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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

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ABSTRACT

For the purpose of understanding the environmental fate of microcystins (MCs) and the potential health risks caused by toxic cyanobacterial blooms in Lake Taihu, a systematic investigation was carried out from February 2005 to January 2006. The distribution of MCs in the water column, and toxin bioaccumulations in aquatic organisms were surveyed. The results suggested that Lake Taihu is heavily polluted during summer months by toxic cyanobacterial blooms (with a maximum biovolume of 6.7×10^8 cells/L) and MCs. The maximum concentration of cell-bound toxins was 1.81 mg/g (DW) and the dissolved MCs reached a maximum level of 6.69 μg/L. Dissolved MCs were always found in the entire water column at all sampling sites throughout the year. Our results emphasized the need for tracking MCs not only in the entire water column but also at the interface between water and sediment. Seasonal changes of MC concentrations in four species of hydrophytes (*Eichhornic crassipes*, *Potamogeton maackianus*, *Alternanthera philoxeroides* and *Myriophyllum spicatum*) ranged from 129 to 1317, 147 to 1534, 169 to 3945 and 124 to 956 ng/g (DW), respectively. Toxin accumulations in four aquatic species (*Carassius auratus auratu*, *Macrobrachium nipponensis*, *Bellamya aeruginosa* and *Cristaria plicata*) were also analyzed. Maximum toxin concentrations in the edible organs and non-edible visceral organs ranged from 378 to 730 and 754 to 3629 ng/g (DW), respectively. Based on field studies in Lake Taihu, risk assessments were carried out, taking into account the WHO guidelines and the tolerable daily intake (TDI) for MCs. Our findings suggest that the third largest lake in China poses serious health threats when serving as a source of drinking water and for recreational use. In addition, it is likely to be unsafe to consume aquatic species harvested in Lake Taihu due to the high-concentrations of accumulated MCs.

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1. Introduction

The occurrence of toxic cyanobacterial blooms in eutrophic lakes, reservoirs and recreational waters has become a worldwide problem (Carmichael, 1992). Some of the cyanobacterial genera such as *Microcystis*, *Anabaena*, *Nostoc* and *Aphanizomenon* can produce a wide range of potent toxins, including a family of hepatotoxins called microcystins (MCs) that comprise the most frequently encountered cyanotoxins in fresh water (Harada, 1996; Chorus and Bartram, 1999; Akcaalan et al., 2006). MCs are produced mainly by freshwater cyanobacterial blooms. Exposure to these hepatotoxic compounds can lead to liver failure in wild animals, livestock and aquatic life (Carmichael, 1992; Sivonen and Jones, 1999; Carmichael, 2001), as well as human illnesses and mortality (Azevedo et al., 2002). Some reports suggest that the incidence of human primary liver cancer in the eastern region of China is related to the presence of MCs found in drinking water (Yu, 1989; Ueno et al., 1996).

Freshwater contamination by toxic cyanobacterial blooms and MCs has received increased attention following the report by Jochimsen and his colleagues regarding the deaths of 50 patients exposed to MC-contaminated water during a dialysis treatment (Jochimsen et al., 1998). To reduce risks caused by MCs, the WHO has published protocols concerning their detection and recommendations for maximum permitted concentrations (1 μg/L) in water destined for human consumption and recreational use (WHO, 1998). Furthermore, a tolerable daily intake (TDI) of 0.04 μg total MCs per kg body weight per day has been proposed as a provisional guideline (Chorus and Bartram, 1999).

Generally, MCs are retained in cyanobacterial cells during the growth and steady phase of blooms (Sivonen, 1990; Rapala et al., 1997). However, these cell-bound toxins are eventually released into the surrounding water body by senescence of the blooms which leads to the creation of dissolved MCs in the water column (Park et al., 1993; Sivonen and Jones, 1999). To assess the health implications of exposure, it is crucial to pursue MCs under field conditions. As proposed in the literature, toxin bioaccumulation in aquatic organisms might be one of the critical pathways which contribute to the natural fates of MCs (Pires et al., 2004a; Gkelis et al., 2006). Therefore, the early warning detection and toxin monitoring in water columns and aquatic animals, as well as the subsequent risk assessment are becoming more essential in the regions where a dense bloom has occurred.

Lake Taihu (119°54′–120°36′ N, 30°56′–31°33′ E), the third largest freshwater lake in China, is located in the highly developed and densely populated Yangtze Delta (Pu et al., 1998). Its water depth ranges from 1 to 2.5 m (average 1.89 m) with a total water surface area of about 2338 km^2, and a mean water volume of approximately 4.43×10^{12} L. The 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 an area called Meiliang Bay in the northern region of the lake (Shen et al., 2003). To our knowledge, little information is available on the fate of MCs and the potential risks associated with toxin occurrences during both the warm bloom period and the cold seasons in Lake Taihu. Furthermore, as the lake is an important drinking water source for many developing cities around the Taihu drainage area, collection of preliminary data on the current status of toxic cyanobacterial blooms in Lake Taihu is urgently needed.

In the present study, a systematic investigation was performed to survey the fate of MCs in the water column and toxin bioaccumulations in hydrophytes and aquatic species during 2005. The objective of this study was to evaluate the potential risks caused by MC occurrence in the heavily polluted water area of Lake Taihu based on toxin distribution and bioaccumulation tests. In addition, this study traces the fate of MCs in the aquatic environment and aims to support strategies to reduce or avoid potential health risks.

2. Material and methods

2.1. Toxin standards and reagents

MC standards (microcystin-RR, YR, LR) were obtained from Pure Chemical Industries (Osaka, Japan) and [Dha^7] LR was isolated from a laboratory mass culture of *Microcystis aeruginosa* DS from Lake Dianchi using an improved Ramanan method (Chen et al., 2005). The purities of the MC standards were over 95% as determined by HPLC-DAD and high-performance liquid chromatography/tandem electrospray ionization mass spectrometry (LC/MS). ODS Sep-pak cartridges were manufactured by Waters (Milford, MA, USA, Part No. Wat051910). Methanol (HPLC grade) used as the HPLC mobile phase and for extractions was purchased from Fisher (Loughborough, UK). All other chemicals used in the study were of analytical grade and were purchased from standard sources.

2.2. Sample collection and preparation

2.2.1. Study area

Four sampling sites were selected for the present study by taking into consideration Lake Taihu's eutrophication status as documented in the past (Shen et al., 2003), Site S (31° 31.758′N, 120° 13.713′E) is located in Wuli Lake, which had been the most eutrophied bay in Lake Taihu. The other three sites (Site D 31° 31.538′N, 120° 12.626′E; Site O 31° 31.141′N 120° 13.098′E; Site M 31° 29.085′N 120° 13.334′E) are located in Meiliang Bay, where many well-known scenic sites and recreation centers can be found. In addition, site O had been used as a freshwater source for many years. Monthly sampling of the four sites in Meiliang Bay of Lake Taihu was conducted over the period from February 2005 to January 2006.

2.2.2. Samples for phytoplankton species composition

Water samples were taken in each site of the four sampling sites, from zero (surface) to 0.5-m depth, using a vertical

sampler, and fixed with Lugol's iodine solution. Sampling for phytoplankton species was conducted each month over 2005. Fixed phytoplankton were identified and enumerated by light microscope, according to commonly used monographs on phytoplankton (Utermöhl, 1958; Watanabe et al., 1992).

2.2.3. *Sampling of cyanobacterial blooms and lake water and preparation for toxin analysis*

Duplicate bloom samples were collected monthly in each site of the four sampling sites, from the surface water using a phytoplankton net (40 μm mesh), and stored in 250 mL polyethylene bottles. In the laboratory, the cyanobacterial bloom samples were settled overnight in a refrigerator at 4 °C to separate the zooplankton according to Watanabe's (Watanabe et al., 1992) method. The bloom samples were lyophilized and extracted in a solution of 5% (v/v) acetic acid and 80% (v/v) aqueous methanol. After centrifugation and evaporation of the methanol, the extracted solutions were cleaned using Sep-pak cartridges, and the eluted solutions were stored at −20 °C for HPLC analysis (Barco et al., 2005). Duplicate lake water samples were also collected at each site from the top (0–0.5 m; surface water) and the bottom (0–10 cm over the sediment; over-sediment water) of the water column. Both the surface water samples and the over-sediment water samples were centrifuged (3000*g*, 5 min) and filtered through Whatman™ GF/C filter papers prior to storage at −20 °C for the subsequent ELISA and LC/MS assays.

2.2.4. *Samplings of hydrophytes and aquatic species and preparation for toxin analysis*

Four species of hydrophytes (*Eichhornic crassipes*, *Potamogeton maackianus*, *Alternanthera philoxeroides* and *Myriophyllum spicatum*) were collected from site O (occasionally, no samples could be collected due to seasonal variations or changes of hydrological conditions). Samples were stored at 0 °C prior to transport to the laboratory. Duplicate hydrophyte samples were lyophilized and processed using the above extraction procedure for bloom samples. Native aquatic species (*Carassius auratus auratu* (fish), *Macrobrachium nipponensis* (freshwater shrimp), *Bellamya aeruginosa* (snail) and *Cristaria plicata* (mussel)) were caught by traps and other special tools, anatomized, frozen and transported, in insulated containers, to the laboratory. For sample preparation, 3–5 individuals of fish and mussel (more than 1 year old) were needed, and for shrimp and snail, more than 50 adult individuals were needed. The same organ isolated from different individuals were mixed together and duplicate of the different organs (the muscle and liver for fish, the abdomen and cephalothorax for shrimp, the foot and visceral sac for snail and mussel) including edible parts of the aquatic animals (0.5–1.0 g (DW) for each sample) were extracted, using 15 mL of 85% (v/v) aqueous methanol with stirring by magnetic stirrer for 1 h, followed by centrifugation at 3000*g* for 10 min at 10 °C. The supernatant was transferred to a clean glass flask and the residue was re-extracted with 10 mL of 85% (v/v) methanol (Moreno et al., 2005). The extract was evaporated to dryness at 35 °C using a rotary-evaporator (Büchi, Germany), and redissolved in 15 mL of Milli-Q water (Millipore Co., MA, USA). After processing the samples with Sep-park cartridges to remove impurities, the eluted methanol fractions were evaporated and redissolved in 0.5 mL of Milli-Q water. The extracted solutions were stored at −20 °C until use and subjected to the subsequent enzyme-linked immunosorbent assay (ELISA) and LC/MS assay (below).

2.3. *MC determination in the laboratory*

For the bloom samples (which contained high concentrations of MCs), the HPLC was employed to quantify the intracellular toxins. All samples containing low concentrations of MCs, such as, water samples for dissolved toxins and samples for the investigation of bioaccumulation, were first screened by ELISA, and samples testing positive were further identified and quantified using the LC/MS.

2.3.1. *Determination of MCs by HPLC method*

HPLC tests were performed using a Shimadzu LC-10A system with two LC-10A pumps and a UV detector. Elution conditions using a Shimadzu shim-pack (CLO-ODS 6.0 × 150) column were 60% of solution A (100% methanol) and 40% solution B (0.05 M KH_2PO_4, pH3) over 20 min at a flow rate of 1 mL/min. Column temperature was maintained at 40 °C and the injection volume was 10 μL (Chen et al., 2005)

2.3.2. *Determination of MCs by ELISA method*

For screening samples containing low concentrations of toxins, ELISA was used. The sensitivity of this method was 0.1 ng/mL (Lei et al., 2004). Briefly, microtiter plates were coated with MAB (monoclonal antibodies, 4.0 μg/mL) and incubated overnight at 4 °C, then blocked with blocking buffer 170 μL (0.5% (w/v) gelatin in phosphate-buffered saline (PBS)) by incubating for either 2 h in a microplate incubator at 37 °C or overnight at 4 °C. Aliquots (70 μL) of various concentrations of MC-LR and samples were preincubated at 37 °C, for 30 min, and an equal volume of biotinylated MC MAB (25 ng/mL) was added to the coated wells for 30 min. The plates were washed thoroughly, three times, with PBS-T using an Immunowash apparatus. HRP-streptavidin (Sigma) diluted 10,000 × in dilution buffer (PBS containing 0.5% (w/v) gelatin and 0.05% (v/v) Tween 20) was added and incubated for 30 min at 37 °C. Enzyme reaction was initiated by adding a substrate solution (0.1 M sodium acetate buffer (pH 5.0) containing 100 μg/mL of TMBZ and 0.005% (v/v) H_2O_2) and stopped with 1 M H_2SO_4. Absorbance at 450 nm was measured with a microtiter plate reader (Ueno et al., 1996; Lei et al., 2004).

2.3.3. *LC/MC determination of MCs*

LC/MS analyses were performed using a Hewlett Packard (Palo Alto, CA, USA) HP-1100 Series system equipped with a binary solvent pump, an autosampler and a mass spectrometry detector (MSD) coupled to an analytical work station. A Kromasil ODS column (150 mm × 4.6 mm i.d., 5 μm, Sweden) was used for LC separation. The column oven temperature was 40 °C, the flow rate was 0.4 mL/min and the injection volume was 10 μL. Methanol and water, containing 0.1% formic acid were used as the mobile phases. The fraction of methanol was linearly increased from 35% to 90% over 25 min, then increased to 100% over 0.5 min and held for 4 min, followed by a return to 35% where it was held for 5 min prior to the next injection. The positive mode electrospray

ionization in multiple-reaction monitoring mode (MRM) was operated at 350 °C gas temperature, 10.0 L/min drying gas flow, 60 Psi nebulizer gas pressure and 4000 V capillary voltage. MS detection was recorded from 0 to 22 min, m/z 519.9 for MC-RR, m/z 1045.5 for MC-YR and m/z 995.6 for MC-LR (Moreno et al., 2005). The detection limits for the three MC variants ranged from 0.05 to 0.1 μg/g for biological samples, and 0.05 to 0.15 μg/L for water samples, respectively. Data for water and biological samples were expressed as means and standard deviations derived from two independent experiments using duplicate determinations for each sample ($n = 4$).

2.4. Statistical analysis

The correlation matrix between toxin concentrations in algae blooms and the physical-chemical parameters were calculated by SPSS software, version 13.0 for Windows (Chicago, USA). Toxin concentration comparisons in different organs of aquatic animals were conducted by one-way analysis of variance (ANOVA), followed by Bonferroni tests to identify the sources of detected significance. In all cases, comparisons that showed a p value <0.05 were considered significant.

3. Results

3.1. Distributions of intracellular and dissolved MCS in water columns

Cyanobacteria, composed mainly of colonial *Microcystis* were dominant in phytoplankton samples collected from Lake Taihu during the bloom period (May–October). As indicated in Fig. 1, the cyanobacteria biomass ranged from 6.4×10^6–6.7

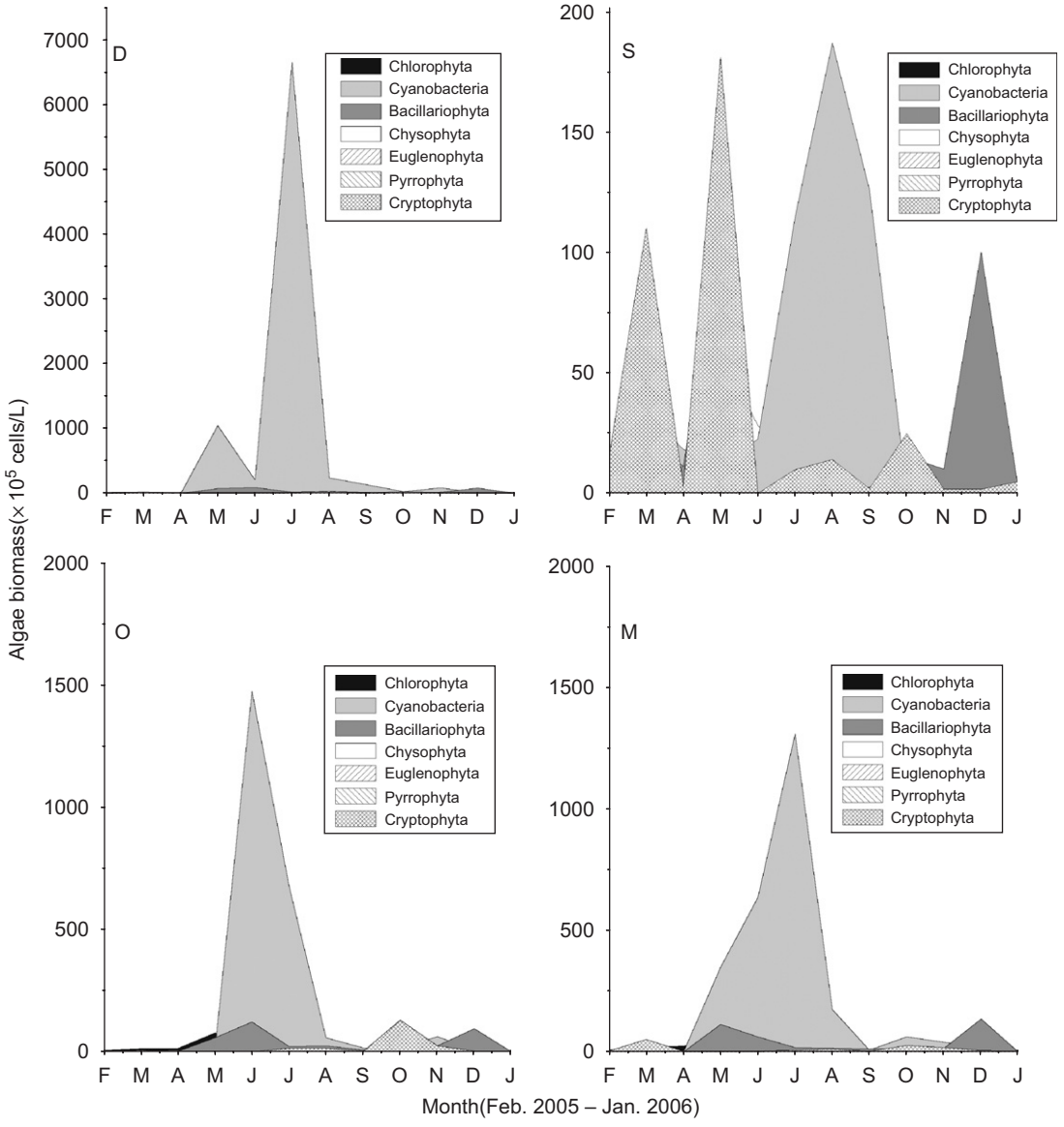

Fig. 1 – Abundance and composition of phytoplankton in Meiliang Bay, Lake Taihu, from February 2005 to January 2006. D, S, O and M represented the four sampling sites (Sites S, D, O and M), respectively.

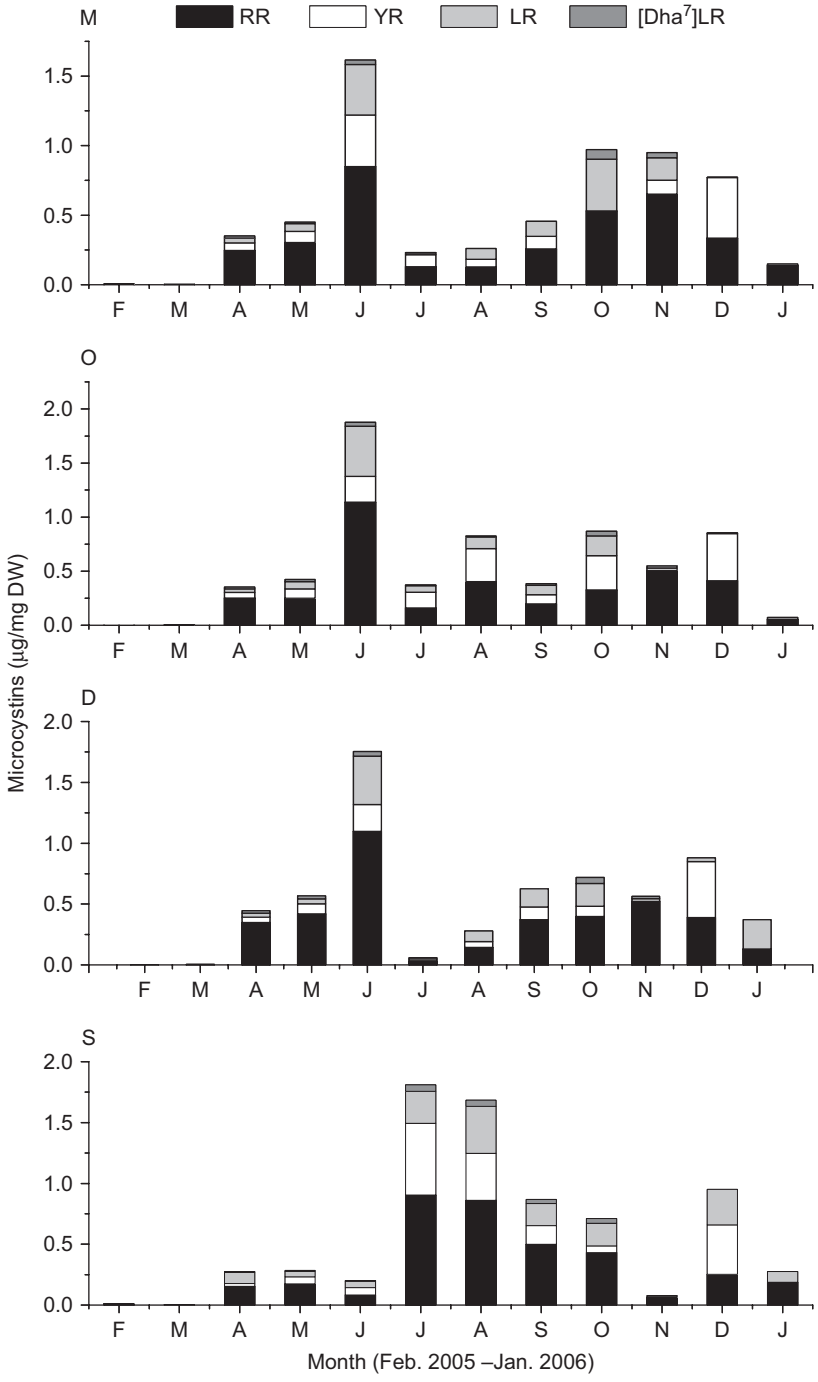

Fig. 2 – Concentrations of four microcystin variants (RR, YR, LR and Dha^7 LR) in the naturally occurring algal blooms in Meiliang Bay, Lake Taihu, from February 2005 to January 2006. Detection limit of HPLC method in the present study is 0.05 μg/ml. Data are expressed as the mean value of two independent experiments with duplicate determinations. D, S, O and M represented the four sampling sites (Sites S, D, O and M), respectively.

$\times 10^8$ cells/L for the phytoplankton samples in site D, 1.3×10^5–1.8×10^7 cells/L in site S, 1.8×10^7–1.7×10^8 cells/L in site O and 3.4×10^6–1.3×10^8 cells/L in site M. Heavy cyanobacterial blooms were primarily attributed to *Microcystis* spp. (90% or more), and only a few other species coexisted except occasionally, during the cool season, when bacillariophyta and cryptophyta were dominant. Annual variations of intracellular MCs in the algae blooms from Meiliang Bay of Lake Taihu during 2005 are shown in Fig. 2. In all four sampling sites, MC-RR, MC-LR and MC-YR are the main toxin species, and, in most cases, the proportion of MC-RR is more than 50%. The highest concentrations of MCs occurred in June from sampling sites D, O and M, at 1.75, 1.87 and 1.62 mg/g (DW), respectively. The highest

concentration for sampling site S was recorded in July (1.81 mg/g (DW). Toxin species and concentrations varied considerably each month. To learn the mechanisms of the frequent variation, the correlations between toxin concentrations and the physical-chemical parameters were analyzed (data not shown). Results showed that significant positive correlations ($p<0.01$) could be found between the concentrations of each toxin variant and water temperature, chlorophyll *a*. Simultaneously, toxin concentrations are significantly correlated ($p<0.01$) with total concentration of phosphorus.

Toxin distributions in the surface water as well as at the bottom of the water column were tracked in the large, shallow lake during the course of both bloom and non-bloom seasons. Dissolved MCs were always found in the entire water column in all sampling sites throughout 2005, and toxins were detected in both surface water and over-sediment water (Fig. 3), Surface water toxin concentrations were measured at 0–2.71, 0–6.69, 0–2.84 and 0–2.3 μg/L for sites S, D, O and M, respectively. Over-sediment water toxin concentrations were 0–1.64, 0–3.59, 0–1.62 and 0–3.56 μg/L for samples from site S, D, O and M, respectively. Concentrations of dissolved MCs in site D were evidently higher than in others during bloom seasons. Furthermore, for each site, toxin concentrations in surface water were slightly higher than in over-sediment water samples.

3.2. *Bioaccumulation of MCs in aquatic organisms from Meiliang Bay, Lake Taihu*

The accumulated MCs in the four species of hydrophytes (*E. crassipes*, *Potamogeton maackianus*, *A. philoxeroides* and *Myriophyllum spicatum*) collected from site O, ranged from 129.4 to 1316.9, 146.7 to 1534.1, 169.1 to 3944.7 and 124.2 to 955.6 ng/g (DW), respectively, in 2005 (Table 1). From February 2005 to January 2006, the concentration of the accumulated MCs in the liver of *Carassius auratus auratus Linnaeus* (fish) ranged from 461.8 to 3628.6 ng/g (DW), and in the muscle, ranged from no detectable concentration to 377.8 ng/g (DW) (Fig. 4A). Accumulated MC concentrations in fish liver and muscle varied from month to month, reaching the highest concentration in either June or July, and the accumulated toxin concentrations in the liver of this species were always significantly higher ($p<0.05$) than those accumulated in the muscle (several times to more than forty times, Fig. 4A). Fig. 4B shows the accumulated MCs concentrations in the abdomen and cephalothorax of *Macrobrachium nipponensis* (shrimp), with maximum values of 388.9 and 754.3 ng/g (DW), respectively. In some months, toxin concentrations in the cephalothorax were significantly higher than those in the abdomen of the *Macrobrachium nipponensis* collected from Lake Taihu ($p<0.05$). MCs concentrations accumulated in *B. aeruginosa* (snail) are presented in Fig. 4C. In the foot, toxin

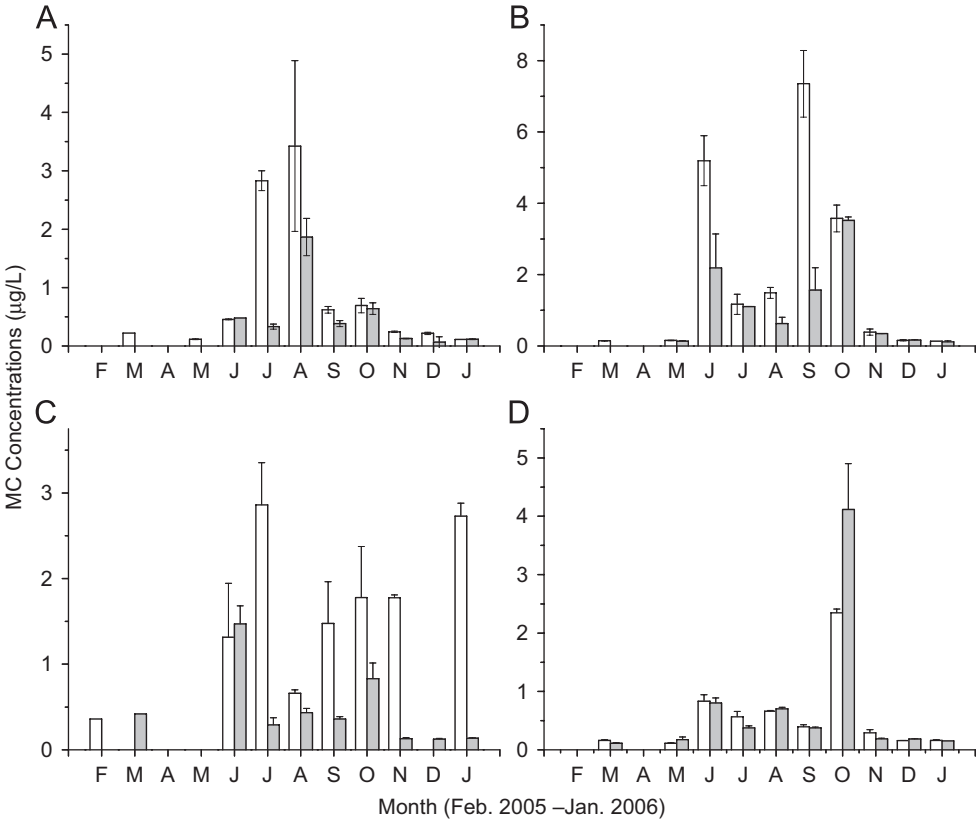

Fig. 3 – Distributions and variations of dissolved microcystins in surface water (open bars) and over-sediment water (gray bars) in Lake Taihu from February 2005 to January 2006. Data were expressed as the mean value and standard deviations of four replicate determinations. A, B, C and D represented the results from the four sampling sites (Sites S, D, O and M), respectively.

Table 1 – Accumulation of microcystins in aquatic macrophytes collected from Meiliang Bay, Lake Taihu, from February 2005 to January 2006

Sapling date	Aquatic plant	MCs concentration (ng/g DW)
05-02-26	*Myriophyllum spicatum*	124.2±19.1
05-03-27	*Myriophyllum spicatum*	436.4±44.5
05-05-27	*Myriophyllum spicatum*	157.1±6.7
05-06-27	*Myriophyllum spicatum*	197.8±31.4
05-07-27	*Myriophyllum spicatum*	589.2±23.4
05-08-28	*Myriophyllum spicatum*	715.8±89.3
05-09-27	*Myriophyllum spicatum*	955.6±52.5
05-10-28	*Myriophyllum spicatum*	252.5±13.9
05-11-27	*Myriophyllum spicatum*	172.3±39.1
05-12-27	*Myriophyllum spicatum*	553.8±174.1
05-05-27	*Alternanthera philoxeroides*	481.9±44.3
05-06-27	*Alternanthera philoxeroides*	3944.7±409.3
05-08-28	*Alternanthera philoxeroides*	650.2±13.1
05-11-27	*Alternanthera philoxeroides*	270.7±10.3
05-12-26	*Alternanthera philoxeroides*	169.1±18.7
05-02-26	*Potamogeton maackianus*	129.4±12.7
05-05-27	*Potamogeton maackianus*	183.3±14.7
05-08-28	*Potamogeton maackianus*	1316.9±414.7
05-11-27	*Potamogeton maackianus*	160.3±47.8
05-08-28	*Eichhornic crassipes*	670.6±20.8
05-09-27	*Eichhornic crassipes*	1534.1±130.1
05-10-28	*Eichhornic crassipes*	476.8±18.4
05-11-27	*Eichhornic crassipes*	146.7±28.3

Toxin concentrations in aquatic macrophytes are expressed as mean±S.D. of four replicate determinations.

concentrations ranged from below detectable levels to 380.8 ng/g (DW). More toxins were detected in the visceral sac, with the maximum concentration reaching 2315.4 ng/g (DW). In another native species of clam, *Cristaria plicata*, the accumulated MCs in the foot reached 730.3 ng/g (DW) in July, and high concentrations of MCs could be detected in nearly every sample during 2005 (Fig. 4D).

3.3. *Potential risks of toxic blooms and MCs in Meiliang Bay, Lake Taihu*

To evaluate the potential health risks caused by MCs, we adopted the WHO provisional guideline value of 1.0 μg/L for drinking water. Most of the water samples collected during boom seasons from the large bay of Lake Taihu, as is indicated in Fig. 3, contained high concentrations of dissolved MCs (several times higher than the guideline value by WHO), with a maximum of 7.4 μg/L in the surface water from site D in September. Moreover, a great number of cyanobacterial cells (containing intracellular toxins) in lake water have not been calculated yet (Fig. 2). To learn the potential health risks of aquatic product consumption based on Chinese dietary habits, risk assessments were carried out on the edible organs of four native aquatic animals. The estimated daily intake of MCs for an adult weighing 60 kg ingesting 300 g of edible organs of aquatic animals is shown in Fig. 5. Compared with the TDI, 0.04 μg/kg body weight per day, 89.5% of aquatic animals in Lake Taihu were not deemed safe for consumption (Fig. 5) due to their having concentrations of MCs that were 3–22.8 times higher than the TDI.

4. Discussion

4.1. *Environmental fate*

The present study clearly demonstrated the extensive occurrence of MC-producing cyanobacteria in the seriously eutrophic bay of Lake Taihu. Current results suggest that Lake Taihu is widely affected by cyanobacterial blooms, except where interventions are being carried out to reduce contamination. To reduce toxin levels, strategies such as sediment dredging, planting aquatic macrophytes and stocking benthic animals have been implemented in the water area of Site S in the past few years. As is indicated from Fig. 1, the cyanobacterial biomass was considerably reduced comparing with the other three sites.

We detected strange phenomenon that frequent variations of toxin species and toxin concentrations in bloom samples from the same sampling site, even within short-term period, which could be also observed in previous studies (Watanabe et al., 1992; Park et al., 1993; Vezie et al., 1997). However, laboratory culture experiments performed to investigate the relationship between toxin production and cultural conditions in unicellular or colony *Microcystis* (Utkilen and Gjolme, 1995; Lehtimäki et al., 1997) do not explain why these frequent and substantial variations occur for either toxin concentrations or toxin species in the natural aquatic environment. In the present study, we have found that temperature and the total concentrations of phosphorus were significantly correlated ($p<0.01$) with toxin production when the effects of chemical, physical and climate conditions

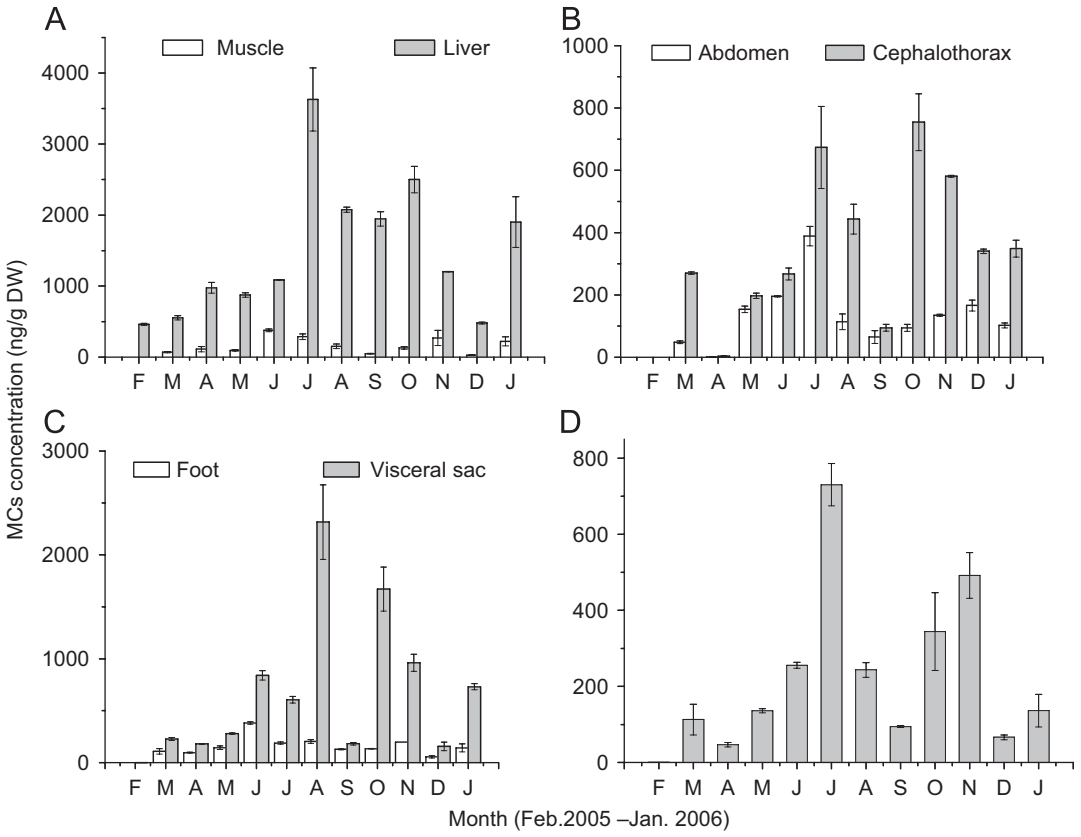

Fig. 4 – Accumulations of MCs (A) in the liver and muscle of *Carassius auratus auratus Linnaeus*; (B) in the abdomen and cephalothorax of *Macrobrachium nipponensis*; (C) in the foot and visceral sac of *Bellamya aeruginosa*; (D) in the foot of *Cristaria plicata* in Meiliang Bay, Lake Taihu, from February 2005 to January 2006. Data were expressed as the mean value and standard deviations of four replicate determinations.

are taken into consideration. However, further investigations into the effects of field conditions are still needed to understand why these frequent variations occur.

In previous reports, the concentration of MCs in bloom samples ranged from several μg/g (DW) to several mg/g DW (Sivonen and Jones, 1999). Park et al. (1993) detected a concentration of 622 μg/g (DW) MCs using HPLC, in cyanobacterium-dominant bloom samples collected in a Japanese lake. In another Japanese lake (Lake Kasumigaura), MC concentrations in the *Microcystis* blooms (the percentage of *Microcystis* biomass is more than 99% in bloom samples during warm seasons) also determined by HPLC, ranged from 160 to 950 μg/g (DW), during 1990 (Watanabe et al., 1992). In the current study, nearly 1.9 mg/g DW was detected in cyanobacterial blooms in Lake Taihu, relatively high toxin concentrations compared to MC-concentrations detected in Chinese and Japanese freshwaters so far. Moreover, this was the same lake in which Shen et al. (2003) reported concentrations of cell-bound MCs ranging from 54.91 to 96.58 μg/g (DW) during bloom seasons. After only 4 years, the MC concentration in cyanobacterial blooms is now nearly 20 times higher. However, due to the lack of phytoplankton data in Shen's investigation, it is difficult to estimate whether the increased MC concentration was caused by the changes in the toxin-producing ability of *Microcystis* or the changes in phytoplankton species composition.

High toxin concentrations for dissolved MCs were recorded from September to October immediately after the breakdown of a major bloom, and this observation is consistent with previous studies (Lindholm and Meriluoto, 1991; Jones and Orr, 1994; Ueno et al., 1996; Aboal and Puig, 2005). In the field, healthy bloom populations produced few extracellular toxins. Jones and Orr (1994) proposed that in freshwater lakes or rivers, once toxins are released from cells, they would be rapidly diluted by the large volume of water, especially if the mixing of water by wind action or currents was vigorous. This might explain why the measured range of concentrations for dissolved cyanotoxins usually falls between 0.1 to several μg/L, in most cases, remaining below 1 μg/L (Jones and Orr, 1994; Chorus and Bartram, 1999; Aboal and Puig, 2005) even in seriously polluted water bodies. Compared with toxin concentrations in other large freshwater bodies throughout the world, the nearly 7 μg/L of dissolved MCs in Lake Taihu merit an urgent calling for more attention to control water quality and reduce potential health risks. In this study, toxins were always detected in both the surface and bottom of the water column, indicating that, when tracking the environmental fate of MCs, the entire water column along with the interface between water and sediment should be taken into consideration. Moreover, differences in toxin distribution between the surface and over-sediment water seemed to imply that the

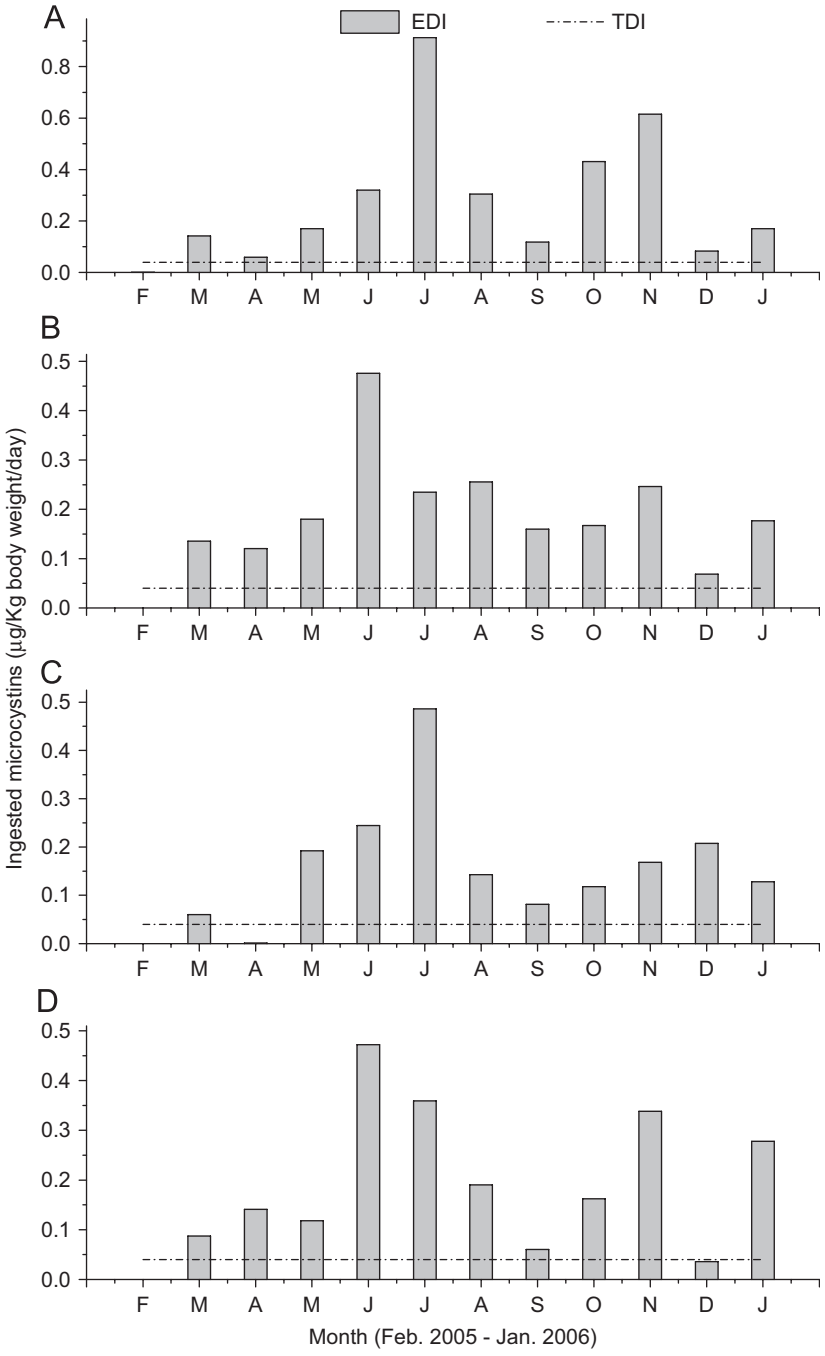

Fig. 5 – Estimated daily intake of microcystins (EDI) by a person consuming 300 g of esculent organs: (A) the foot of *Cristaria plicata* (*Leach*), (B) the foot of *Bellamya aeruginosa* (*peere*), (C) the abdomen of *Macrobrachium nipponensis*, (D) the muscle of *Carassius auratus auratus linnaeus* in aquatic products in Lake Taihu during 2005, The horizontal line indicates the maximum tolerable daily intake for human (TDI, tolerable daily intake, 0.04 μg kg^{-1} day^{-1}) proposed by the WHO (Chorus and Bartram, 1999).

sediment interface may have an effect on the natural elimination of MCs.

In a similar pattern to the detection of dissolved or cell-bound toxins, high concentrations of toxins were detected in the hydrophytes during bloom seasons. Toxin accumulations detected in the current investigation were close to those reported in past studies. For example, Pflugmacher et al. (1999) found that the submerged macrophyte *C. demersum* took up 1.98 ng/mg fresh wt. after 7 days exposure. In another study, Pflugmacher et al. (1998a) demonstrated the uptake of between 0.6% and 1.75% of the applied radiolabeled MC-LR in three rooted aquatic plants. MC-LR was taken up in a time-dependent manner with toxin found primarily in the leaf and shoot tissues, and less in the stem and roots. Other laboratory

and field studies on toxin accumulation in terrestrial and aquatic plants (Codd, et al., 1999; Mitrovic et al., 2005), suggested that dissolved MCs could be assimilated by plants. In addition, Pflugmacher et al. (1998b) described the detoxification of MCs using enzymes extracted from aquatic plants in laboratory and found that glutathione-S-transferase could react with MCs and degrade the added toxins. Consequently, we suggest that planting hydrophytes in eutrophic lakes and reservoirs might help reduce contaminations caused by cyanobacterial blooms and/or toxins, especially in the case of dissolved toxins.

In bioaccumulation studies, toxin concentrations in different organs of four aquatic animals harvested from Lake Taihu were examined. As the target organ of aquatic vertebrate (such as fish), the liver always accumulated most of the assimilated MCs (Malbrouck et al., 2003). In this study, it was also found that toxin accumulation in fish liver was significantly higher than that in muscle.

Similarly, since more visceral organs, including the stomach, are found in the cephalothorax, higher concentrations of accumulated toxins were found in the cephalothorax than that in the abdomen of freshwater shrimp *Macrobrachium nipponensis*. In a recent similar study of another eutrophic lake, Chen and Xie (2005) analyzed the toxin accumulations in different organs of freshwater shrimps *Palaemon modestus* and *Macrobrachium nipponensis* to determine the target organ. They also found that more than 10 μg/g (DW) of MCs in their stomachs, considerably more than we found in our test shrimps in Lake Taihu. These data indicate that the cephalothorax of shrimps should be removed prior to cooking in order to reduce health risks.

Freshwater mussels are probably among the most important filter feeding grazers in the lake ecosystem and as such, they are considered to be a useful tool in the restoration of shallow eutrophic lakes (Eriksson et al., 1989; Pires et al., 2004a). Not only can they assimilate dissolved toxins in the water column, but they can also remove cell-bound toxins through grazing on toxic cyanobacteria (Prepas et al., 1997; Pires et al., 2004b). Thus, toxin accumulation and depuration in aquatic organisms are considered to be very important mechanisms for natural elimination of MCs in the water column (Cazenave et al., 2005). However, this process is fairly limited in Lake Taihu due to the low biomass of aquatic plants and animals in the highly polluted bay. In another study of Lake Taihu during the summer of 2005, we found that photodegradation process by sunlight in field conditions could not efficiently eliminate dissolved toxins due to weak photo irradiation and dense blooms in the water column (unpublished data). Therefore, more attention should be focused on the bacterial degradation process in order to trace the environmental fate of MCs in field conditions. As indicated in Fig. 4, MCs were still detectable in aquatic organisms, even when the concentrations of dissolved toxins were very low and very few cyanobacteria could be detected. This phenomenon suggests that the accumulated toxins could persist for a long time in aquatic organisms, although complex detoxification systems exist (Pflugmacher et al., 1998b, 1999). Furthermore, long-term exposure to MCs in the field environment is expected to have adverse effects on the detoxification capabilities of aquatic organisms (Chen et al., 2005). The concentration of accumulated toxins in the present study is similar to those in the previous reports (Eriksson et al., 1989; Vasconcelos, 1995; de Magalhães, et al., 2001; Soares et al., 2004; Chen and Xie, 2005).

4.2. *Risk assessment*

The main pollutants in the large lake are nutritive substances, including nitrogen, phosphorus and organic carbons, as opposed to industrial chemicals (Pu et al., 1998). In China, nearly all drinking water plants used the traditional procedures for water treatment (flocculation, deposition, percolation and disinfection (primarily ClO_2)), and water from water supplies cannot be consumed directly without boiling. However, it is well known that neither the above treatment procedures nor boiling can efficiently remove MCs from drinking water. Therefore, it is crucial to perform risk assessments to avoid potential health risks. For drinking water, a provisional guideline value of 1.0 μg/L has been adopted by WHO for MC-LR to protect public health (WHO, 1998). In order to derive the guideline value, a TDI with a value of 0.04 μg/kg body weight per day, an average adult body weight of 60 kg and an average water intake for adults of 2 L/day were used (Falconer et al., 1999; Falconer, 1998). Based on these results, water from our sampling sites can no longer be used as a source of drinking except in areas where water treatment is taking place and cyanobacterial cells (without rupture) as well as cyanotoxins are removed. However, Lake Taihu is currently a major drinking water resource for many cities around the Taihu basin, and this is regarded unsafe for long-term consumption. In addition to this, Lake Taihu also serves as an important recreational water facility for entertainments and sports, such as swimming and water-skiing. For recreational water, a density of 100,000,000 cyanobacterial cells per liter in the water column (which is equivalent to approximately 50 μg/L of chlorophyll *a* if cyanobacteria dominate) is a guideline for health risk alerts in the water columns (Falconer et al., 1999). The detected cyanobacterial cells in some water samples, especially for the samples in site D, far exceeded the guideline value, reaching amounts as high as 6.7×10^8 cells/L during the summer months in 2005 (Fig. 1). Thus, potential health risks may exist where recreational users are exposed to the water in areas around the sampling sites in Lake Taihu.

Many studies had been carried out in the past to investigate toxin accumulations in aquatic vertebrates and invertebrates, but most studies were of toxicological concern with the main objectives of determining the target organs in aquatic organisms (Carbis et al., 1997; Chen and Xie, 2005). Whether the levels of MC accumulation in aquatic products (fish or shellfish) were sufficient to pose health risks to human consumers remained uncertain until now. Water resource authorities generally recommended that the visceral parts of freshwater lake fish and shellfish should not be eaten. Freshwater clams and snails are more popular food sources in China than in most other countries (Ozawa et al., 2003), therefore, more attention should be focused on the potential health risks associated with the consumption of these food items. Risk assessments addressing toxin accumulations in the edible organs of aquatic products indicate that it is likely

to be unsafe to consume aquatic species harvested from seriously polluted areas of Lake Taihu due to the high-concentration accumulation of MCs.

5. Conclusions

1. Results from this study suggest that Lake Taihu is heavily affected by toxic cyanobacterial blooms during summer months. During 2005, high concentration of intracellular and extracellular MCs could be detected in Meiliang Bay, Lake Taihu. Also a considerable amount of toxins were found to be accumulated in native species of hydrophytes and aquatic animals.
2. Based on the findings from this study, the large lake, in its current condition, is losing the original function as an important natural resources and natural filtering system. When serving as a water resource for drinking water or recreational use, potential health risks must be taken into consideration. In addition, it is most likely unsafe to consume aquatic animals harvested in heavily polluted regions of Lake Taihu due to the high concentration of accumulated MCs found in the organisms.
3. In order to reduce or avoid the health risks, continuous monitoring of toxins and toxic cyanobacterial blooms in both water columns as well as in aquatic organisms should be increased and maintained. Up to date monitoring results on toxin contamination should be provided for water suppliers and visitors to recreational sites. On the other hand, strategies for algae control, toxin removal, and ecosystem restoration must be improved and implemented to control further deterioration of the water quality in Lake Taihu.

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