCTTGTTTACGCGACA-3') and PCαR(5'-AGTACCACCAGCAACTAA-3'); Micr 16S F(5'-ATGTGCCGCGAGGTGAAACCTAAT-3') and Micr 16S R(5'-TTACAA(C/T)CCAA(G/A)(G/A)(G/A)CCTTCCTCCC-3'); MBF(5'-AGGAACAAGTTGCACAGAATCCGCA-3') and MBR(5'-ACTAATCCCTATCTAAACACAGTAACTCA-3'). All of the primers are synthesized by Sangon Co., Ltd.

The RTQ-PCR mixture contains 12.5 μL of SYBR green master mix-plus, 2.5 μL of plus solution, 10 pmol of each primer, 10 ng of genomic DNA, and water to a final volume of 25 μL. The RTQ-PCR programs consist of an initial warm-up step of 50°C for 5 min, followed by 40 cycles of 95 ℃ for 1 min, 95 ℃ for 15 s, 55 ℃(57 ℃ for *Microcystis*) for 15 s, and 72 ℃ for 45 s in an ABI 7300 real-time PCR thermocycler.

### 1.6 Standard curves of cyanobacteria, *Microcystis*, toxin-producing gene[10]

Standard curves of the cyanobacteria, *Microcystis*, toxin-producing gene are constructed with the log value of different 10-fold serial dilutions of copy numbers $C$ as abscissa, $C_t$ value as ordinate. The formulae for standard curves are shown in Tab. 1.

**Tab. 1** Standard curves of cyanobacteria, *Microcystis*, toxin-producing gene

| Gene | Standard curve | $R^2$ |
|---|---|---|
| PC-IGS | $C_t = -3.1466 \lg C + 45.816$ | 0.994 1 |
| Micr 16S DNA | $C_t = -3.0369 \lg C + 42.551$ | 0.993 6 |
| Toxin-producing gene | $C_t = -3.312 \lg C + 31.297$ | 0.995 1 |

### 1.7 Calculation of environmental abundance of *Microcystis* and toxic *Microcystis*

In accordance with the above-mentioned methods, the extracted genomic DNA of environmental samples from Lake Taihu is amplified by RTQ-PCR with three pairs of primers, respectively. The copy numbers are calculated by the formulae shown in Tab. 1. The environmental abundance of *Microcystis* is demonstrated by the copy number of *Microcystis* and cyanobacteria, and the abundance of toxic *Microcystis* is demonstrated by the copy number of toxic *Microcystis* and cyanobacteria.

### 1.8 Extraction and detection of MC-LR[11]

Algal cells which are collected by the Millipore vacuum pump from water samples are washed by deionized water, fixed volume to 200 mL by adding 5% of glacial acetic acid and stored at 4 ℃ overnight. MC-LR is extracted by solid-phase extraction column after the algal liquid has been filtered by a 0.45 μm membrane filter. The solid-phase extraction column is activated with 5 mL of methanol and 20 mL of deionized water twice, respectively. In order to remove impurities, 40 mL of deionized water, 20 mL of 10% methanol and 20 mL of 20% methanol elution are used to wash the solid-phase extraction column. The velocity is 5 mL/min. MC-LR is obtained by eluting the solid-phase extraction column with 5 mL of 0.1% trifluoroacetic acid methanol solution, and is concentrated with a water bath at 45 ℃. MC-LR condensates are clarified by centrifugation at 12 000*g* followed by filtration through a 0.45 μm membrane filter and stored at −20 ℃. Concentrations of MC-LR are determined by HPLC. The mobile phase is methanol and 0.05% (volume fraction) TFA aqueous solution. The parameters are as follows: The velocity is 1 mL/min; the wavelength is 238 nm; the sample volume is 20 μL and the column temperature is 40 ℃. The MC-LR concentration is quantitatively analyzed by the external standard method of the chromatographic peak area, that is, according to the retention time in the chromatogram and the maximum absorption wavelength in the spectrogram compared with the standard sample for qualitative analysis.

## 2 Results

### 2.1 Water quality

The indicators of water quality in the Nanquan region of Lake Taihu from May to December in 2009 are shown in Tab. 2. The results show that the water temperature is relatively stable during the period from May to October with an average value of 26.2 ℃. In November, the water temperature starts to decrease and falls to 5.5 ℃ in December. The algal density ranges from $1.2 \times 10^4$ to $2.6 \times 10^9$ cell/L, which is over the threshold of bloom except for December.

**Tab. 2** Detection results of water quality in Nanquan region of Lake Taihu

| Month | Algal density/(cell·L$^{-1}$) | pH | $COD_{Mn}$/(mg·L$^{-1}$) | $\varphi(NH_3\text{-}N)$/(mg·L$^{-1}$) | Water temperature/℃ |
|---|---|---|---|---|---|
| May | $1.5 \times 10^8$ | 6.5 | 6.11 | 0.18 | 24.0 |
| June | $2.9 \times 10^8$ | 6.5 | 5.95 | 0.21 | 28.0 |
| July | $1.8 \times 10^9$ | 8.5 | 5.67 | 0.36 | 29.6 |
| August | $1.4 \times 10^9$ | 8.0 | 6.98 | 1.03 | 25.3 |
| September | $2.6 \times 10^9$ | 9.0 | 4.96 | 0.59 | 27.5 |
| October | $4.8 \times 10^8$ | 7.0 | 6.30 | 0.44 | 22.8 |
| November | $2.1 \times 10^7$ | 7.0 | 7.09 | 0.25 | 14.5 |
| December | $1.2 \times 10^4$ | 6.5 | 6.80 | 0.11 | 5.5 |

As shown in Tab. 2, the values of pH, $COD_{Mn}$ and the $NH_3$-N concentration do not have an obvious seasonal variation. It indicates that the water resource reaches a situation of a moderate degree of pollution and eutrophication according to the eutrophication evaluation method of lakes.

## 2.2 Environmental abundance of toxic *Microcystis*

The environmental abundance of toxic *Microcystis* means the percentage of toxic *Microcystis* among total cyanobacteria. From May to October, the abundance of toxic *Microcystis* is about 40% to 50%, which appears to be at a maximum in June and drops to 27.86% in November, 5.66% in December (see Fig. 2). It indicates that toxic *Microcystis* bloom is dominant in the Nanquan region of Lake Taihu. In summer and fall, the environmental abundance of toxic *Microcystis* has no seasonal difference. However, it decreases significantly in winter.

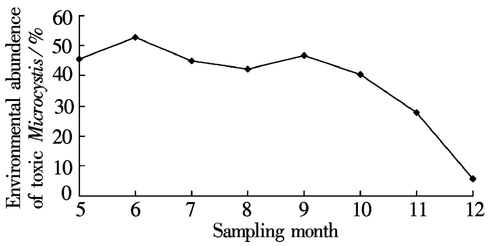


**Fig. 2** Environmental abundance of toxic *Microcystis*

## 2.3 MC-LR production ability of total algae and toxic *Microcytis*

The MC-LR production ability of total algae is the MC-LR content per $10^8$ cells of algae and the MC-LR production ability of toxic *Microcystis* is the MC-LR content per $10^8$ cells of toxic *Microcystis*. The average MC-LR production ability of total algae is 0.917 μg/$10^8$ cells in the investigated period. It reaches the highest value in June and November, and it presents a relatively low and stable value of 0.75 μg/$10^8$ cells from July to September, which is similar to the microcystin studies in Lake Dianchi and Lake Xingyunhu[12]. The variation of the MC-LR production of total algae in Lake Taihu is consistent with the variation of the environmental abundance of toxic *Microcystis* except in November (see Fig. 3). Furthermore, the variation of the MC-LR production ability of toxic *Microcystis* ranges from 1.661 to 9.293 μg/$10^8$ cells. The average MC-LR content produced by per $10^8$ cells of toxic *Microcystis* is moderated to be relatively stable from May to October and increases significantly from November. It reaches the highest value of 9.293 μg/$10^8$ cells in December (see Fig. 4).

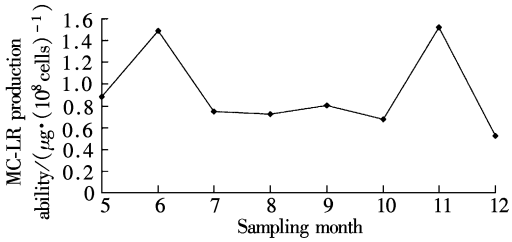


**Fig. 3** MC-LR production ability of total algae

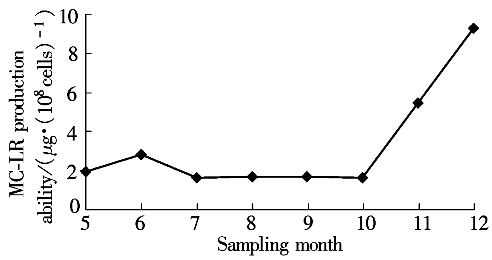


**Fig. 4** MC-LR production ability of toxic *Microcystis*

## 2.4 Relationship between environmental abundance and MC-LR production ability of toxic *Microcystis*

Based on the results, the algal density and environmental abundance of toxic *Microcystis* are found to be not dependent on water temperature, $COD_{Mn}$ and the $NH_3$-N concentration but on the pH value. However, the algal density and environmental abundance of toxic *Microcystis* dramatically decline with water temperature falling from October to December. It is concluded that the growth of algae is not influenced by water temperature over 23 ℃. However, water temperature has a significant constraint on the growth of algae if it becomes low enough. In June and November, the MC-LR production ability of total algae is higher than that in the other months, which results from the sharp changes in water temperature. However, with the significant drop in water temperature and algae density from November to December, the MC-LR production ability of toxic *Microcystis* to increase is substantial.

# 3 Discussion and Conclusion

Algal blooms have become a common concern of environmental issues all over the world. It is very important to understand the eutrophic status and the distribution pattern of toxic *Microcystis*, which prevent and govern the bloom aiming to improve water quality. Usually, *Microcystis* is the dominant algae of cyanobacteria blooms. Meanwhile, microcystins produced by toxic *Microcystis* can cause acute and chronic toxicity, especially suspected to play a strong role in promoting liver cancer. Thus, the analysis of the environmental abundance, properties and the microcystin production ability of toxic *Microcystis* will be helpful for further study of its toxic effects and mechanisms.

In this study, the real-time quantitative PCR and the high performance liquid chromatography are adopted to analyze the variations in the environmental abundance and the microcystin-LR production ability of toxic *Microcystis* in the Nanquan region of Lake Taihu. The study reveals that Lake Taihu has been in bloom according to the algal density during the investigation period from May to November and the growth of algae is influenced by the changes in water temperature. The water quality in the Nanquan region of Lake Taihu presents a moderate degree of pollution and eutrophication during most investigation periods. Cyanobacteria bloom in Lake Taihu is mainly *Microcystis* bloom, in which the environmental abundance of toxic *Microcystis* is over 40%. The range of the MC-LR production ability of toxic *Microcystis* varies from 1.661 to 9.293 μg/$10^8$ cells. It can be seen that water temperature has a certain influence on the environmental abundance and the MC-LR production ability of toxic *Microcystis*. With a rapid decline in water temperature, both the total algae and the toxic *Microcystis* density decrease sharply, but, on the contrary, the MC-LR production ability of toxic *Microcystis* is increased substantially from November to December, which indicates that fewer cells of toxic *Microcystis* with strong microcystin production ability might survive for the future proliferation due to natural environmental selection. It is suggested that the effective control of toxic *Microcystis* should be considered not only in the bloom period but also in the non-bloom period in winter.

To sum up, the water quality presents moderate pollution and eutrophication in which the *Microcystis* bloom is the most dominant in the Nanquan region of Lake Taihu during of the most period of the observation. The environmental abundance and the MC-LR production ability of toxic *Microcystis* have a close relationship with water temperature. The mechanism of the MC-LR production ability changes with temperature should be further studied to support the control of eutrophication.

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# 太湖南泉水域产毒微囊藻环境丰度和微囊藻毒素-LR产毒能力

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**摘要：**采用实时荧光定量PCR和高效液相色谱技术对太湖南泉水域2009年5月至12月产毒微囊藻的环境丰度及其微囊藻毒素-LR产毒能力进行研究，并监测水质和富营养化程度. 研究结果表明：南泉水域的水污染程度与富营养化程度均为中度；5月至11月蓝藻密度均超过水华的阈值；5月至10月产毒微囊藻的环境丰度均高于40%，随着温度的降低，产毒微囊藻的环境丰度迅速降低并在12月降到了5.66%；5月至12月产毒微囊藻的微囊藻毒素-LR产毒能力为1.661 ~9.293 μg/亿细胞. 11月至12月随着水温和藻密度的急速下降，产毒微囊藻的微囊藻毒素-LR产毒能力显著增强. 结果提示太湖南泉水域全年大部分时间存在水华，产毒微囊藻为其优势藻，产毒微囊藻的环境丰度及其微囊藻毒素-LR产毒能力的动态变化和水温密切相关. 冬季产毒微囊藻产毒能力的显著增强也提示在水华和非水华期内对其防制都应予以重视.

**关键词：**富营养化；湖泊污染；藻类；微囊藻毒素；环境丰度；太湖

**中图分类号：**X171