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Cyanobacteria-/cyanotoxin-contaminations and eutrophication status before Wuxi Drinking Water Crisis in Lake Taihu, China

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Abstract

After the appalling "Wuxi Drinking Water Crisis", increasing investigations concerning the contaminations of cyanobacterial blooms and their toxins in Lake Taihu have been performed and reported in the last two years. However, information regarding these issues before the crisis in 2007 remained insufficient. To provide some background data for further comparisons, the present study reported our investigations conducted in 2004, associated with the cyanotoxin contaminations as well as the eutrophication status in Lake Taihu. Results from the one-year-study near a drinking water resource for Wuxi City indicated that, unlike the status in recent two years, cyanobacteria and chlorophyta are the co-dominance species throughout the year. The highest toxin concentration (34.2 ng/mL) in water columns occurred in August. In bloom biomass, the peak value of intracellular toxin (0.59 μg/mg DW) was determined in October, which was lag behind that in water column. In addition, MC-RR was the major toxin variant throughout the year. During the study period, nutrients levels of total nitrogen and phosphorus were also recorded monthly. Results from the present study will lead to a better understanding of the eutrophication status and the potential risks before "Wuxi Drinking Water Crisis".

Key words: cyanobacterial blooms; eutrophication; microcystins; Lake Taihu

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Introduction

Lake Taihu, located in the eastern China, around the lower reaches of Yangtz River, is the third largest freshwater lake in China. It has a water surface area of 2427.8 km^2 and an average water depth of 1.9 m. The annual average air temperature is 14.9–16.2°C and the climate is an SE-NW monsoon climate. There are six large cities located around this lake and the population in Taihu drainage area is approximately 31.182 million. This lake serves as an important resource for drinking water, irrigation, aquaculture and industrial waters, in addition to being a popular recreational and tourist attraction. Due to rapid economical development and the intensive use of water resources, the lake water is becoming more seriously polluted (Pu et al., 1998). The occurrence of heavy cyanobacterial blooms in warm seasons has increased in frequency and intensity in recent years, especially in Meiliang Bay in the northern region of the lake (Zhang and Qin, 2001).

Cyanobacterial blooms, a common feature of eutrophication, occur worldwide and have been well documented in the past years (Skulberg et al., 1984; Liu et al., 2002). Cyanobacteria may produce and release toxic compounds, which can bring disaster effects on aquatic organisms, wild life, domestic animals and humans upon drinking or contacting with water containing these compounds. Microcystins (MCs) are one of the most frequently occurring cyanotoxins produced by cyanobacteria (usually by *Microcystis* sp.) in freshwaters globally (Chorus, 2001). As a family of cyclic peptide of hepatotoxins, MCs have differential functions as the inhibitors of several serine/threonine protein phosphatases including PP1 and PP2A, and can lead to the disruption of normal cell metabolism and their functions (Yoshizawa, 1990; Mackintosh, 1990). Exposure to MCs can lead to liver failure in wild animals, livestock and aquatic life (Sivonen and Jones, 1999; Carmichael, 2001), as well as human illnesses and mortality (Azevedo et al., 2002). Some reports suggested that the incidence of human primary liver cancer in the eastern region of China was related to the presence of MCs found in drinking water (Yu, 1989; Ueno et al., 1996).

In late May 2007, a drinking water crisis took place in Wuxi, the city around Meiliang Bay, following a massive bloom of the toxin-producing cyanobacteria *Microcystis* spp. in Lake Taihu. As Lake Taihu was the city's sole water supply, approximately two million people have no drinking water for at least one week, and can only rely

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on bottled water (Qin et al., 2009). After the crisis, increasing safety concerns from both the government and the public have been focused on the further contaminations of cyanobacterial blooms, and the control of eutrophication in large freshwaters as well. In the past two years, many strategies for ecosystem restoration with the aim to reduce the nutrients were implemented in this lake.

To evaluate the effect of these strategies and predict the potential disaster trends in the sensitive water area in Lake Taihu, more comparion studies, associated with the dynamic of cynobacterial species and biomass, intracellular and extracellular microcystins, and nutrient levels before and after the crisis are urgently needed. However, to our knowledge, little information regarding this issue was available before the crisis. For this purpose, the present study reported our investigations conducted in 2004, related to the cyanotoxin contaminations as well as the eutrophication status in Lake Taihu. Results from this study will provide important data for future comparisons. Also as a part of "bio-manipulate project-2002", the present study was conducted to investigate the background data of toxic cyaobacteria and nutrients, and further provide information for evaluating the effects of bio-controlling techniques in Meiliang Bay.

1 Material and methods

1.1 Sample collections

The sampling stations were set in the area targeted as algal material enrichment and cleanup project undertaken. Sites A and C were located near the intake of Chongshan and Qianlongkou waterworks, and site B was located at the middle of the enclosure, where aimed as a bio-controlling area (Fig. 1). Samples were taken monthly. For phytoplankton identification, two parallel samples were taken with a phytoplankton net. One was for living material observation and the other was for immediate fixation in lugol's solution and for further identification. One or two hundred-milliliter lake water was taken directly for the determination of Chlorophyll contents, and the results

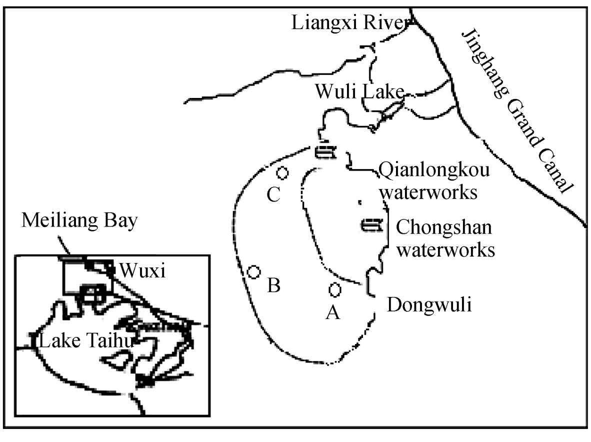

Fig. 1 Sampling map. A, B and C are the three sampling stations in Meiliang Bay in Taihu. Enclosure composed of broken lines was the area of bio-manipulate on cyanobacteria. Sites A , C are distributed near the intake of Chongshan and Qianlongkou waterworks for drinking water supply for this region, and site B is at the center of the enclosure.

represented the phytoplankton biomass in lake water. MCs contents, both in water (free) and in the algal material (cell-bound) were measured. For measurement of free MCs, samples were taken directly from the water columns and kept on ice during the transportation. For the measurement of cell-bound MCs, the bloom materials were collected with a phytoplankton net and lyophilized, and then stored at –20°C until analysis. Nutrients including total/dissolved nitrogen (TN/DTN) and phosphorus (TP/DTP) in the water column were determined. All the samples were mixtures from the surface and 0.5 m depth in the water columns.

1.2 Sample preparation

Bloom samples collected for MCs analysis were lyophilized in a freeze-drier (Yamato Scientific Co., Ltd., Japan). One hundred milligram of dried biomass was extracted with 15 mL of 5% acetic acid, and then further extracted with 100% methanol twice. Extracts from methanol extraction were evaporated and redissolved in water, and then combined with the extracts from acetic acid extractions. The crude extracts were purified using preconditioned solid-phase-extraction cartridge (SPE cartridges, Part No. WAT 051910, Waters, USA). The methanol elution was evaporated and redissolved in 60% methanol, kept under –20°C until analysis. For the determination of free MCs in water columns, sample was centrifuged (4°C, 8000 r/min, 5 min) or filtrated through a filter paper (GF/C, Part No. 1822-047, Whatman, England) to remove phytoplankton cells and suspended particles, and then analyzed with enzyme-linked immunosorbent assay (ELISA) according to the methods described by Ueno et al. (1996). Water samples for DTN and DTP measurement were filtered through a GF/C glass microfibre filter.

1.3 Analysis

1.3.1 Phytoplankton identification

Phytoplankton samples were observed with microscope for phytoplankton community identification and density estimation.

1.3.2 Determination of Chlorophyll *a*

Water samples of 100 to 300 mL (according to the phytoplankton biomass) were filtered through GF/C filters, extracted with ethanol (95%, 4°C, overnight) and centrifuged. The supernatants were measured at 649 and 665 nm. Chlorophyll *a* (Chl-*a*) content (C, μg/mL) was calculated as follows:

$$C = 13.7A_{665\ \mathrm{nm}} - 5.76A_{649\ \mathrm{nm}} \quad (1)$$

where, $A_{665\ \mathrm{nm}}$ and $A_{649\ \mathrm{nm}}$ represent the optical density of the extracts respectively (Wintermans and de Mots, 1965).

1.3.3 Determination of MCs in algal cells

Extracts from the algal material was analyzed with high performance liquid chromatography equipped with UV-detector (HPLC-UV). Toxin separation was performed on a reversed phase Silica based C-18 bonded column (5 μm, 4.6×150 mm, Shimadzu, Japan). The mobile phase was 60% methanol:0.05 mol/L phosphate buffer (pH 3) (6:4, *V/V*) and flow rate was set at 1.0 mL/min. The absorbance at 238 nm was continuously detected after separation on the column. Toxin contents were achieved by comparing the retention time and peak area with the corresponding standards of MC-RR, LR and YR.

1.3.4 Determination of MCs in water column

Free MCs concentrations were determined by ELISA method according to the procedures described previously (Song et al., 2007; Ueno et al., 1996). The sensitivity of this method was 0.1 ng/mL (Lei et al., 2004).

1.3.5 Determination of nutrients levels

Contents of total nitrogen (TN) and total phosphorus (TP) in the original samples were measured according to the national standards (GB11894-89 and GB11893-89). Lake water filtered through a fiberglass membrane was used for determination of the DTN and DTP levels in the lake. The methods and calculations were the same as described previously for TN and TP determination.

1.3.6 Chemicals and reagents

All organic solvents used in this experiment were of HPLC grade and purchased from Fisher Scientific (USA). Reagents used for nitrogen and phosphorus determination were of analytical grade. Toxin standards of microcystins (MC-LR, MC-RR and MC-YR) were from Wako Chemicals (Japan). Pure water used in this study was newly prepared through a Milli-Q water purification system (Millipore Co., USA).

1.3.7 Statistical analysis

All the data in this article were expressed as mean ± SD of two or three individual sample analyses. Correlation analysis was performed by SPSS for windows (version 11.5, USA) and all conclusions were based on at least the 5% level of significance ($P < 0.05$).

2 Results and discussion

2.1 Variations of phytoplankton communities and Chlorophyll concentrations in Meiliang Bay

Cyanophyta and Chlorphyta were the most frequent occurrence in the phytoplankton samples collected from the Meiliang Bay (Table 1). Throughout the year, chlorophyta was identified at high densities in Meiliang Bay, especially in spring and winter, which was quite different from the investigations by Song et al. (2007), after the crisis in the same water area. In their study, *Microcystis* was the predominance in most months throughout the year. Unlike their findings, we found that, in 2004 before the crisis, chlorophyta represented by *Chlorella* and *Scenedesmus*, and cyanobacteria by *Microcystis* and *Anabaena*, could be the co-occurring populations and by turns, be the predominances in cooler and warmer seasons respectively. Furthermore, algal of bacillarophyta and xanthophyta could also be inspected at large scales in the relative cool seasons. When compared with previous studies, it was easy to find that the biodiversity of phytoplankton populations decreased dramatically in the past years with the aggravation of contamination in Lake Taihu (Shen et al., 2003; Song et al., 2007), which may result in the single dominance of *Microcysits* in most of a year.

Levels of Chl-*a* at the three sampling sites differed spatio-temporally during the experiment (Fig. 2). Chl-*a* concentrations maintained at low levels (less than 50 μg/mL) before Jun, but increased dramatically after Jul, and reached the peak values in Aug at sites B and C. However, at site A, no obvious increase of Chl-*a* concentrations in water columns could be observed even in hot summer, which may be caused by the implementation of mechanical collection of cyanobacterial biomass and biocontrol methods applied in this area. Site A was near the intake of Chongshan drinking water sources, one of the important drinking water supplies in Wuxi City. By using several control techniques, such as gravitational shaking, rotating shaking and centrifuging, the surface water was cleaned effectively from being condensed of phytoplankton. Results indicated that mechanical collection of cyanobacterial biomass was an effective approach in the hyper-eutrophicated water area to reduce the occurrences of cyanobacterial blooms, and further avoid or reduce the health risk caused by the toxic blooms and/or their harmful metabolites.

2.2 Variations of free and total cell-bound microcystins (TCB MCs) in water columns

Free MCs concentrations were detected at high levels from May to Oct (Fig. 3). The highest free MCs

Table 1 Variations of phytoplankton communities in Meiliang Bay in 2004

Sampling month	Cyanophyta	Chlorophyta	Bacillarophyta	Xanthophyta	Chrysophyta	Phaeophyta	Pyrrohyta
Jan	+	+++	+	+	ND	ND	ND
Feb	+	+++	+	+	+	ND	ND
Mar	+	+++	+	ND	ND	+	ND
Apr	+	+++	+	+	ND	+	ND
May	++	+++	++	+	ND	+	+
Jun	+++	++	+	+	ND	ND	ND
Jul	+++	++	ND	+	ND	+	ND
Aug	+++	++	+	+	ND	ND	ND
Sep	+++	++	+	+	ND	ND	+
Oct	+++	++	+	+	ND	ND	ND
Nov	++	+++	+	+	ND	++	ND
Dec	++	+++	ND	ND	ND	++	ND

"+": the relative ratio of each kind of algae in the phytoplankton detected under a optical microscope; ND: not detected.

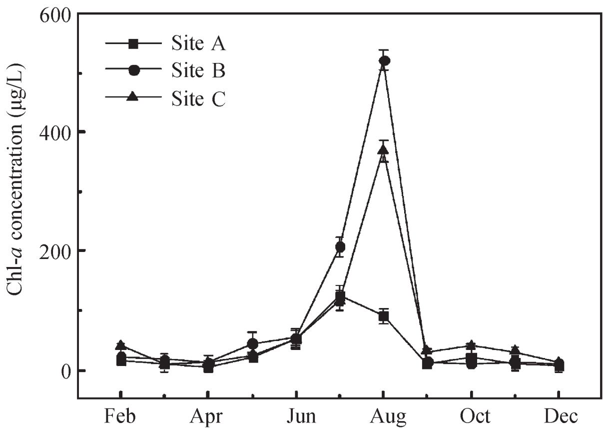

Fig. 2 Annual variations of Chl-*a* concentrations from sites A, B and C in Meiliang Bay in Lake Taihu during 2004. Data are presented as mean values ±SD (n = 3).

concentration occurred between Aug and Sep, with the value of 35 ng/L in the water column. In spring and winter, when cyanobacteria presented at relatively low ratio, the concentrations of dissolved toxins maintained at very low levels. Usually, cyanotoxins, such as microcystins were restrained inside the cells, only very small proportion of the intracellular toxins could be released into the surroundings during the normal growth stage. However, most of the cell-bound toxins could be released and formed free toxins in the water columns after lyses of the cyanobacterial cells (Sivonen, 1990). High levels of dissolved toxins detected in Aug and Sep most probably due to the cell death of cyanobacterial blooms. Results also indicated that the trends for free toxins were well consistent with the variations of phytoplankton compositions. Similar correlation was reported by Song et al. (2007). However, in their study the toxin concentrations were almost three hundred times higher than that in our investigation. This tremendous difference probably was due to the rapid proliferation of *Microcystis* cells in 2007. As an aggravating result of the increase in toxic cyanobacteria and their toxins in water, some species were confronted more stress to survive and water quality was deteriorated gradually. More seriously, high level of dissolved toxins in the source water, has been proved, could not be effectively removed with the applied treatment technologies for drinking water. Thus, MCs contamination posed high potential health risks to the citizens around this area. According to Song et al. (2007), free MCs in Lake Taihu has increased dramatically from 35 ng/L to 6.69 μg/L during past several years. It was almost 200 times higher in 2007 than that in 2004, and has far exceeded the safe level of 1 μg/L established by World Healthy Organization. Comparison data suggested that contaminations of MCs must be taken into consideration when Lake Taihu, particularly Meiliang Bay was selected as the drinking water sources.

From the results, we can also find that consistent with the trend of Chl-*a* peak value of free toxins in site A was obviously lower than those in sites B and C in Aug and Sept. This decrease may owe to the mechanical collection and elimination of cyanobacterial biomass. These controlling methods could effectively reduce the accumulation of algal cells on surface water and consequentely, decrease cell lysis and toxins release into water column.

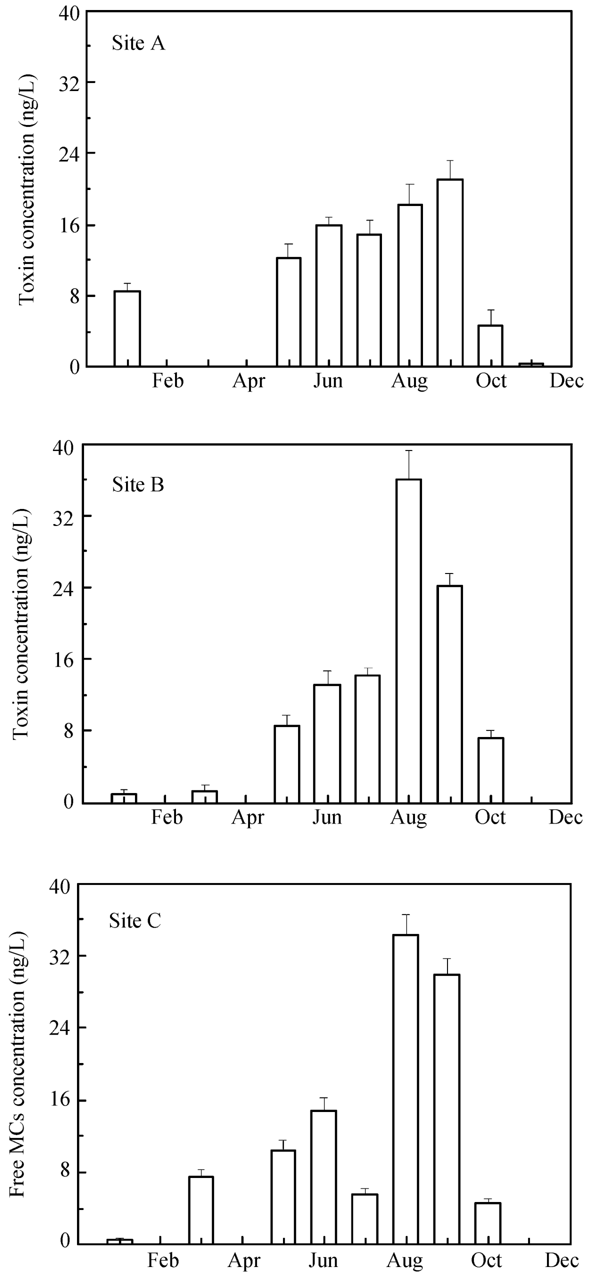

Fig. 3 Variations of free MCs contents in water columns from three sampling sites in Meiliang Bay, Lake Taihu during 2004. Data are presented as mean values ± SD (n = 2).

Intracellular toxins were monitored from May to Oct in 2004, and there were no data for the other months because the collected phytoplankton biomass was not enough for the subsequent toxin extraction and HPLC analysis. Results from Fig. 4 indicated that the concentrations of intracellular toxins changed with sampling sites and sampling months quickly, even there was no big difference between the bloom biomass in the water columns. Similar results were also obtained in studies after the water crisis (Song et al., 2007; Chen et al., 2009), in which the quick variations of MCs concentrations were related with the changes of different *Microcystis* colonies. In the present study, the concentrations of intracellular toxins in May, Aug and Sep were significantly lower than that in Jun, Jul and Oct. In addition to the colony variation-reliant theory, it can be concluded thought that the different growth stages of cyanobacteria and phytoplankton compositions might contribute to the variation of toxin concentrations in different months. A good example was that when green alga and cyanobacteria were the co-dominative phytoplankton in May, there was a remarkable decrease in toxin concentrations in the collected blooms. On the other hand, the growth stage of cyanobacterial cells might also influence the toxin production. For the toxin synthesis in some filamentous cyanobacteria and dinoflagellate, it has been reported that maximal toxin production occurred at the stationary growth stage when the growth rate was very low (White, 1986; Oshima and Yasumoto, 1979; Liu et al., 2006), and similar phenomenon was also observed in *Microcystis* (Sivonen, 1990). Additionally, water temperature is an important factor for the reduction of intracellular toxin productions in cyanobacteria. Due to the high water temperature and high density of cyanobacteria on the surface water in Aug and Sep, cell lysis may be easily induced and subsequently, the release of inner toxins would probably occur. As a result, the concentrations of determined intracellular toxins would be correspondingly lower than that in cool seasons. Our results were consistent with the studies after the crisis (Song et al., 2007; Chen et al., 2009), but somewhat different from the previous investigation by Shen et al. (2003). In their study, high temperature would increase the concentrations of cell-bound toxins, and the maximum toxin production for the blooms occurred in Aug and Sep.

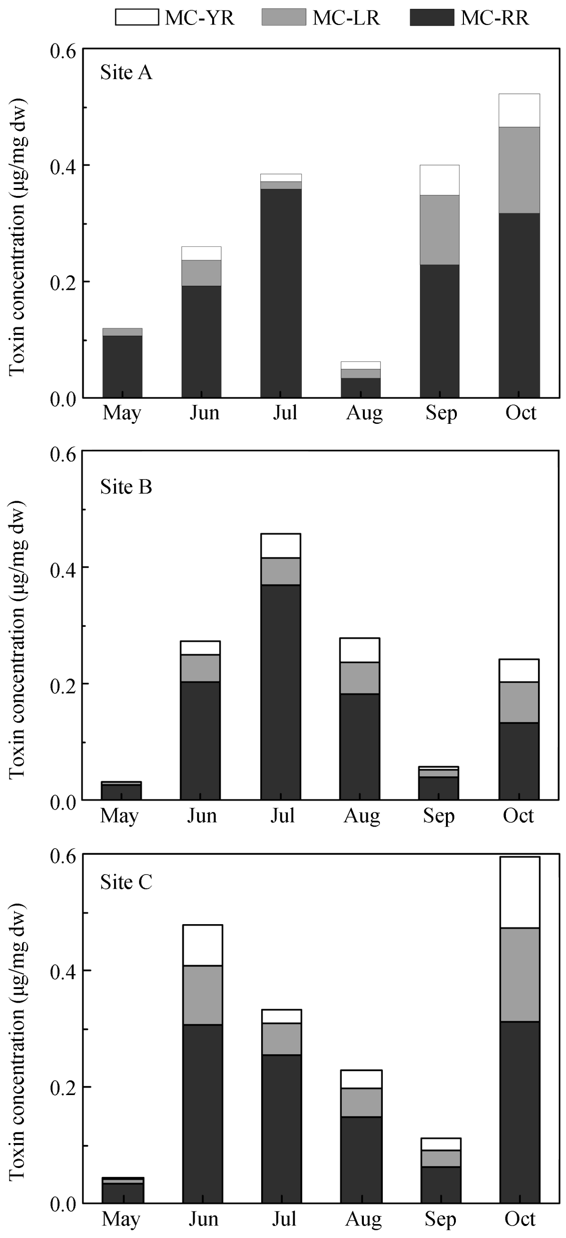

Fig. 4 Concentrations of MCs and each of toxin isomer in cyanobacterial blooms at the three sampling sites in Meiliang Bay, Lake Taihu from May to October in 2004. Data are presented as mean values ± SD (*n* = 2).

Results indicated that MC-RR was the major toxin variant in Lake Taihu (the highest proportion, 93.5%), followed by MC-LR, with the highest proportion of 28.3%. This was a little different from the previous report on Lake Dianchi, another high-eutrophicated freshwater Lake in China, in which the major toxin variant in the mixed blooms was MC-LR (Deng, 2000).

2.3 Nutrient levels and the correlations with MC concentrations

To provide more information for future comparisons, the annual variations of nutrient levels were also investigated in Lake Taihu in 2004 (Fig. 5). It was observed that TN concentration in lake water ranged from 2 to 4 mg/L at sites A and C. Exceptively, in area around site B, higher values with a peak of 4.8 mg/L were determined and which may be related to the industrial activities around the station. In most months, TP concentrations maintained at levels below 0.5 mg/L, but a higher value of 0.6 mg/L was recorded at sites B and C in hot Aug, which may be caused by the increased influx of wastewater rich of phosphorus from human activities. Fluctuation of DTP was similar to that of TP (Fig. 5), but DTN was somewhat different. Results indicated that DTN gradually decreased from Jun and reached their lowest levels of 0.5–0.7 mg/L in Aug at the three sites. In Taihu, as investigated, the non-nitrogen-fixative *Microcystis* would reproduced rapidly in summer, which would take lots of nitrogen from the surroundings, thus, significant decrease in DTN levels would take place. Moreover, with the coming of slower reproductive rate of algal cells in mid/late period of the bloom, a decreased nitrogen demands would result in a re-increase in DTN levels. In fact, this difference in nutritional dynamics may be synthetically resulted from the algal community, hydrology and human activities as well. For more comprehensive understanding of the function of nutrients in the occurrence of algal blooms, further studies involving field and laboratory works should be performed.

Although it was common to associate the massive proliferation of cyanobacteria and their blooms with the

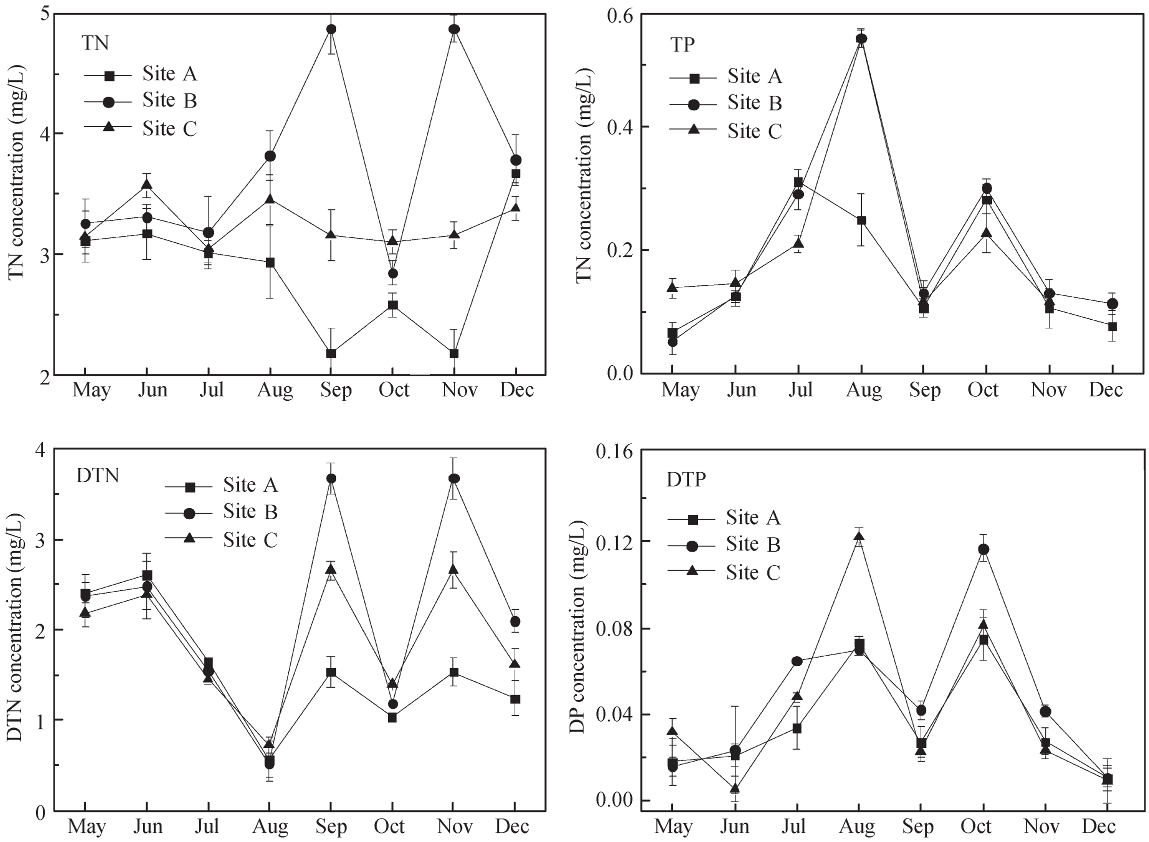

Fig. 5 Variations of nutrient levels at sites A, B, and C in Mayling Bay in Lake Taihu from May to Oct in 2004. Data are presented as mean values ±SD (n = 3).

increases in nutrients, it remained inadequate to establish a logical relationship between nutrient levels and toxin production. In some publications, it was even indicated that the production of toxins was not necessarily related to freshwater eutrophication (Willén and Mattsson, 1995). In this study, the similar results were obtained, and no obvious correlations was found between the production of each toxin variants, together with the total toxins and the nutrient levels in Lake Taihu (Table 2). Results from the present study were somewhat different from that of Chen et al. (2009) who reported that toxin concentration was negatively correlated not only with N:P ratio, but also with the TN contents. In our study, similar correlations were only observed between the concentrations of total intracellular MCs and the N:P ratios. As to the functions of nutrients in the course of the toxin synthesis in toxic cyanobacteria, there were several explanations. Some studies proposed that high phosphate concentration did not seem to enhance the toxic colonies or toxin concentrations in natural blooms from field studies (Chen et al., 2009; Briand et al., 2009). However, some other laboratory studies investigated toxin-producing abilities by using toxic and nontoxic *Microcystis* strains, suggesting that phosphate concentrations were beneficial to the growth of toxic strains but detrimental to nontoxic strains (Vézie et al., 2002). Xie et al. (2003) also proposed that reactive phosphorus, rather than a low N:P ratio, was the key regulatory factor for establishing the dominance of the non-nitrogen-fixing cyanobacteria. Free MCs in lake water were found correlated with the Chl-*a* levels in this study. In Lake Taihu, cyanobacteria presented mainly by *Microcystis* sp. and the predominance increased rapidly after July. High densities of the bloom biomass and high temperatures in the summer may potentially cause the lyses of the blooms, which would ultimately lead to the formations of dissolved MCs in water columns.

Table 2 Correlation matrix between microcystins and nutrient levels

	MC-RR	MC-LR	MC-YR	TCB MCs	Free MCs	Chl-*a*
Chl-*a*	0.140	–0.100	0.054	0.076	0.641**	1
TN	–0.017	–0.144	–0.048	–0.059	0.260	0.201
TP	0.267	0.134	0.238	0.256	0.457	0.854**
DTN	–0.287	–0.335	–0.341	–0.342	–0.076	–0.564*
DTP	0.078	0.274	0.317	0.181	0.107	0.425
TN/TP	–0.469*	–0.371	–0.419	–0.487*	–0.152	–0.429

* Correlation is significant at the 0.05 level (2-tailed); ** correlation is significant at the 0.01 level (2-tailed).

3 Conclusions

In comparison with the investigations after the crisis, eutrophication of Lake Taihu has become more and more serious, and water qualities had deteriorated continuously. Therefore, contaminations of toxic cyanobacteria and their toxins must be taken into consideration in the management of Lake Taihu, especially in Mayliang Bay, when it serves as resource for drinking water, aquaculture as well as recreation area. To avoid or reduce the potential risks associated with human exposure to the toxins, investigations regarding the prediction of the occurrences of toxic blooms and their toxins must be implemented and strengthened in the future.

To elucidate the key factors which can affect the eutrophication processes and the occurrences of toxic blooms in eutrophicated lakes, more comparison studies are urgently needed. Based on this consideration, the present study provided important background data for future comparisons in Taihu before 2007 Drinking Water Crisis.

Acknowledgments

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References

Azevedo S M F O, Carmichael W W, Jochimsen E M, Rinehart K L, Lau S, Shaw G R et al., 2002. Human intoxication by microcystins during renal dialysis treatment in Caruaru-Brazil. *Toxicology*, 181-182: 441–446.

Briand E, Escoffier N, Straub C, Sabart M, Quiblier C, Humbert J F, 2009. Spatiotemporal changes in the genetic diversity of a bloom-forming *Microcystis aeruginosa* (cyanobacteria) population. *The ISME Journal*, 3: 419–429.

Carmichael W W, 2001. Health effects of toxin-producing cyanobacteria: the CyanoHABs. *Humman Ecololgy Risk and Assessment*, 7: 1393–1407.

Chen W, Peng L, Wan N, Song L R, 2009. Mechanism study on the frequent variations of cell-bound microcystins in cyanobacterial blooms in Lake Taihu: Implications for water quality monitoring and assessments. *Chemosphere*, 77: 1585–1593.

Chorus I, 2001. Introduction: cyanotoxins-research for environment safety and human health. In: Cyanotoxins (Chorus I, ed.). Sringer Press, Germany. 1–4.

Deng Y M, 2000. Study on microcystins in Dianchi Lake. *Yunnan Environmental Science*, 19: 117–119.

Liu Y D, Song L R, Li X Y, Liu T M, 2002. The toxic effects of microcystin-LR on embryo-larval and juvenile development of loach, *Misguruns mizolepis* Gunthe. *Toxicon*, 40: 395–399.

Liu Y M, Chen W, Li D H, Shen Y W, Liu Y D, Song L R, 2006. Analysis of paralytic shellfish toxins in *Aphanizomenon* DC-1 from Lake Dianchi, China. *Environmental Toxicology*, 21: 289–295.

Lei L M, Wu Y S, Gan N Q, Song L R, 2004. An ELISA-like timeresolved fluorescence immunoassay for microcystin detection. *Clinical Chemica Acta*, 348: 177–180.

Mackintosh C, Beattie K A, Klumpp S, Cojen P, Codd G A, 1990. Cyanobacterial microcystin LR is a potent and specific inhibitor of protein phosphatase 1 and 2A from both mammals and high plants. *FEBS Letters*, 264: 187–192.

Oshima Y, Yasumoto T, 1979. Analysis of toxins in cultured *Gonyaulax excavate* cells originating in Ofunato Bay, Japan. In: Toxic Dinoflagellate Blooms (Taylor D L, Seliger H H, eds.). Elsevier North Holland, New York. 377–380.

Pu P M, Hu W P, Yan J S, Wang G X, Hu C G, 1998. A physicoecological engineering experiment for water treatment in a hypertrophic lake in China. *Ecological Engineering*, 10: 79–90.

Qin B Q, Zhu G W, Gao G, Zhang Y L, Li W, Paerl H W et al., 2009. A drinking water crisis in Lake Taihu, China: Linkage to climatic variability and lake management. *Environmental Management*. DOI: 10.1007/s00267-009-9393-6.

Shen P P, Shi Q, Hua Z C, Kong F X, Wang Z G, Zhuang S X et al., 2003. Analysis of microcystins in cyanobacteria blooms and surface water samples from Meiliang Bay, Taihu Lake, China. *Environment International*, 29: 641–647.

Sivonen K, Jones G, 1999. Cyanobacterial toxins. In: Toxic Cyanobacteria in Water: a Guide to Their Public Health Consequences, Monitoring and Management (Chorus I, Bartram J, eds.). E & FN Spon, London. 41–111, 416.

Sivonen K, 1990. Effects of light, temperature, nitrate, orthophosphate, and bacteria on growth of and hepatotoxin production by *Oscillatoria agardhii* strains. *Applied Environment Microbiology*, 56: 2658–2666.

Skulberg O M, Codd G A, Carmichael W W, 1984. Blue-green algal (cyanobacteria) toxins: water quality and health problem in Europe. *Journal of the Human Environment*, 10: 244–247.

Song L R, Chen W, Peng L, Wan N, Gan N Q, Zhang X M, 2007. Distribution and bioaccumulation of microcystins in water columns: A systematic investigation into the environmental fate and the risks associated with microcystins in Meiliang Bay, Lake Taihu. *Water Research*, 41: 2853–2864.

Ueno Y, Nagata S, Tsutsumi T, Hasegawa A, Watanabe M F, Park H D et al., 1996. Detection of microcystins, a blue-green algal hepatotoxin, in drinking water sampled in Haimen and Fusui, endemic areas of primary liver cancer in China, by highly sensitive immunoassay. *Carcinogenesis*, 17: 1317–1321.

Vézie C C, Rapala J, Vaitomaa A, Seitsonen J, Sivonen K, 2002. Effect of nitrogen and phosphorus on growth of toxic and nontoxic *Microcystis* strains and on intracellular microcystin concentrations. *Microbial Ecology*, 43: 443–454.

Wintermans J F, de Mots A, 1965. Spectrophotometric characteristics of chlorophyll *a* and *b* and their pheophytins in ethanol. *Biochemical and Biophysical Acta*, 109: 448–453.

White A W, 1986. Salinity effects on growth and toxin content of *Gonyaulax excavata*, a marine dinoflagellate causing paralytic shellfish poison. *Journal of Phycology*, 14: 475–479.

Willén T, Mattsson R, 1995. Water-blooming toxin producing cyanobacteria in Swedish fresh and brackish waters, 1981–1995. *Hydrobiologia*, 353: 181–192.

Xie L Q, Xie P, Li S, Tang H, Liu H, 2003. The low TN:TP ratio, a cause or a result of *Microcystis* blooms? *Water Research*, 37: 2073–2080.

Yoshizawa S, Matsushima R, Watanabe M F, Harada K I, Ichiara A, Carmichael W W et al., 1990. Inhibition of protein phosphatases by microcystins and nodularin associated with hepatotoxicity. *Journal of Cancer Research Clinical Oncology*, 116: 609–614.

Yu S Z, 1989. Drinking water and primary liver cancer. In: Primary Liver Cancer (Tang Z Y, Wu M C, Xia S S, eds.). Springer, Berlin and China Academic Publications, Beijing. 30–37.

Zhang Y P, Qin B Q, 2001. Study on the evolvement of water environment in Taihu Lake. *Transactions of Oceanology and Limnology*, 2: 8–15.